

ORIGINAL RESEARCH

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

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Abstract

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. We 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 (a) body size, (b) hydration status, and (c) chosen microenvironments influence CT_{max} . We further tested a fourth hypothesis that native species are particularly physiologically vulnerable in urban environments. We 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 size. There was a moderate phylogenetic signal influencing CT_{max} , and 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 hr) reduced CT_{max} and body water content in some species while longer-term water limitation (32 hr) was required to reduce these traits in other species. However, CT_{max} was not related to the temperatures chosen by ants during activity. Further, we found support for our fourth hypothesis because CT_{max} and estimates of thermal safety margin in native species were more sensitive to water availability relative to non-native species. In sum, we provide evidence of links between heat tolerance and water availability, which will become critically important in an increasingly warm, dry, and urbanized world that others have shown may be selecting for smaller (not larger) body size.

KEYWORDS

critical temperature, knock-down, thermal maximum, urban heat island, water availability

1 | INTRODUCTION

Temperatures are increasing globally due to climate change (IPCC, 2014; Oreskes, 2004), and high temperatures may alter survival,

growth, and reproduction in animals (Angilletta, 2009; Angilletta et al., 2007; Huey & Stevenson, 1979; Savage, Gillooly, Brown, West, & Charnov, 2004). The sensitivity of animals to high temperatures can be determined by features of the thermal performance curve,

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including the critical thermal maximum (CT_{max} , the temperature at which an animal loses essential motor function: reviewed in Angilletta, 2009). The CT_{max} metric is an established proxy for assessing heat tolerance (Lutterschmidt & Hutchison, 1997) that links whole-animal performance to organismal fitness, species' distribution, and outcomes of interspecific interactions (Angilletta et al., 2007; Diamond, Chick, Penick, et al., 2017; Huey & Stevenson, 1979; Wiens, Graham, Moen, Smith, & Reeder, 2006). It has been used to assess heat tolerance in both invertebrates and vertebrates (Baudier, Mudd, Erickson, & O'Donnell, 2015; Geerts et al., 2015; Zhang & Kieffer, 2014) from a diversity of habitat types (e.g., aquatic, tropical, and urban environments: Diamond, Chick, Perez, Strickler, & Martin, 2017; Geerts et al., 2015; Nguyen et al., 2017). Further, it can be used to understand an animal's thermal safety margin (herein, 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 (Khaliq, Hof, Prinzinger, Bohning-Gaese, & Pfenninger, 2014; Sinclair et al., 2016; Sunday et al., 2014).

Variation in heat tolerance of terrestrial animals 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} perhaps due to increased heat shock protein (Hsp) synthesis and/or reduced thermal conductance of integument (Galushko et al., 2005; Gehring & Wehner, 1995; Hood & Tschinkel, 1990). 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 heat loss, and warming may select for smaller body size (e.g., temperature-size rule and Bergmann's rule: reviewed in Angilletta, 2009; Gardner, Peters, Kearney, Joseph, & Heinsohn, 2011; but see Horne, Hirst, & Atkinson, 2015). Second, variation in CT_{max} may also be explained by animals' adaptations to local microenvironments, which are changing with climate change (Stillman & Somero, 2000; Sunday et al., 2014) and may be linked to body size (Kaspari, 1993). For example, animals living in warmer microenvironments may be adapted to have higher CT_{max} values than those from cooler microenvironments (Baudier et al., 2015) due to variation in membrane composition, or in the production of isoenzymes or Hsps (Gabriel & Lynch, 1992; Stillman & Somero, 2000; Gabriel, Luttbeg, Sih, & Tollrian, 2005; Pincebourde & Casas, 2019; reviewed in Angilletta, 2009; Hochachka & Somero, 2002). Third, phylogeny can influence animal physiology (Cahan et al., 2017; Gutierrez-Pesquera et al., 2016; Rezende, Bozinovic, & Garland, 2004), and closely related species may, therefore, exhibit similar CT_{max} values regardless of differences in morphology or microenvironment preferences. 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 (Andrew, Hart, Jung, Hemmings, & Terblanche, 2013; Oke, 1973; Pincebourde, Murdock, Vickers, & Sears, 2016; Youngsteadt, Dale, Terando, Dunn, & Frank, 2015). Consequently,

