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Water loss and metabolic activity in bed bug eggs (*Cimex lectularius*)

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Abstract. Few studies have evaluated water loss and respiratory activity of insect eggs, particularly insects that are known to live within indoor environments. The present study quantifies water loss and respiratory activity for the eggs of a re-emerging indoor pest of human environments *Cimex lectularius* (L.). Water loss is measured gravimetrically and calculated as a function of chorion permeability. For these studies, bed bug eggs are placed at 0% relative humidity and repeatedly weighed over 48 h. Temperature effects and bed bug strain differences on the standard metabolic rate (SMR) and respiratory quotient are measured using closed system respirometry. The SMR ($\dot{V}o_2$; mL g^{-1} h^{-1}) is measured for two field strain bed bugs and compared with a laboratory strain held at one temperature (25 °C). The standard metabolic rate is measured for Harlan (laboratory) strain bed bug eggs at six different temperatures (15, 20, 25, 30, 35 and 39 °C). Total water loss is not significantly different between all three strains. However, water loss across the chorion (chorion permeability) is significantly different between the Harlan laboratory strain and the two field collected strains. Standard metabolic rates for Harlan (laboratory) strain bed bug eggs increase with temperatures from 15 to 35 °C but decline at 39 °C. Overall, the Harlan bed bug eggs have the largest standard metabolic rates (0.18 ± 0.05 mL g^{-1} h^{-1}) compared with the Epic Center strain eggs (0.14 ± 0.03 mL g^{-1} h^{-1}) and Richmond strain eggs (0.16 ± 0.04 mL g^{-1} h^{-1}), although this difference is not significant.

Key words. Chorion permeability, common bed bug, egg, oxygen consumption, respiration, respirometry.

Introduction

Most insect eggs do not obtain water from their surrounding environment; instead, they contain all of the water required for development at the time of oviposition (Hinton, 1981). Furthermore, insect eggs are small in size and consequently have high surface area to volume ratios (Hinton, 1981). These high ratios make insect eggs particularly vulnerable to desiccation, complicating the balance between respiration activity and water conservation. The insect eggshell is comprised of an inner waxy layer and a crystalline structure to prevent water loss, although the many physiological components within the eggshell that contribute to its ability to conserve water are not fully understood. To date, it has been suggested that the thick outer chorion layer and the inner waxy and crystalline chorion layers assist in gas flux and water loss resistance (Woods, 2010).

Standard metabolic rates are quantified for a number of insect species, although few studies have focused on the egg stage. However, metabolic rates are quantified for eggs of certain insects: moths (Woods & Hill, 2004; Zrubek & Woods, 2006); milkweed bugs, beetles, grasshoppers and flies (Richards, 1964); and locusts (Slama, 2000; Kambule *et al.*, 2011). Most recently, Kambule *et al.* (2011) reported metabolic rates of diapause and nondiapause locust eggs (*Locustana pardalina*). Diapause eggs have low, stable metabolic rates, whereas nondiapause

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eggs have increased metabolic rates throughout development (Kambule *et al.*, 2011). Richards (1964) reported that developmental stage and temperature influence egg oxygen consumption rates in six different insect species. In general, all insect eggs that are evaluated consume more oxygen as the embryo develops (Richards, 1964). Slama (2000) reports that there is a 20-fold increase in oxygen consumed between freshly oviposited eggs compared with eggs nearing hatch in *Shistocerca gregaria*. This probably contributes to the high metabolic demands required during embryonic development that would slow as the eggs near hatching and become fully developed. Woods & Hill (2004) determined that oxygen availability affects the metabolic rates in moth eggs, thus influencing development time and subsequent survival. When eggs are exposed to higher temperatures, hyperoxia and hypoxia conditions greatly reduce survival.

The common bed bug is a recently re-emerging pest of the human environment (Hwang *et al.*, 2005; Anderson & Leffler, 2008; Doggett *et al.*, 2011; Wang *et al.*, 2013). This species is rapidly increasing its presence in human homes throughout the world. Modern human dwellings are typically equipped with indoor heating and cooling systems. These systems are known to contribute to the rapid desiccation of outdoor insect species that become accidently trapped indoors (Robinson, 1996). However, the common bed bug is able to survive and reproduce successfully under these stressful ambient conditions.

