Journal of Comparative Physiology B
https://doi.org/10.1007/s00360-026-01697-6

ORIGINAL PAPER

# Latitudinal variation in cold tolerance, metabolism, and locomotor performance of green anole lizards (*Anolis carolinensis*)

**Jerry F. Husak[1] · Margaret A. Duerwachter[1] · Jamie R. Marks[2] · Mahaut V. Sorlin[3] · Simon P. Lailvaux[3]**

Received: 2 March 2026 / Accepted: 30 June 2026
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2026

**Abstract**
Range expansion into cooler climates frequently drives phenotypic changes within a species to increase thermal tolerance. The oxygen and capacity-limited thermal tolerance (OCLTT) hypothesis maintains that thermal tolerance changes when animals move from one climate to a cooler or warmer one based on rates of oxygen demand and supply in the new environments. We tested central tenets of the OCLTT hypothesis across the north-south distribution of the green anole lizard (*Anolis carolinensis*) in North America, predicting that northern populations in cooler climates would have higher cold tolerance due to decreased standard metabolic rates (SMR; decreased oxygen demand), greater heart ventricle mass or hemoglobin concentrations (increased oxygen supply), or both. We further predicted that the adaptations leading to greater cold tolerance would also increase endurance capacity, with a corresponding decrease in sprint speed and exertion. Our results provided mixed support for the OCLTT hypothesis. Heart ventricle mass of the northern populations was greater, but only for females. There was no difference in SMR across populations, and hemoglobin concentrations did not follow a latitudinal pattern. However, performance traits generally matched our predictions, as endurance increased with latitude and sprint speed decreased, likely due to physiological tradeoffs associated with increased aerobic capacity. There are clearly selection pressures other than temperature acting on these populations to cause patterns differing from those expected under the OCLTT hypothesis.

**Keywords** Cold tolerance · Endurance · Lizard · Oxygen and capacity-limited thermal tolerance hypothesis · Sprint speed · Standard metabolic rate

## Introduction

The distributions of organisms are functions not only of their evolutionary histories, but also of the current and historical environmental contexts in which they exist. Factors such as temperature, habitat type, and resource availability, among others, exert selection pressures which, over evolutionary timescales, prompt adaptation to local conditions and ultimately drive variation in the expression of organismal phenotypes. Trends such as Bergmann's (Ashton et al. 2000; Meiri and Dayan 2003) and Allen's rules (Nudds and Oswald 2007; Symonds and Tattersall 2010), which describe patterns of increasing size and more compact body shapes, respectively, across increasing latitudinal gradients in endotherms, are exemplars of the powerful influence of temperature on animal functional and morphological traits. However, ectotherms frequently buck these trends, and Bergman's rule specifically is often subverted in non-avian reptiles (Ashton and Feldman 2003; Pincheira-Donoso et al. 2008) and amphibians (Adams and Church 2007), which exhibit more complex relationships with environmental temperature than do birds and mammals. Nevertheless, when tropical ectotherms expand northward to colonize higher latitudes, climatic differences levy physiological challenges. Lower minimum temperatures and stronger

Communicated by Kathrin Dausmann

✉ Jerry F. Husak
jerry.husak@stthomas.edu

[1] Department of Biology, University of St. Thomas, 2115 Summit Avenue, St Paul, MN 55105, USA

[2] Department of Integrative Biology, Kellogg Biological Station, Michigan State University, 3700 E. Gull Lake Rd., Hickory Corners, MI 49060, USA

[3] Department of Biological Sciences, The University of New Orleans, 2000 Lakeshore Drive, New Orleans, LA 70148, USA

Published online: 27 July 2026

seasonality may impose strong selection on thermal tolerance and other related physiological systems (Snyder and Weathers 1975; Bozinovic et al. 2011; Bennett et al. 2021). When temperatures drop below the range of body temperatures that are optimal for whole-organism performance, there is a progressive decline in performance (Huey and Kingsolver 1989), which may ultimately decrease Darwinian fitness (Bennett 1980; Angilletta 2009). Thus, movement to cooler climates is expected to be associated with adaptations that allow performance to be maintained at optimal levels during steady-state activities (Pörtner et al. 2017).

According to the oxygen and capacity-limited thermal tolerance (OCLTT) hypothesis, an organism's thermal limits are reached when its ability to deliver oxygen to tissues can no longer keep pace with increasing metabolic demand. As temperatures rise or fall beyond optimal levels, metabolism changes and the cardiovascular and respiratory systems must change to supply oxygen. Beyond critical temperatures the organism's oxygen transport capacity becomes insufficient, leading to reduced performance and ultimately loss of survival at extreme temperatures (Pörtner 2001). More simply, this hypothesis predicts that thermal limits of ectotherms manifest due to a mismatch between the rates of oxygen supply and metabolic demand at extreme hot and cold temperatures (Pörtner 2001). This mismatch results in decreased whole-organism performance and, presumably, decreased Darwinian fitness (Pörtner et al. 2017). The OCLTT has been used to predict the impacts of warming climates on populations (Pörtner 2021), but it can also be applied to understand how organisms that have expanded to colder climates have acclimatized or adapted (Campbell-Staton et al. 2018). To evolve greater cold tolerance and maintain optimal performance at cooler temperatures, populations in colder climates may decrease oxygen demand or increase oxygen delivery to tissues, or both (Schulte 2015). This can be accomplished by reducing maintenance costs (e.g., decreased standard metabolic rates, SMR) or via adaptations to the cardiovascular system, such as increased hematocrit or higher cardiac output via greater heart rate or stroke volume (Pörtner et al. 2017). Partially supporting this, green anole lizards (*Anolis carolinensis*) at higher latitudes along a latitudinal gradient in the southwestern portion of their distribution had greater cold tolerance and lower oxygen consumption rates, but neither larger hearts nor higher concentrations of hemoglobin (Campbell-Staton et al. 2018). In this case, oxygen demand decreased in cooler climates, but oxygen delivery was seemingly unaffected. Although the OCLTT has been supported for marine ectotherms that live in water with lower partial pressures of oxygen compared to air, results are mixed for terrestrial ectotherms (Schulte 2015). The mechanism of oxygen delivery for a terrestrial species likely impacts how important that delivery is to thermal tolerance, with a greater role for vertebrates that use convective oxygen transport compared to insects that rely on diffusion (Klok et al. 2004). Furthermore, critical thermal maximum (CTmax) was explicitly defined by Cowles and Bogert (1944) as the high temperature at which locomotor activity ceases, and was based on locomotor disruption due to muscle fibers becoming damaged by warm temperatures. It is difficult to determine with certainty that failing cardiovascular function causes decreased metabolic rates as thermal limits are approached, because under these conditions everything else is failing as well. The mechanisms defining upper and lower thermal limits do not necessarily have to be the same, and perhaps this is why evidence supporting the OCLTT for terrestrial animals has been equivocal.

