Independent influence of thermoregulatory cost on the lower and upper set-points of a heliothermic lizard

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ABSTRACT

Studies on ectothermic vertebrates generally lead to average indicators of thermal preferences measured in the laboratory, which do not say about responses to natural environmental change and may not inform about individual variation and its triggering mechanisms. We studied whether and how changes in costs of thermoregulation influence the preferred temperature (T_p) of individual lizards and their energetic investment in thermoregulation by exposing specimens to three treatments of increasing costs, recording body temperature (T_b) and distance walked (energetic investment). Moderate costs induced an investment trade-off between energy-investment in thermoregulation and T_b, and highlighted individual variation that decreased with higher costs. Lower average T_p's were observed in the high costs trials as a result of the decreased values of the lower and upper voluntary T_b's. As costs increased, lizards walked a shorter overall distance and accepted lower T_b's, but lizards still engaged in costly thermoregulation. Individual variation resulted in two main thermoregulatory patterns that in certain ecological contexts may have an important role in decision-making and adjusting to temperatures that are far from optimal temperatures for performance and physiological processes.

1. Introduction

The thermal ecology of ectothermic tetrapods emerged as a discipline with the remarkable discovery that several reptile taxa could use behaviour to maintain body temperature (T_b) within narrower ranges than those of air temperature (T_a; Cowles and Bogert, 1944). Further theoretical progress led to the expectation that, during activity, field T_b's should fit the range of temperatures optimizing behavioural and physiological performance (T_o; Dawson, 1975; Huey and Slatkin, 1976). A later and more general proposition was that, among taxa that thermoregulate behaviourally, co-adaptation should exist among the various traits of ecological significance in thermal biology (Huey and Bennett, 1987; Garland et al., 1991; Angilletta et al., 2006), including those related to thermoregulatory behaviour. The ecological significance, repeatability, and variation of thermal preferences under laboratory conditions may differ among lineages, yet such preferences have been demonstrated in numerous studies with various fish species (Beitinger et al., 1975; Williams and Brauer, 1987), amphibians comprising both anurans (Lillywhite, 1971; Bradford, 1984; Freidenburg and Skelly, 2004) and salamanders (Feder, 1982), crocodiles (Johnson et al., 1976), amphisbaena (López et al., 2002), and squamates. Among the last mentioned, comparatively fewer examples exist on snakes

(Blouin-Demers et al., 2000; Lelièvre et al., 2011) and many exist among lizards (Huey, 1982; Angilletta, 2009; Qu et al., 2011). For some small heliothermic lizards, the evolutionary and ecological significance of preferred body temperature (T_p) is well established. Under no ecological restrictions, such lineages are expected to display a strong thermoregulatory drive in the field, and a T_p matching typical activity T_b and T_o (Angilletta et al., 2006).

Because T_b's are usually measured under experimental conditions, recent studies have delved into the extent, scope and conditions under which thermal preferences measured in the laboratory become realized in the field (Sears and Angilletta, 2015; Sears et al., 2016; Camacho and Rusch, 2018), as to better understand the significance of thermal preferences as a component of thermal co-adaptation in heliothermic lizards (Vanberkum et al., 1986; Van Damme et al., 1990; Bauwens et al., 1995). Whereas laboratory studies show that short-time thermal preferences may be population-specific and repeatable, and possibly attributable to natural selection (Gilbert and Miles, 2017), preferred body temperatures may also vary within days among individuals within a population, and may display low repeatability (Clusella Trullas et al., 2007). Thus, the role of natural selection on thermal preferences apparently varies among lineages, and not necessarily the values expressed under certain laboratory conditions reflect dominant

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behavioural drives in the field. Given the patterns reported, extreme care is needed before generalizing about the evolutionary role of preferred temperatures as normally measured. Understanding both the sources of variation in thermal preferences and the nature of such variation seems an essential demand of the field.

Several sources of variation in the thermal preferences in squamates have been identified. Thermal preferences are prone to physiologically adjustment (Seebacher, 2009) and may change with season (Christian and Bedford, 1995; Truter et al., 2014; Baez and Cortes, 1990), oxygen availability (Cadena and Tattersall, 2009a), health (Kluger et al., 1975) or exercise status (Wagner and Gleeson, 1997), feeding condition (Wall and Shine, 2008; Schuler et al., 2011), thermal history (acclimation; Corn, 1971), and quality of the thermal environment (Row and Blouin-Demers, 2006). Indeed, from a neurotheological viewpoint the quality of the thermal environment matters, for individual lizards may respond to integrated field information (Heath, 1970). This premise gained early empirical field support derived from population trends seemingly based on individual decisions. Early examples include the observation that *Anolis oculatus* is both eurytopic and eurythermic, and that their T_b differ among populations and according to weather (Ruibal and Philibosian, 1970); and that *A. sagrei* exhibit lower field temperatures in environments considered more costly for thermoregulation (Lee, 1980). Likewise, habitat quality, possibly in terms of food availability or predation, influences the T_b of conspecific lizards (*Psammodromus algirus*) across environmental gradients (Diaz, 1997).

The notion that field differences in thermal ecology reflect individual thermoregulatory decisions is supported by experimental and mechanistic approaches. Several cases of lizard thermoregulation fit what was termed as *the coupled on-off model* (Heath, 1970; Cadena and Tattersall, 2009b) under which two voluntary temperatures, lower (T_{sl}) and upper (T_{sh}) escape temperatures, signal the voluntary extremes of a thermoregulation range. This model received empirical backing with the findings that, in *Dipsosaurus dorsalis*, the statistically compared variance (by level of confidence) in T_{sl} is higher than in T_{sh}, and that these two extremes act as interacting, yet independent systems (Berk and Heath, 1975). For example, lizards exposed to enhanced costs of thermoregulation (progressively shorter heating time after a minimum displacement of 1 m), display lower T_p paralleled by decreased precision of thermoregulation, with dramatic effects in experiments performed at low environmental temperatures (Withers and Campbell, 1985). Contrasts between field and laboratory behaviour lend further support to this view, for example in the lizard *Elgaria multicarinata*, who thermoregulates precisely under controlled conditions but may display eurythermy in the field (Kingsbury, 1993). Additionally, experimental manipulation of the thermal environment can induce thermoregulation or thermoconformity in some lizard species, including *Zootoca vivipara* (Herczeg et al., 2006), not only when facing changes in the thermal environment, but also under conditions reproducing predation risk (Herczeg et al., 2008). These findings generally suggest enhanced behavioural thermoregulation in cost-benefit favourable environments (Huey and Slatkin, 1976). Furthermore, the accuracy of thermoregulation may also be determined by the thermal environment, which generally varies along the distribution range of a species (Sears and Angilletta, 2015; Sears et al., 2016). However, the experimental study of individual lizard thermoregulation has received comparatively less attention in the context of individual variation and responses.

