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RESEARCH ARTICLE

Endurance training does not affect maximum exertion/distance capacity in *Anolis carolinensis* lizards

Mahaut V. Sorlin*, Jamie R. Marks and Simon P. Lailvaux

ABSTRACT

Locomotor performance abilities are key predictors of survival and reproductive success in animals and understanding how selection targets them can provide insights into how morphology and physiology relate to fitness. But despite the large body of work on performance traits, along with well-established protocols to measure them, performance can be challenging to measure. Endurance, for instance, is commonly measured by recording how long an animal can run at a set pace until exhaustion, which is time consuming and requires dedicated equipment. Consequently, exertion or distance capacity, measured as distance run until exhaustion when chased, is often used as a proxy for endurance, but the relationship between these two metrics has never been assessed even though they likely rely on different underlying physiological mechanisms. We tested experimentally for a relationship between endurance and exertion by training green anole lizards for sprinting and endurance and measuring whether exertion capacity responds to either type of training. Prior to training and across treatments, males displayed a mean (±s.d.) exertion capacity of 14.08±0.29 m and females 12.03±3.52 m; after training, this was 14.78±3.57 m and 12.19±2.21 m, respectively. We found that exertion capacity was unaffected by either type of training in green anoles. We also show that a positive relationship between endurance and exertion capacity pre-training exists only in females and that this relationship is inconsistent among studies. Exertion should be studied as a locomotor trait in its own right and not as a proxy for endurance.

INTRODUCTION

Locomotor capacities are key targets of selection and important predictors of fitness in a variety of animal species (reviewed in Husak and Fox, 2008; Irschick et al., 2008). Animals employ many different types of whole-organism performance (defined as measures of animals conducting dynamic, physically challenging and ecologically relevant fitness tasks, of which locomotor abilities are a subset; see Irschick et al., 2008; Lailvaux and Irschick, 2006) in their daily lives and those different types of performance may trade-off against both each other (Cameron et al., 2013; Pasi and Carrier, 2003) and other components of the integrated organismal phenotype (Ghalambor et al., 2003; 2004; Lailvaux and Husak,

Department of Biology, University of New Orleans, 2000 Lakeshore Dr., New Orleans, LA 70148, USA.

*Author for correspondence (mvsorlin@uno.edu)

M.V.S., 0000-0003-3098-0073; J.R.M., 0000-0003-2384-9333; S.P.L., 0000-0002-2737-8682

2014). These trade-offs are driven at least in part by the underlying mechanisms bolstering the expression of specific performance capacities and those mechanisms may vary depending on the performance trait in question (Husak and Lailvaux, 2022). Understanding the morphological and physiological pathways driving the expression of a given performance trait is therefore important for understanding both the conditions under which that trait can be maximally expressed and its relationship to other components of the integrated organismal phenotype.

Over the last 40 years, performance researchers have developed protocols for measuring the maximum capacities of different kinds of whole-organism performance traits with the goal of standardizing performance measures to facilitate comparisons both within and among species (Adolph and Pickering, 2008; Losos et al., 2002). Just as important, however, is the specific method that is used to measure a particular maximum performance capacity. For example, maximum sprint speed is an anaerobically supported burst performance trait and as such it is commonly measured in small vertebrates by encouraging animals to sprint down a racetrack, where speed is measured either with photocells spaced at known intervals (Cox et al., 2009; Husak et al., 2015) or using high-speed cameras (Foster and Higham, 2012). However, unlike short-term burst performance, endurance capacity is generally understood to support sustainable activity over longer periods of time (Garland, 1999) and as such is specifically and explicitly tied to aerobic capacity (Garland, 1993; John-Alder et al., 1983; John-Alder and Bennett, 1981). Endurance capacity is therefore most commonly measured as the time it takes for an animal to become exhausted using a treadmill set to a speed that is below the maximum speed that the animal is able to maintain aerobically (John-Alder et al., 1983; Bennett and Huey, 1990).

However, despite this distinction between anaerobically supported sprinting and aerobically supported endurance, researchers commonly assess endurance by measuring a different trait, variously termed exertion or distance capacity, that is arguably not a function of aerobic capacity. Unlike treadmill endurance where the organism is forced to conform to the speed of the belt on which it runs, exertion is typically measured by chasing individuals around a circular track (∼1 m in circumference for small vertebrates, but larger or smaller tracks appropriate to the size of the organism are also used) until the animal becomes exhausted (e.g. Lailvaux et al., 2005; Leal, 1999; but see Ramos et al., 2004, for a different approach), with the total distance run taken to be the measure of exertion. Exertion trials are easier to conduct than treadmill endurance, as they require minimal equipment, tend to be of shorter duration than endurance measures, and are far less dependent on the prolonged cooperation of the organism in question, all of which likely explains why researchers might measure exertion instead of endurance. But while exertion is an interesting and potentially informative metric in and of itself, it is likely to be a poor proxy for aerobic endurance capacity for at least two reasons.