Adaptations to increase oxygen delivery to tissues should not only track enhanced thermal tolerance in cooler climates, but there should also be consequences to tissue function and the whole-organism performance capacities that are linked to oxygen delivery (Heine and Hood 2020). Whole-organism performance traits such as sprint speed and endurance capacity are key determinants of Darwinian fitness because of their use in capturing prey, escaping predators, and acquiring mates (Irschick et al. 2008; Husak 2016). The OCLTT hypothesis is built around the impact of temperature on routine performance, but adaptations to increase cold tolerance may impact performance traits themselves (Pörtner et al. 2017). Additionally, changes to one type of performance trait may have cascading impacts on other traits that are physiologically linked. For example, endurance capacity is often linked to increased hematocrit and heart size and function (Garland 1984), as well as a higher proportion of slow-oxidative muscle fibers with a high concentration of mitochondria to support sustained locomotion (Jayne et al. 1990; Bonine et al. 2005; Scales et al. 2009; Albuquerque et al. 2023). Specialization for endurance performance is predicted to induce a physiological tradeoff (Vanhooydonck et al. 2001, 2014) resulting in reduced anaerobic sprint speed, which is determined by the presence of fast-twitch muscle fibers (Bonine et al. 2005; Scales et al. 2009; Albuquerque et al. 2015). Thus, adaptations that enhance endurance are likely to result in a decrease in anaerobic sprinting capacities. Unlike endurance capacity and its aerobic underpinnings, sprint speed and its anaerobic foundations are not expected to change directly as a result of adaptations to cooler temperatures (Telemeco et al. 2022). Because of the complex nature of how locomotor performance traits are linked physiologically, we sought to determine whether potential changes in oxygen carrying capacity in colder climates are associated with whole-organism performance traits that are important to fitness. If so, are there performance trade-offs due to those phenotypic changes, with endurance and sprint speed affected in opposite directions? Because locomotor

performance is key to fitness, any trickle-down effects on a particular performance trait, such as sprint speed, may be disadvantageous, which could limit the extent to which adaptations for cold tolerance are related to oxygen delivery instead of oxygen demand (Telemeco et al. 2022).

Green anole lizards are well-suited to test how whole-organism performance is affected by cooler climates within the OCLTT framework. They relatively recently colonized south Florida (USA) from Cuba between 6 and 12 million years ago and gradually expanded northward to Tennessee and westward to Texas (Campbell-Staton et al. 2012). They also show increased cold tolerance at higher latitudes (Wilson and Echternacht 1987). Previous work on a latitudinal gradient from southern Texas to southern Oklahoma revealed that green anoles had greater cold tolerance at higher latitudes and lower standard metabolic rates (SMR) in environments with more variable diurnal temperatures, but reported no latitudinal effect on SMR, heart size, or hemoglobin concentrations (Campbell-Staton et al. 2018). Although it is possible that cardiovascular changes are not important for green anole adaption to colder climates, this study did not cover the northernmost extent of the species' distribution. Thus, we sought to test aspects of the OCLTT hypothesis across a larger latitudinal gradient from southwest Florida to Tennessee. Knowing the south-to-north colonization history of the species allows us to examine the underlying physiological factors affecting cold tolerance and decreasing critical thermal minimum (CTmin; the lowest temperature at which motor function control ceases; Cowles and Bogert 1944) at higher latitudes as lizards moved from warmer to cooler climates. We therefore measured physiological and performance traits in green anoles across the latitudinal extent of their distribution.

We predicted that anoles at higher latitudes would have greater cold tolerance; lower oxygen demand (lower SMR); higher oxygen delivery (larger hearts, higher hemoglobin); and, as a result, higher endurance but lower sprint speed. In addition to sprinting and endurance, we also measured maximum exertion, a commonly measured performance trait that is often used as a proxy for endurance but that shows distinctly different responses to endurance training, and whose precise physiological underpinnings are unclear (Sorlin et al. 2022). We predicted that latitudinal exertion patterns will be more similar to those of sprint speed, with which exertion may share some physiological similarities (Garland 1984). We also determined what physiological traits best predicted each performance trait. This will allow a better understanding of how physiological adaptations associated with cold tolerance might impact performance.

## Materials and methods

### Ethical statement

The experimental procedures and animal handling were conducted after obtaining approval from the University of St. Thomas Institutional Animal Care and Use Committee (2022, protocol 112).

