



# Immune activation affects whole-organism performance in male but not female green anole lizards (*Anolis carolinensis*)

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## Abstract

Immune responses are intuitively beneficial, but they can incur a variety of costs, many of which are poorly understood. The nature and extent of trade-offs between immune activity and other components of the integrated phenotype can vary, and depend on the type of immune challenge, as well as the energetic costs of simultaneously expressing other traits. There may also be sex differences in both immune activity and immunity-induced trade-offs, particularly in the case of trade-offs involving functional traits such as whole-organism performance capacities that might be of different fitness value to males and females. We tested the response of three performance traits (sprinting, endurance, and biting) to two different immune challenges (LPS injection and wound healing) in both male and female *Anolis carolinensis* lizards. We found clear differences in how male and female performance capacities were affected by immune activation. LPS injection and wound healing had interactive effects on all three performance traits in males, but immune activation did not affect female performance. We also found that the degree of wound healing exhibited complex interactive effects involving sex and type of immune activation that varied depending on the performance trait in question. These results demonstrate that male and female green anoles experience different consequences of immune responses, and also that the type and extent of that activation can drive trait-specific performance trade-offs.

**Keywords** Bite force · Endurance · Immunity · Life history · Sprint speed · Trade-off

## Introduction

Pathogens can exert strong selection on hosts, both through the nature of an infection and the costs of mounting an effective immune response (Lochmiller and Deerenberg 2000). The energetic costs involved are often substantial (Martin et al. 2003), enough so that they also divert acquired energetic resources from being allocated towards other fitness-enhancing tasks and traits (Demas et al. 2012; Bonneaud et al. 2003; Sapolsky et al. 2000). Understanding the direction, nature, and consequences of the resulting trade-offs is one of the central goals of ecoimmunology, and there is

still a lack of tests for how different components of immune defense covary with the expression of key fitness-related traits (Bonneaud et al. 2003; Lee 2006; Brock et al. 2014; Ardia et al. 2011). Studies have focused primarily on trade-offs involving reproduction and reproductive traits (Uller et al. 2006; East et al. 2015), but relatively little attention has been paid to fitness components comprising functional, ecological, and physiological phenotypes that relate to survival.

Both the effects of parasites and pathogens on hosts and the costs of immune defense are variable. Components of the induced innate response, for example, not only affect metabolic activity and requirements (Smith and French 2017), but the resulting systemic inflammation also inflicts indirect physiological costs such as autoimmune damage to tissues (Lee 2006). These general costs may be context-dependent and contingent on the nutritional or reproductive state of the organism (French et al. 2009). For instance, wound healing, an integrated immune response, is suppressed during vitellogenesis specifically in female *Urosaurus ornatus* lizards (French and Moore 2008). Indeed, the sex of an organism can further interact with these factors to affect

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immune activity for several reasons, ranging from differential reproductive investment in males and females (Vincent and Gwynne 2014) and mounting of an immune response to the presence of male ejaculate in females (reviewed in Morrow and Innocenti 2012), to potential prioritization of specific immune system components in one sex relative to the other (Lee 2006; Kelly et al. 2018). These sex differences in immune activity can drive further sex-specific trade-offs in fitness components under similar conditions. For example, the swelling response to phytohaemagglutinin in zebra finches (*Taeniopygia guttata*) reduced reproductive effort in females that were under a low-quality diet, but did not do so in males (Love et al. 2008). Thus, despite the general expectation that enhanced immune activation will result in phenotypic trade-offs, both the circumstances of immune activation and the nature of the consequent trade-offs can be sex-specific.

Although sex differences in immune function could be attributed in part to the influence of sex hormones (Foo et al. 2016), a further possibility is that either immune function or traits that are linked to immunity via a common energetic resource pool are subject to intralocus sexual conflict. Specifically, fitness optima for males and females might differ such that trait expression is favored in different ways in similar contexts (Parker 1979; Bonduriansky and Chenoweth 2009; Metcalf et al. 2020). Males are often assumed to have lower investment in immune function compared to females (Rolff 2002), but this is a coarse prediction because not all individual components of the immune system are equally susceptible to trade-offs (López-Pérez et al. 2021; Neuman-Lee and French 2014), and some components may be differentially advantageous in one sex over the other. For example, in many species females are predicted to have lower inflammatory/non-specific/innate immune responses and higher antibody responses relative to males, especially during times of energetic stress (Metcalf and Graham 2018; Lee 2006). This is because systemic inflammatory responses are especially costly, and reducing this response is likely to have a larger effect on fitness in reproductively active females compared to an equivalent reduction in males (Lee 2006). These differing responses in turn can have distinct effects on individual fitness in males and females, particularly when they involve the linked expression of costly traits that are important to fitness in other ways, such as those that increase survival via predator escape or avoidance.

