Lizards paid a greater opportunity cost to thermoregulate in a less heterogeneous environment

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Summary

1. The theory of thermoregulation has developed slowly, hampering efforts to predict how individuals can buffer climate change through behaviour. Mixed results of field and laboratory experiments underscore the need to test hypotheses about thermoregulation explicitly, while measuring costs and benefits in different thermal landscapes.

2. We simulated body temperature and energy expenditure of a virtual lizard that either thermoregulates optimally or thermoconforms in a landscape of either low or high quality (one or four basking sites, respectively). We then compare the predicted values in each landscape with the observed values for real lizards in experimental arenas.

3. Lizards thermoregulated more accurately in the high-quality landscape than they did on the low-quality landscape, albeit only slightly so, but spent similar amounts of energy in these landscapes. Basking, rather than shuttling between heat sources, accounted for the majority of the energy consumed in both landscapes.

4. These results did not support the predictions of our model. In the low-quality landscape, real lizards thermoregulated intensely despite the potential to save energy by thermoconforming. In the high-quality landscape, lizards moved more than expected, suggesting that lizards explored their surroundings despite being able to thermoregulate without doing so.

5. Our results suggest that non-energetic benefits drive thermoregulatory behaviour in costly environments, despite the missed opportunities arising from thermoregulation. We propose that energetic costs associated with thermoregulatory movement will become substantial in homogeneous environments such as flat plains and dense forests. The theory of thermoregulation should incorporate these aspects if biologists wish to predict responses of ectotherms to changing climates and habitats.

Key-words: climate change, cost–benefit model, ectotherm, energy budget, microclimate, null model, performance, survival

Introduction

Many organisms actively maintain temperatures to maximize physiological functions that enhance fitness (Sinervo & Adolph 1989; Angilletta 2009). Despite the central role of behaviour in ecological and evolutionary processes, a theory of behavioural thermoregulation has developed slowly. Forty years have passed since Huey & Slatkin (1976) formulated the first mathematical model of optimal thermoregulation. Yet few experimental tests of this model have been conducted (reviewed by Angilletta 2009; see also Vickers, Manicom & Schwarzkopf 2011). This slow pace of theoretical development hampers efforts to predict

whether species will buffer climate change through behaviour (Kearney, Shine & Porter 2009; Huey *et al.* 2012; Sunday *et al.* 2014; Buckley, Ehrenberger & Angilletta 2015). Furthermore, because thermoregulatory strategies are tightly linked to the landscape of microclimates (Clusella-Trullas & Chown 2011; Sears & Angilletta 2015), we need a theoretical understanding to assess the potential for thermoregulation in disturbed habitats, such as those fragmented by human activities or invaded by alien plants (Schreuder & Clusella-Trullas 2016). Thus, a theory of thermoregulation has implications for patterns at higher levels of biological organization, such as communities and ecosystems (e.g. Tuff, Tuff & Davies 2016).

Huey & Slatkin's (1976) model predicts the optimal amount of thermoregulation given an energetic cost and

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Table 1. Predicted effects of various costs on the thermoregulatory behaviour of animals based on the optimality model of thermoregulation

Environment	Energetic cost of deviation from preferred temperature	Opportunities missed while using thermal resources	Risk of mortality from predators attracted to movement
Low quality	↓Thermoregulation	↓Thermoregulation	↓Thermoregulation
High quality	↑Thermoregulation	No effect	Little or no effect

benefit. According to this model, an organism should thermoregulate precisely only when the net benefit outweighs the net benefit of responding passively to the thermal heterogeneity of the environment. Costs should be low in landscapes where an abundance of optimal microclimates reduces the distance that animals must travel between sun and shade (Sears *et al.* 2016). By contrast, when the landscape imposes a high energetic cost, animals should thermoconform rather than thermoregulate (Table 1). Huey and Slatkin were motivated by patterns of thermoregulation in lizards, but their model applies to any ectothermic animal and has even been applied to endothermic animals (Angilletta 2010). Therefore, one might be surprised to learn that few, if any, experiments have adequately tested the model (reviewed by Angilletta 2009).

Comparative studies of thermoregulation in natural environments, which typically do not account for key factors, provide mixed support for Huey & Slatkin's model. Field studies of tropical anoles have shown that lizards in forested habitats are more likely to thermoconform than are lizards in patchier habitats, which seems consistent with Huey & Slatkin's model (Huey 1974; Huey & Webster 1976; Lee 1980). Vickers, Manicom & Schwarzkopf (2011), however, found that tropical lizards thermoregulate more precisely when thermoconforming imposes a severe risk of overheating. In extreme climates, where most of the landscape deviates from the preferred microclimate, lizards invest substantial energy in thermoregulation (e.g. Herczeg *et al.* 2003; Blouin-Demers & Nadeau 2005). Such patterns might occur because animals would suffer a great loss of performance when thermoconforming in extreme environments (Blouin-Demers & Nadeau 2005). In which case, a rigorous test of the model requires one to control the benefits of thermoregulation while manipulating the costs experimentally.

Consistent with this conclusion, experimental studies in simple arenas show that lizards thermoregulate less intensely when thermoregulation requires more energy. Specifically, animals that have to shuttle more frequently to thermoregulate allow their body temperature to fall closer to the ambient temperature (Withers & Campbell 1985; Cadena & Tattersall 2009). Costs or constraints not only reduce thermoregulatory performance (Herczeg *et al.* 2006) but also physiological performances such as growth (Sinervo & Adolph 1994). In some cases, however, lizards exert more effort to thermoregulate in costly environments (Besson & Cree 2010; Brewster, Sikes & Gifford 2013). Presumably, some unobserved benefit of thermoregulation offsets

the energetic cost in such cases. The mixed results of experiments underscore the need to model costs and benefits explicitly when testing hypotheses about thermoregulation.

