



# Black widows on an urban heat island: extreme heat affects spider development and behaviour from egg to adulthood

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Urbanization rapidly alters the environment, often leading to decreased biodiversity. One abiotic selection pressure uniquely associated with urbanization is the urban heat island (UHI) effect—wherein built structures (e.g. paved surfaces) capture heat during the day, retain it through the dark cycle and result in significantly elevated night-time temperatures. Relatively few studies have asked what effects the UHI might have on the development and behaviour of urban animals, and even fewer have asked this question of urban arthropod pests who rely on external heat sources and can experience explosive urban population growth rates. In particular, behavioural plasticity is often cited as a critical phenotype for organisms to thrive after rapid environmental change such as urbanization. Here, the relationship between elevated urban temperatures and behaviour was examined in the western black widow spider, *Latrodectus hesperus*. With regard to the UHI, young spiderlings raised at urban, elevated temperatures showed significantly reduced life span, reduced body mass, elevated web building, heightened voracity towards heterospecific prey and sibling cannibalism. In contrast, males studied later in the final pre-reproductive moults and raised at urban, elevated temperatures showed reduced life span, decreased adult body condition and increased voracity towards prey. We found no effects of temperature on late-stage male web building or courtship behaviour. UHI temperatures simulated in the laboratory present black widow spiders with a myriad of developmental problems, but our data suggest these spiders use temperature-sensitive behavioural plasticity to attempt to accommodate this stressor.

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Humans dramatically alter ecosystems, from resource use to land conversion (Ojima, Galvin, & Turner, 1994). Urban areas represent the fastest growing habitat type worldwide (Collins et al., 2000). In particular, urbanization can drive local extinctions, ultimately reducing native biodiversity (Kowarik, 2001; Marzluff, 2001). However, a subset of taxa can tolerate urban environments, exploiting this growing niche (Blair, 1996). Adaptive behavioural plasticity is often cited as necessary to tolerate rapid human-induced stresses such as urbanization because it acts on a shorter timescale than evolutionary adaptations (Ditchkoff, Saalfeld, & Gibson, 2006). For example, urban coyotes, *Canis latrans*, in Chicago show plastic foraging behaviour by utilizing anthropogenic resources in highly disturbed habitats where natural prey have become less available (Newsome, Garbe, Wilson, & Gehrt, 2015). The extent to which the success of urban species is the product of plasticity, local adaptation to the new environment

or the evolution of plasticity continues to be debated (Diamond & Martin, 2016).

Another challenge that faces species residing in urbanized areas is the increased heat associated with urbanization. The urban heat island (UHI) effect refers to a phenomenon in which built structures (e.g. concrete paved surfaces) replace native soils and vegetation, subsequently trapping heat from the sun during the day, resulting in elevated night-time temperatures (Hawkins, Brazel, Stefanov, Bigler, & Saffell, 2004). Phoenix, Arizona, U.S.A., for example, has been found to have an 11.0 °C UHI effect when compared to the surrounding Sonoran Desert (Hawkins et al., 2004). Rising city temperatures are a novel challenge that organisms must cope with in order to continue living in urbanized areas (Heinl et al., 2015). Organismal response to the UHI is, perhaps, one of the many factors leading to decreased biodiversity in urban areas (Munzi et al., 2014).

The impact of urban heat on arthropods is of particular interest, as they play critical roles in ecosystems and sometimes experience explosive population growth leading to urban infestations. Elevated temperatures could benefit urban ectotherms and urban pest infestations (e.g. Meineke, Dunn, Sexton, & Frank, 2013), as

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ectotherms cannot internally regulate body temperature; thus, their bodies are subject to the temperature of the surrounding environment, which has been shown to exponentially elevate metabolism (Clarke & Johnston, 1999). For example, increased prey abundance and the extended breeding window associated with warmer urban temperatures increases survivorship in the orb-weaving spider, *Nephila plumipes*, when they are transplanted from rural sites to urban sites (Lowe, Wilder, & Hochuli, 2016).

The unique challenges associated with the UHI may often require animals to alter behavioural traits to survive and reproduce in cities. Indeed, behaviour is often considered to be a highly plastic trait and is often cited as an important determinant of success in rapidly changing environments (e.g. see coyote example above). Alternatively, urban success may require rapid evolution in the city. For example, the water flea, *Daphnia magna*, provides evidence of adaptive thermal evolution wherein urban *Daphnia* and individuals raised at higher temperatures have higher thermal tolerances, and population level genetic variation in thermal tolerance exists (Brans, Stoks, & De Meester, 2017). Similar results suggest that acorn ants (*Temnothorax curvispinosus*) have also evolved an adaptive increase in their thermal tolerance to the UHI (Diamond, Chick, Perez, Strickler, & Martin, 2017).

