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Effects Of Thermal Acclimation On The Critical Thermal Maxima Of The Tropical Cockroaches: Blaptica Dubia, Eublaberus Posticus And Blaberus Discoidalis (blaberidae)

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Bachelor of Science
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DEDICATION

This thesis is dedicated to my son, Vincent Sebastian Mershon, the light of my life. Thank you for your inspiration and love.

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I would like to thank my thesis advisor, Dr.Paul Cupp, for his guidance and patience from the development through the completion of this project. I would also like to thank my committee members, Dr. Ronald Jones, who has consistently and patiently supported me throughout my academic endeavors and Dr. Amy Braccia, for her feedback and assistance in the preparation of this thesis.   I would also thank the faculty, students and staff of the Eastern Kentucky University Biology Department for their numerous and varied contributions to my education.

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ABSTRACT

The Critical Thermal Maxima (CTM_{ax}) is a measure of upper thermal tolerance. The physiological response upon reaching CTM_{ax} is similar across taxa, making CTM_{ax} useful in comparative studies. The CTM_{ax} defines the ecological lethal temperature of an organism and has been used to predict the effects of global climate change. CTM_{ax} was determined for adults and first instar nymphs of three species of tropical cockroaches: *Blaptica dubia*, *Eublaberus posticus*, and *Blaberus discoidalis.* Adult cockroaches were acclimated to temperatures of 10°C, 15°C, 20°C, 25°C, 31°C and 37°C for a period of seven days. *Blaptica dubia* was the only species to survive the acclimation period at temperatures of 10°C and 37°C. All three species survived at 15°C, 20°C, 25°C and 31°C and at each temperature there were significant differences in CTM_{ax} between two or more species. The average CTM_{ax} values at 25°C were significantly different between all species, *Blaptica dubia* (47.82°C ± 0.53°C), *Eublaberus posticus* (45.57°C ± 0.42°C) and *Blaberus discoidalis* (44.49°C ± 0.44°C).

Across acclimation temperatures, the response of the CTM_{ax} varied within each species. *Blaptica dubia* exhibited a significantly higher CTM_{ax} value at 37°C than all other acclimation temperatures (49.18°C ±0.80°C) and *Eublaberus posticus* had significantly higher CTM_{ax} values at 15°C (46.89°C ± 0.35°C) and 31°C (47.27°C ± 0.64°C). *Blaberus discoidalis* did not exhibit any significant changes in CTM_{ax} at any acclimation temperature.

First instar nymphs of each species were acclimated at 25°C. *Eublaberus posticus* nymphs (44.77°C ± 1.01°C) had significantly different CTM_{ax} values than adults. No significant differences in CTM_{ax} were detected between first instar nymphs and adults in *Blaptica dubia* and *Blaberus discoidalis.*

The rate of the acclimation response was tested in *Blaptica dubia* for roaches acclimated at 10°C for a period of seven days then exposed to 37°C. The reverse response was also tested. CTM_{ax} values significantly increased when cockroaches

acclimated to 10°C (47.06°C ± 0.63°C) were exposed to 37°C for a period of six hours (49.18°C ± 1.13°C), after 96 hours (48.38°C ± 0.79°C) CTM_{ax} values returned to those at 10°C. Animals acclimated at 37°C and moved to 10°C showed no changes in CTM_{ax} values.

The findings of this study suggest that tropical cockroaches are limited in their ability to shift their upper thermal tolerance when exposed to novel acclimation temperatures. The CTM_{ax} values determined in this study are consistent with previous studies of cockroach CTM_{ax} and can be applied to future modeling of the effects of climate change.

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I. INTRODUCTION

Temperature has a direct and at times profound influence on virtually all life history parameters of insects including; foraging, habitat selection, reproduction, development and movement (Angilletta et al. 2002, Chown and Nicolson 2004, Hanna and Cobb 2007, Ribeiro et al. 2012). Relationships between performance and body temperature (T_b) over a range of temperature exposure are described using an asymmetrical function, the thermal performance curve (Figure 1)(Angilletta 2002). Maximum performance occurs at an intermediate temperature on the curve, the optimum body temperature (T_o), and performance is limited by the critical thermal minima (CTM_{in}) and maxima (CTM_{ax}) (Angilletta et al. 2002, Huey and Stevenson 1979). Tests of the critical thermal limits provide valuable insight into how climate affects the physiology, distribution and overall ecology of an organism (Lutterschmidt and Hutchison 1997*a*). Data from investigations of an organism's thermal tolerance can also be used to determine the scope for an organism's ability to respond to ongoing climate changes and extreme temperature events (Somero 2005).

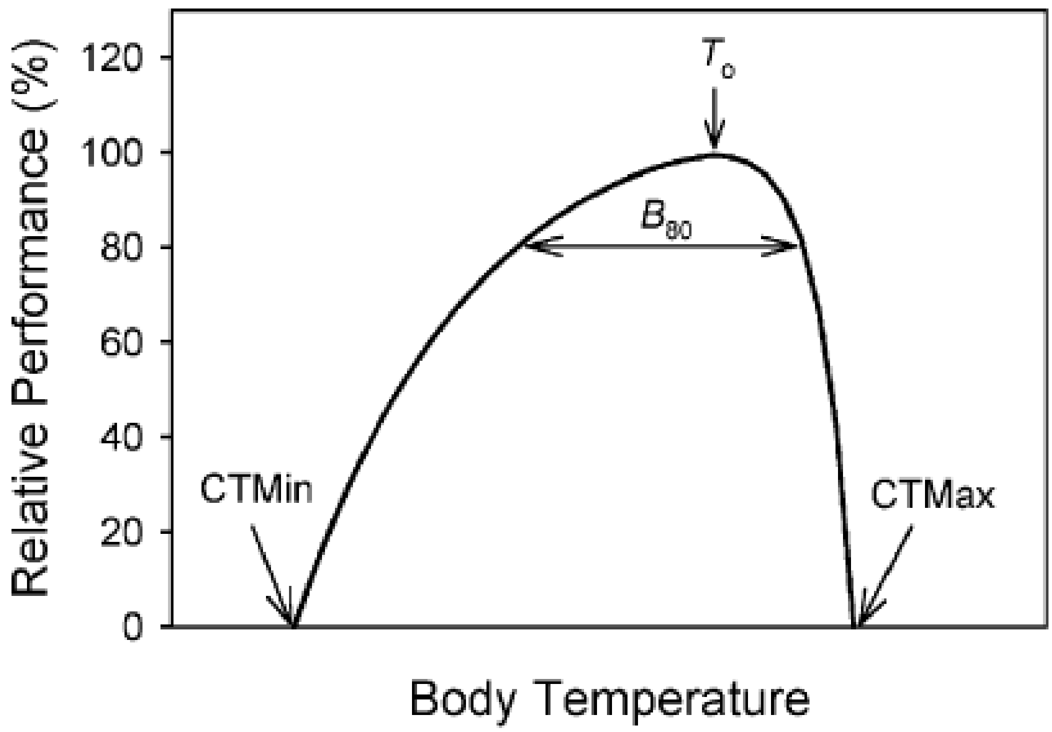

Figure 1: The Thermal Performance Curve. *Source:* Angilletta Jr., M.J., Niewiarowski, P.H., Navas, C.A. 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. 27, 249-268

The Critical Thermal Maximum (CTM_{ax}) is a widely used index for evaluation of the upper thermal requirements of an organism. Despite variation in temperature of CTM_{ax}, the behavioral response upon reaching CTM_{ax} is similar across taxa (Lutterschmidt and Hutchison 1997*a*). The CTM_{ax} was originally defined by Cowles and Bogert (1944) as, "The thermal point at which locomotor activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death." The definition of CTM_{ax} has since been modified to include, statistical variation (Lowe and Vance 1955), standardized methods and defined endpoints (Hutchison 1961). The onset of spasms is the recommended end point for defining CTM_{ax} (Lutterschmidt and Hutchison 1997*b*). The critical thermal limits are the ecologically lethal temperatures for an organism. The critical thermal limits are found using the *dynamic* method of thermal tolerance assessment which increases the temperature to which the organism is exposed at a constant rate until an endpoint is reached (Hutchison 1961, Lutterschmidt and Hutchison 1997*a*).

Acclimation is a measure of phenotypic plasticity, the ability of an organism to exhibit a change in phenotype (physiological, biochemical or anatomical) in response to an exposure to a new environmental condition (Chown and Nicolson 2004). It is important to understand the way in which organisms respond to environmental variability over short term and long term time scales. Such data are crucial to studies of physiology, ecology and conservation in the light of global climate change (Chown and Terblanche 2007). The extent of the phenotypic plasticity of thermal tolerance can provide insight to an organism's ability to withstand changes in environmental temperatures. That data can also be used to predict effects of current and future climate change (Deutsch et al. 2008). The ability of ectothermic animals to acclimate to varying temperature regimes has received attention in recent publications (Allen et al. 2012, Arribas et al. 2012, Dulger et al. 2012, Kumlu et al. 2010, Jumbam et al. 2008). The known studies of cockroach CTM_{ax} did not assess the acclimatory ability of any species tested (Appel and Sponsler 1989, Appel et al. 1983, Appel 1991). Despite the usability of data regarding acclimatory ability of an organism, the range of taxa investigated is small and skewed toward temperate species (Allen et al. 2012, Chown et al. 2002).

Understanding the rate of the acclimation response is also important in studies of thermal tolerance, as it is a better indicator of how phenotypic plasticity of thermal limits enables insects to cope with changes in temperature over a daily or rapid time frame (Chown and Terblanche 2007, Weldon et al. 2011). In insects, studies of acclimation rate and reversal thereof are lacking and the rate of acclimation has only been investigated in 14 species of insects (Allen et al. 2012, Weldon et al. 2011). The rate of acclimation has been assessed for one species of cockroach, *Blatta orientalis* (Blattidae) (Mellanby 1939).