First, because the speed of the animal cannot be controlled during exertion trials, animals frequently run at speeds that, even if not necessarily maximal, are significantly above the maximum aerobic speed. Bennett and Huey (1990) specifically distinguish between endurance and exertion and argue for the anaerobic nature of exertion specifically and Garland (1984) showed that variation in endurance and exertion (termed 'maximal distance run') between individuals of the iguanid lizard (*Ctenosaura similis*) correlated with different physiological variables. Second, exertion trials are often characterized by intermittent movement whereby individuals stop running briefly at semi-regular intervals. Intermittent running is especially common in lizards whose sprawling gait limits their ability to breathe while in motion (Carrier, 1987). Using ghost crabs (*Ocypode quadrata*), Weinstein and Full (1992) showed that such intermittent locomotion (before fatigue) allowed crabs to run 5.8 times farther than conspecifics prevented from intermittent pausing, and Edwards and Gleeson (2001) found that intermittent running also lowered energetic expenditure compared with continuous running over the same time period in *Mus domesticus* mice. These studies show that differences in measurement protocol can affect performance outcomes and raise further questions regarding the use of exertion as a proxy for endurance.

Before using any performance trait as a proxy for another, ostensibly related trait, the nature of the relationship between those two traits should be rigorously assessed. However, with the exception of Garland (1984), no studies to our knowledge have tested for a common physiological basis of endurance and exertion, nor have any demonstrated a direct relationship between them. Baxter-Gilbert et al. (2017) compared three common methods for measuring endurance capacity in a lizard (*Intellagama lesueuri*) by making animals: (1) run on a stationary track; (2) run on a moving platform (treadmill); or (3) swim. Although they found that the two running methods elicited similar results, they also found that the rank order of performance was different for each, indicating potentially important variation between the two protocols. Baxter-Gilbert et al. (2017) also showed that all three methods lacked repeatability. This lack of repeatability represents a further difficulty for measuring the relationship between endurance and other traits through correlations because within- and among-individual variation in performance traits can mask each other. This renders phenotypic correlations that do not partition within- and among-individual variation misleading (Careau and Wilson, 2017a,b) and a lack of among-individual repeatability means that among-individual level relationships between endurance and other traits cannot be assessed (see Lailvaux et al., 2019, for an example involving lizard endurance). However, a growing body of literature shows that endurance capacity can be enhanced in non-human animals through exercise training (Husak et al., 2015, 2017), suggesting that the effect of increasing endurance on exertion can be assessed experimentally. Specifically, if endurance and exertion are related, then increasing endurance capacity via training might be expected to increase exertion as well. Sprint training also increases sprint speed (González-Ortega et al., 2021), and increases muscle mass and the proportion of fast glycolytic muscle fibers in lizards (Husak et al., 2015), meaning that the relationship between exertion and sprinting can be experimentally assessed using training as well.

We tested the null hypothesis that maximum endurance capacity and maximum exertion/distance capacity are unrelated. To do so, we trained *Anolis carolinensis* lizards for endurance and measured exertion both before and after training. Green anoles are a suitable species for testing this hypothesis because their endurance

capacities are easily measured and respond to exercise training (Husak et al., 2015; 2017; Husak and Lailvaux, 2019; Lailvaux et al., 2018; Wang and Husak, 2020). Because existing evidence suggests that exertion is likely anaerobic, we also sprint-trained animals to test the ancillary hypothesis that sprinting and exertion are related. Specifically, we predicted that lizards trained for endurance should exhibit no change in exertion compared with untrained controls, but that sprint training should increase exertion compared with both endurance-trained and control lizards.

MATERIALS AND METHODS

Adult individuals (n=49 male, n=50 female) were captured either by hand or using a noose (McDiarmid et al., 2012) around New Orleans parish between 28 March 2019 and 17 April 2019, and subsequently housed in individual terraria (30 cm×20 cm×19 cm W×D×H) equipped with substrate and a perch mimicking their natural environment. The terraria were placed under UV and halogen lights to allow for thermoregulation. The humidity level in the husbandry was maintained at 62.9±6.4% (mean±s.d.) and temperature was kept at 28.16±1.43°C in both the husbandry and experiment room so that performance would not be impacted by a temperature change (Bennett, 1980). All individuals were fed *ad libitum* [2–3 crickets (*Acheta domesticus*) supplemented with vitamins, 3 times per week] and misted at least once a day (Lailvaux et al., 2012; Husak et al., 2015). After capture, the lizards were randomly assigned to one of three groups: control (n=15 male and n=16 female), endurance training (n=17 male and n=17 female) and sprint training (n=17 male and n=17 female). All procedures were approved by the UNO Institutional Animal Care and Use Committee #19-002.