Here, we introduce a model of thermoregulation and test hypotheses about optimal thermoregulation in different thermal landscapes (Table 1). Our model describes a small ectothermic animal moving in a spatially explicit landscape with either one or several sites for basking. This model was used to simulate patterns of body temperature and energy expenditure for a thermoregulator or a thermoconformer. We then compared the expected strategy in each environment to the behaviour of real lizards (*Cordylus oelofseni*, Fig. 1) in thermal arenas. For each individual, we constructed time and energy budgets to estimate costs of thermoregulation precisely. We then inferred the intensity and effectiveness of thermoregulation by comparing the behaviours of virtual lizards to those of real lizards. We show that lizards were effective thermoregulators in both low- and high-quality landscapes, despite the large opportunity cost paid to thermoregulate in low-quality landscapes. This finding underscores the need to develop a theory of thermoregulation that considers non-energetic costs and benefits.

Materials and methods

EXPERIMENTAL ARENA

Measurements were made in a small thermal arena (180 × 180 cm) positioned in a temperature-controlled room. The floor of the arena was divided into squares (5 × 5 cm) with adhesive tape. A total of nine basking infrared bulbs (175 W) were suspended *ca.* 30 cm from the floor and equally spaced to create a 3 × 3 matrix (see Fig. S1, Supporting Information). Bulbs were manipulated temporally and spatially to simulate four conditions: (i) low-quality homogeneous ('LQ homog'), (ii) low-quality

Fig. 1. The model organism, *Cordylus oelofseni*, inhabits rock outcrops where it is frequently seen basking but also ventures into vegetated areas where it hunts for prey.

heterogeneous ('LQ heterog'), (iii) high-quality homogeneous ('HQ homog') and (iv) high-quality heterogeneous ('HQ heterog'). For the LQ homog treatment, the climate room was set at 15 °C and all infrared bulbs were switched off. This temperature matches the mean of the minimum daytime temperature experienced by lizards in the field (see field T_e data in Basson & Clusella-Trullas 2015). For the LQ heterog treatment, the room was maintained at 15 °C and a single bulb (among the nine bulbs) was switched on randomly every hour forcing a lizard to move to a different single bulb to maintain its preferred body temperature. For the HQ homog treatment, two small heaters were used to keep the room at 30 °C and all infrared bulbs were switched off. For the HQ heterog treatment, the room was kept at 15 °C and four infrared bulbs were activated simultaneously and alternated randomly every hour. In this arena, the preferred temperature could be maintained by shuttling between heated areas under the bulbs and distances between optimal microsites were shorter and basking opportunities more abundant compared to the LQ heterog treatment (Fig. 2).

We mapped the thermal environment of the arena by estimating operative temperatures (T_es) with hollow copper electroforms that resembled a small lizard. These models had a similar size, shape and reflectance as live individuals of *C. oelofseni* (see Clusella-Trullas, van Wyk & Spotila 2009 for details). The air temperatures inside the models were recorded every 5 min with a datalogger (CR 1000; Campbell Scientific, Logan, UT, USA). To establish the mean thermal quality for each treatment, we subdivided the arena into nine grids (60 × 60 cm), each corresponding to one bulb at the centre of the grid and monitored T_e in the grids. Copper models of lizards were placed at 5-cm intervals from activated bulbs (Fig. S2). Additionally, 13 models were randomly distributed in the remainder of the arena to map T_e in cold areas. In LQ heterog and HQ heterog treatments, the T_es away from the area under the bulbs were 16·2 and 18·0 °C, respectively. This ~2 °C difference reflects the higher T_e in cold grids when four bulbs are activated simultaneously (HQ heterog) versus a single bulb (LQ heterog). Thermal maps and T_e distributions were generated by assigning seven T_es within each grid for all of the nine arena grids (LQ heterog had a single warm grid corresponding to seven T_es in warm and 56 T_es in cold grids, HQ heterog had 28 T_es in warm and 35 T_es in cold grids, and LQ and HQ homog had 63 T_es each). In the homogeneous arenas, seven random draws from the T_e data simulated random positions by lizards (LQ homog: 16·3 ± 0·3 °C and HQ homog: 30·4 ± 0·5 °C). To avoid non-independence of data in the statistical analyses, a mean T_e for each position in the arena was calculated for the entire 6-h recording.

We used a general linear model to compare mean T_e among thermal treatments. Since distributions of T_e for heterogeneous arenas were heavily skewed, we log-transformed T_es before fitting the model. As intended, heterogeneous treatments had more variation in T_e than did homogeneous treatments (Fig. 2). Mean T_e in the HQ heterog treatment (22·7 ± 7·3 °C) was lower than in the HQ homog treatment (30·4 ± 0·5 °C; t_{251} = 10·95, P < 0·001) and higher than in the LQ heterog treatment (17·6 ± 4·7 °C, t_{251} = −7·81, P < 0·001). Mean T_e did not differ significantly (t_{251} = 1·62, P = 0·1) between the LQ heterog (17·6 ± 4·7 °C) and LQ homog (16·4 ± 0·3 °C) treatments.

