Level and Precision of Behavioural Thermoregulation in the Bearded Dragon,

Pogona vitticeps: Effects of Hypoxia and Environmental Thermal Quality.
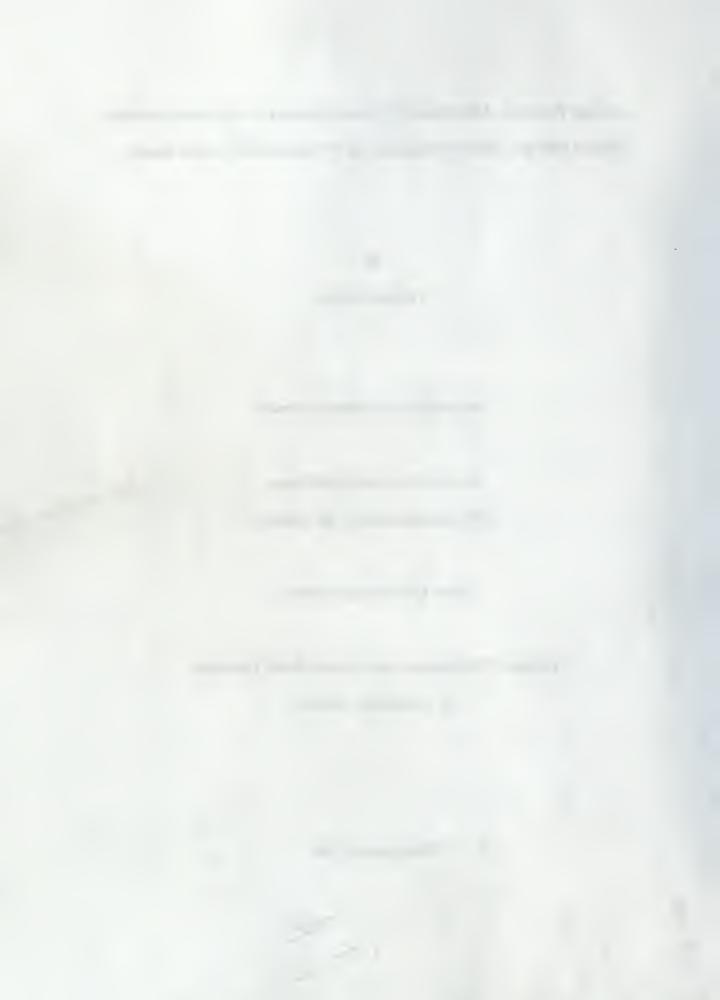

By

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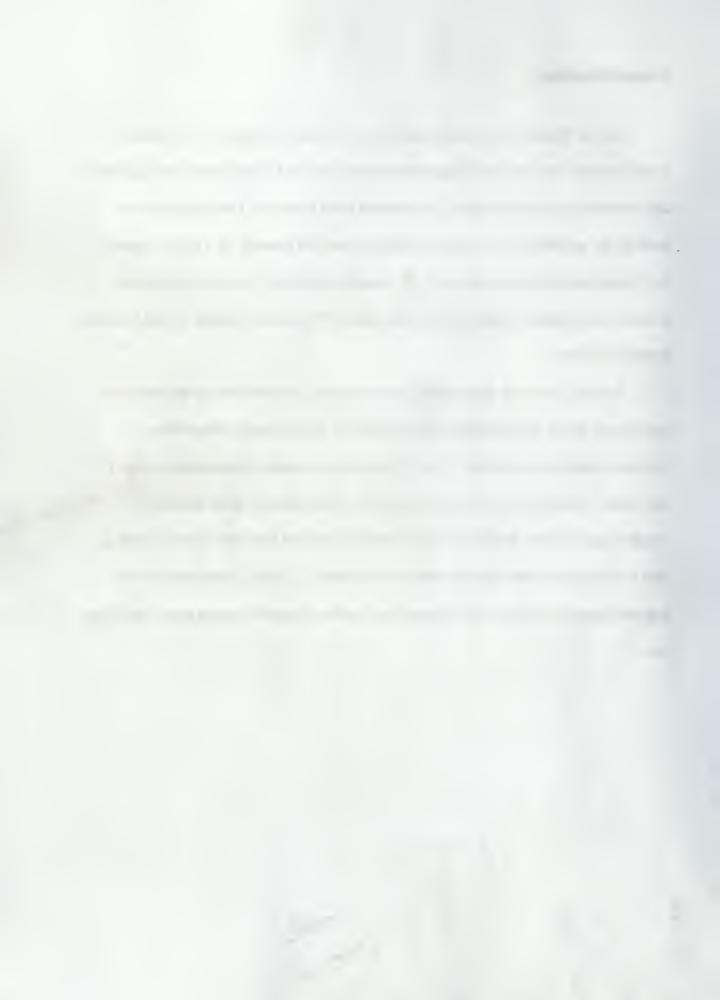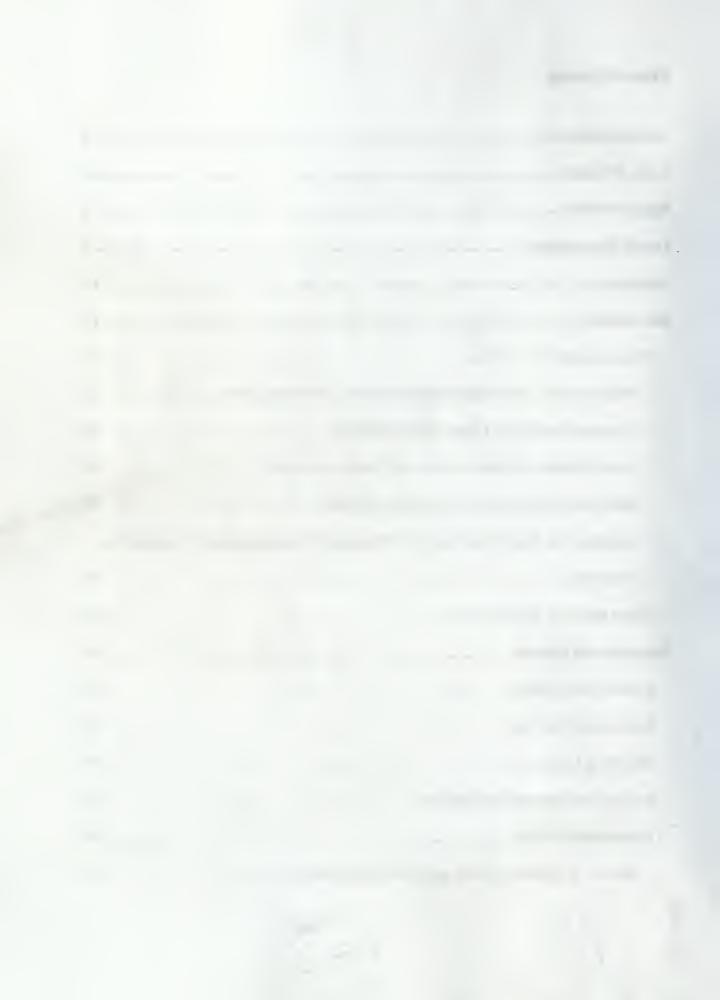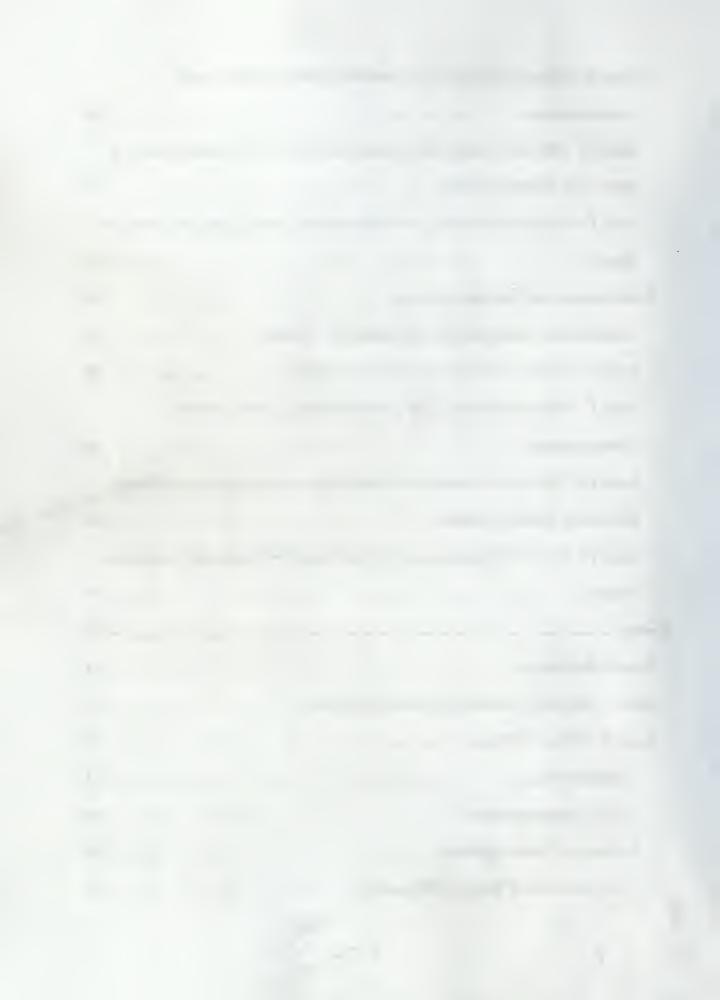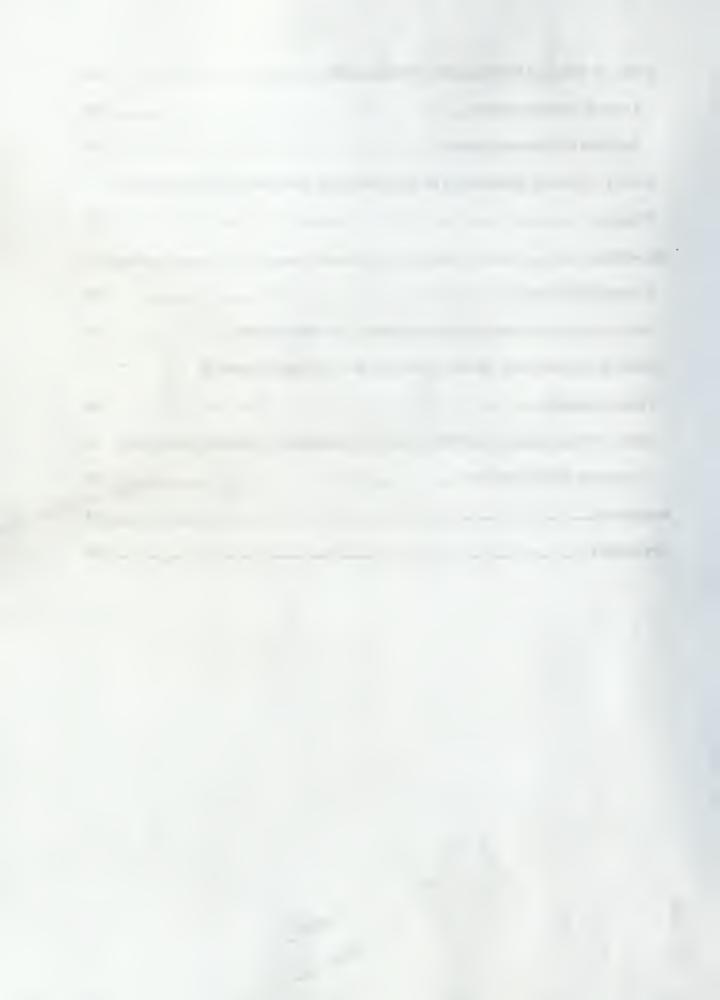
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CC: The cooling compartment of the electronic shuttle box used in this study.

ΔT_a: Ambient temperature range. The range of ambient temperatures within the central 68% of the ambient temperature distribution.

δT_a: Rates of temperature change in the electronic shuttle box. Temperature change rates of 0.7, 0.4 and 0.1°C/min were used in this study.

ΔT_b: Preferred body temperature range. The range of body temperatures within the central 68% of the body temperature distribution.

ES: "Exploratory Shuttling". Movement that occurred between the two compartments of the shuttle box, presumed to be unrelated to temperature regulation.

HC: The heating compartment of the electronic shuttle box used in this study.

HT_aL: The high limit of the ΔT_a – i.e. the upper limit of the range of ambient temperatures within the central 68% of the ambient temperature distribution.

HT_bL: The high limit of the ΔT_b – i.e. the upper limit of the range of body temperatures within the central 68% of the body temperature distribution.

LET_a: Lower escape ambient temperature. The ambient temperature at which a lizard exited the cold side of the shuttle box. Typically, median values of numerous exit events were used to define the LET_a.

LSP: Lower body temperature set-point. The lower tolerable body temperature also referred to as lower T_b threshold.

LT_aL: The low limit of the ΔT_a – i.e. the lower limit of the range of ambient temperatures within the central 68% of the ambient temperature distribution.

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LT$_b$L: The low limit of the ΔT_b – i.e. the lower limit of the range of body temperatures within the central 68% of the body temperature distribution.

RZ: Refractory zone. Range of body temperatures between the upper and lower T_b set-points (USP and LSP, respectively).

T$_b$: Internal body temperature

T$_a$: Ambient temperature; typically the ambient temperature the lizard was experiencing.

UET$_a$: Upper escape ambient temperature. The ambient temperature at which a lizard exited the hot side of the shuttle box. Typically, median values of numerous exit events were used to define the UET$_a$.

USP: Upper body temperature set-point. The highest tolerable body temperature also referred to as upper T_b threshold.

Abstract

Most metabolic functions are optimized within a narrow range of body temperatures, which is why thermoregulation is of great importance for the survival and overall fitness of an animal. It has been proposed that lizards will thermoregulate less precisely in low thermal quality environments, where the costs associated with thermoregulation are high; in the case of lizards, whose thermoregulation is mainly behavioural, the primary costs of thermoregulation are those derived from locomotion. Decreasing thermoregulatory precision in costly situations is a strategy that enhances fitness by allowing lizards to be more flexible to changing environmental conditions. It allows animals to maximize the benefits of maintaining a relatively high body temperature while minimizing energy expenditure. In situations where oxygen concentration is low, the costs of thermoregulation are relatively high (i.e. in relation to the amount of oxygen available for metabolic functions). As a result, it is likely that exposures to hypoxic conditions induce a decrease in the precision of thermoregulation. This study evaluated the effects of hypoxia and low environmental thermal quality, two energetically costly conditions, on the precision and level of thermoregulation in the bearded dragon, *Pogona vitticeps*, in an electronic temperature-choice shuttle box.

Four levels of hypoxia (10, 7, 5 and 4% O_2) were tested. Environmental thermal quality was manipulated by varying the rate of temperature change (δT_a) in an electronic temperature-choice shuttle box. Higher δT_a's translate into more thermally challenging environments, since under these conditions the animals are forced to move a greater number of times (and hence invest more energy in locomotion) to maintain similar temperatures than at lower δT_a's. In addition, lizards were tested in an "extreme

temperatures" treatment during which air temperatures of the hot and cold compartments of the shuttle box were maintained at a constant 50 and 15 °C respectively. This was considered the most thermally challenging environment. The selected ambient (T_a) and internal body temperatures (T_b) of bearded dragons, as well as the thermoregulatory precision (measured by the central 68% of the T_a and T_b distribution) were evaluated. The thermoregulatory response was similar to both conditions. A significant increase in the size of the T_b range, reflecting a decrease in thermoregulatory precision, and a drop in preferred body temperature of ~2 °C, were observed at both 4% oxygen and at the environment of lowest thermal quality. The present study suggests that in energetically costly situations, such as the ones tested in this study, the bearded dragon reduces energy expenditure by decreasing preferred body temperature and minimizing locomotion, at the expense of precise behavioural thermoregulation. The close similarity of the behavioural thermoregulatory response to two very different stimuli suggests a possible common mechanism and neuronal pathway to the thermoregulatory response.

Introduction

Thermoregulation in Reptiles

Most biochemical and physiological functions are optimal within a narrow range of body temperatures (T_b) (Hutchison and Dupré, 1992; Peterson *et al.*, 1993). Physiological, behavioural and ecological processes such as the ability of reptiles to avoid predators (Christian and Tracy, 1981), feeding behaviour (Vandamme *et al.*, 1991) and embryonic development (Blumberg *et al.*, 2002) have been demonstrated to exhibit strong temperature dependency. As a result, the regulation of body temperature has direct implications on the survival and overall fitness of an animal. This is particularly important in ectothermic animals, such as reptiles, in which T_b is primarily determined by behaviour (see section below on *Behavioural and Physiological Mechanisms of Thermoregulation*). Only an animal that is capable to maintain its T_b at or near the "optimal physiological temperature" (i.e. The T_b at which most physiological functions are optimal) will be able to achieve maximum performance.

Behavioural and Physiological Mechanisms of Thermoregulation

Unlike endotherms (i.e. mammals and birds), ectotherms do not generally rely on metabolically generated heat to maintain a high body temperature. Instead, ectotherms rely primarily on external heat sources (hence the name *ecto*therm) such as radiation from the sun or conductive heat transfer from the substrate to regulate their temperature. Despite being ectothermic, reptiles are capable of regulating their T_b within a narrow range, where 'regulation' refers to the homeostatic potential for maintaining T_b, rather than the potential

to generate internal body heat. The regulation of a relatively constant body temperature, typically higher than that of their surrounding environment, is made possible by a series of behavioural and physiological adaptations that exploit temporal and spatial gradients in thermal variability (Cowles and Bogert, 1944; Bennett and Ruben, 1979; reviewed in Seebacher and Franklin, 2005).

