**Tick-tock, racing the clock: Parasitism is associated with decreased sprint performance in the Eastern Fence Lizard**

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**Abstract (200 words):**

Host-parasite relationships are important components of ecological systems, influencing the evolution of both hosts and parasites. High levels of ectoparasitic tick infections can disrupt host homeostasis, causing adverse effects on host health and performance. In this study, we examined the interplay between tick parasitism and host characteristics (sex, body size) on body condition and locomotor performance in Eastern fence lizards (*Sceloporus undulatus*). We found a higher prevalence of tick infections in male lizards relative to females, with larger males being more likely to experience tick infection. Infected lizards appear to exhibit an energetic trade-off between increased immune function and reduced locomotor performance, which is consistent with the immunocompetence-handicap hypothesis (ICHH). Higher prevalence of tick infections in adult male lizards may be explained by age as well as the immunosuppressive effects of testosterone. However, tick infection did not appear to reduce overall body condition of the lizards. Our findings shed light on the interplay between ectoparasitic infection, host characteristics, and locomotor performance in natural conditions. Such insights are crucial for understanding host-parasite dynamics and managing ectoparasite prevalence in ecological contexts.

# 1| Introduction:

Host-parasite relationships have been a well-documented phenomenon across taxa and are a fundamental aspect of ecological systems that shape the evolutionary trajectories of both hosts and parasites 1,2. Parasites exploit resources from their host, and have the potential to disrupt host homeostasis, which can ultimately result in adverse effects on host health influencing survival and reproduction3–6.

A complex interplay of factors shapes ectoparasite prevalence including host, sex, age, health, and habitat. Host sex can influence parasite load, as hormonal variations may affect immune responses and susceptibility to infection7,8. Developmental processes can dictate physiological changes across different life stages and alter host vulnerability to parasites9,10. For instance, acquired immunity, in which the immune system becomes more robust over time with increasing exposure to pathogens, can significantly decrease individual susceptibility to parasites, particularly in organisms with longer lifespans11. Body condition, reflecting the overall health and nutritional status of the host, can also be negatively impacted by parasitic infections12,13. Lastly, habitat can affect exposure to parasites and the host's ability to evade or cope with them12.

However, parasitic relationships often involve complex trade-offs1. For instance, a host's investment in growth or reproduction might compromise its immune function, increasing susceptibility to parasites14,15. These trade-offs are central to the Immunocompetence-Handicap Hypothesis (ICHH), which postulates that the expression of sexually selected traits, driven by hormones like testosterone, can negatively impact an organism's immune function, thereby increasing vulnerability to parasitism16,17. In particular, in reptilian hosts, meta-analytic and experimental manipulations have supported ICHH, where testosterone reduces immunocompetence and increases the incidence or severity of parasitism14,18. This may result in additional trade-offs for other sexually selected traits involving testosterone. For example, in lizards, locomotor performance is a sexually selected trait19, which is regulated by testosterone levels in some species20,21. Therefore, it's plausible that enhanced locomotor performance, driven in part by testosterone, may be counterbalanced by increased susceptibility to parasites such as ticks, illustrating the intricate balance between sexual selection, performance, and survival.

Most studies investigating the influence of tick parasitism on health and performance are from experimental manipulation of tick prevalence on hosts22,23 or through hormonal manipulations13,24,25. Under natural conditions, there is limited information on how the host-parasite relationship varies with factors such as sex and age, and whether infection influences host physiological traits. Here, we investigate how tick infection varies across sex and body size, and test if locomotor performance is affected in Eastern Fence Lizards (*Sceloporus undulatus*). This species demonstrates sex differences in hormonal profiles, including corticosterone and testosterone25,26, and hormonal manipulations in wild males (testosterone-implanted) have been shown to increase tick frequencies27. Suggesting that trade-offs associated with the Immunocompetence Handicap Hypothesis (ICHH) may be at play, where testosterone reduces immunocompetence and increases parasitism. Despite the wealth of knowledge derived from experimental manipulations, there remains a significant lack in our understanding of how these host-parasite dynamics unfold under natural conditions, particularly in relation to the influence of sex and age on natural infection rates and the subsequent impact on host physiological traits. This study aims to address this gap in understanding by investigating the interplay between tick parasitism, host characteristics, and locomotor performance in *S. undulatus* in under natural conditions. Such data will provide a more comprehensive and ecologically relevant perspective on host-parasite interactions.

**2| Methods**

Field research was conducted at Land Between the Lakes National Recreation Area in Kentucky (United States), where *Dermacentor variabilis (*American Dog Tick)and *Amblyomma americanum* (Lone Star Tick) are common ectoparasites of *S. undulatus*.During the Spring and Summer of 2014 and 2015, adult *S. undulatus* were captured by hand or by noosing. Morphological characteristics including enlarged base of the tail, femoral pores, and ventral colouration were used to determine sex. 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. 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 again 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 30 min. The incubator maintained (33°C ±1.0) which is the preferred temperature of *S. undulatus* (Angilletta, 2001). After 30 min, each lizard was placed on a race track (2.4 x 0.2m) and encouraged to run by prodding with a soft-bristle paintbrush. The race track floor was covered by Astroturf that was marked into 25cm segments. Each trial was recorded with a camera that was mounted 3 m above the center of the race track. The camera recorded at a rate of 35 frames s-1. Lizards were raced three times, with trials separated by at least 30 min for recovery. The quality of each sprinting trial was classified as “poor” or “good”28. 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 25 cm interval of the trials, and maximum 2-meter run speed was the single fastest continuous 2-meter run 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 & Gienger29. Lizards were then marked with a unique toe clip and released back at their location of capture within 24 h of initial capture.

