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ORIGINAL PAPER

Thermal ecology of three coexistent desert lizards: Implications for habitat divergence and thermal vulnerability

Shu-Ran Li[1,2] · Yang Wang[1,2] · Liang Ma[1,2] · Zhi-Gao Zeng[1] · Jun-Huai Bi[3] · Wei-Guo Du[1]

Abstract How ectotherms exploit thermal resources has important implications for their habitat utilization and thermal vulnerability to climate warming. To address this issue, we investigated thermal relations of three sympatric lizard species (*Eremias argus, Eremias multiocellata*, and *Phrynocephalus przewalskii*) in the desert steppe of Inner Mongolia, China. We determined the thermoregulatory behavior, body temperature (T_b), operative temperature (T_e), selected body temperature (T_{sel}), and critical thermal maximum (CT_{max}) of adult lizards. Based on these physiological parameters, we quantified the accuracy and effectiveness of thermoregulation as well as thermal-safety margin for these species. The three species were accurate and effective thermoregulators. The *P. przewalskii* preferred open habitats, and had a higher T_b than the two *Eremias* lizards, which preferred shade habitats and shuttled more frequently between the shade and sun. This indicated that the three sympatric lizards have different thermoregulatory behavior and thermal physiology, which might facilitate their coexistence in the desert steppe ecosystem. In addition, the *P. przewalskii* had higher T_{sel} and CT_{max}, and a wider thermal-safety margin than the two *Eremias* lizards,

suggesting that the two *Eremias* lizards would be more vulnerable to climate warming than *P. przewalskii*.

Introduction

Unlike endotherms that maintain a high and relatively constant body temperature via metabolic heat production, ectotherms mainly regulate their body temperature by behaviorally exploiting thermal resources. As a result, the body temperature of ectotherms is strongly influenced by the thermal quality of microhabitats (Hertz et al. 1993; Scheers and Van Damme 2002; Besson and Cree 2010; Shen et al. 2010). Moreover, temperature is a dominant factor that can dramatically determine behavioral and physiological processes (e.g., locomotion, foraging, growth, and reproduction) in most ectotherms (Bennett 1980; Huey 1982; Vandamme et al. 1991; Angilletta et al. 2002). Therefore, the thermal heterogeneity of the habitat is an essential resource for ectotherms, in addition to other resources such as water and food (Magnuson et al. 1979; Hertz 1992a). The partitioning of these resources may facilitate the coexistence among sympatric organisms, which has long been a central topic in ecological studies and has attracted great scientific attention (Ruibal 1960; Schoener 1974; Daly et al. 2008).

Thermoregulation plays an important role in habitat selection and, therefore, the distribution of terrestrial ectotherms, such as reptiles (Ruibal 1960; Rocha and Vrcibradic 1996; Melville and Schulte 2001; Blouin-Demers and Weatherhead 2001a, 2002). Some studies have found that sympatric reptiles differing in thermal preferences usually occupy different thermal habitats (Ruibal 1960;

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Scheers and Van Damme 2002; Du et al. 2006; Row and Blouin-Demers 2006; Lelievre et al. 2011; Sears and Angilletta 2015; but see; Huey 1982). For example, species that show a preference for high body temperatures select open and warm habitats, whereas those that prefer low body temperatures choose shade and cold habitats (Ruibal 1960; do Amaral et al. 2002; Scheers and Van Damme 2002; Shen et al. 2010; Hertz et al. 2013). However, as the thermal resource partitioning is confounded by microhabitat divergence in sympatric species that occupied distinctive microhabitats with different thermal quality, such studies could not decouple the respective effects of habitat preferences and thermal requirements on species coexistence. Direct evidence of the impact of thermal requirements on habitat preferences comes from studies on syntopic species within a smaller area where they share a microhabitat with many similar properties, such as vegetation cover, food, and water. For example, microhabitat use is related to differences in thermoregulation in syntopic lizards; thermoregulatory requirements may promote species coexistence or microhabitat divergence if the species have similar or different thermal preferences (Adolph 1990; Hertz 1992a, b; Martinvallejo et al. 1995; Grover 1996; Corbalan et al. 2013). Therefore, syntopic reptiles provide excellent opportunities for studying the influence of thermoregulatory behavior on habitat selection, which has important implications for how similar species may coexist.

Lizards have been considered as an ideal research model to study the thermal biology of ectotherms (Huey et al. 1974; Pianka 1986). In the field, most lizards can maintain their body temperatures within a narrow range to optimize their physiological performances by behavioral thermoregulation, such as basking, shuttling between sun and shade patches, and altering activity time (Adolph 1990; Hertz 1992b; Angilletta 2009; Dubois et al. 2009). Desert lizards have aroused great interest from thermal ecologists, because they experience unique thermal environments (e.g., extremely hot temperatures in summer and cold in winter, high fluctuation in daily temperature) (Grant and Dunham 1988; Bauwens et al. 1999; Sartorius et al. 2002; Lara-Resendiz et al. 2015). Moreover, desert lizards are vulnerable to climate warming as they are already facing extreme temperatures in open habitats that approach or exceed their physiological thermal tolerance and shaded habitats providing suitable thermal microenvironment for thermoregulation are limited (Huey et al. 2009, 2012; Sunday et al. 2014). Heatwole (1970) developed the concept of thermal-safety margin (TSM) that has recently been used for assessing the vulnerability of ectotherms to climate warming (Deutsch et al. 2008; Clusella-Trullas et al. 2011; Sunday et al. 2014). The TSM is defined as the difference between a species' thermal tolerance and its body temperature (Heatwole 1970), the difference between a species' thermal tolerance and its ambient temperature (Sunday et al. 2014), or the difference between a species' thermal optimum and its ambient temperature (Deutsch et al. 2008; Huey et al. 2009; Clusella-Trullas et al. 2011). All else being equal, those species with a narrower TSM are likely to be more vulnerable to climate warming (Deutsch et al. 2008; Sunday et al. 2014).