SIMULATIONS OF THERMOREGULATORY BEHAVIOUR

To predict whether lizards should thermoregulate or thermoconform, we simulated the energetic consequences of these two main strategies in the four experimental treatments. A virtual landscape of the same size as the experimental arenas (as described above) was configured to match the distribution of T_e recorded in the four treatment arenas. Virtual lizards either moved between patches according to their body temperature (thermoregulators) or chose a random location and remained there throughout the simulation (thermoconformers). For a thermoregulator, we modelled the chance of moving with a beta function (equations 15–17 in Landsberg 1977; and see Appendix S1 for details on the code), with a 10% chance when the body temperature equalled the preferred temperature (32·6 °C) and a 100% chance when the body temperature was outside the preferred range (30·3–34 °C, Basson & Clusella-Trullas 2015). If an animal moved, a new patch was randomly chosen and body temperature was set to the operative temperature. We chose not to model gradual heating and cooling because our lizards were small enough (<7 g) to limit thermal inertia effects (Stevenson 1985). For each interval of 30 s, we determined whether an animal moved, updated its body temperature and calculated its energy loss. Rates of energy consumption for both resting and active states were based on empirical data obtained for lizards of *C. oelofseni* (see section on the 'Energetic Cost of Thermoregulation'). We summed the energy consumption for thermoregulators and thermoconformers for a simulated
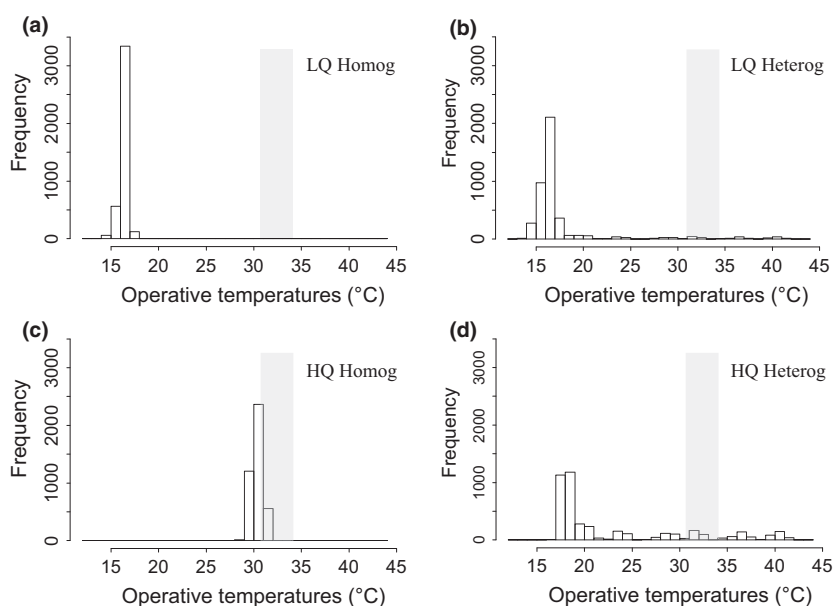

Fig. 2. Frequency distributions of operative temperatures (T_e) available in each of the treatments during 6-h trials: (a) low-quality homogeneous (LQ homog), (b) low-quality heterogeneous (LQ heterog), (c) high-quality homogeneous (HQ homog) and (d) high-quality heterogeneous (HQ heterog). Grey shaded bars are the preferred body temperature, 32·6 ± 1·8 °C (SD), for the model species, *Cordylus oelofseni*. Operative temperatures were sampled every 5 min.

period of 6 h to match the duration of experiments with real lizards. We ran 10 000 replicate simulations for each treatment. For each simulation, we recorded the mean body temperature, the absolute deviation of body temperature from the preferred temperature of *C. oelofseni*, the duration of time spent moving, the distance moved and the total energy consumed.

THERMOREGULATORY TRIALS WITH LIZARDS

To test our model, we quantified the thermoregulatory behaviours of lizards (*C. oelofseni*) collected from isolated rock outcrops in the Hottentots Holland Mountains of South Africa (1200 m elevation). Adult males (6.9 ± 0.8 g, $n = 7$) were obtained in November of 2011 and 2012 and brought to the laboratory. Here, lizards were housed in groups of 2–4 in plastic containers with mesh tops (9·5 L) and kept in a temperature-controlled incubator (Sanyo Cooled Incubator, MIR-254; Sanyo Electric Co., Ltd., Moriguchi, Japan) set at daily cycles of 25 °C 12 h L: 15 °C 12 h D which matches field conditions (Basson & Clusella-Trullas 2015). Artificial refuges and sand and stone substrates were available at all times. Food (crickets and superworms) was provided twice a week and water provided *ad libitum*. These conditions were maintained for at least 1 week before our experiment, and animals were returned to these conditions in between the trials described below.

Thermoregulatory trials occurred between 10:00 and 16:00 daily, with a single lizard being randomly exposed to each thermal treatment on a separate day. A week prior to trials, lizards were introduced to the arena for 4 h to increase familiarity with the environment and observe their behaviour. Lizards were fasted for 24 h before each trial. During a trial, water was provided *ad libitum* in each corner of the arena, about 10 cm from the edge. The arena was lit homogeneously by a single fluorescent light bulb fixed in the ceiling. Each trial was filmed with a webcam (Chat Pack CNR-CP12; Canyon, Limassol, Cyprus) and videos were analysed to obtain the distance travelled, time spent travelling, time spent resting away from bulbs and time spent basking under lit bulbs. In the heterogeneous treatments, a lizard was considered 'basking' if it was sitting within a warm zone of 20×20 cm surrounding the light. By contrast, a lizard was considered 'resting' away from a bulb if sitting outside of the warm zone. Pilot trials indicated that the temperatures within the warm zones favoured basking close to the bulbs but periodically forced lizards to visit cooler places. Lizard body mass was measured before and after each trial (± 0.1 mg; AX504, Mettler Toledo International Inc., Leicester, UK) to ensure no loss of body condition during trials and to use as a covariate in statistical analyses.

Body temperature was obtained by recording the position of a lizard every 30 s and we assumed that body temperature equalled the T_e of the arena in that position (Fig. S2; validation of T_e models in Clusella-Trullas, van Wyk & Spotila 2009). We used body temperature to calculate an index of thermoregulatory accuracy (d_b) (Hertz, Huey & Stevenson 1993) at 30-s intervals.