A few experimental studies have already demonstrated that locomotory costs, a proxy for energy cost, influence the thermoregulatory behaviour of lizards in laboratory settings. Experimental changes in the thermal environment causes lizards to escape from thermal extremes more or less often, according to conditions (Campbell, 1985; Withers and Campbell, 1985; Cadena and Tattersall, 2009b). These studies prove that extreme temperature conditions impact thermoregulation, yet they lack details on individual variation on thermoregulatory behaviour. Such details are needed for a full understanding of lizard thermoregulation, and as a link between natural selection on

thermoregulation leading to broad evolutionary trends. Given this scenario, we investigated whether and how changes in the cost of thermoregulation influence the T_p of individual lizards, and their energetic investment in thermoregulation under various treatments. We manipulated cost of thermoregulation in a system inspired in the shuttle-box approach, which offered access to intermittent sources of heat to the experimental animals. By changing the timing, the minimum distance a lizard had to move for effective thermoregulation could be manipulated between low and moderate costs (the latter requiring more movement in the oval to access heat sources). In an additional experiment, we enhanced cost further by increasing the drag of locomotion. Therefore, in the context of our design, distance moved becomes a quantifiable indicator of energetic investment in thermoregulation. As a study animal we selected the heliothermic lizard *Tropidurus torquatus* (Squamata, Tropiduridae), a lizard known to thermoregulate under both laboratory (Ribeiro et al., 2008; Piantoni, 2015) and field conditions (Kohlsdorf and Navas, 2006; Arruda et al., 2008; Piantoni, 2015; Piantoni et al., 2016). In addition, their thermoregulation behaviour is resilient to minor experimental manipulations: when granted access to energy sources, specimens show a similar T_b to the T_p registered in the laboratory, and their T_p in thermal gradients surpass 34 °C in all studies to date (for example Kohlsdorf and Navas, 2006; Ribeiro et al., 2008).

Because *T. torquatus* display precise thermoregulation under sunny conditions and laboratory conditions (see citations in previous paragraph), we anticipated that enhanced cost of thermoregulation would elicit precise, yet more costly thermoregulation (same T_p and increased distances). Although this hypothesis was supported by the theory and field observations presented in this Introduction, alternative responses could emerge, particularly at very high costs of thermoregulation. For example, even in the context of unknown aspects of thermoregulatory drive and individual variation in this species (and in small heliothermic lizard in general), we anticipated that thermoregulatory effort would decreased if the costs associated with thermoregulation became too high. However, we could neither determine an a priori threshold, nor could we know whether or not such a threshold would be attained in the planned experiment.

2. Materials and methods

2.1. Natural history and care of study animals

Tropidurus torquatus is a common lizard that inhabits in different types of environments across Brazil, Argentina and Uruguay (de Carvalho et al., 2013). They feed on invertebrates and plant material, and exhibit sexual dimorphism so that adult males are larger than females (Pinto et al., 2005). We must emphasize that the name *T. torquatus* applies to several populations widely distributed in South America, and comprehensive ongoing phylogenetic studies suggest that it represents a species complex embracing several independent lineages. Thus, we herein take refuge under the name *T. torquatus* for the populations approached in our work, identified with the aid of experts; nonetheless, future studies may provide arguments in order to revise our nomenclatural decision. For this study six individuals (five females and one male) were captured in Piracicaba, SP, Brazil (22°43′30″ S, 47°38′56″ W, April 2011) and 13 (eight females and five males) in Arinos, MG, Brazil (15°55′01″ S, 46°06′21″ W, March 2012). All individuals were maintained in plastic boxes (60–70 l, 66 × 40 x 33 cm) fit as terraria and placed at the Ectothermic Animals Facility at the Biosciences Institute, University of São Paulo. Each terrarium had vermiculite as substrate and bricks as shelters, and housed four to five lizards with ad libitum sources of water and food (cockroaches and crickets). To facilitate thermoregulation, terraria were also fit with both a UV lamp and a 100 W incandescent spotlight placed at 25–30 cm above substrate level. The room photoperiod was set on from 8:00 h to 18:00 h and the room temperature was not controlled varying between 20 and 30 °C. The experiments were run between February 2nd, 2012
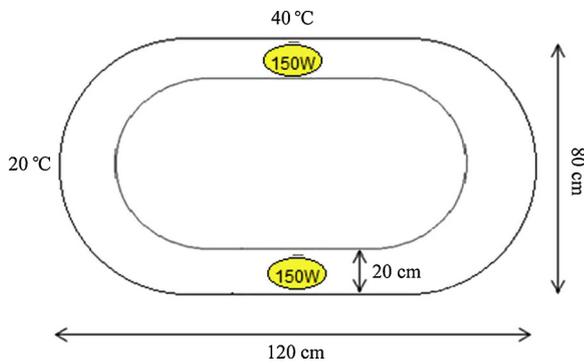
Fig. 1. Sketch of the oval track used to perform the experiments. The temperature varies from 40 °C (under the 150 W lamps) to 20 °C (farthest from the lamps). This particular drawing represents the state with both lamps on; when only one of the lamps was on, the opposite side of the track was at 20 °C. Lizards were always placed at the mid-distance between lamps.

and July 19th, 2012. Posterior to the experiments the specimens were required for alternative studies, after which they will be donated to the Museum of Zoology of the Universidade de São Paulo (MZUSP) after the systematic status is once defined.

2.2. Experimental cost of thermoregulation

Our procedure included one experimental situation aimed the sole analysis of behaviour in the absence of thermal gradients (e.g., without sources for thermoregulating) and three treatments that were applied to all tested lizards in a custom designed transparent oval track (120 X 80 cm) with a sandy substrate. The entire set-up was placed in a walk-in thermal chamber permanently set at 20 °C ± 1 °C. The track had two 150 W incandescent lamps positioned at 25 cm above the track floor at opposite sides of the oval (Fig. 1). These lamps, when permanently on, generated a temperature gradient ranging from 40 °C (substrate under the lamps) to 20 °C (farthest point from the lamps within the track). This set-up, together with further manipulations, allowed for four experimental treatments differing in cost of thermoregulation:

Treatment 1: Minimum experimental cost of thermoregulation (L): Both spotlights were permanently on, so that lizards had access to a stable gradient between 20 °C and 40 °C. This treatment mimics typical conditions offered to lizards in thermal gradients where the cost of thermoregulation is minimal.

Treatment 2: Moderate cost of thermoregulation (M): Spotlights alternate between on and off, every 20 min, and never simultaneously. According to preliminary tests, the 20 min lapse allowed episodic rising of T_b. However, maintaining elevated T_b required periodic movement through the oval track.

