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## Water Loss Rates and Desiccation Tolerances for Spiders and Crickets

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## Water loss rates and desiccation tolerances for Spiders and Crickets

Matt Zach and Kevin E. McCluney

### Abstract

Despite the importance of water to living organisms, access varies across the globe with high variability over space and time. Seventy-five percent of the world's freshwater alone is ice while 99% of unfrozen freshwater is underground (Winter et al. 1998). Understanding how terrestrial species respond to water availability and compensate for water stress can provide insight to their behavior, ecology and physiology. The goal of this research was to examine the differences in the evaporative water loss rates and desiccation tolerances of *Hogna carolinensis* and *Acheta domesticus*. First, an experiment was conducted to quantify the rate of evaporative water loss of the species *Hogna carolinensis* and *Acheta domesticus*. This was done by desiccating twenty specimens of *Hogna carolinensis* and forty specimens of *Acheta domesticus* within an environmental chamber set at 30%RH and 30C and measuring their masses until death. Another experiment was conducted to explore how prey hydration influenced predation of *Hogna carolinensis*. Thirty-nine female specimens of *Hogna carolinensis* were housed within the environmental chamber under the same conditions, treated to a four day period of starvation and desiccation then fed eight specimens of *Acheta domesticus* from one of three hydration treatments. Initial observations concluded that there was little difference between how much mass was consumed by the predators across the three treatments.

### Introduction

Water is essential to living organisms as it plays a vital role in countless physiological processes. It is a strong determinant of species range, and a significant correlate to species

richness for many ecosystems (Hawkins et al. 2003). Despite its importance, access to water varies across the globe with high variability over space and time. Water can be a fairly limited resource as seventy-five percent of the world's freshwater alone is ice while 99% of unfrozen freshwater is underground (Winter et al. 1998). Regardless of where water comes from its importance to terrestrial ecosystems is crucial. This is especially true for dry climates, which account for 1/3 of the world's landmass (Reynolds et al. 2007). Within these water stressed environments, water accessibility modulates ecosystem processes and animal consumption patterns (McCluney et al. 2009; Sponseller et al. 2013). Because current climate models predict a higher frequency of droughts and varied precipitation patterns worldwide (IPCC 2014), the role of water availability influencing terrestrial ecosystems and species should not be understated (Allen et al. 2013).

Investigating how species respond and compensate for water stress provides potential insight into their ecology. Past research has found moisture levels indirectly influencing the activity of spiders and partitioning microhabitats amongst species (DeVito et al. 2004; Shultz et al. 2006). While field crickets and spiders have been observed altering rates of consumption of moist food to the addition of water (McCluney et al. 2009). Not all species respond similarly to water availability and thus investigating how species differ in desiccation tolerance and water loss rate could be relevant for understanding variable distributions of taxa as well as potential responses to climate change (Chown et al. 2011).

Model organisms for studying the effects of water stress are terrestrial arthropods. Their relatively high surface area to volume body ratio gives terrestrial invertebrates a high rate of evaporation (Hadley 1994). Even though the wax layer of their cuticle helps prevent water loss, it loses functionality when a certain critical temperature is reached and evaporation increases

(Wigglesworth 1945). These factors combine to make water limitation an important obstacle for terrestrial invertebrates to overcome, and a strong likelihood for influencing their ecology. For example, the habitat used by species of wandering spiders has been shown to be associated with significant differences in desiccation resistance and varying water loss rates (Lapinski et al. 2014).

Here, we seek to investigate how water is lost in two invertebrate species and how they tolerate desiccation. The wolf spider (*Hogna carolinensis*) and the common house cricket (*Acheta domesticus*) were selected for this experiment due to their potential relationship as predator and prey, and their availability and ease of care.

Water limitation affects predator-prey interactions as consumer species are profoundly affected by water availability (McCluney et al. 2009). This revelation is not to be taken lightly, as predators can be responsible for the stability and complexity of food webs (Lowe et al. 2014) and water may play a role in these “trophic cascades” (McCluney et al. 2016). And these effects may have important implications. For example, an increase in invertebrate predators can reduce pest populations in agricultural or alter the overall biodiversity (Riechert et al. 1997; Lowe et al. 2014). While the significance of altered predator-prey interactions is well understood, there is little scientific research conducted on how predator-prey interactions are influenced by limited water availability (McCluney et al. 2012). The work that has been done suggests that acquiring more information about physiological traits relevant to water balance will be especially useful in understanding how water influences food webs, especially in the light of climate change (Chown et al. 2011; McCluney et al. 2012).

Often in scientific studies concerning terrestrial food webs, researchers focus upon energy or nutrients as the driving force of species interactions (McCluney and Sabo 2009). They

have observed that predators will actively consume prey that are rich in the nutrition they need to maintain a steady balance of nutrients (Mayntz et al. 2005). Water is rarely considered by animal ecologists, making this vital resource an unexplored topic in terms of pressures regulating terrestrial food webs (McCluney et al. 2012). Thus, we followed our examinations of water loss and desiccation tolerance with an experiment designed to investigate how prey hydration influences predator-prey interactions. We expected a hump shaped relationship between prey hydration and rates of predation as water provided by the crickets will not make up for the deficiency created by the spiders' activity or the conservation of the ingested food to feces (Hadley 1994). There is an inherent trade-off between activity and conservation of resources, as increased activity will increase water loss and spend energy (Chown 2002). Invertebrate predators will actively avoid this trade-off when possible.

