Effects of Starvation and Molting on the Metabolic Rate of the Bed Bug (*Cimex lectularius* L.)

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ABSTRACT

The bed bug (*Cimex lectularius* L.) is a common hematophagous pest in the urban environment and is capable of surviving extended periods of starvation. However, the relationship between starvation and metabolism in bed bugs is not well understood. To better understand this relationship, we measured the metabolism of all life stages for >900 h after feeding (starvation) using closed-system respirometry. Measurements were made around molting for the immature life stages, which occurs only after a blood meal. In addition, both mated and unmated adults were measured. Starvation and molting had significant effects on the metabolism of the bed bug. Mass-specific metabolic rate ($\dot{V}O_2$; mL g^{-1} h^{-1}) declined in a curvilinear fashion with the period of starvation for adults and with the postmolting period for immature bed bugs (used to standardize all immature life stages). A standard curve was developed to depict the generalized pattern of metabolic decline observed in all life stages that molted. Individual metabolic comparisons among life stages that molted revealed some differences in metabolic rate between unmated males and females. In addition, the mass scaling coefficient was found to decline with starvation time (postmolting time) for all life stages that molted. In most life stages, the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$ (respiratory exchange ratio) declined over time, indicating a change in metabolic substrate with starvation. Finally, daily percent loss in body mass declined in a pattern similar to that of $\dot{V}O_2$. The observed patterns in metabolic decline are evaluated in relation to the life history of bed bugs. In addition, the evolutionary development of these patterns is discussed. The metabolic pattern after feeding was also found to share several similarities with that of other ectothermic species.

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Introduction

Starvation presents a difficult physiological challenge for those animals that experience it. During starvation, most animals are incapable of producing their own energy and therefore must rely on previously acquired and stored energy reservoirs. The ability of an organism to survive starvation is determined primarily by three factors: the amount of energy available at the onset of starvation, the rate of energy use during starvation (metabolism), and the energy reserve state below which physiological functions can no longer occur (Wang et al. 2006). Of these factors, only metabolic rate can be modified by organisms entering unanticipated periods of starvation. Metabolic comparisons among ectothermic species are generally made via the standard metabolic rate, a measure of the metabolic rate of an ectothermic species that is in a postabsorptive state (no longer digesting food), at rest, and at a defined temperature (Lighton and Fielden 1995; Vogt and Appel 1999; Moyes and Schulte 2008; DeVries and Appel 2013). Unfortunately, ectothermic species that commonly experience prolonged periods of starvation are often extremely difficult to define as being postabsorptive. Furthermore, these species often experience metabolic depression during periods of starvation, making standardized metabolic measurements even more difficult because these depressions are not always consistent among species (Zwicky and Wigglesworth 1956; Bradley et al. 2003; Schimpf et al. 2012). Metabolic depression during starvation may also be responsible for the inconsistent results of metabolic studies attempting to understand the relationship between metabolic rate and survival during starvation (Rixon and Stevenson 1957; Schimpf et al. 2012; DeVries and Appel 2013).

In addition to starvation, molting may also impact the metabolism of ectothermic species. Molting is required for growth and development and occurs across a number of animal taxa. Molting has discontinuous energetic costs because of associated events such as hormone production, apolysis, digestion of old cuticle, and production of new cuticle (Chapman 1998). In addition, this process must occur rapidly for the organism to avoid both predation and desiccation. Because of the association between molting and feeding in many arthropods, it is important to consider molting and starvation together when measuring metabolism in any nonmature arthropod.

Several studies have investigated the effects of fasting on metabolism in a variety of ectothermic species (Young and

Block 1980; Yang and Somero 1993; Mehner and Wieser 1994; Fielden et al. 1999; Bedford and Christian 2001; Trzcionka et al. 2008). However, at present few ectothermic species have had their metabolism fully characterized during periods of starvation or molting. Zwicky and Wigglesworth (1956) measured the metabolic rate of a hematophagous insect, *Rhodnius prolixus* Stål, during periods of feeding, molting, and starvation. Their results indicated large increases in metabolism after feeding and during molting followed by a rapid decline after molting. Bradley et al. (2003) also measured the metabolism of *R. prolixus* after feeding, finding results similar to those of Zwicky and Wigglesworth (1956). However, Bradley et al.'s (2003) use of CO_2 to measure metabolic rates complicates their results because the conversion of CO_2 production to energy is more substrate dependent than the conversion of O_2 consumption to energy, and metabolic substrate has been shown to change during starvation (Sinclair et al. 2011). In another study, Defur (1990) measured the metabolic rate of the blue crab (*Callinectes sapidus* Rathbun), an omnivorous aquatic arthropod. Defur (1990) found a peak in metabolism during molting, which is important to all arthropods because of the ubiquity of molting in these taxa. Finally, Secor and Diamond (1997) measured the metabolism of the Burmese python (*Python molurus* L.), a sit-and-wait predator. Their work characterized the changes in metabolism after feeding on meals of various sizes. The effects of starvation and molting on the metabolism of the bed bug (*Cimex lectularius* L.) have not been measured.

Bed bugs are ectoparasites of endothermic species and are capable of surviving prolonged starvation (Usinger 1966). The length of survival during starvation depends on life stage, temperature, and relative humidity and can range from a month to over a year (Usinger 1966). Bed bugs cycle through successive feeding and molting events, with a blood meal required for molting to occur (Usinger 1966). Reproduction is also closely associated with feeding; a blood meal is required for continued egg and sperm production (Usinger 1966). While experiencing long-term starvation, bed bugs alter their behavior, including a reluctance to aggregate over time (Olson et al. 2009) and decreased activity during the scotophase (Romero et al. 2010). These behaviors likely prolong bed bug survival by reducing nonessential energy costs (aggregation/random host searching) and focusing more energy into stimulated host searches (Olson et al. 2009; Romero et al. 2010; Reis and Miler 2011). However, it is still unclear what occurs metabolically to accompany these behavioral changes that enable bed bug survival during extended periods of starvation. Bed bug metabolism has received very little attention; Mellanby (1932) and Rao (1973) are the only studies of bed bug metabolism. However, neither of these studies addressed changes in metabolic rate during starvation and molting.

In the present study, we measured the metabolic rate of bed bugs for >900 h (37.5 d) of starvation. This period included molting and hatching in nymphal bed bugs. Changes in O_2 consumption, CO_2 production, and respiratory exchange ratio (RER) were calculated. Also, we corrected for bed bug movement, so only individuals that exhibited little to no movement were included in the study because movement can impact respiratory measurements considerably (Bartholomew et al. 1985; Lighton and Feener 1989; Lighton and Duncan 1995). In addition, the effects of starvation on mass loss were determined.

