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ORIGINAL ARTICLE

Embryonic and post-embryonic responses to high-elevation hypoxia in a low-elevation lizard

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Abstract

Low-elevation species can migrate toward higher elevations to survive in a warming world. However, animals' responses to hypoxia when migrating to high elevations have rarely been addressed. To identify the response of low-elevation lizards to high-elevation hypoxia, we collected field body temperatures (T_{fb}) and operative temperatures (T_e) of lizards (*Eremias argus*) from a low-elevation population (1036 m) and a high-elevation population (2036 m), and then determined adult thermal physiology, embryonic development, and hatchling phenotypes after acclimating low-elevation lizards and incubating their eggs in conditions mimicking the low-elevation oxygen condition (18.5% O_2) and high-elevation oxygen (hypoxic) condition (16.5% O_2). Our study revealed that T_{fb} and T_e were higher for the low-elevation population compared to the high-elevation population. We also found adults from low elevation acclimated to hypoxia preferred lower body temperatures, but did not show changes in locomotor performance or growth. In addition, hypoxia did not affect embryonic development (hatching time and success) or hatchling phenotypes (body size and locomotor performance). These results suggest that adult lizards from low elevations can respond to hypoxia-induced stress when migrating to high elevations by behaviorally thermoregulating to lower body temperatures in order to sustain normal functions. Similarly, low-elevation embryos can develop normally (with unchanged hatching success and offspring phenotypes) under the high-elevation hypoxic condition. This study highlights that low-elevation populations of a species that inhabits a range of elevations can buffer the impact of high-elevation hypoxic conditions to some degree and thus attain similar fitness to the source population.

Key words: climate warming, high elevation, hypoxia, oviparous lizard, thermal preference

INTRODUCTION

Global warming is expanding its impacts, covering almost all organisms and ecosystems (Gonzalez *et al*. 2010; Pacifici *et al*. 2015). Ectotherms are extremely vulnerable to climate warming because they are highly subject to environmental temperatures (Deutsch *et al*. 2008; Huey *et al*. 2012). In response to climate warming, ectotherms depend on a wide variety of behavioral and physiological strategies (Rodriguez-Trelles *et al*. 2013; Ma *et al*. 2014; Seebacher *et al*. 2015; Wong & Candolin 2015).

Species or populations at low elevations are predicted to disperse to higher elevations in mountains to survive

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in a warming world (Parmesan & Yohe 2003; Sinervo *et al*. 2010; Chen *et al*. 2011; Pauchard *et al*. 2016), and upward shifts of distribution range have already been observed in many taxa, including plants (Morueta-Holme *et al*. 2015), insects (Moret *et al*. 2016), and birds (Flousek *et al*. 2015). Ascending to higher elevations certainly provides a way for threatened species to persist in more favorable temperatures; however, this ascent may also increase challenges from factors such as radiation, vegetation cover, and low partial pressure of oxygen (PO_2), which are characteristics on high elevations (e.g. Scheinfeldt & Tishkoff 2010; Storz *et al*. 2010; Gangloff *et al*. 2019).

Hypoxia may limit the functions, or even the survival and reproduction of ectotherms. For example, when environmental temperatures approach upper thermal limits, hypoxic environments cannot supply sufficient oxygen to the tissues, which causes increased reliance on anaerobic metabolism, and eventually death according to "oxygen and capacity limitation of thermal tolerance" (OCLTT) (Hicks & Wood 1985; Pörtner 2001; Pörtner 2002). This hypothesis has been recently expanded by a unified framework combining thermal performance curve paradigm, "hierarchical mechanisms of thermal limitation" (HMTL) (Gangloff & Telemeco 2018). HMTL predicts that hypoxia at high elevation will lower both maximal performance and optimum temperature for performance in terrestrial ectotherms. For amphibians and reptiles, hypoxia largely limits their functional performance (Wood & Gonzales 1996; Hicks & Wang 2004; Sun *et al*. 2014; Li *et al*. 2016). Under hypoxic conditions, some lizards even reduce heating from solar radiation by moving pigments within chromatophore cells to lighten their bodies (de Velasco & Tattersall 2008). Hypoxia also reduces the metabolic and growth rates of embryos (Kam 1993; Warburton *et al*. 1995; Liang *et al*. 2015), induces cardiac hypertrophy, and increases heart rate, vascular density on the chorioallantoic membrane, and hematocrit levels in oviparous amniotes (Corona & Warburton 2000; Crossley & Altimiras 2005; Du *et al*. 2010). However, most of the research done in the last few decades are relevant to extreme hypoxia, very few have studied animals' responses to ecological hypoxia when migrating to high elevations, especially along life history cycles (but see Cordero *et al*. 2017; Kouyoumdjian *et al*. 2019).

