

**Orientation Preference and Behavioural Thermoregulatory Coordination in
Pogona vitticeps.**

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Table of Contents

Acknowledgments	3
Index of Figures	6
Index of Tables	7
Abstract	10
Chapter 1 General Introduction	11
The Impact of Environment on an Organism	11
Importance of Temperature	13
Maintaining Thermal Optimality.....	15
Thermoregulatory Behaviours	20
Thermoregulatory Precision	30
Thermoregulatory Control Mechanisms.....	33
Abiotic Influences on Thermoregulation.....	37
Examining Behavioural Thermoregulation in Ectotherms in a Laboratory Environment	41
Thesis Rational and Objectives	45
Chapter 2 Thermoregulatory Behaviour in Adult and Neonatal Bearded Dragons	46
Introduction	46
Methods	50
Animal Husbandry	50
Thermal Preference and Orientation Measurement in Adults Protocol.....	51
Neonatal Thermoregulatory Behaviours Protocol	52
Experimental Design.....	52
Data Analysis	53
Results	56
Series I: Behavioural Responses in Adult Bearded Dragons.....	56
Series II: Behavioural Responses in Neonatal Bearded Dragons	61
Discussion.....	64
Ancillary Experimental Effects: Mass, Time, Sex, and Movement	65
Main Experimental Effects: Orientation and Selected Temperature	65
Conclusions: Thermoregulatory Orientation	66
Chapter 3 Orientation Preference and Influences on Adult Bearded Dragon Thermoregulation	69
Introduction	69
Methods	72
Animal Husbandry	72
Thermal Preference and Orientation Measurement Protocol.....	72

Tail Insulation Protocol.....	74
Mouth Sealing Protocol	74
Experimental Design.....	75
Data Analysis	78
Results	80
Series III: Rates of Heat Gain	80
Series IV: Behavioural Responses in Tail Insulated and Sham Treatments	83
Series V: Behavioural Responses in Gaping Inhibited and Gaping Inhibition Sham Treatments.....	89
Discussion.....	95
Ancillary Experimental Effects: Mass, Time, Sex, and Movement	96
Main Experimental Effects: Selected Temperature, Gaping, and Orientation	98
Conclusion: Thermoregulatory Coordination	101
Chapter 4: Summary and Future Directions	103
Summary	103
Future Directions	103
Work Cited	105
Appendix.....	114

Index of Figures

Figure 1.1 Graphical representation of a performance curve.....	16
Figure 1.2 Graphical representation of the change in body and brain temperature over time.....	26
Figure 2.1 Influence of time, sex, and orientation on selected ambient temperature in adult bearded dragons	57
Figure 2.2 Model predicted probability that adult bearded dragons will orient towards a heat source	59
Figure 2.3 Model predicted probability that adult bearded dragons will move	60
Figure 2.4 Selected ambient temperature in neonatal bearded dragons.....	62
Figure 2.5 Model predicted probability that neonatal bearded dragons will face a heat source...	63
Figure 3.1 Effect of mass and sex on the rate of heat gain in adult bearded dragons.....	82
Figure 3.2 Selected ambient temperature in adult bearded dragons during treatments TI and SH	84
Figure 3.3 Model predicted probability that adult bearded dragons gape	86
Figure 3.4 Chances that TI and SH adult bearded dragons will face a heat source.....	87
Figure 3.5 Model predicted probability that TI and SH adult bearded dragons will move	88
Figure 3.6 Selected ambient temperature in adult bearded dragons during IG and GS treatments	90
Figure 3.7 Model predicted probability that GS bearded dragons will gape	91
Figure 3.8 Model predicted probability that adult bearded dragons will face a heat source	93
Figure 3.9 Model predicted probability that adult bearded dragons will move.....	94

Index of Tables

Table 2.1 Top four models, nested in the global model run through a LME analyzing selected ambient temperature in adult bearded dragons	114
Table 2.2 Type II Wald’s analysis of deviance on the top model, selected from table 2.1, analyzing selected ambient temperature in adult bearded dragons.....	114
Table 2.3 Top four models, nested in the global model run through a GLMM analyzing preferred orientation in adult bearded dragons.....	115
Table 2.4 Type II Wald’s analysis of deviance on the top model, selected from Table 2.3, analyzing the preferred orientation in adult bearded dragons	115
Table 2.5 Top four models, nested in the global model run through a GLMM, analyzing movement in adult bearded dragons	116
Table 2.6 Type II Wald’s analysis of deviance on the top model, shown in Table 2.5, analyzing the movement in adult bearded dragons	116
Table 2.7 Top four models, nested in the global model run through a LME, analyzing selected ambient temperature in neonatal bearded dragons	116
Table 2.8 Type II Wald’s analysis of deviance on the top model, shown in Table 2.7, analyzing the selected ambient temperature in neonatal bearded dragons	117
Table 2.9 Top four models, nested in the global model run through a GLMM, analyzing preferred orientation in neonatal bearded dragons	117
Table 2.10 Type II Wald’s analysis of deviance on the top model, shown in Table 2.9, analyzing the orientation preference in neonatal bearded dragons	117
Table 3.1 Top four models, nested in the global model run through a LME, analyzing rate of heat gain in adult bearded dragons	118
Table 3.2 Type II Wald’s analysis of deviance on the top model, shown in Table 3.1, analyzing the rate of heat gain in adult bearded dragons	118

Table 3.3 Top four models, nested in the global model run through a LME, analyzing selected ambient temperature in TC and TS adult bearded dragons	119
Table 3.4 Type II Wald’s analysis of deviance on the top model, shown in Table 3.3, analyzing the selected temperature in TC and TS adult bearded dragons	119
Table 3.5 Top four models, nested in the global model run through a GLMM analyzing probability of gaping in TC and TS adult bearded dragons	120
Table 3.6 Type II Wald’s analysis of deviance on the top mode, shown in Table 3.5, analyzing the chance of gaping in TC and TS adult bearded dragons	120
Table 3.7 Top four models, nested in the global model run through a GLMM, analyzing probability of facing a heat source in TC and TS adult bearded dragons	120
Table 3.8 Type II Wald’s analysis of deviance on the top model, shown in Table 3.7, analyzing the chances of facing a heat source in TC and TS adult bearded dragons	121
Table 3.9 Top four models, nested in the global model run through a GLMM, analyzing probability of movement in TC and TS adult bearded dragons	121
Table 3.10 Type II Wald’s analysis of deviance on the top model, shown in Table 3.9, analyzing the chances of movement in TC and TS adult bearded dragons	121
Table 3.11 Top four models, nested in the global model run through a LME, analyzing selected ambient temperature in IG and GS adult bearded dragons	122
Table 3.12 Type II Wald’s analysis of deviance on the top model, shown in Table 3.11, analyzing the selected temperature in IG and GS adult bearded dragons	122
Table 3.13 Type II Wald’s analysis of deviance on the global model analyzing the chance of gaping in IG and GS adult bearded dragons	122
Table 3.14 Top four models, nested in the global model run through a GLMM, analyzing probability of facing a heat source in IG and GS adult bearded dragons	123

Table 3.15 Type II Wald’s analysis of deviance on the top model, shown in Table 3.14, analyzing the chances of facing a heat source in IG and GS adult bearded dragons123

Table 3.16 Top four models, nested in the global model run through a GLMM, analyzing probability of movement in IG and GS adult bearded dragons123

Table 3.17 Type II Wald’s analysis of deviance on the top model, shown in Table 3.16, analyzing the chances of movement in IG and GS adult bearded dragons124

Abstract

Regulating body temperature is a critical function for vertebrates and many invertebrates. Vertebrates that rely on ambient temperature as a heat source (ectotherms) make use of multiple voluntary and involuntary behaviours to thermoregulate, including body orientation. The purpose of this work was to examine orientation behaviour in bearded dragons (*Pogona vitticeps*) as well as possible coordination between thermoregulatory responses. Both adult and two week old neonatal bearded dragons were placed in a thermal gradient and left to thermoregulate behaviourally in order to observe the presence of a thermoregulatory orientation preference. Since a thermal orientation bias could manifest from a preference for favoring rostral versus caudal traits, animals were subsequently subjected to a separate experiment, in which either heat exchange across the tail or respiratory heat exchange from gaping was inhibited. Changes in thermoregulatory behaviour were then observed in the manipulated bearded dragons in order to assess the potential contributors to thermal preferences as well as isolate potential coordination between behaviours. Both adult and two week old neonatal dragons displayed a non-random orientation preference for facing a heat source that strengthened with time exposed to a thermal gradient, supporting this behaviour as a thermoregulatory response. Bearded dragons also exhibited changes in orientation preference and gaping behaviour when the tail was insulated and when gaping was inhibited, but no changes to selected ambient temperature, indicating that the changes to gaping and orientation behaviours are compensatory responses that support the presence of coordination between thermoregulatory behaviours.

Chapter 1 General Introduction

The Impact of Environment on an Organism

All animals are affected by, and have an effect on, their environment. Any effect that an organism's surroundings have on the organism can be either negative or positive, and animals must be able to adjust to these effects, to avoid negative consequences. While the relationship between an animal and its environment is a fundamental aspect of biology, the relationship is not necessarily simple. A single, seemingly stable environment will have multiple environmental factors that will impact an organism; these factors range from abiotic (such as temperature, oxygen concentration and humidity) to biotic (such as predator and prey abundance) (Cadena & Tattersall, 2009a; DeWitt, 1967; Hicks & Wood, 1985; Huey & Webster, 1976; Malvin & Wood, 2005; Scarpellini, Bicego, & Tattersall, 2015; Wood, 1995). The animal in question will be exposed to most of these factors simultaneously and must respond to them simultaneously as well. In the natural world, there are countless different environments and they are never completely stable, further complicating an animal's interaction with its surroundings. The type and severity of an animal's response to environmental factors can also vary between different species (Bowker & Johnson, 1980; Blouin-Demers & Weatherhead, 2001).

There are two different overarching environments that relate to animals, their external environment (consisting of everything outside the animal's body) and their internal environment (consisting of everything inside the animal's body) (Bernard, 1878, as seen in Mrosovsky, 1990). As the external environmental factors fluctuate and influence an organism's internal environment, animals either make adjustments to keep their internal environment relatively constant, also referred to as maintaining homeostasis (these animals are regulators), or they allow their internal environmental factors to shift to that of the external environment (these animals are conformers) (Sanborn, 2005). Only regulators react to changes in their external and internal

environments. A change in an external environmental factor leads to a change in an internal parameter, which, once detected by a sensory process, causes the animal to evoke a behavioural or physiological response to correct any deviation of the internal environment (Angilletta, 2009; Jessen, 2001; Mrosovsky, 1990; Sanborn, 2005). If the effect of an external environmental factor has a negative impact on the internal environment of the animal, then the response will most likely be a compensatory one that aids in re-establishing homeostasis of the internal environment (Angilletta, 2009; Jessen, 2001; Mrosovsky, 1990;). In nature, there are no perfect conformers or regulators in the animal kingdom; instead, most species can be placed on a spectrum between the two extremes (Angilletta, 2009).

At the cellular level, the effects of external and internal environmental factors can be seen as changes in the interactions between different organic macromolecules as well as between macromolecules and ions. Since most individual organic macromolecules and ions found in animals are part of larger chains of macromolecules and ions, called biochemical pathways, changes to an animal's surroundings can also affect the functions of that animal's biochemical pathways (Dawson, 1975, as seen in Vandamme, Bauwens, & Verheyen, 1991). Changes to the internal environment of an animal can cause many pathways to be sped up or slowed down. In extreme cases, pathways can even stop functioning completely (Angilletta, 2009). Most pathways have an optimal rate of performance that is reached and sustained with aid from environmental factors being at or within specific, optimal levels. Should environmental factors deviate too far from this optimum level, the pathways that are affected can be shut down (Angilletta, 2009).

Temperature is an abiotic environmental factor that plays an important role in almost every aspect of any animal's life, including reproductive fitness (Blumberg, Lewis, & Sokoloff, 2002; Christian & Tracy, 1981; Vandamme et al., 1991). Temperature changes can influence predation escape responses (Christian & Tracy, 1981), feeding (Vandamme, et al., 1991), and

reproduction (Blumberg et al., 2002), showing a clear impact on fitness. As with any environmental factor, animals will either regulate their internal environmental temperature with regards to the external ambient temperature to maintain a relatively stable body temperature (homeotherms), or their body temperature will conform to that of the ambient temperature (poikilotherms) (Moyes & Schulte, 2008; Jessen, 2001; Sanborn, 2005; Tattersall, et al., 2012). The process of regulating an animal's internal environmental temperature (body temperature), with regards to the ambient temperature, is called thermoregulation (Jessen, 2001; Moyes & Schulte, 2008; Sanborn, 2005).

Importance of Temperature

All animals, regardless of phylogeny, are impacted by a variety of different environmental factors that they must address. Temperature is one such factor that can have a significant impact on performance and fitness. In order to account for changes that can affect body temperature, organisms have developed numerous ways to either survive extreme core temperature changes, or to regulate core temperature despite ambient fluctuations (Bale, 1987; Bennett, 2004; Cowles & Bogert, 1944; Dzialowski & O'Connor, 2001). Some more poikilothermic animals, such as some invertebrates and select amphibians (Bale, 1987; Layne Jr. & Jones, 2001; Lee Jr., Costanzo, Davidson, & Layne Jr., 1992), have evolved the means to survive extreme core temperature, while animals that are relatively homeothermic are present amongst both invertebrates and most vertebrates (Angilletta, 2009; Cowles & Bogert, 1944; Jessen, 2001; Moyes & Schulte, 2008; Tattersall, et al., 2012). Regardless of phylogeny, all animals that thermoregulate must dedicate varying degrees of time and energy to regulating body temperature (Jessen, 2001; Moyes & Schulte, 2008; Sanborn, 2005).

The consequences of no thermoregulation, or failed thermoregulation, in an animal depend heavily on the species and the severity of the ambient temperature they experience. In

extreme cases, should an organism get so cold that the body begins to freeze, extensive damage can occur. The formation of ice crystals can pierce cell membranes and destroy cellular structures, eventually leading to loss of function (Bank, 1973; Bank, 1974; Bank & Mazur, 1973; Diller, 1975; Mazur, 1977). Despite this, many animals have developed the means to tolerate or resist freezing, i.e.: antifreeze proteins, cryoprotectants and super cooling (Bale, 1987; Layne Jr. & Jones, 2001; Storey & Storey, 1986). A prime example of this is *Rana sylvatica*, a species of frog capable of surviving a significant percentage of body water being frozen (Lee Jr. et al., 1992). Similarly, in extreme cases where the temperature gets too hot, animals can be faced with cell damage and denaturation of proteins (Leach, Peters, & Rossiter, 1943; Lee & Astumian, 1996). Other animals have found ways to cope with these extremes as well. Heat shock proteins, for example, help many species deal with thermal stress when their body temperature strays too far from tolerable levels (Feder & Hofmann, 1999; Hartl, 1996; Morimoto & Santoro, 1998; Pelham, 1986; Pirkkala, Nykanen, & Sistonen, 2001).

For animals that cannot survive overheating or freezing, preventing body temperature from reaching a deadly extreme is clearly important; however, temperatures that cause such high damage are generally less common in habitats with high species diversity and density, such as tropical or temperate regions (Allen, Brown, & Gillooly, 2002; Buckley, Hurlbert, & Jetz, 2012). While habitats with high species diversity and density rarely experience temperature extremes (Buckley et al., 2012), temperature can still have a significant impact on the life cycles of many animals in these habitats. An example of this can be seen during the developmental stages of most animals' lives. Reptiles, for example, can have an interesting temperature influence on their sex phenotype. Many reptiles show the typical genetic-dependent sex determination (GSD), but others express a temperature-dependent sex determination (TSD), either in addition to or at the expense of GSD (Janzen, 1991; Pough, et al., 2016; Radder, Quinn, Georges, Sarre, & Shine, 2008; Shine, Elphick, & Donnellan, 2002). Traditionally reptile sex determination was thought to

be either GSD or TSD exclusively (Janzen, 1991), but it has been recently shown that there is a third possibility (Quinn, et al., 2007; Radder et al., 2008; Shine et al., 2002). Quinn et al. (2007) have shown that some reptiles that exhibit GSD can also have their sex influenced by temperature. The presence of both GSD and TSD in a single animal suggests that these mechanisms are not as separate as was first thought, and that animals originally considered TSD may have a genetic component to their sex determination as well (Quinn, et al., 2007).

As with many environmental factors, there is an optimal range of temperatures at which biochemical pathways and metabolic rate show relatively higher performance (Huey & Stevenson, 1979; Moyes & Schulte, 2008; Tattersall, et al., 2012). Specifically, temperature impacts the kinetic energy of biochemical pathways, causing their performance rates to change, which in turn impacts an animal's performance as a whole (Moyes & Schulte, 2008). Keeping pathways within their optimal temperature range can have noticeable benefits, while operating outside these temperature ranges can have a negative impact (Moyes & Schulte, 2008; Tattersall, et al., 2012). Remaining within this "thermally optimal" range is important enough for most animals to invest energy into some form of thermoregulation.

Maintaining Thermal Optimality

There is usually a temperature for any given metabolic and physiological process that yields a maximum performance (Huey & Stevenson, 1979). This performance maximum (P_{\max}) is found within a temperature range that encompasses a maximal percentage of performance, called the performance breadth (Figure 1.1) (Huey & Stevenson, 1979). Typically, the performance breadth is defined as the range of temperatures over which physiological performance remains at or above a predetermined percentile of the maximum, often 80% although this can change depending on the model (Huey & Stevenson, 1979). In addition, there will also be a critical maximum (CT_{\max}) and minimum (CT_{\min}) temperature which, if surpassed,

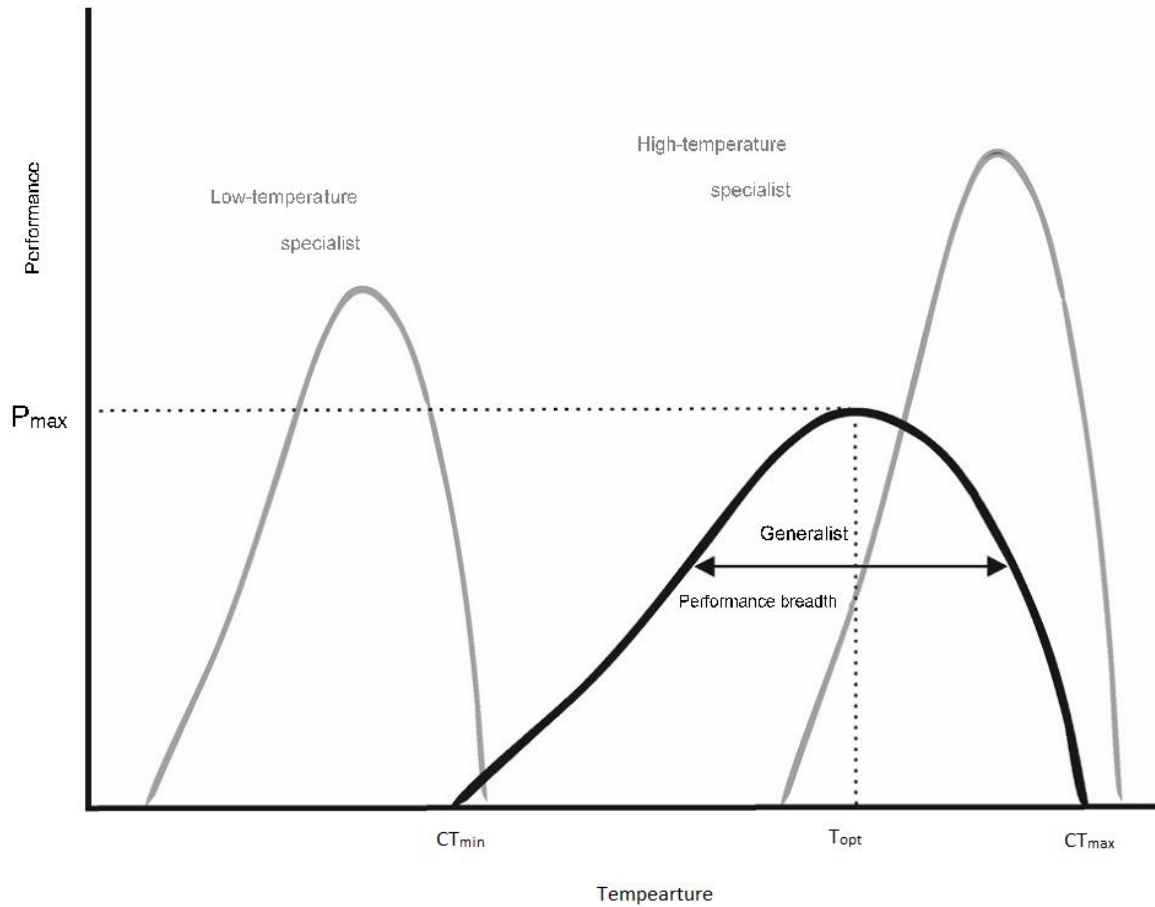


Figure 1.1. Graphical representation of a performance curve, focusing on thermal generalists (dark curve) and comparing to both low and high-temperature specialists (grey curves). Also shown, for the thermal generalist curve, is the performance maximum (P_{max}) as well as the optimal temperature (T_{opt}), the performance breadth, the critical minimum temperature (CT_{min}), and the critical maximum temperature (CT_{max}). Adapted from Tattersall et al. 2012.

will prevent the pathway from working at all (Huey & Stevenson, 1979). As shown in Figure 1.1, the P_{\max} combined with a critical maximum and minimum temperature creates a curve which indicates that performance rises with temperature from the critical minimum, until an optimal temperature (T_{opt}) is reached, after which performance falls until the critical maximum is reached (Huey & Stevenson, 1979; Tattersall, et al., 2012). In the relationship between temperature and performance, the slope always takes on this general shape, rising from the CT_{\min} to the T_{opt} then dropping to the CT_{\max} (Tattersall, et al., 2012). As temperature rises past the CT_{\min} value, the kinetic energy of the pathway under examination rises, thus making the likelihood of interaction between the different molecules and structures of the pathway higher (Moyes & Schulte, 2008). The increased interaction causes performance to rise as the pathways complete faster, until the T_{opt} is reached. Once the T_{opt} is surpassed, performance of the pathway begins to decline until the CT_{\max} . The drop in performance is due to the temperature being so high that the proteins involved in the pathway either denature or lose effectiveness (Moyes & Schulte, 2008). A loss of effectiveness in proteins, due to high temperature, can be caused by multiple factors, such as a loss of activation sites, alterations in ionization state for critical amino acids or an alteration in the enzyme's ability to undergo conformational changes (Moyes & Schulte, 2008). Therefore, thermoregulation is not simply a response to life threatening temperatures, but rather a continuous process that works to keep body temperature within optimal ranges, as much as possible (Angilletta, 2009).

While the basic shape of temperature related performance curves is consistent, many of the specifics can vary from species to species and from pathway to pathway (Huey & Stevenson, 1979; Tattersall, et al., 2012). Figure 1.1 shows examples of three different types of performance curves: low-temperature specialist, temperature generalist, and high-temperature specialist. High and low-temperature specialists have higher performance around a smaller range of temperatures in relation to generalists (Huey & Stevenson, 1979; Tattersall, et al., 2012). The benefit of being

a specialist is that a relatively higher P_{\max} can be achieved, but body temperature must remain closer to the T_{opt} in comparison to generalists. Specialists would, therefore, likely thermoregulate more precisely than generalists to maintain body temperature within this tighter range (Huey & Stevenson, 1979; Tattersall, et al., 2012).

Organisms can be broadly classified as being either endotherms (animals that generate metabolic heat) or ectotherms (animals that rely on the ambient temperature as a heat source) (Moyes & Schulte, 2008; Tattersall, et al., 2012). Most animals, however, also exhibit some form of thermoregulation, either through behaviours or through physiological processes, and therefore can be further categorised as being either homeotherms (animals with a relatively constant body temperature) or poikilotherms (animals with a variable body temperature which changes in response to the environment) (Moyes & Schulte, 2008; Tattersall, et al., 2012). Therefore, an ectothermic species that inhabits a region with a constant ambient temperature might be considered homeothermic simply because the environment does not fluctuate enough to change core temperature. While the term homeothermic is still commonly used (Mrosovsky, 1990), the term poikilothermic is less common and is often incorrectly used interchangeably with ectothermic (Tattersall, et al., 2012). The majority of invertebrates as well as many reptiles and amphibians are ectotherms and often fall more on the poikilothermic side of the spectrum.

It is generally accepted that ectothermy is the ancestral condition with respect to thermogenic heat production (e.g. endothermy) and is shared by all living invertebrates and most of the non-mammalian and non-avian vertebrates (Grigg, Beard, & Augee, 2004). A connection between the ancestral condition and ectothermy is supported by the fact that ectothermy is often present in taxa that are common ancestors to ectothermic and endothermic species (Grigg et al., 2004). Ectothermic animals, which range from sea sponges to reptiles, use behaviour and environment selection for thermoregulation (Angilletta, 2009; Bennett, 2004; Berk & Heath, 1975b; Cowles & Bogert, 1944; Heath, 1970; Moyes & Schulte, 2008; Pough, et al., 2016;

Tattersall, et al., 2012). Additionally, the inability to generate metabolic heat allows metabolic costs to be much lower in ectotherms (Tattersall, et al., 2012). This means that ectotherms often have lower resource demands put on them for the sake of temperature regulation (Nagy, 2005; Pough, 1980).

