2019

City limits: Heat tolerance is influenced by body size and hydration state in an urban ant community

Dustin Jerald Johnson
University of the Pacific, djohnson4@pacific.edu

CITY LIMITS: HEAT TOLERANCE IS INFLUENCED BY BODY SIZE AND HYDRATION
STATE IN AN URBAN ANT COMMUNITY

by

Dustin Jerald Johnson

A Thesis Submitted to the

Graduate School

In Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

College of the Pacific
Biological Sciences

University of the Pacific
Stockton, CA

2019

CITY LIMITS: HEAT TOLERANCE IS INFLUENCED BY BODY SIZE AND HYDRATION
STATE IN AN URBAN ANT COMMUNITY

by

Dustin Jerald Johnson

APPROVED BY

Thesis Advisor: Zach Stahlschmidt, Ph.D.

Committee Member: Ryan Hill, Ph.D.

Committee Member: Eric Thomas, Ph.D.

Department Chair: Eric Thomas, Ph.D.

Dean of Graduate School: Thomas Naehr, Ph.D.

DEDICATION

I dedicate my thesis to my aunt, Linda, for being the parent that she did not have to be; always giving me the support that I needed and pushing me to always be the best me, no matter what. Secondly, I would like to dedicate my thesis to my father, Ken, and my brother Aaron, for being there for me since day one; they have both held me up to allow me to become the person that I am.

AKNOWLEDGMENTS

City Limits: Heat Tolerance Is Influenced by Body Size and Hydration State in an Urban Ant
Community

Abstract

By Dustin Jerald Johnson

University of the Pacific
2019

Cities are rapidly expanding, and global warming is intensified in urban environments

due to the urban heat island effect. Therefore, urban animals may be particularly susceptible to

warming associated with ongoing climate change. Thus, I used a comparative and manipulative

approach to test three related hypotheses about the determinants of heat tolerance or critical

thermal maximum (CT_{max}) in urban ants—specifically, that (1) body size, (2) hydration status,

and (3) preferred micro-environments influence CT_{max}. I further tested a fourth hypothesis that

native species are particularly physiologically vulnerable in urban environments. I manipulated

water access and determined CT_{max} for 11 species common to cities in California's Central

Valley that exhibit nearly 300-fold variation in body mass. Inter- (but not intra-) specific

variation in body size influenced CT_{max} where larger species had higher CT_{max}. The sensitivity

of ants' CT_{max} to water availability exhibited species-specific thresholds where short-term water

limitation (8 h) reduced CT_{max} in some species while longer-term water limitation (32 h) was

required to reduce CT_{max} in other species. However, CT_{max} was not influenced by the preferred

foraging temperatures of ants. Further, I did not find support for my fourth hypothesis because

native species did not exhibit reduced thermal safety margins, or exhibit CT_{max} values that were more sensitive to water limitation relative to non-native species. In sum, understanding the links between heat tolerance and water availability will become critically important in an increasingly warm, dry, and urbanized world that may be selecting for smaller (not larger) body size.

TABLE OF CONTENTS

LIST OF FIGURES

Figure

LIST OF ABBREVIATIONS

CT_{max} – Critical thermal maximum

Hsps – Heat shock proteins

T_{active} – Active temperature

PGLS – phylogenetically generalized least squares methods

CHCs – Cuticular Hydrocarbons

Chapter 1: Introduction

Temperatures are increasing globally due to climate change (Oreskes, 2004; IPCC, 2014), and high temperatures may alter patterns of survival, growth, and reproduction in animals (Huey and Stevenson, 1979; Savage et al., 2004; Angilletta et al., 2007). The sensitivity of animals to high temperatures can be determined by the critical thermal maximum (CT_{max}), the temperature at which an animal loses essential motor function. The CT_{max} metric is an established method of assessing the upper limits of animals' thermal tolerance (Lutterschmidt and Hutchison, 1997) that links whole-animal performance to organismal fitness, species' distribution, and outcomes of interspecific interactions (Huey and Stevenson, 1979; Wiens et al., 2006; Angilletta et al., 2007; Diamond et al. 2017a). It has been used to assess heat tolerance in both invertebrates and vertebrates (Zhang and Kieffer, 2014; Baudier et al., 2015; Geerts et al., 2015) from a diversity of habitat types (e.g., aquatic, tropical, and urban environments: Geerts et al., 2015; Diamond et al., 2017b; Nguyen et al., 2017). Further, it can be used to understand an animal's thermal safety margin (i.e., the difference between an animal's CT_{max} and the maximal temperature of its environment), which is an important metric for predicting animals' responses to ongoing climate change (Sunday et al., 2014; Khaliq et al., 2014; Sinclair et al., 2016).

Variation in heat tolerance may be driven by a range of factors. First, body size may influence CT_{max} variation where large body size may lead to higher CT_{max} due to increased water stores and, hence, greater evaporative cooling potential (Baudier et al., 2015; Bujan et al., 2016; Brans et al., 2017; but see Clark et al., 2017). On the other hand, smaller body size may be associated with higher CT_{max} because a smaller body size increases the relative surface area available for evaporative heat loss (Bujan et al., 2016; Brans et al., 2017), and warming may

select for smaller body size (e.g., temperature-size rule and Bergmann's rule: Bergmann, 1847; reviewed in Angilletta, 2009; Gardner et al., 2011). Second, variation in CT_{max} may also be explained by animals' adaptations to local micro-environments, which are changing with climate change (Sunday et al., 2014). For example, animals living in warmer micro-environments may be adapted to have higher CT_{max} values than those from cooler micro-environments (Gabriel and Lynch, 1992; Stillman and Somero, 2000; Gabriel et al., 2005). Third, phylogeny can influence animal physiology (Rezende et al., 2004; Gutiérrez-Pesquera et al., 2016; Cahan et al., 2017), and closely related species may therefore exhibit similar CT_{max} values regardless of differences in morphology or micro-environment preferences (Stillman and Somero, 2000). Thus, it is crucial to account for body size, local adaptation, and phylogeny when determining this important metric of thermal sensitivity.

Examining the determinants of heat tolerance in urban animals is critical because cities are rapidly expanding (Grimm et al., 2008), and global warming is intensified in urban environments due to the urban heat island effect (Oke, 1973; Angilletta, 2009; Andrew et al., 2013; Pincebourde et al., 2016). The urban heat island effect occurs when densely populated urban areas are much warmer then surrounding rural areas. This phenomenon is driven by urban environments' relatively high occurrence of impervious surfaces (i.e. concrete, brick, asphalt, etc.) that absorb heat and the relative low incidence of vegetation (Oke, 1973; Li et al., 2011). Consequently, urban environments can reduce animals' thermal safety margins, giving animals little buffer to further increases in environmental temperature (Chown and Duffy, 2015; Diamond et al., 2017b). However, the thermal hazard of the urban heat island effect may be offset due to increased availability of water because many cities are subsidized with water, especially in more arid regions exhibiting rapid human population growth (McCarthy et al.,

2010; Vahmani and Jones, 2017). Hydration state plays a critical role in CT_{max}, body temperature, and homeostasis (Popkin et al., 2010; McWhorter et al., 2018; Smit et al., 2018). Desiccation can increase aspects of the heat shock response in some species (flies: Benoit et al., 2010; Gotcha et al., 2018); yet, in other species, it reduces CT_{max} and does not increase the up-regulation of inducible heat shock proteins (Hsps) during a heat shock (ants: Nguyen et al., 2017). Thus, a comparative examination of the effects of body size, thermal life history, and water availability on CT_{max} is required, and such a comprehensive approach may also provide insight into community dynamics. For example, overlapping thermal and hydric niches explain the success of invasions by multiple species of fruit flies and the concomitant decline in a native species of fruit fly (reviewed in Duyck et al., 2006). Thus, species-specific variation in thermal safety margin or CT_{max} sensitivity to hydration may predict interspecific competition outcomes in warming urban environments.

I used two experiments to first test a set of three hypotheses related to determinants of heat tolerance—specifically, that (1) body size, (2) preferred micro-environments, and (3) hydration status influence CT_{max}. For my first hypothesis, I predicted that larger animals would have relatively high CT_{max} values due to access to more water stores (Harrison et al., 2012; Smith and Lyons, 2013). Secondly, I predicted that animals using warmer micro-environments would have higher CT_{max} values (*sensu* coadaptation of thermal physiology and thermoregulatory behavior: reviewed in Angilletta 2009) because these animals regularly experience higher temperatures (Baudier et al., 2015; Belasen et al., 2017; Hemmings and Andrew, 2017). Thirdly, I predicted that those that were well-hydrated would have relatively high CT_{max} values as they will have access to more water stores for evaporative cooling (Harrison et al., 2012).