Material and Methods

Experimental Animals

Insecticide-susceptible bed bugs originally obtained from i2L Research (Baltimore, MD) were reared at the University of Minnesota, Twin Cities, as described by Olson et al. (2009). In brief, bed bugs were reared at 23° ± 2°C, 55% ± 5% relative humidity, and 14L : 10D in 0.5-L glass jars that contained filter paper harborage and a mesh top to allow ventilation. Bed bugs were fed human blood (1 : 1 combination of red blood cells and plasma), purchased from the American Red Cross (St. Paul, MN), that had expired for human use. Feeders supplied blood through a Parafilm membrane (American National Can, Chicago, IL).

Bed bugs were shipped to Auburn, Alabama, via overnight delivery immediately after feeding. Once the bed bugs arrived, they were placed into respirometry chambers and allowed a minimum of 24 h to acclimate. In between experiments, bed bugs were kept in respirometry chambers under conditions identical to those described above. Masses of bed bugs changed throughout the experiment (with starvation); however, the masses of bed bugs tested ranged from 0.41 mg (groups of younger bed bugs) to 7.93 mg (single, fed adult bed bugs).

Respirometry Measurements

Different instars were received and tested in a randomized order by instar. Once received, bed bugs were immediately placed into respirometry chambers constructed of 3-mL plastic syringes that contained one small (0.775 × 0.375 mm^2) piece of cardstock paper to provide harborage and keep bed bugs quiescent. The syringes had six holes (1.4 mm in diameter) drilled past the last gradation. After the 24-h acclimation period, the syringes were placed on a manifold where dry, CO_2-free air was forced through at a rate of 230 mL min^{-1} for a minimum of 6 min. This process purged CO_2 and H_2O from the syringe, reducing their respective concentrations to unmeasurably low levels. After purging was complete, a 26-gauge needle was attached to the syringe, and the volume was adjusted to a known level (depending on the life stage and starvation time) by pushing the plunger past the drilled holes to the desired volume. The syringe was then sealed by attaching a rubber stopper (size 000) to the needle. Each syringe was then placed into an incubator at 25°C. Incubation times varied widely (2–30 h), depending on the size (mass) of the bed bug and the postfeeding period. The incubator was illuminated with a red light (20 W), and animals were moni-

tored for movement via a video camera (DCR-SX85; Sony, Tokyo). Video recordings (MPEGs) were reviewed manually at a rate of 1 min of video per second of viewing time with Windows Media Player Classic (ver. 6.4.9.1). After incubation was complete, an air sample from each syringe was injected into the respirometry system (described below) that permitted measurement of O_2 consumption and CO_2 production. After testing, each animal was weighed to the nearest 0.01 mg using a digital balance (AX205; Mettler-Toledo, Greifensee, Switzerland). To adjust for any possible leakages, especially during the longer incubation periods, we used control syringes with every group tested. These syringes were treated identically to the experimental syringes except that they contained no animals. After injections were complete, any residual leakage of O_2 or CO_2 into or out of the control syringes was used to adjust the values in the experimental syringes. On average, CO_2 leakage was less than 5% of the total measured CO_2 in experimental syringes, and O_2 leakage was not measurable. In addition, the partial pressure changes of the gasses in the syringes showed little change during the experiments. CO_2 rose from 0% (start) to an average of 0.46%, while O_2 began at 20.95% (initial, room air) and fell to an average of 20.28%.

The respirometry system used to measure O_2 and CO_2 was similar to that described by DeVries and Appel (2013) and was operated as follows. Before entering the respirometry chamber, air was forced into a purge gas generator (Whatman; Haverhill, MA), where it was scrubbed of both CO_2 and H_2O. Next, the dry, CO_2-free air was forced into a 340-L mixing tank and then into an open manifold, where it equalized to atmospheric pressure. The air was then pulled through a Drierite-Ascarite-Drierite column (W. A. Hammond Drierite Company, Xenia, OH; Thomas Scientific, Swedesboro, NJ) to ensure that the air stream was free of water vapor and CO_2. Next, the air was pulled past an injection port, where air samples were injected from each syringe after incubation was complete. This air then flowed through a Li-6251 CO_2 analyzer (LI-COR; Lincoln, NE) and another Drierite-Ascarite-Drierite column to remove CO_2 and water vapor before measuring O_2. Next, the air traveled to an Oxzilla II O_2 analyzer (Sable Systems, Henderson, NV) and a mass flow system (MFS2; Sable Systems), which pulled air through the system at a rate of 100 mL min^{-1} at STP. The exact time of incubation (from the removal of the respirometry chamber from the manifold to when the air sample was injected) was recorded for each bed bug.

Data were recorded and analyzed using Datacan V acquisition and analysis software (Sable Systems). Specifically, CO_2 and O_2 were converted to units of milliliters per hour, and the resulting peaks from the injections were integrated to determine the total CO_2 production and O_2 consumption for each animal. For more details and specific equations, please refer to Lighton (1991).

The Li-6251 CO_2 analyzer was calibrated using dry, CO_2-free air to 0 and 100-ppm CO_2 to span. The Oxzilla II O_2 analyzer (Sable Systems) was spanned to 20.95% using room air and was internally zeroed automatically.

Repeated-Measures Testing Schedule

Bed bug O_2 consumption and CO_2 production were measured daily from the day after they were received until 9 days after feeding; after this period they were tested intermittently for 30 d, although this time varied depending on survival. In addition, first instars were measured beginning with their egg stage, and they were measured two or more days apart because of the extended incubation time required to obtain measurable results. Approximately 10 replicates were measured repeatedly for each of the five nymphal instars and for adults of defined mating status and sex (e.g., unmated males, unmated females, mated males, mated females). Preliminary results revealed no differences between those bugs that had been tested multiple times and those that had been tested only once, so when bed bugs died, they were replaced by bed bugs of the same age and similar weight that had been starved an equal amount of time and allowed at least 24 h to acclimate in a respirometry chamber. This ensured that sample sizes remained relatively consistent throughout the experiments.

Metabolic Calculations

Several additional variables were calculated from the recorded data. Metabolic rate was measured and reported as $\dot{V}_{O_2}$ in milliliters per hour as well as in milliliters per gram per hour (mass specific). RER was calculated by dividing total CO_2 production by total O_2 consumption and is reported as a unitless value. Finally, to assess the changes in body composition, changes in mass were reported as percent mass loss per day. Use of percentages standardizes the measurement for all life stages and allowed for comparisons among all life stages.