With the aim to reveal the responses of ectotherms to hypoxia when dispersing to high elevations, we combined field and lab experiments to evaluate the effects of hypoxia both on adults and embryos, with Mongolian racerunner (*Eremias argus* Peters, 1869) as study system. *E. argus* is a small oviparous lacertid lizard that generally occupies grassy sand dunes and thickets, and is widely distributed at altitudes ranging from 1000 to 3000 m in northern China. During the reproductive season (May–July), females lay 2–5 eggs per clutch (Sun *et al*. 2013; Zhao 1999; Zeng *et al*. 2016; Zeng *et al*. 2016). First, we collected field body temperatures and operative temperatures of *E. argus* from a low-elevation population (1036 m) and a high-elevation population (2036 m). Then, we experimentally exposed the adults and embryos of *E. argus* from the low-elevation population to lab conditions mimicking the low-elevation oxygen condition (18.5% O_2, control) and high-elevation oxygen condition (16.5% O_2, hypoxia) to determine how fitness-related traits of ectotherms would be impacted by warming-driven migrations to high elevations at different life stages, under the assumption that ranges of low-elevation populations would shift 1000 m higher to elevations that would keep ambient temperature unchanged if average air temperature rose 6 °C (Dillon *et al*. 2006). We aim to answer the following questions: (1) how would the behavior and physiology (e.g. thermal preference, growth, and locomotion) of low-elevation adult lizards change in response to high-elevation hypoxia; (2) how would embryonic development (e.g. hatching time and success, and offspring phenotypes) change in response to high-elevation hypoxia? As hypoxia affects the thermal performance curve shape of reptiles (Gangloff & Telemeco 2018), we predicted lizards exposed to hypoxia would lower their thermal preferences, or changed their thermal performance curves of growth or locomotion to maintain normal functions. Considering hypoxia reduces metabolism and growth rates of embryos, we expected embryos incubated in hypoxic conditions would prolong development time. With these physiological or developmental responses, we predicted embryos could suffer from reduced oxygen availability, and yet sustain key phenotypes like body size and locomotion at hatching.

MATERIALS AND METHODS

Field body temperatures (T_{fb}), operative temperatures (T_e), and animal collection

From July 1st to 10th, we measured the field body temperatures (T_{fb}) of lizards of the Inner Mongolia (40.2°N, 111.1°E, elevation 1036 m, IM) and the Qinghai (36.0°N, 100.7°E, elevation 2600 m, QH) populations, respectively. On sunny days, we searched for active adult lizards in the field. Once found, field body temperatures (T_{fb}) of the lizards were measured immediately (±0.1 °C) by inserting the probe of electronic thermal couple

(UNT-325, Shenzhen Meter Instruments, China) about 5 mm into their cloaca. For each individual, we also recorded the time of day and the sex before releasing the lizard at the site of capture.

We also collected operative temperatures (T_e) using 10 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 had a data logger (iButton, DS1921, MAXIM Integrated Products, Ltd., USA) inserted into it to record temperatures hourly. Ten models were set in the field to collect the surface temperatures under sun and shade randomly.

After field body temperatures and operative temperatures were collected (i.e. T_{fb} and T_e), 10 adult males and 12 gravid females with oviduct eggs (i.e. closest to oviposition) from IM were transported to the lab (40.0° N, 116.4° E, elevation 48 m), where they were toe-clipped for individual identification, measured (snout-vent length [SVL], ± 0.01 mm, 52.2 ± 0.6 mm [mean ± SEM]), weighed (Body mass [BM], ± 0.001 g, 3.140 ± 0.110 g [mean ± SEM]) and acclimated under different oxygen treatments after reproduction.

