# Testosterone, body size, and sexual signals predict parasite load in Yarrow's Spiny Lizards (Sceloporus jarrovii)

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# ARTICLE

# Testosterone, body size, and sexual signals predict parasite load in Yarrow's Spiny Lizards (*Sceloporus jarrovii*)

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**Abstract:** Parasite load significantly impacts host health and fitness and may vary substantially among individuals within a population. The immunocompetence handicap hypothesis posits that sexual signals are honest indicators of male quality because they are maintained by testosterone, an immunosuppressant that yields higher parasite loads. Additionally, testosterone may influence parasite load by increasing activity levels. We examined these two hypotheses in a wild population of Yarrow's Spiny Lizards (*Sceloporus jarrovii* Cope, 1875) in Arizona. We (*i*) compared fecal testosterone levels to ectoparasite and haemoparasite loads, (*ii*) tested if sexual signals (total coloured area, aggression, and head size), locomotor activity, and body size correlated with testosterone levels, and (*iii*) compared sexual signals, locomotor activity, and body size to parasite load. Ectoparasite loads increased with total coloured area and tended to increase with testosterone, but this latter relationship was only nearly significant. Parasite loads increased with body size. Thus, we found some support for the immunocompetence handicap hypothesis and none for the activity hypothesis. Our results are consistent with an alternative hypothesis that larger individuals have more parasites because they have more surface area and (or) have had longer to accumulate parasites. Future studies should examine the relative contributions of testosterone and glucocorticoids in driving variation in parasite loads.

*Key words:* behaviour, chiggers, immunocompetence handicap hypothesis, locomotor activity, *Plasmodium*, Yarrow's Spiny Lizard, *Sceloporus jarrovii*.

**Résumé :** La charge parasitaire a une incidence significative sur la santé et l'aptitude de l'hôte et peut varier considérablement d'un individu à l'autre au sein d'une même population. L'hypothèse du handicap d'immunocompétence postule que les signaux sexuels sont des indicateurs fiables de la qualité des mâles parce qu'ils sont maintenus par la testostérone, un immunosuppresseur qui entraîne des charges parasitaires accrues. En outre, la testostérone pourrait influencer la charge parasitaire en accroissant les niveaux d'activité. Nous avons examiné ces deux hypothèses dans une population sauvage de lézards épineux de Yarrow (*Sceloporus jarrovii* Cope, 1875), en Arizona. Nous avons (*i*) comparé les concentrations de testostérone fécale aux charges d'ectoparasite et d'hémoparasites, (*ii*) vérifié si les signaux sexuels (superficie colorée totale, agressivité et taille de la tête), l'activité locomotrice et la taille du corps étaient corrélés aux concentrations de testostérone et (*iii*) comparé les signaux sexuels, l'activité locomotrice et la taille du corps à la charge parasitaire. Les charges d'ectoparasites augmentaient parallèlement à la superficie colorée totale et tendaient à augmenter parallèlement à la concentration de testostérone, cette dernière relation n'étant que presque significative. Les charges parasitaires augmentaient parallèlement à la taille du corps. Si certains résultats appuient donc l'hypothèse du handicap d'immunocompétence, aucun n'appuie l'hypothèse de l'activité. Nos résultats concordent avec une autre hypothèse selon laquelle les individus plus grands auraient plus de parasites parce qu'ils ont une plus grande superficie et/ou ont eu plus de temps pour accumuler des parasites. Des études futures devraient se pencher sur les contributions relatives de la testostérone et des glucocorticoïdes comme causes de variabilité de la charge parasitaire. [Traduit par la Rédaction]

*Mots-clés :* comportement, lepte automnal, hypothèse du handicap d'immunocompétence, activité locomotrice, *Plasmodium*, lézard épineux de Yarrow, *Sceloporus jarrovii*.

## Introduction

Parasitism can influence the health and fitness-related traits of hosts (reviewed in Møller 1997). Parasites may decrease the energy available to the host directly or indirectly (Khokhlova et al. 2002) and hinder movement, copulation, feeding, or respiration (Schall 1990; Walter and Proctor 1999). Parasites may also diminish an individual's attractiveness to potential mates (Zuk et al. 1990) and affect reproductive success (Schall 1983, 1990) and population dy-

namics of hosts (Begon et al. 1990). Parasite loads differ between the sexes in a variety of vertebrate taxa, including mammals, reptiles, birds, amphibians, and fishes (reviewed in Klein 2004). In the majority of cases, the intensity and prevalence of parasitic infections is higher in males than in females (Klein 2004). Males may be more susceptible to parasites for various reasons, depending on taxa; for example, having a larger body size (Moore and Wilson 2002), covering more area and moving more, which may

increase exposure to parasites (Klein 2000; Nunn and Dokey 2006), or having a weaker immune response than females (Zuk and McKean 1996; Klein 2000).

