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Dehydration resistance and tolerance in the brush-legged wolf spider (*Schizocosa ocreata*): A comparison of survivorship, critical body water content, and water loss rates between sexes.

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Dehydration resistance and tolerance in the wolf spider *Schizocosa ocreata*: A comparison of survivorship, critical body water content, and water loss rates between sexes.

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Small-bodied terrestrial animals like spiders face challenges maintaining water reserves essential for homeostasis. They may experience dry microclimates and/or seasonal variation in water availability, so dehydration is a common stressor that may help explain movement, foraging, and other behaviors. This study examines aspects of dehydration resistance and tolerance in the brush-legged wolf spider, *Schizocosa ocreata* (Hentz, 1844) (Aranaea: Lycosidae), commonly found in the leaf litter of eastern deciduous forests of North America. Sexes differ in morphology, physiology, reproductive strategy, and life history, likely resulting in divergent abilities to resist and tolerate dehydration. We used humidity-controlled chambers to examine relative survivorship by sex under varying humidity regimes, water loss rates, body water content, and critical water mass. Spiders survived significantly longer in higher humidity regimes ($\geq 50\%$ RH). Females had significantly better survivorship overall, lower body loss rates, and lower critical mass, though males had greater percent body water content, indicating that females have greater dehydration resistance and tolerance than males. Although sex based differences in survival time and water loss rates are likely an effect of relative body mass, females should be more successful than males during periods of episodic drought, and are likely selected to survive later in the season while caring for offspring.

Keywords: Wolf spider, water balance, stress, ecophysiology, dehydration

Introduction

Across all terrestrial organisms, water is an essential component for maintaining proper body function. It is required for ionic regulation in body fluids and tissues (Le Rudulier et al. 1984), aids in gas exchange (Feder and Burggren 1985), and in many cases provides structural support and motion (Foelix 2011; Kier 2012). Because of these vital roles, access to water and maintenance of water balance (dehydration prevention) are potential challenges for any terrestrial animal. Small-bodied terrestrial invertebrates are particularly susceptible to issues of water balance and rapid dehydration due to their high surface area to volume ratio (SA:V) (Hadley 1994). They can lose water to their environment via cuticular evaporative water loss (CEWL), respiratory water loss (RWL), and excretion, with CEWL and RWL being the primary routes of water loss (Pulz 1987). Given the critical nature of maintaining water balance, it is unsurprising that physiological and behavioral adaptations that prevent water loss are well documented in arthropods. A thick waxy cuticle on many arthropods living in xeric conditions reduces the amount of water lost to the environment via evaporative water loss (Hadley 1980; Punzo and Jellies 1983). Some small arthropods, such as mites and ticks (Arlian and Wharton 1974; McMullen et al. 1976) or even flesh fly pupae (Yoder and Denlinger 1991), have evolved the ability to absorb water vapor from the air to compensate for water loss. Insects and spiders can reduce respiratory water loss through controlling the opening and closing of their spiracles (Loveridge 1968; Finke and Paul 1989; Chown 2011). Many animals also show behavioral responses to reduce water lost to the environment. Both mesic and arid species of spiders choose microhabitats with more moisture or higher humidity (Humphreys 1975; Uetz 1979; Cady 1983). Modifications to foraging behaviors, such as periods of fasting, may also reduce water loss. Prey

can be an important part of water gain in spiders, as those in a water-limited environment tend to consume more prey than those in an environment where water is plentiful (McCluney and Sabo 2009). Conversely, some arthropods, such as locusts, may consume less if only dry food is available, presumably to limit fecal water loss if it exceeds water gain from food (Edney 1977).

Despite a reasonably broad understanding of adaptations to minimize water loss, dehydration resistance (the ability to reduce water loss to the environment) and dehydration tolerance (the ability to withstand a non-lethal amount of water loss) remain poorly understood for many arthropods. This is unfortunate since physiological limits can provide crucial information about the biology and ecology of the organism and even help explain emergent behaviors (Lubin and Henschel 1990; Klok et al. 2004). Investigations into critical dehydration limits may help to understand habitat partitioning, food choice, and responses to acute and chronic environmental disturbance, such as drought (Hadley 1994; DeVito et al. 2004; McCluney and Sabo 2009).