Although metabolism and subsequent water loss are quantified for bed bugs nymphs and adults (Benoit *et al.*, 2007; DeVries *et al.*, 2013, 2015), the metabolism and water loss in bed bug eggs remains to be evaluated. The low humidity and warm temperature of the indoor urban environment coupled with the inability to feed (acquire water) makes bed bug eggs particularly vulnerable to desiccation. Therefore, the present study aims to measure the standard metabolic rate and water loss rates of bed bug eggs. In the present study, standard metabolic rates are quantified by measuring the amount of oxygen consumed per egg mass over time ($mL^{-1}\,h^{-1}\,g^{-1}$). We also calculate eggshell permeability values to determine the amount of water lost through the eggshell.

Materials and methods

Experimental insects

All bed bug strains were reared and maintained in the Dodson Urban Pest Management Laboratory on the Virginia Tech campus in Blacksburg, Virginia. All strains were fed weekly with defibrinated rabbit blood (Hemostat, Dixon, Calfornia) using an artificial feeding system similar to that described in Montes *et al.* (2002). The bed bug strains were contained in plastic rearing jars (60 mL; Thermo Fisher Scientific, Waltham, Massachusetts) provided with cardboard pieces for harbourage, and enclosed with mesh at one end to allow for feeding. All rearing jars were stored in an environmental chamber under an LD 12 : 12 h photocycle at approximately 27 °C and 60% relative humidity (RH),.

Three bed bug strains were used for the water loss and chorion assays (Harlan, Richmond and Royal Oaks), as well as three strains for comparing standard metabolic rates (SMR) between strains (Harlan, Richmond and Epic Center). The Epic Center field strain was used to replace the Royal Oaks field strain in the SMR experiments because the Royal Oaks colony collapsed before further experiments could be conducted. Only one strain was evaluated for the effects of temperature on SMR because previous assays had shown there were no differences between strains and their SMR.

The laboratory strain bed bugs were acquired from Dr Harold Harlan (National Pest Management Association, Fairfax, Virginia) in February 2005. Dr Harlan had collected this strain from Fort Dix, New Jersey, in 1973 and maintained this population in colony prior to sending a portion of the colony to the Dodson laboratory. In addition, three field strain populations (Richmond, Royal Oaks and Epic Center) of bed bugs were also evaluated. The Richmond strain was collected from an elderly group home located in Richmond, Virginia, in 2008. The Epic Center strain was collected in 2008 in an apartment complex in Cincinnati, Ohio. The Royal Oaks strain was collected in Royal Oaks, Michigan, in 2006. Resistance to deltamethrin (0.06%) had been determined in all of the field collected strains [resistance ratios = Richmond (>391), Epic Center (>340) and Royal Oaks (>400; T. McCoy, unpublished observations), when these strains were evaluated in the laboratory, and compared with the (susceptible) Harlan strain (Polanco *et al.*, 2011).

For all studies, eggs were acquired from each strain by placing recently fed female bed bugs (30 groups of 10) into plastic Petri dishes (60 × 15 mm; Thermo Fisher Scientific) provided with a clean piece of filter paper (Whatman #1; GE Healthcare, U.K.) for oviposition daily. Filter papers containing eggs that were 24 h old were removed and allowed to age for two additional days (3 days old at beginning of experiments). Prior to the bioassay, eggs were gently removed from the filter papers using soft-tip forceps.

Water loss and chorion permeability

One laboratory strain of bed bug eggs (Harlan) and two field-collected strains (Richmond and Royal Oaks) were used for these assays. Groups of five eggs from each strain were weighed to the nearest 0.1 μg on a Cahn C-35 Microbalance (Thermo Fisher Scientific) and then placed inside an aluminium weigh boat. After the initial weight was recorded, groups of eggs were weighed again at 2, 4, 6, 8, 24 and 48 h. Between each weighing period, aluminium weigh boats containing eggs were placed inside sealed plastic containers (Rubbermaid, Fairlawn, Ohio) and maintained in an environmental chamber at 25 °C. Containers were prepared for egg storage by adding magnesium perchlorate desiccant crystals (Thermo Fisher Scientific) to the bottom of the containers to maintain a constant RH of 0% inside the containers (Winston & Bates, 1960). To minimize any container effects, each container held six egg replicates (two replicates from each strain) in a randomized complete block design. After the 48-h weighing period, each egg replication was dried in an Isotemp oven (Model 655F; Thermo Fisher Scientific) at 50 °C to the point of desiccation. After drying, the eggs were re-weighed on the microbalance to determine dry mass.

