Dear Professor Karen Sears and Editorial Team,

We would like to thank you and the reviewers for providing extremely constructive feedback on our manuscript entitled: “**Tick-tock, racing the clock: Parasitism is associated with decreased sprint performance in the Eastern Fence Lizard”.** We are glad yourself and Reviewers found the paper to be well written and of general interest.

We have now carefully considered all the comments and revised our main manuscript and supplementary materials to deal with the comments. Below we provide a line-by-line response (in ‘blue’) to each of the comments raised by the two reviewers (in ‘black’). Where relevant, we have pasted the section of our manuscript we have edited to provide clarity to what we have done to address comments. We believe that our revised manuscript is significantly improved following yours and the reviewer critiques.

We hope that you now find it suitable for publication in *Biological Journal of the Linnean Society*.

Sincerely,

Kristoffer Wild (on behalf of all authors)

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**Editor and editorial team**

 I invite you to respond to their comments and revise your manuscript accordingly. I would then be happy to consider it formally for publication in the Biological Journal of the Linnean Society. Please provide details in the Author's Response box of:  
\* The changes you have made; and  
\* Any of the comments that you have not accepted with the reason/s.

**Response**: We have provided a saved pdf that shows the in-line track changes form the original submission and have provided in detail our responses to each comment from the reviewers.

On the editorial side, authors are asked to ensure the following:  
\* The manuscript must conform exactly to the Biological Journal house-style, checking that all reference citations in the text match those in the References, and all figures, tables and appendices are cited in the text. For a copy of the Instructions to Authors, please visit <https://academic.oup.com/biolinnean/pages/General_Instructions>

- This has been done.   
\* Please check carefully the formats, especially concerning headings. Please see some recent issues or papers of the journal for style.  
- This has been done.

\* All figures should be a minimum of 300 dpi; any text in these should be in vector format.  
- This has been done within the document and they have been saved individually and attached.

\* Please check that photographs making up figures are all numbered consecutively

- This has been done.

**Reviewers' Comments to Author**:

**Referee: 1**

Comments to the Author(s)

Line 38: any example reference around the idea that ecto(parasite) abundances can be influenced by multiple factors?

**Response:** Yes this is a good point, we have added a sentence, with references, to this paragraph which includes the extrinsic mechanisms that drive abundances anddisease prevalence that are associated with ectoparasites.

“*Finally extrinsic mechanisms such as habitat modification, fire, and rainfall can facilitate the abundance of ectoparasites, and in some cases facilitate disease prevalence associated with ectoparasites (Berger et al., 2014; Diuk-Wasser, Vanacker, & Fernandez, 2021; Gallagher et al., 2022).”*

Refs:

* *Gallagher, M. R., Kreye, J. K., Machtinger, E. T., Everland, A., Schmidt, N., & Skowronski, N. S. (2022). Can restoration of fire‐dependent ecosystems reduce ticks and tick‐borne disease prevalence in the eastern United States?. Ecological Applications, 32(7), e2637*
* Berger, K. A., Ginsberg, H. S., Dugas, K. D., Hamel, L. H., & Mather, T. N. (2014). Adverse moisture events predict seasonal abundance of Lyme disease vector ticks (Ixodes scapularis). *Parasites & vectors*, *7*(1), 1-8
* Diuk-Wasser, M. A., VanAcker, M. C., & Fernandez, M. P. (2021). Impact of land use changes and habitat fragmentation on the eco-epidemiology of tick-borne diseases. *Journal of Medical Entomology*, *58*(4), 1546-1564

Line 51: a recent review of the H&Z hypothesis in lizards supporting the statement here is (Megía-Palma, R., Barrientos, R., Gallardo, M., Martínez, J., & Merino, S. (2021). Brighter is darker: the Hamilton–Zuk hypothesis revisited in lizards. Biological Journal of the Linnean Society, 134(2), 461-473.).

**Response:** Thank you for more recent reference, it has been used here and other places within the in the manuscript.   
  
Lines 53-54: “where testosterone reduces immunocompetence and increases the incidence or severity of parasitism” I would be careful citing here Roberts et al 2004 because in that paper the authors clearly say that the IHH is not demonstrated in reptiles and blood parasites (although it is fulfilled with ectoparasites).  
**Response:** Agreed. This reference has been removed and the reference the reviewer suggested (Megía-Palma et al., 2021) has been used.

