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Effect of Temperature and Light on Burrowing Activity in Wolf Spider (*Rabidosa punctulata*)

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ABSTRACT

Many organisms possess adaptations that allow them to survive in variable environments.In particular, species that reside in the Midwest are exposed to environments that widely fluctuate across a number of factors, especially temperature. How do ectotherms living in these habitats deal with continuously fluctuating environments? One taxa, wolf spiders, commonly burrow underground as a tactic to get out of harm's way. Here, we tested what environmental factors, temperature or light levels, influenced the activity levels and burrowing activity in the wolf spider *Rabidosa punctulata*. Our experiment used a fully crossed design varying both temperature (low: 4.4° C vs. high: 26.7° C) and light levels (light vs. dark). In total, we conducted 80 trials (*N* = 20/treatment).The spider's movement, burrowing, and use of an artificial tent were monitored. Both temperature and light levels influenced the spider's activities. We found that both temperature and light affected spider activity, while only temperature affected burrowing behavior. Spiders were the most active when warm and in the dark, and they were more likely to burrow in the cold. This study provides insight into how various organisms, like ectotherms, may alter behaviors to deal with extremely variable habitats especially those that rely on the environment for various bodily processes.

Keywords: *Rabidosa punctulata, temperature, light, activity, burrowing*

INTRODUCTION

It is no overstatement to claim that spiders inhabit an incredible variety of terrestrial habitats, being found on every continent except Antarctica (Turnbull, 1973). Each of these environments varies considerably across a number of different environmental conditions (e.g. temperature, light intensity, wind, moisture, predators etc.; (Foelix, 1996). This spatial variation in habitats has selected for incredible diversity in adaptive traits across spider species for a number of traits (e.g. life history, morphology, behavior) (Gibb, 2002). While many spider species inhabit regions with relatively stable or constant environments, environmental variables such as light (day vs. night), humidity, and temperature can also fluctuate on relatively short temporal scales (daily, hourly, seasonally) that are relevant to short-lived arthropods. For example, the wolf spider (Lycosidae), *Pardosapullata* lives on top of sphagnum moss, and experiences large fluctuations in temperature, whereas the wolf spider, *Piratapiraticus* lives more in the stem region of the moss, which is a more constant temperature environment (Norgaard, 1951). High humidity greatly affects the webs of *Argiope* and *Nephila* prey capture performance significantly increased for *Argiope* while the improvement was less dramatic for *Nephila*. (Boutry and Blackledge 2013) Jumping spiders truly value that quality and quantity of light while searching and attacking prey, such as with the jumping spider genus *Plexippus,* where the green sensitive photoreceptor cells in the eye to judge the distance of prey. (Nagata et. al. 2012) In courting success, the *Habronattuspyrrithrix* male jumping spiders experience greater success in their courtship with their brilliant ornaments, but only in the sun. (Taylor and McGraw 2013) How does this constantly fluctuating environment affect a spider's biology, ecology, behavior, and activity levels and have spiders continuously exposed to this variability evolved specialized behaviors to help cope with their consistently inconsistent environment.

Spider biology, ecology and behaviors have been found to be sensitive to a variety of fluctuating environmental factors. In particular, large-scale fluctuations in temperature are particularly important for arthropods, since they are ectothermic.

Temperature is known to influence a variety of aspects of spider biology, such as metabolism, blood pressure, reaction time, activity levels, and other behaviors (Foelix, 1996). For example, spiders in warmer sites were found to be more aggressive towards prey, were more active, and more tolerant to conspecifics than spiders found in colder sites. (Pruitt et.al. 2011) In fact, if spiders are left unprotected from the extreme heat, the water loss will be fatal and the spider will die (Schmalhofer, 1999). Because of this intimate connection between temperature to spider's lifestyle and the dramatic consequences of reaching temperature extremes in body temperature, spiders have evolved a number of ways to deal with the seasonal change of temperature in their environment. Spiders that remain in these conditions have acquired several different physiological and behavioral adaptations to survive daily extreme changes in temperatures (Foelix, 1996). Many spiders enter a dormancy phase, reducing metabolism and activity, in response to lower temperatures (Roberts 1978), This behavior coupled with natural anti-freeze molecules in theirhemolymph help spiders avoid being frozen to death, also found in other athropods, like beetles (Roberts, 1978) (Duman 2001) (Liou et. al. 1999). One way to enhance this survival technique is the choice of appropriate microhabitats (e.g. leaf-litter; Foelix 1996) or locations that act as insulated zones that shelter the spiders from extreme changes in temperature and also desiccation (Edgar and Loenen, 1974). One such location that could enhance survival is underground burrows.

