THE BEHAVIOURAL THERMOREGULATION AND ECOPHYSIOLOGY OF THE LEOPARD TORTOISE (*GEOCHELONE PARDALIS*) IN THE NAMA-KAROO

MEGAN KAY MCMASTER

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Pietermaritzburg

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Preface

The experimental and laboratory work described in this dissertation was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, from January 2003 to December 2004, under the supervision of Prof. Colleen T. Downs.

This study is the original work of the author and has not been submitted in any form for any diploma or degree to another university. Where use has been made of the work of others, it is duly acknowledged in the text. Each chapter is written in the format of the journal stated at the beginning of each, to which it will be submitted.

Megan K. McMaster

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Megan Kay McMaster 　　　　　　　　　　　 Prof. Colleen T. Downs

Pietermaritzburg 　　　　　　　　　　　　　 Pietermaritzburg

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ABSTRACT

The leopard tortoise (*Geochelone pardalis*) is the largest of the southern African tortoise species and has a wide distribution range. However, there is a lack of ecological and physiological information about the species, especially arid and semi-arid regions. The Nama-Karoo, an arid region of South Africa, is subject to large fluctuations in rainfall, food availability and ambient temperatures (T_a). This study focused on the thermal behaviour, thermoregulatory, digestive and metabolic plasticity of the leopard tortoise within the Nama-Karoo biome.

Seasonal changes in activity patterns and body temperature (T_b) were investigated in free ranging leopard tortoises in the Nama-Karoo. Leopard tortoises had unimodal daily activity patterns in winter, bimodal in summer, and there were daily and seasonal differences in the extent to which certain behaviours were practiced. Daily activity behaviours were executed at lower T_b and at lower T_a in winter compared to summer. In summer, core T_b of all tortoises oscillated on a daily basis well below maximum T_a, while core T_b of all tortoises in winter oscillated well above the daily T_a range. Tortoises were therefore able to maintain their T_b independently of T_a. Differences in T_b as measured from various positions on the tortoises body was investigated in relation to T_a. There was a strong seasonal and temporal influence on the relationship between various T_b's, with the skin and external shell temperatures being more variable in response to fluctuating T_a's compared with cloacal and core T_b. Cloacal temperatures were significantly different to other T_b measurements suggesting that it should be treated with circumspection as an exclusive measure of T_b.

Heating and cooling rates of leopard tortoises were investigated in the field and under controlled laboratory conditions to determine if the tortoises maximise operational daily activity periods, and to determine the effect of behaviour and size on the rate of heat flux. In the laboratory, cooling rates were faster than heating rates in summer and winter for all size

classes and decreased with increasing body mass. Leopard tortoises had significantly faster heating and cooling rates in winter than in summer. Free-ranging leopard tortoises had faster heating rates than cooling rates and their heat flux was largely independent of T_a. Heating and cooling rates were dependant on body mass and surface area-to-volume ratio of individuals. Under experimental conditions, tortoises physiologically adjusted their rate of heat flux, while free-ranging tortoises used physiological and behavioural mechanisms to minimise the risk of overheating, to aid thermal inertia and maximise operative activity time.

Seasonal climatic cycles and fluctuating daily temperatures influence the oxygen consumption (VO_2) of reptiles, however the result of these effects on metabolism in chelonians is poorly understood. The effect of seasonal and daily differences in T_a on VO_2 was investigated. Leopard tortoises' VO_2 was slightly higher than reported for other chelonians. There were significant differences in tortoise VO_2 at different T_a's during the day and night and in different seasons. This metabolic plasticity is possibly an adaptive mechanism to cope with unpredictable environmental conditions.

Unpredictable climatic conditions lead to unpredictable food and water availability. Little is known how tortoises adjust dietary parameters in response to food type and water availability, and if this affects body mass, energy and water balance. Therefore this study also considered whether leopard tortoises adjusted food transit rate, food intake and water loss to cope with a diet fluctuating in fibre and water content, and whether body mass, energy and water balance were maintained. Leopard tortoises fed a high fibre, low water content diet had lower food intake rates, longer food transit times, but lower daily energy assimilation compared with tortoises fed a low fibre, high water content diet. Tortoises fed a high fibre, low water content diet had lower urine osmolality, but similar total water loss to those fed a high fibre, low water content diet. The results indicate that tortoises can adjust digestive

parameters according to diet composition and exercise some control over energy and water balance.

It is concluded that leopard tortoises show a high degree of plasticity in their thermal behaviour and physiology which allows survival in an unpredictable environment, particularly where there are fluctuations in rainfall, food availability and T_a's. Seasonal and daily variation in thermoregulation, metabolic rate and the uptake of energy allows the leopard tortoise to maximise the duration of operative temperature, to minimise energy loss and to use variable and unpredictable seasonal resources.

Acknowledgements

LIST OF ABBREVIATIONS:

AE = assimilation efficiency

BM = body mass

DEA = daily energy assimilated

FE = faecal energy

FI = food intake

FWL = faecal water loss

g = grams

GEI = gross energy intake

ha = hectare

ibuttons = Thermocron iButtons^{TM}

Kg = kilograms

KJ = kilojoules

T = temperature

T_a = ambient temperature

T_b = body temperature

T_e = operative temperature

TWL = total water loss

UWL = urinary water loss

WI = water intake

CONTENTS

CHAPTER 1

Introduction

The family Testudinidae (Suborder Cryptodira) is represented by 14 species in southern Africa, with ten species endemic to South Africa (Boycott and Bourquin, 2000). The largest of the Southern African species is the leopard tortoise (*Geochelone pardalis*). The species has a wide distribution range throughout much of sub-Saharan Africa and into the arid regions of southern Africa, including the Nama-Karoo (Figure 1).

The Nama-Karoo biome occurs on the central plateau of the western half of South Africa and is the second-largest biome in the region. The distribution of this biome is determined primarily by rainfall (Low and Rebelo, 1996). The average annual rainfall in the Nama-Karoo is 200 - 400 mm but varies between 100 and 520 mm per year, with the highest rainfall in late summer and autumn. Daily ambient temperatures (T_a) are highly variable and range from 5° to 39°C in spring and summer, and from -5° to 26°C in autumn and winter. Whilst daily temperature ranges are extreme, they are also unpredictable within a season with aseasonal warm or cold periods. In southern Africa unpredictable environmental conditions are also a result of the effects of El Niño South Oscillation (ENSO) events that occur every two to seven years (Mason, 2001; Kruger, 2004). In southern Africa, the periodicity of ENSO and the duration of each ENSO event remains unpredictable, as does the extent to which the southern African climate will be affected (Ramage, 1986; Koen, 1992; Mason, 2001; Kruger 2004). ENSO affects both rainfall and temperature patterns in South Africa, particularly in summer, and causes extreme temperature variation and below-average rainfall (Mason, 2001; Kruger, 2004).

Such environmental unpredictability poses different ecological and physiological problems, particularly in semi-arid and arid areas. Regular rainfall is particularly important to tortoises as it provides drinking water and sustains ephemeral plants that are high in preformed water content (Nagy and Medica, 1986). Following rainfall events, tortoises greatly increase their activity and foraging periods, show increased field metabolic rates and have increased reproductive outputs (Turner *et al*., 1986; Henen *et al*., 1998). Unpredictable rainfall, as found in southern Africa, can cause droughts which have been shown to result in low reproductive rates, low recruitment and high mortality in other xeric tortoise species (Henen, 1997).

Temperature extremes are also the product of unpredictable climatic events and high temperatures can further acerbate drought conditions. High temperatures have been shown to be more dangerous to some tortoise species than cooler temperatures (Swingland and Frazier, 1979; Meek, 1984; Peterson, 1994; Hailey and Coulson, 1996). Extreme weather events with higher than average temperatures could affect tortoises directly through increased mortality (Peterson, 1994), but also indirectly by limiting the amount of time that a tortoise is able to be active during the day. Decreased activity periods will limit food and water intake, reproductive output and ultimately recruitment (Henen *et al.*, 1998). Any herbivorous ectotherm found in areas affected by ENSO must therefore exhibit a large degree of thermoregulatory and dietary plasticity if they are to be successful.

Although all tortoises living in semi-arid to arid environments face challenges in resource availability, not all arid regions are affected by ENSO and are therefore temporally predictable. Although the ecophysiology of many Mediterranean tortoise species is well studied (e.g. Lambert, 1981; Meek, 1984; Wright *et al.*, 1988; Geffen and Mendelssohn, 1989), these tortoises do not live in unpredictable environments. The desert tortoises (*Gopherus agassizii*) of California are subject to unpredictable climatic events (Henen *et al.,*

1998) and may therefore have a comparable ecophysiology to tortoises living in southern African deserts. However, little is known about the thermal behaviour, digestive and metabolic plasticity that would enable leopard tortoises to live in such an unpredictable environment. Therefore this study focussed on this paucity of knowledge and the effect of seasons on these aspects of their physiology and behaviour.

Body temperature (T_b) is the most important variable affecting the behaviour and physiology of reptiles and the regulation of T_b is an effective method of dealing with spatial and temporal heterogeneity in the thermal environment (Angilletta *et al*., 2002). Consistently over the past 40 years, cloacal temperatures have been the favoured method of measuring T_b in reptiles (Avery, 1982; Cloudsley-Thompson, 1991). Although other T_b measurements have been used (reviewed in chapter 2), the cloacal measurement remains the most popular, despite some doubts being expressed about its relevance (Johnson *et al*., 1978; Lambert, 1981; Seebacher *et al*., 1999). The relevance of the cloacal T_b is extremely important, especially in a reptile which is insulated by an exterior shell and where the adult can reach a mass of 25 kg. Both the size and the shell ameliorate the effects of outside temperatures and can lead to thermal gradients within the body of the animal. This would not be registered by taking the cloacal T_b in isolation. Therefore, the first chapter of this study considers other methods of measuring internal/core T_b in a large insulated ectotherm. In particular, the efficacy of taking T_b readings from non-cloacal positions on the body and implanted internal techniques of T_b measurement, and comparing these to the cloacal T_b was investigated. The hypothesis considered in this chapter was that there is a difference between cloacal, non-cloacal or core T_b of leopard tortoises.

Reptiles employ physiological and behavioural processes to thermoregulate, however physiological thermoregulation is energy-consuming and behavioural thermoregulation is time-consuming. This reduces energy and time that could otherwise be dedicated to foraging

and social activities (Gvozdik, 2002). Maintenance of T_b and/or control of the rate of heat flux would maximise energy conservation and minimise the time spent in thermoregulation. Therefore, in chapter three, the ability and the extent to which leopard tortoises are able to maintain their T_b in natural conditions was investigated. Particular emphasis was placed on individual's ability to maintain T_b in fluctuating and extreme daily and seasonal T_a. It was expected that individual leopard tortoises do not maintain their T_b independently of T_a. In chapter four, the ability of individual leopard tortoises to manipulate the rate of heating and cooling of their T_b was investigated, particularly under controlled laboratory conditions. The hypothesis was that leopard tortoises differ in the rates of heating and cooling, and that these rates are the same as an inert model simulating live individuals.

Evolution of body size is a trait of great significance in both ectotherms and endotherms in terms of ecophysiology as shown by numerous allometric studies (Chown *et al.*, 2003). Increased body mass can lead to increasingly stable body temperatures and reduced heat flux, however, the response time of T_b to changes in the thermal environment is much longer in larger reptiles (Seebacher *et al.*, 1999). The influence of body size on heat flux in juvenile and hatchling leopard tortoises was considered in the captive studies. Hatchlings and juveniles were rarely found in the wild and the available tracking devices and iButton^{TM} s were too large to use on them. As such, only adult tortoise heat flux was studied in the wild.

Energy is a universal currency and its management is closely tied to all aspects of an individual's life history, including thermoregulation, dietary requirement and allocation of time (Ricklefs, 1996). Minimising the thermoregulatory costs for the maintenance of energy budgets requires various adaptations, which may include lowered metabolic rates. Seasonal and daily temperature fluctuations are vitally important in influencing the metabolism of reptiles (Hailey and Loveridge, 1997). As has been stated, leopard tortoises in the Nama-

Karoo are subjected to wide temperature fluctuations both daily and seasonally, in conjunction with unpredictable rainfall resulting in unpredictable food and water resources. The ability of tortoises living in such areas to lower their metabolism in times of adverse conditions and show plasticity in their metabolic response to varying temperatures, would allow them to save energy and minimise thermoregulatory costs. Therefore, in chapter five, the change in metabolic rate of different sized tortoises was measured in relation to T_a, time of day and season. It was hypothesised that T_a and seasonal differences would have an effect on the metabolic rate of the tortoises.

In order to meet their energy demands and cope with unpredictable food resources and fluctuating ambient temperatures, herbivorous ectotherms have to compromise diet quality, transit times and assimilation efficiency (Waldschmidt *et al*., 1987). However, little is known about what digestive constraints prevail in environments where food and water resources are generally patchy in time and space. Therefore, in chapter six, the degree of digestive plasticity shown by leopard tortoises was investigated. It was hypothesised that tortoises fed two diets differing in preformed water and fibre content would have differing food intake, gut transit rate, assimilation efficiency, and faecal and urinary water and would alter digestive parameters to maintain energy and water balance.

