

Analysis of resting status reveals distinct elevational variation in metabolisms of lizards

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Abstract

Animals spend a considerable proportion of their life span at rest. However, resting status has often been overlooked when investigating how species respond to environmental conditions. This may induce a large bias in understanding the local adaptation of species across environmental gradients and their vulnerability to potential environmental change. Here, we conducted an empirical study on montane agamid lizards, combined with mechanistic modeling, to compare elevational variations in body temperature and metabolisms (cumulative digestion and maintenance cost) between resting and active status. Our study on three populations of an agamid lizard along an elevational gradient revealed a trend of decreasing body temperature toward higher elevations, the main contributor of which was resting status of the lizards. Using population-specific reaction norms, we predicted greater elevational variation in hourly and cumulative digestion for resting lizards than for active lizards. Climate-change impacts, estimated as the change in cumulative digestion, also show greater elevational variation when resting status is factored into the analysis. Further, our global analysis of 98 agamid species revealed that in about half of their combined distributional range, the contribution of resting status in determining the elevational variation in cumulative digestion and maintenance cost of lizards was greater than the contribution made by a lizard's active status. Our study highlights the importance of considering resting status when investigating how species respond to environmental conditions, especially for those distributed over tropical and subtropical mountain areas.

KEY WORDS

active, climate change, elevational adaptation, inactive, metabolism, physiological response, reptile

INTRODUCTION

Liang Ma and Wei-Guo Du share equal leadership contributions as leaders of multidisciplinary research teams that worked in collaboration on this project.

The rotation of the earth creates daily fluctuations in solar radiation and temperature, which, in turn, induce daily cycles of animal activity and rest (Rusak & Zucker, 1975). Environmental gradients exert various

selective forces on both active and resting animals by generating physiological consequences, such as changes in body temperature and energy dynamics (Deutsch et al., 2008; Dillon et al., 2010). However, in contrast to the numerous studies on how environmental conditions affect active animals (e.g., Cowles & Bogert, 1944; Deutsch et al., 2015; Huey & Stevenson, 1979; Penn & Deutsch, 2022), studies on the resting status of animals have received much less attention (but see Assefa et al., 2015; Bai et al., 2019; Huey, 1982, 1991; Huey et al., 1989; Kayser et al., 2014). This leaves a huge gap in our understanding of the role that resting status plays in determining the physiological constraints on species across their distributional ranges. Hence, it is important to expand our knowledge of the effects of resting status on physiological constraints of species across their distribution ranges for several reasons. Firstly, contingent on local climate and species behavior and physiology, animals may spend a large proportion of their life span in resting status (Campbell & Tobler, 1984; Huey et al., 1989). Secondly, due to distinct temperature profiles experienced during resting versus active status and nonlinear thermal reaction norms of species' physiology, resting animals show distinct physiological responses compared with active animals (Sinclair et al., 2016). Thirdly, the spatial and temporal patterns of climate conditions experienced by active animals may differ from those experienced by resting animals (Huey, 1982; Huey et al., 2021). Failing to include resting status in studies on animal physiology may induce large biases in understanding how species adapt to local environmental gradients and in estimating species' vulnerability to climate change.

Metabolism includes two important aspects: cumulative digestion and maintenance cost, which respectively represent the energy input and energy output for heterotrophic organisms during resting and active status. These processes profoundly influence an animal's energy budget and are therefore of central importance in determining species fitness in certain environmental conditions (Dunham et al., 1989; Kooijman, 2010; Porter & Gates, 1969). Cumulative digestion reflects energy assimilation (Angilletta, 2001), while maintenance cost supports an organism's basic life functions (Kooijman, 2010; Mueller & Diamond, 2001). The digestion rate and resting metabolic rate (RMR in short: the rate of producing maintenance cost) are largely influenced by body temperature, which can be quantified as thermal performance curves, especially for ectotherms (Huey, 1982; Huey & Kingsolver, 1989; Sinclair et al., 2016). Active ectotherms thermoregulate according to the operative temperatures available in their microhabitats, whereas the body temperatures of resting ectotherms are highly dependent on

the temperature profile of their retreats (Colinet et al., 2015; Rutschmann et al., 2021). Therefore, higher body temperatures of ectotherms during activity are closely aligned to their thermal preferences, enabling faster digestion and inducing higher maintenance cost, in contrast to the lower body temperatures of resting ectotherms. Moreover, distinct thermal performance curves of digestion rate and resting metabolic rate collectively determine how metabolism in animals change with body temperature (Sinclair et al., 2016). The thermal performance curve of digestion rate is usually bell-shaped, where the slope tends to be steep at low temperatures and flattens out at preferred temperatures. The thermal performance curve of resting metabolic rate is very different, increasing exponentially with body temperature (Sinclair et al., 2016). Therefore, temperature-driven changes in physiological functions at resting body temperatures (usually lower than those experienced by active individuals) may be more pronounced for cumulative digestion than for maintenance cost. For some ectotherms, resting status is prolonged at areas where aboveground operative temperatures are below the temperature range required for activity (Enriquez-Urzelai et al., 2020; Sinervo et al., 2010); at these areas, ectotherms may complete a significant proportion of their daily digestion and maintenance cost during rest (Christian et al., 1996; Huey, 1982). It follows that variation in thermal conditions experienced by resting ectotherms play a major role in the pattern of metabolism across geographic gradients.

