

Body size, not age, affects parasite load in Clark's Spiny Lizards (*Sceloporus clarkii*)

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Abstract

Understanding the factors that influence parasite load in hosts is a fundamental goal of parasitology and of epidemiology. Body size often influences parasite load in reptiles, and has commonly been used as a proxy for age in studies on parasitism because size and age are correlated. To the best of my knowledge, however, there are currently no studies on reptiles that disentangled the influence of body size and of age on parasite load. The use of body size alone as a predictor of parasite load makes it difficult to determine whether higher levels of parasitism are a result of greater surface area of individuals (a simple function of size), or of longer periods of exposure to parasites (a function of age). Using skeletochronology in a wild population of Clark's Spiny Lizards (*Sceloporus clarkii*) in Arizona, I tested the competing, but not mutually exclusive, hypotheses that (i) larger individuals have higher parasite loads due to increased surface area available for colonization by parasites and their vectors and that (ii) older individuals have higher parasite loads because they have had longer exposure to parasites and their vectors. I predicted that parasite load should increase with body size and with age while controlling for the effect of sex. Males harboured more parasites than females. Males and females differed in how body size influence parasite load; larger males harboured more ectoparasites than smaller males, but this was not the case in females. Age did not affect parasite load in either sex. These results emphasize the importance of disentangling the effects of size and age in models of parasitism to gain a clearer understanding of intraspecific variation in parasite load.

Introduction

Understanding the factors that influence parasite load in hosts is a fundamental goal of parasitology and of epidemiology. Parasitism is a ubiquitous phenomenon across taxa, and high levels of parasitism can have varying fitness effects on the hosts, ranging from apparently benign to very severe (reviewed in Moller *et al.*, 1999). The intimate host-parasite relationship can act as a powerful driver for selection and, as such, understanding the mechanisms underlying differences in host susceptibility is an important goal in evolutionary ecology (Anderson and May, 1982; Poulin, 2007).

Within populations, there can be major differences in the intensity of infection between individuals. One of the most common sources of intraspecific variation in parasite load is sex. In general, males tend to carry more parasites than females (reviewed in Klein, 2004). The exact mechanism underlying this observed pattern is unclear, and several competing hypotheses have been proposed to explain it. These hypotheses generally fall into one of two categories, explaining differences between the sexes either in terms of exposure or in terms of susceptibility (Klein, 2004). In many species, males are larger than females, increasing the surface area available for colonization by parasites or parasite vectors (Blanckenhorn, 2000). In addition to greater surface areas, males often face increased exposure to parasites due to higher activity levels than females (Klein, 2000). This activity hypothesis is generally attributed to higher testosterone levels in males than in females (Fuxjager *et al.*, 2011). Testosterone is also thought to be the source of greater susceptibility to parasitism in males; high levels of circulating steroid hormones (such as testosterone) may suppress the immune system (Folstad and Karter, 1992). These hypotheses have been tested repeatedly in reptiles, with males consistently

harbouring more parasites than females (e.g. Halliday *et al.*, 2014; Klukowski and Nelson, 2001; Lumbad *et al.*, 2011).

Another major source of variation in parasite load is body size. As larger organisms have an increased surface area and detectability, this increases the risk of infection (Blanckenhorn, 2000). Just as differences in body size between the sexes have been suggested as a mechanism for the observed differences in parasite loads in males and females, differences in body size between individuals may also account for variation within the sexes. The other mechanism commonly suggested as driving increased parasite load with increased size is age; given that older individuals have had longer exposure to parasites and parasite vectors compared to younger individuals, this could result in higher intensities of parasitism if individuals do not demonstrate an acquired immune response over time (Raffel *et al.*, 2009). Current tests of this hypothesis, however, hinge on the common assumption that size is strongly correlated with age. For reptiles, which generally exhibit indeterminate growth, this assumption has long been considered valid (Halliday and Verrell, 1988). As such, body size of individuals is often used as a proxy for age (e.g., Raffel *et al.*, 2009; Leinwand *et al.*, 2005). Consequently, studies concerning the proximate causes of increased parasite load in relation to exposure to parasites tend to focus on body size, treating age as either a categorical variable (i.e. juvenile or adult) or ignoring it (e.g., Izhar and Ben-Ami, 2015; Irschick *et al.*, 2006).