Treatment 3: High cost of thermoregulation (H): Same M protocol applied, but an additional weight was attached with an adhesive stripe to the abdomen of the lizard by a 5 cm cut of thread. The extra mass consisted of a piece of dry epoxy weighing as much as each lizard. For this, lizards were weighted two days before the first treatment using a Pesola spring scales of 10 and 100 (± 0.5) g. Body mass was continuously measured during the five months of experiments. This load and design were chosen after diverse preliminary tests because lizards so treated displayed seemingly normal behaviour despite clear increase in the cost of locomotion. Under treatments L, M and NT (see below) lizards were also fit with a 5 cm thread tied to the abdomen (as an additional control to guarantee that observations under the H treatment were explained only by the extra mass added).

Treatment 4: No thermoregulation (NT): No spotlights were on and lizard T_b was 20–26 °C. Thermoregulation at such low temperature was not possible but T_b did not impair activity nor alter locomotion of the individuals during the trials.

We opted to replicate each treatment for any given individual, as to

reduce the impact of technical problems, changes in lizard condition or obviously abnormal behaviours (none of which turned out to be observed), and to evaluate possible impact of a previous experiment in the system. For this, we compared the highest and lowest values of T_b of both replicates for each treatment. Given this design, a full experimental cycle for each individual lizard consisted of eight tests, two for each treatment listed above. However, the final analyses included only the data from the replica generating the highest mean T_b for an individual under a given treatment. All tests started with lizards placed at a marked point in the track, at the midpoint between spotlights (coldest part of the track). A previous study with *T. torquatus* demonstrated that this species remains active and bold since the start of the trials and that after a few hours the animals would become more inactive, particularly after reaching a suitable T_b (Piantoni, 2015; Piantoni et al., 2016). Therefore, in order to obtain more accurate results, the duration of the trials was set to last 100 min, and the order of treatments was randomized for each individual. Lizards were tested only once a day, and never two days in a row.

2.3. Thermal preferences

All experiments were performed from 8:00 h and 18:00 h which corresponds to the activity hours of the lizards in the field (Piantoni, 2015). The T_b was recorded every minute using an ultra-thin catheter thermocouple (1 mm, Omega Engineering Inc., Product code: 5SC-TT-T-40-72) inserted into the cloaca of the experimental lizards. Previous experience with this method, and preliminary tests on the specific set-up here described, confirmed that such thin wire did not cause any perceivable alteration of behaviour during the experimental period.

The T_p was calculated as the mean of the T_b values for each individual lizard/treatment. Additionally, T_{sl} and T_{sh} were defined as the lower and higher "escape" T_b values registered for each individual lizard within a given treatment. The temperature of NT lizards did vary from a maximum (pre-experimental room temperature ~26 °C) to a minimum (experimental chamber temperature ~20 °C), and this variation was unavoidable by behavioural responses. Therefore, these T_b bear no relationship with T_{sl} and T_{sh}, and are referred to as maximum ($T_{b\ max}$) and minimum ($T_{b\ min}$), a nomenclature used only for the NT treatment.

2.4. Locomotion

To quantify locomotion under the diverse treatments we used an observational approach under which an observer recorded the distance of shuttling in individual lizards every time the animal moved. For these records, any possible care was taken not to influence animal behaviour, including a location where the observer would be barely perceptible to test animals. A folding ruler with large numbers was fixed around the track and aided in the estimation of distances moved. Although formally we measured distance over time, we report only distances given that all observation periods were of the same duration.

2.5. Statistical analyses

We used multivariate generalized linear mixed effects models in R statistical environment (R Development Core Team, 2011) to analyse the variation of T_p, T_{sl} and T_{sh} and walked distances across treatments and using as fixed predictors the order in which the treatment took place, the sex, and site where the lizards was collected with "LizardID" as a random intercept. To analyze differences of T_p, T_{sl}, T_{sh} and walked distance across treatments we calculated the lower and upper values for the confident intervals using the *emmeans* package in R as post-hoc test. We further explored inter-individual variation in thermoregulatory responses for T_p, T_{sl} e T_{sh} via a cluster analysis using the hierarchic clustering algorithm in Excel (cluster add-in). For the graphical models, the best-fit curves for the data were chosen based on the number of

Table 1

Type III Analysis of Variance table with Satterthwaite's method for the variation of T_p, T_{sl}, T_{sh} and walked distances of lizards across the treatments with low (L), moderate (M), high (H) and no thermoregulation (NT) cost.

	Variables	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr (> F)
T_p	order	0.02	2.21	1	129	0.0029	0.9570
	sex	0.42	0.1	1	16	0.0531	0.8208
	site	2.74	40.19	1	16	0.3430	0.5663
	treatment	1890.34	630.11	3	129	78.7608	< 2e-16
T_{sl}	order	0.44	0.435	1	129	0.1415	0.7074
	sex	0.02	0.02	1	16	0.0066	0.9364
	site	0.73	0.732	1	16	0.2379	0.6324
	treatment	401.06	133.688	3	129	43.4579	< 2e-16
T_{sh}	order	2.21	2.21	1	129	0.1395	0.7094
	sex	0.1	0.1	1	16	0.0065	0.9367
	site	40.19	40.19	1	16	2.5365	0.1308
	treatment	1925.71	641.9	3	129	40.5094	< 2e-16
Walked distance	order	364273	364273	1	53.868	1.1382	0.2907
	sex	97408	97408	1	15.988	0.3044	0.5888
	site	183907	183907	1	16.007	0.5746	0.4594
	treatment	7274874	2424958	3	53.067	7.577	< 2e-16

parameters and AIC values of all possible curves obtained with the software TableCurve.

3. Results

3.1. Body temperature and cost of thermoregulation

Only cost of thermoregulation had a significant effect on lizards (Table 1). Neither the order in which the treatments were performed, the sex or the site of the lizards were significant predictors for T_b and distance (Table 1). A predominant response of *T. torquatus* was to reduce T_b T_{sl} and T_{sh}, and walked distance with enhanced cost of thermoregulation (Fig. 2; Tables 1 and 2) but significant differences existed only when comparing the values between the treatments L and H, including overall range and quartile distribution of T_p (Q_3-Q_1; L: 4.2 °C, M: 7.1 °C, H: 5.2 °C; Fig. 2; Tables 1 and 2). In contrast, all three variables were similar when comparing treatments M and H (Fig. 2; Tables 1 and 2).
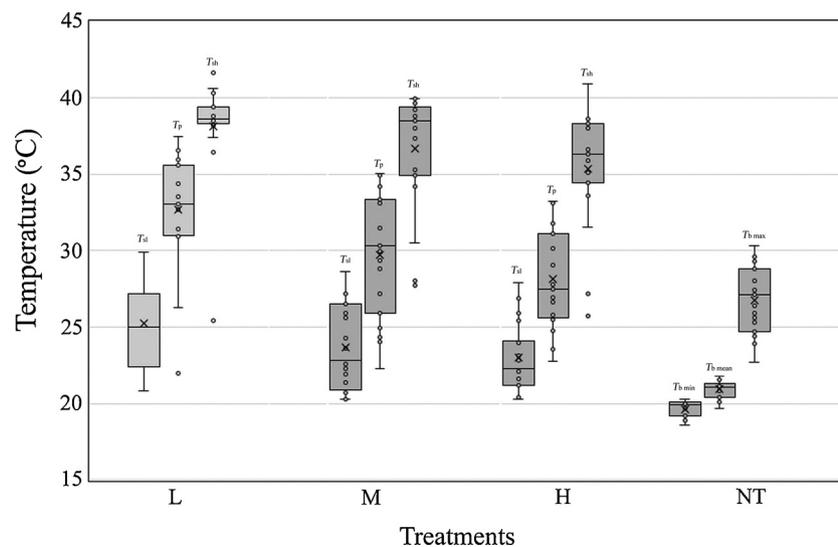
3.2. Graphical models