The long term success and survivorship of a species over successive generations is dependent upon the limits of the least tolerant life stage of the species in question (Piyaphongkul et al. 2012), thus when assessing thermal tolerance it is important to explore various life stages of the species in question. Piyaphongkul et al. (2012) assessed thermal tolerance of Brown Plant hoppers (*Nilaparvata lugens*), a tropical insect, and found significant differences in the CTM_{ax} and upper thermal limits (ULL) of first instar nymphs compared to adult males and females. Vorhees and Bradley (2012) demonstrated that the larval and pupal stages of the mealworm beetle (*Tenebrio molitor*) had significantly lower CTM_{ax} than adult beetles. Other recent publications of thermal tolerance in insects have not addressed differences between life stages (Allen et al. 2012, Jumbam et al. 2008).

Studies of thermal tolerance of a species provide data for understanding the effects of global climate change across taxa, as heat stress is a significant proximate effect of climate change. Although global climate change is expected to be most pronounced in the temperate regions, tropical species may be more at risk (Chown et al. 2002). Studies indicate that tropical and subtropical ectotherms may currently be living closer to their optimum temperature range and may have reduced phenotypic plasticity for adapting to shifts in thermal regimes from ongoing climate change (Piyaphongkul et al.2012, Sinervo et al. 2010, Somero 2005). Deutsch et al. (2008) developed a model of the impact of climate change on terrestrial ectotherms. The results indicate that the warming tolerance of tropical species is an average of one-fifth that of temperate insects and may cause a decrease of up to 20% in intrinsic rates of population growth. Despite the evidence that climate change may have a greater impact in the tropics, the majority of thermal tolerance studies have focused on species of the Holarctic region, leaving tropical species underrepresented (Chown et al. 2002) The concept of warming tolerance (WT) is an approximation of the amount of warming an ectotherm can tolerate before performance drops to fatal levels. WT is measured as the difference between the CTM_{ax} and the habitat temperature (T_{hab}), considered as the mean annual surface temperature of the organisms range, (WT= CTM_{ax}-T_{hab}) (Deutsch et al. 2008). CTM_{ax} assessments provide important data to climate change modeling.

Insects comprise the largest class of Arthropods, constituting the majority of terrestrial biodiversity with over 1,000,000 named species (Wilson 1999). Over 4,500 insect species are classified in the order Blattodea, the cockroaches (Beccaloni 2007). Cockroaches are grouped into six recongnized families: Polyphagidae, Cryptocercidae, Nocticolidae, Blattidae, Blattellidae and Blaberidae (Bell et al. 2007). The majority of cockroach species live in tropical habitats throughout the old and new world. Cockroaches are important decomposers in terrestrial ecosystems, recycling dead animals, plants and excrement thus, releasing and returning nutrients to the soil (Bell et al. 2007).

A number of recent studies have been conducted to determine the critical thermal maximum of insects including; beetles (Allen et al. 2012, Vorhees and Bradley 2012), green lynx spiders (Hanna and Cobb 2007), Argentine ants (Jumbam et al. 2008), and brown plant hoppers (Piyaphongkul et al. 2012). CTM_{ax} has also been extensively investigated in vertebrates including, Atlantic stingrays (Fangue and Bennett 2003), salientian amphibians (Cupp 1980), and small mammals (Erskine and Hutchison 1982). Studies of the critical thermal maxima of cockroaches demonstrated a range of CTM_{ax} values from 40.9°C for *Cryptocercus punctulatus* (Cryptocercidae) a primitive cockroach (Appel and Sponsler 1989) to 51.4°C for *Supella longipalpa* (Blattellidae) (Appel et al. 1983). Within the family Blaberidae, observed CTM_{ax} values ranged from 43.20°C in *Diploptera punctata* to 49.50°C in *Blaberus cranifer* (Appel et al. 1983, Appel 1991).

Investigations into the Critical Thermal Limits of Cockroach species have been limited to less than 20 species (Appel and Sponsler 1981, Appel et al. 1983, Appel 1991). Those studies did not look at the ability of the species to acclimate to various temperatures regimes. Nor did the studies investigate the differences in CTM_{ax} between adult cockroaches and any of the instar stages of a cockroaches incomplete metamorphosis pattern (Borror et al. 1989). The majority of cockroach species occur in tropical regions across the globe and are important decomposers in those ecosystems (Bell et al. 2007). It is important to understand how current and future changes in the climate will affect species, thermal limits provide one method for researchers to predict the effect these changes will have on affected species. The thermal limits of the three cockroach species used in this study have not yet been determined. Each species is of tropical orgin. The natural range of *Blaptica dubia* extends from Argentina to Paraguay and Uruguay, where it occurs in terrestrial habitats (Beccaloni 2007). *Eublaberus posticus* is found throughout Central and South America from Costa Rica into Peru and primarily lives in the moist inner sections of caves (Beccaloni 2007, Bell et al. 2007). *Blaberus discoidalis* occurs in terrestrial habitats from Columbia and Venezula, across the Carribbean to Florida, USA (Beccaloni, 2007).

Objectives

The objective of this study was to investigate the critical thermal maximum of new world tropical cockroaches, *Blaptica dubia, Eublaberus posticus* and *Blaberus discoidalis* (Blaberidae). The specific objectives for the study are:

(1.) Determine the Critical Thermal Maxima of adult cockroaches exposed to a series of acclimation temperatures

(2.) Determine the Critical Thermal Maxima of first instar nymphs of each cockroach species maintained at 25°C.

(3.) Determine the rate of acclimation from highest acclimation temperature, 37°C to lowest acclimation temperature, 10°C, and the reversal thereof for adult *Blaptica dubia*.

II. MATERIALS AND METHODS

Study Species

The species addressed in this study, *Blaberus discoidalis*, *Blaptica dubia* and *Eublaberus posticus* are popular feeder insects and pets in the exotic pet trade. They are commonly reared in captivity and readily available from online dealers (Greg's Exotic Inverts, Yucca Valley, CA and Aaron Pauling, www.aaronpauling.com). All individuals used in this study were from colonies maintained with stock from commercial suppliers (Greg's Exotic Inverts, Yucca Valley, CA and Aaron Pauling).

Acclimation

Small, separate colonies of adult roaches of each species were maintained for testing purposes. Colonies were fed rat pellets and carrots ad lib and provided with dampened paper towels as a water source. Colonies of adults of each species were acclimated at six test temperatures: 10°C, 15°C 20°C, 25°C, 31°C and 37°C for a period of at least seven days before CTM_{ax} assessments were conducted. When possible, CTM_{ax} was tested for groups of 20 adults at each acclimation temperature.

To obtain first instar nymphs, adult females were isolated in plastic containers, maintained as test colonies and observed daily for presence of nymphs. Once nymphs were present, small colonies of nymphs were held at 25°C, maintained as test colonies and CTM_{ax} assessed after a period of 7-10 days. CTM_{ax} was assessed for twenty first instar nymphs of each species.

Determination of Critical Thermal Maximum

Prior to CTM_{ax} testing each cockroach was weighed on a balance to the nearest 0.1mg (Mettler-Toledo pb3002s). The starting body temperature was then determined by gently touching the thermocouple to the membrane between the cockroach's mesoand metathoracic coxae (Appel and Sponsler 1989). It was then placed into the upper jar of the testing apparatus (Figure 2). The temperature inside the apparatus at the beginning of the test must be within 1°C of the acclimation temperature, for this reason, separate testing apparatuses were kept in the acclimation chambers for use in respective CTM_{ax} tests. The test temperature began at +/- 1°C of the acclimation temperature and increased continuously at a rate of approximately 1°C per minute to allow body temperature to follow chamber temperature (Lutterschmidt and Hutchison, 1997*a*). Temperature inside the apparatus and body temperature of the roach was monitored using a Type-T thermocouple attached to a digital thermometer (BAT-10, Physitemp inc. NJ, USA).

The critical thermal maximum (CTM_{ax}) of individual roaches was determined using similar methodologies to Appel (1991). To determine the CTM_{ax}, a 118.29 ml glass jar (Ball, USA) was filled with 115ml of water, sealed with metal lid and placed into a 470 ml glass jar (Ball, USA). An additional 118.29 ml glass jar, lined with dampened filter paper (Lab Nerd), was placed on top of the inner jar and 65 milliliters of water was added to the space between the jars. The 470ml jar was sealed with a metal band and lid with a 1/4 inch hole drilled for the insertion of a temperature probe. The entire apparatus (Figure 2) was then placed on a hotplate (Thermoscientific, SP131325) set such that the temperature inside the upper 118.29ml jar increased at an average rate of 1°C per minute to avoid heat shock or partial acclimation during the trial (Lutterschmidt and Hutchison 1997*a*, Chown and Terblanche 2007).