Lines 57-59: yes, but the distance travelled by the lizards per se can also explain exposure to ectoparasites (two examples supporting my comment:  
- Wieczorek, M., Rektor, R., Najbar, B., & Morelli, F. (2020). Tick parasitism is associated with home range area in the sand lizard, Lacerta agilis. Amphibia-Reptilia, 41(4), 479-488.  
- Barrientos, R., & Megía-Palma, R. (2021). Associated costs of mitigation-driven translocation in small lizards. Amphibia-Reptilia, 42(3), 275-282.  
**Response:** We have added a caveat of increased locomotion or poor health of host influencing susceptibility of parasite infection.

“This enhancement in locomotion, however, may also increase the risk of parasite infestation, with host health and mobility adding further complexity to the vulnerability (Wieczorek *et al.*, 2020; Barrientos & Megía-Palma, 2021).”

Lines 60-61: “Most studies investigating the influence of tick parasitism on health and performance have been from experimental manipulation of tick prevalence” a more recent reference:

- Megía-Palma, R., Martínez, J., & Merino, S. (2018). Manipulation of parasite load induces significant changes in the structural-based throat color of male Iberian green lizards. Current Zoology, 64(3), 293-302.

- Lanser, D. M., Vredevoe, L. K., & Kolluru, G. R. (2021). Tick parasitism impairs contest behavior in the western fence lizard (Sceloporus occidentalis). Behavioral Ecology and Sociobiology, 75, 1-15.  
**Response:** We have added the suggested references and appreciate the more recent literature suggestions.

Lines 63-65: 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. The many works of Bull are example of this:  
- Main, A. R., & Bull, C. M. (2000). The impact of tick parasites on the behaviour of the lizard Tiliqua rugosa. Oecologia, 122, 574-581.

- “Megía-Palma, R., Paranjpe, D., Blaimont, P.,Cooper, R., & Sinervo, B. (2020). To cool or not to cool? Intestinal coccidians disrupt the behavioral hypothermia of lizards in response to tick infestation. Ticks and tick-borne diseases, 11(1), 101275.”  
**Response:** We have reworded this section to accurately reflect the current literature on the topic and the point we are trying to address. As for the Main and Bull reference, they did use adult lizards collected from the field but they were held in captivity for 6 months and tick load was manipulated on lizard host. We have added the Megía-Palma, et al., study as these animals were measured ~2d. We clarified our point about the lack information on how tick prevalence directly influences physiological traits.

“Under natural settings, there is how the host-parasite relationship varies with factors such as sex and age (Amo *et al.*, 2007; Dudek *et al.*, 2016; Pollock & John-Alder, 2020), but limited information on how parasites can directly influence host physiological traits for hosts in situ (*but see* Megía-Palma et al., 2020).”

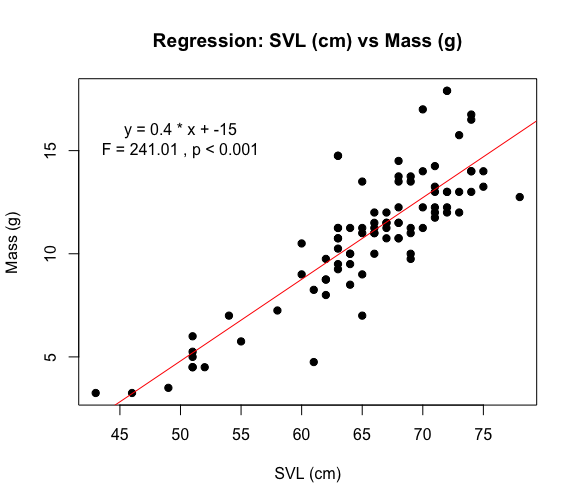
Line 76: an example supporting this prediction could be also the paper mentioned in the previous line of this revision.  
**Response:** We have added the reference to the paragraph prior which introduces the information that is known on this topic and the areas of limited understanding. The start of the paragraph we used references for these points to then outline our predications. We feel it would be redundant to have citations for our predictions that were outlined in the previous part of the paragraph.