The final chapter discusses the plasticity of leopard tortoises in the Nama-Karoo and how they compare to other chelonians living in unpredictable climates.

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Figure 1: Distribution of *G. pardalis* in Africa and in southern Africa (enlarged) after Branch, 1988.

CHAPTER 2

Thermal Variability in Body Temperature in a Large Ectotherm: Are Cloacal Temperatures Good Indicators of Tortoise Body Temperature?

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ABSTRACT

Historically, studies of reptilian thermal biology have compared ambient temperatures (T_a) to body temperatures (T_b) from the animal under study, with T_b usually taken from the cloaca and various instruments being used to measure T_b. The advent of surgically implanted miniature temperature loggers, have offered the opportunity to test the efficacy of cloacal T_b as a measurement in thermoregulatory studies. We predicted that there was no difference between cloacal, non-cloacal or internal T_b's. T_b's were measured from various positions on leopard tortoises (*Geochelone pardalis)* using thermocouples and miniature temperature loggers, including surgically implanted temperature loggers. Measurements of T_b from various positions on the tortoise were significantly different from T_a, although some variation was shown between cloacal T_b and T_a. Significant differences were found between cloacal T_b and all other body T_b measurements. In addition, significant differences were found between measures of T_b from other parts of the body. The discrepancy between core T_b, cloacal T_b and other measures of T_b indicates that there may be large thermal gradients within the body of an ectotherm at any given time and cloacal T_b may not be an accurate measure of core T_b.

KEYWORDS: behaviour, thermoregulation, core body temperature, cloacal body temperature, skin temperature, leopard tortoise, *Geochelone pardalis*.

INTRODUCTION

Various thermal characteristics and evidence of thermoregulation have been studied in tortoises (Craig, 1973; Perrin and Campbell, 1981; Chelazzi and Calzolai, 1986; Meek, 1988; Hailey and Coulson, 1996a; Hailey and Loveridge, 1998), however, body temperatures (T_b) are often only measured as part of an ecological study (Cloudsley-Thompson, 1970; Lambert, 1981; Branch, 1984; Meek, 1984; Wright et al., 1988).

Most studies of reptilian thermal biology have compared ambient temperature (T_a) with T_b for the animal under study, with the T_b typically taken from the cloaca (e.g. Spencer and Grimmond 1994; Hailey and Coulson, 1996a,b; Angilletta et al., 2002; Southwood et al., 2003). Only a few studies have compared measured T_b from various positions on or in the animals' body, Johnson et al. (1978) used deep cloacal, head, heart, subcutaneous and lumbar temperatures to investigate thermoregulation in the American alligator (*Alligator mississipiensis*), while Lambert (1981) measured cloacal, skin and shell temperatures of the spur-thighed tortoise (*Testudo graeca*).

Whilst the latter two studies used different temperature measuring techniques: temperature sensitive radio-transmitters and thermocouples, respectively, in recent years, a miniature temperature logger, the Thermocron iButton^{TM} (Dallas Semiconductors, Texas, USA) has become available. This is small enough to be implanted into larger tortoises and allows constant temperature monitoring of deep core T_b (described and reviewed by Angilletta and Krochmal, 2003).

In this study, thermocouples and iButton^{TM}s were used as the temperature measuring devices. The following T_b measurements were compared: core, cloacal, external (underside of the shell) and skin. T_a was measured concurrently using a thermocouple and iButton^{TM}s.

The hypothesis considered was that there is a difference between cloacal, non-cloacal or internal T_b's.

MATERIALS AND METHODS

Field-work was conducted on a 5500 ha area of a 26000 ha mixed commercial sheep and game farm in the De Aar District, Nama-Karoo biome, South Africa (31°04'S, 23°41'E). The vegetation is classified as grassy dwarf shrubland (Palmer and Hoffman, 1997). Average annual rainfall is low (200-400 mm) and the area has its highest rainfall in late summer and autumn. Daily temperatures range from 5 to 39 °C in spring (September to November) and summer (December to February) and from -5 to 26 °C in autumn (March to May) and winter (June to August) (Kruger, 2004).

Leopard tortoises were located by riding transects on horseback through the study area. In total, four males and six females (in summer and winter 2002) and four males and five females (in summer and winter 2003) were fitted with unique-frequency radio transmitters. These transmitters (mass 60g, <1% body mass), were powered by a lithium battery (AA), had a ¾ wavelength stainless steel tracer wire antenna, were potted in moulded PVC tubing, and were attached to the carapace with dental acrylic.

For both telemetered tortoises and for any other conspecific tortoises sighted, date, time, T_a's, cloacal and skin temperatures were recorded. Skin temperatures for all tortoises were obtained by inserting the thermocouple deep into the folds of skin in the junction between the neck and foreleg (termed "front skin" temperature), and in the folds of skin of the upper thigh (termed the "rear skin" temperature). Cloacal T_b's were taken by inserting the thermocouple 50 mm into the cloaca. T_a's were recorded in the shade close to each tortoise found using a fine-gauge thermocouple thermometer (Cole-Palmer Digi-Sense®). In addition, daily T_a's were recorded every 20 min using iButton^{TM} s placed 20 cm above the ground in full shade. All temperature recording equipment was calibrated against an Ever

Ready electronic reference thermometer by the comparison method using thermally stabilised liquid baths (following Nicholas and White, 2001), and a calibration curve generated. The correction values were calculated for the observed temperatures using the generated calibration curve, and the temperature recording equipment corrected (following Nicholas and White, 2001).

Telemetered leopard tortoises were located at least twice daily during February and July 2002 and 2003. In addition, at least two tortoises of each gender were followed for a continuous 12 h period once every month, and the behaviour, ambient, cloacal and skin temperatures were recorded every 20 min. For each month of field sampling, iButton^{TM} s were attached to each telemetered tortoise and programmed to take a temperature recording every 20 minutes. The iButton^{TM} s were attached (using dental acrylic) to the ventral surface of the upper carapace inside the rear leg cavity of the tortoise and are referred to as "external" temperature recordings.

To measure core T_b, iButton^{TM} s were programmed to record temperature every 20 min and were surgically implanted into the sub-clavial cavities of 2 male and 2 female, and 3 male and 2 female telemetered tortoises for summer and winter 2003, respectively. Prior to implantation, tortoises were anaesthetised using Fluorothane gas and surgery performed by a veterinary surgeon.

T-tests were run to compare temperatures (STATISTICA software (Statsoft, USA). In order to control for family-wise Type 1 errors, significance levels for each t-test were adjusted downwards using the sequential Bonferroni procedure (Holm, 1979). The P values of each test were ranked and the smallest tested at $^\alpha/_c$, the next at $^\alpha/_{c-1}$, the next at $^\alpha/_{c-2}$ and so on, where $\alpha = P$ value and c = number of tests. A number of studies dealing with data of a similar nature have arranged the data into units of time periods, averaged across individuals (Meek, 1988; Christian and Weavers, 1996; Shine et al., 2000; Sartorius et al., 2002;

Whitaker and Shine, 2002). This is not valid and introduces pseudoreplication into the statistical analysis. In order to compare the method and site of temperature measurement and avoid pseudoreplication, the data was arranged so that the units were individuals, averaged across time periods.

RESULTS

Comparison of T_a

The relationship between T_a measured by thermocouples and iButton^{TM}s was tested using regression analysis. The high values of the coefficient of determination (r^2) indicated that the regression line gave a significant goodness of fit and subsequent testing of the data showed there was no statistically significant difference between the two forms of measurement (summer 2002 $r^2 = 0.77$, n = 146, $p > 0.05$; winter 2002 $r^2 = 0.76$, n = 114, $p > 0.05$; summer 2003 $r^2 = 0.73$, n = 235, $p > 0.05$ and winter 2003 $r^2 = 0.88$, n = 183, $p > 0.05$ (Figure 1)). As such, only the iButton^{TM} measurement for T_a was used for further comparison with T_b between different parts of the body.

Comparison of T_a and T_b

During the winter and summer of 2002, T_a, as measured by iButton^{TM}s, was not significantly different from T_b as measured from the cloaca (summer 2002, $r^2 = 0.49$, p > 0.05; winter 2002, $r^2 = 0.34$, p > 0.05). Comparison of these two measurements in 2003 were significantly different (summer 2003, $r^2 = 0.17$, p < 0.05; winter 2993, $r^2 = 0.16$, p < 0.05) (Tables 1 and 2; Figures 2-5). External T_b's and front and rear skin T_b's were all significantly different from T_a in all seasons (Tables 1 and 2; Figures 2-5). Similarly, internal T_b's, measured with iButton^{TM}s in July 2003, were significantly different from T_a (Table 2; Figure 5).

Comparison of T_b from different positions on the body

In comparing the T_b's as measured on different parts of the body, the cloacal T_b was significantly different to the external T_b, the rear and front skin in both seasons and in both years (Tables 1 and 2). This was also the case when comparing the external T_b to the rear and front skin T_b over both seasons in both years (Table 1 and 2). Comparison of the rear skin T_b and the front skin T_b also shows no significant difference during summer of both years (Table 1). However, interestingly, there was a significant difference between the rear and front skin T_b in winter of both years (Table 2). Internal T_b which was only measured in July, 2003 was significantly different from all other T_b's except external temperature (Table 2).

DISCUSSION

There was little variation in the measurement of T_a's using the fine-gauge thermocouple thermometer and iButton^{TM}s. At higher daily T_a iButton^{TM}s delivered a lower T_a than the thermocouple but at lower daily T_a, the position was reversed and iButton^{TM}s returned a slightly higher T_a than the thermocouples. This could have been because the thermocouples are more sensitive to air movement compared with iButton^{TM}s which are enclosed in metal casing (Angilletta and Krochmal, 2003).

Over the two seasons in 2002, no significant difference was found between the cloacal T_b of tortoises and T_a, while over the two seasons in 2003, the results were significantly different. It is difficult to explain this trend. As can be seen the cloacal temperatures are lower in 2002 than in the corresponding seasons in 2003. In winter 2002, the maximum was 25 °C, while in winter 2003 the maximum was 30 °C. This was also the case in summer where the maximum cloacal temperature measured in 2002 was around 32

°C, while in 2003 it was around 36 °C. It is interesting then that the T_b, as measured by the cloaca, is not significantly different from T_a when the daily temperature range is relatively narrow, but when there is a wider range of daily temperatures, the difference between the T_b measured by the cloaca and the T_a measured by the iButton^{TM} was significantly different. This anomaly would render the cloacal measurement of T_b unreliable (Nussear et al., 2002).

Measurements of T_b taken from other parts of the body were all significantly different from T_a in both seasons and for both years. Other reptiles have skin and external temperatures that fluctuate widely in response to T_a (Spotila et al., 1973; McNab and Auffenburg, 1976; Johnson et al., 1978; Lambert, 1981; Seebacher et al., 1999). Meek (1984) found that the cloacal T_b's of Hermann's tortoise (*Testudo hermanni*) showed no significant correlation with T_a in summer, with T_b being higher than both air and substrate temperatures, while the cloacal T_b of *Chersina angulata* were consistently 2-6 °C above T_a (Branch, 1984). Lambert (1981) reported that plastral surface temperature of the shell of *T. graeca* fluctuated widely in response to T_a, less so for the carapace surface and skin, while cloacal temperatures remained constant, although all these temperatures were higher than T_a's.

Cloacal T_b's in this study were significantly different from external T_b's, and front and rear skin T_b's. Similarly, external T_b's were significantly different from all other T_b measurements taken, except the internal T_b measured in July 2003 and rear skin and front skin T_b were significantly different in the winters of both years, but not in the summers.

Differences between T_b measurements from the rear and front skin of leopard tortoises showed strong seasonal variation, most of which may have resulted from seasonal behavioural differences. In winter leopard tortoises remain within shelters for long periods of time and push themselves well into the shelter, such that the front half of the tortoise (and shell) is completely covered by vegetation, while the rear half of the tortoise is orientated to the sun for passive basking (McMaster and Downs, 2006). However in summer, leopard

tortoises usually bask for short periods of time outside their shelters before starting activity and eventually seeking shade (McMaster, 2002, McMaster and Downs, 2006). Therefore in winter, tortoises would receive more solar radiation on the rear half of their shells, while in summer solar radiation would be more evenly spread across the shell surface. This difference in exposure to direct solar radiation would account for the significant difference between the front and rear skin T_b's of leopard tortoises in winter, and the similarity between them in summer.