One of the most important mechanisms for reptile thermoregulation is the careful selection of thermally favourable habitats (both at the micro-habitat and macro-habitat scales) (Cowles and Bogert, 1944; Row and Blouin-Demers, 2006). Other common mechanisms are the regulation of activity times (i.e. times of day in which the animals are active) (Cowles and Bogert, 1944) and changes in body posture and orientation in relation to the sun. These postural changes enable them to modify the amount of body surface exposed to heat sources (i.e. heated substrates and the sun) allowing the animal to maximize heat absorption (Schmidt-Nielsen, 1964). However, the most effective thermoregulatory behaviour used by reptiles in the wild is moving between sun and shade. This movement is referred to as shuttling behaviour. Diurnal reptiles emerge from their burrows in the morning and spend long periods of time basking in the sun. Once they have achieved their preferred T_b they start shuttling between sunny and shaded areas to maintain their T_b within a narrow range (Schmidt-Nielsen, 1964). While not as effective as shuttling, panting is also an important thermoregulatory strategy available to some species of reptiles. By means of evaporative cooling, panting reduces head temperature (Crawford *et al.*, 1977). This allows animals to bask for longer periods of time and thus to achieve preferred T_b levels while preventing the brain from reaching lethal temperatures (Crawford *et al.*, 1977; Spotila *et al.*, 1977; Hertz *et al.*, 1993; Tattersall *et al.*, 2006). Due to the

importance of the brain as the control center for autonomic and behavioural processes, the maintenance of a constant head temperature is of utmost importance. Indeed, the presence of highly temperature sensitive neurons in the hypothalamus allows reptiles to regulate brain temperature more tightly than body temperature. As a result, reptiles often exhibit head temperatures higher than body temperatures at low ambient temperatures (T_a), whereas maintaining cooler brain temperatures at high T_a conditions (reviewed in Tattersall *et al.*, 2006).

Although behavioural mechanisms such as basking or shuttling between sun and shade are the primary determinants of T_b in reptiles, physiological mechanisms are also important components of reptilian thermoregulation. These mechanisms act as modulators of T_b, increasing the efficacy of the behavioural response. The most important of these mechanisms is the capability of reptiles to control the rates of heating and cooling through a series of cardiovascular adjustments and alterations in peripheral blood flow (Cowles, 1958; Bartholomew, 1963). The increase in peripheral blood flow (by cutaneous vasodilation) and heart rate exhibited by reptiles during heating promotes heat transfer between the animal and its surrounding environment allowing it to heat faster (Seebacher, 2000). Conversely, reptiles reduce the perfusion of blood to the periphery and their heart rate during cooling, thus decreasing the rate of heat loss (Bartholomew, 1963; Dzialowski and O'Connor, 2001). This difference in heart rate during heating and cooling is known as heart rate hysteresis and is thought to be of functional significance. It allows reptiles in heterothermic habitats to maintain their temperature within their preferred range for longer periods of time, keeping energetically expensive or potentially dangerous (in terms of

predation risk) thermoregulatory mechanisms (i.e. shuttling and basking) to a minimum (Bartholomew, 1963; Heath, 1970; Seebacher, 2000; Seebacher and Grigg, 2001).

Level and Precision of Lizard Thermoregulation

When studying thermoregulation in reptiles (and ectotherms in general), it is useful to evaluate the following two principal aspects to obtain a complete depiction of the animal's thermoregulatory behaviour; level and precision: The "level" of thermoregulation refers to the preferred T_b of an animal either in the field or in a laboratory setting. Under ideal laboratory conditions this temperature is thought to be at or near the optimal physiological temperature. The level of thermoregulation is generally expressed as either the mean or the median of all observed T_b's during a determined time period. The second of these parameters deals with the variability of T_b across time and is referred to as the "precision" of thermoregulation. It is commonly measured as the variance, the standard deviation or the preferred T_b range. This last one is described as the range of temperatures within which a certain percentage of the observed T_b's are found (Dewitt, 1967).

Huey and Slatkin (1976) proposed a mathematical cost-benefit model for thermoregulation in lizards. The model predicts that lizards will only thermoregulate (i.e. exhibit behaviours consistent with an attempt to maintain near constant T_b) if the costs associated with this behaviour are lower than the benefits obtained from it. In an ideal environment, a lizard is assumed to thermoregulate at a temperature in which its ability to gather and process energy is optimal. This temperature is often called the physiological "optimal temperature" (Huey and Slatkin, 1976). In the field, however, lizards encounter a number of contingencies that can make thermoregulation expensive. When shuttling, for

example, lizards expend energy and time on locomotion and expose themselves to potential predation. Thermoregulatory behaviour can also interfere with social, antipredatory, territorial or feeding activities (Dewitt, 1967; Huey and Slatkin, 1976). In a series of field and laboratory experiments, Dewitt (1967) found that the level and precision of thermoregulation of the lizard *Dipsosaurus dorsalis* is influenced by both physical and biological factors. The temperature distribution in the environment was the most important physical factor influencing the thermoregulation of *D. dorsalis*, while fights between individuals and the presence of predators were the most significant biological factors affecting this species behavioural thermoregulation. Territorial fights involve long periods of sun exposure causing T_b to rise above preferred temperatures, while the presence of predators forces the lizards to remain motionless in order to remain undetected (Dewitt, 1967). Dewitt (1967) reported unusual increases in the T_b's of *D. dorsalis* (46.5 °C vs. an upper threshold of 38.5 °C in a thermal gradient) associated with the nearby presence of an observer (i.e. potential predator).

If thermoregulation were to be "too precise" around one specific T_b, the animal would be an excellent thermoregulator but it would not have time to engage in other activities. In this sense, careful or overly precise thermoregulation can sometimes be maladaptative (Huey, 1974), and would be expected to be selected against. As a result, T_b and the mechanisms by which lizards achieve it, should be a compromise between the optimal physiological temperature and ecological reality of the animal. The cost-benefit model of Huey and Slatkin (1976) proposes that the costs of thermoregulation should be subtracted from its gross physiological benefits to obtain the net benefits of maintaining a particular T_b.

Huey (1974) measured the costs of thermoregulation in the Puerto Rican lizard, *Anolis cristatellus*, in terms of availability and distance of basking sites. He compared the thermoregulatory strategies of lizards living in an open habitat versus lizards living in an adjacent forest. Because the amount of basking sites and the proximity between them was significantly lower in the forest than in the open habitat, the amount of energy and time lizards must invest in thermoregulatory activities is increased in the forest habitat. As a consequence, lizards in the forest thermoregulate less carefully, tolerating lower and more variable T_b's than lizards in the open habitat (Huey, 1974). Similar results have been found in laboratory conditions where the level (i.e. the T_b at which a lizard regulates) and precision (i.e. how much T_b deviates from the preferred T_b; T_b variance or T_b range) of thermoregulation is decreased at low thermal quality environments (Campbell, 1985; Withers and Campbell, 1985).

Other factors such as dehydration and low water availability in the environment also have an effect on the thermoregulation of reptiles (Lorenzon *et al.*, 1999; Ladyman and Bradshaw, 2003) and should be considered as part of the costs of maintaining an "optimal physiological" T_b. Ladyman and Bradshaw (2003) studied the influence of hydration state on the temperature selection of two different populations of tiger snakes; one population from a semi-arid environment and the other population from a wetland. They found that dehydrated snakes select significantly lower temperatures in a photo-thermal gradient than hydrated ones and that snakes from the semi-arid population exhibit a greater decrease in T_b when dehydrated, than wetland snakes. Since a decrease in temperature can reduce water loss, the more pronounced effect of dehydration on temperature selection observed in the semi-arid snake population suggests that their survival is associated with thermoregulatory

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adaptations that allow them to conserve water in habitats where this is a very limited resource (Ladyman and Bradshaw, 2003).

The nature of the costs and benefits of thermoregulation is diverse and can be measured in terms of energetics, such as oxygen consumption, water loss, calories expended or time employed, or through non-energetic, but "life-or-death" responses, such as predation risk. Because the costs of thermoregulation can be high, temperature selection in a reptile is a trade-off between many variables such as reproductive status, costs of locomotion, food and water intake, oxygen concentration, phylogeny and morphology to name a few. In order to optimize the net benefits of thermoregulation, animals in the field are expected to balance the costs and benefits of maintaining a certain body temperature and adjust their thermoregulatory behaviour according to this balance. In laboratory conditions, the costs of thermoregulation are almost entirely those dictated by the experimental methodology. Animals would be expected to thermoregulate accordingly, exhibiting more precise thermoregulation in experimental setups which require less "thermoregulatory effort" (i.e. energy expenditure) to maintain a certain T_b.

Control Models of Lizard Behavioural Thermoregulation

Behavioural thermoregulatory mechanisms are thought to operate under two principal control systems: proportional control and on-off control (Heath, 1970). Proportional control systems are mainly used to finely tune T_b regulation. As the name implies, in proportional control systems the extent of the behavioural thermoregulatory response is proportional to the deviation of T_b from the preferred T_b (i.e. optimal physiological temperature) (Heath, 1970). An example of a proportionally controlled

behaviour is gaping (Tattersall and Gerlach, 2005). Bearded dragons, for example, exhibit a wider gape at higher temperatures than they do at lower temperatures and the amount of time the lizard maintains its mouth open is proportional to the thermal stimulus (Tattersall and Gerlach, 2005). Other behaviours such as postural changes or body orientation with respect to the sun are also examples of proportionally controlled behaviours (Heath, 1970).

On-off control systems are present in behavioural responses where there is an "all or none response". Many behavioural responses include two or more on-off type control behaviours that are coupled together to maintain a narrow T_b range (Heath, 1970). An example of such type of control is the shuttling thermoregulatory behaviour. During shuttling, lizards expose themselves to direct sunlight until they reach an upper T_b threshold or set-point (USP). Once this happens, lizards seek shade and cool down until T_b has dropped to a lower set-point (LSP). Shuttling is an on-off control type system because the behaviour evoked is either "move to the sun" or "move to the shade" without an intermediate type of behaviour (Heath, 1970).

As a consequence of the above, reptiles do not usually thermoregulate around one specific body temperature; they are thought to thermoregulate in a manner that is consistent with a dual set-point thermoregulatory mechanism (Barber and Crawford, 1977). A reptile will avoid its T_b to rise above an USP, as well as allowing T_b to fall below a LSP (by shuttling between sun and shade) rather than defending a particular optimal T_b (Figure 1). When body temperature is between the USP and the LSP, the animal is relatively unresponsive to thermal stimuli only using comparatively inexpensive (in terms of time and energy expenditure) thermoregulatory mechanisms such as panting or cardiovascular adjustments. The range of temperatures between the upper and lower set-point is referred

Figure 1. Thermoregulatory shuttling behaviour in a lizard. During the activity period a lizard will shuttle back and forth between sun and shade in an effort to maintain a relatively constant body temperature (T_b). In order to warm up, the lizard exposes itself to direct sunlight until T_b reaches a high threshold or upper set-point (USP). Once this happens, the lizard seeks shade and cools down until T_b has dropped to a lower set-point (LSP). The T_b zone between the USP and the LST is the refractory zone (RZ) in which the lizard can carry out other activities without the need to engage in thermoregulatory behaviour. (Modified from Heath, 1970).

to as the "refractory zone" and it enables the animal to carry out other activities such as predator avoidance, courtship, territorial defence or foraging, without the constraints of continuous thermoregulatory behaviour (Heath, 1970). This strategy allows them to be more flexible to environmental conditions, minimizes the energetic requirements associated with a precisely regulated T_b, and hence, enhances their overall fitness.

The T_b set-point is a theoretical concept that implies the existence of a reference value to which the actual T_b (i.e., of the body, brain, or periphery) can be compared. If a deviation from this value or set-point occurs, one or more thermoregulatory responses are evoked in the opposite direction to the deviation (i.e. "feedback"), to return T_b to the set-point value (Figure 2) (Hammel, 1965 reviewed in Mitchell *et al.*, 1970; Hammel, 1972; reviewed in Mrosovsky, 1990). Whether the set-point is a reality or only an analogy for the control of T_b is still a matter of debate. Mitchell *et al* (1970) proposed a similar control system without a reference value or set-point. In this type of control T_b is the result of a balance in the activity between hot and cold sensors (i.e. thermosensitive neurons). In other words, a decrease in T_b increases the activity of cold sensing neurons and decreases the activity of neurons sensitive to warm temperatures. The feedback from these sensors is then compared centrally, generating an "unbalanced signal". Because the signal indicates dominance in the activity of the cold sensitive neurons, a thermoregulatory response is activated to correct for the imbalance (Figure 3). The opposite occurs when the activity of the warm sensitive neurons predominates. A similar balance model describes T_b as the result of the balance between active and passive processes in any given response (Romanovsky, 2004). The implication in this model is that the regulated value of T_b depends on the particular conditions experienced by the animal at any given time. All of

Figure 2. Schematic diagram of a negative feedback control system of temperature regulation. The controlled system (i.e. body of the animal) is subjected to a disturbance (e.g. environmental temperature) which causes a change in the controlled variable body temperature (T_b). This departure of T_b from the set-point is detected by feedback elements (i.e. peripheral and internal temperature sensors) and a feedback signal is transmitted and compared to the reference value (set-point). Any departure of T_b from the reference is corrected by the control elements which activate one or more control actions (i.e. thermoregulatory responses; behavioural and/or physiological). The thermoregulatory response evoked by the initial disturbance is opposite and proportional to the magnitude of the deviation of T_b from the set-point. (Modified from Hammel, 1965, reviewed in Mitchell *et al.*, 1970)

Figure 3. Schematic diagram of a negative feedback control system without a set-point. The controlled system (i.e. body of the animal) is subjected to a disturbance (e.g. environmental temperature) which causes a change in the controlled variable body temperature (T_b). This increases the activity of one of the two types of feedback elements (warm or cold sensitive neurons) while causing a decrease in the activity of the other. The feedback signals are transmitted and compared. Any imbalance in cold vs. hot sensor activity is corrected by the control elements which activate one or more control actions (i.e. thermoregulatory responses; behavioural and/or physiological). The thermoregulatory response evoked by the initial disturbance is opposite and proportional to the magnitude of the imbalance. (Mitchell *et al.*, 1970).

these neuronal models fit well into the concept of rheostasis, proposed by Mrosovsky (1990). The term rheostasis is described as a "condition or state in which, at any one instant, homeostatic defences are still present but over a span of time there is a change in the regulated level". This concept is useful in the study of temperature regulation as it takes into account changes in set-point (whether real or analogical) as the normal response to certain environmental conditions. For example, it has been shown that rodents regulate their T_b at a lower level when exposed to toxins; this is believed to be a normal response that protects tissues against toxic insult (Gordon, 1988). Similarly, many vertebrates decrease their T_b when exposed to low oxygen concentrations, as a protective mechanism from tissue damage due to oxygen depletion (Hicks and Wang, 2004).

The regulation of temperature in vertebrates is controlled by neural centers located in the hypothalamus (Cabanac *et al.*, 1967; Hammel *et al.*, 1967; Myhre and Hammel, 1969; Berk and Heath, 1975a; Berk and Heath, 1975b). Numerous studies have shown that manipulation of hypothalamic temperatures affects preferred body temperatures of reptiles. Heating the hypothalamus induces blue-tongued lizards to exit the hot environment of a shuttle box at colonic temperatures 1 to 2 °C lower than control lizards, while cooling of the hypothalamus elicits 1 to 2 °C higher exit colonic temperatures from the hot side of a shuttle box (Hammel *et al.*, 1967; Myhre and Hammel, 1969). Similar findings have been observed in the lizard *Dipsosaurus dorsalis* in which lesions in the nucleus of the anterior hypothalamus cause a significant reduction in the mean exit body temperature when shuttling from the cold to the hot side of a shuttle box, demonstrating once again the importance of hypothalamic centres in the control of reptilian behavioural thermoregulatory responses (Berk and Heath, 1975b).

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Warm and cold sensitive neurons similar to those found in endothermic species, have been found in the anterior hypothalamus of reptiles (Cabanac *et al.*, 1967). In the case of reptiles, the hypothalamic centers receive photic information from the environment by the pineal complex (consisting of the parietal and pineal organs) and the lateral eyes (Firth and Heatwole, 1976; Firth, 1979). This has been demonstrated by experiments in which removal of the parietal and/or the pineal organ resulted in a decrease in the panting threshold of *Amphibolorus muricatus* (Firth and Heatwole, 1976). In species such as geckos which do not posses a parietal organ, the function of the parietal organ is replaced by the lateral eyes (Firth, 1979).

Influences of Hypoxia on Temperature Regulation

Oxygen is essential for the proper physiological functioning of aerobic organisms. In environments where oxygen concentration is low, most animals decrease their T_b (Wood, 1995). The ubiquity of this thermoregulatory response to hypoxia across protozoa and metazoa has been confirmed by its presence in a wide variety of organisms among which *Paramecium caudatum*, fish, lizards, birds and mammals are included (Kadono and Besch, 1974; Hicks and Wood, 1985; Schurmann *et al.*, 1991; Dupré and Owen, 1992; Malvin and Wood, 1992). The effect of oxygen concentration in the air on lizard thermoregulation has been extensively studied and is known to have a significant lowering effect on the preferred T_b (Hicks and Wood, 1985; Petersen *et al.*, 2003; Tattersall and Gerlach, 2005). Similar thermoregulatory responses have been observed in situations such as anaemia and exhaustive exercise, both conditions where oxygen content in the blood is low (Hicks and Wood, 1985; Petersen *et al.*, 2003).