urban environments can reduce animals' thermal safety margins, giving animals little buffer to further increases in environmental temperature (Chown & Duffy, 2015; Diamond, Chick, Perez, et al., 2017). However, the thermal hazard of the urban heat island effect may be offset by an increased availability of water because many cities are subsidized with water, especially in warmer or more arid regions exhibiting rapid human population growth (McCarthy, Best, & Betts, 2010; McCluney, Burdine, & Frank, 2017; Vahmani & Jones, 2017). The availability of water constrains terrestrial life, and hydration state plays a critical role in CT_{max} , body temperature, and homeostasis (Da Lage, Cappy, & David, 1989; Manenti, Cunha, Sørensen, & Loeschcke, 2018; Nguyen et al., 2017; Smit et al., 2018). Desiccation can enhance the physiological heat shock response in some species (flies: Benoit et al., 2010; Gotcha, Terblanche, & Nyamukondiwa, 2018); yet, in other species, it reduces CT_{max} and does not increase the upregulation of inducible 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} in terrestrial animals is required, and such a comprehensive approach may also provide insight into community dynamics associated with invasion biology. For example, overlapping thermal and hygric 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, David, & Quilici, 2006). Yet, the invasive Argentine ant may be particularly vulnerable to desiccation, which may limit its success in warmer, drier habitats (Schilman, Lighton, & Holway, 2007). Therefore, species-specific variation in thermal safety margin or CT_{max} sensitivity to hydration may predict competition outcomes between native and non-native species in warming urban environments.

We used two experiments to first test a set of three hypotheses related to determinants of heat tolerance within and among species—specifically, that (a) body size, (b) chosen microenvironments, and (c) hydration status influence CT_{max} . For our first hypothesis, we predicted that larger animals would have relatively high CT_{max} values. Second, we predicted that animals using warmer microenvironments would have higher CT_{max} values because these animals regularly experience higher temperatures (sensu coadaptation of thermal physiology and thermoregulatory behavior: reviewed in Angilletta, 2009). Third, we predicted that well-hydrated animals would have relatively high CT_{max} values. Although our study examined a community of ants from western North America (see below), these first three predictions are based on work in ants from other regions (Cerdeira & Retana, 2000; Clemencet, Cournault, Odent, & Doums, 2010: western Europe; Ribeiro, Camacho, & Navas, 2012; Baudier et al., 2015: neotropics; Nguyen et al., 2017: eastern North America; but see Hemmings & Andrew, 2017: Australia). We also tested a fourth hypothesis that native species are particularly physiologically vulnerable in urban environments. Specifically, we predicted that native species would exhibit reduced thermal safety margins and CT_{max} values and exhibit CT_{max} thermal safety margins, 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 or climate change (Buczkowski & Richmond, 2012; Lejeune,

Latchere, Petit, Rico, & Green, 2014; Menke et al., 2011; Vonshak & Gordon, 2015; Zerebecki & Sorte, 2011).

To test our hypotheses, we 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 microenvironments (surface temperatures chosen by ants during activity). Recent work comparing CT_{max} values in ants across urban and rural populations has improved our understanding of how urban environments influence the evolution of thermal tolerance traits (Angilletta et al., 2007; Diamond, Chick, Perez, et al., 2017; Diamond, Chick, Perez, Strickler, & Martin, 2018). However, our study used a multi-species approach to comprehensively examine the factors influencing an important metric of heat tolerance in urban 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). Thus, our study offers unique insight into the role of water availability in heat tolerance across a community, which is important in an increasingly warm, dry, and urbanized world (Angilletta, 2009; Grimm et al., 2008; Oke, 1973; Pincebourde et al., 2016; Sarhadi, Ausín, Wiper, Touma, & Diffenbaugh, 2018).

2 | MATERIALS AND METHODS

2.1 | Research system

Ants are abundant and important components of terrestrial ecosystems (Underwood & Fisher, 2006), including urban ecosystems (e.g., Menke et al., 2011; Penick, Savage, & Dunn, 2015; Stahlschmidt & Johnson, 2018). They are effective behavioral thermoregulators and, thus, are adapted and sensitive to a wide range of temperatures (Angilletta et al., 2007; Chick, Perez, & Diamond, 2017; Jumbam, Jackson, Terblanche, McGeoch, & Chown, 2008; Lighton & Turner, 2004; Underwood & Fisher, 2006). Also, shifts in microenvironments due to climate change are expected to be particularly important to small-bodied animals, such as ants (Hemmings & Andrew, 2017; Pincebourde et al., 2016; Pincebourde & Suppo, 2016; Scheffers, Edwards, Diesmos, Williams, & Evans, 2014).

Because populations near the edge of a species' range are expected to be at the extreme end of the environmental stress gradient (Gaston, 2009; Han et al., 2019; Magi, Semchenko, Kalamees, & Zobel, 2011; Sexton, McIntyre, Angert, & Rice, 2009), sampling such edge populations may misrepresent species-wide thermal physiology and thereby confound comparative analyses. Therefore, the populations of all species used in the experiments were well within species' geographical and/or latitudinal ranges (AntWeb). Ants used in the experiments (Figure S1; Table S1) were collected on sunny days in June–August in Stockton or Lodi, California, which are cities characterized by a hot-summer Mediterranean climate (Kottek, Grieser, Beck, Rudolf, & Rubel, 2006).

