

Hypoxia progressively lowers thermal gaping thresholds in bearded dragons, *Pogona vitticeps*

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Accepted 30 June 2005

Summary

Most animals, including reptiles, lower body temperature (T_b) under hypoxic conditions. Numerous physiological and behavioural traits significant to the regulation of T_b are altered by hypoxia in ways that suggest an orchestrated adjustment of T_b at a new and lower regulated level. We examined this matter in bearded dragons, *Pogona vitticeps*, a species of reptile that naturally exhibits open mouth gaping at high temperatures, presumably in order to promote evaporation and thus prevent or avoid further increases in T_b . The threshold for the onset of gaping (assessed as the temperature at which lizards spent 50% of their time gaping) was reduced from 36.9°C in normoxia to 35.5°C at 10% and 34.3°C at 6% O₂. The overall magnitude or degree of gaping, measured qualitatively, was more pronounced at lower temperatures in hypoxia. Females

consistently had lower gaping threshold temperatures than did males, and this difference was retained throughout exposure to hypoxia. In addition to gaping, evaporative water loss from the cloaca may also play a significant role in temperature regulation, since the ambient temperature at which cloacal discharge occurred was also reduced significantly in hypoxia. The results reported herein strongly support the view that hypoxia reduces temperature set-point in lizards and that such changes are coordinated by specific behavioural thermoeffectors that modulate evaporative water loss and thus facilitate a high potential for controlling or modifying T_b .

Key words: body temperature, evaporative heat loss, hypoxia, panting, reptile, set-point, sex differences, thermoregulation.

Introduction

The bearded dragon, *Pogona vitticeps* (Ahl 1926), is an agamid lizard that naturally inhabits the inland regions of Australia. In the wild, it prefers temperatures of approximately 33°C (Melville and Schulte, 2001) and will adopt behavioural and physiological strategies to achieve this preferred body temperature. Lizards achieve these kinds of preferred temperatures in the wild through shuttling between warm and cold environments (Brattstrom, 1971), modifying heart rates during heat and cooling (Seebacher and Franklin, 2001), altering peripheral blood flow (Grigg and Seebacher, 1999), changing cloacal evaporative water loss (DeNardo et al., 2004) and *via* ventilatory mechanisms, manifesting as gaping or panting responses (Heatwole et al., 1973). Numerous lizard species open the mouth widely when heated. The temperature at which this gaping occurs in some lizard species is often well above the preferred temperature, acting as a last-ditch attempt to survive nearly lethal temperatures (Veron and Heatwole, 1970; Webb et al., 1972). Many lizards, however, begin panting at temperatures at or very close to their preferred body temperature (Heatwole et al., 1973), suggesting that ventilatory heat loss through evaporation is an important route for fine-tuning body temperature (T_b) regulation in these species. Australian bearded dragons are lizards that employ the latter

strategy, spending much of their time basking and gaping, rather than shuttling back and forth between warm and cool environments.

Numerous physical factors have been shown to alter the temperature at which lizards gape or pant. Panting thresholds have been shown to be altered by dehydration (Parmenter and Heatwole, 1975), circadian rhythms (Chong et al., 1973) and the rate or source of heating (Heatwole et al., 1973). The direction of these changes reflects the constraints of other physiological processes on thermoregulation; to conserve water, lizards do not gape until higher temperatures, and, since daily metabolic demand decreases at night in many reptiles (Rismiller and Heldmaier, 1987), a concomitant decline in the threshold for gaping occurs as the thermoregulatory set-point (T_{set}) is reduced along with the reduced metabolic requirements (Rismiller and Heldmaier, 1982).

It is well established that low oxygen causes many animals, including reptiles, to lower T_b (Wood and Gonzales, 1996). Changes in preferred temperatures are often taken as proof of a change in temperature set-point in the brain, since preferred temperature selection is a behavioural and thus, ultimately, a neurophysiological phenomenon. Numerous lizards (e.g. genera *Iguana*, *Dipsosaurus* and *Anolis*) select temperatures in

hypoxia approximately 10°C lower than normoxic preferred temperatures (Hicks and Wood, 1985; Petersen et al., 2003). If the T_{set} is decreased in hypoxia, then the thresholds for any autonomic or behavioural response that results in a decline or an attempt to decrease T_b should be decreased. Since many lizards exhibit panting or gaping responses as ambient temperature rises (Crawford and Barber, 1974; Crawford and Gatz, 1974; Crawford et al., 1977; Heatwole et al., 1973; Pough and McFarland, 1976), this is taken to imply that reptiles exhibit some degree of control of T_b , albeit manifesting as a behavioural response.

With respect to behavioural thresholds for thermoregulation, Dupré et al. (1986) showed that the evaporative cooling threshold is diminished in hypoxic lizards; however, no attempt to assess the magnitude or persistence of the gaping response was made. Lizards that gape or pant may do so intermittently. It would be fruitful to assess the entire gaping response across a wide range of temperatures in order to determine whether the response is sustained, is proportional to temperature and the severity of hypoxia and is dramatic enough to have an effect on T_b regulation.

Studying the control of T_b in lizards under hypoxic conditions allows for the exploration of the existence of thermal threshold responses and thus makes inferences regarding the presence of thermoregulatory set-points. The objectives of this study, however, were to examine whether the gaping behaviour was proportionately related to ambient temperature and whether the threshold temperature at which gaping occurred was proportionately lowered in hypoxia. Understanding these two questions will shed light on the mechanism of thermoregulation in reptiles and other vertebrates and will provide evidence for whether hypoxia elicits a reduction in a hitherto seldom-studied behavioural response in reptiles. For the purpose of this study, we examined the gaping response (i.e. simple mouth opening) rather than panting (i.e. an altered breathing pattern). It is plausible that bearded dragons actually adopt panting, where the breathing frequency rises and tidal volume declines; however, assessing tidal volume accurately is difficult to do in lizards that open their mouths. Since simply opening the mouth may achieve a similar result of eliciting evaporative water loss across the mucosa within the mouth and throat, it is not necessarily required that panting accompanies gaping behaviour.