Immunological differences between males and females are often attributed to the level of circulating steroid hormones (reviewed in Klein 2004). According to the immunocompetence handicap hypothesis, testosterone (T) enhances the expression of sexual signals in male vertebrates while also suppressing the immune system and thus increasing the susceptibility of individuals to parasitic infection (Folstad and Karter 1992; reviewed in Roberts et al. 2004). While some studies have shown that T increases parasite load (e.g., Weatherhead et al. 1993; Saino et al. 1995; Salvador et al. 1996; Klukowski and Nelson 2001; Cox et al. 2005; Cox and John-Alder 2007), others have failed to find a relationship (e.g., Veiga et al. 1998; Buttemer and Astheimer 2000; Oppliger et al. 2004). This may be due to differences in the types of parasites examined (Klein 2004; Roberts et al. 2004; Fuxjager et al. 2011). Resistance to different parasites may occur via different mechanisms (e.g., antibody production versus other cellular immune responses) and T may affect each differently (Klein 2004; Fuxjager et al. 2011).

The Yarrow's Spiny Lizard (*Sceloporus jarrovii* Cope, 1875) is a small, insectivorous mountain lizard that is strongly territorial (Simon 1975). As is typical, males have higher levels of T than females (Moore 1986), and experimentally elevated T increases aggression in both sexes (Marler and Moore 1989; Woodley and Moore 1999), as well as activity and territorial patrolling behaviour in males (Marler and Moore 1989). By manipulating T levels, Fuxjager et al. (2011) found that T increased ectoparasite load, decreased intestinal parasite load, and had no effect on blood parasite load in *S. jarrovii*. Fuxjager et al. (2011) proposed that individuals with more T have higher ectoparasite loads because they are more active and therefore pick up more parasites, rather than because of the immunosuppressive effects of T. In support of this hypothesis, lizards injected with T have higher ectoparasite loads and are more mobile than sham-treated individuals (Olsson et al. 2000). However, whether elevated parasite loads were due to increased mobility or suppression of the immune system was not determined. To the best of our knowledge, the activity hypothesis has not been tested in lizards with natural levels of circulating T.

Using *S. jarrovii*, we tested two alternative hypotheses (Fig. 1) to explain variation in parasite loads. First, we tested several of the predictions of the immunocompetence handicap hypothesis (Fig. 1; Folstad and Karter 1992), including (*i*) parasite load increases with T levels, (*ii*) parasite load increases with the level of sexual signals (aggressive behaviour, relative head width, and the area of colour patches), and (*iii*) male lizards have higher parasite loads than female lizards because males have more T than females (Moore 1986). Aggression (Marler and Moore 1989; Woodley and Moore 1999), head width (Husak et al. 2007), and coloured area (Cox et al. 2008) are driven by T in lizards and influence success in courtship behaviours and intraspecific dyadic encounters (e.g., Fox 1983; Olsson 1994; Perry et al. 2004). We did not directly test the prediction that T suppresses immune function. Second, we tested the hypothesis that T increases parasite load via an increased activity level (Fig. 1), because more active lizards are more likely to pick up parasites in the environment (Fuxjager et al. 2011). We predicted positive relationships between locomotor activity level, T levels, and parasite load. This is the first study, to the best of our knowledge, teasing apart the effects of T and locomotor activity level on two classes of parasites in lizards.

## Materials and methods

### Study site and species

*Sceloporus jarrovii* is a relatively small (mean = 13 g), heliothermic, insectivorous lizard that lives on talus slopes and other rocky habitats in mountains in the southwestern USA and northern Mexico. We sampled 100 *S. jarrovii* (21 female juveniles, 30 male

juveniles, 23 female adults, 26 male adults) on three talus slopes (38 individuals from each of the first two slopes and 24 individuals from the third slope) at Barfoot Park (elevation 2550 m) in Coronado National Forest in the Chiricahua Mountains, near Portal, Arizona, from 28 September to 3 October 2013, during the breeding season. This research was conducted with a State of Arizona Scientific Collection Permit (No. SP635290) and approved by the University of Ottawa's Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care (#BL-282).

### Field measurements

We caught lizards by noose. Upon capture, we attempted to collect a fecal sample, ran activity and aggression trials (see below), measured their mass, determined their sex using secondary sexual characteristics (enlarged femoral pores, postanal scales, enlarged tail base, and blue patches on the throat and sides of the belly in males), and photographed (using a digital camera; 12.3 megapixels) their ventral side with a reference scale object. Fecal samples were collected by rubbing the lizard's venter after capture. After fecal sample collection, lizards were placed in individual cloth bags until the behavioural trials. We analysed each picture using ImageJ (Abramoff et al. 2004) and measured snout–vent length (SVL; correlated with age), head width, and total coloured area (TCA; sum of coloured throat patch and ventral side patch areas). For ectoparasite load, we counted the number of chiggers (Acari: Trombiculidae) on the entire body of each individual with the help of a hand lens. We toe-clipped each individual and created one blood smear per individual on glass slides for analysis of haemoparasites (see below). Finally, we conducted behavioural trials examining the activity level and aggression of each individual. We released each lizard at its point of capture within 4 h of capture.