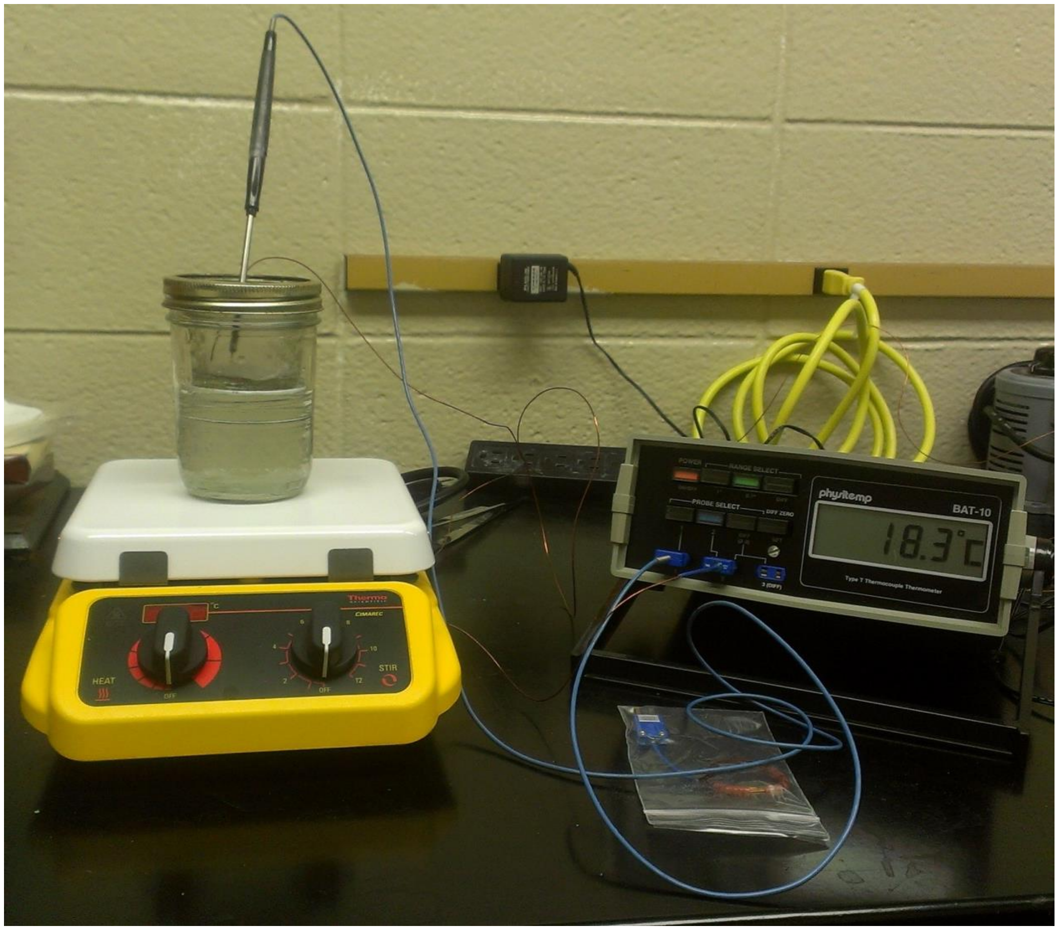

Figure 2: Critical Thermal Maxima Testing Apparatus

Definition of the endpoint

CTM_{ax} of adult cockroaches was determined by the inability of the cockroach to right its self, followed by the onset of muscular spasms. The onset of spasms is thought to be a more biologically precise and meaningful endpoint for CTM_{ax} determination (Lutterschmidt and Hutchison 1997*b*). Upon the onset of spasms, the test temperature was recorded and the cockroach was promptly removed from the test chamber and placed into the recovery chamber (22°C). Recovery of individuals was determined based upon the cockroach's ability to right itself and move about the recovery chamber. Upon recovery, the datum from the CTM_{ax} assessment was added to the dataset. Due to their comparatively small size, CTM_{ax} of first instar nymphs was determined to be the temperature at which the cockroach was unable to move when mechanically disturbed.

Acclimation rate

The rate of acclimation was assessed using only adult *Blaptica dubia*. To test the rate of acclimation from high to low temperatures, cockroaches acclimated at 37°C for a period of seven days were transferred to the 10°C acclimation chamber. To test the acclimation rate from low to high temperatures cockroaches acclimated at 10°C for a period of seven days were transferred to 37°C. When possible, ten individual CTM_{ax} tests were performed at 0, 6, 12, 24, 48, 72, 96, and 120 hours from temperature transfer for both low to high and high to low test groups. CTM_{ax} was determined as previously described.

Data Analysis

Data was analyzed using SPSS (IBM). Levene's test was used to determine if data were normal. A one-way analysis of variance (ANOVA) was conducted to determine if there were significant differences in CTM_{ax} between different acclimation groups and between species. Independent sample t-tests were used to detect significant diffecences between adult cockroaches and first instar nymphs. Tukey's post-hoc comparisons were used to determine which groups differed significantly (Zar 2010). The rate of acclimation was assessed using a one-way ANOVA, contingent upon normalcy of data. Independent sample T-tests were used to compare first instar nymphs and adults of each species. Statistical analyses were considered significant when p-values are less than 0.05.

III. RESULTS

Acclimation of the Critical Thermal Maxima of Blaptica dubia, Eublaberus posticus and Blaberus discoidalis in response to changes in temperature exposure

The CTM_{ax} of each cockroach species was assessed following an acclimation period to test temperatures. 244 total CTM_{ax} assessments were conducted including: 105 *Blaptica dubia,* 70 *Eublaberus posticus* and 69 *Blaberus discoidalis*. The mean CTM_{ax} value of each species at each acclimation temperature is listed in Table 1.

Table 1: Mean Critical Thermal Maxima (±95%CI) of Three Species of Cockroaches Across Acclimation Temperatures

Acclimation Temperature (°C)	Species	*n*	Average CTMax ± 95% CI
10°C	*Blaptica dubia* *Eublaberus posticus* *Blaberus discoidalis*	20	47.06 ± 0.63 No Survival No Survival
15°C	*Blaptica dubia* *Eublaberus posticus* *Blaberus discoidalis*	20 19 20	47.59 ± 0.52 46.89 ± 0.35 45.11 ± 0.76
20°C	*Blaptica dubia* *Eublaberus posticus* *Blaberus discoidalis*	10 17 14	47.64 ± 0.80 45.88 ± 0.42 45.64 ± 0.84
25°	*Blaptica dubia* *Eublaberus posticus* *Blaberus discoidalis*	19 20 18	47.82 ± 0.53 45.57 ± 0.42 44.49 ± 0.44
31°C	*Blaptica dubia* *Eublaberus posticus* *Blaberus discoidalis*	18 14 17	47.78 ± 0.64 47.27 ± 0.64 44.86 ± 0.47
37°C	*Blaptica dubia* *Eublaberus posticus* *Blaberus discoidalis*	18	49.18 ± 0.80 No Survival No Survival

The mean CTM_{ax} of *Blaptica dubia* (Table 1) was significantly different when individuals were acclimated to varied temperature regimes, (ANOVA F (5,99) = 4.854, p= 0.001). Post-hoc Tukey's HSD tests indicate that mean CTM_{ax} remained the same in cockroaches acclimated at 10°C to 31°C and cockroaches acclimated at 37°C exhibited a significantly higher mean CTM_{ax} than other acclimation groups (Figure 3).

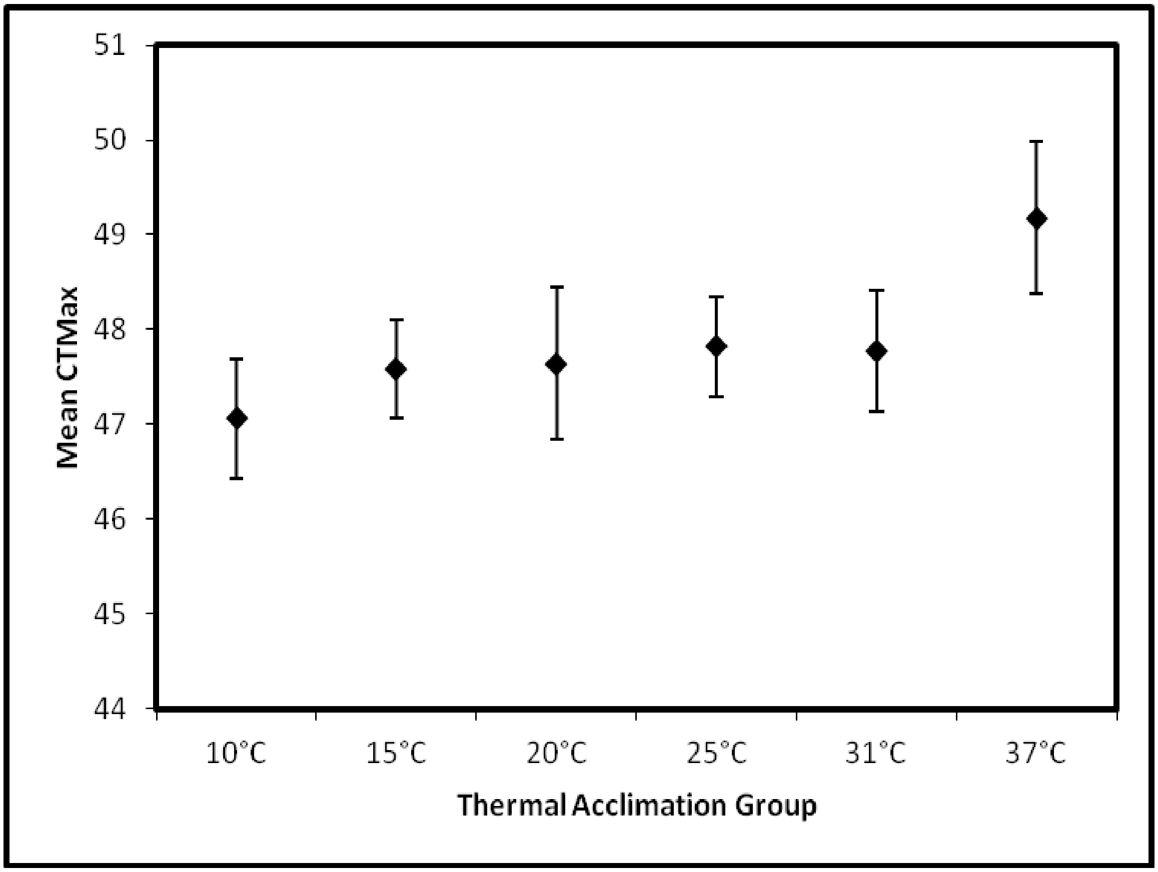

Figure 3: Mean Critical Thermal Maxima of *Blaptica dubia* across acclimation temperatures. Error bars represent 95% confidence interval of the mean.

Eublaberus posticus was unable to survive exposure to 10°C or 37°C acclimation temperatures for more than 5 consecutive days, thus, those temperatures were not included in tests of CTM_{ax} (Table 1). The remaining acclimation temperature groups exhibited significant differences in mean CTM_{ax} values (ANOVA, F(3,66)= 12.261, p=0.0000018). Post Hoc Tukey's HSD tests indicate mean CTM_{ax} of 15°C and 31°C acclimation groups were significantly higher than CTM_{ax} of 20°C and 25°C acclimation groups (Figure 4).