### Animals and procedures

We captured 209 adult male and female green anole lizards during May – July of 2022 from four different locations making up a latitudinal gradient in the continental United States: Punta Gorda, Florida (PG: 26°51'; $N$=30 males, 25 females); New Orleans, Louisiana (NOLA: 29°56'; $N$=26 males, 26 females); Charleston, South Carolina (SC: 32°47'; 29 males, 25 females); and Monroe County, Tennessee (TN: 35°34'; $N$=26 males, 22 females). The four chosen populations span the entire north-south continental distribution of green anoles across the eastern United States, and were recently compared to test the effect of latitudinal variation on sexually-selected trait expression in green anoles (Lailvaux et al. 2026). The populations sampled are not in a straight north-south transect, because doing so would have made it very difficult to capture enough genetic diversity of this broadly distributed species (Campbell-Staton et al. 2012). Instead, we targeted sites that would give good representation of major genetic clusters in this species (Bourgeois and Boissinot 2019), while focusing on populations previously studied (see Discussion below and Lailvaux et al. 2026). The sites differ in average monthly minimum (PG=12 °C, NOLA=7 °C, SC=3 °C, TN = -3 °C), but not maximum (PG=33 °C, NOLA=33 °C, SC=33 °C, TN=31 °C) temperature, with the minimum temperatures being notably different along the north-south gradient. Animals were taken to the laboratory at the University of St. Thomas where they were housed in climate-controlled cages (28–30° C) following standard protocols in the laboratory throughout the duration of the project (Husak et al. 2021). We then measured morphological and physiological traits according to standard methods, except for exertion, which was measured on site as described below. Measurements were taken in an order that would minimize impact on other measurements. Thus, after two weeks of acclimatization to the laboratory, on day 1 of experiments we measured standard metabolic rate (SMR); CTmin on day 2; sprint speed on day 7; and endurance on day 14. Bite force was measured between the measurement of CTmin and sprint speed for a different study, and while we do not present those results here, they are discussed in Lailvaux et al. (2026). Body mass was measured with a Pesola spring scale at capture, and with a

digital balance when SMRs were measured and again at the end of the study. Snout-vent length (SVL) was measured with digital calipers at capture and at the end of the study. We did not measure SMR, CTmin, or performance traits before and after laboratory acclimatization, because we did not want the measurement of these traits to impact measurements done later. Campbell-Staton et al. (2018) found that population differences in cold tolerance and metabolism remain after extended acclimatization.

Phenotypic measures followed standard published protocols. We measured SMR of fasted lizards (48 h without food) during their normal period of inactivity (2000–0100 h). Lizards were gently put into a flow-through metabolic chamber (60-mL syringe barrel) that was then placed into an incubator set to 28 °C (Orrell et al. 2004). They were left in the chamber for 45 min, and we used the lowest 5 min of $CO_2$ production as SMR (following Lailvaux et al. 2018). $CO_2$ production was measured with a Qubit S151 h $CO_2$ analyzer. Air was drawn from an undisturbed area of the laboratory, dried with Drierite, and scrubbed of $CO_2$ with soda lime. We measured CTmin by placing lizards on ice and periodically flipping them onto their back to see if they could right themselves. Body temperature was continuously monitored with a 36-gauge digital thermocouple (type T, Omega Engineering) inserted approximately 5 mm into the cloaca and taped in place. Body temperatures started at room temperature and cooled 1 °C per minute (Campbell-Staton et al. 2018). CTmin was the temperature at which righting response was lost (Lutterschmidt and Hutchinson 1997). We measured sprint speed on a 2 m-long, 5 cm-diameter dowel racetrack that was covered in cork and sitting at a 45° angle. We chased lizards up the track with a paintbrush tapping their tails, and as they ran they broke the infrared beams of vertically paired infrared photocells positioned 0.25 m apart along the length of the track. TrackMate software (Trackmate Racing, Surrey, British Columbia, Canada) determined times that each gate was broken. We ran lizards four times, with each run separated by 1 h, and used the fastest 0.25-m interval as maximum speed (Husak et al. 2021). We measured endurance by running the lizards until exhaustion (loss of righting response) on a motorized pet treadmill (PetRun model PR700 modified for lower speeds) set to 0.3 km/h (Perry et al. 2004; Cox et al. 2009; Husak et al. 2015). Exertion was measured in the field the day after capture by continuously chasing lizards around a 2-m circular track (Sorlin et al. 2022). The distance run on the track until exhaustion (loss of righting response) was recorded as exertion. Performance measures were taken at 28 °C.

After all measurements were completed, we euthanized lizards with rapid decapitation, collected trunk blood, and removed hearts. A portion of the blood was used to determine hemoglobin concentration using a Hemocue® Hb 801 Analyzer. After atria were dissected from the hearts, ventricles were dried overnight at 60 °C and weighed to the nearest 0.001 mg with an ultra-microbalance (Mettler Toledo UMX2).

### Statistics

We used Box-Cox (endurance, heart mass, SMR) or log transformations (exertion, sprint speed, CTmin) to transform dependent variables to meet assumptions of normality as required. We used a MANCOVA with mass at capture as a covariate to test for effects of population and sex and the interaction between population and sex on the measured traits. To test the general influence of the measured physiological traits (heart mass, SMR, hematocrit) on the four performance traits (sprinting, endurance, exertion) across all populations, we fit separate general linear mixed models for each performance trait with heart mass, hematocrit, and SMR as predictor variables; mass and sex as covariates; and population as a random factor. Because p-values associated with fixed factors in mixed-models are approximate and potentially unreliable (Bolker et al. 2009; Luke 2017), we used log-likelihood ratio reduction tests to find the minimum adequate model (i.e. the simplest model that explains the most amount of variation) for each performance trait (Zuur et al. 2009; Crawley 1993). We then re-fit each final model using REML. We used the DHARMa package to check the fit of mixed models. In all cases, we visualized marginal effects and calculated contrasts using the emmeans package (Lenth and Piaskowski 2025). We used R 4.4.2 for all analyses (R Core Team 2024).