Materials and methods

Animals

Fourteen (eight male and six female) inland bearded dragons, *P. vitticeps*, were used in this study. All were captive-bred individuals ranging in age from approximately 6 to 12 months, as well as one adult lizard (>5 years of age). The lizards ranged in mass from approximately 50 g to 220 g, with the adult weighing 350 g. The animals were fed a daily diet consisting of crickets and mealworms and were provided with chopped vegetables *ad libitum*. T_b in the home cages (using a non-contact infrared thermometer) and body masses were

monitored once a week at 11.00 h. Lizards had access to a 100 W light bulb, which could allow their T_b s to rise as high as 39°C or as low as 28°C in the housing environment. Animal experimentation was approved by the Brock University Animal Care Committee (ACUC Protocol #031001).

Experimental set-up

Throughout experimentation, an individual lizard was housed in a 24×24×40 cm clear acrylic box, which was blacked out on three sides to prevent distractions and to minimise reflections. Once inside the box, the lizard sat elevated 3.5 cm from the bottom on a perforated, clear, acrylic platform, which allowed gas to be pumped into the box from a tube below the platform and helped to collect urine and faeces. The box was located within an environmental chamber (Thermo Forma; Marietta, OH, USA) to enable changing temperatures between 30 and 40°C. A non-radiant heat source was chosen in order to simplify the exposure regime. Since lizards reacted to human presence, a small surveillance camera was affixed to the environmental chamber inside wall and aimed towards the acrylic box to allow for observation of the undisturbed lizard's behaviour on a video monitor outside the chamber. An infrared (IR) thermal imaging camera (Mikron 7515; Oakland, NJ, USA) was positioned on top of the box, looking down onto the lizard, to obtain body surface temperature data. The IR imager was hooked up to a computer outside the chamber to obtain computerised images. In order to allow for varying oxygen levels inside the box, the IR camera rested on a set of 'bellows', sealed to the box with weather stripping, making the box relatively air-tight. A small tube was inserted beneath the bellows into the box to allow for gas sampling during hypoxic conditions to verify oxygen levels of 21, 10 and 6%.

Data collection

The animals were placed in the chamber to start experimentation in the morning, preferably before feeding. Since it has been shown that lizards have different gaping thresholds between night and day (Chong et al., 1973), we elected to perform the gaping measurements during the same time of the day, between 10.00 h and 16.00 h. Lizards were given time to acclimate to the new temperature, as well as for the box to reach hypoxic levels if necessary. This usually required 20–30 min.

Assessing gaping behaviour

The lizard was observed for 15 min at each temperature of interest (30, 32, 34, 36, 38, 40°C), and the time spent gaping was recorded together with the degree or type of opening of the lizard's mouth (Fig. 1). Type I represented a barely open mouth, Type II was a typical gape and Type III was a wide open mouth, usually accompanied by a head-back posture, with the tongue partially protruding and a puffing out of the throat. The observation periods were started once the lizard's dorsal surface temperature was within 0.5°C of the ambient temperature of interest. The only exceptions were the

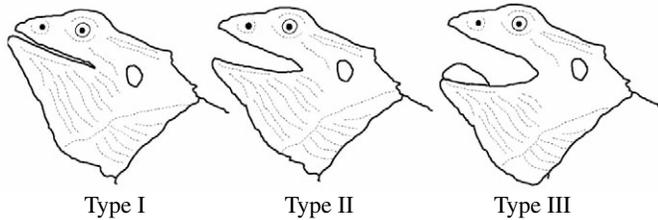


Fig. 1. The three types of gaping categorised in this study. Type I was ascribed to situations when the mouth was visibly, but barely, open (i.e. there was no obvious sealing of the upper jaw with the lower jaw), which is readily distinguished from a normal closed mouth. Type II was ascribed to situations when the mouth was obviously open by more than a few millimetres. Type III was easily distinguished from other types as the lizard's tongue was easily visible and the throat was obviously distended due to the open mouth.

observation periods at 40°C (in normoxia and 10% oxygen) and 38°C (at 6% oxygen), when the animal was observed before it had reached ambient temperature. This was due to the long time required for the animal to reach the highest temperatures. The animals were observed sooner in order to prevent heat damage, since at this temperature all animals were already gaping maximally.

Assessing animal surface temperatures

The camera software, MikroSpec RT Version 2.1394 (Oakland, NJ, USA), was set to capture an image of the thermal data every minute, up to a maximum of 500 frames. Image capturing was started upon the beginning of heating to 32°C (or 30°C at 6% oxygen). Images from the time during the 15 min observation period were analyzed, and the lizard surface temperatures recorded every minute. The mean temperature of a circular region of interest was recorded from the lizard's back, head (between the eyes), tip of the nose, tongue tip (if visible when gaping) and eye.

Experimental design and test conditions

The animals were each tested under normoxic and two hypoxic conditions (10 and 6% O₂), which was accomplished by mixing air with nitrogen to achieve the desired oxygen level. In all cases, gases entered the box at 5 l min⁻¹. Since no previous data were known on the effects of hypoxia in *P. vitticeps*, initial observations were made of the animals at oxygen levels between 5 and 10%. An oxygen level of 6% was chosen as appropriate for this experiment, since this placed a significant enough stress on the animal while not seriously risking damage to the animal after the 6 h of exposure required (i.e. lizards appeared distressed at levels below 6% O₂ whereas at 6% O₂ or above they remained calm throughout the procedures). Throughout the trials, the oxygen level was kept to within ±0.2% of the desired level. One animal was tested per day at each oxygen level. Two to three weeks passed before the performance of another experiment on the same individual at a new level of oxygen. At each level of oxygen, lizards were tested at 4–5 different ambient

temperatures between 30 and 40°C (32, 34, 36, 38 and 40 for 21 and 10% O₂ and 30, 32, 34, 36 and 38°C for 6% O₂). Lizards were exposed to these temperatures in a step-wise fashion, with the 2°C increments lasting either 1 h each or as long as it took to achieve skin surface temperature equilibration with ambient temperature. It usually took approximately 1 h for the lizard's body surface temperature to come into equilibrium with the environment. The highest temperature (40°C) was not used in the 6% O₂ group out of concern for the lizard's survival. Previous studies have shown that lizards held at high temperatures under hypoxic conditions will die (Hicks and Wood, 1985).