The hypoxic induced decrease in T_b protects organisms from oxygen depletion, particularly in life sustaining organs that are typically more sensitive to changes in O_2 concentrations, such as the heart or the brain. This fall in T_b can potentially reduce oxygen demands by up to 50% (Hicks and Wood, 1985) through a combination of lowered metabolic rate via Q_{10} effects (i.e. the effect of temperature on the rate of biochemical reaction, with increasing rates at higher temperatures) (Wood and Gonzales, 1996), an accompanying reduction in ventilatory costs, and an increase in the oxygen loading capacity of the lungs. The protective effect of the thermoregulatory response to hypoxia has been confirmed in mammals, lizards and even protozoa. Hicks and Wood (1985) observed a 100% survival of lizards when allowed to decrease their T_b during hypoxia, compared to 100% mortality of those animals prevented from cooling down. Survival of hypoxic paramecia has also been shown to increase significantly at lower temperatures (Malvin and Wood, 1992) as well as that of anaemic rabbits (Gollan and Aono, 1973) and newborn mammals (reviewed in Mortola, 1999). A regulated decrease in T_b has also been shown to protect the brain from the oxidative stress that normally occurs during reperfusion from hypoxic ischemia (Katz *et al.*, 2004).

The T_b reduction observed in animals as a response to hypoxic exposure is achieved through both behavioural and physiological means. Although behavioural mechanisms play an important role in the hypoxic thermoregulatory response in mammals (Dupré and Owen, 1992), the mechanisms used by these animals are primarily physiological. These animals decrease heat production by inhibiting normal responses to possible hypothermia such as shivering and non-shivering thermogenesis (Dupré *et al.*, 1988) and by increasing heat loss by redirecting blood flow to the periphery (Tattersall and Milsom, 2003).

Ectotherms, such as lizards, use behavioural mechanisms as their main means of reducing T_b. These include cold seeking behaviour and a decrease in the gaping threshold when exposed to hypoxic environments (Hicks and Wood, 1985; Dupré *et al.*, 1986; Tattersall and Gerlach, 2005). Although behavioural mechanisms are the direct determinants of an ectotherm's T_b, the physiological mechanisms help enhance the decrease in T_b observed during exposures to hypoxic environments. In normoxia, the presence of a hysteresis of the heating and cooling curves (for a detailed description see section on *Behavioural and Physiological Mechanisms of Thermoregulation* above) allows lizards to maintain a high body temperature for longer periods of time in thermally variable environments. This thermal hysteresis is also abolished and is even reversed with exposures to extreme levels of hypoxia, such that under these conditions lizards heat more slowly than they cool (Hicks and Wood, 1985; M. Skinner, *pers. comm.*). This suggests that multiple thermoeffectors are coordinated to achieve the lower T_b's observed in hypoxic reptiles.

The decrease in T_b in response to hypoxia (and other stimuli such as toxins or dehydration) has received a number of names. Gordon *et al.* (1988) proposed the term "regulated hypothermia" to describe what he postulates is a regulated decrease of T_b observed in rodents exposed to toxins. This term was used to distinguish a regulated decrease in T_b from a pathological hypothermia in which the decrease in T_b occurs as a result of an inability of the animal to maintain a higher T_b. In 1987 (and in a later edition in 2003) the IUPS Commission for Thermal Physiology proposed the term anapyrexia and defined it as a "pathological condition in which there is a regulated decrease in core temperature. Anapyrexia is distinct from hypothermia in that thermoregulatory responses

indicate a defence of the anapyretic level of core temperature". Both of these terms imply a pathological but regulated and defended decrease in T_b (a paradoxical definition in itself).

Given that the decrease in T_b observed as a response to stressors (such as hypoxia and intoxication) has been demonstrated to have a protective effect, the pathological implications of the terms described above result in an inaccurate description of the thermoregulatory response. To describe more accurately the decrease in T_b observed as a response of a stressor stimulus (whether it be low O_2 concentrations, toxicity, water availability, etc.) I adopt here the term cryexia. Although this term was proposed by Lagerspetz and Väätäinen (1987) to describe the regulated decrease in T_b observed in young mice when infected with bacteria, the word cryexia *per se* (from Greek *kruos*, frost), does not possess a pathological or regulatory connotation (therefore not excluding the possibility of an non-regulated component of the thermoregulatory response) and therefore it is an unbiased description of the thermoregulatory response. Furthermore, the terms "regulated hypothermia" and anapyrexia suggest a tightly regulated decrease in T_b set-point. However, data from studies of thermoregulation in mammals suggest that the precision of T_b control in hypoxia may be lower than that observed in normoxia (Dupré *et al.*, 1988; Barros *et al.*, 2001), implying that cryexia more accurately describes the response.

Using an ectotherm model to study the effect of hypoxia on thermoregulation has the obvious advantage of eliminating the factor of metabolically generated heat that occurs in endothermic species. This means that T_b is primarily dictated by behavioural mechanisms that can be easily assessed in laboratory settings (see section below on *Laboratory Methods for the Study of Behavioural Thermoregulation in Ectothermic*

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Vertebrates) without the confounding factors of metabolic heat production, a process which is oxygen-dependent. By studying behavioural thermoregulation of an ectothermic animal it is possible to determine the effect of hypoxia on actual selected or "preferred T_b" (i.e. changes in set-point), and thus shed light on the question: is the lowering of T_b observed under hypoxic conditions the result of a regulated decrease in T_b (as has been extensively suggested), or of a pathological response (reflected as a lack in the precision of thermoregulation) or of both?

Laboratory Methods for the Study of Behavioural Thermoregulation in Ectothermic Vertebrates

One of the most popular techniques used in laboratories to determine the thermoregulatory preferences of reptiles is the shuttle box. In a typical shuttle box experiment, the animal is placed inside a chamber where it is given two different ambient temperature (T_a) choices (hot and cold). To maintain T_b within its preferred range, the animal needs to shuttle back and forth between the two chambers. Shuttle box experiments intend to mimic shuttling behavioural thermoregulation in the field, thus reinforcing the on-off notion of reptile thermoregulation discussed earlier in the section *Control Models of Lizard Behavioural Thermoregulation.* Shuttle boxes allow researchers to manipulate different conditions of an animal's environment while evaluating modifications to their thermoregulatory behaviour. Furthermore, they allow one to measure these responses repeatedly throughout a trial, thus providing useful and accurate information on upper and lower temperature thresholds (i.e. set-points) of individual species as well as preferred T_b.

In the shuttle boxes traditionally used in reptile behavioural thermoregulation studies, a choice of two extreme temperatures is given to the animals (Myhre and Hammel, 1969; Berk and Heath, 1975a; Berk and Heath, 1975b; Hicks and Wood, 1985; Blumberg *et al.*, 2002). A problem with this design is that the hot and cold compartments need to be well above and below the animal's normal thermoregulatory zone, and thus movement in and out of the compartments may represent distress responses rather than thermoregulatory ones. A second, purely experimental problem is that animals will often elect to situate themselves half-way between the two sides, exhibiting a stable T_b typically halfway between the two extremes, but with no behavioural indicators (i.e. no movements, escape responses) of thermoregulation. Since an implicit assumption of any assessment of thermal preference is that the thermal location an animal 'chooses' is actually that which it prefers, and would return to if disturbed, static shuttle boxes do not easily allow for one to distinguish this response. Furthermore, since the motivational state of an animal cannot be known, but only inferred, static or extreme temperature shuttle boxes may not be reliable indicators of a preferred or regulated T_b. The actual T_b recorded may thus be the result of coincidence rather than preference, making it difficult for analysis of thermoregulation. A further disadvantage with the static shuttle box is that the role of peripheral or external temperature sensations of the lizard cannot be inferred since the animal essentially escapes the hot side or cold side at fixed ambient temperatures (T_a's).

A more complex type of shuttle box design is the electronically controlled shuttle box, commonly used in fish studies (McCauley, 1977; Reynolds and Casterlin, 1979; Schurmann *et al.*, 1991; Staaks *et al.*, 1999; Petersen and Steffensen, 2003). These shuttle boxes develop a temporal and spatial temperature gradient controlled by the operant

responses of the animal. The temperature of the environment (i.e. water, in the case of fish) is controlled by movements of the animal between chambers and a constant temperature differential between the two chambers establishes a spatial gradient that guides the movements of the animals to preferred temperatures (Neill *et al.*, 1972); thus, by shuttling back and forth between the two chambers, the animal is able to control T_a and thus its own T_b. As opposed to the extreme fixed temperatures shuttle boxes described above, the slow changes in temperature characteristic of the electronic shuttle box provide the animals with a thermal environment that more closely resembles that encountered in nature. Another advantage of this system over the traditional ones is that it allows for the determination of the upper and lower escape ambient temperatures (ambient temperature at which the animal exits the hot or cold chamber of a shuttle box; UET_a and LET_a respectively) along with core body temperature allowing for the determination of the possible role of peripheral thermal sensors (assessed via the ambient escape temperatures) in central thermoregulatory control.

Another experimental mechanism widely used in the study of thermoregulation in ectotherms is the linear thermal gradient (Hicks and Wood, 1985; Arad *et al.*, 1989; Jarling *et al.*, 1989; Branco and Steiner, 1999; Branco *et al.*, 2000; Bennett, 2004). Thermal gradients allow the animals to position themselves at a "comfortable" environmental temperature within the gradient. The preferred T_b of the animal is determined by some measure of central tendency (i.e. mode, median, mean) of the T_b measurements obtained over time. Other devices such as circular, temporal or vertical gradients are modifications of this basic linear gradient design.

In contrast to shuttle boxes, temperature profiles of thermal gradients are static in nature and do not allow for testing of upper and lower temperature thresholds. T_a selection

is also often inferred from animal location within the gradient, which can be complicated if the animal in question is physically large enough to be straddling a range of possible T_a's, rather than one fixed T_a. Furthermore, the existence of a refractory zone in ectothermic animals (see section on *Control Models of Lizard Behavioural Thermoregulation* above) allows the animal to place itself anywhere within this zone inside the temperature gradient without the need to change positions or make any corrections to its T_b. If this is the case, the resulting estimated preferred T_b could then be biased to a low or high value, making it a less robust method for the evaluation of T_b regulation.

As in the static shuttle box, motivational state of the animal cannot be fully known in the thermal gradient, and a simple recording of a stationary lizard at a particular part of the thermal gradient cannot be taken as proof of a preferred temperature. Instead, the ultimate location that the lizard exhibits may reflect an active 'choice' not to thermoregulate due to altered motivational states. On the other hand, the regulation of a constant body temperature in a thermal gradient device requires very little energy expenditure (when compared to a shuttle box, which requires regular locomotory efforts). This lower cost of behavioural thermoregulation should probably be reflected in a higher precision of the regulation of T_b (Huey and Slatkin, 1976). Solving these potential dilemmas can likely only be achieved through differing thermal qualities of the choice chamber (i.e. changing the metabolic requirement of behavioural thermoregulation inside the chamber by, for example, changing the rates of temperature change in an electronic shuttle box) in order to 'dissect' out the role of thermoregulatory drive from other spontaneous behaviours.

Thesis Rationale and Objectives

Lizards use a wide array of behavioural and physiological adaptations to maintain their T_b within the small range in which most physiological functions are optimal. This range has been studied extensively and is commonly referred to as the "preferred T_b range" (Heath, 1970; Barber and Crawford, 1977). The capability of reptiles to maintain their T_b within this range has direct implications on their fitness which is why thermoregulation is of chief importance in the life history of an animal.

Every thermoregulatory act has potential costs associated with it and these reduce the final potential physiological gains. The costs of thermoregulation are varied and can depend on factors such as thermal quality of the habitat (i.e. availability of basking spots), the presence of predators or water availability (Huey and Slatkin, 1976; Ladyman and Bradshaw, 2003). To maximize the net benefits of thermoregulation, animals should carefully weigh the costs and benefits of maintaining a certain body temperature and adjust their thermoregulatory behaviour according to the magnitude and direction of the net effect (Huey, 1974; Huey and Slatkin, 1976). If the costs associated with thermoregulation are high, less precise thermoregulation is expected. This should be reflected in a widening of the selected temperature range.

At low oxygen concentrations, lizards significantly lower their preferred T_b (Hicks and Wood, 1985). This deviation from the presumed optimal T_b in normoxia is thought to protect organs from oxygen depletion, and thus could be considered a new, albeit lower, optimal T_b for hypoxic stress. A lower T_b reduces ventilatory costs, induces an increase in the oxygen loading capacity in the lungs, and lowers metabolic rate which in turn leads to a decrease in oxygen consumption (Wood and Gonzales, 1996). This thermoregulatory

adaptation to hypoxia has been described in a wide array of organisms across the animal kingdom and is even present in species that do not experience hypoxia in nature (Kadono and Besch, 1974; Hicks and Wood, 1985; Schurmann *et al.*, 1991; Dupré and Owen, 1992; Malvin and Wood, 1992). The ubiquity of this thermoregulatory response suggests a possible common origin in the evolution of metazoa and therefore a common neural control system for the regulation of the response. Since hypoxia can be presumed to be a highly costly environment (i.e. in relation to the amount of oxygen available for metabolic functions), it is possible that the decrease in T_b is, at least partly, the consequence of a decrease in the precision of temperature regulation and not exclusively the result of a regulated decline in the T_b set-point.

The purpose of this study is to evaluate the effect of oxygen concentration and environmental thermal quality on the level and precision of behavioural thermoregulation of the bearded dragon (*Pogona vitticeps*) in a controlled laboratory setting. An electronic shuttle box was used to evaluate these thermoregulatory parameters. In such a device, where a lizard needs to move continually to maintain an "optimal" T_b, the costs of thermoregulation are primarily those derived from locomotion. In conditions where oxygen is a limited resource, locomotion can be presumed to be highly costly to the animal. Similarly, metabolic costs are increased if the thermal quality of the habitat is low and the lizard is forced to move more often to maintain its preferred T_b.

I hypothesize that under severely hypoxic conditions or in environments where the thermal quality is low, the bearded dragon (*Pogona vitticeps*) will minimize locomotory costs at the expense of precise behavioural thermoregulation and thus forego maintaining an "optimal physiological temperature".

Materials and Methods

Experimental Animals

A total of twelve (eight male and four female) bearded dragons, *P. vitticeps*, were used for all aspects of this study. All animals were raised in captivity from eggs and were one year of age or older at the time of experiments. Mass of the experimental animals ranged from 219.5 to 417.1 g. The lizards were housed in sand-lined terraria (93 x 70 x 35 cm), each containing a 100 W light bulb placed at one end for thermoregulation. The terraria were further enriched with small logs and a plastic container (34 x 26 x 18 cm) that provided shade and shelter. The dragons were kept on a 12:12 light:dark photoperiod and were fed a daily diet of chopped vegetables. A source of protein such as crickets, mealworms or cat food was provided at least once a week. All procedures involving the use of these animals were approved by the Brock University Animal Care and Use Committee (protocol # 041001).

Experimental Set-Up

To determine the thermal preferences of the lizards, each individual was allowed to behaviourally thermoregulate inside an electronic shuttle box (Figure 4). This device consisted of a wooden chamber (119 X 61 X 45 cm) divided into two identical compartments by a Plexiglass® partition. An 11.5 X 14 cm hole at the bottom of the partition communicated the two compartments allowing for shuttling behaviour. Internal wooden walls in each of the compartments ran from each of the corners of the box to the

Figure 4. Photograph (A) and schematic drawing (B) of the electronic shuttle box used in this study (internal view). The box consisted of a wooden chamber divided into two identical compartments by a Plexiglass® partition. A hole at the bottom of the partition connected the two compartments allowing for shuttling behaviour. Internal wooden walls in each of the compartments ran from each of the corners of the box to the edge of the opening in the partition. This created a "funnelling" effect that facilitated shuttling behaviour between compartments. Infrared cameras installed inside the box allowed for continuous behavioural monitoring. A treadle-switch located on the floor between the two compartments indicated the location of the lizard inside the box (heating or cooling compartment) activating accordingly the heating or cooling sources positioned at each end of the box. Thermometers inside each compartment allowed for continuous air temperature monitoring and were fed back to the computerised control system.

edge of the opening in the partition. This created a "funnel" that guided lizards to the opening between compartments and thus facilitated shuttling. The enclosure was symmetrically illuminated by two 13W compact fluorescent light bulbs. Infrared cameras installed inside the box allowed for continuous behavioural monitoring without disturbance from the observer.

By stepping on a treadle-switch located on the floor under the partition separating the two compartments, lizards regulated the temperature inside the box each time they switched compartments, and could thus regulate their own body temperature. Heating and cooling of the box was regulated by an automated electronic system. The cooling sources consisted of a radiator located at each end of the box through which cold antifreeze (i.e. ethylene glycol) was circulated from a refrigerated water bath. Heating elements were also positioned at each end of the box. Fans located behind the heating and cooling sources blew the cold or warm air inside the box and continually mixed the air, maintaining similar temperatures and oxygen levels throughout each compartment. The treadle-switch indicated the location of the lizard inside the box (left or right compartment) and activated the automated electronic control system which activated accordingly the heating or cooling sources.

Once a lizard was positioned inside the "heating compartment" (HC) the air inside the box was automatically heated at a fixed rate of 0.7 °C/min. This rate could be modified to accommodate the specifications of the different experimental protocols used in the study (see Series III experiments). The temperature continued climbing until the lizard moved to the "cooling compartment" (CC), at which time the air inside the box was cooled at a fixed rate of 0.7 °C/min. Maximum and minimum achievable temperatures inside the box were

set at 46.5 and 10 °C respectively, as a safety precaution for the wellbeing of the animals. A 10 °C differential between the two compartments was maintained at all times during the experiments, creating a spatial temperature contrast that guided the lizards towards an instantly warmer or cooler temperature. In this manner, the lizards were able to behaviourally thermoregulate by shuttling back and forth between the two compartments.

