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Experimentally enhanced performance decreases survival in nature

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Superior locomotor performance confers advantages in terms of male combat success, survival and fitness in a variety of organisms. In humans, investment in increased performance via the exercise response is also associated with numerous health benefits, and aerobic capacity is an important predictor of longevity. Although the response to exercise is conserved across vertebrates, no studies have tested whether non-human animals that invest in increased athletic performance through exercise realize a survival advantage in nature. Green anole lizards respond to exercise training, and enhanced performance drives trade-offs with reproduction and immunocompetence. We released sprint-trained, endurance-trained and untrained-control male and female green anole lizards into an isolated, urban island in New Orleans, LA, USA and monitored their survival. Sedentary controls realized a significant survivorship advantage compared to trained lizards. Our results suggest that locomotor capacity is currently optimized to maximize survival in green anoles, and that forcing additional investment in performance moves them into a suboptimal phenotypic space relative to their current environmental demands.

1. Introduction

Locomotor performance is a key determinant of Darwinian fitness in many species [1,2] and predicts success in ecological contexts ranging from male combat [3,4] and dispersal [5] to predator escape [6]. Enhancing performance might therefore be expected to increase fitness even further. However, performance is a life-history trait and part of the integrated organismal phenotype [7], and as such is linked developmentally, functionally and energetically with a variety of other traits that also affect fitness [8]. Because resources are typically limited for non-human animals, increased investment in performance is frequently associated with trade-offs in the expression of other important life-history traits [9] and, under certain environmental conditions, could lead to a fitness or survival decrement instead of an overall fitness advantage.

Investment in locomotor performance is increased via exercise training in several animal taxa, suggesting that organisms can augment their locomotor performance capacities through additional activity. Recent work on *Anolis carolinensis* lizards shows that the exercise response enhances locomotor performance in both males and females, but also drives trade-offs in several key life-history traits. For example, eight weeks of endurance training in green anoles significantly increased endurance capacities [10], but also suppressed reproductive investment [11] and immune responses [12]. Despite these trade-offs, trained green anoles realized energetic savings in terms of decreased resting metabolic rates relative to untrained controls [13]. Thus, in addition to superior locomotor performance, trained animals expend energy at a lower rate when inactive. Given the substantial energetic costs of reproduction for both male and female green anoles and the consequent poor condition of individuals who survive the breeding season [14], this reduced energetic expenditure alone could enhance survival, particularly in the post-breeding period. However, the relative survival benefits of reduced energetic expenditure versus reduced immunocompetence are poorly understood, especially within the context of investment in locomotor performance [15].

We tested the hypothesis that enhancing performance prolongs survival in nature relative to untrained-control individuals by training marked *A. carolinensis* lizards for two types of locomotor performance (sprinting and endurance), releasing them into an urban island of habitat empty of conspecifics and recapturing them repeatedly over the next year. We also measured several potential predictors of survival at the end of the training period and conducted behavioural observations of released trained and control individuals to test the hypothesis that training increases activity in free-ranging green anoles.

2. Materials and methods

(a) Training

We acquired 90 wild-caught adult *A. carolinensis* lizards (45 males and 45 females) from a Louisiana population 45 km away from our study site (Candy's Quality Reptiles, Laplace, LA, USA). We assigned them to either a control, sprint-trained, or endurance-trained group (equal number of males and females). Sprint animals were forced to run the length of a 45°-inclined 2 m long racetrack three times per week for six weeks [10]. After two and four weeks, training intensity was increased by hanging weights (pipette tips filled with clay) off the lizards. Weights equalled first one-quarter, then one-half the average body weight of males or females. We trained endurance animals for 30 min at a speed of 0.18 km h^{-1} three times per week over six weeks, with intensity increased by increasing the incline of the treadmill after two and four weeks [12]. Control lizards were removed from their cages three times per week for six weeks to simulate handling stress experienced by the trained lizards.

(b) Post-training

Following training, we measured each lizard for sprint speed; endurance capacity (time to exhaustion on a treadmill at 0.3 km h^{-1} [12]); immune response as assessed by the extent of swelling at the site of injection of phytohemagglutinin (PHA), which induces innate and acquired immune defences [11,16]; and snout-vent length (SVL). We calculated individual condition as residuals from a regression of mass against SVL.

(c) Mark–recapture

We marked each lizard permanently and uniquely by injecting the ventral side of the limb elements with specific colour combinations of Visual Implant Elastomer (Northwest Marine Technology, Inc.) that fluoresce under UV light [17]. In June 2017, one week after training ended, we released the lizards into a 30 × 100 m urban island of habitat in Orleans parish, Louisiana from which all resident lizards were removed in the few days prior. Experimental lizards were all equally naive to the new habitat and were placed in a density comparable to other *A. carolinensis* populations in the area (approx. 0.3 lizards m^{-2}; S. P. Lailvaux 2014, unpublished data). Because the experimental habitat was not an artificial or semi-natural enclosure, it possessed the full complement of predators, pathogens and potential competitors also present in any other urban green anole population.

