

Decreased precision contributes to the hypoxic thermoregulatory response in lizards

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SUMMARY

The decrease in body temperature (T_b) observed in most vertebrate classes in response to hypoxia has been attributed to a regulated decrease in set-point, protecting organs against tissue death due to oxygen depletion. Hypoxia, however, imparts particular challenges to metabolic function which may, in turn, affect thermoregulation. In ectotherms, where thermoregulation is mainly behavioural, stressors that influence the propensity to move and respond to temperature gradients are expected to have an impact on thermoregulatory control. Using low oxygen as a potent stressor, we evaluated the variability and level of thermoregulation of inland bearded dragons. To examine the source of thermoregulatory variability, we studied their behaviour in an electronically controlled temperature-choice shuttle box, a constant temperature dual-choice shuttle box, and a linear thermal gradient. A significant increase in the size of the T_b range was observed at the lowest oxygen concentration (4% O_2), reflecting a decrease in thermoregulatory precision in the temperature-choice shuttle box. This was also accompanied by a drop of $\sim 2\text{--}4^\circ\text{C}$ in T_b , the drop being greatest in situations where T_b must be actively defended. Situations that force the lizards to continually choose temperatures, rather than passively remain at a given temperature, lead to an increase in the variability in the manifested T_b , which is further exaggerated in hypoxia. This study reveals that a decrease in thermoregulatory precision caused by a diminished propensity to move or effect appropriate thermoregulatory responses may be a contributing component in the lowering of selected body temperatures observed in many hypoxic ectotherms.

Key words: hypoxia, behavioural thermoregulation, thermosensitivity, thermoregulatory effort, variability, anapnoea.

INTRODUCTION

Body temperature (T_b) is one of the most important variables in an ectotherm's life history, since it determines the rate at which most physiological functions take place. Optimal performance of these functions occurs within a narrow range, and the capability of an animal to regulate T_b within this range ultimately determines its ability to escape predators, grow and reproduce. Low oxygen, however, causes reptiles to select lower temperatures in the laboratory (Hicks and Wood, 1985) as well as in the field (Rollinson et al., 2008). Indeed, it is common for most animals studied to reduce T_b in hypoxia (Wood, 1995; Wood and Gonzales, 1996), an inherent regulatory response that protects tissues against oxygen depletion, particularly in life-sustaining organs such as the heart and the brain. This fall in T_b can reduce oxygen demands by up to 50% (Hicks and Wood, 1985) through a combination of lowered metabolic rate *via* Q_{10} effects [as well as metabolic depression (Bickler and Buck, 2007; Hicks and Wang, 2004)], an accompanying reduction in ventilatory costs, and an increase in the oxygen-loading capacity of the lungs (Wood and Gonzales, 1996).

It is not uncommon for reptiles in nature to encounter situations which can cause hypoxaemia. Infections from blood-borne parasites are known to reduce the oxygen-carrying capacity of the blood (Saint-Girons, 1970; Schall et al., 1982); anaemia and exhaustive exercise can also cause temporary hypoxaemia. These conditions are known to induce decreases in T_b similar to those induced by external hypoxia (Hicks and Wood, 1985; Petersen et al., 2003). A fall in T_b also commonly occurs in response to numerous stressors in addition to hypoxia [e.g. dehydration, hypoglycaemia, toxins (Kozak, 1997)], suggesting a common regulatory process that responds to alterations in the neurochemistry of the brain (Bicego et al., 2007). Thus, the relevance of examining hypoxia-induced

alterations in T_b also lies in its potential for shedding light on the thermoregulatory defence mechanisms common to a number of stressors.

The natural and laboratory T_b values of reptiles have a skewed distribution (Dewitt and Friedman, 1979), dropping off sharply at higher temperatures with a wider distribution at lower temperatures. One explanation for this may be the decrease in locomotor performance observed in reptiles when exposed to low temperatures, prolonging the time animals spend at low T_b values. Higher temperatures, on the other hand, are thought to be typically avoided due to lethal effects and potential cardio-respiratory limitations (Wood, 1984). Any factor that decreases the responsiveness to cold could increase the overall variability in T_b , because animals will not respond as readily to lower temperatures. The net result of encountering a wider distribution of low temperatures is an overall fall in selected T_b . Although hypoxia has been shown to lower the preferred T_b along with other thermoeffectors [e.g. panting, skin reflectivity (de Velasco and Tattersall, 2008; Hicks and Wood, 1985; Petersen et al., 2003; Tattersall and Gerlach, 2005)], the precision of thermoregulation has been largely overlooked. If the decrease in T_b observed in conditions like hypoxia is associated with a decrease in thermoregulatory precision, it is possible that changes to the underlying thermosensation may be significant contributors to the change in T_b (McKemy, 2007; Sayeed and Benzer, 1996).

On the other hand, severe hypoxia can limit the capacity for aerobic metabolism (Hicks and Wang, 2004; Wood and Glass, 1991) and therefore the potential for aerobic activity. Given that ectotherms commonly reduce metabolic expenditure in response to hypoxia (Bickler and Buck, 2007), it is expected that lizards exposed to low oxygen concentrations will be less apt to exhibit movement than those in normoxia. If extensive movement is required for

thermoregulation, it is plausible that hypoxia will induce a decrease in thermoregulatory precision. To test this prediction we took advantage of the different metabolic efforts associated with three different techniques (Withers and Campbell, 1985) commonly used to assess thermal preference.

