Spring 2020

Ticks on Lizards: Parasite-Host Interactions of the Southern Alligator Lizard (Elgaria multicarinata) in Washington State

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Senior Capstone

Submitted in Partial Fulfillment of the Requirements for Graduation from

The William O. Douglas Honors College

Central Washington University

June, 2020

Accepted by:

_______________________________ June 11, 2020

Committee Chair (Dr. Daniel Beck, Professor, Biology) Date

_______________________________ June 11, 2020

Committee Member (Dr. Gabrielle Stryker, Professor, Biology) Date

_______________________________ 6/18/2020

Director, William O. Douglas Honors College Date

ABSTRACT

I investigated interactions between ectoparasites (ticks) and their host, the southern alligator lizard (*Elgaria multicarinata*). Alligator lizards are capable of complement-mediated killing of the Lyme disease spirochete carried by ticks and may potentially reduce Lyme disease prevalence by cleansing pathogenic organisms from ticks. Despite this, little is known about host-parasite dynamics in alligator lizards. My goals were to 1) assess patterns of tick presence (i.e. parasite load) on alligator lizards and 2) investigate potential negative effects of ticks on alligator lizards. I sampled lizards during the summer of 2019 near Catherine Creek, along the Columbia River Gorge in southern Washington. Ticks were counted on all lizards captured, removed with tweezers, and stored for later analysis. Lizards were weighed, measured (snout-to-vent length, tail length), and released on the study site. A "body condition index" was determined for each lizard and compared to its parasite load to test the hypothesis that ticks are associated with reduced lizard fitness (Jakob et al. 1996). Parasite load averaged 0.4 ticks/lizard (range: 0-2), with 25% of 16 lizards sampled having at least 1 tick. Ticks showed a preference for lizards with longer tails, a result which matches observations of other studies of *E. multicarinata*. I found no relationship between tick presence and lizard body condition. Our research is ongoing, using molecular techniques such as diagnostic PCR to determine the tick species involved (possibly *Ixodes pacificus*, the western black-legged tick) and whether *E. multicarinata* could reduce the amount of Lyme disease and Rocky Mountain spotted fever in Washington's ticks.

Table of Contents

Introduction

Ectoparasites, parasites that live on the outer surface of the body such as ticks and mites, use powerful mouthparts to attach to their hosts and draw blood (Fig 1). They can be found on a wide variety of animals, including humans, and are capable of transmitting diseases ranging from the flea-borne bubonic plague to the tick-borne Lyme disease (De la Fuente et al. 2017).

Figure 1. A southern alligator lizard from southern WA infested with several ticks (arrow). Photo by Dan Beck.

Individual tick species, such as the brown dog tick (*Rhipicephalus sanguineus*) and blacklegged tick (*Ixodes* spp.), are also capable of transmitting specific diseases to humans such as the aforementioned Lyme disease and other diseases such anaplasmosis, ehrlichiosis, babesiosis, and Rocky Mountain spotted fever (Eisen et al. 2018). In addition to the transmission of pathogens, ectoparasites draw energy and resources from their hosts and can reduce their reproductive success (Anderson and May, 1978). This can also be referred to as a reduction in biological fitness (Jakob et al. 1996). Despite their importance as disease vectors, there is little known about the host-parasite relationships in animals other than humans.

In terms of human hosts, Lyme disease, which is an illness caused by a bacterium form known as a spirochete, is recognized globally as a threat to human health and has been steadily increasing in prevalence within the United States (Steere et al. 2004). Unfortunately, there are varied symptoms that accompany this illness that complicate diagnosis. Lyme disease was first misidentified as a form of naturally occurring arthritis but was eventually found to be a form of arthritis caused by the bite of a tick; being one of the many pain-related symptoms that can develop in this systemic illness (Steere et al. 2004). Fortunately, chronic symptoms such as arthritis only occur in absence of timely treatment. Different courses of antibiotics are available for the many different stages of Lyme disease infection, however they become less effective with infection time length and can leave late-stage patients with chronic symptoms (Steere et al. 2004). Therefore, treatment success is highly dependent on discovery and identification of the tick bite. Another source of study in the potential reduction of Lyme disease incidence is in the study of primary hosts of infected ticks, particularly lizards.