Individual variation in growth rate can result in a large range of body sizes for individuals of a given age (Halliday and Verrell, 1988). Therefore, without measuring the age of individuals directly, it is impossible to disentangle the effects of size and age on parasitism from one another. If the hypothesis that longer exposure to parasites and their

vectors results in higher parasite loads is true, older individuals should have higher parasite loads independent of their size. Recently, several studies on amphibians have examined age independently as a potential factor affecting parasite load. Using skeletochronology to determine age, Gustafson *et al.* (2015) found that both age and size had significant effects on the intensity of parasitic infection. However, both the magnitude and direction of these effects differed depending on the species of parasite, as well as the sex of the host. Although reptiles do not exhibit the same distinct life stages that are hypothesized to be a driving force behind the observed impacts of age on parasite load in amphibians (Gustafson *et al.*, 2015), it is still possible that both age and size play a role in parasitism.

While skeletochronological analyses have been conducted in reptiles before, most of these studies focused on growth rates and did not examine the effects of age on parasite load (e.g., Yasumiba *et al.*, 2016; Piantoni *et al.*, 2006; Dubey *et al.*, 2013). One recent study using skeletochronology examined parasitism in the lizard *Lacerta schreiberi*, but this determination of age was used to create an index of body condition rather than examined as a factor directly influencing parasite load (Rodrigo *et al.*, 2016). As such, there is currently a gap in our understanding of how age may be influencing parasite load in reptiles.

In this study, I used natural populations of Clark's Spiny Lizards (*Sceloporus clarkii*) to test the competing, but not mutually exclusive, hypotheses that (i) larger individuals have higher parasite loads due to increased surface area available for colonization by parasites and their vectors and that (ii) older individuals have higher parasite loads because they have had longer exposure to parasites and their vectors.

Specifically, I tested the predictions that parasite load should increase with both body size and age while controlling for the effect of sex. While parasite load commonly increases with body size in other lizards (e.g., Halliday *et al.*, 2014; Irschick *et al.*, 2006; Garrido and Pérez-Mellado, 2013), the incorporation of an independent measure of age should allow for a clearer understanding of whether these patterns are based on the actual size of the individual, or if they exist simply because larger individuals are older. To the best of my knowledge, this is the first study to distinguish between the effects of age and size on parasite load in reptiles with skeletochronology.

Methods

Study site and species

The Clark's Spiny Lizard (*Sceloporus clarkii* Baird and Gerard, 1852; Figure 1) is a medium-sized (mean adult mass = 34.8 g), insectivorous lizard that occurs in wooded habitats at low elevations in the mountains of southwestern USA and western Mexico. They are semi-arboreal and can be found along the edges of creek beds and in the surrounding forests. I sampled 86 *S. clarkii* (12 female juveniles, 13 male juveniles, 24 female adults, 37 male adults) from 13 sites (Table 1; Figure 2) in Coronado National Forest in the Chiricahua Mountains of Arizona, USA from 18 May to 1 August 2016. This research was conducted with a State of Arizona Scientific Collection Permit (No. SP740592) and approved by the University of Ottawa's Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care (#BL-2300-A1).

Field measurements

Using a telescopic fishing pole, I caught lizards by noose. Upon capture, I gave each individual a unique identifying code (UIC) with a medical cauterizer (Ekner *et al.*, 2011). I determined the sex of the lizards by observing secondary sexual characteristics

(colouration, femoral pore size, and postanal scales) measured snout-vent length (SVL) with digital calipers. I used a hand lens to count the number of chiggers (Acari: Trombiculidae) and ticks (Acari: Ixodida) to determine parasite load. To obtain a bone sample for skeletochronological analysis, I toe-clipped each individual, taking a single digit from the back right foot. Prior to clipping, I disinfected both the toe and the scissors using chlorhexidine. I placed the toe in an Eppendorf tube containing 95% ethanol, where it remained until return to the laboratory. I then used one drop of blood from the toe to create one blood smear per individual on a glass slide, then fixed each slide in methanol.