2.2 | Experiment 1

In 2017, an interspecific comparison was used to examine the effects of body size, microenvironmental 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 (Figure S1; Table S1). From 10:00 to 14:00, ants were collected via an aspirator along foraging trails on both impervious and nonimpervious surfaces (e.g., bare soil and concrete, respectively) in shaded and unshaded conditions as described previously (Stahlschmidt & Johnson, 2018). At each colony, six different temperature readings of ground surface were taken using an infrared thermometer (Fluke 62 MAX) at the time of sampling. To estimate the temperatures of microenvironments chosen by ants during activity (T_{active}), three temperature readings were taken on each ant trail approximately 1 m from one another. To estimate the range of ants' thermal options, three temperature readings were also taken near the ant trail ($T_{available}$) 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 (i.e., T_{active} and $T_{available}$) was used to estimate each ant's thermal safety margin (i.e., the difference between its CT_{max} [see below] and the maximal temperature of its environment).

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) in 470 ml round glass storage containers. Granulated table sugar was provided as a food source even though mild food limitation (e.g., 1 day of starvation) does not affect thermal tolerance in other insects, including ants (Bubliy, Kristensen, Kellermann, & Loeschcke, 2012; Nguyen et al., 2017; Overgaard, Kristensen, & Sørensen, 2012). Ants were kept in these open-top containers (i.e., unsealed containers with no lid; $n = 1$ –30 ants per group replicate depending on ant body size; $n = 2$ –28 group replicates per species; see Table S1 for details) 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: unlimited 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 below). Preliminary trials indicated that this duration of water deprivation did not influence mortality across our study species for Experiment 1. Live body mass, CT_{max} , dry body mass, and live water content were determined as described in CT_{max} Trials below.

2.3 | 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

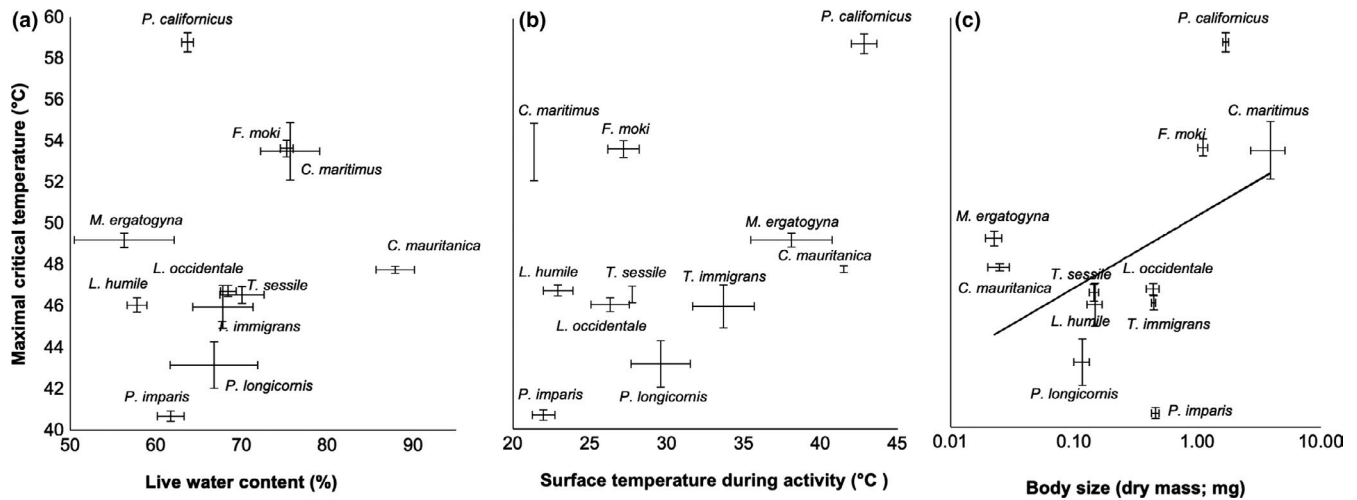


FIGURE 1 Relationships between maximal critical temperature (CT_{max}) and (a) live water content, (b) active temperature (temperatures of microenvironments chosen during activity), and (c) body size for a community of urban ants in California's Central Valley (11 species; $n = 683$) in Experiment 1. Values are displayed as mean \pm SEM across group replicates, and include CT_{max} values for data pooled across both water treatment groups (11 species; $n = 683$ ants; see text for details). As indicated by the regression line, only body size was significantly correlated with CT_{max} after accounting for phylogeny

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 pretrial live mass. Then, each group replicate ($n = 2$ –28 per species; see Table S1) was placed into an open-top 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; see below). An empty open-top 236 ml (Experiment 1) or 30 ml (Experiment 2; see below) 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 (estimated T_{body}) in real-time. After 30 min of acclimation, the water bath was heated and estimated T_{body} increased 0.5°C/min until all of the ants were knocked down. The CT_{max} for each ant was determined by its knock-down temperature, which was the estimated T_{body} at which an ant lost the ability to right itself (mean: <50 min). Before they could recover from knock-down, the group replicates of ants (Experiment 1) or individual ants (Experiment 2; see below) were all euthanized by placing them into a 50°C drying oven. After ≥ 24 hr, ants were reweighed to estimate ant body size (dry mass) and relative (%) live water content.

