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

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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}.

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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

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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

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