The majority of studies on the thermal biology of desert lizards focus on species from Africa (Huey and Pianka 1977; Huey et al. 1977; Bowker 1984; Bauwens et al. 1999), America (Cowles and Bogert 1944; Grant and Dunham 1988; Sartorius et al. 2002), and Australia (Heatwole 1970; Pianka 1971; Bennett and John-Alder 1986; Melville and Schulte 2001). However, such studies, despite receiving increasing attentions in recent times (Luo et al. 2005; Li et al. 2009; Qu et al. 2011; Tang et al. 2013), are still rare in Asia, where the world's largest arid area is located (Middleton and Thomas 1997). Studies from Asian deserts not only deepen our understanding of the thermal requirements and habitat use of desert lizards through extensively comparative studies among different continents (Cowles and Bogert 1944; Pianka 1989), but also provide important implications for the management and conservation of local species (Lara-Resendiz et al. 2015). In this study, we compared the thermal ecology of three lizard species (*Eremias argus, Eremias multiocellata*, and *Phrynocephalus przewalskii*) occupying the same microhabitat in the eastern edge of the Chinese Hobq Desert. Previous studies have determined the preferred body temperature and thermal tolerance of these species from different localities (Luo et al. 2005; Li et al. 2009; Qu et al. 2011; Tang et al. 2013). Nonetheless, how thermal biology differs among these species and its implications for resource partitioning, coexistence, and thermal vulnerability are largely unexplored. We conducted both field and laboratory experiments to determine thermal quality of microhabitat, thermoregulation, and thermal physiology in these three lizards. Our aim was to understand the contribution of the interspecific difference in thermal biology to microhabitat differences and the vulnerability of these species to climate warming. We hypothesized that (1) lizard species preferring higher temperatures would select more open habitats with higher operative temperatures than those preferring lower temperatures and (2) lizard species with lower thermal tolerance and narrower TSM would be more vulnerable to climate warming compared with those sympatric species with higher thermal tolerance and wider TSM in that same habitat.

Materials and methods

Study site and species

Our study was conducted at Shierliancheng Field Station, Institute of Grassland Research of the Chinese Academy of

Agricultural Sciences (40.2N, 111.1E; elevation 1036 m). This area is an arid zone with average annual precipitation of 300–380 mm. The maximum mean monthly air temperature is recorded in July (39.1 °C) and the minimum in January (−32.8 °C). In this arid area, there are mainly three ground-dwelling lizard species. The Mongolian racerunner (*Eremias argus*) is an oviparous lacertid lizard [adult snout–vent length (SVL) 51.9–65.4 mm, body mass (BM) 3.2–4.5 g] that generally occupies grassy sand dunes and thickets. The multiocellated racerunner (*Eremias multiocellata*) is a viviparous lacertid lizard (adult SVL 54.0–67.3 mm, BM 4.7–8.2 g) that generally occupies arid or semi-arid regions. The steppe toad-headed agama (*Phrynocephalus przewalskii*) is oviparous (adult SVL 43.6–55.5 mm, BM 3.3–7.0 g) and generally inhabits desert, semi-desert, or grassland habitats (Zhao 1999). These three species coexist in the natural habitat of desert steppe (mainly covered with *Artemisia ordosica* shrub) in Inner Mongolia of China, including our study site.

Thermal resources utilization

We observed the thermoregulatory behavior of the lizards in 12 square enclosures ($5 \times 5 \times 0.5$ m, $L \times W \times H$). These enclosures were constructed using iron sheets in the natural habitat, keeping the vegetation in the enclosures intact. The environmental conditions inside the enclosures were similar to those outside the enclosures in terms of ground surface temperatures (35.5 ± 1.0 vs. 35.4 ± 1.5 °C), relative humidity (50.2 ± 0.4 vs. $48.4 \pm 10.2\%$), and light intensity (23371.8 ± 1438.0 vs. 23167.8 ± 1474.8 lx). Sixteen lizards (sex ratio = 1:1) of each species were placed in four separate enclosures with four lizards in each enclosure. The SVL and BM of lizards were 56.3 ± 0.9 mm and 4.2 ± 0.1 g for *E. argus* ($n = 16$), 62.2 ± 0.9 mm and 6.3 ± 0.2 g for *E. multiocellata* ($n = 16$), and 49.1 ± 0.7 mm and 4.6 ± 0.1 g for *P. przewalskii* ($n = 16$). For identification, we painted a number on the back of lizards with a marker pen. Prior to being observed, lizards were kept in the enclosure for 72 h, so that the lizards could become familiar with the enclosures and the observer. During the study, the observer stood 2 m away from the edge of the enclosure. The study was conducted from August 4 to 19, 2012 and observations were made between 08:00 and 18:00, 2 h after sunrise and 1 h prior to sunset, on sunny days. In every 20-min observation period, we recorded the species, lizard identity, substrate (noted as bare ground, grass cluster, edge of grass cluster, or burrow), and whether lizards were on substrates in full sun, full shade, or filtered sun. We calculated the percentages of time that the lizards spent in different solar conditions and the frequencies at which the lizards shuttled between different substrates in each observation period. These percentages were calculated for the following

time intervals for each day: morning (08:00–11:00), midday (11:00–14:00), early afternoon (14:00–16:00), and late afternoon (16:00–18:00).

Operative temperatures and field body temperatures

From August 12 to August 31, 2013, we measured operative temperatures (T_e) using 16 copper models (sealed pipes with a diameter of 15 mm, and a length of 70 mm) following the protocol of Hertz et al. (1993). Each model was inserted with an iButton (DS1921, MAXIM Integrated Products Ltd., USA) to record temperatures. T_es were calibrated against one lizard of each species under a heat lamp (temperature ranging from 23 to 48 °C, $n = 21$) (Hertz 1992b; Bakken and Angilletta 2014). There was a significant linear regression relationship between lizard body temperatures (T_bs) and T_es (*E. argus*: slope = 0.983, intercept = 1.258, $R^2 = 0.997$, $P < 0.001$; *E. multiocellata*: slope = 0.991, intercept = 0.954, $R^2 = 0.998$, $P < 0.001$; *P. przewalskii*: slope = 0.993, intercept = 0.869, $R^2 = 0.997$, $P < 0.001$). The average T_es calibrated by T_bs of the three species were used to evaluate the operative thermal environment in our study site. We placed the models randomly on the ground to record temperatures every 30 min. The models were exposed to different solar conditions including full sun, full shade, and filtered sun. Along with T_e, field T_bs were collected on sunny days. For this, we captured adult lizards by hand in the field, ensuring that we did not chase the lizards longer than 20 s. Once caught, T_bs of the lizards were measured immediately to the nearest 0.1 °C by inserting a probe of UT325 electronic thermal meter (Shenzhen Meter Instruments, Shenzhen, China) into cloacals (about 5 mm). For each individual lizard, we also recorded the time of day, species, and the sex before releasing the lizard at the site of capture.

