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SYMPOSIUM ARTICLE

Developmental Temperature Does Not Influence Thermal Preferences but Affects Age at Sexual Maturity in the Tropical Lizard, *Anolis cybotes*

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Synopsis As rising environmental temperatures can negatively affect some ectothermic animals, it is imperative to anticipate whether and how these organisms could cope with new thermal conditions. Several groups of animals that face constraints to behavioral thermoregulation must rely on phenotypic plasticity to adjust to rising temperatures. Evaluating whether fitness-related traits could plastically change in warmer conditions is critical for predicting how these thermoconformer ectotherms could navigate a changing world. Here, we experimentally manipulated short-term and developmental thermal environments in a thermoconformer population of the Hispaniolan stout anole (*Anolis cybotes*) to address two complementary goals. We first examined how short-term and/or full development exposure to warmer conditions shape lizards' preferred body temperatures to test for potential plasticity in this fitness-related trait. We then evaluated the potential effects of elevated developmental temperature on growth rate and the age at sexual maturity in females to evaluate how temperature shapes life history traits. We found that neither short-term exposure to elevated temperatures nor developmental temperatures affected the preferred body temperatures of *A. cybotes*, suggesting that some fitness-related physiological processes are organized within a non-plastic thermal range. By contrast, warmer developmental temperature delayed growth rate and age at sexual maturity in females, which could affect population processes by extending generation times and limiting annual fecundity. Together, this study demonstrates a fitness-related cost associated with warmer temperatures in *A. cybotes* and suggests that the capacity for tropical ectotherms to plastically buffer rising temperatures may be relatively limited.

Dado que el incremento de la temperatura ambiental puede afectar negativamente a algunos animales ectotermos, es necesario anticipar cómo estos organismos podrían afrontar las nuevas condiciones térmicas. Diferentes grupos de animales que enfrentan restricciones ecológicas, físicas, o fisiológicas para termorregular dependerían de la plasticidad fenotípica para ajustarse al incremento de la temperatura. Por ello, evaluar si características fenotípicas asociadas con adecuación podrían cambiar plásticamente en condiciones más cálidas es crítico para predecir cómo ectotermos termoconformistas podrían sobrevivir en un mundo que se calienta. En este trabajo manipulamos experimentalmente las condiciones térmicas a corto plazo y durante el desarrollo en una población termoconformista de la lagartija *Anolis cybotes* de La Española para abordar dos objetivos complementarios. Primero, evaluamos si una exposición breve y/o el desarrollo a temperaturas altas moldean las preferencias térmicas de las lagartijas para evaluar plasticidad en ese rasgo asociado con adecuación. Posteriormente, evaluamos los efectos potenciales de temperaturas elevadas en el crecimiento y la edad a la madurez sexual de hembras para evaluar cómo la temperatura moldea características de historias de vida. Encontramos que temperaturas altas a la adultez o durante el desarrollo no cambian las preferencias térmicas de *A. cybotes*, indicando que algunos procesos fisiológicos están

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organizados dentro de un intervalo térmico con plasticidad limitada. En contraste, temperaturas altas durante el desarrollo retrasaron el crecimiento y la edad a la madurez sexual en hembras, lo cual podría afectar negativamente procesos poblacionales al extender el tiempo generacional y limitar la fecundidad anual. En conjunto, este estudio demuestra un costo asociado de temperaturas altas en la adecuación de *A. cybotes*, y sugiere que la capacidad de ectotermos tropicales para amortiguar plásticamente el incremento de la temperatura ambiental podría estar relativamente limitada.