Considerable work has demonstrated effects of urban heat on development (e.g. spiders: Johnson, Urcuyo, Moen, & Stevens, 2019; insects: Kaiser, Merckx, & Van Dyck, 2016; lizards: Hall & Warner, 2018), physiology (spiders: Moen, Johnson, & Hackney-Price, 2019; lizards: Hall & Warner, 2018; Campbell-Staton et al., 2020), life history (insects: Chick, Strickler, Perez, Martin, & Diamond, 2019; lizards: Hall & Warner, 2018) and mortality (spiders: Johnson et al., 2019; Lowe et al., 2016; insects: Kaiser et al., 2016; lizards: Hall & Warner, 2018). Surprisingly few studies have isolated the behavioural mechanisms that may be responsible for allowing organisms to tolerate elevated temperatures such as the UHI and potentially global climate change. Nevertheless, as suggested above, the biology of ectotherms intuitively suggests the UHI should speed development, unless temperatures exceed a threshold. Faster development should be accompanied by the exhaustion of energy reserves and perhaps behavioural accommodations. For example, Zhao and Feng (2015) showed that, as temperature increases, activity, aggressiveness and boldness are all increased in crayfish. Presumably these behavioural accommodations result in increased foraging success at elevated temperatures, but they may also be costly in terms of exposure to predation risk. Furthermore, we know of no empirical demonstration of the costs and benefits of such behavioural accommodations to spiders. Lubin and Henschel (1990) documented the ability of desert ground-dwelling spiders to hunt at extreme temperatures by behaviourally moving to and from a cooler retreat. Additionally, these authors found that aggression towards prey increased at higher temperatures. An understanding of the behavioural phenotypes of urban organisms, and how they respond to novel urban selection pressures such as the UHI, will better allow us to conserve biodiversity as urban ecosystems grow worldwide.

Foraging behaviour is not the only trait that may be affected by the UHI, ultimately affecting an organism's ability to thrive in urban habitats. Sexually selected traits (e.g. male courtship/competition intensity) may be similarly shaped by temperature (Nedim, Lin, & Stoks, 2017). While urban noise has recently been shown to affect sexual selection (Phillips & Derryberry, 2018), Diamond and Martin's (2020) recent review of urban evolution notes the lack of work on urban heat and sexual selection. Intuitively, the assertions above that activity increases with temperature can be applied to sexual activity leading to the prediction that urban male spiders might build webs, attack prey and court more intensely due to UHI conditions. However, it remains unclear how urban heat will

interact with other salient urban selection pressures. For example, urban deserts are characterized by both UHI conditions (Hawkins et al., 2004) and heightened productivity (e.g. prey abundance; Shochat, Stefanov, Whitehouse, & Faeth, 2004) via supplemental water use. As spider predators get a large percentage of their water from their prey (Foelix, 2010), urban prey abundance may interact with heat to minimize the exhaustion of energy and water reserves predicted to result from elevated temperatures. This hypothesized buffering effect of urban prey abundance may also have the effect of tempering any behavioural shifts resulting from urban heat.

Here we study the western black widow spider, *Latrodectus hesperus*, a superabundant arthropod pest species found throughout urban habitats of Phoenix, Arizona, U.S.A. This species is native to the surrounding Sonoran Desert where it is found at low population density (Johnson, Trubl, & Miles, 2012). Widow spiders are troublesome urban pests both because their venom is toxic to humans (Vetter & Isbister, 2008) and because they appear to thrive in urban ecosystems, forming dense urban infestations (Johnson et al., 2012), often leading to widespread (largely ineffectual) pesticide applications (J. C. Johnson, personal observation). Recent work has shown that *L. hesperus* is affected by a dramatic UHI effect in urban Phoenix habitats. Ibuttons placed in the refuges of black widows from replicate urban and desert sites demonstrate a 6 °C difference averaged across the month of July (urban = 33 °C versus desert = 27 °C; Johnson et al., 2019). At least under laboratory conditions, this six-degree difference has a dramatic influence on the development of black widow spiderlings. Indeed, following 34 urban lineages across early development, these UHI conditions slow development to the third moult by almost 5 full days and reduce body mass at day 105 by just under 50% (Johnson et al., 2019). Additionally, the limited data collected so far suggest that UHI temperatures can increase spiderling voracity but decrease late-stage female web building (Johnson et al., 2019). Thus, developmentally, UHI appears to have negative rather than positive consequences for urban black widows, but it remains to be established whether black widows compensate for these conditions with behavioural shifts.

We tested the hypothesis that the success of urban black widows, and their ability to overcome the developmental delays resulting from UHI conditions, may be explained, in part, by the behavioural shifts they mount in response to extreme heat. Specifically, in experiment 1, we tested newly hatched full sibling spiderlings from nine different urban families across a 6-week early developmental period to look for behavioural repeatability (intra-individual consistency) and behavioural differences between full siblings raised at UHI and desert temperatures. Based on our pilot data and the limited literature available (see above), we predicted that spiders raised at the UHI temperature of 33 °C would (1) develop slower, (2) be, on average, more active in web building, voracity and cannibalism than their sibling counterparts raised at the lower (desert-like) temperatures of 27 °C and 30 °C. In experiment 2, we followed some of these same individuals and focused on males across their final two juvenile moults and the adult phase. We predicted that male spiders raised at the UHI temperature of 33 °C would (1) also develop slower, and (2) also be, on average, more active in web building, more aggressive towards prey and more eager to court females than their sibling counterparts raised at the lower (desert-like) temperatures of 27 °C and 30 °C. Lastly, we tested the idea that prey abundance slows the exhaustion of energy reserves, thus dampening the effects of UHI temperatures, making well-fed males (1) grow faster, and (2) be, on average, less active in web building, less aggressive towards prey and less eager to court females than their sibling counterparts raised with abundant prey. Conversely, we predicted that low prey levels compounded by UHI conditions may force males to develop

dangerously slowly, perhaps resulting in extreme behavioural compensations like increased web building and aggression in order to survive.

