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Cover Page Footnote

We thank Carmen, Garth, and Mikayla Brown for assisting us with laboratory equipment, and Angie Pytel for editing an earlier version of the manuscript. Research costs were supported by the Hillsdale College biology department. This paper won the best student presentation award at the 64th Annual Meeting of the Michigan Entomological Society. It is paper #21 of the G.H. Gordon BioStation Research Series.

Effects of Laboratory Heat Stress on Mortality and Web Mass of the Common House Spider, *Parasteatoda tepidariorum* (Koch 1841) (Araneae: Theridiidae)

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Abstract

We determined the effects of laboratory heat stress on web construction of *Parasteatoda tepidariorum* (Koch) (Araneae: Theridiidae) by measuring the survival and web mass of specimens after a 48-h period within a temperature chamber at 21, 30, 35, 40, or 50°C. The 21, 30 and 35°C treatments had the highest mean survival rate (100%), the 50°C treatment had the lowest (0%), and the 40°C treatment was intermediate (58%). The 21, 30, and 35°C treatments had the highest mean web mass, and the 40 and 50°C treatments had the lowest. Web mass did not correlate with spider mass for specimens across all temperature treatments. While acclimation temperature and humidity fluctuated throughout the 3 weeks of the study, neither variable affected web mass. This study demonstrates the sublethal effect of temperature on web construction, an effect that may ultimately be lethal in nature if a spider was unable to construct its web.

Keywords: Spider, *Parasteatoda tepidariorum*, heat, stress, sublethal, web

Ambient temperature is widely known to have many important effects on spiders and other organisms. Because spiders are ectothermic, their metabolic rate and subsequent activity level is directly tied to temperature. Moreover, because many spiders must engage in a specific physiological behavior—web construction—in order to feed and mate, any temperature that affects this behavior will be ultimately be lethal, even if that temperature does not directly cause death.

Despite the importance of spiders in controlling pest insects in both natural and agricultural ecosystems (Nyffeler and Benz 1987, Uetz et al. 1999), the study of spider thermal ecology has historically gotten little attention. As of the last comprehensive review, thermal tolerance values were known for <0.1% of species (Humphreys 1987). Several studies, however, have shown that spiders actively seek out habitats with preferred temperatures, and may position their web to optimize sun exposure and temperature within a habitat (Biere and Uetz 1981, Lubin et al. 1991, Lubin et al. 1993). Spider species found in relatively cool natural environments tend to mature at a slower rate when exposed to high temperatures in the laboratory. The reverse is true for spiders that inhabit warm environments when exposed to colder temperatures (Li and Jackson 1996). Due to concerns about anthropogenic

temperature increase, and the importance of spiders in both natural and agricultural systems, it is necessary to study the effects of high temperatures on spiders, especially subtle effects on web construction.

The common house spider, *Parasteatoda tepidariorum* (Koch 1841) (Araneae: Theridiidae) is thought to be native to South America and is currently established throughout much of the world, where it is associated with both natural environments and human dwellings (Edwards 2001). The species builds a tangle-style web that houses both males and females, making it crucial for feeding and reproduction. House spiders are increasingly being used as model organisms for genetic, embryological development, and evolutionary studies (McGregor et al. 2008, Mittmann and Wolff 2012, Posenien et al. 2014, Schomburg et al. 2015). Tanaka (1989, 1991, 1992) determined its life cycle in Japan, as well as the effects of temperature and photoperiod on its maturation and reproduction.

Barghusen et al. (1997) studied the effects of laboratory temperatures between 5–30°C on *P. tepidariorum* web construction over 6-d periods and found the highest web mass was produced by specimens housed at 20°C, although there was statistical overlap between groups housed between 15–30°C. That study did not test spider mortality and, thus, utilized fairly low temperatures.

Table 1. Dates of trials and the randomly-assigned order of temperature (°C) treatments for each trial.

Trial round	Date begun	Date ended	Temperature treatment order				
1	14 May	23 May	50	30	35	40	21
2	24 May	02 June	35	30	40	21	50
3	03 June	12 June	40	30	21	35	50

The objective of the current study was to compare both mortality and web mass of *P. tepidariorum* based on temperatures warmer than those used by Barghusen et al. (1997).