Introduction

Temperature influences organismal performance and fitness, particularly in ectothermic animals (*e.g.*, amphibians, insects, and reptiles) that rely on the environment to regulate body temperature (Angilletta et al. 2002; Angilletta 2009; Lahondère 2023). As the environment warms, the body temperatures of ectotherms may increase and potentially exceed their optimal temperature range, imposing costs on fitness-related activities, such as burst speed, digestive efficiency, and growth rates (Huey and Kingsolver 1989; Tewksbury et al. 2008; Huey et al. 2009). Consequently, physiological performance and Darwinian fitness in ectotherms may be negatively affected by rising temperatures (Dillon et al. 2010; Sinervo et al. 2010; Jørgensen et al. 2022). For example, higher body temperatures could induce morphological abnormalities in the embryos of some lizard species (Beuchat 1988; Sanger et al. 2021) or cause direct mortality from overheating in some amphibian populations (Cicchino et al. 2023; Pottier et al. 2025). Two intragenerational compensatory responses that ectotherms can use to cope with rising temperatures are thermoregulatory behavior (*e.g.*, shuttling between thermal microhabitats, retreating to thermal refugia) and phenotypic plasticity (*e.g.*, acclimatization, heat hardening) (Kearney et al. 2009; Chevin et al. 2010; Huey et al. 2012; Deery et al. 2021). Nevertheless, behavioral responses to elevated temperatures are not universally accessible to all ectotherms: thermoregulatory behavior is constrained when ectotherms are sessile, inhabit spatially homogeneous thermal environments, or when the cost of thermoregulation exceeds the benefits (Huey 1974; Huey et al. 2012; Lowe et al. 2021; Lahondère 2023). Thus, thermoconforming ectotherms are expected to rely primarily on phenotypic plasticity to respond within an individual's lifespan to warming (Huey et al. 2012; Seebacher et al. 2015; Neel et al. 2021).

Studies of acclimation (in the laboratory) and acclimatization (in the field) have demonstrated that ectotherms can adjust their physiological functions and maintain their biological activities at higher body temperatures, supporting that phenotypic plasticity is a mechanism of response to global warming (Kaufmann and Bennett 1989; Palumbi et al. 2014; van den Berg et al. 2015; Domínguez-Guerrero et al. 2021) but see Gunderson and Stillman (2015). For example, the Antarctic fish *Pagothenia borchgrevinki* can adjust its cardiac function when experimentally maintained at temperatures warmer than those in its natural habitat (Franklin et al. 2007). However, assessments of physiological plasticity have primarily focused on adult ectotherms exposed to elevated temperatures over short time periods, with reproductive traits rarely considered. Understanding how developmental temperatures shape fitness-related physiological traits and affect life history traits is equally important (Angilletta et al. 2004; Palumbi et al. 2014; Gunderson et al. 2020; Muell et al. 2025). For instance, growth and sexual maturity in ectotherms are expected to be accelerated within an optimal temperature range but delayed when body temperatures exceed this range (Huey and Kingsolver 1989; Angilletta, Niewiarowski, et al. 2002; Hall et al. 2024). Furthermore, warmer developmental temperatures can result in smaller body sizes at adulthood (*i.e.*, temperature-size rule), which can have downstream consequences for survivorship and reproduction in the wild (Atkinson 1996; Angilletta 2009). By improving our understanding of when phenotypic plasticity arises and how elevated temperatures affect growth and reproductive traits, we can better anticipate patterns of response and vulnerability in ectotherms under global warming.

Here, we experimentally manipulated thermal environments using the Hispaniolan stout anole (*Anolis cybotes*) as a model system to investigate patterns of phenotypic plasticity associated with short-term acclimation and developmental temperatures. Anole lizards are a well-established model system for studying organismal responses to environmental change. Recent work has shown that temperature influences multiple aspects of anole biology, including thermal physiology and reproduction (Campbell-Staton et al. 2020; Muñoz et al. 2023), making the group especially useful for understanding biological responses to climate warming. *Anolis cybotes* is a medium-size lizard (snout-vent length [SVL] around 65 mm in females and 80 mm in males) widely distributed across the Caribbean island of Hispaniola, where the species spans diverse elevational and environmental gradients (Henderson and Powell 2009). The thermoregulatory behavior of this species varies across the island, with some populations exhibiting active thermoregulation and others exhibiting thermoconformity (Hertz and Huey 1981; Muñoz and Losos

