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Original Article

Altitudinal variation in thermal vulnerability of Qinghai-Tibetan Plateau lizards under climate warming

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Abstract

The survival of ectotherms worldwide is threatened by climate change. Whether increasing temperatures increase the vulnerability of ectotherms inhabiting temperate plateau areas remains unclear. To understand altitudinal variation in the vulnerability of plateau ectotherms to climate warming, Qinghai toad-headed lizards (*Phrynocephalus vlangalii*) were subjected to semi-natural enclosure experiments with simulated warming at high (2,600 m) and superhigh (3,600 m) elevations of the Dangjin Mountain, China. Our results revealed that the thermoregulatory effectiveness and warming tolerance (WT) of the toad-headed lizards were significantly affected by climate warming at both elevations, but their thermal sensitivity remained unchanged. After warming, the thermoregulatory effectiveness of lizards at superhigh elevations decreased because of the improved environmental thermal quality, whereas that of lizards at high-elevation conditions increased. Although the body temperature selected by high-elevation lizards was also significantly increased, the proportion of their active body temperature falling within the set-point temperature range decreased. This indicates that it is difficult for high-elevation lizards to adjust their body temperatures within a comfortable range under climate warming. Variations in the WT and thermal safety margin (TSM) under climate warming revealed that lizards at the superhigh elevation benefited from improved environmental thermal quality, whereas those at the high elevation originally on the edge of the TSM faced more severe threats and became more vulnerable. Our study highlights the importance of thermal biological traits in evaluating the vulnerability of ectotherms in temperate plateau regions.

Key words: climate change, *Phrynocephalus vlangalii*, plateau ectotherm, thermoregulatory effectiveness, thermal sensitivity, vulnerability.

Climate change is one of the most important problems worldwide (IPCC 2022), leading to biodiversity declines and potential species extinctions (Whitfield et al. 2007; Sinervo et al. 2010). Ectotherms are highly vulnerable to climate change as their body temperatures (T_bs), and consequently their physiological processes, are directly related to environmental temperature (Deutsch et al. 2008; Kearney et al. 2009; Huey et al. 2012). Tropical ectotherms are considered more vulnerable to the physiological and ecological effects of climate warming than temperate ectotherms (Huey et al. 2009; Logan et al. 2013; Kubisch et al. 2016). Ectotherms inhabiting high-altitude or high-latitude habitats exhibit larger thermal tolerance breadths, and warming may increase their fitness (Deutsch et al. 2008; Valdecantos et al. 2013). However, high-altitude species forced into a more limited range due to increasing temperatures may be particularly vulnerable (Freeman et al. 2018; Feldmeier et al. 2020). Furthermore, increasing interspecific competition when lowland species move up the mountain also enhances the vulnerability of species (Freeman et al. 2019). Clarification of altitudinal variation in the thermal vulnerability of ectotherms is essential for understanding how they respond adaptively to climate warming.

Behavioral thermoregulation of ectotherms is key to their adapting to plateau microhabitats under global warming and can help maintain their body temperature (T_b) within the set-point temperature range (T_{set}), which is best estimated as preferred or selected T_bs in the laboratory (Hertz et al. 1993). The operative environmental temperature (T_e: the T_b that a non-thermoregulating animal would experience; Hertz et al. 1993) of a plateau decreases with increasing altitude, whereas the T_b of ectotherms does not decrease or decreases only slightly with increasing altitude because of thermoregulation (Díaz de la Vega-Pérez et al. 2019; Gilbert and Miles 2019). In microenvironments with low thermal quality estimated from T_e (Hertz et al. 1993), ectotherms need to enhance their thermoregulatory effectiveness to maintain a constant T_b as required for activity (Hertz et al. 1993; Blouin-Demers and Nadeau 2005). Thermoregulatory effectiveness is defined as the extent to which an individual maintains its T_b closer to T_{set} than allowed by the thermal quality of its environment (Row and Blouin-Demers 2006). The thermoregulatory effectiveness of ectotherms in plateau environments is positively correlated with altitude (Lymburner and Blouin-Demers 2020). As thermal quality diminishes with increasing altitude,

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effective thermoregulation requires increased energy and time, rendering it more costly. The cost of thermoregulation primarily thus depends on the habitat's thermal quality and the degree to which T_e differs from T_{set} (Blouin-Demers and Nadeau 2005). Climate warming has increased average environmental temperatures and the frequency of extreme events, especially in high-latitude and high-altitude regions (Griffiths et al. 2005; Buckley and Huey 2016). The thermoregulation cost in high-temperature environments increases with higher T_e to avoid overheating risks (Blouin-Demers and Nadeau 2005; Vickers et al. 2011; Stark et al. 2023). However, with the increase of temperature in a cold environment, the thermoregulatory effectiveness of ectotherms decreases, thus reducing their thermoregulation costs. Thermoregulatory effectiveness in plateau environments can indicate the cost of thermoregulation for ectotherms (Huey and Slatkin 1976; Vickers et al. 2011). More active T_b values falling within the T_{set} indicate that behavioral thermoregulation is more effective. The proportion of T_b values within the T_{set} can be used as an index of vulnerability. A decrease in the proportion of suitable T_b values after improving thermoregulatory effectiveness indicates increasing vulnerability.

The thermal sensitivity of ectotherms describes the degree to which their physiological functions depend on temperature (Hertz et al. 1983; Huey and Kingsolver 1989; Angilletta et al. 2002), which ultimately determines their vulnerability to climate warming (Buckley et al. 2022). Their capacity to perform these functions at different temperatures is typically examined by fitting a thermal performance curve (TPC) (Huey and Stevenson 1979). Thermal performance breadth can reflect the thermal sensitivity of ectotherms to temperature (Huey and Stevenson 1979; Huey and Kingsolver 1989). The narrower the thermal performance breadth, the higher the thermal sensitivity of the ectotherms, and the earlier their response to warming temperatures. Thermal sensitivity is typically measured using proxy indices such as sprint speed, metabolic rate, and growth rate (Angilletta 2009). Generally, tropical ectotherms inhabit environments where temperatures are closer to their upper thermal thresholds, limiting their potential to buffer against temperature increases and making them more sensitive to even small changes in temperature (Deutsch et al. 2008; Huey et al. 2009; Sun et al. 2022). In addition, ectotherms residing at high altitudes tend to be better adapted to cold environments, resulting in a greater decline in performance within a narrower temperature breadth when the T_b exceeds the optimum level (Martin and Huey 2008; Huey et al. 2012; Gunderson and Stillman 2015). Therefore, a narrower thermal performance breadth, reflecting higher sensitivity, suggests increased vulnerability for ectotherms to climate warming.

