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Standard metabolic rate of the bed bug, *Cimex lectularius*: Effects of temperature, mass, and life stage

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ABSTRACT

Metabolic rates provide important information about the biology of organisms. For ectothermic species such as insects, factors such as temperature and mass heavily influence metabolism, but these effects differ considerably between species. In this study we examined the standard metabolic rate of the bed bug, *Cimex lectularius* L. We used closed system respirometry and measured both O_2 consumption and CO_2 production across a range of temperatures (10, 20, 25, 30, 35 °C) and life stages, while also accounting for activity. Temperature had a stronger effect on the mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) of mated males (Q_{10} = 3.29), mated females (Q_{10} = 3.19), unmated males (Q_{10} = 3.09), and nymphs that hatched (first instars, Q_{10} = 3.05) than on unmated females (Q_{10} = 2.77) and nymphs that molted (second through fifth instars, Q_{10} = 2.78). First instars had significantly lower respiratory quotients (RQ) than all other life stages. RQ of all stages was not affected by temperature. $\dot{V}_{O_2}$ (ml h^{-1}) scaled more with mass than values previously reported for other arthropods or that would be predicted by the 3/4-power law. The results are used to understand the biology and ecology of the bed bug.

1. Introduction

Metabolic rates provide important information for understanding the biology of all animal life. Respiratory physiology has been used to understand the metabolism of a wide variety of arthropods including ants (Lighton, 1988; Vogt and Appel, 1999), beetles (Burges, 1960), cockroaches (Dingha et al., 2009), crickets (Hack, 1997), fleas (Fielden et al., 2001), moths (Schneiderman and Williams, 1953), spiders (Anderson, 1970), termites (Shelton and Appel, 2001), ticks (Fielden et al., 1999; Lighton and Fielden, 1995), and thysanurans (DeVries and Appel, 2013). Metabolic measurements provide useful information on the rate of energy expenditure and often incorporate the effects of temperature and mass (Lighton and Fielden, 1995; Vogt and Appel, 1999). Mass is of particular importance because of the ongoing debate regarding mass scaling. Specifically, the 3/4-power law is currently being challenged by other models such as the cell-size model which predicts a wider range of mass scaling coefficients (Chown et al., 2007; Glazier, 2005; Kozlowski et al., 2003; West et al., 1997, 2002). Metabolic rates also provide insight into what nutrients are being metabolized (Kells et al., 1999; Vogt and Appel, 1999). Even though metabolic rates have been described in a number of taxa, bed bugs have received limited attention.

Bed bugs present a very interesting biological system in the study of metabolism. First, bed bugs can survive a wide range of temperatures, despite the relatively constant temperature of their usual environment (Benoit et al., 2009; Kells and Goblirsch, 2011). In addition, Benoit (2007) showed bed bugs are very resistant to desiccation. Bed bugs are capable of surviving extended periods of starvation, with some reports indicating survival after more than a year without feeding (Usinger, 1966). Prolonged starvation can also significantly impact their metabolism, with different life stages responding differently to starvation (DeVries, 2013). In addition to their interesting biology, a better understanding of bed bug biology is important because they are a major pest in the urban environment, capable of causing both physical and psychological harm to their victims (Delaunay et al., 2011; Goddard and deShazo, 2009; Reinhardt and Siva-Jothy, 2007).

Despite the importance of bed bugs and the insight that metabolic measurements could provide into their biology and life history, little information is currently available on bed bug metabolism. Mellanby (1932) performed the first study on bed bug metabolism, although little information on metabolic rates was presented. This study focused largely on the energy source used over time by examining bed bugs before and after starvation for their chemical composition. Rao (1973) also conducted a study on bed bug metabolism. This study indicated a difference in

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metabolic rate between mated and unmated bed bugs, and focused largely on the role of sperm in increasing metabolic rate. However, Rao (1973) did not measure differences or similarities among any other life stages. With the current lack of information on bed bug metabolism, we determined that a complete study of the effects of temperature, mass, and life stage on bed bug metabolism was necessary. In the present study, we used closed system respirometry to measure the standard metabolic rate of the bed bug across a range of temperatures, masses, and life stages. The results are related to bed bug biology and compared with other arthropods (DeVries and Appel, 2013; Lighton and Fielden, 1995).

2. Materials and methods

2.1. Experimental animals

Bed bugs were reared and maintained at the University of Minnesota, Twin Cities, MN as described by Olson et al. (2009). Briefly, we used an insecticide susceptible laboratory strain that was maintained at 23 ± 2 °C and 55 ± 5% RH on a 14:10 light:dark cycle. Colonies were contained in modified 0.5 l glass jars with filter paper for harborage and screen tops to allow feeding. Bed bugs were fed human blood from the American Red Cross which had expired for medical use. Blood was fed weekly as a 1:1 combination of red blood cells and plasma.

