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Estimating the critical thermal maximum (CT_{max}) of bed bugs, *Cimex lectularius*: Comparing thermolimit respirometry with traditional visual methods

Zachary C. DeVries [a,b,*], Stephen A. Kells [c], Arthur G. Appel [a]

[a] Department of Entomology and Plant Pathology, 301 Funchess Hall, Auburn University, Auburn, AL 36849, USA
[b] Department of Entomology, Campus Box 7613, North Carolina State University, Raleigh, NC 27695-7613, USA
[c] Department of Entomology, 219 Hodson Hall, 1980 Folwell Ave., University of Minnesota, St. Paul, MN 55108, USA

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ABSTRACT

Evaluating the critical thermal maximum (CT_{max}) in insects has provided a number of challenges. Visual observations of endpoints (onset of spasms, loss of righting response, etc.) can be difficult to measure consistently, especially with smaller insects. To resolve this problem, Lighton and Turner (2004) developed a new technique: thermolimit respirometry (TLR). TLR combines real time measurements of both metabolism ($\dot{V}_{CO2}$) and activity to provide two independent, objective measures of CT_{max}. However, several questions still remain regarding the precision of TLR and how accurate it is in relation to traditional methods. Therefore, we evaluated CT_{max} of bed bugs using both traditional (visual) methods and TLR at three important metabolic periods following feeding (1 d, 9 d, and 21 d). Both methods provided similar estimates of CT_{max}, although traditional methods produced consistently lower values (0.7–1 °C lower than TLR). Despite similar levels of precision, TLR provided a more complete profile of thermal tolerance, describing changes in metabolism and activity leading up to the CT_{max}, not available through traditional methods. In addition, feeding status had a significant effect on bed bug CT_{max}, with bed bugs starved 9 d (45.19[±0.20] °C) having the greatest thermal tolerance, followed by bed bugs starved 1 d (44.64[±0.28] °C), and finally bed bugs starved 21 d (44.12[±0.28] °C). Accuracy of traditional visual methods in relation to TLR is highly dependent on the selected endpoint; however, when performed correctly, both methods provide precise, accurate, and reliable estimations of CT_{max}.

1. Introduction

Temperature is a critical factor underlying the abundance and distribution of organisms (Molles, 2012; Price et al., 2011). In particular, understanding the critical thermal maximum (CT_{max}) of organisms is important as temperatures continue to increase and climate change produces greater temperature variability (Cox et al., 2000; Walther et al., 2002). CT_{max} has been defined as, "the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death" (Cowles and Bogert, 1944). CT_{max} has been measured for a variety of insects, showing a considerably wide range from <30 °C to >50 °C (Araújo et al., 2013; Hoffmann et al., 2013; Kellermann et al., 2012). Understanding CT_{max} is not only important in relation to climate change, but it is also critical for pests associated with the indoor urban environment, which are often shielded from the effects of climate change. In the urban environment, temperature is commonly used in control efforts, particularly with bed bugs (Cooper, 2011; Kells, 2006; Kells and Goblirsch, 2011). It is also worth noting that even in the indoor settings, CT_{max} is still positively correlated with adaptation to warm environments (Appel et al., 1983).

Despite its importance, there are still a plethora of problems associated with both the measurement of CT_{max} and the consistency of these measurements (Lutterschmidt and Hutchison, 1997; Terblanche et al., 2011). In particular, measurements of CT_{max} have been confounded by the selection of an appropriate endpoint. The most common parameters used to estimate CT_{max} are loss of righting response (LRR) and the onset of muscular spasms (OS) (Lutterschmidt and Hutchison, 1997). These parameters can be difficult to assess in small arthropods, therefore many authors have estimated the upper lethal limit using a static method, where groups of animals are exposed for varying times to target temperatures and mortality is assessed (ULL, Lutterschmidt and Hutchison, 1997). However, the static method requires a large number of insects which are not always available, does not provide information on an individual scale, and does not truly address the CT_{max}. To further complicate CT_{max} estimation, there is currently an ongoing debate with some authors criticizing the validity of measurements made using the dynamic method (Rezende et al., 2011; Santos et al., 2011) and others finding these

* Corresponding author at: Department of Entomology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695, USA.
E-mail address: zcdevrie@ncsu.edu (Z.C. DeVries).

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methods to be appropriate for estimating CT_{max} (Overgaard et al., 2012; Terblanche et al., 2011).