Eggshell permeability values were quantified from water loss measurements taken between the first 2- and 4-h period, similar to cutaneous measurements reported in Appel *et al.* (1991). Water loss measurements between 2 and 4 h of desiccation were used to determine eggshell permeability to avoid changes in eggshell shape that may occur rapidly in response to desiccation. To calculate chorion permeability (surface area × water loss × saturation deficit), we first had to calculate egg surface area for each strain. Bed bug eggs are cylindrical; therefore, we calculated the surface area of the egg using the formula for calculating the surface area of a cylinder ($2\pi r^2 + 2\pi rh$) where 'r' was $^1/_2$ the width of an egg and 'h' was the length an egg. A microscope fitted with a micrometer was used to measure mean egg length and width of 12 bed bug eggs from each strain. The saturation deficit (3.17 kPa) was held constant because all experiments were conducted under the same humidity and temperature conditions.

Standard metabolic rates

Groups of bed bug eggs (7–20) from three strains (Harlan, Richmond and Epic Center) were placed into plastic syringes (3 mL; Becton, Dickinson and Company, Rutherford, New Jersey) that had two holes (diameter 1.4 mm) drilled into the syringe barrel. Each syringe was then attached to a manifold and purged of CO_2 and water and then the syringe plunger was pulled above the drilled holes to allow dry CO_2 to flow through the syringe. After removal of water vapour and CO_2, each syringe was removed from the manifold and set to a final volume of 0.7 mL. A needle (26-gauge intradermal bevel needle; Becton, Dickinson and Company) was attached to each syringe and inserted into a rubber stopper to prevent the exchange of atmospheric gases and water. The syringes containing bed bug eggs were then placed inside of an environmental chamber.

One strain (Harlan) of bed bug eggs was used to determine the effects of temperature on SMR. Eggs held inside syringes were incubated at one of six temperatures (15, 20, 25, 30, 35 and 39 °C) for different periods of time (depending on the incubation temperature) ranging from 12 to 60 h and allowed to respire. Extended time periods (up to 60 h) were necessary to obtain oxygen consumption values at low temperatures when insect egg metabolism was slowed. The exact time of incubation was recorded, starting from the time the syringe was sealed. To determine whether there were any differences between strains and SMR, oxygen consumption was measured among three strains (Harlan, Richmond and Epic Center) at one temperature (25 °C).

Each syringe was considered as a replicate with a minimum of seven eggs per syringe. A minimum of 10 replicates were used for each temperature with two control syringes. The control syringes contained no eggs but were subjected to the same procedures as the syringes containing eggs. After incubation, a bed bug egg air sample (0.5 mL) from inside each syringe barrel was injected into the respirometry system for analysis to determine O_2 depletion and CO_2 production. Control syringes were injected into the system that had been purged of CO_2 and water with a known O_2 concentration.

Table 1. Water characteristics (mean ± SE) of a laboratory strain (Harlan) and two field-collected strains (Richmond and Royal Oaks).

Strain	n	Initial mass (μg)	Dry mass (μg)	Water mass (μg)	Water content (%)
Harlan	60	673.0 ± 24.7	211.2 ± 11.8	463.8 ± 25.5	68.8 ± 2.0
Richmond	60	665.3 ± 13.7	215.5 ± 2.8	449.8 ± 8.8	67.6 ± 0.2
Royal Oaks	60	692.7 ± 9.3	230.1 ± 6.0	462.6 ± 12.6	66.8 ± 0.7

The system used atmospheric air in the room and forced it through a purge gas generator (Whatman Inc., Haverhill, Massachusetts) to remove CO_2 and H_2O. The purged air then equilibrated in large barrels (340 L) to reduce the pressure of the air being pulled into the rest of the respirometry system. After equilibration, the air was pulled through a Drierite-Ascarite-Drierite [Drierite (WA Hammond Drierite Co., Ltd, Xenia, Ohio); Ascarite (Thomas Scientific, Swedesboro, New Jersey)] column to further remove any traces of water or CO_2. The column air was then pulled past an injection port, where the syringe samples (control and bed bug air samples) were injected. Both the atmospheric air and the syringe sample were then drawn through a Li-6251 CO_2 analyzer (Li-COR Inc., Lincoln, Nebraska) and an Oxzilla II O_2 analyzer (Sable Systems, Henderson, Nevada). A mass flow generator (MFS2; Sable Systems) was used to pull and maintain air through the entire system at a constant rate of 100 mL min^{-1}. All syringe sample data were recorded using DATACAN V (Sable Systems).