Many spider taxa are known to use burrows, either by constructing their own, or finding a pre-existing one, such as the *Lycosacarolinensis* Walckenaer (Araneae, Lycosidae) (Shook 1978). The size and structure of burrows differs considerably. The burrows from certain spiders could be from 4-18 cm deep, depending on the size of the spider and season. (Humphreys 1973) The *G. hubbelli* always builds a turret from leaf litter at the entrance to its burrow but *G. xeraarchboldi* does not build any kind of turret at all, while the genus *Geolycosa* will construct their burrow in a nearly vertical, cylindrical tunnels. (Wallace, 1942). Burrows may offer a reprieve from unsuitable conditions (i.e. lack of water), and may maintain temperatures more suitable for the spider than at the surface. (Shook 1978) (Humphreys 1975). However, spiders all around the world utilize burrows suggesting many other potential benefits to burrowing (Richter, 1971; Blanke, 1973a). Given all these potential benefits, further research is needed to explore what environmental factors promote burrowing behaviors (i.e. production of and/or use of a burrow).

One group of spiders that may lend some insight into this question, is the wolf spider, *Rabidosa punctulata.* These spiders are distributed throughout the South and Mid-West regions of the United States. While, *R. punctulata*, is known to create its own small burrow with an average depth of 4 cm (Nicholas, 2005), it is also a facultative burrower, using preexisting underground holes or structures (pers. communication with G. Stratton). Spiders found throughout the Midwest deal with continuously fluctuating temperature, both seasonally (range: 41.7˚ C to -17.8˚ C) and daily. During this time of year, spiders could experience daily temperatures fluctuations as great as 15.6˚ C in a single day. In addition to temperature fluctuations, *R. punctulata* also deal with natural light fluctuations, as they have been found to be active during both day and night (pers. observation). Thus, changes in either factor could influence the use of burrows by this spider. Here, we investigated the relative influence of light and temperature on the frequency of burrow use and activity levels in the wolf spider, *R. punctulata*.

MATERIALS AND METHODS

I collected 318 immature spiders from Lancaster County, NE on August 24-25, 2012. By collecting the spiders at this time, they will have matured into virgin spiders under supervision. I only used only 80 virgin females that have not had been exposed to males. All spiders were caged separately due to the fact the spiders are cannibalistic in nature and each spider were provided water ad libitum and fed 3 crickets per week.

All trials were run from October 7, 2012 – November 12, 2012, which coincides with the time of year they are naturally active. October is also usually the time where the temperature starts to fluctuate from the warmer side of the thermometer (roughly 26.89° Celsius) to the colder side of the thermometer (roughly 7.22° Celsius) in the Midwest. (weather.com) Using the extreme ends of temperatures the spider could face during this time of year, the temperature used for the hot setting was 26.67° C and the cold temperature was 4.44° C.

Females used in the trial were 15-27 days old after their maturation date. On the day of the trial, they were first dotted with a white Elmer's® Painters paint marker on the lower part of the cephalothorax and at times, upper part of the opisthosoma. This was done to track the spider better during the dark setting of the trials. All spiders were dotted to maintain consistence throughout the experiment. Next, we weighed each spider using a glass vial on a scale (brand, type). After this, they were introduced into the trial arena to begin trials. This trial arena was a 22 cm diameter by 8 cm high Circular Plastic Arena (CPA) with an open top. There were clear walls as to see what the spider's activities are. The floor of the CPA was completely covered with Greensmix® sphagnum peat moss that was roughly 3.5 inches thick for the spider to walk on, rather than the smoother plastic floor of the CPA to simulate a more natural walking surface. In the peat moss, two artificial holes or burrows were constructed out of two glass tubes with black electrical tape around to not let light in and were partially submerged in the peat moss at opposite ends of the CPA. These burrows were put at an angle so the spider may easily crawl in and out when it felt the need to do so. Also a 6 cm by 5.5 cm paper tent was folded up and placed in the middle of the CPA in case the spiders do not wish to use the burrows, but still want to get out of the light.