Immediately after feeding, bed bugs were shipped to Auburn, AL overnight in 1.5 ml centrifuge tubes with filter paper for harborage and a small hole in the top for air ventilation (screening was present to prevent escape). Once the bed bugs arrived, they were held until testing in the same 1.5 ml centrifuge tubes in identical conditions as those used for rearing. The filter paper was replaced as needed and bugs were sometimes divided into multiple 1.5 ml centrifuge tubes to prevent overcrowding.

Testing was performed on average between 216 and 264 h (9–11 d) post feeding for mated adults (no molting) and 108–150 h (4.5–6.3 d) post molting for all life stages which molted. These times provided a short period of relatively little change in metabolic rate before the animals entered into a starvation metabolic rate. This period was determined by an extensive series of preliminary experiments used to measure the effects of starvation on bed bug metabolism (DeVries, 2013). Post-molting times were approximately equal to post-feeding times that mated adults were tested, allowing for comparison of all life stages, regardless of whether or not they molted.

2.2. Respirometry equipment

After bed bugs reached the correct time either post-feeding or post-molting, they were placed into individual respirometry chambers constructed of 3 ml plastic syringes (Becton, Dickinson and Company, Rutherford, NJ, USA). Syringes were modified to permit air flow while preventing the bed bugs from escaping. This was done by drilling six 1.4 mm diameter holes in each syringe barrel past the last gradation where the plunger enters the barrel. Bed bugs were placed into individual syringes (respirometry chambers) along with a 0.32 cm^2 piece of cardstock paper (0.81 × 0.40 cm) and allowed to acclimate in the respirometry chamber overnight. Bed bugs were either measured as individuals or in groups (up to 12 bugs per syringe). Groups were used to reduce incubation time and preliminary results indicated no difference in bed bug metabolic rates when measured in groups or individually. After acclimation, the syringe plungers were adjusted so that the drilled holes remained open, but the syringe was sealed off to the environment. Syringes were then placed onto a manifold which pushed dry, CO_2 free air into the syringe tip, through the syringe, and out the drilled holes at the top to remove all CO_2 and water. Air flowed into the manifold at a rate of 230 ml min^{-1} before entering the syringes. Immediately after purging, a 26 gauge intradermal bevel needle (Becton, Dickinson and Company, Rutherford, NJ, USA) was placed on the end of each syringe and the syringe volume was adjusted to 0.7 ml. Then the needle was inserted into a rubber stopper (size 000) to seal the respirometry chamber off from atmospheric gasses. Syringes were then placed into incubators (Thermo Fisher Scientific, Marietta, OH, USA) at one of 5 temperatures (10, 20, 25, 30, 35 °C) and allowed to incubate for various times, depending on temperature and life stage being tested (2–18 h, except first instars which required more time). Preliminary results showed incubation time had no effect on bed bug metabolism over the time course measured. Animals were observed for movement under red light (20 W) using a SONY® DCR-SX85 video camera (Sony Corporation, Minato, Tokyo, Japan). Windows® Media Player Classic version 6.4.9.1 (© 2002–2009) was used to manually review all videos at a rate of approximately 1 min video recording per sec for movement of bed bugs. Post incubation, an air sample (0.5 ml) was injected from each syringe into the respirometry system which measured O_2 depletion and CO_2 enrichment. The total time of incubation was recorded after the injection and bed bugs were weighted to the nearest 10^{-5} g on a digital balance (Mettler-Toledo AX205, Mettler-Toledo GmbH, Greifensee, Switzerland). With every experiment, two control syringes were subject to the same procedure as the above experimental chambers, except they contained no animals. After injections, data from experimental syringes were adjusted accordingly based on measurements from the control syringes. Changes in CO_2 in the control syringes accounted for less than 5% of the total CO_2 measured in the experimental syringes, and there was no change in O_2 levels from the control syringes.