Data Analysis

Because of the complex relationship between starvation and metabolism, we were unable to mathematically model this relationship. However, a detailed description of the pattern of metabolic decline was used to understand the effects of starvation on bed bug metabolic rate. Analysis of covariance (ANCOVA) was used to compare mated adult male and female $\dot{V}_{O_2}$ (mL h^{-1}) at several times after feeding, with mass serving as a covariate (Packard and Boardman 1999). Slopes relating $\dot{V}_{O_2}$ to mass for both sexes were confirmed to be homogenous at all postfeeding times compared: 48 h ($F_{1,18} = 2.70$, $P = 0.1176$), 192 h ($F_{1,18} = 1.27$, $P = 0.2761$), and 370 h ($F_{1,18} = 0.12$, $P = 0.7374$). ANCOVA was also used to compare $\dot{V}_{O_2}$ among all life stages that either hatched (first-instar nymphs) or molted (second- through fifth-instar nymphs, unmated adults) at several times after hatching/molting, with mass serving as a covariate (Packard and Boardman 1999). Specific differences among life stages were determined using the Tukey-Kramer

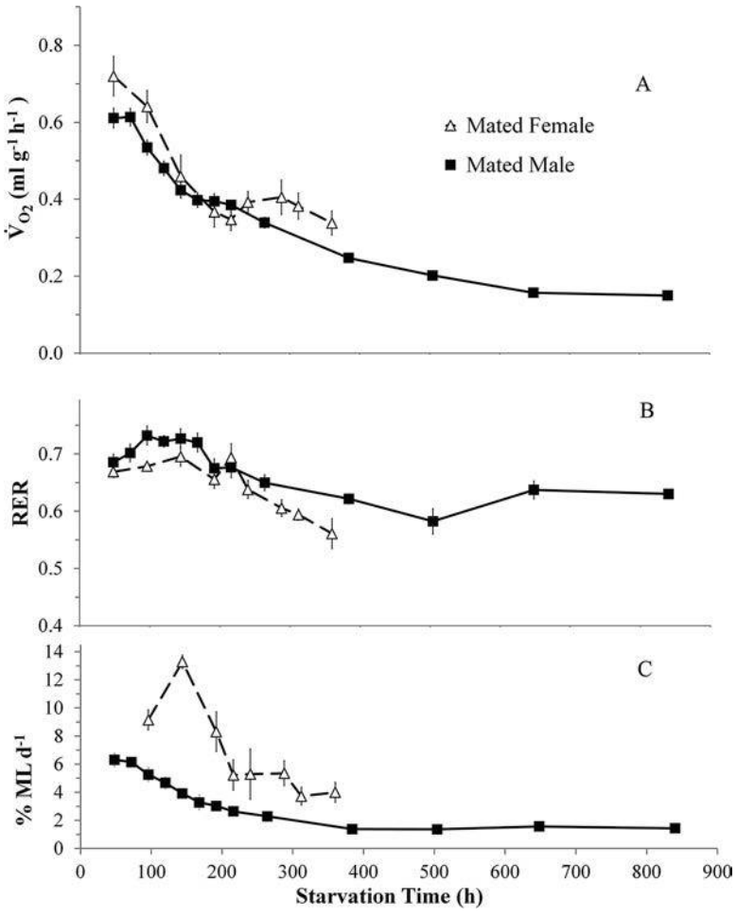

Figure 1. Effects of starvation time on several factors for mated adult males and females. Factors include $\dot{V}O_2$ (*A*), respiratory exchange ratio (RER; *B*), and percent mass loss per day (% ML d^{-1}; *C*). All values are reported as means ± SE.

multiple comparison test. Slopes relating $\dot{V}O_2$ to mass for all life stages that either hatched or molted were confirmed to be homogenous at all postmolting times compared: from −9 to 11 h ($F_{6,45} = 1.55$, $P = 0.1850$), from 128 to 155 h ($F_{6,48} = 0.50$, $P = 0.8056$), from 216 to 248 h ($F_{6,49} = 1.64$, $P = 0.1573$), and from 400 to 800 h ($F_{5,56} = 1.22$, $P = 0.3128$). In addition, metabolic rates ($\dot{V}O_2$) for all life stages that either hatched or molted were related to mass using simple linear equations. It should also be noted that both metabolic rate ($\dot{V}O_2$) and mass were log transformed before any statistical analysis.

RER and percent mass loss per day are reported and compared among life stages at specific times after feeding or molting by either a *t*-test or an ANOVA with a least significant difference (LSD) mean comparison test. All statistical tests were performed using SAS (ver. 9.1; SAS Institute 1985).

To track the effects of starvation and molting on metabolic rate, specific life stages were grouped to include the period from the end of the previous instar through molting and during the postmolt (starvation) period. For example, third-instar nymphs included the following: fed second-instar nymphs before molt, those molted to third instar, and those subjected to starvation while a third-instar nymph. Exceptions to this stage-specific classification were mated adult males and females (fed as adults, do not molt) and first-instar nymphs (never fed, hatched from eggs). Unmated adults were grouped by age in the same fashion as nymphal instars 2 through 5 but were isolated after feeding as fifth instars to ensure that no mating occurred once they eclosed to adults.

Results

Activity

A previous study by DeVries et al. (2013) showed that movement of <50 mm h^{-1} did not significantly affect bed bug metabolism. Therefore, all bed bugs that moved less than 50 mm h^{-1} were included in the study. When given at least 24 h to acclimate to their respirometry chamber, bed bugs generally had little to no movement, and only 22 samples (2.1% of all samples) were excluded because of movement.