Selected body temperatures (T_{sel}) and critical thermal maximum (CT_{max})

To measure the selected body temperatures of lizards, we transferred 59 adult lizards (21 *E. argus*, 21 *E. multiocellata*, and 17 *P. przewalskii*) to our laboratory in the Institute of Zoology (Beijing) in September 2013. The animals were housed individually in plastic terraria ($60 \times 30 \times 40$ cm, $L \times W \times H$). Two 100-W incandescent lamps (20189.7 ± 39.3 lx) were suspended above one end of the terraria to create a temperature gradient from 20 to 45 °C from 7:00 to 19:00. The mean night-time temperature (20 °C) in the terraria was similar to that in the field (20.6 °C). Photoperiod was provided by fluorescent lamps from 6:00 to 19:00. Food (mealworm, *Tenebrio molitor*) and water were provided *ad libitum*. We recorded body temperatures of the active lizards by inserting a probe of UT325 electronic thermal meter

into cloacals every 2 h from 08:00 to 18:00 on two consecutive days. The selected temperature range was defined as the central 80% of all temperature recordings for each lizard (Bauwens et al. 1996). The average values of all the upper and lower limits of the selected temperatures for a species represented its set point temperature range (Hertz et al. 1993).

Next, we measured the critical thermal maximum (CT_{max}) of lizards in an incubator (Binder KB 240, Binder GmbH, Tuttlingen, Germany). Lizards were maintained at 28 °C for 2 h and then heated at the rate of 0.1 °C min^{-1}. We monitored the behavior of lizards during heating, and measured cloacal temperatures of lizards once they lost righting response. The mean cloacal temperatures were calculated as CT_{max} (Du et al. 2000; Qu et al. 2011). All lizards recovered.

Thermoregulatory accuracy and effectiveness

Following the procedure of Hertz et al. (1993), we calculated the following indices: d_b (the accuracy of thermoregulation) and d_e (the thermal quality of the habitat). When T_b or T_e is within the set point range, the corresponding d_b or d_e equals zero. When T_b or T_e is below the range, d_b and d_e are calculated as the difference between the lower limit of T_{sel} and T_b, and T_e, respectively. When T_b or T_e is above the range, d_b and d_e are calculated as the difference between T_b (and T_e in the case of d_e) and the upper limit of T_{sel}.

To estimate the thermoregulatory effectiveness of lizards, we used two indices: E (calculated as $1 - $ mean d_b/mean d_e) and $d_e - d_b$. The index of E approaches zero when animals do not thermoregulate, and tends to a value of one when animals thermoregulate effectively (Hertz et al. 1993). The $d_e - d_b$ index measures the degree to which animals depart from thermoconformity (Blouin-Demers and Weatherhead 2001b).

Thermal-safety margins

We calculated thermal-safety margin (TSM) of each species in the following four ways.

According to Sunday et al. (2014), the TSM is calculated as the difference between an organism's critical thermal maximum (CT_{max}) and maximum operative temperatures ($T_{e, max}$), which is the mean maximum hourly T_e:

$$TSM = CT_{max} - T_{e, max}. \tag{1}$$

According to Clusella-Trullas et al. (2011), the TSM is calculated as the difference between an organism's thermal optimum (T_{opt}, represented by the mean T_{sel}) and habitat temperatures (T_{hab}, represented by the mean temperature of the warmest season):

$$TSM = T_{opt} - T_{hab, \text{mean of the warmest season}}. \tag{2}$$

According to Deutsch et al. (2008), the TSM is calculated as the difference between an organism's thermal

optimum (T_{opt}, represented by the mean T_{sel}) and habitat temperatures (T_{hab}, represented by the mean annual air temperature):

$$TSM = T_{opt} - T_{hab, \text{annual mean}}. \tag{3}$$

According to Heatwole (1970), the TSM is calculated as the difference between an organism's critical thermal maximum (CT_{max}) and mean body temperatures ($T_{b, mean}$), which is the mean body temperature during the daily active period from 9:00 to 17:00:

$$TSM = CT_{max} - T_{b, mean}. \tag{4}$$

Statistical analyses

All data were analyzed with IBM SPSS Statistics Version 20 (IBM Corp). Dependent variables were checked for normality with Shapiro–Wilk test and homogeneity of variances with Levene's test. Data were transformed to achieve the assumptions of parametric tests when necessary. If the data did not meet the assumptions of parametric tests, non-parametric tests were used. Significant effects were taken as $P \leq 0.05$, and all values are represented as mean ± SE. Linear mixed-effects models (LMMs) were used to evaluate thermoregulatory behavior (the percentages of time that lizards spent in different sunlight conditions and the number of lizards shuttling between different substrates), with species, sex, time of day and their interactions (species × sex, species × time, species × sex × time) as fixed factors, and lizard identity and enclosure as random factors. The degrees of freedom were calculated by Satterthwaite approximation. The percentages of time spending under different sunlight conditions were arcsine-square root transformed and shuttling frequencies (the number of shuttling between different substrates) were $\log(x+1)$ transformed. We used repeated-measures ANOVA to compare T_e among microhabitats with time of day as within-subject variable. Mauchly test was used to check the sphericity assumption, and the Huynh–Feldt correction was used to adjust for sphericity violations when necessary. A univariate general linear model was used to analyze T_b with species, sex, time, and their interactions (species × sex, species × time, and species × sex × time) as fixed factors. We used one-way ANOVA to detect the among-species difference in T_{sel} and CT_{max}. Bonferroni test was used for multiple comparisons. In addition, we performed Kruskal–Wallis test to identify the differences of d_b and d_e among species.

Results

Thermal resources utilization

The steppe toad-headed agama (*P. przewalskii*) spent more time in full sun sites than the two *Eremias* species ($F_{2, 10.061} = 8.526$, $P = 0.007$) (Fig. 1a). Active time in filtered sun sites was similar among species ($F_{2, 9.450} = 0.344$, $P = 0.718$), and did not change through the day ($F_{3, 47.190} = 0.425$, $P = 0.736$) (Fig. 1b). The two *Eremias* lizards preferred occupying full shade sites compared to *P. przewalskii* ($F_{2, 11.426} = 23.411$, $P < 0.001$) (Fig. 1c). In addition, *E. argus* and *E. multiocellata* shuttled between sun and shade more frequently than *P. przewalskii* ($F_{2, 9.643} = 9.066$, $P = 0.006$), with more shuttling movements noted during the mid-day observation period ($F_{3, 39.420} = 12.396$, $P < 0.001$) (Fig. 2). In addition, there was no sexual difference in the utilization of thermal resources in each species (All $P > 0.200$).

Operative temperatures and field body temperatures

T_es differed among microhabitats ($F_{2, 13} = 22.867$, $P < 0.001$), increasing from shade to sun sites (full shade, 28.9 ± 1.3 °C; filtered sun, 36.5 ± 0.7 °C; full sun, 41.2 ± 1.3 °C). T_es fluctuated dramatically during the day (Huynh–Feldt correction, $F_{1.998, 25.977} = 72.767$, $P < 0.001$). The significant interaction (Huynh–Feldt correction, $F_{3.997, 25.977} = 4.9637$, $P = 0.004$) indicated lower fluctuation of T_es at sites with more shade (Fig. 3).