Montane areas across the globe reside elevational thermal gradients, which may induce divergent metabolisms for species that are distributed along those gradients (Enriquez-Urzelai et al., 2020; Jiang et al., 2023). However, resting ectotherms may show a different elevational pattern in their metabolisms than active ectotherms because of two reasons. First, resting ectotherms may be subject to greater elevational variation in their thermal environment than active ectotherms due to the lack of opportunities for behavioral thermoregulation. For example, at cold high-elevations sites, active ectotherms may achieve body temperatures as high as those available at low elevations by actively thermoregulating between available heat resources (Beaupre et al., 1993; Lu et al., 2018; Zamora-Camacho et al., 2016), while resting ectotherms are confined to the range of temperatures available within their retreats (burrows, under rocks) (Colinet et al., 2015; Rutschmann et al., 2021). Second, the elevational variation in resting body temperatures may be further enlarged by the low oxygen level at high-elevation sites as resting ectotherms may have to use shallow yet cold retreats to avoid hypoxia (Weissberger et al., 2009). It is noteworthy that the

relative contribution of resting status versus active status in determining the overall elevational variation in metabolisms may vary geographically. This is primarily due to geographical variations in both the delineation of activity time, which distinguishes between active and resting status, and the ectotherms' body temperature, which determines their metabolisms (Buckley et al., 2012; Dunham et al., 1989; Grigg & Buckley, 2013).

Here, we combined an empirical study with mechanistic niche modeling on an agamid lizard distributed along a large elevational gradient at Qinghai-Tibet Plateau to examine their metabolisms (i.e., cumulative digestion and maintenance cost). We then extrapolated our results for agamid lizards distributed across montane areas around the globe. We aimed to explore the contribution of resting status in determining elevational variation in metabolisms of ectotherms. Specifically, we address the following three questions: (1) How does the physical environment and consequent body temperatures of resting lizards vary across elevations? (2) How does the resting status of lizards contribute to elevational variation in metabolisms and how will they shift under climate change? (3) How does the contribution of resting status to elevational variation in metabolisms vary across a global scale?

METHODS

Study system

In our empirical study, we used an agamid lizard (*Phrynocephalus vlangalii*; Agamidae) widely distributed at ~2000–4500 m above sea level (asl) on Qinghai-Tibet Plateau as our study system (Zhao et al., 1999). The lizard is a diurnal species and retreats into underground burrows when resting. We collected physiological traits of lizards from three populations, hereafter defined as follows: 4200 m (34°45' N, 98°08' E, ~4208–4306 m asl); 3400 m (36°39' N, 100°52' E, ~3405–3514 m asl); and 2600 m (36°04' N, 100°41' E, ~2599–2700 m asl). Protocols for lizard collection, handling, and husbandry were approved by the Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences (IOZ-IACUC-2022-135). Additionally, to explore whether the patterns found in this species exist on a broader scale, we collected and analyzed data on agamid lizards from around the globe. We conducted all statistical analyses in R v.4.0.3 (R Core Team, 2020) and used Tukey post hoc tests for all traits to analyze pair-wise differences between the three lizard populations ("emmeans" function in "emmeans" package; Lenth, 2021).

Physiological responses data

We used data from our previous study of physiological responses (i.e., active body temperatures, thermal tolerance, RMR) in this species (Jiang et al., 2023). Linear mixed-effects models ("nlme" package; Pinheiro et al., 2020) were used to analyze the differences in active body temperatures of lizards from the three populations, with population as the fixed factor, and measurement time and date as random factors. We found no difference in physiological traits of lizards between 2600 and 3400 m. However, lizards from 4200 m had lower preferred body temperatures, higher RMR, and greater cold tolerance than congeners from the 2600- and 3400-m populations. Thus, we amalgamated the data from lizards at 2600 and 3400 m in terms of body temperatures, thermal tolerance, and RMR for the parameters of biophysical models. Resting body temperatures, resting depth, and food passage times of lizards were collected in this study. All measurements were collected during the peak activity season of lizards (July and August) in 2020 and 2021.

Resting body temperatures

We recorded resting body temperature of lizards from 20:00 to 07:00. To do so, we dug into their burrows and immediately measured the cloacal temperatures of resting lizards using an electronic thermometer (UNT, T-325; with an accuracy of $\pm 0.1^\circ\text{C}$). The depth at which the lizards were resting in their burrows was also recorded. To reduce the amount of disturbance to the lizards, we minimized the time for digging and measuring body temperatures to approx. 1–2 min. All lizards were inactive when we found and were released during the daytime. We used ANOVA to estimate the variations in resting depth between the three lizard populations, with population as the fixed factor and lizard snout-vent length (SVL) as the covariate. Generalized least squares models ("nlme" package; Pinheiro et al., 2020) were used to test for the effects of lizard population on resting body temperatures, using population as the fixed factor and measurement time as the covariate.

Food passage time

We recorded the food passage time of lizards from the three populations in three climate-controlled rooms that were located at the corresponding three elevation sites. The distance between the climate-controlled rooms and the field sites was <20 km, and the difference in elevation was <100 m. Lizards were fasted for 2 days before

feeding them a mealworm attached to a 5-mm indigestible blue rope (0.2 mm diameter). In our experimental setup, each lizard was individually housed in separate plastic containers ($17 \times 11.7 \times 4$ cm). They were then subjected to five constant temperature treatments: 20, 25, 30, 35, and 40°C. The temperature gradients were achieved using five temperature-controlled incubators (XHC-25-AC/DC; Changzhou, China), with each temperature treatment containing five male and five female lizards. Every hour, we monitored the lizard to determine whether it had defecated and whether the excreted feces contained blue rope. Due to the 2-day fasting period followed by feeding only one mealworm, lizards defecated only once, with blue rope present in the feces. Consequently, we defined the period from swallowing to appearance of the blue rope was defined as the food passage time, which was counted to the reciprocal as the digestion rate. Linear models were used to analyze differences in food passage time between the three lizard populations, with population, body temperature, and the interaction between the two as fixed factors.

Metabolism parameters

Calculating cumulative digestion and maintenance cost

We calculated cumulative digestion and maintenance cost as the sum of digestion rates or RMR over a period of time. We fitted reaction norms of RMR (V ; in milliliters of CO₂ per hour) with body temperature (T ; in degrees Celsius) and body mass (B ; in grams) as independent variables for each of the three lizard populations: $V = M_1 \times B^{M_2} \times 10^{M_3 \times T}$ (Andrews & Pough, 1985). A multiple regression model was used to fit the parameters: M_1 , M_2 , and M_3 . To model digestion rate (R ; in percentage per hour) as a function of body temperature (T ; in degrees Celsius), we utilized a second degree polynomial function to fit food passage time (in hours) data. The reciprocal of this function is represented as $R = 1/F_1 \times T + F_2 \times T^2 + F_3$ (Angilletta, 2001), F_1 , F_2 and F_3 are the digestion rate parameters.