I also tested a fourth hypothesis that native species are particularly physiologically vulnerable in urban environments. In particular, I predicted that native species would exhibit reduced thermal safety margins and CT_{max} values, and exhibit CT_{max} and whole-body water content values that are more sensitive to water availability relative to non-native species. This prediction is based on work demonstrating that invasive species may benefit from urbanization and climate change (Zerebecki and Sorte, 2011; Menke et al., 2011; Buczkowski and Richmond, 2012; Lejeusne et al., 2014) I determined CT_{max} in ants common to cities in California's Central Valley after manipulating and quantifying hydration state (i.e., via water limitation and measuring animals' water content), and accounting for variation in body size (nearly 300-fold variation in live mass), phylogeny (11 species), and local micro-environments (surface temperatures chosen by ants during activity). My study comprehensively determines the factors influencing an important metric of heat tolerance in animals that may be particularly adapted for a reliance on water to reduce thermal hazards—the study area is characterized by hot, dry summers, as well as water subsidization (i.e., regular irrigation).

Chapter 2: Methods

Research System

Ants are abundant and important components of terrestrial ecosystems (Underwood and Fisher, 2006), including urban ecosystems (e.g. Penick et al., 2015; Stahlschmidt and Johnson, 2018). They are effective behavioral thermoregulators and, thus, are adapted and sensitive to a wide range of temperatures (Angilletta et al., 2007; Underwood and Fisher, 2006; Chick et al., 2017). Also, shifts in micro-environments due to climate change are expected to be particularly important to small-bodied animals, such as ants (Scheffers et al., 2014; Pincebourde et al., 2016; Pincebourde and Suppo, 2016; Hemmings and Andrew, 2017). Ants used in the experiments were collected in June-August in Stockton or Lodi, California, which are cities characterized by a hot-summer Mediterranean climate (Kottek et al., 2006).

Experiment 1

In 2017, an interspecific comparison was used to examine the effects of body size, micro-environmental temperature, and water availability on ants' CT_{max} values. A total of 683 individuals from 11 species (seven native species, and four non-native species) across 37 colonies were collected (Fig. 1). Ants were collected 10:00 – 14:00 using an aspirator. At each colony, six different temperature readings of ground surface were taken using an infrared thermometer (Fluke 62 MAX, Everett, Washington, USA) at the time of sampling. To estimate the temperatures of micro-environments chosen by ants during activity (T_{active}), three temperature readings were taken on each ant trail approximately approx. 1 m from one another.
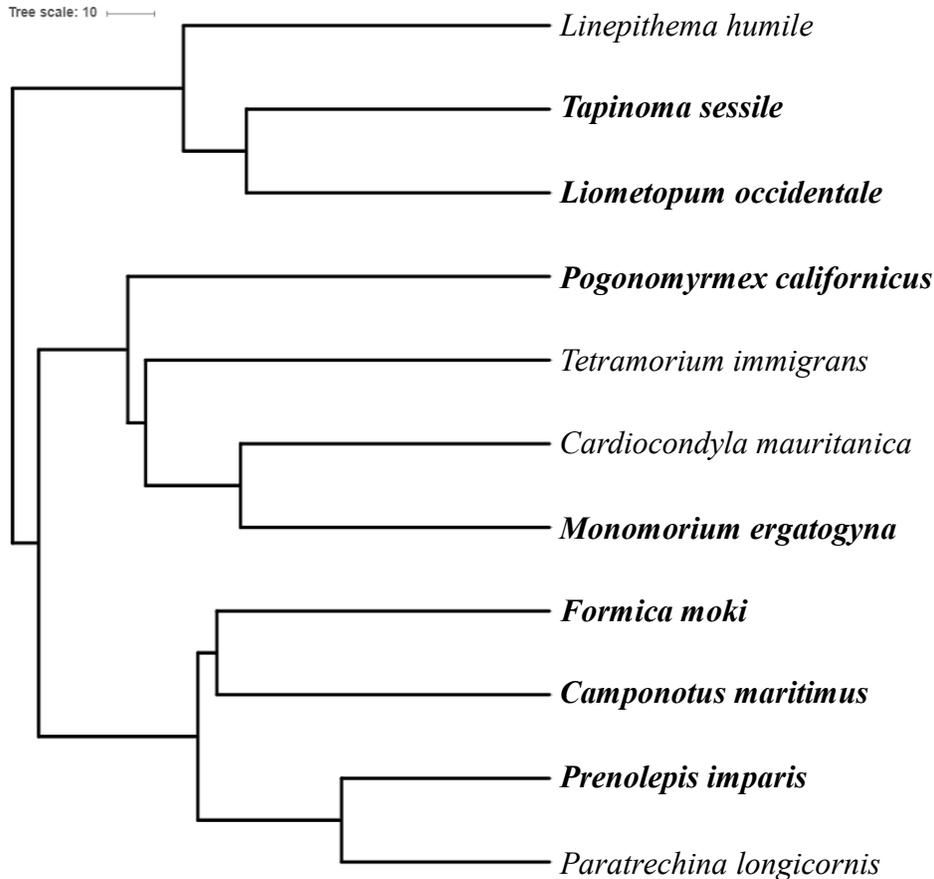
Figure 1: Phylogenetic tree demonstrating the relative relationships among ant species. Estimated minimum branch lengths were used in Experiment 1 based on Janda et al. (2004), Moreau et al. (2006), and Ward et al. (2015). Only *P. imparis* and *F. moki* were used in Experiment 2. Native species' names are bolded, and non-native species' names are not bolded, and the time scale is denoted (Mya).

To estimate the range of ants' thermal options, three temperature readings were also taken near the ant trail where directionality ($0 - 360°$) and distance ($1 - 8$ m) from each ant trail were determined via a random number generator. The maximal temperature of these six readings was used to estimate each ant's thermal safety margin (i.e., the difference between its CT_{max} [see *CT_{max} Trials* below] and the maximal temperature of its environment). My estimates of thermal

safety margins were consistent with the findings of others across various study systems (e.g. Sunday et al., 2014; reviewed in Rohr et al., 2017).

Collected ants were brought back to the University of the Pacific in Stockton, CA, and they were provided *ad libitum* water (water-filled shell vials with cotton plugs) and food (granulated table sugar) in 470 ml round glass storage containers. Ants were kept in these containers (1 – 30 ants per container depending on ant body size) overnight at room temperature (~21°C) and a 14:10 light:dark cycle, which approximates the mean summer temperature and light:dark cycle for Stockton, CA (National Weather Service). At 8:00 the next morning, ants were assigned to one of two water treatment groups: *ad libitum* or limited access to water, where the latter treatment group had water-filled vials replaced with empty vials until CT_{max} trials later in the day (see *CT_{max} Trials* below). Preliminary trials indicated that this duration of water deprivation did not influence mortality across My study species for Experiment 1.

Experiment 2

To better understand how CT_{max} was affected by water limitation, T_{active}, and intraspecific variation in body size, CT_{max} was determined in 2018 for two focal, native species: winter ant, *Prenolepis imparis* (n=118) and field ant, *Formica moki* (n=114). *Prenolepis imparis* is readily found throughout the contiguous United States whereas *F. moki* is found in the western United States (Sanders et al., 2001). The two species are relatively similar in body size (within ~0.5 mg dry mass) and are fairly sympatric as both are common in wooded urban environments. Despite these similarities, results from Experiment 1 indicated that these species varied greatly in T_{active} and CT_{max} (*P. imparis*: 22°C and 41°C; *F. moki*: 28°C and 53°C; respectively) and their CT_{max} values responded differently to water limitation (i.e., 8 h of water limitation reduced CT_{max} in *P. imparis*, but not in *F. moki*) (Fig. 2).
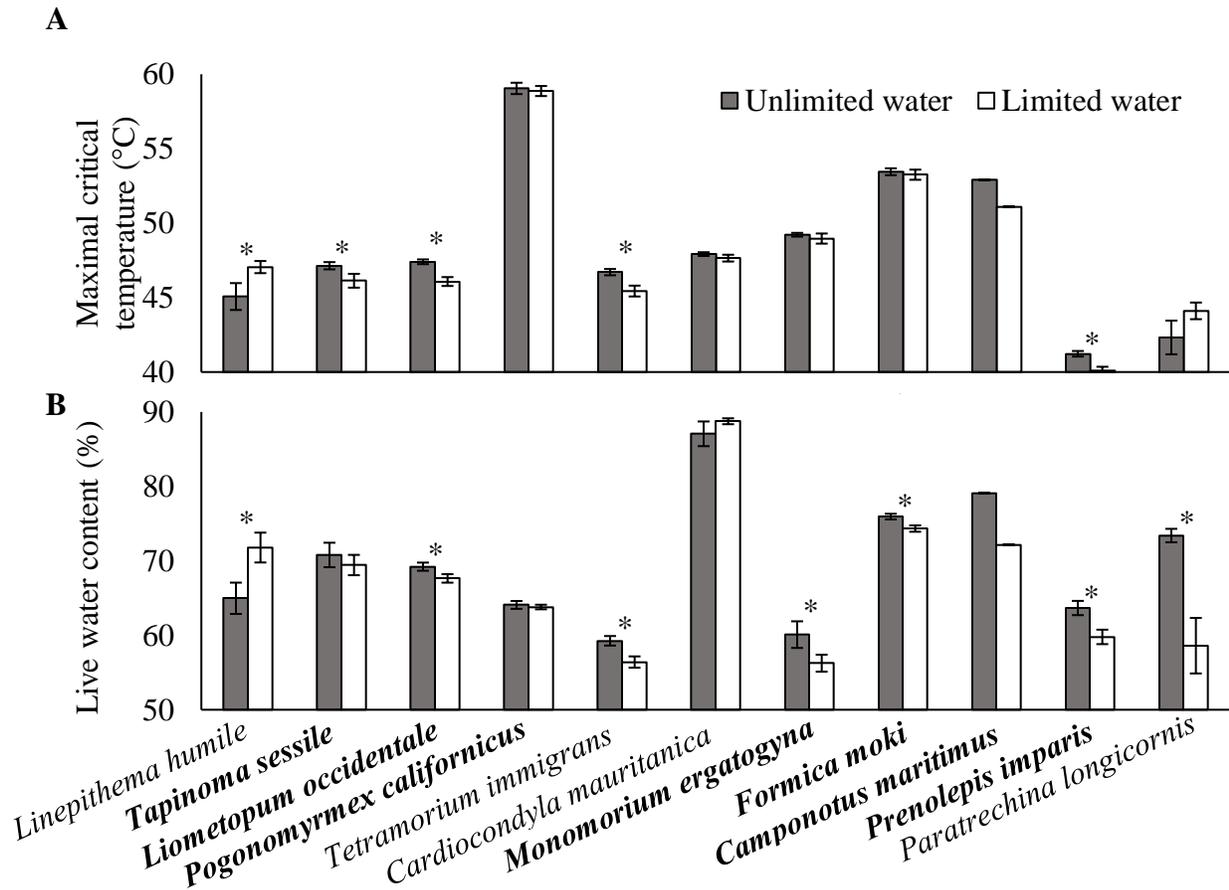
Figure 2: Effects of species and water treatment on maximal critical temperature and live water content in urban ants. Effects of species and water treatment (white: 8 h water limitation; gray: unlimited water) on (A) maximal critical temperature and (B) live water content in a community of urban ants in California's Central Valley (n=683 individuals) in Experiment 1. Values are displayed as mean±s.e.m. Asterisks denote significant effects of water treatment for each species, where native species' names are bolded and non-native species' names are not bolded. Note: the effect of water treatment on *C. maritimus* could not be analyzed due to low sample size (n=1 for each treatment).