T_bs fluctuated through time of day ($F_{8, 279} = 10.708$, $P < 0.001$), with no interaction between species and time ($F_{14, 279} = 1.575$, $P = 0.086$) (Fig. 3). In addition, T_bs significantly differed among species ($F_{2, 279} = 21.628$, $P < 0.001$), with higher T_b noted in *P. przewalskii* (38.1 ± 0.2 °C, $n = 129$) and *E. argus* (37.5 ± 0.2 °C, $n = 84$) than in *E. multiocellata* (36.6 ± 0.2 °C, $n = 116$), but did not differ between sexes ($F_{1, 279} = 1.149$, $P = 0.285$) (Fig. 4). There was no interaction between species and sex on T_bs ($F_{2, 279} = 1.315$, $P = 0.270$).

Selected body temperatures and critical thermal maximum

The two *Eremias* lizards had similar mean T_{sel}s, which were lower than that of *P. przewalskii* (Table 1). The upper
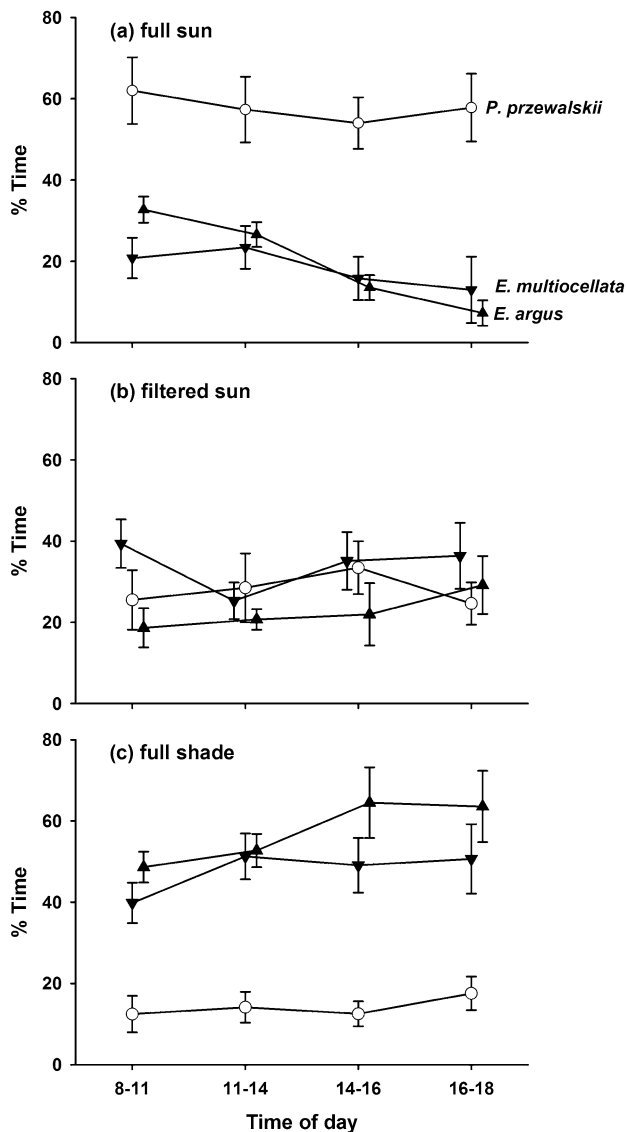

Fig. 1 Diurnal variation in the use of solar microclimates, **a** full sun, **b** filtered sun, and **c** full shade, by the three species of lizards (*Eremias argus, E. multiocellata*, and *Phrynocephalus przewalskii*) in the desert steppe of Inner Mongolia, China
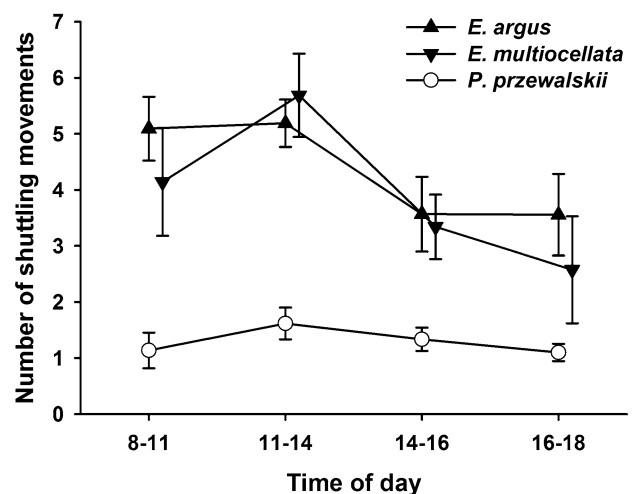