Heat gain by solar radiation (heliothermy) is the primary mode of heating in many species of lizards, snakes and chelonians (reviewed by Belliure and Carrascal, 2002), and as such, differences in exposure to solar radiation would cause differences in the heat gain across the body (Webb and Heatwole, 1971; Whitaker and Shine, 2002). In addition, the shell of chelonians acts as a buffer against solar radiation (Cloudsley-Thompson, 1991). Reiber et al. (1999) reported that over a wide range of T_a's, the shell temperatures of juvenile desert tortoises (*G. agassizii*) were consistently higher than cloacal temperatures by at least 3 ºC, while in adults of the same species, McGinnis and Voigt (1971) measured a temperature difference of more than 10 ºC between the shell and cloacal T_b at midday. Lambert (1981) also found the shell temperatures of the spur-thighed tortoise (*T. graeca*) to differ markedly from cloacal and skin temperatures, depending on whether the tortoise was basking, partially shaded or fully shaded, with the shell temperature oscillating in response to solar radiation and cloacal temperatures remaining fairly constant. Therefore, cloacal T_b may be consistently underestimating the internal temperature of a tortoise and does not reflect the temporal oscillations in core T_b in relation to T_a.

Internal T_b, measured in July 2003, was significantly different from all other T_b's except external temperature. Core T_b's in large reptiles can differ markedly from surface (shell or skin) temperatures (McGinnis and Voigt, 1971; Webb and Heatwole, 1971; Spotila

et al., 1973; Johnson et al., 1978; Lambert, 1981; Smith et al., 1981; Ayers and Shine, 1997; Seebacher et al., 1999). In addition, core T_b of large reptiles exhibit thermal inertia compared to ambient and other external temperatures, allowing for the maintenance of T_b that are buffered and largely independent of the external variations in temperature extremes (Webb and Heatwole, 1971; Spotila et al., 1973; McNab and Auffenburg, 1976; Johnson et al., 1978; Stevenson, 1985; Seebacher et al., 1999). In contrast, head temperatures are usually lower and more stable than core T_b, despite the smaller mass of the head and resultant mass relations (Heath, 1964; Dewitt, 1967; Johnson et al., 1978). Studies of smaller reptiles show no differences between cloacal or surface temperatures and core T_b (Russo, 1972; Chelazzi and Calzolai, 1986; Geffen and Mendelssohn, 1989; Alexander et al., 1999; do Amaral et al., 2002; Whitaker and Shine, 2002).

Differences in T_b in different parts of the body can be accentuated or reduced through various physiological mechanisms (Bartholomew and Tucker, 1964; Bakken, 1976). Bartholomew and Tucker (1963) reported that the primary physiological mechanism used to transfer heat within a reptile is circulation, and suggests that circulation augments heat exchange during heating and diminishes it during cooling. Vasoconstriction and counter-current exchange systems within the body can also lead to temperature differences being maintained within a reptile (Heath, 1964; Bakken, 1976). Heating of the skin during basking results in cutaneous vasodilatation, a factor favouring more rapid transport of heat from the surface to the core (White, 1973). As a reptile gains heat during the day, circulation of blood from the core to the body wall and skin would induce cutaneous vasodilatation as the skin is heated from within and heat would be lost through conduction to the air or a cooler substrate (White, 1973). To prevent rapid cooling, cutaneous vasoconstriction would reduce the conductance of heat to the skin surface and heat would be retained within the body (White, 1973). Bakken (1976) noted that vasoconstriction and vasodilatation are important in

adjusting heating and cooling rates in tortoises, while blood vessels in the head and limbs can be cooled by evaporative cooling during copious salivation (Cloudsley-Thompson, 1974). Vascular shunting has been recorded in *T. graeca* and *Geochelone elephantopus* where a heat-exchange transfer system causes heat to be dissipated through the plastron (MacKay, 1964; Lambert, 1981). Warm blood shunted from elsewhere in the body is passed through capillaries connecting the underlying bone tissue of the plastron when in contact with a cool ground surface causing heat to be lost through conductance (MacKay, 1964; Lambert, 1981).

Discrepancies between core T_b, cloacal T_b and other measures of T_b indicate that at any given time and at any given T_a, there are large thermal differences within the body of leopard tortoises. Factors such as the amount of direct solar radiation received on different areas of the body, seasonal changes in behaviour and physiological mechanisms can cause variable relationships between the temperatures of different parts of the body. Tortoises in particular have large gradients of temperature within the body, and will constantly be in thermal transit due their large size and the insulation buffering afforded by their shell.

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Table 1: Results of pair-wise *t*-tests conducted between ambient and body temperatures for summer 2002, where $n = 26$, $df = 25$ and for summer 2003 in italics, where $n = 25$, *d.f.* = 24. *T*-values are shown in the table and those marked by an asterisk (*) are significant at $P < 0.05$. A sequential Bonferroni procedure was used to control for family-wise Type 1 errors (Holm, 1979).

Summer 2002 *Summer 2003*	T_a iButton*TM*	T_b Cloacal	T_b External	T_b Rear Skin	T_b Front Skin
T_a iButton*TM*		-0.15	-12.09*	-6.70*	-6.48*
T_b Cloacal	*6.37**		-13.77*	-10.17*	-9.23*
T_b External	*5.21**	*-12.72**		-9.42*	-8.60*
T_b Rear Skin	*3.34**	*-12.67**	*7.98**		0.01
T_b Front Skin	*3.70**	*-10.72**	*-8.29**	*1.64*	

Table 2: Results of pair-wise *t*-tests conducted between ambient and body temperatures for winter 2002, where $n = 22$, $df = 21$; and for winter 2003 in italics, where $n = 23$, $d.f. = 22$. *T*-values are shown in the Table and those marked by an asterisk are significant at $P < 0.05$. A sequential Bonferroni procedure was used to control for family-wise Type 1 errors (Holm, 1979).

Winter 2002 *Winter 2003*	T_a iButton*TM*	T_b Cloacal	T_b External	T_b Rear Skin	T_b Front Skin
T_a iButton*TM*		-2.11	-10.22*	-5.29*	-2.72*
T_b Cloacal	*-3.01*		-8.21*	-8.77*	-1.22
T_b External	*-9.24*	*-4.77*		5.21*	7.37*
T_b Rear Skin	*-5.50*	*-4.43*	*2.63*		5.38*
T_b Front Skin	*-3.98*	*1.73*	*3.09*	*2.87*	
T_b Internal iButton*TM*	*4.53*	*7.19*	*0.34*	*3.43*	*9.15*

FIGURE LEGENDS

FIGURE 1: Plots of ambient temperature (T_a) measured by iButton^{TM}s on T_a measured by thermocouples. A to D = four separate seasons; A = summer 2002, B = winter 2002, C = summer 2003, D = winter 2003. Fitted regression line given as dashed line (A: y = 0.6013x + 9.955, r^2 = 0.77, n = 146, $p > 0.05$; B: y = 0.7845x + 2.3325, r^2 = 0.74, n = 114, $p > 0.05$; C: y = 1.2012x − 4.7716, r^2 = 0.73, n = 235, $p > 0.05$; D: y = 0.8653x + 2.6505, r^2 = 0.88, n = 183, $p > 0.01$).

FIGURE 2: Plots of body temperature (T_b) on ambient temperature ($T_{a)}$ measured by iButton^{TM}s during summer 2002. A = T_b cloacal, B = T_b rear skin, C = T_b front skin, D = T_b external. Fitted regression line given as dashed line; n = 146 (A: y = 1.0393x − 1.001, r^2 = 0.49, $p > 0.05$, B; y = 1.0426x + 0.6699, r^2 = 0.56, $p < 0.05$, C; y = 1.1789x − 2.9626, r^2 = 0.62, $p < 0.05$, D; y = 1.2892x -3.74, r^2 = 0.59, $p < 0.05$).

FIGURE 3: Plots of body temperature (T_b) on ambient temperature (T_a) measured by iButton^{TM}s during winter 2002. A = T_b cloacal, B = T_b rear skin, C = T_b front skin, D = T_b external. Fitted regression line given as dashed line; n = 114 (A; y = 0.9440x +1.6397, r^2 = 0.34, $p < 0.05$, B; y = 0.9371x + 3.1372, r^2 = 0.35, $p < 0.05$ C; y = 0.9996x +1.2036, r^2 = 0.39, $p < 0.05$, D; y = 0.9389x +4.8702, r^2 = 0.41, $p < 0.05$).

FIGURE 4: Plots of body temperature (T_b) on ambient temperature (T_a) measured by iButton^{TM}s during summer 2003. A = T_b cloacal, B = T_b rear skin, C = T_b front skin, D = T_b external. Fitted regression line given as dashed line; n = 235 (A; y = 0.2777x +20.3850, r^2 = 0.17, $p < 0.05$, B; y = 0.2926x + 21.0970, r^2 = 0.21, $p < 0.05$, C; y = 0.2873x +21.1320, r^2 = 0.22, $p < 0.05$, D; y = 0.4763x +16.8980, r^2 = 0.45, $p < 0.05$).

FIGURE 5: Plots of body temperature (T_b) on ambient temperature (T_a) measured by iButton^{TM} s during winter 2003. A = T_b cloacal, B = T_b rear skin, C = T_b front skin, D = T_b external, E = T_b internal. Fitted regression line given as dashed line; n = 183 (A; y = 0.6763x + 7.0259, r^2 = 0.16, $p < 0.05$, B; y = 0.7817x + 6.2034, r^2 = 0.27, $p < 0.05$, C; y = 0.8354x +4.9022, r^2 = 0.26, $p < 0.05$, D; y = 0.9349x +4.6420, r^2 = 0.51, $p < 0.05$, E; y = 0.7682x + 7.4853, r^2 = 0.11, $p < 0.05$).

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FIGURE 2: Plots of body temperature (T_b) on ambient temperature (T_a) measured by iButton[TM]s during summer 2002. A = T_b cloacal, B = T_b rear skin, C = T_b front skin, D = T_b external. Fitted regression line given as dashed line; $n = 146$ (A: $y = 1.0393x - 1.001$, $r^2 = 0.49$, $p > 0.05$, B; $y = 1.0426x + 0.6699$, $r^2 = 0.56$, $p < 0.05$, C; $y = 1.1789x - 2.9626$, $r^2 = 0.62$, $p < 0.05$, D; $y = 1.2892x - 3.74$, $r^2 = 0.59$, $p < 0.05$).

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FIGURE 4: Plots of body temperature (T$_b$) on ambient temperature (T$_a$) measured by iButton^{TM} s during summer 2003. A = T$_b$ cloacal, B = T$_b$ rear skin, C = T$_b$ front skin, D = T$_b$ external. Fitted regression line given as dashed line; n = 235 (A; y = 0.2777x +20.3850, r^2 = 0.17, p < 0.05, B; y = 0.2926x + 21.0970, r^2 = 0.21, p < 0.05, C; y = 0.2873x +21.1320, r^2 = 0.22, p < 0.05, D; y = 0.4763x +16.8980, r^2 = 0.45, p < 0.05).
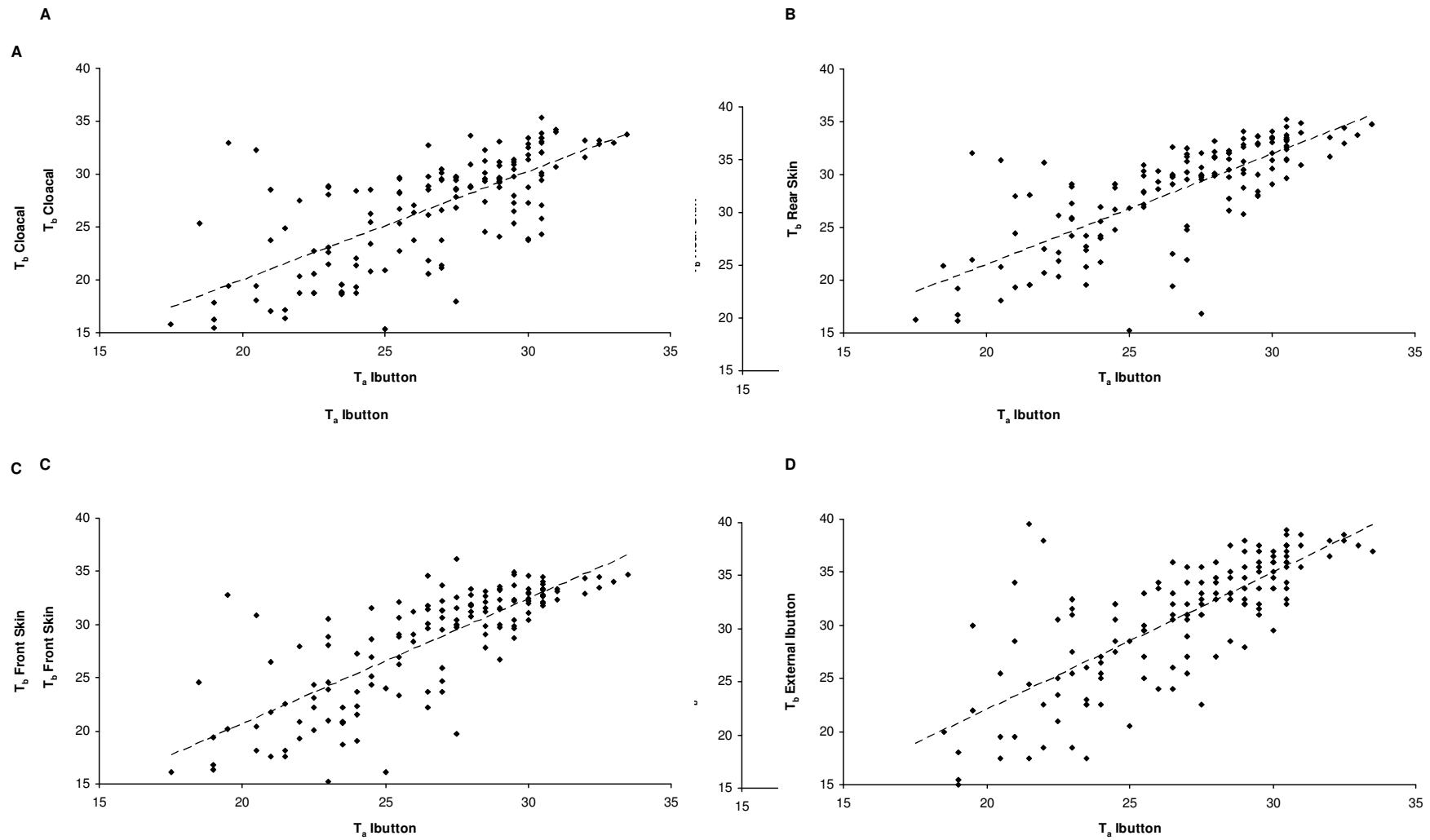
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CHAPTER 3

Thermal behaviour and maintenance of stable body temperatures by leopard tortoises

(*Geochelone pardalis)* in the Nama-Karoo.