Whole-organism performance capacities such as locomotion are important determinants of fitness in contexts ranging from predator escape and foraging to territory defense and male combat (Lailvaux and Irschick 2006; Husak and Fox 2008; Irschick and Garland 2001) and consequently are key targets of selection (Irschick et al. 2008; Husak 2016). But because performance is itself energetically expensive (Speakman and Selman 2003; Christian et al. 1997),

significant increases in immune system activation can result in decreased performance abilities (Lailvaux and Husak 2014). Parasitized individuals show diminished burst performance in lizards (Schall et al. 1982; Oppliger et al. 1996; Main and Bull 2000), amphibians (Chatfield et al. 2013), birds (Lindström et al. 2003), and mammals (Alzaga et al. 2008). However, both performance and immune function are multivariate phenomena; and just as different aspects of immune function incur distinct costs, separate performance traits that are supported by specific morphology and physiology could be subject to distinct sets of trade-offs. For example, sprinting and endurance are bolstered by separate metabolic pathways and exhibit markedly different trade-offs with a suite of life-history traits in 25 species of phrynosomatid lizards (Husak and Lailvaux 2017). Similarly, sex can moderate the relationship between different aspects of immunity and different types of performance just as it affects immunity alone, albeit in variable ways; in green anole lizards, increasing investment in endurance via training specifically reduces immune function as assessed by the swelling response to phytohaemagglutinin (PHA) injections to a greater extent in females than in males, yet percentage of bacterial killing by blood is similarly suppressed by training in both sexes (Husak et al. 2016). On the other hand, injection with lipopolysaccharide reduces sprint speed in male *Psammodromus algirus* lizards, but not females (Zamora-Camacho et al. 2015). The inter- and intraspecific variation in both performance and immune function, coupled with the complexity of the immune response, means that we currently lack a proper understanding of the sex-specific nature of functional immune trade-offs in any species.

Green anole lizards (*Anolis carolinensis*) are an ideal species for considering potential sex-specific trade-offs between immune function and performance because performance traits such as sprinting, endurance, and biting are easily and routinely measured, and because previous studies have documented sexual dimorphism in both immune function (Husak et al. 2016) and performance (Lailvaux and Irschick 2007; Lailvaux et al. 2019). Nonetheless, we have no clear picture of the effects of immune activation on the expression of multiple whole-organism performance traits in green anoles, or in lizards in general, at least in part because the immune strategies and costs of reptiles remain understudied (Zimmerman 2020; Zimmerman et al. 2010). We experimentally challenged the immune systems of adult male and female green anoles to varying degrees to test for sex-specific trade-offs with three distinct whole-organism performance traits. We used lipopolysaccharide (LPS), a pathogen-associated molecular pattern (PAMP) from naturally occurring lizard pathogens (*E. coli*) to induce an innate immune response in adult male and female green anole lizards (*Anolis carolinensis*). LPS treatment induces the release of cytokines, an energetically expensive acute phase response, without

a pathogen, allowing measurement of the effects of host immune activation exclusive of any effects of a pathogen or host–pathogen interactions. We also investigated the impact of healing a wound. Interactions between immune activity induced via wound healing and other aspects of the immune system are likely highly relevant given that compromised wound healing renders organisms susceptible to co-infection from other pathogens or parasites (Rojas et al. 2002). Indeed, individuals exhibiting scarring from previous wounding are common within green anole populations in nature (Irschick et al. 2006).

We tested two explicit hypotheses: (1) Performance trade-offs in immune challenged green anoles are sex-specific. Specifically, because of the likely differential investment in innate immunity between the sexes, we predicted that males would have reduced performance capacities (sprint speed, endurance, and bite force) compared to females. (2) Performance trade-offs differ depending on the nature and extent of the immune challenge. Here, we predicted that a combination of immune challenges (wound healing + LPS) would result in greater decrements in performance than with either challenge alone. Furthermore, we predicted that animals exhibiting less wound healing would also be more compromised performers, and that deleterious effects on performance would be alleviated as the degree of wound healing progresses. Alternatively, individuals might invest less in wound healing to allow maintenance of performance during the wound healing process such that lizards with less wound healing have higher performance compared to those investing more.

## Materials and methods

Adult male ( $N=40$ ) and female ( $N=40$ ) green anoles (*Anolis carolinensis*), were obtained (Candy's Quality Reptiles, Laplace, LA, USA) and housed in a climate-controlled room for 2 weeks of acclimation at 28–31 °C on a 12:12 h light:dark cycle. Each cage had a male–female pair so that reproduction and reproductive behavior would be maintained. Lizards were each fed four crickets 3 days a week, and crickets were dusted with calcium and vitamin D supplements on one of those days. This diet is not ad libitum, and has been shown to result in phenotypic trade-offs in previous studies (Husak et al. 2016, 2017; Wang and Husak, unpublished data). Cages were sprayed twice daily to provide water. All procedures were in accordance with approved protocols by the institutional animal care and use committee.