Measurements of O_2 consumption and CO_2 production from the injected air samples were made using a respirometry system following the methods of DeVries and Appel (2013), Lighton (1991), and Vogt and Appel (1999). To summarize: room air was forced into a Whatman purge-gas generator (Whatman Inc., Haverhill, MA, USA) where both CO_2 and water were removed from the air. The air then passed through a 340 l mixing tank and into an open mixing tank (30 l) where the air equalized to atmospheric pressure. Next, the air was pulled from the open mixing tank through a Drierite®-Ascarite®-Drierite® column (Drierite-W.A. Hammond Drierite Company Ltd., Xenia, OH, USA; Ascarite-Thomas Scientific, Swedesboro, NJ, USA) to remove any minute traces of water or CO_2. The air was then pulled through an injection port where air samples were injected after incubation was complete. The air then passed through a Li-6251 CO_2 analyzer (Li-COR Inc., Lincoln, NE, USA), another a Drierite®-Ascarite®-Drierite® column, and a Sable Systems Oxzilla II O_2 analyzer (Sable Systems, Henderson, NV, USA). The air finally passed through a Sable Systems mass flow system MFS2 (Sable Systems, Henderson, NV, USA), which controlled the air flow (pulled the air) at a rate of 100 ml min^{-1} at STP. All data were recorded and analyzed using Datacan V (Sable Systems, Henderson, NV, USA). Analysis was performed by converting the data into units of ml h^{-1} and then subsequently integrating peaks to calculate the total CO_2 production or O_2 consumption per syringe. For specific equation and more details see Lighton (1991).

2.3. Metabolic Calculations

Several important metabolic variables were calculated from these recorded metabolic measurements. Metabolic rates ($\dot{V}_{O_2}$) are reported as both non-mass-specific (ml h^{-1}) and mass-specific (ml g^{-1} h^{-1}). In addition, metabolic rates are also reported in μW, where 1 W is equal to 1 J s^{-1} and 1 ml of O_2 is equal to 20.1 J (Lighton and Wehner, 1993). Respiratory quotient (RQ) was calculated

from metabolic measurements of O_2 and CO_2, the result of dividing total CO_2 production by total O_2consumption. Q_{10} is a measure of the change in metabolic rate with a 10 °C change in temperature. Q_{10} is calculated by multiplying the slope of the equation relating temperature to mass specific $\dot{V}_{O_2}$ (in log form) and then taking the antilogarithm of the product (Lighton, 1989). However, this is only the case if the equation relating $Log_{10}\dot{V}_{O_2}$ to temperature is linear.

2.4. Statistical analysis

To control for error in metabolic rates because of movement, a *t*-test (PROC TTEST, SAS Institute, 1985) comparing metabolic rates of animals which did and did not move determined what maximum level of activity could occur before metabolic rate measurements were affected. Life stages were then divided into groups based on sex, mating status, and growth/development to aid in comparisons. Adults were divided into four groups: mated males (MM), mated females (MF), unmated males (UM), and unmated females (UF). Nymphal instars were grouped based on whether they hatched (first instars) or molted (second through fifth instars). The relationship between metabolism, temperature, and mass of nymphs that molted was explained through multiple regression, because this was the only life stage group with a wide enough mass range to model using mass (PROC GLM, SAS Institute, 1985). The relationship between temperature and metabolism was explained through linear regression for all groups (PROC GLM, SAS Institute, 1985).The effects of bed bug group (main effect) and temperature (covariate) on metabolism was assessed via analysis of covariance (PROC GLM, SAS Institute, 1985). Comparisons of mass specific oxygen consumption ($\dot{V}_{O_2}$, ml g^{-1} h^{-1}) and RQ among all bed bug groups were made using analysis of variance (PROC GLM, SAS Institute, 1985), with an LSD test to determine differences among the means. Modeling metabolic rate (MR, in μW) versus mass for all bed bugs was accomplished by least square regression and a power function was fitted to this model. This equation was also log-transformed into a linear equation and compared with mass scaling equations for other arthropods using analysis of covariance (arthropod group as main effect, mass as covariate). All means are reported with standard errors (±SE).

3. Results

3.1. Activity

Bed bugs which had activity (movement) that significantly affected SMR measurements were removed from the study. Movement which significantly affected bed bug SMR was determined using a *t*-test comparing mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) between individuals which did not move to those which moved between 1 and 50 mm h^{-1} at 25 °C. There were no significant differences in mass specific $\dot{V}_{O_2}$ due to movement <50 mm h^{-1} for adults ($t_{1,37} = -1.49$, p = 0.1066) and nymphs($t_{1,56} = 0.95$, $p = 0.3460$) and this permitted inclusion of all bed bugs moving <50 mm h^{-1}. When allowed enough time to acclimate, bed bugs showed little to no movement, requiring exclusion of only 6 samples (1.3% of all tested) because of movement.

