

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

H.V. Watkins and G. Blouin-Demers

Abstract: Determining the factors that influence parasite load is a fundamental goal of parasitology. Body size often influences parasite load in reptiles, but it is unclear whether higher levels of parasitism are a result of greater surface area of individuals (a 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* Baird and Girard, 1852), we tested the 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. Males harboured more ectoparasites than females. Males and females differed in how body size influenced chigger (Acari: Trombiculidae) load; larger males harboured more chiggers than smaller males, but this was not the case in females. Age did not affect ectoparasite 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.

Key words: age, body size, Clark's Spiny Lizard, *Sceloporus clarkii*, Trombiculidae, Ixodida, *Plasmodium*.

Résumé : La détermination des facteurs qui influencent la charge parasitaire constitue un objectif fondamental de la parasitologie. La taille du corps exerce souvent une influence sur la charge parasitaire chez les reptiles, mais il n'est pas bien établi que des degrés élevés de parasitisme sont le résultat d'une plus grande surface des spécimens (une fonction de la taille) ou de plus longues périodes d'exposition aux parasites (une fonction de l'âge). Nous avons utilisé la squeletochronologie dans une population sauvage de scélopores *Sceloporus clarkii* Baird et Girard, 1852, pour vérifier les hypothèses voulant (i) que les spécimens plus grands ont des charges parasitaires plus importantes en raison de la plus grande surface disponible pour la colonisation par des parasites et leurs vecteurs et (ii) que les spécimens plus vieux ont des charges parasitaires plus grandes parce qu'ils ont eu une plus longue exposition aux parasites et à leurs vecteurs. Les mâles comptaient plus d'ectoparasites que les femelles. L'influence de la taille du corps sur la charge d'aotats (acarien : trombiculidés) était différente pour les mâles et les femelles; les mâles plus grands comptaient plus d'aotats que les mâles plus petits, ce qui n'était pas le cas pour les femelles. L'âge n'avait pas d'incidence sur la charge d'ectoparasites chez l'un ou l'autre des sexes. Ces résultats soulignent l'importance de bien départager les effets de la taille et de l'âge dans les modèles de parasitisme pour en arriver à une compréhension plus claire des variations intrasécifiques de la charge parasitaire. [Traduit par la Rédaction]

Mots-clés : âge, taille du corps, scélopore, *Sceloporus clarkii*, trombiculidés, ixodidés, *Plasmodium*.

Introduction

Determining the factors that influence parasite load in hosts is a fundamental goal of parasitology. Parasitism is ubiquitous across taxa and high levels of parasitism can have marked fitness effects on the hosts (reviewed in Møller et al. 1999). The intimate host–parasite relationship can be a powerful driver for selection and, as such, determining 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 by parasites among 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, in-

creasing 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; Nunn and Dokey 2006). This activity hypothesis is generally attributed to higher testosterone levels in males than in females (Marler and Moore 1989; Fuxjager et al. 2011). Testosterone is also thought to be the source of greater susceptibility to parasitism in males, in which high levels of circulating steroid hormones (such as testosterone) may suppress the immune system (Folstad and Karter 1992; Schalk and Forbes 1997; but see Roberts and Peters 2009; Desprat et al. 2015). These hypotheses have been tested repeatedly in reptiles, with males consistently harbouring more parasites than females (e.g., Klukowski and Nelson 2001; Lumbad et al. 2011; Halliday et al. 2014).

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

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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 exposure time. Given that older individuals have had longer lifetime exposure to parasites and parasite vectors compared with younger individuals, this could result in higher intensities of parasitism, provided that parasites persist within the host for a long period of time and that individuals do not demonstrate an acquired immune response over time (Lo et al. 1998; 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., Christian and Bedford 1995; Leinwand et al. 2005; Brown and Symondson 2014). 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 excluding it when accurate estimates cannot be obtained (e.g., Irschick et al. 2006; Garrido and Pérez-Mellado 2013).

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. This can have consequences for how we interpret the mechanisms driving variation in parasite load. If the hypothesis that longer exposure to parasites and their vectors results in higher parasite loads is true, then older individuals should have higher parasite loads independent of their size. However, if host age has no effect on parasite load (as would be expected for parasites that do not persist on or within the host for long periods of time), then the use of size as a proxy for age may lead to a false conclusion that host age actually does play a role. Recently, a study on amphibians demonstrated the importance of examining age independently from size 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 in Wood Frogs (*Lithobates sylvaticus* (LeConte, 1825)). However, both the magnitude and the 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.