Thermal tolerance affects the vulnerability of ectotherms to climate warming. The critical thermal minimum (CT_{min}) of ectotherms decreases with increasing elevation or latitude (Sunday et al. 2019), but their critical thermal maximum (CT_{max}) is relatively consistent because most such organisms are unable to prevent the adverse impact of high temperatures on cell membrane integrity and protein structure (Araújo et al. 2013). As altitude increases, temperature fluctuations become more noticeable, and the thermal tolerance breadth of ectotherms broadens (Deutsch et al. 2008; Sunday et al. 2011; Weeks and Espinoza 2013; Bozinovic et al. 2014). The fate of ectotherms largely depends on their thermal tolerance and adaptive capacity to buffer the adverse effects of extreme local temperatures (Pincebourde and Casas 2015; Nowakowski et al. 2018; Montejo-Kovacevich et al. 2020). Regarding physiological performance, warming tolerance (WT: the difference between CT_{max} and the maximum T_e), and the thermal safety margin (TSM: the difference between optimal temperature and mean T_e) are typically employed to evaluate the vulnerability of ectotherms to climate change (Hertz et al. 1993; Deutsch et al. 2008; Sunday et al. 2014). A narrower WT and TSM of ectotherms indicate greater vulnerability to climate warming (Logan et al. 2013; Sunday et al. 2014; Kubisch et al. 2016), with their vulnerability increasing when the environmental thermal quality approaches or exceeds their thermal tolerance limits (Sunday et al. 2014).

The Qinghai toad-headed lizard (*Phrynocephalus vlangalii*) is widely distributed on the Qinghai-Tibetan Plateau at an altitude of 2,000 to 4,500 m (Zhao et al. 1999). Its broad altitudinal distribution makes it an ideal ectothermic species for examining the relationship between climate change and thermal biological traits (Sunday et al. 2014). *Phrynocephalus vlangalii* exhibits no intersexual differences in thermal preference and tolerance related to body size and habitat use (Shu et al. 2010; Wang et al. 2013), but low-elevation populations are considered to have a narrower performance breadth and higher thermal sensitivity than high-elevation populations (Wu et al. 2018). Based on thermoregulation, Sinervo et al. (2018) modeled the extinction risk of *Phrynocephalus* lizards under climate change and found that the extinction risk (2070 RCP 8.5 scenario) across species was generally higher at low-elevation sites; however, to date, the understanding of the influence of climate warming on the thermoregulation of *P. vlangalii* remains incomplete, and differences in vulnerability among populations occurring at different altitudes under climate warming remain unclear.

In this study, we conducted semi-natural enclosure experiments at superhigh (3,600 m) and high (2,600 m) elevations on a mountain and explored how changes in thermal environments influenced thermoregulation and vulnerability to climate warming. We hypothesized that *P. vlangalii* would show altitude-dependent thermoregulation and vulnerability because of large changes in thermal environments. Lizards at high altitudes tend to show higher thermoregulatory effectiveness and greater WT than those at low altitudes in cold environments of high mountains (Zamora-Camacho et al. 2016; Guerra-Correa et al. 2020). We further predicted that the high-altitude population would become more vulnerable than the superhigh-elevation population because of their increased thermoregulatory effectiveness, narrower WT, and higher thermal sensitivity in a warming climate. The objective was to understand altitudinal changes in the vulnerability of the Qinghai-Tibet Plateau lizard to global warming.

Materials and Methods

Study area and animals

The study was conducted in the middle section of Dangjin Mountain (39°18′–25′ N, 94°14′–16′ E) in Aksai Kazakh Autonomous County, Gansu province, China. The average annual temperature here is <3.9 °C with a mean maximum of 11–16 °C in July and a mean minimum of −9 to −20 °C in January (Han et al. 1999). The superhigh-elevation site (39°18′53″N, 94°15′40″E) was in an area of alpine steppe at an elevation of 3,600 m near the mountain's southern peak, whereas the high-elevation site (39°24′42″N, 94°14′12″E) was a temperate steppe located at an elevation of 2,600 m near the mountain's northern foot. The area experiences a temperate continental climate. The average air temperature between the 2 study sites decreased at a rate of 6.81 °C with a 1,000-m increase in elevation from June to October 2019 (Supplementary Table S1).

Phrynocephalus vlangalii is a small, diurnal, ground-dwelling, viviparous lacertid (47–64 mm snout-vent length). This species shows obvious sexual dimorphism, as adult females are larger than males (Zhang et al. 2005). Body size increases with altitude (Li et al. 2014; Yu et al. 2023). Females produce a single litter each year from June to September (Li et al. 2014; Yu et al. 2023). Lizards from superhigh-elevation habitats produce fewer but larger offspring than those from high-elevation habitats (Yu et al. 2023).