## METHODS

### *Experiment 1: Spiderling Development and Behaviour at UHI Temperatures*

Between March and June of 2017, we collected adult female black widows from eight different urban sites around Phoenix, Arizona (33°26'54"N, 112°4'26"W). Each spider was kept in the laboratory in individual clear plastic boxes (10 × 10 × 12 cm) with an X-shaped structure built with wooden sticks (17 cm) upon which they could build web. Boxes were checked daily for eggsacs, which were collected the day they were laid (day 0) and processed by day 3. Processing consisted of weighing the eggsac (mg) and opening it to count the number of eggs. We weighed (μg) 75 eggs from the first eggsac laid by each of nine black widows from five different urban sites on a Cahn C-30 microbalance, then digitally imaged the eggs and separated them into individual boxes (4 × 4 × 4 cm) each containing an X-shaped toothpick structure (6.5 cm) on which to build a web after hatching. Eggs and spiderlings were housed in a closet on a reverse 12:12 h photoperiod. After 30 days (when larvae first begin to chew their way out of the eggsac; J. C. Johnson, personal observation), spiderlings were fed two *Drosophila melanogaster* each twice a week. At day 44, for each family, we placed 25 spiderlings into each of three different incubators/temperature treatments. We used our field data (Johnson et al., 2019) to construct the three temperature treatments of 27 °C, 30 °C and 33 °C, simulating desert, intermediate and urban temperatures, respectively. Because we were limited to the use of incubators that could not fluctuate temperature across the day, each treatment experienced their temperature across the entire day. Throughout development, spiders were checked daily for moults and deaths. Spiderling mass (mg) was recorded on day 105.

After processing the initial 75 eggs, we also stored another 110 nonexperimental eggs from each eggsac individually on a reverse 12:12 h photoperiod to be used later in spiderling cannibalism trials (see below). Throughout development these 'nonexperimental' spiderlings were fed only two flies once per week in order to maintain a smaller body size than experimental spiders to ensure they would become prey in cannibalism trials.

#### *Behavioural assays*

Beginning on day 55 we assayed three different behavioural contexts (foraging, web building and cannibalism) across three repeated measures, which were separated by an interval of 14 days. During trials measuring these three behavioural contexts, spiders were left at their treatment temperature to best determine the influence of temperature on behaviour. Foraging trials were always conducted first, followed by web building the subsequent day, and cannibalism on the third day. Foraging trials consisted of placing two flies in each spiderling's box and recording how many flies had been preyed upon every 5 min for the first 30 min, then every 10 min until 1 h. In the second hour of foraging trials, spiders were checked every 15 min. Thus, voracity was scored as the latency to take prey across a 2 h trial. All prey were taken within the 2 h period. Latency to take the second prey item began at the point the first prey was taken. Thus, these two measures are not necessarily correlated. We opted for the occasional checks described above because spiders needed to be held in treatment incubators, and we wanted to ensure that spiders were experiencing the designated heat treatment.

For the web-building trials, we placed spiderlings in a new clean box (4 × 4 × 4 cm) with a fresh toothpick structure, and we recorded whether they were moving (i.e. web building), as well as their location within the box, every 5 min for a total of 2 h. In addition to actually seeing the spider moving, we scored a spider as web building if it was inactive at the scan but was found in a quadrant of the box that it was not in during the previous scan. To do this, spider location was determined by visually separating the box into four equal quadrants and recording whether the spiderling was in the upper right, upper left, lower right or lower left quadrant at the time of each check. If a spiderling was in a different quadrant but not currently moving during a check, it was considered to have moved for that interval. Thus, web-building behaviour was scored as the proportion of scans scored as active (either moving at the scan sample or assumed to have built a web in the interval because it had changed locations in between scan samples) across the 2 h trial.

Finally, we staged cannibalism trials within 1 week after day 105 when spiderling mass was first measured. In cannibalism trials, we introduced a nonexperimental sibling to the focal spiderling's existing box and recorded the latency to cannibalism. Spiderlings were checked every 10 min for 1 h, and then every hour for another 2 h. After the first 3 h of day 1, we checked spiders every 24 h for 5 more days. In our experience, cannibalism can take anywhere from seconds to weeks to occur (Johnson, Kitchen, & Andrade, 2010). Due to heightened mortality among the food-restricted nonexperimental spiders, cannibalism trials were only conducted on a subset of the experimental spiderlings. When necessary, we used spiderlings from the second eggsac laid by the same female as non-experimentals to boost the sample size of cannibalism trials for a family. Regardless, for a nonexperimental spider to be used in a trial, it had to appear to be 50–75% of the focal spider's body mass to ensure the identity of the spiderlings. We never found cannibalism to be committed by the smaller spiderling. Thus, we scored cannibalism as the latency to prey upon the smaller conspecific across a 5-day period.

### *Experiment 2: Male Development and Behaviour at UHI Temperatures*

Males in experiment 2 experienced the rearing regime described above. To examine male behaviour, we used 143 males from experiment 1 and another 141 male spiders reared subsequently under the same conditions. In total, males were derived from 16 different females collected from six urban locations. On day 89, we increased food to all spiders from two flies twice per week to three flies twice per week. When a male spider experienced his penultimate moult, the moult leading to the final interval before maturity, he was placed into one of two food treatments: three flies twice per week (low food = 6 flies per week) or four flies three times per week (high food = 12 flies per week). This created six treatment groups for study: low temperature and low food, low temperature and high food, intermediate temperature and low food, intermediate temperature and high food, high temperature and low food, and high temperature and high food. To track development, each male was weighed (mg) after every moult starting from the penultimate moult; additionally, spiders were weighed (mg) before every trial (see below).