Morphology

Snout–vent length, hindlimb length, forelimb length, head length, head width and head depth were measured to the nearest 0.01 mm with digital calipers after capture.

All performance types were recorded in each individual from all three treatment groups (untrained control, endurance trained and sprint trained) at two different time points: one week prior to training (T1) and within 3 days after the end of training (T2). Lizards from the control group were not trained but were removed from their cages on training days and briefly handled to match the level of handling the lizards from the training groups were subjected to, as well as any associated handling stress (Husak et al., 2015; Lailvaux et al., 2018).

Exertion capacity

Exertion capacity was measured using a circular track of 1 m diameter, separated into 10 equal parts of 10 cm. Lizards were chased around the track by hand until reaching exhaustion (loss of righting reflex) as previously described (Brodie, 1993; Cullum, 1998; Lailvaux et al., 2003, 2005; Leal, 1999; Simon et al., 2022). We measured total distance run by counting the number of times the lizard travelled the entire track plus the number of segments past the starting segment the lizard reached before exhaustion. The number of segments was then converted to distance in meters. In addition, we measured using a stopwatch the total time of each exertion trial from the time a lizard started running until it became exhausted.

Endurance capacity

We measured endurance capacity by running lizards to exhaustion, again determined by loss of righting reflex (Husak et al., 2015), on a motorized treadmill rotating at 0.3 km h^{-1} (Cox et al., 2009; Perry

et al., 2004). Lizards were placed on the moving treadmill and encouraged to run by gentle tapping of the tail and hindlimbs with a paintbrush. The duration from initiation of the trial to loss of righting reflex was recorded as the measure of endurance.

Endurance training consisted of sustained low-speed running exercise on a custom-built treadmill (speed 0.3 km h^{-1}) 3 times a week for 6 weeks. For the first 2 weeks, the treadmill was horizontal, then a 10 deg incline was used for weeks 3–4 and a 20 deg incline was used for weeks 5–6. This training regime has been used in several comparable studies on green anoles and has proven effective in eliciting increases in endurance capacity (Husak et al., 2015; Lailvaux et al., 2018) without harmful side-effects, such as joint damage, caused by more strenuous regimes (e.g. Garland et al., 1987).

Sprint speed

Sprint performance was measured on a racetrack at T1 and T2. At both time points, the anoles performed three trials, each separated by at least 1 h. The racetrack consisted of a 2 m long, 5 cm dowel covered in cork (for traction) and was angled at 45 deg because anoles tend to hop on horizontal surfaces (Cox et al., 2009; Losos and Irschick, 1996). It was equipped with vertically paired infrared photocells (Trackmate Racing, Surrey, BC, Canada) at 25 cm intervals so that a running lizard breaks the beams sequentially and the elapsed time (ms) for each interval is recorded by computer. The fastest speed measured for a 25 cm interval was recorded for each trial. This is a highly repeatable, standard procedure for lizards, including green anoles (Cox et al., 2009; Huey et al., 1990; Husak et al., 2015; Lailvaux et al., 2003; 2019).

For sprint training, lizards were trained on the same racetrack used during the performance trials. They trained 3 times a week for 6 weeks, with each trial consisting of 4 runs separated by 1 h. Starting on week 3, training intensity increased by hanging weights (centrifuge tubes filled with clay) off the lizards. The weights equaled one-quarter (weeks 3 and 4), then one-half (weeks 5 and 6) of the body weight of each lizard. In each trial, lizards were taken out of their cage and immediately chased up the racetrack into a black bag. This training regime has also been shown to be effective and not overly strenuous for green anoles (Husak and Lailvaux, 2019; Lailvaux et al., 2018).

Statistical analysis
Pre-training

To test whether exertion capacity differed among training groups independent of size prior to any training being conducted, we used an ANCOVA (car R package v3.0.12; Fox and Weisberg, 2019) with pre-training exertion capacity as the dependent variable; sex, training group and the interaction between sex and training group as independent variables; and pre-training mass as a covariate.

We tested whether exertion was associated with either sprinting or endurance pre-training using separate multiple regressions for each performance type, with exertion as the dependent variable and mass, sex, performance (either maximum speed or maximum endurance capacity) and the interaction between sex and performance as predictor variables. We also fitted sex-specific models for clarity.