Prior to treatment assignment, we measured mass (to the nearest 0.1 g; mean ( $\pm$  SEM) for males =  $4.39 \pm 0.06$  g, females =  $2.46 \pm 0.03$  g) with a digital balance and snout-vent length (SVL to the nearest 0.01 mm; mean ( $\pm$  SEM) for males =  $63.67 \pm 0.28$  mm, females =  $52.5 \pm 0.25$  mm) with

digital calipers. We also measured pre-treatment values of maximum bite force, sprint speed, and endurance following standard protocols (Perry et al. 2004; Cox et al. 2009; Husak et al. 2015). Bite force was measured using a Kistler isometric force transducer with padded stainless-steel bite plates (Husak et al. 2016). Lizards bit six times, which consisted of two trials of three bites, with an hour between trials for rest, and the hardest bite was used as maximal. Sprint speed was measured on a 2 m-long, 5 cm-diameter dowel racetrack that was covered in cork and sitting at a 45° angle. There were vertically paired infrared photocells positioned 0.25 m apart along the length of the track. We chased lizards up the track with a paintbrush tapping their tails, and as they ran they broke the infrared beams. TrackMate software (Trackmate Racing, Surrey, British Columbia, Canada) determined times that each gate was broken. We ran lizards three times and used the fastest 0.25-m interval as maximum speed. Endurance was measured by running the lizards until exhaustion on a motorized pet treadmill (PetRun model PR700 modified for lower speeds) at 0.3 km/h. Exhaustion was defined as the lizards' loss of righting reflex (Perry et al. 2004; Cox et al. 2009; Husak et al. 2015). Different performance measures were taken with 24 h between them to allow time for recovery, with bite force measured first, then sprint speed, and endurance last.

Lizards were then randomly assigned to four different treatment groups with ten lizards per sex and per group. Treatment groups were: (1) control with PBS injection, (2) LPS injection only, (3) cutaneous wound with a PBS injection, and (4) cutaneous wound with LPS injection. After lizards were anesthetized using surface-induced deep hypothermia (French et al. 2006) we created wounds with a sterile 3.5-mm biopsy punch that made a cutaneous circular wound anterior to the tail and on the dorsal surface of the lizards (French et al. 2006). The biopsy tool was gently moved in a circular motion to cut the skin, and the flap of skin was removed with sterile forceps. Animals without a wound had the plastic end of the biopsy punch rubbed on the same area to simulate the procedure but not cause a wound. The wound was immediately photographed using a dissecting microscope with a ruler in the field of view for reference. 3 days after the wounds were created, experimental lizards received a subcutaneous injection with 0.1 mg LPS (Sigma L2630) in 0.01 mL PBS, and control lizards received an injection with 0.01 mL PBS.

We measured post-treatment performance variables in the same manner as pre-treatment measures described above over the next several days (see also Table 1). Since we could not measure sprint speed and endurance on the same day, we staggered the order of measurements so that half of the lizards had endurance measured first, and the other half had sprint speed measured first (Table 1). This scheme was used to control for any recovery effects that might have existed

**Table 1** Timeline of experimental treatments

	Set 1	Set 2
Day 0	Wound or control	Wound or control
Day 3	LPS or PBS	LPS or PBS
Day 4	BF + SS	Endurance
Day 5	Endurance	BF + SS
Day 9	LPS or PBS	LPS or PBS
Day 10	Endurance	BF + SS
Day 11	BF + SS	Endurance

*BF* bite force, *SS* sprint speed, *LPS* lipopolysaccharide injection, *PBS* saline injection

Sets represent half of the lizards in each treatment, which were kept consistent for each measurement period

‘Order’ identity was included in statistical models, but it was retained in none (i.e., it was not significant)

Pre-treatment measurements (not shown) represent performance measurement session 1, whereas days 4 and 5 correspond to measurement session 2, and days 10 and 11 represent session 3

after endurance measurements (see also Lailvaux et al. 2019 for a similar approach). On the fourth day, half of the lizards were tested for bite force and sprint speed while the other half were tested for endurance. On the fifth day, the lizards that were tested for bite force and sprint speed the day prior were then tested for endurance. The other group of lizards that were tested for endurance the day before were then tested for bite force and sprint speed. The lizards were then given 3 days of rest so that on the ninth day, LPS and PBS injections could be re-administered. On the tenth day, following LPS and PBS injections, half of the lizards were tested for endurance and the other half were tested for bite force and sprint speed, and vice versa for each lizard half-group on the eleventh day (Table 1). Individuals that were in each half were held consistent over the course of measurements, and we refer to this ordering as ‘order’ identity in analyses. After performance measures were completed, we re-measured mass and SVL.

## Statistical analyses

We used R version 3.6.0 for all analyses (R Core Team 2019). To test the hypothesis that immune activation affects performance differently in males and females, we fit sex-specific general linear mixed-models to bite force, sprint speed, and endurance using the R package *lme4* (Bates et al. 2015). Although a direct test of this hypothesis would ideally include sex as a factor in each model, preliminary analyses showed that sex-specific models are a better fit to the data than models with sex as a factor due to sex-specific mean and variance for bite force specifically; grouped patterns in the residuals of some models that included sex as a factor; and insufficient power to test for three-way interactions

involving sex. We, therefore, fit separate male and female mixed-models for each performance trait and consider any differences in retention of immune treatments as significant factors following model reduction as evidence for sex-specific immune effects on performance. Fixed factors in all cases were measurement session, order identity, mass, LPS treatment, and wounding, whereas individual was included as a random effect to account for the repeated measurements across sessions. Random slopes for session caused convergence problems in several models, and thus were excluded. Dependent variables were transformed as required using Box–Cox transformations to meet model assumptions (noted with exponents on sprint speed and endurance in Table 2). We used log-likelihood ratio tests with maximum likelihood to identify variables that do not explain significant amounts of variation in each mode, and re-fitted the simplified final models with restricted-estimate maximum likelihood (REML).