#### *Behavioural assays*

Foraging trials were conducted on males three times during their penultimate moult, with three days in between each trial. The protocol for foraging trials was identical to that described in experiment 1, except for the increase in prey availability described above. Web-building trials took place the day after foraging trials.



Web-building trials were conducted with males three times during their penultimate moult, with 3 days in between each trial. Web-building assays were identical to that described above for spiderlings, except that they lasted only 1 h.

Nonvirgin females were exclusively used in courtship trials because virgin and nonvirgin *Latrodectus* females are courted at different intensities (Stoltz, McNeil, & Andrade, 2007). However, we made sure that females had not been mated within 2 months, to ensure that mated females did not retain active pheromones from their last partnering (Stoltz et al., 2007). As males will court readily on an active female web where the female has been recently removed (Ross & Smith, 1979; J. C. Johnson, personal observation), we opted to quantify courtship in the absence of the female to remove confounding variation stemming from female receptivity or signals (e.g. sexual cannibalism attempts). Males were not paired with their mother's web. Courtship trials began 7 days following the male's adult moult. These trials were repeated three times for each male, with 3 days in between each trial. We drew females from a population of 65 spiders. We ensured that males never saw the same female twice and, after a female was used, we moved to the next spider, thus minimizing the number of times any one female was used. Given the healthy number of females used, and the three replicates for each male with a different female, we feel confident that our data are not the result of a bias introduced by any certain females.

On the day that a male black widow moulted into his adult form, a field-caught nonvirgin female was placed into a clean plastic box and given 1 week to build a web. Boxes (10 × 10 × 12 cm) were equipped with two crisscrossed wooden dowels (17 cm), which were attached to the side of the box with putty. After females built web for 1 week, we removed the females from the boxes. Then, we weighed (mg) the males and placed one male into each box containing female web and recorded courtship activity ('slow walking' and 'leg waving'; Ross & Smith, 1979) continuously for the first 5 min. We then returned each male to his designated incubator and scored courtship activity every 5 min for 1.5 h, at which point trials ended. Thus, we scored male courtship intensity as the proportion of intervals during which a male was active and leg waving across a 90 min period. Each male used in the experiment courted for at least one interval.

Adult male size was determined using the third leg of the male. Legs are easily preserved, digitized and measured after death, allowing us to not harass spiders during trials. Also, we chose leg III as front legs are often suggested to be under sexual selection (Huber, 2005) and therefore are poorer indicators of body size. Once a male died we removed the third leg, digitally photographed the leg and measured the leg using ImageJ v1.34 (National Institutes of Health, Bethesda, MD, U.S.A., <http://rsbweb.nih.gov/ij/>).

#### Statistical Analysis

Linear mixed models were performed in R (R Development Core Team, 2008). For all dependent variables, a rank-based inverse normal transformation was applied to approximate a Gaussian distribution. To account for repeated measures, each model included temperature as a fixed factor and individual identity (i.e. spider ID) nested within family ( $N = 9$  for experiment 1,  $N = 16$  for experiment 2) as random factors. Dependent measurements included latency to attack fly 1 and fly 2 ( $N = 485$ ), web-building activity ( $N = 483$ ), latency to cannibalize siblings for spiderlings in experiment 1 ( $N = 168$ ) and male latency to court in experiment 2 ( $N = 279$ ). Where a significant main effect of temperature was detected, a We used Tukey post hoc test to investigate pairwise comparisons. To determine differences in body condition of spiders in the three temperature treatments, we calculated body condition

using the residuals produced from a linear regression of body mass and leg length, which is the preferred method when looking for variation within groups from the same population (Jakob, Marshall, & Uetz, 1996).

## RESULTS

### Experiment 1: Spiderling Development and Behaviour at UHI Temperatures

#### Development

Mixed model ANOVA showed that family of origin had a strong effect on spiderling mass at day 105 (ANOVA:  $F_{8,483} = 5.06$ ,  $P = 0.002$ ). In addition, temperature treatment significantly influenced mass at day 105 ( $F_{2,483} = 16.65$ ,  $P < 0.001$ ). Paired contrasts among temperature treatments were all significantly different from each other, showing that mass significantly decreased as temperature increased ( $z$  test: all  $z > 16.00$ , all  $P < 0.0001$ ; Fig. 1a). Spiderling survivorship was marginally affected by family (ANOVA:  $F_{8,483} = 2.450$ ,  $P = 0.06$ ), but was nevertheless strongly reduced by increasing temperature ( $F_{2,483} = 39.43$ ,  $P < 0.0001$ ; Fig. 1b). Paired contrasts again showed significant differences between all treatments ( $z$  test: 27 °C and 30 °C:  $z = 0.05$ ,  $P = 0.015$ ; 27 °C and 33 °C:  $z = 0.11$ ,  $P < 0.0001$ ; 30 °C and 33 °C:  $z = 0.09$ ,  $P < 0.0001$ ). Lastly, family affected development speed to the second moult (ANOVA:  $F_{8,483} = 14.47$ ,  $P < 0.0001$ ), but temperature treatments did not significantly affect this measure ( $F_{2,483} = 0.645$ ,  $P = 0.537$ ).