Post-training

We tested the effectiveness of the endurance training in two different ways. First, we used an ANCOVA with post-training endurance capacity as the dependent variable; sex, training group and the interaction between sex and training as independent

variables; and post-training mass as a covariate. Then, we fitted a general linear mixed model to all of the endurance data, both pre- and post-training, with endurance capacity as the dependent variable; sex, treatment, time point (pre- and post-training) and their three-way interaction as fixed effects; mass as covariate; and, finally, individual as a random factor to account for repeated endurance measures before and after training.

Previous training studies have shown that green anoles often do not increase their measured sprint speed following training, most likely because of decreased motivation to sprint maximally resulting from habituation over the course of the training regime (Husak et al., 2015; Lailvaux et al., 2020). However, we also know from earlier studies that our sprint training regime does prompt clear physiological changes associated with anaerobic performance in green anoles, including an increased proportion of fast-twitch glycolytic muscle fibers (Husak et al., 2015), an increase in active metabolic rates and a decrease in standard metabolic rates (Lailvaux et al., 2018). Consequently, we did not test for an effect of training on sprint speed here, as these data are unlikely to be meaningful either way.

To test whether maximum exertion was affected by training, we fitted a general linear mixed model to all measured exertion data, as above, with: mass as covariate; sex, treatment, time point (pre- and post-training) and the three-way interaction between sex, treatment and time as fixed factors; and individual as a random factor to account for the repeated measure of exertion both before and after training. We also performed an ANCOVA analysis with post-training exertion capacity as the dependent variable; sex, training group and the interaction between sex and training as independent variables; and post-training mass as a covariate.

For all mixed models, we used log likelihood ratio deletion tests to determine the minimum adequate model (i.e. the simplest model that explains the greatest amount of variation) (Crawley, 1993; Venables and Ripley, 2013). All analyses were performed in R Statistical Software (v4.1.1; http://www.R-project.org/).

RESULTS
Pre-training

After accounting for mass we found no significant differences in exertion capacity among treatment groups ($F_{2,92}$=0.564, P=0.57), nor was there any significant two-way interaction between sex and treatment ($F_{2,92}$=0.188, P=0.82; see Fig. 1). Across treatments, males displayed a mean (±s.d.) exertion capacity of 14.08±0.29 m and females 12.03±3.52 m. Preliminary analyses identified two datapoints in the endurance group as outliers (Cook's distances >1). Consequently, we repeated these analyses with those datapoints removed and found the results to be qualitatively unchanged (treatment: $F_{2,83}$=2.292, P=0.1; sex×treatment interaction: $F_{2,83}$=0.85, P=0.43).

While multiple regression of performance types against exertion showed that sprint speed does not predict exertion capacity overall ($F_{4,94}$=5192, adjusted R^2=0.1461, ddf=94, P=0.95), we did find a significant positive correlation between endurance and exertion capacity after controlling for mass, albeit in females only, not males ($F_{4,94}$=5.192, adjusted R^2=0.2943, ddf=94, P<0.001) (Table 1, Fig. 2).

The sex-specific models confirmed these results. In the female regression model ($F_{2,47}$=10.36, adjusted R^2=0.276, ddf=47, P<0.01) the variables accounted for 27.65% of the variance, with endurance treatment significantly predicting exertion capacity (P=0.004). No significant relationship was observed in the male model ($F_{2,46}$=2.913, adjusted R^2=0.073, d.f.=46, P=0.06).

Table 1. Results of the multiple regression analysis for pre-training sprint and endurance performance predicting exertion capacity

Model	Variable	Coefficient	s.e.	T-statistic	P-value
Endurance	Intercept	5.160	1.392	3.705	0.001***
	Mass	0.433	0.301	1.438	0.15
	Sex(M)	4.955	2.05	2.417	0.018*
	Endurance	0.062	0.014	4.292	0.001***
	Sex(M)×Endurance	−0.046	0.018	−2.571	0.012*
Sprint	Intercept	9.879	1.199	8.237	0.001***
	Mass	0.888	0.309	2.873	0.005**
	Sex(M)	−0.021	1.632	−0.013	0.99
	Speed	0.072	1.051	0.068	0.95
	Sex(M)×Speed	−0.068	1.568	−0.044	0.97

Asterisks indicate significance. M, male.