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2018; Bodensteiner et al. 2024). We focused this study on a low-elevation, thermoconforming population of *Anolis cybotes* from Los Patos, Dominican Republic (Muñoz and Losos 2018). We transported wild-caught lizards to an environmental chamber at a constant temperature of 28°C, where females laid eggs. In the same environmental chamber, we incubated some eggs and reared the emerged hatchlings until adulthood. We began by measuring preferred body temperatures (T_{pref}) of adult lizards reared at 28°C and then transferred them to 31°C for a month to evaluate the potential effects of relatively short-term exposure to warmer temperatures on their thermal preferences (*i.e.*, we tested for acclimation). We focused on T_{pref}, as it is a reasonable proxy in diurnal lizards (like these anoles) for the optimal temperature range and, therefore, for their maximal physiological performance (Huey and Kingsolver 1989; Angilletta 2001; Gilbert and Miles 2017). Subsequently, we incubated another group of eggs and reared the emerged hatchlings until adulthood at 31°C. We measured the T_{pref} of adults reared at 31°C to compare with their siblings reared at 28°C and evaluate whether developmental temperature shapes thermal preferences of *A. cybotes*. Warmer thermal preferences in lizards exposed to elevated temperatures during development or adulthood suggests plastic adjustments can occur in response to rising temperatures (Huey et al. 2012). By contrast, no shifts in thermal preferences or lower thermal preferences in lizards exposed to elevated temperatures would indicate that *A. cybotes* exhibit a more limited capacity to plastically adjust to increasing temperatures (Gilbert and Miles 2016; Huey and Kingsolver 2019). Lastly, we compared growth rate and the age at which females in each thermal treatment (28 and 31°C) reached the minimum size for sexual maturity to evaluate whether these life-history traits vary under different developmental temperatures. Similar growth and ages at sexual maturity between thermal treatments would suggest that warmer experimental temperatures do not affect physiological processes (such as digestion) in females. It is also likely that 28 and 31°C fall within the optimal developmental temperature range of *A. cybotes*, and thus we would not expect differences in body size between groups, as observed in other anoles (Hall et al. 2024). Differences in growth and in age at sexual maturity would indicate that physiological performance is higher at the temperature at which females reach sexual maturity earlier. Here, we find that the mean thermal preferences of *A. cybotes* do not change with short-term exposure to elevated temperatures or developmental thermal environment. However, developmental temperatures influence growth rate and age at sexual maturity in females of *A. cybotes*, with potential implications for fecundity as temperatures rise.

Methods

Ethic statement

We captured adult lizards of *Anolis cybotes* in the Dominican Republic and exported them to the United States of America in accordance with the permits DJ-C-1–2023-00,038 and VAPB-11,787 provided by the Ministerio de Medio Ambiente y Recursos Naturales de la República Dominicana. Animal care and thermal preference experiments (described below) were conducted in accordance with the Institutional Animal Care and Use Committee policies (Yale IACUC 2022–20,297).

Field work

We conducted field work from 7:00 to 18:00 (activity time of *A. cybotes*) on September 09, 2024, in a tropical shaded forest located in Los Patos, Barahona Province, Dominican Republic (17.956°N, -71.189°W; 45 m elevation). Based on previous work at the same locality, we focused on capturing females larger than 50 mm SVL and males larger than 55 mm SVL, as they should be reproductively active (Muñoz and Losos 2018). We captured 14 females and 15 males by a lasso attached to an extendable panfish pole. We placed each lizard individually within a plastic container (15 cm L × 10 cm W × 8 cm H) lined with damp paper towel to prevent dehydration, and the plastic containers were placed within a thermally insulated cooler for transport back to the laboratory.

Experimental thermal treatments

We used two walk-in environmental chambers (each 8′ × 8′) located at Yale University to regulate developmental temperature in *Anolis cybotes*. Both environmental chambers were programmed to maintain a constant humidity of 70% (±5%) and a 12:12 D light cycle but were set at different temperatures. One of the environmental chambers was programmed to maintain a constant temperature of 28°C (±1°C) and the other to maintain a constant temperature of 31°C (±1°C). Temperatures of 28 and 31°C represent the lower and upper range of thermal preferences measured in *A. cybotes* from Los Patos (Muñoz and Losos 2018). Based on our previous experience, we maintained the wild-caught lizards at 28°C to minimize thermal stress and increase the likelihood of reproduction (Muñoz and Losos 2018). We housed female-male pairs in a plastic container (38 cm L × 23 cm W × 28 cm H) with a felt cage carpet, a mesh hammock and wood dowel for perching, and a small pot with moist soil for laying eggs. Lizards were misted twice daily with spring water and fed twice a week with 0.5-inch banded crickets (*Gryllodes sigillatus*) dusted with multivitamins and calcium. We checked the