In an effort to improve CT_{max} estimation, Lighton and Turner (2004) explored a new technique termed thermolimit respirometry (TLR). This technique allows for the simultaneous measurement of respiration and activity in response to increasing temperature. Their results on the thermophilic desert ant, *Pogonomyrmex spp.*, indicated an extremely high level of precision in estimating CT_{max} by both activity and respiration (Lighton and Turner, 2004). However, Klok et al. (2004) did not find the same level of precision when using TLR on both a terrestrial isopod (*Armadillidium vulgare*) and a tenebrionid beetle (*Gonocephalum simplex*). In addition, Stevens et al. (2010) found low, but comparable, levels of precision between traditional (visual) methods and TLR, with traditional methods estimating higher CT_{max} values than TLR. These studies suggest that although TLR may provide a more objective estimate of CT_{max}, precision may not be better than traditional methods. Thus, further investigation into the differences between traditional methods and TLR is required.

To compliment these questions regarding estimation of CT_{max}, the effect of heat on bed bugs has not been estimated using an objective dynamic method such as TLR. Heat is a common method used to control bed bugs because they have developed high levels of resistance to many commonly used insecticides (Adelman et al., 2011; Kells, 2006; Zhu et al., 2010). Recent studies have evaluated thermal tolerance in bed bugs using the static method (Benoit et al., 2009; Kells and Goblirsch, 2011; Pereira et al., 2009). Of the three most recent studies, only one calculated an LT_{50} (i.e., lethal temperature, 43.5 °C; Kells and Goblirsch, 2011). The other two studies only report percent mortality at a range of temperatures (Benoit et al., 2009; Pereira et al., 2009). These studies provide useful information on bed bug thermal tolerance, particularly in terms of bed bug management; however, they make comparisons among studies difficult. Bed bug metabolism has also been evaluated, but only at temperatures below the described ULL (DeVries et al., 2013). In addition, when measured at 25 °C, DeVries et al. (2015a) found starvation to have significant yet characteristic effects on bed bug metabolism. Specifically, DeVries et al. (2015a) found metabolic rate peaked at ~1 d after feeding, declined rapidly until 7 d, where it remained stable (plateaued) for 2 d. After this plateau period, metabolic rate continues to decline slowly in an exponential decay form. Therefore, because we know how starvation affects metabolism, it would be useful to evaluate how starvation affects thermal tolerance.

In this study we evaluated CT_{max} in bed bugs starved for a range of times. To estimate CT_{max}, both traditional methods using video recordings as well as TLR were employed. Both methods (traditional visual and TLR) were compared and CT_{max} was estimated among feeding statuses. The results are discussed in relation to CT_{max} estimation methodology and bed bug thermal tolerance.

2. Materials and methods

2.1. Experimental animals

An insecticide susceptible strain of bed bugs originally obtained from i2L Research (Baltimore, MD) was reared at the University of Minnesota. Bugs were maintained in 0.5 L glass jars with mesh tops at 23 ± 2 °C and 55 ± 5% RH on a 14L:10D light cycle. Bed bugs were fed 1:1 combination of human red blood cells and plasma, obtained from expired stocks provided by the American Red Cross (St. Paul, MN), through an artificial feeding system as described by Montes et al. (2002). Bed bugs were shipped to Auburn, AL, immediately following feeding as needed. Upon arrival, insects were housed under identical conditions until they reached one of three starvation times: 1 d, 9 d, or 21 d, reflecting three distinct metabolic periods experienced by bed bugs (DeVries et al., 2015a). Adult males were used for all experiments, and masses ranged from 2.42 mg (21 d starved) to 7.80 mg (1 d starved).

2.2. Traditional CT_{max} determination

Bed bugs were weighed with a digital balance (AX205; Mettler-Toledo, Greifensee, Switzerland) and then placed onto a Peltier temperature controlled plate controlled by a Pelt-5 temperature controller (Sable Systems International, Henderson, NV, U.S.A.—hereafter termed Sable Systems) at room relative humidity (20 ± 5%). A plastic Petri dish (diameter = 4 cm; Falcon Plastics, Brookings, SD, USA) was inverted and placed over the bed bugs to hold them within the Peltier plate boundaries. After placing bed bugs onto the plate, the following program was initiated: start and hold at 30 °C for 5 min then ramp at 0.5 °C·min^{-1} to 50 °C. This temperature ramp rate was used to ensure that bugs did not acclimate while simultaneously preventing a lag time between body temperature and ambient temperature (Lighton and Turner, 2004), and had been shown to be effective when used with mosquitoes of similar mass (Vorhees et al., 2013). Throughout the experiment, temperature was measured independently via a copper constantan bead thermocouple placed directly on the hot plate and connected to a TC-2000 Type-T thermocouple meter (Sable Systems), to verify temperature and subsequent rate of increase. A minimum of 10 replicates were performed for each feeding status. Bed bugs were weighed and examined in groups of 2 due to the size of the heating arena. However, the results from bed bugs in groups of 2 were averaged and treated as 1 replicate to avoid pseudo-replication.