ANALYSES OF THERMOREGULATORY PERFORMANCE

To test for the effects of thermal environment on thermoregulatory responses, we used general linear models with mixed effects (nlme package, Pinheiro *et al*. 2013). The response variables were body temperature, distance travelled, time spent travelling, time spent basking, time spent resting away from bulbs or energy consumed. Thermal treatment and time of day were fixed predictors and individual was a random factor. The index of thermoregulatory accuracy (d_b) was calculated as the absolute deviation of body temperature from the preferred temperature (32·6 °C) for each 30-s interval. To account for the heterogeneity of the variances between treatments, we modelled a separate variance of body temperature in each thermal treatment; an improved fit of

this model over a pooled variance would indicate that thermal treatment affected the precision of thermoregulation. Mixed effects models were compared to generalized least squares models to verify that models with a random intercept were superior. Model selection followed Zuur *et al*. (2009). Plots of residuals versus fitted values were checked for any severe violations of model assumptions.

Models that accounted for differences between individuals and for heterogeneity of variances were the best models of those tested for all dependent variables except time travelled, for which a constant variance model was a better fit. As mass is confounded by individuals and the latter was included in the best models as a random factor, mass was not included in the mixed effects models. Rather, simple linear regressions were used to test for mass effects on response variables within each treatment, with mass as a continuous variable and treatment as a categorical factor. All statistical analyses were conducted in R (v. 3.1.0, R Development Core Team, 2012). All results presented are for minimal adequate models based on Akaike information criteria (Burnham & Anderson 2004). Means and standard deviations were estimated from the most likely statistical model for each dependent variable.

ENERGETIC COST OF THERMOREGULATION

To quantify the energetic cost of thermoregulation in each trial, our observations were combined with estimates of resting and active metabolic rates (RMR and AMR, respectively) for *C. oelofseni*. We used RMR data of post-absorptive adult males recorded at 10, 20 and 30 °C published in Basson & Clusella-Trullas (2015). The mean AMR of post-absorptive lizards were estimated on a motorized treadmill, with seven lizards acclimated to 25/15 °C for at least a week under 14L:10D. The treadmill consisted of a rubber belt (50 cm long) rotated by a DC motor (45 rpm, 24V, RS Components, Midrand, South Africa) around two axles and placed within a Perspex chamber (volume = 573·7 mL, Fig. S3). A speed of 0.22 ± 0.009 m s^{-1} was maintained with a regulator (6–15V, RS Components). The front end of the chamber was coated black to simulate a crevice, encouraging lizards to move towards it. The treadmill was placed in an incubator (Sanyo Cooled Incubator, MIR-153; Sanyo Electric Co., Ltd.) set at one of four temperatures: 15, 20, 25 or 30 °C (verified using a thermocouple and recorder, TC 1000; Sable Systems, Las Vegas, NV, USA). The incubator was kept within 1 °C of the target temperature.

We measured the metabolic rate of each lizard while walking on the treadmill. The chamber surrounding the treadmill was connected to a LI-COR infrared CO_2/H_2O analyser (Li-7000; LI-COR Inc., Lincoln, NE, USA) set in gas flow (push-mode) configuration and plumbed in differential mode (Lighton 2008). Air was supplied using an air pump (OPTIMA, Hagen Air Pumps, Durban, SA) that flowed through a column of soda lime and a column of silica gel and drierite (50:50) to eliminate CO_2 and water, respectively, from the air. Thereafter, air flowed through a mass-flow valve (Sierra Instruments, Side-Track Model 840, Monterey, CA, USA) connected to a mass-flow controller (Sable Systems, MFC-2, Las Vegas, NV, USA) set at 150 mL min^{-1}. Before and after lizards were placed in the chamber, a V_{CO_2} baseline was recorded for 5 min. After the initial baseline, a lizard was placed on the treadmill and given 10 min to equilibrate to the test temperature. During this period, the chamber was flushed with air until CO_2 levels reached a steady state. The treadmill was then activated by slowly increasing the speed of the treadmill from 0 to 0·22 m s^{-1} – the mean walking speed of lizards in our arenas (0.30 ± 0.10 m s^{-1}, $n = 28$). The lizard was encouraged to walk on the treadmill by tapping the sides of the chamber and was kept in the chamber until it ran steadily for at least a minute. Each lizard ran once at each temperature. We weighed each lizard before and after each trial.

We use a general mixed effects model to estimate how RMR (and AMR) varies with temperature and included individuals as a random factor to account for the repeated measurements in the experiment. Active metabolic rates did not differ between males and females ($t = -2.53$, d.f. = 5, $P > 0.05$) and we only used data from males for RMR (Basson & Clusella-Trullas 2015). We used the relationships between temperature and RMR (Fig. S4a) and AMR (Fig. S4b) to estimate the energetic costs during each of the thermoregulatory trials, based on body mass, and times spent active and resting. Metabolic data were then converted from mL CO_2 to joules, assuming a respiratory quotient of 0.71 for post-absorptive animals, and that oxygen consumption can be converted to energy use by assuming 20 J mL O_2^{-1} (Congdon, Ballinger & Nagy 1979).

Results

Our simulations confirmed the expected shift in optimal strategy from thermoregulating to thermoconforming as the quality of the environment declined (Fig. 3). Compared to virtual thermoregulators in a LQ heterog landscape, those in the HQ heterog landscape moved less and thermoregulated more accurately. Compared to thermoconformers, thermoregulators in the HQ heterog landscape reduced the deviation between body temperature and the preferred temperature by 49%. In the LQ heterog landscape, however, thermoregulating barely provided a benefit over thermoconforming; the average deviation from the preferred temperature was only 12% lower for thermoregulators than for thermoconformers (Fig. 3a). Yet, thermoregulators in the low-quality landscape spent 43% more energy while moving more often and much farther than thermoconformers did (Fig. 3b, c). Both this energetic cost and a perceived risk of predation would favour thermoconformers in the low-quality landscape, in spite of the potential to thermoregulate. Obviously, the optimal strategy in any homogeneous environment would be to thermoconform, because no amount of movement can alter body temperature.