Fig. 2 Diurnal variation of movements in the three species of lizards (*Eremias argus, E. multiocellata*, and *Phrynocephalus przewalskii*) from the desert steppe of Inner Mongolia, China
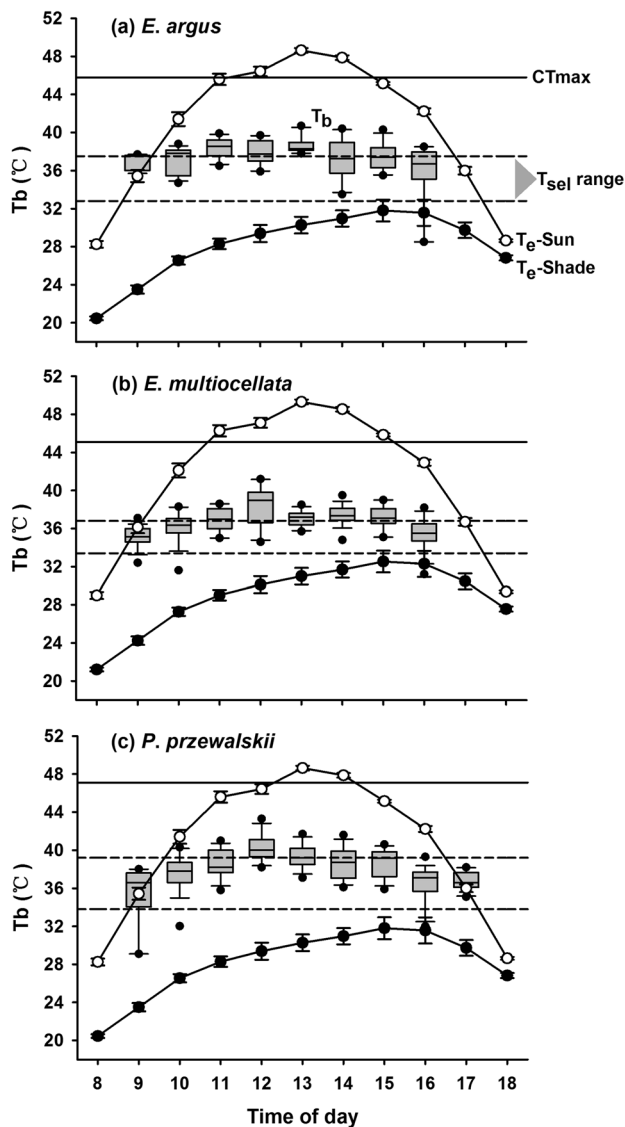
Fig. 3 Body temperature, selected body temperature (T_{sel}) range, sun and shade operative temperatures (T_e), and critical thermal maximum (CT_{max}) of the three lizards **a** *Eremias argus*, **b** *E. multiocellata*, and **c** *Phrynocephalus przewalskii* in the desert steppe of Inner Mongolia, China
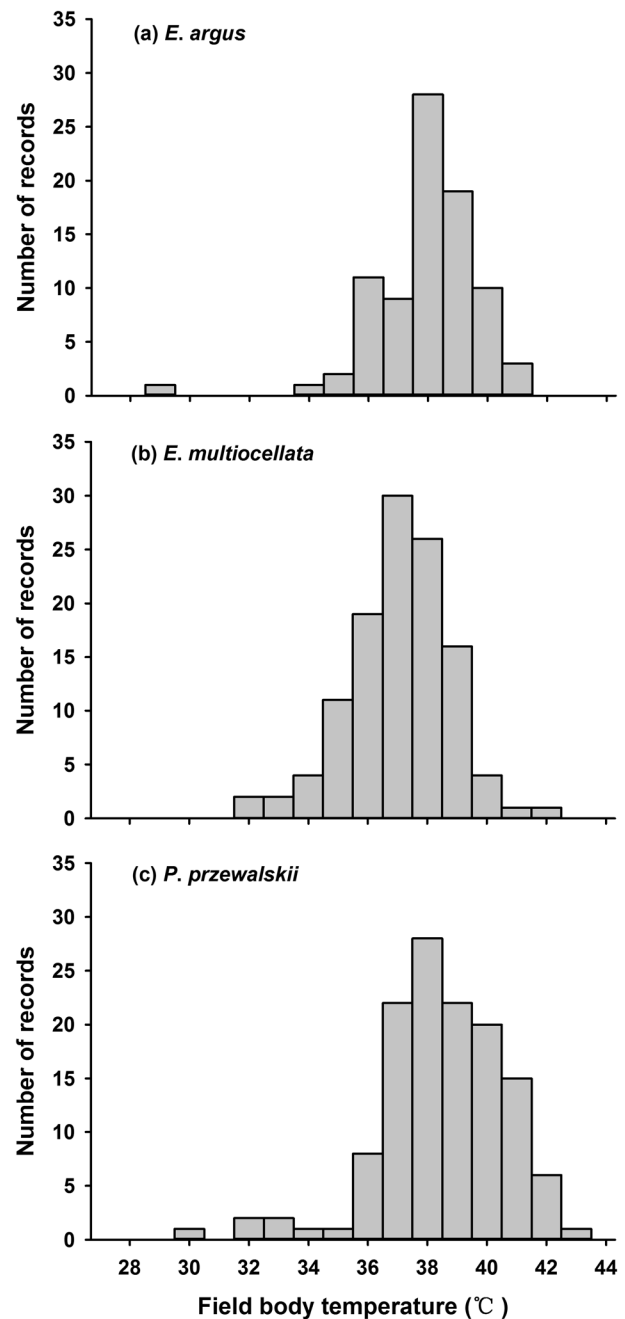

Fig. 4 Distribution of field body temperatures (T_b) in the three lizards **a** *Eremias argus*, **b** *E. multiocellata*, and **c** *Phrynocephalus przewalskii* from the desert steppe of Inner Mongolia, China

limits of T_{sel} differed significantly among species, but the lower limits of T_{sel} did not (Table 1). CT_{max} of lizards was the highest in *P. przewalskii* and the lowest in *E. multiocellata*, with *E. argus* in between (Table 1).

Thermoregulatory accuracy and effectiveness

Thermoregulatory accuracy (d_b) differed among species, with lower d_b and thus higher thermoregulatory accuracy in *P. przewalskii* than in the two *Eremias* lizards ($\chi^2 = 10.555$, $df = 2$, $P = 0.005$; Table 2). The thermal quality of the habitat (d_e) did not differ among species

($\chi^2 = 3.158$, $df = 2$, $P = 0.206$; Table 2). The two thermoregulatory indices presented different patterns of between-species difference in thermoregulatory effectiveness. Amongst the three species, the most effective thermoregulator was *E. multiocellata* according to the $d_e - d_b$ index, but was *P. przewalskii* according to the index of E (Table 2).

Table 1 Selected body temperatures and critical thermal maximum (CT_{max}) of the three species of lizards (*Eremias argus, E. multiocellata*, and *Phrynocephalus przewalskii*) in the desert steppe of Inner Mongolia, China

Species	n	Mean (°C)	Upper limit (°C)	Lower limit (°C)	CT_{max} (°C)
E. argus	21	35.3 ± 0.2^a	37.5 ± 0.2^b	32.8 ± 0.3	45.8 ± 0.1^b
E. multiocellata	21	35.2 ± 0.2^a	36.8 ± 0.1^a	33.4 ± 0.2	45.1 ± 0.1^a
P. przewalskii	17	36.6 ± 0.2^b	39.2 ± 0.1^c	33.9 ± 0.3	47.1 ± 0.2^c
Statistical significance		$F_{2,56} = 16.044$, $P < 0.001$	$F_{2,56} = 53.642$, $P < 0.001$	$F_{2,56} = 3.087$, $P = 0.053$	$F_{2,56} = 65.071$, $P < 0.001$

Different superscript letters denote significant differences between species in post-hoc analyses

Table 2 Thermoregulatory accuracy and effectiveness of the three species of lizards (*Eremias argus, E. multiocellata* and *Phrynocephalus przewalskii*) in the desert steppe of Inner Mongolia, China

Species	d_b (°C)	d_e (°C)	$d_e - d_b$ (°C)	E
E. argus	0.65 ± 0.10	4.94 ± 0.39	4.29	0.87
E. multiocellata	0.60 ± 0.08	5.50 ± 0.40	4.90	0.89
P. przewalskii	0.42 ± 0.07	4.65 ± 0.41	4.23	0.91

Table 3 Thermal-safety margins (TSM) of the three species of lizards (*Eremias argus, E. multiocellata*, and *Phrynocephalus przewalskii*) in the desert steppe of Inner Mongolia, China

Species	TSM^a (°C)	TSM^b (°C)	TSM^c (°C)	TSM^d (°C)
E. argus	−3.9	27.5	11.6	8.3
E. multiocellata	−4.6	27.4	11.5	8.5
P. przewalskii	−2.6	28.8	12.9	9