Ectotherms rely on the environment for increasing or lowering body temperature, which means that there is a heavy behavioural component to their thermoregulation (Huey, 1982). The dependence of ectotherms on the environmental temperature could be disadvantageous as animals generally have no control over environment. This only becomes a problem in a few extreme cases, however, since most vertebrate ectotherms live in areas with relatively stable and high temperatures, such as near the equator (Buckley et al., 2012), and use behaviour to adjust body temperature as needed (Tattersall, et al., 2012).

A smaller group of animals avoid a high dependence on environmental temperature by generating their own body heat through their metabolism (Jessen, 2001; Moyes & Schulte, 2008). The animals that do this, referred to as endotherms (Moyes & Schulte, 2008), include mammals and birds and are somewhat more wide spread across the globe and are influenced more by resource demand than temperature (Allen et al., 2002; Aragon, Lobo, Olalla-Tarraga, & Rodriguez, 2010; Buckley et al., 2012). Endotherms are not more successful than ectotherms but they do have more flexibility in their choice of ambient temperature (Aragon et al., 2010), and many extant endothermic species can grow to larger sizes than extant ectotherms (Buckley et al., 2012; Nagy, 2005; Pough, 1980). Many endotherms, for example, live in temperate environments where there is a significant drop in ambient temperature, during the winter months, to below 0°C (Buckley et al., 2012; Scholander, Hock, Walters, & Irving, 1950; Stirling, Lunn, Iacozza, Elliott, & Obbard, 2004). The presence of metabolic heat production gives endotherms the opportunity to exploit areas where there is less competition (Buckley et al., 2012).

Endotherms often have some form of insulation that prevents generated body heat from escaping,

either in the form of fur, feathers, hair, or fat (Scholander et al., 1950). The presence of this insulation is beneficial as the cost of generating body heat is quite high and requires a higher metabolic rate than occurs in ectotherms (Buckley et al., 2012).

Thermoregulatory Behaviours

In the case of endotherms, sizable amounts of energy are dedicated to metabolic heat production, which allows for adjustments to body temperature (T_b) despite fluctuations in the ambient temperature. Ambient temperature (T_a) does still have an impact, and it is assumed that homeothermic endotherms will try to remain in an environment where energy costs of maintaining T_b are minimal. Ambient temperatures that are close enough to the desired T_b of an endotherm will have relatively little impact on metabolic rate (MR). The range of temperatures that shows little effect on MR is called the thermoneutral zone (TNZ). Ambient temperatures that are outside the TNZ, however, cause significant MR increases with changing T_a (Buckley et al., 2012).

Endotherms also have thermoregulatory behaviours that are used in conjunction with metabolic heat production to maintain homeothermy. Thermoregulatory behaviours can be divided into two categories: voluntary, and involuntary. When an animal actively tries to remain within its TNZ, by moving from one T_a to another, it is voluntarily thermoregulating. Heath (1970) refers to voluntary thermoregulation as involving directed movements and calls it a complex of behaviours related to moving between available yet varying thermal environments. Metabolic rate increases can be affected by involuntary thermoregulatory responses such as shivering or sweating (West, 1965). Shivering causes an increase in ATP use as antagonistic muscles contract asynchronously and repeatedly. As ATP demand rises so does ATP formation, thus increasing metabolic rate (Rolfe & Brown, 1997). Sweating, on the other hand, is the release of liquid, mostly water, through pores in the epidermis. Once exposed to the environment, this

liquid leads to evaporative heat loss, often cooling the animal despite metabolic increases (Finch, Bennett, & Holmes, 2009; Shirreffs et al., 2005). A consequence of any rise in metabolic rate is a relative increase in metabolic heat being produced, which raises core body temperature if heat loss does not rise simultaneously. Animals in the midst of sweating offset this heat generation through larger heat loss due to evaporation, leading to a lowering of body heat. Shivering, on the other hand, allows the higher metabolic rate and heat production to increase body temperature. In order for endothermy to be viable, however, there must be some mechanism to store the metabolically produced heat (Angilletta, 2009; West, 1965). Endotherms make use of insulation, such as fur, feathers or fat, to help keep their own metabolically produced heat from being lost to convection (Scholander, Walters, Hock, & Irving, 1950), and to separate them from overly cool or warm environments. Finally, increasing or decreasing blood flow, through vasodilation and constriction, to body regions exposed to the environment, is another means of regulating body temperature (Sessler, et al., 1990). Vasomotor control is an autonomic response rather than a voluntary one. While the production of metabolic heat on such a large scale does have a high energetic cost, it does yield a higher metabolic rate which has thermoregulatory benefits, among many others (Wieser, 1985). By being “warm,” endotherms achieve higher physiological performance and can expand their temporal and spatial activity ranges, compensating for heat loss with metabolic heat production (Angilletta, 2009).

Ectotherms also have a range of thermally optimal body temperatures; however, they do not rely on metabolic heat production to maintain it. Instead, should ectotherms chose to thermoregulate they must do so using voluntary and involuntary responses as well as using the ambient temperature and the environment (Huey, 1982). This is done using a variety of different approaches such as basking, gaping, orientation, posture, and vasodilation or constriction (Bartholomew, 1966; Crawford Jr., 1972; Huey, 1982). Usually, ectotherms use solar radiation or conduction of heat emanating from the substrate as their primary heat source when one is

needed. By remaining at a desirable ambient temperature or by exposing themselves to a heat source, ectotherms can regulate their T_b (Seebacher & Franklin, 2005; Smith, 1979). While these techniques may be effective, there are challenges that ectotherms must overcome. Should the environmental temperature fluctuate too rapidly and drastically it may be difficult for the animal to remain within a thermally optimal range. For many ectotherms, remaining in relatively stable environments, such as marine or tropical ones near the equator, helps avoid large temperature fluctuations (Buckley et al., 2012). Ectotherms at lower latitudes tend to have narrower thermal optimal ranges and lower metabolic rates, as they are temperature specialists rather than generalists (Figure 1.1) (Woods, Dillon, & Pincebourde, 2014). It has also been suggested that the distribution of ectotherms is primarily influenced by average ambient temperature, while resources like food and mate availability are the driving forces for endotherm distribution (Aragon et al., 2010; Buckley et al., 2012). Regardless of latitude, many vertebrate ectotherms use behavioural thermoregulation to compensate for the lack of metabolic temperature regulation (Huey, 1982).

The behaviour that usually has the largest impact on T_b is microenvironment selection. Finding an area with a perfect, unchanging T_a is unlikely, however, and animals will usually shuttle between multiple microenvironments depending on their needs (Dreisig, 1984). Should an organism choose to warm up or cool down, then it must carefully select the appropriate habitat to allow this (Bennett, 2004; Cowles & Bogert, 1944). As the day progresses, any selected habitat's T_a is likely to change. Therefore, if a microenvironment becomes too warm or too cold for an organism to perform optimally, that individual must shuttle to a more desirable environment. In nature, for example, this can be observed as animals moving back and forth between shaded and sunny areas (Bennett, 2004; Cowles & Bogert, 1944). Shuttling can also occur between a large variety of microenvironments, such as above ground and underground, between a gradient of several temperatures, or between aquatic and terrestrial regions

(Bartholomew, 1966; Cowles & Bogert, 1944; Smith, 1979). Crocodiles, for example, will often alternate between terrestrial microenvironments to heat up and cooler aquatic areas to cool down (Smith, 1979). In any case, should an animal choose to make significant changes to its body temperature; it must shuttle to an appropriate location. This means that most thermoregulation is voluntary for ectotherms, as opposed to endotherms where it is often involuntary.

Working in concert with shuttling behaviours are basking behaviours. Basking describes the process when an animal exposes itself to a heat source, like the sun, for extended periods to absorb heat energy (Bartholomew, 1966; Cowles & Bogert, 1944). Animals usually bask in open regions or on warm rocks or similar structures. By remaining on a warm substrate, animals can absorb additional heat from the ground through conduction. It is common to see reptiles basking extensively during different parts of the day depending on their needs. Many desert animals take advantage of the early morning sun to warm up and then avoid the sun, often by burrowing underground, during midday to avoid overheating (Buckley et al., 2012; Cowles & Bogert, 1944). Others bask extensively before entering cooler hunting or foraging regions (Bartholomew, 1966). Both of these two behaviours, shuttling and basking, represent the primary means by which many vertebrate ectotherms (especially reptiles) thermoregulate on a day-to-day basis.

Environment selection, shuttling and basking are not the only voluntary techniques at an animal's disposal. While basking and shuttling are the most effective methods of causing a relatively large change in core temperature of an ectotherm (Cowles & Bogert, 1944; DeWitt, 1967), other behaviours allow for more fine temperature control. Showing preferential orientation to the sun and gaping both allow an animal to make slight adjustments to body temperature whilst still remaining within a single micro-habitat (Bartholomew, 1966; Crawford Jr., 1972; Gibson, Penniket, & Cree, 2015; Sartorius, do Amaral, Durtsche, Deen, & Lutterschmidt, 2002). Many animals, such as the marine iguana, *Amblyhynchus cristatus*, use

orientation as a means of thermoregulating (Bartholomew, 1966). By facing the sun they minimise the surface area exposed to direct solar radiation. The barren lava rocks on which *A. cristatus* are often found basking have surface temperatures that can exceed 50°C, yet the iguana's core temperature is usually below 40°C (Bartholomew, 1966). The marine iguanas maintain a lower T_b relative to their substrate with the aid of posture and orientation (Bartholomew, 1966). By limiting their exposure to the sun light and maximising exposure to cooler trade winds, marine iguanas can prevent overheating while they bask on land (Bartholomew, 1966). In this way, orientation is a behavioural form of thermoregulation. Animals also use posture to aid in body temperature manipulation, in much the same way they use orientation. By choosing which body parts are in contact with hot surfaces, they can slightly control rates of heat exchange (Bartholomew, 1966). The use of posture as a thermoregulatory strategy has been seen in the nocturnal gecko *Christinus marmoratus* which helps the individuals thermoregulate to an extent comparable to that of diurnal animals (Kearney & Predavec, 2000). In contrast, by flattening their bodies, many lizards, such as the marine iguana and the bearded dragon, can expose more surface area to the sun, further increasing the rate at which they absorb heat (Bartholomew, 1966; Cowles & Bogert, 1944). The use of posture and orientation for thermoregulation has also been seen in the New Zealand gecko, *Woodworthia* Otago/Southland and in two lizard species from the Phrynosomatidae family, *Uta stansburiana* and *Sceloporus arenicolus* (Gibson et al., 2015; Sartorius et al., 2002). The Otago/Southland gecko will bask by exposing either all or one side of its abdomen to the sun, depending on its thermoregulatory needs. Additionally, Otago/Southland geckos will occasionally bask "on-toes" or with their abdomens off the substrate depending on thermoregulatory needs (Gibson et al., 2015). Gibson et al. (2015) also noted that female geckos show basking behaviour more than males do during the spring and summer months (roughly 40% higher incidence of basking in females). Female geckos were also selecting higher temperatures on average (roughly 5°C higher) when pregnant.

Despite female geckos selecting higher temperatures during pregnancy, Gibson et al. (2015) saw no impact of reproductive condition on incidence of basking in geckos.

Another behaviour used for fine temperature control in reptiles, as well as several other animals, is respiratory cooling (Crawford Jr., 1972; Tattersall, Cadena, & Skinner 2006; Tattersall & Gerlach, 2005). Evaporative water loss, occurring in the upper airways during respiration, causes a slight cooling effect due to the transfer of heat when liquid water turns into a vapour (Borrell, LaDuc, & Dudley, 2005; Tattersall et al., 2006). Reptiles will alternate between different respiratory patterns to further regulate water loss and, as a consequence, heat loss (Tattersall et al., 2006). The main respiratory patterns related to cooling seen in most species are relaxed breathing through the nares, more rapid breathing through the nares (similar to panting), and breathing during an open mouthed gape (Tattersall et al., 2006). Typically the first two patterns allow for very limited heat and water loss, and reptiles use these patterns the most (Tattersall et al., 2006). Gaping is usually only seen as a means to lower head temperature by several degrees (Tattersall et al., 2006). By opening the mouth, gaping animals increase the surface area for evaporative heat loss significantly, allowing for a noticeable drop in head temperature compared to ambient temperature (Crawford Jr., 1972; Spotila, Terpin, & Dodson, 1977; Tattersall, et al., 2012). Crawford (1972) found that gaping allows the head temperature of *Sauromalus obesus* to be 2.7°C lower than the ambient temperature (Figure 1.2). During periods of gaping, head temperature also drops below body temperature in many animals (Crawford Jr., 1972; Webb, Johnson, & Firth, 1972). At lower ambient temperatures, head temperature tends to be higher than T_b (Webb et al., 1972). This holds true until T_a rises to a critical temperature, sometimes called the panting threshold, after which breathing becomes more rapid and head temperature drops to or below T_b (Webb et al., 1972). Technically, gaping is simply the opening of the mouth for an extended period of time, and while both gaping and lizard “panting,” or rapid

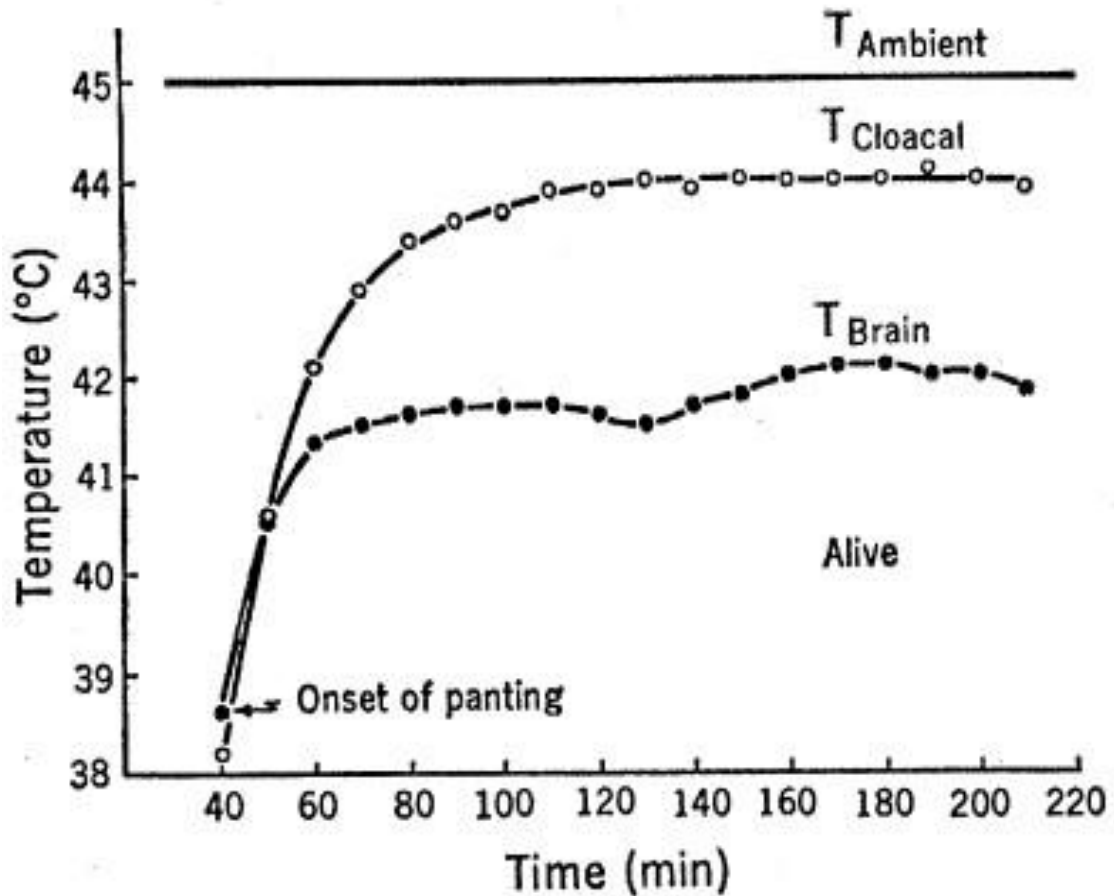


Figure 1.2. Graphical representation of the change in body temperature ($T_{Cloacal}$) and brain temperature (T_{Brain}) with regards to time in *Sauromalus obesus*. Animals showed a significantly lower T_{Brain} in comparison to $T_{Cloacal}$ and both temperatures were lower than the ambient temperature ($T_{Ambient}$). This is in contrast to a non-living organism, which approached the $T_{Ambient}$ asymptote in both cases. Adapted from Crawford, 1972.

breathing, have a cooling effect on head temperature, gaping itself is not an increase in breathing tempo, but is simply an increase in exposed surface area for heat exchange (Crawford Jr., 1972; (Tattersall et al., 2006). Whether breathing rates increase or fluctuate during periods of gaping is still widely unknown in many species. While head-body temperature differences have been observed both in the laboratory and in the field, Webb et al. (1972) noted that the differences are much smaller outside of the laboratory environment. It does appear that respiratory cooling could be an evolved response to aid in preventing high brain temperatures in reptiles (Tattersall et al., 2006). Understanding how efficient these breathing patterns are, as well as elucidating the specifics of the control mechanisms for head temperature and core temperature regulation with regards to each other, could prove to be valuable for understanding the evolution of thermoregulatory control. Despite this, these areas are not as well researched as other aspects of ectothermal thermoregulation.

During gaping, heat is exchanged with the environment through evaporative heat loss, much like panting in mammals (Jessen, 2001); it is possible, therefore, that gaping could lead to a trade-off between thermoregulatory and osmoregulatory needs (Wolf & Walsberg, 1996). Recent work by Scarpellini, Bicego, & Tattersall (2015), using salt-loading to mimic increased plasma osmolality, has shown that *Pogona vitticeps* have lower metabolic rate after salt-loading when compared with before. Examining the ratio of evaporative water loss/metabolic rate indicated that gaping does contribute to water loss. Lizards that had been injected with hypertonic saline solutions also spent less time gaping, depending on the saline concentration; there was 90% less gaping at the highest concentration and 50% less gaping with the lowest dose used. The notable drop in time spent gaping suggests that thermoregulatory behaviours that rely on water loss, such as gaping, show sensitivity to dehydration (Scarpellini et al., 2015).

All the behaviours mentioned above are used to facilitate thermoregulation in reptiles to maintain thermal optimality. While basking and shuttling certainly have the greatest effects,

other behaviours, such as orientation and gaping, are still expressed often enough to suggest a similar level of importance with regards to temperature regulation. While these voluntary behaviours are very important, and represent the main means by which reptiles thermoregulate, there are certain autonomic responses which help as well. Responses such as changes in heart rate, or constriction and dilation of blood vessels will inevitably have an effect on heat exchange rates (Bartholomew & Lasiewski, 1965; Dzialowski & O'Connor, 2001). Since the extremities are where most heat is gained or lost in ectotherms, increasing or decreasing blood flow to and from the skin can effectively “speed up” or “slow down” their rates of heating and cooling. Therefore, during both an animal’s active and inactive periods of the day, there may be an autonomic response to increase blood flow while the animal is in warmer areas to speed up heating, and to decrease blood flow while the animal is in cooler areas to slow heat loss. In other words, the rate at which an animal warms its body may not be the same as the rate at which it cools its body (Bartholomew & Tucker, 1963; Cowles, 1958). This is called a hysteresis effect or a “thermal hysteresis” in this case.

A thermal hysteresis effect can be influenced by several factors. A study done on five small species of scincid lizards by Fraser and Grigg (1984) found that physiological control over thermal conductance is, essentially, insignificant in small reptiles (<20g). Reptiles under 20g in mass should be unable to control heat exchange with the environment through changes in peripheral blood flow and must instead rely more on behavioural thermoregulation (Fraser & Grigg, 1984; Turner, 1987). For alterations in peripheral blood flow to be effective in controlling heat exchange, conductive resistance within the body must be high while external heat exchange resistance must be low (Turner, 1987). As a consequence, very large reptiles (> 5kg in the case of alligators) should be unable to use heart rate and blood flow to control heat exchange unless they are exposed to certain environmental conditions, such as water, high winds or extensive heat radiation (Turner, 1987). Turner (1987) also speculated that there should be an optimal body size

for the usefulness of blood flow in heat exchange. In alligators, the optimal body size is 5 kg in air, but could be substantially larger for reptiles thermoregulating in water (Turner, 1987). Dzialowski and O'Connor (2001) noted a similar impact of size on the effectiveness of blood flow with regards to warming and cooling in their work, but they also observed that heat exchange rates were only noticeably changed during periods of simulated basking, as opposed to shuttling. While working with the crocodile *Crocodylus porosus*, Franklin and Seebacher (2003) observed a clear hysteresis effect on both T_b and heart rate during later stages of heating and cooling; however, they also saw large changes in heart rate with little to no changes in body temperature during initial stages of adding or removing a heat source. Franklin and Seebacher (2003) indicated that changes in heart rate represent a thermoregulatory mechanism in these animals in response to their thermal environment, but that heart rate is also partially controlled independently of T_b during heating and cooling. Finally, once reptiles reach a certain temperature threshold, they may undergo a reverse hysteresis pattern. A study done with *Pogona barbata* found that when placed in an environment that put the lizards at risk of overheating, their heart rate dropped significantly once T_b reached a critical temperature ($>40^\circ\text{C}$), rather than continuing to rise. In comparison, heart rate rose significantly during the cooling phase, facilitating heat loss, until T_b dropped below the critical temperature (Grigg & Seebacher, 1999). This decline in heart rate at high temperatures was argued to be a protective response to prevent further heating, which would be exacerbated by enhancing peripheral blood flow.

A more visible reaction to temperature can be seen in the form of pigmentation of an animal's skin (King, Hauff, & Phillips, 1994). The phenomenon of changing pigmentation usually occurs over a longer time scale in comparison to other thermoregulatory changes, but does have a noticeable impact. When a reptile is below its preferred T_b , it will adopt a darker skin tone within minutes, allowing higher rates of solar heat absorption (Carey, 1978; Cole, 1943; Cowles, 1958; King et al., 1994; Trullas, van Wyk, & Spotila, 2007). Conversely, upon

reaching or surpassing the desired T_b , these same organisms are often capable of changing their skin pigmentation to a lighter shade, thus becoming more reflective to solar heat radiation (Cole, 1943; Cowles & Bogert, 1944). The use of melanism in heat absorption has given rise to the thermal melanism hypothesis (TMH), which predicts that darker individuals are at greater advantage in cooler climates, as they absorb heat faster than lighter individuals (Porter & Gates, 1969; Trullas, Terblanche, Blackburn, & Chown, 2008; Watt, 1968). Work on TMH has yielded mixed results, but there seems to be support for this hypothesis at a broader geographic scale (Trullas et al., 2008). Changing pigmentation is not exclusively a thermoregulatory response, and is often used for defense, social interaction or other necessities (King et al., 1994). Finally, there are also trace amounts of heat generated through all metabolic processes. In ectotherms, however, this typically has an almost negligible impact on core temperature (Seebacher & Franklin, 2005; Tattersall, et al., 2012). In lizards, thermoregulation can be attributed, almost exclusively, to voluntary behaviours, such as basking or gaping, and to certain involuntary mechanisms, such as changes in blood flow or pigmentation (Seebacher & Franklin, 2005; Tattersall, et al., 2012).

Thermoregulatory precision

When examining thermoregulation in ectotherms, two notable points of interest are the preferred T_b of an individual, usually shown as the mean or median body temperature recorded during a certain time interval, and how precise an animal is at reaching its desired T_b (DeWitt, 1967). In an ideal scenario, one would expect the preferred temperature to match up with the optimal physiological temperature of that organism. In reality, however, there is often variability in T_b over time, showing that animals have differing levels of precision when trying to reach their preferred temperature (Bowker & Johnson, 1980; DeWitt, 1967). Precision is often measured as the variance or standard deviation from the mean body temperature, or is described

as the temperature range within which a number of T_{bs} can be found (DeWitt, 1967). DeWitt (1967) calculated precision to be the temperature range comprised of the central 50% of the measured T_b in *Dipsosaurus dorsalis*.

Thermal optimality and precision have been further explained with the aid of mathematical models that make numerous predictions. The first such model is that the physiologically optimal temperature and the ecologically optimal temperature may not be the same (Huey & Slatkin, 1976). The second model is that thermoregulation is only favorable when the costs associated with it are lower than the benefits gained by it (Huey & Slatkin, 1976). In an ideal environment one would expect the cost of thermoregulating to be low or non-existent, and therefore one would anticipate reptiles to try to reach their thermally optimal T_b . However, ideal environments don't exist in the natural world, and costs of thermoregulation must be taken into account. Huey and Slatkin (1976) predicted that, when associated costs are low, reptiles will be thermoregulators, but if metabolic costs are high, reptiles will instead conform to the ambient temperature (thermoconformers). The model put forth by Huey and Slatkin (1976) went on to predict that reptiles living in environmental extremes, where precise thermoregulation is costly, will be thermoconformers. More recent work has found this prediction to be unsatisfactory and looks to clarify the cost-benefit model. A study done on black rat snakes (*Elaphe obsoleta*) found that, despite their study population being at the northern extreme of the snake's distribution, these black rat snakes were moderate thermoregulators similar to other species in less costly environments (Blouin-Demers & Weatherhead, 2001). Further work has supported this, showing that the costs of thermoconformity are higher than the costs of thermoregulation in regions of poor thermal quality (Blouin-Demers & Nadeau, 2005). Thermoconformity can be beneficial in tropical regions where more active thermoregulating may be unnecessary since the thermal environment is relatively benign (Blouin-Demers & Weatherhead, 2001; Huey & Slatkin, 1976). There are numerous factors that will increase the expense of thermoregulation, not the least of

which is the energetic cost of shuttling, or balancing the need to thermoregulate and the need to eat, mate, or engage in social interaction.