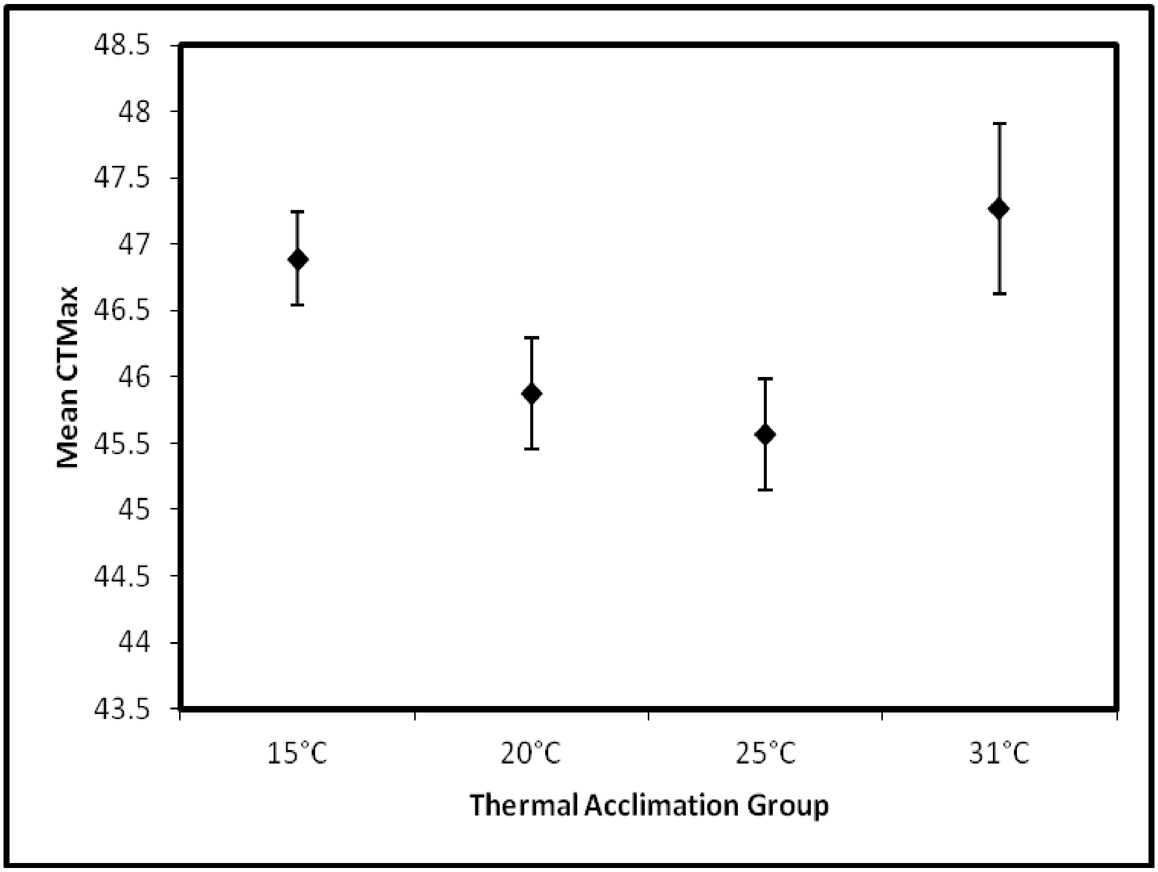

Figure 4: Mean Critical Thermal Maxima of *Eublaberus posticus* across acclimation temperatures. Error bars represent 95% confidence interval of the mean.

Blaberus discoidalis was unable to survive exposure to 10°C or 37°C acclimation temperatures for more than 5 consecutive days, thus those acclimation temperatures were not included in tests of CTM_{ax} (Table 1). There was no significant differences in mean CTM_{ax} between the remaining acclimation groups, (ANOVA $F(3,65) = 1.961$, $p=0.129$) (Figure 5).

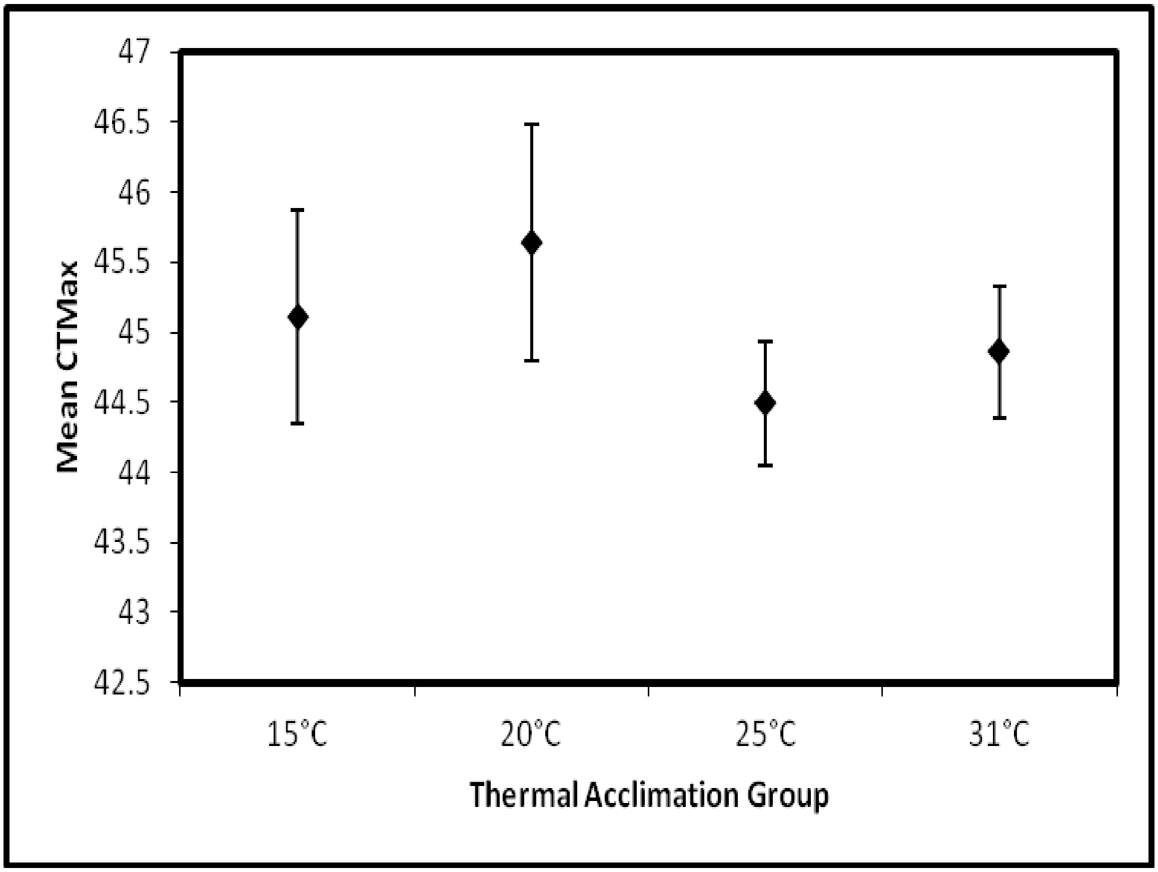

Figure 5: Mean Critical Thermal Maxima of *Blaberus discoidalis* across acclimation temperatures. Error bars represent 95% confidence interval of the mean.

Comparison of Critical Thermal Maxima between species at each acclimation temperature

Blaptica dubia was the only species able to survive the seven day acclimation period in 10°C and 37°C acclimation temperatures. *Eublaberus posticus* and *Blaberus discoidalis* died within 1-5 days of exposure to 10°C and 37°C acclimation chambers. No comparisons between species were conducted at 10°C and 37°C.

Between the three species of roaches acclimated at 15°C there was a significant difference in mean CTM_{ax} (ANOVA, $F(2,56) = 19.450$, $p=0.000$). Post Hoc Tukey's HSD tests indicate that mean CTM_{ax} of *Blaberus discoidalis* was significantly lower than the mean CTM_{ax} of either *Eublaberus posticus* or *Blaptica dubia* (Figure 6).

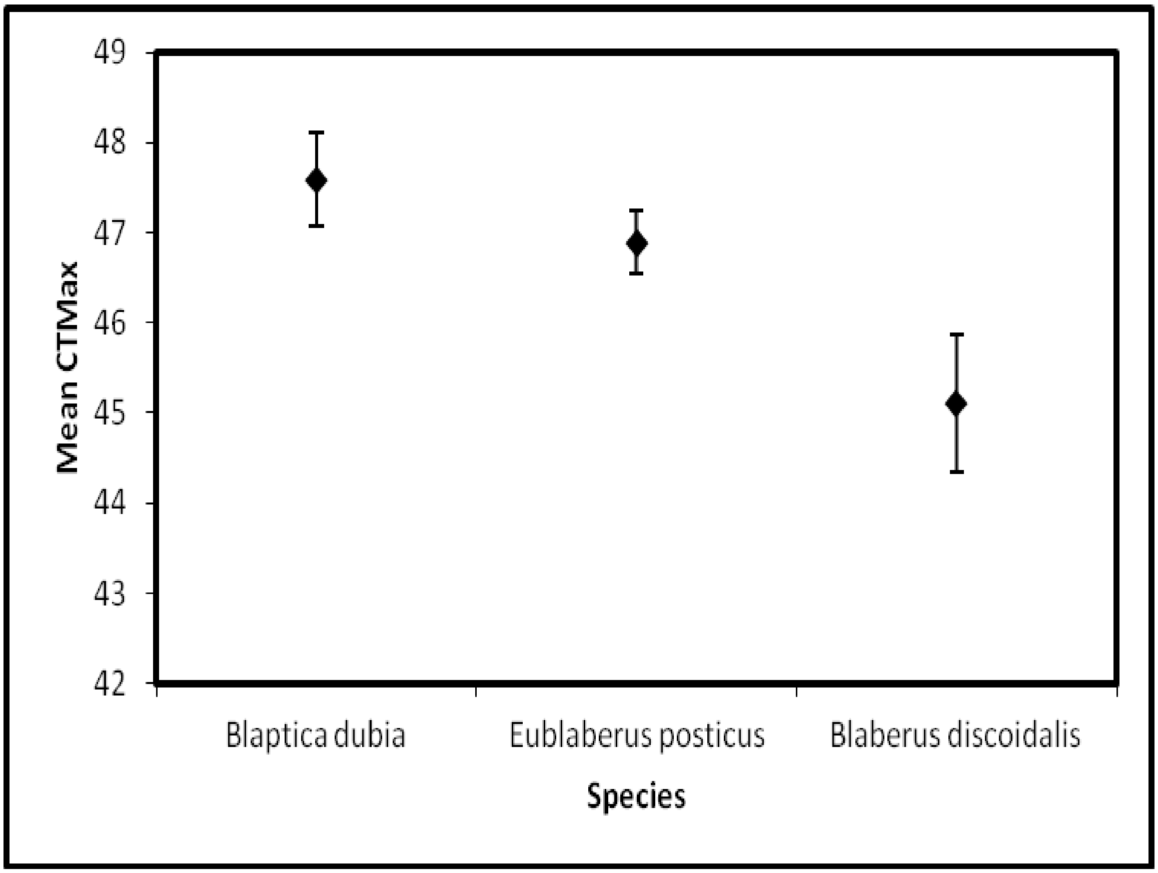

Figure 6: Mean Critical Thermal Maxima of Cockroaches Acclimated at 15°C. Error bars represent 95% confidence interval of the mean.