[a] $TSM = CT_{max} - T_{e, max}$, according to Sunday et al. (2014)

[b] $TSM = T_{opt} - T_{hab, mean\ of\ the\ warmest\ season}$, according to Clusella-Trullas et al. (2011)

[c] $TSM = T_{opt} - T_{hab, annual\ mean}$, according to Deutsch et al. (2008)

[d] $TSM = CT_{max} - T_{b, mean}$, according to Heatwole (1970)

Thermal-safety margins

Thermal-safety margins differed among species, with a broader TSM in *P. przewalskii* than the two *Eremias* species (Table 3). Correspondingly, in the field, *P. przewalskii* had less proportion of T_bs that exceeded the upper limits of its T_{sel} [31.0% (40/129)] when compared to the two *Eremias* species [58.3% (49/84) for *E. argus*, and 46.6% (54/116) for *E. multiocellata*] ($\chi^2 = 16.153$, $P < 0.001$; Fig. 3). In addition, the percentage of T_es that were higher than CT_{max} was lower in *P. przewalskii* [17.7% (311/1760)] than that in the two *Eremias* species [20.7% (364/1760) for *E. argus* and 22.5% (396/1760) for *E. multiocellata*] ($\chi^2 = 12.952$, $P = 0.002$).

Discussion

Behavioral thermoregulation is very important for desert lizards to adapt to extreme environments (Grant and Dunham 1988; Sartorius et al. 2002). Both indices of thermoregulatory effectiveness indicated that all the species could maintain T_bs close to their selected body temperature ranges (Table 2). This suggests that the three species were effective thermoregulators as reported for many other reptiles (Hertz et al. 1993; Scheers and Van Damme 2002; Besson and Cree 2010; Corbalan et al. 2013; Lara-Resendiz et al. 2015). The two *Eremias* lizards had similar mean T_{sel}, spent more than half of the day in the shade, and shuttled frequently between sun and shade patches, although they differed in T_b and CT_{max} (Figs. 1, 2; Table 1). In addition, T_{sel} is similar not only among species but also among different populations in the two *Eremias* lizards (Luo et al. 2005; Li et al. 2009; Tang et al. 2013). These similarities in thermal requirements and strategies among these close-related species and among different populations within a species coincide with the conservative view of thermal physiology (Bogert 1949; Hertz et al. 1983; Araújo et al. 2013; Corbalan et al. 2013; Grigg and Buckley 2013).

Despite the similarity in thermal requirements among species from the same genus, thermal behavior and physiology differed significantly among species from different genus, as reported in other studies (Hertz 1992b; Sartorius et al. 2002; Du et al. 2006; Corbalan et al. 2013). The *P. przewalskii* had higher field body temperatures and thermal tolerance than the two *Eremias* lizards (Fig. 3; Table 1). This result begs the question—what is the contribution of this between-lineage difference in thermal biology to microhabitat divergence among these species? Thermal heterogeneity is likely fine-grained (i.e., high thermal variability among microsites) in our study site with relatively homogeneous habitat structure (Zeng et al. 2016). Such thermally fine-grained environments may facilitate lizards to thermoregulate accurately and decrease the energetic costs of behavioral thermoregulation (Sears and Angilletta 2015; Sears et al. 2016), and would thus allow different species to coexist with higher spatial overlap (Huey 1982;

Hertz 1992a). Our results show that partitioning of thermal resources among species was consistent with the thermal biology of theses lizards. Corresponding to the between-lineage difference in thermal biology, *P. przewalskii* preferred open habitat with high ambient temperature, whereas the *Eremias* lizards spent more time in shade habitat (Fig. 1). These suggest that thermal divergence may play an important role in the interspecific difference in microhabitats. Alternatively, the evolutionary and biogeographic history of these species may contribute to the interspecific divergence in microhabitat use (Wiens and Donoghue 2004). Consistent with their respective microhabitat use, the *Eremias* lizards originated from Eastern Asia, where they generally occupy arid or semi-arid regions, whereas the steppe toad-headed agama (*P. przewalskii*) originated from Alashan Plateau temperate desert, where they generally inhabit the desert habitats (Guo and Wang 2007; Lixia et al. 2007). In addition, biotic factors such as interspecific competition and predation avoidance may also affect microhabitat use by lizards (Rummel and Roughgarden 1985; Lopez and Martin 2013; Zeng et al. 2016).

Ectotherms in desert are vulnerable to high ambient temperatures due to sparse vegetation cover and, therefore, limited opportunities of thermoregulation to keep the body temperature low (Kearney et al. 2009). More severely, these arid regions including our study region have become hotter since 1950, and this warming trend is expected to continue in this century (Pachauri et al. 2014; Wang et al. 2016). A previous study showed that ectotherms in arid mid-latitude zones have narrow thermal-safety margins, and therefore, are at risk from future climate warming (Clusella-Trullas et al. 2011). Our study further suggests that the risk of desert ectotherms to climate warming differs among species, with higher risk for those species having narrower thermal-safety margins. The two *Eremias* lizards had narrower thermal-safety margin than *P. przewalskii* (Table 3). In addition, the two *Eremias* lizards were experiencing potentially lethal operative temperatures when on the bare ground during the mid-day period (Fig. 3). To avoid the lethal high temperatures, they moved more frequently between sun and shade patches to reduce active time in the sun patches (Figs. 1, 2). In contrast, *P. przewalskii* experienced less thermal stress, because they had higher heat tolerance (CT_{max}), higher thermoregulatory accuracy (lower d_b), and milder thermal environment (lower d_e) when compared to the two *Eremias* lizards (Table 2). Therefore, it is reasonable to predict that, if all else is equal, the *Eremias* lizards would be more vulnerable to climate warming than *P. przewalskii*. Nonetheless, it is noteworthy that other factors, such as species interaction, may complicate the fate of species under the context of global warming. For example, the competition for shade patches between these lizards is expected to occur under the scenario of global warming, as

found in tropical forest lizards (Huey et al. 2009; Huey and Tewksbury 2009; Kearney et al. 2009).

In future studies, it would be important to evaluate and unify the different definitions of TSM that have been proposed in previous studies (Heatwole 1970; Deutsch et al. 2008; Huey et al. 2009; Clusella-Trullas et al. 2011). For example, TSM was defined as the difference between a species' thermal tolerance and its ambient or body temperature (Heatwole 1970; Sunday et al. 2014). However, the utilization of thermal resources and body temperature of ectotherms may be affected by behavioral thermoregulation and physiological plasticity (Angiletta 2009; Gunderson and Stillman 2015). Then, how behavioral thermoregulation and physiological plasticity would affect the TSM of a species? Can we take these phenotypic plasticities into account when defining TSM? For example, all else being equal, the enhancement of T_{opt} or CT_{max} induced by behavioral or physiological adjustment may increase the TSM. Such evaluations are important, because a clear and consistent definition of TSM would make the data comparable and greatly improve the usage of TSM in thermal physiology of ectotherms.