Animal husbandry

In 2019, we built 60 semi-natural circular enclosures (radius r = 0.85 m) with transparent plastic sheets at each study site. Half of the enclosures were covered with open-top plastic hoods to increase the temperature and simulate a warming climate, and the others without open-top plastic hoods were used as natural-temperature control enclosures to represent the ambient climate. Our study included four groups of enclosures with 2 altitudinal populations × 2 heating treatments: superhigh elevation control (SH-C), superhigh-elevation warming (SH-W), high-elevation control (H-C), and high elevation warming (H-W) treatments. Three enclosures were randomly selected from each group to measure the temperature in 3 microenvironments (T_{air}: air temperature 10 cm above ground, T_{sub}: substrate temperature, and T_{und}: underground temperature 30 cm below ground). A total of 36 miniature iButton data loggers (DS1921, MAXIM Integrated Products Ltd., San Jose, CA, USA) were placed in the enclosures to record hourly temperatures to the nearest 0.5 °C every day from June to October. Compared to the control enclosures, the warming enclosures with open-top plastic hoods exhibited significant warming effects. Daily average T_{air} and T_{sub} increased by 3.36 °C and 4.83 °C, respectively, at the 2,600-m high elevations, and by 3.63 °C and 4.78 °C, respectively, at the 3,600-m superhigh elevations from June to October 2019 (Supplementary Table S1). This was equivalent to the predicted warming level near the end of the 21st century under the high-warming SSP5-8.5 scenario, that is, warming 3.3–5.7 °C above the temperatures of the 1850–1900 period (IPCC 2022). A total of 240 adult lizards were placed in the enclosures across both sites (Supplementary Table S2), with one male and one female per enclosure. The 120 lizards at each site were captured around the enclosures at this site. After being captured, lizards were measured for snout-vent length using digital vernier calipers (PD-151, Pro'sKit) and for body mass using an electronic balance (ES-08B, Hochoice), and then toe-clipped for permanent identification (Bull and Williamson 1996). Initial body size at superhigh elevations was larger than that at high elevations ($P < 0.001$), with no significant difference at the same elevation between the warming and control groups ($P = 0.353$; Supplementary Table S2). We fed the lizards with the same quantity of mealworms twice per week in the active period of 2019–2020 (May–September).

T_e and T_b

The T_e refers to the temperature of a stationary object that is similar in size, shape, and radiation traits to animals under the same environmental conditions (Hertz 1992; Seebacher and Shine 2004). From July to August 2019, we measured T_es in microenvironments of the enclosures using iButton loggers, each iButton logger was embedded in a copper model (sealed pipe with an inner diameter of 15 mm and length of 55 mm to approximate the size of adult *P. vlangalii*; Hertz et al. 1993). Five groups of warming and control enclosures were randomly selected at each study site and a set of 3 copper models was randomly placed in 3 microenvironments (full sun, full shade, and burrow) in each enclosure. The temperature data recorded by the iButton loggers were calibrated against a dead lizard under full-spectrum bulbs (50 W, UVA + UVB; temperature ranging from 16.5 to 42.0 °C, $n = 47$; Hertz 1992; Bakken et al. 2014). Accordingly, we established a significant linear regression relationship between lizard T_b and T_e (slope = 0.8475, intercept = 0.7783, $R^2 = 0.9987$, $P < 0.001$). The average T_e values calibrated using the T_b values were used to evaluate the operative thermal environment in the enclosures.

From July to August 2019, we measured the active T_bs of the lizards after being acclimated for 1 month in the enclosures at the 2 study sites. Every hour during the daytime (10:00–18:00), we slowly closed the enclosures, captured individuals within 30s, and immediately inserted the probe of an infrared thermometer (Testo 835-T1, Testo Instruments (Shenzhen) Co., Ltd., China) into the cloaca to measure T_bs. We recorded the lizards' identity, sex, time, and date before releasing them into their original enclosures.

Selected body temperature (T_{sel}) and T_{set}

During July to August 2020, T_{sel}s were measured in a thermal gradient with temperatures from 20 °C to 60 °C in a plastic box (1,200 × 400 × 500 mm). A total of 119 lizards (SH-C:SH-W:H-C:H-W = 30:29:30:30) were randomly selected from the enclosures for testing. The plastic box was placed in a car near each study site so that lizards were tested in their local oxygen environment. The bottom of the box was covered with sand and soil (thickness = 5 cm), which was replaced after each experiment to avoid any potential effect from previously tested individuals. Two full-spectrum bulbs (50 W, UVA + UVB) were suspended on one side of the box (400 mm above the box substrate), and the air conditioning of the car was opened on the other side to maintain the thermal gradient during the experimental process from 10:00 to 18:00. The bulbs and air conditioner were powered by a portable mobile power supply and an external solar panel power supply system (STD-T3048120, Shenzhen Sturdy New Energy Technology Co., Ltd., China). Five lizards (2 individuals from the warming enclosures and 3 individuals from the control enclosures, or vice versa) were placed in the low-temperature end of the plastic box one by one for each test. After acclimatizing to the box microenvironment for 1 h, the skin temperature ($T_{surface}$) of each lizard was measured using an infrared thermometer. Each lizard was examined 10 times, at 15-min intervals. In addition, 28 lizards from the 4 groups were randomly selected to measure both their skin surface temperatures and cloacal temperatures. They were placed in a vehicle refrigerator (652 × 365 × 300 mm; Alpicool C15; Electric Appliance Co., Ltd., Foshan, China) for cold acclimation at 15 °C. Then, all individuals were transferred to a plastic storage bin (670 × 490 × 400 mm) placed in the field to let their T_bs increase naturally. Based on 287 pairs of cloacal and skin temperature data, their correlation was determined using linear regression ($T_{\mathrm{cloaca}} = 0.9665 \times T_{\mathrm{surface}} + 1.8842$, $R^2 = 0.9839$, $P < 0.001$). The skin temperature values, calibrated using the cloacal temperature values, were used to calculate T_{sel}s. The recorded temperatures for each lizard were averaged as its T_{sel}, and its T_{set} was estimated as the central 50% (25–75% quartile) of the recorded temperatures (Hertz et al. 1993).

Critical thermal maximum and minimum

From August to September 2020, 121 lizards (SH-C:SH-W:H-C:H-W = 30:31:30:30) were grouped into a vehicle-mounted incubator (400 × 280 × 290 mm; Yuan Tian Fu Kang, Nanjing, China). The incubator's working temperature ranged from 20 to 65 °C. Up to 5 lizards per group were allowed to acclimatize for 30 min in the incubator at 28 °C, after which it was heated at 1 °C min^{-1}. During the experiment, the top cover of the incubator was half open to observe the behavior changes of lizards from lifting their limbs to panting and collapsing with the temperature rising. Once a lizard lost its righting response, its T_{b} was measured by the thermometer. The mean cloacal temperature of all measured lizards was calculated to obtain the $\mathrm{CT}_{\mathrm{max}}$ (Du et al. 2000; Li et al. 2017). A total of 120 lizards (SH-C:SH-W:H-C:H-W = 30:31:30:29) were measured in the vehicle refrigerator to obtain the data of $\mathrm{CT}_{\mathrm{min}}$. The refrigerator's working temperature ranged from −20 to 20 °C. Five lizards per group were allowed to acclimatize for 30 min at 15 °C, after which the refrigerator was cooled at 1 °C min^{-1}. During this process, the refrigerator door was opened at an angle to observe the status of lizards. We ultimately estimated $\mathrm{CT}_{\mathrm{min}}$ as the average T_{b} of lizards when they lost their righting response.