Post-training

We saw a clear treatment effect of training on maximum endurance capacity. Lizards trained for endurance showed significantly better endurance times compared with control and sprint-trained lizards (Fig. 3) with an 18.49% increase in endurance capacity between the two time points (on average 97.03±28.18 s at T1 and 114.97 ±20.89 s at T2 in endurance-trained lizards). After accounting for mass, we found a significant difference in endurance capacity among treatment groups ($F_{2,92}$=19.332, P<0.001). Because preliminary analysis identified 9 datapoints in the endurance group as outliers (Cook's distances >1), we repeated our analysis

on log-transformed endurance capacity data and found the results to be similar to our first model (treatment: $F_{2,191}$=3.535, P=0.03).

The mixed model corroborated these results, showing that every parameter but the 3-way interaction between sex, treatment and time point was retained in the final model explaining variation in post-training endurance capacity (AIC=1837.15, n=7 parameters; see Table 2).

By contrast we found no significant differences in maximum exertion capacity between training groups after the training took place (Table 3 and Fig. 4).

Finally, following the same steps as for the endurance mixed model, we looked at the variation in exertion capacity between the two time points. Only mass was retained in the final mixed model (AIC=978.8, n=1 parameter), suggesting that neither endurance nor sprint training had any significant effect on variation in exertion capacity (see Table 4).

To summarize, following training, endurance-trained animals exhibited significantly better endurance capacities compared with controls and sprint-trained lizards. In contrast, exertion did not differ among training treatments. Finally, using all the data in a linear mixed model, we saw no evidence that exertion capacity changed over the course of the experiment in response to training of any kind.
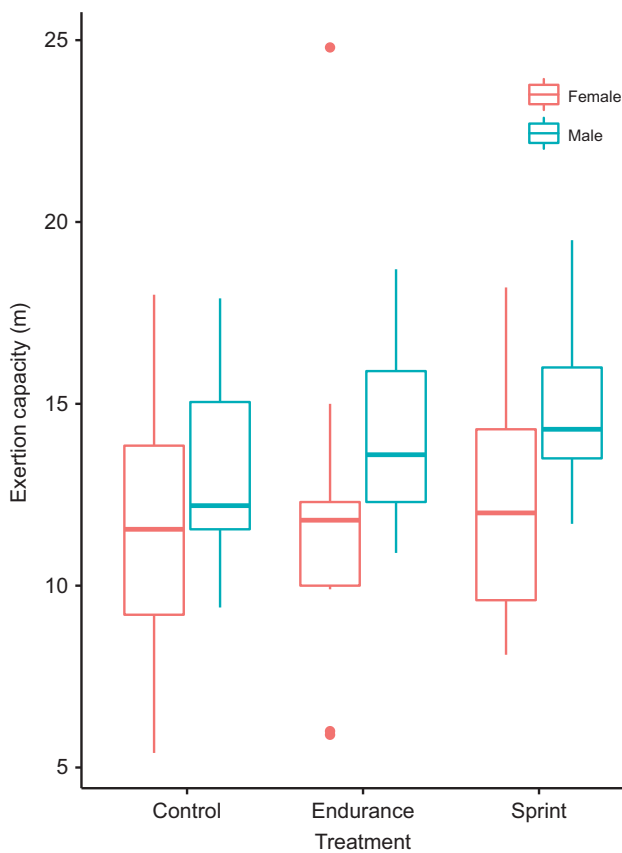

Fig. 1. Exertion capacity of green anoles, *Anolis carolinensis*, before training. Boxplot [with minimum, first quartile (Q1), median, third quartile (Q3) and maximum] of exertion capacity prior to training according to treatment (control, endurance training and sprint training) and sex. Circles depict outliers. For females: n=16 control, n=17 endurance, n=17 sprint; and for males: n=15 control, n=17 endurance, n=17 sprint.
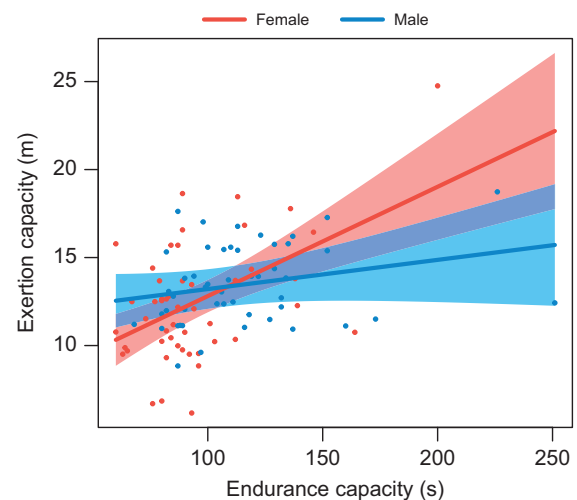