Although similar to Experiment 1, the methods of Experiment 2 were modified in three ways.

First, the effects of intraspecific variation in body size on CT_{max} was determined (i.e., the mass of

each ant was determined, rather than estimating each ant's mass by determining the mass of

multiple-ant replicates in Experiment 1: see *CT$_{max}$ Trials* below). Second, multiple water

limitation treatments groups were used (8 and 32 h of water limitation, rather than only 8 h in

Experiment 1). Third, a more comprehensive estimate of T$_{active}$ was achieved in Experiment 2 by

taking temperature measurements three times during activity (across 2 h intervals) each sampling

day, rather than just once at the time of sampling in Experiment 1.

CT$_{max}$ Trials

Starting at 15:00 – 16:00 (i.e., the warmest time of day in the field), ants underwent

CT$_{max}$ trials. Prior to each trial, the live body mass of ants was recorded. Due to limitations of

the available analytical balance (±0.1 mg), ants were typically pooled together as a group

replicate (e.g., five ants) and weighed in Experiment 1 to determine an average value of pre-trial

live mass. Then, each group replicate was placed into a 236 ml round glass storage container in

a 24°C water bath (note: each individual ant was weighed and then placed in a 30 ml glass

container for Experiment 2). An empty 236 ml (Experiment 1) or 30 ml (Experiment 2)

container was also placed into the water bath, and a thermocouple was attached to the floor of

each empty container to estimate ant body temperature in real-time. After 30 min. of

acclimation, the water bath temperature was raised 0.5°C · min^{-1} until all of the ants were

knocked down. The CT$_{max}$ for each ant was determined by its knock-down temperature, which

was the temperature at which an ant lost the ability to right itself. After ants were knocked down

(mean: < 50 min.), the group replicates of ants (Experiment 1) or individual ants (Experiment 2)

were placed into a 50°C drying oven for ≥24 h before weighing to estimate ant body size (dry

mass) and live water content.

Statistical Analyses

To determine relationships between variables of interest (e.g., body size [dry mass] and CT_{max}) across study taxa in Experiment 1 (i.e., to test my first set of hypotheses), a software for comparative analyses (COMPARE: ver. 4.6b, open-access) was used. Linear regression analyses on both raw data (not accounting for phylogeny) and phylogenetically controlled data were performed using both phylogenetically independent contrasts and phylogenetically generalized least squares methods (PGLS). The maximum likelihood estimate of alpha, the parameter of phylogenetic dependence, for each pair of variables was determined on a scale from 0 to 15.5 (Freckleton et al., 2002). For PGLS, a low alpha (near 0) suggests data are highly dependent on phylogeny, whereas a high alpha suggests data are reasonably independent of phylogeny. The phylogenetic tree for my study taxa included estimated minimum branch lengths and was constructed from established taxonomic sources (Janda et al., 2004; Moreau et al., 2006; Ward et al., 2015).

In addition, several linear models were performed in SPSS (ver. 22, IBM Corp.), data were log-transformed when necessary, and two-tailed significance was determined at $\alpha=0.05$ unless otherwise noted. All models included species, water treatment (*ad lib.* or limited), and a species×treatment interaction as fixed effects, as well as nest identity as a random effect, unless otherwise noted. For all individuals in Experiment 1, a model included CT_{max} as the dependent variable. To test my fourth hypothesis, a one-tailed, unpaired *t* test was first performed on each species' CT_{max} to inform whether water availability improved CT_{max} more in native species relative to non-native species. Results from these tests were then used to determine whether native status (i.e., native vs. non-native) influenced the sensitivity of CT_{max} to water availability across species via a binary logistic generalized linear model (fixed effect: native status;

dependent variable: sensitivity of CT_{max} to water availability coded as 0=not sensitive or 1=sensitive). Also, to test my fourth hypothesis, linear mixed models were performed on data for each ant's thermal safety margin (i.e., pooling across water treatment groups) and CT_{max} where native status was included as a fixed effect and species was included as a random effect.

Water content was also analyzed to determine whether, for example, water treatment influenced the water content of animals. Thus, for group replicates in Experiment 1, two additional models also included T_{active} (average T_{active} for each group replicate) and body size (total dry mass of each replicate / number of ants in each replicate) as covariates, and estimated per capita absolute water content (mg) and relative live water content (%) for each replicate were the dependent variables. To test my fourth hypothesis, a one-tailed, unpaired *t* test was first performed on each species' relative water content to inform whether water availability promoted water balance more in native species relative to non-native species. Results from these tests were then used to determine whether native status influenced the sensitivity of relative water content to water availability across species via a binary logistic generalized linear model (fixed effect: native status; dependent variable: sensitivity of water content to water availability coded as 0=not sensitive or 1=sensitive). In Experiment 1, another model included mortality rate for each group replicate during acclimation prior to CT_{max} trials.

For Experiment 2, a model for CT_{max} in each species included water treatment (*ad lib.* or limited), time since water treatments were assigned (8 or 32 h), and a treatment×time interaction as fixed effects, and body size (dry mass) and T_{active} as covariates. Another model for absolute (mg) live water content in each species also included water treatment, time, and a treatment×time interaction as fixed effects, and body size (dry mass) as a covariate. A similar model (same fixed

effects as previous model) for relative (%) live water content in each species was also performed,

but it excluded body size as a covariate.

Chapter 3: Results

Experiment 1

Comparative regression analyses on data from Experiment 1 indicated moderate

phylogenetic dependence (alpha values ranged from 2.2 to 3.4). Results (e.g., significance levels

and regression coefficients) were similar across data sets from both water treatment groups—

thus, displayed data and reported results from Experiment 1 represent the entire data set (i.e.,

pooled across both water treatment groups). In Experiment 1, CT_{max} was significantly influenced

by body size (Fig. 2A; R^2 ranged from 0.31 to 0.33, depending on method of accounting for

phylogeny). However, CT_{max} was not influenced by T_{active} (Fig. 2B; R^2 range: 0.17 – 0.21) or

relative live water content (Fig. 2C; R^2 range: 0.027 – 0.034).

Mixed model analyses indicated that CT_{max} was influenced by species ($F_{10,27}=31$,

$P<0.001$) and a species×water treatment interaction ($F_{10,644}=31$, $P<0.001$), but not by water

treatment independently ($F_{1,642}=2.8$, $P=0.095$) (Fig. 3A). The sensitivity of CT_{max} to water

availability was not influenced by native status (Wald $\chi^2=0.60$, df=1, $P=0.44$; Fig. 2A).

Similarly, estimated thermal safety margins were similar between native and non-native ants

(mean: 12.1°C and 8.6°C, respectively; $F_{1,8}=1.4$, $P=0.27$), as were CT_{max} values (native mean:

50.0°C; non-native mean: 45.7°C; $F_{1,9}=1.6$, $P=0.24$).