Throughout the experiment, bed bugs were monitored via a Sony handycam video camera (DCR-SX86; Sony, Tokyo, Japan). Video recordings were viewed and analyzed with Windows® media player (Microsoft, Redmond, Washington, U.S.A.). Videos were assessed and CT_{max} was determined when body movement ceased. Temperature data from TC-2000 Type-T thermocouple meter was recorded simultaneously with the video time so that CT_{max} could be determined at any time during the video.

2.3. Thermolimit respirometry

The methods employed for TLR were modified from the protocol outlined by Lighton and Turner (2004). Bed bugs were weighed individually as above and placed into a 30 mL glass respirometry chamber (Sable Systems). Respirometry chambers were placed onto an AD-1 activity detector housed within a temperature controlled cabinet and controlled by a Pelt-5 temperature controller (Sable Systems). The activity detector measured fluctuations in infrared light (ca. 900 nm) caused by movement (Lighton, 1988). The temperature controlled cabinet was programmed to start and hold at 30 °C for 5 min then increased by 0.5 °C·min^{-1} to 50 °C. Rate of temperature increase was determined by a thermocouple inserted into the respirometry chamber and connected to TC-2000 Type-T thermocouple meter (Sable Systems) which was used to validate the temperature ramp rate.

Metabolic measurements were made using a flow-through respirometry system. An electric air compressor (Kobalt 2-HP 30-Gallon 155-PSI 120-Volt Vertical Electric Air Compressor, Lowe's Companies Inc., Mooresville, NC, USA) delivered room air into a Whatman purge-gas generator (Whatman Inc., Haverhill, MA, USA) that removed CO_2 and H_2O. The air then moved into a 340 L mixing tank followed by a 30 L manifold to permit equilibration to atmospheric pressure. A mass flow system (MFS2; Sable Systems) controlled the air flow (i.e., pulled the air) from the manifold through the rest of the apparatus at a rate of 75 mL min^{-1} at STP (as confirmed by a calibrated glass and metal ball rotameter). From the manifold, this air flowed through a Drierite®-Ascarite®-Drierite® column (Drierite-W.A. Hammond Drierite Company Ltd., Xenia, OH, USA; Ascarite-Thomas Scientific, Swedesboro, NJ, USA) to ensure the air was dry and CO_2-free. The air then flowed through a 2 m copper coil (i.d. = 3 mm) housed within the temperature controlled cabinet. Next the air was pulled through the respirometry chamber, a CO_2 analyzer (Li-6251; Li-COR Inc., Lincoln, NE, USA) and then finally through the mass flow controller. Data were