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small pots daily for eggs, which were individually placed in a Petri dish (10 cm in diameter) containing 20 g of vermiculite mixed with 20 mL of spring water. From the fourteen adult females captured in the field, we collected eggs from twelve lizards. We incubated 79 eggs (∼6 per female) at 28°C between September 2024 and May 2025, and 33 eggs (∼3 per female) at 31°C between March and May 2025. Differences in dates of incubation (and T_{pref} experiments between siblings) were necessary given the logistics of environmental chamber availability (the warmer environmental chamber was available for experiments 6 months after the other environmental chamber). In the same environmental chamber where eggs were incubated, the emerged hatchlings were reared to adulthood. Upon emergence, each hatchling was measured and housed individually in a plastic container (31 cm L × 20 cm W × 20 cm H) with a felt cage carpet, and a mesh hammock and wood dowel for perching. Hatchlings were misted twice daily with spring water and fed daily with 10–15 pinhead banded crickets dusted with multivitamins and calcium. Once juveniles reached a size of ∼30 mm SVL, they were fed with ∼10 banded crickets (0.25 inch) every other day. In total, we reared 53 juveniles at 28°C and 10 at 31°C. We randomly selected 10 juveniles reared at 28°C (offspring from 10 wild-caught females) and included all individuals reared at 31°C in our experiments described below.

Comparison of preferred body temperatures

We were interested in evaluating whether short-term exposure and/or full-development at higher temperatures shape thermal preferences of *A. cybotes*. On October 26, 2025, we measured the preferred body temperatures of 10 lizards reared at 28°C (mean SVL = 45 mm and a mean age of 8 months; offspring from 10 wild-caught females). We then transferred these lizards from 28 to 31°C for a month (from November 03 to December 07, 2025) and re-measured their T_{pref} to test for acclimation to warmer temperatures. On February 18, 2026, we measured T_{pref} of 8 lizards reared at 31°C (mean SVL = 43 mm and a mean age of 9 months; offspring from 5 wild-caught females) to compare with their siblings reared at 28°C. We measured preferred body temperatures using a laboratory thermal gradient with the following specifications. The gradient consisted of a polypropylene box with six tracks and dimensions of 91 cm (length) × 91 cm (width) × 15 cm (height). We installed wire heating coils (50 W; Zoo Med) that increased in density toward one end of the gradient. The substrate consisted of 2 cm of coarse sand, which was misted with spring water to a packed consistency, covering the heating coils. The thermal gradient was installed in a room maintained at an ambient temperature of 20°C to create a temperature gradient from 20°C on the cool end to 40°C at the warm end. We placed one lizard within each track and allowed lizards to explore the gradient for 20 min before starting temperature recordings. To record body temperatures, we used a type T thermocouple (5SC-TT-T-40–72, Omega Engineering) inserted ∼5 mm into the cloaca of each lizard and fastened with medical tape. The thermocouple, in turn, was connected to an automatic digital thermometer (HH378, Omega Engineering) that recorded temperature every 5 min for a set period of 3 h within the typical active hours of the *A. cybotes* (8:00 to 18:00). Each experiment was performed with 5 lizards in the gradient at any given time (each lizard in its own track), and during the experiments, the gradient was covered with black mesh netting to prevent lizards from escaping. The preferred temperature for each lizard was estimated as the average of the central 50% of temperatures recorded within the 3-hour period (Hertz et al. 1993). We compared preferred body temperatures of lizards reared at 28 before and after acclimation to 31°C using a linear mixed effect model (LMM) with T_{pref} as the dependent variable, SVL as the continuous predictor, temperature as the categorical predictor, and lizard ID as a random effect to account for repeated measurements. We then compared thermal preferences between lizards reared at 28 and 31°C using a LMM with T_{pref} as the dependent variable, SVL as the continuous predictor, developmental temperature as the categorical predictor, and maternal ID as a random effect to account for repeated measurements. We did not include sex within the analyses, as this variable usually does not influence T_{pref} of anoles (Logan et al. 2021). Despite differences in the dates when we estimated thermal preferences in lizards reared at 28 and 31°C, we consider that the temporal gap should not have affected our results because, aside from temperature, lizards were reared under identical conditions, including humidity (70%) and a 12:12 h light-dark cycle.

Comparison of life history traits

We were interested in testing whether developmental temperatures affect two reproductive traits: growth rate and age at sexual maturity. To address this goal, we selected seven pairs of female siblings (offspring from seven wild-caught females), with one sibling reared at 28°C and the other at 31°C. Using sibling pairs allowed us to control for potential genetic effects. We restricted the analysis to females because we did not have adult males reared at 31°C. We started by measuring body size (SVL) at hatching and continued measuring this trait every three weeks (on average) during ∼9 months for lizards reared at 28°C and during ∼8 months for lizards reared at 31°C. Hatchlings from both groups exhibited