Contrary to our model, real lizards did not shift from thermoregulating in high-quality landscapes to thermoconforming in low-quality landscapes. Lizards in the LQ heterog treatment had lower body temperatures (28.3 ± 10.5 °C) and higher deviations from preferred temperature (d_b, Fig. 5a) than in the HQ heterog treatment (29.4 ± 9.8 °C; $t_{10,072} = -8.02$, $P < 0.001$) and this difference was much lower than in the simulation (Fig. 3a). The body temperature in the LQ heterog treatment varied more than in the HQ heterog treatment (Fig. 4), particularly early in the day (interaction between treatment and time of day: $t_{10,072} = 6.16$, $P < 0.001$). Not surprisingly, lizards in the homogeneous landscapes experienced a narrow range of body temperatures (30.3 ± 0.6 °C and 16.4 ± 0.4 °C for HQ and LQ, respectively), as dictated by the uniformity of their environment (see Fig. 2).

The poorer performance of lizards in the low-quality landscape was likely driven by environmental constraints on thermoregulation instead of a shift in thermoregulatory behaviour. In our simulations, virtual thermoregulators
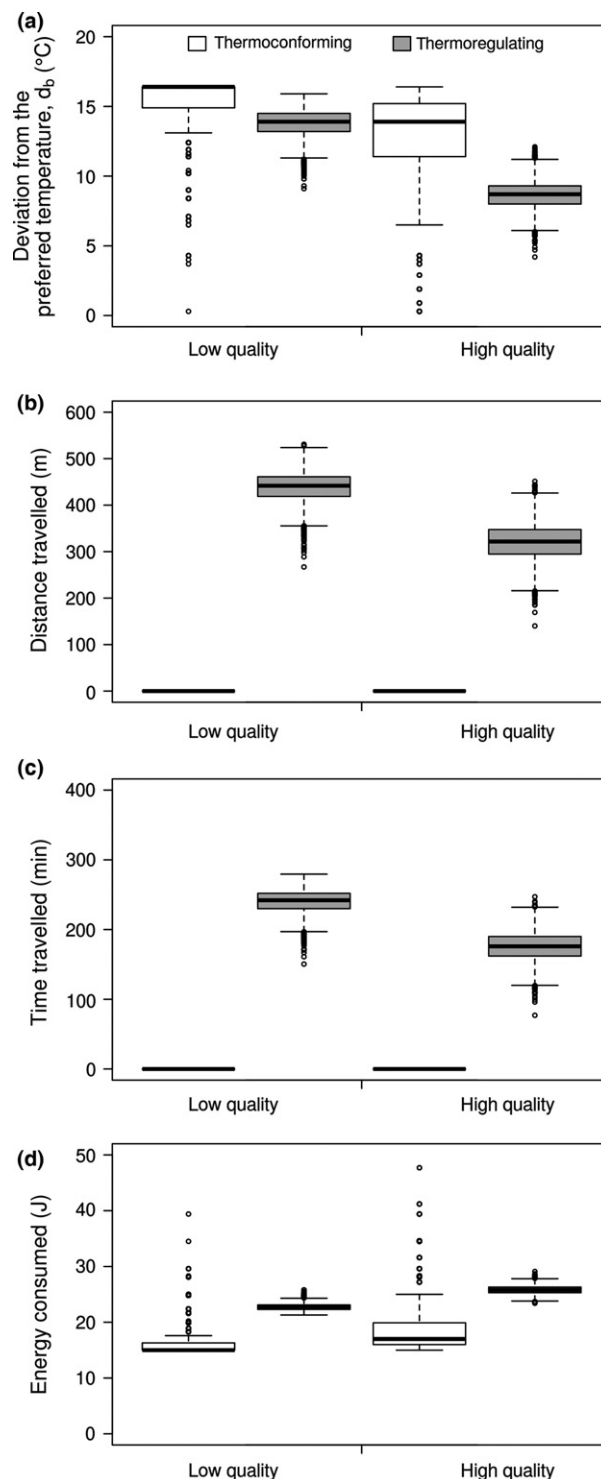

Fig. 3. Both the strategy of thermoregulation and the quality of the thermal environment influenced behaviour and performance of virtual lizards: (a) accuracy of thermoregulation, (b) distance travelled, (c) time travelled and (d) energy expenditure. Virtual lizards were programmed to either thermoregulate or thermoconform in homogeneous or heterogeneous environments of low quality (LQ) or high quality (HQ).

moved more frequently and traversed a greater distance in the lower quality landscape. By contrast, real lizards moved less frequently and traversed a shorter distance in
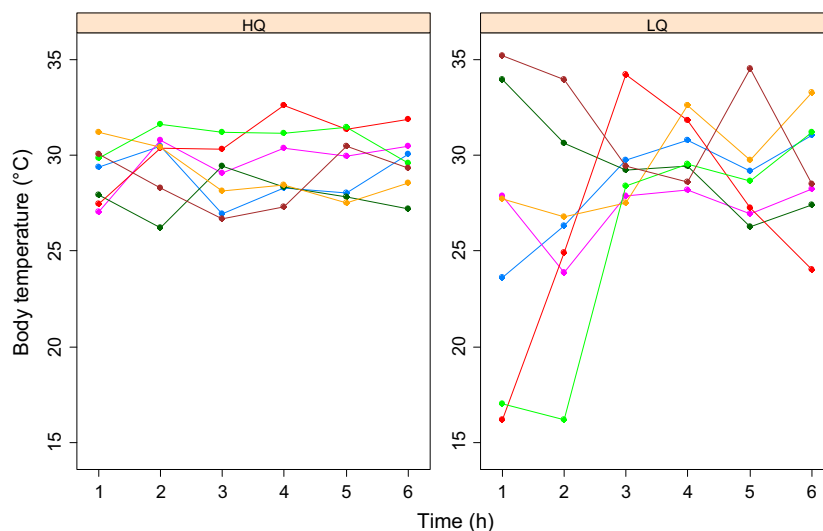
Fig. 4. Mean hourly body temperature of seven lizards tested in a high-quality arena (HQ) where four bulbs were active but changed hourly and a low-quality arena (LQ) where only one bulb was active at a time. Each colour represents a different lizard.