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Abstract

The ability of reptiles to thermoregulate is enhanced by adopting a variety of behavioural mechanisms. Different behavioural postures, the use of retreat sites and selection of microhabitats enable reptiles to maintain their core body temperatures (T_b) above that of ambient temperatures (T_a). This study describes the daily activity patterns of leopard tortoises (*Geochelone pardalis*) in relation to T_b and T_a, and the extent to which leopard tortoises can manipulate their T_b in response to seasonal changes in T_a. Ten and nine leopard tortoises were radio-tracked in 2002 and 2003, respectively and cloacal T_b and behaviours observed. Core T_b's were measured using Thermocron iButtons^{TM} surgically implanted into the body cavities of 4 and 5 adult telemetered tortoises for summer and winter 2003, respectively. There were seasonal differences in the extent to which certain behaviours were practiced and the time of day that these occurred. Leopard tortoises had unimodal activity patterns in winter and bimodal in summer. In winter tortoises were active at lower preferred T_b, and at lower T_a, than in summer. Tortoises maintained their core T_b well below operative temperatures (T_e) in summer and well above T_e in winter. Core T_b closely followed the increase in T_e in the mornings, however tortoises exhibited an extended thermal lag when T_e cooled overnight, with core T_b cooling more slowly than T_e. Consequently by using different

behavioural mechanisms in summer and winter, leopard tortoises maintained their T_b compared with T_a, allowing them to maximize their daily activity periods.

Keywords: activity, behaviour, thermoregulation, core body temperature, Leopard tortoise, *Geochelone pardalis*.

Introduction

The ability of reptiles to thermoregulate is enhanced by adopting a variety of behavioural mechanisms. Stevenson (1985) calculated that a 1 kg dry-skinned ectotherm could modulate its body temperature (T_b) by 30 to 50 °C by changing activity time or habitat, compared to 5 to 15 °C using postural adjustments, and only 1-5 °C by physiological mechanisms. Different behavioural postures, the use of retreat sites and selection of microhabitats enable reptiles to maintain their core T_b above that of T_a (Chelazzi and Calzolai, 1986; Huey *et al*., 1989; Kearney, 2001; Belliure and Carrascal, 2002; Gvozdik, 2002; Kearney, 2002; Pearson *et al*., 2003).

Since the rigid shells of tortoises limit the effectiveness of postural changes during behavioural thermoregulation (Heath, 1964; White, 1973; Huey *et al*., 1989; Kearney, 2001; Pearson *et al*., 2003), one would expect the main thermoregulatory mechanism used by tortoises to be a change in daily activity period and behaviour, as was found for the tortoise *Kinixys spekii* (Hailey and Coulson, 1996b).

Experimental investigations into the preferred T_b and activity patterns of captive tortoises are limited (Craig, 1973; Perrin and Campbell, 1981; Hailey and Loveridge, 1998). However, behavioural thermoregulation and activity have been reported in free-ranging tortoises, with behavioural patterns being compared over 24 hours, between seasons, between age classes, or to environmental conditions (Cloudsley-Thompson, 1970; McGinnis and

Voigt, 1971; Judd and Rose, 1977; Lambert, 1981; Meek, 1984; Pulford, *et al.*, 1984; Chelazzi and Calzolai, 1986; Meek, 1988; Loehr, 2002).

In the few studies that have measured the T_b of tortoises, these temperatures were monitored in association with behavioural activity in the field (Lambert, 1981; Pulford *et al.*, 1984; Wright *et al.*, 1988; Hailey and Coulson, 1996b), without investigating whether tortoises are able to maintain relatively high and stable T_b or establishing how effective their thermoregulation is in maintaining core T_b.

Since behavioural thermoregulation is time-consuming and reduces energy and time that could otherwise be dedicated to foraging and social activities (Gvozdik, 2002), the ability and the extent to which leopard tortoises are able to maintain their T_b in natural conditions was investigated. Particular emphasis was placed on individual's ability to maintain T_b in fluctuating and extreme daily and seasonal T_a. It was hypothesised that leopard tortoises maintain their T_b independently of T_a. It was predicted that tortoises would alter their daily and seasonal activity patterns in order to maintain their T_b independently of T_a, while their large size would be expected to result in a degree of thermal inertia, allowing for the maintenance of independent T_b.

Materials and Methods

Field work was conducted on a 5500 ha area of a 26000 ha mixed commercial sheep and game farm in the De Aar District, Nama-Karoo biome, South Africa (31°04'S, 23°41'E). Vegetation here is classified as grassy dwarf shrubland (Palmer and Hoffman, 1997). Average annual rainfall is low (200-400 mm) and the area has its highest rainfall in late summer and autumn. Daily temperatures range from 5 to 39 °C in spring (Sept-Nov) and summer (Dec-Feb), and from -5 to 26 °C in autumn (Mar-May) and winter (June-Aug).

Daily T_a's were recorded in the shade every 20 minutes using Thermocron iButtons^{TM} Temperature-Logger (Dallas Semiconductors, Dallas, Texas, U.S.A) accurate to 0.5 °C. Following Vitt and Sartorious (1999), iButton^{TM}s were placed in various positions in the field to get an indication of the range of daily T_a's available to leopard tortoises, defined as operative temperatures (T_e) (Webb and Shine, 1998). iButton^{TM}s were placed 20 cm above the ground in full sun, full shade and on the north, east, west and south sides of shrubs to represent positions that leopard tortoises use in the field.

Leopard tortoises were located by riding transects on horseback through the study area. In total, four males and six females in summer 2002, and four males and five females in summer 2003 (See Appendix I for their body mass and morphometrics) were fitted with unique-frequency radio transmitters[1]. These were 60g (<1% body mass) and powered by a lithium battery (AA), with a ¾ wavelength stainless steel tracer wire antennae, potted in moulded PVC tubing and attached to the carapace with dental acrylic.

For both telemetered tortoises and for any other tortoises sighted, the date, time, T_a, sex, individual marking, morphometrics, body mass (measured using a spring balance accurate to 500g), type of activity and cloacal T_b were recorded. Cloacal T_b were taken by inserting a fine-gauge thermocouple thermometer (Cole-Palmer Digi-Sense®) 50 mm into the cloaca of adult tortoises.

All temperature recording equipment was calibrated comparatively against an Ever Ready electronic reference thermometer using thermally stabilised liquid baths and a calibration curve generated. The correction values were calculated for the observed temperatures using the generated calibration curve, and the temperature recording equipment corrected (following Nicholas and White, 2001).

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[1] Locally made by Mr Cliff Dearden; 6 Isabelle-Beardmore Drive, Scottsville, 3209.

Upon sighting, leopard tortoises were classified either as inactive or active. Inactive tortoises were those found in a refuge with their head and legs retracted into their shells. Refuges were used overnight or for protection from cold weather or sun. Those tortoises that were active were performing one of seven types of activity (following Els, 1989): i) alert: tortoise was in, or just out of its form, with its head and/or legs out of its shell; ii) basking: a tortoise orientated to expose the maximum shell surface area to the sun, often with limbs and neck extended; iii) walking: tortoise actively walking in the open; v) feeding; vi) drinking; vii) shading: tortoise is in the shade of a bush or other shelter, often with head and limbs extended; and vii) courtship and mating.

Telemetered leopard tortoises were located at least twice daily for a one month period in February 2002, 2003 and July 2002, 2003. In addition, at least two of these tortoises (of each gender) were followed for a continuous 12 h period once every month, and behaviour and cloacal T_b were recorded every 20 minutes.

For each telemetered tortoise in each month of field sampling, ibuttons were attached (using dental acrylic) to the ventral surface of the upper carapace inside the rear leg cavity and programmed to take a temperature recording every 20 minutes. Temperature recordings from these iButton^{TM}s are referred to as "external" T_b recordings.

To measure core T_b, iButton^{TM}s (programmed to record temperature every 20 minutes) were surgically implanted into the body cavities of 2 male and 2 female, and 3 male and 2 female telemetered tortoises for summer and winter 2003, respectively. Tortoises were anaesthetised using Fluorothane gas and iButton^{TM}s surgically implanted by a qualified by a veterinary surgeon.

Results

Minimum and maximum T_a's and cloacal T_b's and respective observed behaviours for each tortoise are shown in Figures 1-2 for summer 2002 and 2003, and winter 2002 and 2003. During the summer, the most common behaviour at low T_b's was basking (Figure 1). As T_a and cloacal T_b increased, tortoises discontinued basking in favour of walking (Figure 1). At cloacal T_b's between 25 and 35 °C, with corresponding minimum T_a's of 25 to 35 °C and maximum T_a's of 30 to 45 °C, the majority of tortoises observed were walking (Figure 1). At T_a's over 25 °C, most tortoises were observed in shaded areas (Figure 1).

The behavioural response of tortoises occurred over a much broader temperature range in winter than in summer, for instance, tortoises were observed as being in shelter (either inactive or alert) at a range of cloacal T_b's from 2 to 25 °C, and at minimum T_a's of 5 to 20 °C (Figure 2). Basking in winter 2002 was observed over a lower absolute range of T_a's (3 to 20 °C) as opposed to winter 2003 (15 to 30 °C) (Figure 2). Observations of tortoises walking were limited, however, all tortoises observed walking had cloacal T_b's over 20 and 25 °C in winter 2002 and 2003, respectively and were walking at T_a's of 15 to 20 °C in winter 2002 and over 25 °C in winter 2003 (Figure 2).

A finer scale analysis of daily behaviour for individual tortoises in summer 2002 and 2003, and winter 2002 and 2003 are shown as Figures 3 to 6. Individual leopard tortoises were alert in the early morning when T_a minimum approximated T_a maximum (Figures 3-6). As minimum and maximum T_e's began to diversify, tortoises left overnight shelters and began periods of basking (Figures 3-6).

In summer, tortoises started to actively bask from 09h00 for a short period, and then became fully active, walking and feeding, before midday (Figures 3 and 4). However, they all sought shade by 12h00, and remained in shade until late afternoon, when activity was

once again resumed (Figures 3 and 4). As such, the summer activity pattern shown by leopard tortoises was bimodal.

During winter, activity patterns of leopard tortoises were limited to short bouts of walking just after midday, with tortoises staying alert in their overnight refuges for most of the morning and basking over the middle of the day and into the early afternoon (Figures 5 and 6). Consequently, during winter leopard tortoises showed a unimodal pattern of activity.

In general, all individually monitored leopard tortoises in summer 2002 and 2003 had cloacal T_b's lower than their external T_b's, and lower than that of T_e over the course of a day (Figures 3 and 4). External T_b's were similar to T_e in the early morning, with temperatures increasing steadily towards midday. Towards late afternoon, T_e and external T_b's decreased, but cloacal T_b decreased at a slower rate resulting in the cloacal T_b's of tortoises being higher than T_e for the early part of the night (Figures 3 and 4).

In winter, the cloacal T_b of individually identified tortoises was generally higher than T_a minimum but as in summer was consistently lower than their external T_b (Figures 5 and 6).