Oxygen treatment and body temperatures (T_b) at the beginning of acclimation

Low/high-elevation oxygen concentration was set by an "air-pressure-at-altitude-calculator" to calculate atmospheric pressure at different altitudes at a specific temperature, with oxygen concentration at every altitude being 20.9 %. According to "NicheMapR" output and analysis results (Kearney & Porter 2017), the average air temperature for the IM population from July to August is 26 °C (range 23∼29 °C). Atmospheric pressures of low elevation (1036 m) and high elevation (2036 m) are 89.9 kPa and 79.9 kPa, respectively. Converting the reduced oxygen availability due to reduced atmospheric pressure at high elevations results in oxygen concentrations of 18.5% (control) and 16.5% (hypoxia) under normobaric (sea level) conditions.

Adult lizards were randomly assigned to low-elevation oxygen (control) concentration (18.5% O_2, 1036 m) and high-elevation oxygen (hypoxic) concentration (16.5% O_2, 2036 m) treatments for acclimation for two months, according to natural oxygen environment at IM and 1000 m higher (i.e. 2036 m, elevation), respectively. Lizards were housed in custom-made oxygen control chambers (Huaxi Electronics, Changsha, China), with a photoperiod of 10:14 (light: dark). For each chamber, we placed the lizards into three terraria (47 × 35 × 24 mm), with three or four lizards contained in each terrarium. We used full-spectrum bulbs (50 W, UVA + UVB) as a heat source for the lizards' thermoregulation from 0800 to 1800. Food (mealworms and crickets dusted with vitamins and minerals) and water were provided ad libitum. The oxygen environments inside the chambers were controlled automatically by a PID system (Huaxi Electronics, Changsha, China).

At the beginning (the third day) of acclimation, we measured active T_b of lizards with the same method as used in the field in the terraria every 2 h from 0700 to 1900. We also placed iButtons at the hot end (below the lamp) and cold end (away from the lamp) respectively in each terrarium to record surface temperatures hourly. One escaped male from hypoxia group was excluded in the following test even though it was recaptured two days later during acclimation.

Selected body temperatures (T_{sel}), growth, and locomotor performance

We measured selected body temperatures (T_{sel}) of the lizards after 45 days of acclimation. A plastic tank (950 × 600 × 400 mm) with sand substrate was placed inside a room with air temperatures maintained at 20 °C. Two heating lamps (50 W) were suspended at one end to create a thermal gradient of 20–50 °C in the tank. We introduced four or five lizards to the cold end and measured the body temperatures of each lizard 3 h later. Then we recorded three body temperatures with the same method as used in the field for each lizard at 15 min intervals in half an hour. Each measurement was finished in 15 sec. The selected body temperature was calculated as the average of three records for each lizard.

During acclimation, the body size of each lizard was measured twice to determine their growth. We measured SVL (± 0.01 mm) and BM (± 0.001 g) when the lizards were acclimated for one month and two months, respectively. After 2 months of acclimation and body size measurement, locomotor performance was tested from 18–38 °C with a randomized sequence (i.e. 26, 18, 30, 38, 22, 34 °C). All lizards were tested in a random order during each trail. We acclimated lizards in an incubator at test temperatures for 2 h prior to each trial. Lizards were then introduced into the racetrack (1000 × 100 × 150 mm) and stimulated to run along the track with a paintbrush. Each lizard ran twice, in 30 min intervals. Distance scales of 200 mm intervals were printed on the racetrack to determine lizard-running speeds. Each 200 mm interval was equipped with an infrared tube, which converged at the end to an LED screen to record the amount of time lizard had taken to run through each 200 mm segment automatically. Sprint speed was calculated as the fastest speed through each 200 mm interval, and average speed was calculated as the speed across the entire length of the track (1000 mm).