We computed cumulative digestion and maintenance cost of the three lizard populations at every hour throughout a day, by inputting active and resting body temperatures obtained from fieldwork and published data into the two reaction norms. Wilcoxon signed-rank exact tests were used to estimate the differences in cumulative digestion and maintenance cost between the three lizard populations during resting and activity periods, respectively. Additionally, when assessing the effect of

climate change on metabolisms and calculating metabolisms of global agamid species, we incorporated body temperatures as predicted by biophysical models generated by NicheMapR (see *Potential effects of climate change on resting metabolisms*).

Potential effects of climate change on resting metabolisms

We integrated microclimate models, plus biophysical data and reaction norms to predict the changes in cumulative digestion and maintenance cost of lizards at resting status under climate change, from current values (2020–2021) to predicted values a 100 years into the future. The protocol for using reaction norms follows *Calculating cumulative digestion and maintenance cost*.

We used microclimate models (function “*micro_era5*” in “NicheMapR” package; Kearney et al., 2020) to generate hourly estimations of microclimate variables (e.g., solar radiation, air temperature, soil temperature, wind speed and relative humidity) for the three elevational populations in 2020 and 2021. These estimations were generated using climate data obtained from the ERA5 climate dataset, which we obtained with the “*mcera5*” package (Klinges et al., 2022). The resolution of the data was 0.25°. Based on the degree of warming, we set the “warm” parameter in the “*micro_era5*” function to simulate the different future climate scenarios. The highest degree of warming at our three study elevations is approximately 6°C after 100 years (Appendix S1: Section S1). A recent study suggested the temperature increase at nighttime is 1.2°C higher than during the daytime in Qinghai-Tibet Plateau (Cox et al., 2020). Therefore, we considered a symmetry warming scenario by assuming a uniform 6°C warming, and an asymmetry warming scenario by assuming a 5.4°C warming during the day and 6.6°C warming at night.

We used biophysical models (function “*ectotherm*” in NicheMapR; Kearney & Porter, 2020) to estimate body temperature with physiological traits and microclimate data (Kearney & Porter, 2020). We used the parameters of biophysical models for our three study populations from our recent work (Jiang et al., 2023), which suggested that physiological modeling could accurately predict active body temperatures of *P. vlangalii*. Because the thermoregulatory behavior of resting ectotherm is restricted and their body temperatures at rest are largely influenced by their surrounding environment (Colinet et al., 2015; Rutschmann et al., 2021), we used hourly soil temperatures recorded at various depths from three elevational study sites to validate the accuracy of our biophysical model’s predictions of

resting lizard body temperatures. We used the “*cor.test*” and “*rmse*” function (“stats” package) to perform a Spearman’s rank correlation on observed and predicted values of environmental temperatures. Root mean squared error (RMSE) and correlation coefficients indicated good model performance in predicting resting body temperatures (Appendix S1: Figure S1).

Global patterns of elevational variations in metabolisms for agamid lizards

To maximize the predictability of our empirical data, we selected diurnal and terrestrial agamid lizards, such as *P. vlangalii*, from a comprehensive global database of lizard traits (Meiri, 2018), and obtained distribution information for our focal species from a reptile distribution database (Roll et al., 2017). We combined the distributional ranges of 98 diurnal and terrestrial agamid species (a list of species is in Appendix S1: Table S3) and divided the combined distributional range into 1355 grid cells with a resolution of $2^\circ \times 2^\circ$. For each cell, we calculated the differences in cumulative digestion and maintenance cost over the hottest month (July for the Northern Hemisphere and January for the Southern Hemisphere) between the highest and lowest elevations within a grid cell. Grid cells with maximum elevational difference <500 m were removed from the analysis.

We extracted current (1986–2015) monthly climate data at a resolution of 0.04° from TerraClimate. We down-scaled these data into hourly microclimate data (air temperature, humidity, soil temperature, humidity, solar radiation, wind speed, etc.) using microclimate models (“*micro_terra*” in the “NicheMapR” R package) for each distribution region of these agamid lizards. To generate metabolisms for all our focal species, we used the parameters of biophysical and reaction norms from our *P. vlangalii* populations below 3400 m asl, which corresponds to the elevational range of most agamid lizards, as detailed in *Calculating cumulative digestion and maintenance cost* and *Potential effects of climate change on resting metabolisms*.

RESULTS

Lizards at 3400 and 2600 m asl rest at similar depths (46.63 ± 1.10 cm and 47.22 ± 1.13 cm, respectively), while lizards at 4200 m rest at a significantly shallower depth (33.96 ± 0.98 cm; $F_{2,346} = 41.71$; $p < 0.001$; Figure 1). Body temperatures are lower in lizards from populations at higher elevations, and thermal differentials are larger for lizards in resting status (Figure 2a,b). The whole-day body temperatures of lizards from the three populations

all exhibit bi-modal distributions (Figure 2c). Although the three populations share a similar body temperature range during activity, lizards from the population at 2600 m experienced a completely different temperature profile (i.e., higher body temperatures) than lizards from the other two populations during rest. By comparing whole-day body temperatures of lizards with thermal tolerances reported in our previous study (Jiang et al., 2023), we found that all three lizard populations have a large thermal safety margin ($>9.4^\circ\text{C}$) at both cold and hot extremes (Figure 2a,b; Appendix S1: Table S1).

