**Tick infection is sex-specific and lowers locomotor performance in the Eastern Fence Lizard**

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# Abstract:

Parasitic infections are known to affect physiological traits directly linked to host fitness can lead to direct or indirect effects on their hosts. Here we focus on two ecologically relevant traits, locomotor performance measurements and body condition indices, that have been shown to have direct links to an individual's health and survival. To understand the relationship between ectoparasites and their impacts on host physiology, we investigated the impact of tick infection on locomotor performance and body condition in their lizard hosts. Here we show that parasite presence differs by sex, body size, and on host performance. Our results indicate that male lizards are more likely to be infected than their female counterparts, and larger male individuals are infected than smaller individuals. Overall performance was reduced in male lizards that were parasitized than ones that were not. These results, along with the lack of differences in body condition, indicate trade-offs are occurring across life stages in this species.

These data suggest that parasite prevalence can negatively impact physiology, which can lead to indirect consequences on host fitness. These results provide an example of how exposure to ectoparasite infection is strongly sex-dependent and shows the potential physiological consequences of parasitism to hosts.

# 1| Introduction:

Host-parasite relationships have been a well-documented phenomenon across taxa, and understanding the dynamics and trade-offs between hosts and parasites has been the subject of many studies investigating consequences associated with consequences of parasitism (REFS). In brief, parasites exploit resources from their host, and during this exchange, parasites disrupt the host's homeostasis, which can ultimately result in negative effects on the host's health (Barnard & Behnke, 1990; Moore, 2002). A spectrum of host responses to parasites does exist, where infection can have little to no effect on host health (REFS) or where infection can result in deleterious effects to host health (REFS). This relationship between hosts and parasites is usually quantified in laboratory experiments and is often overlooked in field studies (REFS). This is because in nature the host-parasite relationship can be multi-dimensional, where parasitic infection can be driven by host sex, life stage, weather and habitat (Minchella & Scott, 1991;XXX). There is a need to quantify this interaction under natural settings further to understand the role parasites play in shaping host selection processes in nature.

**Hormones as mediators of fitness**

**2| Methods**

Study location was at the Land Between the Lakes National Recreation Area (LBL) in Trigg County, Kentucky (United States), where *Dermacentor variabilis (*American dog tick)and *Amblyomma americanum* (lone star tick) are common ectoparasites of *Sceloporus undulatus* (eastern fence lizard). During the Spring and Summer of 2014 and 2015, adult male and female *S. undulatus* were captured by hand or by noosing. Sex was determined by morphological characteristics and ventral colouration (Cooper and Burns, 1987). Upon capture, snout-to-vent length (SVL), body mass, and hindlimb length were measured for all individuals. Hindlimb length was defined as the greatest distance on the outstretched leg from the distal tip of the fourth toe to the point of insertion in the body wall (Pianka, 1969). Lizards were measured to the nearest 0.1 mm for length and 0.25 g for mass. Capture locations were recorded with a handheld GPS (Garmin Fēnix® GPS). The number of ticks infecting each captured lizard was recorded in the field before each animal was placed in a cloth bag and transported to Hancock Biological Station (Murray, KY), where the ticks were recounted before laboratory locomotor performance trials.

All locomotor performance trials were conducted within 24h of capture. Each lizard was placed individually into copper containers (repurposed autoclave pipette boxes; 4cm x 6cm x 25cm) which was then placed inside a lighted incubator (Percival I30-BLL) for 30min. The incubator maintained (33°C ±1.0) which is the preferred temperature of *S. undulatus* (Angilletta, 2001). After 30min, each lizard was encouraged to run on a race track (2.4 x 0.2m) by prodding with a soft-bristle paintbrush. The race track floor was covered by Astro turf that was maked into 25cm segments. Each trial was recorded with a camera that was mounted 3m above the center of the race track to ensure visibility of the whole race track. The camera recorded at a rate of 35 frames s-1. Lizards were raced three times, with trials separated by at least 30min for recovery. The quality of each sprinting trial was classified as “poor” or “good” (Van Berkum et al., 1989). Where a poor trial was defined as a pause or reversal run by a lizard, and a good trial was defined as a continuous run by the lizard. A minimum of two good trials were needed for an individual to be included in analyses. Maximum sprint speed was defined as the single fastest 25cm interval of the trials, and maximum 2-meter run speed (2 meter run) was the single fastest continuous 2 meter run speed of the trials. Videos were analysed using Tracker Video Software (version 4.85; www.cabrillo.edu/tracker). Further details on video data collection can be found in Wild & Gienger1. Lizards were then marked with a unique toe clip and released back at their location of capture within 24h of initial capture.