Egg collection and incubation

Before oxygen acclimation, gravid females were maintained in egg-laying cages (310 × 210 × 180 mm) individually, with 20 mm moist sand. We checked egg-laying cages four times per day for freshly laid eggs. If found, the eggs were weighed (average egg mass 0.489 ± 0.0031 g [mean ± SEM]) and incubated in plastic boxes (220 × 100 × 80 mm) containing moist vermiculite with a water potential of −220 kPa. The eggs from each female were assigned to control and hypoxic treatments respectively, with a split-clutch design. Each plastic box was sealed in a 30 L polybag and supplied with 18.5% or 16.5% O_2. The polybags were then transferred to an environmental chamber set to a fluctuating temperature 26 ± 3 °C.

Oxygen concentrations were achieved by mixing compressed oxygen with nitrogen, using air cylinders with an established method (Liang *et al*. 2015). In brief, each polybag was filled with the mixed gas and checked twice per day to ensure the eggs were under slight positive pressure from the mixed gas supply. The gas in the polybag was renewed every day. Meanwhile, the boxes were weighed and water was added to compensate for water loss due to evaporation and absorption by the eggs to keep the water potential of the incubation substrate relatively constant. All gravid females laid eggs within one week after they were collected, and 12 clutches of 26 fertilized eggs were collected and incubated in total.

Hatchling morphology, locomotor performance, and residual yolk

During the end of incubation, we checked for hatchlings four times per day. If found, we measured initial SVL (±0.01 mm) and BM (±0.001 g) of hatchlings immediately. Incubation periods were calculated as the number of days between oviposition date and hatching date. After body size determination, we tested the locomotor performance of the hatchlings at 34 °C, with same protocols used to measure adults (see details above). After the running trials, the residual yolk of hatchlings was separated from their abdomens and weighed (±0.00001 g).

Ethics

Lizard collection, handling, and husbandry were approved by Animal Ethics Committees at the Institute of Zoology, Chinese Academy of Sciences (IOZ14001).

Statistical analysis

All the statistical analysis was performed using R software (version 3.3.1; R Core Team, 2016) and SPSS (version 20.0, IBM). We used repeated-measures ANOVA to detect the differences of T_e between populations from 0800 to 1800 when the lizards were active. A Greenhouse–Geisser correction was used to adjust the result because the sphericity assumption was violated. We ran linear mixed effects models (“lmer” and “lmerTest: anova” in library “lmerTest”) to test for differences in field body temperatures between the IM and the QH populations, and to test whether the growth rate, T_b and T_{sel} of adults differed among oxygen treatments with treatment, using testing time and sex as fixed factors and plastic terrarium as a random factor. We also tested the effects of oxygen treatment on locomotor performance of adults by conducting repeated measures ANOVA, with treatment and sex as fixed factors, testing temperature as repeated factors and plastic terrarium as a random factor. We used generalized linear mixed model to detect the effect of oxygen treatment on hatching success with clutch as random factor. We ran linear mixed models to test for between-treatments differences in incubation period, hatchling SVL, BM, and residual yolk mass with clutch as random factor. When we tested the differences in hatchling SVL, BM, and residual yolk mass, we also used initial egg mass as a covariate. Linear mixed effects models were also used to test the effects of treatment on hatchling locomotor performance with hatching SVL as a covariate, and clutch as random factor. Degrees of freedom for F-tests were corrected by the Satterthwaite method. Data are presented as mean ± SEM.

RESULTS

In the field, T_e fluctuated through the daytime ($F_{2.16,\,38.93} = 148.295$, $P < 0.001$), and as expected, low-elevation site was warmer than high-elevation site (IM: 37.0 ± 1.1 °C; QH: 33.9 ± 1.1 °C; $F_{1,\,18} = 4.082$, $P = 0.058$). The T_{fb} of lizards were in between the range of max T_e (i.e. sun) and min T_e (i.e. shade) in both the IM and the QH populations. Field body temperatures were higher in the IM population compared to the QH population ($F_{1,\,172.21} = 6.796$, $P < 0.01$) (Fig. 1a,b), with the