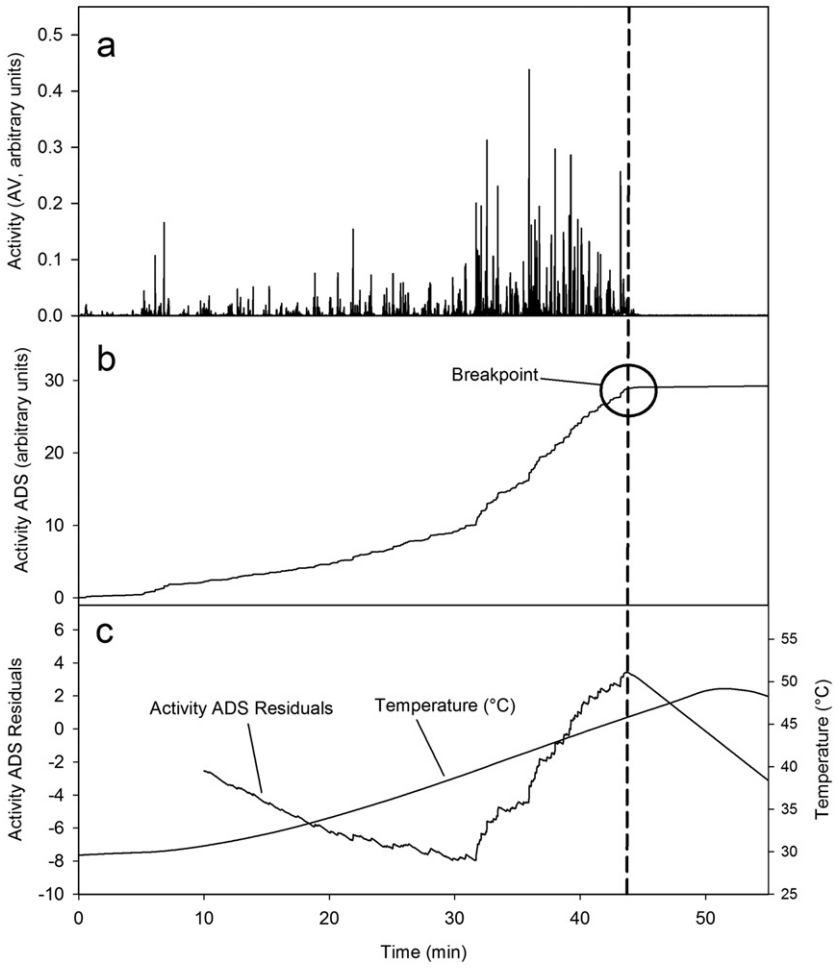

Fig. 1. Example bed bug recording showing: a) the absolute value of raw activity (arbitrary units), b) activity ADS (arbitrary units) with the breakpoint indicated, and c) the residuals of the regression line around the breakpoint (unitless). Temperature (°C) is shown in (c) to display how CT_{max} would be inferred from this data and a dotted line is displayed vertically through the breakpoint. The intersection between the dotted line and temperature is the activity based CT_{max}.

acquired using Datacan V software and analyzed in ExpeData software (Sable Systems).

2.4. Data analysis

To determine CT_{max} values from TLR data, we followed the procedures outlined by Lighton and Turner (2004) with minor modifications. Briefly, absolute difference sums (ADS) were calculated for both activity and $\dot{V}_{CO2}$. ADS values were calculated by sequentially adding the absolute differences between adjacent data points, creating a picture of variability and change for a recorded parameter (Lighton and Turner, 2004). ADS data were then analyzed by regression around the breakpoint, that is, the point at which there is a sudden change in the slope of the ADS trace line (ca. 5 min, Fig. 1, b). Further analysis of the linear regression residuals revealed a clear inflection point for both activity ADS residuals and $\dot{V}_{CO2}$ ADS residuals (Fig. 1, activity). The temperatures (°C) at the inflection points were then recorded as either the activity ADS CT_{max} or the $\dot{V}_{CO2}$ ADS CT_{max}. Both CT_{max} values were then averaged for each individual to generate an average CT_{max}. For a visual representation of this process please refer to Fig. 1 (representative example of activity) and for more information on this process, please refer to Lighton and Turner (2004).

Two $\dot{V}_{CO2}$ measurements were made, one during the equilibration plateau period and the second immediately preceding the combined CT_{max}. The equilibrium plateau $\dot{V}_{CO2}$ was measured as the average of the first 5 min of the TLR measurement, and the CT_{max} $\dot{V}_{CO2}$ was measured as the average of 15 s preceding the combined CT_{max}. All TLR calculations were made using ExpeData software (Sable Systems).

All means are reported with ± SEM. All CT_{max} and $\dot{V}_{CO2}$ comparisons (both traditional visual and TLR) among feeding statuses and between traditional visual and TLR methods were made using analysis of variance (SAS Institute, 1985). Means were further compared using the least significant difference mean comparison test and significance was determined at the $p < 0.05$ level. Linear regression was used to understand the relationships among equilibration plateau $\dot{V}_{CO2}$ (μL h^{-1}), CT_{max} $\dot{V}_{CO2}$ (μL h^{-1}), and combined CT_{max} (°C) (SAS Institute, 1985).

Table 1
Traditional CT_{max} measurements for bed bugs starved 1 d, 9 d, and 21 d. All values reported are means accompanied by (±SEM).

	Starvation time		
	1 d	9 d	21 d
Sample size	9	10	10
Body mass (mg)	6.21(±0.20)	4.73(±0.14)	3.09(±0.13)
Visual CT_{max} (°C)	43.95(±0.07) A	44.21(±0.29) A	43.21(±0.12) B

Note: Statistical comparisons are made within rows, and means followed by the same letter are not significantly different according to the least significant difference mean comparison test at the $p < 0.05$ level.