## Results

### Population differences in phenotypic traits

The overall MANCOVA found significant effects of mass (Pillai's trace = 0.86, $F_{7,126}$ = 110.2, $P < 0.001$), sex (Pillai's trace = 0.251, $F_{7,126}$ = 6.0, $P < 0.001$), and population (Pillai's trace = 1.20, $F_{21,384}$ = 12.3, $P < 0.001$) on the measured traits, and there was a significant multivariate sex-by-population interaction (Pillai's trace = 0.283, $F_{21,384}$ = 1.9, $P = 0.01$). We report significant interaction and main effects for individual traits from this model, but contrasts are structured from the significant multivariate interaction. We further note that all of the results presented here are conditioned on the entire MANCOVA model, and thus relative to body size. There was a sex-by-population interaction for heart mass ($F_{3,132}$ = 3.58, $P = 0.02$) and hemoglobin ($F_{3,132}$ = 4.06, $P = 0.008$). Significant main effects of sex were evident for sprint speed ($F_{1,132}$ = 8.00, $P = 0.005$); heart mass

($F_{1,114} = 12.544$, $P < 0.001$); SMR ($F_{1,114} = 16.36$, $P < 0.001$); and hemoglobin ($F_{1,114} = 5.13$, $P = 0.03$), but not for exertion ($F_{1,114} = 3.07$, $P = 0.08$). Where those differences exist, they are almost always in the direction of higher relative values for males compared with females (Fig. 1). Figure 1 shows marginal means, which reflect remaining variation after other variables are accounted for in the model. Thus, interpreting the patterns visually should be tempered by the realization that other covariates are already accounted for in the calculations of marginal means.

The effect of population on measured traits was more complex (Fig. 1). Significant main effects of population were evident for CTmin ($F_{3,132} = 29.28$, $P < 0.001$); heart mass ($F_{3,132} = 8.86$, $P < 0.001$); and hemoglobin ($F_{3,132} = 9.78$, $P < 0.001$); endurance ($F_{3,132} = 48.87$, $P < 0.001$); sprint speed ($F_{3,132} = 4.08$, $P = 0.008$); exertion ($F_{3,132} = 26.14$, $P < 0.001$), but not for SMR ($F_{3,132} = 1.20$, $P = 0.31$). In general, differences were characterized by a trend towards higher values at lower latitudes for sprint speed (Fig. 1f) and CTmin (Fig. 1a), and an opposite trend with higher values at higher latitudes in exertion (Fig. 1g) and endurance (Fig. 1e).

## Performance mixed models

The minimum adequate mixed model that best described exertion over the four measured green anole populations retained effects of mass and heart size, such that there was a generally positive relationship between heart mass and exertion capacity (Table 1). The model that best described endurance capacity also retained effects of body mass and heart mass, as well as an effect of hematocrit. Here, the