Data analysis

All values reported are means ± S.E.M., unless otherwise stated. The percentage time spent gaping at the different ambient temperatures was initially analysed to determine a temperature at which 50% of the animal's time was spent gaping. This was done by fitting individual Hill equations to each animal using:

$$P_{\text{gape}} = 100 \times \left\{ (T_a)^N / [(T_a)^N + (ET_{50})^N] \right\}, \quad (1)$$

where P_{gape} refers to the percentage of time spent gaping, T_a is the ambient temperature, ET_{50} is the effective temperature at which 50% of the animal's time was spent gaping, and the exponent, N , is Hill's constant (a larger N indicates a steeper sigmoidal relationship between percentage gaping time and T_a). The parameters that minimised the root mean square error were determined using an iterative procedure facilitated by Microsoft™ Excel's solver tool.

Data (either time spent gaping or surface temperatures) were analysed using repeated-measures two-way ANOVA, with oxygen and ambient temperature as the two treatments. On occasions where normality was not met, log transformations were performed and the test repeated. In all cases, residuals from the individual ANOVAs were examined to verify a normal distribution. If residuals were non-normal, a non-parametric repeated-measures ANOVA of ranks was performed. We were able to examine the effects of gender once we had calculated the ET_{50} s and N . On that occasion, we used two-way repeated-measures ANOVA to test for significant ET_{50} and N , with oxygen level and sex as treatments. In cases where ANOVAs yielded significant effects, *post-hoc* multiple comparisons were performed using the Holm-Sidak method. All statistics were considered significant at $P < 0.05$.

Results

General observations in hypoxia

Lizards initially responded to the test chambers by turning around and searching their environments, settling down within 30 min, moving only occasionally thereafter. At the lower levels of oxygen, lizards would often close their eyes, appearing to fall asleep, though we did not make attempts to quantify this. Most lizards defecated or urinated in the test chamber during the course of observations (Fig. 2), even

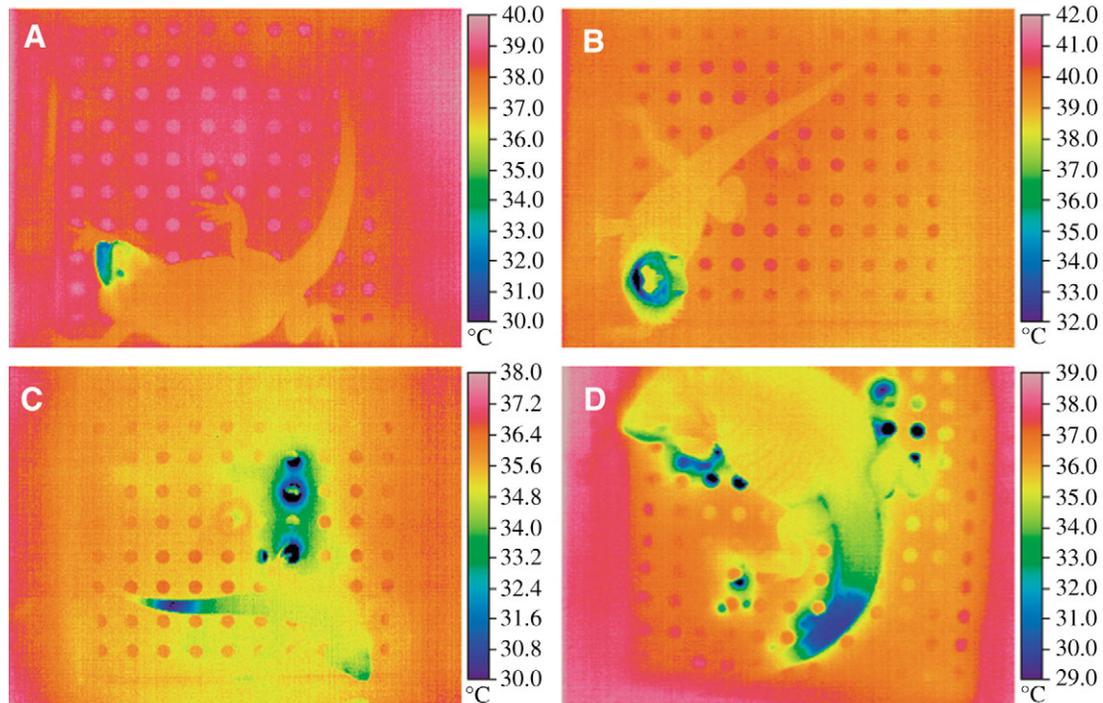


Fig. 2. Infrared thermal images of lizards at different ambient temperatures showing Type II gaping (A), Type III gaping with the inside of the mouth clearly visible (B) and cloacal discharge in two different lizards (C, in hypoxia; D, in normoxia). In all cases, regional temperature differences can be observed across the body surfaces. Note the different temperature keys to the right of each image.

though they had been fasted for 16 h prior to experimentation. This discharge led to a dramatic and often prolonged decline in tail surface temperature (Fig. 2). Lizards (10/14=71.4% of the individuals) kept under normoxic conditions first discharged faeces and urine (cloacal discharge) at $36.1 \pm 0.6^\circ\text{C}$. At 10% O_2 , the cloacal discharge temperature (T_{CD} ; back surface temperature at which cloacal discharge first occurs) fell significantly ($P=0.02$) to $34.4 \pm 0.8^\circ\text{C}$ in 71.4% of lizards, and at 6% O_2 the T_{CD} was also significantly lower ($P=0.02$) than normoxia at $33.2 \pm 0.4^\circ\text{C}$ (Table 1) in 78.6% of individuals. There was no significant difference in the T_{CD} between 10 and 6% O_2 . The lizards that did not exhibit cloacal discharge (21.4–28.6% of individuals) were not factored into the calculations for T_{CD} . Of those lizards that exhibited cloacal discharge during exposure to temperatures between 30 and 40°C , 1.8 ± 0.2 discharges occurred at 21% O_2 , 1.5 ± 0.3 discharges occurred at 10% O_2 , and 3.4 ± 0.4 discharges occurred at 6% O_2 . The latter value was significantly higher than the values at 21% O_2 ($P<0.05$; Table 1).