Although skeletochronological analyses have been conducted in reptiles, most studies have focused on growth rates and have not examined the effects of age on parasite load (e.g., Piantoni et al. 2006; Dubey et al. 2013; Yasumiba et al. 2016). In a recent study involving skeletochronology, parasitism in the Iberian Emerald Lizard (*Lacerta schreiberi* Bedriaga, 1878) was examined, but this estimation 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, we used wild Clark's Spiny Lizards (*Sceloporus clarkii* Baird and Girard, 1852) to test the distinct but interrelated 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 lifetime exposure to parasites and their vectors. Specifically, we tested the predictions that parasite load should increase both with body size and with age while controlling for the effect of sex. Although parasite load commonly increases with body size in other lizards (e.g., Irschick et al. 2006; Garrido and Pérez-Mellado 2013; Halliday et al. 2014),

the incorporation of an independent measure of age should allow for a clearer determination 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 our knowledge, this is the first study to distinguish between the effects of age and size on parasite load in reptiles with skeletochronology.

Materials and methods

Study site and species

Sceloporus clarkii 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. *Sceloporus clarkii* are semi-arboreal and can be found along the edges of creek beds and in the surrounding forests. We sampled 86 *S. clarkii* (12 female juveniles, 13 male juveniles, 24 female adults, 37 male adults) from 13 sites 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-R1).

Field measurements

We caught lizards by noose and gave each individual a unique identifying code with a medical cauterizer (Ekner et al. 2011). We determined the sex of the lizards by observing secondary sexual characteristics (colouration, femoral pore size, and postanal scales) and measured snout-vent length (SVL) with digital calipers. We used a hand lens to count the number of chiggers (Acari: Trombiculidae) and ticks (Acari: Ixodida) to determine ectoparasite load. To obtain a bone sample for skeletochronological analysis, we toe-clipped each individual, taking a single digit from the back right foot. Prior to clipping, we disinfected both the toe and the scissors with chlorhexidine. We placed the toe in an Eppendorf tube containing 95% ethanol, where it remained until return to the laboratory. We 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

Plasmodium chiricahuae Telford, 1970 (Telford 1970), a type of malarial parasite, is present in *S. clarkii* in the Chiricahua Mountains and in the sympatric Yarrow's Spiny Lizard (*Sceloporus jarrovi* Cope, 1875) (Bulté et al. 2009). To quantify the level of haemoparasitic infection in the lizards, we examined the blood smears collected in the field using a compound microscope. We stained the smears in the laboratory using Wright-Giemsa stain (Fisher Scientific Company, Middleton, Virginia, USA), then observed each at 400× magnification for 20 min to determine the presence or absence of *Plasmodium* infection.

Skeletochronology

We followed the skeletochronology methods outlined in the U.S. Geological Survey Protocol (McCreary et al. 2008) to determine the age of the lizards. Whereas the femur is the best bone to use for skeletochronological determination of age, Comas et al. (2016) demonstrated that the non-lethal method of using phalanges is equally reliable. Skeletochronology has been validated in a closely related species to *S. clarkii*, the Ornate Tree Lizard (*Urosaurus ornatus* (Baird and Girard, 1852)), in the same study plots as the current study using individuals of known age (Paterson and Blouin-Demers 2018).

We used a scalpel to isolate the second and third phalanges to be used for sectioning. We decalcified toes in Cal-Ex solution (Fisher Scientific Company, Middleton, Virginia, USA) overnight, then rinsed them in deionized water for 8 h. For sectioning, we placed each toe in optimal cutting temperature compound and froze it at -20 °C. We sectioned each toe in a cryostat at 20 µm and

collected sections from the epiphysis portion of the toe on a microscope slide. After fixing each slide in methanol for 1 min, we stained each slide with Harris hematoxylin (Fisher Scientific, Middleton, Virginia, USA) for 2 min and then rinsed the excess stain with deionized water. When dry, we rehydrated each bone section with deionized water and observed sections using a compound microscope at 100× magnification. We photographed sections that displayed the most prominent lines of arrested growth (LAGs). Two independent observers examined each picture (Sagor et al. 1998) and reached a consensus on the number of LAGs when the scores differed.

Statistical analyses

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

We began by running a linear model to ensure age and size were not so highly correlated that their effects would be indistinguishable statistically. We then ran the full models for each parasite type, including all main effects and relevant two-way interactions (i.e., interactions between sex and each of the continuous variables). We determined the best models with the bias-corrected Akaike's information criterion (AIC_c ; package: MuMIn; function: dredge; Bartoń 2016) and averaged the models with $\Delta AIC_c < 2$ when compared with the model with the lowest AIC_c (package: MuMIn; functions: get.models, avg.model; Bartoń 2016).