All statistical analysis were conducted using the R environment, ver. 4.1.0 ([www.r.-project.org](http://www.r.-project.org)), and significance was accepted at an α level of 0.05. Chi-square with Yates’ correction was used to assess the independence of the proportion of ticks observed between males and females. A logistic regression was used to test if body size (SVL) predicted the number of ticks found on an individual. Body condition index (BCI) was calculated from the residuals of an ordinary least squares linear regression of mass (g) on length (SVL), and an ANOVA was used to compare BCI measurements between uninfected lizards and infected lizards (1 ≥ ticks). An Analysis of Covariance (ANCOVA) was used to compare individual performance measurements (maximum sprint seed and 2-meter run) between lizards infected (1 ≥ ticks) and lizards uninfected with ticks. Hindlimb length was used as a covariate to remove the potential effects of body size on performance (Tsuji et al. 1989).

**3| Results**

A total of 92 lizards were captured (females n= 38; males 54) during the 2014 and 2015 field seasons. The frequency of tick infections was highly sex-specific, with the frequency of tick infection being over 2 times higher in males (n = 20; 37%) than in females (n = 5; 13%). Females were therefore precluded from further statistical analysis because the difference in tick infections was significantly different between sex (x = 9; df = 1; n = 92; p = 0.003). Infection rate for males ranged one to seven ticks per individual. There was a positive relationship between male body size, and the number of ticks (F = 0.103, p = 0.045), where larger males had a higher probability of tick infection than smaller males (Fig. 1). Maximum sprint speed was significantly higher in uninfected lizards (LS mean = 2.741m/sec) in comparison to infected lizards (LS mean = 2.48m/sec; F = 16.12; p = 0.016; Fig. 2a). Maximum 2-meter run speed was significantly higher in uninfected lizards (LS mean = 1.942m/sec) than in infected lizards (LS mean = 1.613m/sec; F = 15.01; p = 0.003; Fig. 2b). There were no differences in body condition indices between uninfected and infected lizards (F = 0.025; p = 0.875).

**4|Discussion**

Our study demonstrates that ectoparasite prevalence differed between sexes, and lizards infected with parasites showed reduced locomotor performance in comparison to uninfected lizards. Specifically, there was a negative relationship between parasite prevalence and two estimates for locomotor performance for male *S. undulatus.* Together these clear differences in the probability of infection between sex and performance may be driven by behavioural and physiological differences between male and female lizards. The lack of detection of body condition differences may suggest that infection may not affect overall health status long-term or the lack of differences does not support individuals in poor condition are more likely to be infected. However, it appears there are short-term consequences of reduced performance and differences between age class (SVL) suggest there are trade-offs occurring that may have fitness consequences in male lizards.

There are physiological differences associated with differing endocrine systems between male and female *S. undulatus*, where males have higher testosterone levels than females (Cox et al., 2005). Immunosuppression associated with differences of testosterone in male lizards has shown to attribute to higher intensities of ticks found in males with higher levels of testosterone (Olsson et al., 2000). Specifically, lizards with elevated testosterone have been shown to have higher mortality rates, decrease mass, and suppressed immune function in comparison to individuals with lower testosterone (Salvador et al., 1996; Klukowski & Nelson, 2001). There may be possible trade-offs between behavioural traits that promote high life-history productivity and the risk of parasitism.