Gaping times in normoxia and hypoxia

Overall gaping time was significantly affected by inspired oxygen level ($P<0.001$) and by temperature ($P<0.001$; Fig. 3). In all cases, the total gaping time increased sigmoidally at the higher ambient temperatures; however, there was no significant interaction between oxygen and temperature ($P=0.112$). The mean ET_{50} values for 21%, 10% and 6% O_2 were $36.9 \pm 0.2^\circ\text{C}$, $35.5 \pm 0.4^\circ\text{C}$, and $34.3 \pm 0.4^\circ\text{C}$, respectively (Table 1), and the

Table 1. Estimates of ET_{50} , Hill constant (N) and cloacal discharge temperature (T_{CD}) from lizards breathing 21, 10 and 6% O_2

	ET_{50}	N	T_{CD} ($^\circ\text{C}$)	No. of cloacal discharges [†]
21% O_2	36.9 ± 0.2	69.8 ± 11.0	36.1 ± 0.6	1.8 ± 0.2 (71.4)
Male	37.2 ± 0.3	–	–	–
Female	36.6 ± 0.4	–	–	–
10% O_2	$35.5 \pm 0.4^*$	78.4 ± 10.8	$34.4 \pm 0.8^*$	1.5 ± 0.3 (71.4)
Male	35.8 ± 0.5	–	–	–
Female	35.0 ± 0.6	–	–	–
6% O_2	$34.3 \pm 0.4^*$	$113.4 \pm 15.6^*$	$33.2 \pm 0.4^*$	3.4 ± 0.4 (78.6)*
Male	34.8 ± 0.4	–	–	–
Female	33.6 ± 0.6	–	–	–

Values are means \pm S.E.M. Mean male and female values are shown for those parameters in which there was a significant difference between sexes using repeated-measures ANOVA, with oxygen level and sex as factors.

*Significant difference ($P<0.05$) from 21% O_2 values.

[†]Numbers in parentheses refer to the percentage of lizards exhibiting cloacal discharge.

effect of hypoxia (10 and 6% O_2) was found to be significantly lower than normoxia. There was also a significant trend for the Hill constant, N , to be higher in hypoxia, although this effect

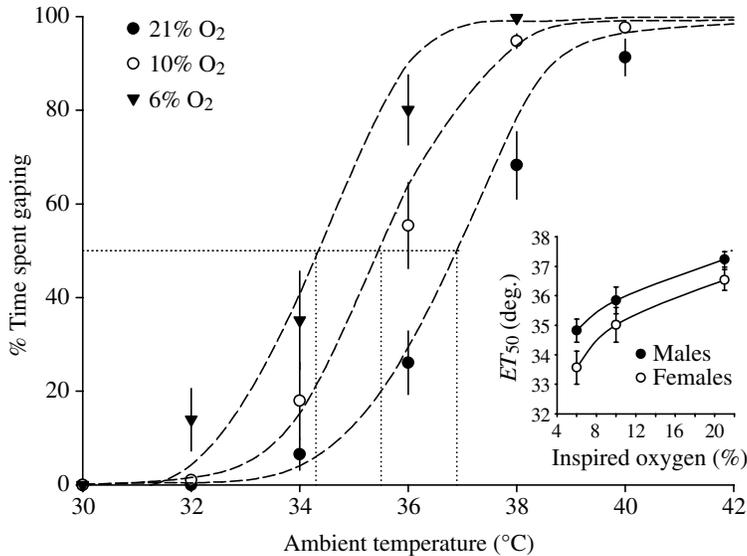


Fig. 3. Mean times (\pm S.E.M.) spent gaping in lizards at three different levels of oxygen – 21% (filled circles), 10% (open circles) and 6% O_2 (filled triangles) – during 15 min periods of observations of lizards that were in thermal equilibrium with different environmental temperatures (30–40°C). The broken lines represent Hill equations (see equation 1) fitted through the mean data. The vertical dotted lines represent the mean time at which lizards spent 50% of their time gaping (ET_{50}) at each level of oxygen inspired, as calculated from each lizard. The inset graph refers to the ET_{50} values for male (filled circles) and female lizards (open circles) at 21, 10 and 6% O_2 .

was only significant at 6% O_2 ($P=0.05$). N was 69.8 ± 11.0 at 21% O_2 , 78.4 ± 10.8 at 10% O_2 and 113.4 ± 15.6 at 6% O_2 (Table 1). The appropriateness of ET_{50} as an overall estimate of the threshold temperature is demonstrated by the good fit ($r^2=0.873$, $P<0.001$) of the regression of ET_{50} versus the total time spent gaping between 30 and 38°C (i.e. the experimental duration).

Effect of sex on thermal preferences and ET_{50}

Using a two-way repeated-measures ANOVA to test ET_{50} with oxygen and sex as the treatments, there was no significant interaction ($P=0.49$), although sex and oxygen level each had a significant effect ($P=0.046$ and $P<0.001$, respectively). Male lizards exhibited significantly higher ET_{50} values than females at all levels of oxygen tested (Table 1). Interestingly, this sex difference was also borne out in the mean preferred temperatures of lizards in their home cages during the 3-month period of experimentation; just as in the ET_{50} estimates, there was a significant effect of sex on home cage preferred temperature ($P=0.02$). Males exhibited a slightly, though significantly, higher T_b of 35.2 ± 0.17 versus 34.2 ± 0.34 °C in females.