Once in the arena, the spiders were subjected one of four potential environmental treatments. I varied both the temperature (Warm: 26.7° C, Cold: 4.4° C) and light levels (Light vs. Dark).I performed all possible combinations of these treatments (i.e. Light/Warm, Light/Cold, Dark/Warm, Dark/Cold).Temperature levels for each treatment was determined based on the spiders experience during the month of October. All trials were performed in a Percival® Intellus environmental controller Model: E-36L, which allowed us to simultaneously control both environmental variables. The CPA was put on top of a piece of 3.3 cm thick foam padding and a 2 cm thick piece of granite, to reduce vibration of the arena.

Trials lasted 30 minutes. Each trial was recorded by a Sony® Handycam HDR-CX12. Trials performed in complete darkness were recorded using the night-vision option. Each trial began once the spider was introduced to the arena. Trials were scored from the video for each of the following behaviors: time in motion (TIM), number of times under the paper tent (UT), time in tent (TIT), times burrowed (#B), and time in burrow (TIB). I made sure that I always followed the order of hot/light, hot/dark, cold/light, cold/dark to try and minimize factors of the time of day, and day tested. After each trial, the peat moss and paper tent were disposed of, and the CPA and the glass vial wrapped with black electrical tape were rinsed with water, then 70% ethyl alcohol, once again with water, and finished by drying of a paper towel, so the CPA is clean each time of spider excreta and silk cues.

After the data was collected, a logistic regression was done on the burrow probability to the environmental treatments as well as the tent use probability. A Kruskal-Wallis test was done on the non parametric data of the time in burrow relative to treatments. Time in motion required a least squares regression test. The data for time in motion was square rooted to make it show more of a parametric distribution. All results are presented as mean ± standard error.

RESULTS

In total, 80 trials were conducted (N=20 per treatment). There were no differences across the groups in either female age or weight. (Age: F=0..8388, P = 0.4768 Weight: F = 0.0680, P = 0.97768). In all trials, spiders actively moved throughout the circular plastic arena (CPA). The spiders tended to keep close to the walls of the CPA and many, if not all, the spiders attempted "escape attempts" multiple times during their trial run. Spiders used the burrow in 53% percent of trials, but rarely used the artificial tent structure (5% of trials). A few spiders immediately found the burrow and used it, while the vast majority moved around the CPA before burrowing. Interestingly, facultative burrowing in natural substrates was witnessed in one trial, where the spider found a small hole in the peat moss substrate of the CPA and stayed there the remainder of the trial. This was not counted as a "burrowing attempt".

The environmental treatments influenced the likelihood of a spider entering a burrow during a trial (Overall Logistic Regression Model: χ_3^2= 59.84785, P=<0.0001). The temperature of the arena significantly influenced burrowing activity (Likelihood ratio, χ_1^2= 59.6472684, P = <0.0001; Fig. 1), while light levels did not affect burrowing (Likelihood ratio, χ_1^2 = 0.59675884, P = 0.4398; Fig. 1). The spiders that experienced the cold treatment were much more likely to enter the burrow than the spiders in the hot treatments (Fig. 1). In addition, the overall time spent in the burrow during a trial followed the same overall patterns. (Kruskal-Wallis test, Overall Model: χ_3^2 = 48.81, *P* <0.001; Cold/Dark N=20 б =1043.30±142.27; Cold/Light N=20 б =1368.95±99.65; Hot/Dark N=20 б =49.50±49.08; Hot/Light N=20 б =196.95±110.29)

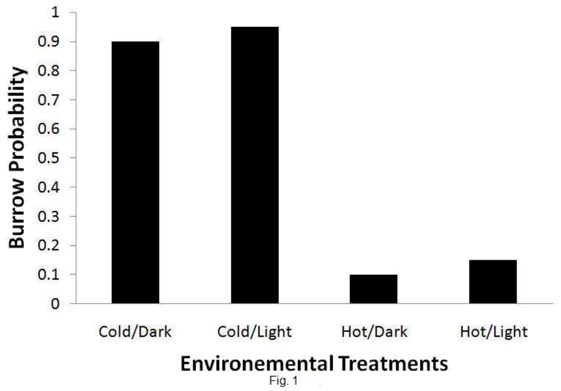

Fig. 1

The environmental treatments not only affected the burrowing behaviors but also the overall activity of the spiders during a trial (Overall Model: F=5.6780, P=0.0015). The overall time in motion (TIM; square-root transformed) was affected by both environmental factors independently, but not via an interaction between the two(Temperature F = 5.1138, P = 0.0265; Light F = 10.6954, P = 0.0016; Temperature*Light P= 0.76 Fig. 2) Spiders spent more time in motion in the hot treatment compared to cold and likewise spent more time in motion in the dark treatment compared to the light (Fig. 2).