Sprint speed and thermal performance curve (TPC)

Twelve lizards (half males and half females) from each treatment group were randomly selected for sprint speed measurements on a customized wooden track (1,000 × 120 × 150 mm, with intervals marked every 200 mm). Laser sensors (Omron) were installed at 200 mm intervals to sense the passage of the lizard. The other end of the sensor was connected to a Siemens LOGO (Siemens LOGO 12/24RCE) equipped with a custom-made program for reading time. Each lizard was placed on the runway inside the tent and stimulated with a brush at one end to encourage it to cross the runway. Each lizard was tested twice with a 1 h rest interval between tests at the 5 test temperatures from 20 to 40 °C (20, 25, 30, 35, and 40 °C). Before the test, the lizards were acclimated at each test temperature for 2 h in the vehicle-mounted incubator. The sprint speed was used to estimate the locomotory performance of lizards and measured at only one test temperature per day. The maximum running speed of each lizard was calculated by a minimum elapsed time recorded at 200 mm intervals. The sprint speed was used to measure the thermal sensitivity of lizards by fitting a sprint-running performance curve, that is, a TPC (Hertz et al. 1983). TPC associated with the sprint speed was fitted by a mechanistic function, which describes an exponential increase in biochemical reaction rates because of thermodynamics and a quadratic decline due to denaturation (Rezende & Bozinovic 2019). We used nonlinear least-squares to estimate the function parameters and extracted 4 indices from TPC, including thermal sensitivity (Q_{10}), maximal sprint speed (V_{Max}), optimal temperature (T_{opt}), and 80% thermal performance breadth (B_{80}) (Bozinovic et al. 2020; Rezende and Bozinovic 2019; Table S3). We used the $\mathrm{CT}_{\mathrm{min}}$ and $\mathrm{CT}_{\mathrm{max}}$ of all lizards as the start and end points of the TPC, respectively. Q_{10} is the fold change in performance resulting from increasing the temperature by 10 °C. V_{Max} was the speed at the top point of the TPC. T_{opt} is the temperature at the top point of the TPC, and B_{80} was the temperature range over which a lizard moved at 80% of the V_{Max}.

WT and TSM

WT and TSM are 2 simple indices used to evaluate ectotherm vulnerability (Deutsch et al. 2008). Here, WT and TSM were used to evaluate the vulnerability of lizards in different elevations of the northern Qinghai-Tibetan Plateau. WT is the difference between $\mathrm{CT}_{\mathrm{max}}$ and the mean maximum operative temperature per day ($T_{\mathrm{e,\ max}}$), and TSM is calculated as T_{opt} minus the mean operative temperature ($T_{\mathrm{e,\ mean}}$; Clusella-Trullas et al. 2021).

Accuracy and effectiveness of thermoregulation

We calculated 2 indices of the thermal quality of the microhabitat (d_{e}) in the enclosures and thermoregulatory accuracy (d_{b}), according to the program of Hertz et al. (1993). If T_{b} or T_{e} is within the T_{set}, the corresponding d_{b} or d_{e} equals zero; if T_{b} or T_{e} is higher than the upper limit of T_{set}, d_{b} or d_{e} was calculated as the difference between T_{b} or T_{e} and the upper limit; if T_{b} or T_{e} is lower than the lower limit of T_{set}, d_{b} or d_{e} was calculated as the difference between the lower limit and T_{b} or T_{e}. Therefore, the higher the d_{b} and d_{e} values, the lower the thermoregulatory accuracy and thermal quality of the microhabitats. We used 2 indices of d_{e}–d_{b} and $E(1 - \bar{d}_b/\bar{d}_e)$ to evaluate the thermoregulatory effectiveness of lizards. The maximum value of E was 1. The larger the E index, the higher the thermoregulatory effectiveness of the lizards (Hertz et al. 1993). The d_{e}–d_{b} was used to measure the extent of thermoconformity (Blouin-Demers and Nadeau 2005).

Statistical analyses

We examined the normality of data using the Shapiro–Wilk test and the homogeneity of variances using Levene's test. When both assumptions were violated, nonparametric tests were used for data analysis. The Kruskal–Wallis test (R package car; function kruskal.test) was used to analyze the differences in T_{air}, T_{Sub}, T_{und}, and T_{e}s among the different microhabitats of the enclosures. The Dunn test (R package PMCMRplus) was used for multiple comparisons of T_{e}s (Pohlert 2018). A generalized linear mixed model (R package lme4) was used to compare the differences in T_{b}s of lizards inhabiting different microhabitats, with elevation, thermal treatment, time of day, and their interactions (elevation × treatment, elevation × time, treatment × time, and elevation × treatment × time) as fixed factors and enclosure number as a random factor (Bates et al. 2015). Linear mixed-effects models were used to analyze the differences in T_{sel}, $\mathrm{CT}_{\mathrm{max}}$, and $\mathrm{CT}_{\mathrm{min}}$ among different microhabitats (R package lme4), with thermal treatment, elevation, and their interactions (elevation × treatment) as fixed factors and enclosure number as a random factor (Bates et al. 2015). The Scheirer-Ray-Hare test (R package rcompanion) was used to compare differences in d_{b} and d_{e} among the microhabitats (Mangiafico 2020). A two-way ANOVA was used to compare differences in body mass, snout-vent length, T_{opt}, V_{Max}, and B_{80}. Repeated-measures ANOVA (R package ez) was used to calculate the difference in sprint speed, with population as the between-subjects factor (elevation × treatment) and test temperature as the within-subjects factor (Lawrence 2016).