Mated Adults: Metabolic Rates

Mated adult males and females were analyzed separately from their unmated counterparts (i.e., unmated adult males, un-

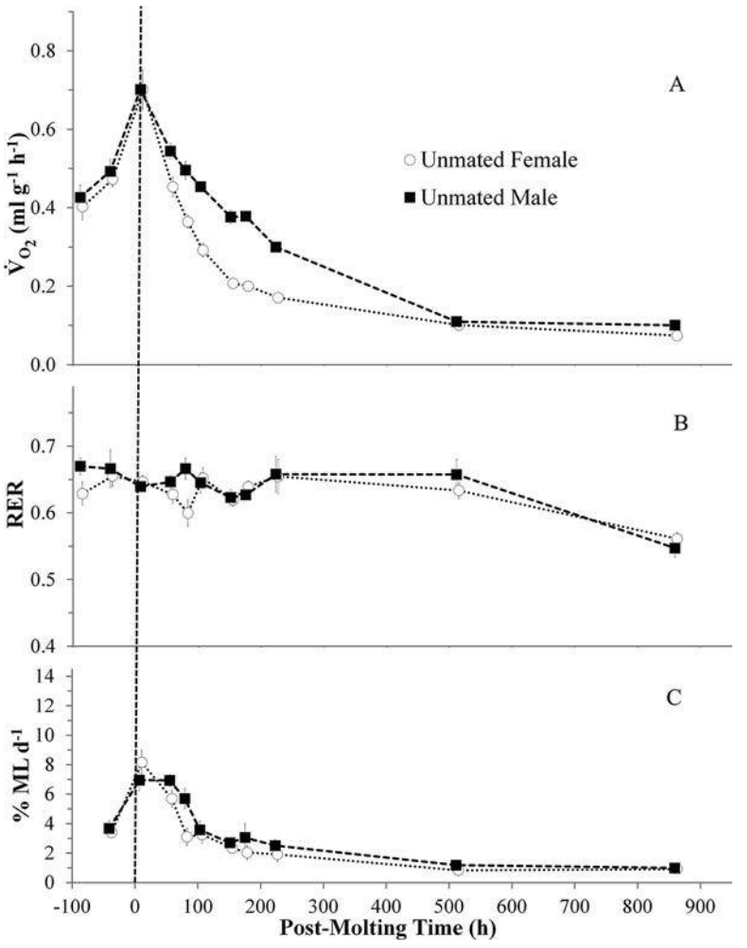

Figure 2. Effects of postmolting time on several factors for unmated adult males and females. Factors include $\dot{V}O_2$ (*A*), respiratory exchange ratio (RER; *B*), and percent mass loss per day (% ML d^{-1}; *C*). All values are reported as means ± SE.

mated adult females) and all nymphal instars because they were the only life stages measured that did not molt. The relationships between metabolic rate and starvation time can be described as a curvilinear decline (fig. 1*A*). However, closer examination reveals periods during starvation that depart from this curvilinear pattern (fig. 1*A*). The general metabolic pattern can be described as a peak in $\dot{V}O_2$ 0–48 h after feeding, followed by a rapid decline in metabolism until 168 (males) or 192 (females) h of starvation. At those respective times, both sexes showed an approximately 48-h period of stable $\dot{V}O_2$. After this period of stability, mated male $\dot{V}O_2$ continued to decline again, while mated female $\dot{V}O_2$ did not decline and remained relatively constant until 400 h (17 d), when the last measurement was recorded. After this time, >50% of mated females died, preventing further testing.

ANCOVA between mated adult males and females (sex as main effect, log of mass as covariate) showed that at 48 h after feeding (first recorded metabolic rate) females had significantly greater $\dot{V}O_2$ than males ($F_{1,19} = 5.88$, $P = 0.0255$). At 192 ($F_{1,18} = 0.57$, $P = 0.4601$) and 370 ($F_{1,19} = 0.31$, $P = 0.5849$) h after feeding, there were no significant differences between the sexes. At 370 h after feeding, a 59.5% reduction in $\dot{V}O_2$ for adult males and a 53.0% reduction in $\dot{V}O_2$ for adult females from $\dot{V}O_2$ measurements at 48 h was recorded.

Mated Adults: RER

RER decreased over time for both mated adult males and females, showing a negative relationship with time after feeding (fig. 1*B*). A *t*-test to compare mated adult males and female RER at specific times during starvation revealed no significant differences ($P > 0.05$) in RER between the sexes at any of the times compared (48, 192, and 370 h of starvation).

Mated Adults: Change in Body Mass

Percent mass loss per day showed different relationships with starvation time between mated males and females. The relationship for mated males showed a gradual decline in the rate of percent body mass loss with starvation time (fig. 1*C*). This trend displayed a pattern similar to that for the change in $\dot{V}O_2$ with starvation time for males. However, females displayed a

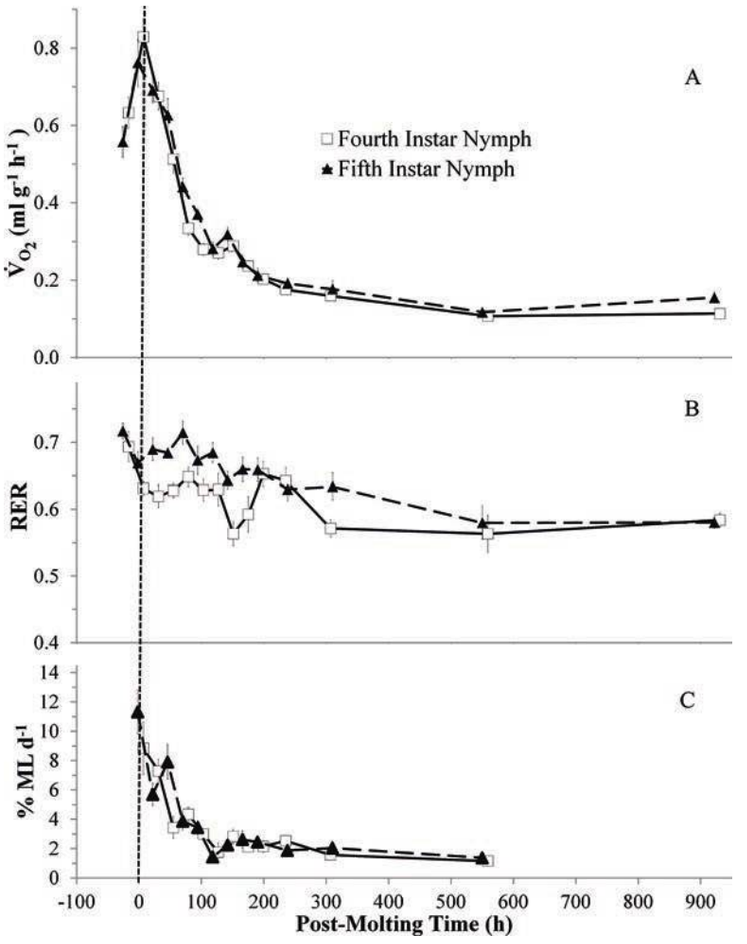

Figure 3. Effects of postmolting time on several factors for fourth- and fifth-instar nymphs. Factors include $\dot{V}O_2$ (*A*), respiratory exchange ratio (RER; *B*), and percent mass loss per day (% ML d^{-1}; *C*). All values are reported as means ± SE.

different relationship, characterized by a steep increase in percent mass loss per day at 144 h of starvation (fig. 1*C*). It should be noted that female mass loss included that associated with egg laying, and therefore no specific comparisons were made between the sexes.