The value of understanding lizard-tick interactions has been shown through research on the western fence lizard, *Sceloporus occidentalis*, which commonly serves as a host to western blacklegged ticks, *Ixodes pacificus* (a vector for Lyme disease), in California (Lane et al. 2006). These lizards effectively cleanse attached ticks of any Lyme disease through “complement-mediated killing”, a process that enhances the ability of the immune system to clear the body of microbes such as the spirochete bacteria that causes Lyme disease (Lane et al. 2006). Any ticks at a juvenile life stage will then molt into adult ticks that are permanently free of Lyme disease as the lizards’ blood renders the ticks’ gut microbiome unsuitable for the spirochete to survive (Swei and Kwan, 2017). The implications of these findings have led to debate among researchers regarding the impact of the presence of lizards with spirochete-killing abilities on the prevalence of Lyme disease within an area. Some believe these lizards increase the presence of Lyme disease within an area, as they are primary hosts of the tick (Swei et al. 2011). Others believe these lizards decrease the transmission of Lyme disease because the lizard attracts ticks away from hosts lacking spirochete-killing abilities and cleanses the pathogen from the tick population. Areas in California where western fence lizards are abundant have a notably lower prevalence of Lyme disease (Lane et al. 2006; Swei and Kwan, 2017; Tälleklint-Eisen and Eisen, 1999).

The southern alligator lizard, *Elgaria multicarinata,* has been found to have the same ability of complement-mediated killing of the Lyme disease spirochete in California, however little else is known about the lizard or its tick-host relationship (Kuo et al. 2000; Wright et al. 1998). What is known about *E. multicarinata* is primarily observation-based. This includes use of shrubs and trees for shelter, possible use of their long tail for climbing, and avoidance of temperature extremes (Cunningham 1955). Additionally, the lizards are observed to reside in Mexico and the west coast of the United States from Washington to California (Hammerson and Hollingsworth 2007). *Elgaria multicarinata* are locally abundant in oak woodland habitats along the Columbia River Gorge in Washington, and are often locally infested with many ticks (Fig. 1; Observation by Daniel Beck). Despite this wealth of potential data, no studies have previously been conducted on interactions between alligator lizards and their ectoparasites in Washington.

Uncovering the processes behind this lizard-parasite relationship will benefit scientific understanding of tick-lizard interactions including determination of how ticks with Lyme disease impact their lizard host. As previously noted, it has been established that parasites such as ticks will reduce fitness of their host (Anderson and May, 1978; Jakob et al. 1996). Further, Mugabo et al. (2015) suggested that different immune responses to parasite infection by lizards can result in different impacts on the lizard's fitness. However, it is still unknown how the additional investment of energy into more cleansing immune responses, specifically complement-mediated killing of the Lyme disease spirochete, impacts lizard fitness. In turn, this project emphasizes an investigation into this relationship, as well as several other topics of interest.

Due to the combined complexity and lack of study on this subject, many potential topics are available for research. Producing more studies on *E. multicarinata* could assist in building the available knowledge in hopes that future research might lead to more effective cures, or reduction in the incidence of Lyme disease in other host species, such as humans. Specifically, this project aimed to determine whether *E. multicarinata* is a host for *Ixodes pacificus* (the western black-legged tick and principle vector of Lyme disease in our region), the level of infestation on the lizards in the population, and provide information on whether *E. multicarinata* could reduce the amount of Lyme disease and Rocky Mountain spotted fever in ticks in Washington (Eisen R. J. et al. 2016).

In order to determine host status and the level of infestation, the general ectoparasite load on the southern alligator lizard population at the study site needed to be assessed. I hypothesized that lizards would be heavily parasitized by ticks (primarily *Ixodes pacificus*) at the study site, based on prior observations of ectoparasite load in the area by Dr. Daniel Beck. True assessment of this complex question was broken down into several smaller evaluations including sampling of the lizard population and assessment of the proportion of lizards that carry ticks, how many ticks lizards carry (parasite load), and differences in parasite load among males, females and juvenile lizards.

As a means to evaluate the tick-host relationship, as an essential question was whether ticks had a measurable effect on their lizard hosts. To answer this question, I weighed and measured all lizards captured to create an indicator of fitness and test the hypothesis that lizards with higher parasite loads show reduced fitness.