3.2. Adult metabolic rates

All adult groups were modeled separately throughout the study. Each adult group was modeled for the effects of temperature on mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) due to the relatively small mass ranges within adult groups. Mated male mass specific $\dot{V}_{O_2}$ had the following relationship with temperature (°C):

Mated Males :

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.772(\pm 0.048) + 0.052(\pm 0.002)\mathrm{Temperature}$$

($F_{1,46} = 785.4$, $p < 0.0001$, $r^2 = 0.9447$) (Fig. 1). Mated female mass specific $\dot{V}_{O_2}$ had the following relationship with temperature:

Mated Females :

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.791(\pm 0.068) + 0.050(\pm 0.003)$$

($F_{1,43} = 337.5$, $p < 0.0001$, $r^2 = 0.8870$) (Fig. 1). Unmated male mass specific $\dot{V}_{O_2}$ had the following relationship with temperature:

Unmated Males :

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.713(\pm 0.043) + 0.049(\pm 0.002)\mathit{Temperature}$$

($F_{1,46} = 870.7$, $p < 0.0001$, $r^2 = 0.9498$) (Fig. 1). Unmated female mass specific $\dot{V}_{O_2}$ had the following relationship with temperature:

Unmated Females :

$$\mathit{Log}_{10}\dot{V}_{O_2} = -1.796(\pm 0.049) + 0.044(\pm 0.002)\mathrm{Temperature}$$

($F_{1,45} = 550.8$, $p < 0.0001$, $r^2 = 0.9245$)(Fig. 1).Analysis of covariance (adult group as main effect, temperature as covariate) revealed no significance differences by which $\dot{V}_{O_2}$ of adult groups scaled with temperature ($F_{3,184} = 2.37$, $p = 0.0719$). However, closer examination revealed that the variable for unmated adult females was significant in the model ($p = 0.0414$), despite the overall interaction not being significant.

3.3. Adult Q_{10} and RQ

Mean Q_{10} values were 3.29(±0.15) for mated males, 3.19(±0.20) for mated females, 3.09(±0.12) for unmated males, and 2.77(±0.12)

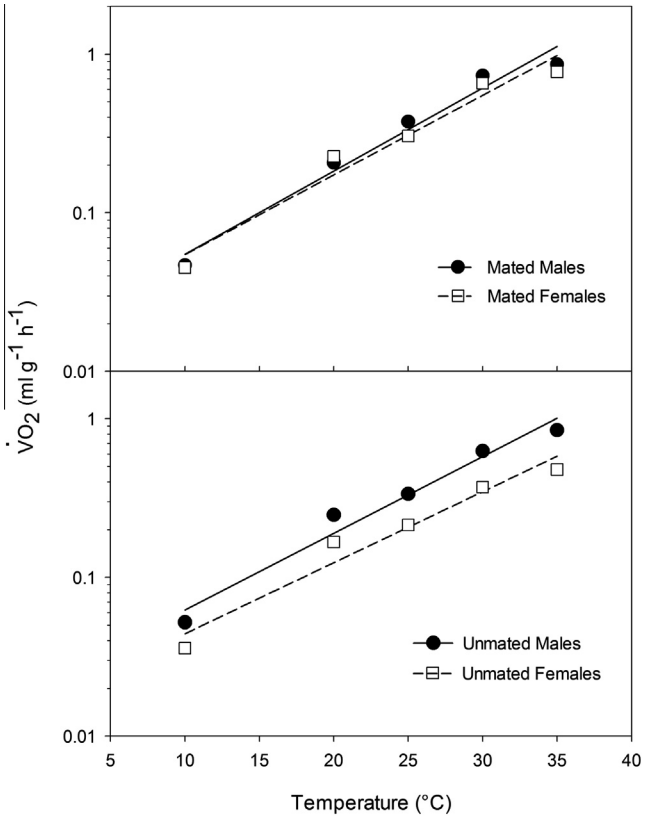

Fig. 1. Mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) for mated males, mated females, unmated males, and unmated females across a range of temperatures. See text for equations.

for unmated females. Also, because the linear equations relating adult $Log_{10}V_{O2}$to temperature are good fits ($r^2 > 0.88$) Q_{10} can be assumed to be constant across the measured temperature range.

Respiratory quotient showed little change with temperature and therefore was not modeled. Average RQ is reported for each adult group (Table 1). Analysis of variance revealed RQ was not significantly different among adult groups ($F_{3,184} = 0.10$, $p = 0.9568$).

3.4. Nymphal metabolic rates

Both nymphal groups (nymphal instars 2–5 that molted and first instar nymphs that hatched), were modeled separately throughout the study. Multiple regression analysis was used to determine the relationship between $\dot{V}_{O_2}$ (ml h^{-1}), temperature (°C), and mass (g) in the nymphal group that molted:

Molted Nymphs :

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.738(\pm 0.056) + 0.045(\pm 0.001)\mathrm{Temperature} + 1.022(\pm 0.019)\mathrm{Log}_{10}\mathrm{Mass}$$

($F_{2,211} = 2671.5$, $p < 0.0001$, $r^2 = 0.9620$). Coefficients for temperature and Log_{10}Mass were both significant ($p < 0.0001$). Nymphs that hatched consisted only of first instars and had a very narrow mass range. Therefore, first instar nymphs were not modeled using both variables of mass and temperature, but were used in a subsequent analysis evaluating the singular effect of temperature.