Male bias in parasite prevalence or parasite abundance has been documented in other lizards (Tälleklint-Eisen & Eisen, 1999; Vaclav et al., 2007; Salkeld & Schwarzkopf, 2005). Our results clearly demonstrate that male lizards are more likely to be infected with ticks than females. Along with physiological differences between sex, it is possible behavior differences in this species between male and females could explain tick prevalence (Cooper & Burns, 1989; Haenel et al., 2001) and physiological differences (Cox et al., 2005) between males and females. For example, male *S. undulatus* have larger home range and territory size than females (Haenel et al., 2001). This behavioral difference could explain why there are differences in infection prevalence and intensity between sex, where males spend more time actively moving to defend and maintain territories which would increase probability of potential encounters with ticks and (or) encountering infected conspecifics within their home range.

Few studies have investigated the influence ticks have on relevant individual performance metrics, such as endurance and sprint speed, yet in reasonable numbers, it is conceivable that ticks can reduce performance. Parasitized lizards in this study ranged from one to eight ticks, with an average three ticks on each infected lizard. On average, an engorged female tick (*Amblyomma spp.*) takes about 7 to 12 days to engorge up to 11 mg (Bullard et al., 2016). If blood makes up about 5-8% of a lizard's body mass (Prosser and Brown 1973), then a small-bodied lizard can lose a large amount of blood for each engorging female. For averge sized lizard in our study (9.5g) could potentially lose 1-2% of blood for each engorged tick. This blood loss can have significant physiological consequences, such as anemia that can reduce performance (Lehmann, 1993). In an experimental study where ticks were allowed to attach and engorge on lizard hosts, ticks had a significant reduction in sprint and endurance performance than lizards with no ticks (Main & Bull, 2000). To our knowledge, this is the only experimental manipulation study to show a negative correlation between individual sprint and endurance performance and tick prevalence. Our results support the findings of Main & Bull (2000); however, *Tiliqua rugosa* are large-bodied lizards (18-34 cm) and have relatively few predators as adults (Bull, 1995). Whereas adult *S. undulatus* are considerably smaller (5-8 cm) and are frequently preyed upon by thermophilic snakes and birds (Crowley, 1985), and lower performance may leave parasitized lizards more vulnerable to predation.

Contrary to our findings, other studies have shown that ectoparasite infestation negatively affected body condition in reptiles (Dunlap & Mathies, 1993; Madsen et al., 2005; Olsson et al., 2000). However, some research suggest that host parasite associations should have low to minimal fitness costs due to co-evolutionary dynamics between hosts and parasites (Anderson & May, 1982; Stjerman et al., 2008). Ticks that are vectors for malaria-like diseases have shown to negatively affect body condition of lizard hosts (Dunlap & Mathies, 1993). Though the two genera of ticks found at our sites carry *Borrelia. spp.* (Lyme disease) and *R*ickettsia spp. (rocky mountain spotted fever) (James et al., 2015; Trout Fryxell et al, 2017). Tick paralysis is associated with *Borrelia spp.* is an illness that commonly occurs in the genus *Amblyomma*. Salivary neurotoxins released from a feeding tick can cause a partial loss or complete loss of muscle movement in hosts (Masina & Broady, 1999). Depending on the duration of attachment, the removal of the tick can improve or even reverse symptoms of paralysis (Gothe, 1984; Hanson et al., 2007). To our knowledge, here is one documented case of tick paralysis occurring in reptiles (*Coluber constrictor*) that has shown to a direct link to impairing locomotor ability and immediate improvement (within hours) of movement after removal of the parasite (Hanson et. al., 2007). It is possible that early stages of tick paralysis could cause a short-term effect on host mobility and may not affect the long-term health or body condition of the host.



Figure 1. Relationship between the probability of tick infection and body size (SVL) for male lizards. The line represents the probability function from logistic regression, and light grey bands represent 95CI of model fit. Raw data points are shown with circles that distinguish if lizards were infected by ticks (yellow) or lizards that were not (grey).



Figure 2. ANCOVA results of maximum sprint speed (a) and two-meter run speed (b) of male lizards. Hindlimb length (mm) was used as a covariate to remove the effect of size on performance. The presence of ticks (yellow) significantly reduced maximum sprint speed (p < 0.01) and two-meter run speed (p = 0.02) in comparison to lizards with no ticks (grey).