Fig. 2. Exertion capacity versus endurance capacity of green anoles. Cross-sectional plot of a multiple regression model, using partial residuals and adjusted means, depicting the sex-dependent relationship between endurance and exertion capacity prior to exercise training and adjusting for mass. n=50 female and n=49 male. Shaded areas represent 95% confidence intervals.
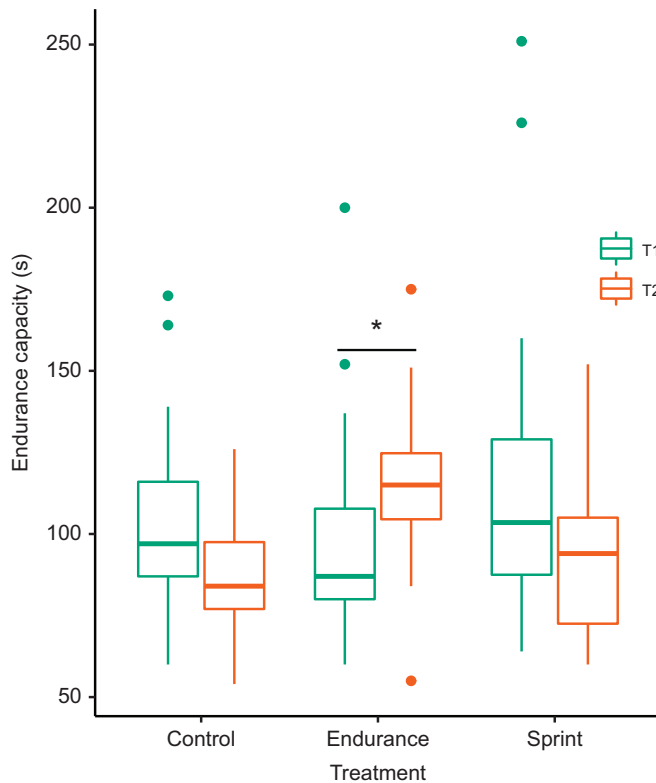
Fig. 3. Endurance capacity of green anoles before and after training.
Boxplot (with minimum, Q1, median, Q3 and maximum) of endurance capacity by treatment group (control, endurance and sprint) pre- and post-training (time points T1 and T2). Significant results are indicated by an asterisk (*P*<0.001). *n*=31 control, *n*=34 endurance, *n*=34 sprint.

DISCUSSION

Endurance capacity is an important and ecologically relevant performance trait in a variety of invertebrate (Full and Herreid, 1984; Full and Tullis, 1990) and vertebrate animal species (Garland, 1999; Lindstedt et al., 1991), but measuring endurance is time consuming and requires specialized equipment. We tested the hypothesis that a commonly measured proxy for endurance, called exertion or distance capacity, is actually unrelated to endurance, by measuring whether green anole lizards trained for endurance exhibit increased exertion capacity as well.

Table 2. Minimum adequate model describing variation in endurance capacity (s) between time points by sex and treatment (endurance training, sprint training and untrained control), controlling for mass (g)

Traits	Coefficient	s.e.
Intercept	68.847	8.559
Mass	10.666	2.321
Sex(M)	−8.487	10.047
Treatment	−3.830	8.511
Treatment(Sprint)	−1.884	8.505
Time point 2	−15.335	7.987
Treatment×Time point 2	31.932	11.013
Treatment(Sprint)×Time point 2	−0.654	11.022
Sex(M)×Treatment	−3.326	12.132
Sex(M)×Treatment(Sprint)	18.731	12.145
Sex(M)×Time point 2	−10.637	11.363

Reported values give estimated change in endurance between the baseline treatment level and the level shown in the table. The baseline category for sex is female (F) and for treatment is control.

Table 3. Analysis of covariance (ANCOVA) summary table for exertion capacity by mass, training regime, sex and the interaction between sex and training regime

Variable	d.f.	SS	MS	*F*	*P*-value
Mass	1	177.33	177.33	20.46	<0.001***
Sex	1	10.11	10.11	1.16	0.28
Training	2	19.18	9.59	1.1	0.33
Sex×Training	2	21.22	10.61	1.22	0.29
Residuals	92	797.2	8.66		

SS, sum of squares; MS, mean square.