Recording Temperature

Air temperatures (T_a) in the HC and the CC were obtained from thermometers suspended at each end of the box and automatically recorded to a custom-built software (Brock University Electronics Shop) every 30 s and whenever the lizard moved from one compartment to the other (i.e. at their UET_a and LET_a). Simultaneous records of the location of the lizard (HC or CC) were also obtained throughout the duration of the experiments. Body temperature (T_b) was obtained at 30 s intervals throughout all the experiments, from small (12 g) telemeters (model TA11CTA-F40 or TA10CTA-F40, Data Sciences International PhysioTel® and Multiplus™ implant) implanted in the abdominal cavity of the lizards. The telemeters were originally designed for use in rats but worked well within the temperature range experienced by the lizards during the experiments. These transmitters emit frequencies directly proportional to temperature; the telemeter signal was amplified by a series of antennae located beneath the shuttle box and transmitted to a receiver. This signal was relayed to a computer in an adjacent room where the frequencies were decoded using empirically determined 2nd order polynomials (i.e. the best fit relationship between frequency and temperature), by a custom made software program and converted to body temperature values.

Surgery and Telemeter Implantation

In order to measure core T_b, telemeters were surgically implanted in the abdominal cavity of the lizards. This method of acquiring body temperature presents obvious advantages over traditional methods (i.e. cloacal thermocouples or probes) since it allows for continuous T_b readings without disturbing the animal, and thus eliminates interfering with thermoregulatory behaviour itself.

To prepare animals for surgery, anaesthesia was rapidly induced by placing the lizards in a container with small pieces of gauze soaked with halothane. Once the animal had lost its righting ability, it was intubated via a tube placed into the glottis, administered with 3 to 4% isoflurane, and ventilated with a small rodent ventilator (Model INSPIR Asv.) at 7-10 breaths per min and a tidal volume of 3-5 mL. Isoflurane concentration was then lowered to 1 to 2% until the end of the surgery to maintain an appropriate plane of anaesthesia. Surgery was initiated when the response to pinching of the hind foot had ceased. The skin was sterilized subsequently using alcohol, betadine scrub and betadine solution. A small incision (1.5 to 2 cm) lateral to the midline was then made through the skin and the muscle wall, and the telemeter was implanted inside the abdominal cavity of the lizard. Finally, the muscle wall and the skin were sutured and glued with tissue adhesive (3M Vetbond ^{TM}). The lizards were allowed at least three weeks of recovery from surgery before experiments were initiated. All animals recovered well and appeared healthy the day after surgery. Most males resumed their typical courtship and territorial display behaviour on the days following surgery (sometimes even hours after surgery), suggesting that the procedure was not debilitating and did not adversely affect the animals.

Telemeters have since remained within the animals for more than 18 months with no fatalities or complications.

Experimental Design

Lizards were fasted for a period of 12 h prior to the experiments. All experiments were run from 8:00 am to 8:00 pm. At the beginning of each experiment a lizard was placed in the right chamber of the shuttle box which was designated the "Heating Compartment" for thermoregulation experiments. Placing the lizards inside the cooling compartment at the beginning of the day, when the animal was already cold and had just emerged from its nocturnal shelter would have further cooled the animal and induced lethargy. This would have hindered the animal's movement capacity and thus its capability to explore, shuttle, learn, and thermoregulate inside the box. At the end of each experiment lizards were removed from the box and returned to their housing facilities.

Series I: Exploratory Shuttling (Control Experiments)

The purpose of this series of experiments was twofold. Firstly, to test possible bias or preferences for one of the two compartments of the box in the absence of thermal stimuli; secondly, to determine the amount of movement that would occur between the two compartments unrelated to temperature regulation ("exploratory shuttling", ES) throughout the day. This information allowed for the determination of a period of time after which ES was considerably diminished or completely abolished. Determining the length of this initial acclimation period guaranteed that in subsequent thermoregulatory experiments, all

or at least most of the shuttles occurring after the acclimation period would be evoked by a thermal stimulus (i.e. were thermoregulatory in nature), rather than representative of spontaneous exploratory behaviour.

Twelve lizards were used in Series I experiments. During these experiments both sides of the shuttle box were maintained at a constant air temperature of 34.5 °C. This temperature is well within the range of T_b's and T_a's for active bearded dragons in the field (Bartholomew, 1963) and is not expected to evoke any thermoregulatory responses in the lizards (i.e. the movements of the lizard inside the box should not be evoked by the necessity of the lizards to search for a more "comfortable" temperature). During the experiments, the time and location (HC or CC) of the lizard was recorded automatically every time the lizard moved from one compartment of the box to the other. This allowed for the calculation of ES frequency and time spent in each compartment, throughout the experiment.

Series II: Effects of Hypoxia on the Level and Precision of Behavioural Thermoregulation

To examine the effects of hypoxia on the behavioural thermoregulation of bearded dragons, each lizard (n = 12) was tested under five different oxygen concentrations (21, 10, 7, 5 and 4% O_2). Lizards were exposed to these treatments in a haphazard order. At the beginning of each experiment a lizard was placed inside the shuttle box and left undisturbed for a period of 4 h under normoxic conditions. The length of this acclimation period was considered appropriate given the results from the control experiments (Series I) which indicated a considerable decline of putative non-thermoregulatory activity ("exploratory shuttling") after this 4 h period (see results section for exploratory shuttling experiments).

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After this initial acclimation period, hypoxic conditions were initiated. Oxygen concentrations were manipulated by delivering nitrogen into the shuttle box and flushing out the air, until the desired level of oxygen was reached (10, 7, 5 or 4% O_2). This was usually achieved within 30 min, after which the oxygen level was maintained at a constant level ($\pm$ 0.2% O_2) for the remainder of the experiment. This half hour following the initiation of hypoxia was not included in the data analysis. Oxygen concentrations were regulated and maintained using an oxygen controller (Pro-Ox, model 110, BioSpherix Ltd., Redfield, New York, USA). In the cases where normoxic levels were required (21% O_2), no gases were added to the box since the shuttle box was considerably large and porous to gases. T_a and T_b were recorded at 30 s intervals throughout the duration of the experiments, as well as time and temperature at the moment the lizard exited either compartment of the box (i.e. UET_a and LET_a).

Series III: Effect of Environmental Thermal Quality on the Level and Precision of Behavioural Thermoregulation

To examine the effect of the thermal quality of the environment on the precision and level of thermoregulation, lizards were exposed to four different levels of thermally challenging environments. This was done by varying the rate of temperature change ($\delta T_a/\delta t$ = change of temperature per time unit; hereafter denoted δT_a for simplification) in the electronic shuttle box. Higher δT_a's translate into more thermally challenging environments since under these conditions the animal is forced to shuttle a greater number of times (and hence invest more energy in locomotion) to maintain a similar T_b range than under conditions of lower δT_a. The δT_a used in these experiments were 0.7, 0.4, and

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0.1 °C/min. Since all of the experiments for Series II were run at a δT_a of 0.7 °C/min, the experiments at normoxic conditions for Series II were also common to the 0.7 °C/min δT_a conditions for this set (Series III) of experiments (Appendix A); only the data from the 10 lizards used in the rest of Series III experiments were used in the analysis of the effect of environmental thermal quality on behavioural thermoregulation. Additionally, lizards were exposed to an "extreme temperatures" treatment during which air temperature of the HC was maintained a constant 50 °C while the CC was held at a constant 15 °C. This was considered the most thermally challenging environment.

Ten lizards were used in Series III experiments. At the beginning of each experiment a lizard was placed inside the shuttle box to acclimate. To remain consistent throughout the different series of experiments, particularly the hypoxic series, only data from the last 7.5 h of experiment were used in the analysis; T_b and T_a were recorded every 30 s. LET_a and UET_a were also recorded.

Series IV: Effect of Methodology on the Behavioural Thermoregulatory response to Hypoxia

Studies in the past have used "extreme temperatures" shuttle boxes to evaluate different aspects of lizard behavioural thermoregulation (Berk and Heath, 1975a; Campbell, 1985; Hicks and Wood, 1985). Similarly to the "extreme temperatures" treatment of Series III experiments, these shuttle boxes provide the lizards with the choice of two extreme temperatures, well above and below the preferred temperature range of the species in question. In 1985, Hicks and Wood made a study of the effect of hypoxia on the behavioural thermoregulation of different species of lizards, using this type of shuttle box.

The purpose of this series of experiments was to compare the effects of hypoxia on the behavioural thermoregulation of bearded dragons between an "extreme temperatures" shuttle box, and the "Changing Temperatures" shuttle box used in Series II experiments. This will provide an indication of the usefulness and accuracy of the results obtained through these two different methodologies. For Series IV, 10 lizards were tested at "extreme temperatures" (as in Series III experiments) at 4% oxygen concentrations, using the same protocol as in Series II and III. These data were used together with the "extreme temperatures" treatment of Series III experiments as the normoxic (21% O_2) comparison.

Data Processing and Statistical Analysis

Statistical analyses for all experiments were performed using SigmaStat statistical software (version 3.0). In the cases where electrical noise interfered with the temperature telemeter signal, data points were reconstructed using a double parabolic interpolation using temperature points before and after the 'noise'. Temperature points were only interpolated when the temperature trend of the interpolated range was clear and did not exceed 10 min, or when the direction of temperature change did not vary within the interpolated range (i.e. where temperature either only increased or decreased). This could be determined by the direction of the slopes of the temperature traces before and after the interpolated range (i.e. data were only interpolated if both the section before and the section after the interpolated range had similar slopes). Experiments that did not fulfil these requirements were excluded from the analysis and repeated on a later date. Interpolations were performed using the XLXtrFun Excel Extra Functions for Microsoft® Add-In software.

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Assessment and Description of Thermoregulatory Variables

The T_b distribution of lizards both in the field and in laboratory settings is characterized by a negative skewness (Dewitt, 1967; Dewitt and Friedman, 1979), with greater variability below the mean than above the mean. For this reason, Dewitt (1967) proposed that the level of thermoregulation be expressed by the median instead of the mean of the distribution, and the precision as "the range of temperature within which a certain percentage of all observations are found". Consequently, the preferred T_b and selected T_a (i.e. level of thermoregulation) are expressed in this study by the medians of the T_b and selected T_a distributions of each lizard, respectively. Following Dewitt (1967), the precision of thermoregulation was evaluated by comparing the "preferred T_b range" (ΔT_b) across experimental treatments. ΔT_b is delimited by the high and low limits (HT_bL and LT_bL respectively) of the central 68% range of the T_b distribution (Dewitt, 1967; Dewitt and Friedman, 1979; Hertz *et al.*, 1993), such that $\Delta T_b = HT_bL - LT_bL$ (Figure 5). Although other measurements of central tendency have also been proposed to describe the range of preferred T_b (i.e. the central 50%, 80% or 95%), much of the existing literature on behavioural thermoregulation in the laboratory studies (Hammel *et al.*, 1967; Campbell, 1985; Hicks and Wood, 1985; Withers and Campbell, 1985; Arad *et al.*, 1989; Ladyman and Bradshaw, 2003; Petersen and Steffensen, 2003) uses the standard deviation as a measure of variation in T_b. Since the central 68% of observed temperatures is the equivalent of $\pm 1SD$ in normally distributed data, the use of this range allows for comparisons between studies.

The precision of selected T_a was evaluated using the size of the "selected T_a range" (ΔT_a) delimited by the high and low limits (HT_aL and LT_aL respectively) of the central

Figure 5. Representative trace of the body temperature (T_b), lower escape temperature (LET_a) and upper escape temperature (UET_a) distributions of one lizard displaying the thermoregulatory variables measured in this study. UET_a and LET_a as well as preferred T_b and selected T_a were determined by the median of the corresponding distributions. The preferred T_b range (ΔT_b) and selected T_a range (ΔT_a) were delimited by the high and low limits of the central 68% of the T_b (HT_bL and LT_bL, respectively) and T_a (HT_aL and LT_aL, respectively) distributions.

68% range of the T_a distribution ($\Delta T_a = HT_aL - LT_aL$) (Figure 5). Because the UET_a and LET_a (Figure 5) determine the absolute range of T_a's experienced by the lizards, these values also provided an indication of thermoregulatory precision. Furthermore, the measurement of these variables allowed the evaluation of the precision of selected T_a thresholds.

Being able to obtain simultaneous measurements of different parameters of T_a and T_b allows for the evaluation of the importance of peripheral thermal receptors on core body temperature regulation. Moreover, it sheds light on the relative importance of physiological mechanisms in the maintenance of a certain level or precision of thermoregulation as a response to the different environmental stimuli examined.

Unless stated otherwise, values for UET_a, LET_a, T_a and T_b are presented as the mean, for all lizards, of the medians of the corresponding distributions of each individual lizard ± SE. All other parameters are mean values of individual animals' means ± SE. In all statistical tests, a significance level of $P \leq 0.05$ was used.

Series I: Exploratory Shuttling (Control Experiments)

The amount of time spent in each compartment of the box was compared by subtracting the total amount of time spent in the left side from the total amount of time spent in the right side. A positive value indicates that on average the lizards spent a greater amount of time in the right compartment; a negative value indicates that the lizards spent a greater amount of time in the left compartment, and a value equal or close to zero indicates that lizards were using both compartment similar amounts of time. Departure from zero of this value was examined using a one way t-test. For each experiment, the 12 hours of data

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were binned into 30 min intervals. The number of shuttling events in each 30 min bin was then converted into percentages of total daily shuttles. This was done by dividing the number of shuttling events at each 30 min bin by the total number of shuttles throughout the day and multiplying by 100. These daily percentages were averaged for the 12 lizards and the appropriate acclimation period for Series II, III and IV was determined as the time when at least 90% of all shuttling events had taken place. The conversion of frequencies into daily percentages allowed for comparisons between experiments eliminating possible bias incurred by lizards that exhibited a much higher or lower frequency of ES than others.

Series II: Effects of Hypoxia on the Level and Precision of Behavioural Thermoregulation

The number of times each lizard shuttled was also calculated over the 7.5 h period and compared among oxygen levels. RM ANOVA was used to test statistical differences in the number of shuttles between oxygen levels. Medians for T_b, T_a, UET_a and LET_a and means for ΔT_b and ΔT_a across the 7.5 h of experimental conditions, at the different oxygen levels, were compared using a RM ANOVA. To account for the possible correlation existing between the different T_a and T_b variables (i.e. T_b, T_a, UET_a, LET_a, ΔT_b and ΔT_b), a Bonferroni correction was performed on all statistical tests. Time of day effects were evaluated comparing the median T_b's for the first and last 75 min of exposure to experimental conditions (from 12:30-13:45 h and from 18:45-20:00 h). A two way RM ANOVA with time and oxygen level as factors was used for this purpose.

Whenever significant differences were detected by the RM ANOVAs, differences between 21% oxygen and the hypoxic groups were further examined using Holm-Sidak

procedure as a *post-hoc* method. This method was preferred over the Tukey's test since it allows for both pair-wise comparisons and versus a control group.

Series III: Effect of Environmental Thermal Quality on the Level and Precision of Behavioural Thermoregulation

Analyses of T_b, T_a, UET_a, LET_a, ΔT_b and ΔT_a at the different levels of thermal quality were performed in the same manner as described above for Series II experiments.

Series IV: Effect of Methodology on the Behavioural Thermoregulatory response to Hypoxia

T_b, and the different aspects of the preferred T_b range were compared for 0.7 °C/min δT_a and the "extremes temperatures" treatment at 21 and 4% oxygen, using a two way RM ANOVA, using environmental thermal quality and oxygen concentration as factors. Significant differences were further compared using a Holm-Sidak *post-hoc* test.

The number of times each lizard shuttled at the "extreme temperatures" conditions was calculated though the 7.5 h period and evaluated for statistical differences between exposures to 21 and 4% oxygen using a paired t-test.

Results

General Observations

Five of the 12 lizards (3 males, 2 females) appeared to be "poor thermoregulators" moving during the initial period of the experiments (first 1-3 hours of experimental conditions) and then retreating to one corner inside the cold side of the box. After having spent at least 1.5 h in this position, lizards were not considered to be actively thermoregulating, and were removed from the shuttle box and returned to their housing facilities. The data from these experiments were not used in the analysis. These animals were exposed to the experimental conditions repeatedly until they were able to successfully thermoregulate inside the shuttle box (i.e. shuttle back and forth rather than remain in the cold compartment for 1.5 h or more). This happened regardless of oxygen concentration or thermal quality, suggesting the effect was specific to the animal and not the treatment. All other individuals (5 males, 2 females) were able to thermoregulate continuously inside the electronic shuttle box, exhibiting shuttling behaviour throughout the length of the experiments without the need for more than one or two trials per experiment/treatment. A summary of the number of trials required for each lizard at each oxygen and environmental thermal quality level is presented in Appendix A.

The values for the different thermoregulatory variables, obtained from animals requiring more than two trials at the different treatments fell within the range of those obtained for animals that required only one or two trials. In other words, there was no apparent difference between the data obtained between the two groups of animals (i.e. animals that required more than two trials and animals that only required one or two

trials to exhibit active thermoregulation inside the shuttle box) and therefore, data from all animals were pooled for the analysis.

To account for the possible correlation existing between the different T_a and T_b variables, a Bonferroni correction was performed on all statistical tests. There were no differences in significance after the Bonferroni correction was performed.

Series I: Exploratory Shuttling (Control Experiments)

All of the lizards engaged in exploratory shuttling shortly after being placed inside the box (within half an hour). Inter-individual variability was high, with lizards exhibiting from a total of 2 to 405 shuttles during the 12 hours of experiment. The back and forth exploratory behaviour continued for approximately four hours, at which point, $90.3 \pm 5.4\%$ (mean of the cumulative percentages of ES of 12 lizards) of all shuttling observations had already occurred. Lizards shuttled 22.7 ± 34.2 times per hour during these first 4 hours. After the initial 4 hours of exploratory behaviour ES was rare or absent (Figure 6), occurring at a rate of approximately 1.1 ± 4.3 shuttles per hour. Based on these results, a four hour acclimation period was considered appropriate for all subsequent experiments.