To test the hypothesis that performance is affected by the extent of wound healing, we fit separate general linear models for sprinting, log-transformed biting, and log-transformed endurance within the subset of the wounded lizards from measurement session 3, which is the only session for which wound-healing data were available. Dependent variables were sex, mass, LPS treatment, proportion of wound healed, and interactions between sex and LPS treatment; sex and proportion of wound healed; and LPS treatment and proportion of wound healed. We again used log-likelihood tests to determine the significance and individual factors and to simplify the models. Finally, we used the *visreg* R package (Breheny and Burchett 2017) to visualize relationships between dependent variables and specific factors from models of interest while holding other factors in those models constant.

## Results

### Bite force

The best fit mixed-models describing bite force over the three measurement sessions were different for males and females (Table 2). The best fit model for male bite force was the saturated model (excluding ‘order’, which was never significant in any performance model throughout), with parameter estimates indicating that mass and session both affect bite force, with heavier lizards biting harder and bite force decreasing over the three measurement sessions. The interaction between LPS treatment and wounding shows that male green anoles that were both wounded and had LPS treatment exhibited lower bite forces than control males (Table 2). By contrast, the best fit mixed-models for green anole female bite force harbored similar mass and

**Table 2** Best-fit models describing the variation in (a) bite force; (b) (sprint speed)<sup>0.6</sup>; and (c) (endurance time)<sup>0.38</sup> for males (left) and females (right)

Males			Females		
Model term	Coefficient	SE	Model term	Coefficient	SE
<b>(a) Bite force</b>					
Intercept	6.51	0.385	Intercept	2.456	0.054
Session 2	- 0.815	0.2	Session 2	- 0.149	0.037
Session 3	- 0.764	0.2	Session 3	- 0.11	0.037
Mass	0.881	0.167	Mass	0.246	0.033
LPS (yes)	1.381	0.524			
Wound (yes)	0.718	0.519			
LPS (yes):Wound (yes)	- 1.9	0.73			
<b>(b) Sprint speed<sup>0.6</sup></b>					
Intercept	0.703	0.059	Intercept	0.624	0.029
Session 2	- 0.155	0.029	Session 2	- 0.074	0.031
Session 3	- 0.14	0.03	Session 3	- 0.101	0.031
Mass	0.074	0.026	Mass	0.096	0.02
LPS (yes)	0.134	0.081			
Wound (yes)	0.089	0.081			
LPS (yes):Wound (yes)	- 0.262	0.112			
<b>(c) Endurance<sup>0.38</sup></b>					
Intercept	7.832	0.34	Intercept	6.91	0.141
Session 2	- 0.47	0.212	Mass	0.36	0.121
Session 3	- 0.48	0.212			
Mass	0.339	0.152			
LPS (yes)	0.818	0.45			
Wound (yes)	1.08	0.45			
LPS (yes):Wound (yes)	- 1.617	0.622			

The reported values give estimated change in the dependent variable between the category named in the table and the baseline category

The baseline category for session is session 1; for LPS is LPS (no); and for wound is wound (no)

session effects, but retained neither an LPS: wound interaction nor any main treatment effects. The best fit session three-only model testing the effect of wound healing on log-transformed bite force contained an effect of mass as well as interactions between sex and proportion of wound healed, and between LPS treatment and proportion of wound healed (Table 3). The first interaction indicates opposite effects of wound healing on bite force in males and females, such that male bite force decreases as wounds heal, whereas female bite forces increase (Fig. 1a). The second interaction points to a markedly more positive relationship between proportion of wound healing and bite force in LPS treated lizards overall compared with control animals (Fig. 1b).

### Sprint speed

Parameter estimates for the best fit mixed-models for sprint speed were also sex-specific (Table 2). In addition to similar effects of session and mass as obtained for bite force, the male model again yielded an interaction between LPS and wounding, such that wounded, LPS-treated males were

slower compared to unwounded, untreated control males. The best fit mixed-model for female sprint speed contained only negative effects of session and a positive effect of mass, comparable to the female bite force model (Table 2). The best fit session three-only model testing the effect of wound healing on sprint speed yielded positive effects of proportion of wound healed (Fig. 2a) and mass as well as an interaction between sex and LPS treatment such that male, LPS treated lizards were slower than untreated females (Fig. 2b), recapitulating the result of the mixed-models (above).