Cockroaches acclimated at 20°C had significant differences in mean CTM_{ax} values, (ANOVA $F(2,38) = 8.392$, $p=0.001$). Post Hoc Tukey's HSD tests indicate that mean CTM_{ax} of *Blaptica dubia* was significantly higher than CTM_{ax} of *Eublaberus posticus* or *Blaberus discoidalis* (Figure 7).

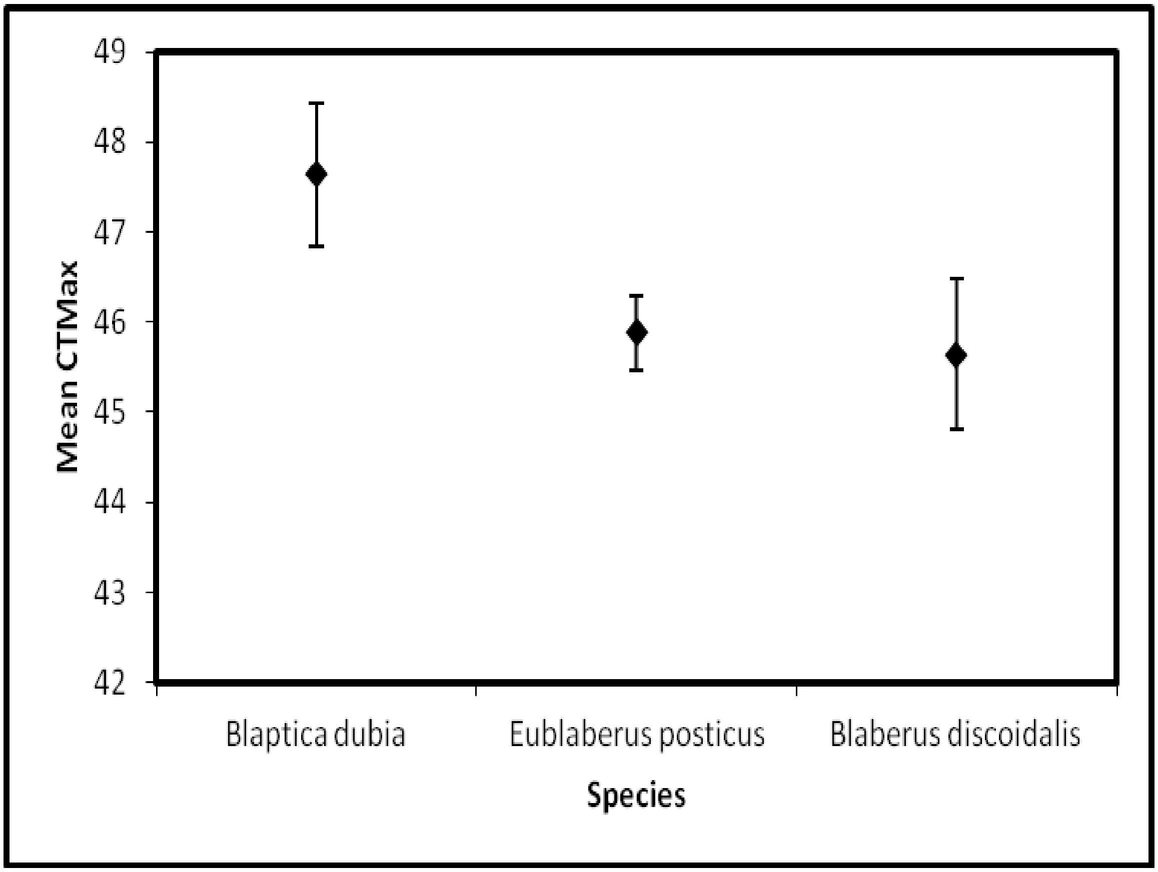

Figure 7: Mean Critical Thermal Maxima of Cockroaches Acclimated at 20°C. Error bars represent 95% confidence interval of the mean.

Cockroaches acclimated at 25°C had significantly different mean CTM_{ax} values (ANOVA, $F(2,54) = 49.298$, $p=0.000$). Post Hoc Tukey's HSD tests indicated that each species was significantly different from the others (Figure 8), mean CTM_{ax} *Blaptica dubia* (47.82°C ± 0.53°C), *Eublaberus posticus* (45.57°C ± 0.42°C) and *Blaberus discoidalis* (44.49°C ± 0.44°C).

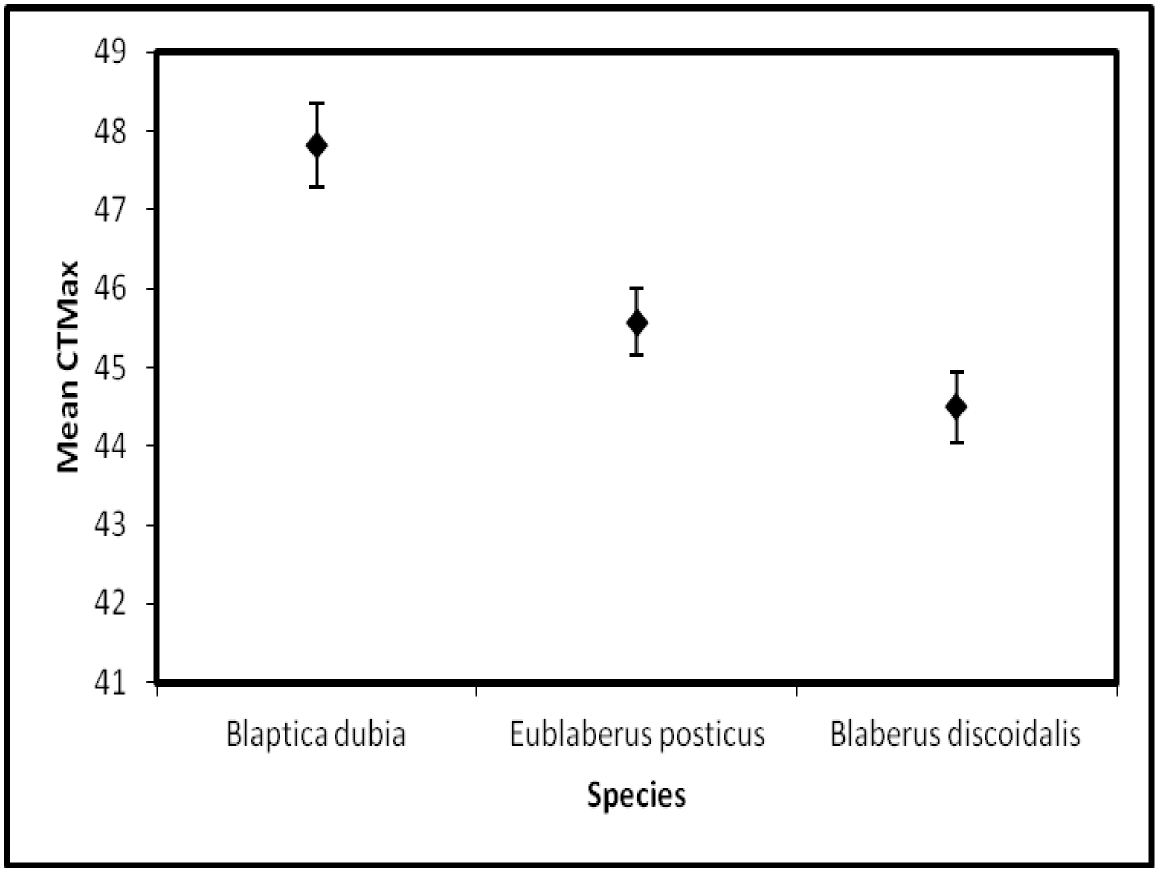

Figure 8: Mean Critical Thermal Maxima of Cockroaches Acclimated at 25°C. Error bars represent 95% confidence interval of the mean.

Cockroaches acclimated at 31°C had significantly different mean CTM_{ax} values (ANOVA, $F(2,46) = 28.364$, $p=0.000$). Post Hoc Tukey's HSD tests indicate that *Blaberus discoidalis* had significantly lower mean CTM_{ax} values than *Eublaberus posticus* or *Blaptica dubia* (Figure 9).