2.4 | 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* ($n = 118$ ants; $n = 5$ colonies) and field ant, *Formica moki* ($n = 114$ ants; $n = 5$ colonies). *Prenolepis imparis* is readily found throughout the contiguous United States whereas *F. moki* is found in the western United States (Sanders, Barton, & Gordon, 2001; AntWeb). The two species 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} , and their CT_{max} values responded differently to water limitation (i.e., 8 hr of water limitation reduced CT_{max} in *P. imparis*, but not in *F. moki*; see Section 3). Thus, examining both species allowed us to examine the roles of water limitation and intraspecific variation in body size in species with dissimilar thermal biology.

Although similar to Experiment 1, the methods of Experiment 2 were modified in three ways. First, the effect of intraspecific variation in body size on CT_{max} was determined because the mass of each ant could be determined (mean live mass: 2.6 mg), rather than relying on group replicate data for body mass as in Experiment 1. Second, multiple water limitation treatment levels were used (8 and 32 hr of water limitation, rather than only 8 hr in Experiment 1). Captive housing may influence CT_{max} independent of water availability (e.g., ants housed for 32 hr with unlimited water may exhibit different CT_{max} values than those housed for 8 hr with unlimited water). Therefore, water-limited and -unlimited ants were assessed for CT_{max} at each time point to control for captive housing effects. Third, a more comprehensive estimate of T_{active} was achieved in Experiment 2 by taking the six temperature measurements as in Experiment 1 three times during activity (across 2 hr intervals) each sampling day, rather than just once at the time of sampling as in Experiment 1. Dependent variables (e.g., CT_{max} and T_{active}) were similar across sampling years for *P. imparis* and *F. moki* (Figures 1a,b, 2, and 3).

2.5 | 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 our first set of three hypotheses), two analytical methods were used. First, a software for comparative analyses (COMPARE: ver. 4.6b, open-access) was used to perform linear regression analyses

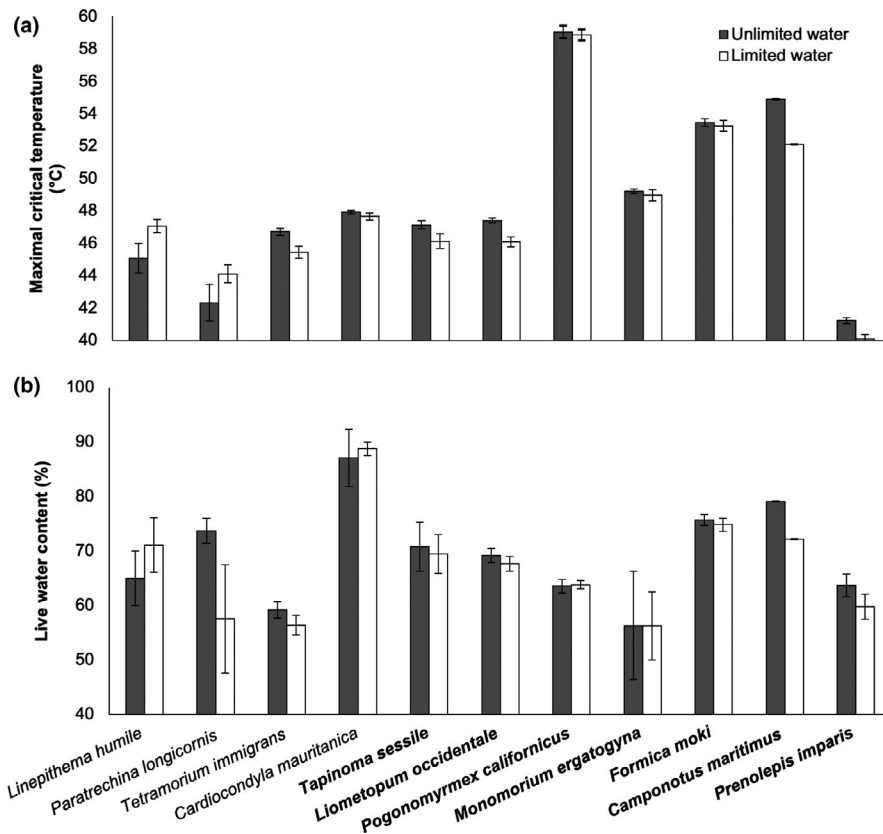


FIGURE 2 Effects of water treatment (white: 8 hr water limitation; gray: unlimited water) on (a) maximal critical temperature (CT_{max}) and (b) live water content in a community of urban ants in California's Central Valley ($n = 683$ individuals) in Experiment 1. Values are displayed as mean \pm SEM across individuals for CT_{max} and across group replicates for live water content (see text for details). Native species' names are bolded