Haemoparasites

Past studies have found *Plasmodium chiricahuae*, a type of malarial parasite, present in *S. clarkii* in the Chiricahua Mountains (Telford, 1970) and in the sympatric *Sceloporus jarrovi* (Bulté *et al.*, 2009). To quantify the level of haemoparasitic infection in the lizards, I examined the blood smears collected in the field using a compound microscope (Figure 3). I stained the smears in the laboratory using Wright-Giemsa stain (Fisher Scientific Company, Middleton, Virginia, USA), then observed each at 400X magnification for 20 minutes to determine the presence or absence of *Plasmodium* infection.

Skeletochronology

I followed the skeletochronology methods outlined in the U.S. Geological Survey Protocol (McCreary *et al.*, 2008) to determine the age of the lizards. While the femur is considered to be the best bone to use for skeletochronological determination of age, Comas *et al.* (2016) have demonstrated that using phalanges is an adequate method for this procedure that does not require killing the individual.

I used a scalpel to isolate the second and third phalanges to be used for sectioning. I decalcified toes in Cal-Ex solution overnight, then rinsed them in deionized water for 8 h. For sectioning, I placed each toe in optimal cutting temperature (OCT) compound, and froze it at -20°C . I sectioned each toe in the cryostat at $20\ \mu\text{m}$ thickness and collected sections from the epiphysis portion of the toe on a microscope slide. After fixing each slide in methanol for one minute, I stained each slide with Harris' Haemotoxylin for two minutes then rinsed the excess stain off with deionized water. When dry, I rehydrated each section with deionized water delivered via an eyedropper, and observed sections using a compound microscope at 100X magnification (Figure 4). I photographed sections that displayed the most prominent lines of arrested growth (LAGs). Two independent observers examined each picture, as there is a level of subjectivity in determining the number of LAGs present in a sample (Sagor *et al.*, 1998). When there was interobserver variation in the counts, we examined the sections together to make a unanimous decision.

Statistical analyses

To determine the effects of sex, size, and age on parasite load, I ran a generalized linear model in R version 3.3.3 (R Core Team, 2017). As the chigger data are count data that were positively skewed, I chose to run a negative binomial regression (package: MASS; function: glm.nb; Venables and Ripley, 2002). For lizards caught more than once during the summer, I used the data from the first capture in the analysis to avoid pseudoreplication. Age, SVL, and sex were used as independent variables in the model. I also included date of capture as a covariate in the model since previous studies in other lizards have found that ectoparasite load tends to increase over the course of the active season (e.g., Klukowski, 2004; Huyghe *et al.*, 2010). Tick counts and haemoparasite

presence were not included in the analysis because too few individuals were infected with these parasites.

I began by running a linear model to determine the relationship between age and size, to ensure that these factors were not so highly correlated that their effects would be indistinguishable statistically. I then ran the full model for the chigger data, including all main effects and relevant two-way interactions (i.e. interactions between sex and each of the continuous variables). I determined the models with the best relative fit to the data using bias-corrected Akaike Information Criterion (AIC_c ; package: MuMIn; function: dredge; Bartón, 2016) and averaged the models within 2 AIC_c of the model with the lowest AIC_c (package: MuMIn; functions: get.models, avg.model; Bartón, 2016).