the lower quality landscape, suggesting that they put less effort into locating sources of heat (Fig. 5b, c, Tables 2 and S1). The temperatures and movements of lizards in the LQ heterog landscape were somewhere in between those of thermoregulators and thermoconformers in our simulations. However, real lizards spent similar times basking in the HQ and LQ treatments (Table S1) and expended similar amounts of energy (Table 3; Fig. 5d). From these patterns, we draw two inferences about the impact of low-quality landscape on the behaviour of lizards. First, lizards explored their arenas more when presented with several heat sources, but traded off opportunities to explore their environment for opportunities to bask when only one source of heat was available. Secondly, lizards thermoregulated poorly at the low-quality landscape because they could not track sources of heat as quickly as they could in the high-quality landscape. For a given environmental quality, lizards in the homogeneous treatment moved significantly less than did lizards in the heterogeneous treatment (Fig. 5b; Table 2). Since movement has no thermal advantage in the homogeneous set-ups, it may represent the mere tendency of lizards to explore their environments.

Body mass influenced most behaviours only slightly. The scaling of basking time and resting time with body mass differed between the LQ heterog and HQ heterog treatments (homogeneity of slopes test, $F_{12,1} = 6\cdot27$, $P < 0\cdot05$ and $F_{12,1} = 7\cdot09$, $P < 0\cdot05$). In the LQ heterog treatment, larger lizards spent more time basking but less time resting away from bulbs than smaller lizards, contrasting weaker mass scaling relationships in the HQ treatment (Figs S5d and S5e).

Discussion

Our model of optimal thermoregulation based strictly on energetics failed to predict the behaviour of lizards in a low-quality environment. Recently, other researchers showed how the abundance and distribution of resources

affects the effectiveness of thermoregulation (Sears & Angilletta 2015; Vickers & Schwarzkopf 2016). In particular, animals were predicted to thermoregulate poorly when preferred microclimates are rare or concentrated in space (Sears & Angilletta 2015). Our simulations captured this effect by comparing virtual lizards in landscapes with either one or four sources of heat, which shifted throughout the day. In the environment with only one source of heat, lizards should have abandoned thermoregulation to save energy while suffering a minor loss of thermoregulatory performance. In contrast to this expectation, real lizards thermoregulated and spent as much time basking in the low-quality treatment as they did in the high-quality treatment. By constructing an energy budget (Table 3), we discovered that basking, rather than locomotion, accounted for 94–95% of energy consumed in both treatments. The energetic costs were nearly identical because energy expenditure resulted primarily from the high temperatures during basking instead of locomotion. Routine activity typically demands far less energy than maximal aerobic activity (Schmidt-Nielsen 1997) and distances between basking sites in the heterogeneous treatments were small, despite being ecologically relevant. Thus, the energetic cost of thermoregulation in our low-quality treatment was insufficient to outweigh the perceived benefits of thermoregulation. These results agree with those of several previous studies, in which animals exerted more effort to thermoregulate in more challenging environments (Gvoždík 2002; Herczeg *et al.* 2003; Blouin-Demers & Nadeau 2005).

By thermoregulating, an animal accepts a cost of predation risk (see Table 1; Herczeg *et al.* 2008). An animal that moves a greater distance or basks more often could attract the attention of a predator, leading to injury or death. In another study, lizards traded the ability to bask for safety following a simulated risk of predation (Polo, López & Martín 2005). In our experiment, lizards in the high-quality treatment could have moved far less than they did, because the abundance of and distance between heat
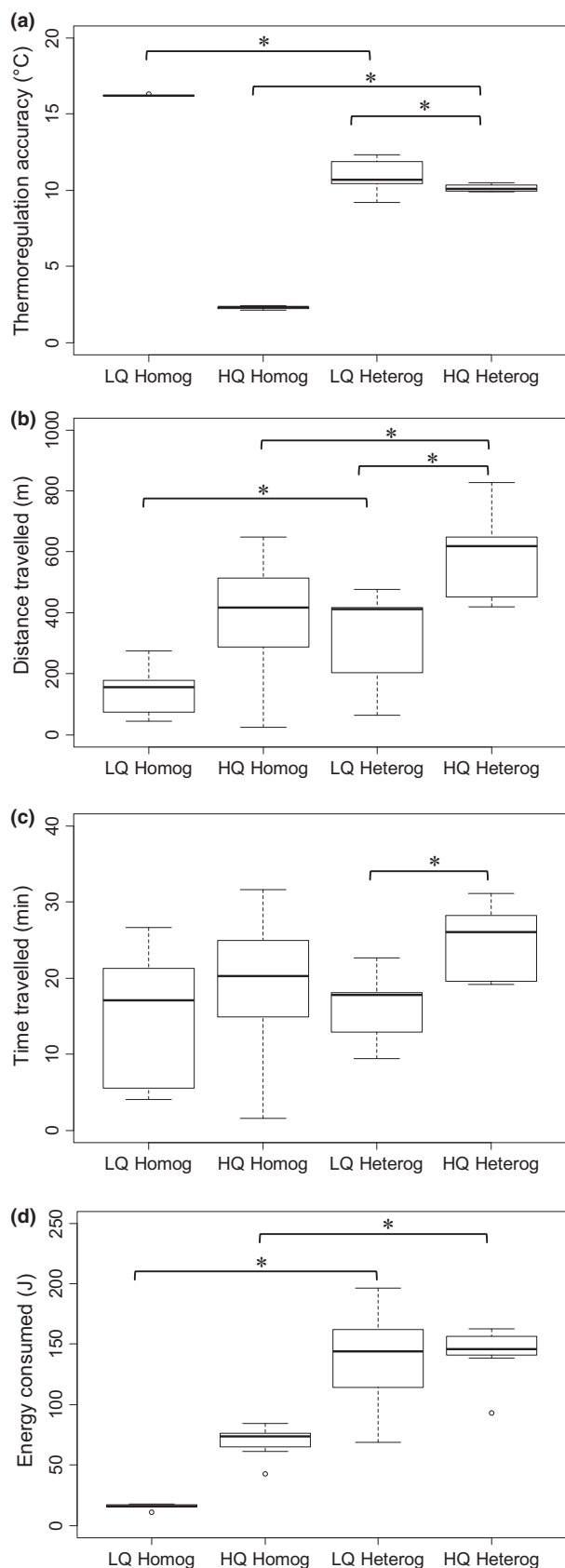
(a)