All analyses were carried out using R software (version 4.3.2; R Core Team 2023); statistical significance is reported at $\alpha < 0.05$. Shown are the means ± standard error of measured data.

Results

T_es and T_bs

T_es in each type of microhabitat became higher under simulated climate warming and were higher at the high elevation than at the superhigh elevation (Figure 1, Supplementary Table S4). At the superhigh elevation site, T_es of full sun and shade were significantly higher in the warming enclosures than in the control enclosures (Bonferroni-adjusted $P < 0.001$) and even for T_es of burrows (Bonferroni-adjusted $P = 0.018$; Figures 1a,1b, Supplementary Table S4). At the high-elevation site, T_es of full sun and burrow were significantly higher in the warming enclosures than in the control enclosures (Bonferroni-adjusted $P < 0.001$), but not for T_es of full shade (Bonferroni-adjusted $P = 0.088$; Figures 1c,1d, Supplementary Table S4). Warming treatment significantly increased T_es of microenvironments.

T_bs of lizards became higher under simulated climate warming ($P < 0.001$) and were higher at the high elevation than at the superhigh elevation ($P < 0.001$) and also changed with time of day ($P < 0.001$), with no significant interactions between populations and treatments ($P = 0.832$) or between treatments and time of day (Figure 1, Supplementary Table S4).

T_{sel} and critical thermal temperatures

T_{sel} of lizards were higher at the high elevation than at the superhigh elevation ($F_{1,87.145} = 11.166$, $P = 0.001$) and became higher under simulated climate warming ($F_{1,106.805} = 9.330$, $P = 0.002$), with no significant interaction among them ($F_{1,106.805} = 2.818$, $P = 0.096$). Warming significantly increased

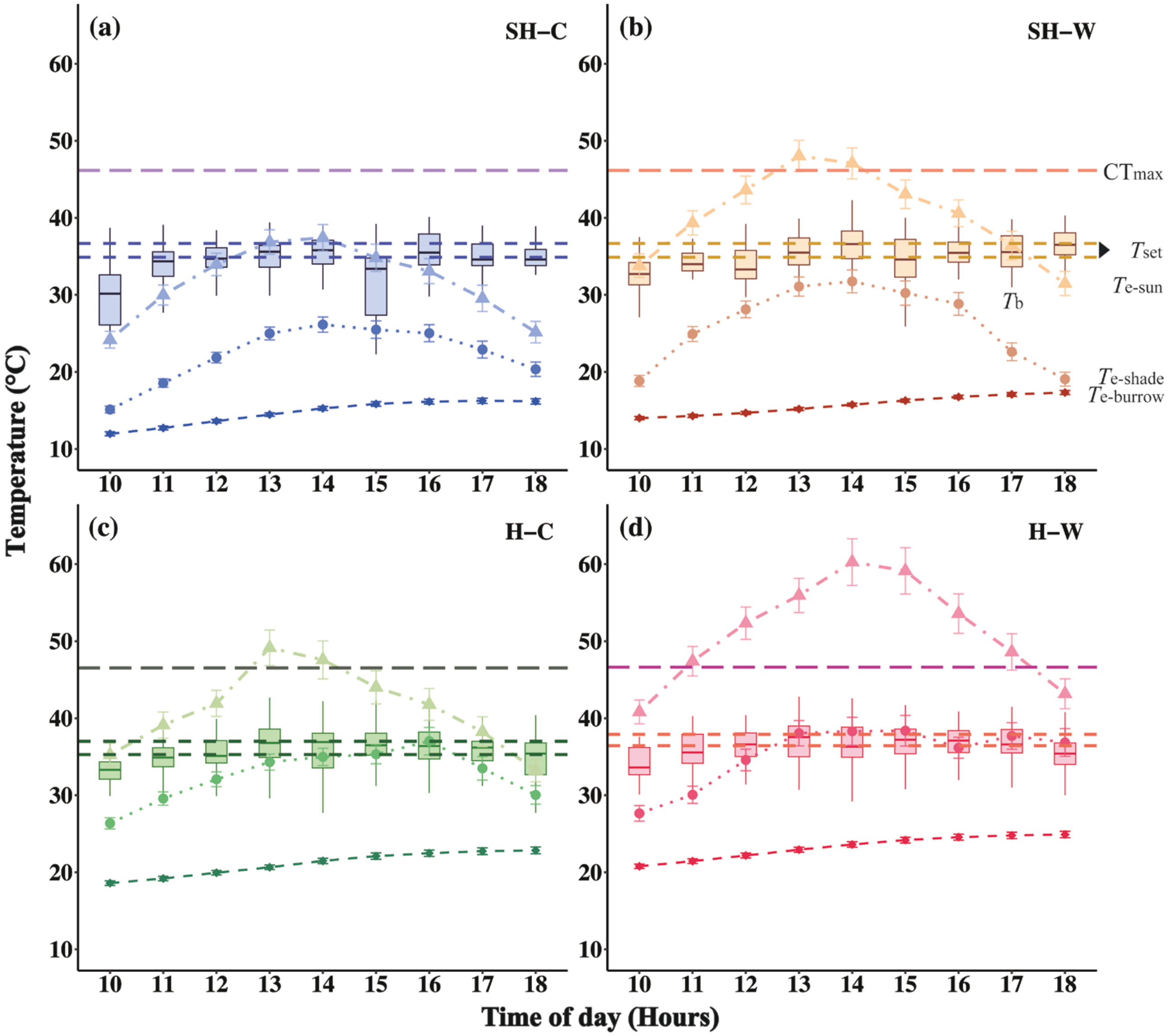

Figure 1 Body temperature (T_b), set-point temperature range (T_{set}), operative temperatures in full-sun (T_e-sun), full-shade (T_e-shade) and burrow (T_e-burrow), and critical thermal maximum (CT_{max}) of the Qinghai toad-headed lizard (*Phrynocephalus vlangalii*) over their daily active period. Boxplots represent a median line, interquartile range (IQR boxes) and 1.5 IQR (whiskers) of lizard T_bs. SH-C: control treatment at the superhigh elevation; SH-W: warming treatment at the superhigh elevation; H-C: control treatment at the high elevation; H-W: warming treatment at the high elevation.

the T_{sel} of lizards at the high elevation ($F_{1,55.478} = 11.493$, $P = 0.001$) but not at the superhigh elevation ($F_{1,53.101} = 0.846$, $P = 0.362$; Figure 2A).