Results

Only 33 lizards harboured ticks (mean = 3.43 ticks) and only 17 lizards were infected by malaria. Four individuals were missing toe samples or were without high quality bone sections. In the 82 remaining lizards, chigger parasite load varied from 0 to 97 chiggers per individual. Older lizards were indeed larger ($df = 80$, $r^2 = 0.43$, $p < 0.001$), but the relationship was nonlinear and there was high variability in SVL at any given age (Figure 5). Males were not significantly larger than females ($t = 1.613$, $p = 0.112$). Variance inflation factors for the full model without interactions were all below 2, so multicollinearity was not an issue.

The averaged model for predicting variation in chigger load in both sexes included date, sex, SVL, and the interaction between sex and SVL, but only date (coefficient = 0.008, $p = 0.032$) and the interaction term (coefficient = 0.332, $p = 0.019$) were significant predictors of parasite load. Given that the sexes differed in how body

size affected parasite load (Figure 6), I ran separate models to look at the effects of date, age, and SVL in each sex. In females, the averaged model included date, age, and SVL, but none of these variables had a significant effect on parasite load. In males, the averaged model included date and SVL, with parasite load increasing with body size (coefficient = 0.242, $p = 0.011$). Date of capture had a nearly significant positive effect on parasite load (coefficient = 0.010, $p = 0.090$).

Discussion

In this study, I tested two competing hypotheses (body size and age hypotheses) to determine why *S. clarkii* individuals differ in their intensity of infection by ectoparasites. I found some support for the hypothesis that larger individuals have greater ectoparasite loads due to an increased surface area available for colonization by parasites and their vectors, but only in males. As I predicted, large males harboured more ectoparasites than smaller ones, but this was not the case for females. I found no support for the hypothesis that older individuals have more parasites due to increased exposure, as there was no effect of age on parasite load in either sex.

In males, larger individuals tended to have higher parasite loads, regardless of age, providing support for the body size hypothesis. These results are consistent with other studies in lizards (e.g., Halliday *et al.*, 2014; Garrido and Pérez-Mellado, 2013; Schall, 1996). Eliminating age as a potential source of variation in parasite load improves our understanding of the causal mechanism underlying this pattern; while in these previous studies increased exposure to parasites was suggested as an explanation for why larger individuals have greater parasite loads, my results suggest that it is actually the larger surface area of these individuals that is responsible for this pattern.

On the other hand, none of the factors of interest appeared to have any effect on ectoparasite load in female lizards. This could be due to at least two reasons. First, the sample size of female lizards was smaller than that of male lizards ($n = 33$ and $n = 49$, respectively) and as such, I simply may not have been able to detect the effects of size, age or date in females. In this case, it is still possible that the same mechanisms acting on male parasite load could also be acting on female parasite load. Alternatively, the proximate causes underlying variation in female parasite load may genuinely be different than those in males.

Activity levels tend to increase with testosterone in spiny lizards (Fuxjager *et al.*, 2011; Marler and Moore, 1989), and males generally move around more and have larger home range sizes than females (Perry and Garland, 2002). Consequently, males are likely to have an increased detectability and increased exposure to parasites (Klein, 2000). For larger males, body size may be interacting with activity levels to create a greater risk of infection. For females, who are more stationary, it is possible that differences in body size may not have a significant impact on parasite load because they are less likely to be exposed to parasites in the first place.

The interaction between sex and body size also indicated a significant difference in parasite load between the sexes. Although there does not appear to be any difference in parasite load between the sexes at small body sizes, for the range of SVLs included in this study, males appear to have more ectoparasites than do females (Figure 6). This result supports the general trend seen across taxa (Klein, 2004), although I was unable to directly test the mechanism underlying this pattern. Males were not larger than females, suggesting that differences in parasitism between the sexes may be attributed to higher

activity levels in males (Klein, 2000) or reduced immune function as a consequence of higher circulating testosterone levels (Folstad and Karter, 2002), rather than differences in surface area available for parasites.