This study aimed to answer whether the hypoxia-induced lowering of T_b consists solely of a regulated decrease in T_b (i.e. decrease in set-point), as has been extensively suggested, or whether the decrease in T_b is accompanied by a decrease in thermoregulatory precision (i.e. an increase in load error). In addition, this study examined the importance of 'effort' (i.e. amount of locomotion required to maintain a constant T_b) on the variability and magnitude of the hypoxic thermoregulatory response. The recognition of a decrease in thermoregulatory precision during hypoxia would shed light on the potential for a reduction in thermosensitivity and/or a diminished propensity to move as contributing factors to the hypoxic decline in T_b .

MATERIALS AND METHODS

Experimental animals

Twelve bearded dragons (eight male and four female; mass 323.9 ± 63.2 g, age 2–3 years), *Pogona vitticeps* (Ahl 1926), raised from eggs at Brock University were used in the study. A detailed description of the animals' housing and care conditions is given elsewhere (Cadena and Tattersall, 2009). All procedures involving the use of these animals were approved by the Brock University Animal Care and Use Committee (protocol no. 041001).

Experimental set-up

To examine the effect of thermoregulatory effort on the variability and magnitude of the hypoxic thermoregulatory response, we used two configurations of an electronic temperature shuttle box (see below for details), as well as a thermal gradient [all methods are similar to those described in another publication (Cadena and Tattersall, 2009)]. The temperature on each side of the shuttle box and the thermal gradient were measured using platinum thermistor wires and thermocouples calibrated against a known temperature standard. Body temperatures of lizards were monitored using implanted telemeters (model TA11CTA-F40 or TA10CTA-F40, Data Sciences International PhysioTel® and Multiplus™ implant; St Paul, MN, USA), calibrated to the same source. Both air temperature (T_a) and T_b were thus recorded to an accuracy of $\pm 0.1^\circ\text{C}$. The shuttle box was essentially a two-choice chamber, where the two chambers were held at different temperatures. We used the shuttle box in two configurations: (1) ramping temperatures and (2) extreme temperatures. The ramping temperature protocol was designed such that a 10°C differential was always maintained between the two chambers, using electronically controlled radiator fans and heater coils. Temperatures inside the box changed dynamically according to the location of the lizard (either the heating or the cooling compartment), so that T_a rose at a fixed rate of $0.7^\circ\text{C min}^{-1}$ whenever the lizard moved to the heating compartment and cooled at this same rate when the lizard moved to the cooling compartment. A detailed description of the shuttle box specifications is provided in another study (Cadena and Tattersall, 2009). Previous experiments showed that this rate of heating and cooling provides estimates of preferred T_b and T_b precision equivalent to those obtained in a thermal gradient (Cadena and Tattersall, 2009). The extreme temperatures protocol used the same shuttle box with one compartment held at a constant 15°C while the other was held at 50°C . In this kind of shuttle box the temperatures inside the chambers are maintained well above and below the animals' preferred

temperatures (Berk and Heath, 1975a; Berk and Heath, 1975b; Blumberg et al., 2002; Hicks and Wood, 1985; Myhre and Hammel, 1969), relying on the animal shuttling at higher rates than in either the thermal gradient or the ramping shuttle box to achieve a desired T_b . In the extreme temperatures shuttle box, extra energetic costs are added to the thermoregulatory behaviour of lizards, which will potentially influence variability (Cadena and Tattersall, 2009); this was consistent with the objectives of the study. Finally, a thermal gradient was utilised to test thermoregulatory preference in a low effort environment. The gradient [described elsewhere (Cadena and Tattersall, 2009)] consisted of a flat, copper sheet with one end maintained at 15°C and the other at 50°C . Fans at either end of the gradient helped to ensure a linear gradient in air temperatures similar to floor temperature, as well as circulating the gases during hypoxic exposure.

Experimental design

Lizards were fasted for a period of 12 h prior to the experiments. Experiments were run from 08:00 to 20:00 h, with the first 4 h consisting of a habituation period (in normoxia). Previous experiments indicated a considerable decline of putative non-thermoregulatory activity ('exploratory shuttling') after an initial 4 h period inside the shuttle box (Cadena and Tattersall, 2009). At the beginning of the day the animals were cold (T_b at 08:00 h, $28.5 \pm 0.4^\circ\text{C}$) and had just emerged from their nocturnal shelter. To avoid further cooling-induced lethargy, individual lizards were placed on the warm side of the experimental apparatus at the start of each experiment. Upon completion of the experiment, lizards were returned to their housing facilities.

Series I: influence of hypoxia on thermoregulatory behaviour in a ramping shuttle box

Each individual lizard was exposed to five different oxygen concentrations (4, 5, 7, 10 and 21% O_2) in a random order, one oxygen level per day. After the initial habituation period, the oxygen concentration was manipulated using an oxygen controller (Pro-Ox, model 110, BioSpherix, Redfield, NY, USA). This was done by delivering nitrogen into the shuttle box and flushing out the air, until the desired level of oxygen ($\pm 0.2\%$ O_2) was reached, usually within 30 min. This half hour following the initiation of hypoxia was not included in the data analysis, leaving a total of 7.5 h of analysable data. Normoxic levels (21% O_2) were produced by leaving the shuttle box open to room atmosphere. T_a and T_b were recorded at 30 s intervals throughout the experiments. The time and temperature at the moment the lizard exited either compartment of the box were also recorded. The number of times each lizard shuttled was also calculated over the 7.5 h period as an indication of thermoregulatory effort and activity.