### Activity and aggression

We placed each lizard in an arena (50 cm × 35 cm × 50 cm) that acted as a novel environment (similar to Rodriguez-Prieto et al. 2011) for 5 min to assess locomotor activity level. Behaviour in a novel environment is often used as a rapid assessment of locomotor activity, particularly in animal temperament research (e.g., Mikheev and Andreev 1993; Boon et al. 2007; Rodriguez-Prieto et al. 2011). Short-term locomotor activity may be influenced by environmental factors such as interactions with conspecifics (Downes and Shine 1998; Aragón et al. 2003) and predators (Lima and Dill 1990; Downes and Shine 1998), and placement of the lizards into an arena removes these potentially confounding variables. Behavioural trials occurred before measurements and toe-clipping to reduce the effect of handling on behavioural responses. The arena contained a large flat rock in the middle that functioned as a display rock, and we fixed a mirror (30 cm × 30 cm) to one end of the arena, but kept it covered with cardboard during the locomotor activity trial. We recorded each trial using a digital video camera (at 15 frames/s) and we later watched the video for analysis. All trials occurred between the hours of 0900 and 1600 in full sun at the base of the slope being sampled. We measured the number of movement bouts performed, the amount of time active (walking, running, and climbing), and the amount of time spent hiding beneath the rock during the trial.

Following the 5 min locomotor activity trial, we removed the cover from the mirror and continued recording the lizard for another 5 min to score aggression. Mirrors have been successfully used to elicit aggressive responses in other iguanids (e.g., Hurd 2004). Following the trial, we recorded the surface body temperature (±0.1 °C) of the lizard using an infrared thermometer (Raynger ST; Raytek Corp, Santa Cruz, California, USA). We then removed the lizard and wiped down the arena with water to remove scent cues (Bastiaans et al. 2013). During the video analysis,

**Fig. 1.** Flowchart representing two alternative hypotheses that explain the relationship between testosterone and parasite load in Yarrow's Spiny Lizard (*Sceloporus jarrovii*). On the left is the immunocompetence handicap hypothesis, where testosterone directly causes an increase in parasite load due to immunosuppression. Individual indicators, such as sex and sexual signals (head width, total coloured area, and aggression) are correlated with parasite load due to the relationship between testosterone and these factors. On the right is the activity hypothesis, where testosterone only indirectly affects parasite load through its effect on locomotor activity. Testosterone causes an increase in locomotor activity, and more active individuals are then more likely to encounter parasites.
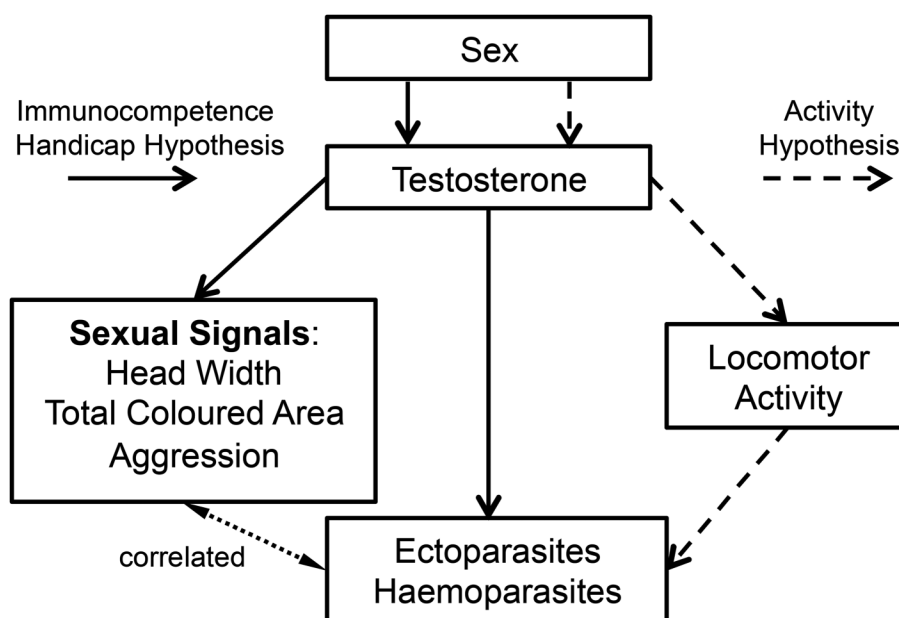


we recorded the number of aggressive behaviours as the number of push-ups, lateral compressions, bites, and lunges, and the number of submissive behaviours as the number of retreats (Bastiaans et al. 2013). We also recorded the time to the first movement, whether a lizard saw the mirror, and the time spent hiding. We created a hiding score by classifying 0 s spent hiding as 0, less than or equal to 150 s hiding as 1, and greater than 150 s as 2. One hundred and fifty seconds was chosen because it represents the middle of the 300 s behavioural trial. Using these data, we calculated an aggression score for each individual by adding the number of different aggressive behaviours and subtracting the number of submissive behaviours (retreats and hiding score; Table 1).

### Haemoparasites

To quantify haemoparasites, we created one blood smear per lizard. Bulté et al. (2009) and Fuxjager et al. (2011) previously found a high prevalence of *Plasmodium chiricahuae* Telford, 1970 in this species. We fixed the smears in methanol for 1 min and stored the smears in silica gel until we analysed the smears in the laboratory. We stained the smears in the laboratory using Wright–Giemsa stain (Fisher Scientific Company, Middleton, Virginia, USA). We viewed the smear from each lizard at 400× magnification and counted the number of infected red blood cells for every 5000 counted. We then multiplied our infected cell counts by two to enable comparison with the standard 10 000 cell count used in other studies (Oppliger et al. 2004).