We surveyed the population and recorded the presence/absence of each lizard four times over the next year: late breeding season (August–September 2017); post-breeding season (October 2017); over-winter (March 2018); mid-breeding season (June 2018).

(d) Movement

We marked treatment groups differently on the dorsal base of the tail with coloured ink prior to release for treatment identification during focals. These markings are lost when the skin is next shed. Following the release of individuals, we used a Sony DCR-SR87 Handycam to film lizards for at least 5 min or until the animal disappeared from view. To avoid filming the same animals more than once, the site was canvassed systematically such that each part of the habitat was visited only once over the course of the focals. Unique head markings also made it possible in many cases to recognize individual lizards, which further assured us that we were not repeatedly filming the same animals. Our necessarily opportunistic approach gave us an overview of activity in the entire population, and we were able to record focals on 32 different animals (11 control, 11 endurance and 10 sprint-trained), or 36% of the released lizards. We extracted total number of moves per minutes (MPM) and the percentage of recorded time spent moving (PTM) for each individual. The same investigator (SPL) recorded and analysed all videos.

(e) Analyses

We performed analyses in R v.3.5.1 (R Core Team 2018). We tested for sex and treatment effects on sprint speed, (log10) endurance and PHA swelling using two-way ANOVA. We included body mass as a covariate in ANOVAs for sprint speed and endurance. We used the *survminer* package [18] to fit Kaplan–Meier curves to the raw survival data simultaneously for the control, endurance and sprint-trained lizards, and to test, using log-rank tests, whether curves differed. Post hoc pairwise comparisons to determine specific differences among trained/control groups were performed using the pairwise_survdiff command in the *survival* [19] package. To test the effect of additional factors on survival, we fit a Cox proportionate hazards regression to the survival data with treatment, condition and PHA response as factors and sex as a covariate. We analysed PTM as a generalized linear model with sex, treatment and a sex-by-treatment interaction as factors and quasipoisson errors. We used a general linear model to test for effects of sex, treatment and a sex-by-treatment interaction on MPM (transformed to satisfy assumptions of normality). We conducted model simplification of general(ized) linear and proportional hazard models using log-likelihood ratio deletion tests.

3. Results

Training significantly enhanced sprint and endurance for the respective treatments relative to controls (endurance: $F_{2,83} = 21.66$, $p < 0.001$, all treatments differing from each other with Tukey's HSD, $p < 0.03$ for all; sprint speed: $F_{2,83} = 6.77$, $p = 0.002$, sprint greater than endurance and control with Tukey's HSD, $p < 0.02$ for both; figure 1), but the response to PHA was significantly muted in trained lizards ($F_{2,84} = 27.1$, $p < 0.001$; all treatments differing from each other with Tukey's HSD, $p < 0.003$ for all; figure 1). There were no significant treatment-by-sex interactions for any variable ($p > 0.2$ for all). Kaplan–Meier survival curves differed significantly among treatments ($p < 0.008$; figure 2); specifically, survival of endurance-trained lizards was significantly lower than that of untrained controls ($p < 0.004$), whereas survival of sprint-trained lizards was not