Series II: influence of methodology on the assessment of thermoregulatory behaviour in hypoxia

We used nine lizards in these experiments and exposed them to 21% and 4% O_2 [normoxic data are derived from our previous study (Cadena and Tattersall, 2009)]. As in series I, individual lizards were placed in the experimental apparatus for an initial 4 h habituation period and 30 min were required for oxygen to reach the desired level of 4% O_2 . Lizards were then allowed to thermoregulate inside the experimental apparatus for an additional 7.5 h.

Data recording and analysis

Assessment of the thermoregulatory variables, data acquisition, recording and processing followed the methodology described

elsewhere (Cadena and Tattersall, 2009). Significant differences between groups detected by a repeated measures analysis of variance (RM ANOVA) (see below for details) were further explored using the Holm–Sidak procedure as a *post-hoc* method. Differences were considered significant at $P \leq 0.05$. Whenever data were not normally distributed, log transformations were applied to comply with the homoscedasticity requirements of the statistical tests. An analysis of the residuals on the log-transformed data indicated these were normally distributed. On occasions where normality could not be achieved, an RM ANOVA on ranks was used instead. All statistical analyses were performed using SigmaStat statistical software (version 3.0.1, Systat Software Inc., San Jose, CA, USA).

Series I: influence of hypoxia on thermoregulatory behaviour in a ramping shuttle box

Medians for upper escape ambient temperature (ambient temperature at which a lizard exited the hot side of the shuttle box; UET_a), lower escape ambient temperature (ambient temperature at which a lizard exited the cold side of the shuttle box; LET_a), T_b and T_a and means for the T_b range (central 68% range of the T_b distribution; RT_b), as well as coefficients of variation (c.v.) of UET_a , LET_a , upper escape T_b (body temperature at which a lizard exited the hot side of the shuttle box; UET_b) and lower escape T_b (body temperature at which a lizard exited the cold side of the shuttle box; LET_b) across the 7.5 h of experimental conditions were compared between treatments using RM ANOVA. The c.v. was used as an indicator of intra-individual variability since it standardises differences in variability that may result from the level of the values. RT_b was described using the central 68% of the data range, as it is analogous to the standard deviation around the median (see Dewitt, 1967). Due to the intrinsic dependence between RT_b and the corresponding high and low limits of this range (HT_bL and LT_bL), statistical analysis was only performed on RT_b .

A one-way RM ANOVA was also used to test differences in the number of shuttles between oxygen levels. To account for possible confounds between the multiple test procedures performed on the T_a and T_b variables (i.e. T_b , T_a , UET_a , LET_a and RT_b and also between c.v. of escape temperatures) Bonferroni–Holm procedures (Holm, 1979) were performed on the P -values.

Series II: influence of methodology on the assessment of thermoregulatory behaviour in hypoxia

The median values for T_b from each individual ($N=9$) were calculated. T_b and RT_b were compared between the three different methodologies (i.e. ramping shuttle box, extreme temperatures shuttle box and thermal gradient) and oxygen levels using a two-way RM ANOVA, with methodology and oxygen level as factors.

The number of shuttles that occurred in the extreme trials was compared between 21% and 4% O_2 using Student's paired t -test. Additionally, in the extreme temperatures shuttle box, animals were noted to spend time straddling the cold and warm compartments. The proportion of time spent in the cold, hot and 'middle' (straddling the two compartments) was calculated for both oxygen concentrations, but to avoid type I error only the proportion of time spent in the middle was compared between 21% and 4% O_2 using an RM ANOVA. For the thermal gradient experiments, 'movement' was approximated by summing up the number of times that animals moved greater than 20 cm in a 1 min period. This value was subsequently compared between the 21% and 4% O_2 treatments. P values for movement estimates in the extreme temperatures shuttle box and the thermal gradient and for time spent in the middle between compartments of the shuttle box were Bonferroni–Holm corrected.

RESULTS

Series I: influence of hypoxia on thermoregulatory behaviour in a ramping shuttle box

By continuously shuttling back and forth between the heating and the cooling compartment of the box, lizards were able to maintain a relatively constant T_b at all oxygen levels (Fig. 1). Five of the twelve lizards (three males, two females) required at least three repetitions to exhibit active thermoregulation throughout the entire 7.5 h period. This was unrelated to oxygen treatment. Data obtained from these five lizards fell within the range of data obtained from the other seven lizards and therefore data from all lizards were pooled for the analysis. Despite several repetitions (>6) four lizards did not thermoregulate at one of the experimental conditions presented to them (one at 5%, one at 7% and two at 21% oxygen). These experiments comprised only 4% of the total of 96 experiments conducted, and were excluded from the analysis.

UET_a exhibited no change with oxygen levels, although there was a trend to increase at lower oxygen concentrations ($F_{4,40}=2.42$, $P=0.064$). LET_a decreased significantly with exposure to 4% and 5% oxygen ($F_{4,40}=10.65$, $P<0.001$; Fig. 2; Table 1) with respect to normoxia.