Statistical analysis

Water loss was gravimetrically measured over time and compared among bed bug strains (Harlan, Richmond and Royal Oaks) using a repeated measures multivariate analysis of variance (JMP, version 9.0; SAS Institute Inc., Cary, North Carolina). Values of $\alpha < 0.05$ were used to indicate significance. Water loss measurements were then compared between strains (Harlan, Richmond and Royal Oaks). Water loss was determined for each bed bug strain by subtracting the egg weight (μg) at each time period (2, 4, 6, 8, 24 and 48 h) from the initial egg weight (μg). The percentage water content of each egg was calculated by subtracting the weight of the dry egg (μg) from that of the initial egg weight (μg) and dividing that number by the initial egg weight (μg) (Table 1). Chorion permeability values and surface area values were compared between strains using analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant. The strain means then were separated using a Tukey–Kramer honestly significant difference test (JMP, version 9.0).

The metabolic data were analyzed by measuring the area below the gas volume peaks output from DATACAN V. The control syringes were purged of CO_2 and H_2O with a known amount of O_2; therefore, the depletion of O_2 in the bed bug air syringes was calculated from the known O_2 concentration in the control syringes. The gas volume data were then converted to mL h^{-1} to obtain standard metabolic rates.

Standard metabolic rates were compared among strains held at a constant temperature (25 °C) using ANOVA ($P \leq 0.05$ was

considered statistically significant). Means were separated using Tukey–Kramer significant difference test. Metabolic rates for Harlan strain eggs at different temperatures were analyzed using linear regression analysis. All statistical analyses were conducted using JMP PRO, version 10.0.0 (SAS Institute Inc.)

Respiratory quotients (RQs) were calculated by dividing total CO_2 production by total O_2 consumption. Q_{10} values quantify increasing $\dot{V}o_2$ with a 10° temperature increase (Lighton, 1989). These values were quantified by first log-transforming $\dot{V}O_2$ and then regressing log-transformed $\dot{V}O_2$ against temperature. The resultant slope was then multiplied by 10 and the antilogarithm of that value yields the mean Q_{10} (Lighton, 1989).

Results

Water loss rates and chorion permeability values

Eggs from the tested strains were determined to contain approximately 70% water and were similar in their water balance characteristics (Table 1). There were no significant differences in water loss between strains held at the same temperature (repeated measures ANOVA, $F = 0.2367$, d.f. = 2,12; $P = 0.79$) (Fig. 1).

Chorion permeability values ($\mu g\,h^{-1}\,mm^{2-1}\,mmHg^{-1}$) were significantly different among strains ($F = 36.9$, d.f. = 2,35; $P \leq 0.0001$). Eggs from the Harlan strain had significantly higher mean chorion permeability values ($64.5 \pm 0.46\,\mu g\,h^{-1}\,mm^{-2}\,mmHg^{-1}$) during the first 2 h desiccation period compared with the two field strains (Richmond: $43.6 \pm 0.45\,\mu g\,h^{-1}\,mm^{-2}\,mmHg^{-1}$; Royal Oaks: $57.7 \pm 0.60\,\mu g\,h^{-1}\,mm^{-2}\,mmHg^{-1}$). The mean separation test indicated that the chorion permeability values of all three strains were significantly different. The surface areas of Harlan strain and Richmond eggs were not significantly different (1.81 and 1.83 mm^2, respectively), although both strains were significantly larger from the Royal Oak strain eggs (1.61 mm^2) (ANOVA, $F = 4.32$, d.f. = 2,35; $P = 0.02$).