In addition to determining the effects of both mass and temperature, we also assessed the effects of temperature individually on mass specific $\dot{V}_{O_2}$ (mlg^{-1} h^{-1}) Mass specific $\dot{V}_{O_2}$ of nymphs that molted had the following relationship with temperature (°C):

Molted Nymphs :

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.803(\pm 0.0231) + 0.044(\pm 0.001)\mathrm{Temperature}$$

($F_{1,212} = 2659.6$, $p < 0.0001$, $r^2 = 0.9262$) (Fig. 2). Mass specific $\dot{V}_{O_2}$ of nymphs that hatched had the following relationship with temperature:

Hatched Nymphs :

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.844(\pm 0.045) + 0.048(\pm 0.002)\mathrm{Temperature}$$

($F_{1,50} = 746.4$, $p < 0.0001$, $r^2 = 0.9372$) (Fig. 2). Analysis of covariance using molting versus hatching as main effect, with temperature as covariate, revealed the interaction term (molting versus hatching * temperature) to be significant ($F_{1,264} = 14.2$, $p = 0.0002$). This significant interaction indicated that mass specific $\dot{V}_{O_2}$ scaled differently with temperature between bed bug nymphs that molted (second-fifth instars) and bed bug nymphs that hatched (first instars).

3.5. Nymphal Q_{10} and RQ

Nymphal instars 2–5 that molted had a Q_{10} of 2.78(±0.06), compared to a Q_{10} of 3.05(±0.13) for first instar nymphs that hatched.

Table 1
Mean respiratory quotient (RQ) and mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}, at 25 °C) for all bed bug groups. Means within columns which differ significantly according to the LSD test are indicated by different letters ($p < 0.05$).

	RQ		$\dot{V}_{O_2}$ at 25 °C	
Bed bug group	N	Mean (±SE)	N	Mean (±SE)
Mated male	48	0.64(±0.01) A	10	0.375 (±0.025) A
Mated female	45	0.64(±0.01) A	9	0.305 (±0.023) B
Unmated male	48	0.65(±0.01) A	10	0.336 (±0.014) AB
Unmated female	47	0.65(±0.01) A	10	0.214 (±0.010) C
Nymphs that molt	214	0.61(±0.01) B	40	0.245 (±0.008) C
Nymphs that hatch	52	0.53(±0.01) C	18	0.239 (±0.014) C

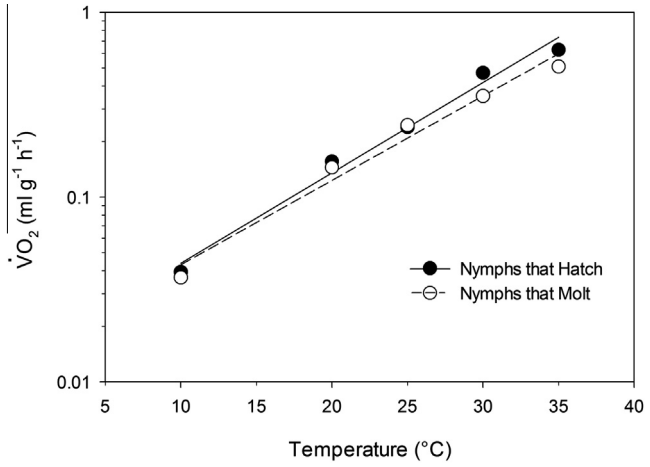

Fig. 2. Mass specific V_{O2} (ml g^{-1} h^{-1}) for nymphs that molted (second through fifth instars) and nymphs that hatched (first instars). See text for equations.

Due to the good fit of the linear equations relating $Log_{10}\dot{V}_{O_2}$ to temperature ($r^2 > 0.92$), these values can be assumed to be constant across the measured temperature range.

Respiratory quotient changed little with temperature and therefore was not modeled. Average RQ is reported for both nymphs that hatched and nymphs that molted (Table 1). A *t*-test revealed RQ was significantly different between both nymphal groups ($t_{1,264} = -7.6$, $p < 0.0001$), with nymphs that hatch having a significantly lower RQ.