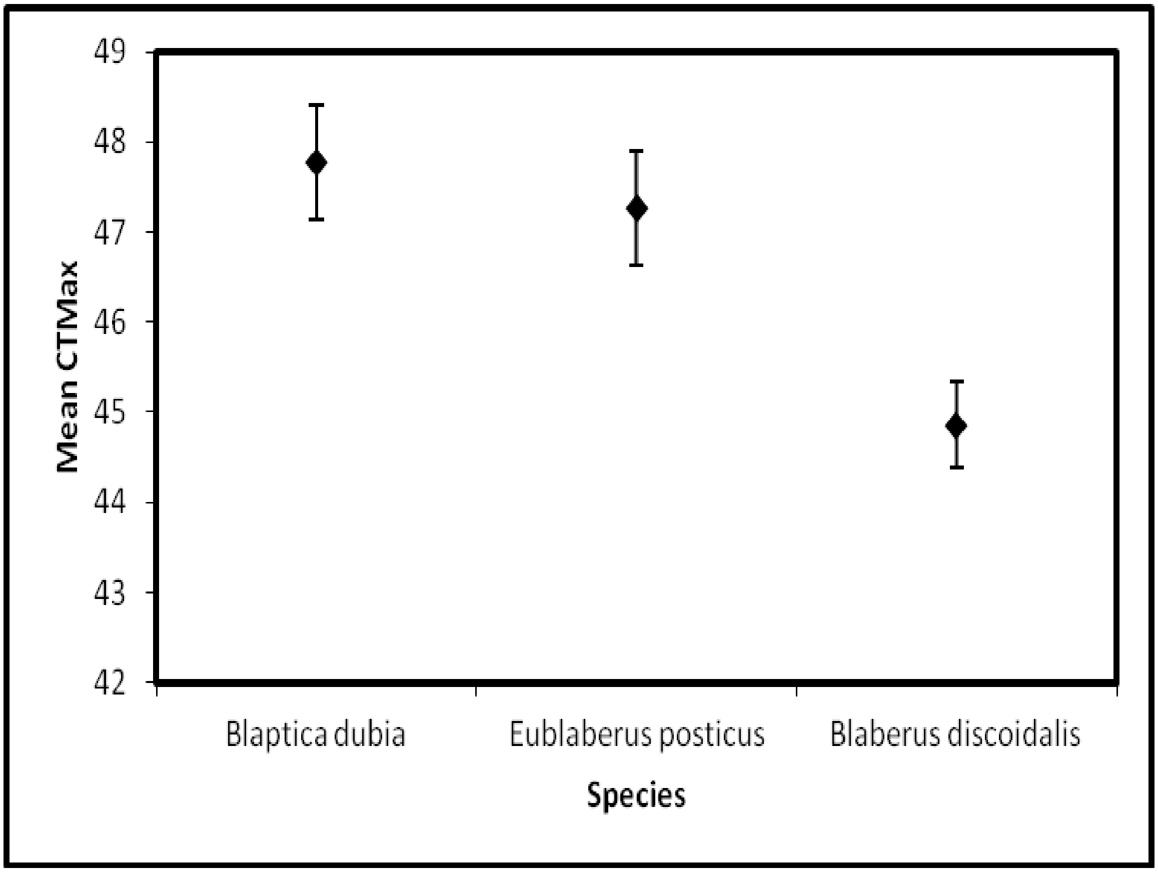

Figure 9: Mean Critical Thermal Maxima of Cockroaches Acclimated at 31°C. Error bars represent 95% confidence interval of the mean.

Comparison of Critical Thermal Maxima of Adult Cockroaches and First Instar Nymphs

Independent sample t-tests were used to determine if significant differences occurred between mean CTM_{ax} values of adults and first instar nymphs of each species (Table 2). Mean CTM_{ax} values did not significantly differ between *Blaptica dubia* adults and first instar nymphs, (t-= 1.440, df = 36, sig = 0.158). *Blaberus discoidalis* adults and first instar nymphs did not significantly differ in mean CTM_{ax} values (t= -1.200, df = 36, sig= 0.238). Mean CTM_{ax} values were significantly different between adults and first instar nymphs of *Eublaberus posticus*, (t=2.651, df = 39, sig= 0.012).

Table 2: Mean Critical Thermal Maximum (±95%CI) of Adults and First Instar Nymphs

Species	Life Stage	*n*	Average CTMax ± 95%CI
Blaptica dubia	Adult	19	47.82 ± 0.53
	Nymph	19	47.36 ± 0.34
Eublaberus posticus	Adult	20	45.57 ± 0.42
	Nymph	21	44.76 ± 0.43
Blaberus discoidalis	Adult	18	44.49 ± 0.46
	Nymph	20	44.91 ± 0.41

Rate of Acclimation Response of Blaptica dubia acclimated to 37°C and shifted to 10°C

A total of 52 adult *Blaptica dubia* were tested in the assessment of rate of acclimation response when shifted from 37°C to 10°C (Table 3). CTM_{ax} for ten individual roaches was assessed at removal from 37°C with no exposure to 10°C. The remaining individuals were transferred to the 10°C acclimation chamber and CTM_{ax} tested in groups of six individuals at 6, 12, 24,48,72,96 and 120 hours post transfer . The mean CTM_{ax} values for *Blaptica dubia* adults acclimated at 37°C did not significantly change at anytime following transfer to 10°C (ANOVA $F(7,52)= 0.861$, $p= 0.543$) (Figure 10).

Table 3: Acclimation of CTM_{ax} from 37°C to 10°C in *Blaptica dubia*

Exposure to 10°C (Hours)	Mean CTMax ± 95%CI
0	49.18 ± 1.08
6	47.62 ± 0.87
12	48.73 ± 1.59
24	48.62 ± 1.58
48	48.80 ± 0.87
72	48.02 ± 1.34
96	49.07 ± 1.06
120	48.65 ± 0.70

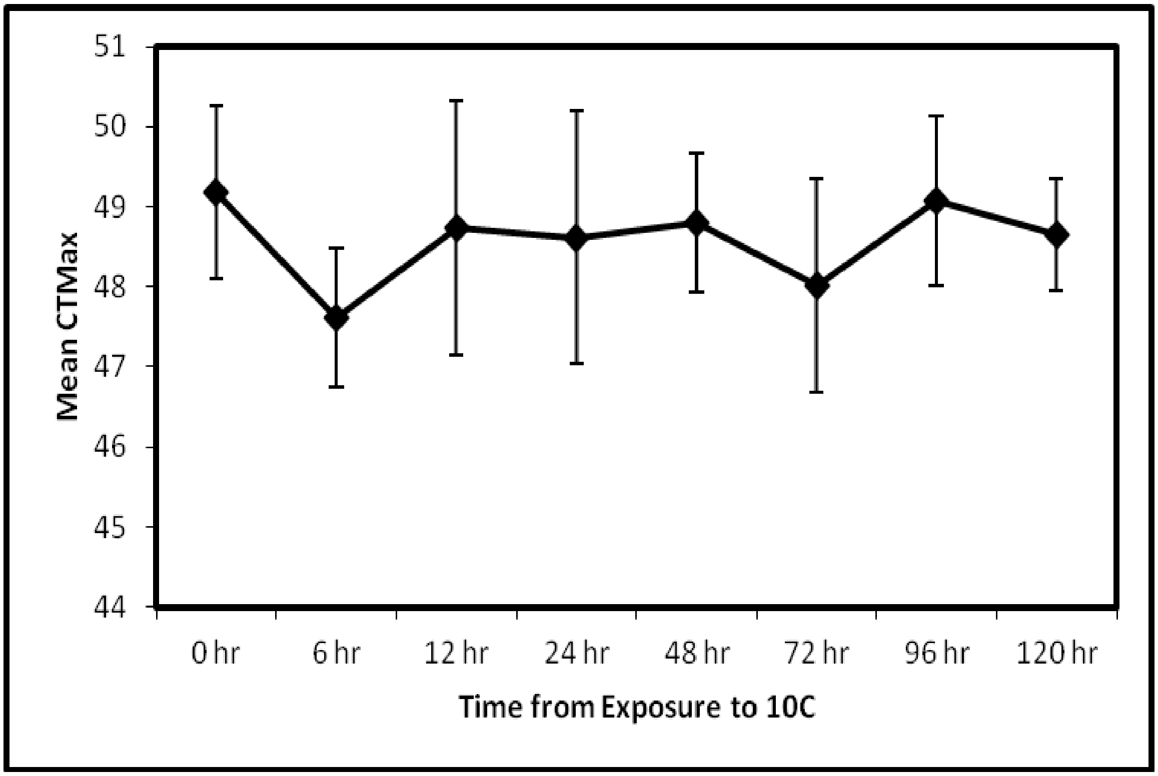

Figure 10: Rate of Acclimation of CTM_{ax} from 37°C to 10°C in *Blaptica dubia.* Error bars represent 95% confidence interval of the mean.

Rate of Acclimation Response of Blaptica dubia when acclimated to 10°C and shifted to 37°C

A total of 81 adult *Blaptica dubia* were tested in the assessment of rate of the acclimation response when acclimated to 10°C and shifted to 37°C. CTM_{ax} of 20 individuals was assessed at 10°C with no exposure to 37°C. The *Blaptica dubia* were transferred to 37°C and CTM_{ax} assessed in groups of ten at 6,12,24,48,72,96,and 120 hours post transfer (Table 4). The mean CTM_{ax} values of *Blaptica dubia* adults acclimated at 10°C was significantly different than the average CTM_{ax} of individuals exposed to 37°C, (ANOVA $F(7,81)= 3.742$, $p=0.001$)(Figure 12). Post-hoc Tukey's HSD tests indicate that cockroaches acclimated at 10°C had significantly different CTM_{ax} values than individuals exposed to 37°C for six, twelve, twenty four and 72 hours. Mean CTM_{ax} values of cockroaches exposed to 37°C for 96 and 120 hours were not significantly different from mean CTM_{ax} values of the 10°C acclimation group.