Only for heuristic purposes, we built graphic models presenting alternative scenarios of thermoregulatory behaviour that could emerge

Table 2

Values for the preferred (T_p), the lowest (T_{sl}) and highest (T_{sh}) body temperature (°C) and moved distances (cm) for overall specimens exposed to the control (NT), low (L), moderate (M), and high (H) costs of thermoregulation treatments. Thermal data are expressed as means ± SD and lower and upper CL whereas distance data is expressed as median (interquartile range).

Variables	Treatments	Mean ± SD / Mean (range)	lower CL	upper CL
T_p	NT	21 ± 0.6	19.4	28.4
	L	32.7 ± 3.7	29.8	32.9
	M	29.7 ± 4	26.7	29.8
	H	28.1 ± 3.3	25.3	28.4
T_{sl}	NT	19.6 ± 0.5	18.7	20.7
	L	25.3 ± 2.8	23.5	25.4
	M	23.6 ± 2.8	22	24
	H	23 ± 2.2	21.5	23.5
T_{sh}	NT	26.8 ± 2.2	25.4	28.5
	L	38.1 ± 3.3	35.5	38.5
	M	36.7 ± 3.9	33.8	36.9
	H	35.4 ± 3.8	32	35.1
Distance	NT	315 (0-1330)	35.4	755
	L	525 (0-4095)	664	1372
	M	595 (140-3500)	596.3	1332
	H	210 (70-980)	−100.8	613

Fig. 2. Box-plot of the variation of the mean (T_p), lower (T_{sl}) and higher (T_{sh}) escape temperatures (°C) across the treatment where no thermoregulation is needed (NT) and where cost is low (L), moderate (M) and high (H).
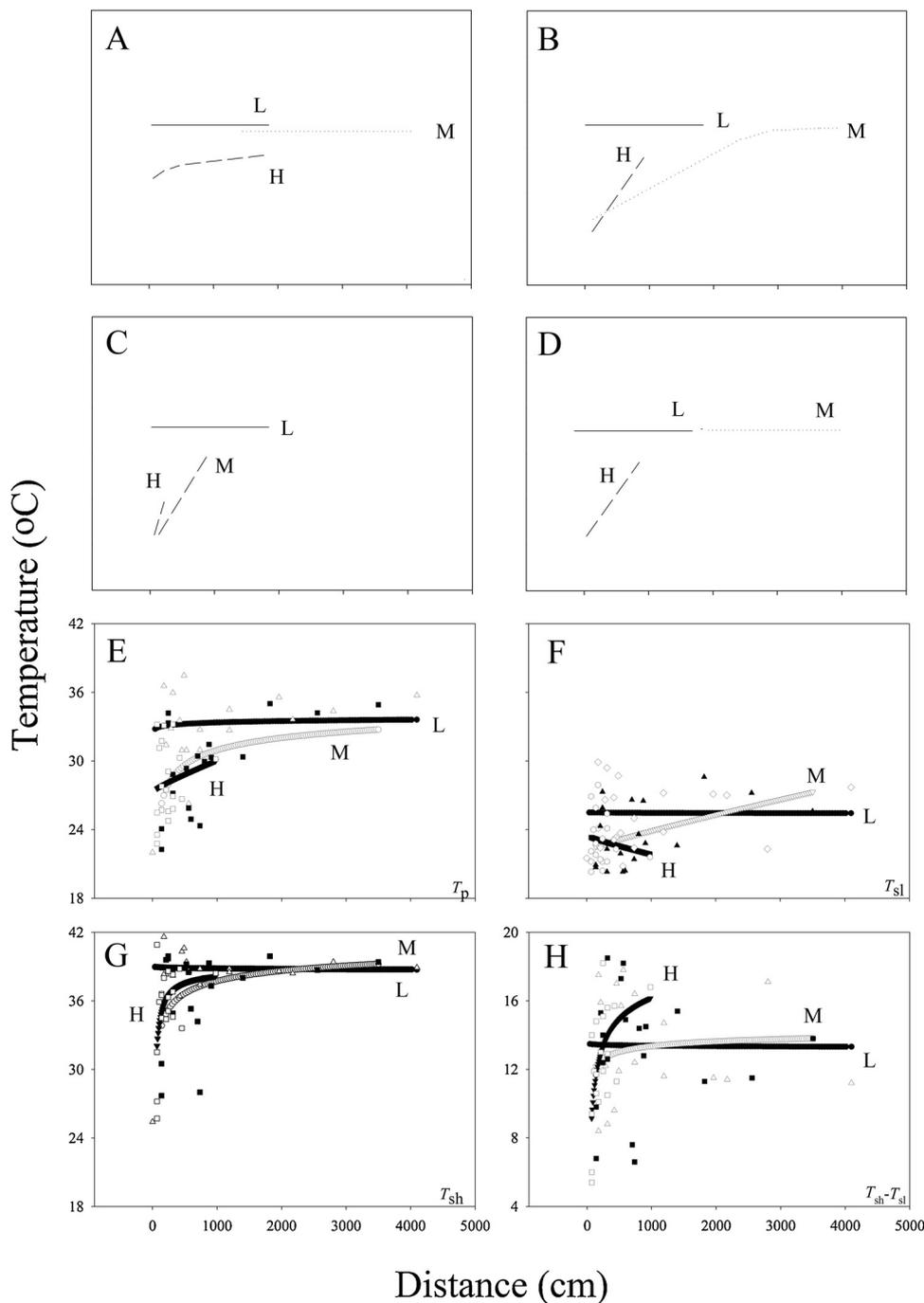
Fig. 3. (A–D) Graphical models exploring possible scenarios of response to cost of thermoregulation (H = high, M = moderate and L = low) in the system used. These models are used for heuristic purposes and have no theoretical pretention. Scenario 1A: consistent behaviour among individuals. At L lizards move less and maintain typical T_p, whereas at M lizards also attain T_p, but at a cost in movement. At H movement is reduced with a toll on T_p. Scenario 1B: Enhanced variation among individuals. Thermoregulation under L remains the same but under M lizards vary the response across a continuum. H lizards tend to abandon thermoregulation. Scenario1C: Weak thermoregulatory responses. Only at L thermoregulation is preserved, but lizards tend not to thermoregulate actively at either M or H. Scenario 1D: Moderate thermoregulatory response in all individuals. Similar to 1B, but all lizards under M attain typical T_p enhancing distance moved. Figs. 2(E–H): Observed variation of the mean (T_p, A), lower (T_{sl}, B) and higher (T_{sh}, C) selected temperatures and range between the lower and higher selected temperature (T_{sl} and T_{sh}; D) as a function of distance moved (m) under L, M and H conditions.