Results

Four individuals were missing toe samples or did not yield high-quality bone sections. In the 82 remaining lizards, ectoparasite load varied from 0 to 97 chiggers and from 0 to 36 ticks. Older lizards were indeed larger ($r^2 = 0.43$, $df = 80$, $p < 0.001$), but the relationship was nonlinear and there was high variability in SVL at any given age (Fig. 1). Repeating the analysis separately by sex confirmed the pattern in both males ($r^2 = 0.40$, $df = 47$, $p < 0.001$) and females ($r^2 = 0.46$, $df = 31$, $p < 0.001$). 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 (Supplementary Table S1),¹ but only date (coefficient = 0.008, $p = 0.032$) and the interaction term (coefficient = 0.332, $p = 0.019$) were significant (Supplementary Table S2).¹ Given that the sexes differed in how body size affected chigger load (Fig. 2), we 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 (Supplementary Table S3),¹ but none of these variables had a significant effect on chigger load (Supplementary Table S4).¹ In males, the averaged model included date and SVL (Supplementary Table S5),¹ with chigger load increasing with body size (coefficient = 0.242, $p = 0.011$; Supplementary Table S6).¹ Date of capture had a nearly significant positive effect on chigger load (coefficient = 0.010, $p = 0.090$; Supplementary Table S6).¹

Fig. 1. Body size increases with age in Clark's Spiny Lizards (*Sceloporus clarkii*) in southeastern Arizona, USA, but there is high variability within age classes. Solid line indicates the predicted values estimated by the von Bertalanffy growth model for males ($L(t) = 10.08[1 - e^{-2.498(t-0.504)}]$; package: FSA; function: vbFuns, vbStarts; Ogle 2017). Broken line indicates the predicted values estimated by the von Bertalanffy growth model for females ($L(t) = 10.14[1 - e^{-1.143(t-0.026)}]$).

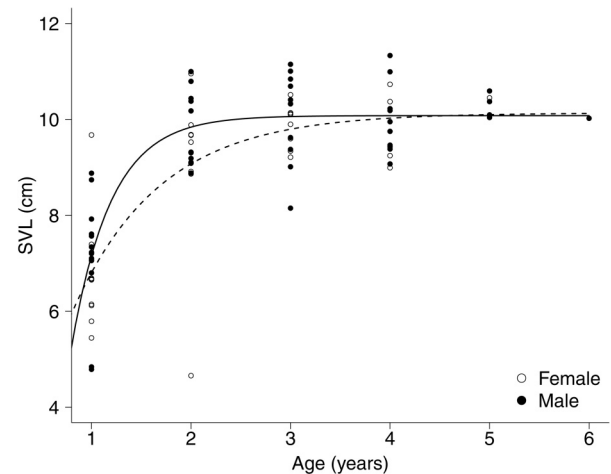
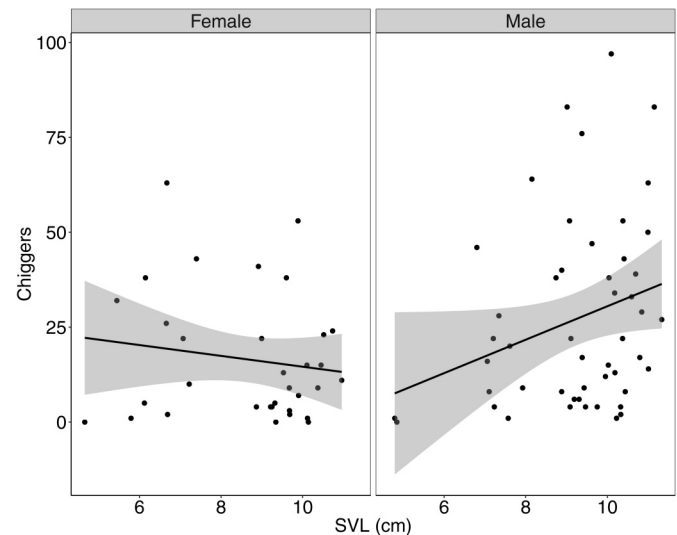


Fig. 2. Chigger (Acari: Trombiculidae) load does not vary with size in females, but increases with size in male Clark's Spiny Lizards (*Sceloporus clarkii*) in southeastern Arizona, USA. Male parasite load is higher than female parasite load. Data are fit with a linear regression for visualization purposes.



The averaged model for predicting variation in tick load in both sexes included date, sex, age, and SVL (Supplementary Table S7),¹ with males having significantly more ticks than females (coefficient = 1.772, $p < 0.001$) and intensity of tick infection increasing throughout the season (coefficient = 0.062, $p < 0.001$; Supplementary Table S8).¹ Neither age nor body size had a significant effect on tick load. The averaged model for predicting *P. chiricahuae* presence included sex, age, and SVL (Supplementary Table S9),¹ but none of these variables had a significant effect on *P. chiricahuae* presence (Supplementary Table S10).¹

¹Supplementary tables are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2017-0328>.