that accounted for the effects of phylogeny. Previous work on heat tolerance indicates that using phylogenetically informed models results in consistently better fits of the data than noninformed models (Leiva, Calosi, & Verberk, 2019). Thus, CT_{max} was regressed on each dependent variable from Hypotheses 1–3 (dry body mass, T_{active} , and relative [%] body water content, respectively) using three linear regression analyses (i.e., one analysis for each independent variable) and phylogenetically generalized least squares methods (PGLS). The maximum likelihood estimate of alpha, the parameter of phylogenetic dependence based on the Ornstein-Uhlenbeck model for trait evolution, for each pair of variables was determined on a scale from 0 to 15.5 (Freckleton, Harvey, & Pagel, 2002; Hansen, Pinaar, & Orzack, 2008; Martins & Hansen, 1997). For PGLS, a low alpha (near 0) suggests data are highly dependent on phylogeny, whereas a high alpha suggests data are generally independent of phylogeny. The phylogenetic tree (Figure S1) for our study taxa included estimated minimum branch lengths and was constructed from established taxonomic sources (Janda, Folková, & Zrzavý, 2004; Moreau, Bell, Vila, Archibald, & Pierce, 2006; Ward, Brady, Fisher, & Schultz, 2015).

Second, several linear mixed models were run in SPSS (ver. 22, IBM Corp.) to test all four hypotheses in Experiment 1. In these models, data were log-transformed when necessary (e.g., to achieve normally distributed residuals), and two-tailed significance was determined at $\alpha = 0.05$ using Satterthwaite approximations of p -values. Species and nest identity were included as random effects in all linear mixed models on data from Experiment 1. For group replicates in Experiment 1, one model included mean CT_{max} of group replicates as the dependent variable to test Hypotheses 1–3. In this model, water

treatment (unlimited or limited) was included as a fixed effect to test Hypothesis 3, and body size (mean dry mass of group replicates) and mean T_{active} (i.e., mean of three temperature readings of group replicates; see above) were included as covariates to test Hypotheses 1 and 2, respectively. That is, Hypotheses 1–3 were tested by phylogenetically informed, single-factor models (see above), as well as by one multi-factor mixed model that accounted for phylogeny by including species as a random effect. To test Hypothesis 4, three additional models were fit where CT_{max} , estimated thermal safety margin, and relative (%) live water content were each included as a dependent variable. The model on relative live water content used data from mean values of group replicates while those on CT_{max} and estimated thermal safety margin used data from individual ants. In these models, water treatment, native status, and treatment \times native interactions were included as fixed effects.

For Experiment 2, a model for CT_{max} in each species included water treatment (unlimited or limited), time since water treatments were assigned (8 or 32 hr), and a treatment \times time interaction as fixed effects to test Hypothesis 3, and body size (dry mass) and mean T_{active} as covariates to test Hypotheses 1 and 2, respectively. To determine the effect of water treatments on live water content, another mixed model was run on relative (%) live water content for each species with water treatment, time, and a treatment \times time interaction as fixed effects. Water availability can facilitate food intake in insects (Raubenheimer & Gade, 1994, 1996; Padda & Stahlschmidt, in revision), meaning that effects of water limitation on CT_{max} may have been driven by the effects of food limitation. Therefore, to determine whether water-unlimited ants ate more