in an experimental system such as ours (Fig. 2A–D). Fig. 2 represents the actual data relating thermoregulation variables to and distance moved at the three costs of thermoregulation high (H), moderate (M) and low (L), and reflects the graphical model in 2B: At L all individuals maintained T_p around a mean (~33 °C, Fig. 2E). At M diverse responses occurred given individual variation in thermoregulating behaviour. Some individuals moved long distances to enhance thermoregulation, thus approaching a T_b higher than 30 °C with only a slight reduction in T_p (highest values ~30 °C) relative to L. In a continuum of responses, other individuals remained stationary or moved over short distances, thus displayed T_b well below L T_p's. At the highest cost of thermoregulation (H) the overall range of distances moved decreased sharply, and consequently, T_b varied among individuals according to lizard position in the track. However, some individuals did move over slightly longer distances than others, which granted them comparatively higher

T_b within the H treatment. Particularly at M and H, the overall ranges of T_{sl}, T_p and T_{sh} (i.e., ranges between the minimum and maximum values), and the mean T_p values decreased as lizards walked over shorter distances (Table 1) (Fig. 3).

3.3. Impact of cost of thermoregulation on voluntary thermal extremes

Voluntary thermal extremes varied independently among treatments. The observed decrease in T_p under H and M (relative to L) was mainly due to the reduced T_{sl} (Table 1). Under L, all lizards maintain T_{sl} and T_{sh} closely around the general mean, independently of the distances moved by lizards (Fig. 2E–G). Under M, lizards approached the T_{sh} reached under L (Fig. 2G), but the individuals that relaxed thermoregulation displayed lower T_{sh} and moved less, as most individuals under H. Therefore, although the maximum T_{sh} was similar under the

three treatments, within-treatment variation in T_{sh} was particularly high at L, and to a lower extent, at M (Fig. 2G). Thermoregulating lizards under M treatment moved more and maintained higher T_{sl} that those that did not thermoregulate, which moved less and displayed low T_{sl} (Fig. 2F). At high cost of thermoregulation lizards maintained T_{sl}'s near the lowest range of the M treatment, yet with a tendency to further decrease T_{sl} even in lizards that moved within the treatment (see Fig. 2F). In other words, overall, when costs were high lizards moved more and reached elevated T_{sh}, but their T_b dropped significantly when experiencing the thermoregulation extremes challenge. This trend was conspicuous when analyzing the total range of T_b under experimental conditions (Fig. 2H): L and most M lizards varied T_b (T_{sh}-T_{sl}) by some 13 °C, whereas few M and more H lizards presented ranges of T_b above 17 °C. The observed increase in range was due mainly to a reduction in T_{sl}.

3.4. Movement in the absence of thermal gradients

Lizards in the "no thermoregulation" (NT) treatment shuttled comparatively shorter distances than thermoregulating lizards (Table 2).

3.5. Individual variation in responses to enhanced cost of thermoregulation

Our cluster analyses did not define any fix number of clusters, so that the split of lizards into two *a posteriori* groups based on thermoregulatory traits was itself a finding. The hierarchical algorithm used by the cluster analysis estimated the degree of separation among the lizards (considering the individual T_{sl}, T_p and T_{sh} for each treatment) creating a dendogram (Figure S1). Based on the overall traits observed, we named these groups "Relaxed Thermoregulators" (RT, $n = 8$) and "Precise Thermoregulators" (PT, $n = 11$). Both groups included lizards from Arinos and Piracicaba as well as females and males. With the exception of T_{sh} under the L treatment, all main thermal variables T_{sl}, T_p and T_{sh} differed between these two groups (Fig. 1E). RT thermoregulated with high variance in T_p, reached high T_b only at the lowest thermoregulation cost, displayed very low T_{sl}, and dropped T_{sh} substantially at higher thermoregulation costs (Table 3). In contrast, PT grouped individuals that thermoregulated with high precision, reached high T_b at any thermoregulation cost, and responded to enhanced cost of thermoregulation with a small and symmetrical drop in both T_{sl} and T_{sh} (Table 3). RT and ST lizards also differed in distances moved, with RT lizards moving over longer distances at all treatments (Table 3).

4. Discussion

Huey and Slatkin (1976) suggested that costs and benefits determine optimal thermoregulatory behaviour in lizards and that this trade-off generates predictions applicable at various levels of biological organization. These predictions can be tested experimentally. For example, prediction II breads "*Depending on the shape and the position of the composite functions, the optimal amount of thermoregulation can increase, decrease, or switch abruptly along a gradient of ambient temperatures*". Our data matter for they support this prediction and add a notion of intra- and inter-individual variation in responses. So, the "*optimal amount of thermoregulation*" varies with cost and thermal environments, but may vary also among individuals within a population. Our laboratory experiments show unequivocally that lizards in the same population may take different thermoregulatory decisions under similar circumstances, and that this individual variation is not linked to sex, size or locality of origin. A strong thermoregulatory drive, evident by the longest distances travelled by individual lizards, was evident only at moderate costs of locomotion. At highest costs, some individuals do not make thermoregulation a priority, and reduce movement at the cost of body temperature. This strongly suggests that, even for small heliothermic lizards, the individual drive to thermoregulate is heavily influenced by

cost of thermoregulation. We acknowledge that multiple and non-exclusive neurophysiological mechanisms may be involved. For example, one reviewer of this paper has posed that differential learning about the system may enhance individual differences in thermoregulatory responses. We cannot formally discard this or other possible factors, but hypothesize that the dominant basis underlying this pattern root from intrinsic neurophysiological differences among individuals. We propose that when thermoregulatory conditions are challenging, neural physiology, expressed as behaviour in the context of environmental perception and decision-making, may generate a type of thermoregulation that, even if active and unambiguous, may convey T_p's lower than those regarded as optimal (usually those obtained at low cost of thermoregulation). We shall discuss, then, how this modified T_p's are attained.