## Materials and Methods

### *Evaporative water loss rates and desiccation tolerance*

The evaporative water loss rates and desiccation tolerances of the spider and the cricket were quantified through the use of gravimetric techniques. We recorded masses of 20 adult female wolf spiders and 40 juvenile house crickets while desiccating them inside an environmental chamber set at 30°C and 30%RH. The wolf spiders were wild caught from Arizona and shipped to the lab by an online retailer (Spider Pharm Inc Yarnell, AZ). The crickets were provided by the Bowling Green State University Herpetology Lab.

Each individual specimen was placed inside their own vial. The spiders were kept inside the plastic vial they were shipped in while the crickets were kept inside a 20ml scintillation vial. These vials were covered with pieces of bridal veil held down by a rubber band. A drying cabinet

was placed inside the environmental chamber, and all specimens were kept there throughout the duration of the experiment. Inside the drying cabinet, 8 handfuls of drierite wrapped in nylon stockings were scattered amidst the vials. Also, drierite was poured onto a tray and set directly beneath all the vials of the drying cabinet. The drierite was replaced every 5-7 days. Thus, the animals were maintained at approximately 0% relative humidity.

Cricket and spider masses were at stepped time increments, starting with measurements every 3hrs, but with longer intervals as their rate of mass loss reduced and stabilized. Masses were measured until death, upon which they were transferred to a desiccator cabinet kept at 55°C for 7-9 days and weighed once more to acquire a final dry mass. This dry weight was then used to quantify the hydration and water loss rate of all spiders and crickets at each time interval ( $mass_{tot} - mass_{dry} = mass_{water}$ ). Instantaneous water loss rate was then quantified in milligrams of water lost per hour. Desiccation tolerance was then quantified using the last wet mass taken before death.

### *Calculations*

Mean water loss rate was the summation of water loss for each individual up until the last time interval before any specimen died divided by the total number of hours passed. Mass specific water loss rate was calculated by taking the mean water loss rate for each individual and dividing it by their corresponding dry mass. Critical water content was calculated by taking the final mg H<sub>2</sub>O and dividing it by the corresponding wet weight for each individual. The time until death was calculated as a simple average time of death.

### *Prey hydration influences predation*

The second experiment was conducted under similar conditions as the first. There were 39 wolf spiders from the same distributor, all were sexed and identified as adult females. They were once again set inside the environmental chamber at 30°C and 30%RH. Each individual spider was housed in their own container (15.24cm x 23.2cm x 16.8cm). Spiders received a 30 day acclimation period before being subjected to a simple feeding trial. Throughout this period they were maintained inside the environmental chamber, given free access to water and fed 2-3 quarter inch crickets every two days.

Once the spiders were fully acclimatized they were subjected to the feeding trial. Each spider had their water and remaining food items removed. They were treated to 4 days of no food and water. After this starvation and desiccation period they were all fed 8 approximately fifteen mm house crickets from 1 of 3 hydration treatments (low, medium, high). The hydration treatments for the crickets were accomplished by desiccating the crickets under similar conditions for 22hrs, 12hrs, or 2hrs which comprised the low, medium, and high hydration treatments respectively. Sixteen crickets from each treatment were used as a subsample to validate that the hydration of each treatment was significantly different from one another. This was done by treating them to the same conditions as the other crickets but freezing them to death at -15°C for 12hrs and then desiccating them at 55°C. This was done to quantify the hydration state of each treatment in mg of water.

The crickets from the main sample were weighed before being fed to the spiders. Once they were given to the spiders 2 hours were allotted for predation to occur. After the 2-hour period, whatever crickets and cricket remains left in the cage were weighed. Also, the number of crickets killed by the spider was recorded.

*Calculations*

The feeding trial was analyzed by taking the average mg of cricket mass consumed which was calculated by finding the difference between the mass before and after the 2 hour feeding period. As for the subsample hydration state test, this was calculated by taking the difference between the wet mass and the dry mass over the wet mass, which was kept as a proportion of H<sub>2</sub>O.

### *Statistical Analysis*

The effects of body size (dry mass) on water loss rates and desiccation tolerance was assessed with an ANCOVA, examining potential differences between species. Responses included mean water loss rate vs dry mass, mass specific water loss rate vs dry mass, and critical water content vs dry mass. Data were transformed in all statistical tests in order to better fit model assumptions. Also, a t-test was conducted on the average time until death between the two species.

To examine potential differences in predation between prey hydration treatments simple ANOVA was conducted examining how the mass consumed by the spiders varied by treatment. We also used this approach to determine if hydration was successfully altered. All data in both studies were transformed using various approaches and analyzed in R statistical program.

### Results

Water loss for the spiders varied greatly early on during the desiccation period, then ultimately stabilized until death. Cricket water loss rate was relatively constant throughout the desiccation period. Water loss for both species was at its highest in the beginning. Spiders



greatly outlasted the crickets, with individual spiders living more than 900 hours longer than the longest lasting crickets.