In terms of dehydration, spiders face the same challenges as any small invertebrate due to their large SA:V and therefore comparatively high CEWL. In addition to these challenges, spiders may be especially vulnerable to dehydration due to their hydraulically-mediated leg extension (Foelix 2011). Without sufficient hydration, spiders cannot fully extend their legs, and therefore would have trouble moving about to forage or escape predation (Anderson and Prestwich 1975). Wolf spiders of the genus *Schizocosa* are important models in behavioral ecology studies (Hebets et al. 2013; Uetz et al. 2013; Uetz et al. 2016), yet little is known about their underlying physiology or behavioral responses to physiological stressors, such as

dehydration. The goal of this study is to establish the dehydration limits of the wolf spider *Schizocosa ocreata* (Hentz, 1844) (Araneae: Lycosidae), and as a result, better understand how these spiders will respond to dehydration stress both behaviorally and physiologically. *Schizocosa ocreata* is an ideal species for this study as they are common wolf spiders in deciduous leaf litter of eastern North America (Dondale and Redner 1990), and individuals are easily maintained in a lab. These, like many wolf spiders, are cursorial, potentially traveling large distances and experiencing various microclimates within their lifetime (Cady 1983; Walker et al. 1999; Samu et al. 2003). Additionally, the deciduous forest habitats where they are found commonly experience random episodic and/or seasonal drought (Hanson and Weltzin 2000), meaning that these spiders are likely to experience acute or chronic dehydration at some point across their lifespan.

In this study we investigated dehydration resistance and tolerance in *S. ocreata* by focusing on three key aspects: survivorship under varying relative humidity regimes, water loss rate, and critical water loss. We hypothesized that due to the morphological and ecological differences between males and females, dehydration resistance and tolerance would vary between the two sexes reflecting these differences. Males typically have less mass than females (Dondale and Redner 1990), higher metabolic rates (Kotiaho 1998), and different reproductive strategies than the females (Foelix 2011), and we predicted that males would have less dehydration resistance and tolerance than the females due to their larger SA:V. We expected females to survive longer, have lower water loss rates, and greater critical water loss values.

Materials and Methods

Animal collection and care

We collected spiders as juveniles in spring and fall of 2011 at The Dawes Arboretum in Newark, Ohio, USA (N 39.973863, W -82.40128). We returned all individuals to the lab where they were reared to adulthood in individual 500 ml round, plastic containers, each with a moistened peat moss substrate to provide constant access to moisture. We maintained the spiders on a 13 hour light: 11 hour dark cycle. All spiders were fed a mixed diet of three to four *Drosophila spp.* and/or pinhead crickets (*Acheta domestica*), as appropriate by individual size, twice a week. Adult spiders no older than four weeks past their final molt were randomly selected to participate in each of the trials. All of these studies involved destructive sampling of spiders, so experimental methods were designed to minimize total sample sizes where possible.

Humidity Chambers

We used humidity chambers (consisting of closed, 6 liter clear-plastic containers) to establish treatment groups for all experiments described here. Following the methods of Winston and Bates (1960), we used DrieRite desiccant or saturated salt solutions (as appropriate) to create and maintain relatively stable humidity levels in each chamber, and we placed a platform within each chamber but above the chemical/solution in order to keep spiders away from treatment solutions. The treatments were as follows: 4% RH (DrieRite), 33% RH (CaCl_2), 55% RH (MgNO_3), 75% RH (NaCl), and 90% RH (KNO_3) (all reagents obtained from Fisher Chemical, USA). We confirmed that we could establish appropriate humidity levels using these solutions

approximately three weeks prior to starting experiments, then periodically checked the humidity levels during the experiment phase using a HOBO data logger (Onset® model # U-DT-2) with a Temperature/RH Smart Sensor (Onset® model# S-THB-M00x). We used all of these chambers for the survivorship experiment, while we only used the 4% RH chambers for the critical water mass and water loss rate experiments. The ambient temperatures within the chambers were maintained between 22 and 23°C for the duration of experiments.