#### The effect of temperature on spiderling behaviour

Mixed models showed statistically significant effects of individual spiders on web building (ANOVA:  $r = 0.08$ ,  $F_{8,483} = 5.75$ ,  $P = 0.02$ ), but not voracity ( $r = 0.05$ ,  $F_{8,483} = 2.86$ ,  $P = 0.09$ ) or cannibalism ( $r = 0.001$ ,  $F_{8,483} = 0$ ,  $P = 1$ ). Statistically significant family effects were observed for voracity ( $r = 0.03$ ,  $F_{8,483} = 22.3$ ,  $P < 0.001$ ), web building ( $r = 0.06$ ,  $F_{8,483} = 30.3$ ,  $P < 0.001$ ) and cannibalism ( $r = 0.05$ ,  $F_{8,483} = 11.71$ ,  $P < 0.001$ ).

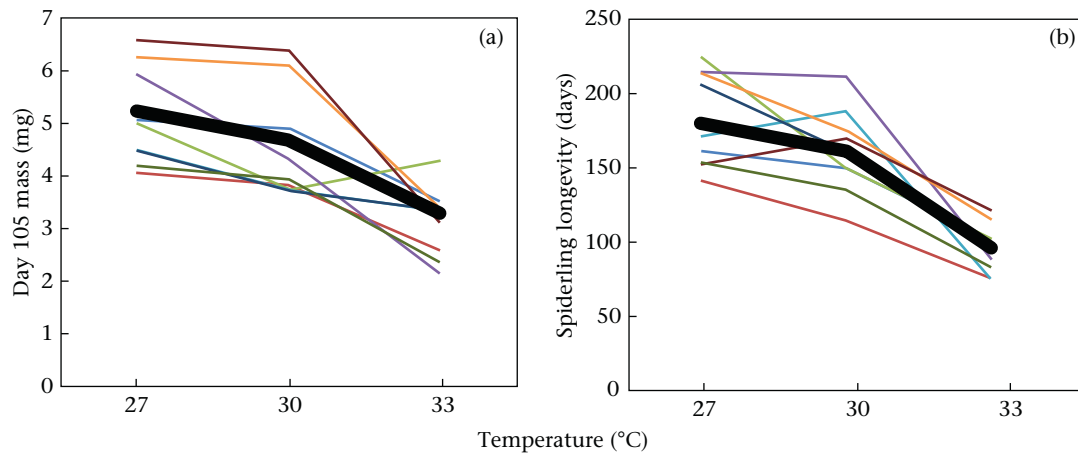
Temperature had a significant influence on voracity (ANOVA: latency to kill fly 1:  $F_{2,483} = 17.8$ ,  $P < 0.001$ ; latency to kill fly 2:  $F_{2,483} = 19.9$ ,  $P < 0.001$ ; Fig. 2a, b). Specifically, the UHI treatment (33 °C) heightened voracity towards fly 1 relative to the 27 °C treatment ( $z$  test:  $z = 4.13$ ,  $P < 0.001$ ) and the 30 °C treatment ( $z = 3.02$ ,  $P = 0.007$ ). However, no significant difference was found between the 27 °C and 30 °C treatments ( $z = 1.12$ ,  $P = 0.55$ ). Similarly, the latency to attack the second fly was shorter for the 33 °C treatment compared to the 27 °C treatment ( $z = 3.90$ ,  $P < 0.001$ ) and the 30 °C treatment ( $z = 4.93$ ,  $P < 0.001$ ). No significant differences were found between 27 °C and 30 °C ( $z = 1.01$ ,  $P = 0.618$ ).

Web-building activity was significantly affected by temperature (ANOVA:  $F_{2,481} = 176.82$ ,  $P < 0.001$ ). A significant difference was observed between each of the temperature treatments ( $z$  test: all  $z > 6.42$ , all  $P < 0.001$ ). Notably, web building was lowest in the 30 °C treatment and highest in the 33 °C treatment (Fig. 2c). Sibling cannibalism was significantly influenced by temperature treatment (ANOVA:  $F_{2,166} = 6.28$ ,  $P = 0.04$ ; Fig. 2d). Sibling cannibalism occurred significantly sooner at 33 °C than at 27 °C ( $z$  test:  $z = 2.47$ ,  $P = 0.04$ ). However, no significant difference in cannibalism behaviour was detected between 33 °C and 30 °C ( $z = 1.67$ ,  $P = 0.22$ ), or between 27 °C and 30 °C ( $z = 1.12$ ,  $P = 0.55$ ).