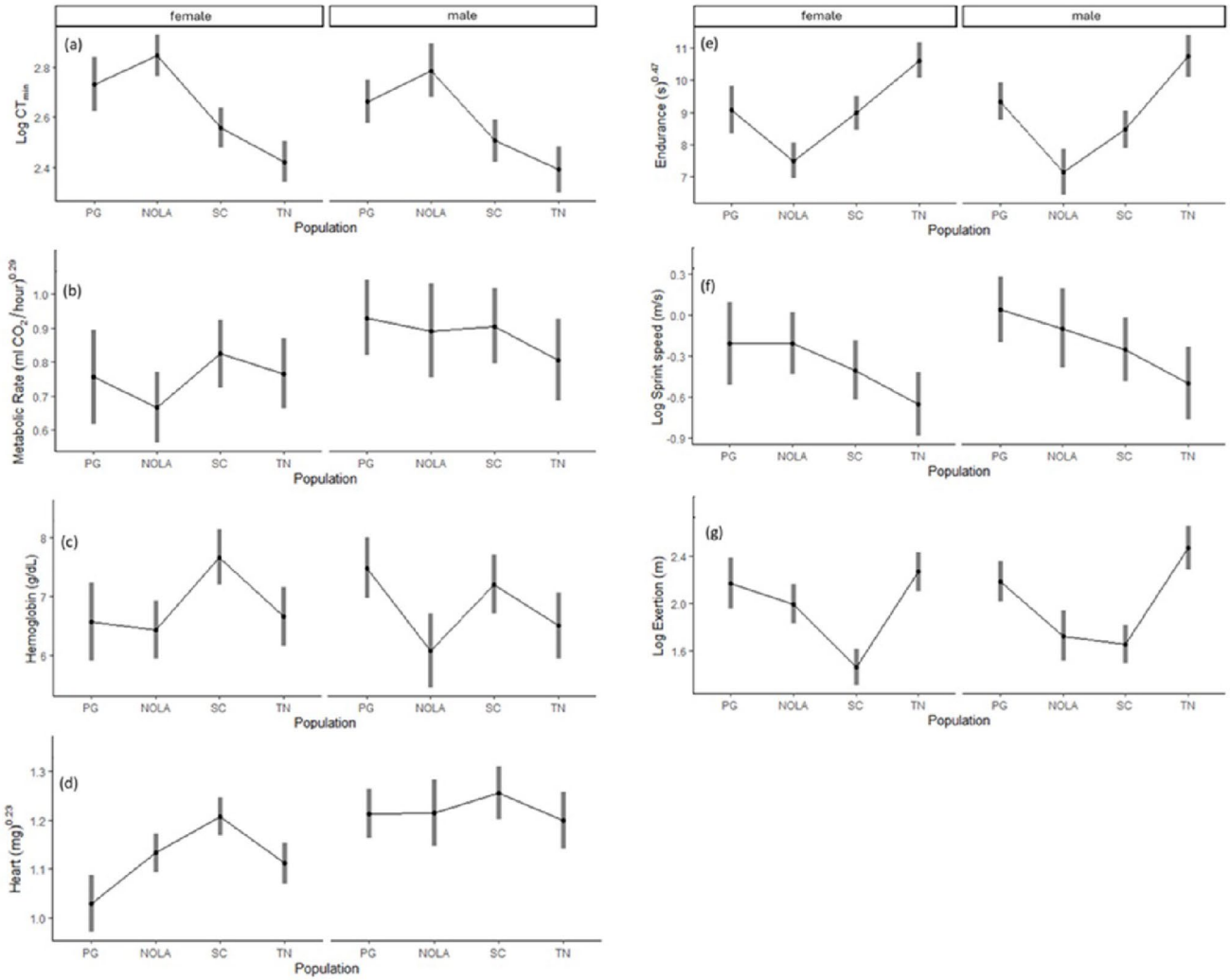


**Fig. 1** Latitudinal variation in transformed population- and sex-specific estimated marginal means ± 95% confidence intervals for **a** CTmin; **b** standard metabolic rate; **c** heart mass; **d** hemoglobin concentration; **e** endurance capacity; **f** sprint speed; and **g** exertion capacity for adult males and females from four *A. carolinensis* populations across the continental United States. Populations are arranged on the x-axis in order of increasing latitude from left to right: *PG* Punta Gorda, FL, *NOLA* New Orleans, LA, *SC* Charleston, South Carolina, *TN* Greenback, TN. Superscripts on axis labels for metabolic rate, heart mass, and endurance indicate Box-Cox transformations

**Table 1** Minimum adequate mixed-models describing the physiological predictors of (a) maximum exertion; (b) maximum endurance capacity; and (c) maximum sprint speed across all four of the measured green anole populations

| | Trait | d.f. | Coefficient | S.E. |
|---|---|---|---|---|
| (a) Log Maximum exertion (m) | Intercept | 138 | 1.24 | 0.22 |
| | Mass | 138 | 0.15 | 0.06 |
| | Heart mass | 138 | 0.14 | 0.11 |
| (b) Log Endurance (m) | Intercept | 136 | 4.26 | 0.02 |
| | Mass | 136 | 0.09 | 0.05 |
| | Heart mass | 136 | -0.14 | 0.09 |
| | Hemoglobin | 136 | 0.054 | 0.03 |
| (c) Log Sprint speed (m/s) | Intercept | 135 | -0.55 | 0.16 |
| | Heart mass | 135 | 0.07 | 0.07 |
| | SMR | 135 | 0.23 | 0.07 |

Reported values are the estimated change in the respective dependent variable between the baseline predictor level and the level shown in the table. The baseline category for sex is female (F)

marginal relationship between heart mass and endurance capacity was negative, whereas the marginal relationship between endurance and hemoglobin was positive (Table 1). The minimum adequate model for sprint speed retained effects of heart mass; and standard metabolic rate (Table 1). The marginal relationships between sprint speed and each of these main effects were all positive, such that the fastest animals were associated with relatively high SMR and relatively large heart masses.

## Discussion

Our results from four populations of green anoles that vary in latitude partially supported the OCLTT hypothesis. Cold tolerance was greater at higher latitudes (lower CTmin), which is consistent with previous findings for this species (Wilson and Echternacht 1987; Campbell-Staton et al. 2018). However, there were no population differences in SMR, and hemoglobin concentrations did not follow a latitudinal pattern. Heart ventricle mass showed a trend to be larger at higher latitudes, but this was only true in females, not males. Nevertheless, endurance capacity tended to be greater at higher latitudes, while sprint speed showed the opposite pattern, as predicted. We note that these patterns are not as clean as expected, with one population typically breaking the trend for each trait examined. For example, PG lizards from the lowest latitude had higher endurance than NOLA lizards and similar values as SC lizards. TN lizards had relatively small heart ventricles, thus breaking any latitudinal pattern. Taken as a whole, cold tolerance and performance traits mostly followed our predicted patterns, but measures of oxygen demand (SMR) and oxygen delivery (heart ventricle mass, hemoglobin concentration) did not. Clearly, there are many selective forces at work in these populations on the examined traits beyond just the thermal environment.

According to the OCLTT hypothesis, evolving greater cold tolerance (lower CTmin) can be accomplished by either a decrease in demand for oxygen (lower SMR), an increase in supply via oxygen delivery by the cardiovascular system, or both (Campbell-Staton et al. 2018). Our results showed no change in oxygen demand, with no detectable differences in SMR at 28 °C. We also did not see a consistent increase in potential oxygen delivery with increasing latitude. However, we did not measure heart rate, which may also alter oxygen delivery. With no apparent change in either oxygen demand or supply, a third option for increasing cold tolerance is more efficient use of oxygen by tissues. This possibility would explain the pattern we saw for endurance, with performance increasing at higher latitudes, though this difference could be due to shifts in whole thermal performance curves (Telemeco et al. 2022). Endurance is determined by aerobic capacity and effective oxygen consumption (Garland 1984; Coyle 1999; Albuquerque et al. 2023). It is possible that there is no direct selection for better endurance in the populations at higher latitudes, but instead higher values are a side effect of selection for more efficient use of oxygen by tissues to deal with thermal challenges. Under this scenario, the inverse would be true for sprint speed – better aerobic capacity would result in reduced anaerobic sprint performance as a physiological tradeoff (Vanhooydonck et al. 2001, 2014), which is what we found. This hypothesis leads to clear testable predictions for our populations. If colder, high-latitude populations have greater cold tolerance that is not due to oxygen demand or supply, but instead efficient oxygen use, we should see specific mitochondrial adaptations for increased efficiency (Sokolova 2023), such as increased mitochondrial density, reduced proton leak, modifications to the electron transport chain protein complexes for greater oxidative phosphorylation, or some combination of these (Pörtner 2021; Filice et al. 2026). Such mitochondrial adaptations could also be due to selection for decreased oxidative damage that occurs with cold exposure and rewarming (Haussmann et al. 2025). Exertion had no clear relationship with latitude, which is not surprising given its unclear physiological underpinnings. In a previous study where green anoles underwent specialized training for endurance or sprinting, exertion was unaffected by either training regime (Sorlin et al. 2022), suggesting that it is not determined solely by aerobic or anaerobic capacity. Our results show different physiological predictors of exertion compared to sprint speed and endurance, and this is consistent with previous work on other species (Garland 1984).