Gaping type in normoxia and hypoxia

There was a significant effect of temperature on the percentage time spent in Type I gaping ($P=0.03$), but no significant effect of oxygen ($P=0.31$) nor a significant

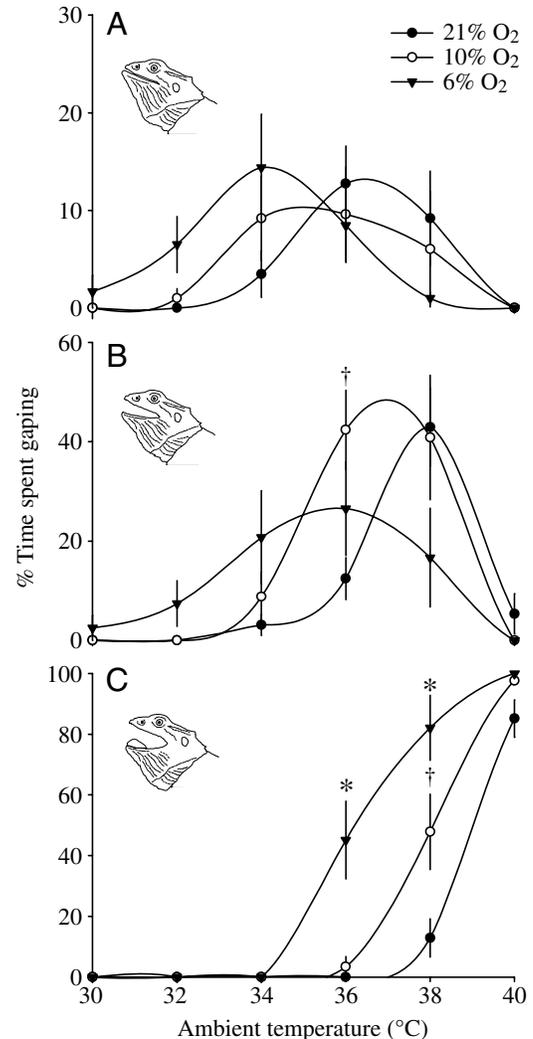


Fig. 4. Mean times (\pm S.E.M.) spent engaged in (A) Type I, (B) Type II and (C) Type III gaping at 21, 10 and 6% O_2 . Data for 40°C are shown for comparison, although not included in statistical analysis, since data points were not available at 6% O_2 . † refers to a significant difference between 10 and 21% O_2 , and * refers to a significant difference between 6 and 21% O_2 with *post-hoc* tests.

interaction effect ($P=0.18$). At all three levels of oxygen tested, the percentage time spent in Type I gaping gradually increased with increasing temperature before falling back down to 0% at the highest temperatures (Fig. 4).

Type II gaping was significantly affected by inspired oxygen ($P=0.02$) and ambient temperature ($P<0.001$). Furthermore, there was a significant interaction between oxygen and temperature ($P<0.001$). Type II gaping predominated at lower temperatures at 6% O_2 . At all three levels of oxygen, Type II gaping initially increased with higher temperatures before decreasing at the highest temperatures (Fig. 4).

There was a significant effect of oxygen and temperature on the percentage time spent in Type III gaping ($P<0.001$ for oxygen and $P<0.001$ for temperature) and a significant interaction between oxygen and temperature ($P<0.001$).

Qualitatively, Type III gaping was initiated earlier (i.e. at lower temperatures) in hypoxia than in normoxia (Fig. 4). Indeed, at temperatures above 36°C, significantly more time (>50%) was spent engaged in Type III gaping at 6% O₂ than at 21% O₂.

Effect of body mass on gaping times

There was no significant effect of body mass on the *ET*₅₀ estimates at 21, 10 or 6% O₂, despite the large range of body masses examined ($P=0.18, 0.29$ and 0.45 and $r^2=0.14, 0.09$ and 0.05 , respectively, determined through linear regressions). Furthermore, body mass had no significant effect on *N* estimates for 21 and 10% O₂ ($P=0.26$ and 0.76 and $r^2=0.10$ and 0.008 , respectively); however, *N* estimates were significantly and negatively correlated with body mass in the lizards at 6% O₂ ($P=0.0005$ and $r^2=0.66$), demonstrating that

small lizards exhibited a more rapid transition to continuous gaping as temperature increased.

Effect of ambient temperature and oxygen on surface temperatures

Ambient temperature significantly affected all surface temperatures (body, head, nose, eye; $P<0.001$ for all values), except for tongue surface temperature, where there was a nearly significant difference ($P=0.062$). Oxygen level had no significant effect on any of the surface temperatures (except tongue) nor was there a significant interaction between oxygen and temperature for either body, head, nose or eye temperature ($P=0.466, 0.10, 0.011$ and 0.261 , respectively; Fig. 5).

The only meaningful significant effects were on the tongue temperature. To simplify comparisons and account for some inter-individual responses, we also examined the body surface–tongue surface temperature difference. Oxygen level and ambient temperature had significant effects ($P=0.011$ and $P<0.001$, respectively) on the body surface–tongue surface temperatures, although there was not a significant interaction between oxygen and ambient temperature ($P=0.92$; Fig. 6). Overall, the body–tongue difference was greater at 6% O₂ than at 21% O₂, an effect that was most apparent at the lower ambient temperatures.

Heating times in normoxia and hypoxia

Although not specifically controlled for, the times required to heat body surface temperature from 30 to 38°C over the course of the entire experiment were significantly affected by inspired oxygen ($P=0.003$). It took 225.6 ± 12.7 min in