Core T_b's of tortoises were plotted against the daily minimum and maximum T_a's in summer (Figure 7) and winter (Figure 8), where continuous minimum and maximum temperatures were obtained from iButton^{TM}s placed in the coolest and hottest positions, respectively. In summer, core T_b's of all tortoises oscillated on a daily basis between 25 - 35 °C, with a minimum of 17°C and a maximum of 38°C, independently of T_e (Figure 7). In the morning, the tortoises were thermoconformers and their T_b rose at the same incremental level and at the same rate as T_e until T_b reached around 35 to 36 °C (Figure 7). At this temperature the tortoise T_b became independent of T_e, which could rise to over 50 °C. As T_e cooled in the afternoon and overnight, the T_b of the tortoise lagged against T_e, such that the tortoise

became a thermoregulator; although T_a minimum reached below 10 °C, the tortoise T_b never reached below 22 °C (Figure 7).

In winter, core T_b of all tortoises oscillated above T_e's on a daily basis between 8 - 30°C on most days, with a minimum recorded T_b of 3 °C and a maximum of 32 °C (Figure 8).

In winter, leopard tortoises exhibited little thermal inertia when heating, with core T_b following the increase in T_e closely, and continuing to rise above T_e at midday (Figure 8). As in summer, the tortoises exhibited an extended thermal lag when T_e cooled from midday and overnight. The core T_b cooled more slowly than T_e, and never decreased to the minimum T_a (Figure 8).

Discussion

Leopard tortoises followed the classic daily pattern of chelonian thermoregulatory behaviour (Cloudsley-Thompson, 1970; McGinnis and Voight, 1971; Lambert, 1981; Meek, 1984; Loehr, 2002), exhibiting distinctly bimodal activity patterns in summer and unimodal activity patterns in winter. The switch from bimodal activity in summer to unimodal activity under cooler conditions has been widely reported in many chelonians (Lambert, 1981; Meek, 1988; Geffen and Mendelssohn, 1989; Hailey and Coulson, 1996b; Hailey and Loveridge, 1998; Loehr, 2002; Ramsay *et al.*, 2002). Higher summer T_a's allow for a reduction in basking time and an increase in the amount of time available for locomotory activity, however tortoises must seek shade at midday to avoid internal T_b's reaching critical thermal maxima. Swingland and Frazier (1979) found that heat-death, as a result of over-exposure to midday temperatures, was a major cause of death in the Aldabran giant tortoise (*Geochelone gigantea*). Other tortoise species living in hot climates seek shade over midday, and thus exhibit a bimodal activity pattern in summer (Cloudsley-Thompson, 1970; McGinnis and

Voight, 1971; Rose and Judd, 1975; Lambert, 1981; Meek 1984; Meek, 1988). In this study it was not uncommon for midday temperatures to exceed 40 ^{o}C.

In winter, extensive periods of basking are required until T_b's are sufficiently elevated to support activity. As such, activity behaviour is typically unimodal and towards midday, as found in other tortoise species when under cooler conditions (Hailey *et al*., 1984; Meek, 1984; Meek, 1988; Geffen and Mendelssohn, 1989; Hailey and Loveridge, 1998; Loehr, 2002; Ramsay *et al*., 2002). By extension, if T_e's remain low during the day, minimum preferred T_b may not necessarily be reached by tortoises, in which case they remain basking or alert in their refuges for the entire day, as was found in other tortoises at lower T_e (Lambert, 1981; Hailey *et al.,* 1984; Meek, 1988; Loehr, 2002).

Leopard tortoises in summer had lower T_b's than T_e's over the course of a day. T_b's were similar to T_a's in the early morning, while towards late afternoon T_b decreased at a slower rate than T_e resulting in T_b's of tortoises being higher than T_a overnight. In winter, the T_b of tortoises was generally higher than T_e. Similarly, Meek (1984; 1988) found that Hermann's tortoises (*Testudo hermanni*) showed a sharp increase in cloacal T_b's during the morning in spring, summer and autumn. Cloacal T_b's were higher than those of T_e's in the early morning in summer, and for the course of the day in autumn, but lower than T_e's during midday and afternoon in spring and summer (Meek, 1988).

In winter Leopard tortoises were active at lower T_b, and at lower T_e's, than in summer. Leopard tortoises would become alert and start basking and walking when their T_b's were 10 – 15 °C lower in winter than in summer. It has been widely reported that locomotory activity in tortoises is not supported until the tortoise has a reached preferred T_b and that tortoises will remain inactive or continue basking until these T_b have been reached (Branch, 1984; Pulford *et al*., 1984; Meek, 1988; Geffen and Mendelssohn, 1989; Hailey and Coulson, 1996b; Loehr, 2002; Ramsay *et al*., 2002). Other studies have quantified this figure. Perrin

and Campbell (1981) have suggested 28.7 °C as a preferred temperature, while Hailey and Loveridge (1998) reported that leopard tortoises had a preferred T_b of 32.6 °C in a warm climate and 29.1 °C in a cooler high altitude climate. This study found no evidence to support the precision of these claims. Rather, it was found that the tortoises began locomotory activity at a range of temperatures which were dependant on the operative temperature available. This is similar to the conclusions of Meek (1988) who argued that seasonal changes in recorded T_bs of *T. hermanni* are unlikely to indicate seasonal differences in preferred means or ranges, but more probably reflect the T_b's tolerated for activity. Therefore, in a climate where environmental temperatures fluctuate widely, they represent a compromise between physiological optima and ecological reality.

High T_a's have a large and often primary influence on the activity patterns of tortoises (Rose and Judd, 1975). Various tortoise species seek shade above a range of upper limits of T_a, (e.g. Ramsay *et al*., 2002; Hailey and Coulson, 1996a, 1996b; Geffen and Mendelssohn, 1989; Lambert 1981, Meek 1988; and Cloudsley-Thompson, 1971). In this study, it was found that leopard tortoises began to move to shade when T_e approached 30 °C in summer.

Thermal homeostasis has been recorded in tortoise species and is usually achieved through behavioural means (Cloudsley-Thompson, 1971; McGinnis and Voigt, 1971; Cloudsley-Thompson, 1972). However, in this study, leopard tortoises did not maintain or attempt to defend a particular T_b. Instead, leopard tortoises oscillated between a narrow range of T_b using different behaviour in summer and winter. In summer these behaviours included shuttling between shade and sun, while in winter extended periods of basking and passive thermoregulation were utilized to maintain T_b (McMaster and Downs, 2006). In conjunction with behaviour, some physiological mechanisms such as vascular shunting, vasoconstriction and vasodilatation, can be used to manipulate T_b's to a greater extent allowing for further T_b stability (Lambert, 1981). The adoption of these mechanisms maximizes the time the tortoise

is within its preferred T_b, while avoiding exposure to ecologically lethal temperatures (Swingland and Frazier, 1979, Lambert, 1981, Meek, 1984).

An additional adaptation towards T_b maintenance is the thermal lag illustrated in Figures 7 and 8. Although, heating is closely correlated with rising T_e in the morning, this correlation is broken as T_e decreases and the rate of cooling of T_e is significantly greater than the rate of cooling of T_b. Thermal inertia aided by the selection of overnight refuges and tortoises' orientation within the refuges (McMaster and Downs, 2006), and by physiological mechanisms such as vasoconstriction (Lambert, 1981), prevented the tortoises' T_b's from decreasing to the extent of T_e's.

Leopard tortoises in this study showed seasonal differences in their daily activity patterns, the T_e and core T_b at which certain behaviours can be performed, and the ability to cope with large variations in daily and seasonal T_a's. For example, at very low T_a's in winter, leopard tortoises showed tolerance of low T_b by becoming active at lower T_b, and therefore were able to increase the period over which walking and feeding could take place. In addition, through thermoregulatory behaviour, tortoises were able to maintain core T_b's that were independent of extreme T_a's in summer and winter, thus maximizing the time spent at their preferred T_b and allowing for longer daily activity periods.

In conclusion leopard tortoises can maintain their T_b independently of T_a, and alter their daily and seasonal activity patterns to do so. This is in conjunction with a degree of thermal inertia.

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FIGURE LEGENDS

Figure 1: Plots of behavioural activity of individual leopard tortoises at various cloacal temperatures (T_b) against ambient temperature (T_a) maximum and minimum for summer 2002 (top) and summer 2003 (bottom). The types of behavioural activity being performed are basking (open square), walking (solid circle) and shading (open triangles).

Figure 2: Plots of behavioural activity of individual leopard tortoises at various cloacal temperatures (T_b) against ambient temperature (T_a) maximum and minimum for winter 2002 (top) and winter 2003 (bottom). The types of behavioural activity being performed are basking (open square), walking (solid circle) and shading (open triangles).

Figure 3: Plots of ambient operative temperatures (T_e) (shaded) and external body temperatures (T_b) (dotted) of individual leopard tortoises, with focal samples of cloacal (T_b) (solid squares), against time of day during summer 2002. Types of behavioural activity being performed by individual tortoises over the times of the day are indicated next to each focal cloacal T_b; A = alert; B = basking; W = walking; F= feeding; S = shade.

Figure 4: Plots of ambient operative temperatures (T_e) (shaded) and external body temperatures (T_b) (dotted) of individual leopard tortoises, with focal samples of cloacal (T_b) (solid squares), against time of day during summer 2003. Types of

behavioural activity being performed by individual tortoises over the times of the day are indicated next to each focal cloacal T_b; A = alert; B = basking; I = inactive.

Figure 5: Plots of ambient operative temperatures (T_e) (shaded) and external body temperatures (T_b) (dotted) of individual leopard tortoises, with focal samples of cloacal T_b (solid squares), against time of day during winter 2002. Types of behavioural activity being performed by individual tortoises over the times of the day are indicated next to each focal cloacal T_b; A = alert; B = basking; W = walking; F= feeding; S = shade; I = inactive.

Figure 6: Plots of ambient operative temperatures (T_e) (shaded), external body temperatures (T_b) (dotted) and internal core T_b (dot-dash) of individual leopard tortoises, with focal samples of cloacal T_b (solid squares), against time of day during winter 2003. Types of behavioural activity being performed by individual tortoises over the times of the day are indicated next to each focal cloacal T_b; A = alert; B = basking; W = walking; F= feeding; S = shade; I = inactive.

Figure 7: Plots of continuous daily minimum (dot-dash) and maximum (dotted) ambient temperatures (T_a) against internal core body temperatures (T_b) (solid) of individual leopard tortoises, over the summer sampling period in 2003.

Figure 8: Plots of continuous daily minimum (dot-dash) and maximum (dotted) ambient temperatures (T_a) against internal core body temperatures (T_b) (solid) of individual leopard tortoises, over the winter sampling period in 2003.

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Figure 2: Plots of behavioural activity of individual leopard tortoises at various cloacal temperatures (T_b) against ambient temperature (T_a) maximum and minimum for winter 2002 (top) and winter 2003 (bottom). The types of behavioural activity being performed are basking (open square), walking (solid circle) and shading (open triangles)

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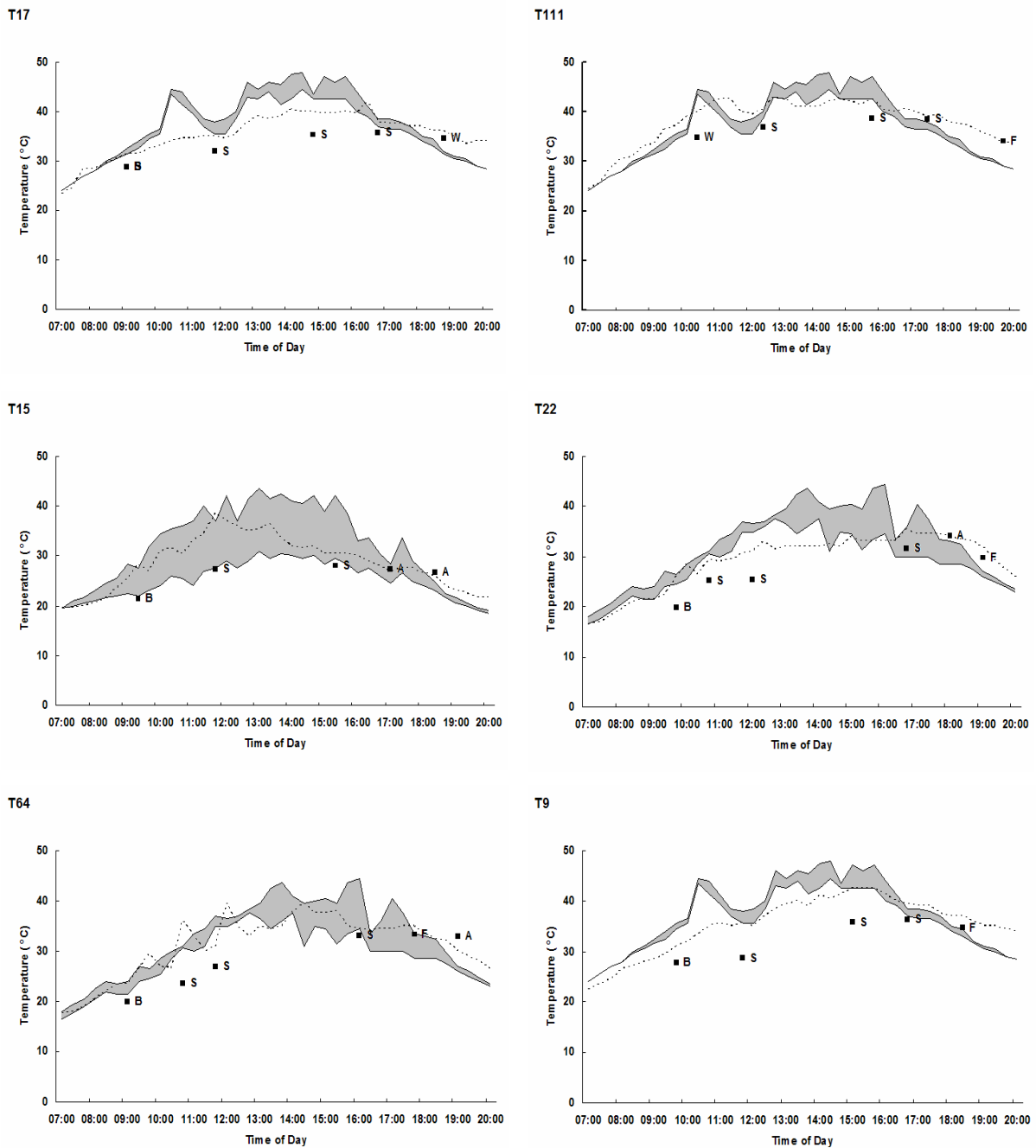
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Figure 8: Plots of continuous daily minimum (dot-dash) and maximum (dotted) ambient temperatures (T_a) and internal core body temperatures (T_b) (solid) of individual leopard tortoises, over the winter sampling period in 2003.