Bowker and Johnson, (1980) reported variation in thermoregulatory precision among three species of whiptail lizard by examining sinusoidal-like fluctuations of body temperature with time. Reptiles exhibiting small amplitude fluctuations (in this case less than 1.33°C) and short average periods (less than 4 minutes) were considered to have high thermoregulatory precision. Precision is also impacted by physical factors such as temperature distribution within the environment (DeWitt, 1967; Huey & Webster, 1976). Dewitt's (1967) work also examined some impacts of biological factors on *Dipsosaurus dorsalis*, such as predation and competition. Both of these factors, physical and biological, impact the level of precision a reptile has with regards to thermoregulation (DeWitt, 1967). During warmer months of the year, *D. dorsalis* shows higher than normal body temperature during active periods away from shelter. Typically, the ambient temperature on or above ground level never drops down to the desired ambient temperature of the animal during warmer months. For this reason, despite thermoregulatory behaviours, the lizards are forced to maintain a T_b 3-4 degrees higher than their preferred T_b when they are above ground. Therefore, *D. dorsalis* would usually try to remain in their burrows where it is cooler, but this is not always possible. The biological and behavioural factors that most strongly impacted thermoregulation and forced these animals from their cooler dwellings were territorial behaviour and predator response. Smaller changes to precision were caused by feeding demands, while courtship behaviours showed no significant impact. During territorial disputes in *D. dorsalis*, there are often lengthy periods of threat displays and occasional fights between lizards. These displays and fights often last several minutes and are commonly above ground in exposed areas. Dewitt (1967) observed that in several cases, the animals' T_b would be, on average, 6-8 degrees higher than their preferred T_b immediately after a fight. The high body temperature suggests that these animals compromise thermoregulation for territory protection

and competition. During periods of predation threat, these animals will often remain as still as possible to avoid detection. When this occurs in exposed areas during warm periods it can lead to overheating as well. This behaviour is more common when the potential predator is between the organism and its burrow (DeWitt, 1967). Changes to thermoregulatory precision caused by physical or biological factors can be seen across many species and varies slightly between them (Cadena & Tattersall, 2009b; DeWitt, 1967; Huey & Webster, 1976)

Thermoregulatory Control Mechanisms

The simplest form of thermoregulatory control, or control of any homeostatic system, can be understood with the aid of control theory (Mrosovsky, 1990). Control theory states that all behavioural response pathways can be regulated with either a feedback or feedforward loop (Modell, et al., 2015; Mrosovsky, 1990; Schneck, 1987). A feedback loop is a pathway wherein a stimulus leads to corresponding response. Essentially, a stimulus causes a reaction in a sensor which sends a signal to the system's controller. This in turn leads to an opposing response, which either counters the stimulus and reduces the sensory response or acts as an agonist and maintains the signal from the sensor. If the effector response counters the initial signal from the sensor, this model is called a negative feedback loop (Schneck, 1987). In the case of vertebrate temperature regulation, the sensor would be a thermoreceptor, which signals to the controller the direction of the error signal, which then activates a thermoregulatory response.

One primary mechanism used in neurons responsible for peripheral and internal temperature sensation involves transient receptor potential ion channels (TRP channels) (Caterina, 2007). These TRP channels have recently been shown to respond strongly (with Q_{10} values vastly different from other ion channels) and predictably to temperature, with different TRP channels responding to different temperature ranges (Caterina, 2007; Romanovsky, 2007). The TRP channels respond to a large range of temperatures, including both noxious and

innocuous temperatures (Romanovsky, 2007). Seebacher and Murray (2007) identified the expression of vanilloid TRP channel TRPV1 in numerous ectotherms including the crocodile (*Crocodylus porosus*). Additionally, Seebacher and Murray (2007) suggested that TRPV1 and melastasin TRP channel (TRPM8) are used as both internal and peripheral temperature sensors.

There is extensive documentation that points to neural centers in the hypothalamus being responsible for control of temperature regulation (Berk & Heath, 1975a; Cabanac, Hammel, & Hardy, 1967; Hammel, Caldwell, & Abrams, 1967; Hellstrom & Hammel, 1967; Myhre & Hammel, 1969). Hellstrom and Hammel (1967) showed that by increasing the hypothalamic temperature in dogs a cooling thermoregulatory reaction is stimulated; they also noted that the inverse reaction occurred when the dog hypothalamus was cooled. Work done in blue-tongued lizards (*Tiliqua scincoides*) showed similar trends, such that warming or cooling the hypothalamus induced a corresponding change (i.e., behaviours in opposition to the direction of temperature change) in shuttling behaviour (Hammel et al., 1967). Berk and Heath (1975a) further observed that lesions in the nucleus of the anterior hypothalamus of *Dipsosaurus dorsalis* caused a significant drop in mean exit T_b when the animals were shuttling from the cold side to the hot side, lending additional support to the importance of hypothalamic centers with regards to thermoregulation and thermoregulatory behaviour.

While a basic negative feedback loop does help explain some aspects of thermoregulatory control, it is not complex enough to account for all the different responses and behaviours that maintain homeothermy and homeostasis in general. Mrosovsky (1990) brings together several different works in his review that show that, in truth, negative feedback loops are only a small part of the story. To maintain homeothermy, and homeostasis, several different control mechanisms are used. In addition to negative feedback, feedforward loops are needed (Kissileff, 1969; Ruiter & Wiepkema, 1969; Thomas & Robin, 1977; Wiepkema, 1971). Negative feedback loops are reactionary; they respond to a stimulus until the stimulus stops or the controller is

inhibited (McFarland, 1971; Ruiter & Wiepkema, 1969). In many cases, a reactionary response could be wasteful. If the stimulus activates the controller relatively frequently, such as for eating or drinking, then it may be beneficial to buffer against the signal by responding longer than needed to shut off a negative feedback pathway. Positive feedback is a signaling loop where the response stimulates the controller to respond again rather than terminating the original stimulus. An example of this is seen in Wiepkema's (1971) work, which showed that rats continue to eat even after consuming enough food to account for their immediate needs. By eating a larger meal rather than eating small amounts of food every few minutes, animals avoid needlessly switching between tasks. A feedforward loop might stimulate changes in behaviour depending on the animal's current environment, but in advance of actual changes in the controller temperature. Budgell (1970) found that dehydrated pigeons exposed to water in a warm test area drank more than those exposed to water in a cooler test area. Since warmer areas are more conducive to future dehydration, pigeons in these areas would be benefited by a feedforward loop that stimulates more consumption than normally needed. Positive feedback loops are not typically used in thermoregulatory responses, unlike negative feedback loops. Feedforward loops are occasionally found in thermoregulatory responses such as in cold-defence responses to skin cooling in rats and some other endotherms (Nakamura & Morrison, 2008).

Negative feedback loops rely on the assumption of a set-point from which the controlled variable, such as T_b , is compared. Set-points are constructs that help explain how thermoregulatory homeostasis might work and are used to produce testable hypotheses about negative feedback control. Whether set-points are real or just an analogy for T_b control is still under considerable debate (Cabanac, 2006; Romanovsky, 2004). Mitchell, Snellen, & Atkins (1970) suggested a control system that did not make use of set-points, but rather used an activity balance between multiple sensors. In a control system like the one Mitchell et al. (1970) proposed, the controlled variable, in this case T_b , is a result of an activity balance between both

hot and cold thermoreceptors. A drop in T_b causes a corresponding rise in cold thermoreceptor activity and a decrease in hot thermoreceptor activity. This “unbalanced signal” causes a thermoregulatory response to increase T_b and thus balance out the thermoreceptor signaling again (Mitchell et al., 1970; Romanovsky, 2004).

In ectotherms, there are two principal control mechanisms for behavioural thermoregulation, which are: proportional control, used for fine tuning body temperature, and on-off control, used for more broad scale thermoregulation (Heath, 1970). Systems that use proportional control use subtler thermoregulatory behaviours to address relatively smaller deviations from the regulated T_b . The level of the thermoregulatory response is proportional to the amount of deviation in the body temperature (Heath, 1970). Behaviours such as gaping, orientation changes and posture adjustments are all likely regulated by proportional control mechanisms (Heath, 1970). Depending on the relative need, specific thermolytic or heat seeking behaviours will be expressed at different levels. In the case of gaping this could be represented by how wide the mouth is opened and the time spent gaping. Bearded dragons have been shown to have a wider gape at higher temperatures compared to lower ones, as well as an increase in the amount of time spent gaping depending on the thermal stimulus (Tattersall & Gerlach, 2005).

In more pronounced behaviours, such as shuttling, the on-off control system appears to be present. This leads to an all or nothing behavioural reaction, meaning that the behaviour occurs or it does not; there is no intermediate step. In the shuttling model, lizards will alternate between two responses in order to maintain a relatively narrow temperature range. Lizards will either move into a warm region to heat up, or into a cooler region to cool off. A typical example of this is an animal shuttling between shade and sunlight (Heath, 1970). An animal shuttling between a warmer and cooler region has two on-off systems at work; one reacts when the lizard warms up too much and the animal reaches its upper T_b set-point (USP). When a lizard reaches this point it moves to a cooler location and stays there until it reaches its lower T_b set-point

(LSP). The ambient temperature that stimulates the animal to move from one environment to another is called the escape temperature (Cadena & Tattersall, 2009a). The upper escape temperature (UET) is the highest temperature reached by the lizard before moving from a warm to colder environment. Conversely, the lower escape temperature (LET) is the lowest temperature reached by a lizard before moving from a colder to a warmer environment. At present, it is not known to what extent peripheral vs. core temperature sensation drives shuttling behaviours.

These control mechanisms do not really allow the animal to reach its desired temperature *per se*, but rather allows it to fluctuate around its preferred T_b . This means that reptiles will strive to prevent their T_b from rising above the USP or falling below the LSP, thereby keeping them within the optimal range of temperatures. This allows them to take advantage of other thermoregulatory responses, such as gaping or orientation, whose associated costs are far lower. Conversely, lizards forced to shuttle repeatedly will be less likely to show orientation and gaping behaviours related to thermoregulation. By remaining within the zone between the USP and LSP, called the refractory zone, reptiles do not need to dedicate all their time to costly shuttling thermoregulatory behaviours (Heath, 1970).

Abiotic Influences on Thermoregulation

Metabolism and most physiological processes are not just impacted by temperature and temperature regulation but can also impact thermoregulatory behaviours. While it is true that changing T_b has an impact on metabolism and physiology, the relationship is more complex than that. Behaviours such as hibernation or abiotic factors like oxygen levels or the presence of toxins can lead to an apparent change in the upper and lower temperature set-points of an animal. Mrosovsky (1990) noted that these significant and often sudden changes were difficult to explain with the traditional concept of homeothermy and feedback control systems. He instead observed

that all the neuronal models (feedback, feedforward, and balanced models) fit into a concept he named rheostasis. Rheostasis is defined 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 proves useful when examining thermoregulation as it allows for changes in set-point as the typical response to numerous environmental fluctuations. Changes in oxygen levels or plasma osmolarity within an animal or the environment, for example, can cause changes in what would otherwise be normal behaviour. In hypoxic environments, where oxygen concentration is low the majority of animals will show a decrease in T_b (Wood, 1995). Lizards will also show a lower preferred T_b in the presence of hypoxia (Cadena & Tattersall, 2009a). In nature, similar hypoxia can be brought about by exercise or some parasitic infections. It is suggested that by lowering body temperature, animals protect themselves from oxygen depletion in critical organs. Hicks and Wood (1985) found that a lower T_b (by about six to eight degrees) can drop oxygen demand by around 50%. Lizards that are allowed to cool down below their normal preferred T_b show a survival rate of 100% during hypoxia, as opposed to a mortality rate of 100% in lizards that are forced to remain at their normal preferred T_b during hypoxia (Hicks & Wood, 1985). The observations made by Hicks and Wood (1985) have led to the suggestion that thermoregulatory control is malleable, and that, during times of metabolic compromise (such as hypoxia), animals that normally regulate T_b at higher temperatures will switch to lower, but still regulated, temperatures. The response of adopting a lower regulated temperature has been referred to as anapyrexia (the opposite of fever), to reflect the fact that thermoregulatory defence is still functional, but reset to a lower value.

In addition to hypoxia, plasma or cellular osmolarity can also impact behaviours and preferred T_b (Harrison, Edwards, & Fennessy, 1978; Ladyman & Bradshaw, 2003; Malvin & Wood, 2005). *Bufo marinus*, has been shown to select lower ambient temperatures, and thus have lower T_b , when placed in dry conditions (Malvin & Wood, 2005). Selecting a lower temperature

reduced the water loss due to evaporation by 42% in these animals. A similar situation is seen in the Western Tiger snake *Notechis scutatus*. When these snakes are dehydrated, they select a lower preferred T_b (around 19.7 °C) than their hydrated counterparts (around 26 °C) (Ladyman & Bradshaw, 2003). Dehydration is an important point of concern since many reptiles live in deserts or dry environments, and water conservation is critical in these habitats. Despite this, loss of water through evaporation can be an effective means of cooling off, and evaporative heat loss, through the tongue or skin, is an integral part of an animal's thermoregulatory strategy (DeNardo, Zubal, & Hoffman, 2004). The work done by Scarpellini et al. (2015) in the desert animal bearded dragon (*Pogona vitticeps*) showed that when injected with hypertonic saline (to rapidly mimic dehydration without the debilitating health effects), *P. vitticeps* spend proportionally less time gaping as saline concentration increases. Bearded dragons also showed altered UET and LET after being injected with the highest saline concentration, which was attributed to a decrease in the animals' propensity to move, and a suppression of the normal thermoregulatory response (Scarpellini et al., 2015). By gaping less, dehydrated animals avoid water loss through evaporation, but they also lose this as an avenue of heat loss. The consequence of this is that head temperature rises with dehydration.

Physiological processes are not the only mechanisms that can influence thermoregulatory behaviours. Other physical changes can have a dramatic impact. Since ectotherms draw heat from their environment, a physical barrier that provides insulation would be counterproductive, in most cases. Ectotherms, such as reptiles, need to be able to readily absorb or lose heat, and this would be impeded by insulation (Cowles & Bogert, 1944; Scholander et al., 1950). In some cases, certain morphological structures can have several secondary uses such as providing more surface area for heat exchange. Osteoderms, for example, are often highlighted as being primarily for defence, however, Seidel (1979) noted extensive vascularization in the osteodermal layer of the alligator species *Alligator mississippiensis*, and proposed that this could play a role

in thermoregulation. Seidel's work was further complemented by work done by Richardson, Webb, & Manolis (2002) who reported similar vascularization and the appearance of a role in heat exchange in crocodilian osteoderms. Other more pronounced structures have also been suggested as having a role in thermoregulation. The plates of the thyreophoran dinosaur *Stegosaurus* could have been used for heat exchange in much the same way that the osteoderms in *Alligators* are. Farlow, Hayashi, & Tattersall (2010) observed what appeared to be tubes for vascularization of the dorsal plates in *Stegosaurus* fossils. Like the vascularized alligator and crocodilian osteoderms, *Stegosaurus* plates could also have served a thermoregulatory function.

Since ectotherms do not rely on metabolic heat production, they are a useful model for examining and modelling thermoregulation and coordination between thermoregulatory structures and behaviours. Coordination is defined in this work as the organization of different elements of a complex body that enables them to work together effectively and efficiently. In the case of temperature regulation, this would mean that all thermoregulatory elements, whether structures or behaviours, would act in a universally controlled fashion. A coordinated thermoregulatory set of responses operates with either a common set-point or in an ordered fashion, such that thresholds for each response occur at different body temperatures. Therefore, less costly responses are evoked at less extreme temperatures, and more costly responses occur at extreme temperatures. Additionally, if one behaviour or response is inhibited or eliminated, then the other behaviours should be enhanced as they are required to compensate. It may be unreasonable to assume that all thermoregulatory responses originate from the same controlling region or specific center of the brain, such as the hypothalamus. In recent work it has been noted that, in endothermic models, different thermoregulatory responses have different controlling regions as well as different efferent pathways (McAllen, Tanaka, Ootsuka, & McKinley, 2009). Despite the independent pathways attributed to different thermoregulation responses, McAllen et al. (2009) did acknowledge that there is apparent coordination between behaviours. By

examining an ectothermic model that shows clear thermoregulatory behaviours, it may be possible to uncover the nature and extent of this coordination. Another advantage to studying behaviours is that they can be less invasively examined compared to physiological responses. Since reptilian thermoregulatory behaviours are clear and easily observable, quantifying these mechanisms and how they interact with each other becomes much simpler than it would be in endothermic models. By examining these interactions, we can begin to study whether or not there is coordination in thermoregulatory behaviours that allows for adjustments and compensation between them. It is important to note that thermoregulatory control is a conceptual model for homeostasis, not a practical one, from which predictions can be made.

Examining Behavioural Thermoregulation in Ectotherms in a Laboratory Environment

Studying thermoregulation in reptiles is commonly done using a selection of different methods, including shuttle boxes, thermal chambers, and thermal gradients. Typically, in a shuttle box experiment, an animal is given a choice between two ambient temperatures (T_a) and must shuttle between them to maintain a desired T_b (Berk & Heath, 1975b; Blumberg et al., 2002; Cadena & Tattersall, 2009a; Hicks & Wood, 1985). The utility of shuttle boxes, as opposed to thermal gradients and chambers, is that they aid in determining the proximate thermal limits that may evoke thermoregulatory behaviours. By simulating two changing microenvironments, upper and lower escape temperatures in animals can be determined, and shuttling behaviour can be observed. Shuttle boxes are limited, however, and other subtler behaviours such as orientation or gaping are often not observed as readily. In addition, shuttle boxes do not allow an animal the choice of a constant preferred T_a further limiting this technique to focusing primarily on active or locomotory behaviours. While preferred temperature can be accurately estimated with a shuttle box, precision of thermoregulatory behaviour is influenced by the amount of locomotory work

the animals must use (Cadena & Tattersall, 2009b). For analyzing other thermoregulatory responses, the use of thermal chambers and thermal gradients can prove more useful.

A thermal chamber (or incubator), is simply a room that is separated from the ambient environment. The size of the thermal chamber varies with respect to the experiment and the organism being studied; the environment of the chamber can then be manipulated for the purposes of the experiment. Many basic studies can be done with this design. By changing the ambient temperature of the chamber a researcher can observe the set-points for certain thermoregulatory behaviours (e.g., gaping as described by Crawford Jr. (1972), or posture changes for example), or how those set-points change after manipulations to the animal (dehydration, for example). Thermal chambers allow an investigator to examine select behaviours and physiological responses. Using probes or other thermal sensing equipment, one can examine the rate a reptile absorbs heat from, or loses heat to, the environment. Cloacal probes are commonly used to infer core body temperature and the rate at which that temperature changes in different ambient temperatures.

While thermal chambers can be used effectively to observe select behaviours and to track rates of heat exchange, the animals are still forced into a predetermined temperature. In order to truly examine an animal's behavioural thermoregulation and account for multiple different mechanisms, a thermal gradient must be employed (Arad, Raber, & Werner, 1989; Bennett, 2004; Hicks & Wood, 1985). Thermal gradients are devices with a floor or air temperature that ranges from a warm temperature on one side to a cooler temperature on the other, which allows animals to select their desired T_a through adjustments in physical locomotion within the gradient. Animals are free to shuttle to cooler or warmer temperatures as well as gape as often as needed. Finally, animals can orient in whichever direction is preferable, though this has never been examined in a thermal gradient before.

Thermal gradients are used to determine what temperatures an animal prefers and to observe how other thermoregulatory behaviours influence this selection. Preferred T_a is inferred by the reptiles' location within the gradient itself. Inferring preferred temperature in this way can be complicated slightly if the animal in question is large enough to occupy a large swath of different temperatures along the chamber. A final concern arises when researchers are trying to estimate T_b using this method. Due to the existence of a refractory zone in ectotherms, T_b estimates can be less precise when compared to other T_b estimation techniques. In a thermal gradient, the organism would cease most voluntary thermoregulation upon reaching the refractory zone (Heath, 1970) and, since the zone can span up to several degrees, this makes estimating the specific desired T_a and T_b more difficult.

By working with an ectothermic animal, complex involuntary thermoregulatory behaviours, such as metabolic heat production, can be assumed to be negligible, thus simplifying the study of voluntary thermoregulatory responses. Additionally, by studying animals that retain ancestral thermoregulatory traits and lack the derived traits (i.e. endothermy) present in birds and mammals, insight into the evolutionary origins of thermoregulatory control can be examined, as well as the coordination between behaviours. The Australian bearded dragon (*Pogona vitticeps*) is a suitable organism for the study of thermoregulation. Previous work on these animals has documented their preferred body and ambient temperature at around 34°C (Cadena & Tattersall, 2009b), and isolated their upper and lower escape temperatures for shuttling behaviour (Cadena & Tattersall, 2009b). Gaping has also been studied in *P. vitticeps*, suggesting an impact on head temperature as well as how gaping is potentially impacted by dehydration (Scarpellini et al., 2015). Gaping may also have a direct influence on a lizard's propensity to shuttle or alter selected temperature, if the head and brain contain sensitive thermosensory responses. An area that has not been so well documented, however, is the use of orientation in the thermoregulation of bearded dragons, though it has been documented in some other reptiles (Bartholomew, 1966;

Gibson et al.,2015). Nor has there been much work on how a thermoregulatory behaviour coordinates its response with other thermoregulatory behaviours.

Thesis Rationale and Objectives

Maintaining T_b within the physiologically optimal range is critical for all lizards. In order to maintain thermal optimality, lizards use numerous behavioural and physiological traits to regulate their body temperature. The preferred temperature ranges of many lizards and the aforementioned behaviours have been fairly well studied (Huey & Stevenson, 1979; Tattersall, et al., 2012). Maintaining a lizard's T_b within the optimal range has direct impact on an animal's overall fitness, suggesting that thermoregulation has high level of importance in an animal's life history (Angilletta, Niewiarowski, & Navas, 2002).

Every behaviour or demand has potential costs, and if the costs outweigh the benefits then those demands may be tolerated (Huey & Slatkin, 1976). With thermoregulation, costs can vary and can be cumulative, but usually revolve around habitat quality, resource availability, and predation risk (Buckley et al., 2012; DeWitt, 1967). Behaviour is therefore adjusted to make sure that the cost is less than the benefit as much as possible (DeWitt, 1967). As cost increases, precision of thermoregulation drops (Cadena & Tattersall, 2009a; DeWitt, 1967). In most cases this means a widening of selected temperature ranges (Buckley et al., 2012; Cadena & Tattersall, 2009a; DeWitt, 1967). In addition, the impact these situations have on individual behaviours is well understood. However, what, if any, connections do these behaviours show with each other? Are they independent choices of one another, or are they more coordinated? In the Australian bearded dragon, behaviours such as shuttling and gaping have been well documented (Cadena & Tattersall, 2009a; 2009b). Orientation, as a method of regulating temperature, has not been demonstrated but its presence in other animals suggests it may be seen in bearded dragons (Bartholomew, 1966; Gibson et al., 2015; Sartorius, et al., 2002). By observing bearded dragon

thermoregulatory behaviour before and after inhibition of gaping behaviour and blocking heat exchange through the tail, I examine whether there is coordination across all thermoregulatory behaviours.

The purpose of this study is to examine coordination between thermoregulatory behaviours and to observe the effect of manipulating one thermal effector, or thermoregulatory structure, with respect to other structures or behaviours. The intent is to tease out coordination across the different behaviours and see if influencing one would induce compensation by another during normal thermoregulatory responses. This work was done using thermal gradients so that selected ambient temperature could be included as a potential thermoregulatory behaviour. In addition this allowed examination of orientation choices as well as gaping and movement.

I hypothesized that thermoregulatory behaviours in bearded dragons are coordinated to produce a regulated thermoregulatory response. I predicted that when one behaviour is manipulated or inhibited, others will be used to compensate, in an attempt to facilitate or maintain a constant body temperature.

Chapter 2 Thermoregulatory Behaviour in Adult and Neonatal Bearded Dragons

Introduction

Animals will commit time and energy to remain within their thermally optimal range, and thus keep metabolic processes operating at, or near, peak conditions (DeWitt, 1967; Huey & Slatkin, 1976). For ectotherms which depend on environmental heat absorption, this means using thermoregulatory behaviours to adjust body temperature in a changing environment (Cowles & Bogert, 1944). How thermoregulatory behaviours originally present themselves in an individual is not clear, however. Shuttling and basking behaviours, which have a relatively high impact on body temperature, are likely present from an animal's first exposure to a novel environment. It is possible though, that shuttling and basking behaviours show lower precision in more naïve reptiles (Cadena & Tattersall, 2009b). That lizards dedicate time to exploring/habituating to a thermal gradient in the lab suggests that some learning is involved in thermoregulating in novel environments. Other subtler thermoregulatory responses might, therefore, also require time and may even need to be learned well after hatching.