Exertion capacity did not differ among treatment groups prior to the initiation of our training regimes (Fig. 1). Exertion was also unrelated to sprinting in untrained lizards, but did predict endurance ability in untrained female, but not male, green anoles (Fig. 2, Table 1). These results contrast with those of Simon et al. (2022), who found a positive correlation between exertion and endurance in males but not females using a larger sample size than that of the present study. Furthermore, Simon et al. (2022) also found evidence for an influence of locomotor muscle mass on female exertion, but no such effect on males. These conflicting results are difficult to reconcile and again it is important to note that phenotypic correlations that do not partition out the effects of within- versus among-individual correlations can be misleading (Careau and Wilson, 2017a). However, these findings do collectively illustrate
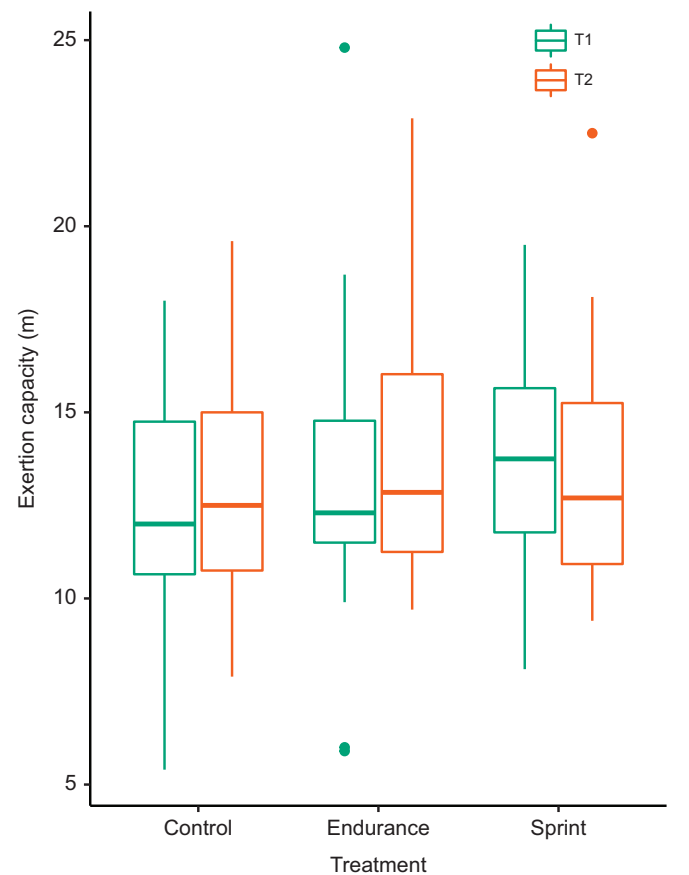

Fig. 4. Exertion capacity of green anoles before and after treatment.
Box plot (with minimum, Q1, median, Q3 and maximum) of exertion capacity by treatment group (control, endurance and sprint) pre- and post-training (T1 and T2). *n*=31 control, *n*=34 endurance, *n*=34 sprint.

Table 4. Minimum adequate model describing variation in exertion capacity (m) between time points by sex and treatment (endurance training, sprint training and untrained control), controlling for mass (g)

Model term	Coefficient	s.e.
Intercept	10.118	0.992
Mass	0.749	0.274

that exertion is not a consistently reliable proxy for endurance in *A. carolinensis* and likely in other lizard species as well.

Our first prediction, that endurance training would not increase exertion capacity, was supported (Fig. 4, Tables 3 and 4). Endurance-trained lizards significantly increased their endurance capacity compared with control and sprint-trained lizards, consistent with the results of earlier studies (e.g. Husak et al., 2015, 2017; Husak and Lailvaux, 2019). However, we found no effect of training on exertion capacity in the current dataset. If maximum exertion capacity is driven by the same underlying physiological pathways as endurance capacity, then we would have expected a significant increase in exertion in those lizards that improved their endurance abilities through training as well. The lack of any such effect in our dataset suggests that endurance and exertion are in fact decoupled and lends support to earlier studies suggesting different underlying mechanisms driving endurance and exertion (Bennett, 1994; Bennett and Huey, 1990; Garland, 1984). Specifically, Garland (1984) found that almost 90% of inter-individual variability in endurance capacity in the black iguana (*Ctenosaura similis*) could be explained by variability in thigh and heart muscle mass, maximum oxygen consumption rate ($\dot{V}_{O_2}$) and citrate synthase activity; all metrics typically involved in the aerobic pathway. Variability in 'maximum distance capacity' (exertion), in contrast, could be predicted by changes in maximum carbon dioxide production rate ($\dot{V}_{CO_2}$) and thigh pyruvate kinase activity, which is an indicator of a tissue's anaerobic capacity. Garland's (1984) findings are consistent with patterns observed in other taxa. In several lacertid species, endurance is correlated with the aerobic pathway whereas exertion correlates with anaerobic capacity (Bennett et al., 1984); an experiment in frogs (Cummings, 1979) showed that the main responses to endurance training involved changes in the aerobic mechanism (i.e. better lactate removal efficiency and an increase in citrate synthase activity). Similarly, responses to endurance training in mammals include muscle hypertrophy, an increase in enzyme activity in the Krebs cycle and an increase in aerobic pathway efficiency (Dohm et al., 1973; Holloszy and Booth, 1976; Vigelsø et al., 2014). The results of our study further suggest that using exertion as a more easily measured proxy for endurance is likely to be misleading.