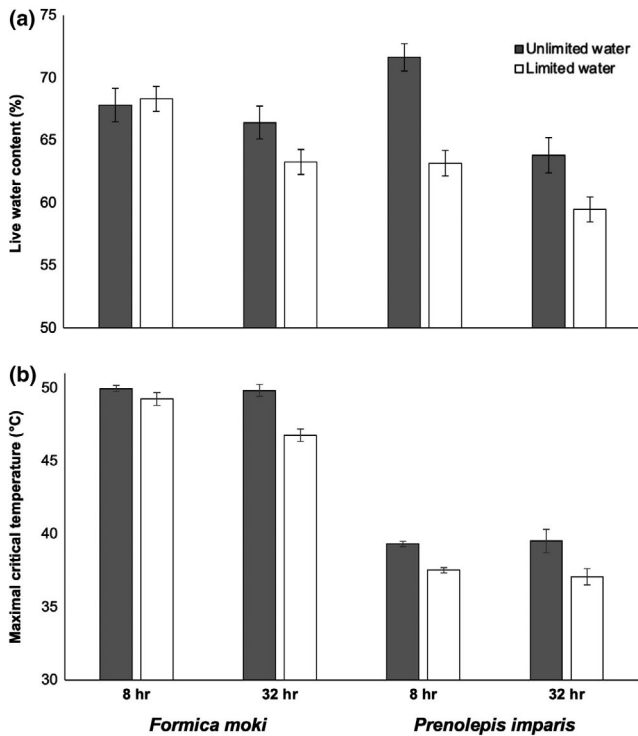


FIGURE 3 Effects of species, water treatment (white: water limitation; gray: unlimited water), and duration of water treatment on (a) live water content and (b) maximal critical temperature in two species of native urban ants (*Formica moki* and *Prenolepis imparis*) in California's Central Valley ($n = 232$ individuals) in Experiment 2. Values are displayed as estimated marginal mean \pm SEM because body size (dry mass) and mean T_{active} were each included as a covariate (see text for details)

food than water-limited ants during the water-manipulation period, a mixed model was also run on body size (dry mass, which would increase with food intake) for each species with water treatment, time, treatment \times time interaction as fixed effects, and nest identity as a random effect.

3 | RESULTS

3.1 | Experiment 1

Comparative regression analyses (phylogenetically generalized least squares methods [PGLS]) on data from Experiment 1 indicated moderate phylogenetic dependence (alpha values ranged from 2.2 to 3.3). 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 (Figure 1a; $F_{1,9} = 5.6$; $p = .042$; $R^2 = .34$). However, there was not a detected effect of T_{active} on CT_{max} (Figure 1b; $F_{1,9} = 1.6$; $p = .21$; $R^2 = .20$) or relative (%) live water content (Figure 1c; $F_{1,9} = 0.32$; $p = .59$; $R^2 = .028$). Mixed model analysis

on Experiment 1 data agreed with results from the PGLS analyses: CT_{max} was influenced by body size ($F_{1,100} = 4.7$, $p = .032$), but there was not a detected effect of T_{active} on CT_{max} ($F_{1,32} = 0.56$, $p = .46$). Mixed model analysis also indicated that CT_{max} was affected by water treatment where water limitation reduced heat tolerance ($F_{1,94} = 4.4$, $p = .038$; Figure 2a).

There was not a detected effect of native status on CT_{max} ($F_{1,9} = 1.9$, $p = .21$), but CT_{max} was influenced by the interaction between native status and water treatment ($F_{1,653} = 6.8$, $p = .010$) where CT_{max} in native ants was more sensitive to water availability (Figure 2a). There was not a detected effect of native status on estimated thermal safety margin ($F_{1,5} = 4.2$, $p = .10$), but estimated thermal safety margin was affected by water treatment where margins were greater for water-unlimited individuals ($F_{1,650} = 7.5$, $p = .0063$) and a native \times treatment interaction (safety margins in native ants were more sensitive to water availability; $F_{1,649} = 5.1$, $p = .025$). Relative (%) live water content was influenced by water treatment ($F_{1,92} = 4.1$, $p = .046$), but there was not a detected effect of native status ($F_{1,6} = 0.012$, $p = .92$) or a native \times treatment interaction on relative live water content ($F_{1,92} = 0.22$, $p = .64$; Figure 2b).

3.2 | Experiment 2

In *P. imparis*, CT_{max} was influenced by water treatment ($F_{1,108} = 32$, $p < .001$), but there was not a detected effect on CT_{max} due to the time since water treatments were assigned (i.e., 8 or 32 hr; $F_{1,109} = 0.32$, $p = .57$), a time \times water treatment interaction ($F_{1,108} = 0.72$, $p = .40$), T_{active} ($F_{1,104} = 1.9$, $p = .18$), or body size (dry mass; $F_{1,111} = 0.33$, $p = .57$; Figure 3a). The relative (%) live water content of *P. imparis* was influenced by water treatment ($F_{1,110} = 4.0$, $p = .047$) and time ($F_{1,110} = 4.1$, $p = .045$), but there was not a detected effect of a time \times water treatment interaction on relative live water content ($F_{1,110} = 0.094$, $p = .76$; Figure 3b). There was not a detected effect on dry mass due to water treatment ($F_{1,110} = 0.060$, $p = .81$), the time since water treatments were assigned ($F_{1,110} = 0.030$, $p = .86$), or a time \times water treatment interaction ($F_{1,110} = 0.026$, $p = .87$).