There were no significant differences in the c.v. of UET_a and UET_b ($F_{4,40}=1.45$, $P=0.235$ and $F_{4,40}=2.22$, $P=0.167$, respectively) between oxygen treatments. The c.v. of LET_a and LET_b increased significantly at the lowest oxygen concentrations. When compared with normoxia, the c.v. of LET_a was significantly higher at 4%, 5%

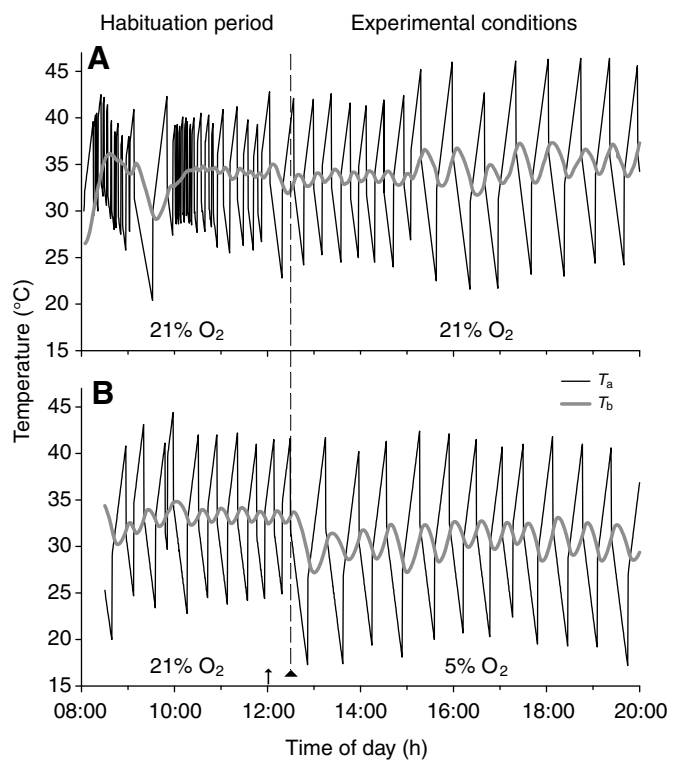


Fig. 1. Representative trace of body temperature (T_b) and selected ambient temperature (T_a) of two different lizards (*P. vitticeps*) allowed to thermoregulate inside an electronic temperature-choice shuttle box. Traces are plotted for the 4.5 h acclimation period and the subsequent 7.5 h of exposure to (A) 21% O_2 and (B) 5% O_2 . Arrow denotes the onset of hypoxia at 12:00 h. Arrowhead represents the moment at which 5% oxygen was reached (12:30 h).

Table 1. Thermoregulatory variables of bearded dragons during exposure to different oxygen concentrations in the ramping shuttle box trials

O ₂ (%)	UET _a (°C)	LET _a (°C)	T _a (°C)	HT _b L (°C)	LT _b L (°C)	RT _b (°C)	T _b (°C)
21	43.0±2.4	25.4±2.6	34.4±0.6	35.9±0.5	33.4±1.0	2.3±1.0	34.7±0.7
10	43.0±2.9	24.8±1.8	34.1±0.8	35.9±0.7	33.3±0.9	2.6±0.6	34.7±0.6
7	44.1±2.0	24.4±2.1	33.6±0.9	35.4±0.8	32.6±1.0	2.8±1.1	34.2±0.7
5	43.6±1.5	23.0±2.0*	32.9±1.2*	34.8±1.5	31.6±1.6	3.2±1.4	33.5±1.2*
4	44.7±1.3	20.6±2.7*	32.1±2.0*	34.7±1.5	30.1±2.4	4.6±1.8*	32.5±1.7*

Values are means of medians (±s.d.) of 12 lizards during a 7.5 h period. *Significant differences relative to 21% oxygen, with the Holm–Sidak *post-hoc* test ($P<0.05$). UET_a, upper escape ambient temperature; LET_a, lower escape ambient temperature; T_a, ambient temperature; HT_bL and LT_bL, high and low body temperature limits, respectively (limits of the central 68% range of the body temperature distribution); RT_b, ambient temperature range (HT_bL–LT_bL); T_b, body temperature. Statistical analysis was not performed on the HT_bL and LT_bL to minimize type I error due to the intrinsic dependence between these variables and RT_b.

and 7% oxygen ($F_{4,40}=8.71$, $P<0.001$) and the c.v. of LET_b rose to reach significance at 4% oxygen ($F_{4,40}=5.65$, $P=0.003$; Fig. 3).

The pronounced decrease in LET_a was reflected in the resulting preferred RT_b; both the high (HT_bL) and low (LT_bL) limits of RT_b decreased with lower oxygen concentrations (Fig. 2; Table 1). Because LT_bL exhibited a more pronounced decrease than HT_bL, RT_b increased significantly at 4% oxygen ($F_{4,40}=5.92$, $P<0.001$; Table 1).

Exposure to 5% and 4% oxygen led to significantly lower T_a values ($F_{4,40}=9.91$, $P<0.001$) than exposure to 21% oxygen (Table 1). Preferred T_b followed a similar pattern, presenting slightly higher values than T_a at all levels of oxygen (Fig. 2; Table 1). T_b was 34.7±0.7°C under normoxic conditions. T_b decreased significantly at 4% and 5% O₂ ($F_{4,40}=15.99$, $P<0.001$) compared with normoxia (Fig. 1; Table 1).