One of the most striking patterns we found was for heart ventricle mass. Females generally followed predictions of the OCLTT hypothesis that heart ventricles should be larger

at higher latitudes, whereas males did not. One plausible explanation for this is that males in all populations have evolved large heart ventricles to maximize oxygen delivery for their particular energetic demands that differ from those of females. That is, female hearts vary among populations because of thermal constraints on oxygen supply and demand, but male hearts face over-riding selection pressures, especially at lower latitudes where smaller hearts are predicted. PG lizards show greater sexual size dimorphism and higher relative bite-force performance compared to higher latitudes (Lailvaux et al. 2026), suggesting strong selection for traits that enhance mating success. During the breeding season, male green anoles have high energy expenditure due to patrolling of territories and displaying at females and rival males (Orrell et al. 2004). That results in locomotor activity being a greater proportion of the time budget and total energy expenditure of free-living males compared to females (Orrell et al. 2004). Females can perhaps compensate for their higher reproductive energy requirements with increased food intake, but males do not appear to compensate for their heightened activity, as evidenced by significant loss of body mass over the breeding season (Ruby 1984). Such high energy expenditure on activity and resultant mass loss was associated with 75% of marked males disappearing in a SC population during a 12-week period of the breeding season (Ruby 1984). Indeed, in another SC population, males had higher SMRs even during winter months outside of the breeding season (Jenssen et al. 1996), suggesting a higher oxygen demand that can be supplied with larger hearts, but which may also be because of those larger, energetically expensive hearts. These observations are consistent with our finding that males had higher mass-specific SMRs than females across all populations. Although measuring heart ventricle mass can say something about stroke volume, it does not include the other important component of cardiac output that can vary – heart rate. Future studies should consider latitudinal variation in heart rate, because that is another way to increase oxygen delivery without altering stroke volume (Du et al. 2010).

Our results on physiological predictors of performance traits across populations emphasize the likely complex selection on heart ventricle size. While the three performance traits we studied had different predictors in their final models, heart ventricle mass was retained in the model for each performance trait. Surprisingly, ventricle mass was negatively related to endurance when other variables were accounted for. However, we note that hemoglobin concentration was the other significant predictor aside from body mass. This suggests that having more hemoglobin but with a smaller heart to deliver it is better for endurance. Sprint speed was positively predicted by SMR, which is not surprising. Sprint speed is determined by force production of leg muscles (Farley 1997; Curtin et al. 2005; Anderson and Roberts 2020), so our results are likely due to faster lizards having greater skeletal muscle mass (Husak et al. 2015) that contributes to a higher SMR. The only variables retained in the exertion model were body mass and heart ventricle mass, both positively affecting exertion. Exertion is a complex trait that is not due solely to aerobic or anaerobic capacity, but probably both (Garland 1984). Typically, lizards run fast at the beginning of a trial, at speeds where breathing is not possible (Carrier 1987; see also Owerkowicz et al. 1999), and then slow down to speeds that allow ventilation before exhaustion. Exertion capacity is also strongly and positively related to lactate levels in the blood after exhaustion, whereas endurance and sprint speed are not (J. Husak, unpublished data), suggesting different underlying mechanisms for the three traits. More work is necessary to determine how the three traits are physiologically connected.

As others have suggested, the OCLTT hypothesis may not be as applicable to terrestrial species as it is for aquatic organisms (Schulte 2015; Rubalcaba et al. 2020; Pörtner 2021). The ability of terrestrial ectotherms to more effectively behaviorally thermoregulate than aquatic ectotherms (Carter et al. 2023) may partly explain this, making other selective pressures on physiology, such as microhabitat availability or predation pressure, more pronounced. In a study of green anoles in a more limited latitudinal gradient at the western edge of the species' distribution, there was mixed support for the OCLTT hypothesis (Campbell-Staton et al. 2018), and our study across a larger gradient similarly offered mixed support. In populations of the garden skink *Lampropholis guichenoti* across an elevational gradient, lizards at higher elevations, and colder temperatures, had lower CTmin, higher SMR, and broader sprint speed thermal performance curves compared to lower elevations (Anderson et al. 2022). These results are not completely consistent with OCLTT predictions for cold climate adaptations, yet they do not match our results in green anole populations either. Clearly more studies are needed on populations of terrestrial ectotherms to determine what physiological adaptations allow success when invading cooler climates, but especially how rising temperatures due to global climate change will impact those adaptations (Mi et al. 2022; Sun et al. 2022, 2025). Will populations at high latitudes be able to evolve physiological traits that better match their warmer ancestral conditions as their climates warm? Genomic studies of species that have expanded northward from warm climates into large distributions that span cooler climates, such as the case in green anoles, will help to understand this question better.

**Acknowledgements** We thank M. Chopra, M. Melchior, and R. Yusuf for help with laboratory data collection.

**Author contributions** J.H., M.D., and S.L. conceived of the study and

designed the experiments. J.H., M.D., J.M., M.S., and S.L. collected data and conducted the experiments, J.H. and S.L. completed the statistical analyses, interpreted the data, prepared the figures, and drafted the manuscript. All authors edited the manuscript.