Lines 113-114: “used to assess the independence of the proportion of ticks observed between males and females” Please, clarify what you mean with “proportion of ticks”. Do you mean number of ticks or tick load? Or proportion of males and females infected with ticks?  
**Response:** Yes we mean number of ticks between male and females. The Chi-square with Yate’s correction is used to test for a difference in proportions between categories. So it accounts for the sample size difference between the two categories. Specifically, it evaluates whether the distribution of counts across the categories of a 2x2 contingency table is different from what would be expected if the two variables were independent of each other.We apologise for the inconsistency of our wording here and have updated it so that it reads clearer.

“Chi-square with Yates’ correction was used to assess the independence of the number of ticks observed between males and females while accounting for the observations for each sex”

Lines 114-115: “Body condition index (BCI) was calculated from the residuals of an ordinary least squares linear regression of mass (g) on length (SVL)” Was this relationship linear? Or should the authors log-transform SVL and mass prior to performing the regression? Do females and males in Sceloporus undulatus show sexual dimorphism or have asynchronous reproductive cycles with storage of fat bodies in different seasons as in other lizard and Sceloporus species so BCI should be calculated for males and females separately?  
**Response:** Good points here. First you are correct that *S. undulatus* show sexual dimorphism and this would effect storage of fat bodies and they would need to be analysed separately. However, in the results section females were removed from further analysis due of the low frequency of parasitism.

As for the linearity mass (g) on length (SVL) the relationship was linear without transformation (see below). We have added the following in the final paragraph in the methods section “We assessed the data for homogeneity of variances and normal distribution where relevant. If the data did not conform to these assumptions, we applied transformations to achieve approximate normality and variance homogeneity”



Line 132: Please, provide the final number of lizards that were included in the analyses of speed performance because reading the Methods that you describe I assume some of the males were lost because they did not perform “good trials”.  
**Response:** The sample size can be found using the F ratio for each of our sprint performance measurements but we have added the final number of lizards that were used for locomotor performance analysis.

“A total of 54 male lizards were used in locomotor performance analysis”

Line 144-149: I find this explanation quite speculative. Please, check these two papers to see the negative effect of tick infestation on the hematocrit of Sceloporus lizards. Could this be a more plausible explanation of the negative effect of tick infestation on speed performance? Because, this said, I would expect that testosterone would increase the running performance of the lizards.  
**-** “Lanser, D. M., Vredevoe, L. K., & Kolluru, G. R. (2021). Tick parasitism impairs contest behavior in the western fence lizard (Sceloporus occidentalis). Behavioral Ecology and Sociobiology, 75, 1-15.”

- Dunlap, K. D., & Mathies, T. (1993). Effects of nymphal ticks and their interaction with malaria on the physiology of male fence lizards. Copeia, 1045-1048.  
**Response:** We have reworded this paragraph that provides evidence that supports ICHH may be driving differences. We have also aknowdge that we did no directly measure immune function but provided that with evidence from the literature on this species that ICHH maybe one possibility. We also added that other physiological parameters such as hematocrit levels could explain the negative effects of locomotor performance. We go into detail in paragraph 2/3 for support for ICHHH and discuss other possibilities, such as hematocrit levels, in paragraph 4.

“Our findings are congruent with predictions of the Immunocompetence Handicap Hypothesis (ICHH), with male lizards exhibiting a higher tick infestation rate than females, which may be indicative of the immunosuppressive effects of testosterone(Olsson *et al.*, 2000; Roberts, Buchanan, & Evans, 2004). While we did not directly measure immune function or testosterone levels, our results are supported by a body of literature that establishes a relationship between hormone levels, immune function, and parasite prevalence in this species (Klukowski & Nelson, 2001; Cox *et al.*, 2005b; John-Alder *et al.*, 2009). Other physiological parameters, such as reduction in hematocrit levels, could explain the negative effect of locomotor performance from tick infestation (Dunlap & Mathies, 1993; Lanser *et al.*, 2021). Together this suggests there may be a functional trade-off in parasitized hosts, which may be a product of immune function differences between sexes and differences across age classes.”

Line 158: a reference in a different format (35) was placed here. Please correct this typo.  
**Response:** Thanks, this instance, and others, have been corrected throughout the manuscript.