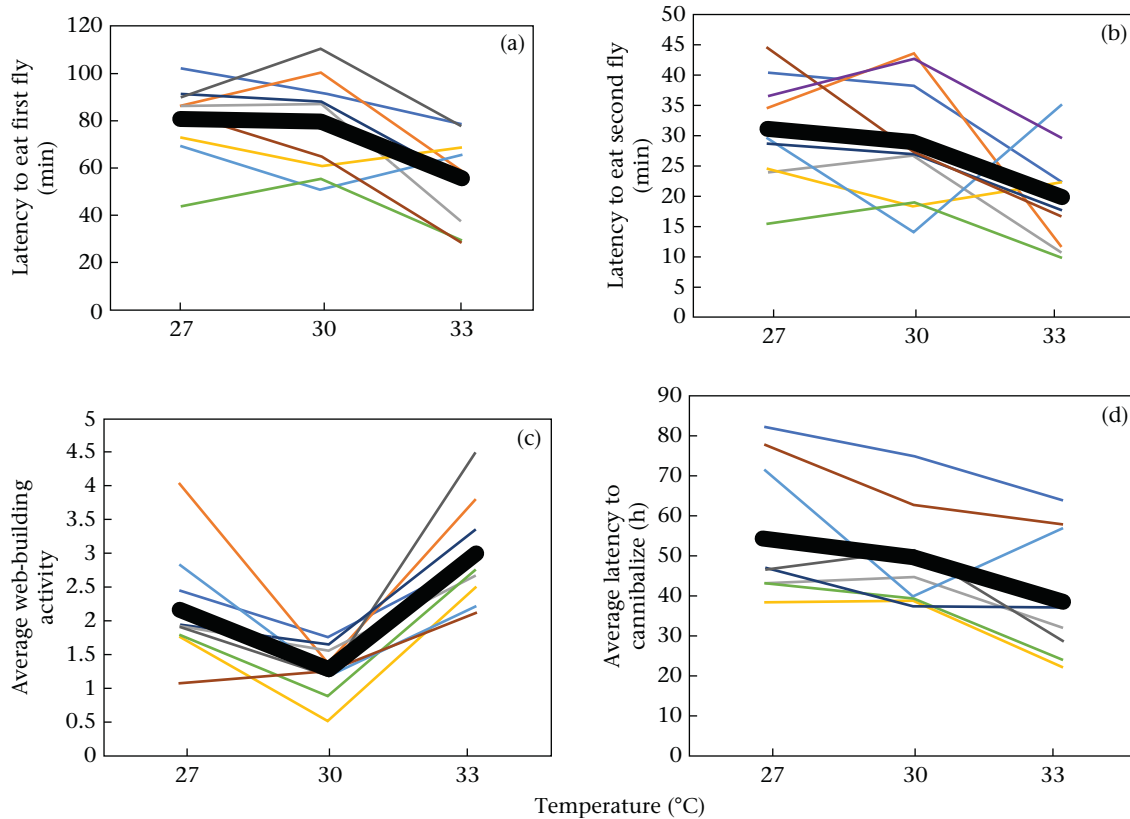
### Experiment 2: Male Behaviour

#### Body condition

Statistically significant family effects were observed for male body condition (ANOVA:  $r = 0.09$ ,  $F_{8,117} = 22.1$ ,  $P < 0.001$ ). The body condition of males was significantly affected by temperature



**Figure 1.** Effect of family (separate lines) and temperature on (a) body mass and (b) longevity of western black widow spider, *Latrodectus hesperus*, spiderlings. Bold line represents treatment average among replicate families.



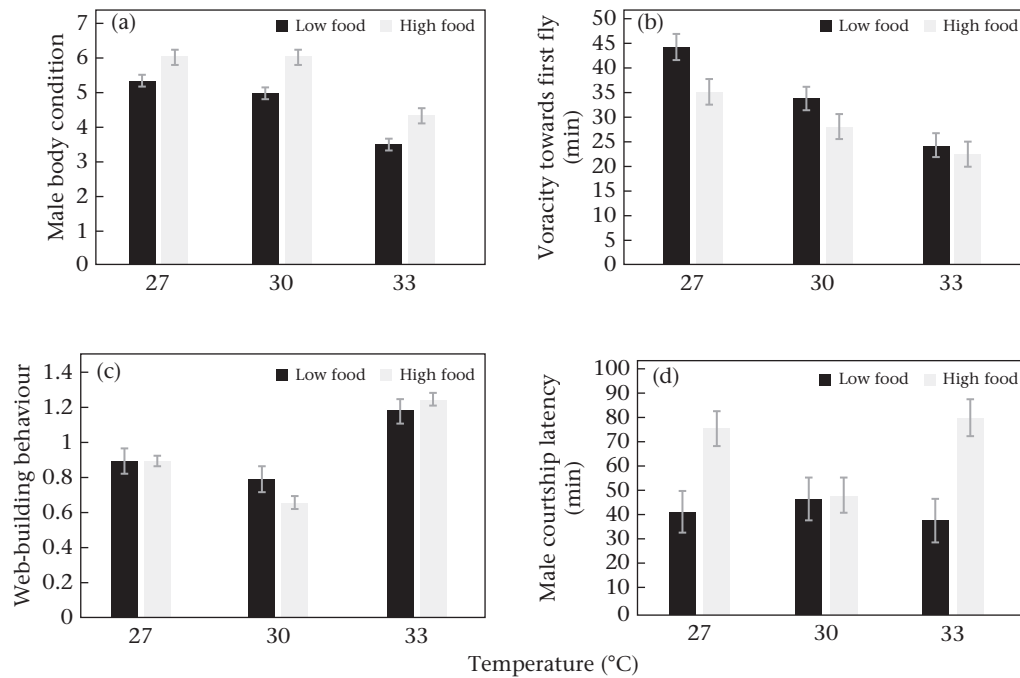
**Figure 2.** Effect of family (separate lines) and temperature on (a, b) voracity, (c) web building and (d) sibling cannibalism of western black widow spider, *Latrodectus hesperus*, spiderlings. Bold line represents treatment average among replicate families.