Survivorship

We explored survivorship in *S. ocreata* as a function of varying humidity by randomly assigned 100 individual spiders (50 adult males and 50 adult females) from the 2011 spring lab population to five different humidity treatments such that each treatment had 10 spiders of each sex. Prior to the experiment, we provided spiders with food and water *ad libitum* for three days. At the start of the experiment, we recorded the mass of each individual, placed them into separate vials with a mesh covering to prevent escape, and then placed vials on the platform in a humidity chamber. Once in the chamber, spiders received no food or water for the duration of the experiment. We checked the spiders daily for survival, and considered a spider “dead” if the legs were curled in toward the ventral surface and the individual was unresponsive to light movement of their vial and gentle prodding by a brush. Once we determined a spider to be dead, we removed it from the chamber, weighed it, and then placed it in a 50°C drying oven over night (approximately 12hrs). The following day, we recorded a final dry mass for each spider.

Body water content, critical water mass and water loss rates

In order to investigate body water content, critical water mass, and water loss rates in *S. ocreata*, we gave 50 spiders (25 adult males and 25 adult females, randomly selected from the 2011 fall lab population) food and water *ad libitum* for three days prior to the experiment. We recorded the weight of each spider before beginning the trials, and placed it in a vial with a mesh covering to prevent escape. We placed each of these vials into one of three 4% RH chambers, and for the first 12 hours, we recorded the mass of each spider every hour. After 12 hours, we left the spiders relatively undisturbed in the chambers until death. We checked the spiders daily, and when a spider had perished (using the same criteria as for the survivorship study), we removed it from the chamber and weighed it. As described previously, we then put the dead spider in a drying oven overnight and weighed them again the next day to get a dry mass.

We calculated the body water content (% body weight) by subtracting the dry mass from the mass at the start of the experiment and dividing that by the total mass. We calculated the critical water mass by subtracting the amount of water left in the body at death (dead mass-dry mass) from the total body water mass at the beginning of the trial.

To measure water loss rates, we determined the water mass for each spider by subtracting the dry mass from the mass taken each hour during the first 12 hours of the experiment. Using the exponential model described in Wharton (1985),

$$m_t = m_0 e^{-kt}$$

and following the methods of Benoit et al. (2005), we calculated the water loss rate by finding the slope of the regression line of the equation $\ln(m_t/m_o)$, where k is the percentage mass lost at time t , m_t is the water mass at any time t and m_o is the initial water mass of the animal. We used the initial mass, mass at death, and dry mass to examine the critical water mass for spiders. Using the dry mass, we calculated proportion of body mass that consists of water and the proportion of total body water that is lost at the time of death. We analyzed data using JMP version 11.0 and Microsoft Office Excel 2007.

Results

Survivorship

Survival times for the spiders were not normally distributed according to a Shapiro-Wilk goodness of fit test, before or after a log transformation, thus we used a nonparametric analysis to compare survival times for each sex. There was differential survivorship under the varying humidity levels for both females (Kruskal Wallis: $\chi^2=37.94$, $df=4$, $p<0.0001$, Figure 1a) and males (Kruskal Wallis: $\chi^2=38.39$, $df=4$, $p<0.0001$, Figure 1b). We used the Steel-Dwass method for post-hoc comparison of humidity levels on survivorship within each sex, and we used the Wilcoxon rank sum post-hoc analysis to compare males and females in each treatment (Table 1). Values for the median time to mortality (T50) for each group are listed in Table 2. Females survived significantly longer than males across all treatments (Figure 2). Females had the greatest survival times in the 75% and 90% RH chambers with a mean survival time of 32.4 ± 2.5 days and 26.7 ± 3.5 days, respectively, and there was no significant difference in survival time

between these treatments (Steel-Dwass: $Z = -1.136$, $p = 0.7874$). There was also no difference among the females between 55% and 90% RH treatments (Steel-Dwass: $Z = 2.389$, $p = 0.1182$). The difference among the females between the 33% and 55% RH treatments was marginally significant (Steel-Dwass: $Z = 2.713$, $p = 0.0520$).