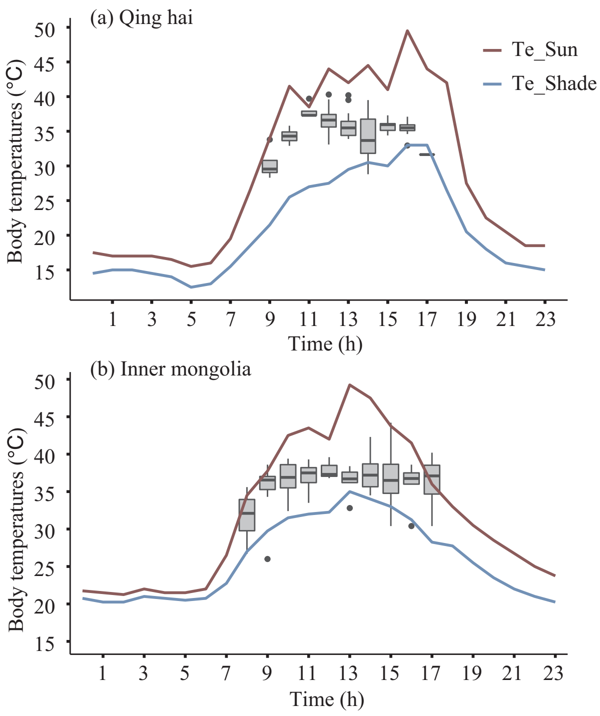

Figure 1 The field body temperatures (T_{fb}), sun and shade operative temperatures (T_e) of the Inner Mongolia and the Qinghai *Eremias argus* population. The horizontal line in the box represents the median. The top and bottom edges of the box indicate the 75th and 25th percentiles, respectively. Whiskers above and below the box represent the 90th and 10th percentiles, respectively. Black circles beyond the whiskers indicate outliers.

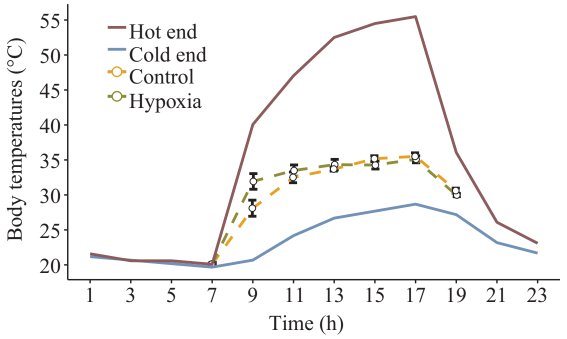

Figure 2 The body temperatures (T_b) of the Inner Mongolia population of *Eremias argus* treated with different oxygen concentration maintained indoors before acclimation and its hot/cold end environment temperatures. Error bars indicate SEM.

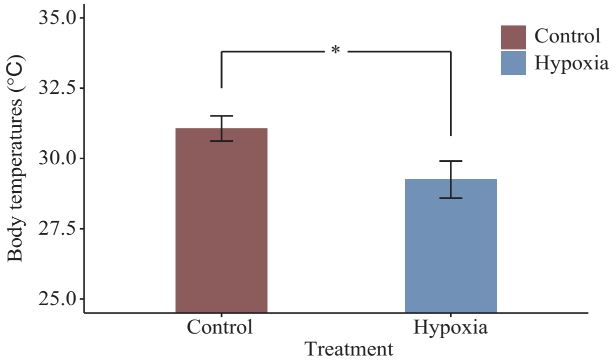

Figure 3 The selected body temperatures (T_{sel}) of the Inner Mongolia *Eremias argus* population treated with different oxygen concentration maintained indoors after short acclimation. Error bars indicate SEM.

mean T_{fb} from 0800 to 1800 of the IM was 36.4 ± 0.3 °C and in the QH population was 35.1 ± 0.3 °C, respectively.

The T_b did not differ between oxygen treatments at the beginning of acclimation ($F_{1,\,143.09} = 2.029$, $P = 0.157$; Fig. 2). Interestingly, hypoxic treatment depressed the T_{sel} of adult lizards, compared to the control treatment ($F_{1,\,58.03} = 5.413$, $P < 0.05$; Fig. 3). However, oxygen acclimation did not affect the growth of SVL and BM (Table 1), sprint speeds ($F_{1,\,110.44} = 2.739$, $P = 0.101$) (Fig. 4a) or average speeds ($F_{1,\,111.55} = 0.468$, $P = 0.496$) (Fig. 4b) in adults.

The hatching success and incubation period were not affected by oxygen treatment. Embryonic oxygen environment did not affect the SVL and BM, residual yolk mass, sprint speeds, or average speeds of hatchlings (Table 2).