Lines 188-189: Yes, but they can also remain sheltered at lower temperature to reduce the negative impact of ectoparasites or the impact of reduced hematocrit on oxygen highly demanding organs such as the brain or heart (Megía-Palma, R., Paranjpe, D., Blaimont, P., Cooper, R., & Sinervo, B. (2020). To cool or not to cool? Intestinal coccidians disrupt the behavioral hypothermia of lizards in response to tick infestation. Ticks and tick-borne diseases, 11(1), 101275.).  
**Response:** This is a good point. We have added that other factors, such as thermoregulation, could be impacted by tick prevalence.

“In contrast, adult S. undulatus are considerably smaller and are frequently killed by fast-moving thermophilic snakes and predatory birds (Crowley, 1985). Thus, smaller lizard species that experience high tick loads may be at higher risk of predation due to a reduction in locomotor performance or may have to adjust other behaviours to avoid parasitism. For example, lizards infested with ticks have shown to select cooler temperatures, which could be a strategy to conserve energy to overcome the tick infestation (Megía-Palma et al., 2020).”

Line 191: Please, correct the typo of a different reference format.  
**Response:** Thanks. We should have fixed all these inconsistencies now.

Lines 191-193: I would like to see first the results after correcting BCI calculations based on my previous comments. In addition, I quite disagree with the statement “It appears that ticks do not influence host energetic status,” because the authors found that infested lizards performed worse than uninfected ones, clearly contradicting this statement written as conclusion remark.  
**Response:** Our body condition results have been clarified (see above). As for the statement, we agree that “energetic status” is too broad. We have changed the wording of the statement:

“It appears that ticks do not influence host health, as evident by the lack of differences in body condition between uninfected and infected lizards”

Lines 193-194: I think this thought goes beyond what authors tested in the current study.  
**Response:** This statement has been removed.

Lines 197-199: Do authors have repeated measurements of the body mass of the lizards at different time points during their stay in the lab? This would be better for testing whether ticks can reduce body mass or BCI of the lizards, a repeated measures analysis rather than a cross-sectional study of an isolated measure in time. For example, this might be circumvented if authors would have data on how long the ticks have been attached to the lizards at the time of weighing the lizards. In sum, to me looks rather unlikely to find a negative relationship with BCI in the way and time BCI measurements were performed.

**Response:** The reviewer brings up an excellent point but it was out of the scope our project. The ethics our project only allowed animals to be housed in the lab for a maximum of 48hrs. Body mass was recorded upon capture and animals were measured for locomotor performance within 24h of capture (*See paragraph 1 & 2)*. The reasoning behind the BCI measurements was to test if “poor” health individuals had higher tick abundances (*see Dunlap et al., 1993; Olsson et al., 2000; Madsen et al., 2005 for examples)*. This poor BCI would in turn effect other physiological traits such as locomotor performance.

* Dunlap, K. D., & Mathies, T. (1993). Effects of nymphal ticks and their interaction with malaria on the physiology of male fence lizards. *Copeia*, 1045-1048.
* Madsen, T., Ujvari, B., & Olsson, M. (2005). Old pythons stay fit; effects of haematozoan infections on life history traits of a large tropical predator. *Oecologia*, *142*, 407-412.
* Olsson, M., Wapstra, E., Madsen, T., & Silverin, B. (2000). Testosterone, ticks and travels: a test of the immunocompetence-handicap hypothesis in free-ranging male sand lizards. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1459), 2339-2343.

**Referee: 2**

Comments to the Author(s)

INTRODUCTION  
First Paragraph: This is a sparse paragraph and could benefit from a bit more background on parasite effects on host fitness. Keep in mind that there are a bunch of studies that find no evidence of parasite effects on host fitness. For example, there is a recent study by Conrad et al. 2023 that show no effects of parasitism on growth and body condition in S. undulatus.  
**Response:** We have adjusted this paragraph to fit the reviewers suggestion to elaborate more on the background of parasite effects on host “fitness” and provided that this isn’t always the case.

*“Host responses to parasites can be nuanced and influenced by life history traits, such as reproductive strategies (Moore & Wilson, 2002) or hormonal fluctuations across different life stages (Foo et al., 2017), which may dictate the degree of physiological investment in parasite defence mechanisms. While these interactions are often critical, they do not always translate into measurable impacts on host fitness (Conrad et al., 2023). This, the complexity of host-parasite dynamics highlights the need to consider a wide array of biological factors and ecological contexts to fully understand their impact on fitness consequences to hosts through time.”*

A complex interplay of factors determines ectoparasite (tick, mites, fleas, or lice

Line 38: By "prevalence", do you mean "load"? Load would be # of parasites, while prevalence can imply # of parasites, but also the % of individuals infected with at least 1 parasite.  
**Response:** Here and in other places we mean tick prevalence \*presence/absence of ticks. Thank you for clarification between the two because the usage in the literature seems inconsistent. We have gone through the whole manuscript and changed prevalence to tick load where appropriate.