There was no statistical difference in the total amount of time spent in each side of the shuttle box when the 12 h of experiment were analysed (t_{11}=1.21, P = 0.253) or when the morning (first 4 h of experiment) and afternoon times were analysed separately (t_{11}=1.37, P = 0.19 and t_{11}=1.04, P = 0.32 for the morning and afternoon analysis respectively). Lizards spent a total of 7.47 ± 1.26 h and 4.45 ± 1.25 h in the left and right compartments respectively, during the 12 h of experimentation. It is possible that the lack of significance between the times spent in the left vs. the right compartment is due to a low

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sample size (power = 0.09 for the all day analysis and, 0.13 and 0.14 for the analysis of morning and afternoon times respectively) instead of a lack of effect. Nine out of the 12 lizards spent more than 50% of the time (more than 6 h of a total of 12 h of experiment) in the left compartment. Both of these parameters (i.e. the total amount of time spent at each compartment and the number of lizards spending more than 50% of the time in the cold compartment) indicate a slight preference for this side of the box.

Series II: Effect of Hypoxia

Qualitative Results

Shortly after being placed in the shuttle box the lizards engaged in shuttling behaviour. As in Series I, shuttling was usually more frequent during the first four hours of being in the box (acclimation period), after which shuttling became more regular and periodic. By continuously shuttling back and forth from the heating and the cooling compartment of the box, the lizards were able to maintain a relatively constant T_b. Typical daily traces of individual lizards for T_a and T_b at 21 and 5% oxygen are presented in Figure 7. Despite several repetitions (>6), two lizards at 21, one at 7 and one at 5% oxygen did not actively thermoregulate inside the shuttle box (Appendix A). All but these experiments were included in the analyses. Lizards spent nearly 50% of the time at each compartment in every experiment performed (see Figure 14 in *Discussion* section).

Figure 6. Frequency and percentage of exploratory shuttling (ES) for 12 individuals throughout 12 hours of activity inside a shuttle box. Air temperature was set at a constant temperature of 34.5 °C. For each experiment, the 12 hours of data were binned into 30 min intervals. Percentage of shuttles were calculated by dividing the number of shuttling events at each 30 min bin by the total number of shuttles throughout the day and multiplying by 100. Values are plotted as the mean values for 12 individuals (+ SE) for every 30 min interval during a 12h period. 90.3 ± 5.4 % (SE) of all shuttling observations occurred before 12:00 h.

Figure 7. 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 shuttle box. Traces are plotted for the 4 h acclimation period and the subsequent 8 h of exposure to A. 21% O_2 and B. 5% O_2. ↑ Denotes the onset of hypoxia at 12:00 h. ↑ Represents the moment at which 5% oxygen was reached (12:30 h). Note the inter-individual variability in the amount of exploratory shuttling (i.e. irregular movements, represented by irregular changes in T_a) that occurred during the 4 h of acclimation.

Level of Thermoregulation

Selected T_a (mean of 7.5-hour medians for all lizards) showed progressively lower values with decreasing oxygen concentrations. T_a was 34.4 ± 0.2 °C under 21% oxygen. Exposures to 5 and 4% oxygen led to significantly lower T_a's ($F_{4,7} = 9.91$, $P < 0.001$) of 32.9 ± 0.4 and 32.1 ± 0.6 °C respectively. Although not significantly different from the 21% oxygen treatment, exposures to 10 and 7% oxygen also resulted in lower selected T_a's (34.1 ± 0.2 and 33.6 ± 0.3 °C respectively) (Figure 8). Preferred T_b (mean of 7.5-hour medians) followed a similar pattern, presenting slightly higher values at all levels of oxygen. Under normoxic conditions T_b was 34.7 ± 0.2 °C. Exposure to 10% oxygen had no effect on the T_b of the lizards (34.7 ± 0.2 °C). At all other levels of oxygen T_b decreased with decreasing oxygen concentrations to values of 34.2 ± 0.2 at 7% oxygen, 33.5 ± 0.4 °C at 5% and 32.5 ± 0.5 °C at 4% oxygen concentration (Figure 8). This decrease was significant at the 4% and 5% oxygen levels ($F_{4,7} = 15.99$, $P < 0.001$).

Precision of Thermoregulation

UET_a (mean of 7.5-hour medians) was slightly but not significantly increased with increasing levels of hypoxia ($F_{4,7} = 2.41$, $P = 0.064$). An opposite and more pronounced trend was observed for LET_a, where exposures to 4% oxygen resulted in a significant decline of LET_a ($F_{4,7} = 9.91$, $P < 0.001$) (Figure 9; Table 1). As a reflection of the pronounced decrease in LET_a, both the high and low limits of the T_a range (i.e. HT_aL and LT_aL respectively) decreased with lower oxygen levels (Figure 9). The temperature decrease was more pronounced for the LT_aL, particularly at the lower oxygen levels (5 and 4%) resulting in a corresponding increase in the size of the T_a range (i.e. ΔT_a)

Figure 8. Selected ambient and internal body temperature (T_a and T_b respectively) of bearded dragons (*P. vitticeps*) for the different levels of oxygen tested. Animals were tested for 7.5 hours in an electronic shuttle box at each of the experimental conditions. T_b was obtained from temperature telemeters implanted in the abdominal cavity of the animals and T_a was obtained though thermometers located at each compartment of the shuttle box. Data plotted as mean of medians ± SE. † Refers to a significant difference in T_b and * refers to a significant difference in T_a relative to 21% oxygen with the Holm-Sidak post-hoc test ($P < 0.05$).

Figure 9. 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$_a$ and T$_b$ ranges (HT$_a$L, LT$_a$L, HT$_b$L and LT$_b$L respectively) and preferred T$_b$ are plotted as the mean of median values of 12 lizards (*P. vitticeps*) ± SE. Animals were tested for 7.5 hours in an electronic shuttle box at each of the experimental conditions. † Refer to a significant effect compared to normoxic values with the Holm-Sidak post-hoc test (P < 0.05).

Table 1. Upper and lower escape ambient temperatures (UET_a and LET_a, respectively) of bearded dragons allowed to thermoregulate inside an electronic shuttle box at different oxygen concentrations. Values are means of medians ($\pm$ SE) of 12 lizards during a 7.5 h period. * indicates significant differences relative to 21% oxygen values with the Holm-Sidak post-hoc test ($P < 0.05$).

O_2 Concentration	UET_a (°C)	LET_a (°C)
21%	43.0 ± 0.8	25.4 ± 0.8
10%	43.0 ± 0.8	24.8 ± 0.5
7%	44.1 ± 0.6	24.4 ± 0.6
5%	43.7 ± 0.5	23.8 ± 0.9
4%	44.7 ± 0.4	$20.6 \pm 0.8*$

with decreasing oxygen concentrations (Table 2). This effect was significant at 4% oxygen ($F_{4,7}$ = 5.12, P = 0.002) where the T_a range increased from 13.5 ± 0.5 °C in normoxia, to 16.5 ± 0.7. The effect of hypoxia on the selected T_a range was reflected in the resulting preferred T_b range, exhibiting similar patterns for HT_bL and LT_bL (Figure 9). The HT_bL remained unaffected at 10% oxygen levels, but progressively decreased at all other oxygen levels. LT_bL decreased with decreasing oxygen concentrations at exposures to all levels of hypoxia. Because LT_bL exhibited a more pronounced decrease than HT_bL with lower oxygen concentrations, ΔT_b increased with higher levels of hypoxia exhibiting a significant effect ($F_{4,7}$ = 5.92, P < 0.001) at exposures of 4% oxygen (Table 3).

RM ANOVA was used to compare the effect of hypoxia on the number of times lizards moved between sides of the shuttle box. Since data were not normally distributed, a log-transformation was applied, in an effort to meet the assumptions of ANOVA. Normality, however, could not be achieved. Nevertheless, ANOVA is a robust test that usually performs well even with departures from normality. Because the log-transformed data had a smaller deviation from normality than the non-transformed data, the RM ANOVA was performed on the transformed data. Hypoxia elicited a progressive decrease in the number of times the lizards moved from one compartment to the other (i.e. number of shuttles) with decreasing oxygen levels (Figure 10). This decrease was significant at 4% oxygen ($F_{4,7}$ = 5.28, P < 0.001) where the lizards shuttled 20.7 ± 1.9 times during a 7.5 hour period versus 59.5 ± 19.4 times in normoxia.

Table 2. High and low limits of the selected ambient temperature (T_a) range (HT_aL and LT_aL, respectively) and size of the T_a range (ΔT_a) of bearded dragons thermoregulating inside an electronic shuttle box at different oxygen concentrations. Values are means of medians ($\pm$ SE) of 12 lizards during a 7.5 h period. * indicate significant differences in ΔT_a relative to 21% oxygen values with the Holm-Sidak post-hoc test ($P < 0.05$).

O_2 Concentration	HT_aL (°C)	LT_aL (°C)	ΔT_a (°C)
21%	41.2 ± 0.2	27.7 ± 0.4	13.5 ± 0.5
10%	41.3 ± 0.3	27.3 ± 0.2	14.1 ± 0.3
7%	41.2 ± 0.4	26.9 ± 0.3	14.3 ± 0.4
5%	40.7 ± 0.6	25.8 ± 0.5	14.8 ± 0.7
4%	40.4 ± 0.7	23.9 ± 0.9	16.5 ± 0.7*

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Table 3. **High and low limits of the selected body temperature (T_b) range (HT$_b$L and LT$_b$L, respectively) and size of the T_b range (ΔT_b) of bearded dragons allowed to thermoregulate inside an electronic shuttle box at different oxygen concentrations.** Values are means of medians ($\pm$ SE) of 12 lizards during a 7.5 h period. * indicate significant differences in ΔT_b relative to 21% oxygen values with the Holm-Sidak post-hoc test ($P < 0.05$).

O_2 Concentration	HT$_b$L (°C)	LT$_b$L (°C)	ΔT_b (°C)
21%	35.9 ± 0.2	33.4 ± 0.3	2.5 ± 0.3
10%	35.9 ± 0.2	33.3 ± 0.3	2.6 ± 0.2
7%	35.4 ± 0.2	32.6 ± 0.3	2.8 ± 0.3
5%	34.9 ± 0.5	31.6 ± 0.5	3.3 ± 0.4
4%	34.7 ± 0.4	30.1 ± 0.7	4.6 ± 0.5*

Figure 10. Mean number of shuttles of 12 lizards, during a 7.5 hour period at different oxygen concentrations when allowed to thermoregulate inside an electronic shuttle box. Plotted values are means ± SE. * Denotes a significant difference vs. 21% oxygen conditions. The number of non-thermoregulatory shuttles during the last 7.5 h of the control experiments (constant 34.5 °C at 21% O_2) is plotted as a dotted line for comparison.

Time Component of Temperature Regulation

T_b values were significantly higher ($F_{4,4} = 8.526$, $P = 0.019$) in the last 75 min of the trial than the initial 75 min of exposure to experimental oxygen levels; a ≈ 1 °C increase was observed at any of the O_2 conditions studied (Table 4). There was no interaction between the effect of oxygen level and the different times of day on T_b ($F_{4,4} = 0.318$, $P = 0.864$).

Series III: Effect of Environmental Thermal Quality

As in Series II, lizards engaged in shuttling behaviour shortly after being placed inside the shuttle box. By continuously shuttling back and forth from the heating and the cooling compartment of the box, the lizards were able to maintain a relatively constant T_b. Similarly to Series II, the percentage of time spent in cold vs. the hot compartment of the shuttle box was nearly 50% for all δT_a conditions (see Figure 14 in *Discussion* section). During the "extreme temperatures" conditions, however, lizards were frequently observed straddling the two compartments. On these occasions, lizards were observed with the lower half of their body (i.e. tail, back legs and abdomen) in the cold compartment while their front legs and head remained in the hot compartment. Five out of the 10 lizards tested under the "extreme temperatures" conditions displayed this type of behaviour at one point or another during the experiment. As a result, lizards spent a total of 1.5 ± 0.5 h astraddle the two compartments while spending 2.8 ± 0.3 and 3.2 ± 0.3 h in the hot and cold compartments respectively (values are means $\pm$ SE of 10 individuals' means) during the 7.5 h of "extreme temperatures" experimental conditions. This type of behaviour was not observed under any of the other thermal quality regimes.

Table 4. Observed T_b at the initial and final 75 min of exposure to experimental conditions at the different oxygen levels. Values are means of T_b medians for 12 animals ($\pm$ SE) allowed to thermoregulate inside an electronic shuttle box at five different levels of oxygen concentrations. * Represent a significant difference ($P < 0.05$) between the initial and the final 75 min of exposure to experimental conditions.

O_2 Concentration	T_b for 12:30-13:45h period (°C)	T_b for 16:15-20:00h period (°C)
21%	34.3 ±0.3	35.2 ±0.2*
10%	33.9 ±0.2	35.1 ±0.2*
7%	33.5 ±0.3	34.7 ±0.3*
5%	33.0 ±0.8	33.7 ±0.5*
4%	32.1 ± 0.5	33.0 ±0.6*

Level of Thermoregulation

T_b (mean of 7.5-hour medians, n = 10) was not affected by the different δT_a's. However, the "extreme temperatures" treatment did have a significant lowering effect on T_b ($F_{3,5}$ = 10.28, P < 0.001) when compared to any of the δT_a treatments. Values for T_b were 34.8 ± 0.5 °C at δT_a of 0.1 °C/min, 35.3 ± 0.4 °C at 0.4 °C/min conditions, and 34.7 ± 0.2 and 32.7 ± 0.7 °C at 0.7 °C/min and the "extreme temperatures" treatments, respectively (Figure 11). Because of the fixed T_a nature of the extreme temperatures treatment, T_a could only be compared for the different δT_a conditions. T_a was not significantly affected by δT_a ($F_{2,7}$ = 1.45, P = 0.267). Selected T_a for 0.7 °C/min conditions was 34.5 ± 0.2 °C/min, 35.0 ± 0.4 for conditions of 0.4 δT_a and 35.4 ± 0.8 °C at 0.1 °C/min (Figure 11).

Precision of Thermoregulation

As mentioned above, the fixed temperature nature of the "extreme temperatures" treatment did not allow for comparisons of UET_a, LET_a or any aspect of the T_a range between this treatment and any of the δT_a conditions. Therefore, these parameters were analysed and compared only within the different δT_a conditions. Higher δT_a's elicited a significant increase in UET_a at both 0.4 °C/min and 0.7 °C/min ($F_{2,7}$ = 9.18, P = 0.002) (Figure 12). An opposite trend was observed for LET_a where δT_a of 0.7 °C/min lead to a significant decrease ($F_{2,7}$ = 4.76, P = 0.007) in LET_a (Figure 12; Table 5).

Figure 11. Selected ambient and internal body temperature (T_a and T_b respectively) of 10 bearded dragons (*P. vitticeps*) for the different levels of environmental thermal quality tested in this study. Animals were tested for 7.5 hours in an electronic shuttle box at each of the experimental conditions. Environmental thermal quality was modified by changing the rate of temperature change (δT_a) inside the box. δT_a's of 0.1, 0.4 and 0.7 °C/min were used in this study. Additionally, lizards were also evaluated under an "extreme temperatures" (ET) treatment in which the cooling and heating compartments of the box were maintained at constant 15 and 50 °C respectively. Data plotted as means of medians ± SE of 7.5 h of exposure to experimental conditions. * Refers to a significant effect on T_b at the ET treatment (Holm-Sidak post-hoc test; $P < 0.05$).

Figure 12. **Values for the different parameters of precision of thermoregulation with exposures to different levels environmental thermal quality.** Upper and lower escape ambient temperatures (UET_a and LET_a respectively), high and low limits of the T_a and T_b ranges (HT_aL, LT_aL, HT_bL and LT_bL respectively) and preferred T_b are plotted as mean values for 10 lizards (*P. vitticeps*) ± SE. Animals were tested for 7.5 hours in an electronic shuttle box at each of the experimental conditions. † Refers to a significant effect in UET_a and LET_a when compared to 0.1 °C/min δT_a values with the Holm-Sidak post-hoc test ($P < 0.05$).

Table 5. Upper and lower escape ambient temperatures (UET$_a$ and LET$_a$, respectively) of bearded dragons allowed to thermoregulate inside an electronic shuttle box at different levels of thermal environmental quality. Values are means of medians ($\pm$ SE) of 10 lizards during a 7.5 h period. * Indicate significant differences relative to values obtained at 0.1 °C/min δT_a with the Holm-Sidak post-hoc test (P < 0.05).

Level of Environmental Thermal Quality (δT_a)	*UET$_a$ (°C)*	*LET$_a$ (°C)*
0.1 °C/min	39.6 ± 0.3	27.9 ± 0.7
0.4 °C/min	42.6 ± 0.8*	26.4 ± 0.8
0.7 °C/min	43.0 ± 0.8*	25.4 ± 0.8*

LT_aL exhibited a slight decrease at the higher rates of temperature change. Conversely, HT_aL exhibited an increase at the higher rates of temperature change (δT_a's of 0.7 and 0.4 °C/min) (Figure 12). As a consequence, the size of the T_a range (ΔT_a) was significantly larger at both 0.7 and 0.4 °C/min δT_a ($F_{2,7} = 11.63$, $P < 0.001$) than at δT_a of 0.1 °C/min (Table 6).

Both the HT_bL and the LT_bL of the T_b range were lowered by the "extreme temperatures" treatment. This lowering effect was more pronounced for the LT_bL (Figure 12) causing the size of the T_b range (ΔT_b) to increase significantly in the "extreme temperatures" treatment ($F_{2,7} = 4.99$, $P = 0.007$) but remain unaffected at any of the δT_a's (Table 7).