As expected, ectoparasite load increased slightly throughout the season. This is consistent with previous findings in other lizards (e.g., Klukowski, 2004; Huyghe *et al.*, 2010, Schall and Marghoob, 1985). This seasonal increase could be attributed to increased activity towards the end of the season, increased presence of chiggers later in the season, or a greater period of time over which to accumulate chiggers (Klukowski, 2004), although the fact that we did not find an increase in parasite load with age casts doubts on the validity of this latter explanation.

It is possible that other types of parasites, such as haemoparasites or gastrointestinal parasites that exhibit different infection dynamics than chiggers, may be impacted differently by host traits. Gustafson *et al.* (2015) suggest that the body size hypothesis should apply to any parasite that infects its host via skin penetration (e.g., *P. chiricahuae*, which is transmitted through penetration of the lizard's skin by psychotid flies; Bromwich and Schall, 1986). However, other factors such as age may play a more important role for these types of parasites. For instance, in red-spotted newts, aquatic exposure period is a significant predictor in parasite load for eight of twelve parasite taxa, including both haemoparasites and helminth parasites (Raffel *et al.*, 2009). For some of these taxa, there was a positive relationship between exposure period and parasite load, while for others, the newts exhibited an acquired immune response evidenced by decreased parasite intensity in older individuals. However, one important caveat in interpreting these results is that skeletochronology was only used to determine age for a

subset of individuals, and the relationship between SVL and age of this subset was used to estimate age for all other individuals. As such, it is possible that these results were really an effect of body size rather than just exposure period as the authors suggest. Regardless, studies like this, which examine multiple types of parasites, demonstrate how different host traits may be drivers of parasite load for different parasite types. This suggests that while body size appears to be a driver of ectoparasite intensity in *S. clarkii*, we should not assume that it can account for variation in all parasite types.

The results in this study provide insight into the actual causal mechanisms underlying the commonly observed pattern of larger lizards having greater parasite loads. Many authors have suggested that since larger individuals tend to be older, increased parasite load in these individuals may be a consequence of increased exposure to parasites over time. However, my results indicate that it is body size, not age, that is impacting ectoparasite load in male lizards. Using skeletochronology I have demonstrated the high level of variability in body size between individuals of the same age, suggesting that body size should not be used as a proxy for age in these lizards. Future studies should examine the effects of body size and age independently for other parasite types.

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Appendices**Table 1.** Location of study sites in the Chiricahua Mountains, Arizona in standard UTM coordinates. Datum: WGS84, Zone: 12R.

Site	Easting	Northing
A	669424	3529260
B	674003	3530789
C	672418	3516186
D	667765	3533390
E	667479	3530629
F	668076	3533754
G	667930	3531034
H	667444	3528036
I	668095	3528323
J	666651	3532270
K	673726	3530526
L	671092	3534073
M	669552	3528463

Table 2. Model selection for an analysis of the number of ectoparasites in Clark’s Spiny Lizards (*Sceloporus clarkii*) from southeastern Arizona using bias-corrected Akaike’s information criteria (AIC_c). Only models within 2 AIC_c of the top model are listed.

Model	<i>k</i>	AIC _c	ΔAIC _c	Weight
Ectoparasites ~ Date + Sex + SVL + Sex:SVL	5	680.46	0	0.53
Ectoparasites ~ Date + Sex	3	681.80	1.34	0.27
Ectoparasites ~ Sex + SVL + Sex:SVL	4	682.40	1.94	0.20

Note: *k* is the number of parameters in the model. ΔAIC_c is the difference between the best model and the comparison model. SVL, snout–vent length.

Table 3. Coefficients and adjusted standard errors for the averaged model.

Independent variable	Coefficient	Adjusted SE	<i>p</i>
Intercept	2.942	0.892	0.001
Date	0.010	0.005	0.032
Sex	-1.677	1.763	0.342
SVL	-0.080	0.103	0.438
Sex:SVL	0.332	0.142	0.019

Table 4. Model selection for an analysis of the number of ectoparasites in female Clark’s Spiny Lizards (*Sceloporus clarkii*) from southeastern Arizona using bias-corrected Akaike’s information criteria (AIC_c). Only models within 2 AIC_c of the top model are listed.