### Endurance time

Best fit mixed-models for endurance were once again different for males and females (Table 2). The female model retained only a positive effect of mass, whereas the male model contained negative effects of session, a positive effect of mass, and an interaction between LPS treatment and wounding such that wounded, LPS treated males had lower endurance than unmanipulated controls (Table 2). The best fit session three-only model testing the effect of

**Table 3** Best-fit models describing variation in (a) log bite force; (b) sprint speed; and (c) log endurance time for only the wounded animals from session 3

	Model term	Coefficient	SE
(a) Log bite force	Intercept	- 0.388	0.217
	Sex (male)	0.468	0.059
	Mass	0.381	0.232
	LPS (yes)	- 0.438	0.22
	Proportion healed	0.249	0.267
	Sex (male): Proportion healed	- 0.589	0.407
	LPS (yes): Proportion healed	0.54	0.394
(b) Sprint speed	Intercept	- 0.064	0.15
	Sex (male)	- 0.207	0.05
	Mass	0.172	0.12
	LPS (yes)	0.025	0.074
	Proportion healed	0.313	0.16
	Sex (male): LPS (yes)	- 0.152	0.108
(c) Log endurance	Intercept	4.4	0.36
	Mass	0.19	0.06
	LPS (yes)	- 1.14	0.47
	Proportion healed	0.741	0.55
	LPS (yes): Proportion healed	1.144	0.82

The reported values give estimated change in the dependent variable between the category named in the table and the baseline category  
The baseline category for Sex is Sex (male) and for LPS is LPS (no)

wound healing on endurance retained an effect of mass and an interaction between LPS and proportion of wound healing in addition to the main effects of each (Table 3), with LPS treated lizards showing a greater improvement in performance with greater wound healing compared to control lizards (Fig. 3).