Discussion

We tested two competing hypotheses (body size and age hypotheses) to determine why *S. clarkii* differ in their intensity of infection by parasites. We 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 we predicted, larger males harboured more chiggers than smaller ones, but this was not the case for females. We found no support for the hypothesis that older individuals have more parasites due to increased lifetime exposure, as there was no effect of age on ectoparasite load in either sex. However, as both chigger and tick loads increased slightly over the course of the season, we found some support for the hypothesis that individuals harbour more ectoparasites due to increased exposure to ectoparasites throughout the season.

In males, larger individuals tended to have higher chigger loads, regardless of age, providing support for the body size hypothesis. These results are consistent with other studies in lizards (e.g., Schall 1996; Garrido and Pérez-Mellado 2013; Halliday et al. 2014). Eliminating age as a potential source of variation in ectoparasite load improves our understanding of the causal mechanism underlying this pattern. Although previous studies used increased exposure to parasites (due to a longer life) as an explanation for why larger individuals have greater parasite loads (e.g., Schall and Marghoob 1995; Leinwand et al. 2005; Brown and Symondson 2014), our results suggest that it is actually the larger surface area of these individuals that is responsible for this pattern, not their age. Given the short life cycle of ticks and chiggers, this apparent lack of relationship between age and parasite load is unsurprising for these species. In their larval form, ticks and chiggers attach to their hosts for several days to several weeks before detaching and transforming into nymphs, which develop into free-living adults that lay their eggs in the soil (Goldberg and Bursley 1991, 1993; Tuegel and Wrenn 1998). As temperature influences the hatching time of the eggs (Sasa 1961), ectoparasite abundance in the environment drops sharply in the winter and eggs are unable to hatch until spring (Klukowski 2004). Consequently, all hosts should have entered the active season with no or very few ectoparasites, regardless of their age.

On the other hand, none of the factors of interest appeared to have any effect on ectoparasite load in females. This could be due to at least two reasons. First, the sample size of females was smaller than that of males ($n = 33$ and $n = 49$, respectively) and, as such, we simply may not have been able to detect the effects of size, age, or date in females. If this were the case, then the same mechanisms acting on male chigger load could also be acting on female chigger 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 (Marler and Moore 1989; Fuxjager et al. 2011) and males generally move more and have larger home ranges than females (Perry and Garland 2002). Consequently, males are likely to have an increased detectability and increased exposure to parasites (Klein 2000; Nunn and Dokey 2006). 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 ectoparasite load between the sexes. Although there does not appear to be any difference in ectoparasite load between the sexes at small body sizes, for the range of sizes included in this study, males appear to have more ectoparasites than females at large body sizes. This result mirrors the general trend seen across taxa (Klein 2004), but we were unable to test

directly 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 to reduced immune function as a consequence of higher circulating testosterone levels (Folstad and Karter 2002), rather than to differences in surface area available for parasites. Additionally, the sampling period of this study encompassed the reproductive months for this species, with some female lizards being noticeably gravid at time of capture. Therefore, it is possible that hormonal differences driven by reproduction may have also been influencing variation in ectoparasite load between the sexes.

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). This seasonal increase could be attributed to increased activity towards the end of the season, to increased presence of chiggers and ticks later in the season, or to a greater period of time over which to accumulate these ectoparasites (Klukowski 2004). For parasites with short life cycles, such as chiggers and ticks, this smaller scale measure of time is more representative of exposure time than host age. As such, it is still possible that increased exposure time to parasites was a driving force in ectoparasite load, but this is unrelated to age of the host for these short-term parasites.

It is possible that other types of parasites, such as haemoparasites or gastrointestinal parasites that exhibit different infection dynamics than chiggers and ticks, may be impacted differently by host traits. In our study, none of the variables tested appeared to have any effect on *P. chiricahuae* presence. However, due to the low number of individuals infected (only 16 out of 82), it is possible that we may simply not have been able to detect an effect due to the small sample of infected individuals. Gustafson et al. (2015) suggested 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 psychodid flies; Bromwich and Schall 1986). However, other factors such as age may play a more important role for these types of long-term parasites. For instance, in a study on three species of tropical fish, larger individuals of all host species harboured more ectoparasites than smaller individuals (Lo et al. 1998). However, this relationship was less clear for endoparasites, with size only affecting some species. Host age, on the other hand, appeared to have an influence on both abundance of long-lived endoparasites and on the overall species diversity of endoparasites. Studies like this, that examine multiple types of parasites, demonstrate how different host traits may be drivers of parasite load for different parasite types. This suggests that although 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.

Our study provides insight into the potential mechanisms underlying the commonly observed pattern that larger lizards harbour higher 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, our results indicate that it is body size, not age, that is impacting chigger load in male lizards. Using skeletochronology, we have uncovered the high 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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