Table 1 The growth of snout-vent length (SVL) and body mass (BM) of adult *Eremias argus* of the Inner Mongolia population after acclimation. Data are shown as mean ± SEM, and statistical significance is defined as $\alpha < 0.05$

	Hypoxia	Control	Statistical analysis
N	10	12	
Growth of SVL (mm)	1.33 ± 0.31	1.13 ± 0.35	$F_{1,\,20} = 0.181$, $P = 0.676$
Growth of BM (g)	0.621 ± 0.128	0.384 ± 0.111	$F_{1,\,20} = 1.979$, $P = 0.175$

Table 2 The hatching success, incubation periods, snout-vent length, body mass, residual yolk, sprint speeds, and average speeds of *Eremias argus* hatchlings incubated at control and hypoxia environments. Data are shown as mean ± SEM, and statistical significance is defined as $\alpha < 0.05$. Degrees of freedom for F-tests were corrected by Satterthwaite method

	Hypoxia	Control	Statistical analysis
N	12	10	
Hatching success	92.31%	76.92%	$F_{1,24} = 1.076$, $P = 0.310$
Incubation period (days)	43.9 ± 0.3	44.8 ± 0.4	$F_{1,\,10.87} = 1.879$, $P = 0.198$
Snout-vent length (mm)	28.86 ± 0.37	28.62 ± 0.63	$F_{1,\,16.67} = 0.518$, $P = 0.482$
Body mass (g)	0.538 ± 0.021	0.540 ± 0.019	$F_{1,\,13.65} = 0.241$, $P = 0.631$
Residual yolk (g)	0.01343 ± 0.00106	0.01497 ± 0.00168	$F_{1,\,18} = 0.063$, $P = 0.804$
Sprint speeds (m·s^{-1})	1.259 ± 0.070	1.146 ± 0.071	$F_{1,\,17.10} = 0.272$, $P = 0.609$
Average speeds (m·s^{-1})	0.799 ± 0.098	0.699 ± 0.046	$F_{1,\,18} = 0.162$, $P = 0.692$

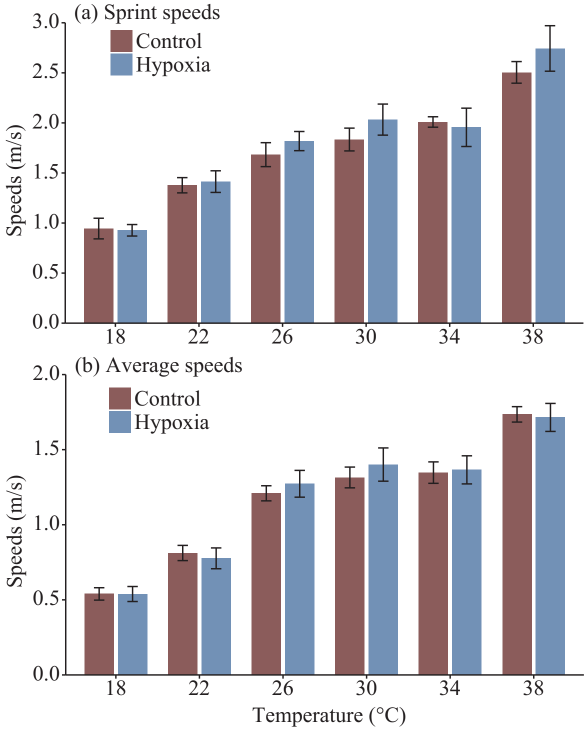

Figure 4 The sprint speeds and average speeds of adult *Eremias argus* of the Inner Mongolia population treated with different oxygen concentrations maintained indoors after short acclimation. Error bars indicate SEM.