Model	k	AIC_c	ΔAIC_c	Weight
Ectoparasites ~ Null	2	256.67	0	0.36
Ectoparasites ~ Date	2	256.96	0.29	0.31
Ectoparasites ~ Age	2	258.08	1.41	0.18
Ectoparasites ~ SVL	2	258.48	1.81	0.15

Note: k is the number of parameters in the model. ΔAIC_c is the difference between the best model and the comparison model. SVL, snout–vent length.

Table 5. Coefficients and adjusted standard errors for the averaged model in the female-only model.

Independent variable	Coefficient	Adjusted SE	p
Intercept	2.824	0.667	<0.001
Date	0.013	0.008	0.144
Age	-0.167	0.181	0.356
SVL	0.092	0.117	0.432

Table 6. Model selection for an analysis of the number of ectoparasites in male Clark's Spiny Lizards (*Sceloporus clarkii*) from southeastern Arizona using bias-corrected Akaike's information criteria (AIC_c). Only models within 2 AIC_c of the top model are listed.

Model	k	AIC_c	ΔAIC_c	Weight
Ectoparasites ~ SVL	2	425.45	0	0.53
Ectoparasites~ Date + SVL	3	425.66	0.21	0.47

Note: k is the number of parameters in the model. ΔAIC_c is the difference between the best model and the comparison model. SVL, snout–vent length.

Table 7. Coefficients and adjusted standard errors for the averaged model in the male-only model.

Independent variable	Coefficient	Adjusted SE	p
Intercept	0.842	0.965	0.383
SVL	0.242	0.095	0.011
Date	0.010	0.007	0.090



Figure 1. Adult male Clark's Spiny Lizard (*Sceloporus clarkii*) in southeastern Arizona.



Figure 2. Map of study sites in the Chiricahua Mountains in southeastern Arizona.