**Data availability** Data are available from the authors upon request.

## Declarations

**Conflict of interest** The authors declare no competing interests.

## References


Adams DC, Church JO (2007) Amphibians do not follow Bergmann's rule. Evolution 62:413–420

Albuquerque RA, Bonine KE, Garland T, Jr (2015) Speed and endurance do not trade off in phrynosomatid lizards. Physiol Biochem Zool 88:634–647. https://doi.org/10.1086/683678

Albuquerque RL, Zani PA, Garland T Jr (2023) Lower-level predictors and behavioral correlates of maximal aerobic capacity and sprint speed among individual lizards. J Exp Biol 226:jeb244676

Anderson CV, Roberts TJ (2020) The need for speed: functional specializations of locomotor and feeding muscles in Anolis lizards. J Exp Biol 223:jeb213397

Anderson RO, Alton LA, White CR, Chapple DG (2022) Ecophysiology of a small ectotherm tracks environmental variation along an elevational cline. J Biogeogr 49:405–415

Angilletta MJ (2009) Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, New York

Ashton KG, Feldman CR (2003) Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. Evolution 57:1151–1163

Ashton KG, Tracy MC, de Queiroz A (2000) Is Bergmann's rule valid for mammals? Am Nat 156:390–415

Bennett AF (1980) The thermal dependence of lizard behaviour. Anim Behav 28:752–762

Bennett JM, Sunday J, Calosi P, Villalobos F, Martínez B, Molina-Venegas R, Araújo MS, Algar AC, Clusella-Trullas S, Hawkins BA, Keith SA, Kühn I, Rahbek C, Rodríguez L, Singer A, Morales-Castillo I, Olalla-Tárraga MA (2021) The evolution of critical thermal limits of life on earth. Nat Commun 12:1198

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135

Bonine KE, Gleeson TT, Garland Jr T (2005) Muscle fiber-type variation in lizards (Squamata) and phylogenetic reconstruction of hypothesized ancestral states. J Exp Biol 208:4529–4547

Bourgeois Y, Boissinot S (2019) Selection at behavioural, developmental and metabolic genes is associated with the northward expansion of a successful tropical colonizer. Mol Ecol 28:3523–3543

Bozinovic F, Calosi P, Spicer JI (2011) Physiological correlates of geographic range in animals. Annu Rev Ecol Evol Syst 42:155–179

Campbell-Staton SC, Goodman RM, Backström N, Edwards SV, Losos JB, Kolbe JJ (2012) Out of Florida: mt DNA reveals patterns of migration and Pleistocene range expansion of the Green Anole lizard (*Anolis carolinensis*). Ecol Evol 2:2274–2284

Campbell-Staton SC, Bare A, Losos JB, Edwards SV, Cheviron ZA (2018) Physiological and regulatory underpinnings of geographic variation in reptilian cold tolerance across a latitudinal cline. Mol Ecol 27:2243–2255

Carrier DR (1987) Lung ventilation during walking and running in four species of lizards. Exp Biol 47:33–42

Carter MJ, Cortes PA, Rezende EL (2023) Temperature variability and metabolic adaptation in terrestrial and aquatic ectotherms. J Therm Biol 115:103565

Cowles R, Bogert C (1944) A preliminary study of the thermal requirements of desert reptiles. Bull Am Mus Nat Hist 83:265–296

Cox RM, Stenquist DS, Henningsen JP, Calsbeek R (2009) Manipulating testosterone to assess links between behavior, morphology, and performance in the brown anole *Anolis sagrei*. Physiol Biochem Zool 82:686–698

Coyle EF (1999) Physiological determinants of endurance exercise performance. J Sci Med Sport 2:181–189

Crawley MJ (1993) GLIM for ecologists. Blackwell Scientific, Oxford

Curtin NA, Woledge RC, Aerts P (2005) Muscle directly meets the vast power demands in agile lizards. Proc R Soc B 272:581–584

Du WG, Warner DA, Langkilde T, Robbins T, Shine R (2010) The physiological basis of geographic variation in rates of embryonic development within a widespread lizard species. Am Nat 176:522–528

Farley CT (1997) Maximum speed and mechanical power output in lizards. J Exp Biol 200:2189–2195

Filice M, Gattuso A, Imbrogno S, Amelio D, Caferro A, Mazza R, Galli G, Shiels HA, Garofalo F, Cerra MC (2026) Mitochondria: at the heart of fish thermal plasticity. J Exp Biol 229:jeb251321

Garland T Jr (1984) Physiological correlates of locomotory performance in a lizard: an allometric approach. Am J Physiol 247:R806–815

Haussmann BD, Joseph NA, Hegdahl TR, Lichtner KE, Woldebirhan RN, Travis BG, Peterson GP, Robbins TR, Haussmann MF (2025) Intraspecific support for the climate variability hypothesis: oxidative damage in lizards after acute temperature exposure. J Exp Biol 228:jeb251040

Heine KB, Hood WR (2020) Mitochondrial behaviour, morphology, and animal performance. Biol Rev 95:730–737

Huey RB, Kingsolver JG (1989) Evolution of thermal sensitivity of ectotherm performance. Trends Ecol Evol 4:131–135

Husak JF (2016) Measuring selection on physiology in the wild and manipulating phenotypes (in terrestrial non-human vertebrates). Compr Physiol 6:63–85

Husak JF, Keith AR, Wittry BN (2015) Making olympic lizards: the effects of specialised exercise training on performance. J Exp Biol 218:899–906

Husak JF, Rohlf CM, Lailvaux SP (2021) Immune activation affects whole-organism performance in male but not female green anole lizards (*Anolis carolinensis*). J Comp Physiol B 191:895–905

Irschick DJ, Meyers JJ, Husak JF, Le Galliard JF (2008) How does selection operate on whole-organism functional performance capacities? A review and synthesis. Evol Ecol Res 10:177–196

Jayne BC, Bennet AF, Lauder GV (1990) Muscle recruitment during terrestrial locomotion: how speed and temperature affect fiber type use in a lizard. J Exp Biol 152:101–128