Unlike birds and mammals, reptiles do not typically display parental care behaviours after their young hatch (Shine, 1988, as seen in Reynolds, Goodwin, & Freckleton, 2002). After hatching, neonates must meet all their needs on their own, but not all behaviours are present during every phase of an animal's life. Certain behaviours are more functional during neonatal or juvenile periods, while others do not appear until later in the adult stage (Barnard, 2004; Dawkins, 1995; Khan, Richardson, & Tattersall, 2010). Any behaviour that is exhibited by an animal can be crudely defined as being either innate or learned (Barnard, 2004; Dawkins, 1995). Innate behaviours are responses that prepare an animal for adaptive reactions to the world around them from the outset, without the need for experience or learning; this is also referred to as

inbuilt adaptiveness (Dawkins, 1995). Innate behaviours are typically present during all life stages with exceptions such as mating or reproduction behaviours. Learned behaviours are responses that develop through the accumulation of experience and the retention of information (Barnard, 2004). While learned behaviour is universally accepted, there is some argument as to the existence of true innate behaviours. For the purpose of this work, it is sufficient to argue that innate thermoregulatory behaviours are those present from the neonatal stage that are not easily influenced by further experience. Examples of both innate and learned responses can be found in all behaviour types (voluntary, involuntary, and autonomic) (Barnard, 2004; Dawkins, 1995). In some cases, classical conditioning can be used to introduce a learned aspect to a typically innate behaviour or reflex (Barnard, 2004). A reflex is one of the simplest forms of behaviour that is an automatic, consistent unit, typically responding to a simple stimulus and does not vary in form. The occurrence of a reflex may be variable with context and habituation, despite the specific form not changing (Barnard, 2004). Any new conditioned response often shows noticeable differences in plasticity and pathways, from the original unconditioned response (Thompson & Krupa, 1994).

Voluntary behaviours display a blend of innate and learned responses (Dawkins, 1995; Thrope, 1958). In many instances, a voluntary behaviour is innate and therefore present without any learning being required. Dawkins (1995) summarises work on crickets (*Teleogryllus*), indicating that mating songs do not change regardless of the environment the animals are raised in, showing this voluntary behaviour to be innate, or virtually formed and unchanging from its first occurrence. Whether a behaviour is innate or learned varies from species to species. Many bird mating calls, for example, require several weeks or months of trial and error before being perfected (Thrope, 1958). The length of time required to learn a behaviour varies depending on the species and the behaviour itself, but voluntary behaviours that are not present in juvenile

stages, or are changed by the adult stage, are likely learned behaviours, especially if they are relatively complex.

Neonatal bearded dragons have several differences from their adult counterparts. Aside from morphological and sexual developmental differences, neonatal dragons also have different social behaviours from adult dragons (Khan et al., 2010). Neonates seem more prone to gregarious behaviour; an example is aggregation. Gregarious behaviour, while common in mammals (Aureli, Cords, & van Schaik, 2002), is less prevalent in reptiles and amphibians and is not necessarily a social response. Instead, aggregation could be related to other fitness demands, such as gestation, oviposition, predation protection, or even thermoregulation (Gautier, Olgun, Uzum, & Miaud, 2006; Graves & Duvall, 1995). Khan et al. (2010), observed aggregation behaviour in neonatal bearded dragons, hoping to gain a better understanding of the interaction between this behaviour and thermoregulation. Both isolated and aggregated neonates were observed to reach a similar skin temperature on average; however, individuals within a group showed lower thermoregulatory precision than those in isolation. Khan et al. (2010) went on to suggest that aggregation in neonates is a potential consequence of mutual attraction to a limited and valuable resource (e.g. basking under a heat source). Furthermore, the drop in precision could be caused by agonistic behaviour among the aggregated neonates (Khan et al., 2010).

Due to the importance of temperature regulation, it is plausible to suggest that most thermoregulatory behaviours will show some level of innateness and therefore be conserved between adults and neonates, despite some differences in other behaviour. Behaviours such as aggregation are likely to be lost at the adult stages due to increased competition between individuals that have reached sexual maturity. Fundamental thermoregulatory behaviours, such as shuttling and basking, however, are likely observed across all life stages. Subtler yet equally important thermoregulatory behaviours, such as gaping and orientation preference, may instead be partially learned. This work seeks to address the presence of a thermoregulatory orientation

bias in bearded dragons in two life stages. Orientation bias, as a thermoregulatory behaviour, has been documented in select species, most notably marine iguanas (Bartholomew, 1966), but it has not been shown in bearded dragons. Sexually mature, adult dragons are the best option for examining the presence of any orientation preference in *P. vitticeps*, as they are larger than neonates and therefore orientation is more likely to have an impact on thermoregulation. Additionally, adults have had time to acquire any learned aspects of the behaviour, if any are present. By determining whether or not orientation preference has an impact on selected ambient temperature, or whether selected temperature impacts orientation in a thermal gradient, a better understanding of bearded dragon thermoregulatory behaviours can be gained. By observing orientation behaviour, or lack thereof, at an earlier life stage (two week old neonates) a better sense of the innateness of orientation as a thermoregulatory strategy may be gained. I hypothesized that orientation would be present as a thermoregulatory mechanism in bearded dragons in that orienting the rostrum towards a heat source would be preferred. I, therefore, expected that bearded dragons would display non-random orientation behaviour in a thermal gradient. Additionally, I predicted they would spend more time choosing to face towards a heat source rather than towards a cold source. If orientation had a significant impact on selected temperature and *vice versa* then orientation as a thermoregulatory behaviour is supported. I also hypothesized that a bias of orienting towards a heat source would be conserved across multiple life stages. I predicted that neonates would display the same orientation bias seen in the adults. Furthermore, if thermoregulatory orientation behaviour is seen in both adult and neonatal dragons, then the innateness of this behaviour will be supported. Conversely, if the neonates demonstrated random orientation behaviour, not favouring facing towards or away from a heat source but rather spending half their time facing one direction and the other half facing the other, then the adult behaviour is more likely learned.

Methods

Animal Husbandry

All animals used in the following experiments were kept at Brock University in a room dedicated exclusively to bearded dragons (*Pogona vitticeps*) and a small cockroach colony. A total of 46 dragons were maintained and used during this time. Of the 46 dragons, 20 were adults (10 Male and 10 Female) and 26 were neonates. Each adult dragon, older than one year, had its own terrarium made by Brock University machine shop. Each terrarium was 76cm x 76cm x 42cm with either a corn cob or a coconut husk bedding and was equipped with a 40W light bulb set over a stone basking plate, which provided a maximum temperature of 45°C compared to a minimum temperature of 25°C at the far side of the terrarium. In addition to a standard light bulb, a UV light (13W Reptisun® 10.0 mini compact fluorescent) was also installed to provide UV required for vitamin D synthesis. All cages were given extra enrichment in the form of cardboard packing material, which was used for shelter or as a climbing surface, and Polyvinyl chloride (PVC) pipe sections, used as hiding spots. The neonatal animals were kept in small cages until they grew large enough to move to an adult cage. These smaller terraria were 45cm x 24cm x 20cm and had paper towel bedding. Heating pads were laid down under the cages and set to provide half of each enclosure with heat. Smaller PVC pipe sections were also supplied as additional enrichment. In addition, neonates were put two to a cage unless they showed aggressive behaviours, e.g., biting or attacking cage mates, in which case they were placed in individual cages.

All lizards were under a 12:12 light:dark cycle and fed cockroaches once or twice a week. In addition, all lizards were fed a diet of assorted chopped fruits and vegetables which they ate *ad libitum* and which were replaced three times a week. Once a week all lizards received a water bath for sanitation and hydration.

Thermal Preference and Orientation Measurement in Adults Protocol

Thermal and orientation preferences were determined using video footage and temperature sensors. During Series I (see below), iButtons™ were affixed to the lizards and gave a measurement of ambient/skin temperature preference for each lizard. All iButtons were calibrated to the computer's clock before being activated and the sampling times were synchronized. Each iButton was set to record every 30 seconds starting immediately without any roll over. Once all the needed iButtons were programmed, two of them were attached to the lizard using 3M Transpore™ tape, one on the ventral side of the abdomen, another on the ventral side of the tail.

In order to examine orientation and any other behaviours, animals were placed in a thermal gradient. The thermal gradient used in Series I had two water baths connected to either end to create a range of temperatures, from 15°C to 45°C along the floor of the experimental chamber. In addition to the floor, an air gradient was also created by using fans, connected to the water baths, at either end of the sheet. The gradient had walls set 18 cm apart and 10 cm high while the length of the gradient itself was 163 cm. This created a somewhat narrow lane that forced each individual lizard to choose a direction to face during the experiment, either cold or hot. The animal was then placed in the center of the gradient facing either left or right, and was allowed to move freely. When placing animals in the gradient, the direction they were facing was randomly alternated, as was the warm and cold sides of the gradient itself. This was true for every series involving thermal gradients.

To ensure a more consistent air gradient within the experiment, Plexiglas was placed on top of the walls to prevent the animal from escaping and to also inhibit heat exchange between the gradient and outside. The gradient set-up allowed the animal more than enough room to manoeuvre but forced it to choose one of two possible orientations when at rest in order to be comfortable. Next, a simple Microsoft LifeCam® VX-1000 web camera was used so that the

animal's movements could be recorded in multiple ways. The camera was placed facing down perpendicular to the gradient. The cameras were set to take an image every 30 seconds and were timed to coincide with the iButtons' temperature sampling.

Neonatal Thermoregulatory Behaviours Protocol

Series II (see below) was set up in a similar fashion to Series I; however, Series II used neonatal bearded dragons. The chosen animals were all two weeks old and, due to their smaller size, were placed in a smaller thermal gradient that was only 53 cm in length (thermal gradient $\sim 0.47^{\circ}\text{C}/\text{cm}$). Furthermore, iButtons were not used for this trial as they were too large to be easily attached to the bodies of the subjects. To collect body temperature, a thermal imaging camera was suspended above the gradient to record skin temperature. Each trial ran four lizards at a time, where each lizard was placed into an individual lane separated by a 10 cm high opaque plastic wall. Once this was set up, the animals were allowed to thermoregulate normally for 6-8 hours.

Experimental Design

Series I: Thermal Preference in Adult Bearded Dragons

In this series, lizards underwent the thermal preference and orientation measurement protocol (outlined above). These animals, which were freely behaving and non-manipulated, gave a baseline for all the other adult series that were run (see Chapter 3). Once an animal was prepared, it was placed in the thermal gradient and allowed to move freely for six to eight hours. The first four hours made up the exploration phase, which allowed the lizards to explore the thermal gradient so they could habituate to their new environment (Cadena & Tattersall, 2009b). After the four hour exploration phase, another two to four hours of data were collected to better capture thermoregulatory behaviour in the absence of exploratory behaviour. Images of the

animals used in this series were digitally captured using a camera mounted above and perpendicular to the gradient to record orientation and movement. Seventeen animals were used, seven females and ten males.

Series II: Thermal Preference in Neonatal Bearded Dragons

This series examined the thermoregulatory behaviour in neonatal bearded dragons, specifically the selected ambient temperature and the presence or absence of orientation bias. To begin, the neonatal thermoregulatory behaviour protocol (outlined above) was followed for a selected lizard. The lizard was then placed in the neonatal thermal gradient and allowed to thermoregulate on its own for six to eight hours. The lengthy time scale allowed the animals' time to explore the gradient, which typically lasted for up to four hours. After the completion of the experiment, all four lizards were taken back to their respective cages. Due to the time constraint on this particular set of experiments, the neonates were tested in batches of 10 at a time over a four month period for a total of 26 animals, and all trials were completed three days after their arrival at Brock University.

Data Analysis

Thermal preference in adults

The temperature data were taken from the iButtons and aligned with the corresponding time point. Next, each frame of the video footage (taken every 30 seconds) was studied to observe orientation and movement. Behaviour was scored in a binary format; orientation preference was scored as either facing heat (H) or facing away from heat (C) per frame, and movement was scored as either moving (Y) or stationary (N) per frame.

Thermal preference in neonatal bearded dragons

Since these trials were performed using a thermal imaging camera (Mikron Model 7515), both the surface temperature of the animal and the orientation and movement were recorded through video. The program MikroSpec-RT was used to study these recorded video files. To calculate neonatal surface temperature, a region of interest (ROI) analysis was used. An ROI analysis determines the mean temperature of a selected region. Using ROIs, an average surface temperature was calculated for each lizard starting from the middle of the head down the body to the base of the tail. As with the adult trials, each frame was studied and orientation was recorded. Orientation and temperature data were scored in the same way as Series I. Similar to the adult trials, the frame rate was set to record every 30 seconds.

Statistical Analysis

Statistical analysis done on Series I data used R (R Development Core Team, 2012) and lme4 (Bates, Maechler, Bolker, & Walker, 2015) to perform three separate linear mixed effects analyses on the impact of multiple terms on selected ambient temperature, orientation preference, and movement. For the analysis of selected temperature, I entered orientation preference, movement, body mass and sex into a global model, including select interactions that were chosen for biologically justifiable reasons, as fixed effects. . For the analysis of orientation preference, I entered selected temperature, movement, body mass and sex into a global model, including select interactions that were chosen for biologically justifiable reasons, as fixed effects. For the analysis of movement, I entered orientation preference, selected temperature, body mass and sex into a global model, including select interactions that were chosen for biologically justifiable reasons, as fixed effects. As random effects, I included only intercepts for animal ID to account for the repeated measures design, into all models.

Statistical analysis done on neonatal data (Series II) used R (R Development Core Team, 2012) and lme4 (Bates et al., 2015) to perform a linear mixed effect analyses (LME) and a generalized linear mixed-effects model (GLMM). The LME was used to analyse the impact of multiple terms on selected ambient temperature, while the GLMM was used to analyse the impact of multiple terms on orientation preference, modelled as a logistic response. For the analysis of selected temperature, I entered orientation preference and time into a global model, as fixed effects. For the analysis of orientation preference, I entered selected temperature and time into a global model, as fixed effects. As random effects, I included only intercepts for animal ID to account for the repeated measures design, into all models. To avoid autocorrelation of the model residuals, the data were resampled at every 10th time point, and an autocorrelation estimation of the residuals was used to confirm.

In both series (Series I and II), I then fit all possible models that nested within the global model, calculated and ranked AICc values (Akaike, 1974), choosing the model that exhibited the highest likelihood of all possible models. Model selection and the information-theoretic approach was implemented using the MuMIn package in R (Barton, 2015). I present model weights as measures of support for parameters; however, when presenting model coefficients (*i.e.* effects), the highest ranked model is presented, which also included superior ranking models. Visual inspection of residuals and QQ plots did not reveal any obvious deviations from homoscedasticity or normality. P-values were obtained using likelihood ratio tests (Type II Wald's chisquare tests) of the full model with the effect in question against the model without the effect in question, using the "car" package in R (Fox & Weisberg, 2011).

Results

Series I: Behavioural Responses in Adult Bearded Dragons

In the adult dragons, three behavioural explanatory variables were examined (orientation, movement and selected temperature) as well as mass, sex, and time. The first behavioural response examined was selected ambient temperature. Model number two (Appendix Table 2.1) supported mass, orientation, sex, time, orientation by sex interaction, orientation by time interaction, and sex by time interaction as the variables that impacted selected ambient temperature in the adult, non-manipulated lizards. A subsequent analysis of deviance showed that selected temperature was negatively associated with orientation to the heat ($B = -1.742$; $p = 1.300 \times 10^{-5}$), but increased positively over time ($B = 0.09172 \text{ }^{\circ}\text{C}/\text{min}$; $p < 1.000 \times 10^{-10}$) (Appendix Table 2.2). The analysis of deviance revealed that selected temperature was positively associated with sex (males) by time interaction ($B = 0.7057 \text{ }^{\circ}\text{C}/\text{min}$; $p = 1.250 \times 10^{-7}$) (Appendix Table 2.2). As selected temperature increased, animals were less likely to face a heat source (Figure 2.1). Selected temperature rose with increasing time and males had a higher slope than females as time increased (Figure 2.1).

The next response variable examined was orientation. Model three (Appendix Table 2.3) supported: movement, mass, selected temperature, sex, time and selected temperature by sex interaction as the variables that impacted preferred orientation in adult bearded dragons. Orientation towards the heat was negatively associated with movement ($B = -0.6160$; $p = 0.001240$) but was positively associated with time ($B = 0.164$; $p = 5.540 \times 10^{-6}$) (Appendix Table 2.4). Orientation towards the heat was negatively associated with selected ambient temperature ($B = -0.1330^{\circ}\text{C}$; $p = 2.270 \times 10^{-6}$) and with selected ambient temperature by sex (males)

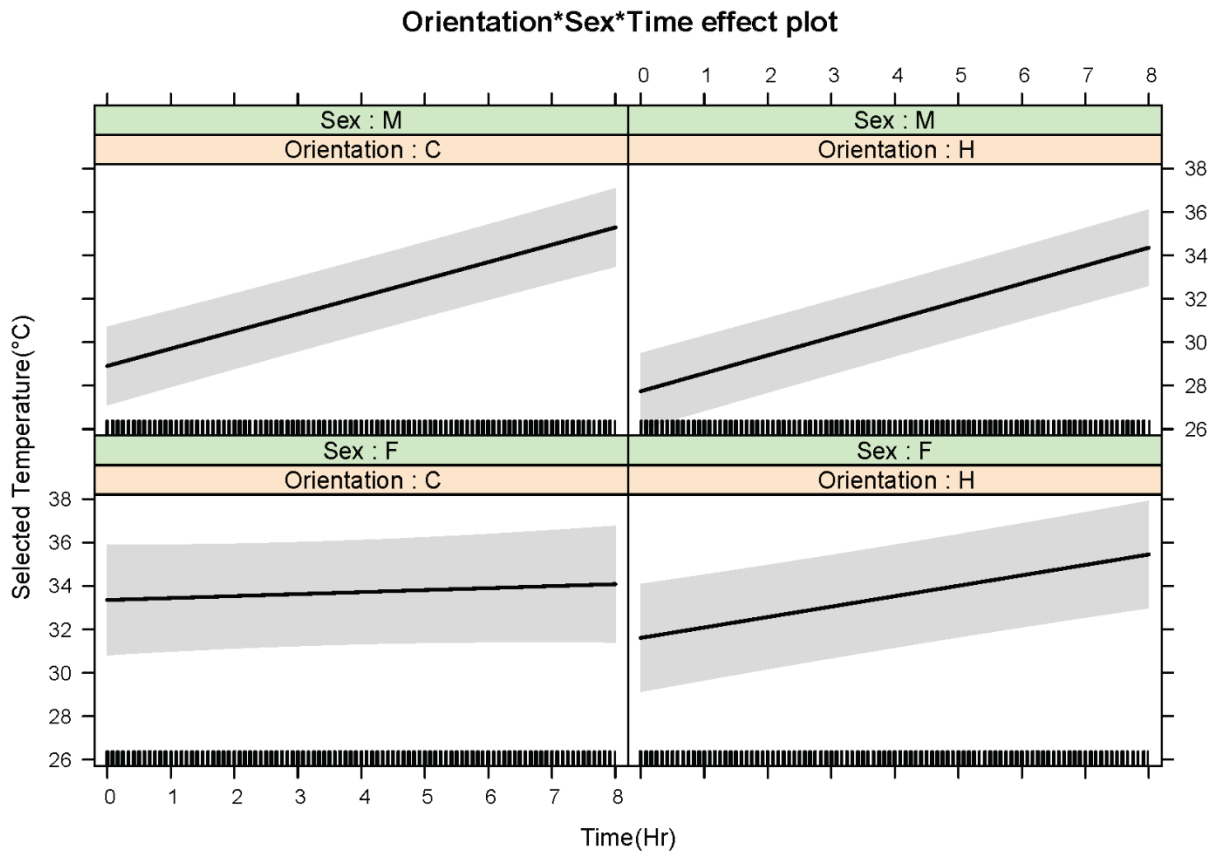


Figure 2.1. Influence of time, sex, and orientation on selected ambient temperature (Temperature) of adult Bearded Dragons (*P. vitticeps*). Time is expressed in hours, while orientation is expressed as facing towards (H) or away from (C) a heat source, and sex is coded as male (M) or female (F). Temperature is expressed in °C, and all animals used were adults over 3 years of age. A total of 17 animals were used, 10 males and 7 females. The grey shade represent the upper and lower 95% confidence.

interaction ($B = -0.4640^{\circ}\text{C}$; $p = 0.03360$). No other terms were found to be significant. The probability that an animal will orient towards a heat source decreased significantly with increased movement (Figure 2.2). As time increased, the probability of facing a heat source rose as well. Higher selected ambient temperature caused animals to be less likely to face a heat source both on its own and by a sex interaction (Figure 2.2).

The next response variable that was examined was the probability of moving. Model two (Appendix Table 2.5) supported orientation, mass, and time as the variables that impacted time spent moving in the adult, non-manipulated lizards. Movement was negatively associated with orientation towards a heat source ($B = -0.6225$; $p = 7.110 \times 10^{-4}$) (Appendix Table 2.6). No other terms were significant. The chance of movement decreased significantly when dragons were facing a heat source in comparison to when they were facing away from a heat source (Figure 2.3).

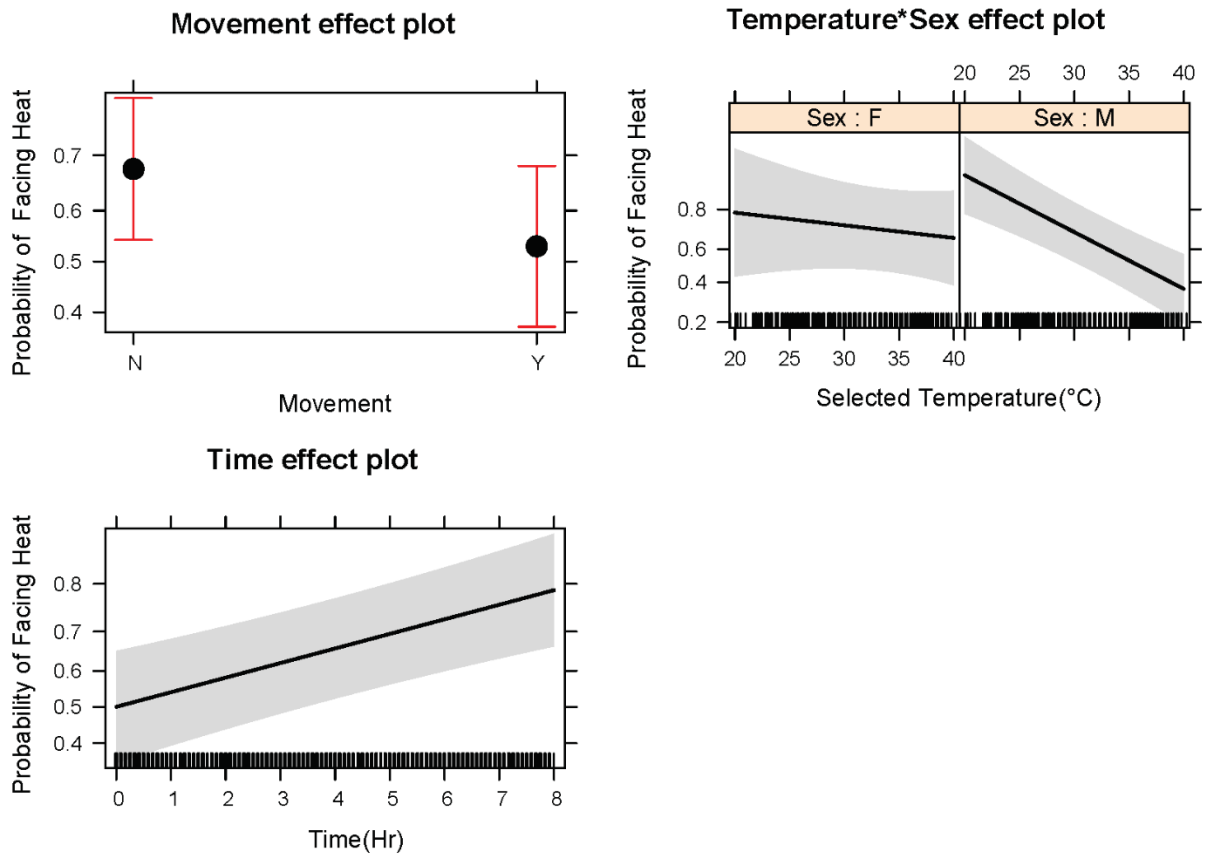


Figure 2.2. The model predicted probability that adult Bearded Dragons (*P. vitticeps*) will orient towards a heat source, against movement (top left), selected ambient temperature by sex interaction (top right), and time (bottom left). Time is expressed in hours, movement is coded as time spent moving (Y) or time spent not moving (N), selected temperature is expressed as degrees Celsius, and sex is coded as either male (M) or female (F). Data were collected from animals in a thermal gradient over a maximum of 8 hours. Orientation is expressed as the probability of an animal facing the warm side of the gradient. A total of 17 animals were used, 10 males and 7 females. The error bars and the grey shade represent the upper and lower 95% confidence.