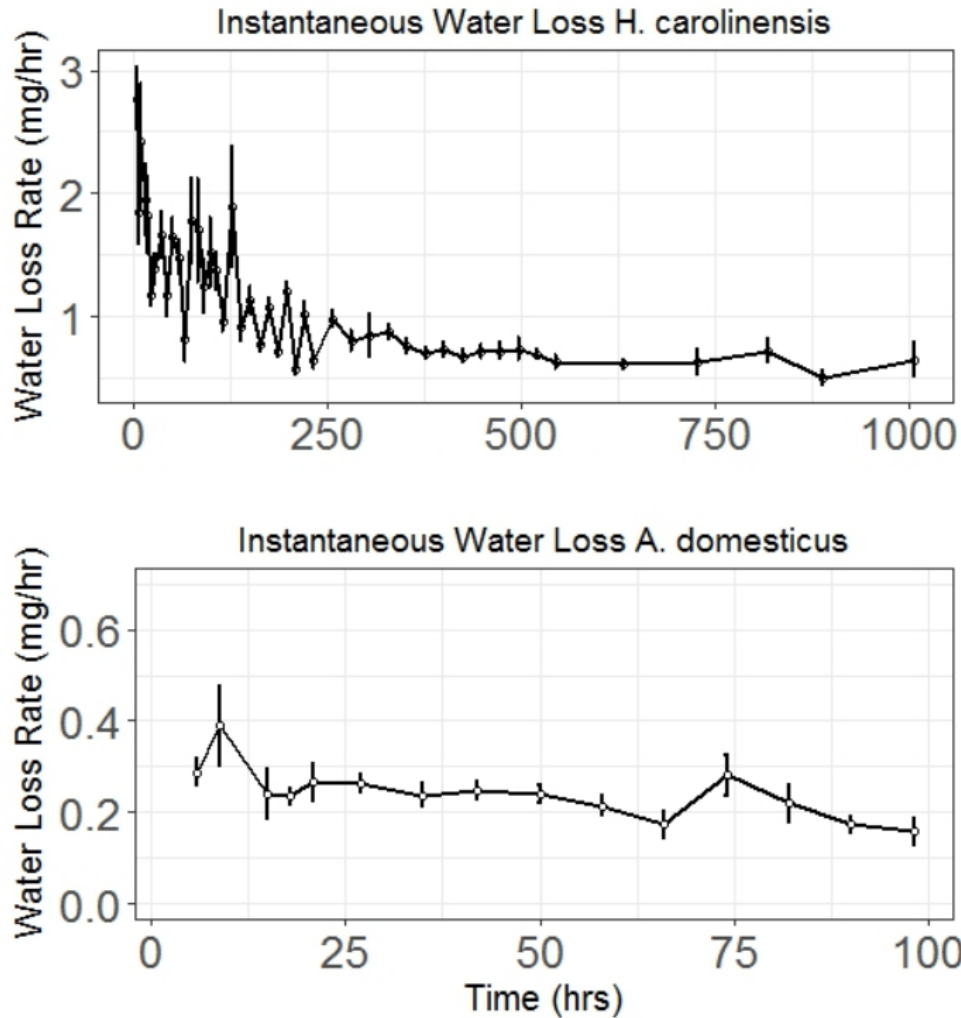
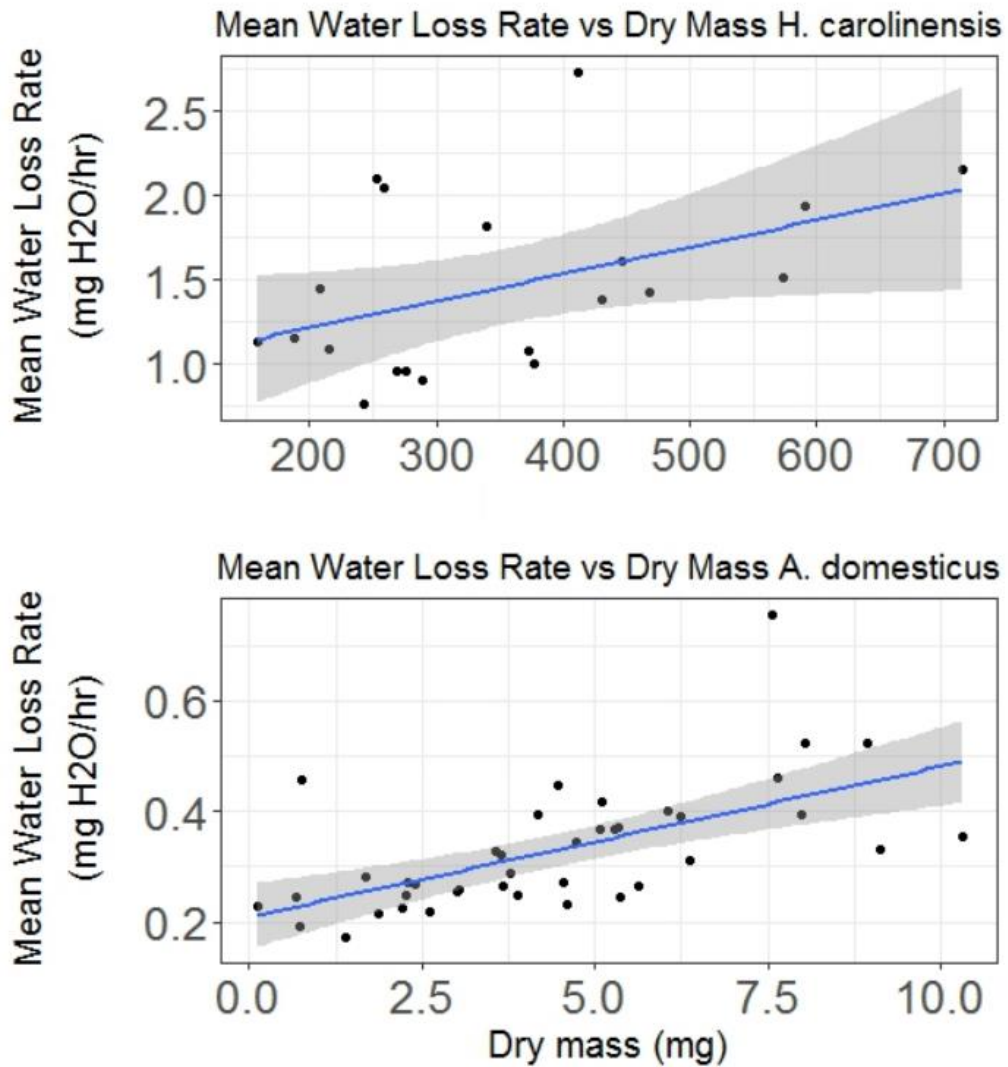


Figure 1 Instantaneous Water Loss for both species

With increasing body mass of either species there was an increase in mean water loss rate (Figure 2). Essentially, this means that crickets and spiders with large masses had greater total water loss over the same time span as smaller individuals. Spiders were on average much larger than crickets, and their mean water loss rates followed suit.