CHAPTER 4

Heating and cooling rates of leopard tortoises (*Geochelone pardalis*) under experimental and natural conditions.

*Formatted for submission to the **Journal of Thermal Biology***

Abstract

This study of heating and cooling rates in the leopard tortoise (*Geochelone pardalis*) compared the effect of body mass on heating and cooling rates across size classes. Ectotherms remain at preferred body temperatures (T_b) for a longer period by reducing the time required to heat up and increasing the time taken to cool down. However, under conditions of high ambient temperatures (T_a), it is advantageous for an animal to retard the rate of heat uptake to avoid overheating; and under cool conditions, it is advantageous to retard heat loss and maintain preferred T_b above that of T_a. Seasonal heating and cooling rates of leopard tortoises were investigated in the laboratory and in the wild to determine their ability to maximise their activity periods, and to determine the effect of behaviour in wild tortoises on the rate of heat flux. Compariso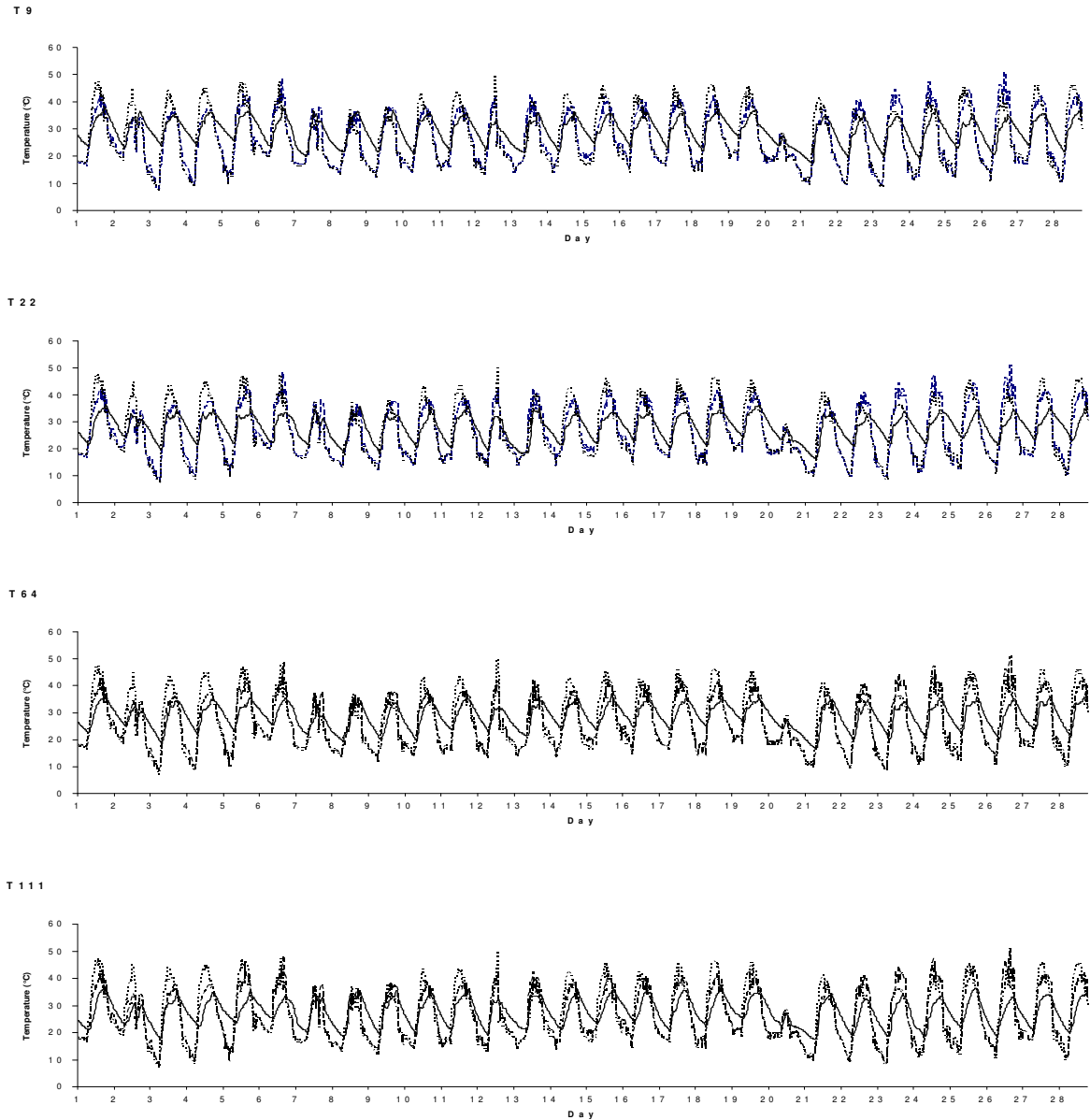ns of heating and cooling rates were made between live tortoises and tortoise models. "Experimental" heating and cooling rates were measured in nine adult, three juvenile and seven hatchling leopard tortoises between 5 and 30 °C using cloacal temperatures. Heating and cooling rates of free-ranging leopard tortoises in the Nama-Karoo were collected using Thermocron iButton^{TM}s. Cooling rates were faster than heating rates in both summer and winter for all age classes and decreased with increasing body mass. Tortoise models had considerably faster heating and cooling rates than live tortoises in summer and winter. Leopard tortoises had higher heating and cooling rates in

winter than in summer. Free-ranging leopard tortoises used physiology and behaviour to further retard heat gain to minimize the risk of overheating in summer and retard heat loss to maximize activity time.

Keywords: heating rate, cooling rate, body mass, thermoregulation, behaviour, optimal temperature, Leopard tortoise, *Geochelone pardalis*.

1. Introduction

Reptiles are ectothermic, and use radiant heat, behavioural and physiological mechanisms to regulate body temperature (T_b) (reviews by Avery, 1982; and Cloudsley-Thompson, 1991). Although ectothermy requires a much lower energy investment than endothermy, behavioural thermoregulation of ectotherms is time-consuming, and may take up a large part of an ectotherm's time budget that could otherwise be dedicated to foraging and social activities (Gvozdik, 2002). The rate at which a reptile heats and cools therefore influences time spent on thermoregulation. Therefore by reducing flux rates, reptiles can lengthen their activity times within a preferred T_b range (Johnson et al., 1978).

Reptiles have faster heating rates than cooling rates in order to remain at preferred temperatures for a longer time period (White, 1973; Voigt and Johnson, 1977; Ellis and Ross, 1978; Johnson et al., 1978; Bartholomew, 1982; Hailey and Davies, 1987; Els et al., 1988). However, in areas of high temperatures, it would be advantageous for an animal to retard the rate of heat uptake and therefore avoid reaching critical thermal maxima (Craig, 1973; Swingland and Frazier, 1979; Perrin and Campbell, 1981; Meek 1984, 1988; Hailey and Coulson, 1996a,b).

Flux rates are largely dependant upon body mass (Grigg et al., 1979; Seebacher et al., 1999). Large reptiles need to bask for very long periods to achieve high T_b, and rely upon long, single basking periods rather than the shuttling heliothermy of smaller reptiles (Pearson et al., 2003). However, a larger body mass increases stability and is advantageous in the subsequent maintenance of thermal constancy once preferred temperatures have been reached (McNab and Auffenburg, 1976; Ayers and Shine, 1997; Seebacher et al., 1999).

Studies of heating and cooling rates in chelonians are of particular interest as some species attain large sizes as adults, but are slow growing, resulting in a large range in body mass from hatchlings to adults. This allows for comparative studies on the effect of mass on flux rates.

This is especially true in the case of the leopard tortoise where adults reach up to 25 kg and may take 50 years to attain that size (Boycott and Bourquin, 2000). Therefore, heating and cooling rates of leopard tortoises of different mass classes were investigated in the laboratory and in the wild. It was hypothesised that leopard tortoises differ in their rates of heating and cooling and that this is independent of mass. Model tortoises were used as controls for laboratory experiments and it was expected that the flux rates of these models would not differ from live animals.

2. Materials and Methods

2.1 Maintenance of captive tortoises

Twelve captive leopard tortoises were loaned for the duration of 2002-2003 from private collectors. The twelve tortoises, of various body masses, comprised six adult females, three adult males, and three juveniles (see Appendix II for morphometric details). These tortoises had been confiscated from illegal pet traders and could not be released back in to the wild.

All had been in captivity for more than a year. In addition to these tortoises, seven hatchling tortoises hatched from nests laid by the adult captive tortoises were used in the study (see Appendix III for morphometric details).

Tortoises were housed in outdoor enclosures measuring 20 m by 12 m at the Animal House of the School of Biological Conservation, University of KwaZulu-Natal (UKZN). The enclosures were predominantly covered with kikuyu grass, but had patches of veld grass and weeds in them.

In addition, tortoises were given supplementary food three times a week consisting of a variety of forbs, succulents and vegetables. Water was always available in shallow trays set into the ground. Tortoises were able to seek artificial shade provided by open-sided, roofed shelters present in the enclosures.

2.2 Experimentation: Cooling and Heating Rates

Heating and cooling rate experiments were conducted at the School of Biological Conservation, (UKZN). Two walk-in Controlled Environment (CE) rooms were used. The first set at 5 °C and the second at 30 °C. Each room was digitally monitored and its temperature maintained to an accuracy of 0.01 °C. Any disturbance (door opening, entrance of investigator) was monitored and ameliorated by computer within 60 seconds. Captive leopard tortoises were collected from their outdoor enclosures and were placed in one of the CE rooms at 20h00 for 12 hours in order to equilibrate their T_b. Tortoises in heating rate experiments were kept overnight the CE room at 5°C and placed into the second CE room at 30 °C immediately prior to the experiment. Tortoises undergoing cooling rate experiments were treated to exactly the opposite procedure. Experiments began at 08h00. Only one tortoise was used in a single experimental run and that tortoise was housed in a wire-mesh cage with a rubber substrate placed on a tiled bench. An initial cloacal T_b was taken for each

tortoise, using a fine-gauge thermocouple thermometer (Cole-Palmer Digi-Sense®), inserted 5.0 cm into adults, 2.0 cm into juveniles and 0.5 cm into hatchlings. Thereafter, cloacal T_bs were measured every 20 minutes. Tortoises were weighed prior to each trial using a spring balance to the nearest 0.1 kg for adults and a desktop digital scale accurate to 0.01 g for juveniles and hatchlings. The trial experiment ran until a tortoise's cloacal T_b had stabilized against the CE room temperature. Tortoises were then returned to their outdoor enclosures and were never experimented on consecutive days. Heating and cooling rates of each captive tortoise was measured in summer and winter respectively to establish seasonal differences.

All temperature recording equipment was calibrated against an Ever Ready electronic reference thermometer using the comparison method in thermally stabilised liquid baths Correction values were calculated for the observed temperatures using the generated calibration curve, and the temperature recording equipment corrected (following Nicholas and White, 2001).

Controls: Tortoise Models

Aluminum foil has been shown to be thermally passive and have the same thermal properties as that of live animal tissue (Walsberg and Weathers, 1986). This material was used in order to make an appropriate model to simulate a leopard tortoise, with the addition of a carapace, to enhance the model's biological significance (Hailey and Loveridge, 1998).