Orientation effect plot

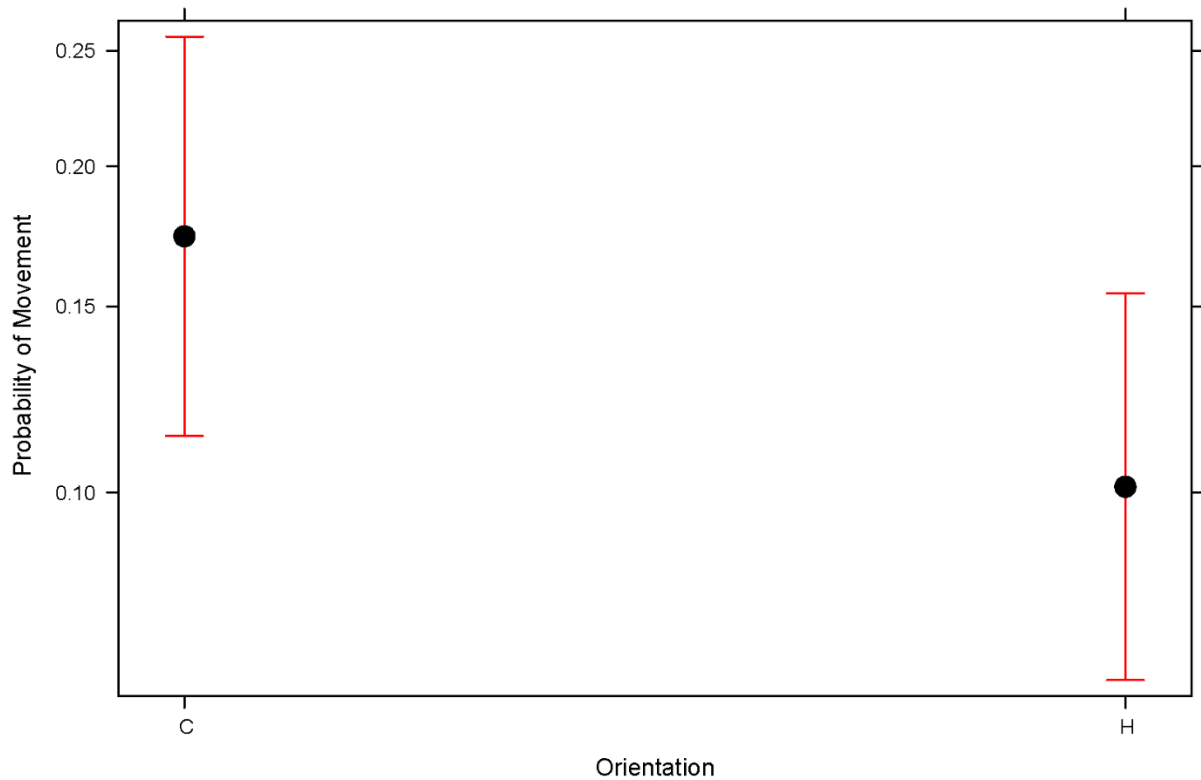


Figure 2.3. The model predicted probability that adult Bearded Dragons (*P. vitticeps*) will move, against orientation. Orientation is coded as facing a heat source (H) or facing away from a heat source (C). Data were collected from animals in a thermal gradient over a maximum of 8 hours. Movement is expressed as the probability of an animal moving. A total of 17 animals were used, 10 males and 7 females. The error bars represent the upper and lower 95% confidence.

Series II: Behavioural Responses in Neonatal Bearded Dragons

In the bearded dragon neonates, two response variables were examined, orientation preference and selected ambient temperature. The explanatory variable, time, was also included in the global models. The first behavioural responses examined was selected ambient temperature. Model one (Appendix Table 2.7) supported time, orientation and orientation by time interaction as the terms that impacted selected ambient temperature in neonatal bearded dragons (Appendix Table 2.7). Selected ambient temperature had a positive association with time ($B = 0.5046^{\circ}\text{C}/\text{min}$; $p < 1.000 \times 10^{-10}$) (Appendix Table 2.8). Selected temperature also, had a negative association with orientation towards a heat source by time interaction ($B = -0.1231^{\circ}\text{C}/\text{min}$; $p = 0.03516$) (Appendix Table 2.8). As time increased, the chances of neonates selecting higher ambient temperatures rose (Figure 2.4). Also, as time increased, neonates were less likely to select higher temperatures when orienting towards a heat source, than when they were facing away from one (Figure 2.4).

The second response variable to be examined was the preferred orientation of neonatal bearded dragons with respect to time. Model one (Appendix Table 2.9) supported time, selected temperature, and selected temperature by time interaction, as the variables that impacted orientation in neonatal bearded dragons. Orientation was negatively associated with selected temperature by time interaction ($B = -0.09100/^{\circ}\text{C}$; $p = 0.0002910$) (Appendix Table 2.10). As time increased, neonates were more likely to face a heat source when they were in cooler temperatures (Figure 2.5). At warmer temperatures, however, neonates were less likely to face a heat source as time increased. Overall, neonates face the heat approximately 69% of the time, which is significantly greater than that predicted by chance (50%).

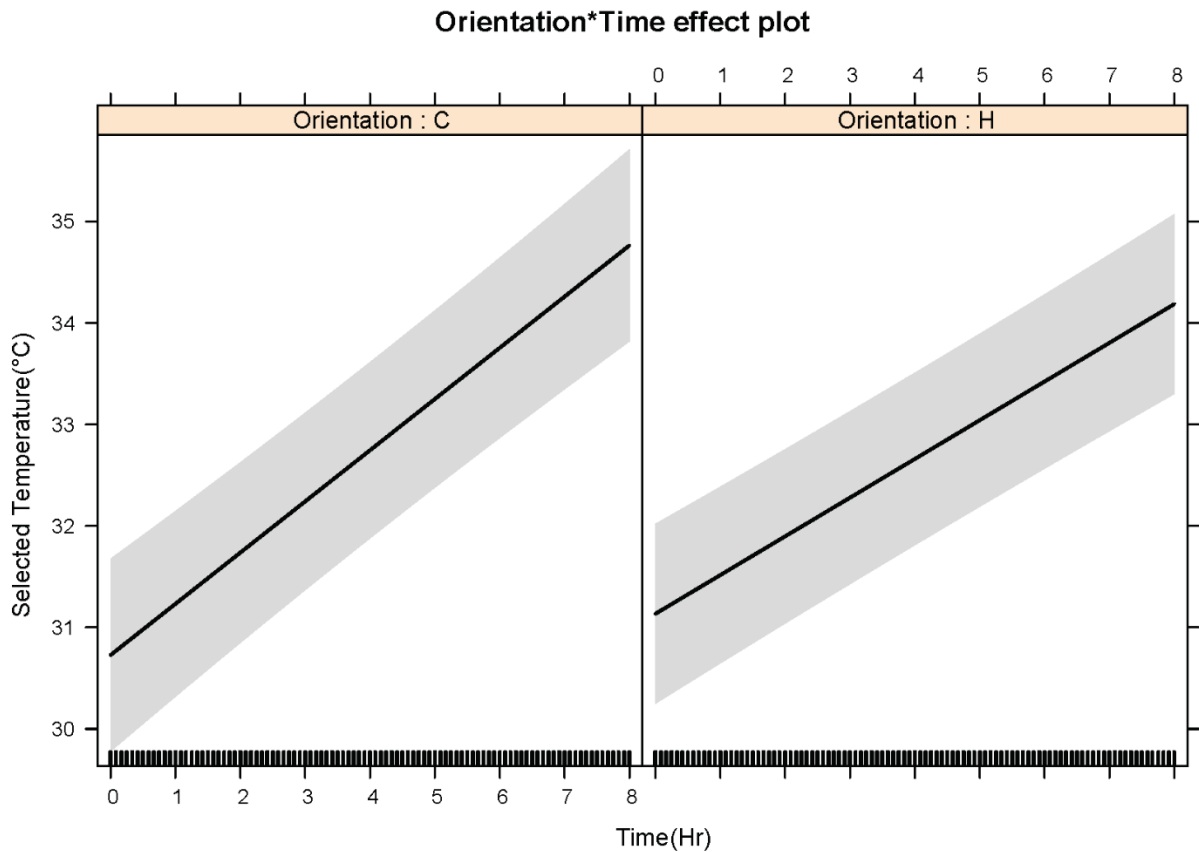


Figure 2.4. The selected ambient temperature (°C) of neonatal Bearded Dragons (*P. vitticeps*), in comparison to preferred orientation by time interaction. Time is expressed in hours and orientation is coded as facing heat (H) or facing away from heat (C). Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the selected ambient temperature while the x-axis shows the explanatory variable. A total of 26 animals were used, all under two weeks old. The grey shade represent the upper and lower 95% confidence.

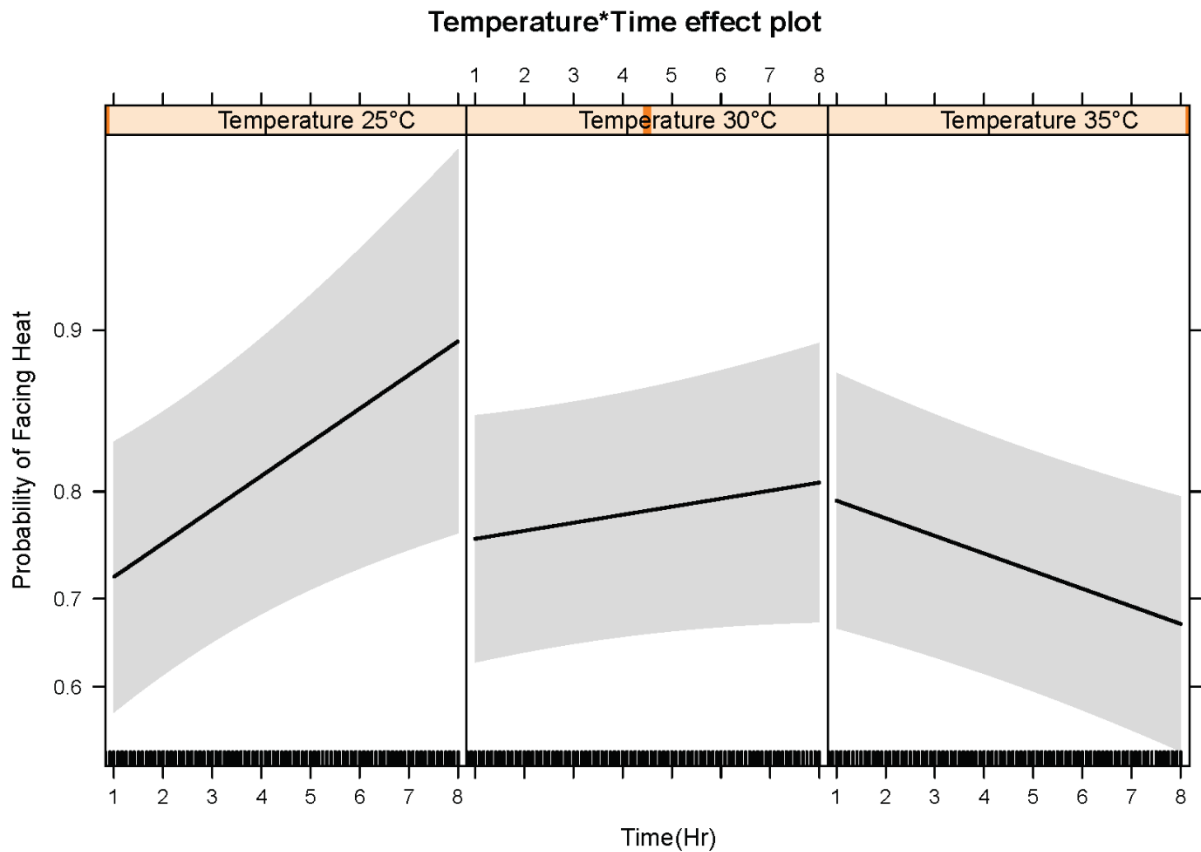


Figure 2.5. The model predicted probability that neonatal Bearded Dragons (*P. vitticeps*) will face a heat source, as a function of time at three select temperatures (since selected temperature was a covariate, to simplify the effects display, three temperatures were chosen that span the range of selected temperatures). Time is expressed in hours and orientation is expressed as a ratio. The temperatures shown are 25°C (left), 30°C (middle), and 35°C (right). Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the selected ambient temperature while the x-axis shows the explanatory variable. A total of 26 animals were used, all under two weeks old. The grey shade represent the upper and lower 95% confidence.

Discussion

I hypothesised that thermal orientation would be present as a thermoregulatory strategy and would be an innate behaviour in bearded dragons. In adult bearded dragons, my data supports chosen orientation as a behaviour related to thermoregulation, as seen in other species of reptile (Bartholomew, 1966; Cowles & Bogert, 1944; Gibson et al., 2015; Sartorius et al., 2002). Marine iguanas are well known for their orientation behaviours in the wild. In the morning and especially during the afternoon, marine iguanas preferentially orient perpendicularly to the sun's rays to avoid overheating and to increase exposure to cooler trade winds (Bartholomew, 1966). In contrast, the Otago/Southland gecko uses orientation to expose portions of the abdomen to direct solar radiation in order to heat those sections up faster (Gibson et al., 2015). The orientation preference displayed by bearded dragons seems to suggest that they either orient to prevent over cooling/heating of the head or to expedite heating and cooling of the posterior tail region. My data support a connection between orientation and other thermoregulatory responses. The presence of thermoregulatory orientation behaviours at relatively young neonatal stages has never been previously described in bearded dragons or in many other reptiles. The neonatal data outlined in this work suggests a high level of importance for orientation behaviours, with regard to thermoregulation, at multiple life stages. The data from Series II also support the possibility that orientation behaviours are innate. Preferential orientation for the purposes of thermoregulation has never been observed in a natural environment in *P. vitticeps*, but due to the larger variety of microenvironments and possible orientation and postural changes available in nature as opposed to the lab, it is plausible to speculate that this behaviour would be more difficult to tease out in nature.

Ancillary Experimental Effects: Mass, Time, Sex, and Movement

The variation in selected ambient temperature that the adult and neonatal bearded dragons showed is consistent with what we would expect to find in behaviourally thermoregulating lizards (Cadena & Tattersall, 2009b). During the earlier time periods of each trial (first four hours) the dragons showed a higher probability of selecting lower ambient temperatures, suggesting that they were exploring the gradient (Figure 2.1; Figure 2.4). As time increased, selected ambient temperature increased, again consistent with what would be expected, suggesting that the bearded dragons were thermoregulating more consistently during the later periods. In addition, the probability of facing a heat source rose with time, suggesting that orientation behaviour may be thermoregulatory as bearded dragons select higher temperatures during the later phase of the trial, suggesting they are thermoregulating (Figure 2.1, Figure 2.2).

Curiously, preferred orientation in adults was also impacted by sex, in that males were less prone to orient towards the heat at higher selected temperatures. The trend for males to face heat less as temperature rises is possibly an artefact of males also being more likely to select higher temperatures than females, as time increases (Figure 2.1), since selecting higher temperatures increases the probability of facing away from a heat source.

Selected temperature does not have an impact on time spent moving. This indicates that increased movement is not a response to temperature but probably represents spontaneous exploratory behaviours.

Main Experimental Effects: Orientation and Selected Temperature

Adult and neonatal bearded dragons selected a higher ambient temperature when facing away from heat (Figure 2.1; Figure 2.4), although this effect is dependent on an interaction with time in neonatal bearded dragons. Facing heat less at high temperatures is possibly a response to cool the head, but this is unclear from this data. Series I and II does show that both adult and

neonatal bearded dragons face a heat source more often than what would be expected from random chance. Additionally, bearded dragons are more likely to face heat at cooler temperatures and more likely to face away from heat at warmer temperatures in both adults and neonates (Figure 2.2: Figure 2.5), although in neonates this effect is, again, dependent on an interaction with time. As orientation both impacts and is impacted by selected ambient temperature, the decision to face towards or away from a heat source is further supported as a thermoregulatory behaviour, but is possibly more dynamic in its response.

The specific utility of orientation as a thermoregulatory behaviour is unclear from the data collected in this series. Since selected temperature can both influence and be influenced by orientation, however, it is plausible that orientation is used for both warming and cooling, and thus integrated with bearded dragons' suite of thermoregulatory responses. The association between orientation and selected temperature, in neonates, being additionally impacted by time interaction suggests that orientation behaviours are less precise in neonates in comparison to adults. In the adults, the design of the thermal gradient effectively forced the lizards to choose a particular orientation, whereas neonates were less constrained to face in a particular direction. The higher variability in neonates is likely explained in part by this methodological difference, but even so, neonatal lizards orient toward the heat ~70% of the time, suggesting a true preference for an anterior to posterior gradient in temperature.

Conclusion: Thermoregulatory Orientation

The importance of thermoregulation is well documented (Angilletta, 2009; Blumberg et al., 2002; Christian & Tracy, 1981; Tattersall, et al., 2012; Vandamme et al., 1991), therefore, it is not surprising that bearded dragons and other reptiles would develop multiple voluntary and involuntary responses to aid in thermoregulation, such as gaping (Crawford Jr., 1972; Tattersall et al., 2006), orientation (Bartholomew, 1966; Gibson et al., 2015), and shuttling (Cowles &

Bogert, 1944). Orientation preference was shown to have an impact on selected ambient temperature in adult bearded dragons, suggesting that these animals do use orientation as a means of thermoregulating. Previous work that has examined orientation as a thermoregulatory behaviour, found that orientation preference is used in tandem with other behaviours, such as basking, and can be used as either a buffer against overheating or overcooling. By using orientation as a buffer, lizards can bask in extremely warm areas without overheating or absorb as much heat as possible in cooler areas (such as from the sun during cooler parts of the day) to prevent overcooling (Bartholomew, 1966; Gibson et al., 2015; Sartorius et al., 2002). The data collected in Series I suggest two possible explanations: either bearded dragons use orientation as a possible means of fine tuning regulation of head or brain temperature, or orientation is used as a means of heating up and cooling down the large posterior tail region of the animal. Intuitively, it seems that, from the data collected, the most likely scenario is that the dragons use orientation to regulate head temperature somewhat separately from body temperature, similar to how certain reptiles use gaping (Crawford Jr., 1972). It is also possible that orientation behaviour is used to prioritise multiple thermoregulatory requirements at the same time, such as keeping the head warmer while losing heat through the tail. Given the concentration of sensory processing in the head, and large surface area of the tail, the optimum way to maintain optimal neural function would be to orient the head towards the heat.

Orientation as a thermoregulatory behaviour in neonatal bearded dragons, while present, does not appear to be as precise or as impactful on selected temperature as it does in adults. The presence of orientation as a thermoregulatory response in neonates suggests that this behaviour is innate, and therefore requires no accumulation of experience (Barnard, 2004), although my results are not definitive. Orientation as a means of thermoregulating could very well be learned at an earlier stage than two weeks old. The stronger interaction between orientation and selected temperature in adults suggests that this behaviour's precision and effectiveness are improved as

the animal ages. While the data collected in Series II are not sufficient to indicate whether orientation is innate or learned, the importance of orientation as a thermoregulatory response is supported as it is present in both life stages studied. It is likely that as size increases, the proximate drivers for orientation behaviours change. Larger animals require longer periods of time to change temperature and may require specific postural orientation in the wild that maximise rates of heating.

Chapter 3 Orientation Preference and Influences on Adult Bearded Dragon Thermoregulation

Introduction

Animals commit time and energy to remain within their thermally optimal range, and thus keep metabolic processes operating at, or near, peak conditions (Angilletta, 2009). For ectotherms that depend on environmental heat absorption, this means using thermoregulatory behaviours to adjust body temperature (T_b) in a changing environment (Bennett, 2004; Cowles & Bogert, 1944; Hammel et al., 1967; Tattersall, et al., 2012). To achieve body temperatures that are sufficiently different from the environment, ectotherms, such as lizards, alternate between shuttling and basking behaviours (Cowles & Bogert, 1944). For smaller, fine tuning of body temperature, behaviours such as gaping, postural adjustments, and orientation changes are used (Bartholomew, 1966; Crawford Jr., 1972; Tattersall et al., 2006). Most reptiles keep their T_b within a specific range during periods when they are active and awake (Cowles & Bogert, 1944; DeWitt, 1967), the precision of which is dependent on several variables, such as latitude, oxygen levels, and habitat quality (Blouin-Demers & Weatherhead, 2001; Buckley et al., 2012; Cadena & Tattersall, 2009a; DeWitt, 1967). Thermoregulation is a large part of an animal's daily behaviours, and balancing the sensory input and eventual output for thermoregulation is an integral component of this.

Once a thermoregulatory response is evoked, reptiles display a voluntary or involuntary behaviour that allows body temperature to be readjusted to desirable levels. In some cases, the consequences of a given thermoregulatory behaviour are clear; gaping enhances evaporative heat loss for example (Crawford Jr., 1972; Tattersall et al., 2006), whereas others, such as orientation and posture, can vary depending on the species and the circumstances. Marine iguanas (*Amblyrhynchus cristatus*), for example, use orientation to expose surface area to cooler winds

rather than the sun to keep body temperature cooler than ambient temperature (Bartholomew, 1966). The Otago/Southland gecko (*Woodworthia* Otago/Southland), which inhabits cooler regions than does the marine iguana, uses orientation to expose more surface area to the sun in order to warm up (Gibson et al., 2015). The use of orientation as a means of thermoregulation in bearded dragons has been previously described (see Chapter 2), but whether this behaviour is used to cool off, warm up, or a mixture of the two is unknown.

Aside from changes to the external or internal temperature of a reptile, thermoregulatory behaviours are also influenced by other factors such as changes in plasma osmolality (Scarpellini et al., 2015), or oxygen levels (Hicks & Wood, 1985). Since coordination is shown between thermoregulation and other biotic and abiotic factors (Cadena & Tattersall, 2009a; DeWitt, 1967; Hicks & Wood, 1985; Scarpellini et al., 2015; Wood, 1995), thermoregulatory behaviours should also show coordination with each other. Therefore, the loss of one thermoregulatory response should cause others to compensate and limit or negate any impact on selected ambient temperature or preferred body temperatures. By impeding specific thermoregulatory structures and behaviours, the presence of coordination can be assessed. Additionally, by blocking cutaneous heat exchange and temperature sensation in the posterior region of bearded dragons, as well as blocking evaporative heat loss through the mouth, a better understanding of the drivers of thermoregulatory orientation behaviour can be gained.

I first hypothesised that orientation was driven by thermal exchange in anterior structures. I predicted that gaping inhibition would cause a change in orientation preference away from facing a heat source. My secondary hypothesis was that orientation was driven by thermal exchange in posterior structures. I anticipated that inhibition of heat exchange across the tail would cause a change in orientation preference away from facing a heat source. I also expected thermoregulatory behaviours would show coordination, meaning that, should one be inhibited, others would compensate for the absence. Specifically, I anticipated that, when the tail is

covered/insulated, the amount of time spent gaping would increase, the time spent orienting towards a heat source would decrease, and the selected ambient temperature may drop. I predicted similar circumstances if gaping is prevented, in that selected ambient temperature may drop and time spent facing a heat source will decrease.

Methods

Animal Husbandry

All animals used in the following experiments were kept at Brock University in a room dedicated exclusively to bearded dragons (*Pogona vitticeps*) and a small cockroach colony. A total of 46 dragons were maintained and used during this time. Of the 46 dragons, 20 were adult bearded dragons (10 male and 10 female). Each adult dragon, older than one year, had its own terrarium made by Brock University machine shop. Each terrarium was 76cm x 76cm x 42cm with either a corn cob or a coconut husk bedding and was equipped with a 40W light bulb set over a stone basking plate, which provided a maximum temperature of 45°C compared to a minimum temperature of 25°C at the far side of the terrarium. In addition to a standard light bulb, a UV light (13W Reptisun® 10.0 mini compact fluorescent) was also installed to provide UV required for vitamin D synthesis. All cages were given extra enrichment in the form of cardboard packing material, which was used for shelter or as a climbing surface, and Polyvinyl chloride (PVC) pipe sections, used as hiding spots.

All lizards were under a 12:12 light:dark cycle and fed cockroaches once or twice a week. In addition, all lizards were fed a diet of assorted chopped fruits and vegetables which they ate *ad libitum* and which were replaced three times a week. Once a week all lizards received a water bath for sanitation and hydration.

Thermal Preference and Orientation Measurement Protocol

Thermal and orientation preferences were determined using video footage and temperature sensors. During Series IV and Series V (see below), iButtons™ were affixed to the lizards and gave a measurement of ambient/skin temperature for each lizard. All iButtons were calibrated to the computer's clock before being activated and the sampling times were synchronized. Each iButton was set to record every 30 seconds starting immediately without any

roll over. Once all the needed iButtons were programed, two of them were attached to the lizard using 3M Transpore™ tape, one on the ventral side of the abdomen, another on the ventral side of the tail.