The relative (%) live water content of ants was influenced by species ($F_{10,23}=5.1$,

$P<0.001$), water treatment ($F_{1,107}=17$, $P<0.001$), and a species×water treatment interaction

($F_{10,86}=3.5$, $P<0.001$), as well as covariates of T_{active} (positive covariation: $F_{1,25}=5.5$, $P=0.028$)

and body size (negative covariation: $F_{1,74}=17$, $P<0.001$) (Fig. 3B). Similarly, absolute (mg)

water content was influenced by species ($F_{10,18}=32$, $P<0.001$), water
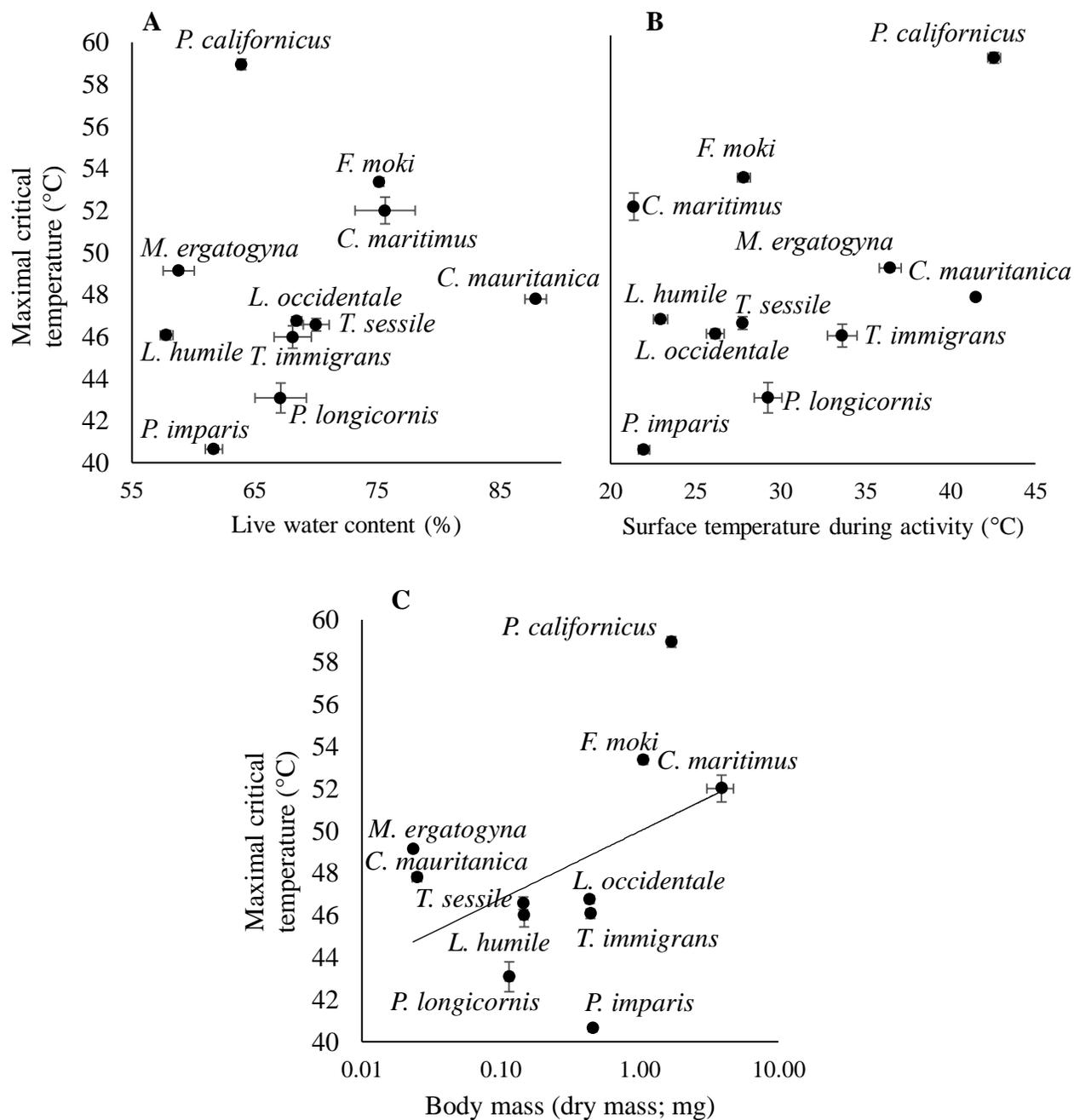
Figure 3: Relationships between maximal critical temperature and body size, active temperature, and live water content for urban ants. Relationships between maximal critical temperature (CT_{max}) and (A) body size, (B) active temperature (temperatures of micro-environments chosen during activity), and (C) live water content for a community of urban ants in California's Central Valley (11 species; n=683) in Experiment 1. Values are displayed as mean±s.e.m., and include CT_{max} values for data pooled across both water treatment groups (11 species; n=683; see text). As indicated by the regression line, only body size was significantly correlated with CT_{max} after accounting for phylogeny.

treatment ($F_{1,103}$=396, P<0.001), a species×water treatment interaction ($F_{10,79}$=54, P<0.001), and body size (positive covariation: $F_{1,102}$=9.3, P=0.0029), but not by T_{active} ($F_{1,21}$=0.72, P=0.41). The sensitivity of relative water content to water availability was not influenced by native status (Wald χ^2=1.7, df=1, P=0.19; Fig. 2B).

Experiment 2

In *P. imparis*, CT_{max} was influenced by water treatment ($F_{1,108}$=32, P<0.001), but not by the time since water treatments were assigned (i.e., 8 or 32 h), a time×water treatment interaction, T_{active}, or body size (dry mass) (all F<1.9, and P>0.17) (Fig. 4A). The relative (%) live water content of *P. imparis* was influenced by water treatment ($F_{1,110}$=4.0, P=0.047) and time ($F_{1,110}$=4.1, P=0.045), but not by a time×water treatment interaction ($F_{1,110}$=0.094, P=0.76) (Fig. 4B). Similarly, the absolute (mg) water content of *P. imparis* was affected by water treatment ($F_{1,113}$=6.5, P=0.012), time ($F_{1,113}$=5.0, P=0.027), and body size (positive covariation: $F_{1,113}$=12, P=0.001), but not by a time×water treatment interaction ($F_{1,113}$=0.25, P=0.62).

In *F. moki*, CT_{max} was influenced by water treatment ($F_{1,105}$=18, P<0.001), the time since water treatments were assigned ($F_{1,105}$=8.2, P=0.005), and a time×water treatment interaction ($F_{1,105}$=6.8, P=0.010), but it was not affected by T_{active} or body size (both F<0.7, and P>0.42) (Fig. 4A). The relative live water content of *F. moki* was influenced by water treatment ($F_{1,106}$=13, P<0.001) and a time×water treatment interaction ($F_{1,106}$=6.2, P=0.015), but not by time alone ($F_{1,107}$=0.25, P=0.62) (Fig. 4B). Similarly, the absolute water content of *F. moki* was affected by water treatment ($F_{1,109}$=28, P<0.001), a time×water treatment interaction ($F_{1,109}$=12, P=0.001), and body size (positive covariation: $F_{1,109}$=73, P<0.001), but it was not influenced by time alone ($F_{1,109}$=1.2, P=0.28).
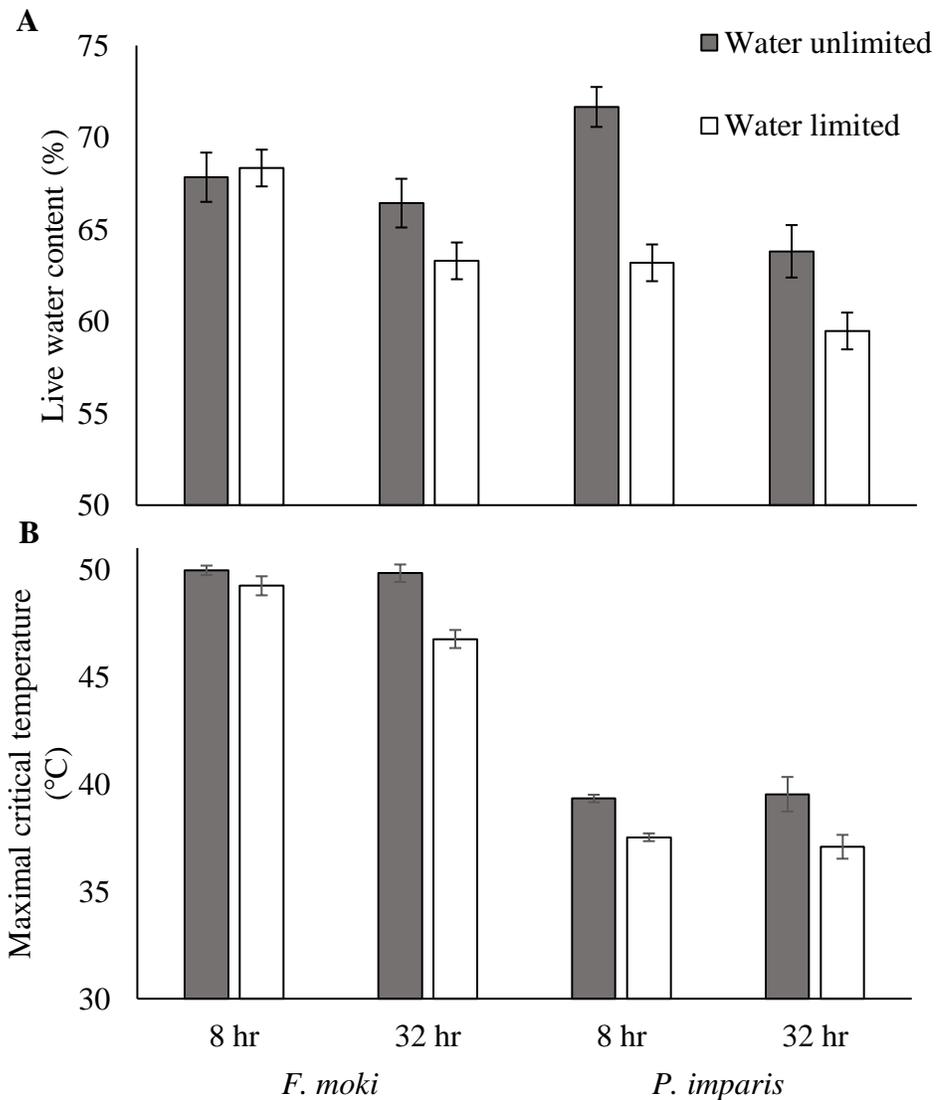
Figure 4: Effects of species, water treatment, and duration of water species in two native urban species. Effects of species, water treatment (white: water limitation; gray: unlimited water), and duration of water treatment on (A) maximal critical temperature and (B) live water content in two species of native urban ants (*Formica moki* and *Prenolepis imparis*) in California's Central Valley (n=232 individuals) in Experiment 2. Values are displayed as mean±s.e.m.