Hypoxia elicited a progressive decrease in the number of shuttles between compartments (Fig. 4). This decrease was significant at 5%

and 4% O₂ ($F_{4,40}=5.69$, $P=0.001$) where lizards shuttled 27.4±7.4 and 20.7±6.7 times, respectively, during a 7.5 h period compared with 59.5±61.2 times in normoxia.

Series II: influence of methodology on the assessment of thermoregulatory behaviour in hypoxia

Methodology had a significant effect on T_b; lizards exhibited significantly lower T_b values at both 21% and 4% O₂ (two-way RM ANOVA, $F_{2,16}=24.20$, $P<0.001$) when evaluated in the extreme temperatures shuttle box compared with both the thermal gradient and the ramping shuttle box. Regardless of the methodology used, however, hypoxia (4% O₂) induced a significant decrease in T_b (two-way RM ANOVA, $F_{1,8}=57.75$, $P<0.001$; Table 2; Fig. 5). LT_bL and HT_bL were affected by hypoxia (4% O₂) in all methodologies used

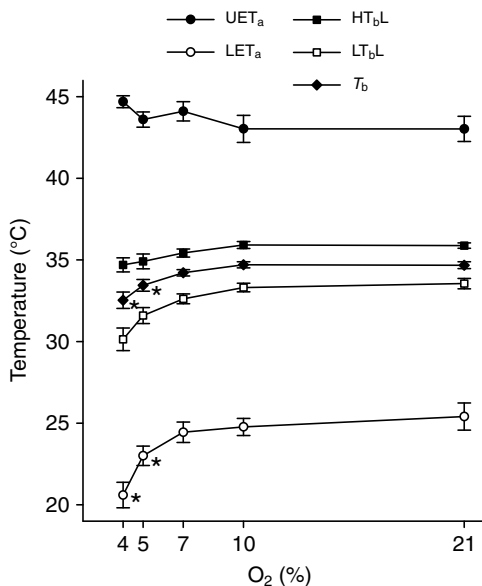


Fig. 2. Values for the different parameters of precision of thermoregulation with exposures to different levels of oxygen. Upper and lower escape ambient temperatures (UET_a and LET_a, respectively), high and low limits of the T_b range (HT_bL and LT_bL) and preferred T_b are plotted as the mean of the 7.5 h median values of 12 lizards (*P. vitticeps*) ±s.e.m. (for visual clarity) instead of s.d. Values for s.d. are reported in Table 1. Animals were tested for 7.5 h in an electronic shuttle box at each of the experimental conditions. *Significant effect compared with normoxic values with the Holm–Sidak *post-hoc* test ($P<0.05$).

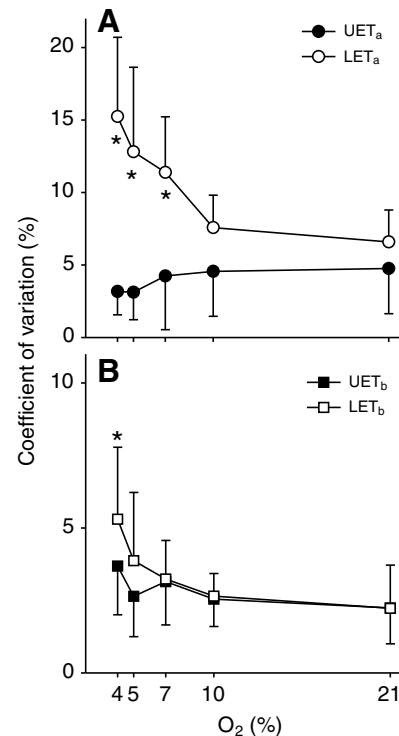


Fig. 3. Coefficients of variation of upper and lower (A) ambient (UET_a and LET_a, respectively) and (B) body (UET_b and LET_b, respectively) escape temperatures of bearded dragons during exposure to different oxygen concentrations. Values are means of 12 lizards during a 7.5 h period. *Significant differences relative to 21% oxygen, with the Holm–Sidak *post-hoc* test ($P<0.05$).

Table 2. Comparison of thermoregulatory variables of bearded dragons during exposure to different oxygen concentrations across the different methodological trials

Treatment	O ₂ (%)	T _b (°C)	ΔT _b 21%–4%	LT _b L (°C)	HT _b L (°C)	RT _b (°C)
Ramping shuttle box	21	34.7±0.7	2.3±1.5	33.5±1.0	35.9±0.5	2.3±1.0
	4	32.5±1.7*		30.1±2.4	34.7±1.5	4.6±1.8*
Extreme temperatures shuttle box	21	33.2±1.4	4.3±2.0†	30.7±2.0	35.0±1.3	4.3±2.1
	4	29.3±2.3*		27.6±2.6	32.2±2.3	4.6±2.4
Thermal gradient	21	34.9±1.6	2.6±2.3	33.4±1.5	36.2±1.2	2.8±1.3
	4	32.4±1.7*		30.6±1.7	34.1±1.5	3.4±1.5

LT_bL and HT_bL were not statistically analysed in order to avoid type I error due to the dependence between these variables and RT_b. †Significant influence of methodology on the decline in T_b during 4% O₂ exposure. *Significant effect of 4% O₂ exposure on the parameter of interest.