Food passage time exhibits a U-shaped relationship with body temperature, decreasing as body temperature increases, reaching a nadir between 30 and 35°C , and then increasing again (Appendix S1: Figure S2 and Table S2), while metabolic rate increases with body temperature monotonically (Appendix S1: Figure S3). Interestingly, compared with lizards from the two lower elevation populations, the digestion of lizards from 4200 m was relatively slower at high temperatures (35°C ; $t = 2.34$, $p = 0.021$; Appendix S1: Figure S2). However, these high-elevation lizards (4200 m asl) have faster maintenance cost rate than the other two lower elevation populations at most temperatures we tested (2600 m: $t = 2.76$, $p = 0.027$; 3400 m: $t = 3.57$, $p = 0.004$; Appendix S1: Figure S3). Hourly and daily cumulative digestion and maintenance cost of lizards during active and resting states were calculated using fitted reaction norms (as above), accounting for activity time periods and body temperatures experienced in the field. Assuming no difference in food availability and gut space between populations, lizards from 2600 m were found to digest more food per day than lizards from the other two populations (Figure 3b), while lizards from 4200 m had higher maintenance costs per day than lizards from the other two populations (Figure 3c,d). It is noteworthy that elevational variation in cumulative digestion of lizards is greater when resting than during activity (Figure 3b), while variation in maintenance cost is greater during activity than during periods of rest (Figure 3d).

Climate-driven changes in cumulative digestion and maintenance cost during activity were larger than those during rest (Figure 4). Interestingly, the elevational variation in symmetric climate-change impact on cumulative digestion was larger for resting status than active status, while the opposite held for the elevational variation in climate change impact on maintenance cost (Figure 4a,c). We also repeated our analyses for a climate change scenario that considered asymmetric warming (SSP5-8.5), where the magnitude of warming is disproportionately higher at night. Under this scenario, the climate-driven changes in

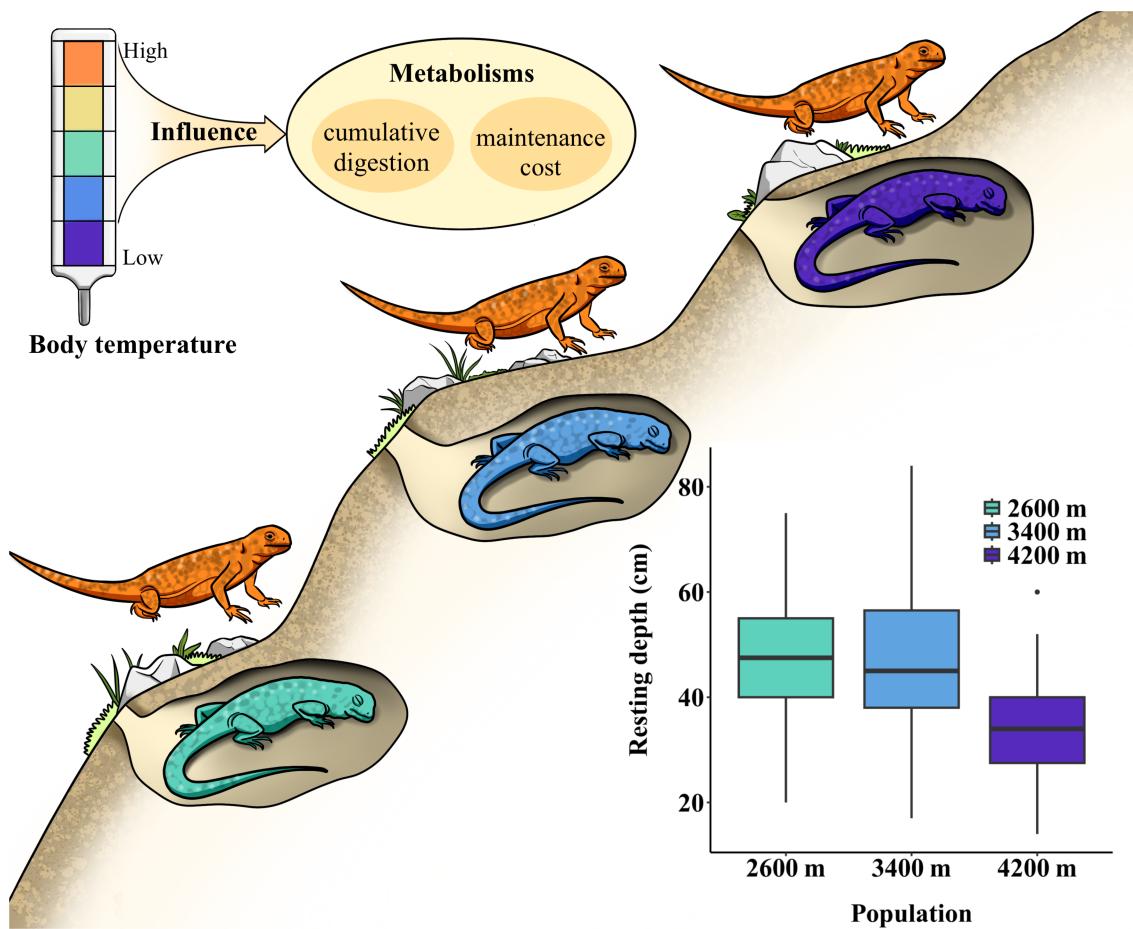


FIGURE 1 A schematic for the study. The boxplot shows the resting depth of *Phrynocephalus vlangalii* at three elevations. The color of lizards represents their body temperature. Lizards exhibit the same color during their active status, suggesting that lizards from different elevations can regulate their body temperature to a similar level through behavioral thermoregulation. Lizards exhibit different colors during their resting status, suggesting that burrowing depth did not buffer the elevational variation in body temperature of resting lizards. Metabolisms of lizards will be influenced by body temperatures. Illustration credits: Zi-Xuan Chen (diagram) and Zhong-Wen Jiang (boxplot).