Materials and Methods

Parasite Load

To assess ectoparasite load on the southern alligator lizard population several sampling trips took place during the spring and summer 2019 to our study site near Catherine Creek, approximately 10 miles west of Lyle, WA (IACUC Protocol #: A041912). Sampling trips totaled approximately 150 hours at the study site. The goal was to obtain a minimum sample of 30 lizards, but the actual n-value was only 17. This was a result of a lack of abundance and environmental conditions (dense poison oak) that made capture difficult. Alligator lizards were captured by noosing and by hand. Noosing involved the construction of a noose made of string attached to a stick which was placed delicately over the head of the lizard before using a sudden upward pull on the stick to tighten the noose. This proved to be less affective, howso lizards were predominantly captured by hand by grabbing the lizards after overturning debris and underbrush to locate them. The lizards were processed individually; placed in individual cloth bags and held for up to 12 hours during periods of continued collection. Several Western fence lizards (*Sceloporus occidentalis*) were captured for comparison purposes. Each lizard was numbered and marked, using non-toxic markers, to avoid repeated sampling of the same lizard, weighed (to nearest 0.1g with a Metler portable balance), measured (mm snout-to- vent length [SVL] and tail length), and sexed (adult males are easily identified by their proportionately wider heads) before being returned to the location where it was captured. Ticks and mites were counted, had their location on the lizard documented, and were removed from each lizard with tweezers and placed in labeled microcentrifuge tubes for later analysis. All statistical analyses were done in R studio.

Impact on Lizard Fitness

To test for impacts of ticks on lizard fitness, measurements of weight and mass were used to derive a "body condition index" for each lizard (Jakob et al. 1996). A correlation analysis was used to test the hypothesis that lizards with more ectoparasites show reduced body mass indices. I made boxplots and mosaic plots to view basic interactions in my data and to see how they could benefit from a general linear model. I found that the 0's and 1's in my dataset resulted in very skewed boxplots that were difficult to interpret (Fig. 2). There were also too many variables with differing data points that made boxplots difficult to read. The many variables also impacted the value of mosaic plots, which only showed basic distributions of males and females or species differences in relation to tick presence/absence and other factors (all independent comparisons). Based on these factors, I decided my biggest issue was heteroscedasticity. Based on this issue of heteroscedasticity, the need for a better way to understand the significance of the data, and the multiple variables involved I decided to utilize a general linear model (binomial test). I adjusted my data so that tick and injury data was in the form of presence/absence instead of numbers so that the general linear model could be used.

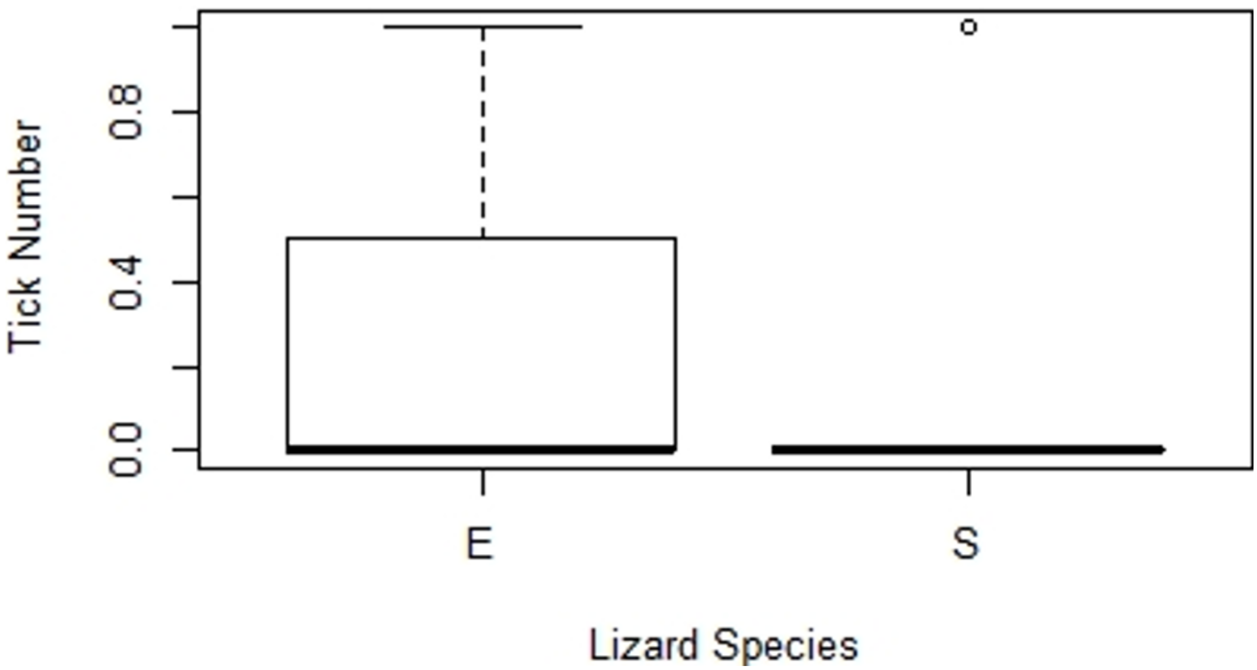

Figure 2. Exploratory boxplot for finalizing statistical methods. Small numbers of lizards caught resulted in skewed data which would suggest ticks were found in similar numbers in both lizard species (E=*E. multicarinata* and S=*S. occidentalis*)