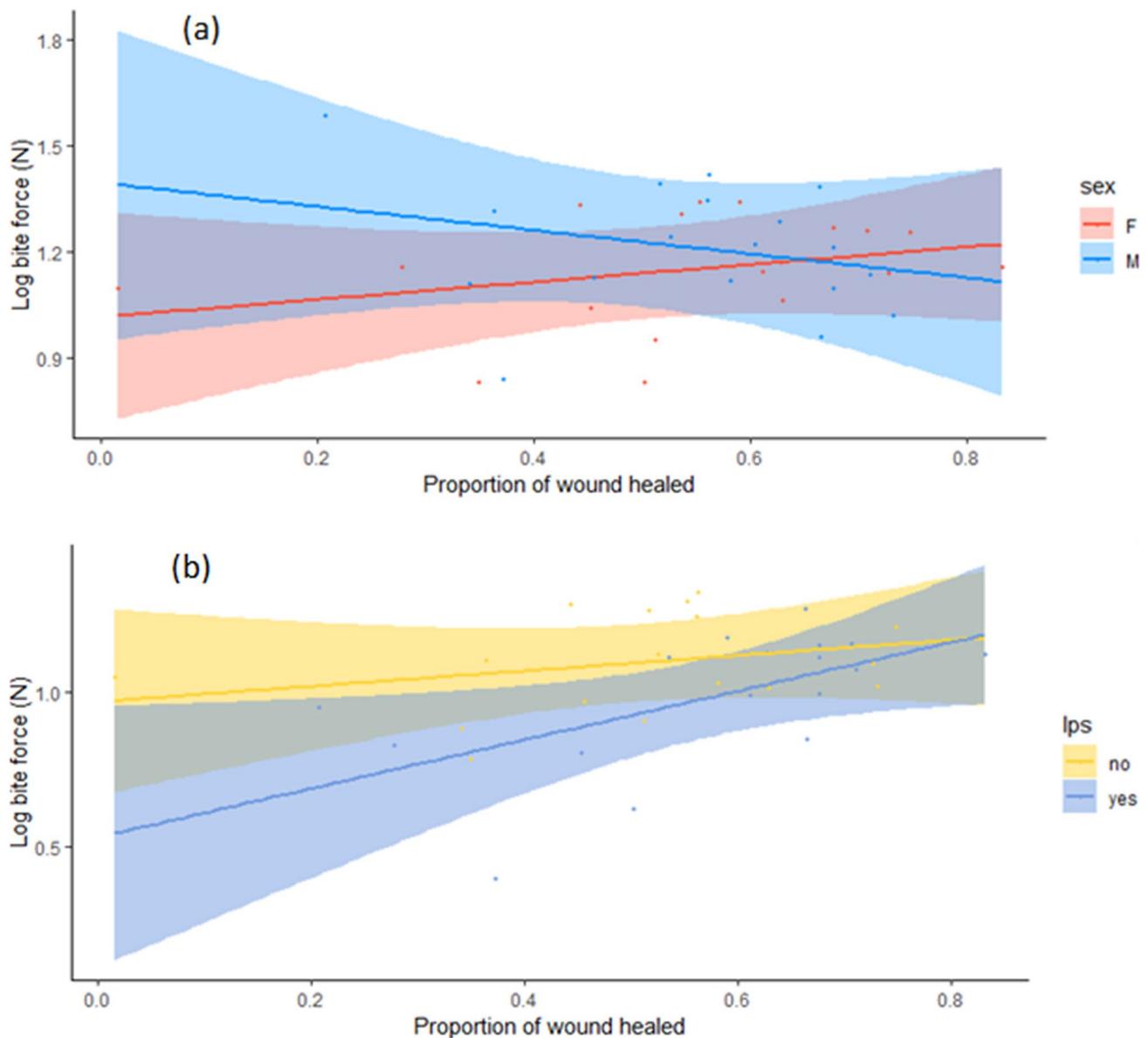
## Discussion

Immune system activation is important to the survival of individuals in a variety of species. However, immune responses can also affect the expression of functional traits that are closely related to fitness, albeit in sex-specific ways. We tested for sex-specific effects of two kinds of immune activation on three different performance traits by applying combinations of LPS injections and wound healing in male and female green anole lizards. We hypothesized that immune activation would induce sex-specific performance trade-offs and that the nature and extent of the immune challenge would have trait-specific effects on performance.

Our first hypothesis was supported. All three measures of performance showed significant reductions in wounded,

LPS injected males compared with controls, but no such effects were seen in females (Table 2). In each case, the male-only models retained interactions between LPS and wound healing. Because interpretation of main effects in the presence of interactions involving those effects is perilous (Engqvist 2005), our results do not allow us to consider the effects of LPS and wound healing separately from each other in males. Nonetheless, the main finding that males are more prone to deleterious consequences of immune activation than females has support from previous studies. Females during breeding seasons are generally expected to invest less in non-specific inflammatory immune responses compared to males due to differences in allocation to reproduction (Lee 2006). The implication, then, is that males should have a higher response to immune activation, thus taking more energy away from other tasks, which is in line with our results. Indeed, Brace et al. (2015) found that male *Anolis sagrei* lizards paid higher costs of immune activation via LPS than did females by allocating more isotopically labeled leucine to their livers with increasing doses of injected LPS compared to females. Our findings extend this result by showing that these costs, although suggested to be of little consequence (Brace et al. 2015), are large enough in green anoles to drive trade-offs with male performance expression, albeit in combination with an effect of wound healing as well. An important caveat, however, is that findings from native green and invasive brown anoles may not be directly comparable given that successful invasive species tend to have altered immune responses in their non-native ranges (White and Perkins 2012). Furthermore, male white-crowned sparrows (*Zonotrichia leucophrys*) also showed a similarly greater decrease in locomotor activity after LPS injection during the breeding season than females, but other metrics of inflammatory responses did not differ between the sexes (Owen-Ashley et al. 2006). Unfortunately, our data do not allow us to rule out the non-mutually exclusive possibility that the higher testosterone levels of males compared with females are responsible, at least in part, for the observed sex differences (immunocompetence handicap hypothesis; Folstad and Karter 1992). However, that hypothesis was not supported in *A. sagrei* (Reedy et al. 2016).