Unmated Adults and Nymphal Instars: Metabolic Rates

The relationships between metabolic rate and time relative to molting for unmated adults and nymphal instars are representative of curvilinear relationships (figs. 2*A*, 3*A*, 4*A*). Because of the similarity among all life stages that molted after feeding, we developed an ideal curve depicting the general effects of time after molting on $\dot{V}O_2$ during starvation (fig. 5). We determined six important events in $\dot{V}O_2$ that occur during the time leading up to and after molting. The first step is an initial postfeeding but premolt increase in metabolic rate (fig. 5, a) leading up to a peak in $\dot{V}O_2$, when the bed bug molts (fig. 5, b). Next, $\dot{V}O_2$ declines rapidly (fig. 5, c) until reaching the initial plateau (fig. 5, d). The initial plateau occurs between 100 and 150 h after molting (slightly later in unmated adult females). After this plateau, $\dot{V}O_2$ declines gradually (fig. 5, e) until reaching the starvation plateau (fig. 5, f). The starvation plateau will persist until either the bed bug feeds and repeats the cycle or dies. Unmated adult males had a small initial plateau, and their curvilinear metabolic decline was less pronounced before and after the plateau (fig. 2*A*), unlike the other stages. First instars that hatched (fig. 4*A*) had a similar pattern but never reached the starvation plateau (fig. 5, f) because of mortality.

During the molt, from −9 to 11 h after molting (fig. 5, b), bed bug mass related to $\dot{V}O_2$ (mL h^{-1}) by the equation

$$\log_{10} \dot{V}O_2 = -0.001\ (\pm 0.090) + 1.052\ (\pm 0.031)\ \log_{10} \text{mass}$$

($F_{1,57} = 1{,}145.1$, $P < 0.0001$, $r^2 = 0.9526$). ANCOVA revealed life stage to have a significant effect on $\dot{V}O_2$ ($F_{6,51} = 2.92$, $P = 0.0159$); however, no pairwise differences were detected.

At the initial plateau (from 128 to 155 h after molting; fig. 5, d), bed bug mass related to $\dot{V}O_2$ by the equation

$$\log_{10} \dot{V}O_2 = -0.792\ (\pm 0.071) + 0.899\ (\pm 0.024)\ \log_{10} \text{mass}$$

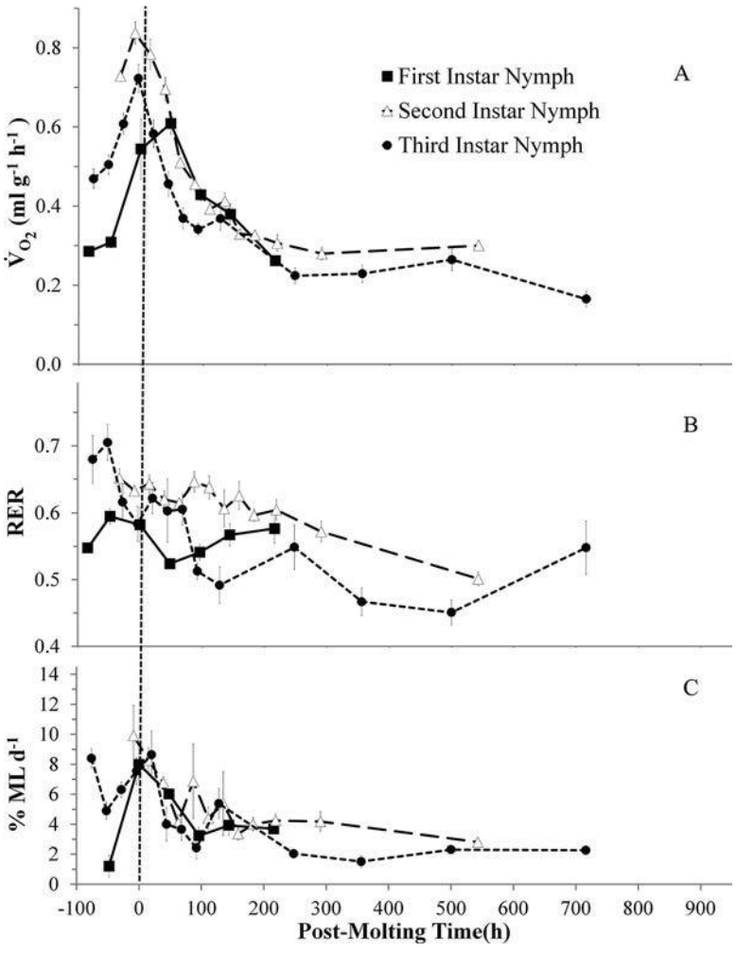

Figure 4. Effects of postmolting time on several factors for first-, second-, and third-instar nymphs. Factors include $\dot{V}_{O_2}$ (*A*), respiratory exchange ratio (RER; *B*), and percent mass loss per day (% ML d^{-1}; *C*). All values are reported as means ± SE.

($F_{1,60} = 1{,}422.2, P < 0.0001, r^2 = 0.9595$). ANCOVA revealed life stage to have a significant effect on $\dot{V}_{O_2}$ ($F_{6,54} = 10.71$, $P < 0.0001$). Pairwise comparisons revealed unmated males to have a significantly greater metabolic rate than unmated females ($P < 0.0001$), with all other life stages showing no significant differences.

During the start of the starvation plateau (from 216 to 248 h after molting; fig. 5, e, f), bed bug mass related to $\dot{V}_{O_2}$ by the equation

$$\log_{10} \dot{V}_{O_2} = -0.890\ (\pm 0.080) + 0.922\ (\pm 0.026)\ \log_{10} \text{mass}$$

($F_{1,61} = 1{,}225.6, P < 0.0001, r^2 = 0.9526$). ANCOVA revealed life stage to have a significant effect on $\dot{V}_{O_2}$ ($F_{6,55} = 12.76$, $P < 0.0001$). Pairwise comparisons revealed unmated males to have a significantly greater metabolic rate than unmated females ($P < 0.0001$), fifth-instar nymphs ($P < 0.0001$), and fourth-instar nymphs ($P = 0.0042$). All other pairwise comparisons were not significant.