These adjustments revolved around meeting the assumptions of a generalized linear model. Assumptions include that there is random (and independent) sampling of Y's at each X value, lizards were randomly selected in the study site and only one of each X value was derived from individual lizards. Ticks were labeled presence/absence so there was only random sampling of ticks from different lizards. Secondly, there must be fixed X values, which is accurate to this dataset. It is highly suggested that there is a linear relationship between X's and Y's, which is another assumption. Finally, there should be normal distribution and equal variance (the last two assumptions) of Y's at each X due to the random sampling and singular presence/absence values taken of ticks (Y).

The formula used became ‘tick number ~ species + SVL + weight + injury'. Tail length and sex were removed for the model to function, which may indicate data errors in sex and tail length. DHARMa was used for model interpretation.

Impact on Ticks

To address the impact of ticks on the lizards, I collected a sample of ticks from our study site that were not yet attached to a host. Ticks were collected by dragging a large square of felt fabric though brush, a technique known as “dragging” (Ginsberg and Ewing. 1989). The majority of ticks, however, were collected after discovery on my person, not the fabric, after dragging through the study site. Ticks were then carefully removed from the fabric and my clothing and placed in small microcentrifuge tubes with 95% ethanol (alcohol) for future analysis through diagnostic PCR and DNA sequence analysis of the cytochrome oxidase c subunit 1 (COI) gene.

Results

Appropriateness of Statistical Model

The KS test of uniformity had a p-value of 0.87 (Fig. 3). The p-value is greater than 0.05, which means there is no significant difference between observed and expected patterns. It is clear from the standardized residuals vs. predicted (Fig. 3) that the values are not close enough to the predicted values. Therefore, through observing the residuals in more detail they are more variable than expected. I then used a cauchit link function to try to linearize the relationship between the log-odds ratio and the linear function of the explanatory variables. My model was finally improved (seen in linearizing of the lines) when limiting explanatory variables to species, SVL, weight, and injury, which may indicate some errors in the data of sex and tail length (Fig. 4). This would have been possible due to misidentification and abnormal lengths due to lizards that had dropped tails. Despite this improvement, there was a decrease in KS test of uniformity to a p-value of 0.53, which is less uniform then previously, however linearization was deemed more important to continue with statistical analysis (Fig. 4). Statistical analyses showed that the data does not violate the assumptions of the generalized linear model that was developed (Fig. 4). Results from the generalized linear model were skewed due to the small sample size which prevented final significance (p-values) from being established (Fig. 6-8).