( $F_{2,117} = 10.7$ ,  $P < 0.001$ ) as well as by food treatment ( $F_{1,117} = 8.27$ ,  $P < 0.001$ ; Fig. 3a). Males raised at 27 °C were in significantly better condition compared to males raised at 33 °C ( $z$  test:  $z = 0.13$ ,  $P < 0.0001$ ). Similarly, males raised at 30 °C were in significantly better condition compared to males raised at 33 °C ( $z = 0.1$ ,  $P < 0.0001$ ). There was no significant difference in body condition of males raised at 27 °C and 30 °C ( $z = 0.03$ ,  $P = 0.105$ ). Not surprisingly, males in the higher food treatment had a significantly higher body condition compared to males in the lower food treatment ( $z = 0.09$ ,  $P = 0.004$ ). There was no interaction between food treatment and temperature on male body condition.

#### Temperature effects on male behaviour

Mixed models showed statistically significant effects of individual spiders on web building (ANOVA:  $r = 0.09$ ,  $F_{8,273} = 6.15$ ,  $P = 0.01$ ) and voracity ( $r = 0.07$ ,  $F_{8,277} = 4.16$ ,  $P = 0.04$ ). Statistically significant family effects were observed for voracity ( $r = 0.11$ ,  $F_{8,277} = 32.3$ ,  $P < 0.001$ ) and web building ( $r = 0.07$ ,  $F_{8,273} = 31.3$ ,  $P < 0.001$ ).

Higher temperatures heightened male voracity towards the first fly (ANOVA:  $F_{2,277} = 4.66$ ,  $P = 0.01$ ; Fig. 3b), with a more marginal effect on voracity towards fly 2 ( $F_{2,277} = 2.81$ ,  $P = 0.06$ ). Voracity towards fly 1 was significantly heightened in the 33 °C treatment



**Figure 3.** Effect of temperature and food treatment on (a) body condition, (b) voracity, (c) web building and (d) courtship of male western black widow spiders, *Latrodectus hesperus*. Error bars indicate standard error around the mean.

relative to both 27 °C ( $z$  test:  $z = 0.08$ ,  $P = 0.009$ ) and 30 °C ( $z = 0.06$ ,  $P = 0.02$ ), whereas voracity towards fly 2 was only significantly heightened for 30 °C relative to 27 °C ( $z = 0.06$ ,  $P = 0.03$ ). We found no significant effects of food treatment and no interaction between food and temperature on voracity measures.

Web-building behaviour in males was affected by temperature treatment (ANOVA:  $F_{2,273} = 4.64$ ,  $P = 0.01$ ) but not by food level ( $F_{1,273} = 0.04$ ,  $P = 0.85$ ; Fig. 3c). Specifically, spiderlings held at 33 °C were significantly more active web builders than their counterparts raised and tested at 27 °C ( $z$  test:  $z = 0.05$ ,  $P = 0.05$ ) and 30 °C ( $z = 0.09$ ,  $P = 0.003$ ). Spiderlings kept at the intermediate temperature of 30 °C were marginally less active web builders than their 27 °C counterparts ( $z = 0.09$ ,  $P = 0.07$ ). Although not statistically significant, low-food males raised at 27 °C and 33 °C courted almost two times more quickly than high-food males (Fig. 3d).

## DISCUSSION

We found that UHI temperatures reduced the body mass and survivorship of spiderlings early in development and compromised later adult male body condition. Most of our measures of spiderling and male behaviour were repeatable across repeated measures and many were typified by strong family effects. Young spiderlings responded to UHI temperatures by increasing voracity, web building and sibling cannibalism. Similarly, males reared at UHI temperatures showed heightened voracity and web building, but not courtship. We found very little effect of our male food level treatment, and no interactions between temperature and food.

### Development

The population success of black widows in urban habitats (Johnson et al., 2012), is not consistent with the high levels of

mortality that our UHI treatment imposed in the laboratory. Indeed, despite our best efforts to measure the microclimate of urban and desert black widows in the field, we have noted elsewhere (Johnson et al., 2019) that it is likely that field conditions allow spiders to tolerate such extremes (e.g. retreating further into a complex refuge during periods of particular heat stress) in ways that laboratory protocols cannot fully mimic. Similarly, our crude laboratory simulation lacked the fluctuations in temperature that are critical to understand in nature. Indeed, Bozinovic et al. (2011) suggested that the interaction between mean temperature and variance in temperature best predicts fitness. Future studies should address the complexity of field refuges from heat, as well as the impact of circadian temperature variation, on the development and behaviour of urban organisms, including arthropod pests.

Noting that our results are based on imperfect laboratory simulations, it is still noteworthy that 33 °C appears to have surpassed a critical threshold that limits the successful development of black widows and triggers a number of behavioural shifts (see below), which may accommodate this abiotic stressor under some circumstances. In particular, our extreme temperature of 33 °C reduced survivorship for spiderlings and resulted in significantly reduced mass and body condition for spiderlings and late-stage males. Note that while this extreme UHI temperature did not slow development to the second moult in the present study, it did significantly slow development to the third moult in a previous study (Johnson et al., 2019), and appears to disrupt the optimal timing of ecdysone peaks leading to moulting (Moen et al., 2019).

