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Brandon Hogland
Harding University, bhogland@harding.edu

Ryan Stork
Harding University, rjstork@harding.edu

Amber Hug
Harding University, ahug1@harding.edu

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Cover Page Footnote

Acknowledgements We would like to thank Harding University for the use of lab space to carry out our research. We would like to thank Noah English for his efforts in collecting spiders and assisting with the data record.

A Description of Variation in Fecundity Between Two Populations of Wolf Spider *Rabidosa rabida* in Searcy Arkansas Using Brood Size Measurements

B.A. Hogland*, R.J. Stork, and A.G. Hug

Department of Biology, Harding University, Searcy, AR 72149

*Correspondence: bhogland@harding.edu

Running title: Fecundity Variation in Local *R. rabida* Populations

Abstract

Fecundity, a very important population variable, can be estimated by measuring the number of juveniles hatching out of individual egg sacs. *Rabidosa rabida* is a large wolf spider that is common in Arkansas and much of the eastern portion of North America. This study attempts to expand previous estimates of variation in fecundity made for this species by Reed and Nicholas in Mississippi. In an attempt to determine baseline variation in a common arthropod predator, we hypothesized that a significant variation would be found in fecundity estimates between two populations of *R. rabida* in Arkansas. We also hypothesized that this variation would be similar to the variation reported in Mississippi. Two populations of *R. rabida* were collected in late August and early September of 2016. The egg sacs were allowed to hatch while both the mothers and juveniles were placed in alcohol, with the exception of twenty from each mother which were photographically measured. A comparison was made between the two populations and between variation measured by Reed and Nicholas. We found significant variation between brood size of the two populations in Arkansas similar in magnitude to what was found in Mississippi. We did not find any significant difference in size of juveniles or mothers between the two locations similar to what was found in Mississippi. Observing patterns in these traits provide a starting point for comparison to future measurements which may aid in quantifying differences in populations. A lack of descriptive data for arthropod species has been a challenge in ecological and conservation studies.

Introduction

Fecundity is defined as "the actual reproductive rate of an organism or population, measured by the number of gametes (eggs), seed set, or asexual propagules by an organism" (Van de Valle 1982). Since fecundity is highly plastic, meaning that it can be manipulated or affected by changes in the environment, it is widely studied in many different organisms. Fecundity studies help researchers gain insight into various life history traits as fecundity is directly related to the amount of energy involved and distributed within certain species (Llodra 2002; Head 1995). Fecundity of Arthropods has been a focus of biology for determining life history traits, including the role of diapause due to climate change (Llodra 2002; Head 1995). Insect and Arthropod life cycles necessitate a dormant phase which occurs during the winter (Bale and Hayward 2010). This period of diapause is important for the cycle of development of various organisms. Spider survivorship and propagation can be influenced by a variety of environmental conditions such as temperature, prey availability (Nyffeler and Birkhofer 2017), and home range from the nesting site. *Rabidosa rabida* is large wolf spider found across eastern North America. This spider is found in the grasslands and areas of low vegetation, (Brady and Mckinley 1994, Eason and Whitcomb 1965). The wolf spider typically has a single reproductive event during which the female produces a large number of offspring. A small number of offspring are predicted to survive until maturity, showing a type III survivorship curve (Edgar 1971). Brood size, or the number of juveniles produced in a single reproductive event, can be seen as a positive correlation with size of the mother. Brood size can also be correlated with environmental influences, such as temperature and elevation which affect overall metabolism, thus affecting energy allocation and overall reproduction (Punzo and Farmer 2006; Bale and Hayward 2010; Bonte *et al*. 2008). Reed and Nicholas (2008) researched gene flow and fecundity within two populations of *R. rabida* geographically located in Mississippi. In our study, we wanted to expand upon previous research by examining the same species for similar fecundity variation in different geographical areas within the described distribution of

R. rabida outside of Mississippi. Our secondary goal was to provide illustrations of the current changes in spider fecundity as well as highlight potential change that could occur in respect to future research on this topic. We hypothesized that there would be significant difference between mother size and brood size with no significant difference in offspring size and location similar to the work done in Mississippi. In our study, we measured fecundity as a means of predicting future applications of life history traits over multiple generations. Descriptive biological research of *R. rabida* is lacking. Further description and research is necessary to describe a better understanding of these fecundity changes during times of environmental change.