DISCUSSION

In response to a warming world, species living at warm temperatures and low elevations are projected to disperse toward to higher elevations (Parmesan & Yohe 2003; Sinervo *et al.* 2010; Pauchard *et al.* 2016), where they should face challenges that come from lower partial pressure of oxygen (Scheinfeldt & Tishkoff 2010; Dahlhoff *et al.* 2019; Gangloff *et al.* 2019; Jacobsen 2020). Given this, the way that a species responds to hypoxia is critical for surviving in a warming world if they disperse to higher elevation. In this study, we mainly focus on the behavioral and physiological responses to oxygen treatment of *E. argus* in embryos and adults. First, we detected the active body temperatures of *E. argus* from low elevation are higher than congeners from high elevation (Fig. 1). Similarly, lizards from the IM population preferred lower T_{sel} after hypoxia acclimation which mimics oxygen availability at 1000 m greater elevation (Fig. 3), suggesting that *E. argus* prefers lower temperatures in response to hypoxic environments, similar to other reptiles (Wood & Gonzales 1996; Hicks & Wang 2004). However, in this study, hypoxia did not affect adults' growth and locomotor performance (Fig. 4a,b) in *E. argus* from the IM population. Previous research suggests embryos are vulnerable to environmental stress when exposed to severe or chronic hypoxia (i.e. 10% O_2) (Kam 1993; Wearing *et al.* 2016). However, embryonic development seems insensitive to mild hypoxia in this study (i.e. 16.5% O_2), indicated by similar hatching success, hatchling morphology and locomotor performances (Table 2). Similar responses of embryos to high-elevation hypoxia were found in low-elevation common wall lizard (*Podarcis muralis*) (Cordero *et al.* 2017; Kouyoumdjian *et al.* 2019), indicating that lizard embryos had high resilience to ecological-relevant hypoxia. Integrating behavioral thermoregulation and physiological responses from adults and embryonic developments, our study suggests that individuals can buffer mild hypoxia-induced stress if they migrate to high elevations by lowering thermal preferences and their high embryonic resilience to high-elevation hypoxia.

Lower thermal preferences usually predicate lower active body temperatures under identical conditions in ectotherms (Hertz *et al*. 1993; Li *et al*. 2017), and then decrease oxygen requirements as metabolic rates decline exponentially with the reduction of body temperatures (i.e. Q_{10} effect) (Gillooly *et al*. 2001; Hicks & Wang 2004). In this case, adult functions such as growth and locomotor performance were not depressed by hypoxia (Tables 1, 2). This suggests that after dispersal to high elevations, ectotherms from low elevations could utilize lowered thermal preferences and thus active body temperatures as an efficient response to hypoxia in order to survive. Behavioral thermoregulation has been investigated for decades and has significant impacts on the fitness of ectotherms (Petraitis 1983; Grant & Dunham 1988; Sartorius *et al*. 2002; Li *et al*. 2017). The T_{fb} of the IM and the QH populations showed that high-elevation populations had lower mean active T_{b} at low environmental temperatures and partial pressures of oxygen. Similarly, tropical *Sceloporus* lizards, had reduced active T_{b} from 35 °C at low elevations to 31 °C at high elevations (Andrews 1998). It is plausible that low T_{b} of *E. argus* from the QH population is induced by the interaction of low environmental temperatures and low oxygen content (Hicks & Wood 1985; Branco *et al*. 1993; Bicego-Nahas *et al*. 2001). In this study, similar T_{b} under hypoxic and control acclimation during the beginning of oxygen acclimation may indicate lizards could not immediately adjust their thermoregulation behavior when exposed to hypoxia (Fig. 2). However, lower T_{sel} in the hypoxic treatment after acclimation suggests that adults will decrease their T_{sel} as a plastic response to hypoxia-induced stress. Similarly, other reptiles and amphibians preferred lower T_{b} after exposure to low oxygen concentrations (Hicks & Wood 1985; Branco *et al*. 1993; Bicego-Nahas *et al*. 2001; He *et al*. 2013). Such hypoxia-induced hypothermia has been considered to be an adaptive plasticity in ectotherms (Wood 1991), as it has some beneficial effects, for example, greatly reducing oxygen demand, slightly influencing systemic oxygen transport ability, protecting oxygen-sensitive organs (e.g. heart and brain) from oxygen limitations, and increasing energetic savings (Wood 1991; Wood & Malvin 1991; Branco *et al*. 1993; Hicks & Wang 2004). However, some negative impacts of selecting a cool body temperature should not be neglected, such as reduction of digestive and assimilation efficiency (Ji *et al*. 1996; McConnachie & Alexander 2004). Overall, more direct empirical evidence is required to detect how hypothermia affects fitness traits, like growth and survival rates in ectotherms exposed to hypoxia.