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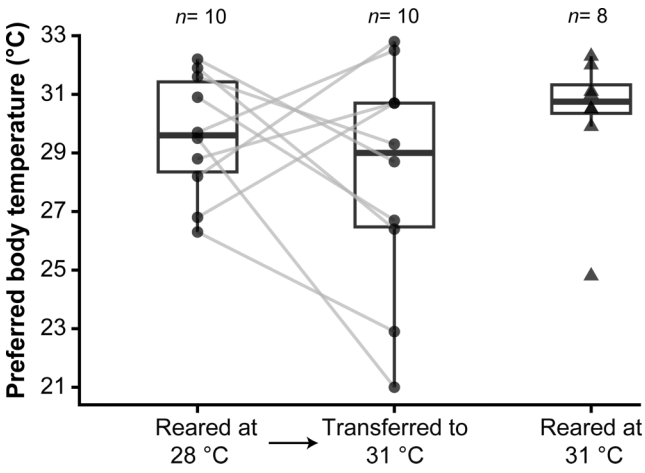

Fig. 1 Mean preferred body temperatures did not change when lizards reared at 28°C (mean T_{pref} = 29.6°C ± 2.0°C) were transferred to 31°C (mean T_{pref} = 28.2°C ± 3.7°C) for a month (t= -0.41; P = 0.69). Lizards showed low repeatability in thermal preferences between treatments (R = 0.005, permutation test P = 0.46), indicating little consistency in this behavioral trait within individuals. Likewise, mean thermal preferences were similar between lizards reared at 28°C (mean T_{pref} = 29.6°C ± 2.2°C) and 31°C (mean T_{pref} = 30.3°C ± 2.2°C) (t = 0.48; P = 0.64). Solid lines connect the same individuals between thermal treatments. Circles represent lizards reared at 28°C, and triangles represent lizards reared at 31°C. Raw data are included as Supplementary Data.

similar body size (mean SVL = 21.3 mm ± 0.5 for those incubated at 28°C, and 21.3 mm ± 0.2 for those incubated at 31°C). We compared growth rates among lizards reared at 28 and 31°C using a LMM with SVL as the dependent variable, age (days) as the continuous predictor, developmental temperature as the categorical predictor, and lizard ID as a random effect to account for repeated measurements. We estimated the age at sexual maturity as the number of days required for females to reach 40 mm SVL from hatching. Forty millimeters of snout-vent length represents the smallest female *A. cybotes* in Barahona (the same province where we collected lizards) containing eggs or yolking follicles (Fobes et al. 1992). Furthermore, in both chambers, we opportunistically observed that only females larger than 40 mm SVL laid unfertilized eggs, consistent with this being the minimum size for reproduction. We compared the estimated age at sexual maturity (40 mm SVL) between developmental temperatures using a LMM, with age as the dependent variable, developmental temperature as the categorical predictor, and maternal ID as a random effect.

Results

We found that neither short-term exposure to warm temperatures (lizards transferred from 28 to 31°C) nor developmental temperature (lizards reared at 28 or 31°C) affected thermal preferences of *Anolis cybotes* (Fig. 1; Table 1). Mean T_{pref} was 29.6°C ± *SE* 2.0 (n = 10) for lizards reared at 28°C, 28.2°C ± 3.7°C (n = 10) for lizards reared at 28°C and transferred to 31°C for a month, and 30.3°C ± 2.2 (n = 8) for lizards reared at 31°C (Fig. 1; Table 1). We found that females reared at 31°C grew more slowly than their siblings reared at 28°C (Fig. 2A; Table 1). On average, females reared at 31°C needed 206 days ± 34 (n = 7) to reach 40 mm SVL (as proxy for sexual maturity), whereas their siblings reared at 28°C needed 130 days ± 34 (n = 7) (Fig. 2B; Table 1).

We conducted statistical power analyses using a conventional power threshold of 0.80 and α = 0.05 to evaluate the robustness of our results (Cohen 1992). Given our sample sizes (10 vs. 8 individuals for thermal preference and 7 vs. 7 individuals for body size), the analyses indicated that our study had sufficient statistical power only to detect relatively large effect sizes (Cohen's d ≈ 1.4–1.6). Therefore, the absence of significant differences in thermal preference could be associated with a small sample size. In contrast, despite the relatively small sample sizes, we detected significant differences in body size, suggesting that the observed effect was sufficiently large relative to within-group variation.

Discussion

While thermoregulatory behavior presents an immediate line of defense against rising temperatures, many animals are thermoconformers, owing either to intrinsic mobility limitations and/or ecological constraints on regulatory behavior (Huey and Slatkin 1976; Huey et al. 2012; Neel et al. 2021; Lahondère 2023). In such organisms, phenotypic plasticity provides an especially important source of protection by enhancing performance and survivorship under warmer conditions (Chevin et al. 2010; Palumbi et al. 2014; Nettle and Bateson 2015).