*Figure 2* Mean Water Loss Rates vs Dry Mass

Smaller individuals of both species lost more water per milligram of dry mass than larger individuals (*Figure 3*). This indicates that larger individuals lost more total water but smaller individuals were losing more water in relation to their body size.

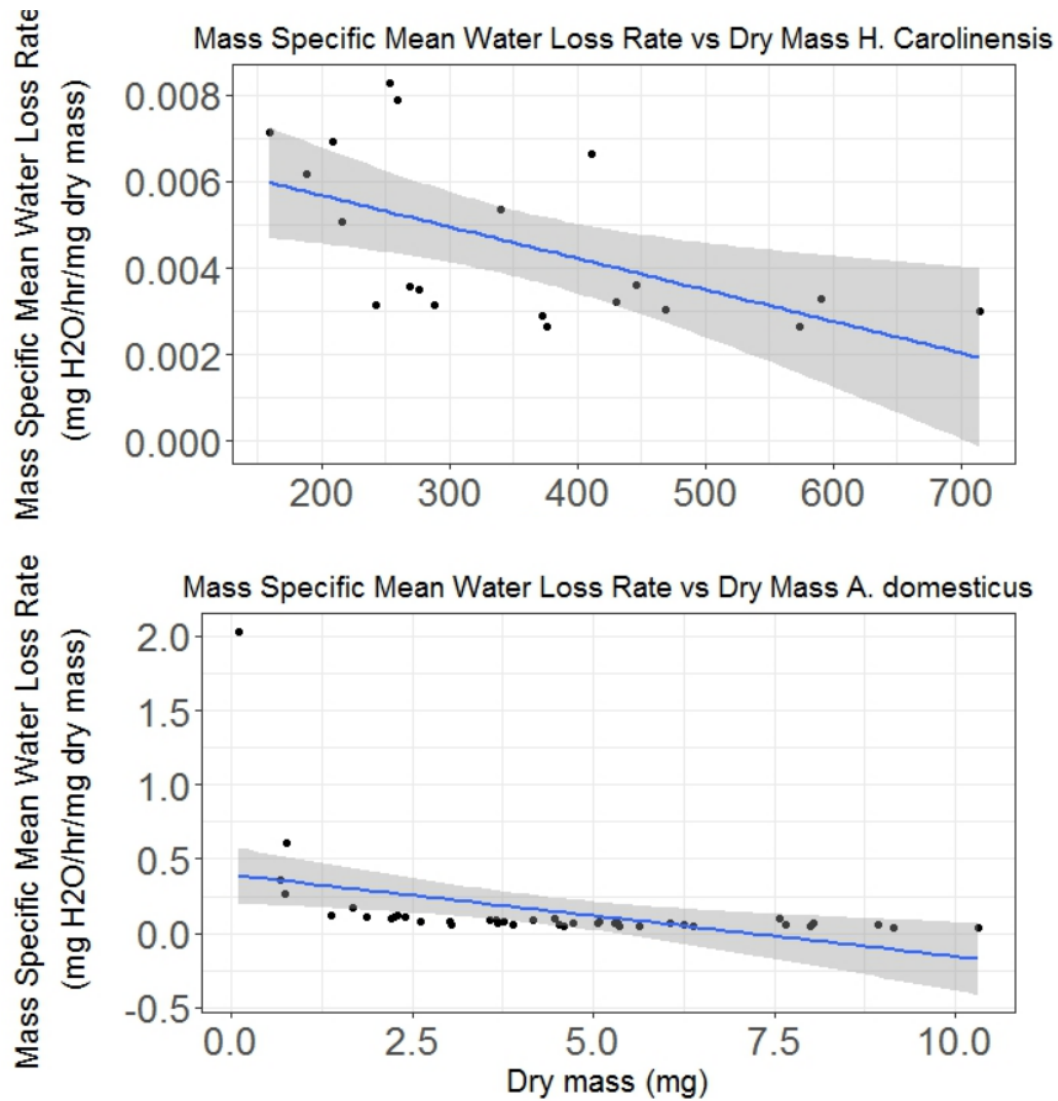


Figure 3 Mass Specific Water Loss Rates vs Dry Mass

Individuals of greater mass tolerated lower water content than smaller individuals for both species (Figure 4). These larger individuals had lower hydration, and better survived desiccation than smaller individuals.