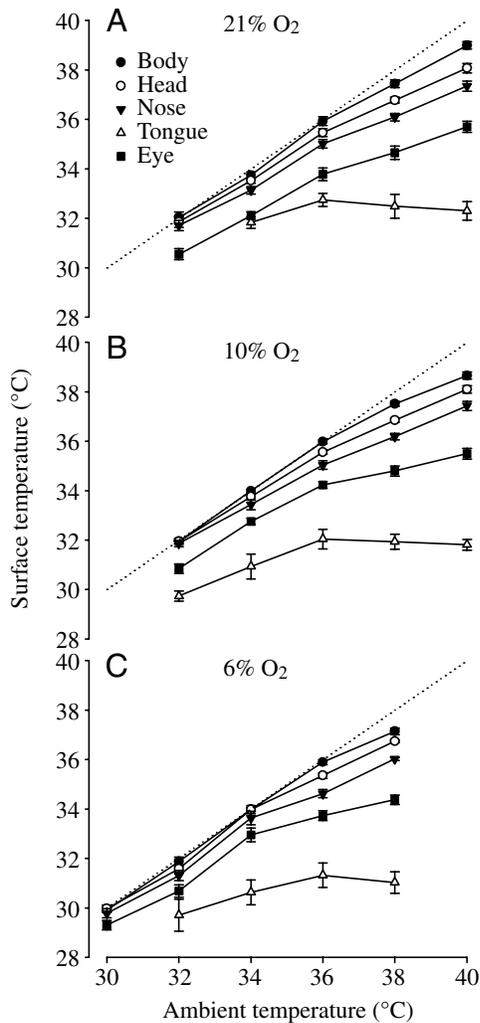


Fig. 5. Mean surface temperatures (\pm S.E.M.) exposed to (A) 21, (B) 10 and (C) 6% O₂ during changes in ambient temperature ranging from 30 to 40°C. Shown are surface temperatures of the head, body, nose, eye and tongue (when visible). The dotted lines represent the line of equality for surface temperature and ambient temperature.

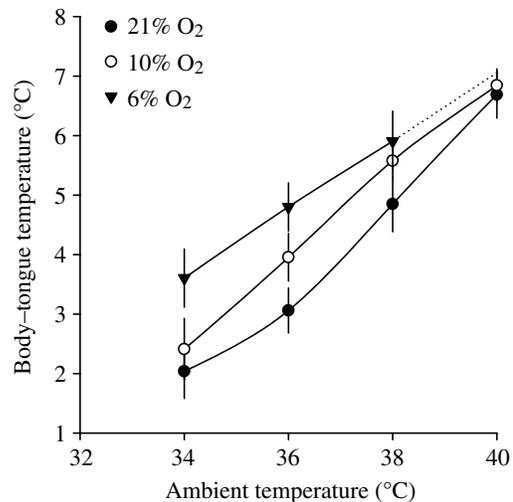


Fig. 6. Body surface temperature minus tongue temperature at 21, 10 and 6% O₂ between ambient temperatures of 32–38°C. There was a significant effect of oxygen and ambient temperature on this difference (two-way repeated-measures ANOVA), suggesting that hypoxic conditions led to a higher value at lower ambient temperatures. The dotted line represents an extrapolation beyond 38°C in the 6% O₂ group.

normoxia to warm body surface temperature to 38°C, 249.4±9.2 min at 10% O₂, and 283.1±9.7 min at 6% O₂ (the latter value being significantly higher than the normoxic value with *post-hoc* comparisons).

Discussion

The results of this study support the notion that the T_b set-point for gaping is reduced in proportion to the severity of hypoxia and that evaporative cooling in the bearded dragon seems critical to normal T_b regulation. That an apparently simple behavioural response such as gaping can be so elegantly controlled, in both magnitude and duration, speaks volumes for the importance of thermoregulation in this species. In terms of behavioural strategies for

thermoregulation, an argument could be made for the importance of this response since the costs of gaping are relatively inexpensive, provided a lizard is hydrated. In general, animal behaviour is believed to exhibit optimality, such that the costs and benefits of a given behaviour balance out. Provided adequate moisture is available, gaping or panting can be an effective strategy that does not require the movement into or out of certain thermal environments. Increased movement in the wild may lead to increased visibility and thus increased risk of predation, so if subtle behavioural adjustments can modulate T_b adequately, then the less expensive behavioural strategies should prevail. Whether the altered gaping response to hypoxia would occur in those lizard species that only utilise gaping and panting as a last resort remains to be seen.