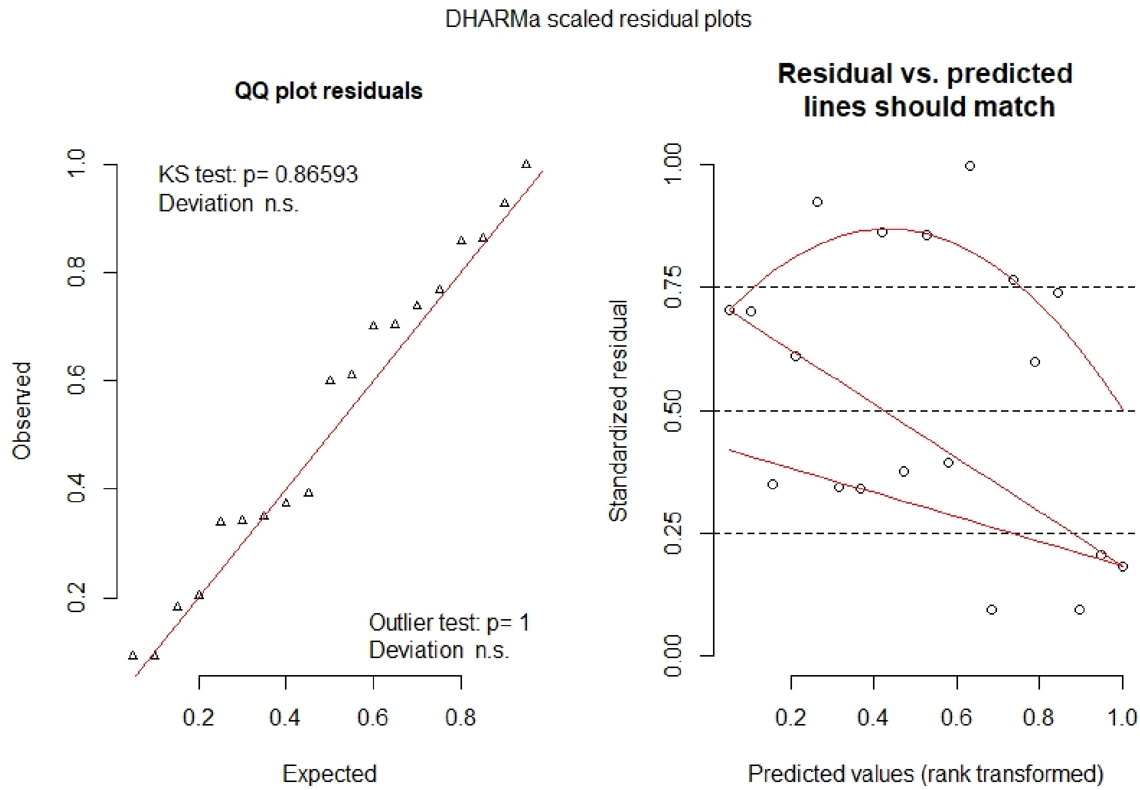

Figure 3. DHARMa scaled residual plots

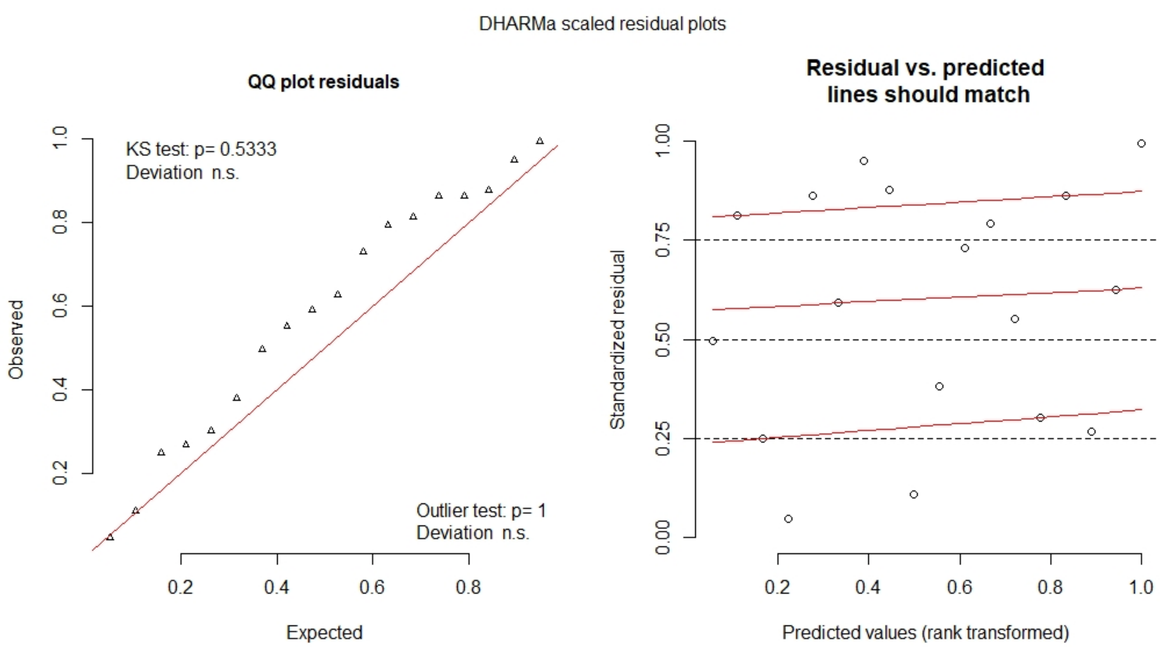

Figure 4. Improved DHARMa scaled residual plots

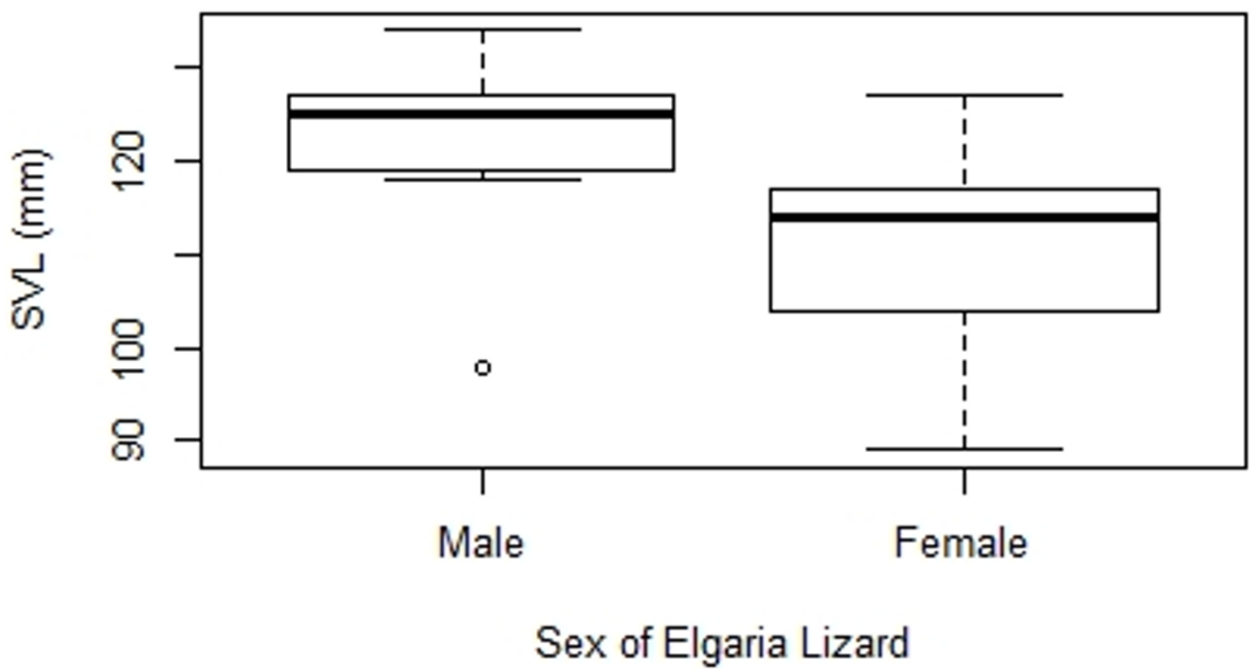