Lizards at the high elevation were less cold tolerant than their counterparts at the superhigh elevation because of significantly higher CT_{min} ($F_{1,116} = 12.082$, $P < 0.001$), whereas thermal treatment did not significantly change the CT_{min} at the 2 elevations ($F_{1,116} = 1.030$, $P = 0.312$), with no significant interaction between them ($F_{1,116} = 0.098$, $P = 0.755$; Figure 2B). Similarly, the CT_{max} of lizards was significantly higher at the high elevation than at the superhigh elevation ($F_{1,117} = 8.036$, $P = 0.005$), and thermal treatment did not significantly change the CT_{max} at the 2 elevations ($F_{1,117} = 0.108$, $P = 0.743$), with no significant interaction between them ($F_{1,117} = 0.147$, $P = 0.702$; Figure 2C).

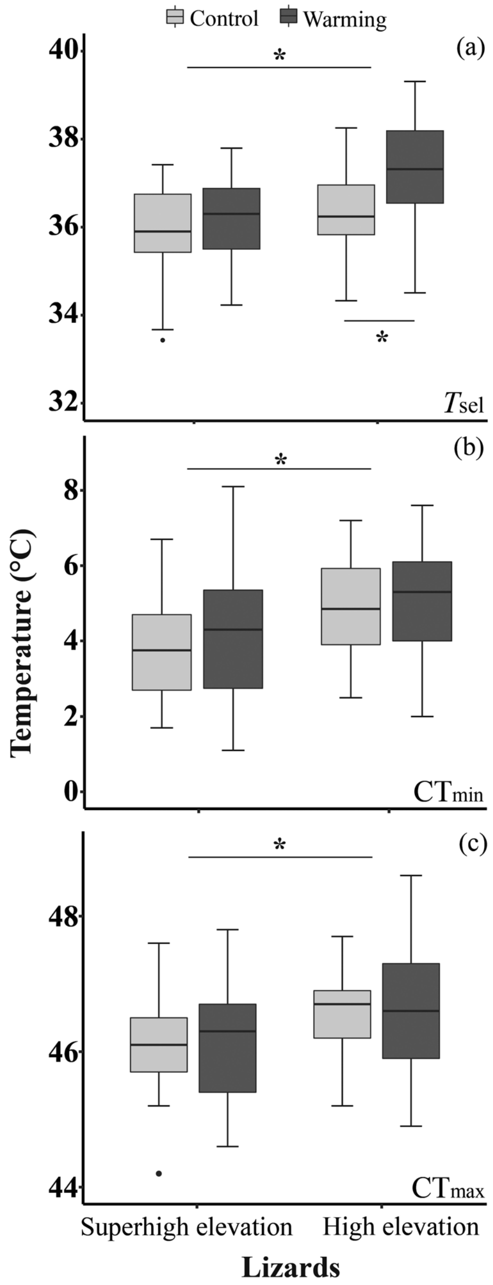

Figure 2 Regional differences in selected body temperature (T_{sel}), critical thermal maximum (CT_{max}), and minimum (CT_{min}) of the Qinghai toad-headed lizard (*Phrynocephalus vlangalii*) in the warming and control enclosures of the Dangjin Mountain, China. Boxplots display a median line, interquartile range (IQR boxes), 1.5IQR (whiskers), and data points (solid circle); *: <0.05.

Sprint speed and TPC

The sprint speed of the lizards was not significantly affected by altitudinal population ($F_{1,42} = 1.275$, $P = 0.265$), thermal treatment ($F_{1,42} = 0.049$, $P = 0.825$), or their interaction ($F_{1,42} = 0.255$, $P = 0.616$); however, the sprint speed increased with experimental temperatures increasing from 20 to 40 °C ($F_{4,168} = 120.676$, $P < 0.001$; Supplementary Figure S1). Their Q_{10}, V_{max}, T_{opt} for sprint speed and B_{80} of TPC were similar between altitudinal populations, or thermal treatments (Supplementary Table S3). Simulated climate warming did not obviously influence TPC (Supplementary Figure S1), indicating that the thermal sensitivity of *P. vlangalii* was similar under different temperature conditions.

Thermoregulation, WT, and TSM

The d_b values were not affected by elevation ($H = 0.170$, $df = 1$, $P = 0.680$), thermal treatment ($H = 1.578$, $df = 1$, $P = 0.209$), or their interaction ($H = 0.399$, $df = 1$, $P = 0.528$); however, simulated climate warming enhanced the accuracy of thermoregulation in the 2 altitudinal populations to a certain extent (Table 1). d_e was significantly affected by altitudinal population ($H = 152.396$, $df = 1$, $P < 0.001$), thermal treatment ($H = 18.987$, $df = 1$, $P < 0.001$), and their interaction ($H = 36.190$, $df = 1$, $P < 0.001$). After eliminating the influence of elevational factor statistically, simulated climate warming significantly increased the thermal quality of habitats at the superhigh elevation ($\chi^2 = 55.269$, $df = 1$, $P < 0.001$), but not at the high elevation ($\chi^2 = 1.990$, $df = 1$, $P = 0.1160$). The 2 indices d_e-d_b and E showed that the thermoregulatory effectiveness of lizards increased at the high elevation but decreased at the superhigh elevation under simulated climate warming (Table 1). However, lizards still needed higher thermoregulatory effectiveness at the superhigh elevation than at the high elevation, even under simulated warming, similar to their needs in the present climate (Table 1).