In *F. moki*, CT_{max} was influenced by water treatment ($F_{1,105} = 18$, $p < .001$), the time since water treatments were assigned ($F_{1,105} = 8.2$, $p = .005$), and a time \times water treatment interaction ($F_{1,105} = 6.8$, $p = .010$), but there was not a detected effect of T_{active} ($F_{1,103} = 0.31$, $p = .62$) or body size ($F_{1,108} = 0.65$, $p = .42$) on CT_{max} (Figure 3a). The relative live water content of *F. moki* was influenced by water treatment ($F_{1,106} = 13$, $p < .001$) and a time \times water treatment interaction ($F_{1,106} = 6.2$, $p = .015$), but there was not a detected effect of time alone on relative live water content ($F_{1,107} = 0.25$, $p = .62$; Figure 3b). Water availability did not appear to influence food intake because there was not a detected effect on dry mass due to water treatment ($F_{1,106} = 3.0$, $p = .086$), the time since water treatments were assigned ($F_{1,106} = 0.26$, $p = .61$), or a time \times water treatment interaction ($F_{1,106} = 1.3$, $p = .27$).

4 | DISCUSSION

Using a comparative and manipulative approach, we demonstrate complex dynamics of temperature sensitivity in a widespread animal taxon. Urban ant species varied in CT_{max} in a body size-dependent fashion (Figure 1c). Although water availability had overall positive effects on body water content and CT_{max} across the ant community, these effects also varied across species (Figures 2 and 3). For example, body water content and CT_{max} in *P. imparis* were strongly dependent on short-term water availability while these variables in *P. californicus* were unaffected by short-term water availability (Figure 2). However, results from Experiment 2 indicate that body water content and CT_{max} can be insensitive to water limitation in the shorter-term in some species, but not in the longer-term (e.g., *F. moki*; Figure 3). 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 (Khaliq et al., 2014; Leiva et al., 2019; Sunday et al., 2014). Last, our results indicate that native ants may be more physiologically vulnerable than non-native ants because the sensitivity of CT_{max} and thermal safety margins to water availability in native ants was greater than in non-native ants (Figure 2a).

An animal's body size influences many aspects of its physiology and ecology—from egg size to population size (Peters, 1986; Savage et al., 2004; Smith & Lyons, 2013). Likewise, body size influenced CT_{max} across species of urban ants in support of our first hypothesis (larger animals have greater heat tolerance: Figure 1c). Similar results have been demonstrated in other ants (Cerdeira & Retana, 2000; Clemencet et al., 2010; Ribeiro et al., 2012; Baudier et al., 2015; but see Hemmings & Andrew, 2017; Baudier et al., 2015) and other insects (Le Lagadec, Chown, & Scholtz, 1998). This may be due to larger animals having a greater thermal inertia (Le Lagadec et al., 1998), more water stores (increased evaporative cooling potential, but see below), or greater Hsp levels (but see Brown et al., 2007; Moreno, Merino, Martinez, Sanz, & Arriero, 2002). Although larger body size may be more beneficial for heat tolerance in terrestrial animals, experimental and biogeographical evidence indicates strong selection for smaller body size due to warming (e.g., temperature-size rule and Bergmann's rule: reviewed in Angilletta, 2009; Gardner et al., 2011; but see Horne et al., 2015). Clearly, future work is required to determine the relative magnitude of these competing selective pressures in terrestrial animals (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., oxygen levels in aquatic environments: Verberk, Leuven, Velde, & Gabel, 2018). There was not an effect of intraspecific variation in body size on CT_{max} , which agrees with other studies examining physiological variation within species (desiccation tolerance: Mogi, Miyagi, Abadi, & Syafruddin, 1996). This is likely due to greater genetic and phenotypic variation across species, rather than within species (Gearty, McClain, & Payne, 2018)—for example, we 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. Future work should examine the effect of body size on heat tolerance using a more accurate balance (e.g., ± 0.01 mg or ± 0.001 mg rather than ± 0.1 mg as in our study) and in additional species because our study only thoroughly investigated its effect in two species (i.e., *F. moki* and *P. imparis*).

For both experiments, our second hypothesis (animals active in warmer microenvironments 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 our study may be due to differences in the methodologies of temperature measurement. In our study, an infrared thermometer was used to collect temperature measurements of surfaces used by ants during activity. In the study by Baudier et al. (2015), miniature temperature data loggers were used to collect measurements, which allowed for continuous temperature data collection (i.e., many temperature measurements). However, we failed to detect an effect of T_{active} on CT_{max} within two focal species 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 our 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 suboptimal temperatures (i.e., nutritional benefits outweigh thermoregulatory costs: Andrew et al., 2013; Andrew & Terblanche, 2013).