Males also had the highest average survival times in the 75% and 90% RH chambers, but with mean survival times of only 7 ± 0.68 days and 8 ± 1.1 days, respectively. As in the females, there was no significant difference between these two treatments for the males (Steel-Dwass: $Z = 0.5779$, $p = 0.9783$). Among the males, there was no difference in survival between the 33% RH and the 55% RH treatments (Steel-Dwass: $Z = 1.4177$, $P = 0.6162$) or between the 4% and 33% RH treatment (Steel-Dwass: $Z = 2.714$, $p = 0.0520$). For both sexes, the lowest average survival time was in the 4% RH chamber, with a mean survival time of 5.8 ± 0.4 days for the females and 1.6 ± 0.2 days for the males.

Body Water Content and Critical Percent Water Mass

During these experiments one male escaped early in the experiment and there was one outlier in the female group, both of which were removed from the analysis for a final sample size of 24 males and 24 females. Females had a mean body water content of $70.8 \pm 0.4\%$, which was significantly lower than the male mean body water content of $74 \pm 0.4\%$ ($t_{(46)} = 5.45$, $p < 0.0001$; Figure 3).

Data for the critical water mass were not normally distributed (Shapiro-Wilk test for goodness of fit), therefore we arcsine square root transformed the data for analysis. The mean

critical percent water mass for females was $37.1 \pm 0.86\%$, which is significantly higher than the mean critical percent water mass for males of $27.8 \pm 1.23\%$ ($t_{(40)} = -6.23$, $p < 0.001$).

Water loss rates

In this analysis it was important that all individuals survive the full 12 hour period, unlike measurements of body water content and critical percent water mass. In addition to the two individuals previously excluded, two males died before the 12 hour period had ended and they were also removed from this analysis, resulting in a final sample size of 24 females and 22 males. The initial mean mass of fed and hydrated spiders was 81.95 ± 4.95 mg for females and 41.62 ± 1.97 mg for males. The females had significantly lower water loss rates (0.64 ± 0.03 %/h) than the males (1.04 ± 0.06 %/h) ($t_{(32)} = 5.9$, $p < 0.0001$).

Discussion

Females had significantly greater survivorship in all treatments than the males. This was expected as the larger body size of the females means they have a lower surface area to volume ratio and subsequently reduced cuticular water loss. As the humidity increases, evaporative water loss should decrease, thus slowing down the rate of dehydration and allowing longer survival times in higher humidity levels. Both males and females had higher survivorship in the higher humidity treatments (75% RH and 95% RH). High humidity clearly provides a benefit to spiders by reducing water loss and thus allowing the spiders to survive for long periods of time without

access to food or drinking water. Interestingly, there was no significant difference in survivorship between the 75% RH treatment and the 90% RH treatment. It is possible that the effects of starvation played a role in this result. Spider starvation tolerance varies widely, but the wolf spider *Pardosa astrigera* has an average survival time of 28.8 days in males and 54.4 days in females with access to water but no food (Nakamura 1987). The spiders in this study had neither food nor drinking water, and it is possible that after a number of days they can no longer survive even at high humidity levels.

Males had significantly higher water content as a percent of body weight than females. With males having an average of 74% body water content and females having an average of 71% body water content, both sexes fall within the range typical for spiders, which is about 60-85% (Pulz 1987). The lower water content of females may be explained by egg production. Female wolf spiders develop eggs within their abdomens for several weeks (are gravid) before depositing the eggs into an egg sac (Foelix 2011). Eggs tend to be lipid-rich, and this may add considerable mass and volume (abdomen size) at the expense of water content, resulting in females having less total water within their bodies than the males (Edney 1977; Carrel 1990).