Simulated climate warming decreased the lizard's WT and TSM at the high and superhigh elevations; however, the WT and TSM values of superhigh-elevation lizards in the warming climate were close to or even larger than those of high-elevation lizards in the present climate. Therefore, the lizard populations had broader WT and TSM at the superhigh elevation than at the high elevation under the present climate, and warming accelerated the vulnerability at both elevations, particularly at the high elevation (Table 1). The T_b proportions of falling within the T_{set} for the treatments of SH-C, SH-W, H-C, and H-W were 23.99% (71/296), 23.91% (94/393), 25.71% (135/525), and 20.48% (119/581), respectively. The proportion of T_bs did not differ among the 4 treatments ($\chi^2 = 4.449$, $df = 3$, $P = 0.220$) but significantly decreased at the high elevation under simulated climate warming ($\chi^2 = 4.288$, $df = 1$, $P = 0.038$; Table 1). The T_e proportions exceeding the CT_{max} for the SH-C, SH-W, H-C, and H-W treatments were 1.91% (16/837), 10.99% (92/837), 12.31% (103/837), and 25.45% (213/837), respectively. The proportion of T_es differed significantly among the 4 treatments ($\chi^2 = 203.597$, $df = 3$, $P < 0.001$), and warming increased its proportion at both elevations (Table 1); however, similar T_e proportions between the H-C and SH-W treatments showed that a simulated warming climate improved the thermal resource environment and reduced the thermoregulatory effectiveness of lizards at the superhigh elevation. In contrast, a simulated warming climate decreases the thermal resource environment and enhances the thermoregulatory effectiveness of lizards at the high elevation (Table 1).

Discussion

Our semi-natural enclosure experiments with simulated warming at 2 contrasting elevations elucidated that the thermal vulnerability of *P. vlangalii* to climate warming depended not only on ambient temperatures but also on its behavioral and physiological response capabilities, which helps us understand the thermoregulation and vulnerability of plateau ectotherms to global warming and altitudinal variation therein. Simulated climate warming did not markedly change the thermal sensitivity and thermal tolerance of *P. vlangalii*; however, warming increased vulnerability of the lizards at 2,600 m elevation because of reduced WT, narrowed TSM and a lower proportion of T_b within the T_{set}. In contrast, the lizards at 3,600 m superhigh elevation retained some buffering capacity after warming.

Climate warming had contrasting effects on the behavioral thermoregulation of *P. vlangalii* at high (2,600 m) and superhigh (3,600 m) elevations. Warming alters the thermal quality of microhabitats and affects the thermoregulatory effectiveness of lizards (Blouin-Demers and Nadeau 2005; Ortega et al. 2016; Moore et al. 2018). Under the present climatic conditions, the environments represented better thermal quality at the high elevation than at the superhigh elevation, and superhigh-elevation lizards would require higher thermoregulatory effectiveness to regulate their T_b (Table 1). Warming increases the microhabitat temperature during the day and the frequency and duration of extremely high temperatures, thus increasing the exposure of ectotherms to high temperatures (Moore et al. 2018). Under simulated climate warming, the thermal quality of the environment at the superhigh elevation improved, and thermoregulatory effectiveness was correspondingly reduced; however, the environmental thermal quality at the high elevation became lower because of warming, and the T_es in full sun were higher than the CT_{max} of lizards at the most active time of the day (Figure 2); therefore, the lizards required higher thermoregulatory effectiveness to regulate their T_bs (Table 1). We also found that T_{sel} of high-elevation lizards significantly increased under simulated climate warming (Figure 2A). T_{sel} is influenced by various factors, including heat source, time, and light conditions (Sievert and Hutchison 1989). Changes in T_{sel} can help lizards adapt to new habitats (Bennett and Lenski 1993). In warming habitats, an increase in T_{sel} can broaden the activity time window for ectotherms and may reduce the costs of behavioral thermoregulation (Gvoždík 2012; Theisinger et al. 2017). Indeed, the costs of thermoregulation largely depend on the thermal quality of habitats. With a decline in thermal quality, effective thermoregulation requires more energy and time, resulting in higher costs (Huey and Slatkin 1976; Blouin-Demers and Nadeau 2005; Row and Blouin-Demers 2006). Although the T_{sel} of high-elevation lizards significantly increased under simulated climate warming in the present study, they still required high thermoregulatory effectiveness to regulate their T_bs values. The high-elevation population buffered the adverse effects of extreme temperature exposure through behavioral thermoregulation under simulated climate warming; however, the proportion of T_bs within the T_{set} significantly decreased (Table 1). This indicates that it is difficult for high-elevation lizards to adjust their T_b within a suitable range when exposed to high temperatures under climate warming. It can be speculated that these lizards face higher costs for behavioral thermoregulation. Therefore, it is evident that climate warming will exert adverse impacts on the behavioral thermoregulation of ectotherms at high elevations on the Qinghai-Tibetan Plateau.

Simulated climate warming had no effect on the thermal tolerance of the plateau lizard *P. vlangalii* but narrowed the WT range and TSM of high-altitude lizards, thus making them more vulnerable. CT_{min} and CT_{max} significantly decreased with elevation, whereas warming did not significantly affect these parameters (Figure 2). The degree of threat to ectotherms can be predicted by matching the states between their physiological traits and environmental temperatures (Heatwole 1970; Deutsch et al. 2008; Sunday et al. 2014). Their WT and TSM tend to expand with increasing latitude or elevation gradients, typically resulting in a larger WT range and higher TSMs at high-latitude or high-elevation areas compared to low-latitude or low-elevation areas (Deutsch et al. 2008; Dillon et al. 2010; Sunday et al. 2014, 2019; Sun et al. 2022). Based on the difference between air temperature and the CT_{max} of animals, climate warming is expected to increase the vulnerability of tropical ectotherms, whereas temperate ectotherms may be less impacted (Deutsch et al. 2008); however, further analyses based on more refined environmental temperatures have revealed that ectotherms in high-latitude regions are also at the edge of being threatened (Sunday et al. 2014). If the

Table 1 The thermal quality of microhabitat (d_e), thermoregulatory accuracy (d_b), and effectiveness (d_e–d_b, and E), warming tolerance (WT), and thermal safety margin (TSM) of the Qinghai toad-headed lizard (*Phrynocephalus vlangalii*) in the warming and control enclosures at superhigh- and high-elevation sites of the Dangjin Mountain, China. SH-C: control enclosure at the superhigh elevation; SH-W: warming enclosure at the superhigh elevation; H-C: control enclosure at the high elevation; H-W: warming enclosure at the high elevation.