The proximate and evolutionary mechanisms of the shift in thermoregulation in response to hypoxia are still largely unknown. However, it may be referred to as behavioral hypothermia or hypoxic anapyrexia, related to changes in body temperatures modified by lactate, adenosine, opioids, and nitric oxide (Steiner & Branco 2001, 2002), which mostly appear to act in the central nervous system in response to a deficit in circulating oxygen (Tamaki & Nakayama 1987; Steiner & Branco 2002). Behavioral hypothermia can prevent an abnormally low concentration of oxygen in the blood. For example, experimental reduction of ATP by NaCN or NaN_3 would cause relative hypoxia internally, and thus drive toads to choose lower temperatures (Branco & Malvin 1996). Alternatively, lower thermal preferences could also be induced by modification of set point of cardiorespiratory physiology as suggested by the oxygen- and capacity-limited thermal tolerance (OCLTT) theory (Gangloff & Telemeco 2018). For example, optimal temperatures and thermal performance curve (TPC) for heart rate of steelhead trout moved toward low temperatures by around 2 °C in respond to hypoxia. By this mechanism, individuals could attain the same heart rates at 2 °C lower body temperatures than congeners in normoxia (Motyka *et al*. 2017).

Hypoxia did not depress fitness-related traits in adults and embryos, including growth and locomotor performance in adults, hatching success of developing embryos, or body size of hatchlings. The modification of thermoregulation under hypoxia may buffer the oxygen availability and result in the normal growth and locomotion in adults. It is not surprising that the sprint speeds were not affected by hypoxia, as sprint performance was almost exclusively fueled by anaerobic respiration (Bennett & Licht 1972; Hicks & Wood 1985; Pörtner 2002). As lizard locomotion increased linearly with increasing test temperatures in current study (Fig. 4), thermal performance curves of locomotion in *E. argus* failed to be estimated, and whether its shape could be affected by hypoxia was unclear. For embryonic development in reptiles, the developmental rate of embryos is demonstrated to be lower in 15% O_2 incubation (Sun *et al*. 2014), and even resulted in retarded growth and depressed metabolism when incubated in 10% O_2 (Kam 1993; Galli *et al*. 2016). However, the 16.5% oxygen concentration used in the current study may not be a stress for embryos, as evidenced by embryonic developmental rate, hatching success, residual yolk mass, and locomotor performance between oxygen treatments. First, in a natural nest, reptile embryos develop underground, where the oxygen content is sometimes lower than in the air (Cheng *et al*. 2015). Second, for the IM population at 1036 m in elevation, the oxygen concentration is lower than that at sea level, which may facilitate adaptation to hypoxia. Moreover, environmental oxygen availability or embryonic oxygen-handling capacity still meet the oxygen demand of embryonic development at a moderate incubation temperature (26 ± 3 °C) in current study, which has been demonstrated in the common wall lizard to affect incubation duration only at higher incubation temperature (28 °C) (Cordero *et al*. 2017; Kouyoumdjian *et al*. 2019). In the future, it would be interesting and necessary to determine the responses of embryonic development with greater oxygen concentration, as some work has been done (2014).

Although our study revealed the insignificant effect of mild hypoxia from high elevation on fitness related traits such as growth and locomotor performance, we are still far away from concluding that *E. argus* would defeat climate warming by dispersal to high elevations. Low elevation (1036 m) adults and embryos were insensitive to hypoxia in the traits tested in the current study when simulating disperse 1000 m higher, but if high-elevation populations (i.e. 2600 m, Qinghai) face the same situation when dispersing to higher elevation, oxygen level is much lower than in the current study, and lizards may face more challenges like lowered hatchling success or even reduced cognition (Sun *et al*. 2014). In the future, it would be necessary and interesting to conduct experiments with more populations from different elevations, or with aggravated hypoxia stress to reveal the physiological responses at multiple levels. It is not only helpful for understanding the responses to hypoxia of organisms, but also critical to predicting the vulnerabilities of species that may migrate toward higher elevation to survive the warming world.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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