Lines 37-38: There are studies out there that show evidence of what factors can affect parasitism. Elaborate a bit more on these. There is also a study that should be included on S. undulatus by Pollock & John-Alder 2019. It shows ectoparasite loads vary based on month of activity season, host sex, host age, and ectoparasite environmental abundance.  
**Response:** This point as been elaborated on in this paragraph and we also used the *Pollock & John-Alder* (2019) citation here and in the discussion. The areas we elaborated in this paragraph are how environment, food availability, and reproductive behaviours can influence exposure to parasitism:

*“Other factors such as food scarcity or reproductive behaviours, can also modulate an individual's susceptibility to parasites, further complicating the dynamics of parasitism (Moore & Wilson, 2002). Finally extrinsic mechanisms such as habitat modification, fire, and rainfall can facilitate the abundance of ectoparasites, and in some cases facilitate disease prevalence associated with ectoparasites (Berger et al., 2014; Diuk-Wasser, Vanacker, & Fernandez, 2021; Gallagher et al., 2022).”*

Lines 40-42: Is there substantial evidence that differences in immune function actually prevent ectoparasitism? To my knowledge, there aren't many and the overall story for this is quite tentative.  
**Response:** We have reworded to fit the reviewers point. There are experimental studies that have shown support for this hypothesis but meta-analytic approaches (Roberts et al., 2004) showed that this was highly species specific in reptiles.

“In reptilian hosts, experimental manipulations have supported the ICHH, where testosterone reduces immunocompetence and increases the incidence or severity of parasitism (Olsson et al., 2000; Megía-Palma et al., 2021), but meta-analytic work has shown support for this in reptiles is species specific (Roberts et al., 2004)”

Line 53: Results of studies on T and parasitism are mixed. Meta-analyses found administration of exogenous T causes an overall increase in parasitism (Roberts et al., 2004; Foo et al., 2017), but parasitism is not usually correlated with T in unmanipulated animals (Foo et al., 2017). I suggest you make this point.  
**Response:**

Line 64: There isn't that much limited information on host-parasite relationships between ages and sexes. See Pollock & John-Alder 2019, studies by RS Lane, and many others.  
**Response:**  Good point and thanks for the papers. We have reworded this section to meet this reviewer’s point and address reviewer 1’s point:

“Under natural settings, there is how the host-parasite relationship varies with factors such as sex and age (Amo et al., 2007; Dudek et al., 2016; Pollock & John-Alder, 2020), but limited information on how parasites can directly influence host physiological traits for hosts in situ (but see Megía-Palma et al., 2020).”

**METHODS**Lines 82-83: Can you be more specific with months (or weeks) that lizards were captured and tick parasitism was quantified? A recent study by Pollock John-Alder 2019, and other prior studies show effects of time of year (i.e., months/weeks of capture) where some times parasitism is significantly lower than others. As such, this could have impacted your results/findings.  
**Response:** The timings were between May and September. We have clarified this in the methods and expanded on this interesting point in the discussion.

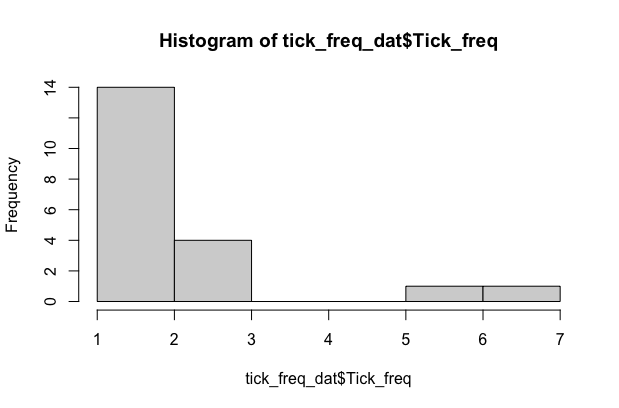
Methods: “From May - September of 2014 and 2015, adult *S. undulatus* were captured by hand or by noosing.”