3.6. Comparison of metabolic rate among different groups

We compared mass specific $\dot{V}_{O_2}$ among all adult and nymphal groups. Analysis of covariance (groups as main effect, temperature as covariate) revealed a significant interaction between the bed bug groups (mated adult males, mated adult females, unmated adult males, unmated adult females, nymph that molt, and nymphs that hatch) and temperature ($F_{5,448} = 38.1$, $p < 0.0001$). This indicated that mass specific $\dot{V}_{O_2}$ of different groups scale differently with temperature. Further evaluation resulted in the formation of two assemblages where the interaction term within each assemblage (bed bug group * temperature) is not significant, indicating that mass specific $\dot{V}_{O_2}$ within these assemblages scales similarly with temperature. Assemblage one consisted of mated adult males, mated adult females, unmated adult males, and nymphs that hatch (first instar) ($F_{3,189} = 0.53$, $p = 0.6602$). The second assemblage included: unmated females and nymphs that molt (second through fifth instars) ($F_{1,259} = 0.00$, $p = 0.9473$).

Comparing mass specific $\dot{V}_{O_2}$ at 25 °C enabled comparisons at the temperature that bed bugs are commonly found. Analysis of variance determined mass specific $\dot{V}_{O_2}$ at 25 °C was significantly different among the individual groups ($F_{5,91} = 15.4$, $p < 0.0001$). An LSD test comparing means indicated that unmated adult females, nymphs that hatched (first instars) and nymphs that molted (second-fifth instars) had significantly lower mass specific $\dot{V}_{O_2}$ than mated adult males, mated adult females, and unmated adult males (Table 1).

Respiratory quotient was also compared among all individual groups and analysis of variance revealed a significant difference in RQ among these groups ($F_{5,448} = 29.2$, $p < 0.0001$).The LSD test for mean comparisons revealed that RQ of nymphs that hatched (first instar) was significantly lower than all other bed bug groups (Table 1). In addition, all adult bed bug groups were significantly greater than nymphal instars 2–5 that molted (Table 1).

3.7. Comparison between bed bugs and other arthropods

In addition to examining the metabolic relationships within bed bugs, we also examined the relationships between bed bugs and other arthropods. All bed bug data were combined and compared with other species, including: ticks; other arthropods (ants, beetles, spiders) and thysanurans (DeVries and Appel, 2013; Lighton and Fielden, 1995). Bed bug mass related to $\dot{V}_{O_2}$ by the following equation at 25 °C:

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -0.421(\pm 0.054) + 1.056(\pm 0.018)\mathrm{Log}_{10}\mathrm{Mass}$$

($F_{1,95} = 3517.2$, $p < 0.0001$, $r^2 = 0.9737$) (Fig. 3). Bed bug metabolic rates were also converted to μW and related to mass (g) by the following equation at 25 °C:

$$\mathrm{MR}\,[\mu W] = 1485.1(\pm 740.3)\mathrm{Mass}_{0.984(\pm 0.090)}$$

($F_{1,95} = 526.3$, $p < 0.0001$, $r^2 = 0.8471$). The effect of mass on metabolic rate (μW) was compared among bed bugs, ticks, thysanurans, and other arthropods (ants, beetles, spiders) (DeVries and Appel, 2013; Lighton and Fielden, 1995). Analysis of covariance (taxa as main effect, mass as covariate) revealed that bed bugs had a significantly greater mass scaling coefficient than all other taxa ($p < 0.0001$). Despite the difference in mass scaling, it is clear that bed bugs, similar to thysanurans, had mass specific metabolic rates which fell in between ticks (lower) and other arthropods (ants beetles, spiders; higher) (Fig. 4). However, it should be noted that bed bug metabolic rates were much closer to other arthropod metabolic rates than they were to tick metabolic rates (Fig. 4).

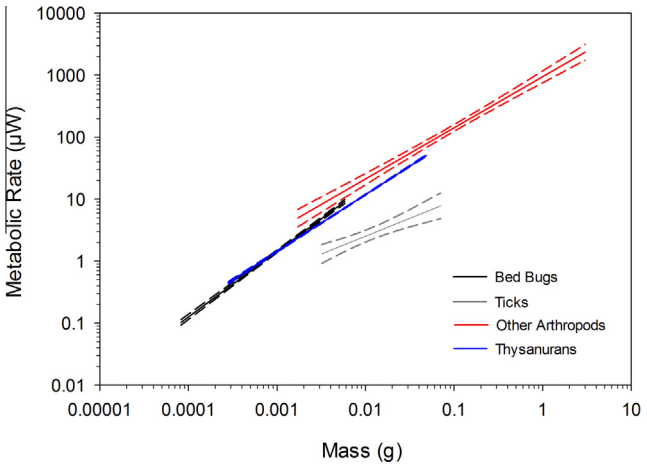

Fig. 4. Metabolic rate (μW) versus mass (g) for bed bugs, thysanurans (DeVries and Appel, 2013), ticks and other arthropods (ants, beetles, spiders) (Lighton and Fielden, 1995).