In order to examine orientation and any other behaviours, animals were placed in a thermal gradient. The thermal gradient used in Series IV and V had two water baths connected to either end to create a range of temperatures, from 15°C to 45°C along the floor of the experimental chamber. In addition to the floor, an air gradient was also created by using fans, connected to the water baths, at either end of the sheet. The gradient had walls set 18 cm apart and 10 cm high while the length of the gradient itself was 163 cm. This created a somewhat narrow lane that forced each individual lizard to choose a direction to face during the experiment, either cold or hot. The animal was then placed in the center of the gradient facing either left or right, and was allowed to move freely. When placing animals in the gradient, the direction it was facing was randomly alternated, as was the warm and cold sides of the gradient itself. This was true for every series involving thermal gradients.

To ensure a more consistent air gradient within the experiment, Plexiglas was placed on top of the walls to prevent the animal from escaping and to also inhibit heat exchange between the gradient and outside. The gradient set-up allowed the animal more than enough room to manoeuver but forced it to choose one of two possible orientations when at rest in order to be comfortable. Next, a simple Microsoft LifeCam® VX-1000 web camera was used so that the animal's movements could be recorded in multiple ways. During series IV and V, two cameras were placed in the gradient itself, one at either end. The cameras were set to take an image every 30 seconds and were timed to coincide with the iButtons temperature sampling.

Tail Inhibition Protocol

In order to properly insulate the posterior region of the animal a simple protocol was followed. First, depending on the experimental series, either the core temperature measurement (see below) or thermal preference and orientation measurement protocol was followed. Once this was done, standard Ap/Armaflex pipe insulation, from Armacell®, was wrapped around the animals' tails covering the tail and iButton completely. The insulation was secured using 3M Transpore™ tape and Velcro straps to tightly bind the fabric to the tail. In addition to this, the insulation was also connected to the tape wrapped around the body to ensure that the lizards did not extricate themselves from the material covering the tail during the trial. This was done by placing a connecting piece of tape from the insulation to the tape wrapped around the abdomen along the dorsal side of the animal. In the case of sham trials, the same protocol was followed with the exception that thin gauze, made by Johnson and Johnson™, was used in the place of insulation. Furthermore, another iButton was placed on the exterior of the insulation or insulation sham on the ventral side, in addition to iButtons being placed on the ventral side of the abdomen and tail, yielding three iButtons total for this experiment.

Mouth Sealing Protocol

In order to properly inhibit the anterior region, animals were prevented from gaping by temporarily muzzling the mouth. To achieve this mouth sealing, dental adhesive (Impregum F™) was applied to the lizard's lip margin in. This was done in such a way as to create a thin, form-fitting muzzle that would be unique to each animal. This muzzle was made to be secure enough that gaping would be inhibited, but care was taken to avoid blocking the nares, the eyes, the pineal eye, or the tympanic membrane. During the sham trial, the animals had Impregum placed around the mouth in such a way that did not prevent gaping, again taking care to avoid any sensory or respiratory structures on the face. Since jaw opener muscles in many reptiles are much

smaller than closer muscles, only small amounts of dental amalgam was required to keep the mouth closed.

Experimental Design

Series III: Rates of temperature change

Core Temperature Measurement

Core temperature was estimated during the trials using a cloacal probe. First, a lizard was given a 10-30 minute water bath in warm water (roughly 20°C) to allow for easier probe insertion and to insure proper hydration. An MSR Electronics® (MSR145) temperature probe was coated in Vaseline™ and then placed in the cloaca of the selected adult lizard. This probe was connected to a sensory pack that was 5.5cm long and 2 cm wide. Both the data pack and its connecting probes were attached to an animal using 3M Transpore™ tape wrapped around the body and the tail. The pack stored all recorded data from the cloacal probe so that it could be extracted once the trial was complete. In addition to this, a second sensor was left exposed to the surrounding environment to record ambient temperature. The second sensor was also an MSR probe attached to the same data pack as the cloacal probe, and both sensors recorded temperature data points every 30 seconds.

Measuring Rates of Temperature Change

Measuring the rate of heat temperature change allowed for an examination of the impact of insulation around the tail and inhibition of gaping on heat exchange with the environment. While core temperature was being measured, the animal randomly underwent one of the five treatments (non-manipulated, tail inhibited, tail inhibited sham, mouth sealed, mouth sealed sham). From here, the animal was placed in one of two thermal chambers (one warm and one cool; see below) and left to equilibrate to the ambient temperature for a maximum of 3 hours.

The two chambers were separated by a sheet of Plexiglas and a section of insulation. Both chambers measured 61cm in length by 50cm in width and were set to a different ambient temperature. The animal was allowed access to only one chamber at a time. After up to three hours had passed the barrier between the chambers was temporarily removed to allow the animal to shuttle into the other chamber. Shuttling occasionally required slight encouragement by gently pushing the animal.

Selected animals were run through this series five separate times, one for each treatment. The ambient temperature for the non-manipulated and the tail inhibited animals and shams, was set to 34°C in the warm chamber and 18°C in the cool chamber. Ambient temperatures for mouth sealed animals were increased to 38 and 22°C respectively. The lizards themselves were allowed to roam freely within the chamber and, aside from moving the lizard between the cold and warm chambers, the animal was not disturbed. Finally, all animals were used multiple times to accommodate all the different treatment paradigms, but a period of at least one week was allowed between each individual lizard's trials. In total, 10 animals were used for each paradigm and the rate of heating was assessed.

Series IV: Thermal Preference in adults: Manipulation of tail heat exchange

The purpose of this series was to examine the consequences of manipulating heat exchange across the tail in adult lizards on thermoregulation. To begin, the thermal preference and orientation protocol and the tail inhibited protocol were followed in order to block heat exchange across the tail of the animal. The lizard was then placed in a thermal gradient and allowed to thermoregulate on its own for six to eight hours. The animals were filmed from two cameras at either end of the gradient in the same plane as the lizards. This allowed for observation of orientation preference as well as movement and gaping. All variables were then compared across the two paradigms (sham and tail insulated). After every animal was run in any

Series, the gradient was cleaned with ethanol or bleach depending on the severity of the mess left by the lizard.

In Series IV, a total of 13 animals were used in tail inhibited and sham trials, six females and seven males. Whenever possible the same animals were used for both tail inhibited and tail inhibition sham paradigms.

Series V: Thermal preference in adults: Manipulation of Gaping

All lizards used in this Series underwent the thermal preference and orientation measurement protocol and the mouth sealing technique protocol, in which either their mouths were sealed with impregum or they received the sham. In both cases, once the adhesive had set, lizards were placed in the thermal gradient to thermoregulate on their own for six to eight hours. The cameras and length of the experiment were the same as in Series IV.

Upon completion of the experiment, all equipment and adhesive was gently removed and the animals were placed back in their cages. To avoid distress, lizards were given a week between the manipulation and sham trial to recover. Unlike the previous Series, the thermal gradient was set to a smaller temperature range, 25°C – 40°C, in order to stimulate the gaping.

The sham and manipulated animals were compared with each other in their corresponding series. As with Series IV, movement and orientation were all recorded, as well as gaping, and compared across the two paradigms (sham and gaping inhibited). After every animal was run in any Series, the gradient was cleaned with ethanol or bleach depending on the severity of the mess left by the lizard. Once again, like the previous series, 13 bearded dragons were run for both the mouth sealed and sham sections, seven males and six females.

Data Analysis

Core Temperature Trial

Once the temperature data were collected they were transformed into a format that could be more readily analysed. Because lizards are ectothermic, body temperature during heating transitions approaches T_a naturally at a nearly exponential rate which can be modelled as follows:

$$dT_i = dT_o e^{-kt}$$

where dT_i is the difference in temperature between cloacal temperature and asymptote temperature at time point “i”, dT_o is the difference between cloacal and asymptote temperature at time point 0, “k” is the rate constant and “t” is time. By taking the natural logarithm of dT_i , the slope of $\ln(dT_i)$ vs. time provides an estimate of k (Tague, Beheshti, & Rees-Potter, 1981). This analysis was completed for warming curves to determine the rate of temperature change as a function of time.

Thermal preference in adults

The temperature data were converted into the average temperature across 15 minute intervals to allow better visualization of the information. Next, each frame of the video footage was studied to observe orientation, movement, and the presence of gaping. This was done for both cameras on either side of the gradient so as to give as complete a picture as possible. Behaviour was scored in a binary format; orientation preference was logged as either facing heat (H) or facing away from heat (C) per frame, movement was logged as either moving (Y) or stationary (N) per frame and gaping was logged as either mouth open (G) or closed (NG) per frame.

Statistical analysis done on Series III, IV, and V data used R (R Development Core Team, 2012) and lme4 (Bates et al., 2015) to perform linear mixed effects analyses (LME) and generalized linear mixed-effects analyses (GLMM) where appropriate. For the data in Series III, an LME was used to analyse the effect of treatment on heating rates. As fixed effects, experimental manipulation (treatment), body mass and sex were entered into a global model, including select interactions that were chosen for biologically justifiable reasons. As random effects, only intercepts for animal ID were included, to account for the repeated measures design into all models.

For statistical analysis done on Series IV data, an LME was used to analyse the impact of multiple terms on selected temperature (due to the numeric nature of the data), while a GLMM was used to analyse the impact of multiple terms on gaping, orientation preference, and movement (due to the binomial nature of the data). For the analysis of selected temperature, experimental manipulation (treatment), orientation preference, presence of gaping, movement, body mass, time and sex, were entered into a global model. For the analysis of orientation preference, experimental manipulation (treatment), selected temperature, presence of gaping, movement, body mass, time and sex were entered into a global model. For the analysis of movement, experimental manipulation (treatment), orientation preference, presence of gaping, selected temperature, body mass, time and sex, were entered into a global model. For the analysis of gaping, experimental manipulation (treatment), orientation preference, movement, selected temperature, body mass, time and sex, were entered into a global model. Additionally, all global models outlined above included select interactions that were chosen for biologically justifiable reasons, as fixed effects. As random effects, the intercepts for animal ID were included, to account for the repeated measures design, into all models.

Statistical analysis on Series V data followed the same steps outlined for the data analysis of Series IV except for the analysis of gaping. The gaping inhibited treatment was not included as this value was always “0” (no gaping) and since this was a constant value it made convergence impossible.

To avoid autocorrelation of the model residuals (in Series IV and V), the data were resampled at every 10th time point and an autocorrelation estimate of the residuals was used to confirm. I then fit all possible models that nested within the global model, calculated and ranked AICc values (Akaike, 1974) choosing the model that exhibited the highest likelihood of all possible models for each test, for each series. Model selection and the information-theoretic approach was implemented using the MuMIn package in R (Barton, 2015). In all series, I present model weights as measures of support for parameters. However, when presenting model coefficients (*i.e.* effects), the highest ranked model that also included superior ranking models is presented. Visual inspection of residuals and QQ plots did not reveal any obvious deviations from homoscedasticity or normality. P-values were obtained using likelihood ratio tests (Type II Wald's chisquare tests) of the full model with the effect in question against the model without the effect in question, using the "car" package in R" (Fox & Weisberg, 2011).

Results

Series III Rates of Temperature Change

The first variable studied was the rate of temperature change. The global model compared rates of temperature change across treatment, sex, mass, and treatment interaction with sex. Model number one (Appendix Table 3.1) supported only sex and mass as the variables that impacted the rate of temperature increase. Rates of temperature change were lower in males ($B = -0.01730$; $p = 0.0001225$) but increased positively with mass ($B = 8.540 \times 10^{-5} \text{ } ^\circ\text{C/g}$; $p = 0.001934$) (Appendix Table 3.2). As mass increases, bearded dragons changed temperature more slowly, although males had a faster rate of temperature change than females (Figure 3.1). Treatment had no effect on rate of temperature change, either independently or as a covariate with sex.

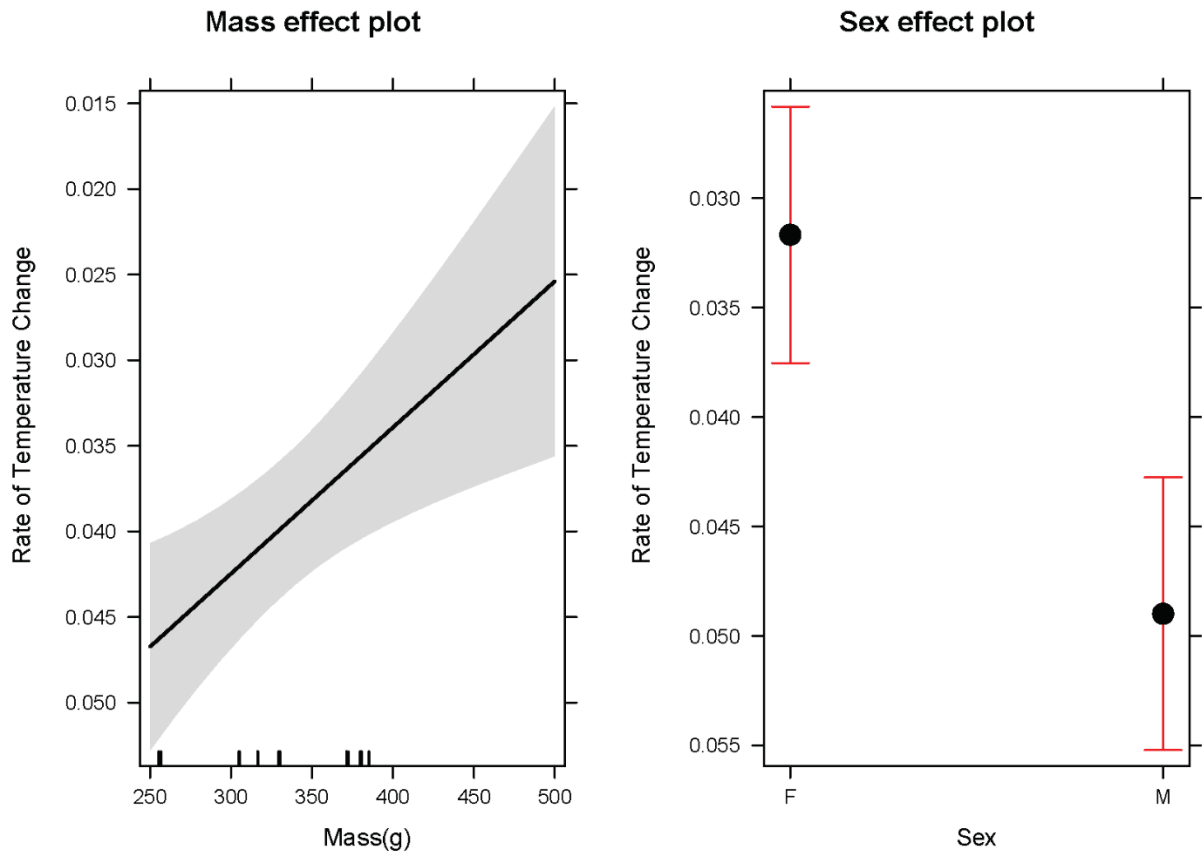


Figure 3.1. The effects of mass (left) and sex (right) on the rate of heat gain in Bearded Dragons (*P. vitticeps*). Mass is recorded in grams and sex is either female (F) or male (M). Data were collected from animals in a thermal chamber over a maximum of 6 hours. Rate of temperature change is the exponential rate constant (k) obtained by estimating the slope of the \ln transformed equilibration of T_b with T_a . All animals used were adults over 3 years of age. A total of 8 animals were used, 4 males and 4 females, for all treatments. The error bars and grey shading represent the upper and lower 95% confidence.

Series IV: Behavioural Responses in Tail Insulated and Sham Treatments

The first behavioural response examined was selected ambient temperature across both treatments. Model number four (Appendix Table 3.3) supported mass, sex, time, treatment, sex by time interaction, and treatment by time interaction as factors that impact selected temperature. An analysis of deviance, showed that selected ambient temperature associated positively with time ($B = 0.4844$ °C/hr; $p < 1.000 \times 10^{-10}$) (Appendix Table 3.4). Selected ambient temperature also had a positive association with time through an interaction with sex ($B = 0.4685$; $p < 1.000 \times 10^{-10}$). No other terms were found to be significant. As time increased, average selected temperature rose with males showing a steeper slope than females (Figure 3.2). At hour zero, average selected temperature was around 28°C which rose steadily until hour eight when temperature reached 34°C. There was no support for tail insulation altering selected temperature.

The next response variable examined was gaping. Model number two (Appendix Table 3.5) supported treatment, sex, selected temperature and mass as the variables that impacted gaping in the adult, tail insulated and tail sham lizards. Gaping was positively associated with tail insulated treatment ($B = 0.9546$; $p = 0.001088$) as was mass ($B = 1.982$; $p = 0.00002730$) and selected temperature ($B = 0.6994$; $p = 0.0004440$) (Appendix Table 3.6). Sex did not have a significant impact on gaping. As mass increased the odds of gaping rose (Figure 3.3). Additionally, as selected ambient temperature rose so too did the chance of gaping. Treatment also showed an impact on gaping, with the odds of gaping being higher in the tail insulated trial as opposed to the tail insulated sham trial (Figure 3.3).

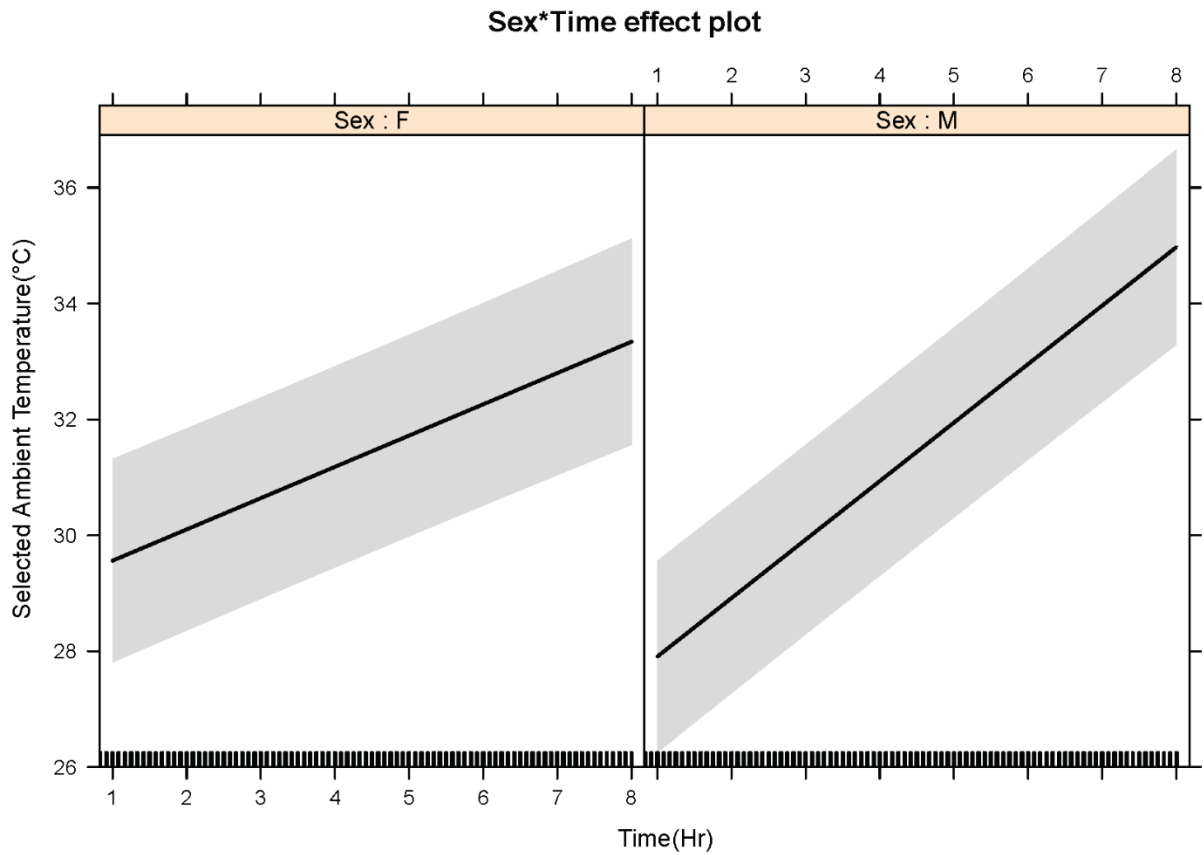


Figure 3.2. The selected ambient temperature for Bearded Dragons (*P. vitticeps*) during treatments tail insulated and tail insulation sham in comparison to sex and time. Temperature is expressed in degrees Celsius, sex is coded as either male (M) or female (F), and time is expressed in hours. Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the selected ambient temperature while the x-axis shows the explanatory variable. A total of 13 animals were used, 6 male and 7 female. The grey shade represent the upper and lower 95% confidence.

The probability of orienting towards a heat source was the next variable to be examined. Model number two (Appendix Table 3.7) supported treatment, sex, and time as the variables that impacted orientation preference in the adult, tail insulated and tail sham lizards. Orientation preference was negatively associated with the tail insulated treatment ($B = -0.6477$; $p = 1.520 \times 10^{-10}$) but increased positively with time ($B = 0.04206$; $p = 0.03125$) (Appendix Table 3.8). Sex did not have a significant impact on orientation preference. The odds of orienting towards a heat source rose with time (Figure 3.4). Treatment also had an impact on orientation behaviour; tail insulated animals were less likely to face a heat source than were sham animals.

The probability of moving was the next variable examined. Model number two (Appendix Table 3.9) supported treatment, selected temperature, and time as the variables that impacted the odds of moving in the adult, tail insulated and tail sham lizards. Movement was positively associated with temperature ($B = 0.2498/^\circ\text{C}$; $p = 0.001153$) as was time ($B = 0.06889/\text{hr}$; $p = 0.01367$) (Appendix Table 3.10). Treatment did not have a significant impact on movement. The odds of movement increased as time increased (Figure 3.5). Selected ambient temperature also had a positive impact on movement.

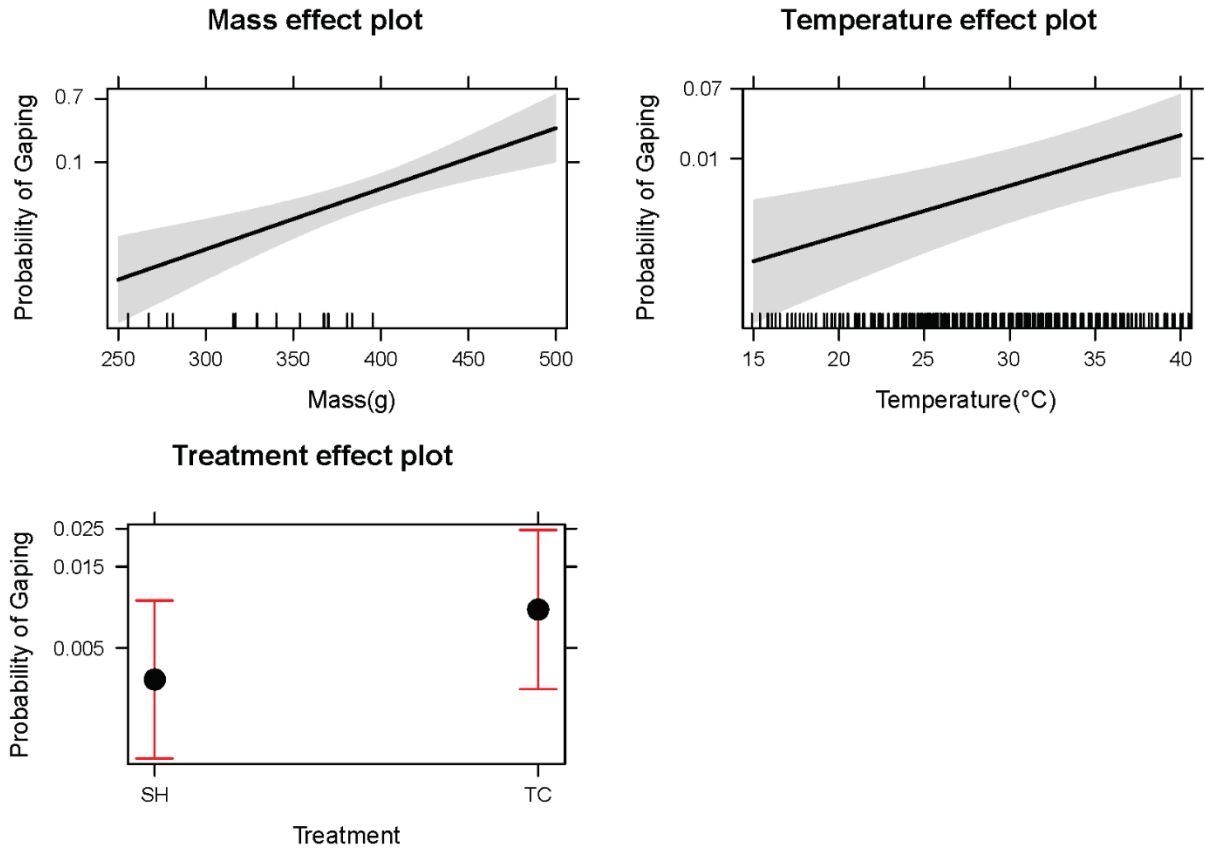


Figure 3.3. The model fit predicted probability that Bearded Dragons (*P. vitticeps*) gape as a function of mass (top left), temperature (top right), and treatment (bottom right). Mass is expressed in grams and temperature is expressed in degrees Celsius. Treatment is coded as either sham tail insulation (SH) or tail insulated (TC). Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the likelihood of gaping while the x-axis shows the explanatory variable. A total of 13 animals were used, 6 male and 7 female. The error bars and grey shade represent the upper and lower 95% confidence.