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Table 1 Linear mixed models evaluating the effects of acclimation or developmental temperature in preferred body temperatures (T_{pref}), growth rate, and age at reaching 40 mm in *Anolis cybotes*.

Comparison	Trait ± SE (*n*)	Effect	df	Statistic	*P*-value
T_{pref} of lizards when reared at 28°C vs. T_{pref} of lizards when transferred to 31°C for a month	Mean T_{pref} Reared at 28 = 29.6°C ± 2.0 (10) Transferred to 31 = 28.2°C ± 3.7 (10)	Temperature	17	t= -0.41	0.69
		SVL	17	t= -1.1	0.28
T_{pref} between lizards reared at 28 and 31°C	Mean T_{pref} Reared at 28 = 29.6°C ± 2.0 (10) Reared at 31 = 30.3°C ± 2.2 (8)	Developmental temperature	15	t = 0.48	0.64
		SVL	15	t= -0.37	0.72
Growth rate between females reared at 28 and 31°C	Rate Reared at 28 = 0.11mm d^{-1} (7) Reared at 31 = 0.09mm d^{-1} (7)	Developmental temperature	12	t= -3.3	0.006
		Age (days)	166	t = 42.6	<0.001
Age at reaching 40 mm SVL between females reared at 28 and 31°C	Mean SVL Reared at 28 = 130 ± 34 (7) Reared at 31 = 206 ± 34 (7)	Developmental temperature	6	t = 4.2	0.006

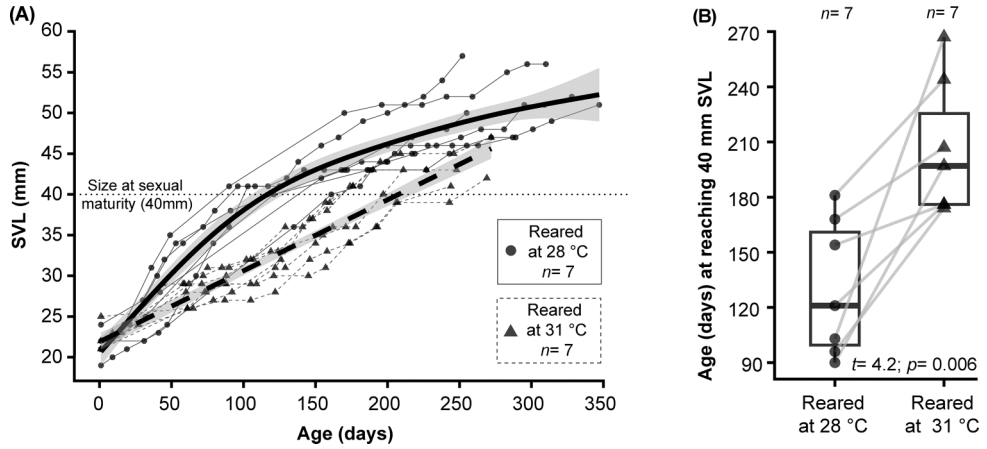

Fig. 2 (**A**) Females reared at 28°C (circles) grew faster than their sibling reared at 31°C (triangles) and therefore reached size at sexual maturity (40 mm SVL) 3 months earlier. Body size changed significantly with age (t = 42.6; $P < 0.001$) and with developmental temperature (t= -3.3; $P = 0.006$). Solid or dashed lines connect the same individual across days. Regression lines are wrapped by 95% confidence bands. (**B**) On average, females reared at 31°C needed 7 months to reach the minimum size at sexual maturity (40 mm SVL), whereas their siblings reared at 28°C did so in 4 months (t = 4.2; $P = 0.006$). Solid lines connect siblings. Raw data are included as Supplementary Data.

Here, we evaluated whether and how temperature affects preferred body temperatures and life history traits of the Hispaniolan stout anole (*Anolis cybotes*), focusing on a population from a shaded habitat where thermal behavior is limited (Muñoz and Losos 2018). We found that short-term acclimation did not influence their preferred temperature: lizards reared at 28°C maintained on average similar thermal preferences even when they were transferred to 31°C for a month (Fig. 1; Table 1). Similarly, developmental temperatures do not influence the preferred temperatures of *A. cybotes*: in both thermal treatments (28 and 31°C) lizards preferred temper-

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atures around 30°C (Fig. 1; Table 1). These results indicate that neither acclimation nor developmental temperature shapes the preferred thermal range of *A. cybotes*. Our findings are similar to those documented in another Caribbean anole, the Cuban brown anole (*Anolis sagrei*), in which incubation temperature does not influence adult heat tolerance (Gunderson et al. 2020).