So why is urban black widow development delayed under UHI conditions? While urban taxa often emerge and show peak abundance earlier in the season than their rural counterparts (reviewed in Chick et al., 2019), the effect of UHI conditions on phenology appears to be highly variable across species. Diamond et al. (2014) studied urban and rural populations of 20 butterfly species across a

latitudinal gradient that reflected a mean temperature (°C) difference of several degrees. One important insight from this work was that while phenology was delayed for geographies experiencing cooler extremes, phenology was also delayed when warm geography interacted with urbanization (Diamond et al., 2014). As such, it may be naïve to expect the UHI to affect all ectotherms equally. For example, arthropods already experiencing warmer conditions because of geography (e.g. lower latitudes such as tropical and desert habitat) may often have their development delayed by UHI conditions. This insight may help explain why we find that black widows from the urban desert of Phoenix, Arizona experience developmental delays (Johnson et al., 2019) and suffer high metabolic costs when reared at UHI temperatures. Future work should take a replicate city approach and test the prediction that urban black widow populations from more moderate climates experience fewer costs (and may benefit) from living at UHI temperatures.

Regarding male development, we were surprised to find no evidence of an interaction between temperature and food availability. We expected high food levels (typical of urban environments) to ease the impact of UHI temperatures, perhaps even allowing spiders in the 33 °C treatment to develop faster while still maintaining a healthy body condition. Instead, elevated food improved body condition similarly across our temperature treatments. One explanation for the lack of interaction is that we started this food manipulation relatively late, such that high-food males only had their final two juvenile moults to stabilize their body condition to a place where elevated temperatures could speed development without exhausting energy reserves. Future work should examine the interaction between UHI temperatures and variation in food availability that spans the entirety of juvenile development.

#### Temperature-dependent Behavioural Differences

Black widow spider behaviour is heavily influenced by temperature and this general flexibility in phenotype may help explain why they succeed so well in the face of rapid disturbances such as urbanization. For example, previous work on *L. hesperus* has shown that female body condition shapes both the female's propensity for sexual cannibalism as well as the male's willingness to court a female (Johnson, Trubl, Blackmore, & Miles, 2011). Even the infamous red hourglass displayed by the black widow is highly plastic, swelling to greater sizes at high body condition (Johnson, Highfill, & Stevens, 2017). Here we show that despite high levels of variation among families, mean behaviour levels were strongly shaped by a spiderling's rearing/testing temperature. Specifically, the UHI treatment of 33 °C significantly heightened web building, voracity towards flies and sibling cannibalism. These results mirror the behavioural responses to elevated temperatures for other ectotherms, such as crayfish (Zhao & Feng, 2015). While we have elsewhere demonstrated that UHI dramatically slows web building in late-stage black widow females (Johnson et al., 2019), it is perhaps a generalizable result that successful urban ectotherms respond to increased heat with behaviours that have the potential to compensate for a heightened metabolism. Current work is looking for correlations among these behaviours and basal metabolic rates for spiders collected from urban and desert habitats and tested at 27 °C and 33 °C.

While our work suggests that urban black widow behaviour is heavily influenced by temperature, urban phenotypic shifts can also be the product of short-term plasticity or local adaptive evolution to the novel urban selection regime, requiring multiple generations to manifest. For example, Winchell, Reynolds, Prado-Irwin, Puente-Rolón, and Revell (2016) showed that urban lizards (*Anolis cristatellus*) have a morphology better suited to climb on the

broader surfaces typically available in urban habitats. Moreover, they demonstrated that laboratory-reared descendants of these urban and rural lizards maintained their habitat-specific phenotype even when raised under common garden laboratory conditions – suggesting this morphology may be the product of local adaptation rather than phenotypic plasticity. Indeed, the field of urban evolutionary ecology currently emphasizes the role of rapid evolution to urban change (Alberti, 2015; Rivkin et al., 2019). Importantly, phenotypic shifts cannot be broken down simply into either plasticity or local adaptation, as plasticity can be viewed as a trait under natural selection in its own right (Ghalambor, McKay, Carroll, & Reznick, 2007). However, we know of very few empirical demonstrations that plasticity itself has evolved in response to urbanization. Exceptions to this include studies of the evolution of thermal tolerance in *Daphnia* (Brans et al., 2017) and acorn ants (Diamond et al., 2017) discussed above, as well as neophobic behaviour in blackbirds (Miranda, Schielzeth, Sonntag, & Partecke, 2013). Given that plasticity is the trait historically and intuitively associated with success in rapidly changing environments, we suggest that disentangling the evolutionary significance of plasticity should be a focus for urban eco/evolutionary behavioural ecologists.

The strong effects of family on phenotype shown here and elsewhere (e.g. Johnson et al., 2010) support the potential existence of heritable differences in behavioural plasticity but are far from sufficient evidence for the evolution of plasticity. We follow Rivkin et al.'s (2019) thinking that researchers need to begin with common garden experiments to establish what traits are maintained as intrinsic to an organism when the environment is held constant. Subsequent transplant experiments can quantify the impact of having a phenotype mismatched to the environment. Furthermore, laboratory studies can isolate key selection pressures and attempt to impose similar selection responses in the laboratory (Rivkin et al., 2019).

As urban ecosystems are the fastest growing habitat, it seems imperative that we accelerate our attempts to understand the mechanisms that shape urban biodiversity patterns. This will include identifying the reasons so many species are quickly lost from urban habitats as well as the reasons some species thrive in the wake of human disturbance. The UHI offers one of the best-studied abiotic forces that characterize many urban settlements. Furthermore, understanding responses to current UHI conditions can shed light on how communities will respond to projected global climate alterations. Thus, urban behavioural ecologists can provide a great service in their attempts to understand the complex feedback between human behaviour, the ecosystems we create and the biodiversity that we so often shape.

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