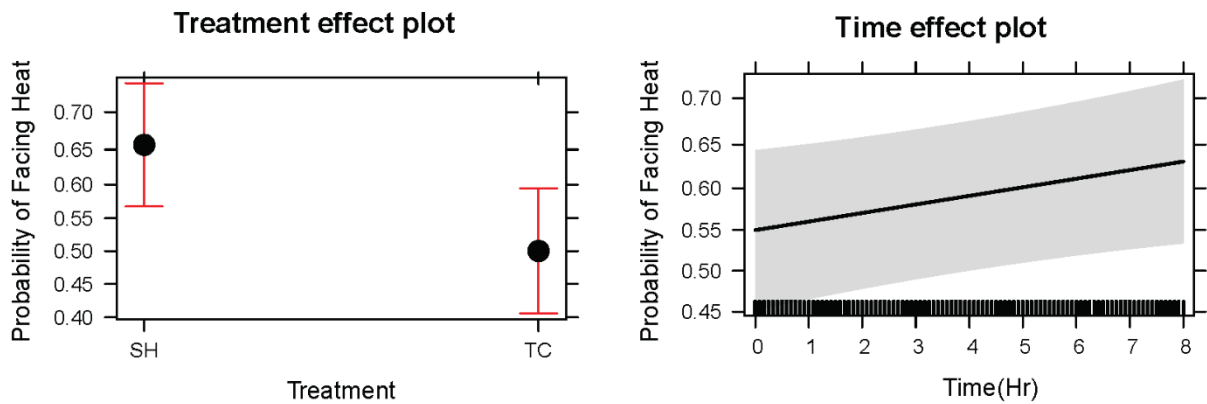


Figure 3.4. The chances that tail insulated and tail insulation sham Bearded Dragons (*P. vitticeps*) will face a heat source, in comparison to treatment (left) and time (right). Time is expressed in hours, and treatment is coded as either sham tail insulation (SH) or tail insulated (TC). Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the probability of orientating towards a heat source while the x-axis shows the explanatory variable. A total of 13 animals were used, 6 male and 7 female. The error bars and grey shade represent the upper and lower 95% confidence.

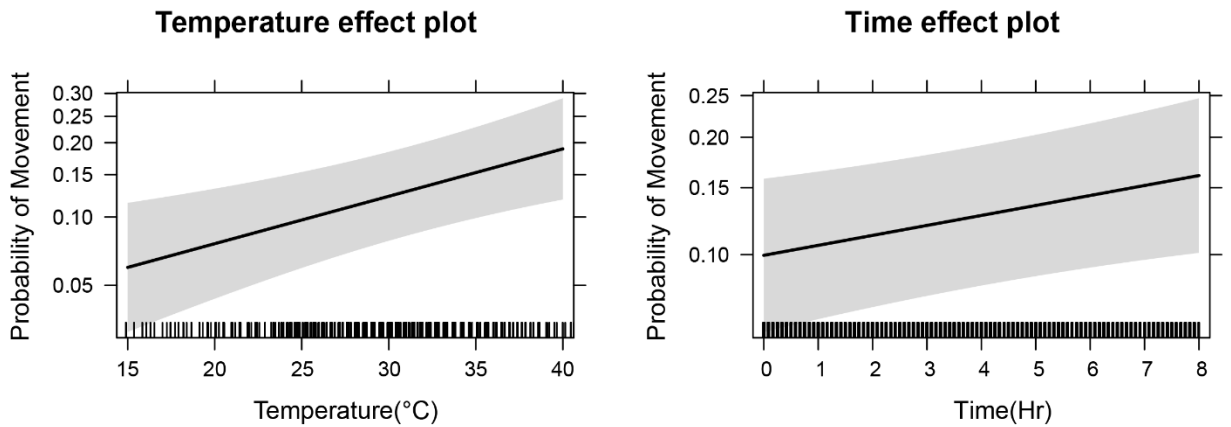


Figure 3.5 The model predicted probability that tail insulated and tail insulation sham Bearded Dragons (*P. vitticeps*) will move, in comparison to selected temperature (left) and time (right). Time is expressed in hours and selected temperature is expressed as degrees Celsius. Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the odds of movement while the x-axis shows the explanatory variable. A total of 13 animals were used, 6 male and 7 female. The grey shade represent the upper and lower 95% confidence.

Series V. Behavioural Responses in Gaping Inhibited and Gaping Inhibition Sham Treatments

The first variable studied was selected ambient temperature. Model number two (Appendix Table 3.11) supported mass, time, treatment, and treatment by time interaction as the variables that impacted selected ambient temperature in the adult, gaping inhibited and gaping inhibition sham lizards. Selected ambient temperature was associated positively with time ($B = 0.3500$ °C/hr; $p < 1.000 \times 10^{-10}$) (Appendix Table 3.12). Selected temperature was also positively associated with gaping inhibited treatment by time interaction ($B = 0.1268$; $p = 0.03370$) (Appendix Table 3.12). Mass had no significant impact on selected ambient temperature. The effect that time and treatment had on selected ambient temperature is shown in Figure 3.6. Time had a significant impact on selected ambient temperature, which increased (from 31°C to 35°C) as time increased (Figure 3.6). The IG treatment animals also had a higher chance of selecting higher temperatures as evidenced by a treatment by time interaction (Figure 3.6).

Next, gaping was examined in gaping inhibition sham adult trials. The global model was used, as the previously used model dredging technique could not converge on solutions for other model fits. The global model supported selected temperature, time, mass, and sex as the variables that impacted gaping in the adult, gaping inhibited sham lizards. Gaping was positively associated with males ($B = 3.619$; $p = 0.02347$) (Appendix Table 3.13). No other terms had a significant impact on gaping. The odds of gaping were higher in males than females (Figure 3.7).

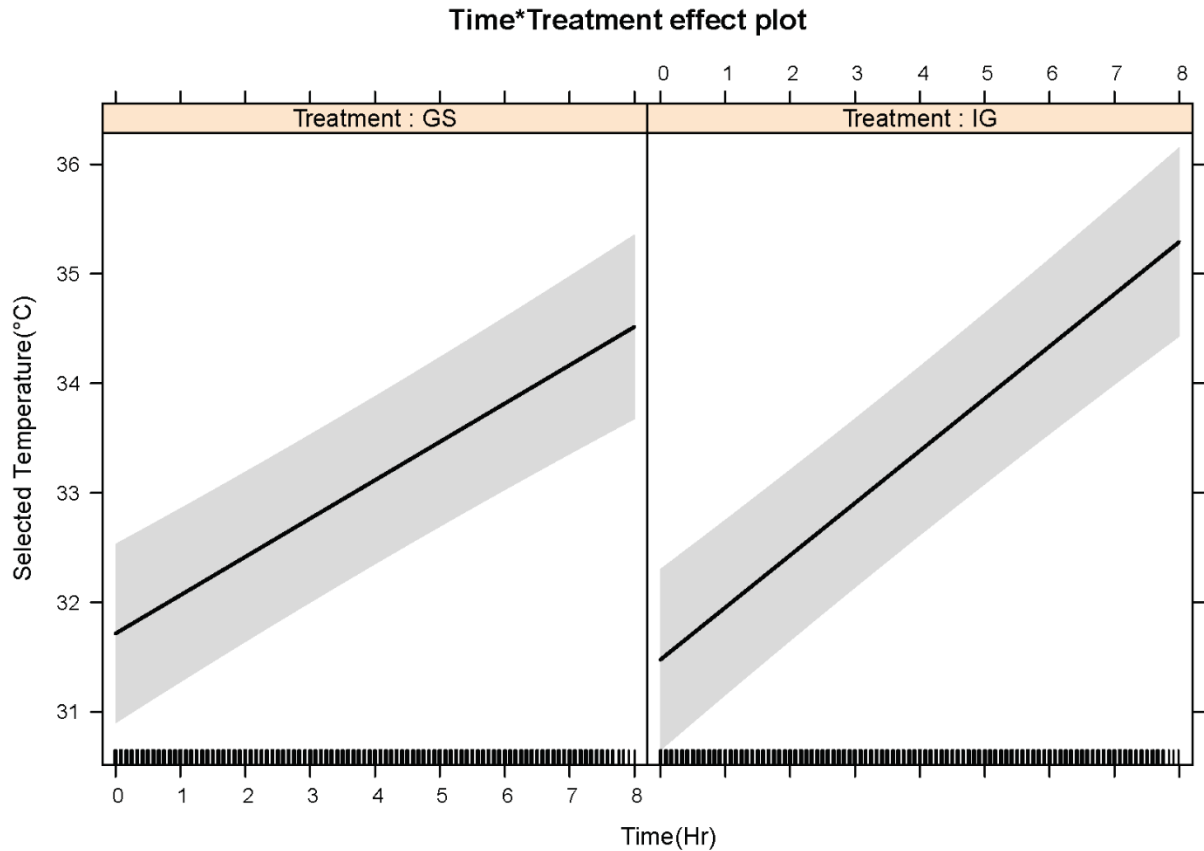


Figure 3.6. The average ambient temperature that gaping inhibited and gaping inhibition sham Bearded Dragons (*P. vitticeps*) selected (°C), in comparison to treatment by time interaction. Time is expressed in hours, and treatment is coded as either gaping inhibited (IG) or gaping inhibition sham (GS). Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the selected ambient temperature while the x-axis shows the explanatory variable. A total of 13 animals were used, 6 male and 7 female. The grey shade represent the upper and lower 95% confidence.

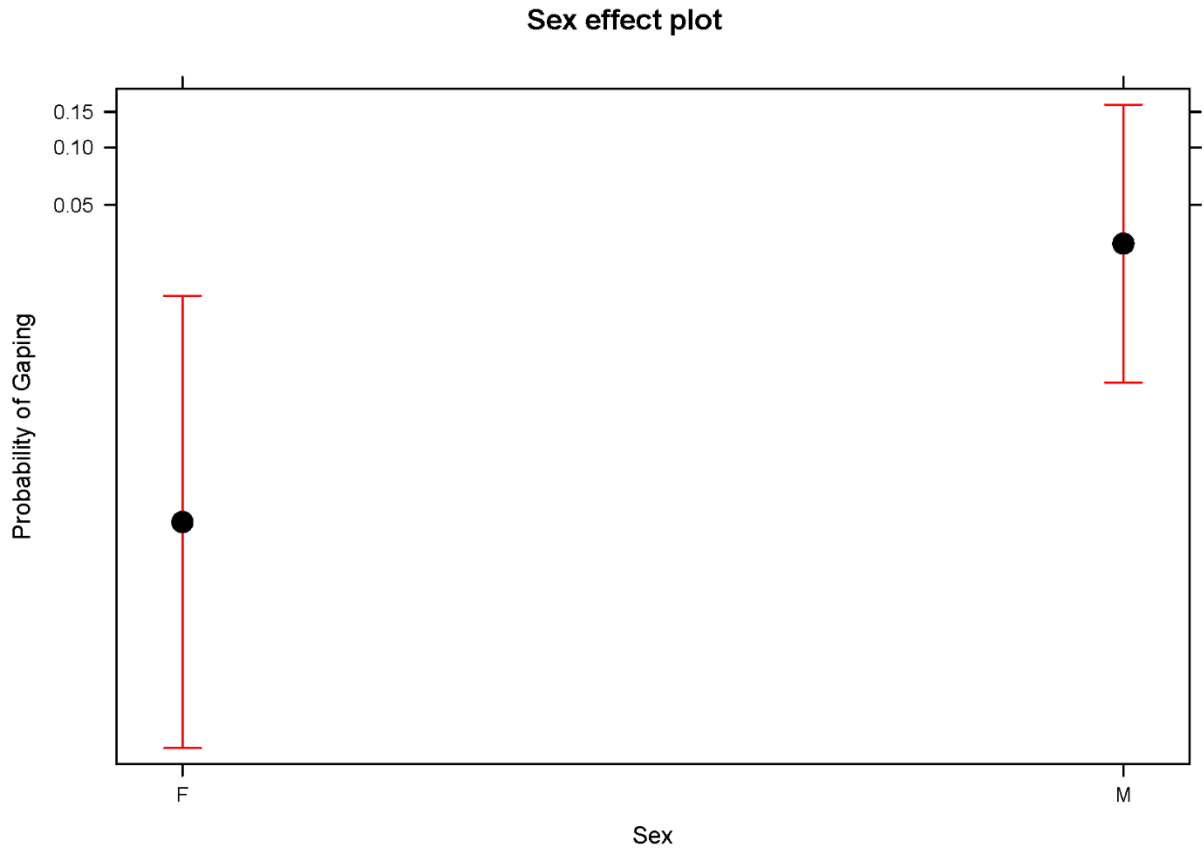


Figure 3.7. The model predicted probability that gaping inhibition sham Bearded Dragons (*P. vitticeps*) will gape in comparison to sex. Sex was coded as either male (M) or female (F). Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the time spent gaping while the x-axis shows the explanatory variable. A total of 13 animals were used, 6 male and 7 female. The error bars represent the upper and lower 95% confidence.

Orientation towards a heat source was the next variable to be examined. Model number two (Appendix Table 3.14) supported treatment, selected temperature, sex, mass, and time as the variables that impacted the odds of orienting towards a heat source in the adult, gaping inhibited and gaping inhibition sham lizards. Orientation towards a heat source was positively associated with mass ($B = 0.3849/\text{g}$; $p = 0.02740$) but was negatively associated with males ($B = -1.094$; $p = 0.003954$). Orientation was also positively associated with the gaping inhibited treatment ($B = 0.3875$; $p = 0.0001801$) (Appendix Table 3.15). Selected temperature and time did not have a significant impact on orientation preference. As mass increased, the odds of an animal orienting towards a heat source rose (Figure 3.8). Females were more likely to orient towards a heat source than males. Lizards in the gaping inhibited treatment were more likely to spend time facing a heat source than lizards in the gaping inhibition sham trial (Figure 3.8).

Finally, the probability of movement was examined. Model three (Appendix Table 3.16) supported treatment, selected temperature, sex, and time as the variables that impacted the movement in the adult, gaping inhibited and gaping inhibition sham lizards. Movement had a positive association with selected temperature ($B = 0.4762/^\circ\text{C}$; $p < 1.000 \times 10^{-10}$) but a negative association with time ($B = -0.1258$; $p = 5.08 \times 10^{-6}$) (Appendix Table 3.17). Sex and treatment did not have a significant impact on movement. The odds of movement increased as selected temperature increased (Figure 3.9). The odds of movement decreased as time increased.

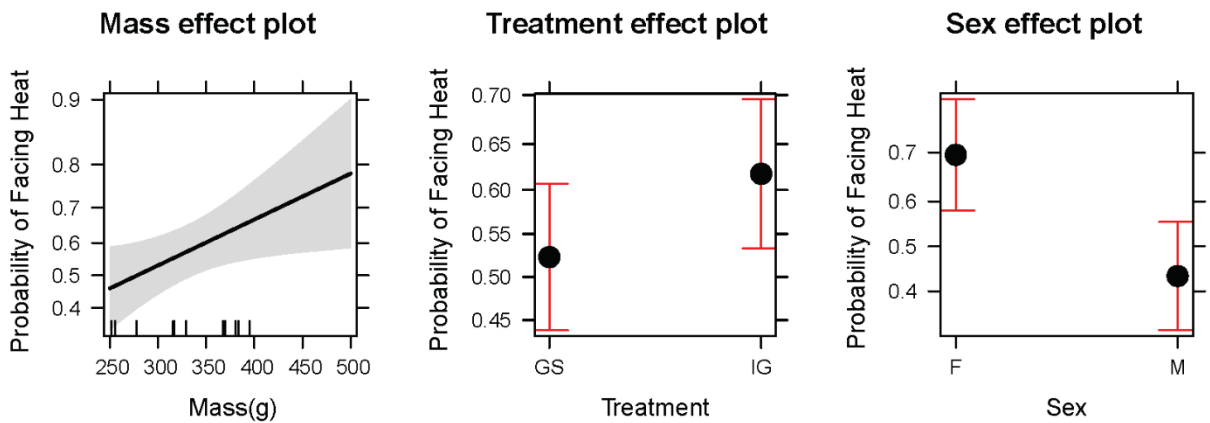


Figure 3.8. The model predicted probability that Bearded Dragons (*P. vitticeps*) will orient towards a heat source, in comparison to mass (left), sex (right), and treatment (middle). Treatment is coded as either gaping inhibited (IG) or gaping inhibition sham (GS), mass is expressed in grams, and sex is coded as either female (F) or male (M). Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the probability of facing a heat source while the x-axis shows the explanatory variable. A total of 13 animals were used, 6 male and 7 female. The error bars and the grey shade represent the upper and lower 95% confidence.

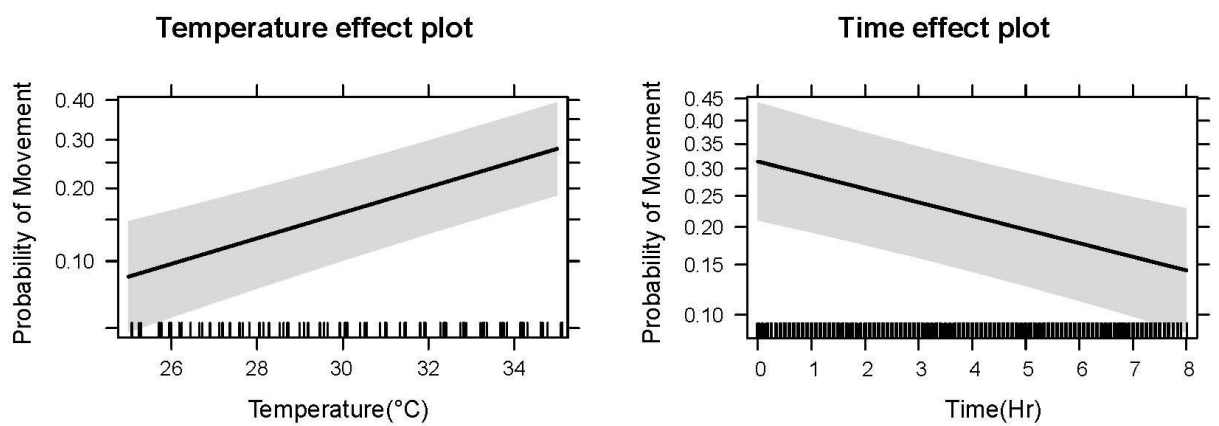


Figure 3.9. The model predicted probability that Series V Bearded Dragons (*P. vitticeps*) will move, with respect to selected temperature (left) and time (right). Time is expressed in hours, selected temperature is expressed as degrees Celsius. Data were collected from animals in a thermal gradient over a maximum of eight hours. The y-axis expresses the probability of moving while the x-axis shows the explanatory variable. A total of 13 animals were used, 6 male and 7 female. The grey shade represent the upper and lower 95% confidence.

Discussion

I hypothesised that orientation would be driven by either anterior or posterior structures. Insulation of the posterior region (tail) of bearded dragons caused a compensatory response to a thermoregulatory imbalance, or at least a perceived one, in the form of increased time spent gaping and a decrease in time spent orienting towards a heat source. Furthermore, the observation that gaping inhibited lizards (IG) were more likely to face a heat source, in comparison to the sham group, suggests that orientation may be a means of cooling more than warming, since in the laboratory thermal gradient, head facing the heat necessarily results in the tail being in contact with a cooler substrate. My data indicates that orientation preference seems to be driven by posterior structures. Therefore, the treatments' (IG and TC) impact on orientation, and gaping for the TC treatment, are not just reactions to a change or perceived change in heat loss, but also evidence of a coordinated response between thermoregulatory behaviours to compensate for the loss of either the tail as a thermoregulatory structure, or the loss of gaping as a thermoregulatory behaviour.

While mass did have an impact on the chance of gaping in Series IV, it did not affect orientation preference. Gaping increasing with mass is logical assuming that larger lizards have a greater thermal inertia. Interestingly, voluntarily thermoregulating lizards will still gape even when they could simply choose a different ambient temperature. Since gaping is a thermoregulatory strategy that also causes water loss, adopting it when the animal could simply move seems counter intuitive. The presence of gaping in animals that are not thermally stressed is counter to what was predicted by Huey and Slatkin (1976), but supports what Tattersall and Gerlach found (2005). That gaping is used in tandem with ambient temperature selection is further evidence that there is coordination of thermoregulatory behaviours acting to maintain homeostasis. The impact of sex on gaping and orientation in Series V suggests that a dragon's behavioural choices, with regards to gaping and orientation, may be related to sexual differences.

The effect of sex also suggests that behavioural thermoregulation may change with sexual maturity in some ways. Orientation preference seems to cool the animals enough to compensate for the loss of gaping in Series V, even in males where gaping is more prevalent, while orientation may be of more importance to female lizards in particular. This supports what Gibson et al. (2015) found in Otago/Southland geckos, that females show orientation and postural behaviours more than males.

Ancillary Experimental Effects: Mass, Time, Sex, and Movement

As size increases, heat transfer between the animal and the environment is influenced by size due to the heat capacity of body tissue as well as the natural insulating properties of tissue (Carey, 1978; Turner, 1987). As mass increases, heat transfer of any object, biological or inert, is expected to slow down. Therefore, rates of heat absorption from the environment decrease with increasing mass of the animal (Figure 3.1) (Carey, 1978; Turner, 1987). Thus, it is not surprising that increases in mass will also lead to a higher probability of gaping (Figure 3.3) since higher mass negatively impacts heat exchange with the environment (Turner, 1987). If larger lizards overheat, they are more likely to recruit thermolytic processes, such as gaping, in order to assist in cooling. By increasing gaping with regards to higher mass, bearded dragons display a compensatory response to an impediment to heat loss, variation in thermal inertia caused by animal size. Mass also affects orientation, with larger animals more likely to face a heat source (Figure 3.8) when gaping is unavailable. The impact of mass on orientation preference supports the use of orientation as a form of thermoregulation and, more specifically, as a means to lose heat through the tail, a much less insulated area.

Interestingly, females showed slower rates of heat gain compared to male bearded dragons (Figure 3.1). Slower heat absorption from the environment could be caused by one sex using thermoregulatory behaviours, such as gaping or orientation, under forced conditions more

readily than the other sex, thus slowing their heat gain (Gibson et al., 2015). Additionally, the difference in heat gain between the sexes could be caused by autonomic changes, such as vasoconstriction or increases in breathing rates, which could impede heating rates. Sex also impacted gaping, with males gaping more than females in the gaping inhibition sham trial (Figure 3.7). That sex had such a noticeable effect on gaping in this trial suggests that males rely on gaping more than females, for thermoregulation. Females, on the other hand, are more likely to orient towards a heat source than males, suggesting that orientation is a thermoregulatory behaviour used more by females than males (Figure 3.8). Work done by Millar, Graham and Blouin-Demers (2012) on the blanding's turtle (*Emydoidea blandingii*) found a similar effect of sex in that females of the species bask more than males. Since orientation is typically most useful during periods of basking, my work lends some support to Millar et al. (2012) and I would expect female bearded dragons to bask more than males. The impact of sex on gaping and orientation suggests that a dragon's behavioural choices, with regards to gaping and orientation, may be related to sexual differences. The effect of sex also suggests that behavioural thermoregulation may change with sexual maturity in some ways. The data collected in this work are not sufficient to make a definitive statement on the effect of sex on thermoregulation, but the presence of a "sex effect," here and in the literature (Kaciuba-Uscilko & Grucza, 2001; Millar et al., 2012), does support the importance of including sex as a fixed effect in the global model when examining thermoregulation.

Additionally, there was a rise in preferred temperature with time, both in Series IV and in Series V, which was most likely caused by animals thermoregulating more and exploring less during the last four hours in comparison to the first four hours (Figure 3.2; Figure 3.6). Bearded dragons exploring less and thermoregulating more in the later portion of a trial (after four hours) has been previously documented by Cadena and Tattersall (2009a). Orientation preference was also impacted by time in a positive fashion. The slight rise in the probability of animals facing a

heat source over time is most likely caused by a decrease in exploration, which would diminish the apparent random orientation as exploration is expected to lead to a 50% time orienting towards either end of the gradient (Figure 3.4).

The slight rise in the probability of moving with increasing selected temperature (Figure 3.5; Figure 3.9) is probably caused by animals moving less at cooler temperatures simply because cooler temperatures are associated with lower muscle performance in ectotherms (Hirano & Rome, 1984; Moberly, 1968; Weinstein, 1998). The impact of temperature on locomotory performance has been shown to be fairly minimal. Therefore it is not surprising that higher selected temperatures did not have a larger impact on movement (Angilletta et al., 2002). Interestingly, animals moved more with time in Series IV, than in Series I and Series V, where animals moved less with time (Figure 2.3; Figure 3.5; Figure 3.9). The difference in the movement results over time between Series I, IV and V is most likely caused by the tail insulating fabric stimulating attempts at removal from slight irritation (Figure 3.5). Because treatment had no effect on movement, however, it is safe to conclude that this slight irritation does not confound the conclusions drawn from Series IV.