The problem of *how* lizard thermoregulation adjusts to environmental factors was first analysed from the perspective of precision of thermoregulation (DeWitt, 1967). DeWitt exposed *Dipsosaurus dorsalis* to a concentric thermal gradient and noticed individual variation with "*low and high extremes of thermoregulatory precision*", with consistency among individuals. A crucial aspect of DeWitt's data is that the T_b distribution was skewed, so that low T_b displayed a wider range and, importantly, occurred at various times of the day. So, DeWitt's data compares to ours in that the voluntary minimum temperatures are more variable than the maxima counterparts. This may be a general pattern for thermoregulating lizards, according to a later compilation (DeWitt and Friedman, 1979). On this matter, and based on studies in the same species (*D. dorsalis*), Withers and Campbell (1985) investigated T_p in relationship with the metabolic cost of thermoregulation and proved that lizard shuttling relates negatively with precision of thermoregulation. Similarly, as the costs of thermoregulation increase, *T. torquatus* allows for lower body temperatures before seeking energy sources, and the modified thermoregulatory pattern is based chiefly on longer and deeper thermal drops. Wither and Campbell (1985) describe somewhat consistent drops in lower and upper T_p's, whereas in *T. torquatus* the temperature triggering heat-seeking behaviour ("T_{bask}", analogous to the "minimum voluntary temperature" - herein T_{sl} - described by Cowles and Bogert, 1944) seems comparatively more flexible. Additionally, Cadena and Tattersall (2009b) reporting on bearded dragons *Pogona vitticeps* showed that high shuttling environments lead to lower T_p and lowered T_{sl} and T_{sh}, with stronger effects on T_{sl}. Care is needed comparing studies because of methodological differences, and because seemingly minor details may modify lizard thermoregulatory responses in experimental settings. However, a common denominator of these papers is higher variability on the low range of thermoregulating temperatures and a decrease in the *minimum* temperature achieved.

We recognise that laboratory results require careful considerations before a conceptual expansion to field conditions, but identify two contexts in which our data may help to understand field observations. First, the findings here reported are compatible with the idea that disparity in the thermal environment may lead to differences among populations in thermoregulatory behaviour. Such differences among populations do exist and may be partially explained by individual responses. For example, in *Tropidurus* (Piantoni et al., 2016), some populations show a higher efficiency of thermoregulation in open and heterogeneous sites preliminarily judged as less costly for thermoregulation relative to forests and other thermally homogeneous environments. An analogue notion applies to temporal variation in thermoregulatory responses within a population such as seasonality (see references in Huey, 1982, p.38). For example, the major source of variation in panting threshold in the agamid lizard *Amphibolurus muricatus* is day-to-day change within individuals (Heatwole et al., 1973). Further studies found that individuals of this species also have a higher voluntary maximum temperature in spring and summer than in autumn (Heatwole and Firth, 1982), and that the T_p of the cordylid *Ouroborus cataphractus* varies between 26.8 °C and 32.6 °C across seasons (Truter et al., 2014). Furthermore, during the Kalahari winter more individual

Table 3

Contrast between relaxed thermoregulators (RT) and strong thermoregulators (ST) groups. Means $\pm$ SD and medians (min-max range) of the preferred (T_p), lower (T_{sl}) and higher (T_{sh}) body temperatures (°C), and means $\pm$ SD and medians (interquartile range) the total shuttled distance (cm) of lizards from the relaxed (RT) and the precise (PT) thermoregulators groups during treatments of low (L), moderate (M), and high (H) cost of thermoregulation. The preferred (T_p), minimum (T_{min}), maximum (T_{max}) body temperatures and the total shuttled distance (cm) of lizards from the two groups are also expressed for the control treatment (NT), where the heat source was absent. The coefficient of variation (CV) of the total shuttling was calculated with the equation $\sigma/\mu \times 100$.

Treatments	Variables		RT ($n = 11$)		PT ($n = 8$)	
			Mean $\pm$ SD	Median (range)	Mean $\pm$ SD	Median (range)
L	Body temperatures (°C)	T_p	31 $\pm$ 3.8	31.4 (22-34.5)	35 $\pm$ 1.8	35.6 (32.7-37.4)
		T_{sl}	23.3 $\pm$ 1.7	23.3 (20.8-27)	27.9 $\pm$ 1.3	27.5 (26.6-29.9)
		T_{sh}	37.8 $\pm$ 4.3	38.6 (25.4-41.6)	38.6 $\pm$ 1.2	38.7 (36.4-40.6)
	Distance (cm)		869 $\pm$ 873	560 (271.25-1076.25)	1116 $\pm$ 1346	455 (300-1575)
	CV		100.5		120.6	
M	Body temperatures (°C)	T_p	27.9 $\pm$ 3	27.2 (22.2-31.5)	33.2 $\pm$ 1.9	33.8 (30.3-35)
		T_{sl}	21.9 $\pm$ 1.9	21.4 (20.3-26.5)	26 $\pm$ 1.8	26.3 (22.8-28.6)
		T_{sh}	35.3 $\pm$ 4.5	38 (27.7-39.9)	38.6 $\pm$ 2	39.5 (34.2-39.9)
	Distance (cm)		582 $\pm$ 370	560 (315-787.5)	1273 $\pm$ 1231	805 (245-2187.5)
	CV		63.5		96.7	
H	Body temperatures (°C)	T_p	25.7 $\pm$ 1.6	25.7 (22.8-27.8)	31.5 $\pm$ 1.6	31.4 (29-33.2)
		T_{sl}	21.7 $\pm$ 1.1	21.4 (20.3-24.1)	24.8 $\pm$ 2.1	24.7 (21.6-27.9)
		T_{sh}	33.6 $\pm$ 4	34.5 (25.7-38.6)	37.7 $\pm$ 1.8	38.15 (35.1-40.9)
	Distance (cm)		207 $\pm$ 124	210 (87.5-297.5)	306 $\pm$ 296	210 (122.5-367.5)
	CV		59.8		96.5	
NT	Body temperatures (°C)	T_p	20.9 $\pm$ 0.4	20.9 (20-21.3)	20.5 $\pm$ 0.7	20.5 (19.2-21.4)
		$T_{b\ min}$	19.5 $\pm$ 0.5	19.7 (18.6-20.1)	19.8 $\pm$ 0.5	20 (18.9-20.3)
		$T_{b\ max}$	26.7 $\pm$ 2.3	27.1 (22.7-30.3)	26.9 $\pm$ 2.3	26.8 (23.9-29.6)
	Distance (cm)		363 $\pm$ 417	175 (70-455)	446 $\pm$ 234	420 (245-630)
	CV		114.9		52.5	

lizards of this species perch under the sun, and do so during longer periods of time (Huey and Pianka, 1977). The mechanisms responsible for such variation may involve cyclic shifts in decision-making, but also day-to-day adjustment in the T_p of lizards. The latter response would be related to what is reported in this study.

For what has been exposed, a key element of lizard thermoregulation is the minimum voluntary temperature (Cowles and Bogert, 1944), T_{bask}, which must have some independence from its maximum counterpart. If we conceive thermoregulation as based on a neurophysiological control system, and such a system generates distributions of upper and lower volunteer limits that are skewed; then the system cannot have basis on a single and proportional set-point structure (Barber and Crawford, 1977). Then, the data here presented are compatible with a lizard thermoregulatory system that, on its neurophysiological basis, requires either a non-linear control mechanism (DeWitt and Friedman, 1979) or two independent controllers respectively associated to minimum (T_{sl}) and maximum (T_{sh}) voluntary temperatures, with a non-thermoregulatory zone in the middle (Heath, 1970). More specifically, our data offer strong empirical support to the *two fully independent systems* view, because both symmetrical and skewed thermoregulatory shifts were induced by increased cost of thermoregulation.