Materials and Methods

Spiders were collected from 2 locations in Searcy, Arkansas in White Co. using the spotlight method (Wallace 1937; Eason and Whitcomb 1965). Spider collection sites were chosen based on the proximity to the university lab and legal permissions available to collect samples. Ecologically these sites were similar to sites studied by Reed and Nicholas. The first location was along a bike path running through Berryhill park (35.260680, -91.718951) and the second location was a powerline right of way on land owned by Harding University on the west side of Searcy (35.354529, -91.641870). Beginning in late August, 47 spiders without egg sacs were collected and kept in the lab at Harding University in 16x14.5x8 cm clear plastic boxes with water provided ad libitum via shell vials stoppered with cotton. These spiders produced egg sacs while in captivity. As soon as juveniles emerged and the egg sac was dropped, the mother and offspring were placed in isopropyl alcohol and the date of egg sac creation, hatching, and preservation were recorded for later analysis. Photograph measurements of the juvenile spiders were taken utilizing a millimeter ruler underneath a clear petri dish in which the spider was placed (Figure 1).

In early September, we went back to Berryhill park and Gilliam farm and collected spiders that had already put out egg sacs and brought them to the lab. Juveniles emerged and were placed in isopropyl alcohol following the same methods as described above. When 47 spiders had been preserved, we counted the number of juvenile spiders in alcohol and added the number kept out of alcohol for measurements and recorded these numbers. Measurements of the juveniles from each mother were also taken from the photographs.

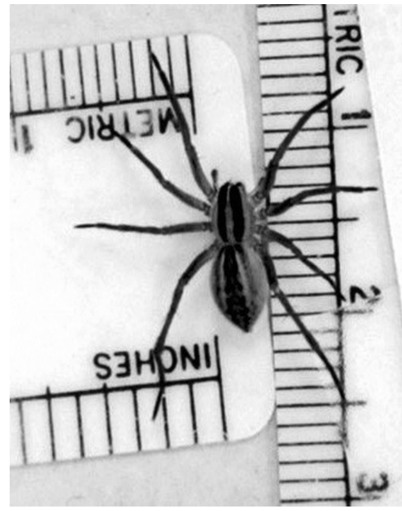

Figure 1. Photograph measurement of juvenile carapace length (millimeters).

We then used calipers to measure the carapace, length, width, and body length of each mother. We compared brood size between spiders who produced eggs sacs in the lab and in the field. We also compared carapace length of mothers from each sample location.

We performed an ANCOVA using brood size as the dependent variable, the location of spider capture as the independent variable, and the mother's carapace length as the covariate. Carapace length (CL) was used rather than the more traditional carapace width (CW) due to CL having a greater significant impact than CW (Stork 2011). A second ANCOVA was performed using the mean CW of the juvenile spiders from each mother as the dependent variable, the location of mother's collection as an independent variable, and mother's CL as a covariate. We performed an ANOVA using mother carapace length as the dependent variable and location as the independent variable. Descriptive statistics for brood size and juvenile size were calculated and graphed. SYSTAT software was used for all statistical analysis guided by James F. Rohlf (2001). Results were compared to data previously reported by Reed and Nicholas (2008).

Results and Discussion

We found significant difference in the brood size between locations (ANCOVA: p=0.004, MS =66,805.366, N=47). The mean brood size per egg sac for the Berryhill Park population was 330.391 $\pm$ 108.612 and 262.375 $\pm$ 77.366 for the Gilliam Property population (Figure 2).

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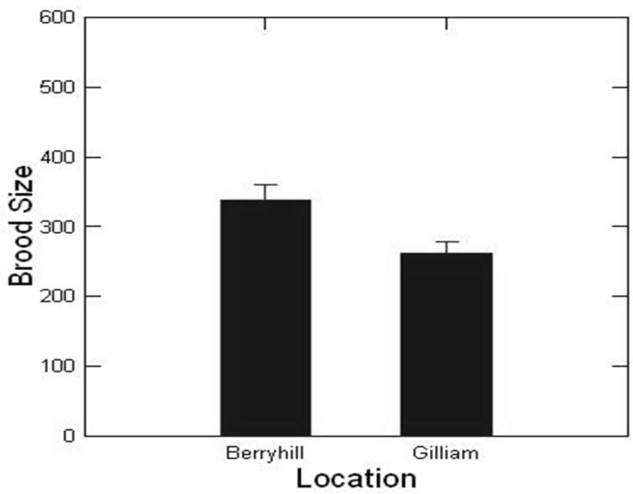

Figure 2. Differences in Brood Size based on location. Standard deviation bars are indicated.