Series IV: Effect of Methodology on the Behavioural Thermoregulatory response to Hypoxia

As in Series III experiments, exposures to 4% oxygen elicited a significant lowering effect ($F_{3,6} = 26.59$, $P < 0.001$) on T_b (mean of 7.5 h medians, n= 10) at the "extreme temperatures" concentrations, exhibiting values of 29.3 ± 0.7 °C compared to the 33.1 ± 0.4 °C T_b obtained in normoxia (mean of 7.5 h medians, n = 10) (Figure 13). There was a significant interaction between level of environmental thermal quality (0.7 °C/min δT_a and "extreme temperatures" conditions) and oxygen level ($F_{3,6} = 8.24$, $P = 0.02$), with exposures to 4% oxygen, eliciting a more pronounced decrease in T_b at the "extreme temperatures" treatment than at 0.7 °C/min δT_a conditions. Indeed, T_b exhibited a decrease of 1.9 ± 0.4 °C from normoxia to 4% oxygen at 0.7 °C/min δT_a conditions compared to the

Table 6. **High and low limits of the selected ambient temperature (T_a) range (HT_aL and LT_aL, respectively) and size of the T_a range (ΔT_a) of bearded dragons allowed to thermoregulate inside an electronic shuttle box at different levels of environmental thermal quality.** Values are means of medians ($\pm$ SE) of 10 lizards during a 7.5 h period. * Indicate significant differences in ΔT_a relative to values obtained at 0.1 °C/min δT_a with the Holm-Sidak post-hoc test ($P < 0.05$).

Level of Environmental Thermal Quality (δT_a)	H_aL (°C)	LT_aL (°C)	ΔT_a (°C)
0.1 °C/min	39.7 ± 0.4	28.5 ± 0.6	11.2 ± 0.2
0.4 °C/min	41.3 ± 0.3	28.2 ± 0.6	13.5 ± 0.5*
0.7 °C/min	41.2 ± 0.2	27.7 ± 0.4	13.2 ± 0.5*

Table 7. High and low limits of the selected T_b range (HT_bL and LT_bL, respectively) and size of the T_b range (ΔT_b) of bearded dragons allowed to thermoregulate inside an electronic shuttle box at different levels of environmental thermal quality. Values are means of medians ($\pm$ SE) of 10 lizards during a 7.5 h period. * Indicate significant differences in ΔT_b relative to values obtained at 0.1 °C/min δT_a with the Holm-Sidak post-hoc test ($P < 0.05$).

Level of Environmental Thermal Quality (δT_a)	*H_bL (°C)*	*LT_bL (°C)*	*ΔT_b (°C)*
0.1 °C/min	36.2 ± 0.4	33.3 ± 0.5	2.9 ± 0.3
0.4 °C/min	36.8 ± 0.4	33.8 ± 0.5	3.0 ± 0.4
0.7 °C/min	35.9 ± 0.2	33.4 ± 0.3	2.5 ± 0.3
"extreme temperatures"	34.7 ± 0.5	29.4 ± 0.9	5.3 ± 1.1*

Figure 13. Values for the different parameters of precision of thermoregulation at two environmental quality levels ("extreme temperatures"_ET, and δT_a of 0.7 °C/min) with exposures to 21 and 4% oxygen concentrations. Preferred body temperature (T_b) and high and low limits of the preferred T_b range (HT_bL and LT_bL respectively) are plotted as the means of medians ($\pm$ SE) for 10 individuals. Animals were tested for 7.5 hours in an electronic shuttle box at each of the experimental conditions. † Refers to a significant effect at 4% oxygen when compared to normoxia with the Holm-Sidak post-hoc test ($P < 0.05$).

greater decrease in T_b of 3.8 ± 0.8 °C observed from normoxia to 4% oxygen at the "extreme temperatures" conditions.

Both the HT_bL and the LT_bL were lowered by environmental thermal quality and by oxygen level (Figure 13). At the "extreme temperatures" treatment, HT_bL was 34.9 ± 0.4 °C in normoxia and 32.2 ± 0.7 °C at 4% oxygen and LT_bL was 29.8 ± 0.9 °C in normoxia and 27.6 ± 0.9 °C at 4% oxygen. The size of the preferred temperature range (ΔT_b) at the "extreme temperatures" did not exhibit a significant difference (paired $t_9 = -0.533$, $P = 0.607$) between exposures to 4 and 21% conditions (5.1 ± 1.1 and 4.6 ± 0.7 °C, respectively).

During the "extreme temperatures" conditions, hypoxia elicited a nearly significant decrease in the number of times the lizards shuttled between the two compartments (paired t-test; $t_9 = 2.12$, $P = 0.063$). Since the power of the test was low (0.38), the lack of significance is likely due to a low sample size and not to a lack of effect. In normoxia, lizards shuttled 78.3 ± 21.9 times during a 7.5 h period, while shuttling only 26.6 ± 8.4 times at 4% oxygen conditions. Seven out of ten lizards displayed a straddling behaviour during exposures to hypoxia (i.e. were observed straddling the cold and hot compartments, with the back legs, tail and abdomen in the cold compartment and the front legs and head inside the hot compartment). This value is higher than that observed for the normoxic experiments where only five out of ten lizards exhibited this behaviour. Lizards spent a total of 3.7 ± 0.9 h astraddle the two compartments while spending 1.5 ± 0.4 and 2.2 ± 0.6 h in the hot and cold compartments respectively (values are means $\pm$ SE of 10 individuals' means) during the 7.5 h of "extreme temperatures" experimental conditions. Lizards spent nearly 50% of their time in the hot and cold compartment at either 21 or 4% oxygen at the

"extreme temperatures" conditions (see Figure 14 in *Discussion* section). Nevertheless,

lizards spent a significantly greater amount of time astraddle the two compartments at 4%

oxygen conditions than in normoxia (paired t-test; t_{11} = 3.45, P = 0.005).

Discussion

General Observations

The behavioural thermoregulation of the bearded dragons was affected in a similar manner by hypoxia and thermal quality of the environment, both eliciting a decrease in the level and precision of thermoregulation. Both of these environmental conditions imply changes in the metabolic costs of thermoregulation; the former in terms of oxygen available for metabolic requirements and the latter in terms of metabolic requirements for the available oxygen. These observations conform to the cost-benefit model of lizard thermoregulation proposed by Huey and Slatkin (1976) that predicts lower thermoregulatory precision when the costs of thermoregulation are high.

Several other factors are known to elicit similar responses in the thermoregulation of reptiles. Dehydration and low water availability cause a decrease in the preferred temperature of tiger snakes and common lizards (Lorenzon *et al.*, 1999; Ladyman and Bradshaw, 2003), and other ecological factors such as the presence of predators and territorial fighting behaviour can also interfere with the level and precision of thermoregulation of desert iguanas (Dewitt, 1967). That different aspects of thermoregulation are affected in a similar manner by a wide variety of stressors suggests a common mechanism to the cryexic (decrease in body temperature) thermoregulatory response.

Some lizards appeared to be better thermoregulators than others, indicating inter-individual variability. Five out of 12 lizards (58.3%) required more than two repetitions at one or more of the treatments before they were able to successfully thermoregulate inside the shuttle box (Appendix A). Despite this, in only 41 out of a total of 129 treatment/lizard

Figure 14. Percentage of time spent by lizards in each compartment or straddling the two compartments (Middle) of an electronic shuttle box when exposed to the different treatments. Environmental thermal quality was modified by changing the rate of temperature change (δT_a) inside the box. δT_a's of 0.1, 0.4 and 0.7 °C/min were used in this study. Additionally, lizards were evaluated under an "extreme temperatures" (ET) treatment in which the cooling and heating compartments of the box were maintained at constant 15 and 50 °C respectively. Experiments were also performed at "extreme temperatures" conditions at 4% oxygen concentrations. During the control experiments lizards were placed inside the electronic shuttle box at a constant temperature environment of 34.5 °C inside both chambers. Values for the different environmental quality levels (n = 10), exposures to the different oxygen concentrations (n = 12) and for the control experiments (n = 12) are plotted as the mean of 7.5 h of exposure to experimental conditions ± SE.

Effect of Hypoxia on the Level and Precision of Thermoregulation

This study shows a proportional effect of hypoxia on both the level and precision of behavioural thermoregulation of the bearded dragon (*P. vitticeps*). Preferred T_b significantly dropped under conditions of severe hypoxia (5% and 4% O_2); this was caused by a decrease in selected T_a. Nevertheless, preferred T_b presented slightly higher values than T_a at all levels of oxygen (Figure 8), suggesting a possible physiological control of thermoregulation. Reptiles are capable of controlling the rates of heating and cooling by adjustments in heart rate, cardiac shunts and changes in peripheral blood flow (Dzialowski and O'Connor, 2001; Seebacher and Franklin, 2001; Seebacher and Grigg, 2001; Seebacher and Franklin, 2004). Such physiological mechanisms allow reptiles to maintain preferred T_b's for longer periods of time and could allow for the differences between preferred T_b and selected T_a observed in this study. It is noteworthy that the percentage of time spent in the heating compartment was slightly greater than 50% (Figure 14) at all O_2 levels and δT_a treatments; over the period of 7.5 hours one would predict that lizards would never fully cool off during the slightly shorter periods spent in the cooling compartment.

The cryexic effect of hypoxia on lizard thermoregulation was previously described by Hicks and Wood (1985), who showed significant decreases in preferred body T_b in four different species of lizards exposed to 7% oxygen conditions. This was accompanied by a decrease in both the upper and lower escape T_b's (UET_b and LET_b respectively) when allowed to thermoregulate in a shuttle box (Hicks and Wood, 1985), suggesting a decline in the upper and lower temperature thresholds. Escape T_b's and T_b thresholds (i.e. T_b set-points), however, are not necessarily analogous. Since T_b usually lags behind T_a, the T_b of even small animals will continue to climb or decline for several minutes after the initial

thermal escape response, thus achieving higher and lower values than their corresponding T_b exit temperatures (i.e. T_b's at which a lizard exits either the cold or the hot side of a shuttle box). In the present study, the T_b lag meant that, in some cases, at the highest rate of temperature change (i.e. 0.7 °C/min δT_a) the upper escape body temperature of an individual lizard at a given time (T_b at which a lizard exits the hot side of a shuttle box; UET_b) was equal to or even lower than the lower escape body temperature (T_b at which a lizard exists the cold side of a shuttle box; LET_b), even when UET_a was considerably higher than LET_a (Figure 15).

To circumvent this difficulty (i.e. the inappropriateness of using UET_b and LET_b as equivalents for upper and lower set-points), ambient upper and lower escape temperatures were used in this study. The size of the preferred T_b and selected T_a ranges (the central 68% of the distribution as described in Dewitt (1967)) were also used as indications of the precision of thermoregulation. Interestingly, UET_a climbed slightly (and nearly significantly, $P = 0.064$), while LET_a declined significantly with lower oxygen concentrations (5 and 4%). The increase in UET_a and decrease in LET_a was the consequence of the lizards shuttling less at lower oxygen levels (Figure 10) and thus spending, on average, more time in each compartment between shuttles. The pronounced decrease in the LET_a under the most severe hypoxic conditions, led to a slight decrease in the HT_aL and a greater decrease in the LT_aL. The T_b response followed the T_a distribution, where both the HT_bL and the LT_bL declined at 5 and 4% oxygen conditions. Because the decline was more pronounced for the LT_bL, the resulting T_b range was significantly increased at 4% oxygen. In the end, the drastic decline of the LET_a over the slight increase

Figure 15. Selected ambient temperature (T_a) and preferred body temperature (T_b) traces of a lizard, while thermoregulating at normoxic conditions inside an electronic shuttle box. A considerable T_b lag is observed in this trace, with T_b's continuing to climb (or decline) for up to 10 min after the lizard has shuttled to the heating compartment (or cooling compartment) of the box. As a consequence, UET_b is similar or in some cases even lower than the following LET_b.

of UET_a caused a more pronounced decrease of the LT_bL than HT_bL, thus producing the general decrease in T_b mentioned above. In other words, by allowing the shuttle box to cool to dramatically lower T_a's before escaping the cold side, the lizards decreased the lower end of the T_b distribution in hypoxia, and thus exhibited less precise and lower T_b's.

The cryexic effect of hypoxia on the behavioural thermoregulation of lizards has, in the past, been attributed to a regulated decrease in preferred T_b (change in T_b set-point) (Hicks and Wood, 1985). This decrease in the T_b set-point can potentially reduce oxygen demands by up to 50% by lowering the metabolic rate, significantly reducing ventilatory costs and inducing an increase in the oxygen loading capacity in the lungs (Hicks and Wood, 1985). Although the present study does not exclude a regulated decrease in preferred temperature, it reveals another component of the cryexic response to hypoxia: the effect exerted on the precision of behavioural thermoregulation.

The cost-benefit model for thermoregulation in lizards proposed by Huey and Slatkin (1976) predicts that lizards will only thermoregulate if the associated costs are low. The model only takes into account costs of an ecological nature (i.e. food availability, homogeneity of the thermal environment, accessibility of basking sites, etc), but it can be extended to situations where physiological costs are associated with thermoregulation. In conditions where oxygen concentration is low, oxygen becomes a limited resource and locomotion becomes more expensive, or indeed impossible to conduct aerobically. In addition, if frequent movement is necessary to maintain a narrow T_b range, a lizard is expected to minimize locomotory costs at the expense of precise behavioural thermoregulation.

In the present study, bearded dragons did not abandon temperature regulation at low oxygen conditions. Instead, the lizards reduced locomotory oxygen expenditure by reducing the frequency of shuttles between compartments (Figure 10), which inevitably led to a decrease in the precision of thermoregulation (manifested in the widening of the selected T_a and T_b ranges). The fact that the frequency of shuttles at the most extreme level of hypoxia (~2I) is still well above the predicted number of shuttles were the behaviour to be purely exploratory (~8), provides further evidence that hypoxic lizards are indeed actively thermoregulating, albeit in a blunted manner. This study reveals that the hypoxic cryexic response in reptiles is not only the result of a regulated decrease in preferred T_b, but also a consequence of a decrease in the precision of behavioural thermoregulation particularly at the lower end of the T_b distribution. In other words, their tolerance or permissiveness to low T_b's is increased in hypoxia.

The higher variability at the lower spectrum of the T_b's observed in this study at all oxygen levels has been documented in normoxia by several authors (Dewitt, 1967; Berk and Heath, 1975a; Tosini and Avery, 1993) and is manifested in the negative skewness of the T_b distribution, also observed in this study. This has been attributed to the exponential relationship between physiological activities and body temperature (Dewitt, 1967; Dewitt and Friedman, 1979), the prevalence and increased sensitivity (i.e. responsiveness or slope of firing rate vs. temperature) of warm sensitive neurons compared to neurons sensitive to cold temperatures, and the exponential increase in the firing rate of warm sensitive neurons with increasing temperatures (Cabanac et al., 1967; Firth et al., 1989; reviewed in Nagashima et al., 2000). It is entirely possible that hypoxia is causing decreased sensitivity of the cold-sensitive neurons responsible for thermoregulatory control, leading to the

decreased precision and tendency to select lower T_a's. In addition, optimal temperatures

for physiological functions are often near the lethal limit (Huey and Slatkin, 1976), and

while cold temperatures can induce lethargy in most species of reptiles, it is rarely lethal, at

least in laboratory conditions where the risks associated with the immobile state are

minimal.

The significant difference in T_b (≈ 1 °C) between the beginning and the end of the

experiments observed at all oxygen levels, reveals an inherent circadian effect on the

preferred T_b of bearded dragons, with lower temperatures at noon than at the end of the day.

Although much of the literature on lizard thermoregulation has concentrated on the

difference between diurnal and nocturnal preferred temperatures (Myhre and Hammel,

1969; Chong *et al.*, 1973; Spellerberg, 1974; Cowgell and Underwood, 1979; Firth *et al.*,

1989; Tosini and Menaker, 1995; Tosini and Menaker, 1996) (temperatures usually being

higher during the light phase than during the dark phase of the photoperiod), changes in

preferred T_b within the diurnal cycle have received far less attention. It is possible that the

higher T_b's observed by the end of the day reflect an anticipation to the offset of light when

T_a drops and so, inevitably does T_b. An increase in T_b before the dark cycle begins would

enable lizards to maintain higher T_b's through a larger portion of the night. Much more

research on this respect is needed to elucidate this hypothesis.

Effect of Environmental Thermal Quality on the Level and Precision of Thermoregulation

Environmental thermal quality plays an important role on the precision of

behavioural thermoregulation in lizards; lower thermoregulatory precision occurs in

environments where thermoregulation requires higher energy investments

(e.g. environments where basking spots are scarce or distant) (Dewitt, 1967; Huey, 1974; Huey and Slatkin, 1976; Hertz *et al.*, 1993). Studies in the past have described the effect of thermal quality on the level and precision of behavioural thermoregulation on T_b alone; this is the first study that quantifies the effect of the thermal quality of an environment on the precision of both selected T_a and its effect on the resulting T_b. This information provides useful insight about the relationship between the two, shedding light on other thermoregulatory mechanisms (both behavioural and physiological) possibly involved in lizard thermoregulation; not to mention the potential role for peripheral (which could be estimated from the T_a parameters) and central thermal sensory inputs (estimated from the T_b parameters).