Therefore, two tortoise carapaces complete with scutes were retrieved from the field. The carapaces, 10 and 35 cm straight length respectively, were stuffed solid with aluminum foil. A Thermocron iButton^{TM} Temperature-Logger (Dallas Semiconductor, Dallas, Texas, USA), accurate to 0.5 °C, was placed in the center of each shell to estimate core T_b. These "model" tortoises were subjected to the same experimental procedure as live tortoises and served as controls for heating and cooling rate trials.

Cooling and Heating Rates of Free-ranging Tortoises

Fieldwork was conducted on a 5500 ha area of a 26000 ha mixed commercial sheep and game farm in the De Aar District, Nama-Karoo biome, South Africa (31°04'S, 23°41'E). The vegetation is classified as grassy dwarf shrubland (Palmer and Hoffman 1997). Average annual rainfall is low (200-400 mm) and the area has its highest rainfall in late summer and autumn. Daily temperatures range from means of 5 to 39 °C in spring (September-November) and summer (December-February), and from means of -5 to 26 °C in autumn (March-May) and winter (June-August) (Kruger, 2004).

Leopard tortoises were initially located by riding transects on horseback through the study area. In total, nine adults (in summer and winter 2003) (See Appendix I for morphometric details) were fitted with unique-frequency radio transmitters (mass 60g, <1% body mass, each powered by a lithium battery (AA), with a ¾ wavelength stainless steel tracer wire antennae, potted in moulded PVC tubing and attached to the carapace with dental acrylic). These transmitters allowed the nine marked individuals to be located for their cloacal T_b's measurements. These cloacal T_b's were measured using the protocol for the laboratory tortoises.

In summer 2003, four of the telemetered adults were surgically implanted with iButton^{TM}s programmed to take a temperature recording every 20 minutes. The following winter, five of the telemetered adults were implanted. Tortoises were anaesthetised with Fluothane gas and surgery performed by a qualified veterinary surgeon. Temperature recordings from these iButton^{TM}s are referred to as "core" T_b recordings. T_a's were simultaneously recorded using iButton^{TM}s.

To investigate heating and cooling rates of free-ranging tortoises in the field, a post-hoc method was used. Daily plots of T_a maximum and minimum were examined to find

representative days where the range of T_a most closely mimicked the range of T_a used in the flux rate trials in the laboratory i.e. 5 to 30 ºC. Within those parameters, the heating and cooling rate of core T_b was investigated.

2.5 Calculations and statistics

Representative heating and cooling rates of a hatchling during winter are shown in Figure 1, to illustrate how heating and cooling rates of individual leopard tortoises, both under natural and experimental conditions, were calculated (following Craig, 1973). The change in temperature was calculated as the difference between body and ambient temperature at a given time. The log change in temperature ($\log_{10}\Delta T$) was plotted against time (Figure 1). Inflection points were estimated from the slope of the curve and to avoid subjectivity and possible bias, inflection points were chosen where ΔT is greater than 2 °C.

All descriptive and regression statistical tests were calculated using STATISTICA software (Statsoft, USA).

3. Results

3.1 Experiment: Cooling and Heating Rates

The time taken for the cloacal temperatures of tortoises in heating and cooling experiments to stabilize varied from 1.3 to 2.3 hours for hatchlings, 2.0 to 4.3 hours for juveniles and 4.6 to 7.3 hours in adults.

Cloacal cooling and heating rates ($º\text{Cmin}^{-1}$) of leopard tortoises in summer and winter for hatchlings, juveniles and adults, are shown in Table 1. Hatchlings had both the highest average cooling rate and highest average heating rate, while adults had the lowest average cooling and heating rates in summer and winter respectively (Table 1).

A heating to cooling ratio (H/C ratio) of 1 would be expected if heat transfer were entirely passive, with values above or below 1 indicating physiological control. In winter, the H/C ratios were 0.91 for hatchlings, 0.55 for juveniles and 0.71 for adults, indicating that tortoises of all age classes were cooling slightly faster than they heated. Similarly in summer, tortoises of all size classes also cooled faster than they heated with H/C ratios of 0.83, 0.88 and 0.54 for hatchlings, juveniles and adults, respectively.

To investigate the effects of body mass on the rates of cloacal cooling (Figure 2) and heating (Figure 3) in leopard tortoises in the laboratory, the rates of temperature change (calculated from the slope of the cooling or heating curve prior to inflection) were log transformed and regressed against the log of body mass (in grams) of the tortoises, for each season.

For both cloacal cooling and heating rates, in summer and winter, the rate of temperature change decreased with increasing body mass (Figures 2 and 3). This would be expected from surface area to volume ratio theory, however, the regression line gave a statistically significant goodness of fit in summer (cooling: $y = -0.177x - 0.404$, $r^2 = 0.92$, $n = 18$; heating: $y = -0.268x - 0.275$, $r^2 = 0.89$, $n = 9$) and winter (cooling: $y = -0.220x - 0.251$, $r^2 = 0.85$, $n = 4$; heating: $y = -0.244x - 0.320$, $r^2 = 0.96$, $n = 17$). The temperature of the hatchlings changed at the highest rate, while adult temperature showed the slowest rate of change (Figures 2 and 3).

These results indicate that the rate at which leopard tortoises heated and cooled as reflected by cloacal temperatures was strongly affected by body mass and that the larger adult tortoises heated and cooled much slower than juveniles and hatchlings.

Cooling rates of tortoises were faster in winter ($y = -0.062x + 0.328$, $r^2 = 0.84$) than in summer ($y = -0.049x + 0.274$, $r^2 = 0.87$), but not significantly so (ANOVA, $F_{(14,1)} = 0.161$; $p > 0.05$) (Figure 4a). Similarly, the heating rates of tortoises were faster in winter ($y = -0.062x$

+ 0.290, r^2 = 0.99), but were not significantly different from heating rates in summer (y = - 0.056x + 0.260, r^2 = 0.95) (ANOVA, $F_{(8,1)}$ = 0.296; $p > 0.05$) (Figure 4b). In addition, the cooling rates of tortoises were not significantly faster than the heating rates of tortoises in winter (ANOVA, $F_{(14,8)}$ = 0.194, $p > 0.05$) and summer (ANOVA, $F_{(14,8)}$ = 0.062, $p > 0.05$) (Figure 4).

The large (adult) tortoise model cooled at 0.139 °C min^{-1} and heated at 0.142 °C min^{-1} which results in an H/C ratio of 1.02. Similarly, the small (juvenile) tortoise model had an H/C ratio of 1.04, with a heating rate of 0.308 °C min^{-1} and cooling rate of 0.296 °C min^{-1}. These H/C ratios both indicate passive heat transfer as would be expected from an inert object. The heating rate of the large (adult) model tortoise was 2.9 to 3.6 times faster than in live adult tortoises, while the small (juvenile) model tortoise heated 2.4 to 3.0 times faster in live juvenile tortoises, in summer and winter respectively (Table 1). Similarly, the large tortoise model cooled 1.8 to 2.0 times faster than in live adult tortoises and the cooling rate of the small tortoise model was 1.6 to 2.1 times faster, in summer and winter respectively (Table 1).

3.2. Heating and Cooling Rates of Free-ranging Tortoises

Heating and cooling rates were consistently similar between adult individuals within a season, despite slight differences in body mass (Table 2; Appendix I). Cooling rates (mean ± S.E.; 0.015 ± 0.001 and 0.021 ± 0.0001 °Cmin^{-1} in summer and winter, respectively) and heating rates (mean ± S.E.; 0.044 ± 0.001 and 0.054 ± 0.0001 °Cmin^{-1} in summer and winter, respectively) were slower in summer than in winter for all individual leopard tortoises.

Free-ranging tortoises heated faster than they cooled in both summer and winter, with cooling to heating ratios (C/H ratio) of 0.34 in summer and 0.39 in winter (derived from

Table 2). Free-ranging tortoises were therefore heating three times faster than they were cooling.

Adjustments were made for differences in adult body mass by dividing individual cooling and heating rates by that individual's body mass ($^{\circ}$Cmin^{-1}gBM^{-1}). Differences in cooling and heating rates between the seasons became negligible when body mass was taken into account, however, the heating rates in both seasons were still faster than the cooling rates (Table 2).

Changes in core body temperature over a 24h period in summer and winter are shown for each individual tortoise in Figures 5 and 6, respectively. All individual tortoises showed a similar relationship between ambient temperature and heating or cooling rates in summer and winter. In summer, Leopard tortoises cooled down at a much slower rate compared to ambient temperature, and heated up at a slightly slower rate and from a lower initial body temperature that the corresponding ambient temperature in time (Figure 5). In winter, Leopard tortoises cooled down at a slower rate than the ambient temperature, and than they had in summer, but heated up at a similar rate to the ambient, although from a lower initial body temperature and with a slight thermal lag (Figure 6). Tortoises continued to heat up after ambient temperatures were at a maximum, and therefore attained body temperatures above that of the daily ambient temperature (Figure 6).

These results indicated that free-ranging adult Leopard tortoises were able to regulate both their cooling and heating rates independently of ambient temperature.

4. Discussion

Perrin and Campbell (1981) measured the heating and cooling rates of three species of South African tortoises, including five leopard tortoises ranging in mass from 0.69 Kg to 11.11 Kg.

In agreement with this study, they obtained higher heating and cooling rates in smaller compared to larger individuals, however the heating and cooling rates recorded for the leopard tortoises were considerably lower than those found for leopard tortoises in this study, within the same mass class. Conversely, Craig (1973) reported heating and cooling rates of around 0.1 ºCmin^{-1} for an 810 g angulate tortoise (*Chersina angulata*), while Hailey and Coulson (1996b) estimated a heating rate of 0.3 ºCmin^{-1} for Hingeback tortoises (*Kinixys spekii*) (mean mass 617g) in an outdoor enclosure, both of which were faster rates than those measured in this study for juvenile leopard tortoises of similar mass.

Body mass has been widely reported to be the primary factor influencing the cooling and heating rates of ectotherms, with cooling and heating rates decreasing with increasing body mass (Grigg, et al., 1979; Avery, 1982; Stevenson, 1985; Seebacher et al., 1999; Pearson et al., 2003). In many ways, this is to be expected as a result of surface area to volume ratio theory. The importance of surface area-to-volume ratios was emphasized by Reiber et al. (1999) and Pearson et al. (2003) who both stated that the ratio of surface area-to-volume is critical in determining and regulating the rate of heat exchange, and the thermal profile of an ectotherm. Similarly, Seebacher et al. (1999) and Grigg et al. (1979) agreed that absolute body size determines the rate of heating and cooling in an ectotherm, with Seebacher et al. (1999) going on to say that it is the combination of body mass, surface area-to-volume ratios and the resulting thermal inertia that drives the thermal relationships of ectotherms, especially in the larger species.

However, after scaling for different size classes, the results illustrate that adult leopard tortoises still cool and heat at slower rates than hatchlings. With no behavioural or physiological adaptations, passive heating and cooling occurs where heating rate would equal the cooling rate (Smith et al., 1981). The results of this study clearly show that this is not the case. Although studies have reported differing heating and cooling rates being the result of

physiological control (Perrin and Campbell, 1981; Hailey and Coulson, 1996a; Spotila et al., 1991; Seebacher et al., 1999), the most important of which is circulation (Bartholomew and Tucker, 1963), there is no general agreement on the extent that physiological control influences these differences over behavioural and postural adjustments (Stevenson, 1985; Webb and Shine, 1998; Seebacher et al., 1999; Whitaker and Shine, 2002).

Early attempts at isolating the physiological mechanisms used by ectotherms for temperature control and maintenance focused on circulation and metabolism (Bartholomew and Tucker, 1963), with the authors concluding that the net effect of such control was to accelerate heating rates and slow down cooling rates. Cloudsley-Thompson (1970) did not address the problem of heating rates but did suggest that ectotherms may increase cooling rates by evaporative cooling through increased salivation. Perrin and Campbell (1981) proposed that leopard tortoises control conductance through physiological control, however, importantly, they suggest that physiological control of temperature may be secondary to behavioural and postural adjustments. This conclusion is supported by Hailey and Coulson (1996a), who marginalize physiological mechanisms in favour of changes in daily activity as the major thermoregulatory mechanism.

In laboratory conditions, there is no opportunity for tortoises to use thermoregulatory mechanisms such as behaviour, the selection of microhabitat sites and activity times to influence the rate of heating and cooling. Perrin and Campbell (1981) reported that leopard tortoises show little physiological control over heat flux, especially as body size increases. It may be that larger reptiles, particularly those enclosed in an insulating carapace, exhibit less physiological control of heat flux, instead using behaviour, the advantage of low surface to volume ratios and thermal inertia to adjust heating and cooling rates. Smaller tortoises are more vulnerable to heat flux due to their size and may use physiological control of heat flux to a larger extent, as was found by Perrin and Campbell (1981) in smaller tortoises.