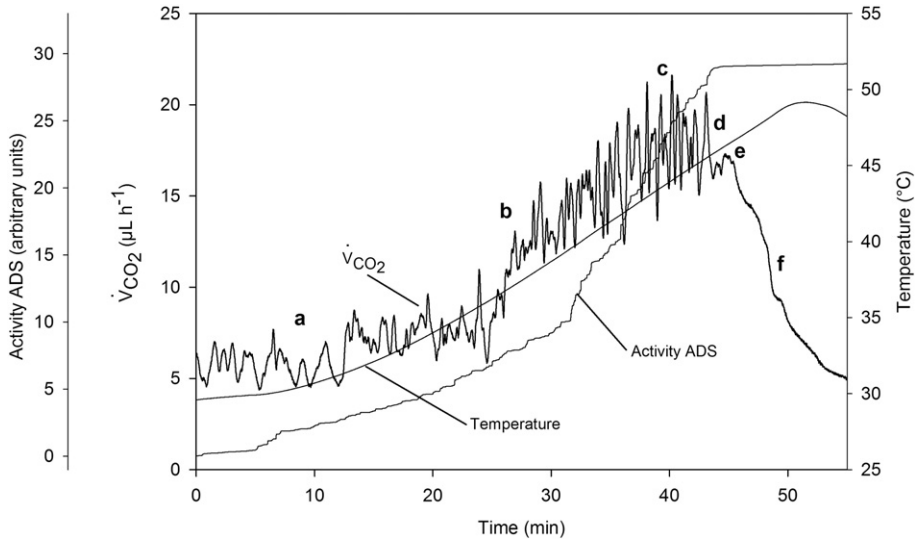

Fig. 2. Characteristic thermolimit respirometry (TLR) recording depicting temperature (°C), $\dot{V}_{CO2}$ (μL h^{-1}), and activity ADS (arbitrary units). This recording is representative of all other TLR recordings. Major regions of the graph that should be noted include: (a) the equilibration plateau; (b) a rapid increase in $\dot{V}_{CO2}$ leading up to (c) the pre-mortal plateau; (d) $\dot{V}_{CO2}$ rapidly declining and leading to (e) the post-mortal plateau; followed by (f) the post-mortal decline.

3. Results

3.1. Traditional (visual) CT_{max}

The rate of temperature increase for traditional visual CT_{max} experiments was measured as 0.499 ± 0.001 °C min^{-1} and no differences were detected among starvation treatment groups ($F_{2,12} = 0.44$, $p = 0.6531$). Visual CT_{max}, as estimated by the observed cessation of movement, was significantly different among feeding groups ($F_{2,26} = 7.58$, $p = 0.0025$). Bed bugs fed recently (24 h) or starved 9 d had significantly greater thermal tolerance than bed bugs starved 21 d (Table 1).

3.2. CT_{max} from thermolimit respirometry

A consistent pattern of respiration ($\dot{V}_{CO2}$, μL h^{-1}) was observed as temperature increased (Fig. 2). This pattern was characterized with an initial equilibration plateau, where $\dot{V}_{CO2}$ remained relatively stable (Fig. 2, a). This was followed by a rapid increase in $\dot{V}_{CO2}$ (Fig. 2, b) leading up to the pre-mortal plateau (Fig. 2, c), a time range where $\dot{V}_{CO2}$ stopped increasing with temperature, as defined by Lighton and Turner (2004). Next, $\dot{V}_{CO2}$ rapidly declined (Fig. 2, d), leading to the post-mortal plateau (Fig. 2, e) where $\dot{V}_{CO2}$ showed very little variation for a short period of time. Finally, a post-mortal decline was observed (Fig. 2, f), where $\dot{V}_{CO2}$ declined in a typical exponential fashion. No post-mortal peak was observed, as described by Lighton and Turner (2004).

The rate of temperature increase for TLR was measured as 0.503 ± 0.004 °C min^{-1} and no differences were detected among treatment groups ($F_{2,32} = 1.22$, $p = 0.3074$). Using the ADS residual method, CT_{max} was estimated based on activity, respiration ($\dot{V}_{CO2}$), and the average of activity and respiration (Table 2). Analysis of variance detected a significant difference in CT_{max} among feeding groups for activity-based ($F_{2,32} = 4.32$, $p = 0.0218$), $\dot{V}_{CO2}$-based ($F_{2,32} = 4.33$, $p = 0.0216$), and average CT_{max} ($F_{2,32} = 4.58$, $p = 0.0179$). In all cases, bed bugs starved 9 d had significantly greater CT_{max} than those starved 21 d; CT_{max} of bed bugs starved 1 d did not differ significantly from either the 9 d or 21 d starved bugs (Table 2).