### Testosterone

We attempted to collect fecal samples from all lizards captured, but some lizards (especially adults, which appear largely aphagic during the mating season; see Discussion) did not provide samples. Thus, we obtained fecal samples from 30 of the 100 lizards (13 female juveniles, 11 male juveniles, 4 female adults, 2 male adults). We weighed each fecal pellet and placed it in 50% ethanol at a ratio of 0.1 g feces to 1.0 mL of ethanol. At the end of each field day, we shook each sample by hand for 5 min and then centri-

fuged each sample at 2600 rev/min for 10 min. Hand shaking is an efficient method to extract steroid hormones from fecal samples in the field (Ziegler and Wittwer 2005). We then poured each sample through filter paper and collected the supernatant of our fecal extract. We sent our samples to the Toronto Zoo Reproductive Physiology Laboratory (Toronto, Ontario, Canada), where samples were analysed for T using enzyme immunoassay (EIA) techniques as in Kummrow et al. (2011), with a detection limit of 3.5 ng of T/g of feces. The laboratory also compared serial dilutions of pooled fecal extract to the standard curve and demonstrated parallel displacement with the standard curve ($r = 0.98$, $p < 0.01$). We used fecal T levels because sample collection is less invasive than obtaining blood from the orbital sinus (Schwarzenberger 2007). Fecal T levels have been quantified in a variety of taxa (reviewed in Schwarzenberger 2007), are correlated to serum T levels (e.g., Walker et al. 2002), and change during reproductive cycles (Kummrow et al. 2011) in several species.

### Statistical analyses

As detailed below, we conducted three sets of statistical analyses to test our hypotheses. We examined the relationships between (*i*) parasites (both ectoparasites and haemoparasites) and T levels, (*ii*) T levels and indicators of body size, sexual signals, aggression, and activity, and (*iii*) parasites (both ectoparasites and haemoparasites), activity, and traits influenced by T (TCA: Cox et al. 2008; aggression: Marler and Moore 1989; Woodley and Moore 1999; head size: Husak et al. 2007) (Fig. 1). All statistical analyses were conducted using the program R version 3.0.2 (R Core Team 2013). We used bias-corrected Akaike's information criteria ($AIC_c$; package: qpcR; function: $AIC_c$; Spiess 2012) to select the final model for each analysis. The model with the lowest $AIC_c$ was considered to be the most supported model as long as the difference in $AIC_c$ ($\Delta AIC_c$) between models was >2; when $\Delta AIC_c$ < 2, we chose the most parsimonious (fewest parameters) model (Burnham and Anderson 2002). Normality (using Shapiro–Wilk tests) and homogeneity of variance (using plots of residuals and fitted values) were

**Table 1.** Behaviours used to calculate the aggression score for Yarrow's Spiny Lizards (*Sceloporus jarrovii*) in mirror trials.

| Behaviour | Description | Score* |
|---|---|---|
| Approach | Count of the number of times the lizard approached the mirror | +1 per approach |
| Lunge | Count of the number of times the lizard lunged at the mirror | +1 per lunge |
| Push-up | Count of the number of times the lizard made push-ups at the mirror | +1 per push-up |
| Lateral compression | Count of the number of times the lizard laterally compressed at the mirror | +1 per lateral compression |
| Substrate tastes | Count of the number of times the lizard licked the floor or rock while facing the mirror | +1 per taste |
| Retreats | Count of the number of times the lizard retreated from the mirror after viewing the mirror | −1 per retreat |
| Hiding score | 0 if time hiding = 0 s; 1 if time hiding ≤ 150 s; 2 if time hiding > 150 s | 0, 1, or 2 |

*The overall aggression score for each lizard is calculated by adding the scores together for each behaviour.

confirmed for each model. We tested whether the lizards from our three sampling sites differed in terms of SVL, mass, head width, or parasite load (see Results). The sampled sites were less than 300 m apart, which is less than the maximum dispersal distance for other *Sceloporus* lizards (over 400 m; Massot et al. 2003). Therefore, site was not included as a random effect in any of our models. We also examined whether time of day (as measured by the order of trials) and the day of sampling had an effect on our behavioural variables.

We first examined whether T predicted the level of parasitism in lizards using multiple linear regression (package: stats; function: lm; R Core Team 2013). The square-root-transformed number of ectoparasites per lizard was the dependent variable and T, SVL, sex, and the interactions SVL × sex and T × sex were independent variables. We included SVL and sex to control for differences that might cause an increase in parasites unrelated to T. We did not include age group as a variable in any of our analyses because age group is based on SVL (juveniles were less than 6.5 cm SVL and adults were longer than 6.5 cm SVL) and SVL thus contains more information than age group. In addition, in preliminary analyses SVL explained more variance in parasite load than an age-group variable. Second, we examined haemoparasite levels using linear mixed effects models (package: nlme; function: lme; Pinheiro et al. 2012). We used the $\log_{10}$-transformed number of infected red blood cells per 10 000 cells counted + 1 as the dependent variable and T, SVL, sex, and the interactions SVL × sex and T × sex as fixed effects. The identity of the person counting haemoparasites was included as a random effect to control for observer bias because there were nearly significant differences between observers (Kruskal–Wallis $\chi^2 = 5.95$, $p = 0.051$). There was no observer bias in our ectoparasite counts because one person counted all ectoparasites.