Material and Methods

Adult female specimens of *P. tepidariorum* were obtained from Spider Pharm, Yarnell, AZ (<https://spiderpharm.com/>) in two batches; the first batch encompassed the first two rounds of trials and the second batch was the third round. Prior to trials, each specimen was maintained in the lab in separate 8 cm H × 7 cm D round clear plastic jars and exposed to ambient photoperiod and indoor temperature. Room temperature (°C) and relative humidity (%) were measured at 9:00 am and 6:00 pm each day and the mean between them recorded. Each specimen jar included 3 round wooden dowels (~5.0 cm L × 0.3 cm D), around which the spiders could spin webs. Specimens were not fed during the holding period except for a single muscoid fly adult—either included with the Spider Pharm order or wild-caught—which was offered 24 hours before that specimen's trial. All spiders caught and wrapped their prey, although we could not determine how much of that prey was consumed.

Three rounds of temperature trials on *P. tepidariorum* were run during the period of mid-May to mid-June 2018 (Table 1). Each round consisted of 5 different temperature treatments: 21, 30, 35, 40 and 50°C. We assumed that 50°C would be lethal to the spiders, whereas the 21°C treatment approximated the 'room temperature' frequently encountered by this synanthropic species, as well as the optimum found by Barghusen et al. (1997). The other temperatures were considered intermediary. Each trial consisted of 4 specimens tested simultaneously at the same temperature. The trials were repeated 3x, for a total sample size of 12 specimens for each temperature treatment.

For each round of trials, specimens were randomized and placed into their temperature treatments. Temperature treatment order within a round was also randomized (Table 1). Each trial consisted of 48 h in a H2200-H MyTemp mini digital incubator (www.benchmarkscientific.com)

set to the appropriate temperature. Prior to treatments, 3 wooden dowels (~21.0 cm L × 0.3 cm D) were weighed on a digital scale at 21°C. These dowels were placed into a 17.8 cm L × 11.4 cm W × 15.2 cm H clear plastic box drilled with 1 mm air holes along with a single specimen. Four of the boxes were then placed into the incubator. Temperature and relative humidity in the incubator were monitored and recorded every 12h throughout the duration of the 48h. Since Barghusen et al. (1997) found no effect of humidity on web construction, we did not attempt to equalize humidity levels at the different temperatures.

After a trial ended, survival or mortality of specimens was determined. Constructed webs were wound around the dowels and weighed after returning to 21°C. The web mass was calculated by subtracting the initial mass of the dowel from the final mass of the dowel and web after the test concluded. Spider specimens were preserved in a pre-weighed vial of ethanol. Spider mass was calculated by subtracting the initial mass of the vial from the final mass of the vial with the specimen. Each specimen was treated as an independent observation.

All data analyses were conducted using Excel for Windows with the RealStats add-in (www.real-statistics.com) with non-parametric tests since no resulting data met parametric assumptions. Mean arcsine transformed percent survival of specimens within the temperature treatments were compared via a Kruskal-Wallis test with post-hoc Dunn test. Mean weight of webs of temperature treatments were also compared via a Kruskal-Wallis test with post-hoc Dunn test. Spearman rank-order correlations were calculated between the mass of spiders and the mass of produced webs for each temperature treatment. Acclimation temperature and acclimation humidity were determined by taking the mean of the 5 previous days that the spider had been exposed to before its temperature test. Spearman rank-order correlations were then calculated between the mass of produced web and both ambient acclimation temperature and relative humidity.

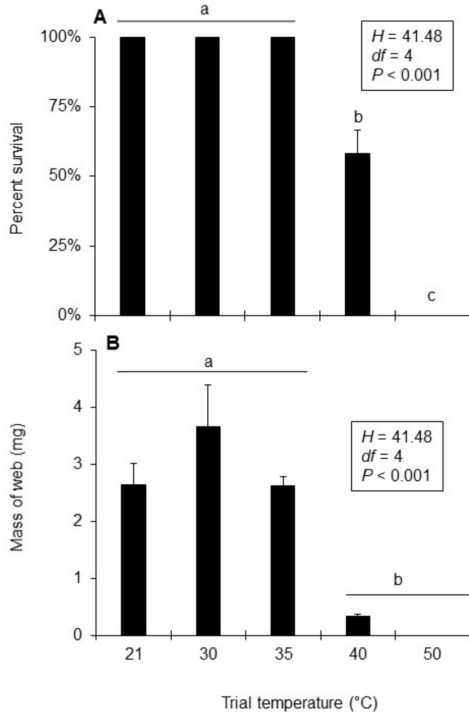


Figure 1. Mean (+SE) percent survival of *Parasteatoda tepidariorum* specimens after 48h at a particular trial temperature (A). Mean (+SE) mass of spider webs of the same specimens after their trials (B). Superscript letters statistically distinct groups based on a Kruskal-Wallis test with post-hoc Dunn test.