(Table 2); however, a significant effect of hypoxia on RT_b was only seen in the ramping shuttle box, but not in either of the other two treatments (two-way RM ANOVA, $F_{1,8}=16.09$, $P=0.003$).

Movement between the cold and hot side of the extreme temperature shuttle box was significantly affected by hypoxia ($t_{11}=2.47$, $P=0.031$). The shuttling frequency over the 7.5 h recording period fell from 77.7±68.9 shuttles in normoxia to 23.7±25.1 shuttles at 4% O₂ (Fig. 4). Similarly, in the thermal gradient trials, movement (assessed as the number of times in 7.5 h that the animals moved more than 20 cm min⁻¹) fell significantly from 76.8±66.5 in normoxia to 25.2±25.6 at 4% O₂ ($t_{10}=3.24$, $P=0.027$). Interestingly, in the extreme trials, lizards spent a considerable amount of time straddling the transition zone between the cold and warm chambers, something not observed in the ramping shuttle box trials. Moreover, at 4% oxygen, lizards spent significantly more time ($F_{1,9}=13.10$, $P=0.018$) inactive in the middle section between the two choice chambers (49.4±36.9% of the time compared with 17.2±23.6% in normoxia; Table 3).

Time course and T_b distributions: comparison of methodology

Low oxygen induced changes in selected T_a, and therefore T_b, within the first hour of exposure in all methodologies (Fig. 6). The decline

in T_b in the ramping shuttle box protocol and thermal gradient was gradual, taking 2–3 h, after which it became more variable, whereas the extreme shuttle box induced a rapid (within 30 min) decline in T_b that was nearly sustained throughout the 7.5 h of measurement. The resulting T_b distribution patterns (Fig. 6) reveal the widening of the T_b distribution that occurred in both shuttle box experiments compared with the thermal gradient trials.

DISCUSSION

Behavioural thermoregulation in hypoxia

This study shows a proportional effect of hypoxia on the level and, in situations where frequent locomotion is required, the precision of behavioural thermoregulation in the bearded dragon (*P. vitticeps*). The cost–benefit model for thermoregulation in lizards proposed by Huey and Slatkin (Huey and Slatkin, 1976) predicts that lizards will actively thermoregulate if the associated costs are low. Although the model was intended to account for costs of an ecological nature (i.e. food availability, homogeneity of the thermal environment, accessibility of basking sites, etc.), it should also be relevant to situations where metabolic or physiological costs are associated with thermoregulation. In severe hypoxia, oxygen becomes a limited resource and locomotion becomes impossible to conduct aerobically.

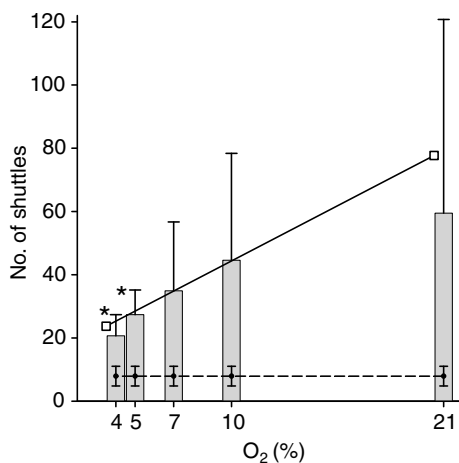


Fig. 4. Number of shuttles at different oxygen concentrations. Plotted values are mean values of 12 lizards \pm s.d. during a 7.5 h period inside an electronically controlled temperature-choice shuttle box. *Significant difference vs 21% oxygen conditions. For comparison, the filled circles show the average number of times a lizard shuttled during the 7.5 h in the absence of a thermal stimulus (constant 34.5°C at 21% O₂) (Cadena and Tattersall, 2009). The open square denotes the average number of shuttles (error bars not shown; see text for details) in the extreme temperatures shuttle box trials at 21% and 4% O₂ (offset slightly for clarity).

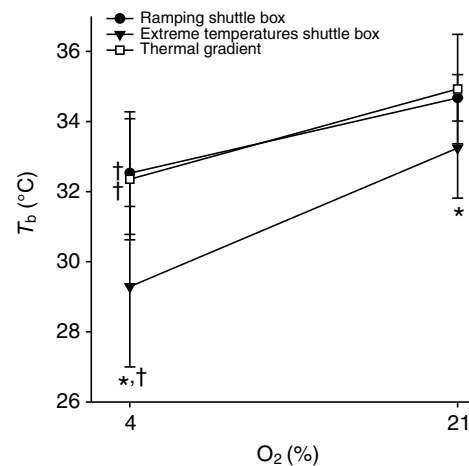


Fig. 5. Effect of methodology on T_b of lizards when exposed to hypoxia. The 'preferred' T_b of nine bearded dragons was determined at 4% and 21% oxygen in an extreme temperatures shuttle box, a thermal gradient and an electronically operated temperature shuttle box (ramping shuttle box). Values are means of nine lizards' median T_b values \pm s.d. *Significant difference between the extreme temperature trials and the other trials. †Significant effect of 4% O₂ on T_b within a trial.