(b)

(c)

(d)

Fig. 5. Both the strategy of thermoregulation and the quality of the thermal environment influenced behaviour and performance of real lizards: (a) accuracy of thermoregulation, (b) distance travelled, (c) time travelled and (d) energy expenditure. 'Heterog' means that either one bulb in the low-quality arena (LQ) or four bulbs in the high-quality arena (HQ) were active. 'Homog' means that no bulb was active and temperature was kept constant across the arenas. The accuracy of thermoregulation (d_b) is presented as individual means ($n = 7$ male lizards) of absolute deviations of body temperature from the preferred temperature (32·6 °C); a lower d_b indicates a higher accuracy of thermoregulation. Bars and stars indicate significant differences between treatments based on a general mixed effects model that accounted for the non-independence of data from the same animal. Boxplots provide the median (black horizontal line), the interquartile range (upper and lower sides of the box) and minimum and maximum values (whiskers), unless outliers were present (open dots).

Table 2. Outcomes of mixed effects models testing for the effects of treatment on the total distance travelled (m), time spent moving (min), time basking (min), time resting (away from bulbs, min) and energy consumption (J) of *Cordylus oelofseni*

Coefficient	Estimate	SE	d.f.	*t*-value	*P*-value
Distance travelled*					
Intercept	580·98	58·25	18	9·97	<0·0001
HQ homog	−196·59	67·04	18	−2·93	<0·01
LQ heterog	−267·58	67·04	18	−3·99	<0·001
LQ homog	−440·66	67·04	18	−6·57	<0·0001
Time moving†					
Intercept	24·57	2·04	18	12·03	<0·0001
HQ homog	−5·58	3·24	18	−1·72	0·10
LQ heterog	−8·59	1·05	18	−8·14	<0·0001
LQ homog	−10·07	4·02	18	−2·50	0·02
Time basking					
Intercept	229·62	4·42	6	51·98	<0·0001
LQ heterog	−6·64	17·59	6	−0·38	0·72
Time resting					
Intercept	105·81	5·15	6	20·55	<0·001
LQ heterog	15·22	17·29	6	0·88	0·41
Energy consumed‡					
Intercept	88·03	6·21	18	14·16	<0·0001
HQ homog	−9·8	2·16	18	−4·56	<0·001
LQ heterog	−4·52	4·10	18	−1·10	0·28
LQ homog	−60·79	4·67	18	−13·02	<0·0001

For the LQ homog and LQ heterog comparisons: *$t(18) = −2·58$, $P = 0·02$; †$t(18) = −0·38$, $P = 0·71$; ‡$t(18) = −10·40$, $P < 0·0001$.

Table 3. Proportion of energy used (mean ± SD%) for each activity observed in the experimental arenas: locomotion, resting away from the bulb in the heterogeneous arenas and basking

Treatment	Locomotion	Resting	Basking
HQ heterog	1·8 ± 0·4	3·8 ± 0·6	94·4 ± 0·7
LQ heterog	1·2 ± 0·4	3·9 ± 2·0	94·9 ± 2·3
HQ homog	4·3 ± 2·2	95·7 ± 2·2	–
LQ homog	8·8 ± 5·4	91·2 ± 5·4	–

sources was much smaller than that in the low-quality treatment. Indeed, our simulations showed that lizards in the high-quality treatment could have thermoregulated more efficiently; the d_b and distance travelled of virtual thermoregulators were 8 °C and 300 m, whereas the d_b and distance travelled of real lizards were 10 °C and

600 m, respectively (compare Figs. 3 and 5). In addition, lizards in the HQ homogeneous treatment moved nearly as much as those in the HQ heterogeneous treatment, despite being able to maintain a preferred temperature with no movement. Thus, lizards seemed to place a premium on exploring their surroundings, perhaps to locate food, water, shelter or mates. We cannot, however, discount that the stress of being in captivity in a simple environment may cause increased exploration, although this was unlikely as lizards in low-quality arenas were equally exposed to this environment.

Lizards in the low-quality heterogeneous treatment were forced to choose between exploring their environment and tracking the single source of heat. These lizards chose to bask most of the time, which precluded exploring the entire arena. In a natural environment, being tied to a single location would limit access to resources distributed throughout space. The cost of missed opportunities during thermoregulation has not been modelled carefully (Angilletta 2009), although the need to shuttle between sun and shade severely restricts movement when preferred microclimates are scarce. Sears & Angilletta (2015) developed a spatially explicit model of thermoregulation that could easily accommodate a trade-off between searching for preferred microclimates and searching for other resources. Biologists will need to combine the costs of energy expenditure, predation risk and missed opportunities to understand when animals should move less than possible or more than needed to thermoregulate.