Temperature effects on standard metabolic rates and RQ

Temperature had a significant effect on respiratory rate (ANOVA, $F = 15.7$, d.f. = 4, 68; $P = 0.0001$) of the Harlan strain bed bugs. The post-hoc comparison test indicated that the bug egg respiratory rate at high temperatures (30 and 35 °C) was significantly greater than at room temperature (25 °C) or at low temperatures (15 and 20 °C) (Table 2). As expected, oxygen consumption increased positively with temperature in Harlan strain bed bug eggs (Fig. 2). We tested Harlan strain bed bugs at 39 °C as well; however, many of the eggs did not survive after being held at this temperature (percentage survival: 15 °C = 84%, 25 °C = 91%, 30 °C = 96%, 35 °C = 90%, 39 °C = 29%) and their SMR rates declined (Table 2). Therefore, we did not include these data in our analyses. Linear regression analysis of $\dot{V}o_2$ and temperature yielded the equation: $\dot{V}_{o_2} = 0.1023$ (Temperature) – 0.08, with a corresponding Q_{10} value of 2.82 (Fig. 2). Harlan bed bug egg RQ values (RQ = carbon dioxide production/oxygen consumption) ranged from 0.54 ± 0.06 to 0.65 ± 0.08 for eggs exposed to the five different temperatures (Table 2).

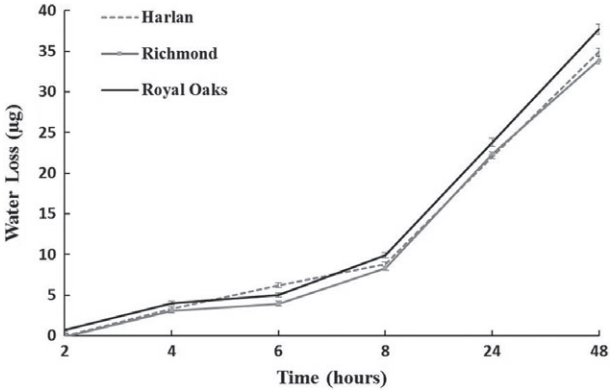

Fig. 1. Water loss of Harlan, Richmond and Royal Oak bed bug eggs over 48 h. There were no significant differences between strains.

Table 2. Comparison of mean ± SE mass specific oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$) and RQ values across six temperatures for the Harlan strain.

Temperature (°C)	*n*	$\dot{V}_{O_2}$	$\dot{V}_{CO_2}$	RQ
15	160	0.068 ± 0.017 a	0.038 ± 0.026 a	0.56 ± 0.05 a
20	120	0.083 ± 0.018 a	0.052 ± 0.008 a	0.65 ± 0.08 b
25	160	0.180 ± 0.056 b	0.097 ± 0.031 b	0.54 ± 0.06 a
30	160	0.367 ± 0.102 c	0.213 ± 0.033 c	0.55 ± 0.05 a
35	91	0.438 ± 0.087 c	0.245 ± 0.031 c	0.57 ± 0.06 a
39	56	0.315 ± 0.100 d	0.153 ± 0.026 d	0.50 ± 0.05 c

Means followed by different lowercase letters are significantly different (analysis of variance; JMP PRO, version 10.0). Level of significance was determined for $P \leq 0.05$. As temperatures reached 39 °C, the bed bug eggs became too stressed and oxygen consumption decreased.

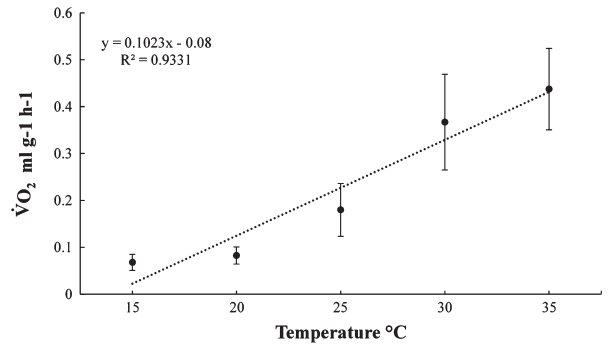

Fig. 2. Harlan strain egg standard metabolic rate (SMR) ($\dot{V}_{o_2}$) at five temperatures (15, 20, 25, 30 and 35 °C).