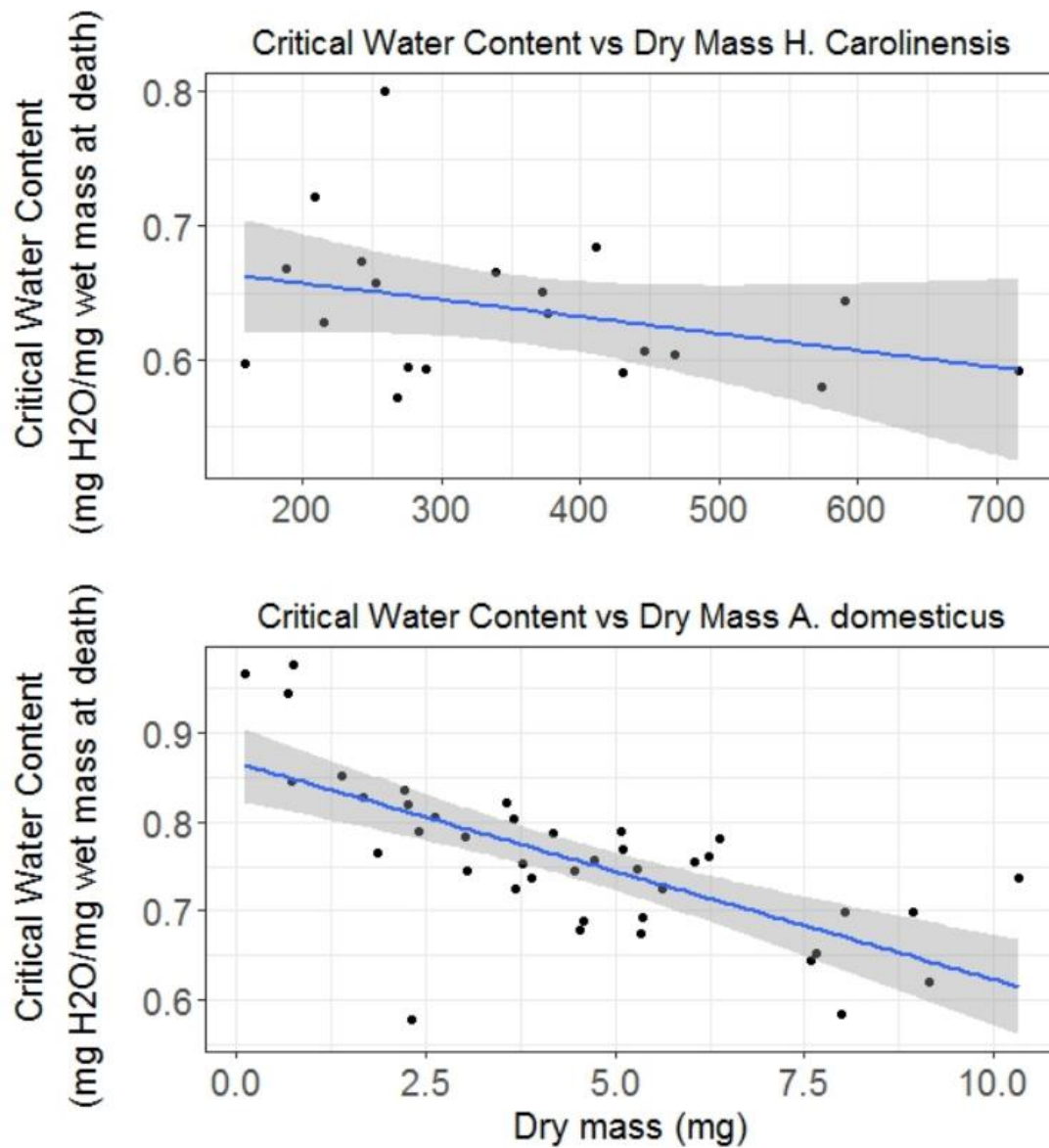


Figure 4 Critical Water Content vs Dry Mass

Only dry mass was a significant predictor of mean water loss rates of both species, indicating that total water loss per hour was not different between the species, only body size changed ( $F = 394.4$ ,  $Df = 1$ ,  $p\text{-value} < 0.0001$ ). As for mass specific mean water loss rate, there was a significant difference between the elevation of the two lines ( $F = 6.5$ ,  $Df = 1$ ,  $p\text{-value} 0.01356$ ) and the dry mass ( $F = 1460$ ,  $Df = 1$ ,  $p\text{-value} < 0.0001$ ). This indicates that in relation to body size species were losing water on different scales, with the spiders losing less water

compared to their body size. For critical water content, there was significant differences between the slopes ( $F = 11.8$ ,  $Df = 1$ ,  $p\text{-value}=0.001124$ ), the elevations of the lines ( $F = 42$ ,  $Df = 1$ ,  $p\text{-value} < 0.0001$ ) and dry mass ( $F = 56.7$ ,  $Df = 1$ ,  $p\text{-value} < 0.0001$ ). The significant difference between the slopes of the lines and their elevations concludes the species tolerated desiccation differently, with spiders tolerating more water loss than crickets in relation to their body size at high body sizes, but with the opposite at very small body sizes.