The observations from this study demonstrate a decrease in the precision of selected T_a with more thermally challenging environments (the "extreme temperatures" treatment or δT_a of 0.7 °C/min as opposed to 0.1 °C/min δT_a conditions); lizards exhibited significantly higher UET_a and lower LET_a with higher δT_a. Despite the significant decrease in precision in the ambient escape temperatures and the selected T_a range, the level and precision of T_b remained unaffected at the different δT_a's used in this study (Figure 12). This maintenance of T_b precision, despite the decrease in precision of selected T_a suggests the use of less energetically expensive behavioural (i.e. panting and changes in posture) and physiological mechanisms (i.e. adjustments in heart rate, cardiac shunts and changes in peripheral blood flow) to maintain preferred T_b levels. The lack of precision in T_b at the "extreme temperatures" treatment and its consequent decrease in the level of thermoregulation (lower T_b) once again confirms the notion of a decrease in the precision of thermoregulation in

thermally challenging environments. The inability to maintain a narrow T_b range (when compared to the δT_a treatments) depicts the limitations of the physiological adjustments, which are able to compensate only partially for the reduction of the use of more effective but more energetically costly behavioural strategies (i.e. shuttling).

A similar response was described by Withers and Campbell (1985) in a study of the behavioural thermoregulation of the desert iguana (*Dipsosaurus dorsalis*). They examined the precision and level of thermoregulation of these lizards inside a shuttling apparatus. This device consisted of two heat sources suspended above the floor of a box, located 1 m apart. The heating sources were on for only a set amount of time after which they were turned off automatically. The amount of time the heat sources were on was varied from 45-180 s, thus altering the frequency at which the animals needed to move to maintain a narrow T_b range (i.e. metabolic costs of locomotion during shuttling). The longer the heat source was on, the higher the thermal quality of the environment. As observed in this study *Dipsosaurus dorsalis* iguanas regulated T_b at a higher level and were more precise thermoregulators in higher thermal quality conditions (i.e. when the heating sources stayed on for longer periods of time). Nevertheless, his methodology did not allow lizards to reach upper temperature thresholds (the heat source was automatically turned off before the lizards had the chance to reach these thresholds) and therefore measurement of the precision of thermoregulation at the upper spectrum of T_b was impossible to evaluate. As a result, it is conceivable that the precision of thermoregulation would have, in fact, been affected and that the change in the level of thermoregulation would have been of smaller magnitude than the one observed in his study. Similar results were found by Campbell (1985).

Effect of Methodology on the Behavioural Thermoregulatory Response to Hypoxia

The "extreme temperatures" condition, comparable to the shuttle boxes used in other studies of behavioural thermoregulation, caused a significant decline in both the level and the precision of thermoregulation. These parameters were further decreased by exposures to hypoxia, exhibiting a more pronounced decrease in T_b at conditions of 4% oxygen at the "extreme temperatures" than at the 0.7 °C/min changing temperatures. Studies using "extreme temperatures" shuttle boxes (Myhre and Hammel, 1969; Berk and Heath, 1975a; Berk and Heath, 1975b; Hicks and Wood, 1985; Blumberg *et al.*, 2002) to study temperature regulation are thus introducing an extra "cost" variable that should be taken into account when drawing conclusions on the level of thermoregulation. The study of thermoregulatory responses to stressors under more costly conditions sets up a scenario where normal thermoregulatory behaviour may not be possible. Furthermore, while a decrease in thermoregulatory precision in hypoxic conditions was evident with the "changing temperatures" methodology (Series II experiments), the "extreme temperatures" methodology did not reveal any changes in the precision of T_b regulation. Nevertheless, the significantly greater amount of time spent by the lizards astraddle the two compartments at 4% oxygen is an indication of a decrease in "thermoregulatory effort" which in turn led to a decrease in T_b. Any study that attempts to describe the effect of hypoxia in preferred body temperature under "extreme temperatures" conditions could lead to the erroneous conclusion that an animal is highly sensitive to changes in ambient oxygen concentrations, when, in fact, the synergistic influences of environmental thermal quality and hypoxia would lead to different conclusions.
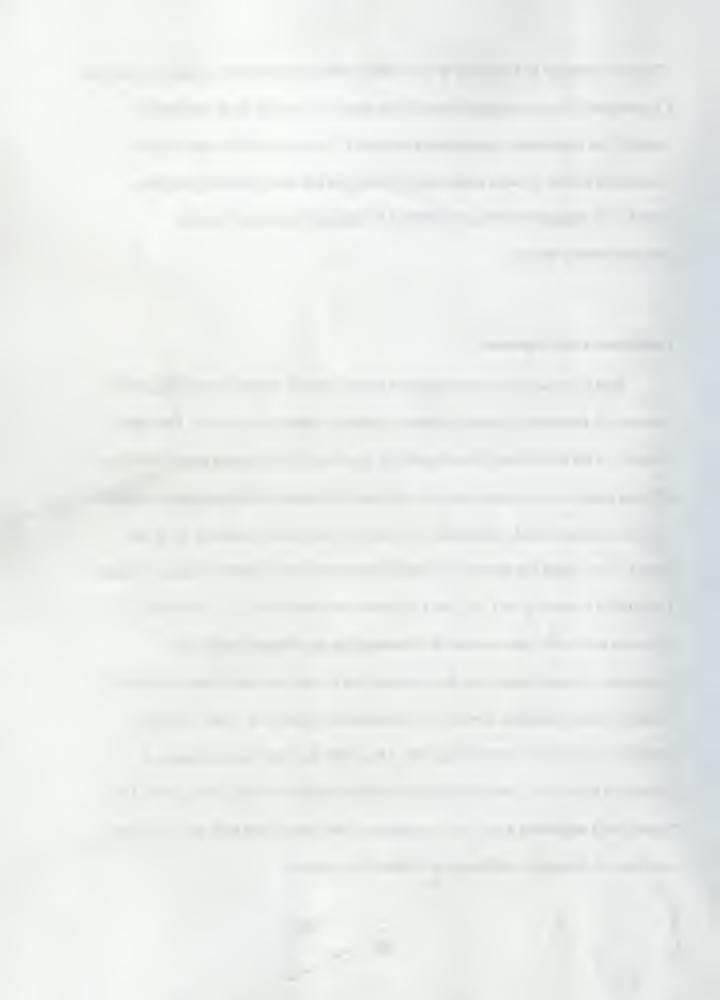
Hicks and Wood (1985) evaluated the effect of hypoxia on the preferred T_b's of five different species of lizards using a thermal gradient. The cryexic effect of hypoxia observed in their study was of a much larger magnitude than the one observed in this study (a decrease of 5 -10 °C when exposed to 7% O_2 vs. the 2.3 °C decrease at exposures to 4% O_2 observed in this study). Although this difference may be attributed to differences between the thermal preferences of the species in question, it is very likely due to methodological differences between the studies. Even though thermal gradients are likely less costly in terms of the energetic locomotory costs of thermoregulation, it is harder to discriminate between thermoregulating lizards and lizards that are not actively thermoregulating, due to lack of knowledge of an animal's motivational state (see section on *Laboratory Methods for the Study of Behavioural Thermoregulation on Ectothermic Vertebrates*). As mentioned above, lizards in a shuttle box do not actively thermoregulate in every experiment and the inclusion of data in which lizards are not actively thermoregulating may lead to a lower estimation of the preferred T_b. If, in addition, lizards in hypoxic conditions tend to decrease the amount of movement required to maintain T_b, as demonstrated by this study (Figure 10), it is possible that in a thermal gradient the animals initially (at the onset of hypoxia) search for a lower T_a within their thermal refractory zone but will not need to make any further adjustments in their position or make any corrections to its T_b. Furthermore, one cannot even be certain that a stationary animal in a thermal gradient is actually preferring a specific temperature or simply electing not to move. In contrast, the shuttle box used in this study forced actively thermoregulating lizards to move to the heating compartment as the temperatures in the cooling compartment kept decreasing and became more and more distressful for the animals. If this is the case, the cryexic effect

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of hypoxia observed by Hicks and Wood (1985) could have been overestimated resulting in a T_b decrease of larger magnitude than the one actually expected. More importantly, however, any conclusions regarding an ectotherm's "T_b set-point" from these kinds of experiments need to be taken cautiously in light of the fact that an actively searching animal is not necessarily moving to different thermal environments for strictly thermoregulatory reasons.

Conclusions and Perspectives

Both hypoxia and low environmental thermal quality elicited a similar decrease in the level and precision of thermoregulation of bearded dragons (Figure 16). The striking similarity of the behavioural thermoregulatory response observed in this study to two very different stimuli (O_2 concentration and environmental thermal quality) suggests a possible common mechanism and neural pathway to the thermoregulatory response. Berk and Heath (1975a) found that lesions in the medial preoptic region of the hypothalamus caused a significant increase in the UET_b and a significant decrease in the LET_b in the lizard *Dipsosaurus dorsalis* when allowed to thermoregulate in a thermal shuttle box. Furthermore, similar studies have demonstrated that the selected temperature of collared lizards and green iguanas is lowered with pinealectomy (Firth *et al*., 1989; Tosini and Menaker, 1996) during the activity period. The results from these studies suggest a decrease in the precision and level of thermoregulation similar to the one observed in the present study suggesting a possible impairment of this region of the brain at low oxygen conditions or thermally challenging environments in general.
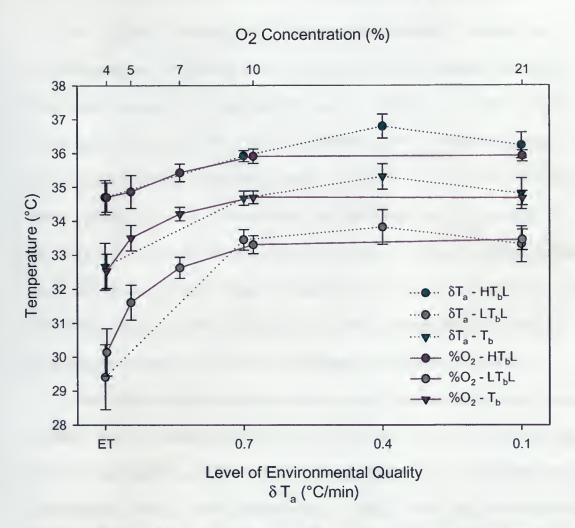
Figure 16. Preferred T_b, and high and low limits of the T_b range (HT_bL and LT_bL, respectively) at the different oxygen conditions (at 0.7 °C δT_a) and levels of environmental thermal quality evaluated in this study. Note the similarity of the thermoregulatory response between both types of stimuli. Values are plotted as means of medians for 12 lizards (± SE) during 7.5 h of exposure to each of the experimental conditions. The same individuals were used throughout the study and exposed to the different treatments.

Based on the observations made in this study, I propose three strategies for lizard thermoregulation in relation to the magnitude of the thermoregulatory costs involved (physiological, such as metabolic costs; or ecological, such as predator avoidance, territorial behaviour or courtship). 1) When the costs of thermoregulation are low, lizards will thermoregulate using almost exclusively very effective but energetically expensive behavioural mechanisms (i.e. shuttling between sun and shade). 2) As the costs of thermoregulation increase, lizards will use other less expensive behavioural mechanisms such changes in posture and panting or gaping. 3) Finally, in environments with very low thermal quality, where the costs associated with thermoregulation are very high, physiological mechanisms such as changes in heart rate, peripheral blood flow, and cardiac shunts are activated. These strategies are transitional and not mutually exclusive as lizards will slowly abandon shuttling to replace it with posture changes and panting and finally increasing the extent to which they use physiological mechanisms (Figure 17).

Because the thermoregulatory mechanisms used in environments where thermal quality is high (i.e. shuttling) are more effective than those used in environments where environmental thermal quality is poor (i.e. panting, changes in posture and physiological mechanisms), thermoregulatory precision is sacrificed in lower quality environments; and because the decrease in precision is mostly manifested at the low end of the T_b distribution (as discussed above), the level of T_b necessarily drops in poor thermal quality habitats. The conceptual model proposed here conforms to the cost-benefit model of lizard thermoregulation proposed by Huey and Slakin (1976) that predicts lower temperatures and less careful thermoregulation in habitats where the costs associated with thermoregulation are high.

Figure 17. Diagrammatic representation of the thermoregulatory mechanisms used by lizards and their relation to the environmental thermal quality. The mechanism used by lizards to thermoregulate, are more expensive (energetically speaking) but also more effective (i.e. shuttling) at environments with higher thermal quality (i.e. environments where the costs of thermoregulation are low). As the thermal quality of the environment decreases, lizards engage in less expensive, but also less effective thermoregulatory mechanisms (i.e panting, changes of posture and physiological adjustments). The line represents the thermoregulatory strategies used by a lizard along with their effectiveness and energetic costs at different levels of environmental thermal quality.