In conclusion, lizards with a divergent use of microhabitats have different thermal behavior and physiology, which might help them to coexist in a small area. Given these interspecific difference in thermal biology, these species are likely to be exposed to different risks in the context of climate warming. Therefore, understanding the thermal biology of coexistent species is a critical step to explore in-depth, how species respond to environmental change (e.g., both physical and biological environments, such as climate and competition).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Research was performed under approvals from the Animal Ethics Committee at the Institute of Zoology, Chinese Academy of Sciences (IOZ14001).

References

Adolph SC (1990) Influence of behavioral thermoregulation on microhabitat use by two *Sceloporus* lizards. Ecology 71:315–327

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

Angilletta MJ, Niewiarowski PH, Navas CA (2002) The evolution of thermal physiology in ectotherms. J Therm Biol 27:249–268

Araújo MB, Ferri-Yáñez F, Bozinovic F, Marquet PA, Valladares F, Chown SL (2013) Heat freezes niche evolution. Ecol lett 16:1206–1219

Bakken GS, Angilletta MJ (2014) How to avoid errors when quantifying thermal environments. Funct Ecol 28(1):96–107

Bauwens D, Hertz PE, Castilla AM (1996) Thermoregulation in a lacertid lizard: the relative contributions of distinct behavioral mechanisms. Ecology 77:1818–1830

Bauwens D, Castilla AM, Mouton PlFN (1999) Field body temperatures, activity levels and opportunities for thermoregulation in an extreme microhabitat specialist. J Zool 249:11–18

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

Bennett AF, Johnalder H (1986) Thermal relations of some Australian skinks (Sauria: Scincidae). Copeia 1986:57–64

Besson AA, Cree A (2010) A cold-adapted reptile becomes a more effective thermoregulator in a thermally challenging environment. Oecologia 163:571–581

Blouin-Demers G, Weatherhead PJ (2001a) An experimental test of the link between foraging, habitat selection and thermoregulation in black rat snakes *Elaphe obsoleta obsoleta*. J Anim Ecol 70(6):1006–1013

Blouin-Demers G, Weatherhead PJ (2001b) Thermal ecology of black rat snakes (*Elaphe obsoleta*) in a thermally challenging environment. Ecology 82:3025–3043

Blouin-Demers G, Weatherhead PJ (2002) Habitat-specific behavioural thermoregulation by black rat snakes (*Elaphe obsoleta obsoleta*). Oikos 97(1):59–68

Bogert CM (1949) Thermoregulation in reptiles, a factor in evolution. Evolution Int J org Evolution 3(3):195–211

Bowker RG (1984) Precision of thermoregulation of some African lizards. Physiol Zool 57:401–412

Clusella-Trullas S, Blackburn TM, Chown SL (2011) Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. Am Nat 177:738–751

Corbalan V, Debandi G, Kubisch E (2013) Thermal ecology of two sympatric saxicolous lizards of the genus *Phymaturus* from the Payunia region (Argentina). J Therm Biol 38:384–389

Cowles RB, Bogert CM (1944) A preliminary study of the thermal requirements of desert reptiles. Bull Am Mus Nat Hist 83:263–296

Daly BG, Dickman CR, Crowther MS (2008) Causes of habitat divergence in two species of agamid lizards in arid central Australia. Ecology 89:65–76

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008) Impacts of climate warming on terrestrial ectotherms across latitude. Proc Natl Acad Sci USA 105:6668–6672

do Amaral JPS, Marvin GA, Hutchison VH (2002) Thermoregulation in the box turtles *Terrapene carolina* and *Terrapene ornata*. Can J Zool 80(5):934–943

Du WG, Yan SJ, Ji X (2000) Selected body temperature, thermal tolerance and thermal dependence of food assimilation and locomotor performance in adult blue-tailed skinks, *Eumeces elegans*. J Therm Biol 25:197–202

Du WG, Shou L, Shen JY (2006) Habitat selection in two sympatric Chinese skinks, *Eumeces elegans* and *Sphenomorphus indicus*: do thermal preferences matter? Can J Zool 84:1300–1306

Dubois Y, Blouin-Demers G, Shipley B, Thomas D (2009) Thermoregulation and habitat selection in wood turtles *Glyptemys insculpta*: chasing the sun slowly. J Anim Ecol 78:1023–1032

Grant BW, Dunham AE (1988) Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. Ecology 69:167–176

Grigg JW, Buckley LB (2013) Conservatism of lizard thermal tolerances and body temperatures across evolutionary history and geography. Biol Lett 9:20121056

Grover MC (1996) Microhabitat use and thermal ecology of two narrowly sympatric *Sceloporus* (Phrynosomatidae) lizards. J Herpetol 30(2):152–160

Gunderson AR, Stillman JH (2015) Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc R Soc B 282(1808):20150401

Guo X, Wang Y (2007) Partitioned Bayesian analyses, dispersal–vicariance analysis, and the biogeography of Chinese toad-headed lizards (Agamidae: *Phrynocephalus*): a re-evaluation. Mol Phylogenet Evol 45:643–662

Heatwole H (1970) Thermal ecology of the desert dragon *Amphibolurus inermis*. Ecol Monogr 40:425–457

Hertz PE (1992a) Evaluating thermal resource partitioning by sympatric lizards *Anolis cooki* and *A. cristatellus*: a field test using null hypotheses. Oecologia 90:127–136

Hertz PE (1992b) Temperature regulation in Puerto Rican *Anolis* lizard: A field test using null hypotheses. Ecology 73:1405–1417

Hertz PE, Huey RB, Nevo E (1983) Homage to Santa-Anita—thermal sensitivity of sprint speed in Agamid lizards. Evol Int J org Evolution 37:1075–1084

Hertz PE, Huey RB, Stevenson RD (1993) Evaluating temperature regulation by field-active ectotherms. Am Nat 142:796–818

Hertz PE, Arima Y, Harrison A, Huey RB, Losos JB, Glor RE (2013) Asynchronous evolution of physiology and morphology in *Anolis* lizards. Evolution Int J org Evol 67:2101–2113

Huey RB (1982) Temperature, physiology, and the ecology of reptiles. In: Gans C, Pough FH (eds) Biology of reptilia, vol 12. Academic, London, pp 25–91

Huey RB, Pianka ER (1977) Patterns of niche overlap among broadly sympatric versus narrowly sympatric Kalahari lizards (Scincidae: *Mabuya*). Ecology 58:119–128