Chapter 4: Discussion

Using a comparative and manipulative approach, I demonstrate complex dynamics of temperature sensitivity in a widespread animal taxon. Urban ant species varied in CT_{max} in a body size-dependent fashion (Fig. 3A), and the effect of water availability on CT_{max} also varied across species (Fig. 2A). For example, *P. imparis* exhibited a low CT_{max} that was strongly dependent on short-term water availability while *P. californicus* had a high CT_{max} that was unaffected by short-term water availability (Fig. 2A). Despite the mixed responses of CT_{max} and body water content to water limitation in Experiment 1 (e.g., Fig. 2), results from Experiment 2 indicate that these physiological variables can be insensitive to water limitation in the shorter-term in some species, but not in the longer-term (Fig. 4A). Thus, studies focusing on individual species or those using limited experimental treatments may yield varying and/or misleading results related to understanding an eco-physiological metric of increasing importance (Sunday et al., 2014; Khaliq et al., 2014; Sinclair et al., 2016). Last, my results do not indicate that native ants are more physiologically vulnerable than urban ants because thermal safety margins and the sensitivity of CT_{max} and relative water content were similar among native and non-native ants in my study system (Fig. 2).

An animal's body size influences many aspects of its physiology and ecology—from egg size to population size (Peters and Peters, 1986; Savage et al., 2004; Smith and Lyons, 2013). Likewise, body size influenced CT_{max} across species of urban ants in support of my first hypothesis (larger animals have greater heat tolerance: Fig. 3A). Similar results have been demonstrated in tropical ants (Baudier et al., 2015), as well as in other insects (Lagadec et al., 1998; Klockmann et al., 2017) and water fleas (Geerts et al., 2015). This may be due to larger

animals having a greater thermal inertia (Lagadec et al., 1998; Klockmann et al., 2017), more water stores (increased evaporative cooling potential, but see below), or greater Hsp levels (but see Moreno et al., 2002; Brown et al., 2007). Although larger body size may be more beneficial for heat tolerance, experimental and biogeographical evidence indicates strong selection for smaller body size due to warming (e.g., temperature-size rule, and Bergmann's rule: Bergmann, 1847; reviewed in Angilletta, 2009; Gardner et al., 2011). Clearly, future work is required to determine the relative magnitude of these competing selective pressures (i.e., for larger body size due to heat tolerance benefits vs. smaller size via temperature-size rule) and the role of other factors that may mediate these pressures, such as phylogenetic constraints or local environmental variation (e.g., water availability). There was not an effect of intra-specific variation in body size on CT_{max}, which agrees with other studies examining physiological variation within species (desiccation tolerance: Mogi et al., 1996). This is likely due to greater genetic and phenotypic variation across species, rather than between species (Gearty et al., 2018)—for example, I detected nearly 300-fold variation in body mass across species in Experiment 1, but only 5-fold variation in body mass within species in Experiment 2.

For both experiments, my second hypothesis (animals active in warmer micro-environments have higher CT_{max} values) was not supported. Microhabitat temperatures have been associated with heat tolerance in other ants (Baudier et al., 2015), and discrepancies between this study and my study may be due to differences in the methodologies of temperature measurement. In my study, an infrared thermometer was used to collect temperature measurements of surfaces used by ants during activity. In other studies, miniature temperature data loggers were used to collect measurements, which allowed for continuous temperature data collection (i.e., many temperature measurements: Baudier et al., 2015). However, I failed to

detect an effect of T_{active} on CT_{max} even after significantly increasing the number of temperature measurements from Experiment 1 to Experiment 2. Coadaptation between thermoregulatory behavior and thermal physiology is not always supported (reviewed in Angilletta 2009), as exemplified by my results testing for the relationship between T_{active} and CT_{max} within and among species. This behavior-physiology mismatch may be due to an acquisition tradeoff between nutritional and thermal resources where animals are obligated to forage in sub-optimal temperatures (i.e., nutritional benefits outweigh thermoregulatory costs: Andrew et al., 2013; Andrew and Terblanche, 2013).

As described above, my third hypothesis (hydration status influences CT_{max}) was partially supported by Experiment 1 and fully supported by Experiment 2. My results indicate that ants have a threshold at which water limitation affects their heat tolerance, and these thresholds vary across species (Figs 2A, 4B). Other physiological metrics (e.g., cold tolerance and stress) also exhibit thresholds, and these thresholds can influence higher levels of biological organization (e.g., species distributions: reviewed in Martínez et al., 2015). Therefore, it is increasingly important to understand such thresholds in the context of global climate change and urbanization. Although body water content was not related to CT_{max} (Fig. 3A), water limitation generally led to a decrease in body water content, which then resulted in reduced heat tolerance (Figs 2, 4). Therefore, water limitation in my study did not facilitate cross-tolerance, which is when exposure to one stressor better equips an animal to tolerate a subsequent and different stressor (reviewed in Harrison et al., 2012). However, other work has shown a link between mechanisms underlying responses to desiccation and heat stress (Benoit et al., 2010; Gotcha et al., 2018), and continued work is required to better understand factors influencing contradictory results (e.g., due to variation in taxon and/or methodology). Life history may play a very important role in

determining how hydration status influences heat tolerance. The sensitivity of other, potentially more susceptible life-history stages (e.g., Vorhees and Bradley, 2012) should be examined with this in mind, as my study focused solely on adult workers.

There are at least two general types of mechanisms that may underlie the benefits of hydration to heat tolerance. First, hydration may confer greater evaporative cooling potential because greater water stores can be deployed (i.e., lost) during periods of heat stress. I can indirectly assess this mechanism in my study by first converting the average difference in absolute water content (mg of H_2O / individual) between the two water treatment groups to energy. For example, after 32 h of treatment, the average water-unlimited *F. moki* contained 1.5 mg more water than the average water-limited *F. moki*, and this water difference translates to water-unlimited *F. moki* having 0.0062 J of additional evaporative cooling due to the specific heat capacity of water (4.184 J g°C^{-1}). Next, I can use the following equation to solve for the temperature difference (i.e., cooling) resulting from this amount of evaporative heat loss.

$\Delta E \ = \ C \ \cdot M \ \cdot \ \Delta T$, where ΔE is the change of energy (J; e.g., 0.0062 J, see above), C is the specific heat capacity of insects (3.3472 J g°C^{-1}: Shinozaki, 1957), M is the mass of the animal (average water-unlimited *F. moki* live mass: 4.9 mg), and ΔT is the change in temperature. Using this approach, I estimated the evaporative cooling advantage of hydration to result in <0.4°C in *F. moki*—yet, the average CT$_{max}$ difference between water-unlimited and water-limited *F. moki* was 3.1°C after 32 h of treatment (Fig. 4A). Note: *F. moki* at 32 h of treatment was used in this example because of the large difference in absolute water content between the two treatment groups. Thus, water treatment-dependent evaporative cooling potential would be relatively high in this case—in contrast, for example, I estimate that water supplementation for *P. imparis* at 32 h of treatment resulted in <0.1°C of additional evaporative

cooling. Together, these calculations reveal that hydration likely did not confer an appreciable evaporative cooling advantage of animals in my study.