The stability in thermal preferences of *A. cybotes* that we observed contrasts with patterns reported in many other lizard species, including alligator lizards (*Abronia* and *Gerrhonotus*), horned lizards (*Phrynosoma*), night lizards (*Xantusia*), knob-scaled lizards (*Xenosaurus*), and spiny lizards (*Sceloporus*) (Kaufmann and Bennett 1989; Domínguez-Guerrero et al. 2019, 2021, 2025; Cardona-Botero et al. 2023). Notably, thermal preference shifts are most often documented in species inhabiting high-elevation and/or high-latitude habitats, where individuals may experience a wider range of environmental temperatures across seasons. By contrast, the population we studied occupies a tropical, lowland forest, where thermal conditions vary little across space and seasons (Muñoz et al. 2014; Muñoz and Bodensteiner 2019). Given that physiological plasticity is energetically costly (Sørensen et al. 2003; Kingsolver and Woods 2016), it is possible that natural selection favors limited thermal plasticity in thermoconformer species from thermally homogeneous environments and greater thermal plasticity in more thermally variable habitats (Pigliucci 2005; Angilletta 2009; Diamond and Martin 2021) but see Seebacher et al. (2015). It is also possible that our sample sizes limited our ability to detect subtle shifts in thermal preference. Indeed, post hoc power analyses indicated sufficient power only to detect relatively large effect sizes. Nevertheless, the similarity in mean thermal preferences across treatments suggests that any plastic response, if present, is likely modest relative to the substantial effect of developmental temperature on growth and maturation.

Of note is that thermal behavior conspicuously varies among populations of *Anolis cybotes*; specifically, low-elevation populations tend to be thermoconformers, but higher-elevation populations thermoregulate more actively (Hertz and Huey 1981; Muñoz and Losos 2018). Therefore, within this species, the degree of plasticity in preferred temperatures may vary across environmental gradients. Although similar thermal preferences have been documented among Caribbean anole populations from different environments (Hertz et al. 1993), it would be informative to examine plasticity of thermal preferences (and other physiological traits) in *A. cybotes* from higher elevations. Furthermore, it remains possible that, rather than through intragenerational plasticity, thermal preferences of thermoconforming ectotherms increase by adaptation (Huey et al. 2012). For example, prior field studies of lizards have shown that, under warming, selection favors individuals with higher preferred body temperatures (Logan et al. 2014; Gilbert and Miles 2017). In this case, however, the preferred thermal range estimated in 2012 (28.3–30.4°C) (Muñoz and Losos 2018) is similar to the preferred range measured in 2025 (Fig. 1), suggesting that adaptive shifts have not occurred in this population.

In diurnal lizards like *Anolis cybotes*, their preferred temperature often correlates with optimal temperatures for performance, such as sprinting (Huey and Kingsolver 1989; Angilletta, Hill, et al. 2002; Gilbert and Miles 2017); stability in the thermal preferences of *A. cybotes* suggests that these lizards perform and maximize their physiological functions within a narrow range. This means that performance of different physiological processes should decrease as body temperature approaches or exceeds the upper limit of their preferred temperature range. The preferred thermal range of *A. cybotes* ranges between 28 and 31°C (Muñoz and Losos 2018) (Fig. 1). Therefore, we could expect that temperatures close to or exceeding 31°C could induce negative effects on *A. cybotes*, likely on growth and reproduction. We found that eggs incubated at different temperatures produced hatchlings with similar body size (21.3 mm SVL), a pattern commonly observed in several Caribbean anoles (Muell et al. 2025). In fact, hatchlings of *A. cybotes* incubated at 28 and 31°C in our study exhibited body sizes similar to those previously reported for hatchlings incubated at 25 and 31°C (Muell et al. 2025). A similarity in hatchling body size is likely because 28 and 31°C fall within the optimal temperature range for embryogenesis, as previously observed in *A. sagrei* and *A. cristatellus* (Hall et al. 2024). Although hatchling females emerged at 28 and 31°C exhibit similar body sizes, we found that lizards reared at 31°C grew more slowly than their siblings reared at 28°C (Fig. 2A). A negative effect of warmer developmental temperatures on growth also has been observed in other lizards. For example, bearded dragons (*Pogona vitticeps*) reared at 30°C are smaller than those reared at 27°C (Siviter et al. 2019). Likewise, an increase in body mass in the European common lizard (*Zootoca vivipara*) is optimized within their preferred range but declines at temperatures above it (Van Damme et al. 1991; Gvoždík and Castilla 2001). Slower growth in *A. cybotes* could be associated with exposure to suboptimal temperatures for digestion, as previously documented in the Eastern fence lizard (*Sceloporus undulatus*) (Angilletta 2001; Angilletta, Hill, et al. 2002). Given that the precision of the environmental chamber is ± 1°C, it is possible that lizards reared at 28°C were frequently exposed at temperatures between 27 and 29°C, whereas their siblings reared at 31°C were commonly exposed at temperatures between