During extended starvation (from 400 to 800 h after molting; fig. 5, f), bed bug mass related to $\dot{V}_{O_2}$ by the equation

$$\log_{10} \dot{V}_{O_2} = -1.884\ (\pm 0.121) + 0.653\ (\pm 0.040)\ \log_{10} \text{mass}$$

($F_{1,61} = 268.3, P < 0.0001, r^2 = 0.8026$). ANCOVA revealed life stage to have a significant effect on $\dot{V}_{O_2}$ ($F_{6,51} = 2.47$, $P = 0.0421$); however; no pairwise differences were detected.

It is also important to compare both mated and unmated adults. This is somewhat complicated because, in the present study, unmated adults molted from fifth instars during the study (fig. 2*A*). However, when evaluated visually the overall pattern of $\dot{V}_{O_2}$ with time after feeding or molting is similar between mated and unmated adult males, but this pattern is different between unmated and mated adult females (figs. 1*A*, 2*A*). Mated and unmated males both decrease in a similar pattern with starvation time and have similar metabolic rates during starvation. Unmated females, though, maintain a low metabolic rate throughout starvation, whereas mated adult females maintain a relatively higher metabolic rate during starvation.

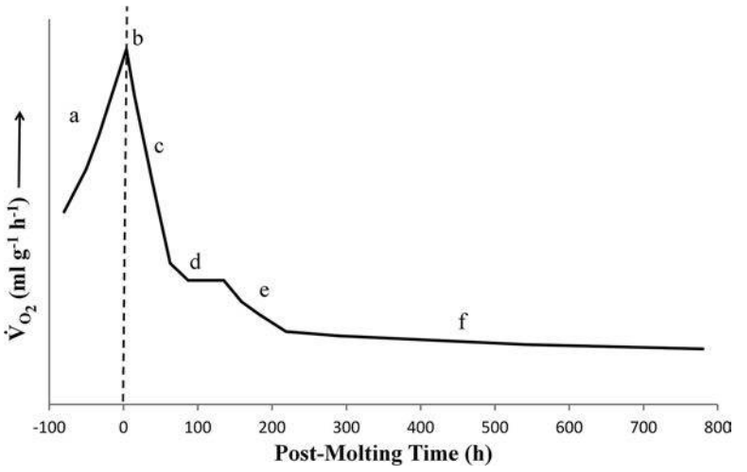

Figure 5. Ideal curve relating $\dot{V}O_2$ to postmolting time for life stages that molt. Different sections of the curve are labeled from a to f and are referenced in the text.

Unmated Adults and Nymphal Instars: RER

RER declined with time after molting for all nymphal instars and unmated adults except first instars (figs. 2*B*, 3*B*, 4*B*). ANOVA was used to test for differences in RER among life stages at different critical times after molting. Significant differences were detected in RER among nymphal instars from −9 to 11 h after molting ($F_{6,52} = 3.61$, $P = 0.0046$), from 128 to 155 h after molting ($F_{6,55} = 8.61$, $P < 0.0001$), from 216 to 248 h after molting ($F_{6,56} = 2.96$, $P = 0.0139$), and from 400 to 800 h after molting ($F_{5,56} = 7.20$, $P < 0.0001$). Further analysis using the LSD mean comparison test revealed a general trend after molting of younger life stages (first-, second-, and third-instar nymphs) having a lower RER than older life stages (fourth- and fifth-instar nymphs, unmated adults; table 1).

Unmated Adults and Nymphal Instars: Change in Body Mass

Percent mass loss per day declined with starvation time for all nymphal instars and unmated adults (figs. 2*C*, 3*C*, 4*C*). However, high variation in percent mass loss per day prevented us from modeling this change. Further examination of the plot of percent mass loss per day versus time after molting revealed the decline in percent mass loss per day to be similar to the decline in $\dot{V}O_2$ versus time after molting for most life stages.

ANOVA was used to test for differences among life stages in percent mass loss per day at selected times after molting. No significant differences were detected from −9 to 11 h after molting. Significant differences were detected in percent mass loss per day among nymphal instars from 128 to 155 h after molting ($F_{6,54} = 2.65$, $P = 0.0250$), from 216 to 248 h after molting ($F_{6,54} = 5.28$, $P = 0.0002$), and from 400 to 800 h after molting ($F_{5,56} = 24.38$, $P < 0.0001$), with the LSD mean comparison test revealing a trend toward higher percent mass loss per day in younger nymphal instars. This trend strengthened with increasing postmolting time (table 2).

Discussion

Metabolic Decline with Time after Feeding/Molting

The observed pattern of metabolic decline during starvation is the first reported for bed bugs. Furthermore, this represents the first time a full characterization of the metabolic decline during starvation has been reported for all life stages of any species. Starvation resulted in a strong reduction in the metabolic rate of all life stages of the bed bug. Although the amount of reduction varied on the basis of life stage, sex, and mating status, the general pattern observed indicates that both processing a blood meal and molting are very energy-expensive activities for bed bugs. The importance of both activities (blood processing and molting) is illustrated by the difference in the time of peak metabolism between adult and immature bed bugs. Adults show a peak in metabolism earlier (48 h after feeding) as opposed to the life stages that molt (during molt, ~120 h after feeding). Since adults do not molt, the peak observed at 48 h is likely due to blood processing. However, at 120 h after feeding most of the blood meal has already been processed, indicating that molting is responsible for the observed increase in metabolism in the immature life stages. In addition, the energetic cost of molting is also supported by the tight linkage between blood meals and molting in bed bugs (Usinger 1966). Without this strategy, bed bugs in an environment with unpredictable or varying frequency of host availability that molted twice after a single blood meal might run out of energy before being able to feed again. This strategy also correlates nicely with the proposed evolutionary hypothesis of bed bugs originally feeding on cave-dwelling bats (Usinger 1966). Under this hypothesis, bed bugs would be subjected to extended periods without food because many spe-

Table 1: Comparison of mean (±SE) respiratory exchange ratio (RER) for immature bed bugs over a range of postmolting times