Table 3. Comparison of time spent in the various compartments within the shuttle box in normoxia and hypoxia

Treatment	O ₂ (%)	Cold (%)	Warm (%)	Middle (%)
Ramping shuttle box	21	48.7±1.7	51.3±1.7	0
	4	49.1±4.2	50.9±4.2	0
Extreme temperatures shuttle box	21	42.9±13.8	39.9±13.5	17.2±23.6
	4	29.9±25.4	20.7±16.0	49.4±36.9 [†]

Note, during the ramping trials lizards were never observed to sit in the middle between the two chambers. [†]Significant effect of hypoxia (within a treatment) on the time spent in the middle between the two compartments (RM ANOVA, $F_{1,9}=3.6$, $P=0.006$).

In addition, if frequent movements are necessary to maintain a narrow T_b range, lizards are expected to minimise locomotion at the expense of precise behavioural thermoregulation.

In the present study, bearded dragons did not abandon temperature regulation at low oxygen conditions, as might be expected if the response was an unregulated (i.e. hypothermic) decrease in T_b . Instead, they reduced locomotory oxygen expenditure by reducing the frequency of shuttles between compartments in the shuttle box (Fig. 4) and overall movement in the thermal gradient. In the case of the ramping shuttle box experiments, this led to a decrease in the precision of thermoregulation, manifested in the widening of the T_b range. The fact that the frequency of shuttles at the most extreme level of hypoxia (~21 shuttles) is still well above the predicted number of shuttles (~8) if the behaviour was purely exploratory (Cadena and Tattersall, 2009) provides further evidence

that hypoxic lizards are indeed still actively thermoregulating. Surprisingly, we did not observe a decrease in thermoregulatory precision in the extreme temperatures shuttle box, where locomotory requirements are expected to be largest. However, animals in this protocol were able to compensate by remaining for longer periods of time straddling the cold and the warm compartments, thus decreasing the amount of locomotion required to maintain a constant T_b (albeit lower, compared with the other methodologies; Fig. 5; Table 2), and avoiding the 'extreme temperatures' that would have otherwise resulted in a decrease in thermoregulatory precision.

The effect of hypoxia on lizard thermoregulation was previously described by Hicks and Wood who showed significant decreases in the preferred T_b of four different species of lizard exposed to 7% oxygen (Hicks and Wood, 1985). Similar decreases in T_b have been observed in situations such as anaemia and exhaustive exercise, both conditions in which the oxygen content of the blood is low (Hicks and Wood, 1985; Petersen et al., 2003), suggesting similar underlying mechanisms to the thermoregulatory response. The response observed by Hicks and Wood, however, was much more pronounced (Hicks and Wood, 1985) than the one observed here (a decrease of 5–7°C vs the ~2–4°C decrease observed by us with exposure to 4% O₂). Although this difference may be attributed to differences between species, it might also be due to methodological differences between the studies. Hicks and Wood used a thermal gradient and an extreme temperatures shuttle box to estimate the preferred T_b of lizards (Hicks and Wood, 1985). Even though thermal gradients require less locomotory 'effort' from the animal, it is hard to discriminate between thermoregulating lizards and lizards that are not actively thermoregulating but remaining stationary, due to lack of knowledge of an animal's motivational state. As we and others (Schurmann and Steffensen, 1994) have observed, ectotherms may not be motivated to actively thermoregulate in every experiment, and the inclusion of these data may lead to a lower (usually) estimation of the preferred T_b (Cadena and Tattersall, 2009). An extreme temperatures shuttle box, on the other hand, is an energetically costly condition since it requires frequent and constant shuttling between compartments to maintain relatively high and constant T_b levels and can therefore cause decreases in normal T_b itself (Fig. 5) (Cadena and Tattersall, 2009). Our results indicate that the use of a dual-choice shuttle box can inflate the effect of hypoxia on T_b by twofold compared with other techniques.

The decrease in T_b of hypoxic lizards observed by Hicks and Wood was accompanied by a decrease in both UET_b and LET_b [when thermoregulating inside an extreme temperatures shuttle box (Hicks and Wood, 1985)], suggesting a decline in the upper and lower temperature set-points (Barber and Crawford, 1977). Escape T_b and T_b set-points, however, are not necessarily analogous, since the thermal inertia of even small animals is usually large enough to cause changes in T_b to lag behind those of T_a , which is why we report different variables in our study (see Table 1). Interestingly, however, the upper escape T_a of