Table 4: Acclimation of CTM_{ax} from 10°C to 37°C in *Blaptica dubia*

Exposure to 37°C (Hours)	Mean CTMax ± 95%CI
0	47.06 ± 0.63
6	49.18 ± 1.13
12	49.22 ± 1.18
24	49.14 ± 1.00
48	48.40 ± 0.65
72	49.41 ± 0.95
96	48.38 ± 0.79
120	48.56 ± 1.14

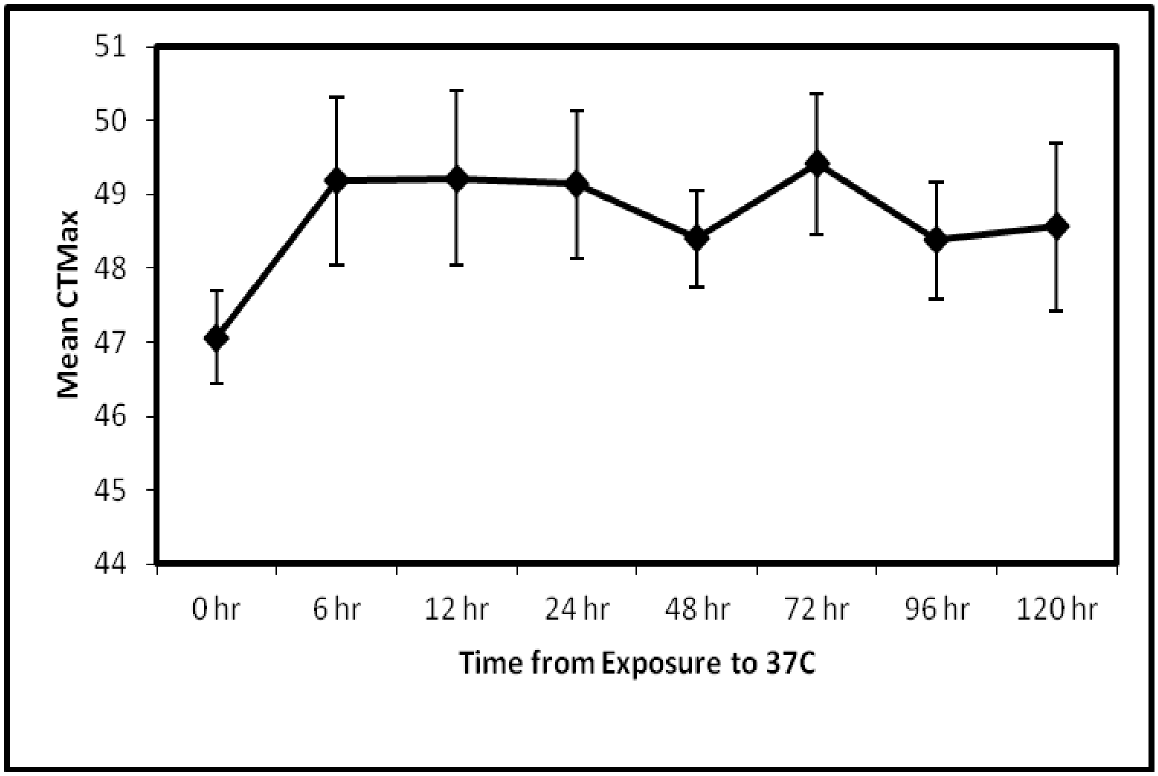

Figure 11: Rate of Acclimation of CTM_{ax} from 10°C to 37°C in *Blaptica dubia.* Error bars represent 95% confidence interval of the mean.

IV. DISCUSSION

The Critical Thermal Maxima

The main objective of my study was to determine the CTM_{ax} of three species of Blaberid cockroaches for which upper thermal limits were previously unexplored, *Blaptica dubia, Eublaberus posticus* and *Blaberus discoidalis*. The mean CTM_{ax} of cockroaches acclimated at 25°C is the most appropriate value to use in comparisons with previous studies of cockroach CTM_{ax}. Other cockroach studies did not focus on acclimation responses and maintained laboratory colonies at 25±2°C (Appel et al. 1983, Appel 1991). The mean CTM_{ax} values for each species in this study acclimated at 25°C were: *Blaptica dubia* 47.82±0.36°C, Eublaberus *posticus* 45.57±0.42°C, and *Blaberus discoidalis* 44.49±0.44°C. These mean CTM_{ax} values fall within the CTM_{ax} range of previously investigated species of Blaberid cockroaches, which ranged from 43.20±0.13°C in *Diploptera punetata* to 49.5±0.36°C in *Blaberus cranifer* (Appel et al. 1983, Appel 1991).

It would be expected that mean CTM_{ax} of *Blaberus discoidalis* determined in my study would be similar to the mean CTM_{ax} of *Blaberus cranifer* determined by Appel et al. (1983), because most variation in CTM_{ax} occurs above the species level (Addo-Bediako et al. 2000) and the two species share similar habitats (Beccaloni 2007). The methodologies used in this study for determining CTM_{ax} followed Appel (1991), using a nested glass jar and hot plate to obtain a 1°C/min heating rate. Appel et al. (1983) used a different testing methodology and found that the rate of heating was 0.75°C/min. The differences in heating rate could explain the observed difference of 5°C in average CTM_{ax} of *Blaberus discoidalis* compared to *Blaberus cranifer.*

Response of CTM_{ax} to Thermal Acclimation

The results of this study indicated different acclimation responses for each species addressed. *Blaptica dubia*, was acclimated at 10°C, 15°C, 20°C, 25°C, 31°C and

37°C and exhibited a significant increase in mean CTM_{ax} when acclimated to 37°C (49.18±0.80°C). The mean $CTM_{ax\ of}$ roaches acclimated at all other temperatures did not significantly differ between groups. In temperate tadpole species Cupp (1980) observed that within a species temperature range, a greater acclimation response occurred in higher temperatures than lower temperatures. *Blaptica dubia* also exhibited the highest overall average CTM_{ax} throughout the study. *Blaberus discoidalis*, which exhibited the lowest overall average CTM_{ax} throughout the study, exhibited no acclimation response of CTM_{ax}. These findings loosely support the concept demonstrated by Calosi et al.(2008) in a study of European diving beetles, *Deronectes sp*., which concluded that the species with the highest upper thermal limits also possesses the greatest acclimatory ability within that trait.

Eublaberus posticus did not survive the acclimation period at 10°C or 37°C, but exhibited significantly lower mean CTM_{ax} values when acclimated at 20°C and 25°C compared to 15°C and 31°C acclimation groups. These results are opposite of what would be expected in thermal acclimation responses. The highest thermal tolerance should occur at optimum temperature (To) and decrease with decreased performance due to exposures outside of the T_o range as consistent with the thermal performance curve (Angilletta et al. 2002, Huey and Stephenson 1979). Therefore, a peak CTM_{ax} should have been observed at one acclimation temperature with CTM_{ax} dropping as acclimation temperatures moved away from optimum temperatures in either direction on the curve as observed by Jumbam et al. in a study of the CTM_{ax} of argentine ants, *Linepithema humile*, which thrive in Mediterranean type habitats. The CTM_{ax} of *Linepithema humile* was highest at 25°C and dropped after acclimation to temperatures higher or lower. However, the overall acclimation response was typically weak with the mean CTM_{ax} only shifting 1°C (Jumbam et al. 2008). Further information regarding the natural history of *Eublaberus posticus* could offer insight into why peak CTM_{ax} would occur at acclimation temperature extremes.

Investigations of the upper thermal limits for tropical species are lacking and the majority of studies have focused on species of the Holarctic region (Chown et al. 2002). Further investigations into the acclimation response of thermal tolerance in tropical species are needed to fully understand the acclimation responses observed in my study.

Comparisons between Species

It has been shown that the majority of variation in physiological traits, including thermal tolerance is found in taxonomic groupings above the species level and that in studies of CTMax, the highest variation occurs at the genus level (Addo-Bediako et al. 2000). This study found significant differences in mean CTM_{ax} between at least two of the three test species at each acclimation temperature. Within every thermal acclimation group, *Blaptica dubia* had the highest CTM_{ax} and *Blaberus discoidalis* had the lowest. At 25°C the mean CTM_{ax} of each species was significantly different from the others.

The statistical differences in the CTM_{ax} of the cockroaches tested occur within a narrow temperature range. It is known that the upper thermal limits of organisms have less variation than lower thermal limits and that upper thermal limits have little variance based on geographic location (Addo-Bediako et. al. 2000). The differences between these species could be attributed to their natural histories. *Eublaberus posticus* is described as frequently occurring in caves, as is *Blaberus discoidalis* (Roth and Willis 1960). Both species also largely occur in Central America and the Caribbean (Beccaloni 2007). *Blaptica dubia* is a terrestrial species, occurring in forested habitats in Paraguay, Uruguay and Argentina (Beccaloni 2007). *Blaptica dubia* could be better adapted to surviving greater temperatures due to its larger, more varied historic habitat and range.

Comparisons between Adults and Nymphs

Previous studies of insect CTM_{ax} demonstrated significant differences in thermal tolerance between developmental stages. In a study of the Brown Planthopper, *Nilaparvata lugens*, Piyaphongkul et al. (2012) found that first instar nymphs had significantly lower CTM_{ax} and ULT_{50} values than adult males or females. Vorhees and Bradley (2012) found that larval and pupal stages of the meal worm beetle *Tenebrio molitor* had significantly lower CTM_{ax} than adult beetles. This study found that first instar nymph *Eublaberus posticus* had significantly lower mean CTM_{ax} than adults. This suggests that the least tolerant life stages of a species are the most important in determining the long term success of a species when exposed to thermal stress.

No significant differences were found between adults and first instar nymphs of *Blaptica dubia* and *Blaberus discoidalis*. The similarity of mean CTM_{ax} values between life stages of these species may be attributed to errors in the assessment of CTM_{ax}. To determine CTM_{ax} of adult roaches the onset of muscular spasms was observed and that temperature point was recorded as the CTM_{ax}. Due to constraints with visibility in the testing apparatus and size of the organisms, CTM_{ax} of first instar nymphs was determined as the cessation of movement when mechanically disturbed. It is likely that this difference in CTM_{ax} determination caused an inflation of the actual upper thermal limits of first instar nymphs. Significant differences may be determined with additional data collection using more precise measuring techniques such as thermolit respirometry technique described by Vorhees and Bradley (2012).