	Postmolting time			
Life stage	−9 to 11 h	128 to 155 h	216 to 248 h	400 to 800 h
First instars	.582 (±.025) [7]^{C}	.567 (±.017) [7]^{BC}	.577 (±.022) [7]^{BC}	. . .
Second instars	.633 (±.008) [7]^{AB}	.607 (±.027) [7]^{ABC}	.605 (±.015) [7]^{ABC}	.501 (±.010) [7]^{C}
Third instars	.584 (±.026) [10]^{BC}	.492 (±.027) [10]^{D}	.549 (±.033) [10]^{C}	.499 (±.024) [22]^{C}
Fourth instars	.631 (±.010) [9]^{ABC}	.563 (±.018) [8]^{C}	.642 (±.020) [9]^{AB}	.563 (±.028) [10]^{BC}
Fifth instars	.669 (±.017) [10]^{A}	.643 (±.013) [10]^{A}	.630 (±.016) [10]^{AB}	.580 (±.025) [9]^{B}
Adult males (unmated)	.639 (±.006) [6]^{A}	.623 (±.011) [10]^{A}	.658 (±.027) [10]^{A}	.657 (±.022) [10]^{A}
Adult females (unmated)	.647 (±.009) [9]^{A}	.620 (±.010) [10]^{AB}	.655 (±.026) [10]^{A}	.633 (±.012) [10]^{AB}

Note. Sample sizes are in brackets. Statistical comparisons are made within a column, and means with the same letter are not significantly different according to the least significant difference mean comparison test at the $P < 0.05$ level.

cies of bats are known to commonly migrate between roosting sites throughout the year, often changing sites due solely to the presence of parasites (Lewis 1995; Fleming and Eby 2003; Reckardt and Kerth 2007; Bartonička and Růžičková 2012). This rapid movement of hosts would favor bed bugs that conserved energy and only molted when food was available.

In addition, we identified an important time for immature bed bugs during starvation, the initial plateau (fig. 5, d). The initial plateau occurred from approximately 100 to 150 h after molting, but for most life stages this was ≈8–9 d after feeding. This is an important period because bed bugs are reported to feed approximately every 7 d at room temperature (20°–27°C; Mellanby 1939; Usinger 1966; Reinhardt and Siva-Jothy 2007). An elevated metabolism would likely give an immature bed bug a better chance of feeding due to an upregulation of genes coding for important proteins and enzymes necessary for host location and blood digestion, but only if a host is present. Thus, if a blood meal is not found during this period, the bed bug's metabolic rate would continue to decline (fig. 5, e), leading to the starvation plateau (fig. 5, f). The second decline is likely due to a downregulation in genes coding for proteins and enzymes associated with nonessential metabolic processes and behaviors, such as aggregation and activity during the scotophase (Olson et al. 2009; Romero et al. 2010). These reductions likely serve to increase the time bed bugs can survive between blood meals.

Comparisons of Specific Postfeeding/Postmolting Metabolic Rates

When comparing mated adults, we found $\dot{V}_{O_2}$ to be significantly greater for mated adult females at 48 h after feeding. This difference is likely due to the increased allocation of energy into reproduction (likely egg production) by females during this critical time after feeding (Ronn et al. 2006; Hayward and Gillooly 2011). When all immature life stages are assessed, ANCOVA reveals an important difference between unmated males and unmated females. At both the initial plateau (from 128 to 155 h after molting; fig. 5, d) and the beginning of the starvation plateau (from 216 to 248 h after molting; fig. 5, e, f), unmated adult males had a significantly greater metabolic rate than unmated adult females. This difference could provide an evolutionary advantage to bed bugs. Evolutionarily, the ultimate objective for female bed bugs is to feed and then reproduce, and thus unmated females would be best served (from a fitness standpoint) to conserve as much energy as possible until both food and males become available. This is also a possible explanation why the initial plateau was greatly reduced in unmated

Table 2: Comparison of mean percent mass loss per day for immature bed bugs over a range of postmolting times

	Postmolting time			
Life stage	−9 to 11 h	128 to 155 h	216 to 248 h	400 to 800 h
First instars	7.99 (±1.02) [7]^{AB}	3.93 (±.68) [7]^{AB}	3.69 (±.32) [7]^{A}	. . .
Second instars	9.92 (±2.02) [7]^{AB}	5.37 (±2.15) [7]^{A}	4.25 (±.43) [7]^{A}	2.81 (±.27) [7]^{A}
Third instars	7.54 (±0.90) [10]^{AB}	5.38 (±1.01) [10]^{A}	2.04 (±0.31) [10]^{B}	2.29 (±0.15) [22]^{B}
Fourth instars	8.85 (±1.78) [9]^{AB}	2.83 (±.49) [8]^{B}	2.53 (±.33) [9]^{B}	1.15 (±.07) [10]^{CD}
Fifth instars	11.32 (±1.51) [10]^{A}	2.25 (±.28) [10]^{B}	1.88 (±.31) [10]^{B}	1.38 (±.15) [9]^{C}
Adult males (unmated)	6.95 (±.71) [6]^{B}	2.70 (±.32) [10]^{B}	2.52 (±.39) [10]^{B}	1.18 (±.11) [10]^{CD}
Adult females (unmated)	8.16 (±.82) [9]^{AB}	2.34 (±.35) [10]^{B}	1.92 (±.48) [10]^{B}	.84 (±.04) [10]^{D}

Note. Sample sizes are in brackets. Statistical comparisons are made within a column, and means with the same letter are not significantly different according to the least significant difference mean comparison test at the $P < 0.05$ level.

adult females, which likely avoid sustaining elevated metabolic rates (even for a few days, as seen in the younger instars) unless feeding and/or reproductive stimuli are present. Under the same circumstances, the primary objective of unmated males is to locate females for mating. To locate females, males likely require an upregulation of genes coding for proteins important in mate location and reproduction, and the energetic cost of upregulation is likely facilitated by an elevated metabolic rate. Finally, the lack of differences in metabolic rates during extended starvation (from 400 to 800 h after molting; fig. 5, f) suggest that at this point all life stages have reduced metabolism to minimum levels, which can be sustained for longer periods during starvation.

The differences observed between mated and unmated adults lie primarily with the females. Mating status had little effect on male metabolism (figs. 1*A*, 2*A*). This is not surprising because males, once mature, have few restrictions that limit them from mating. The difference between mated and unmated females is likely due to the egg production seen only in mated adult females. Unmated females cannot produce viable offspring yet and therefore would benefit from energy conservation until they can start producing eggs. The results support earlier work by Rao (1973), which also showed a large change in metabolic rate between mated and unmated adult female bed bugs.

The mass scaling coefficient relating the log of $\dot{V}O_2$ to the log of mass also changed with starvation time. The mass scaling coefficient declined from 1.052 (during molt) to 0.653 (during extended starvation). This decline indicates that metabolic status can drastically alter intraspecific mass scaling. It is possible that this change in mass scaling is a result of the greater mass change observed in younger instars throughout starvation.