Strain effects on respiration rates

Overall, Harlan strain bed bug eggs ($0.16 \pm 0.01\,O_2^{-1}\,h^{-1}\,g^{-1}$) had larger standard metabolic rates than Epic Center strain bed

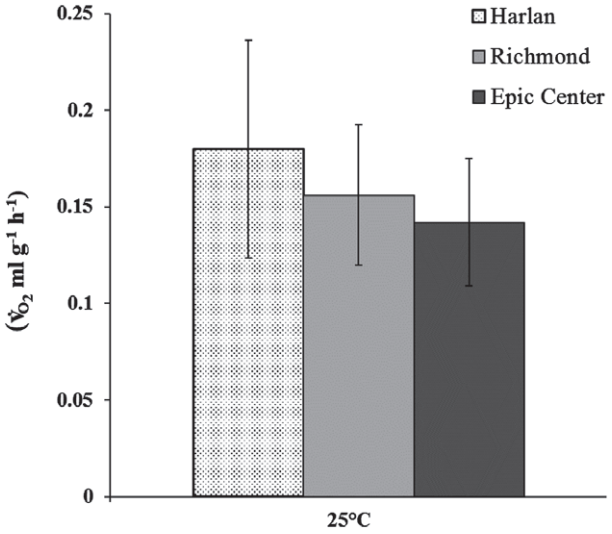

Fig. 3. Rate of oxygen consumption ($\dot{V}_{O_2}$) for three strains of bed bug eggs (Harlan, Richmond and Epic Center) at 25 °C. There were no significant differences in oxygen consumption between strains, as represented by the error bars.

bug eggs ($0.12 \pm 0.02\,mL^{-1}\,h^{-1}\,g^{-1}$) and Richmond bed bug eggs ($0.15 \pm 0.01\,mL^{-1}\,h^{-1}\,g^{-1}$) (Fig. 3). However, metabolic rate, as indicated by oxygen consumption, did not differ significantly ($F_{2,35} = 2.51$, $P = 0.096$) between strains. RQ values (O_2 consumption/CO_2 production) for the three strains were 0.69 ± 0.17 (Harlan), 0.58 ± 0.11 (Richmond) and 0.63 ± 0.15 (Epic Center).

Discussion

A previous study compared water balance characteristics of all post-egg life stages of bed bugs (Benoit *et al.*, 2007). Interestingly, the present study reveals that the water contents of all strains of bed bug eggs are similar to those determined for the post-egg life stages (approximately 70%) as documented previously (Table 1). The present study suggests that bed bug eggs are able to conserve the same percentage of body water that the adult and nymphal life stages maintain through blood consumption. Water conservation is essential for bed bug eggs in an environment of low humidity, particularly because they do not have the possibility of moving to a crack or crevice where they can maintain a suitable microhabitat.

Water loss does not differ between strains; thus, we hypothesize that the ability of eggs to limit water loss throughout the duration of embryonic development is a trait that is highly conserved among different bed bug populations. Genetic selection for desiccation resistance in *Drosophila* species is reported previously for species that dwell in more arid, temperate climates compared with more humid, subtropical environments (Hoffmann & Parsons, 1989). Bed bugs are known to survive long periods of time without consuming a blood meal in the absence of a host and are highly desiccant resistant (Benoit *et al.*, 2007). These post-egg bed bug life stages are shown to have water-conservation characteristics similar to those of desert-adapted insect species (Benoit *et al.*, 2007). Bed bug eggs must be able to survive low humidity and high indoor temperatures, much like insect species that dwell in dry, arid climates. Not surprisingly, bed bug eggs from all of the populations that we test in the present study are similarly able to resist desiccation based on their relatively low water loss at 0% RH.

Although the rate of water loss over time is similar among strains, the Harlan strain eggs lose significantly more water across the chorion compared with the two field-collected strains. Chorion permeability is a function of the egg surface area, and influences the amount of water lost. Hence, an egg that has a larger surface area loses more water than a smaller egg with a lower surface area. Consequently, the Harlan strain of eggs lose significantly more water across the chorion and and exhibit larger surface areas compared with one field strain (Royal Oaks strain). However, the Harlan strain eggs and Richmond strain eggs do not have significantly different surface areas, although the Harlan strain has a significantly higher chorion permeability value. Thus, the overall size and mass of the egg correlates with water loss but there are also differences in water loss characteristics between strains. The difference between strains could be a result of the composition of the eggshell and the thickness, which could be attributed to former exposure of bed bug populations to varied environmental conditions.