In addition to estimating CT_{max} values, $\dot{V}_{CO2}$ was also calculated during the plateau period and at CT_{max} and compared among feeding groups. $\dot{V}_{CO2}$ was significantly different both during the plateau period ($F_{2,32} = 5.00$, $p = 0.0129$) and at CT_{max} ($F_{2,32} = 22.59$, $p < 0.0001$) (Table 2). Bed bugs starved for 1 d had significantly higher $\dot{V}_{CO2}$ (both plateau and CT_{max}) than bed bugs starved for either 9 d or 21 d (Table 2).

Table 2
Thermolimit respirometry measurements for bed bugs starved 1 d, 9 d, and 21 d. All values reported are means accompanied by (±SEM).

	Starvation time		
	1 d	9 d	21 d
Sample size	11	12	12
Body mass (mg)	6.66(±0.19)	4.71(±0.22)	3.62(±0.22)
Plateau $\dot{V}_{CO2}$ (μL h^{-1})	6.28(±0.26) A	5.42(±0.41) B	4.80(±0.30) B
Activity ADS CT_{max} (°C)	44.69(±0.30) AB	45.13(±0.23) A	44.03(±0.28) B
CO_2 ADS CT_{max} (°C)	44.60(±0.28) AB	45.24(±0.17) A	44.21(±0.29) B
Average CT_{max} (°C)	44.64(±0.28) AB	45.19(±0.20) A	44.12(±0.28) B
$\dot{V}_{CO2}$ at CT_{max} (μL h^{-1})	19.81(±0.90) A	17.36(±0.82) B	12.79(±0.48) C

Note: Statistical comparisons are made within rows, and means followed by the same letter are not significantly different according to the least significant difference mean comparison test at the $p < 0.05$ level.

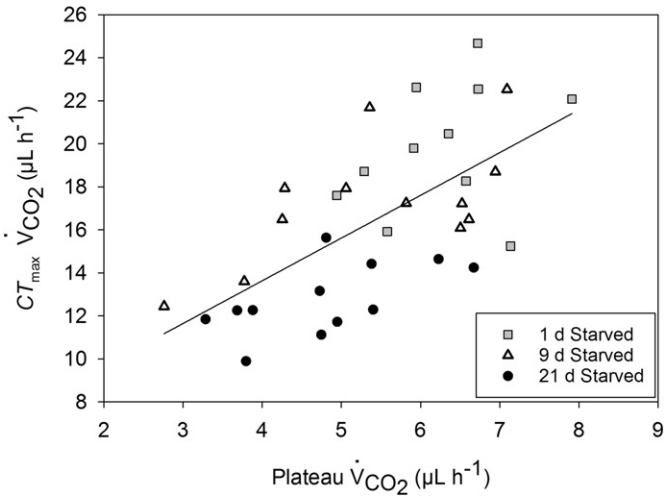

Fig. 3. Relationship between plateau $\dot{V}_{CO2}$ (μL h^{-1}) and $\dot{V}_{CO2}$ (μL h^{-1}) measured at CT_{max} for 1 d, 9 d, and 21 d starved bed bug. The best fit linear regression line is displayed for all data combined, with the equation reported in the text.

Combining data from all feeding groups allowed determination of relationships between several variables, including plateau $\dot{V}_{CO2}$ ($\mu L\ h^{-1}$), CT_{max} $\dot{V}_{CO2}$ ($\mu L\ h^{-1}$), and combined CT_{max} (°C). Plateau $\dot{V}_{CO2}$ was used to predict CT_{max} $\dot{V}_{CO2}$ by the following equation:

$$CT_{max}\dot{V}_{CO2} = 0.441 \pm 0.163 + 0.211 \pm 0.043 * \text{Plateau}\dot{V}_{CO2}$$

($F_{1,33} = 23.77$, $p < 0.0001$, $r^2 = 0.4187$) (Fig. 3). No significant relationship was detected between Plateau $\dot{V}_{CO2}$ ($\mu L\ h^{-1}$) and combined CT_{max} (°C) ($F_{1,33} = 0.31$, $p = 0.5833$, $r^2 = 0.0092$) (Fig. S1), nor CT_{max} $\dot{V}_{CO2}$ ($\mu L\ h^{-1}$) and combined CT_{max} (°C) ($F_{1,33} = 3.23$, $p = 0.0812$, $r^2 = 0.0893$) (Fig. S2).