Comparisons of Postfeeding/Postmolting Metabolic Rates with Other Species

The curvilinear relationships between $\dot{V}O_2$ and starvation or molting time provide for interesting comparisons with a number of different animal taxa. Similar to bed bugs, the kissing bug *Rhodnius prolixus* is a hematophagous ectoparasite of mammals and birds, and later instars require a blood meal to molt (Roberts et al. 2009). Zwicky and Wigglesworth (1956) generated a curve relating $\dot{V}O_2$ (mL h^{-1}) of *R. prolixus* to time after feeding (molting). The pattern they observed was similar to that described here (fig. 5). Zwicky and Wigglesworth (1956) also found a peak in $\dot{V}O_2$ during molting and an extended period with little change in $\dot{V}O_2$, similar to our starvation plateau (fig. 5, f). However, they did not indicate an initial plateau (fig. 5, d), which suggests that an initial plateau in $\dot{V}O_2$ may be specific to bed bugs. Strong similarities in $\dot{V}O_2$ and the molting cycle also exist between bed bugs and the blue crab (*Callinectes sapidus* Rathbun; Defur 1990). Both species have maximal resting metabolic rates during molting, suggesting that molting has similar effects on the metabolism of most arthropods and that a peak in metabolism during this time is likely common.

Another species worth comparing with the bed bug is the Burmese python (*Python molurus* L.). Burmese pythons share some similarities in feeding strategies with bed bugs, particularly the consumption of a large meal followed by an extended period of starvation (Greene 1997). Secor and Diamond (1997) reported a peak in Burmese python oxygen consumption from 24 to 48 h after feeding. Bed bugs showed a similar peak in $\dot{V}O_2$ between 0 and 48 h after feeding (fig. 1*A*). A peak in $\dot{V}O_2$ after feeding is typical among animals (Benedict 1932; Brody 1945 as cited by Secor and Diamond 1997), and our data show that bed bugs are no exception. After the peak in $\dot{V}O_2$ after feeding, Burmese python $\dot{V}O_2$ declines until returning to the baseline standard metabolic rate in a curvilinear fashion (Secor and Diamond 1997), similar to that observed in bed bugs (fig. 1*A*).

Effects of Time after Feeding/Molting on RER

Observed bed bug RER values were lower than those predicted by metabolism of pure compounds (0.7–1.0; Livesey and Elia 1988). However, this is not uncommon among animals, with respiratory quotients (RQs) or RERs below 0.7 reported for a number of species (Fink 1925; Rouland et al. 1993; Walsberg and Wolf 1995; Damcevski et al. 1998; Vogt and Appel 1999; Walsberg and Hoffman 2005; Blossman-Myer and Burggren 2010). To account for these low RER values, we propose that bed bugs may be converting the protein-rich blood meal into glucose using gluconeogenesis (Schutz and Ravussin 1980). Protein is difficult to convert directly to energy (ATP), and for a hematophagous insect like the bed bug, which consumes a meal with a large amount of protein, an intermediate step of gluconeogenesis (RQ = 0.36) could potentially lower the RER. In addition, it is also possible that CO_2 is being sequestered within the hemolymph (Coulson and Hernandez 1983). This process can cause significant declines in respiratory CO_2, drastically altering observed RER values. Both of these hypotheses should be evaluated further.

No differences in RER were found at any of the postfeeding times used to compare mated adults. However, ANOVA revealed some significant differences in RER between the life stages that molted, with larger instars generally having a higher RER for a longer period of time after molting (table 1). This relationship suggests that the blood meals acquired by older instars last for longer periods. First-instar RER showed no relationship with time after hatching (fig. 4*B*). This suggests that first instars never switch metabolic substrate, likely because they never take a blood meal (Usinger 1966).

Effects of Time after Feeding/Molting on Mass Loss

When viewed together, data on both percent mass loss per day and metabolic rate provide useful information in understanding survival during starvation for different bed bug life stages, particularly mated and unmated adult females. Usinger (1966) reported adult females to have one of the lowest mean survival times compared with other instars at 27°C, while Polanco et al. (2011) found adult females to be capable of surviving as long as all other instars for several strains. The differences observed by these authors are likely the result of measuring mated versus unmated adult females, two groups that showed drastically different metabolic rates and mass-loss

rates. Thus, it is reasonable to hypothesize that mated adult females would have the shortest life span (high metabolic rate and percent mass loss per day during starvation) and that unmated females would have the longest life span (low metabolism and percent mass loss per day during starvation).

All immature stages had a peak in percent mass loss per day during molting (figs. 2*C*, 3*C*, 4*C*). Molting leads to large changes in mass due to both loss of the exoskeleton and evaporative water loss during the teneral state of the insect after molting (Wigglesworth and Gillett 1936). Once starvation metabolism was initiated (fig. 5, f), changes in percent mass loss per day become minimal. These values remain relatively low because bed bugs have very little excretory products and are excellent at conserving water (Benoit et al. 2007). However, as time after molting increased, the older instars had lower percent mass loss per day, likely because the older nymphal instars lose less water than the younger nymphal instars (Benoit et al. 2007) and their mass-specific metabolism is lower than the younger instars, as indicated by the $\log_{10}$ mass coefficient being <1 during extended starvation.

Conclusions

Bed bug metabolic rates ($\dot{V}O_2$) were significantly affected by time after feeding and time after molting. Mated adult bed bug metabolic rates had a curvilinear relationship with starvation time. This relationship was similar to that seen in Burmese pythons, a taxonomically different animal sharing only a similar feeding strategy. An ideal curve was developed for the life stages that molted. This curve showed that metabolism increased after feeding leading up to molting and then subsequently declined until reaching an initial plateau (8–9 d after feeding). If no feeding occurs during this initial plateau, the metabolism will continue to decline until reaching a second (starvation) plateau, where it will remain relatively stable until the bed bug either feeds or dies. This pattern shared some similarities with two other arthropods: *R. prolixus* and *C. sapidus*. In additional, unmated males had higher metabolic rates than unmated females, whereas mated females had higher metabolic rates than mated males. RERs declined as time progressed, except for first-instar nymphs, and differences were observed between older and younger nymphal instars. Percent mass loss per day also declined with time after feeding and time after molting and was greater for younger nymphal instars. This study provides a unique perspective on starvation metabolism and also provides the first comprehensive analysis of the effects of starvation on metabolism for all life stages of a species. Furthermore, this study provides evidence for within-species shifts in mass scaling during periods of metabolic depression.

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