Finally, our study was not originally designed to tell apart intra- and inter-individual variation, although certainly, both contribute to explain the findings here reported. The patterns found are compatible with the notion of stochastic elements in lizard thermoregulation. The addition of stochastic elements to a dual set point system would allow for both thermoregulatory and non-thermoregulatory phases, eventually favouring alternative activities in a single population (Barber and Crawford, 1977).

5. Conclusions

When thermoregulation is hindered (i.e., as the distance from heat resources increase) lizards may accept lower temperatures or endeavour to keep themselves warm, but responses may vary among individuals. When the latter response occurs, thermoregulation may become less precise and more energetic. Alternatively, lizards may cover shorter distances and relax thermoregulatory behaviour even to the

point of becoming non-existent. Both responses may occur under same circumstances, thus a full spectrum of energy investment in thermoregulation may coexist in a population. This individual variation may have important repercussions given ecological scenarios where decision-making and adjustment to new or changing environments are necessary.

References

Angilletta, M.J., 2009. Thermal Adaptation. A Theoretical and Empirical Synthesis. University Press, Oxford.

Angilletta, M.J., Bennett, A.F., Guderley, H., Navas, C.A., Seebacher, F., Wilson, R.S., 2006. Coadaptation: a unifying principle in evolutionary thermal biology. Physiol. Biochem. Zool. 79, 282–294.

Baez, C., Cortes, A., 1990. Precision of behavioural thermoregulation in the neotropical lizard *Tropidurus quadrivittatus* (Lacertilia: iguanidae). Rev. Chil. Hist. Nat. 63, 203–210.

Barber, B.J., Crawford Jr., E.C., 1977. A stochastic dual-limit hypothesis for behavioural thermoregulation in lizards. Physiol. Zool. 50, 53–60.

Bauwens, D., Garland, T., Castilla, A.M., Van Damme, R., 1995. Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioural covariation. Evolution 49, 848–863.

Beitinger, T.L., Magnuson, J.J., Neill, W.H., Shaffer, W.R., 1975. Behavioural thermo-regulation and activity patterns in the green sunfish, *Lepomis cyanellus*. Anim. Behav. 23, 222–229.

Berk, M.L., Heath, J.E., 1975. An analysis of behavioural thermoregulation in the lizard, *Dipsosaurus dorsalis*. J. Therm. Biol. 1, 15–22.

Blouin-Demers, G., Kelley, J.K., Weatherhead, P.J., 2000. Plasticity in preferred body temperature of young snakes in response to temperature during development. Copeia 2000, 841–845.

Bradford, D.F., 1984. Temperature modulation in a high-elevation amphibian, *Rana muscosa*. Copeia 1984, 966–976.

Cadena, V., Tattersall, G.J., 2009a. Decreased precision contributes to the hypoxic ther-moregulatory response in lizards. J. Exp. Biol. 212, 137–144.

Cadena, V., Tattersall, G.J., 2009b. The effect of thermal quality on the thermoregulatory behavior of the bearded dragon *Pogona vitticeps*: influences of methodological as-sessment. Physiol. Biochem. Zool. 82 (3), 203–217.

Camacho, A., Rusch, T.W., 2018. Methods and pitfalls of measuring thermal preference and tolerance in lizards. J. Thermal Biol. https://doi.org/10.1016/j.jtherbio.2017.03.010.

Campbell, J.D., 1985. The influence of metabolic cost upon the level and precision of behavioral thermoregulation in an eurythermic lizard. Comp. Biochem. Physiol. A 81, 597–601.

Christian, K.A., Bedford, G.S., 1995. Seasonal changes in thermoregulation by the frill-neck lizard, *Chlamydosaurus kingii*, in tropical Australia. Ecology 76, 124–132.

Clusella Trullas, S., Terblanche, J.S., van Wyk, J.H., Spotila, J.R., 2007. Low repeatability of preferred body temperature in four species of *Cordylid lizards*: temporal variation and implications for adaptive significance. Evol. Ecol. 21 (10), 63–79.

Corn, M.J., 1971. Upper thermal limits and thermal preferenda for three sympatric spe-cies of Anolis. J. Herpetol. 5, 17–21.

Cowles, R.B., Bogert, C.M., 1944. A preliminary study of the thermal requirements of desert reptiles. Bull. Am. Mus. Natm. Hist. 83, 261–296.

Dawson, W.R., 1975. On the physiological significance of the preferred body tempera-tures of reptiles. In: Gates, D.M., Schmerl, R.B. (Eds.), Perspectives in Biophysical Ecology. Springer-Verlag, Berlin, pp. 443–473.

de Carvalho, A.L.G., de Britto, M.R., Fernandes, D.S., 2013. Biogeography of the lizard genus *Tropidurus* wied-Neuwied, 1825 (Squamata: tropiduridae): distribution, en-demism, and area relationships in South America. PLoS One 8 (3), e59736.

DeWitt, C.B., 1967. Precision of thermoregulation and its relation to environmental fac-tors in the desert Iguana, *Dipsosaurus dorsalis*. Physiol. Zool. 40, 49–66.

DeWitt, C.B., Friedman, R.M., 1979. Significance of skewness in ectotherm thermo-regulation. Am. Zool. 19, 195–209.

Diaz, J.A., 1997. Ecological correlates of the thermal quality of an ectotherm's habitat: a comparison between two temperate lizard populations. Funct. Ecol. 11, 79–89.

Feder, M.E., 1982. Thermal ecology of neotropical lungless salamanders (Amphibia: plethodontidae): environmental temperatures and ecological responses. Ecology 63, 1665–1674.

Freidenburg, L.K., Skelly, D.K., 2004. Microgeographical variation in thermal preference by an amphibian. Ecol. Lett. 7, 369–373.

Garland, T., Huey, R.B., Bennett, A.F., 1991. Phylogeny and coadaptation of thermal physiology in lizards: a reanalysis. Evolution 45, 1969–1975.

Gilbert, A.L., Miles, D.B., 2017. Natural selection on thermal preference, critical thermal maxima and locomotor performance. Proc. R. Soc. B 284 20170536.

Heath, J.E., 1970. Behavioural thermoregulation of body temperature in poikilotherms. Physiologist 13, 399–410.

Heatwole, H., Firth, B.T., 1982. Voluntary maximum temperature of the jackie lizard, *Amphibolurus muricatus*. Copeia 1982, 824–829.