Results

The 21, 30, and 35°C treatments had the highest survival rate, the 50°C treatment, had the lowest, and the 40°C treatment was intermediate (Fig. 1). The 21, 30, and 35°C treatments had higher web mass than the 40 and 50°C treatments. Correlations between spider mass and web mass were not significant at any temperature (Fig. 2). There was no difference in mean web mass between spiders that died during a 40°C trial and those that lived for the entire trial (Two-sample *T*-test, *df* = 10, *P* = 0.19). Ambient room indoor temperature varied from 18–25°C during the 30 days of the experiment, and relative humidity ranged from 43–73% (Fig. 3). Correlations between web mass and both temperature and relative humidity, however, were not significant (Fig. 4,5).

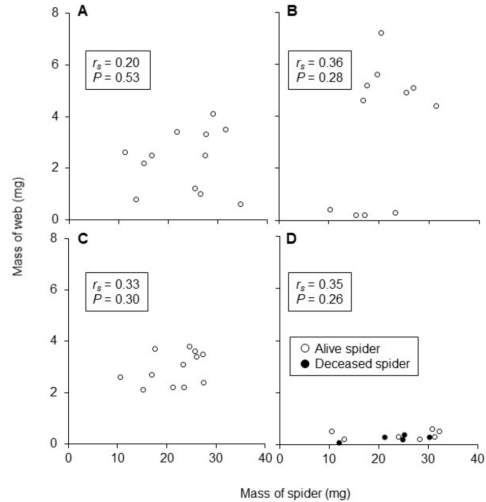


Figure 2. Spearman's rank-order correlation between spider mass and web mass for the 21 (A), 30 (B), 35 (C), and 40°C (D) temperature treatment groups, with resulting r_s and *P*-values for each correlation. *N* = 12 for each treatment group. 'Alive' spiders are those that lived throughout the entire 48-h trial, whereas 'deceased' spiders died at some point during the trial. The 50°C temperature group is not shown since none of those spiders survived or spun any web.

Discussion

This study provides the first preliminary data on 48-h lethal temperature tolerance of adult *P. tepidariorum*. Since 42% of specimens died at 40°C and 100% died at 50°, the actual upper lethal temperature is somewhere between those values. This result is comparable to that of other tested spider species, ~80% of which had an upper temperature limit of 43–48°C in the laboratory (Mouer and Ericksen 1972, Tolbert 1979, Suter 1981, Pulz 1987). Since temperatures this high are unlikely to be encountered in most environments, their primary importance is in comparison to those of other spiders tested under similar circumstances.

This study also provides evidence that *P. tepidariorum* has a fairly wide optimal temperature range for web production. Although they claimed 20°C as the optimal temperature for prey catch, Barghusen et al.'s (1997) study of *P. tepidariorum* demonstrated no statistical difference in web mass between 15–30°C. Similarly, we observed no difference between 21–35°C, suggesting an ability of this cosmopolitan species to effectively construct webs over a temperature range of 15–35°C.

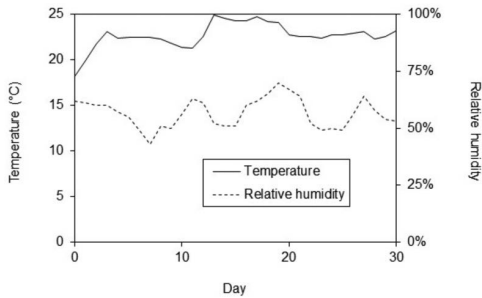


Figure 3. Changes in indoor ambient temperature (primary axis) and relative humidity (secondary axis) throughout the 30d of our experimental trials.

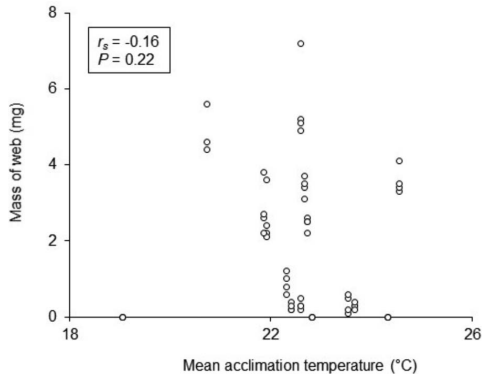


Figure 4. Spearman's rank-order correlation between mean acclimation temperature for the 5d prior to a particular trial and web mass, with resulting r_s and P -values. $N = 60$.