Figure 5. *Elgaria multicarinata* males were significantly larger than *E. multicarinata* females

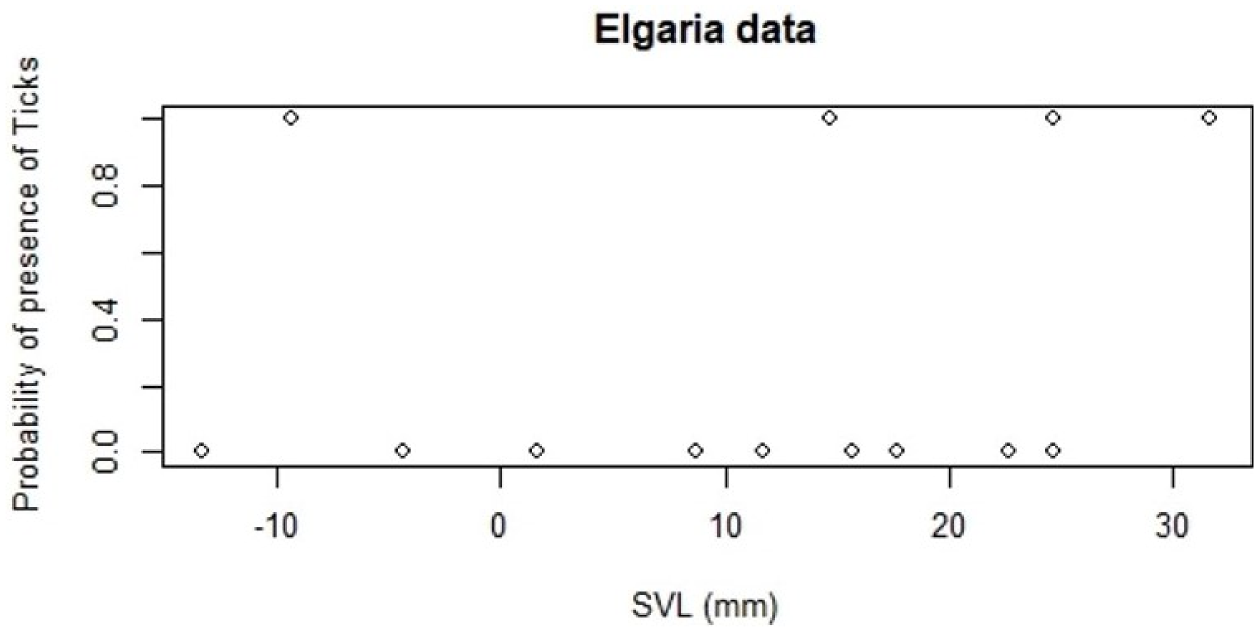

Figure 6. *E. multicarinata* with a longer SVL (mm) have a slightly increased probability of having ticks