We then examined whether aggression, activity, or other individual indicators (e.g., sex, SVL, TCA) predict T levels using multiple linear regression. We used $\log_{10}$-transformed T as the dependent variable and sex, SVL, body condition, TCA, head width, aggression score, time active, time hiding, and all two-way interactions between sex and each continuous variable as independent variables. TCA and head width were both highly correlated with SVL, so we used the residuals of a regression between $\log_{10}$-transformed TCA and SVL and between head width and SVL in our analysis. The residuals of a regression between mass and SVL were used as an index of body condition (Schulte-Hostedde et al. 2005). These residuals provide size-corrected estimates of traits that may be related to individual quality. Finally, time active was correlated with the temperature of the lizard during the trial (but temperature did not affect time hiding or aggression), so a temperature-corrected activity indicator was created using the residuals of the regression between time active and temperature. We refer to these residual variables henceforth as TCA, head width, body condition, and time active.

Finally, we examined whether body size, activity, and sexual signals predict parasite levels. First, we analysed ectoparasite levels using multiple regression. We used the square-root-transformed number of ectoparasites per lizard as the dependent variable and SVL, sex, TCA, head width, condition, aggression score, time active, time hiding, and all two-way interactions between sex and each continuous variable as independent variables. Second, we analysed haemoparasite levels using a linear mixed effects model. The $\log_{10}$-transformed number of infected red blood cells per 10 000 cells counted + 1 was the dependent variable and SVL, sex, TCA, head width, body condition, aggression score, time active, time hiding, and all two-way interactions between sex and each continuous variable were included as fixed effects. The identity of the person counting haemoparasites was included as a random effect to control for observer bias. Values are reported as mean ± SE, unless otherwise noted.

## Results

The 100 *S. jarrovii* used in this study ranged in SVL from 38.3 to 97.3 mm (63.1 ± 1.8 mm) and in mass from 1.0 to 26.0 g (10.3 ± 0.8 g). The number of ectoparasites per individual ranged from 0 to 179 (39.4 ± 4.2) and the number of infected red blood cells per 10 000 cells ranged from 0 to 1042 (84.5 ± 14.5). Of the 100 *S. jarrovii*, 30 provided fecal samples and the mass of fecal samples ranged from 0.10 to 0.20 g (0.11 ± 0.005 g). T concentrations from the fecal samples of these 30 lizards ranged from 3.5 to 121.2 ng/g (22.2 ± 5.0 ng/g). Site of collection had no effect on SVL ($p = 0.51$), mass ($p = 0.27$), head width ($p = 0.10$), or the number of ectoparasites ($p = 0.20$) and haemoparasites ($p = 0.33$). Time of day did not affect time spent active ($p = 0.75$), time spent hiding ($p = 0.99$), or aggression ($p = 0.62$). Similarly, the day of the trial did not affect time spent active ($p = 0.09$), time spent hiding ($p = 0.97$), or aggression ($p = 0.24$).
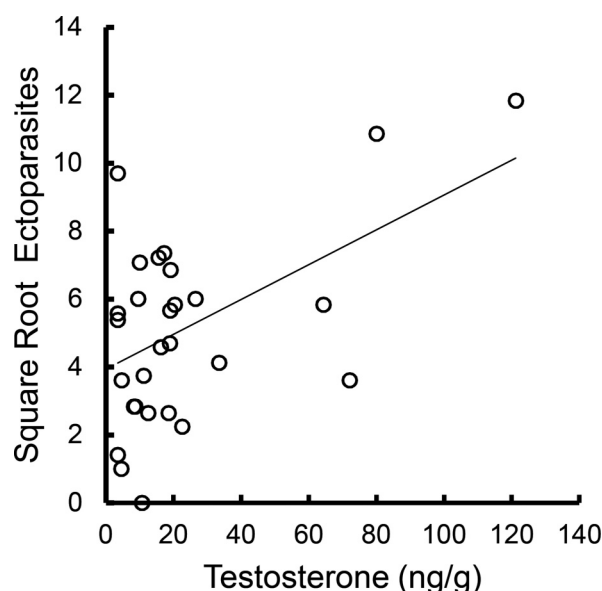
Ectoparasite levels tended to increase, although only nearly significantly, with T (coefficient = 0.03, $t = 1.99$, $p = 0.057$; Fig. 2) and increased significantly with SVL (coefficient = 0.78, $t = 2.15$, $p = 0.041$, whole model df = 27, $R^2 = 0.37$; Supplementary Table S1[1]). Haemoparasite levels did not vary with T (coefficient = − 0.000047, $t = 0.0093$, $p = 0.99$, whole model df = 26; Supplementary Table S2[1]). Sex was not a significant predictor of ectoparasite or haemoparasite load in the most supported models (Supplementary Tables S1[1] and S2[1]).