Second, desiccation or water limitation may lead to shifts in resource use or allocation patterns that result in a weaker heat stress response. For example, dehydration may reduce energy use (i.e., metabolic rate), which, in turn, reduces evaporative water lost through respiration (Marron et al. 2003; reviewed in Chown et al. 2011). Because metabolic rate and Hsp levels may be linked (Dahlhoff et al., 2001; Sammut and Harrison, 2003; Folguera et al., 2011), a reduction in metabolic rate (i.e., energy use) could obligate reduced heat tolerance. Also, cuticular hydrocarbons (CHCs) reduce evaporative water loss in insects (reviewed in Chown et al. 2011), and cuticular changes due to desiccation can occur quickly in some groups (Bazinet et al., 2010). Thus, desiccated insects may allocate resources from other physiological systems (e.g., the heat shock response) to alter CHCs. That said, plasticity in the composition of cuticular hydrocarbons due to desiccation may be limited in ants because CHCs are critical for chemical signaling in this taxon (Martin and Drijfhout, 2009). Related, desiccation may facilitate the allocation of resources from the heat shock response toward other biomolecules associated with desiccation tolerance, such as trehalose, Late Embryonic Abundant proteins, aquaporins, or antioxidants (Chown et al., 2011; Thorat and Nath, 2018). In sum, I recommend future work to examine the links between desiccation and reduced heat tolerance given the increasing likelihood of combined of heat and water stress due to ongoing global climate change (Sarhadi et al., 2018).

Previous research on thermal tolerance has shown that non-native, invasive species can outcompete native species at warmer temperatures (Rahel et al., 2008; Zerebecki and Sorte, 2011; Lejeusne et al., 2014). Thus, I expected similar results in urban areas due to the combined

effects of climate change and the urban heat island effect. Yet, I did not detect a significant effect of native status on thermal safety margins, CT_{max} values, or the water-sensitivities of CT_{max} or whole-body water content values. A possible partial explanation for these negative results is that invasive species may be more reliant on water than native species (Hellmann et al., 2008). In which case, some non-native species may be increasingly favored as urban specialists because they are unable to persist in more natural environments where water is not supplemented or subsidized. Thus, limited water availability and increasing temperatures may favor native (rather than non-native) species in some ecosystems, which is important given environments are expected to become increasingly arid and warm (Sarhadi et al., 2018). Future work on additional taxa is required to better understand the complex interplay among native status, urbanization, and water availability related to heat tolerance.

The availability of water, a vital resource for all animals, continues to be put at risk by a combination of increasing temperatures and drier global climates that could leave animals vulnerable to reduced thermal safety margins (Sunday et al., 2014; Sarhadi et al., 2018). Given the continued natural covariation between elevated temperatures and reduced precipitation (Sarhadi et al. 2018), it is important to continue to consider desiccation resistance as an important physiological metric (Matzkin et al., 2007; Bujan et al., 2016). My results indicate that water subsidization in urban environments may offset the thermal hazards of the urban heat island effect. However, given urban-rural variation in thermal physiology (Angilletta et al. 2007; Chown and Duffy, 2015; Diamond et al., 2017b), similar experimental, comparative studies should be conducted in non-urban environments where water is not subsidized. In sum, understanding the links between heat tolerance and desiccation resistance will become critical in a world that is increasingly warm, dry, and urbanized.

References

Andrew, N.R., Hart, R.A., Jung, M.-P., Hemmings, Z., Terblanche, J.S., 2013. Can temperate

insects take the heat? A case study of the physiological and behavioural responses in a

common ant, Iridomyrmex purpureus (Formicidae), with potential climate change. J.

Insect Physiol. 59, 870–880. https://doi.org/10.1016/j.jinsphys.2013.06.003

Andrew, N.R., Terblanche, J.S., 2013. The response of insects to climate change 14.

Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford

University Press.

Angilletta, M.J., Wilson, R.S., Niehaus, A.C., Sears, M.W., Navas, C.A., Ribeiro, P.L., 2007.

Urban Physiology: City Ants Possess High Heat Tolerance. PLOS ONE 2, e258.

https://doi.org/10.1371/journal.pone.0000258

Baudier, K.M., Mudd, A.E., Erickson, S.C., O'Donnell, S., 2015. Microhabitat and body size

effects on heat tolerance: implications for responses to climate change (army ants:

Formicidae, Ecitoninae). J. Anim. Ecol. 84, 1322–1330. https://doi.org/10.1111/1365-

2656.12388

Bazinet, A.L., Marshall, K.E., MacMillan, H.A., Williams, C.M., Sinclair, B.J., 2010. Rapid

changes in desiccation resistance in Drosophila melanogaster are facilitated by changes in

cuticular permeability. J. Insect Physiol. 56, 2006–2012.

https://doi.org/10.1016/j.jinsphys.2010.09.002

Belasen, A., Brock, K., Li, B., Chremou, D., Valakos, E., Pafilis, P., Sinervo, B., Foufopoulos,

J., 2017. Fine with heat, problems with water: microclimate alters water loss in a

thermally adapted insular lizard. Oikos 126, 447–457. https://doi.org/10.1111/oik.03712

Benoit, J.B., Patrick, K.R., Desai, K., Hardesty, J.J., Krause, T.B., Denlinger, D.L., 2010. Repeated bouts of dehydration deplete nutrient reserves and reduce egg production in the mosquito Culex pipiens. J. Exp. Biol. 213, 2763–2769. https://doi.org/10.1242/jeb.044883

Brans, K.I., Jansen, M., Vanoverbeke, J., Tüzün, N., Stoks, R., De Meester, L., 2017. The heat is on: Genetic adaptation to urbanization mediated by thermal tolerance and body size. Glob. Change Biol. 23, 5218–5227. https://doi.org/10.1111/gcb.13784

Brown, D.A., Johnson, M.S., Armstrong, C.J., Lynch, J.M., Caruso, N.M., Ehlers, L.B., Fleshner, M., Spencer, R.L., Moore, R.L., 2007. Short-term treadmill running in the rat: what kind of stressor is it? J. Appl. Physiol. Bethesda Md 1985 103, 1979–1985. https://doi.org/10.1152/japplphysiol.00706.2007

Buczkowski, G., Richmond, D.S., 2012. The Effect of Urbanization on Ant Abundance and Diversity: A Temporal Examination of Factors Affecting Biodiversity. PLOS ONE 7, e41729. https://doi.org/10.1371/journal.pone.0041729

Bujan, J., Yanoviak, S.P., Kaspari, M., 2016. Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community. Ecol. Evol. 6, 6282–6291. https://doi.org/10.1002/ece3.2355

Cahan, S.H., Nguyen, A.D., Stanton-Geddes, J., Penick, C.A., Hernaiz-Hernandez, Y., DeMarco, B.B., Gotelli, N.J., 2017. Modulation of the heat shock response is associated with acclimation to novel temperatures but not adaptation to climatic variation in the ants Aphaenogaster picea and A. rudis. Comp. Biochem. Physiol. -Mol. Integr. Physiol. 204, 113–120. https://doi.org/10.1016/j.cbpa.2016.11.017

Chick, L.D., Perez, A., Diamond, S.E., 2017. Social dimensions of physiological responses to global climate change: what we can learn from ants (Hymenoptera: Formicidae). Myrmecol. News 25, 29–40.

Chown, S.L., Duffy, G.A., 2015. Thermal physiology and urbanization: perspectives on exit, entry and transformation rules. Funct. Ecol. 29, 902–912. https://doi.org/10.1111/1365-2435.12478

Chown, S.L., Hoffmann, A.A., Kristensen, T.N., Jr, M.J.A., Stenseth, N.C., Pertoldi, C., 2010. Adapting to climate change: a perspective from evolutionary physiology. Clim. Res. 43, 3–15. https://doi.org/10.3354/cr00879

Chown, S.L., Sørensen, J.G., Terblanche, J.S., 2011. Water loss in insects: An environmental change perspective. J. Insect Physiol., "Cold and Desiccation Tolerance" honoring Karl Erik Zachariassen 57, 1070–1084. https://doi.org/10.1016/j.jinsphys.2011.05.004

Clark, T.D., Roche, D.G., Binning, S.A., Speers-Roesch, B., Sundin, J., 2017. Maximum thermal limits of coral reef damselfishes are size dependent and resilient to near-future ocean acidification. J. Exp. Biol. 220, 3519–3526. https://doi.org/10.1242/jeb.162529

Dahlhoff, E.P., Buckley, B.A., Menge, B.A., 2001. Physiology of the Rocky Intertidal Predator Nucella ostrina along an Environmental Stress Gradient. Ecology 82, 2816–2829. https://doi.org/10.2307/2679963

Dammerman, K., Steibel, J., Scribner, K., 2015. Genetic and environmental components of phenotypic and behavioral trait variation during lake sturgeon (Acipenser fulvescens) early ontogeny. Environ. Biol. Fishes 98. https://doi.org/10.1007/s10641-015-0392-6

Diamond, S.E., Chick, L., Penick, C.A., Nichols, L.M., Cahan, S.H., Dunn, R.R., Ellison, A.M., Sanders, N.J., Gotelli, N.J., 2017a. Heat tolerance predicts the importance of species

interaction effects as the climate changes. Integr. Comp. Biol. 57, 112–120. https://doi.org/10.1093/icb/icx008

Diamond, S.E., Chick, L., Perez, A., Strickler, S.A., Martin, R.A., 2017b. Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. Biol. J. Linn. Soc. 121, 248–257. https://doi.org/10.1093/biolinnean/blw047

Duyck, P.-F., David, P., Quilici, S., 2006. Climatic niche partitioning following successive invasions by fruit flies in La Réunion. J. Anim. Ecol. 75, 518–526. https://doi.org/10.1111/j.1365-2656.2006.01072.x

Folguera, G., Bastías, D.A., Caers, J., Rojas, J.M., Piulachs, M.-D., Bellés, X., Bozinovic, F., 2011. An experimental test of the role of environmental temperature variability on ectotherm molecular, physiological and life-history traits: implications for global warming. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 159, 242–246. https://doi.org/10.1016/j.cbpa.2011.03.002

Forman, R.T.T., 2014. Urban Ecology: Science of Cities. Cambridge University Press.