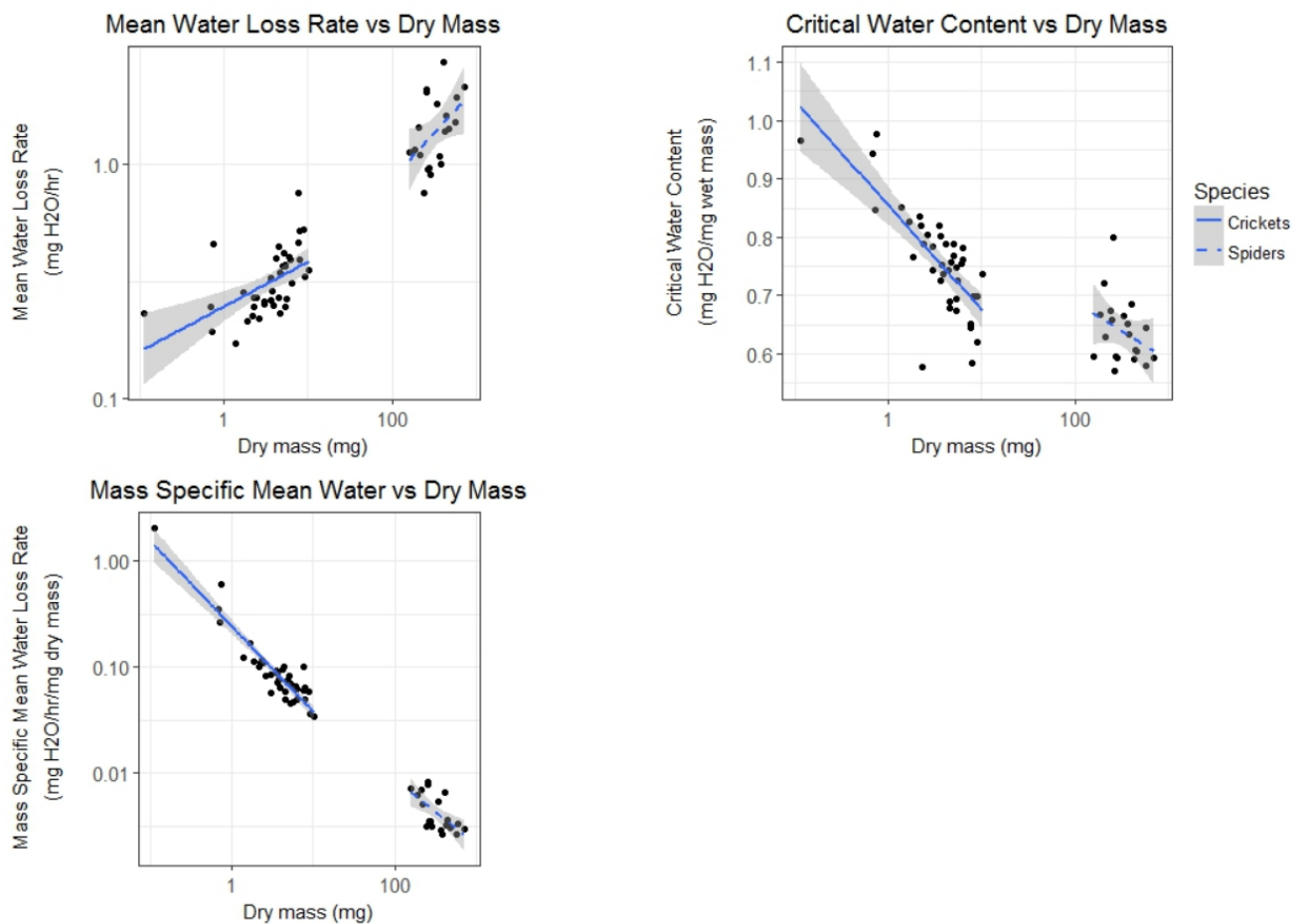
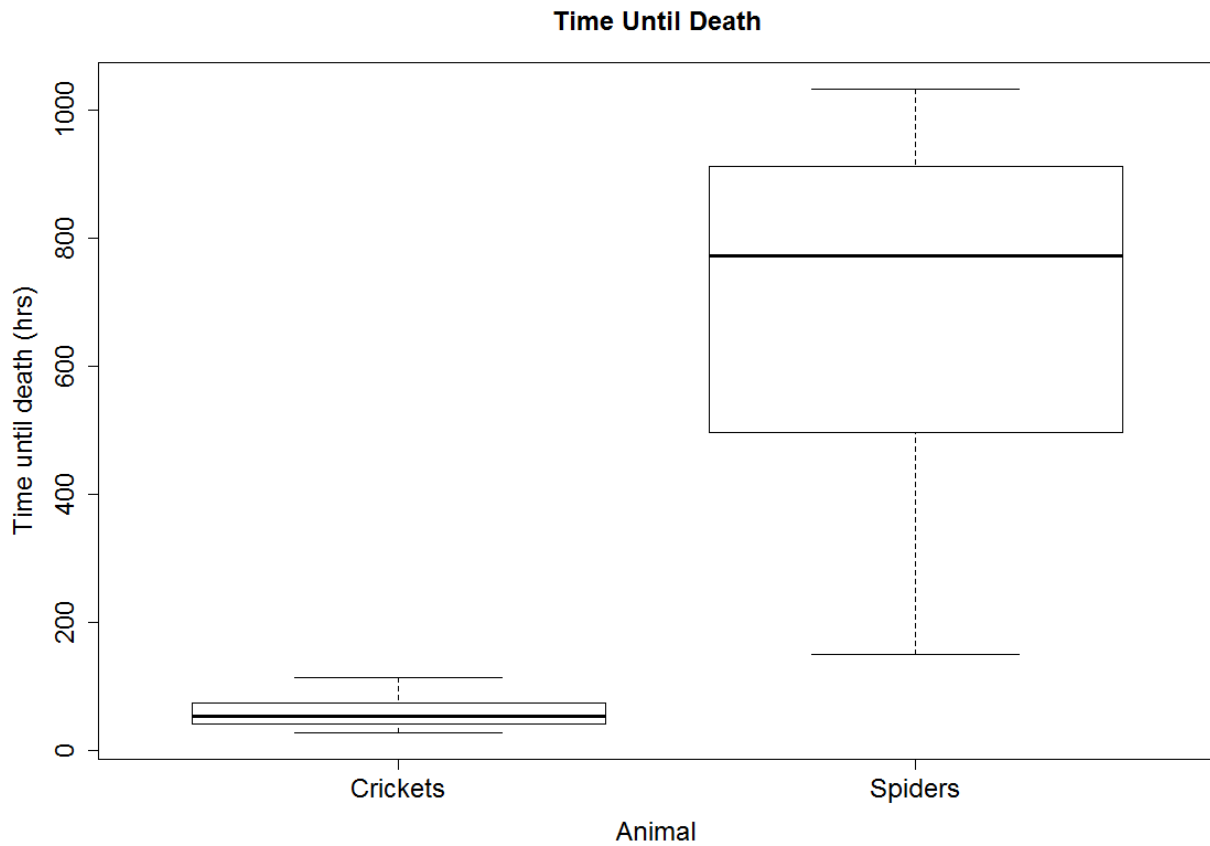