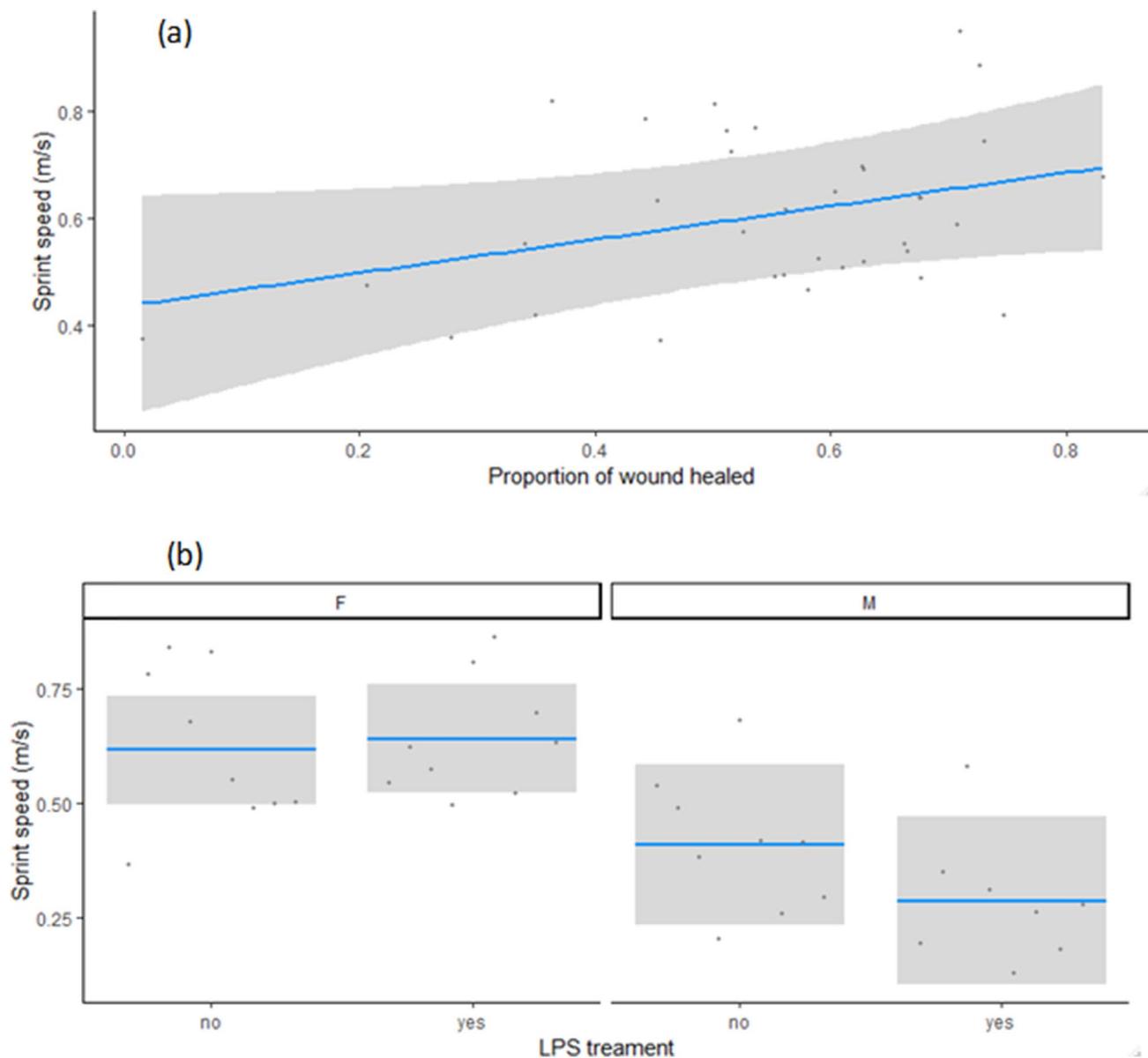
Our second hypothesis was also supported. The extent of wound healing was a consistent significant factor affecting all performance traits, albeit in different ways for each performance type (Table 3). What is clear is that the extent of wound healing, whether in concert with LPS or by itself, is an important predictor of performance in all cases, such that performance usually improves as wounds heal (Table 3; the exception is bite force, where an interaction between sex and extent of wound healing revealed opposite patterns for males and females). These results also show that LPS treatment and extent of wound healing modulate performance expression both independent of



**Fig. 1** Partial residuals from the session 3-only model for log bite force illustrating the interaction between **a** proportion of wound healed and sex, and **b** proportion of wound healed and LPS treatment. Shaded areas indicate 95% confidence intervals

each other (in the case of sprint speed), and in combination with each other (for sprint speed and bite force). Our finding that individuals with a higher proportion of their wounds healed had greater performance may be due to the temporal nature of trade-offs involved with healing a wound. Investment in wound healing is typically at the highest rate within the first 4 days in other lizard species (French et al. 2006; Neuman-Lee and French 2014). If wound healing progresses similarly in green anoles, then it is possible that those individuals who invested more in wound healing early had more resources to devote to performance later. Conversely, those that invested less early in the healing process would have to stretch out investment

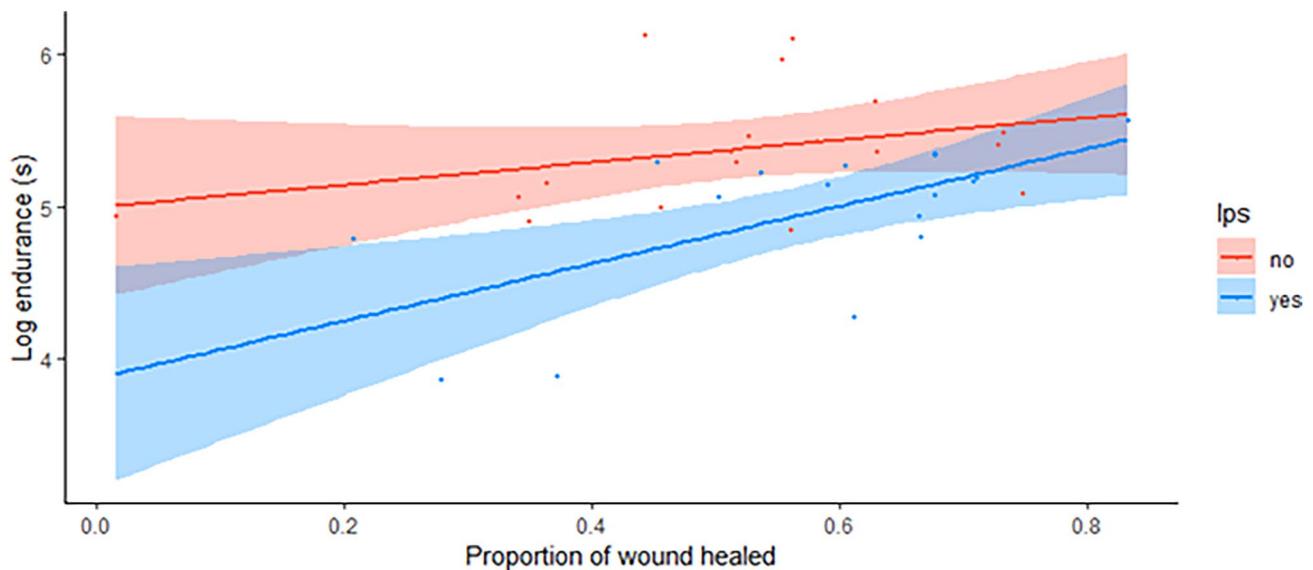
longer, thus taking away resources for performance. This supports the notion that “faster is better” when healing a wound (Neuman-Lee and French 2014), such that quick and efficient healing makes resources and immune cells more quickly available for other purposes. For example, side-blotched lizards with a greater rate of early healing had greater bacterial killing ability at a later time point (Neuman-Lee and French 2014). The lack of limited resources in that study suggests that energy was not limited, but instead immune cells were: faster early healing allowed immune cells to return more quickly to circulation to increase bacterial killing later. Since we did not measure the rate of healing at a fine temporal scale, we cannot rule