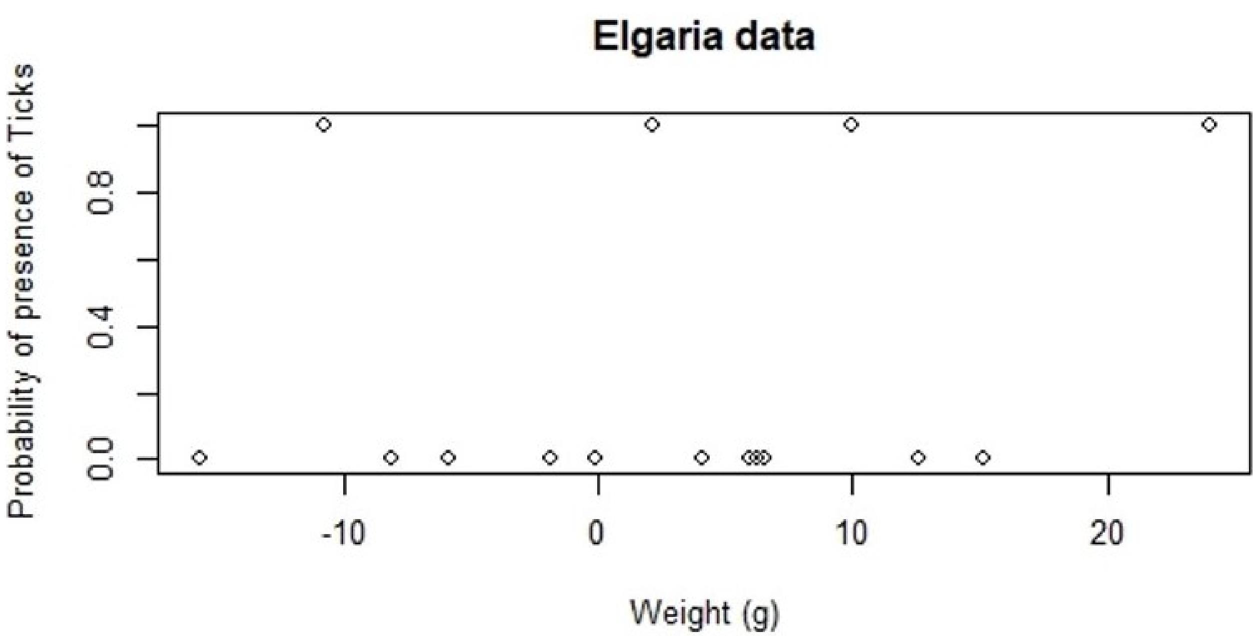

Figure 7. No association between weight (g) and the probability of ticks on *E. multicarinata*