Treatments	d_b (°C)	d_e (°C)	d_e–d_b (°C)	E	WT (°C)	TSM (°C)	$P(T_b)$ (%)	$P(T_e)$ (%)
SH-C	1.88 ± 0.15	12.00 ± 0.33	10.12	0.84	8.73	11.13	23.99	1.91
SH-W	1.66 ± 0.10	8.28 ± 0.35	6.62	0.80	−1.89	6.14	23.91	10.99
H-C	1.61 ± 0.09	5.56 ± 0.29	3.95	0.71	−2.62	1.57	25.71	12.31
H-W	1.53 ± 0.07	6.01 ± 0.29	4.48	0.75	−13.64	−3.34	20.48	25.45

$P(T_b)$ is the percentage of T_bs within the T_{set}; and $P(T_e)$ is the percentage of T_es higher than CT_{max}.

ambient temperatures in temperate regions approach or have already reached the physiological threshold of ectotherms, the impacts of climate warming could prove fatal, as they have little ability to increase these thresholds (Kubisch et al. 2016; Terán-Juárez et al. 2021). Previous studies suggested that climate warming shortens the daily activity window of plateau lizards, while simultaneously increasing their energy expenditure and reducing their survival potential (Doucette et al. 2023); however, the current study suggests that climate warming will benefit superhigh-elevation populations of plateau ectotherms owing to improved environmental thermal quality, but high-elevation populations may still face more severe threats (Table 1). In terms of the vulnerability of plateau lizards, simulated climate warming reduced the WT range and TSM, but the impacts on the 2 altitudinal populations were inconsistent (Table 1). Although simulated warming caused the TSM and WT of superhigh-elevation lizards to become narrower and increase their vulnerability, the values of TSM and WT only became close to those of high-elevation lizards in the ambient climate (Table 1), indicating that lizards at the superhigh elevation are still able to buffer against warmer environments. In addition, compared to lizards at the high elevation, the larger TSM of *P. vlangalii* at the superhigh elevation indicated their potential to buffer the stress of future climate warming (Clusella-Trullas et al. 2021). Therefore, under climate warming, the superhigh elevation population of *P. vlangalii* may benefit from higher habitat temperatures and environmental thermal quality. In contrast, the high-elevation population will face a more severe threat under a warming climate because of the narrowed WT and TSM (Table 1). Obviously, climate warming will have a more detrimental effect on high-elevation Qinghai toad-headed lizards, making them more vulnerable.

The current study revealed that climate warming did not alter the thermal sensitivity measured by the sprint speed of *P. vlangalii*. Sprint speed is an important proxy for the fitness of ectotherms (Johnson et al. 2008; Strobbe et al. 2009), as well as a comprehensive manifestation of their behavioral and physiological capabilities (Huey and Stevenson 1979; Bennett 1980; Hertz et al. 1983). We expected that sprint speed would reflect the fitness of *P. vlangalii* and the impact of climate warming on its thermal sensitivity; however, we did not find any obvious differences in the sprint speeds of *P. vlangalii* between the experimental treatments at different temperatures and altitudes. Some studies suggested interspecific differences in the thermal performance breadth of lizards based on sprint speed, but no such intraspecific differences are known (van Berkum 1988). The sprint performance of lizards is directly influenced by body size and hind limb length (Bonine and Garland 1999; Husak et al. 2006). The structure and type of muscle fibers in the hind limbs determine differences in locomotor performance (Bonine and Garland 1999). In the present study, the lizards in both the warming and control groups had similar body sizes and snout-vent length, which implied that they also had similar hind limb lengths and muscle structure; therefore, their locomotor performance may not be significantly affected by warming. Our study also found that their thermal tolerance breadths at the 2 different elevations were large and that they performed well within a large temperature range (B_{80}), indicating that *P. vlangalii* has low plasticity in thermal acclimation and is less likely to acclimatize to microhabitat warming (Huey et al. 2012). In addition, behavioral thermoregulation can help lizards buffer warming stress. These facts may account for the lack of differences in thermal sensitivity reflected by the sprint speed of *P. vlangalii* among the treatments in this study. In addition to sprint speed, other factors, such as food assimilation rate (Huey and Kingsolver 2019), growth rate (Kingsolver and Woods 2016), and enzyme activity (Feder and Hofmann 1999), can be effective indices of thermal sensitivity. Whether the thermal sensitivity of *P. vlangalii* measured using these indices is affected by climate warming requires further study.

In conclusion, under simulated global warming, 2 populations of *P. vlangalii* from sites at different elevations on the Qinghai-Tibet Plateau showed different variations in thermoregulatory effectiveness and WT, but their thermal sensitivities remained unchanged. Warming improved the thermal quality of superhigh-elevation (3,600 m) habitats, reducing the thermoregulatory effectiveness of *P. vlangalii* and making it easier for them to achieve the required T_b through behavioral thermoregulation; however, it reduced the thermal quality of high-elevation (2,600 m) habitats. Although the high-elevation populations of *P. vlangalii* significantly increased their T_{sel}s, their thermoregulatory effectiveness continued to increase and the proportion of suitable T_b significantly decreased. Warming simultaneously reduced the TSM and WT ranges of *P. vlangalii* at both elevations. It benefited superhigh-elevation lizards with higher environmental temperatures and thermal quality but made high-elevation lizards originally on the TSM edge more vulnerable. These results were obtained under experimental conditions in which the lizards were artificially provided with sufficient food in semi-natural enclosures. Whether global warming will lead to a lack of food resources for these 2 altitudinal populations remains unknown. Therefore, ectotherms inhabiting temperate plateaus may still face significant survival challenges due to global warming.

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Conflict of Interest statement

None declared.

Authors' Contributions

Z.G.Z., Z.Y.Z., and Z.S.L. conceived the ideas and designed the studies; Z.Y.Z., W.Y., X.L.Z., and C.Z. collected data; Z.Y.Z. analyzed data and wrote initial drafts of the manuscript.; Z.G.Z., W.G.D., and Z.S.L. contributed to revisions.

Ethics Statement

The collection and handling of lizards in this study were approved by the Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences (IOZ-IACUC-2023-153).

Supplementary material

Supplementary material can be found at https://academic.oup.com/cz.

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