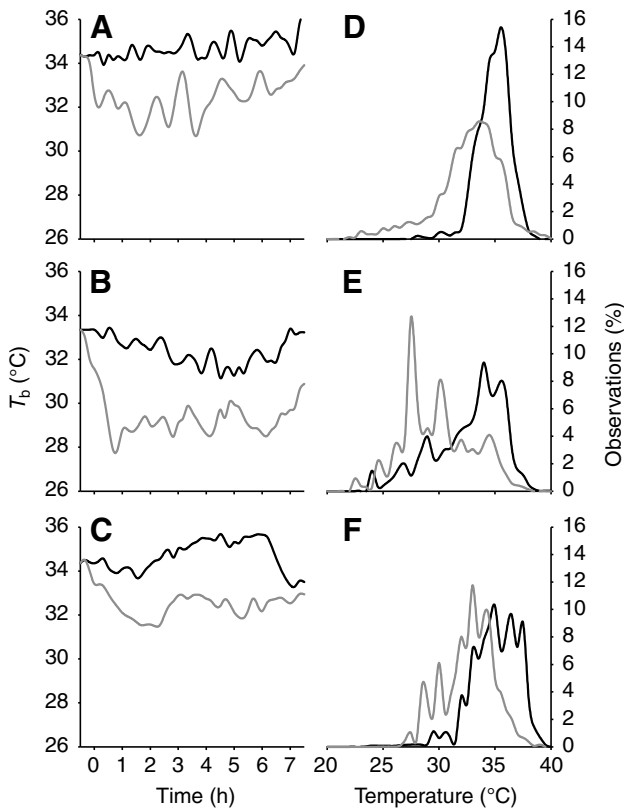


Fig. 6. Influence of methodology on the normoxic (black lines) and hypoxic (grey lines) thermoregulatory responses of bearded dragons. A, B and C show the average change in T_b over the 7.5 h of measurements (starting at 0 h) in the ramping shuttle box, extreme shuttle box and thermal gradient trials, respectively. D, E and F show the corresponding average frequency histograms (expressed as per cent of observations) for lizards in the ramping shuttle box, extreme shuttle box and thermal gradient trials, respectively.

bearded dragons in our study rose slightly (but not significantly), while the lower escape T_a decreased at lower oxygen concentrations. In other words, it was the drastic decline of the lower escape T_a and not a decrease in both the upper and lower temperature set-points that caused the general decrease in T_b . These findings suggest that in conditions in which continuous locomotion is required, hypoxia induces changes in behavioural thermoregulatory precision, a fact hitherto unappreciated.

Implications for the neurophysiological control of T_b

Similar thermoregulatory responses to those reported here have been observed in experiments in which the medial preoptic area of the brain of the lizard *Dipsosaurus dorsalis* was lesioned (Berk and Heath, 1975b). When allowed to thermoregulate inside a thermal shuttle box, lesioned lizards decreased their lower escape temperature and increased their upper escape temperature while significantly reducing their shuttling frequency compared with unoperated control groups. Thus, interference with the thermoregulatory integrative centres of the brain will decrease the precision of thermoregulation at both upper and lower ranges. Interestingly, hypoxia has also been shown to significantly decrease the cutaneous sensitivity of humans to cold but not to warm temperatures (Golja et al., 2004). This is consistent with the observation that rhesus monkeys exposed briefly to anoxia drastically diminished the firing rate of cutaneous cold-sensitive units (Iggo and Paintal, 1977). The firing rate of thermosensitive neurons in the preoptic area of rats has also been shown to change in hypoxia (Tamaki and Nakayama, 1987), although, generally, the main effect is to demonstrate an increase in firing rate at low oxygen levels. It is possible, therefore, that hypoxia causes a decrease in the sensitivity of skin temperature receptors, thereby diminishing peripheral feedback, as well as altering the preoptic thermosensitive neurons responsible for thermoregulatory control in lizards. The tendency for bearded dragons to select lower T_a and the greater variability in their lower escape temperatures in low oxygen is consistent with a greater influence of hypoxia on the cold-sensitive side of thermoregulation. These changes in sensitivity would be manifested in a decrease in locomotory behaviour, as lizards would tolerate higher than and lower than normal temperatures and remain stationary for longer periods of time at T_a values outside their normally preferred range. This is consistent with the observations in this study. Although we do not exclude the possibility that the decrease in T_b observed in hypoxia is a regulated response (i.e. decrease in set-point), the present study reveals another component and potential mechanism of the thermoregulatory response to hypoxia; a decrease in thermoregulatory precision, possibly due to a hypoxia-induced decrease in thermosensitivity.

Conclusions

The evidence presented here provides further information that, in addition to the effect on the level of thermoregulation, the temporal patterns and variance in selected T_b are influenced by the methodology of assessing thermal preference (Fig. 6). These differences are further increased by hypoxia. The integrated behavioural thermoregulatory response to hypoxia could be viewed as resulting from a number of factors. Firstly, severe hypoxia has an impact on the capacity for aerobic activity. This decreases movement and thereby causes decreased precision in behavioural thermoregulation requiring locomotory efforts. Indeed, we observed a decline in the locomotory behaviour of lizards in hypoxia under all experimental conditions, suggesting a reduction in activity.

Secondly, this behavioural response could be manifested through differential effects on the cold- and warm-sensing pathways that influence thermal sensation and effect the changes that accompany behavioural thermoregulation. This is consistent with data from studies of thermoregulation in mammals which also show that the precision of T_b control in hypoxia may be lower than that observed in normoxia (Barros et al., 2001; Dupré et al., 1988; Gordon and Fogelson, 1991; Tattersall and Milsom, 2002).

Other stress stimuli (that in one way or another increase the costs of thermoregulation), such as low environmental thermal quality, risk of predation, territorial defence, or water or food availability have been shown to evoke similar decreases in T_b and/or thermoregulatory precision (Cabanac, 1985; Dewitt, 1967; Huey and Slatkin, 1976; Ladyman and Bradshaw, 2003; Lorenzon et al., 1999; Mathies and Andrews, 1997), suggesting conserved thermoregulatory mechanisms in response to costly conditions. Discovering how these stressors alter the sensation of temperature and the underlying neurophysiological control of T_b remains a challenge for future studies.

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