

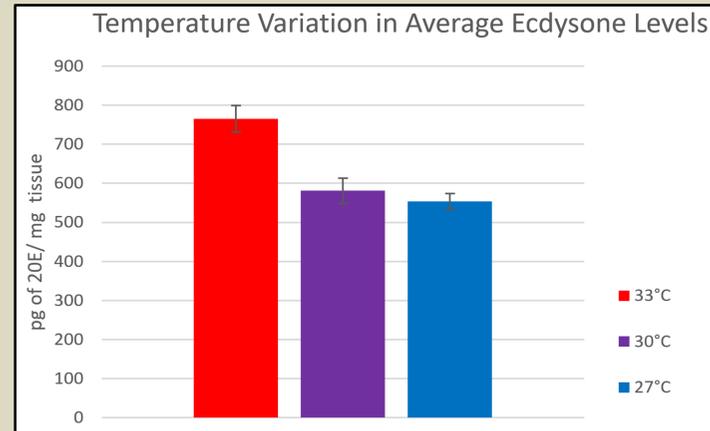
### Introduction

- Rapid urbanization has caused the environment of many organisms to change drastically, and little is known about the potential long-term consequences [1].
- Built structures retain heat, elevating temperatures (Urban Heat Island) [2].
- The Western black widow (*Latrodectus hesperus*), a medically important urban pest species, is 30x more densely populated than desert counterparts [3].
- Surprisingly, current work demonstrates that Phoenix's summer urban-desert temperature differential (33°C vs 27°C, [4]) is associated with higher mortality, developmental delays, and few reaching sexual maturity in *L. hesperus*. [5].
- Here, we hypothesize that UHI temperatures are negatively affecting the ability of *L. hesperus* to regulate the molting hormone 20-hydroxyecdysone (20E) [6].
- Delayed development has been associated with decreased 20E levels in many arthropods including flies, hornworms, and silkworms [7,8,9].
- We predict spiders raised at 33°C will 1) produce lower levels of 20E through development and 2) delay 20E production to trigger molting.

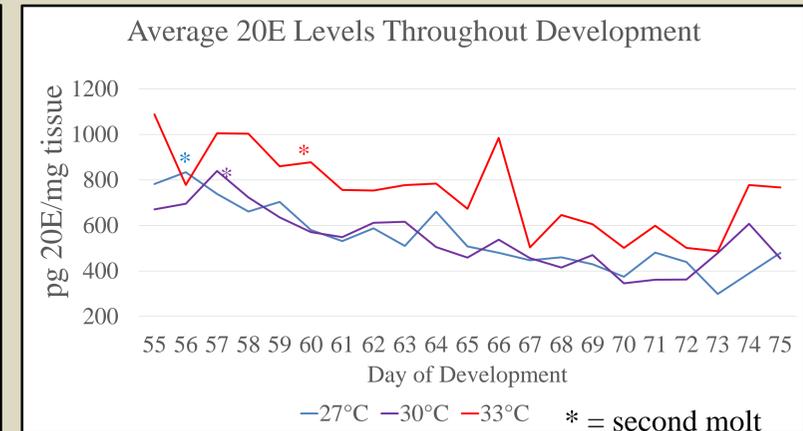
### Methods

- Adult females were collected from six sites across the urban Phoenix Area.
- ~250 eggs from each female's first egg sac were weighed ( $\mu\text{g}$ ), placed in individual boxes (4.13x4.13x5.56 cm), and stored at room temperature.
- On day 44, surviving spiders (ranging from 138 to 227) were divided into 27°C, 30°C, and 33°C incubators.
- One spiderling from each treatment was weighed and frozen from day 55 to 75.
- Each spiderling was ground in methanol and centrifuged to extract 20E.
- Hormone concentrations were determined via an Enzyme Immunoassay based on competition between sample 20E and an enzyme-linked 20E molecule that produces a measurable yellow-colored product (Ellman's Reagent).
- Samples were read on an ELX808IU Ultra Microplate Reader, and 20E concentrations were determined by comparison with a 20E standard curve.

### Results



**Figure 1. Temperature variation in average ecdysone levels.** The 20E levels were averaged across all six families to see if temperature had a significant impact on baseline 20E levels. Temperature was shown to have a significant impact on 20E production throughout development at 33°C ( $p < 0.001$ ). However, there was no significant difference between the cold and intermediate treatments.



**Figure 2. Average 20E levels throughout development.** 20E levels across all six families were averaged to show the general trends in 20E production. At 33°C, significantly more 20E was produced consistently throughout development and a larger delayed peak was produced to initiate the molting process ( $p < 0.001$ ). However, there was no significant difference between the cold and intermediate treatments.

### Discussion

- The elevated temperature of 33°C, but not 30°C, led to 1) a consistently higher production of 20E 2) additional peaks of 20E, and 3) delayed developmental progression (i.e. molts).
  - This suggests that urban temperatures have surpassed the threshold for optimal regulation of 20E causing the hormonal network to drastically increase production of 20E, a common mechanism for times of environmental stress [10,11].
- The 20E stress response is intended to divert energy to essential processes during extreme conditions.
  - Due to the constant elevated temperature, *L. hesperus* is not given sufficient time to recover and this mechanism is ultimately ineffective and harmful.
  - Although the increased abundance of food could counteract some of these effects, leading to the temporary exploitation of the environment, this will not be a sustainable solution moving forward as temperatures continue to rise.
- Future work will focus on using PCR to assess the expression of 20E biosynthesis genes and other key pathways to explore the mechanism by which temperature modulates endocrine function and see how elevated 20E levels impact behavior through 20E microinjections.

### References

- [1] Hawkins et al., 2004. *Applied Meteorology* [2] Kim 1991. *Intl. Remote Sensing* [3] Johnson et al., 2012. *Midland Naturalist* [4] Johnson, unpublished data [5] Johnson et al. in prep [6] Krishnakumaran 1970. *Biol bull* [7] Tennessen & Thummel, 2011. *Current Biology* [8] Schwartz & Truman, 1983. *Developmental Biology* [9] Jindra & Riddiford. 1996 [10] Ishimoto & Kitamoto, 2011. *Fly* [11] Hirashima, Rauschenbach, & Sukhanova 2000. *Bioscience, Biotechnology, and Biochemistry*