This tradeoff between lipid content and water content raises an interesting implication of this study. Documenting hydration state and response to dehydration may influence how we measure and interpret the body condition of spiders. Body condition is typically defined as the energy reserves in an organism, is used as a proxy for an individual's overall quality, and is often measured as a body condition index (Jakob et al. 1996; Moya-Laraño et al. 2008). In wolf spiders, body condition measured in this fashion is correlated with size, egg sac production,

performance of courtship behaviors, and expression of secondary sexual characteristics (Kotiaho et al. 1998; Persons et al. 2002; Uetz et al. 2002). Body condition indices are assumed to reflect nutritional history and current reserves of lipids and/or proteins, with hydration state of the measured individuals rarely considered (Jakob et al. 1996; Uetz et al. 2002; Moya-Laraño et al. 2003; Wrinn and Uetz 2007; Hebets et al. 2008; Rutledge 2010). While there is debate on the best measure of body condition (Jakob et al. 1996; Green 2001), in spiders it often includes some measurement of mass and abdomen size (Moya-Laraño et al. 2008; Rutledge 2010). Both of these measures (mass and abdomen size) can be readily affected by the hydration state of an individual, reflecting only recent access to water instead of providing information about historical or recent foraging success. Currently, there is no accepted, non-destructive technique that teases apart the effects of the animal's hydration state from their overall body condition. Rutledge and Uetz (unpublished) compared starved and dehydrated spiders and found that standard body condition indices did not differ between the two treatments for the first 10 days of the experiments, indicating that typical measurements may not be accurately measuring an animal's energy reserves but rather may also be confounded by the animal's hydration state. An understanding of typical hydration levels and dehydration tolerance in spiders may better help us make inferences from and interpret body condition indices in the future.

Females have a significantly lower critical water mass than males, or alternatively, significantly higher dehydration tolerance. While in females an average loss of 38.7% of their water mass resulted in death, males died after an average of only 27.8% of their body water was lost. Both of these values are high compared to other spiders, which range from about 18%-31% (Pulz 1987). Similarly, females had significantly lower water loss rates (0.63 %/hour) than males

(1.04 %/hour). Although the smaller SA:V may explain females' higher survivorship and lower water loss rates, it doesn't explain why they were able to withstand greater loss of water before perishing. The average water-loss rates of *S. ocreata* males and females are similar to that of other wolf spiders (Davies and Edney 1952; Aspey et al. 1972), although it is important to note that the methods in all of these studies vary. The wolf spiders in these studies are larger, but they are also all mesic species. Water-loss rates may be reflective of the general moisture availability in the animal's habitat; more so than the body water content or critical water loss amount (Vollmer and MacMahon 1974; Addo-Bediako et al. 2001; Mazer and Appel 2001). The data here supports this hypothesis as the water-loss rates for *S. ocreata* are quite high compared to desert arachnids (Hadley 1970), yet the body water content is similar (Pulz 1987). One aspect of water-loss rates that was not addressed in this study was the role in ambient temperature. While our study maintained a consistent ambient temperature throughout the trials, water loss rates likely vary depending on both the temperature and the season (see review by Canals et al. 2015). For example, Figueroa et al. (2010) found that the Chilean mygalomorph *Paraphysa parvula* increased its water-loss rates by a factor of 10 when the ambient temperature was raised from 25°C to 40°C, presumably to allow for evaporative cooling. Although *P. parvula* is a xeric species, because mesic environments may have fluctuating temperatures, it is reasonable to suggest that temperature and seasonality may also impact the water loss rates of *S. ocreata*.

That females have a greater dehydration resistance and tolerance than males is intriguing because males tend to travel greater distances than females and thus are more likely to encounter unfavorable environments (Cady 1983). Similar results were found by DeVito and Formanowicz (2003), when they examined dehydration tolerance in the wolf spider, *Pirata sedentarius*.