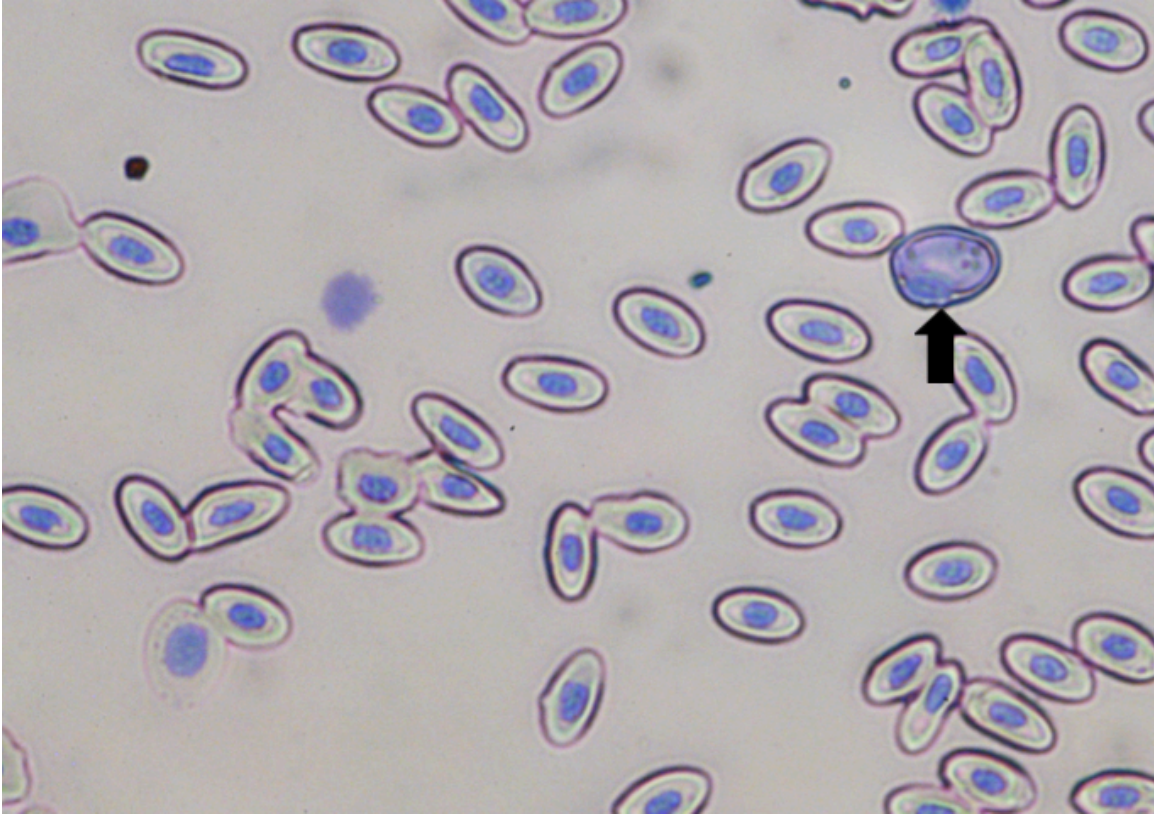


Figure 3. Typical stained blood smear from Clark's Spiny Lizards (*Sceloporus clarkii*) in southeastern Arizona. Arrow indicates cell infected with *Plasmodium chiricahuae*.



Figure 4. Typical 20 μm stained transverse sections from phalanges of a 5-year-old female Clark's Spiny Lizard (*Sceloporus clarkii*) in southeastern Arizona. Arrows indicate LAGs.