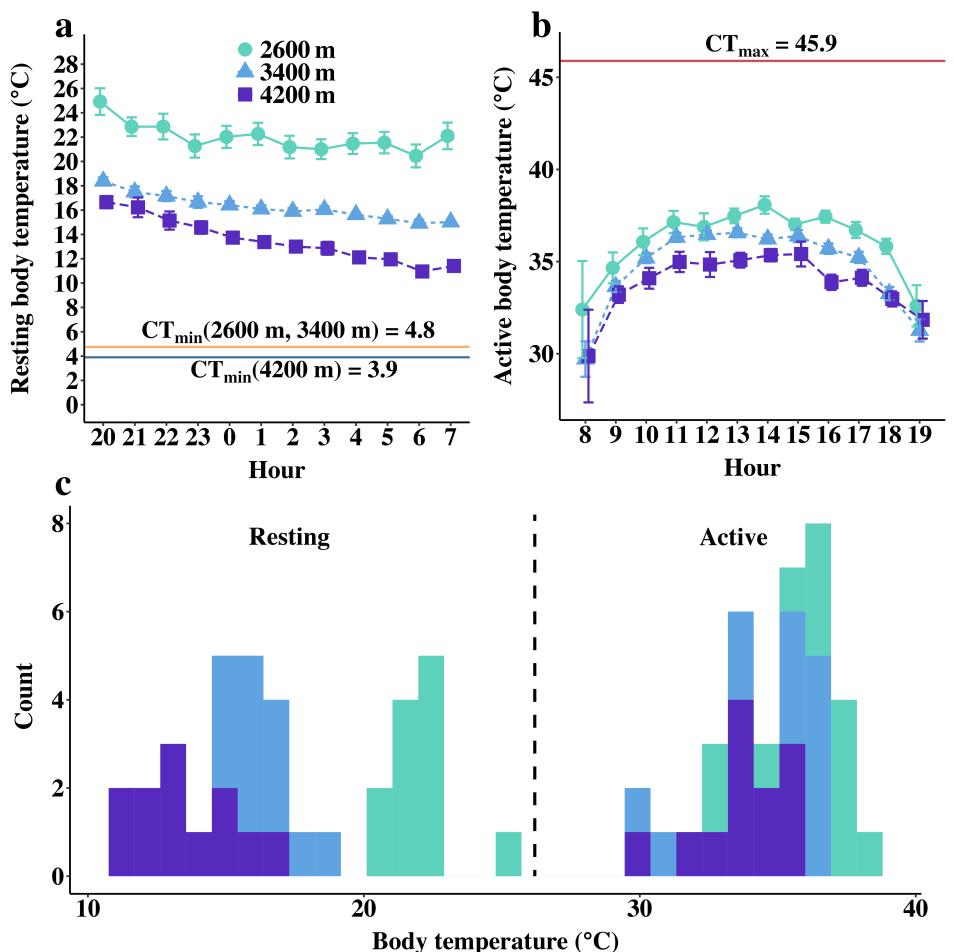
cumulative digestion and maintenance cost during resting status increased by at least 18.7% and at least 16.3%, respectively, compared with those under the symmetric warming scenario (Figure 4b,d).

We conducted a global analysis within a combined distribution range of 98 agamid species for the hottest month. Despite the relatively lower overall contribution of resting status to cumulative digestion and maintenance cost, in 55% of our studied areas (divided by $2^\circ \times 2^\circ$ grid cells), the absolute differences in cumulative digestion between the highest and lowest sites (with an elevation difference of ≥ 500 m) are larger during resting status than during active status. In up to 40% of the areas studied, the absolute difference in maintenance cost between the highest and lowest sites was found to be greater during rest than during activity (Figure 5). Most of mentioned areas above are located at tropical and subtropical plateaus, such as Yunnan–Guizhou Plateau, Deccan

Plateau, Arabian Plateau, Iranian Plateau, and Ahagar Plateau, where mean daily temperature is relatively higher (Appendix S1: Figure S4a,b). It is noteworthy that, at a considerable proportion of areas (30% and 13%; Figure 5 contrast elevational pattern), the relative magnitude of cumulative digestion and maintenance cost between the lowest and highest elevations reversed when resting and active status was considered, respectively (Figure 5), in other words, at these areas, lizards from the lowest elevation digest faster and have higher maintenance cost than those from the highest elevation during rest but the reverse holds during activity.

DISCUSSION

Conducting research on resting animals is challenging for a variety of reasons, but is mainly due to difficulties



in locating resting sites, taking measurements overnight, and transforming the long-standing belief that resting status has a minor impact on ecological processes (Gaston, 2019). However, overcoming these difficulties is necessary to fully understand how both the resting and active status of animals affects ecological patterns. Our study, combining an empirical study on a widespread agamid species with mechanistic niche modeling on global agamid species, revealed that resting status made a major contribution to the trend of decreasing body temperature at higher elevations. We found that there would be larger elevational variation in hourly and cumulative digestion for resting lizards than for active lizards. Climate-change impact, estimated as the change in cumulative digestion, also shows larger elevational variation in resting lizards than active lizards. Our global analysis of 98 agamid

species suggests that in about half of their combined distributional range, the resting status of lizards contributes more to the elevational variation in their metabolisms than does active status.

Elevational variation in resting body temperatures triggered distinct metabolisms of resting lizards across elevations. Lizards at the highest elevations did not rest deeper than those at lower elevations to achieve similar resting body temperatures. On the contrary, they used a shallower average resting depth than the other two populations, which widened the gap in resting body temperatures of lizards between the three populations. The absence of behavioral thermoregulation in determining resting body temperature contrasts with the finding that lizards from all three populations actively thermoregulate to similar body temperatures during their active status.