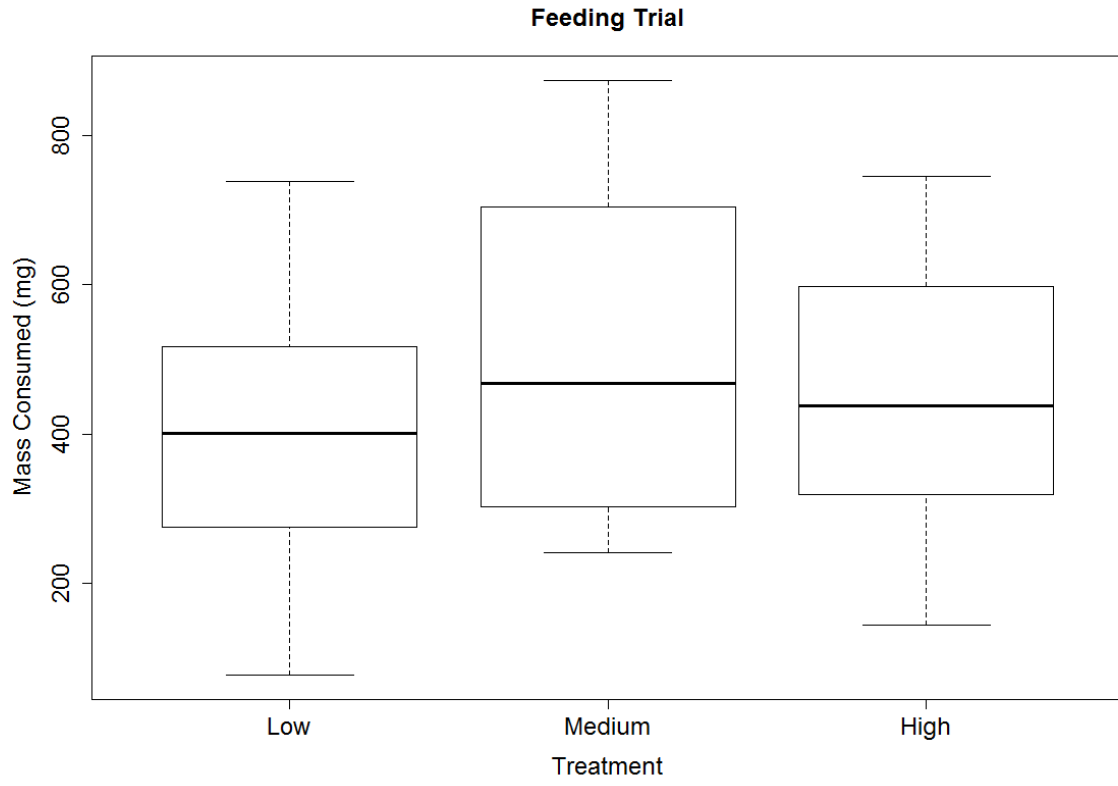