As described above, our third hypothesis (hydration status influences CT_{max}) was supported by mixed model analyses in Experiment 1 and Experiment 2. Our results indicate that ants have a threshold at which water limitation affects their heat tolerance, and these thresholds vary across species (Figures 2a and 3b). 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 Martinez, Arenas, Trilla, Viejo, & Carreno, 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 significantly related to CT_{max} (Figure 1a), water limitation generally led to a decrease in body water content and in reduced heat tolerance (Figures 2 and 3). Therefore, water limitation in our 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, Woods, & Roberts, 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). Continued work is required to better understand factors influencing contradictory results, such as those due to variation in taxon and/or methodology (e.g., life stage of desiccation exposure, or the duration of desiccation or recovery from desiccation). Related, we detected effects of water limitation on body water content and effects of interspecific

variation in body size (dry mass) on heat tolerance—however, the relatively low sensitivity of the analytical balance used in our study (± 0.1 mg) likely introduced error into our data and may have constrained our ability to parse finer-scale effects on these gravimetric variables.

There are at least three general types of mechanisms that may underlie the costs of dehydration to heat tolerance. First, dehydration may confer reduced evaporative cooling potential because fewer water stores can be deployed (i.e., lost to release heat) during periods of heat stress. We indirectly assessed this mechanism in our study (see Appendix S1). Our calculations reveal that hydration likely did not confer an appreciable evaporative cooling advantage of animals in our study. Our experimental design for determining CT_{max} (i.e., using partially submerged glass containers in a water bath) likely reduced evaporative cooling by ants, and recent work similarly indicates that very little water is lost by other small insects during exposure to thermal ramping associated with determining CT_{max} (Manenti et al., 2018).

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, Markow, Kain, & Gibbs, 2003; reviewed in Chown, Sorensen, & Terblanche, 2011). Because metabolic rate and Hsp levels may be linked (Dahlhoff, Buckley, & Menge, 2001; Folguera et al., 2011; Sammut & Harrison, 2003), 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 insects (Bazinet, Marshall, MacMillan, Williams, & Sinclair, 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 & 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 (reviewed in Chown et al., 2011; Thorat & Nath, 2018). Dehydration may also lead to increased catabolism of nutrient reserves (Benoit et al., 2010), which may inhibit an animal's ability to mount a response to heating (Manenti et al., 2018).

Third, desiccation may negatively impact heat tolerance via damage to cellular membranes, inhibition molecular transport, and induction of oxidative stress (reviewed in Alpert, 2006; Minnich, 1982; Toxopeus & Sinclair, 2018). Desiccation-induced damage may reduce an animal's ability to synthesize or employ protein molecular chaperones (e.g., Hsps) during a heat shock because proteins are also sensitive to desiccation (reviewed in Toxopeus & Sinclair, 2018). In sum, we recommend future work to examine the mechanisms underlying the link between desiccation and reduced heat tolerance given the increasing occurrence 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 may outcompete native species at warmer temperatures (Lejeune et al., 2014; Rahel, Bierwagen, & Taniguchi, 2008; Zerebecki & Sorte, 2011; but see Verberk et al., 2018). In our study system, native status did not independently influence estimates of heat tolerance (Figure 2a). Estimates of thermal safety margins in our study were consistent with the findings of others across various study systems (e.g., Sunday et al., 2014; reviewed in Rohr et al., 2017) and sensitive to water availability, but our estimates were also not independently influenced by native status. However, our results indicate that native ants may be more reliant on water subsidization in urban environments because CT_{max} and estimated thermal safety margins were more sensitive to water availability in native ants relative to non-native ants. Thus, limited water availability and increasing temperatures may favor non-native (rather than native) species in some ecosystems, which is important given environments have become increasingly arid and warm (Sarhadi et al., 2018). Future work on additional taxa and more levels of water limitation 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 due to reduced thermal or hygric safety margins (Burdine & McCluney, 2019; Sarhadi et al., 2018; Sunday et al., 2014). 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 (Bujan, Yanoviak, & Kaspari, 2016; Burdine & McCluney, 2019; Matzkin, Watts, & Markow, 2007). Our 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; Diamond, Chick, Perez, et al., 2017; Pincebourde et al., 2016), similar experimental, comparative studies should be conducted in non-urban environments where water is not subsidized. We also advocate for examining the role of hydration state in other aspects of thermal sensitivity, such as its effects on thermal optimum or breadth of performance (Angilletta, 2009). In sum, understanding the links between heat tolerance and desiccation resistance will become critical in a world that is increasingly warm, dry, and urbanized.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

Dustin J. Johnson: Conceptualization (supporting); Data curation (lead); Investigation (lead); Methodology (lead); Project administration (equal); Writing-original draft (lead). **Zachary R. Stahlschmidt:** Conceptualization (lead); Data curation (supporting); Formal analysis (lead); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Resources (lead); Software (equal); Supervision (lead); Writing-original draft (supporting); Writing-review & editing (lead).

DATA AVAILABILITY STATEMENT

The datasets supporting this article can be accessed at: <https://doi.org/10.6084/m9.figshare.11988918>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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