3.3. Comparison between traditional (visual) and TLR CT_{max} estimation

Estimations of CT_{max} were compared between traditional visual and thermolimit methods for bed bugs at all three feeding statuses. CT_{max} estimates using traditional methods were significantly lower for bed bugs of all feeding statuses: 1 d ($t_{19} = 259.59$, $p < 0.0001$), 9 d ($t_{21} = 227.13$, $p < 0.0001$), and 21 d ($t_{21} = 233.38$, $p < 0.0001$).

4. Discussion

While evaluating strictly CT_{max} in bed bugs, we did not find either traditional visual or TLR methods to provide an advantage over the other. Both methods provided similar results with very similar levels of precision, as indicated by the respective standard errors, although the traditional method provided consistently lower CT_{max} values. It is not uncommon for different methodologies to provide slightly different results; however, we hypothesize that the lower CT_{max} values found while using traditional methods are due to an underestimation of the cessation of movement. This is likely the result of better resolution of movement provided by the activity detector compared to the video camera. However, Stevens et al. (2010) found the opposite relationship, with TLR estimated CT_{max} values lower than traditional (knockdown) CT_{max} values. This is likely a result of their selected endpoint (failure to respond to mild physical stimulation) which is not the same as the selected endpoint in TLR (cessation of movement). The differences observed among studies highlights the necessity of defining an endpoint and ensuring the same endpoint is assessed when comparing methodologies. Ultimately TLR afforded us a greater level of confidence in our estimation of bed bug CT_{max}, due to the two simultaneous yet independent measures of CT_{max} (activity- and $\dot{V}_{CO2}$-based).

Thermolimit respirometry provided a greater amount of information about the insect being measured, creating a more complete profile of bed bug thermal stress and tolerance, which is not available through traditional methods (Lighton and Turner, 2004; Lutterschmidt and Hutchison, 1997). This profile not only allows for estimation of CT_{max}, but also an understanding of the animal's behavioral (activity) and metabolic response to thermal stress. This is apparent when TLR metabolic patterns are compared (Folk et al., 2007; Klok et al., 2004; Lighton, 2007; Lighton and Turner, 2004; Stevens et al., 2010; Vorhees and Bradley, 2012; Vorhees et al., 2013). Despite similarities among the TLR metabolic patterns, each shows unique characteristics, particularly around the post-mortal peak (as described by Lighton and Turner, 2004). Mölich et al. (2012) provided evidence to support that this peak appears independent of oxygen. Vorhees and Bradley (2012) further suggest that differences in the post-mortal peak may be due to the development/complexity of the spiracle system. This suggests that the absence of a post-mortal peak in bed bugs may be due to both the simplicity of the bed bug tracheal system and the low standard metabolic rate of bed bugs compared to other arthropods (DeVries et al., 2013; Usinger, 1966).

It should also be noted that the results in the present study and those found by Klok et al. (2004) did not show the same low-level of variance for either CT_{max} or $\dot{V}_{CO2}$ as originally reported by Lighton and Turner (2004). We suspect this may be due to the high genetic relatedness in the ants being studied (likely all sisters). Despite the fact that end points remain subjective, we found that if performed carefully and with the same end point (in this case, cessation of movement), traditional visual CT_{max} measurements can produce results very similar to those obtained using TLR. In addition, our results combined with bed bug biology provide support for the dynamic method of CT_{max} estimation (Overgaard et al., 2012; Terblanche et al., 2011). Bed bugs provide a model insect for dynamic CT_{max} estimation, due to their ability to survive extended periods of starvation (Usinger, 1966) and their ability to resist desiccation (Benoit et al., 2009). Therefore, it is unlikely that starvation or desiccation played a role in influencing bed bug CT_{max}, as previously suggested for other arthropods (Rezende et al., 2011; Santos et al., 2011).