Figure 5 Ancova between species for all three tests



*Figure 6 Time until Death*

There was a significant difference between the mean time of death between species ( $t = -20.6$ ,  $n = 60$ ,  $p\text{-value} < 0.0001$ ). Spiders lived significantly longer than crickets, with the earliest spider death happening 50 hours past the last cricket death (Figure 6).



*Figure 7 Feeding Trial*

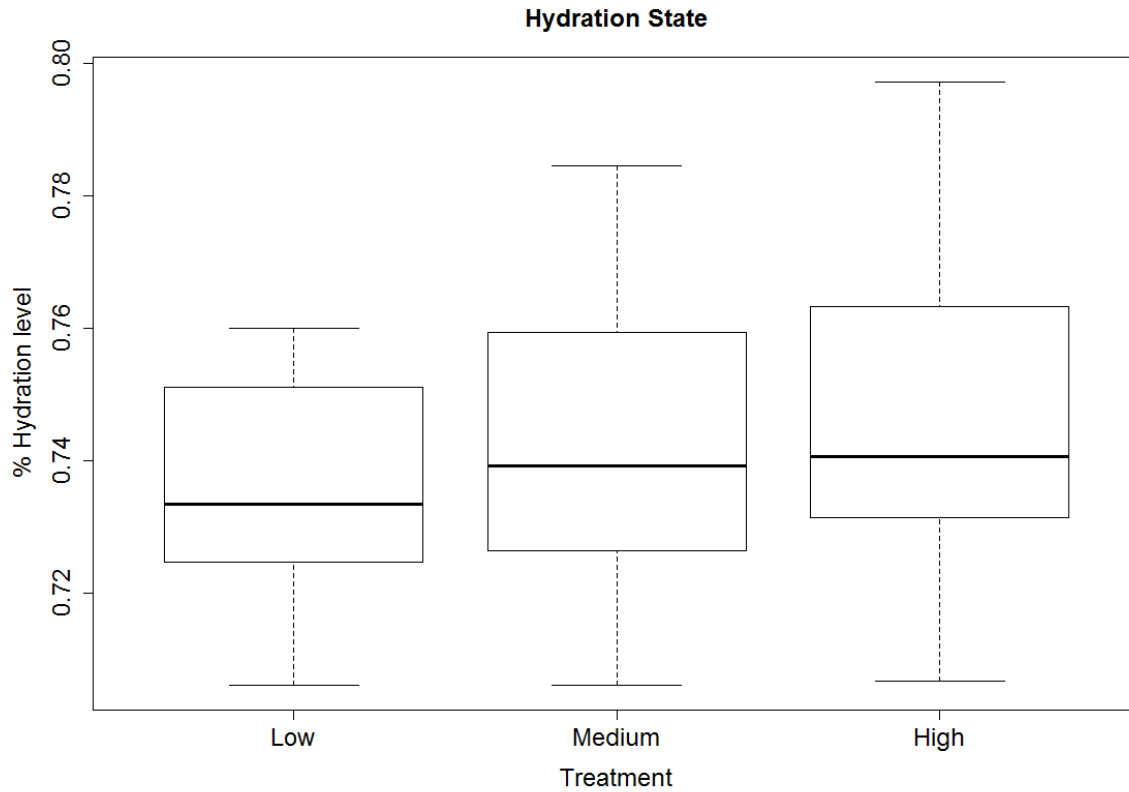


Figure 8 Subsample for hydration state

The statistical analysis conducted on the predation tests found no significant difference between treatments where  $p\text{-value}=0.4113$  (Figure 6). Though, there was a trend with the middle hydration treatment showing greater mass consumption this was ultimately not significant. The subsample also showed no significant differences between treatments ( $p\text{-value}=0.3841$ ), but once again there was a trend following the expected pattern (Figure 7).

### Conclusion



Here we show that differences between water loss rates and desiccation tolerances exist between two species of varying habitat preferences. While these differences may not directly correlate to the studied species' range and distribution, our data does reflect their respective ecological and morphological traits. The wolf spiders were far more desiccation resistant and tolerant compared to the house crickets, which is unsurprising given the fact the spiders were larger and that many desert invertebrates employ respiratory methods to reduce water loss (Bazinet et al. 2010). Though, how the spiders were able to achieve a lower water loss rate in relation to body size compared to the crickets cannot be explained at this time our data does show a significant difference. The desiccation tolerance of the spiders was also greater at higher body sizes, indicating that they were capable of surviving to a lower water content before death. Once again, whether or not this was due to adaptations to varying habitat preferences or simple morphological differences between the species cannot yet be explained.

Our data collected on the instantaneous water loss rates of these two species, to the present knowledge of the authors, appears to be somewhat novel, but it follows expectations acquired from theory. The spiders lasted much longer than the crickets due to a greater desiccation resistance and lower surface area to body volume ratios. Increased desiccation resistance in invertebrates can be achieved by three methods, 1) reducing the rate of water loss, 2) increasing bulk amount of water available to lose, and 3) increasing amount of tolerance to water loss prior to death (Gibbs et al. 1997). The spiders were much larger than the crickets, so it was expected that they had more water to lose in their tissues or stored in molecules such as lipids. Also, the spiders had a lower surface area to body volume ratio compared to the crickets. Evaporative water loss decreases with decreasing surface area to body volume ratio, so larger insects, with more spherical shaped bodies, are more resistant to water loss (Fouet et al. 2012).

Studies conducted on *Drosophila* as well as other tropical insects have also found size-resistance relationships (Hoffmann et al. 1999; Bujan et al. 2016; Parsons 1970). Though, the larger individuals had more water to lose they were ultimately better at resisting desiccation than their smaller counterparts. Our data suggests a similar pattern.

The critical water content of both species point to interesting conclusions that should be further explored in future studies. Theory suggests that smaller organisms are more tolerant to water loss, meaning that smaller organisms can survive with less water prior to death than larger organisms (Alpert 2006). This can be observed in many of the worlds studied organisms that are highly desiccation tolerant (e.g. tardigrades, nematodes). These desiccation tolerant organisms are incredibly small, and can survive extreme periods of low body water content (Alpert 2006). When comparing the critical water content of both species to dry mass it suggests that larger individuals were capable of surviving with lower water content than smaller individuals. This the opposite of what would be expected based on this theory. We don't currently have a hypothesis that could explain this trend, but we believe it requires further investigation.

The differences in mass specific water loss rates and critical water content, in relation to dry mass, reinforces the importance of this study. There are several possible explanations for the differences between taxa. First, the wolf spiders used in this experiment were collected from the desert. Second, spiders and crickets have different body shapes and the more spherical body shape of female spiders could reduce surface area to volume ratios and reduce water loss.

The data collected on prey hydration influencing predation was not significant but a trend does exist that aligns with the hypothesis of this study. There was more consumption of mass by the medium hydration treatment but it was not a significant difference. This could be due to hydration treatments not being significantly different from other another. Future studies should

explore additional methods of altering hydration of invertebrate prey to achieve a greater difference between hydration treatments, which could yield a significant difference between the consumption by the predators according to their treatment.

With the onset of climate change and extreme weather events, our data provides insight on how individuals of these species might respond. These invertebrates will have to adapt to a greater frequency of water stress and unpredictability, potentially altering population dynamics and species interactions. Drying environments might see ecological shifts of invertebrates towards larger sizes. While larger invertebrate species could see expansions in their ranges as drought and altered precipitation patterns affect terrestrial water relationships. Ultimately, larger individuals of these two species are better equipped to handle water stress enacted by climate change, meaning these two species might see dynamic shifts towards greater sizes in the future.

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