Jenssen TA, Congdon JD, Fischer RU, Estes R, Kling D, Edmands S, Berna H (1996) Behavioural, thermal, and metabolic characteristics of a wintering lizard (*Anolis carolinensis*) from South Carolina. Funct Ecol 10:201–209

Klok CJ, Sinclair BJ, Chown SL (2004) Upper thermal tolerance and oxygen limitation in terrestrial arthropods. J Exp Biol 207:2361–2370. https://doi.org/10.1242/jeb.01023

Lailvaux SP, Wang AZ, Husak JF (2018) Energetic costs of performance in trained and untrained *Anolis carolinensis* lizards. J Exp Biol 221:jeb176867

Lailvaux SP, Sorlin M, Marks J, Duerwachter MA, Husak JF (2026) Sexually selected traits vary over a latitudinal gradient in green anole lizards (*Anolis carolinensis*). Biol J Linn Soc 147:blaf141

Lenth R, Piaskowski J (2025) emmeans: estimated marginal means, aka least-squares means. R package version 2.0.1. https://rvlenth.github.io/emmeans/

Luke SG (2017) Evaluating significance in linear mixed-effects models in R. Behav Res Methods 49:1494–1502

Lutterschmidt WI, Hutchinson VI (1997) The critical thermal maximum: history and critique. Can J Zool 75:1561–1574

Meiri S, Dayan T (2003) On the validity of Bergmann's rule. J Biogeogr 30:331–351

Mi C, Ma L, Wang Y, Wu D, Du W, Sun B (2022) Temperate and tropical lizards are vulnerable to climate warming due to increased water loss and heat stress. Proc Biol Sci 289:20221074

Nudds RL, Oswald SA (2007) An interspecific test of Allen's rule: evolutionary implications for endothermic species. Evolution 61:2839–2848

Orrell KS, Congdon JD, Jenssen TA, Michener RH, Kunz TH (2004) Intersexual differences in energy expenditure of *Anolis carolinensis* lizards during breeding and postbreeding seasons. Physiol Biochem Zool 77:50–64

Owerkowicz T, Farmer CG, Hicks JW, Brainerd EL (1999) Contribution of gular pumping to lung ventilation in monitor lizards. Science 284:1662–1663

Perry G, Levering K, Girard I, Garland TJ Jr (2004) Locomotor performance and social dominance in male *Anolis cristatellus*. Anim Behav 67:37–47

Pincheira-Donoso D, Hodgson DJ, Tregenza T (2008) The evolution of body size under environmental gradients in ectotherms: why should Bergmann's rule apply to lizards? BMC Evol Biol 8:13

Pörtner H (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwiss 88:137–146

Pörtner HO (2021) Climate impacts on organisms, ecosystems and human societies: integrating OCLTT into a wider context. J Exp Biol 224:jeb238360

Pörtner HO, Bock C, Mark FC (2017) Oxygen-and capacity-limited thermal tolerance: bridging ecology and physiology. J Exp Biol 220:2685–2696

R Core Team (2024) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rubalcaba JG, Verbeck WCE, Hendricks AJ, Saris B, Woods AH (2020) Oxygen limitation may affect the temperature and size dependence of metabolism in aquatic ectotherms. Proc Natl Acad Sci USA 117:31963–31968

Ruby DE (1984) Male breeding success and differential access to females in *Anolis carolinensis*. Herpetologica 40:272–280

Scales JA, King AA, Butler MA (2009) Running for your life or running for your dinner: what drives fiber-type evolution in lizard locomotor muscles? Am Nat 173:543–553

Schulte PM (2015) The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. J Exp Biol 218:1856–1866

Snyder GK, Weathers WW (1975) Temperature adaptations in amphibians. Am Nat 109:93–101

Sokolova IM (2023) Ectotherm mitochondrial economy and responses to global warming. Acta Physiol 4:e13950

Sorlin MV, Marks JR, Lailvaux SP (2022) Endurance training does not affect maximum exertion/distance capacity in *Anolis carolinensis* lizards. J Exp Biol 225:jeb244576

Sun B, Williams CM, Li T, Speakman JR, Jin Z, Lu H, Luo L, Du W (2022) Higher metabolic plasticity in temperate compared to tropical lizards suggests increased resilience to climate change. Ecol Monogr 92:e1512

Sun B-J, Lu H-L, Cheng K-M, Liu W-L, Han X-Z, Cui L-X, Li X-H, Li S-R, Hao X, Li F, Wu D-Y, Li T, Zhang Y-P, Wang J-C, Liu P, Du W-G (2025) The semi-natural climate chambers across latitudes: a broadly applicable husbandry and experimental system for terrestrial ectotherms under climate change. Adv Sci 12:2414185

Symonds MRE, Tattersall GJ (2010) Geographic variation in bill size across bird species provides evidence for Allen's rule. Am Nat 176:188–197

Telemeco RS, Gangloff EJ, Cordero GA, Rodgers EM, Aubret F (2022) From performance curves to performance surfaces: interactive effects of temperature and oxygen availability on aerobic and anaerobic performance in the common wall lizard. Funct Ecol 36:2544–2557

Vanhooydonck B, Van Damme R, Aerts P (2001) Speed and stamina trade-off in lacertid lizards. Evolution 55:1040–1048

Vanhooydonck B, James RS, Tallis J, Aerts P, Tadic Z, Tolley KA, Measey GJ, Herrel A (2014) Is the whole more than the sum of its parts? Evolutionary trade-offs between burst and sustained locomotion in lacertid lizards. Proc R Soc B 281:20132677

Wilson MA, Echternacht AC (1987) Geographic variation in the critical thermal minimum of the green anole, *Anolis carolinensis* (Sauria: Iguanidae), along a latitudinal gradient. Comp Biochem Physiol Mol Integr Physiol 87:757–760

Zuur AD, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer Science and Business Media, New York

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.