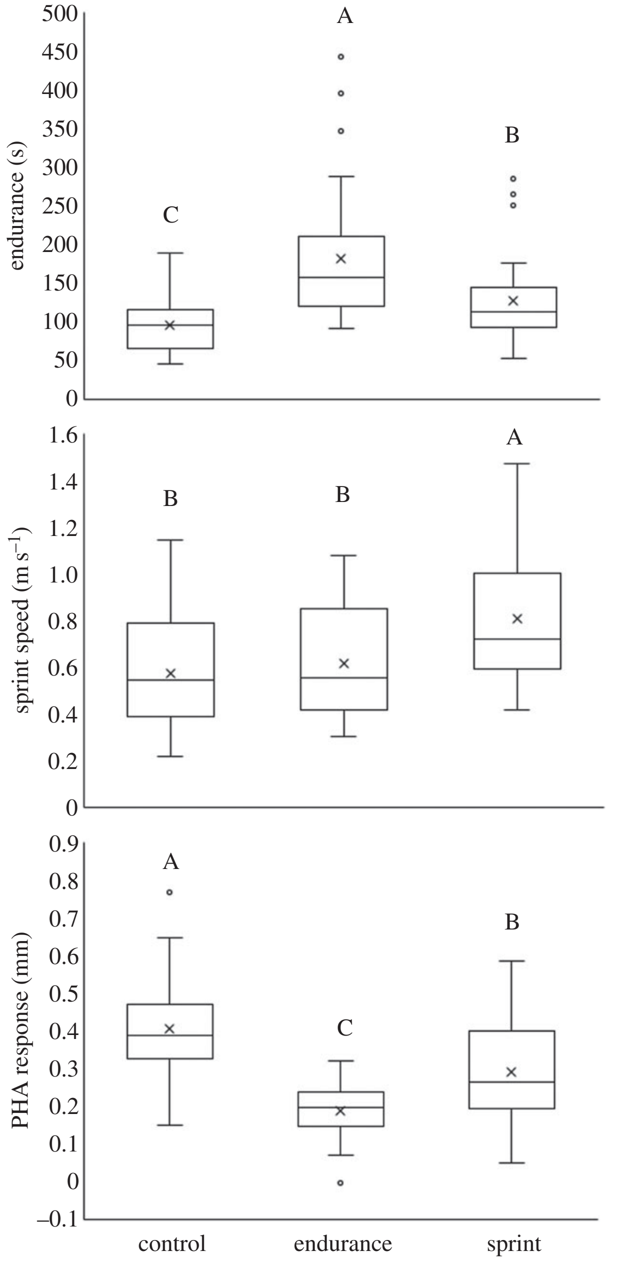

Figure 1. Specialized training for endurance and sprint speed significantly increased endurance capacity and sprint speed relative to controls, respectively, but decreased immunocompetence in *A. carolinensis* lizards. Letters above boxes within a panel represent significant treatment effects. X = mean value; horizontal line = median value; whiskers = minimum and maximum values; circles = outliers.

significantly different from either control ($p = 0.12$) or endurance-trained individuals ($p = 0.12$).

Sex violated the proportional hazards assumption of the Cox regression model and was removed from subsequent models. Following model simplification, the minimum adequate Cox model retained treatment and condition as significant factors. Consistent with the survival analysis, hazard ratios indicated that endurance-trained lizards faced the largest risk of mortality (almost 2.5 times that of controls; table 1), whereas sprint training did not significantly elevate mortality risk relative to controls. Condition was also retained in the minimum adequate Cox regression, with an increase of one ‘unit’ of condition nearly halving the risk of death (table 1).

None of the measured factors were significant predictors of either PTM or MPM, indicating that trained green anoles moved no more or less than untrained individuals. This result holds whether the sexes are pooled or analysed separately.

4. Discussion

This is the first test, to our knowledge, of whether experimentally enhanced performance affects survival in nature. Our hypothesis that training should increase survival was not supported; untrained-control lizards realized a clear survival advantage over endurance-trained green anole lizards when released into a natural setting, with endurance-trained lizards exhibiting a mortality risk roughly twice as high as that of control animals. By contrast, survival was not significantly compromised by sprint training, although the hazard ratio for sprint training trended towards significance.

Studies that have attempted to link endurance to survival in nature have reported mixed results. For example, Clobert *et al*. [9] found no effect of endurance capacity on survival in juvenile *Zootoca vivipara* lizards (see also [20]), whereas Le Galliard & Ferrière [21] reported positive selection on juvenile endurance and weak positive correlation between endurance and reproductive success in adult males of this same species. The discrepancy between these studies and ours could lie either in species differences in the ecology of locomotor performance or in the various costs incurred by the experimental boost in endurance afforded to our adult lizards through the exercise response [15]. Our forced increase in performance resulted in trade-offs that likely shifted the integrated, multivariate phenotype away from an optimal state in the current environment.

One such trade-off that could explain the curtailed survival in endurance-trained lizards is impaired immunocompetence induced through training. Indeed, trained individuals suffered a significant decrease in response to PHA challenge (figure 1), consistent with findings from earlier studies [11,12]. However, PHA response was not retained as a significant predictor of hazard during model reduction, suggesting that mortality is elevated in endurance-trained lizards independently of immune function. Natural selection on immunity in nature is understudied, but is thought to be trait-specific [22]. Future studies should consider more fine-grained immune assays within a design that is powerful enough to estimate the form and intensity of selection on fitness.