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30 and 32°C. Exceeding thermal preferences by even 1°C can have severe consequences, as the breakdown of physiological functions can increase by more than 100% per degree Celsius in ectothermic animals (Jørgensen et al. 2022).

Slower growth in female *A. cybotes* delayed sexual maturity (defined as reaching 40 mm SVL) by approximately 3 months (Fig. 2A). Whereas females reared at 28°C reached adult size at 4 months of age, their siblings reared at 31°C did so at 7 months (Fig. 2B). As age at first reproduction is a major aspect of life history that influences reproductive output (Burger et al. 2019; Healy et al. 2019), we can anticipate negative consequences for the population stability of *A. cybotes* under global warming. Notably, all anoles lay only a single egg per clutch and reproduction stalls during the dry season (Losos 2009; Muñoz et al. 2023): a delayed age at sexual maturity increases generation time and decreases annual fecundity, which could negatively affect population recruitment and demographic dynamics (Brown et al. 2018; Healy et al. 2019). Alternatively, females in the wild could start reproduction at a smaller size, as predicted under the temperature-size rule (Atkinson 1994; Angilletta 2009). While our experimental design did not explicitly test for this possibility, we did opportunistically observe that females did not begin laying until they were 40mm in body size in both treatments, suggesting reproduction at a smaller body size is not occurring. As a caveat of our methodological approach, lizards were exposed to constant temperatures of either 28 or 31°C, whereas in their natural habitat temperatures decrease at night (Muñoz et al. 2014; Muñoz and Bodensteiner 2019). Growth and age at sexual maturity observed in laboratory conditions could change by including temperature fluctuations (*i.e.*, lower nocturnal temperatures). For example, continuous exposure to higher temperature could divert energy into frequent heat shock protein production, negatively affecting growth and reproduction in ectotherms (Sørensen et al. 2003; Kingsolver and Woods 2016). A question that arises from body size patterns in the lab is whether similar patterns are also observed in the field. In the wild, field body temperature of *A. cybotes* from Los Patos has increased by ∼1°C during the past two decades (from 29.5 to 30.5°C; unpublished data); as such, it is possible that body size has likewise changed over the same period.

Together, our results suggest that responses to warming can differ markedly among components of the phenotype. Despite experiencing warmer developmental and acclimatory environments, *Anolis cybotes* maintained stable thermal preferences, suggesting limited plasticity in a trait closely linked to behavioral and physiological performance. Yet, the same temperature increase substantially slowed growth and delayed sexual maturity. These findings suggest that traits involved in temperature regulation and traits underlying life history need not respond in concert to environmental change. Similar contrasts have emerged for *Anolis shrevei*, a closely related species found at higher elevations on Hispaniola: warming is predicted to increase activity opportunities and reduce cold stress in *A. shrevei*, yet this species remains vulnerable through warming-driven changes in habitat distributions and species interactions (Muñoz et al. 2022). Together, these findings suggest that responses to climate change are inherently multidimensional, with apparent resilience in one trait or ecological axis potentially masking vulnerability in another. More broadly, our study highlights a potential vulnerability of thermoconforming ectotherms: temperatures that may be insufficient to shift thermal preferences may nonetheless impose biologically meaningful costs on growth, reproduction, and ultimately population persistence. As climate warming continues, understanding how different dimensions of the phenotype vary in their capacity for plastic adjustment will be critical for predicting species responses to environmental change.

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Supplementary data

Supplementary Data is available at *ICB* online.

Conflict of interest

The authors declare no competing interests.

Data availability

Raw data are included as Supplementary Data.

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