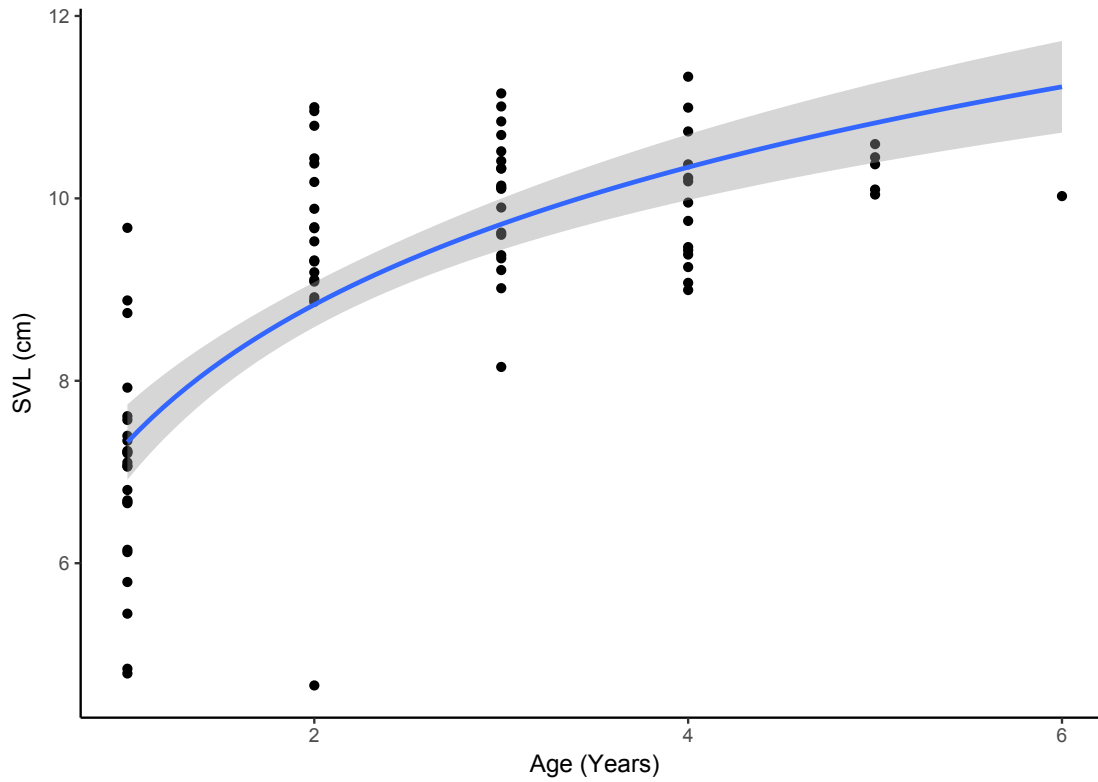


Figure 5. Body size increases with age in Clark's Spiny Lizards (*Sceloporus clarkii*) in southeastern Arizona, but there is a high degree of variability within age classes.

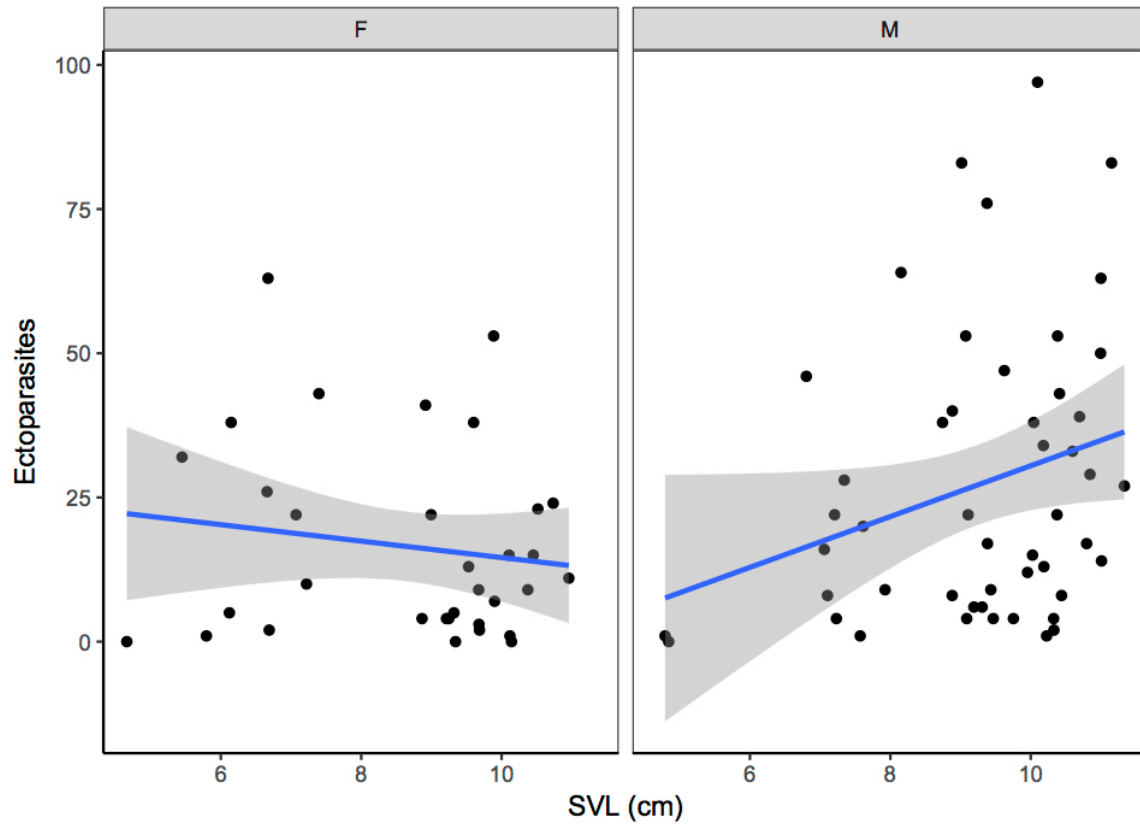


Figure 6. Ectoparasite load does not vary with size in females, but increases with size in male Clark's Spiny Lizards (*Sceloporus clarkii*) in southeastern Arizona. Male parasite load is higher than female parasite load.