4. Discussion

Activity is always important to consider when making metabolic measurements, due to the large effects it can have on O_2 consumption and CO_2 production (Bartholomew et al., 1985; Lighton and Feener, 1989; Lighton and Duncan, 1995). Bed bugs made this problem rather simple to negate, with <2% total number of animals measured moving significantly. This is not surprising from an insect which spends a majority of its lifecycle concealed and motionless (Reinhardt and Siva-Jothy, 2007; Usinger, 1966).

Mass specific $\dot{V}_{O_2}$ showed a strong relationship with temperature for both adults and nymphs ($r^2 > 0.88$). Of the individual groups, the $\dot{V}_{O_2}$ measurements of nymphs which molted (second-fifth instars) and unmated females were less affected by temperature than the $\dot{V}_{O_2}$ measurements of mated adults, unmated adult males, and first instars (Figs. 1 and 2). This relationship was a result of elevated metabolism of some groups at higher temperatures, because all bed bug groups showed very similar metabolism at 10 °C. The cause behind elevated metabolism (mated adults, unmated adult males, and first instars) or depressed metabolism (second-fifth instars and unmated adult females) is still unclear, but we hypothesize it is likely due to the evolutionary benefits of having high versus low metabolism for different groups and the impacts of metabolism on fitness. Nymphs which molt and unmated adult females do not have a need to use energy until feeding becomes an option and, therefore, might have lower mass specific metabolic rates at any given temperature in the absence of foraging and feeding stimulants. However, mated adults and unmated adult males gain few fitness advantages by simply sitting and waiting for food. Therefore, elevated metabolic rates were likely selected for due to the benefits in being constantly ready for locating mates for males, and for females producing eggs and potentially avoiding over-mating (Siva-Jothy, 2006). First instar nymphs were also likely selected to have higher metabolic rates because they do not have the same capacity to survive starvation as other life stages, likely a result of nutrient limitations available upon hatching and the surface area to volume issues resulting in water loss (Benoit et al., 2007; Chapman, 1998; Usinger, 1966). With unpredictable nutrient availability, it would be more favorable to always be alert, and in a state ready to forage and feed to quickly capitalize upon any feeding opportunities. This hypothesis could be tested by assessing which life stages are the first to locate and begin feeding at various post-molting, post-hatching times. Differences in metabolic scaling with temperature within a species are neither new nor uncommon (DeVries and Appel, 2013; Vogt and Appel, 1999). This phenomenon has received little attention in large part because many studies only measure one life stage of insects/arthropods or do not compare different interspecific life stages. It is also worth noting that the low metabolic rates seen in all life stages at 10 °C suggest that metabolic depression could be occurring (Kovac et al., 2007; Withers and Cooper, 2010). However, if metabolic depression is occurring, it appears to be having a rather minor affect (Figs. 1 and 2). Therefore, the linear line describing the relationship between temperature and V_{O2} is still the best fit for the data.

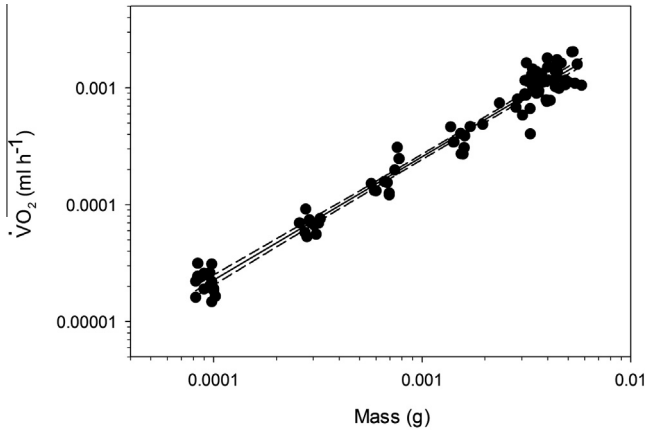

Fig. 3. V_{O2} versus mass for all bed bug groups (ml g^{-1} h^{-1}). See text for equation.

In addition to comparing how different groups scale with temperature, we also compared mass specific $\dot{V}_{O_2}$ for all groups at 25 °C, the temperature at which bed bugs are usually found (Table 1). This comparison revealed a similar scaling pattern between mass specific V_{O2} and temperature, likely a result of the evolutionary benefits of high versus low metabolism for different groups, as discussed above. However, nymphs which hatched (first

instars) had mass specific $\dot{V}_{O_2}$ values that were significantly lower than mated adults and unmated adult males and not significantly different from unmated adult females and nymphs that molted (Table 1). This suggests that despite a higher mass specific $\dot{V}_{O_2}$ scaling factor, first instars have likely been selected to conserve energy at a normal environmental temperature.