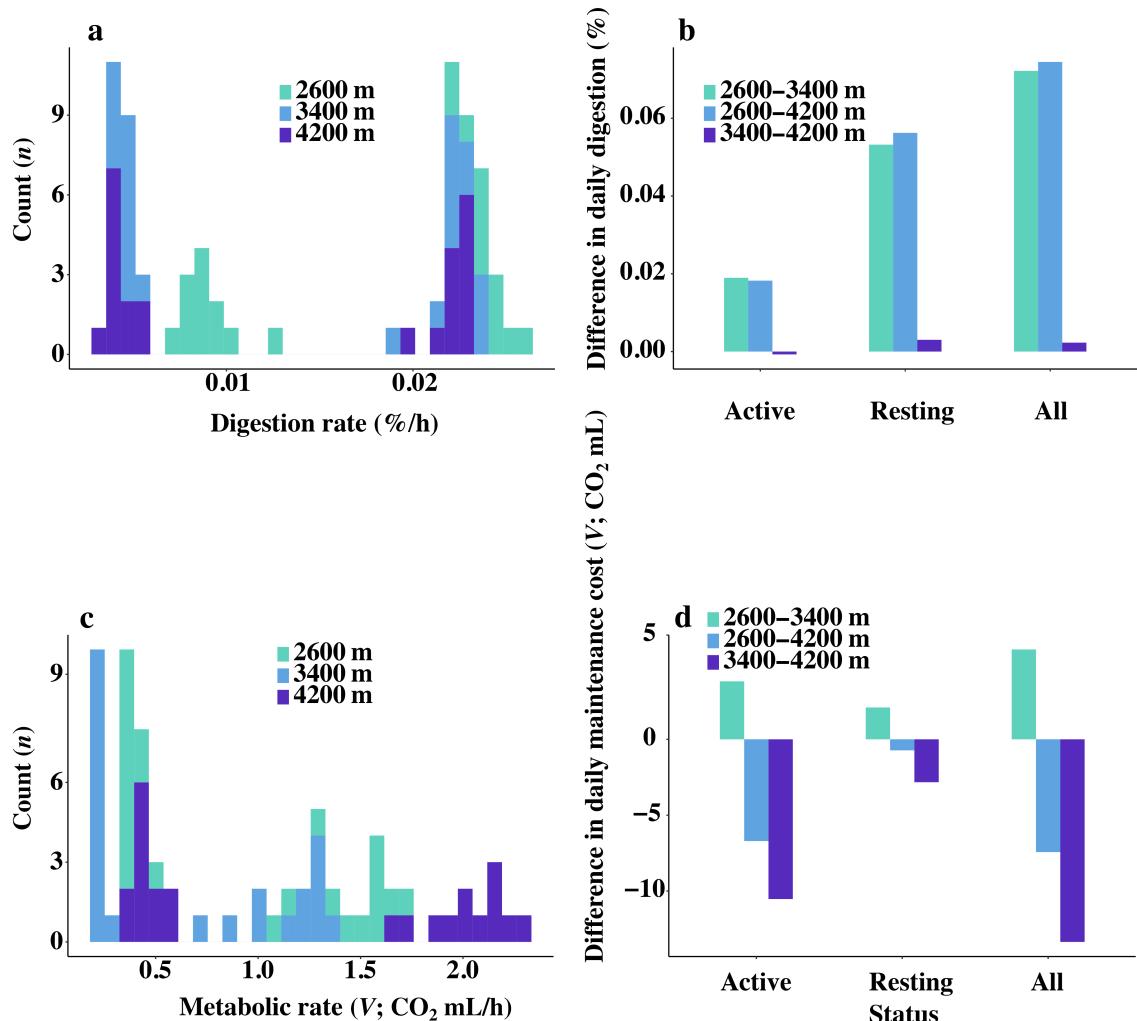


FIGURE 3 Distribution of hourly digestion (a) and maintenance cost (c) throughout a day and between-elevation differences in daily cumulative digestion (b) and maintenance cost (d).

What are the potential reasons for this divergence, we discuss from the perspectives of performance needs and thermoregulation ability. In theory, the functions that active lizards need to maintain (e.g., running speed, stamina, digestion) reach peak performance levels at similar body temperatures (Angilletta, 2001; Sinclair et al., 2016). Controlled experiments have shown that active lizards voluntarily select body temperatures that are close to their thermal optimum within a thermal gradient (Camacho & Rusch, 2017). Active lizards therefore have a convergent target temperature to achieve with thermoregulation. In contrast, resting lizards may meet trade-offs between functional goals (e.g., digestion speed, energy cost, hypothermic risk) with divergent thermal requirements. Like over-wintering lizards, resting lizards may benefit from fast digestion, low energy cost, and low risk of hypothermia (Huey et al., 2021), which requires high, low, and stable (and high) body temperatures, respectively. Resting lizards may therefore lack a unified

and sensible target temperature to achieve via thermoregulation. Moreover, our results showed large hypothermia thermal safety margins (minimum resting body temperature— CT_{\min}) for resting lizards including those at the highest elevation, which indicates that the use of deeper resting sites is not necessary. Resting lizards may also lack the ability to fully control their body temperatures across elevational gradients. Changing the depth of resting sites by digging to regulate resting body temperature may require more energy than achieving preferred body temperature by shuttling between sunlight and shade (Huey & Slatkin, 1976). The digging behavior also becomes more difficult during resting status due to low body temperatures. Elevational variation in environmental temperatures is greater at night (when diurnal lizards are likely to rest) than during the day, which further constrains a lizard's ability to regulate their body temperatures to be similar during resting status (Parton & Logan, 1981; Wang et al., 2017). At high