**Fig. 2** Partial residuals from the session 3, wounded-animal only model for sprint speed illustrating **a** the relationships between proportion of wound healed and sprint speed, and **b** the interaction between sex and LPS treatment. Shaded areas indicate 95% confidence intervals

out that faster wound healers were also better performers such that both traits were equally important and simultaneous investment in both was proportional to the amount of energy they had available. We also cannot definitively say that those with a greater proportion of wound healed at session 3 did most healing early, but our other results are consistent with this explanation. For example, our finding that LPS injection typically made performance worse, even for high-performing individuals, supports the notion that rapid early healing makes energy and immune cells available for other purposes more quickly.

The finding that different performance traits are affected differently by the investment in wound healing points towards distinct costs of trait expression. What is more, these patterns conflict with similar trade-offs prompted by other external stressors. For example, sprint speed is insensitive to dietary restriction in male green anoles (Lailvaux et al. 2020), yet here it is clearly affected by immune activation, consistent with the results of Zamora-Camacho et al. (2015). One possibility is that allocation-based trade-offs involving immune activation are fundamentally different than those caused by dietary restriction, perhaps due to the complexity and expense of systemic immune activation. Martin



**Fig. 3** Partial residuals from the session 3, wounded-animal only model for log endurance, illustrating the interaction between LPS treatment and proportion of wound healed. Shaded areas indicate 95% confidence intervals

et al. (2012) previously found support for the re-allocation hypothesis, which suggests that immune function is suppressed during stressful events to promote allocation towards fitness-enhancing traits, and showed that captivity-stressed house sparrows experienced negative effects on innate immunity, but promoted flight performance. This might explain why sprint speed is unaltered in lizards stressed via dietary restriction (Lailvaux et al. 2020) but changes when only immune activation occurs. However, the varied responses of biting, sprinting, and endurance to immune activation suggest that all performance traits are not affected equally, and it could be that particular traits are prioritized over others depending on both the species and ecological context in question (Zamora-Camacho et al. 2020).

The different performance effects of immune activation on males and females are generally consistent with the notion of sexual conflict over performance expression in green anoles. Bite force has long been known to be sexually dimorphic in this species (Herrel et al. 2007), and Lailvaux et al. (2019) reported that the trade-off between bite force and sprint speed in females is likely due to incomplete compensation for the costs of larger-than-optimal heads in females that is the result of selection on high male bite forces (reviewed in Husak and Swallow 2011). Our findings here that immune activation suppresses performance in males but not females is yet a further indication that priorities regarding performance expression are not aligned between the sexes in green anoles. The existence of intralocus sexual conflict over performance hinges on the different selective utility of these traits in males and females in nature; consequently, performance decrements

due to immunity should ideally be studied in the field as well. Although the effects of infection on other traits in nature is difficult to demonstrate, in part due to selection bias involved in only being able to sample surviving individuals, mark-recapture data suggest that one measure of immune function (swelling response to phytohaemagglutinin, a separate indicator of innate immune function; Martin et al. 2006) was not a predictor of survival in an introduced, experimental population of free-ranging *A. carolinensis* (Husak and Lailvaux 2019). The use of PHA as a proxy for immune function in nature has been criticized on the grounds that host responses to PHA may not replicate the same host's responses to pathogens (Kennedy and Nager 2006; Brock et al. 2014), although this is likely to be true of any measure of immune activity taken out of ecological context. Nonetheless, our lack of knowledge as to how performance and immune activity might interact to influence male and female survival in the wild remains an impediment to understanding the nature and extent of sexual conflict over both immunity and performance, if any, in green anoles.

In conclusion, our data show that LPS and wound healing exhibit sex-specific effects on the expression of biting, sprinting, and endurance in green anole lizards. Furthermore, the effects of immune activation are subtly different among performance traits, particularly with regard to the extent of wound healing, which interacts with both sex and LPS treatment in different ways for each of the three considered performance traits. These results show that immune activation is costly enough to impinge upon traits that are key to survival and reproduction, and raises additional

questions regarding sexual conflict over physiological traits in green anoles.

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