Heatwole, H., Firth, B.T., Webb, G.J.W., 1973. Panting thresholds of lizards. I. Some methodological and internal influences on the panting threshold of an agamid, *Amphibolurus muricatus*. Comp. Biochem. Physiol. 46A, 799–826.

Herczeg, G., Gonda, A., Saarikivi, J., Merila, J., 2006. Experimental support for the cost-benefit model of lizard thermoregulation. Behav. Ecol. Sociobiol. 60, 405–414.

Herczeg, G., Herrero, A., Saarikivi, J., Gonda, A., Jantti, M., Merila, J., 2008. Experimental support for the cost-benefit model of lizard thermoregulation: the ef-fects of predation risk and food supply. Oecologia 155, 1–10.

Huey, R.B., 1982. Temperature, physiology, and the ecology of reptiles. In: In: Gans, C., Pough, F.H. (Eds.), Biology of the Reptilia Vol. 12. Academic Press, New York, pp. 25–74.

Huey, R.B., Bennett, A.B., 1987. Phylogenetic studies of coadaptation: preferred tem-peratures vs. Optimal performance temperatures of lizards. Evolution 41, 1098–1115.

Huey, R.B., Pianka, E.R., 1977. Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. Ecology 58 (5), 1066–1075.

Huey, R.B., Slatkin, M., 1976. Cost and benefits of lizard thermoregulation. Q. Rev. Biol. 51, 363–384.

Johnson, C.R., Webb, G.J.W., Tanner, C., 1976. Thermoregulation in crocodilians—II. A telemetric study of body temperature in the Australian crocodiles, *Crocodylus john-stoni* and *Crocodylus porosus*. Comp. Biochem. Physiol. 53A, 143–146.

Kingsbury, B.A., 1993. Thermoregulatory set points of the eurythermic lizard *Elgaria multicarinata*. J. Herpetol. 27, 241–247.

Kluger, M.J., Ringler, D.H., Anver, M.R., 1975. Fever and survival. Science 188, 166–168.

Kohlsdorf, T., CA Navas, C.A., 2006. Ecological constraints on the evolutionary associa-tion between field and preferred temperatures in Tropidurinae lizards. Evol. Ecol. 20 (6), 549–564.

Lee, J.C., 1980. Comparative thermal ecology of two lizards. Oecologia 44, 171–176.

Lelièvre, 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.

Lillywhite, H.B., 1971. Temperature selection by the bullfrog, *Rana catesbeiana*. Comp. Biochem. Physiol. 401, 213–227.

López, P., Civantos, E., Martin, J., 2002. Body temperature regulation in the am-phisbaenian *Trogonophis wiegmanni*. Can. J. Zool. 80, 42–47.

Piantoni, C., 2015. The Effects of Climate Warming on Populations of the *Tropidurustorquatus* Species Group (Squamata: Tropiduridae) in Brazil. Doctoral dissertation. http://www.teses.usp.br/teses/disponiveis/41/41135/tde-08032016-160411/en.php.

Piantoni, C., Navas, C.A., Ibargüengoytía, N.R., 2016. Vulnerability to climate warming of four genera of New World iguanians based on their thermal ecology. Anim. Conserv. 19, 391–400. https://doi.org/10.1111/acv.12255.

Pinto, A.C.S., Wiederhecker, H.C., Colli, G.R., 2005. Sexual dimorphism in the Neotropical lizard, *Tropidurus torquatus* (Squamata, Tropiduridae). AmphibiaReptilia 26, 127–137.

Qu, Y., Li, H., Gao, J., Xu, X., Ji, X., 2011. Thermal preference, thermal tolerance and the thermal dependence of digestive performance in two *Phrynocephalus* lizards (Agamidae), with a review of species studied. Curr. Zool. 57 (6), 684–700.

R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. ISBN 3-900051-07-0, URL. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Ribeiro, L.B., Gomides, S.C., Santos, A.O., de Sousa, B.M., 2008. Thermoregulatory be-haviour of the saxicolous lizard, *Tropidurus torquatus* (Squamata, Tropiduridae), in a rocky outcrop in Minas Gerais, Brazil. Herpetol. Conserv. Biol. 3 (1), 63–70. http://www.herpconbio.org/Volume_3/Issue_1/Ribeiro_etal_2008.pdf.

Row, J.R., Blouin-Demers, G., 2006. Thermal quality influences habitat selection at multiple spatial scales in milksnakes. Ecoscience 13 (4), 443–450.

Ruibal, R., Philibosian, R., 1970. Eurythermy and niche expansion in lizards. Copeia 1970, 645–653.

Schuler, M.S., Sears, M.W., Angilletta, M.J., 2011. Food consumption does not affect the preferred body temperature of Yarrow's spiny lizard (*Sceloporus jarrovi*). J. Therm. Biol. 36, 112–115.

Sears, M.W., Angilletta, M.J.Jr., 2015. Costs and benefits of thermoregulation revisited: both the heterogeneity and spatial structure of temperature drive energetic costs. Am. Nat. 185 (4), E94–E102.

Sears, M.W., Angilletta, M.J.Jr., Schuler, M.S., Borchert, J., Dilliplane, K.F., Stegman, M., Rusch, T.W., Mitchell, W.A., 2016. Configuration of the thermal landscape de-termines thermoregulatory performance of ectotherms. PNAS 113 (38), 10595–10600.

Seebacher, F., 2009. Responses to temperature variation: integration of thermoregulation and metabolism in vertebrates. J. Exp. Biol. 212, 2885–2891.

Truter, J.C., van Wyk, J.H., Mouton, P.L.N., 2014. An evaluation of daily, seasonal and population-level variation in the thermal preference of a group-living lizard, *Ouroborus cataphractus* (Sauria: cordylidae). AmphibiaReptilia 35, 391–403.

Van Damme, R., Bauwens, D., Castilla, A.M., Verheyen, R.F., 1990. Comparative thermal ecology of the sympatric lizards *Podarcis tiliguerta* and *Podarcis sicula*. Acta Oecol. 11, 503–512.

Vanberkum, F.H., Huey, R.B., Adams, B.A., 1986. Physiological consequences of ther-moregulation in a tropical lizard (*Ameiva festiva*). Physiol. Zool. 59, 464–472.

Wagner, E.L., Gleeson, T.T., 1997. Postexercise thermoregulatory behaviour and recovery from exercise in desert iguanas. Physiol. Behav. 61, 175–180.

Wall, M., Shine, R., 2008. Post-feeding thermophily in lizards (*Lialis burtonis* Gray, Pygopodidae): laboratory studies can provide misleading results. J. Thermal. Biol. 33, 274–279.

Williams, E.E., Brauer, R.W., 1987. Thermoregulatory behaviour and temperature gra-dient perception in a juvenile fish (*Poecilia reticulata*. Can. J. Physiol. Pharmacol. 65, 1389–1394.

Withers, P.C., Campbell, J.D., 1985. Effects of environmental cost on thermoregulation in the desert iguana. Physiol. Zool. 58, 329–339.