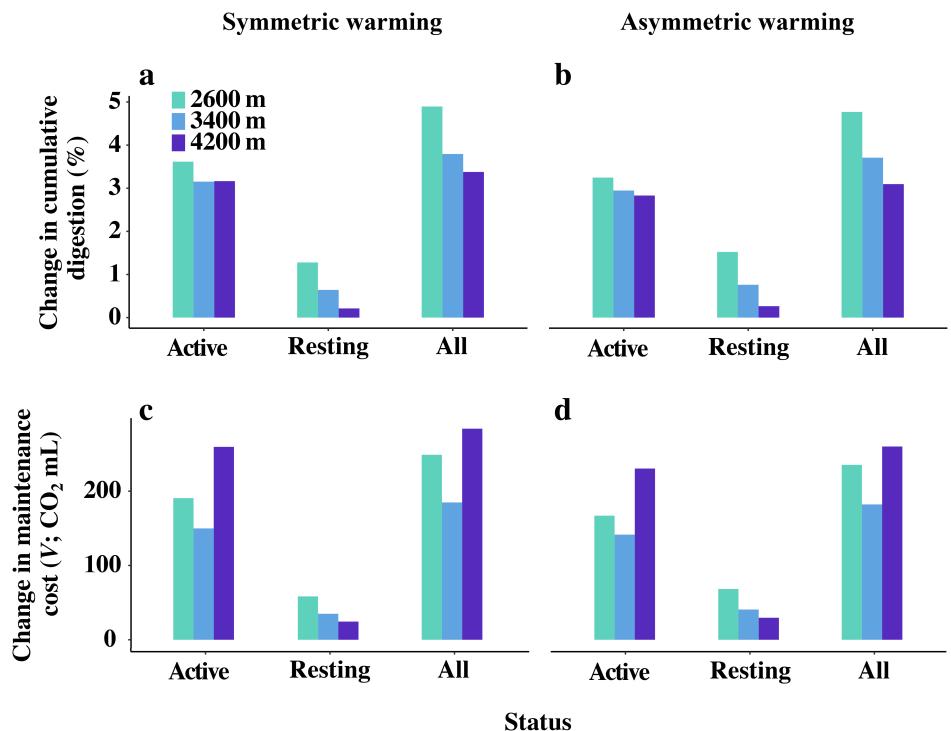


FIGURE 4 Change in cumulative digestion (a, b) and maintenance cost over July under a climate-change scenario (c, d) considering active hours, resting hours, and whole day, respectively. We also considered a symmetric warming (daytime warming = nighttime warming) scenario (a, c) and an asymmetric warming (daytime warming < nighttime warming) scenario (b, d), respectively.

elevations, the hypoxic environment may further prevent resting lizards from using deep sites, as the oxygen level decreases with depth (Hoback & Stanley, 2001; Weissberger et al., 2009).

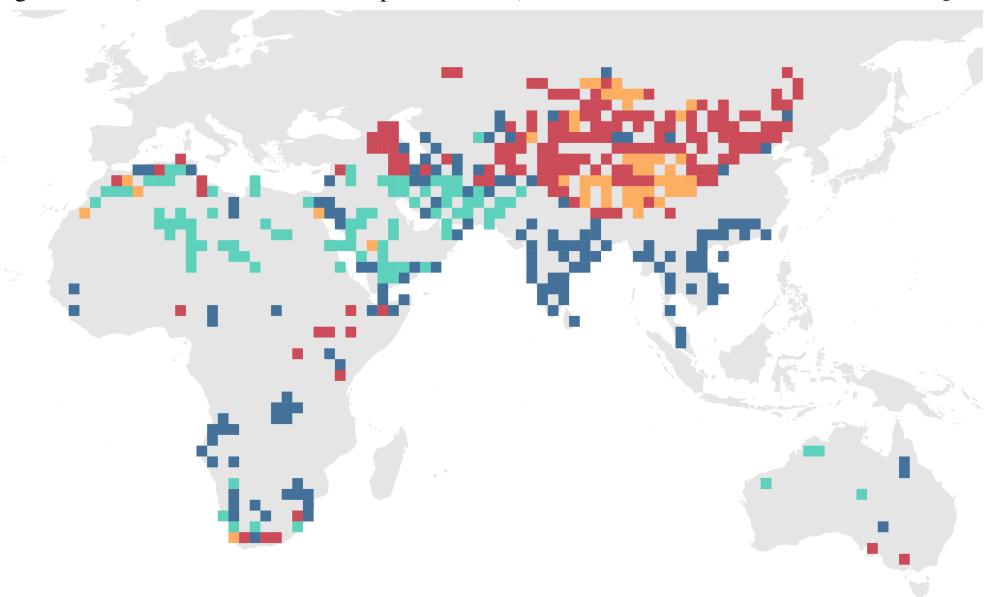
Since feeding rate is restricted by gut capacity (Angilletta, 2001), rapid digestion facilitates energy intake, and may result in a larger energy reserve (Kooijman, 2010), while a stable maintenance cost not only sustains fundamental life functions but also conserves the energy toward growth, reproduction, and other life activities (Congdon & Tinkle, 1982; Kooijman, 2010; Mueller & Diamond, 2001). In this study, cumulative digestion and maintenance cost (calculated as a sum of RMR) have been defined as metabolism, which, in turn, are determined by body temperature via reaction norms of digestion or metabolic rate. Resting lizards experience greater elevational variation in hourly and cumulative digestion than active lizards, while the reverse holds for the hourly and maintenance cost. What are the decisive cause influencing the contribution on variation in metabolism, we discuss from the perspectives of body temperatures and reaction norm. The relatively greater elevational variation in digestion during rest is caused by the relatively greater elevational variation in body temperature during rest. Although lizards at 4200 m experienced the lowest body temperatures among the three populations, they

can metabolize at higher rates than lizards from the other two populations, across a wide range of body temperatures (10–40°C), and especially at high body temperatures (20–35°C), which are often experienced by active lizards. Therefore, relatively high maintenance cost of lizards at 4200 m is the result of their distinct reaction norms. Under climate-change scenarios, the elevational variation in the increment of cumulative digestion was predicted to be larger during resting status than during active status, especially for the asymmetric warming scenario. This highlights the needs of considering resting status when comparing species' vulnerability under climate change across geographical gradients.

Our analysis of global agamid lizards reveals that the relative contribution of resting and active status in determining elevational differences in metabolisms is spatially heterogeneous. In about half (55% for cumulative digestion, 40% for maintenance cost) of the areas studied, the inter-population differences (absolute values; between the lowest and highest elevations) in metabolisms are greater during resting status than during active status. These areas are mainly located at tropical and subtropical plateaus, which provide sufficient solar radiation and high daytime temperatures to facilitate lizards at both low and high elevations to achieve a similar active body temperature. In contrast, the nighttime temperatures in

a Absolute difference in cumulative digestion

- Resting > Active (consistent elevational pattern; 36%) ■ Resting > Active (contrast elevational pattern; 19%)
- Resting < Active (consistent elevational pattern; 34%) ■ Rest < Active (contrast elevational pattern; 11%)



b Absolute difference in maintenance cost

- Resting > Active (consistent elevational pattern; 34%) ■ Resting > Active (contrast elevational pattern; 6%)
- Resting < Active (consistent elevational pattern; 53%) ■ Rest < Active (contrast elevational pattern; 7%)