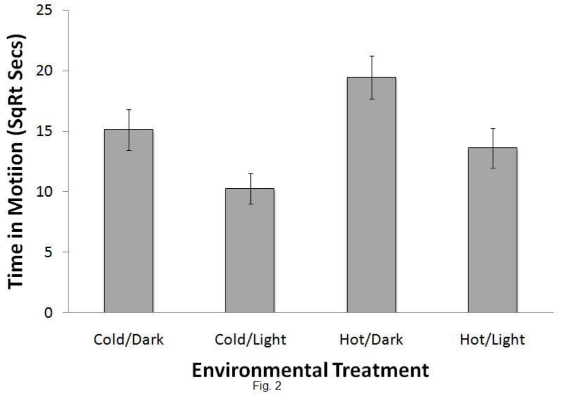

Fig. 2

The artificial tent was used very little. While the use of the tent was not significantly explained by our environmental factors (Overall Logistic Regression Model, χ_3^2= 6.913411, P = 0.0747), some patterns may deem future investigations. Spiders tended to use the tent differently based on light conditions, using it more often in the light when compared to the dark (Likelihood ratio, χ_1^2= 5.756, P= 0.0164), while temperature had no apparent affect. (Likelihood ratio, χ_1^2= 1.099, P= 0.2945).

DISCUSSION

It was clear that the spiders were burrowing with a higher success rate in the variation of temperature rather than the variation in light. The time in burrow was also significantly higher for temperature and not so much for the light. The tent was used minimally used, but when it was used by the spiders. the light was favored over the temperature. However, due to the very small sample size, further research would be needed to confirm this. the time in motion was affected by both environmental factors, but there were no interaction between the two. In general, the changes in light seem to have a greater affect than the change in temperatures on the activity levels.

During trials, spiders tended to use burrows when the environment was colder. There are several possible explanations for *R. Punctulata* to seek burrows when the temperature may be unfavorable to them especially in the colder end of the spectrum. Since they are ectotherms, their blood pressure drops and therefore have a harder time moving about (Tattersall et al., 2012). Sensing that their bodies may no longer be able to escape predators by locomotion, the spider's next best bet is to hide itself completely from view. Another possible explanation may be that the spider is trying to escape the cold by going underground where it is warmer. This practice is not uncommon as other animals engage in burrowing for warmth as well such as G. agassizii tortoises, and *Reticulitermes flavipes* termites. (Morafka et al., 1981, Clarke M.W. et al., 2013)

Light didn't seem to have an effect on burrow use and may have to do with *Rabidosa punctulata* only using the burrows to regulate body temperature and is not really used to actually hide from things such as predators.

Spider movement was affected by variation in both temperature and light. Changes in light levels tended to have a greater effect on *R. Punctulata* activity. One explanation for this is most predators who eat spiders such as R. Punctulata see better in the light compared to the dark like diurnal birds. If the spider is exposed to the light, it makes the predator's job easier to find them. Also, the extra movements of R. Punctulata possibly make it stand out compared to its relatively static environment. The movement affected by temperature is to be expected as ectotherms rely on warmer temperatures to move properly. The colder temperatures have slowed down the movements of the spider to a slow crawl and was not nearly as dynamic and didn't seem to move much once it become conscious of the fact that the temperature was not getting warmer. So, the spider seem to realize that no movement would be better than slow movement that once again, might cause it to stand out from its surroundings.

While the artificial tent was minimally used, this object represents a real option for spiders to use in their natural habitat. The ectotherm may not wish to burrow when it can just as easily hide under a leaf or a branch or even run away. One reason that *R. punctulata* may not have used the tent in this study as much was because of its color. Being white makes this cover object very bright both on the top and underneath, which may not be conducive for a hiding spot when compared to a dark burrow or a brown leaf in their natural environment. Future studies should include a more natural cover object to investigate usage patterns and the environmental variables that affect them..

The thing that I found out most is the potential activity levels of the R. Punctulata. It seems that even though this spider seems to be active during the day, the activity levels at night may be relatively higher than during the daytime. The spider realizes its shortcomings, such as slower movement in colder temperature, or higher exposure to predators during the day and adjusts according to these obstacles. R. Punctulata take whatever they can from their environments to live that extra day. At all comes down to survival, and every living thing tries to thrive to survive.

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