Our results also suggest an inability of *P. tepidariorum* to produce webs beginning at ~ 35 – 40°C . While the web mass of spiders in the 35°C trials approximated that of spiders at lower temperatures, the web mass of spiders at 40°C was the same as that of spiders at 50°C , all of whom died and spun no web (Fig. 1). Moreover, there was no difference in the web mass of spiders that survived to the end of the 40°C trial and those that died at this temperature. This result argues strongly for sublethal web mass decrease, because if spiders were spinning less web due to mortality, then there would have been a difference in web mass between spiders who survived the trials and those that did not.

This result demonstrated the sub-lethal effect of high temperature, as a prolonged exposure to $\sim 40^\circ\text{C}$ in nature might ultimately prove fatal to the species due to its

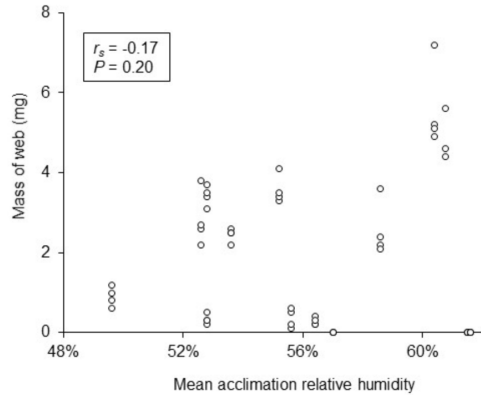


Figure 5. Spearman's rank-order correlation between mean acclimation humidity for the 5d prior to a particular trial and web mass, with resulting r_s and P -values. $N = 60$.

inability to produce a web, even without direct temperature mortality. Small decreases in web mass can have significant decreases in capture efficacy, especially when coupled with the increased metabolic activity and mechanical power of prey insects at warmer temperatures (Hesselberg and Vollrath 2006). Daytime temperatures near 40°C can realistically occur in most temperate environments, albeit for only a portion of the day, and are likely to become more common in the future (e.g., Dai et al. 2016). Since *P. tepidariorum* is a cosmopolitan species with a fairly wide optimal temperature range, it is likely that other spider species may experience sub-lethal high temperature effects at lower temperatures.

The size of our spider specimens appeared to have no effect on the mass of their webs. The relationship between spider mass and web mass is not well understood. Blackledge and Zevenbergen (2007) found that recently fed, and thus larger, specimens of *Latrodectus hesperus* Chamberlin & Ivie constructed heavier webs than did fasted specimens. This result was in contrast to studies of orb weaving spiders (Araneidae), which built smaller webs as they caught more food (Sherman 1994; Venner et al. 2000, 2006), or because they allocated more energy to foraging effort instead of web construction (Venner et al. 2003). Since *P. tepidariorum* is more closely related to *L. hesperus*, and constructs a similar type of web, we suspect it should have a similar relationship between spider mass and web mass. Because we were consistent in our feeding, any effects of hunger should have been similar between specimens and, thus, differences in body mass should have been

due primarily to inherent size difference. Such size differences appeared to have no effect on web mass.

One potential source of error in our study was the fluctuating temperature and associated relative humidity during our acclimation period. The temperature at which an organism is acclimated prior to testing has been shown in numerous studies to have a significant effect on its laboratory-determined thermal tolerance (e.g., Lutterschmidt and Hutchison 1997). We, however, found no correlation between web mass and either temperature or relative humidity based on the mean conditions during the 5 d before a trial, despite such conditions varying by ~5°C. One possible reason for this lack of acclimation is that in intentional laboratory acclimation trials, temperatures are held at a constant temperature for a prescribed period, whereas our temperatures fluctuated based on local weather. Such fluctuations are typical of natural temperate environments, and may lead to an increased temperature tolerance range of specimens exposed to them relative to those exposed to constant temperature (Li and Jackson 1996). Thus, the varying temperatures in our study probably did not strongly acclimate a specimen to a particular temperature, and any minor acclimation effects were insignificant.

Further research is needed to translate our laboratory results to natural environments. Specifically, longer trials at temperatures with differences <5°C between them will help determine specific lethal and sub-lethal threshold temperatures. Because warm temperatures may ultimately harm the spider through starvation, specific experiments on the length of time specimens can live without food at high temperatures are also important, since a long ability to survive without eating would offset our observed sub-lethal effects. Lastly, doing the above experiments with more stenothermic species may suggest how warming environments may affect the important ecological services provided by spiders.

Acknowledgments

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