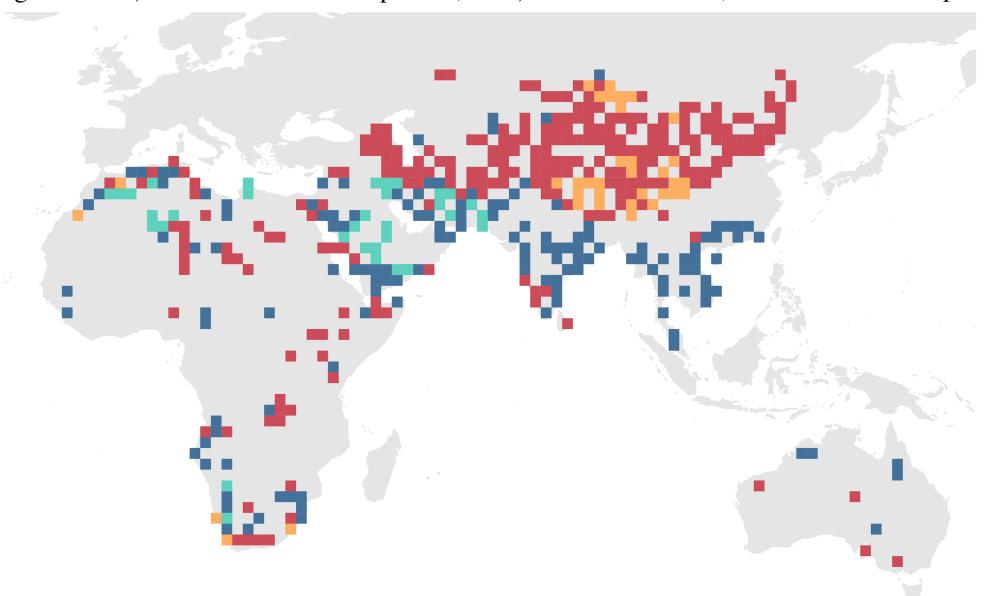


FIGURE 5 Absolute difference between the lowest and highest elevation regions in cumulative digestion and maintenance cost of Agamidae in the world. (a, b) The contribution of resting and active status on absolute difference between the lowest and highest elevation regions in cumulative digestion and maintenance cost of Agamidae in the world. “Resting > Active” represents resting status contributed more than active status in absolute difference, “Resting < Active” represents resting status contributed less than active status in absolute difference. “consistent elevational pattern” represents that both resting and active status show that lizards at the lowest elevation cumulates more or less than that at highest elevation region, but “contrast elevational pattern” represents resting and active status, where only one status shows that the lizards at lowest elevation region cumulate more or less than that at highest elevation region. The percentage values represent the proportion of particular pattern among all patterns. The resolution of every grid is $2^\circ \times 2^\circ$, and the minimum elevation difference is greater than 500 m in each grid.

these areas vary significantly across elevations compared with daytime temperatures, and the temperature gradients available during resting status are relatively narrow (Parton & Logan, 1981; Wang et al., 2017). These factors may limit a lizard's ability to regulate their resting body temperature, leading to greater variation in resting body temperatures of lizards between the lowest and highest elevations. Interestingly, in a small subset of these areas (30% and 13%; Figure 5 contrast elevational pattern), lizards at the lowest elevation might exhibit slower digestion and low maintenance cost than those at the highest elevation during activity, but exhibit faster digestion and high maintenance cost than their high-elevation congeners when resting. This is likely because, at these warm areas, extreme high temperatures during the day may lead to a reduction in activity time for lizards at the lowest elevation (Kearney, 2013; Kingsolver et al., 2013), while increasing activity time for lizards at the highest elevation (Jiang et al., 2023).

We believe that future research merits supplementing and expanding our study in the following aspects. While the use of food passage time as a proxy for assimilation has been widely common in previous studies (Angilletta, 2001; Huey et al., 2021), it remains unclear how much energy can be assimilated per hour during food passage time (Litmer & Beaupre, 2024). In addition, although in our study even the fastest digestion rates required over 24 h (Appendix S1: Figure S2 and Table S2), in other species, the gut may be filled in the morning, followed by significantly faster digestion. While empirical data are lacking, this suggests that digestion can be completed before nighttime, with minimal impact during rest. Our global analysis using uniform modeling lizards provides insights into the impact of geographical climate variation on the patterns at a global scale. However, the lack of species-specific physiological data may make our results deviate quantitatively at a local scale. In the future, the overall energy budget by quantifying energy input and output, along with gathering more information about the activity rhythms of animals (e.g., foraging rhythms) and their resting status (e.g., detailed data on resting locations) would give us a more complete picture on species' adaptation to present and future environments.

By using a combination of empirical work and mechanistic modeling, our study revealed the importance of resting status in determining the elevational variation in metabolisms of diurnal species. We also highlighted the needs to consider spatial heterogeneity when measuring the contribution of resting and active status on the elevational variation in metabolisms. Most importantly, our study suggested that resting status should be considered when investigating the elevational adaptation of species, particularly in regards to some species' traits, whose

geographical variations are largely subject to body temperature, and especially in tropical and subtropical areas.

AUTHOR CONTRIBUTIONS

Zhong-Wen Jiang, Liang Ma, and Wei-Guo Du conceived the idea of the study. Zhong-Wen Jiang, Shi-ang Tao, and Dan-yang Wu collected the data in field works. Zhong-Wen Jiang, Liang Ma, Cheng Wenda, and Chuyu Cheng performed data analysis and built models. Zhong-Wen Jiang, Liang Ma, and Wei-Guo Du led the writing of manuscript, and all co-authors participated in the revision. Liang Ma and Wei-Guo Du share equal leadership contributions as leaders of multidisciplinary research teams that worked in collaboration on this project.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (jzhongwen, 2024) are available in Zenodo at <https://doi.org/10.5281/zenodo.12716029>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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