Our second prediction, that sprint training should increase exertion, was not supported, as sprint training had no effect on exertion capacity in our dataset (Fig. 4, Tables 3 and 4). Given that sprint speed typically does not respond to training in green anoles because of potential issues with habituation of the lizards to researcher presence and consequent decreased motivation to run at maximum speed, one possibility is that exertion capacity measures are subject to similar habituation effects. However, measures of exertion are fundamentally different to those of speed, in that sprint speed measures the time taken for an individual to traverse a given distance, whereas exertion is typically a measure of the distance run prior to the onset of exhaustion (hence the alternative name 'distance capacity'). If habituation did indeed cause lizards to run slower during exertion trials, then one might predict either longer distance capacities in sprint-trained lizards or shorter ones as a result of increased investment in underlying anaerobic capacities. Yet another

possibility is that these occur simultaneously, in effect balancing each other out. Consequently, the relationship between exertion and sprint speed could be complex. Whatever the case, the lack of a consistent significant relationship between exertion and sprint speed either pre- or post-sprint training shows that measures of exertion using the standard exertion protocol do not reflect sprint speed in green anole lizards either. This result is intriguing because it suggests that exertion is neither a strictly aerobic nor a strictly anaerobic capacity, raising the question of exactly what physiological capacities or specific combination thereof are being captured by measures of maximum exertion. Studies that consider the energetic production and maintenance costs of exertion as well the excess post-exercise oxygen consumption induced by exertion activity (Edwards and Gleeson, 2001; Husak and Lailvaux, 2017; Lailvaux et al., 2018) would be useful for identifying the metabolic nature of exertion capacity.

Our results here are consistent with the existing literature showing that differences in measurement protocols for locomotor traits can affect experimental outcomes. In particular, endurance protocols where animals are allowed to choose their own running speed, such as wheel running, can have different biological consequences in certain contexts. In mice, for example, voluntary wheel running and treadmill running induce neuroplasticity in different regions of the brain (Liu et al., 2009) and voluntary wheel running was also less effective than treadmill locomotion in inducing cardioprotective stress responses (Noble et al., 1999). Exertion measures are 'voluntary' only in the sense that the lizards are free to choose the speed at which they run, not both the speed and the amount of time run as in the case of wheel running. Nonetheless, the fact of this voluntary speed component of exertion suggests that it captures different components of the underlying physiological variation than either maximum sprint speed or maximum endurance. It is also of interest to note that equivalent measures of endurance and exertion in animals using other locomotor modalities, although arguably better understood in terms of the underlying metabolism, may be prone to similar ambiguities in interpretation. For example, fish performance researchers distinguish between sustained, prolonged and burst swimming that are also estimated using different methods (Cano-Barbacil et al., 2020), yet studies have shown that even within these categories the choice of equipment can significantly affect the performance estimate obtained (Kern et al., 2018). Although there are many differences between the aquatic and terrestrial media that might affect both the conceptual and logistical choice of performance measure, researchers must nonetheless carefully consider both the nature of the performance trait that they aim to measure and the methods that they use to do so within the context of the research question at hand (Husak and Lailvaux, 2022). Future studies that train lizards or other small terrestrial vertebrates explicitly for maximum exertion would also be useful for determining how investment in exertion affects the physiological capacities driving locomotion in lizards.

A proper understanding of performance evolution demands moving beyond measures of single performance capacities and understanding how organisms use and express multiple types of performance traits (Lailvaux et al., 2022). However, measuring the multivariate performance phenotype has proven to be extremely challenging and few studies have done so comprehensively (Simon et al., 2022). Our results emphasize the importance of understanding the physiological bases of each performance trait being measured and the potential pitfalls of using easily measured traits as a proxy for data that are more difficult to obtain without

first verifying the nature of the relationship between them. Researchers interested in animal locomotion would be advised to select locomotor traits to measure that are appropriate to the species, context and question of interest (Husak and Lailvaux, 2022). Further confusion in the literature could be avoided by using a standardized terminology for each of these traits; for example, by following the historical practice of using 'stamina' for endurance and 'distance capacity' (or 'distance run') for exertion (Garland, 1984, 1999).

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