T levels increased with SVL (coefficient = 0.10, $t = 2.09$, $p = 0.046$; Fig. 3A) and head width (coefficient = 1.55, $t = 2.14$, $p = 0.042$; whole model df = 26; Fig. 3B; Supplementary Table S3[1]) and were higher in males than in females (coefficient (male) = 0.33, $t = 2.49$, $p = 0.02$). Neither aggression score nor activity levels were related to T levels.

Larger lizards harboured more ectoparasites than smaller lizards (coefficient = 0.53, $t = 2.86$, $p < 0.01$; Fig. 4A) and ectoparasite counts increased with TCA in males but not in females (coefficient (male) = 13.80, $t = 3.02$, $p < 0.01$, whole model df = 95, $R^2 = 0.26$; Fig. 4B; Supplementary Table S4[1]). Haemoparasite levels also increased with SVL (coefficient = 0.11, $t = 2.64$, $p = 0.01$, whole model

**Fig. 2.** The relationship between square-root-transformed number of ectoparasites and testosterone in Yarrow's Spiny Lizards (*Sceloporus jarrovii*) from southeastern Arizona. The number of ectoparasites is the total count of ectoparasites found on the scales of a lizard (*N* = 30).



**Fig. 3.** The relationship between $\log_{10}$-transformed testosterone and snout–vent length (A) and between $\log_{10}$-transformed testosterone and residuals of head width (B) between male and female Yarrow's Spiny Lizards (*Sceloporus jarrovii*) from southeastern Arizona. The trend line in each figure pools males and females.



df = 96; Fig. 5; Supplementary Table S5[1]). Neither aggression score nor activity levels affected parasite levels. None of the independent variables were significantly correlated (all $r < 0.46$; Supplementary Table S6[1]).

## Discussion

In this study, we tested two alternative hypotheses (immunocompetence handicap and activity hypotheses) to determine why *S. jarrovii* individuals differ in their intensity of infection by chiggers (ectoparasites) and *Plasmodium* (haemoparasites). We found some support for the immunocompetence handicap hypothesis for ectoparasites. As predicted by this hypothesis, ectoparasite loads tended to be higher in lizards with higher T, but the relationship was only nearly significant. This near significance is likely due to the small sample of lizards that produced a fecal pellet and limited samples with high T. Ectoparasite loads were positively correlated with TCA in males. However, males did not have more parasites than females, even though they had higher levels of testosterone. We found no support for the activity hypothesis, as there was no correlation between locomotor activity and either testosterone or parasite load.

For the immunocompetence handicap hypothesis, we found partial support for two out of three of the predictions for ectoparasites. However, we found no relationship between T and TCA, although ectoparasite load increased both with TCA and (nearly significantly) with T. The lack of relationship between T and TCA is likely caused by the small number of fecal samples from adult males (*n* = 2), and adult males have the highest T levels (Moore 1986) and exhibit the most developed ventral colouration. Expression of ventral colouration in *S. jarrovii* is driven by T (Cox et al. 2008) and is used in intersexual signalling for courtship and intrasexual displays of aggression (Tokarz 1995; Bastiaans et al. 2013). Thus, we believe the relationship between ectoparasite load and TCA in males supports the immunocompetence handicap hypothesis.