Discussion: “A recent study investigating S. undulatus investigated mite parasitism across seasons, and found that mite loads vary seasonally, with the highest loads in the warmer months, and are influenced by environmental mite abundance (Pollock & John-Alder, 2020). More specifically adult females may experience higher mite loads than males during early summer, while yearling males have higher mite loads than females later in the season (Pollock & John-Alder, 2020).”

General Question: Why did you quantify ticks? Are there not chigger mites? If there are chigger mites, then why not quantify both types of ectoparasites?  
**Response:** It’s interesting, we documented if animals had mites but we had less than 5 instances with individuals with mites! Others have suggested that this species is a vector of Lyme disease...?

**RESULTS**  
What were the parasite loads? You describe parasite prevalence (whether a lizard had a tick/ticks), but not loads. Loads would likely be a much better descriptor of how parasitized an individual is and how much of an effect parasitism could have on individuals. For example, 1 tick or 2 ticks might not have much of an effect, but 5 or more ticks could have a strong effect.  
**Response:**

The reason tick load was not used was because it was out of the scope of our project. We did not have the replication/sample size of “tick load groupings” for statistical power to ask those questions. See histogram below of the frequency of lizards with ticks. We agree with this point and have added that tick loads should be investigated in future studies.



**DISCUSSION**  
Lines 144-146: You make this statement, but you did not measure immune function and circulating T levels, so cannot really draw correlations between the two and your results.  
**Response:**

We have reworded this paragraph that acknowledges that we did not directly measure immune function or testosterone levels but our results are supported by a body of literature that establishes a relationship between hormone level. We added that other physiological parameters such as hematocrit levels could explain the negative effects of locomotor performance. We go into detail in paragraph 2/3 for support for ICHHH and discuss other possibilities, such as hematocrit levels, in paragraph 4.

“Our findings are congruent with predictions of the Immunocompetence Handicap Hypothesis (ICHH), with male lizards exhibiting a higher tick infestation rate than females, which may be indicative of the immunosuppressive effects of testosterone(Olsson *et al.*, 2000; Roberts, Buchanan, & Evans, 2004). While we did not directly measure immune function or testosterone levels, our results are supported by a body of literature that establishes a relationship between hormone levels, immune function, and parasite prevalence in this species (Klukowski & Nelson, 2001; Cox *et al.*, 2005b; John-Alder *et al.*, 2009). Other physiological parameters, such as reduction in hematocrit levels, could explain the negative effect of locomotor performance from tick infestation (Dunlap & Mathies, 1993; Lanser *et al.*, 2021). Together this suggests there may be a functional trade-off in parasitized hosts, which may be a product of immune function differences between sexes and differences across age classes.”

Line 148: Did you record the approximate ages of lizards?  
**Response:** Age has been removed from this sentence.

Lines 165-166: The study by Conrad et al. 2023 show that S. undulatus in a NJ population have high tolerance to mite parasitism (with loads as high as 320 mites) and there were no measurable effects of parasitism. Other studies have found no effects of parasitism too (e.g., Paterson & Blouin-Demers 2000).  
**Response:** We’ve added the reviewers point that parasitism may not always affect host health (Introduction paragraph 1 and Discussion paragraph 5). We did not include this in this paragraph because we are discussing why differences in endocrine systems may be driving the prevalence differences in ticks between small male lizards vs large male lizards – as well as the clear differences between female and male lizards. So we do agree this is a point to make but it has been made elsewhere within the manuscript.

Line 175: Average of just 3 ticks? This is quite low, especially compared to other studies on mite parasitism or tick parasitism (see studies on S. occidentalis in CA). Could this really be enough to have effects on a host lizard? Relatedly, is this why you used "probability of infection" and not tick load (actual number of ticks parasitizing a host lizard)?  
**Response:** We agree at first it seems 3 ticks seems like a small number but we go on saying that a lizard can lose up to 2% of blood for each engorged tick which could have a direct physiological effect on performance (via reduction of oxygen-carrying capacity of blood cells). The reason tick load was not used see response in results section (above).

**FIGURE CAPTIONS**You say "yellow" for tick-infested lizard points, but the color is red (or at least orange).

**Response:** Thank you, this has been corrected.