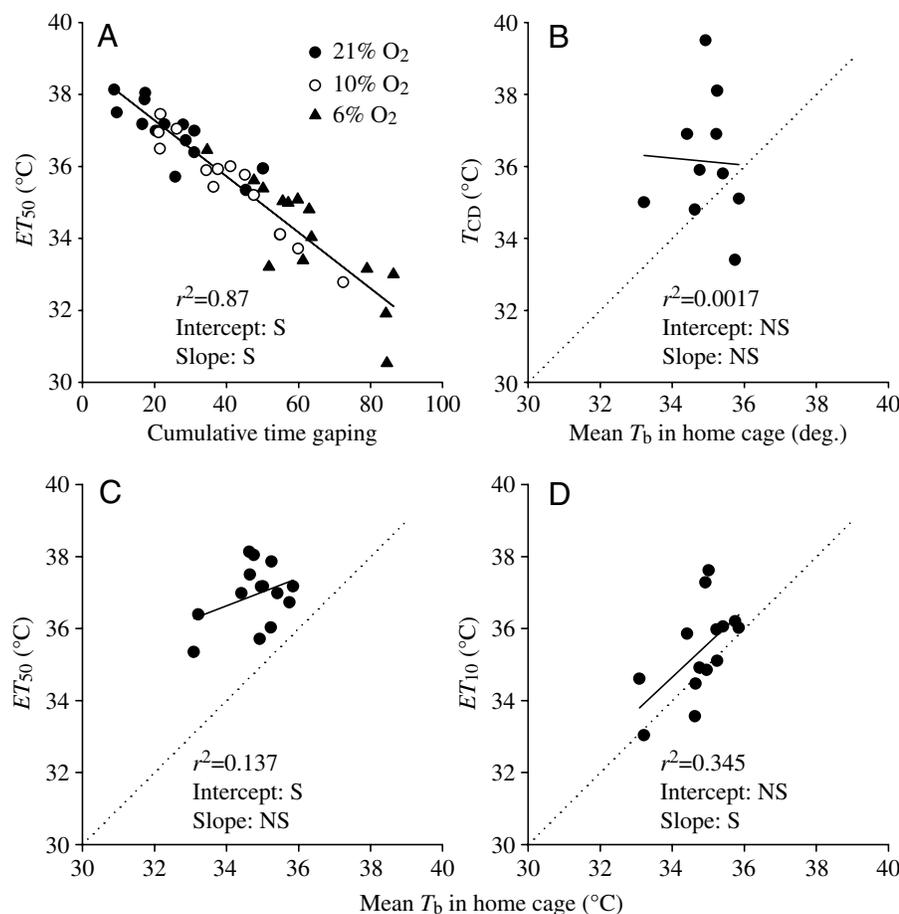


Fig. 7. Various threshold parameters in bearded dragons as they pertain to the appropriateness of thermoregulation. (A) The plot of ET_{50} versus cumulative time spent gaping during experimental procedures demonstrates that ET_{50} estimates provided a good fit across all three levels of O₂. (B) Cloacal discharge threshold (T_{CD}) in normoxia versus mean cage temperature (see Materials and methods) showed no significant relationships. (C) A significant relationship between ET_{50} in normoxia and mean cage temperature was not apparent, although a significant intercept did occur, suggesting that the ET_{50} tends to occur at temperatures higher than preferred cage temperatures. (D) There was a significant relationship between ET_{10} (the temperature at which the lizards were gaping for 10% of the time) and mean cage temperature, although no significant intercept, suggesting an isometric relationship between the two variables.

Applicability of gaping thresholds to reptilian thermoregulation

It is possible that the wide range in body masses (50–350 g) used in this study led to some variability in the data. We found, however, no significant trends with body mass on the ET_{50} estimates, which is consistent with Heatwole et al. (1973), who found no effect of size on the panting threshold in a related species, *Amphibolurus muricatus*. Another possible concern is that we were only measuring surface temperatures of the lizards rather than core T_b . Since core T_b (which should be the regulated variable) will always lag behind peripheral temperatures during changes in ambient temperature, the percentage time estimates for gaping might actually be underestimates since core temperature could still have been rising during our assessments of gaping behaviour. Thus, the ET_{50} values may be slight overestimates (since ET_{50} s are negatively correlated with total time spent gaping; see Fig. 7), although this effect should be consistent across all oxygen levels. The use of ambient temperature as the reference point rather than a particular surface temperature may have circumvented this problem. Furthermore, it could be argued that lizards utilise peripheral thermal information in a feed-forward fashion to predict changes in core T_b . If that were the case, the use of peripheral temperatures in our experiments might be more relevant in the assessment of gaping threshold.

During heating, the periphery would be the first to warm up and, considering the fact that ET_{50} s are always higher than preferred T_b values in normoxia (Fig. 7C,D), this suggests that the majority of gaping occurs at or above the normal T_{set} , acting as an important contributor to T_b regulation when ambient temperatures exceed T_{set} . The fact that, in normoxia, a regression of normoxic ET_{10} versus mean T_b in home cage yielded a significant regression suggests that the gaping thresholds determined here are meaningful values with respect to the individual lizard's normal preferred temperatures and that gaping is not simply a randomly displayed behavioural response.

One final concern was that at the highest temperatures of 38–40°C, lizard surfaces did not come fully into equilibrium with ambient temperature within the experimental time period. This suggests the efficacy with which bearded dragons can defend a lower T_b through increased evaporative cooling, since at 40°C lizards spent 100% of their time gaping. Thus, although the surface temperatures (and thus also T_b) were not in true equilibrium with ambient temperature, this would not have affected the ET_{50} estimates, since the maximum gaping efforts were already reached (i.e. values greater than 100% would not have been possible and thus the Hill equations would not have been affected).

Hypoxia reduces ET_{50} through a proportional regulator

The main hypothesis tested in this study, that hypoxia would lower the gaping threshold in a proportional fashion, was supported by the data. The central hypothalamic thermostat is thought to operate as a proportional controller (Mrosovsky, 1990). In other words, the further the regulated variable deviates from a set-point, the larger will be the corrective response. In this case, as lizards are warmed up, they gape for progressively longer periods of time. This regulator responds, as well, to magnitude; lizards in hypoxia had a greater tendency to exhibit the more pronounced Type II and Type III gaping at lower ambient temperatures than in normoxia (Fig. 4).

One interesting result from this study is that the three levels of O_2 yielded a proportionate reduction in the gaping threshold. Previous work examining the preferred T_b of hypoxic lizards seemed to indicate that a critical level of hypoxia lower than 10% O_2 was required before a proportionate drop in T_b would occur (Hicks and Wood, 1985). There are no data on the preferred temperatures of *P. vitticeps* in hypoxia; however, it is possible that the gaping threshold responds slightly differently to low oxygen than set-point driven behaviour that requires activity or movement (i.e. preferred temperature). Strict or 'precise' thermoregulatory behaviour has been thought only to occur in reptiles when inhabiting 'low-cost' environments with low risk of predation (Huey and Slatkin, 1976). If the costs for thermoregulating (e.g. locomotory costs or risk of predation) are too high, then precise thermoregulation will not occur. In this context, gaping or panting can be viewed as a low-cost strategy for thermoregulation, inasmuch as its instantaneous costs are low. Thus, it might be expected that the threshold for gaping could

more easily be sensitive and responsive to factors that alter set-point than a locomotory means of regulating T_b could.

Regional differences in surface temperatures

During the present experiments, lizards were gradually warmed up from a temperature of 30°C (their surface temperature in the early morning at the beginning of experiments) to a final temperature of 38 or 40°C (depending on O_2 level; see Materials and methods). The overall heating times between temperatures of 30–38°C were progressively longer in the hypoxic trials. This was probably due to their propensity to gape and exhibit cloacal discharge at lower thresholds, and hence the augmented evaporative cooling allowed for greater attempts to defend a lower core T_b at the higher ambient temperatures. Whether other thermoeffectors operate in a similar fashion remains to be shown. Despite the slightly different heating times, most surface temperatures were not affected by hypoxia, except for the tongue surface temperatures, where the changes that occurred strongly suggest that some internal temperatures (i.e. brain) may be differentially controlled in hypoxia.