None of the predictions of the immunocompetence handicap hypothesis were supported for haemoparasite load. There was no relationship between T and haemoparasite load or between sexual signals and haemoparasite load. Our data are consistent with
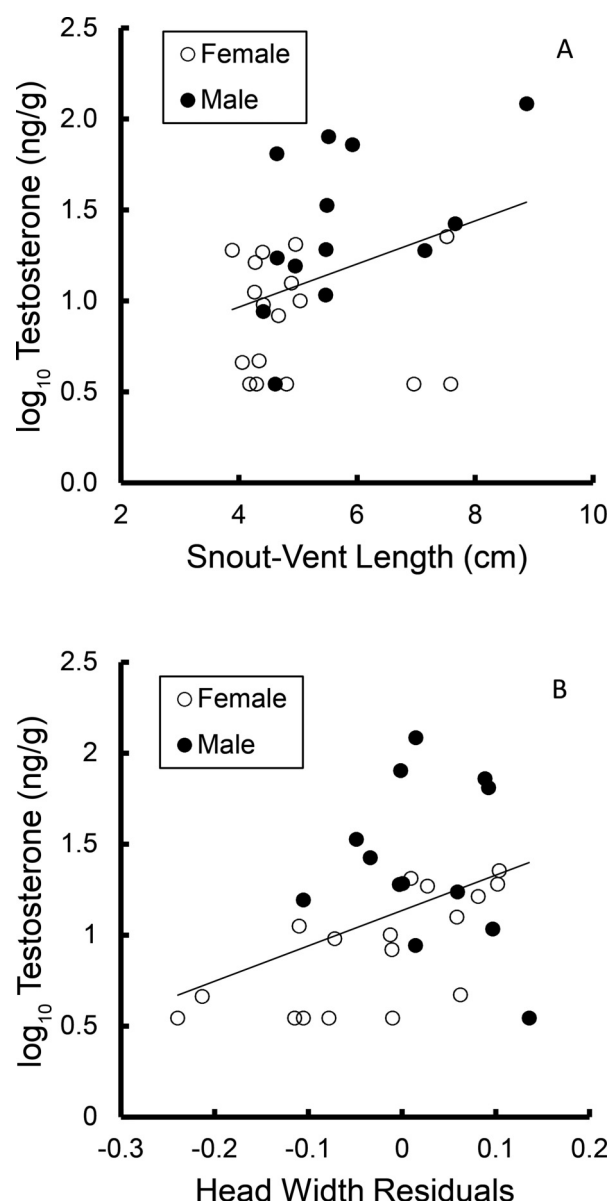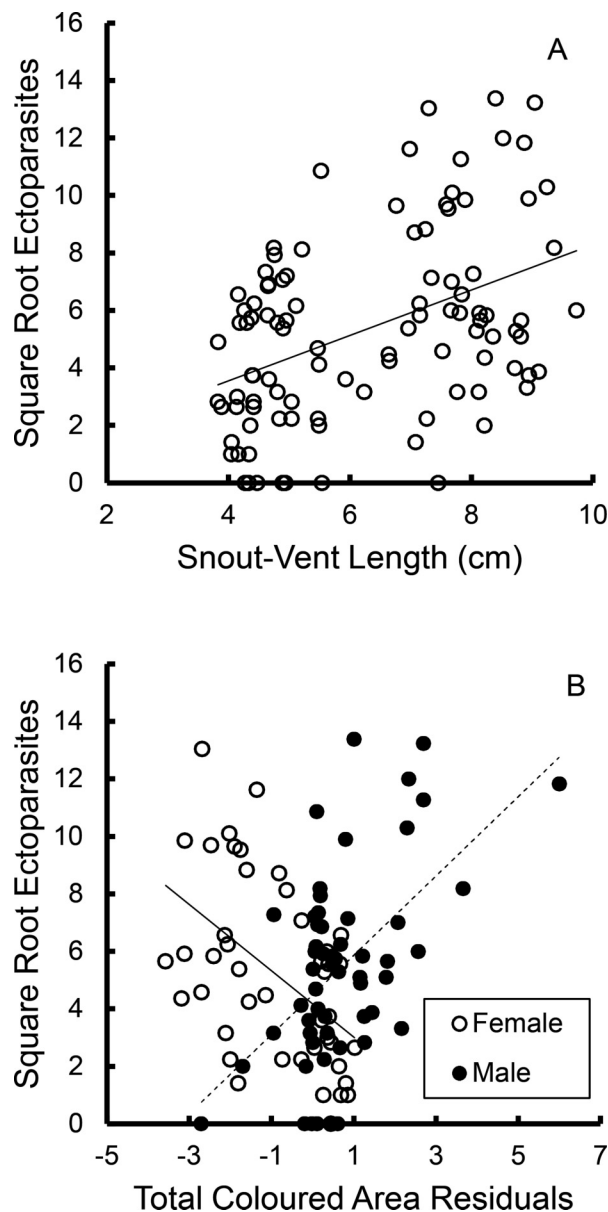
previous findings that T may have an immune-suppressing consequence for ectoparasite load but not for blood parasite abundance (Fuxjager et al. 2011). Although T may have an immunosuppressive effect (Roberts et al. 2004), responses may differ in magnitude and direction depending on the type of parasite (Roberts et al. 2004; Fuxjager et al. 2011; Ezenwa et al. 2012). Positive versus negative associations between T and parasite load may be due to differences in adaptive versus innate immune system response to T (Ezenwa et al. 2012).
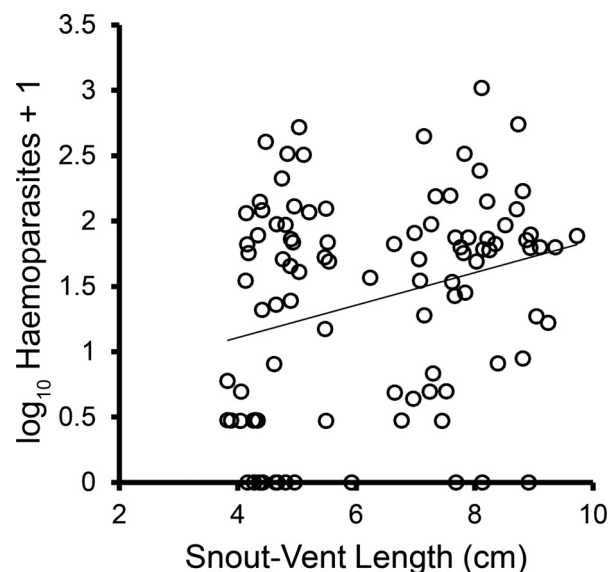
We found no support for the hypothesis that locomotor activity increases parasite load because more active individuals have a greater exposure to parasites in the environment (Fuxjager et al. 2011). Parasite acquisition has been associated with locomotor activity levels in various taxa (Marler and Moore 1989; Poulin et al. 1991; van der Veen 2003; Nunn and Dokey 2006). Male *S. jarrovii* given T implants increase activity and territorial patrolling behav-

**Fig. 4.** The relationship between square-root-transformed number of ectoparasites and snout–vent length (A) and between square-root-transformed number of ectoparasites and interaction between sex and total coloured area (TCA) (B) in Yarrow's Spiny Lizards (*Sceloporus jarrovii*) from southeastern Arizona. The number of ectoparasites is the total count of ectoparasites found on the scales of a lizard. In B, the broken trend line is for males and the solid trend line is for females.