Huey RB, Tewksbury JJ (2009) Can behavior douse the fire of climate warming? Proc Natl Acad Sci USA 106:3647–3648

Huey RB, Pianka ER, Egan ME, Coons LW (1974) Ecological shifts in sympatry—Kalahari fossorial lizards (*Typhlosaurus*). Ecology 55:304–316

Huey RB, Pianka ER, Hoffman JA (1977) Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. Ecology 58:1066–1075

Huey RB, Deutsch CA, Tewksbury JJ, Vitt LJ, Hertz PE, Alvarez Perez HJ, Garland T Jr (2009) Why tropical forest lizards are vulnerable to climate warming. Proc R Soc B 276:1939–1948

Huey RB, Kearney MR, Krockenberger A, Holtum JA, Jess M, Williams SE (2012) Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philos Trans R Soc Lond B Biol Sci 367:1665–1679

Kearney M, Shine R, Porter WP (2009) The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. Proc Natl Acad Sci USA 106:3835–3840

Lara-Resendiz RA, Gadsden H, Rosen PC, Sinervo B, Mendez-De La Cruz FR (2015) Thermoregulation of two sympatric species of horned lizards in the Chihuahuan Desert and their local extinction risk. J Therm Biol 48:1–10

Lelievre H, Blouin-Demers G, Pinaud D, Lisse H, Bonnet X, Lourdais O (2011) Contrasted thermal preferences translate into divergences in habitat use and realized performance in two sympatric snakes. J Zool 284:265–275

Li H, Wang Z, Mei W, Ji X (2009) Temperature acclimation affects thermal preference and tolerance in three *Eremias* lizards. Curr Zool 55:258–265

Lixia W, Shihong S, Yuanting J, Yongfeng Y, Naifa L (2007) Molecular phylogeography of the Chinese lacertids of the genus *Eremias* (Lacertidae) based on 16 S rRNA mitochondrial DNA sequences. Amphibia-Reptilia 28:33–41

Lopez P, Martin J (2013) Effects of microhabitat-dependent predation risk on vigilance during intermittent locomotion in *Psammodromus algirus* Lizards. Ethology 119(4):316–324

Luo LG, Qu YF, Ji X (2005) Thermal dependence of food assimilation and sprint speed in a lacertid lizard (*Eremias argus*) from northern China. Dong wu xue bao.[Acta zoologica Sinica] 52:256–262

Magnuson JJ, Crowder LB, Medvick PA (1979) Temperature as an ecological resource. Am Zool 19:331–343

Martinvallejo J, Garciafernandez J, Perezmellado V, Vicentevillardon JL (1995) Habitat selection and thermal ecology of the sympatric lizards *Podarcis muralis* and *Podarcis hispanica* in a mountain region of central spain. Herpetol J 5(1):181–188

Melville J, Schulte JA (2001) Correlates of active body temperatures and microhabitat occupation in nine species of central Australian agamid lizards. Austral Ecol 26:660–669

Middleton N, Thomas D (1997) World atlas of desertification, 2nd edn. Arnold, London

Pachauri RK et al (2014) Climate change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report. Intergovernmental Panel on Climate Change, Geneva, Switzerland

Pianka ER (1971) Comparative ecology of two lizards. Copeia 1971:129–138

Pianka ER (1986) Ecology and natural history of desert lizards: analyses of the ecological niche and community structure. Princeton University Press, Princeton

Pianka ER (1989) Desert lizard diversity: additional comments and some data. Am Nat 134(3):344–364

Qu YF, Li H, Gao JF, Xu XF, Ji X (2011) Thermal preference, thermal tolerance and the thermal dependence of digestive performance in two coexisting *Phrynocephalus* lizards. Curr Zool 57:684–700

Rocha CFD, Vrcibradic D (1996) Thermal ecology of two sympatric skinks (*Mabuya macrorhyncha* and *Mabuya agilis*) in a Brazilian restinga habitat. Aust J Ecol 21(1):110–113

Row JR, Blouin-Demers G (2006) Thermal quality influences effectiveness of thermoregulation, habitat use, and behaviour in milk snakes. Oecologia 148:1–11

Ruibal R (1960) Thermal relations of five species of tropical lizards. Evol Int J org Evol 15:98–111

Rummel JD, Roughgarden J (1985) Effects of reduced perch-height separation on competition between two *Anolis* lizards. Ecology 66(2):430–444

Sartorius SS, do Amaral JPS, Durtsche RD, Deen CM, Lutterschmidt WI (2002) Thermoregulatory accuracy, precision, and effectiveness in two sand-dwelling lizards under mild environmental conditions. Can J Zool 80:1966–1976

Scheers H, Van Damme R (2002) Micro-scale differences in thermal habitat quality and a possible case of evolutionary flexibility in the thermal physiology of lacertid lizards. Oecologia 132:323–331

Schoener TW (1974) Resource partitioning in ecological communities. Science 185:27–39

Sears MW, Angilletta MJ (2015) Costs and benefits of thermoregulation revisited: both the heterogeneity and spatial structure of temperature drive energetic costs. Am Nat 185:E94–E102

Sears MW, Angilletta MJ Jr, Schuler MS, Borchert J, Dilliplane KF, Stegman M, Rusch TW, Mitchell WA (2016) Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. Proc Natl Acad Sci USA 113:10595–11060

Shen JW, Pike DA, Du WG (2010) Movements and microhabitat use of translocated big-headed turtles (*Platysternon megacephalum*) in southern China. Chel Cons Biol 9:154–161

Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB (2014) Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc Natl Acad Sci USA 111:5610–5615

Tang XL, Yue F, He JZ, Wang NB, Ma M, Mo JR, Chen Q (2013) Ontogenetic and sexual differences of thermal biology and locomotor performance in a lacertid lizard, *Eremias multiocellata*. Zoology 116:331–335

Vandamme R, Bauwens D, Verheyen RF (1991) The thermal dependence of feeding behavior, food consumption and gut passage time in the lizard *Lacerta vivipara* Jacquin. Funct Ecol 5:507–517

Wang Y, Zeng ZG, Li SR, Bi JH, Du WG (2016) Low precipitation aggravates the impact of extreme high temperatures on lizard reproduction. Oecologia 182:961–971

Wiens JJ, Donoghue MJ (2004) Historical biogeography, ecology and species richness. Trends Ecol Evol 19:639–644

Zeng ZG Bi JH, Li SR, Wang Y, Robbins TR, Chen SY, Du WG (2016) Habitat alteration influences a desert steppe lizard community: implications of species-specific preferences and performance. Herpetol Monogr 30:34–48

Zhao KT (1999) Lacertidae. In: Zhao EM, Zhou KY (eds) Sinica Fauna, Reptilia (Squamata: Lacertilia). Science, Beijing, pp 219–242