Rate of Acclimation

This study tested the rate of the acclimation response in *Blaptica dubia*. While roaches acclimated at 37°C did not exhibit significant shifts in mean CTM_{ax} when exposed to 10°C acclimation temperatures. Roaches acclimated at 10°C and subsequently exposed to 37°C exhibited significant increases in mean CTM_{ax} after only 6 hours of exposure. Rapid acclimation response to high temperatures is well documented. Mellanby (1939) observed complete acclimation in the cockroach *Blatta orientalis* in less than 20 hours when moved from 15°C to 30°C. The reverse acclimation of *Blatta orientalis* occurred in 2-3 days. In a study of *Ceratitis* flies acclimation was found to be a rapid process, occurring in less than 24 hours (Weldon et al. 2011). The lack of change in CTM_{ax} when roaches were acclimated at 37°C and moved to 10°C as well as the return of CTM_{ax} to 10°C values after 72 hours at 37°C could indicate that the roaches were exposed to their temperature extremes. If high and low acclimation temperature were extremes, it is likely that the CTM_{ax} values were similar because the roaches were under physiological stress at both acclimation temperatures.

Directions for Future Research

The Critical Thermal Maxima is one measure to determine the thermal limits of a species and can be used to determine how a species group will respond to global climate change. I recommend investigating the mean annual temperatures across each species range and comparing those to the experimentally determined CTM_{ax} values, using the models described by Deutsch et al. (2008). Field-collected cockroach specimens may give a more accurate measure of the thermal tolerance of the species and would determine if slight geographic variance occurred. Seasonal changes in CTM_{ax} may also occur and could be investigated.

To fully understand how temperature affects a cockroach species, CTM_{ax} should be assessed for each developmental stages of a cockroach. Critical differences in CTM_{ax} may occur at different stages or periods of metamorphosis. In addition, for any study which wanted to relate the Critical Thermal Maximum to overall survival, it would be essential to conduct a prolonged reproduction and development study to determine the temperature at which a cockroach would breed, reproduce, and develop.

Recent studies have addressed CTM_{ax} using a variety of deviations from the standards proposed by Lutterschmidt and Hutchison (1997*a*). Future investigations of cockroach CTM_{ax} could explore the effects of the heating rate and the repeatability of the CTM_{ax} values through repeated testing on an individual. Future investigations may also choose to use new techniques to assess CTM_{ax} using physiological responses such as CO^2 output which may be more reliable and repeatable.

Additional studies should seek to investigate less common species of cockroach than those readily available in the pet trade. Those species which are easy to maintain in a lab culture may be more resistant to environmental stressors overall. Rare cockroach species with specific habitat requirements may be less tolerant to thermal stress and therefore more susceptible to future climate changes.

LITERATURE CITED

Addo-Bediako, A., Chown S.L., and Gaston, K.J. 2000. Thermal tolerance, climatic variability and latitude. Proc. R. Soc. Lond. 267, 739-745.

Allen, J.L., Clusella-Trullas, S., Chown, S.L. 2012. The effects of acclimation and rates of temperature change on critical thermal limits in *Tenebrio molitor* (Tenebrionidae) and *Cyrtobagous salviniae* (Curculionidae). J. Insect Physiol. 58, 669-678.

Angilletta Jr., M.J., Niewiarowski, P.H., Navas, C.A. 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. 27, 249-268.

Appel, A.G. 1991. Water relations and thermal sensitivity of several cockroach species (Dicyoptera: Blattidae and Blaberidae). Comp. Biochem. Physiol. 100A(2), 353-356.

Appel, A.G.,Reierson, D.A. and Rust, M.K. 1983. Comparative water relations and temperature sensitivity of cockroaches. Comp. Biochem. Physiol. 74A(2), 357-361.

Appel, A.G. and Sponsler, R.C. 1989. Water and temperature relations of the primitive xylophagous cockroach *Cryptocercus punctulatus* Scudder (Dictyoptera: Cryptocercidae). Proc. Entomol. Soc. Wash. 91(2), 153-157.

Arribas, P., Abellan,P., Velasco, J., Bilton, D.T., Millan, A., Samchez-Fernandez, D. 2012. Evaluating drivers of vulnerability to climate change: a guide for insect conservation strategies. Global Change Biol. 18, 2135-2146.

Beccaloni, G.W. 2007. Blattodea species file online. Version 1.0/4.1. Worldwide web electronic publication. http://blattodea.speciesfile.org [Accessed 9 Oct 2012].

Bell, W.J., Roth, L.M. and Nalepa, C.A. 2007. Cockroaches ecology, behavior and natural history. The John Hopkins University Press, Baltimore, MD.

Borror, D.J, Triplehorn, C.A., and Johnson, N.F. 1989. An Introduction to the Study of Insects. 6th ed. Saunders College Publishing, Philadelphia, PA.

Calosi, P., Bilton, D.T., and Spencer, J.L. 2008. Thermal tolerance, acclimatory capacity and vulnerability to global climate change. Biol. Lett. 4, 99-102.

Chown, S.L., Addo-Bediako, A., Gaston, K.J. 2002. Physiological variation in insects: large scale patterns and their implications. Comp. Biochem and Physiol. Part B 131, 587-602.

Chown, S.L. and Nicolson, S.W. 2004. Insect physiological ecology mechanisms and patterns. Oxford University Press, New York, NY.

Chown, S.L. and Terblanche, J.S. 2007. Physiological diversity in insects: Ecological and evolutionary contexts. Adv. Insect Physiol. 33, 50-152.

Cowles, R.B., Bogert, C.M. 1944. A preliminary study of the thermal requirements of desert reptiles. Bull. Am. Mus. Nat. Hist. 83, 261-296.

Cupp Jr., P.V. 1980. Thermal tolerance of five salentian amphibians during development and metamorphosis. Herpetologica 36(3), 234-244.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak,D.C., and Martin, P.R. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. PNAS 105,18 6668-6672.

Dulger, N., Kumlu, M., Turkmen, S., Olculu, A., Eroldogan, O.T., Yilmaz, H.A., and Ocal, N. 2012. Thermal tolerance of European Sea Bass (*Dicentrarchus labrax*) juveniles acclimated to three temperature levels. J. of Therm. Biol. 37, 79-82.

Erskine, D.J. and Hutchison, V.H. 1982. Critical thermal maxima in small mammals. J. of Mammology 63 (2), 262-273.

Fangue, N.A. and Bennett, W.A. 2003. Thermal tolerance responses of laboratory acclimated and seasonally acclimatized Atlantic stingrays, *Dasyatis sabina*. Copeia 2, 315-325.

Hanna, C.J. and Cobb, V.A. 2007. Critical thermal maximum of the Green Lynx spider, *Peucetia viridians* (Araneae, Oxyopidae). J. Arachn. 35, 193-196.

Huey, R.B. and Stevenson, R.D. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. Am. Zool. 19: 357-366.

Hutchison, V.H. 1961. Critical thermal maxima in salamanders. Physiol. Zool. 34 (2), 92-125.

Jumbam, K.R., Jackson, S., Terblanche, J.S., McGeoch, M.A., and Chown, S.L. 2008. Acclimation effects on critical and thermal limits of workers of the Argentine ant, *Linepithema humile*. J. Insect Physiol. 54, 1008-1014.

Kumlu, M., Turkmen,S., Kumlu, M. 2010. Thermal tolerance of *Litopenaeus vannamei* (Crustacea: Penaeidae) acclimated to four temperatures. J. Therm. Biol. 35, 305-308.

Lowe Jr., C.H., Vance, V.J. 1955. Acclimation of the critical thermal maximum of the reptile *Urosaurus ornatus*. Science (Washington D.C.) 122, 73-74.

Lutterschmidt, W.I. and Hutchison, V.H.. 1997*a*. The critical thermal maximum: history and critique. Can. J. Zool. 75, 1561-1574.

Lutterschmidt, W.I. and Hutchison, V.H. 1997*b*. The critical thermal maximum: data to support the onset of spasms as the definitive end point. Can. J. Zool. 75, 1553-1560.

Mellanby, K. 1939. Low temperature and insect activity. Proc. R. Soc. B 127, 473-487.

Piyaphongkul, J.,Pritchard, J. and Bale, J. 2012. Can Tropical Insects Stand the Heat? A Case Study with the Brown Planthopper *Nilaparvata lugens* (Stal). PLoS ONE 7,1, e29409 1-6.

Ribeiro, P.L., Camacho, A., Navas, C.A. 2012. Considerations for assessing maximum critical temperatures in small ectothermic animals: insights from leaf-cutting ants. Plos ONE 7(2), e32083. Doi:10.1371/journal.pone.0032083.

Roth, L.M. and Willis, E.R.. 1960. The Biotic Associations of Cockroaches. The Lord Baltimore Press, Baltimore, M.D.

Sinervo, B., Mendex-de-la-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Villagran-Santa Cruz, M., Lara-Resendiz, R., Martinez-Mendez, N., Calderón-Espinosa, M.L., Meza-Làzaro, R.N., Gadsen, H., Avila, L.J., Morando, M., De la Riva, I.J., Sepulveda, P.V.,Rocha,C.F.D., Ibargüengoytía, N., Puntriano,C.A., Massot, M., Lepetz,V., Oksanen, T.A., Chapple, D.G., Bauer, A.M., Branch, W.R., Clobert, J., and Sites Jr, J.W. 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science 328, 894-899.

Somero, G.N. 2005. Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. Front. Zool. 2(1), 1-9.

Vorhees, A.S., and Bradley, T.J. 2012. Differences in critical thermal maxima and mortality across life stages of the mealworm beetle *Tenebrio molitor*. J. Exp. Biol. 215, 2319-2326.

Weldon, C.W., JTerblanche, J.S., and Chown, S.L. 2011. Time-course for attainment and reversal of acclimation to constant temperature in two *Ceratitis* species. J. Therm. Biol. 36, 479-485.

Wilson, E.O. 1999. The Diversity of Life *new edition*. W.W. Norton & Company. New York, NY.

Zar, J.H. 2010. Biostastical analysis (5th Ed.) Prentice Hall. Upper Saddle River, NJ.