**Fig. 5.** The relationship between $log_{10}$-transformed ratio of haemoparasites and snout–vent length in Yarrow's Spiny Lizards (*Sceloporus jarrovii*) from southeastern Arizona. The ratio of haemoparasites is the ratio of infected red blood cells per 10 000 red blood cells.



season, whereas other hormones such as glucocorticoids may best predict activity levels in females and juveniles. Indeed, activity levels and dispersal distance increase with corticosterone in a number of lizards (e.g., Cote et al. 2006; but see DeNardo and Sinervo 1994). Nevertheless, we found no relationship between locomotor activity and either ectoparasites or haemoparasites. It is possible that the activity levels observed in the arena were not representative of activity in the field. Behaviour in a novel environment may not always correlate with behaviour in a familiar one and may be influenced by other factors such as boldness, fearfulness, or neophobia (reviewed in Réale et al. 2007). Otherwise, our data suggest that activity is unrelated to ectoparasite or haemoparasite loads in *S. jarrovii*.

We found strong positive relationships between lizard body size and both parasite types. This is consistent with results from fish (Lo et al. 1998) and other lizards (Schall 1996; Garrido and Pérez-Mellado 2013). Possible mechanisms by which size affects parasite loads include increased detectability, larger surface area to support parasite population, or (if size correlates with age) increased time to accumulate parasites (Ruby and Dunham 1984; Lo et al. 1998; Blanckenhorn 2000). Alternatively, larger individuals may be under more stress associated with territorial defense (Schall 1996). If hosts are considered islands for parasites, analogous to the island biogeography theory, then parasite load should increase with both size and age of the host (Kuris et al. 1980). Thus, the advantages of a larger body size in territorial behaviour may be traded off against survival costs of parasitism in *S. jarrovii*.

One potential limitation of our study design is that we used observational data and did not experimentally alter T concentrations through implants (of T or antagonists) or castrations. Therefore, our relationship between T and ectoparasite load may be mediated through mechanisms other than immunosuppression by T such as stress, size, or behaviour. Chronically elevated levels of circulating glucocorticoids can lead to immunosuppression (Hillgarth and Wingfield 1997) by inhibiting macrophage activity, antibody production, and the development and differentiation of T cells used in cell-mediated response (Hall and Goldstein 1984). Thus, individuals with higher levels of corticosterone should have more parasites due to the immunosuppressive properties of this hormone. Our results corroborate Fuxjager et al.'s (2011) findings

iour (Marler and Moore 1989), suggesting that T indirectly increases parasite levels through its effect on activity (Fuxjager et al. 2011). In this study, however, we found no relationship between activity levels and T, ectoparasite load, or haemoparasite load. The lack of association between T and activity in this study, contrary to Marler and Moore (1989), may have occurred because we obtained T samples mainly from juveniles and adult females. Adult female lizards have lower levels of T (Moore 1986), display less territorial behaviour (Ruby and Baird 1994), are less active during the mating season (Aragón et al. 2001), and have smaller home ranges (Simon 1975) than adult males. Juveniles also have smaller home ranges than adults (Simon 1975; Ruby and Baird 1994). Thus, T may best predict activity in adult males during the breeding

that ectoparasite loads increase with T, but haemoparasite loads are unaffected by T, and furthermore suggest that these relationships are not limited to adult males, as the same patterns were found in a sample composed mainly of females and juveniles. On the other hand, an important advantage of an observational study such as ours is that it ensures biological realism, as T manipulation can sometimes elevate T to levels that are not biologically relevant (i.e., supraphysiological; Fusani 2008). Another limitation of our study is that we do not have T values for many adult males, as the majority did not provide a fecal sample before their release. Previous studies have shown that males of this species decrease foraging with increased T (Marler and Moore 1989) and that T is elevated during the breeding season (Moore 1986). A decrease in foraging could account for our difficulty obtaining fecal samples from adult males. Our small number of samples for adult males may explain why we found a correlation between T and relative head width, but not aggression, throat colour patch area, or activity levels, even though TCA interacted with sex when examining ectoparasites. Conversely, our small sample size might have given us significant results that would disappear with a larger sample size if the individuals that we sampled were not representative of the population. Future work should investigate how T, glucocorticoids, and parasite load are related in lizards. T should also be measured across different populations and with a larger sample of adults to confirm our results.

Drivers of parasite load in lizards depend on the sex of the host and on the type of parasite. In both females and males in this study, body size is the main driver of both ectoparasite and haemoparasite loads. Yet with males, ectoparasite load increased with total coloured area, a sexual signal driven by T. Lizards with higher T also tended (with near significance) to have more ectoparasites but not haemoparasites, which suggests that T is driving sex-specific ectoparasite loads. Therefore, the patterns that we observed provide support for the immunocompetence handicap hypothesis for ectoparasites.

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