All statistical analysis were conducted using the R environment, ver. 4.2.0 ([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 Analysis of Variance was used to compare BCI measurements between uninfected lizards and infected lizards (1 ≥ ticks). An Analysis of Covariance was used to compare individual performance measurements (maximum sprint speed 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 performance29.

**3| Results**

A total of 92 lizards were captured (females n= 38; males 54) during the 2014 and 2015 field seasons. There was a positive relationship between male body size, and the probability of tick infection (F = 0.103, p = 0.045), where larger males had a higher probability of tick infection than smaller males (Fig. 1A). For females, there was no relationship between body size and the probability of tick infection (F = -0.008, p = 0.928; Fig. 1B). The probability of tick infection 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 excluded 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. 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 (tick) prevalence differed between sex and body size, and lizards infected with ticks had lower locomotor performance than noninfected lizards. Specifically, there was a negative relationship between parasite prevalence and two estimates of locomotor performance (maximum sprint speed & 2-meter run speed) for male *Sceloporus undulatus.* Our results align with the Immunocompetence Handicap Hypothesis (ICHH) by demonstrating a higher prevalence of tick infections in male lizards relative to females, potentially due to the immunosuppressive effects of testosterone13,30. High testosterone levels in *S. undulatus* are responsible for increasing locomotor performance21. Furthermore, the significant reduction in both sprint performance metrics in infected lizards, regardless of their body condition, could be a consequence of the energy trade-off between maintaining immune function and dealing with the physiological stress of parasitic infection, another key aspect of the ICHH17,31.

The positive correlation between male body size and tick infection rate could be explained by the ICHH, where larger males with higher testosterone levels, may have compromised immune systems, making them more susceptible to parasites30. Physiological differences linked to endocrine systems between juvenile and adult male *S. undulatus* lizards play a significant role in variation in their life-history traits 24,25. More specifically, higher testosterone levels can be found in adults in comparison to juveniles26. Immunosuppression associated with differences in testosterone has been attributed to higher numbers of ticks found in lizards with higher levels of testosterone13.Moreover, in comparison to individuals with low testosterone, lizards with elevated testosterone have been shown to have lower growth, higher mortality rates, higher energetic costs, and higher costs associated with reproduction25,27,32. There is evidence across other taxa (birds, fishes, mammals, and insects) that immunocompetent males generally have higher success in mating and offspring production8. Collectively, these findings suggest the existence of trade-offs in male *S. undulatus*, particularly between behavioural traits that enhance life-history productivity and the increased risk of parasitism. There is evidence that this balance is primarily influenced by the testosterone levels in these lizards, highlighting the intricate relationship between endocrine systems, immune function, and life-history traits.

Male bias in parasite prevalence or parasite abundance has been documented in other lizards33–35. The results of our study show that ticks are more commonly found on male lizards compared to females. Male and female *S. undulatus* have different endocrine systems and behaviours26,27,36, which together could provide a mechanism for the observed differences in tick prevalence between sex. For example, male *S. undulatus* have higher testosterone levels26 and larger home ranges than females36. High testosterone in males may directly suppress the immune response, indirectly influence the host's energy expenditure, or stimulate males to move more frequently or over longer distances 5,37. Consequently, this increased activity could increase exposure to parasites that are seeking hosts38. Additional research is needed to determine the impact of hormonal and behavioural variations on the susceptibility to tick infections to hosts and to determine if these effects vary between the sex.

Indeed, the impact of ticks on individual performance metrics in host animals is an underexplored area in ecological studies (*but see*22), yet in reasonable numbers, it is conceivable that ticks can alter physiological aspects that would result in a reduction in performance. Parasitized lizards in this study ranged from one to seven ticks, with an average of three ticks on each infected lizard. A female tick (*Amblyomma spp.*) takes about 7 to 12 days to become fully engorged, extracting an average of 11 mg of blood39. If blood makes up about 5-8% of a lizard's body mass40, then a small-bodied lizard can lose considerable blood for each engorging female. An average-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, where a reduction of oxygen-carrying capacity of the blood could mechanistically explain the overall lower levels of locomotor performance41. In an experimental study, Main and Bull (2000) allowed ticks to attach and engorge on lizard hosts, and lizards with ticks had a significant reduction in sprint and endurance performance than lizards with no ticks22. Our results support the findings of Main & Bull22; however, *Tiliqua rugosa* are large-bodied lizards (~650g) and have relatively few predators as adults42 and rarely require sprinting to outrun predators. In contrast, adult *S. undulatus* are considerably smaller and are frequently preyed upon by thermophilic snakes and birds43. Thus, smaller lizard species that experience high tick loads could result in lower locomotor performance, leaving them exposed to potential predation.

Contrary to our findings, other studies have shown that ectoparasite infestation negatively affected body condition in reptiles13,44,45. Considering the findings from our study, it appears that ticks do not selectively infect hosts based on their health status or reduce the overall health of hosts. This is evident by the lack of significant differences in body condition between uninfected and infected lizards, even though infected lizards exhibited slower sprint and run speeds. This aligns with the findings of previous work, which suggested that ticks do not preferentially feed on healthier hosts, but the health of their hosts can affect the speed at which ticks could feed23. Experimentally testing how the duration or the frequency of tick infection influences other physiological traits will provide promising insights into the consequences of such effects. Finally, the results from our study further suggest that factors such as the sex and size of lizard hosts may play a more significant role in tick infection rates. Therefore, while tick infection does have an impact on the host speed, it does not appear to be related to the host overall health condition.

Literature cited



Figure 1. Relationship between body size (SVL) and probability of tick infection for male (A) and female (B) Eastern Fence Lizards. The line represents the probability function from logistic regression. 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 body 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).