Feeding status also plays an important role in bed bug thermal tolerance. Despite differences in CT_{max} between traditional (visual) and TLR methods, both showed a clear pattern, with bed bugs starved 9 d having the greatest thermal tolerance, followed by those starved 1 d, and finally followed by those starved 21 d. These times (1 d, 9 d, and 21 d) were selected because they represent important metabolic periods during starvation (DeVries et al., 2015a), although the relationship between metabolism and thermal tolerance is still unclear. Starvation alters thermal tolerance in mosquitoes (*Culex fatigans*) and head lice (*Pediculus humanus*), but only when bugs were evaluated over a longer experimental time (1–24 h), where the authors concluded that bugs tested for 24 h died of starvation (not true CT_{max}) (Mellanby, 1934). In a more recent study, Nyamukondiwa and Terblanche (2009) similarly found feeding status affects CT_{max} in two species of fruit flies, with recently fed flies having significantly higher CT_{max}. Despite the similarities between our results and those of Nyamukondiwa and Terblanche (2009), our results indicate an increase in thermal tolerance following a relatively short period of starvation in bed bugs (9 d). The increase in CT_{max}, suggests that although extended starvation may generally reduce thermal tolerance, the effects can vary greatly between organisms and are relative to what are “short” and “long” periods of starvation for the organisms being tested.

It is unclear what mechanisms are responsible for the observed changes in CT_{max} with feeding status. It is interesting to note the correlation between the lower metabolic rate and lower CT_{max} observed in bed bugs starved 21 d. Because lower metabolism would likely lead to less respiratory water loss, it is possible that the lower metabolic rate observed in 21 d starved bed bugs results in an inability to cool using evaporation (evaporative cooling) and thus the lower CT_{max}. However, this system is likely much more complicated than this, with factors such as the stretched cuticle in bugs starved for 1 d resulting in a greater surface area to obtain heat (a larger heat sink) and bugs starved 21 d displaying an overall decreased ability to resist stressors of any kind (including heat). DeVries et al. (2015b) also found a similar pattern of insecticide tolerance in bed bugs, with the highest levels of tolerance to topically applied deltamethrin reported for bed bugs starved for 9 d.

Heat is a popular alternative management strategy for urban and structural pest species, particularly bed bugs (Cooper, 2011; Kells, 2006; Kells and Goblirsch, 2011). Previous reports of upper lethal temperatures ranged from 43.5 °C to 48 °C (Benoit et al., 2009; Kells and Goblirsch, 2011; Pereira et al., 2009). Our CT_{max} values (independent of feeding status or measurement technique) fell between these ULL values (43.205–45.241 °C), although it should be noted that of the >60 bed bugs measured, the highest CT_{max} value recorded was 46.27 °C. This value is much less than 48 °C, where Benoit et al. (2009) reported survival of some bed bugs after 1 h of exposure and also less than 49 °C, where Pereira et al. (2009) report some survival of bed bugs from 30 s of exposure. We suspect the differences in thermal tolerance reported among studies have to do with both the methods employed and the bugs used. It is likely that the use of multiple

bugs in a single assay left some bugs more exposed to the heat while others were simultaneously insulated. Aggregations are known to provide benefits to bed bugs, as evidence by increased resistance to dehydration (Benoit et al., 2007) and decreased developmental time (Saenz et al., 2014). These effects, although minor, may have led to the increased thermal tolerance reported in previous studies. In addition, it is also possible that the sexes used in different studies (males only, females only, mixed sexes) may have a significant impact on thermal tolerance, with higher values reported for females, although further testing is required to validate this hypothesis. It is important to note that the results obtained here represent true bed bug CT_{max} values, which can be used for intra- and interspecific comparisons of thermal tolerance.

A clear relationship was observed between plateau $\dot{V}_{CO2}$ ($\mu L\ h^{-1}$) and CT_{max} $\dot{V}_{CO2}$ ($\mu L\ h^{-1}$). This relationship was not surprising, with Lighton and Turner (2004) also reporting a strong relationship between these variables. However, despite this relationship, metabolic measurements (both plateau- and CT_{max}-$\dot{V}_{CO2}$) showed no relationship with CT_{max} (°C). Together, these results indicate that resting metabolism and maximal metabolism (as indicated by CT_{max} $\dot{V}_{CO2}$) are linked, but are not predictive of CT_{max} in bed bugs.

Our results indicate that measurements of bed bug CT_{max} made using either traditional visual methods or TLR provide similar results, as long as the selected endpoints are identical. However, measurements made using TLR provide much more information about the insect during thermal stress, not available when using traditional methods. TLR measurements also provide two simultaneous and independent measures of CT_{max}, making this method a better option. Feeding status also significantly affected bed bug CT_{max}, with bed bugs starved 9 d having the greatest CT_{max}, followed by those starved 1 d, and finally followed by those starved 21 d. Future studies should investigate the specific mechanisms involved in controlling thermal tolerance during starvation and how these mechanisms differ among organisms with different feeding strategies and life histories.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpa.2016.03.003.

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