We did not find significant difference in the mean body length of the juvenile spiders measured immediately upon exit from the egg sac between locations (p=0.306, MS=0.495, N=940). The mean body length of the juveniles in each egg sac was 5.113 $\pm$ 0.688 mm for the Berryhill population and 5.465 $\pm$ 0.636 mm for the Gilliam population (Figure 3).

We did not find a significant difference in the mean carapace length of the mothers between locations. (p=0.078, MS=1.541, N=47).

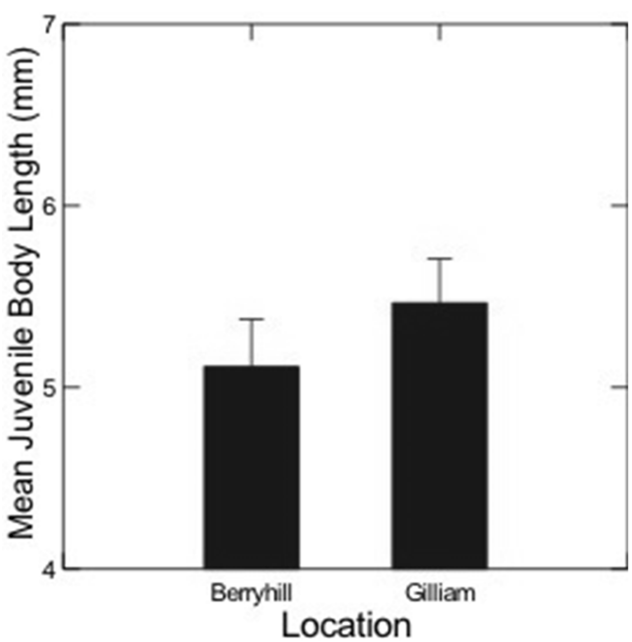

Figure 3. Mean body length of juveniles by location. Standard Error bars are indicated.

Table 1. ANOVA differentiating adult female body mass and brood size between two populations in Mississippi. Modified from Reed and Nicholas 2008.

Adult female mass	**d.f.**	**F-ratio**	**P**
Site	6	4.98	<0.0001
Year	2	7.73	<0.001
Site x Year	12	0.86	=0.59
Brood Size	**d.f.**	**F-ratio**	**P**
Site	6	4.40	<0.0005
Year	2	3.72	<0.03
Site x Year	12	0.65	=0.79

While Reed and Nicholas compared mass of female spiders and mass of juveniles we measured carapace length of females and body length of offspring, similar significant results were observed. Data from the Reed and Nicholas (2008) paper are included here for comparison (Table 1).

Discussion

Our results indicate variation in fecundity between two populations of *R. rabida* sampled in White County Arkansas. This led us to fail to reject our initial hypothesis that there would be significant variation between brood size in different geographic locations. We also failed to reject our hypothesis that female size would differ between locations. Reed and Nicholas used mass as the main variable in comparing female size. We believe that using the same variable, mass, we would have also obtained significant difference in female size. Increasing our sample size would also increase the power of our comparison. (N=47, p=0.07). *R. rabida* has shown a great deal of variation in physiological, behavioral, and life history traits (Reed and Nicholas 2008). This variation appears to be consistent with the variation in brood size that was seen by Reed and Nicholas in Mississippi.

Our results show that brood size variation is consistent at least within the south central United States. future research may identify different patterns of variation across the reported range of this species (Brady and McKinley 1994). We suspect that the large range of this species and the limited gene flow might suggest the presence of multiple species and thus indicate different life history and phenology patterns (Reed and Nicholas 2008).

In our work with *R. rabida*, we often collect hundreds of spiders. We were concerned that removing large numbers of individuals from these populations might influence population size. The large number of juveniles per egg sac suggests that collecting spiders for future tests is not likely to negatively impact the population size due to their ability to produce large numbers of offspring. Future fecundity and juvenile size studies are planned to continue in white county for the next several years. Research on food availability and environmental factors such as precipitation and temperature will also be conducted. In addition, feeding tests will also be conducted in order to see if variation exists that might not be due to food availability alone.

Variation in mother size and fecundity alone are not enough to determine if there is potential for evolution due to changing selective pressures. Reed *et al.* (2007) have suggested that heritability of fecundity traits can be seen through population size and prey availability in an ever-changing environment. Further studies need to be conducted in order to further determine how genetic heritability plays a role in fecundity distribution. Fecundity characteristics such as brood size in arachnid populations provide a glimpse into population dynamics that lead to questions concerning evolutionary strategies of arthropods as their environment changes over time.

Acknowledgements

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