Our second hypothesis, that trained animals would move more than untrained animals in nature, was also not supported. Clobert *et al*. [9] suggested that probability of recapture in *Z. vivipara* was related to activity, not mortality, raising the possibility that our endurance-trained lizards were simply less active and did not necessarily suffer higher mortality. However, the consistency in our recapture results over multiple sampling periods combined with the lack of a significant treatment effect on either MPM or PTM support the notion that our recaptures were not biased by activity levels. Given that Lailvaux *et al*. [13] reported an energetic saving associated with endurance training in green anoles, the explanation that endurance-trained animals change their activity to minimize costs associated with locomotion is unlikely. Furthermore, the study site comprises an urban island of habitat entirely surrounded by roadway. There is also no continuous canopy of vegetation that lizards could use to disperse into surrounding areas, and we searched those nearby areas at each survey period to ensure that this was not occurring. Nonetheless, it is conceivable that some animals may

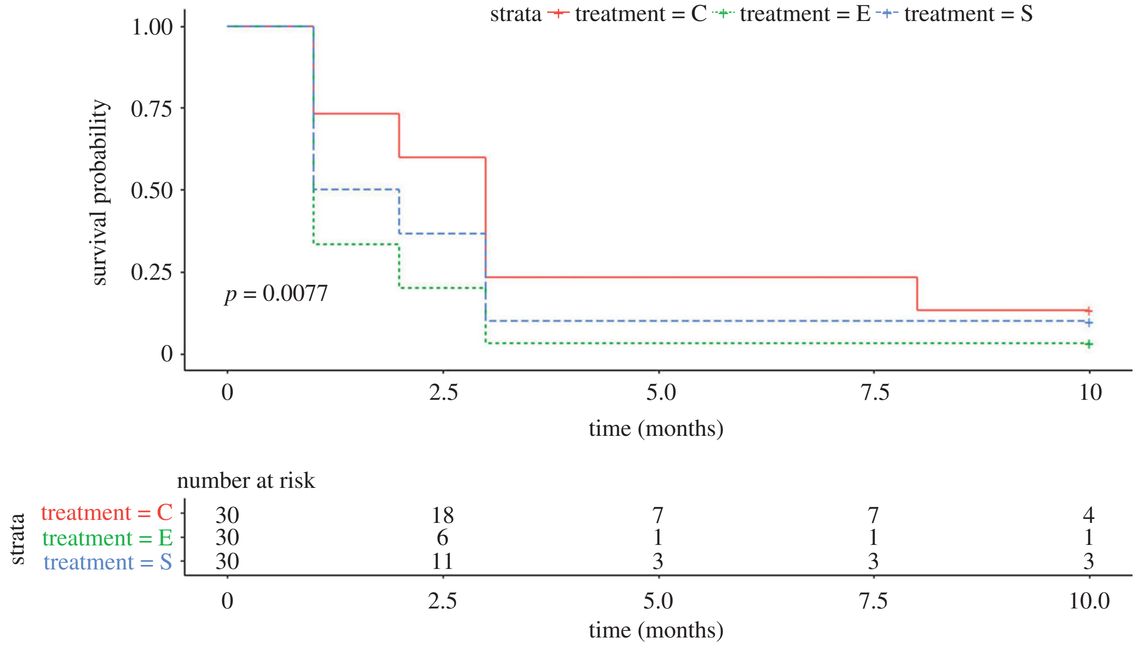

Figure 2. Kaplan–Meier survivorship curves over the course of the study for control (solid red), endurance (dotted green) and sprint trained (dashed blue) *A. carolinensis* lizards. The risk table gives the number of survivors per treatment group at each time sampled. The log rank test for the difference between survivorship curves is significant ($p < 0.0077$; see §3 for *post hoc* results). (Online version in colour.)

Table 1. Hazard ratios and associated 95% confidence interval ranges describing the risk of mortality relative to control animals for terms that explained significant amounts of variation in survival (i.e. endurance training, sprint training and body condition). The positive model coefficients for endurance and sprinting indicate increased mortality risk relative to controls, whereas the negative coefficient for condition indicates that increasing condition reduces the risk of mortality. ($^*p < 0.05$; $^{**}\ p < 0.005$).

	coefficient	hazard ratio	95% CI	*z*-value	*p*-value
endurance	0.909	2.48	1.43–4.31	3.23	0.0012**
sprint	0.545	1.72	0.98–30.3	1.9	0.058
condition	−0.562	0.57	0.33–0.98	−2.05	0.04*

have left the site instead of dying, which could bias our interpretation of the presence/absence data as survival. However, our focal data do not support that scenario. Consequently, there is no reason to suspect that trained animals were leaving the study site at a higher rate than controls.

In conclusion, we show that experimentally increasing investment in locomotor performance via the exercise response does not enhance survival in green anole lizards. Indeed, survival was significantly compromised in endurance-trained lizards relative to untrained controls. Our results stress the importance of considering diverse aspects of the multivariate phenotype when studying life-history evolution and how specific traits impact fitness.

Ethics. Procedures were approved by the University of St Thomas IACUC (protocol # 94).

Data accessibility. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.tt15798 [23].

Authors' contributions. This study was conceived by J.F.H. J.F.H. and S.P.L. designed and executed the study, analysed the data and wrote the manuscript. J.F.H. and S.P.L. approved the final version of the manuscript and agree to be held accountable for the content therein.

Competing interests. We declare we have no competing interests.

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