The cryexic response to hypoxia observed in this and previous studies (Hicks and Wood, 1985) can be partly explained by an increase in the costs of thermoregulation and the consequent decrease in thermoregulatory precision. This explanation may be extended to the cryexic effect evoked by other stress stimuli, such as low environmental thermal quality, predation risks, territorial defence or water availability. It is likely that the neural pathways that control temperature regulation in lizards are affected in a similar way under all these very different stimuli.
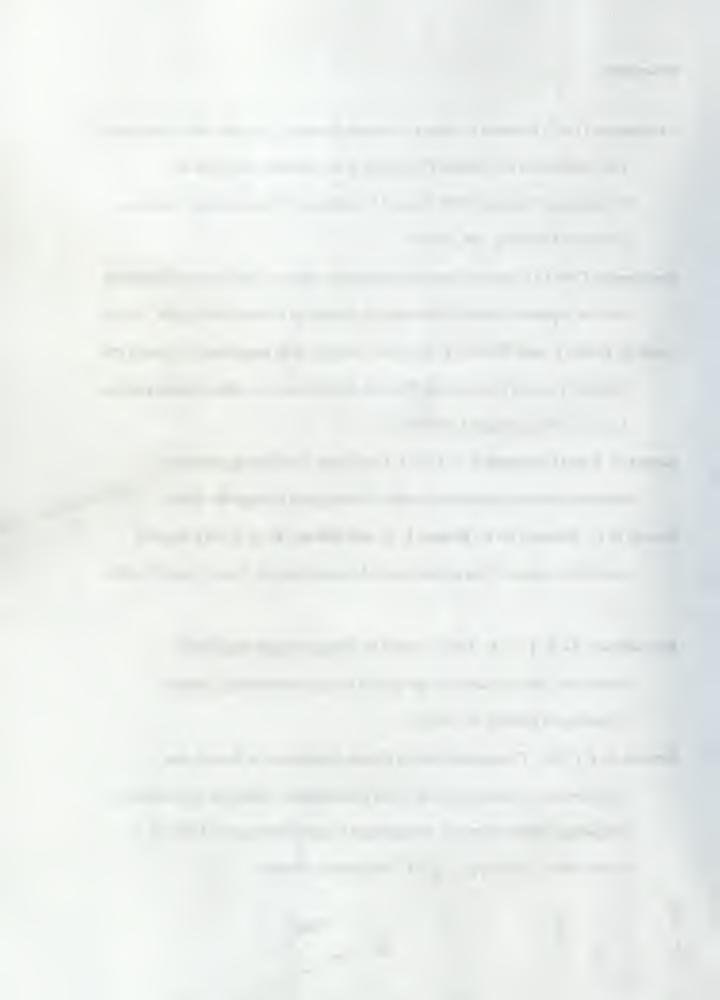
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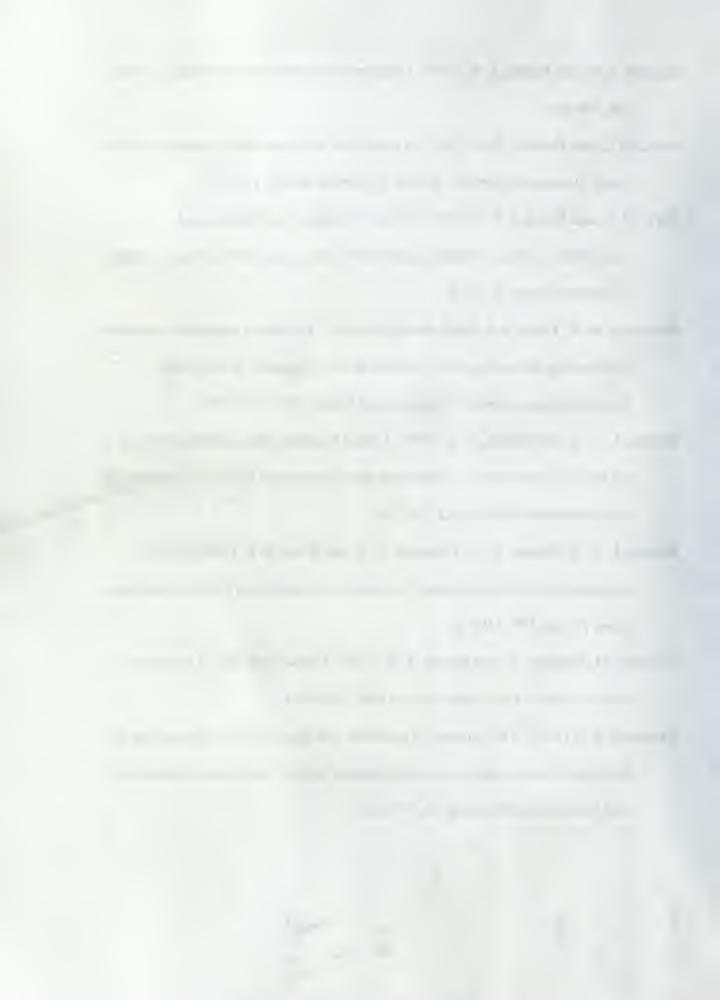**Bennett, A. F.** (2004). Thermoregulation in african chameleons. In *Animals and Environments. Proceedings of the Third International Conference of Comparative Physiology and Biochemistry. International Congress Series*, vol. 1275 eds. S. Morris and A. Vosloo), pp. 234-241. Amsterdam: Elsevier.
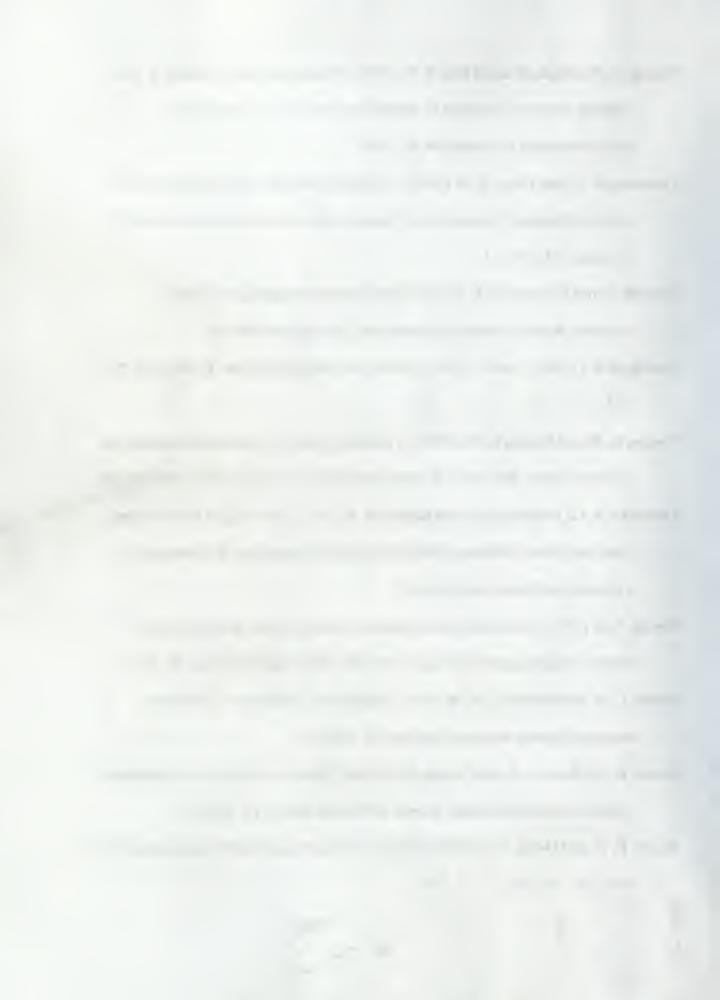
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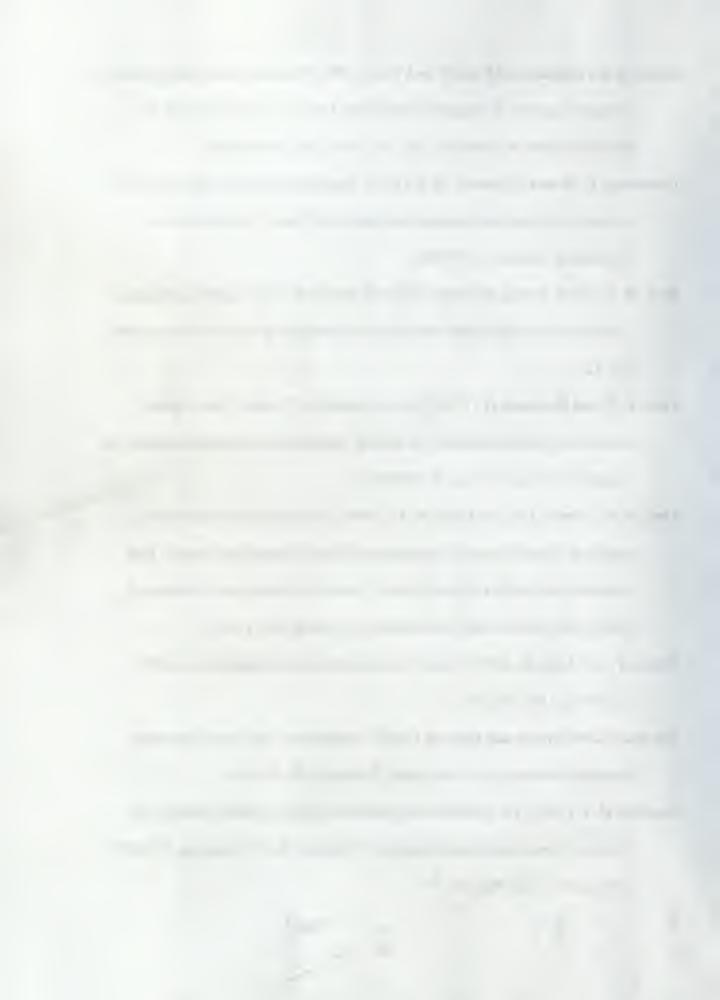
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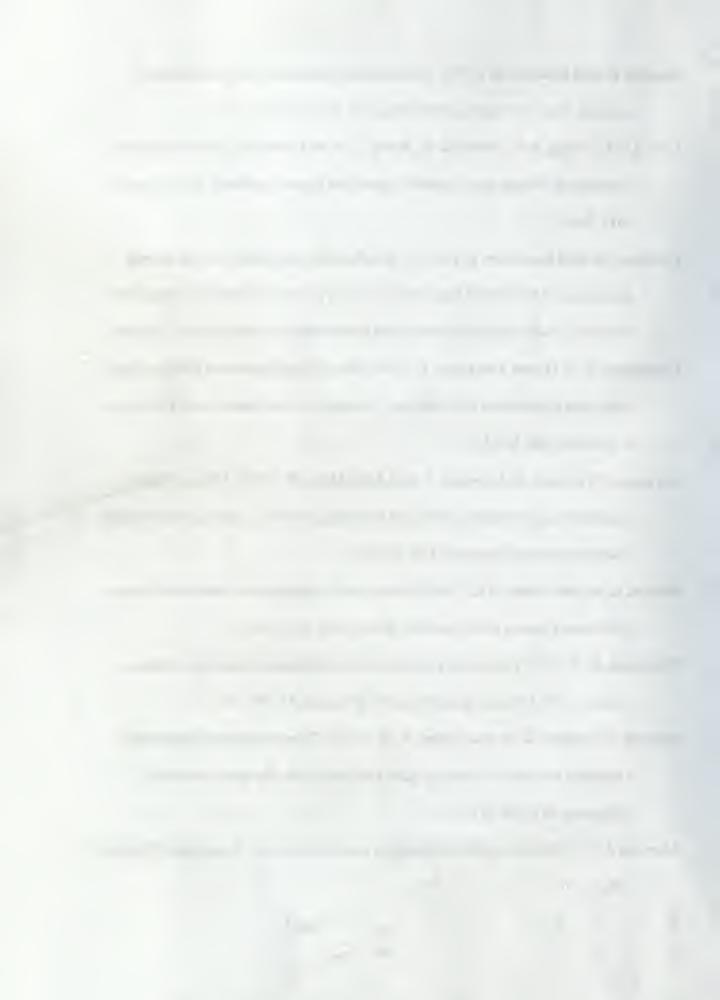
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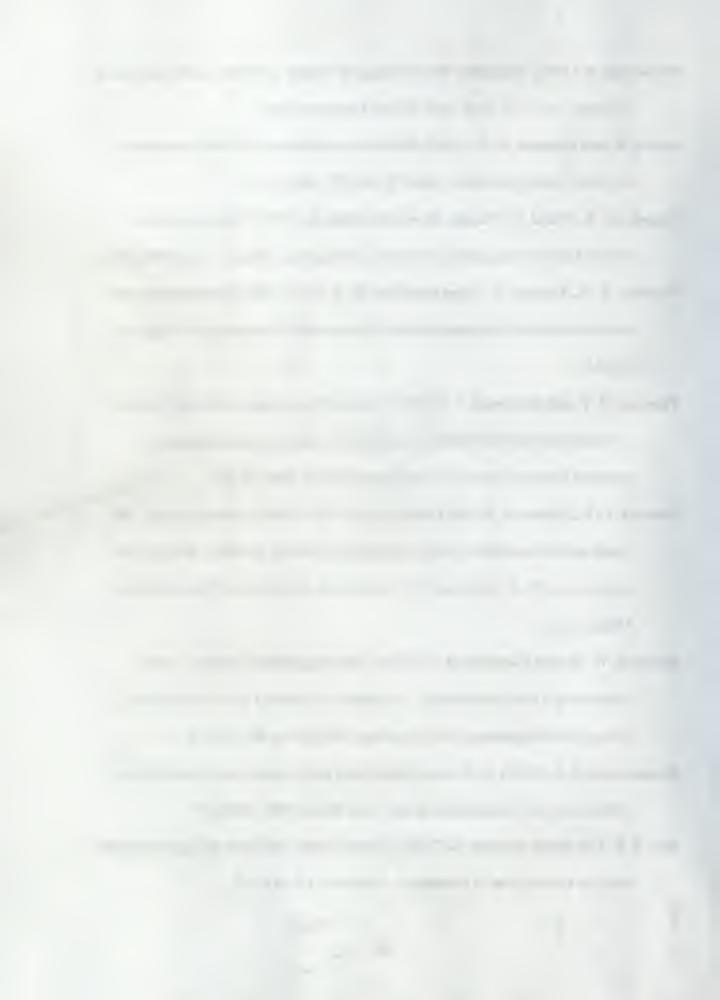
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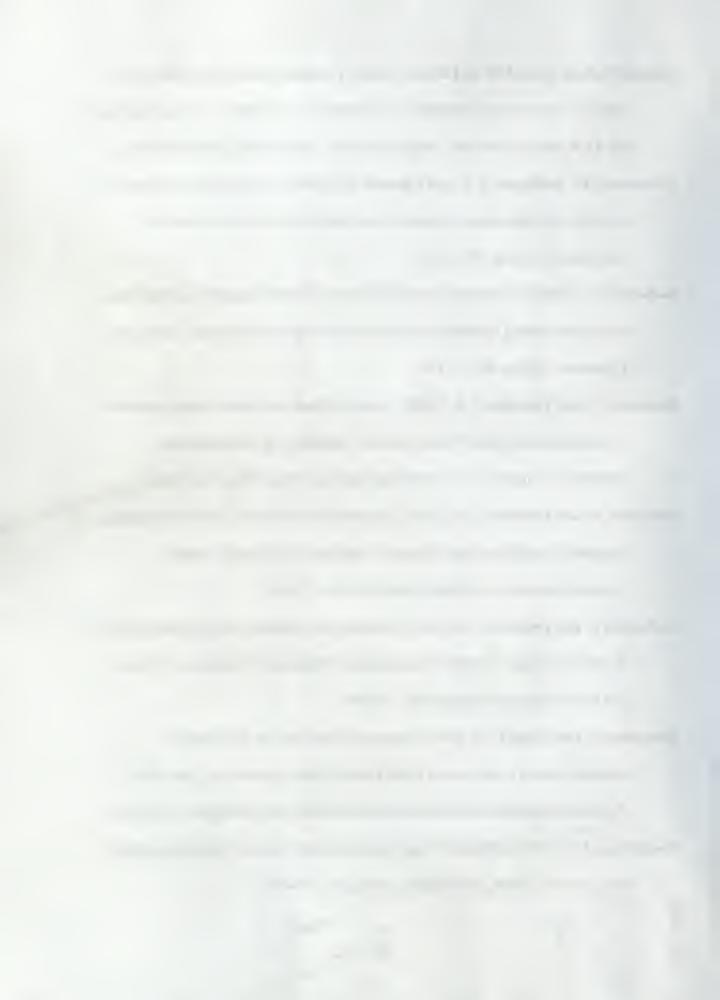McCauley, R. W. (1977). Laboratory methods for determining temperature preference. *Journal of the Fisheries Research Board of Canada* **34**, 749-752.

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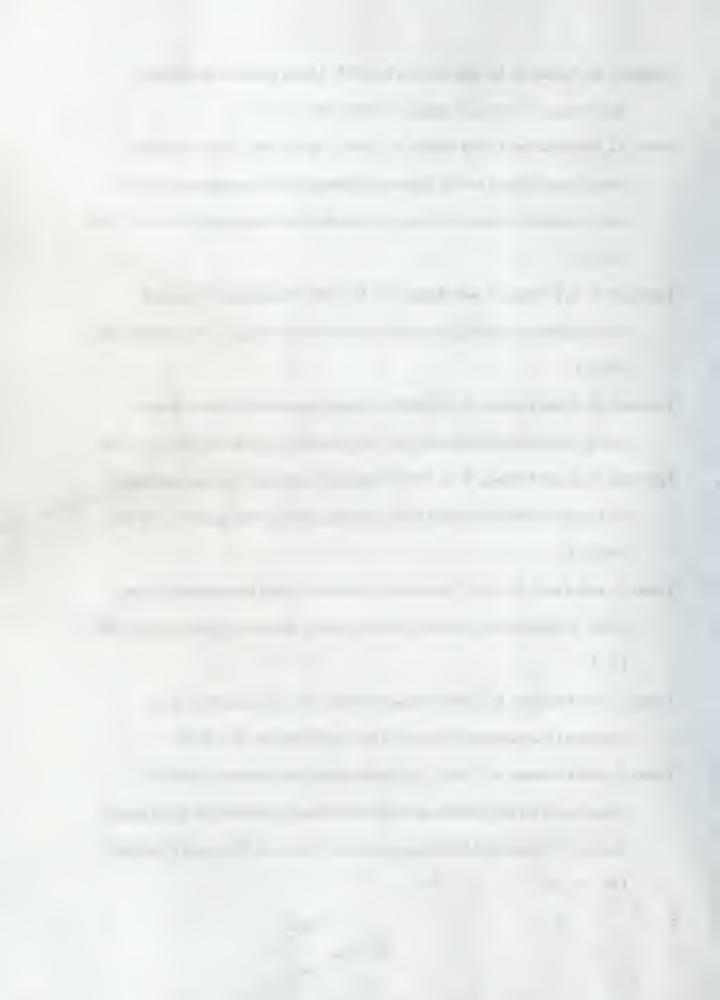Mortola, J. P. (1999). How newborn mammals cope with hypoxia. *Respiration Physiology* **116**, 95-103.

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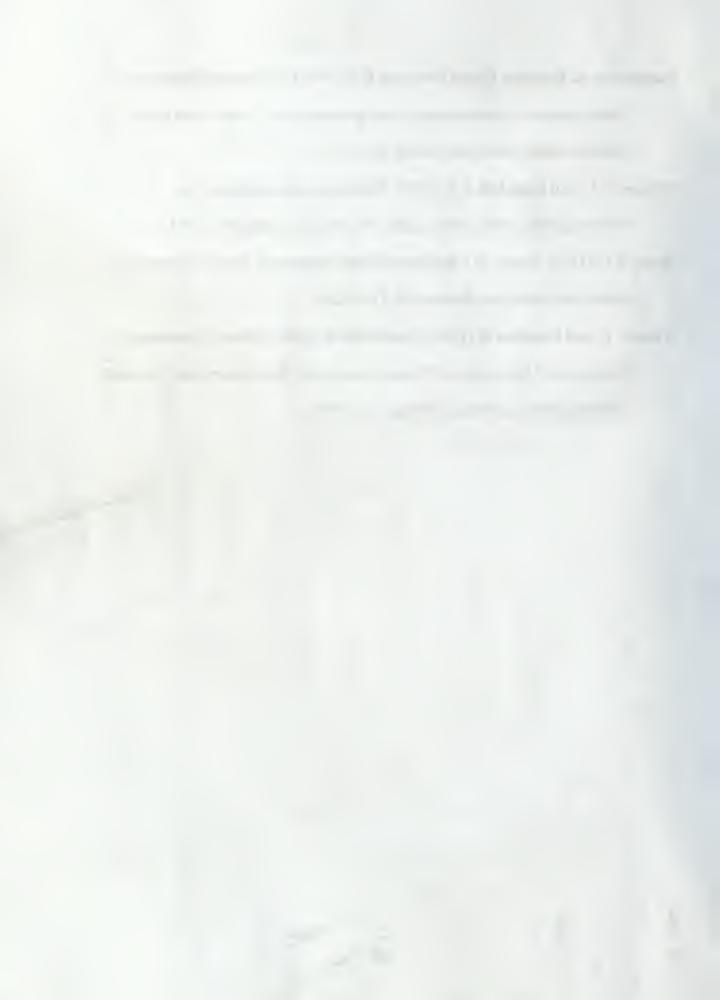
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APPENDIX A

Number of trials (total trials = 167) required for each lizard at each treatment for the different series of experiments performed in this study for active thermoregulation to be observed throughout the experiment (i.e. shuttle back and forth rather than remain in one compartment). * Indicates lizards that required more than two repetitions at one or more of the treatments. As indicated below in the relevant treatment combination row, some treatments were common to more than one series of experiments. Dashes (-) represent a treatment combination for a particular lizard which was abandoned.

Experiment Series		Series II				Series II, III	Series III		Series III, IV	Series IV
Relevant Treatment Combination		Level of O₂				Level of O₂; δTₐ	Level of Environmental Thermal quality		Level of Environmental Thermal quality; Level of O₂	Level of Environmental Thermal quality; Level of O₂
		4%	5%	7%	10%	21%; 0.7 °C/min	0.4 °C/min δTₐ	0.1 °C/min δTₐ	extreme temperatures; 21%	Extreme temperatures; 4%
Lizard ID	Sex									
C1	♂	1	1	1	1	1	1	1	2	1
C2	♂	1	1	1	2	2	2	2	2	1
C3	♂	1	1	1	1	1	2	2	1	1
C4	♂	1	1	1	1	2	1	1	2	2
C5*	♂	3	3	2	1	1	2	1	1	-
C7	♀	2	1	1	2	1	1	2	2	1
C8	♀	2	1	2	1	1	1	1	1	1
D2	♂	1	1	1	1	1	1	1	1	1
E0*	♀	1	2	1	1	>6	-	-	1	-
E5*	♂	1	1	>6	2	4	2	1	1	1
G3*	♂	1	2	2	1	>7	-	-	2	1
H1*	♀	6	1	6	3	2	2	3	2	1

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