Freckleton, R.P., Harvey, P.H., Pagel, M., Losos, A.E.J.B., 2002. Phylogenetic Analysis and Comparative Data: A Test and Review of Evidence. Am. Nat. 160, 712–726. https://doi.org/10.1086/343873

Gabriel, W., Luttbeg, B., Sih, A., Tollrian, R., 2005. Environmental Tolerance, Heterogeneity, and the Evolution of Reversible Plastic Responses. Am. Nat. 166, 339–353. https://doi.org/10.1086/432558

Gabriel, W., Lynch, M., 1992. The selective advantage of reaction norms for environmental tolerance. J. Evol. Biol. 5, 41–59. https://doi.org/10.1046/j.1420-9101.1992.5010041.x

Gardner, J.L., Peters, A., Kearney, M.R., Joseph, L., Heinsohn, R., 2011. Declining body size: a
   third universal response to warming? Trends Ecol. Evol. 26, 285–291.
   https://doi.org/10.1016/j.tree.2011.03.005

Gearty, W., McClain, C.R., Payne, J.L., 2018. Energetic tradeoffs control the size distribution of
   aquatic mammals. Proc. Natl. Acad. Sci. 115, 4194–4199.
   https://doi.org/10.1073/pnas.1712629115

Geerts, A., Vanoverbeke, J., Vanschoenwinkel, B., Van Doorslaer, W., Feuchtmayr, H.,
   Atkinson, D., Moss, B., Davidson, T., D. Sayer, C., De Meester, L., 2015. Rapid
   evolution of thermal tolerance in the water flea Daphnia (vol 5, pg 665, 2015). Nat. Clim.
   Change 5. https://doi.org/10.1038/NCLIMATE2810

Gotcha, N., Terblanche, J.S., Nyamukondiwa, C., 2018. Plasticity and cross-tolerance to
   heterogeneous environments: divergent stress responses co-evolved in an African fruit
   fly. J. Evol. Biol. 31, 98–110. https://doi.org/10.1111/jeb.13201

Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X., Briggs, J.M., 2008.
   Global Change and the Ecology of Cities. Science 319, 756–760.
   https://doi.org/10.1126/science.1150195

Gutiérrez-Pesquera, L.M., Tejedo, M., Olalla-Tárraga, M.Á., Duarte, H., Nicieza, A., Solé, M.,
   2016. Testing the climate variability hypothesis in thermal tolerance limits of tropical and
   temperate tadpoles. J. Biogeogr. 43, 1166–1178. https://doi.org/10.1111/jbi.12700

Harrison, J.F., Woods, H.A., Roberts, S.P., 2012. Ecological and Environmental Physiology of
   Insects. OUP Oxford.

Hellmann, J.J., Byers, J.E., Bierwagen, B.G., Dukes, J.S., 2008. Five Potential Consequences of Climate Change for Invasive Species. Conserv. Biol. 22, 534–543. https://doi.org/10.1111/j.1523-1739.2008.00951.x

Helms Cahan, S., Nguyen, A.D., Stanton-Geddes, J., Penick, C.A., Hernáiz-Hernández, Y., DeMarco, B.B., Gotelli, N.J., 2017. Modulation of the heat shock response is associated with acclimation to novel temperatures but not adaptation to climatic variation in the ants Aphaenogaster picea and A. rudis. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 204, 113–120. https://doi.org/10.1016/j.cbpa.2016.11.017

Hemmings, Z., Andrew, N.R., 2017. Effects of microclimate and species identity on body temperature and thermal tolerance of ants (Hymenoptera: Formicidae). Austral Entomol. 56, 104–114. https://doi.org/10.1111/aen.12215

Huey, R.B., Stevenson, R.D., 1979. Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. Integr. Comp. Biol. 19, 357–366. https://doi.org/10.1093/icb/19.1.357

IPCC, 2014. IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III... | Tribal Climate Change Guide [WWW Document]. URL https://tribalclimateguide.uoregon.edu/literature/ipcc-2014-climate-change-2014-synthesis-report-contribution-working-groups-i-ii-and-iii (accessed 1.21.19).

Janda, M., Folková, D., Zrzavý, J., 2004. Phylogeny of Lasius ants based on mitochondrial DNA and morphology, and the evolution of social parasitism in the Lasiini (Hymenoptera: Formicidae). Mol. Phylogenet. Evol. 33, 595–614. https://doi.org/10.1016/j.ympev.2004.07.012

Kaspari, M., Clay, N.A., Lucas, J., Yanoviak, S.P., Kay, A., 2015. Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. Glob. Change Biol. 21, 1092–1102. https://doi.org/10.1111/gcb.12750

Khaliq, I., Hof, C., Prinzinger, R., Boehning-Gaese, K., Pfenninger, M., 2014. Global variation in thermal tolerances and vulnerability of endotherms to climate change. Proc. R. Soc. B-Biol. Sci. 281, 20141097. https://doi.org/10.1098/rspb.2014.1097

Klockmann, M., Günter, F., Fischer, K., 2017. Heat resistance throughout ontogeny: body size constrains thermal tolerance. Glob. Change Biol. 23, 686–696. https://doi.org/10.1111/gcb.13407

Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F., 2006. World Map of the Köppen-Geiger climate classification updated. Meteorol. Z. 15, 259–263. https://doi.org/10.1127/0941-2948/2006/0130

Lagadec, M.D.L., Chown, S.L., Scholtz, C.H., 1998. Desiccation resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): the influence of body size and habitat. J. Comp. Physiol. B 168, 112–122. https://doi.org/10.1007/s003600050127

Lejeusne, C., Latchere, O., Petit, N., Rico, C., Green, A.J., 2014. Do invaders always perform better? Comparing the response of native and invasive shrimps to temperature and salinity gradients in south-west Spain. Estuar. Coast. Shelf Sci. 136, 102–111. https://doi.org/10.1016/j.ecss.2013.11.014

Li, J., Song, C., Cao, L., Zhu, F., Meng, X., Wu, J., 2011. Impacts of landscape structure on surface urban heat islands: A case study of Shanghai, China. Remote Sens. Environ. 115, 3249–3263. https://doi.org/10.1016/j.rse.2011.07.008

Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: history and critique. Can. J. Zool. 75, 1561–1574. https://doi.org/10.1139/z97-783

Marron, M.T., Markow, T.A., Kain, K.J., Gibbs, A.G., 2003. Effects of starvation and desiccation on energy metabolism in desert and mesic Drosophila. J. Insect Physiol. 49, 261–270.

Martin, S., Drijfhout, F., 2009. A review of ant cuticular hydrocarbons. J. Chem. Ecol. 35, 1151–1161. https://doi.org/10.1007/s10886-009-9695-4

Martínez, B., Arenas, F., Trilla, A., Viejo, R.M., Carreño, F., 2015. Combining physiological threshold knowledge to species distribution models is key to improving forecasts of the future niche for macroalgae. Glob. Change Biol. 21, 1422–1433. https://doi.org/10.1111/gcb.12655

Matzkin, L.M., Watts, T.D., Markow, T.A., 2007. Desiccation resistance in four Drosophila species: sex and population effects. Fly (Austin) 1, 268–273.