In spite of how lizards perceived these costs, they also perceived a benefit of thermoregulation that outweighed those costs. This benefit could have been something other than energy gain, given that real lizards basked intensely in the LQ heterog treatment despite paying much energy to do so (see Fig. 5d). Without knowing how body temperature influences physiological performances of *C. oelofseni* other than metabolism, we cannot partition the relative influence of energetic or non-energetic benefits. However, real lizards in the LQ heterog treatment thermoregulated about 5 °C closer to their preferred temperature than thermoconformers in our simulations. If lizards require a high body temperature to forage and digest, they might have thermoregulated to enhance energy gain. Alternatively, lizards might have sacrificed energy to maintain body temperatures that promote a physical response to threats from predators or competitors. Lizards often maintain body temperatures that enable maximal speeds, even when they rarely resort to sprinting (Hertz, Huey & Garland 1988). Without a shelter for hiding, lizards in our experiment might have felt the need to thermoregulate in case a threat arose. Lizards usually flee from predators when warm but rely on camouflage when cold (Hertz, Huey & Nevo 1982; Crowley & Pietruszka 1983); thus, a lizard foraging with a low body temperature would be vulnerable to a predator. This vulnerability could explain why lizards in LQ homog treatment, which were forced to have a low temperature, moved the least. Other physiological processes that depend on temperature, such as immunity (Butler *et al.* 2013), provide incentives to thermoregulate despite the energetic cost.

Larger lizards spent more time basking in the low-quality treatment, despite the fact that these lizards could travel farther from a heat source before cooling to an undesirable temperature. However, larger lizards likely required longer periods under the infrared lamps to reach the preferred temperature. That said, the small variation in size among our animals would have meant a difference of seconds, rather than minutes, for cooling or warming. A more plausible explanation comes from Regal (1971): a male lizard guarded a heat source more intensely in the presence of a second male of the same species. Once the competing male was removed, the focal lizard reduced its obsession with the heat source. We hypothesize that large males have more incentive to guard a heat source given their potentially high position in a dominance hierarchy. Similarly, large fish also dominate regions of warmer water in the presence of a competitor (Beitinger & Magnuson 1975). What makes this hypothesis compelling is that size had no effect on basking in the HQ heterog treatment, where several sources of light were available (Fig. S5). This hypothesis could be tested by running similar trials with pairs of lizards matched or mismatched according to size. Whether this mechanism or an alternative one explains the pattern, the size dependence of thermoregulatory behaviour in challenging environments should be considered in future models.

Our experiment showed how the abundance of preferred microclimates shapes thermoregulatory behaviour of lizards in the face of conflicting demands. By comparing the movements and activities of animals in heterogeneous and homogeneous environments, we could infer whether behaviours were motivated by thermoregulatory goals or other goals. Our results highlight the importance of measuring the time and energy budgets of individuals along with the body temperatures resulting from these investments. Moreover, these measurements must be analysed in the context of theoretical predictions derived from quantitative models, such as our simulations of thermoregulators and thermoconformers. Thermal sensitivities of activity and metabolism were needed to assess energy expenditure, which should be more closely related to the fitness of an organism (Halsey *et al.* 2015). Our findings support the hypothesis that benefits of thermoregulation drive behaviour in costly environments (Blouin-Demers & Nadeau 2005), despite the likely cost of missed opportunities. This conclusion should guide efforts to model responses of ectotherms to climate change and habitat loss (Lelievre *et al.* 2013; Gunderson & Leal 2015, 2016).

Although the energetic costs of shuttling have been viewed as a principal cost of thermoregulation (Huey & Slatkin 1976; Angilletta 2009; Sears & Angilletta 2015), both laboratory and field studies confirm that animals perceive other factors that influence their thermoregulatory behaviours. The importance of these other factors, relative

to energetic costs, will undoubtedly depend on the environment. Laboratory experiments, in which energetic costs have been controlled and manipulated, involve artificial habitats with little spatial complexity. In natural habitats, temperatures vary at a fine grain because vegetation and terrain create a complex mosaic of operative temperatures for small ectotherms. Such mosaics typically reside within small areas, except in homogeneous environments such as flat plains or dense forests (Bartlett & Gates 1967; Clusella-Trullas & Chown 2011; Sears, Raskin & Angilletta 2011; Goller, Goller & French 2014; Woods, Dillon & Pincebourde 2015). For the latter habitats, energetic costs associated with thermoregulatory movement may become substantial. For most temperate animals, however, that inhabit landscapes with different degrees of thermal heterogeneity, the loss of opportunities to feed, hydrate and mate (and their consequences for fitness) might impose the highest costs of thermoregulation. The theory of thermoregulation should ultimately address these costs and the non-energetic benefits that potentially outweigh them.

Acknowledgements

Data accessibility

Summaries of data are present in the manuscript and its supporting information. Files with the code used in the simulations of thermoregulation behaviour are available in Dryad Digital Repository http://dx.doi.org/10.5061/dryad.p2224 (Basson *et al.* 2016).

Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Additional details on the simulation of thermoregulatory behaviour.

Fig. S1. Illustration of the experimental thermoregulatory arena.

Fig. S2. Operative temperatures measured in the thermoregulation arena.

Fig. S3. Photographs of the treadmill and respirometry set-up.

Fig. S4. Temperature dependence of resting and active metabolic rate in *Cordylus oelofseni*.

Fig. S5. Body size effects on thermoregulatory parameters measured in this study.

Fig. S6. Relationships among the different types of objects in the individual-based model.

Table S1. Summary table of original thermoregulatory parameters measured in this study.