Females of that species have higher survivorship than males when subjected to thermal and dehydration stress. One possible explanation is that male and female spiders have different metabolic rates. A higher metabolic rate leads to a relatively higher respiratory evaporative water loss (Anderson and Preswich 1982; Chown 2002); therefore a higher metabolic rate in males could explain the disproportionately lower dehydration tolerance. However, in both Devito and Formanowicz (2003) and the present study, spiders were kept in enclosures that allowed for limited activity. Spiders are able to close their respiratory spiracles in response to changes in activity (see review by Schmitz 2016). Inactive spiders tend to keep their spiracles closed (Finke and Paul 1989) and so it is unlikely that any differences in metabolic rates played a role in the disparity of responses to water loss seen here. If there does exist a significant difference between male and female metabolic rates, then it is possible that the effects of dehydration stress seen here could be exaggerated in a situation where individuals are active. Currently, the available data for *S. ocreata* is not conclusive on which sex exhibits the higher metabolic rate, although in many of the wolf spider species studied, such as *Pardosa milvina*, males do have a higher metabolic rate than females, and this difference seems to be associated with activity (Kotiaho 1998; Walker and Irwin 2006). Although males travel farther (Cady 1983), it is possible that they encounter enough accessible water along the way that they are unlikely to reach their critical hydration limits, and therefore a higher metabolic rate would benefit males by enabling them to travel long distances.

It is also possible that the female's reproductive state impacts their dehydration resistance and tolerance. Gravid females who have not yet produced an egg sac (the typical condition of females used in this study) may display higher dehydration resistance due to a temporarily

increased SA:V. Additionally, females may have decreased water content after releasing an egg sac as they must invest their own water reserves into that egg sac (Pulz 1987). Females with egg sacs typically have lower metabolic rates than those without egg sacs, and therefore likely have lower evaporative water loss (Canals et al. 2011). Females may have undergone selection towards a higher dehydration tolerance as they must invest their own reserves into producing eggs and carrying egg sacs. Eggsacs require both water and lipids, and females may have to withstand unfavorable conditions in order to maintain the egg sacs at appropriate temperatures (Carrel 1990; Kotiaho 1998). Therefore, the ability to withstand dehydration stress would be particularly beneficial for increased fecundity. Future studies that compare females in different reproductive states can further enhance our understanding of the determinants of dehydration tolerance.

Schizocosa ocreata is an increasingly common model system in behavioral ecology (Hebets 2011; Uetz et al. 2016) and the data from this study provides critical information into the physiological tolerances of both sexes. Spiders are both predators and prey and so have incredible ecological significance in their habitats (Riechert 1974). Because much of their habitat partitioning is dictated by temperature and water availability (Riechert and Bishop 1990), responses to dehydration will help understand how spiders are likely to respond to a changing environment. In order to further investigate the effects of water availability on various behaviors, from microhabitat selection to courtship and mating, baseline information on dehydration tolerance is necessary to understand the influence this environmental parameter has on spider behavior.

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Table 1: Wilcoxon rank summed test of survivorship to compare *S. ocreata* (Hentz, 1844) males and females at each RH level. Asterisk (*) indicates significant difference in survivorship between the sexes.

Treatment RH	Z value	p value
4%	-3.834	0.0001*
33%	-3.823	0.0001*
55%	-3.770	0.0002*
75%	-3.753	0.0002*
90%	-3.154	0.0016*

Table 2: T50 values for *S. ocreata* (Hentz, 1844) females and males, calculated as the median time (days) to mortality.

Treatment	Females	Males
4% RH	6	2
33% RH	8.5	2
55% RH	12	3
75% RH	32.5	7
90% RH	28.5	9

Figure Legends

Figure 1: Survivorship over time for *S. ocreata* (Hentz, 1844) (a) females and (b) males by relative humidity.

Figure 2: Mean survival time ($\pm$ SE) for *S. ocreata* (Hentz, 1844) females and males in five different humidity regimes. Shared letters above the bars indicates no significance by the Steel-Dwass method.

Figure 3: Body water content (as a proportion of body mass) for *S. Ocreata* (Hentz, 1844) males and females.

Draft

(a)

(b)