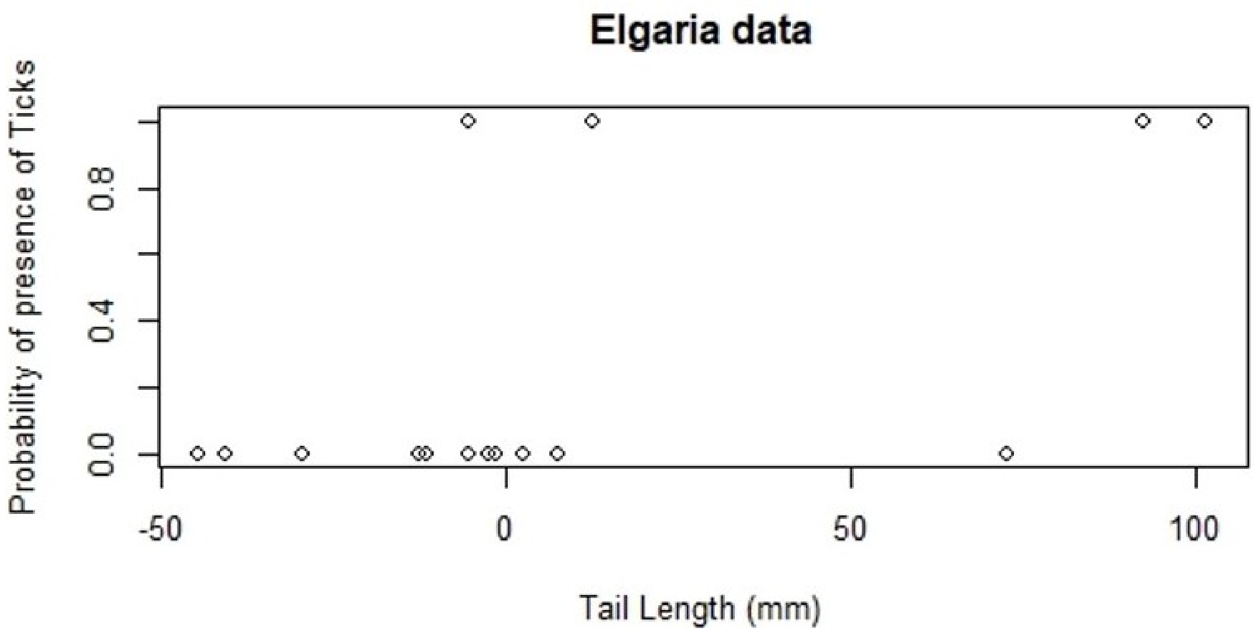

Figure 8. Tail length may be associated with tick presence on *E. multicarinata*

Discussion

My final plots address the relationship between lizard size (tail length, SVL, and weight) on the probability that lizards (*Elgaria multicarinata* and *Sceloporus occidentalis*) will have ticks (Figures 5-8). Due to the small sample size, however, results are limited. Nevertheless, I believe these data are a great starting point for future research. For example, data suggest that male *E. multicarinata* are significantly larger than female *E. multicarinata* (Fig. 5). This is consistent with the variation of traits attributed to sexual dimorphism in lizards (Olsson et al. 2002). Additional results support the hypothesis that parasite load varies among lizards at the study site. Data show that ticks may prefer *E. multicarinata* with longer tails and increased SVL. Specifically, the majority of ticks seem to have a significant preference for *Elgaria multicarinata* with longer tail lengths (Fig. 8). This may be because increased tail length results in increased surface area. Greater amounts of underbrush touched by a lizard resulting from an increase in surface area, results in increased chances of brushing against a questing tick. However, this possible explanation has potential flaws as there is no correlation between weight, which also increases surface area, and tick presence (Fig. 7). This discrepancy may be because the weight of the body does not undulate in the same way as the tail while the lizard is moving, and therefore it does not cover as much of the ground during movement. The long tails of *E. multicarinata* whip back and forth through the tall grass, leaf litter, and other underbrush and debris that are a part of their habitat. Not only are these also the types of environments ticks inhabit, naturally increasing the parasite load of this species, but longer tails would cover larger swaths of ground compared with short tails. Therefore, increased tail length will result in overall increased chances of tick presence. Similarly, this means it is more likely that the correlation of tick presence and tail length is due to an increase in the area of the ground covered by the tail instead of the surface area of the tail.

The hypothesis that lizards with higher parasite loads would show reduced fitness was rejected. This could be due to the lizards having adapted to tick parasitism. The evolutionary arms race among parasites and their hosts results in both becoming progressively less harmful to one another over time (Papkou et al. 2016).

This evolutionary arms race is also the reason that the lizard's immune system has evolved to reduce the impact of pathogens commonly carried by the tick, one such disease potentially being Lyme disease. However, the mechanism of cleansing that allows for the elimination of the Lyme disease spirochete could also have evolved by random chance. The season that the study took place could have influenced these results given that spring is when ticks are most abundant (Salkeld et al. 2014).

This could result in greater variation in parasite load among lizards which could potentially result in differences in fitness. For example, if a small lizard is covered in ticks whereas a large lizard has only 1-2 ticks, the small lizard may be more negatively affected. This is seen in other animals with high tick loads, and can even result in death (Anderson and May, 1978).

Sceloporus occidentalis had very little data as it was not the focus of the study, and as a result reliable interpretations cannot be made (Fig. 2). Based on the sole data available, there is a slight suggestion that larger SVL and tail lengths result in a higher probability of the presence of ticks. This would match the similar results of *Elgaria multicarinata*, and suggest the trend is true for preferences of ticks for lizard hosts.

I look forward to being able to do more complete testing to support or reject these findings in addition to performing different statistical analyses when I obtain more data in the coming months.

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