The effects of mass were assessed on $\dot{V}_{O_2}$ at 25 °C for bed bugs as a group (Fig. 3). Comparison between groups and mass-scaling were not possible due to the limited size range of each group (except nymphs which molted). The mass-scaling coefficient for all bed bugs was mass$^{0.984}$ and this coefficient was greater than mass$^{0.825}$for all arthropods (Lighton and Fielden, 1995), and much greater than would be predicted by the 3/4-power law (West et al., 1997; West et al., 2002). The mass-scaling factor for bed bugs therefore provides support for the cell-size model of mass scaling (Chown et al., 2007; Kozlowski et al., 2003).

Respiratory quotient (RQ) values are used to understand what metabolic substrate is being metabolized, with pure carbohydrate metabolism indicated by and RQ value of 1, pure protein metabolism indicated by an RQ value of 0.835, and pure fat metabolism indicated by an RQ value of 0.7 (Livesey and Elia, 1988). However, these values are the ideal/theoretical values which often differ considerably from measured values. For this reason, RQ is best understood by looking at changes or differences rather than by making comparisons to the theoretical values. In the present study, RQ showed no change across the measured temperature range for any group. This indicates that the metabolic substrate being metabolized did not change from 10 to 35 °C. However, this is not surprising because of the large blood (protein) meals bed bugs take (Usinger, 1966). Mellanby(1932) also found bed bugs to metabolize mostly protein at the same period post-feeding. Comparisons of mean RQ among the different groups revealed first instars to have a significantly lower RQ than all other groups (Table 1). This is not surprising because first instars had not taken a blood meal and therefore did not have the same protein available for metabolism as the other life stages (Usinger, 1966). Therefore, they appear to be relying on fat (vitellogenin) metabolism during this time.

Our results also provide more support for the use of standard metabolic rates to understand and possibly predict survival times during starvation (DeVries and Appel, 2013; Rixon and Stevenson, 1957; Schimpf et al., 2012). When compared with other arthropods (ants, beetles, spiders), ticks, and thysanurans, we found bed bugs to align similarly to thysanurans, above ticks and slightly below other arthropods (Fig. 4). These results make sense in light of the biology of these species. Bed bugs have been reported to survive >1 year without feeding, similar to thysanurans (Lindsay, 1940; Meek, 2011). However, bed bugs do not have the same capacity to survive extended periods of starvation as ticks (Needham and Teel, 1991), but they can generally survive starvation longer than most other arthropods (Mallis, 2011). It is also important to note that bed bug SMRs were measured approximately 8–10 d after feeding. At this time, bed bug metabolism will have reached a plateau, but if they continue to starve their metabolic rates will continue to decline, sometimes >50% (DeVries, 2013). Therefore, it is reasonable to assume that if tested again at a later time, bed bug metabolic rates would be lower than currently reported and likely approaching the metabolic rate of ticks (Fig. 4).

Despite the observed relationship between standard metabolic rate and survival during starvation, it is still unclear what role metabolism plays in determining lifespan. Many authors have suggested that lower metabolism is indicative of a longer lifespan (Pearl, 1928), but this hypothesis has been increasingly challenged by ideas such as the free radical hypothesis (Dowling and Simmons, 2009; Harman, 1992). Niitepold and Hanski (2013) provide interspecific evidence suggesting that peak metabolic rate is positively correlated with lifespan. However, their study was unable to find any relationship between standard (resting) metabolic rate and life span. Life span is still a very complicated variable and the role metabolic rate plays in determining this is still unknown. However, survival during starvation is somewhat less complicated and the evidence provided by the current manuscript and by Lighton and Fielden (1995)and DeVries and Appel (2013) suggest that lower metabolic rates provide an adaptive advantage to arthropods that face long periods of starvation. The relationship between standard metabolic rate and survival during starvation should be further investigated to determine if this relationship holds true for other arthropods and other ectothermic species.

In conclusion, metabolism had a strong relationship with temperature for all stages of the bed bug, *C. lectularius*. Metabolism also had a strong relationship with mass for bed bugs as a group. This information was useful in identifying and characterizing differences among groups, including unmated and mated adults. In addition, our results provide support for the cell-size theory of metabolic scaling, due to the strong deviation from the 3/4 mass scaling factor. Our study also suggests that standard metabolic rate has good potential to help understand and possibly predict longevity during starvation for other arthropods. The results and equations presented in this paper should be useful for future metabolic studies, particularly those dealing with species capable of surviving extended periods of starvation.

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