SMR increases as temperatures are elevated until reaching 39 °C, when it then declines in the Harlan strain. Generally, this positive correlation is documented in other insect species, until the insect egg reaches a thermal limit, when the SMR declines or ceases (Richards, 1964; Slama, 2000). The lethal temperature for bed bug eggs, *C. lectularius*, is 54.8 °C (Kells & Goblirsch, 2011). However, lethal temperatures for a closely related species, *Cimex hemipterus*, are reported as ≥ 39 °C (How & Lee, 2010). Therefore, the observed decrease in metabolic rate at the highest temperature tested (39 °C) is probably a result of embryonic stress as temperatures approached the thermal lethal limit. Consequently, only 29% of eggs that are tested at 39 °C hatch after the metabolic experiments, whereas 90% hatch at the next lowest tested temperature (35 °C). The Harlan strain eggs exhibit the highest SMR at 35 ° C. This temperature is found to be optimal for bed bug egg hatching and is also the temperature that results in the most rapid development and the shortest time to hatch (How & Lee, 2010).

Bed bug populations from across the eastern U.S.A. show high genetic variation (Saenz *et al.*, 2012) and insecticide resistance is variable across bed bug populations dependent upon their previous exposure (Zhu *et al.*, 2010). We expected that bed bug eggs collected from pyrethroid-resistant strains (all the field populations tested) would potentially exhibit higher metabolic rates as a result of their resistance profiles. However, there are no significant differences in standard metabolic rates between strains. The Richmond strain, in particular, is shown to exhibit enhanced detoxification enzyme activity related to resistance (Adelman *et al.*, 2011). We expected that the physiological demands of producing elevated levels of detoxification enzymes would potentially cause an increase in metabolism. However, SMR does not differ between strains and is not a function of insecticide resistance. Our bed bug populations are not exposed

to insecticides in the laboratory once collected from the field; thus, there is the potential that there are no differences in SMR between strains because they are not continually selected for resistance (Kramarz & Kafel, 2003; Dingha *et al.*, 2009).

Metabolic rates can be utilized to determine RQ values. Respiratory quotient values assist with the determination of the substrate (protein, fat or carbohydrates) that an embryo is oxidizing during development (Boell, 1935). The bed bug eggs from the strains that we evaluate in the present study have similar RQ values (near 0.7) when measured at 25 °C. RQ values near 0.7 suggest that the bed bug embryo is utilizing lipids for embryogenesis. The RQ values that we record for bed bug eggs are higher than the values reported previously for bed bug nymphs and adults (DeVries *et al.*, 2013). DeVries *et al.* (2013) report newly hatched nymphs having RQ values of 0.53 ± 0.01. These different RQ values suggest that newly hatched nymphs have different metabolic requirements after eggshell emergence than during embryonic development.

SMR is a function of temperature. Q_{10} values represent the change in SMR for every 10° temperature change. Bed bug eggs have a Q_{10} value of 2.8, demonstrating that SMR in bed bug eggs increases three-fold for every 10 °C increase in temperature. The Q_{10} values for recently hatched first instars are reported to be 3.05 (DeVries *et al.*, 2013), suggesting that bed bug eggs and newly hatched first instars respond in a similar way metabolically to increased temperature. Post-diapause insect eggs exhibit Q_{10} values (approximately 3.0) similar to our reported bed bug egg values over a range of temperatures from 10 to 35 °C (Richards, 1964).

In conclusion, metabolic rates and water loss in bed bug eggs are similar to other post-egg life stages of bed bugs. Water loss between different bed bug strains is not significantly different; however, the surface area of eggs has an effect on the amount of water loss across the eggshell. Harlan strain eggs are larger and have a greater surface area, and thus lose more water across the chorion. SMR does not differ between strains; therefore, there is little difference in the physiological and metabolic requirements of bed bug eggs between populations. Insecticide resistance does not influence metabolic rates; however, future studies should evaluate bed bug egg respiratory behaviour during and shortly after insecticide exposure. These respiration rates would be valuable for determining how bed bug eggs respond physiologically to insecticide exposure. Closely related species, such as *C. hemipterus*, or *C. lectularius* that feed on different hosts (poultry and bats), could also be evaluated in further SMR studies to differentiate the metabolic requirements between species, if present, or to determine whether there is host-associated divergence in metabolic requirements.

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