McCarthy, M.P., Best, M.J., Betts, R.A., 2010. Climate change in cities due to global warming and urban effects. Geophys. Res. Lett. 37. https://doi.org/10.1029/2010GL042845

McWhorter, T.J., Gerson, A.R., Talbot, W.A., Smith, E.K., McKechnie, A.E., Wolf, B.O., 2018. Avian thermoregulation in the heat: evaporative cooling capacity and thermal tolerance in two Australian parrots. J. Exp. Biol. 221, jeb168930. https://doi.org/10.1242/jeb.168930

Menke, S.B., Guénard, B., Sexton, J.O., Weiser, M.D., Dunn, R.R., Silverman, J., 2011. Urban areas may serve as habitat and corridors for dry-adapted, heat tolerant species; an example from ants. Urban Ecosyst. 14, 135–163. https://doi.org/10.1007/s11252-010-0150-7

Mogi, M., Miyagi, I., Abadi, K., 1996. Inter- and Intraspecific Variation in Resistance to Desiccation by Adult Aedes (Stegomyia) spp. (Diptera: Culicidae) from Indonesia. J. Med. Entomol. 33, 53–57. https://doi.org/10.1093/jmedent/33.1.53

Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B., Pierce, N.E., 2006. Phylogeny of the Ants: Diversification in the Age of Angiosperms. Science 312, 101–104. https://doi.org/10.1126/science.1124891

Moreno, J., Merino, S., MartÍnez, J., Sanz, J., Arriero, E., 2002. Heterophil/lymphocyte ratios and heat-shock protein levels are related to growth in nestling birds. Écoscience 9, 434–439. https://doi.org/10.1080/11956860.2002.11682731

Naeem, M., Salam, A., Zuberi, A., 2016. PROXIMATE COMPOSITION OF FRESHWATER RAINBOW TROUT (Oncorhynchus mykiss) IN RELATION TO BODY SIZE AND CONDITION FACTOR FROM PAKISTAN. Pak. J. Agric. Sci. 53, 468–472. https://doi.org/10.21162/PAKJAS/16.2653

Nguyen, A.D., DeNovellis, K., Resendez, S., Pustilnik, J.D., Gotelli, N.J., Parker, J.D., Cahan, S.H., 2017. Effects of desiccation and starvation on thermal tolerance and the heat-shock response in forest ants. J. Comp. Physiol. B 187, 1107–1116. https://doi.org/10.1007/s00360-017-1101-x

Oke, T.R., 1982. The energetic basis of the urban heat island. Q. J. R. Meteorol. Soc. 108, 1–24. https://doi.org/10.1002/qj.49710845502

Oke, T.R., 1973. City size and the urban heat island. Atmospheric Environ. 1967 7, 769–779. https://doi.org/10.1016/0004-6981(73)90140-6

Oreskes, N., 2004. The Scientific Consensus on Climate Change. Science 306, 1686–1686. https://doi.org/10.1126/science.1103618

Penick, C.A., Savage, A.M., Dunn, R.R., 2015. Stable isotopes reveal links between human food inputs and urban ant diets. Proc. Biol. Sci. 282, 20142608. https://doi.org/10.1098/rspb.2014.2608

Peters, Robert H., Peters, Robert Henry, 1986. The Ecological Implications of Body Size. Cambridge University Press.

Pincebourde, S., Murdock, C.C., Vickers, M., Sears, M.W., 2016. Fine-Scale Microclimatic Variation Can Shape the Responses of Organisms to Global Change in Both Natural and Urban Environments. Integr. Comp. Biol. 56, 45–61. https://doi.org/10.1093/icb/icw016

Pincebourde, S., Suppo, C., 2016. The Vulnerability of Tropical Ectotherms to Warming Is Modulated by the Microclimatic Heterogeneity. Integr. Comp. Biol. 56, 85–97. https://doi.org/10.1093/icb/icw014

Popkin, B.M., D'Anci, K.E., Rosenberg, I.H., 2010. Water, Hydration and Health. Nutr. Rev. 68, 439–458. https://doi.org/10.1111/j.1753-4887.2010.00304.x

Rahel, F.J., Bierwagen, B., Taniguchi, Y., 2008. Managing Aquatic Species of Conservation Concern in the Face of Climate Change and Invasive Species. Conserv. Biol. 22, 551–561. https://doi.org/10.1111/j.1523-1739.2008.00953.x

Rezende, E.L., Bozinovic, F., Garland, T., 2004. Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. Evol. Int. J. Org. Evol. 58, 1361–1374.

Rohr, J.R., Civitello, D.J., Cohen, J.M., Roznik, E.A., Sinervo, B., Dell, A.I., 2017. A Global Framework for Estimating Acclimation and Thermal Breadth Predicts Risk from Climate Change. bioRxiv. https://doi.org/10.1101/156026

Sammut, I.A., Harrison, J.C., 2003. Cardiac mitochondrial complex activity is enhanced by heat shock proteins. Clin. Exp. Pharmacol. Physiol. 30, 110–115.

Sanders, N.J., Barton, K.E., Gordon, D.M., 2001. Long-term dynamics of the distribution of the invasive Argentine ant, Linepithema humile, and native ant taxa in northern California. Oecologia 127, 123–130. https://doi.org/10.1007/s004420000572

Sarhadi, A., Ausín, M.C., Wiper, M.P., Touma, D., Diffenbaugh, N.S., 2018. Multidimensional risk in a nonstationary climate: Joint probability of increasingly severe warm and dry conditions. Sci. Adv. 4, eaau3487. https://doi.org/10.1126/sciadv.aau3487

Savage, Van M., Gilloly, J.F., Brown, J.H., Charnov, E.L., 2004. Effects of body size and temperature on population growth. Am. Nat. 163, 429–441. https://doi.org/10.1086/381872

Savage, Van M., Gillooly, J.F., Brown, J.H., West, G.B., Charnov, E.L., 2004. Effects of Body Size and Temperature on Population Growth. Am. Nat. 163, 429–441. https://doi.org/10.1086/381872

Scheffers, B.R., Edwards, D.P., Diesmos, A., Williams, S.E., Evans, T.A., 2014. Microhabitats reduce animal's exposure to climate extremes. Glob. Change Biol. 20, 495–503. https://doi.org/10.1111/gcb.12439

Shinozaki, J., 1957. The Specific Heat of Insects. J. Fac. Sci. HOKKAIDO Univ. Ser. VI Zool. 6.

Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., Dong, Y., Harley, C.D.G., Marshall, D.J., Helmuth, B.S., Huey, R.B., 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? Ecol. Lett. 19, 1372–1385. https://doi.org/10.1111/ele.12686

Smit, B., Whitfield, M.C., Talbot, W.A., Gerson, A.R., McKechnie, A.E., Wolf, B.O., 2018. Avian thermoregulation in the heat: phylogenetic variation among avian orders in

evaporative cooling capacity and heat tolerance. J. Exp. Biol. 221, jeb174870.
https://doi.org/10.1242/jeb.174870

Smith, F.A., Lyons, S.K., 2013. Animal Body Size: Linking Pattern and Process across Space, Time, and Taxonomic Group. University of Chicago Press.

Stahlschmidt, Z.R., Johnson, D., 2018. Moving targets: determinants of nutritional preferences and habitat use in an urban ant community. Urban Ecosyst. 21, 1151–1158. https://doi.org/10.1007/s11252-018-0796-0

Stillman, J.H., Somero, G.N., 2000. A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus Petrolisthes: influences of latitude, vertical zonation, acclimation, and phylogeny. Physiol. Biochem. Zool. PBZ 73, 200–208. https://doi.org/10.1086/316738

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., Huey, R.B., 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc. Natl. Acad. Sci. U. S. A. 111, 5610–5615. https://doi.org/10.1073/pnas.1316145111

Thorat, L., Nath, B.B., 2018. Insects With Survival Kits for Desiccation Tolerance Under Extreme Water Deficits. Front. Physiol. 9. https://doi.org/10.3389/fphys.2018.01843

Underwood, E.C., Fisher, B.L., 2006. The role of ants in conservation monitoring: If, when, and how. Biol. Conserv. 132, 166–182. https://doi.org/10.1016/j.biocon.2006.03.022

Vahmani, P., Jones, A.D., 2017. Water conservation benefits of urban heat mitigation: can cooling strategies reduce water consumption in California? AGU Fall Meet. Abstr. 12.

Vorhees, A.S., 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. https://doi.org/10.1242/jeb.070342

Ward, P.S., Brady, S.G., Fisher, B.L., Schultz, T.R., 2015. The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). Syst. Entomol. 40, 61–81. https://doi.org/10.1111/syen.12090

Weldon, C.W., Nyamukondiwa, C., Karsten, M., Chown, S.L., Terblanche, J.S., 2018. Geographic variation and plasticity in climate stress resistance among southern African populations of Ceratitis capitata (Wiedemann) (Diptera: Tephritidae). Sci. Rep. 8, 9849. https://doi.org/10.1038/s41598-018-28259-3

Wiens, J.J., Graham, C.H., Moen, D.S., Smith, S.A., Reeder, T.W., 2006. Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. Am. Nat. 168, 579–596. https://doi.org/10.1086/507882

Zerebecki, R.A., Sorte, C.J.B., 2011. Temperature Tolerance and Stress Proteins as Mechanisms of Invasive Species Success. PLOS ONE 6, e14806. https://doi.org/10.1371/journal.pone.0014806

Zhang, Y., Kieffer, J., 2014. Critical thermal maximum (CT max) and hematology of shortnose sturgeons (Acipenser brevirostrum) acclimated to three temperatures. Can. J. Zool. 92, 215–221. https://doi.org/10.1139/cjz-2013-0223