Main Experimental Effects: Selected Temperature, Gaping, and Orientation

My first prediction, that gaping inhibited treatment would impact orientation by decreasing the probability of facing a heat source was not supported. Rather, my second prediction that inhibiting heat exchange across the tail would shift orientation preference by decreasing the probability of facing a heat source was supported. Furthermore, my third prediction, that treatment would have no impact on selected temperature, was supported in Series IV, suggesting that treatment did not prevent the animals from selecting their desired T_a and therefore obtaining their preferred core temperature. In Series V, treatment did show an effect by time interaction (Figure 3.6), but, for both GS and IG treatments, selected temperature rose with

time. The increase in temperature with time for both gaping inhibited and sham animals suggests that treatment did not prevent animals from selecting their desired T_b , but that lizards in the IG treatment did select from a broader range of temperatures over time, suggesting a slight change in thermoregulatory precision after the loss of gaping. Curiously, Series III showed that treatment had no significant association with rates of cloacal, or core, heat gain in bearded dragons (Figure 3.1), suggesting that behaviours like gaping and orientation primarily impact their regional temperatures, such as head temperature.

Next I predicted that treatment would impact gaping. My work does show that tail insulated animals would gape more in comparison to their respective sham animals (Figure 3.3). Since gaping is a cooling response, insulation of the tail most likely blocks heat loss from that region. The resulting rise in probability of gaping in the tail insulated animals suggests the potential for overheating in the absence of heat exchange from the tail, which is typically kept orientated towards the cold. Therefore, gaping as a potential compensatory response is also supported by treatment having an impact on gaping (Figure 3.3). It should be noted that gaping is a reasonably low frequency event, and so an apparently large treatment effect is plausible. The results of the tail insulated experiments revealed an interesting relationship that supported the hypothesis of thermoregulatory coordination; as lizards select warmer temperatures, they also engage in more gaping behaviour (Figure 3.3). Gaping has been documented as a thermoregulatory behaviour that responds to elevated head or brain temperatures; furthermore, head/brain temperature in some lizards is maintained at lower levels as a result of thermal gaping and panting (Borrell et al., 2005; Crawford Jr., 1972; Tattersall et al., 2006). An increase in gaping with selected temperature is, therefore, to be expected from the perspective of thermoregulatory control, but, whether bearded dragons use gaping as a thermal stress response or just a normal thermoregulatory one is less clear. Previous work by Tattersall & Gerlach (2005) has shown that animals gape more when forced to conform to a higher temperature. My work

shows that spontaneously behaving animals that can choose their preferred temperature gape more after selecting higher temperatures. My observation that gaping increases at higher temperatures, even when lizards can select a lower ambient temperature when needed, provides some compelling evidence that gaping is a controlled thermoregulatory response rather than a stress response. In Series V, treatment must have an impact on gaping since the gaping inhibited animals could not gape at all.

Preferred orientation was also impacted by treatment, as predicted, as tail insulated animals had a lower probability of facing a heat source in comparison to the sham treatment (Figure 3.4). By orienting more towards the colder side of the gradient when the tail is insulated, bearded dragons show another compensatory response. Changing orientation further supports the possibility that tail insulation causes either an increase in head temperature or a perceived higher ambient temperature in the posterior region. My work shows that a compensatory response is stimulated in the form of both a rise in gaping as well as a shift in orientation preference from facing a heat source to facing away from a heat source when the tail is insulated. Interestingly, treatment had an impact on orientation in Series V in that gaping inhibited animals were more likely to face a heat source than gaping inhibition sham animals (Figure 3.8), which suggests that placing the posterior region of the animal in the cooler area of the gradient is enough of a compensatory response to address any overheating in the head caused by gaping being impeded. There is, however, the possibility that gaping itself provides the lizards with sensory cues on the rate of evaporative cooling. Previous work by Scarpellini et al. (2015) has shown that when gaping is inhibited through salt loading, used to mimic the effects of dehydration, bearded dragons show a shift in their behavioural thermoregulatory responses, suggesting that these animals have compensatory responses to the inhibition of gaping. Furthermore, inhibiting gaping from salt loading leads to head temperatures rising to higher levels than in control saline animals

(Scarpellini et al., 2015), demonstrating the interactions between the homeostatic responses to water balance and body temperature.

Conclusions: Thermoregulatory Coordination

All treatments impacted orientation behaviour; insulation of the tail caused bearded dragons to be more likely to face a heat source and to gape more readily, whereas preventing animals from gaping caused the animals to be more likely to orient towards heat. The increase in gaping and shift in orientation preference caused by tail insulation suggests that orienting the posterior region away from a heat source is used, in non-manipulated animals, as a means to cool the extremities of the animal by placing the tail in a cooler region. That inhibiting gaping increases the odds of facing a heat source is at first glance difficult to explain. It is possible that this response is in fact related to the importance of orientation as a means to stay cool, since the tail will be exposed to cooler temperatures. This form of orientation appears similar to that observed in the wild in marine iguanas (Bartholomew, 1966).

The data collected in Series III suggests that any impact the treatments have on bearded dragons is primarily limited to the corresponding region (*i.e.* the tail and head regions). Gaping primarily influences head temperature regulation rather than core temperature in lizards (Crawford Jr., 1972), further supporting the data in Series III. Additionally, since treatment has a very minimal impact on selected temperature (a proxy for core temperature), it is unlikely that gaping or orientation behaviours are used to regulate core body temperature, but rather are used to regulate the temperature at the animals' extremities. That selected temperature was unaffected by treatment further supports the possibility that gaping and orientation thermoregulatory responses do not strongly influence core temperature, as the dragons were content to keep the bulk of their bodies at the same temperature regardless of treatment, but still changed other "fine tuning" thermoregulatory responses. By increasing gaping, as shown by Crawford Jr (1972) and

Scarpellini et al. (2015), head temperature is likely to drop in relation to T_a whereas body temperature is likely to remain relatively constant.

Orientation preference and gaping show compensation in their responses when gaping is inhibited and when the tail is insulated, which supports my hypothesis that these behaviours are coordinated. That there is coordination between thermoregulatory behaviours is not well documented, but also not surprising. Thermoregulatory behaviours compensating for abiotic changes have been shown previously in reptiles (Cadena & Tattersall, 2009a; Hicks & Wood, 1985; Scarpellini et al., 2015), supporting some level of plasticity in thermoregulatory behaviours. There is still considerable debate as to whether thermoregulation is controlled by a central controlling mechanism or not. Lately the concept of a single controller for thermoregulation has been called into question (Romanovsky, 2007). The results presented in my work suggest coordination between thermoregulatory behaviours, which in turn does support the presence of a central controlling mechanism as a possible explanation for this coordination. In any case, understanding thermoregulatory coordination in animals is critical for understanding thermoregulation in a larger context, beyond simply the individual thermoregulatory responses and how they function in isolation. As thermoregulation is such a complex and important response that is intricately tied with countless other functions and responses in most animals, much of the work examining temperature regulation can be beneficial. Additionally, by gaining a better understanding of coordination in ectotherms that primarily use behaviour for thermoregulation; we gain insight into how these behaviours evolved in taxa that utilise physiological mechanisms for thermoregulation.

Chapter 4 Summary and Future Directions

Summary

Orientation as a thermoregulatory response is present in both adult bearded dragons and two week old neonates, suggesting this behaviour is either innate or learned during the first two weeks of life. Whether orientation is innate or very readily learned, the importance of this behaviour for thermoregulation is supported in *P. vitticeps*. Inhibiting heat exchange with the environment at the extremities (tail and head region) in adult bearded dragons had no impact on core temperature. Bearded dragons showed changes in orientation and gaping behaviour with treatments designed to impair putative heat exchange or impair localised temperature sensation, but no change in selected ambient temperature, suggesting that shifts in orientation preference and the probability of gaping are compensatory responses that allow the animal to remain at its preferred ambient temperature despite impediments to environmental heat exchange. Any impact that treatment has on thermoregulation, either directly or indirectly, is not caused by changes in core temperature, but instead, to changes in the temperature of local regions directly associated with the manipulation or to changes in the animals' ability to sense its environment due to the treatments. Changes to a reptile's thermosensation have been shown to impact thermoregulatory behaviour in crocodiles (Seebacher & Murray, 2007). The data collected in chapters 2 and 3 support my hypothesis that orientation is a thermoregulatory behaviour that shows coordination with other thermoregulatory responses.

Future directions

To further examine thermoregulatory coordination, obtaining the local temperature of select regions, as opposed to only core temperature, on animals that are currently being manipulated could help identify whether or not it is a temperature increase in peripheral regions

that leads to the responses observed in my study. Also, my work indicated slight differences between males and females with regards to thermoregulation, therefore, further examination of a potential sex effect could prove useful. Specifically, it would be worthwhile to examine whether males respond to different external stimuli that relate to temperature absorption in comparison to females. Comparing these reactions, if there are any, could reveal some interesting results with regards to how these responses developed. Studying thermoregulatory coordination in animals that undergo tail autotomy and how the loss of a peripheral structure influences thermoregulatory behaviours would also be a very interesting avenue to explore. Being able to understand how subtle changes in behaviours interact with each other will better inform the research community that attempts to predict how ectotherms will cope with a warming climate.

A more in-depth examination of gaping would be another interesting direction to explore. Observing gaping behaviour in neonates, which has not yet been documented, at different ages could help explain the precision of this common thermoregulatory response and how that precision develops over time. The same could be said for orientation and observing its development as the neonates grow up. Finally, a study that looks to isolate the specific neural pathways related to thermoregulatory behaviours, how these pathways are coordinated, and whether a central regulating mechanism or structure can be isolated could help explain thermoregulation and the sensory input and output in more complex organisms, such as mammals and birds.

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Appendix

Table 2.1 Top four models, nested in the global linear mixed-effect model, analysing selected ambient temperature (SelTemp) in non-manipulated adult bearded dragons (*P. vitticeps*). The global model includes mass, sex, time, orientation preference (Orient), orientation preference by sex and time interaction, movement (Move), as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the log likelihood (logLik), degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (SelTemp~)	DF	logLik	AICc	ΔAICc	weight
1)	Orient + Sex + Time + Orient:Sex + Orient:Time + Sex:Time + Orient:Sex:Time + (1 ID)	10	-3005.73	6031.64	0	0.2229
2)	Orient + Mass + Sex + Time + Orient:Sex + Orient:Time + Sex:Time + Orient:Sex:Time + (1 ID)	11	-3005.50	6033.21	1.576	0.1013
3)	Orient + Sex + Time + Orient:Sex + Orient:Time + Sex:Time + (1 ID)	9	-3007.65	6033.45	1.814	0.08999
4)	Orient + Move + Sex + Time + Orient:Sex + Orient:Time + Sex:Time + Orient:Sex:Time + (1 ID)	11	-3005.72	6033.65	2.012	0.08149

Table 2.2 A type II Wald's analysis of deviance on the top model (model number 2), shown in table 2.1, analysing the selected ambient temperature in non-manipulated control bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr(>Chisq)
Orient	19.01341	1	1.300*10 ⁻⁰⁵
Mass	0.467222	1	0.4943
Sex	2.023361	1	0.1549
Time	336.3805	1	3.920*10 ⁻⁷⁵
Orient:Sex	3.127582	1	0.07698
Orient:Time	2.291844	1	0.1301
Sex:Time	27.94566	1	1.250*10 ⁻⁰⁷
Orient:Sex:Time	3.811486	1	0.05090

Table 2.3 Top four models, nested in the global model run through a GLMM, analysing preferred orientation (Orient) in non-manipulated adult bearded dragons (*P. vitticeps*). The global model includes mass, sex, time, selected ambient temperature (SelTemp), selected temperature by sex interaction, and movement (Move), as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, log likelihood, (LogLik), degrees of freedom (DF) and weight are shown for each model.

Model number	Model (Orient~)	DF	logLik	AICc	ΔAICc	weight
1	Move + SelTemp + Sex + Time + SelTemp:Sex + (1 ID)	7	-670.65	1355.40	0	0.3536
2	Move + SelTemp + Time + (1 ID)	5	-673.03	1356.12	0.7199	0.2467
3	Move + Mass + SelTemp + Sex + Time + SelTemp:Sex + (1 ID)	8	-670.56	1357.25	1.8424	0.1407
4	Move + SelTemp + Sex + Time + (1 ID)	6	-672.81	1357.69	2.292	0.1124

Table 2.4 A type II Wald's analysis of deviance on the top model (model number 3), shown in table 2.3, analysing the preferred orientation in non-manipulated adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr(>Chisq)
Movement	10.42	1	0.00124
Mass	0.1854	1	0.666
SelTemp	22.34	1	2.27*10 ⁻⁶
Sex	0.5198	1	0.470
Time	20.64	1	5.54*10 ⁻⁶
SelTemp:Sex	4.514	1	0.0336

Table 2.5 Top four models, nested in the global model run through a GLMM, analysing movement (Move) in non-manipulated adult bearded dragons (*P. vitticeps*). The global model includes mass, sex, time, selected ambient temperature (SelTemp), orientation (Orient), and selected temperature by sex interaction as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the log likelihood, (LogLik), degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (Move~)	DF	logLik	AICc	ΔAICc	weight
1	Orient + Time + (1 ID)	4	-470.2	948.4	0	0.1829
2	Orient + Mass + Time + (1 ID)	5	-469.9	949.8	1.394	0.09112
3	Orient + (1 ID)	3	-471.9	949.9	1.430	0.08946
4	Orient + Sex + Time + (1 ID)	5	-469.9	950.0	1.543	0.08457

Table 2.6 A type II Wald's analysis of deviance on the top model (model number two), shown in table 2.5, analysing the movement in non-manipulated adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	Df	Pr(>Chisq)
Orientation	11.45	1	7.110*10 ⁻⁴
Mass	0.6341	1	0.4258
Time	3.514	1	0.06083

Table 2.7 Top four models, nested in the global model run through a LME, analysing selected ambient temperature (SelTemp) in neonatal bearded dragons (*P. vitticeps*). The global model includes time, orientation (Orient), and orientation by time interaction as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the log likelihood, (LogLik), degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (SelTemp~)	DF	logLik	AICc	ΔAICc	weight
1)	Orient + Time + Orient:Time + (1 ID)	6	-5319	10650	0	0.4999
2)	Time + (1 ID)	4	-5321	10651	0.7073	0.3510
3)	Orient + Time + (1 ID)	5	-5321	10652	2.421	0.1489
4)	(1 ID)	3	-544.0*10 ⁻¹	10886	236.4	2.220*10 ⁻⁵²

Table 2.8 A type II Wald's analysis of deviance on the top model (model number two), shown in table 2.7, analysing the selected ambient temperature in neonatal bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	Df	Pr(>Chisq)
Orient	0.2950	1	0.5869
Time	251.1	1	1.460*10 ⁻⁵⁶
Orient:Time	4.437	1	0.03516

Table 2.9 Top four models, nested in the global model run through a GLMM, analysing preferred orientation (Orient) in neonatal bearded dragons (*P. vitticeps*). The global model includes time, selected temperature (SelTemp), and selected temperature by time interaction as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the log likelihood (LogLik), degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (Orient~)	DF	logLik	AICc	ΔAICc	weight
1)	SelTemp + Time + SelTemp:Time + (1 ID)	5	-107.0*10 ¹	2150.783	0	0.9796
2)	(1 ID)	2	-1078	2160.612	9.828	0.007191
3)	Time + (1 ID)	3	-1077	2160.786	10.00	0.006593
4)	SelTemp + (1 ID)	3	-1077	2161.839	11.05	0.003894

Table 2.10 A type II Wald's analysis of deviance on the top model (model number one), shown in table 2.8, analysing the orientation preference in neonatal bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	Df	Pr(>Chisq)
SelTemp	0.04391	1	0.8340
Time	1.558	1	0.2118
SelTemp:Time	13.12	1	0.0002910

Table 3.1 Top four models, nested in the global model run through a LME, analysing rate of temperature change (Rate Constant) in adult bearded dragons (*P. vitticeps*). The global model includes experimental manipulation (Treatment), body mass (Mass), sex, and experimental manipulation by sex interaction as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the R squared, (R^2), degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (Rate Constant~)	R ²	DF	AICc	ΔAICc	Weight
1)	Mass + Sex + (1 ID)	0.3683	5	-243.5	0	0.759
2)	Sex + (1 ID)	0.2567	4	-239.5	4.06	0.1
3)	(1 ID)	0.2041	3	-239.1	4.41	0.084
4)	Mass + (1 ID)	0.2206	4	-237.5	6.01	0.038

Table 3.2 A type II Wald's analysis of deviance on the top model (model number one), shown in table 3.1, analysing the rate of temperature change in adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr
Mass	9.6102	1	0.001934
Sex	14.7546	1	0.0001225

Table 3.3 Top four models, nested in the global model run through a LME, analysing selected ambient temperature (SelTemp) in adult bearded dragons (*P. vitticeps*). The global model includes experimental manipulation (Treatment), body mass (Mass), sex, time, and treatment by sex and time interaction as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the log likelihood, (logLik), degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (SelTemp~)	DF	logLik	AICc	ΔAICc	weight
1)	Sex + Time + Sex:Time + (1 ID)	6	-6923.28	13858.6	0	0.2269
2)	Mass + Sex + Time + Sex:Time + (1 ID)	7	-6922.57	13859.19	0.5897	0.1689
3)	Sex + Time + Sex:Time + Treatment + Time:Treatment + (1 ID)	8	-6921.69	13859.44	0.8391	0.1491
4)	Mass + Sex + Time + Sex:Time + Treatment + Time:Treatment + (1 ID)	9	-6920.99	13860.05	1.453	0.1096

Table 3.4 A type II Wald's analysis of deviance on the top model (model number four), shown in table 3.3, analysing the selected ambient temperature in adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr(>Chisq)
Mass	1.45439	1	0.2278
Sex	0.077101	1	0.7812
Time	508.6102	1	1.300*10 ⁻¹¹²
Treatment	0.003777	1	0.9509
Sex:Time	47.25765	1	6.220*10 ⁻¹²
Time:Treatment	3.161582	1	0.07539

Table 3.5 Top four models, nested in the global model run through a GLMM, analysing probability of Gaping (Gaping) in adult bearded dragons (*P. vitticeps*). The global model includes experimental manipulation (Treatment), body mass (Mass), sex, time, and selected temperature (SelTemp) as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (Gaping~)	DF	AICc	ΔAICc	Weight
1)	Treatment + Temp + Mass + (1 ID)	5	485.5	0	0.424
2)	Treatment + Sex + Temp + Mass + (1 ID)	6	486.4	0.96	0.262
3)	Treatment + Time + Temp + Mass + (1 ID)	6	487.1	1.63	0.187
4)	Treatment + Sex + Time + Temp + Mass + (1 ID)	7	488.1	2.65	0.113

Table 3.6 A type II Wald's analysis of deviance on the top model (model number two), shown in table 3.5, analysing the chances of gaping in adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr
Mass	17.5928	1	0.0000273
Temp	12.3372	1	0.000444
Sex	1.1362	1	0.28646
Treatment	10.672	1	0.001088

Table 3.7 Top four models, nested in the global model run through a GLMM, analysing probability of facing a heat source (Orientation) in adult bearded dragons (*P. vitticeps*). The global model includes experimental manipulation (Treatment), body mass (Mass), sex, time, and selected temperature (SelTemp) as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (Orientation~)	DF	AICc	ΔAICc	Weight
1)	Treatment + Time + (1 ID)	4	3191.8	0	0.225
2)	Treatment + Sex + Time + (1 ID)	5	3193	1.14	0.127
3)	Treatment + Time + SelTemp + (1 ID)	5	3193.4	1.62	0.1
4)	Treatment + Time + Mass + (1 ID)	5	3193.5	1.66	0.098

Table 3.8 A type II Wald's analysis of deviance on the top model (model number two), shown in table 3.7, analysing the chances of facing a heat source in adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr
Sex	0.8947	1	0.3442
Time	4.6391	1	0.03125
Treatment	41.0079	1	1.520*10 ⁻¹⁰

Table 3.9 Top four models, nested in the global model run through a GLMM, analysing movement (Movement) in adult bearded dragons (*P. vitticeps*). The global model includes experimental manipulation (Treatment), body mass (Mass), sex, time, and selected temperature (SelTemp) as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (Movement~)	DF	AICc	ΔAICc	Weight
1)	Time + SelTemp + (1 ID)	4	1992	0	0.3
2)	Treatment + Time + SelTemp + (1 ID)	5	1993.5	1.43	0.147
3)	Time + SelTemp + Mass + (1 ID)	5	1993.7	1.68	0.129
4)	Sex + Time + SelTemp + (1 ID)	5	1994	1.96	0.113

Table 3.10 A type II Wald's analysis of deviance on the top model (model number two), shown in table 3.9, analysing the chances of movement in adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr
SelTemp	10.5638	1	0.001153
Time	6.0794	1	0.01367
Treatment	0.5801	1	0.4462

Table 3.11 Top four models, nested in the global model run through an LME, analysing selected ambient temperature (SelTemp) in adult bearded dragons (*P. vitticeps*). The global model includes experimental manipulation (Treatment), body mass (Mass), sex, time, and treatment by sex and time interaction as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the log likelihood, (LogLik), degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (SelTemp~)	DF	logLik	AICc	ΔAICc	weight
1)	Time + Treatment + Time:Treatment + (1 ID)	6	-5338.66	10689.37	0	0.2075
2)	Mass + Time + Treatment + Time:Treatment + (1 ID)	7	-5338.29	10690.64	1.274	0.1097
3)	Sex + Time + Treatment + Time:Treatment + (1 ID)	7	-5338.66	10691.38	2.012	0.07587
4)	Sex + Time + Treatment + Time:Treatment + Sex:Treatment + (1 ID)	8	-5337.75	10691.57	2.206	0.06887

Table 3.12 A type II Wald's analysis of deviance on the top model (model number two), shown in table 3.11, analysing the selected ambient temperature in adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr(>Chisq)
Mass	0.753252	1	0.3854
Time	188.7945	1	5.830*10 ⁻⁴³
Treatment	2.27304	1	0.1316
Time:Treatment	4.509696	1	0.03370

Table 3.13 A type II Wald's analysis of deviance on the global model, analysing the probability of gaping in adult bearded dragons (*P. vitticeps*). The global model includes sex, time, selected ambient temperature and mass as fixed effects and the intercept of animal ID (ID) as the random effect. The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr(>Chisq)
Sex	5.133339	1	0.02347
Time	0.439105	1	0.5075
Temperature	2.162525	1	0.1414
Mass	9.87E-05	1	0.9920

Table 3.14 Top four models, nested in the global model run through an GLMM, analysing preferred orientation (Orient) in adult bearded dragons (*P. vitticeps*). The global model includes experimental manipulation (Treatment), selected temperature (SelTemp), body mass (Mass), sex, and time, as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (Orient~)	DF	AICc	ΔAICc	Weight
1)	Treatment + Sex + SelTemp + Mass + (1 ID)	6	2711.6	0	0.3356
2)	Treatment + Sex + Time + SelTemp + Mass + (1 ID)	7	2713.6	1.939	0.1273
3)	Treatment + Sex + SelTemp + (1 ID)	5	2713.8	2.167	0.1135
4)	Treatment + Sex + Mass + (1 ID)	5	2714	2.351	0.1035

Table 3.15 A type II Wald's analysis of deviance on the top model (model number 2), shown in table 3.14, analysing the probability of facing a heat source in adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr
Mass	4.865	1	0.02740
SelTemp	3.726	1	0.05357
Sex	8.3047	1	0.003954
Time	0.0741	1	0.7854
Treatment	14.0285	1	0.0001801

Table 3.16 Top four models, nested in the global model run through an GLMM, analysing movement (Move) in adult bearded dragons (*P. vitticeps*). The global model includes experimental manipulation (Treatment), selected temperature (SelTemp), body mass (Mass), sex, and time, as fixed effects, and the intercept of animal ID (ID) as the random effect. Models are ranked (Model Number), from one to four, based on the lowest Akaike Index Criterion value (AICc). Additionally, the degrees of freedom (DF) and weight are shown for each model.

Model Number	Model (Movement~)	DF	AICc	ΔAICc	Weight
1)	Treatment + Time + Temp + (1 ID)	5	2145.9	0	0.2395
2)	Time + Temp + (1 ID)	4	2146.2	0.3652	0.1995
3)	Treatment + Sex + Time + Temp + (1 ID)	6	2146.7	0.8473	0.1568
4)	Sex + Time + Temp + (1 ID)	5	2147.2	1.313	0.1241

Table 3.17 A type II Wald's analysis of deviance on the top model (model number 3), shown in table 3.16, analysing the probability of movement in adult bearded dragons (*P. vitticeps*). The terms for the top model are listed and the Chi squared value (Chisq), degrees of freedom (DF) and p value (Pr) are shown for each term.

Terms	Chisq	DF	Pr
Temp	48.2275	1	3.80*10 ⁻¹²
Sex	1.2177	1	0.2698
Time	20.8082	1	5.08*10 ⁻⁶
Treatment	2.4853	1	0.1149