Previously, Pough and McFarland (1976) showed that brain temperatures of lizards housed at temperatures greater than 40°C exhibit a substantial difference from the body, a response that was not observed in dead lizards held at similar temperatures. In extreme cases, lizard brain temperature can be up to 6°C lower than T_b (Crawford and Barber, 1974; Warburg, 1965). It is tempting to ascribe a physiological role (i.e. preferential blood flow that favours brain cooling at high ambient temperature) for this response, although one has yet to be shown. Since we were only examining surface temperatures, we cannot comment on brain temperature or its regulation. Previous work by Webb et al. (1972) showed significant body–head temperature differences in a wide variety of lizards. We did not see very large differences in the present study, although this could be because surface temperatures are not as informative as internal temperatures. Interestingly, a dragon lizard in the Webb et al. (1972) study exhibited panting at high temperatures (usually greater than 40°C), compared with *P. vitticeps* in the present study. This could have been related to the rapidly induced thermal changes, which is quite opposite to the present study. Our study was looking at steady-state changes, where lizards had time to equilibrate with their environmental temperatures, and, as such, we notice lizards gaping at much lower temperatures.

We used body–tongue temperature differences to estimate the degree of evaporative cooling. The difference between body surface temperature and tongue temperature increases at higher ambient temperatures, suggesting a greater degree of evaporative cooling at higher temperatures. It is also apparent that the continuous gaping that occurred in hypoxia, combined with a presumably higher ventilatory rate (i.e. an hypoxic ventilatory response), led to a higher degree of evaporative cooling in the hypoxic lizards across all the test temperatures. This is not surprising given that preservation of brain function would be more critically challenged under hypoxic conditions.

Small changes in brain temperatures have been shown to produce large changes in brain damage in either hypothermic or febrile animals (Herrmann et al., 2003; Kataoka and Yanase, 1998; Katz et al., 2004; Trescher et al., 1997); higher temperatures exacerbate excitotoxic damage, and lower temperatures lead to less damage.

Qualitative aspects of the gaping response

It is possible that using a non-radiant means for changing T_b is not the most appropriate for lizards that tend to bask in the sun. Indeed, Heatwole (1973) showed that thresholds for gaping were less variable in radiantly heated individuals than in non-radiantly heated individuals, even though gaping thresholds were not affected by the source of heat. Variability, however, did not appear to be a problem in our study due to the paired, repeated nature of the experimental design and the fact that we assessed percentage time spent gaping to estimate the ET_{50} threshold (Fig. 2). Previous studies have used the first attempt at gaping as the gaping threshold (Chong et al., 1973; Dupré et al., 1986; Heatwole et al., 1973; Parmenter and Heatwole, 1975). It is possible that assessing a simple value such as the initial gape has much more variability inherent in the estimate, particularly if the response is truly sigmoidal, as we have demonstrated. At the lower temperatures, lizards spend so little time gaping that there is little effect of increasing temperature on the time spent gaping. Furthermore, the magnitude of gaping is much less at lower temperatures (Fig. 4), begging the question of how much evaporative cooling occurs when lizards are using Type I gaping. Using an estimate such as the ET_{50} incorporates the entire response over a range of temperatures and allows for an objective estimate of the animal's overall gaping response to increasing temperature.

There was, however, a significant negative correlation between N and body size at 6% O_2 , suggesting that the smaller lizards exhibited a more typically on-off approach (i.e. a steep sigmoidal relationship between gaping time and ambient temperature) to gaping in severe hypoxia rather than a graded transition into increasingly more time spent gaping. This might reflect the fact that, as the smaller-sized lizards were undergoing more rapid changes in T_b , they responded with a more dramatic increase in time spent gaping as their T_b was raised above T_{set} and evaporative cooling mechanisms were required.

Effect of sex on gaping threshold

Few attempts have been made to examine the role of sex on normal thermoregulatory behaviours in reptiles outside of the breeding season (see Lailvaux et al., 2003). Even with pregnancy, there are inconsistent results regarding whether males and females exhibit consistently different preferred T_b . In one previous study, females had higher mean 24 h T_b than did males (Sievert and Hutchison, 1989), although the reverse or lack of difference occurs just as often, with field data often contradicting laboratory thermal gradient experiments (Lailvaux et al., 2003). Interestingly, we have shown that sex

had a significant effect on the gaping threshold (Table 1). Females consistently initiated gaping at a lower temperature than males, even during hypoxia. Previously, Heatwole et al. (1973) had shown that female Jacky dragons had a tendency toward lower (although not significant) gaping thresholds, a result consistent with the present study.

To the best of our knowledge, this is the first example of a sex difference related to a physiological response to hypoxia in a reptile. If a lowered set-point in hypoxia is truly adaptive, then it could be argued that all lizards, regardless of sex, should lower T_b and gaping threshold to an equal extent or as far as possible. The fact that the difference between males and females is retained in hypoxia suggests that sex differences in T_b regulation are of overriding importance. A corollary of the above is that females will presumably have greater tolerance to hypoxia if their lower gaping thresholds also translate into a lower overall T_b regulation. Wood and Stabenau (1998) showed that female rats exhibit a lower T_b in hypoxia, which helped translate into a greater tolerance to hypoxia. Survival times in hypoxic female rats were also significantly longer than in male rats. Whether the same sensitivities occur in reptiles is unknown.

Concluding remarks

It is apparent from the present study that bearded dragons make use of a subtle behavioural response to effect changes in T_b . As an ectotherm, they may not be able to artificially augment (for exceptions, see Tattersall et al., 2004) or decrease their overall T_b that far from ambient temperature; however, they can use behavioural responses to serve as brakes on thermal changes. The fact that gaping is proportionately controlled in both duration and magnitude with respect to both ambient temperature and oxygen strongly supports the importance of precise thermoregulatory control in as much as other physiological and behavioural constraints will allow. It remains to be shown exactly how effective gaping is at controlling T_b for prolonged periods, how often this behaviour occurs in the wild and whether it is routinely used in response to other stressors in addition to hypoxia. Further information on the altered gaping or panting thresholds in those species of lizards that do not routinely use panting as a 'low-cost' component to thermoregulation could be a fruitful avenue of future research.

We would like to thank Bill Mears for providing us with the bearded dragons, and acknowledge the excellent animal care provided by Dayle Belme, Nicole Handley, and Sara Stewart from Brock University. This project was funded by an NSERC operating grant to Glenn Tattersall and by an NSERC undergraduate research assistantship (USRA) to Rebecca Gerlach.

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