Models can be used to estimate body temperature changes under different thermal environments (Beaupre, 1995), and in this study served to identify whether the cooling and heating rates of live tortoises under experimental conditions were as a result of passive cooling and heating or whether they were actively regulating their change in temperature. The heating and cooling rates of adult and juvenile models were considerably faster than for live tortoises of similar size in all seasons. This could indicate that live tortoises actively retard their heat gain or loss compared to an inert body of similar dimensions. This could be achieved through behavioural, physiological or physical means, such as thermal inertia, which are not available to a model, or due to the medium that made up the model and its properties of thermal conductance (Crawford et al., 1983; Zimmerman et al., 1994; Hailey and Coulson, 1996a,b; Hailey and Loveridge, 1998). Although the models used in the study heated slightly faster than they cooled with heating to cooling ratios of just over 1, the ratio signified that they were thermally appropriate approximations of an inert body. Dead tortoises would serve as more accurate models than man-made simulations, however in this study the use of dead tortoises was not possible for ethical reasons. Dead tortoises were used as models by Perrin and Campbell (1981) who measured the heating and cooling rates of five leopard tortoises under laboratory conditions, killed the tortoises and re-measured the rates of temperature change on the same individual. Perrin and Campbell (1981) found that leopard tortoises heated and cooled at different rates when dead than alive, indicating their ability to retard or augment heat efflux.

Differences in the rates of cooling and heating is a characteristic of most reptiles and has been widely reported in both experimental and free-ranging reptile studies (Voigt and Johnson, 1977; Ellis and Ross, 1978; Perrin and Campbell, 1981; Smith et al., 1981; Els et al., 1988; Pages et al., 1991; Hailey and Coulson, 1996a,b). Leopard tortoises in this study had higher cooling rates than heating rates and heating and cooling rates were higher in

winter than in summer. A higher heating rate in winter would allow tortoises to heat rapidly in the mornings and thus maximise the time spent at preferred body temperatures. The slower heating rate in summer would retard the rate of heat uptake and therefore extend the time that tortoises were in their preferred body temperature before reaching critical thermal maxima in hot conditions.

In agreement with these findings, Pages et al. (1991) and Craig (1973) found that their study species cooled faster than they heated. Craig (1973) concluded that the increased rate of cooling was an active mechanism to prevent overheating in slow, large herbivorous tortoises. This suggests that leopard tortoises are adjusting their rates of heating and cooling to prevent overheating in summer while maximising their heat gain in winter.

In contrast to tortoises in laboratory experiments, free-ranging adult leopard tortoises in this study heated three times faster than they cooled in both summer and winter. This difference is probably due to free-ranging tortoises being able to use behavioural mechanisms to alter their rate of heat flux. The use of retreat sites (Webb and Shine, 1998; Kearney, 2002; McMaster and Downs, 2006), body posture (Kearney, 2001; Bittner et al., 2002), activity periods (Hailey and Davies, 1987; Meek, 1984; Meek 1988; Hailey and Coulson, 1996a,b) and microhabitat selection (Belliure and Carascal, 2002; Gvozdik, 2002; Pearson et al., 2003) would each contribute to the behavioural control of heating and cooling rates and the resulting changes in body temperature.

Many reptiles, especially those from temperate regions, have been found to actively enhance heat influx and typically have a faster heating rate than cooling rate (Voigt and Johnson, 1977; Ellis and Ross, 1978; Perrin and Campbell, 1981; Smith et al., 1981; Hailey and Davies, 1987; Els et al., 1988). By reducing the time required to warm, and by increasing the cooling time, these reptiles can lengthen their activity times at a preferred body temperature (Johnson et al., 1978). However, studies conducted in hotter environments or

under warmer conditions, have found that heat uptake is actively retarded, and cooling rates are faster than heating rates (Craig, 1973; Pages et al., 1991; Hailey and Coulson, 1996a, b). Frequent exposure to high temperatures could result in heat stress and the ability to retard heat uptake and offload heat at a faster cooling rate, will optimize the activity time at a preferred body temperature before overheating (Craig, 1973; Swingland and Frazier, 1979; Perrin and Campbell, 1981; Meek, 1988; Hailey and Davies, 1987; Hailey and Coulson, 1996a, b).

Although there is little doubt that behavioural methods are used in wild leopard tortoises to thermoregulate (McMaster 2002; McMaster, 2006), it appears from these results that the heating and cooling rates of leopard tortoises were influenced by a combination of physiological and behavioural mechanisms and were further dependant on body mass, surface area to volume ratios and the resulting thermal inertia. By adjusting heating and cooling rates, tortoises were able to minimize the risk of overheating and retard heat loss, and so maximize activity time at preferred body temperature.

5. Acknowledgements

6. References

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Table 1: Cloacal cooling and heating rates (change in temperature) (Mean ± S.D.) expressed per minute (°C min^{-1}) in summer and winter for hatchling, juvenile and adult captive leopard tortoises.

Season	Trial Rates	Hatchlings		Juveniles		Adults	
		Mean ± S.D.	*n*	Mean ± S.D.	*n*	Mean ± S.D.	*n*
Summer	Cooling rate (°C min^{-1})	0.206 ± 0.0316	6	0.143 ± 0.012	3	0.074 ± 0.005	9
Summer	Heating rate (°C min^{-1})	0.170 ± 0.011	3	0.126 ± 0.011	3	0.039 ± 0.006	2
Winter	Cooling rate (°C min^{-1})	0.217 ± 0.039	2	0.187 ± 0.014	3	0.069 ± 0.013	9
Winter	Heating rate (°C min^{-1})	0.197 ± 0.018	5	0.102 ± 0.023	3	0.049 ± 0.007	9

Table 2: Cooling and heating rates (change in temperature) expressed per minute ($^{\circ}min^{-1}$) and per gram body mass ($^{\circ}Cmin^{-1}gBM^{-1}$), in summer and winter for adult free-ranging Leopard Tortoises.

Season	Individual Tortoises	Cooling rate ($^{\circ}C\ min^{-1}$)	Cooling rate ($^{\circ}Cmin^{-1}gBM^{-1}$)	Heating rate ($^{\circ}C\ min^{-1}$)	Heating rate ($^{\circ}Cmin^{-1}gBM^{-1}$)
Summer	T9	0.013	0.001	0.027	0.002
Summer	T22	0.017	0.001	0.064	0.005
Summer	T64	0.017	0.001	0.053	0.004
Summer	T111	0.015	0.002	0.034	0.004
Winter	T9	0.020	0.002	0.059	0.005
Winter	T17	0.021	0.001	0.050	0.004
Winter	T22	0.020	0.002	0.059	0.005
Winter	T64	0.020	0.001	0.048	0.003
Winter	T111	0.022	0.002	0.052	0.006

FIGURE LEGENDS

Figure 1: Inflection points of heating (squares) and cooling (triangles) rate for winter for tortoise Hatchling 3. Closed markers denote time prior to inflection, open markers denote time after inflection. The change in temperature (ΔT) was given as an absolute value prior to logarithmic transformation. Regression lines "A" and "a" represent heating prior to (dotted line, $y = -0.1595x + 1.0508$, $r^2 = 0.9361$) and after (dotted line, $y = -0.1952x + 1.3268$, $r^2 = 0.9911$) the inflection point at 160 minutes. Regression lines "B" and "b" represent cooling prior to (solid line, $y = -0.1232x + 0.8648$, $r^2 = 0.9985$) and after (solid line, $y = -0.0374x + 0.0862$, $r^2 = 0.9589$) the inflection point at 150 minutes. Rates of heating or cooling were calculated as the change in temperature over time at slope "A" and "B", respectively.

Figure 2: Log cloacal cooling rates (log °C min^{-1}) over log body mass (log BM g) of all captive leopard tortoises in a. summer ($y = -0.186x - 0.525$, $r^2 = 0.89$) and b. winter ($y = -0.276x - 1.391$, $r^2 = 0.85$).

Figure 3: Log cloacal heating rates (log °C min^{-1}) over log body mass (log BM g) of all captive leopard tortoises in a. summer ($y = -0.259x - 0.483$, $r^2 = 0.87$) and b. winter ($y = -0.236x - 0.50$, $r^2 = 0.71$).

Figure 4: Regression of a. log cooling rate (log °C min^{-1}) for summer ($y = -0.035x + 0.195$, $r^2 = 0.88$) and winter ($y = -0.665x + 0.329$, $r^2 = 0.91$), and b. log heating rate for summer ($y = -0.033x + 0.161$, $r^2 = 0.92$) and winter ($y = -0.042x + 0.196$, $r^2 = 0.87$) against log body mass (log BM g) for captive leopard tortoises.

Figure 5: Change in temperature (ºC) over time comparing ambient temperature to the internal core body temperature of free-ranging adult leopard tortoises a. T9, b. T22 c. T64 and d. T111 in summer.

Figure 6: Change in temperature (ºC) over time comparing ambient temperature to the internal core body temperature of free-ranging adult leopard tortoises a. T9, b. T22 c. T64, d. T111 and e. T17 in winter.

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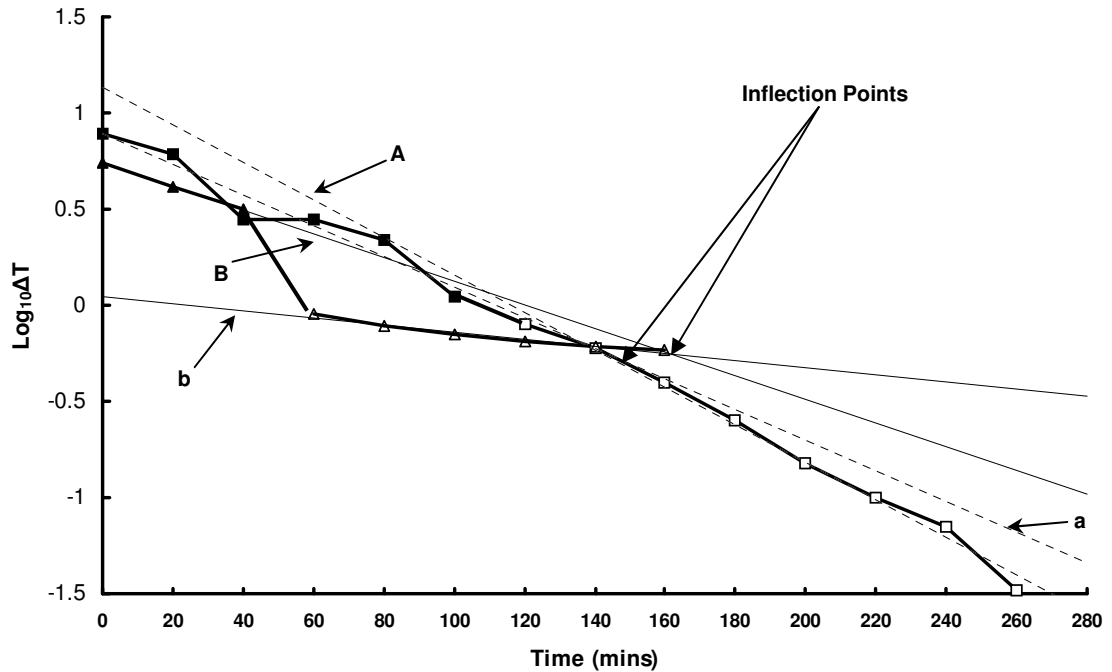
CHAPTER 5

Plasticity in the Metabolic Rate of Juvenile and Hatchling Leopard Tortoises

(*Geochelone pardalis*)

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ABSTRACT

Typically ectotherms show exponential increased oxygen consumption with increasing ambient temperature (T_a). However, seasonal cycles, fluctuating environmental temperatures and aspects of life history influence the oxygen consumption (VO_2) of reptiles. Furthermore, the result of these effects on metabolism in chelonians is poorly understood, particularly in a species that has a broad range in body mass. Oxygen consumption was measured in juvenile and hatchling Leopard tortoises (*Geochelone pardalis*) and it was hypothesised that metabolic rate would differ seasonally and predicted that oxygen consumption would increase with increasing T_a. A lower specific metabolic rate was predicted for juvenile tortoises compared to hatchlings. VO_2 was measured in summer and winter in the laboratory at T_a's of 10, 15, 20, 25, and 30°C using a flow through respirometry system in three juvenile and six hatchling leopard tortoises. Leopard tortoises irrespective of mass showed no distinct daily cycles in VO_2 in either summer or winter, although daily VO_2 was more variable at higher T_a. There were significant differences in VO_2 at different experimental temperatures with some seasonal variation. VO_2 was lower in winter at lower T_a's and increased at higher T_a in both seasons. The relationship between VO_2 and body mass was variable with temperature and between seasons. It was evident that metabolism can vary in response to temperature, season, and photoperiod. This variation is most likely a result of a combination

of these factors at any given time and further exploration of the factors affecting metabolism over a range of body sizes in Leopard tortoises is required to understand chelonian metabolism in an ecological context.

Introduction

Measurement of reptile metabolism in the laboratory has been used in conjunction with data on activity patterns and temperature profiles to estimate the resting metabolic rates of various reptiles in the field (reviewed by Bennett and Dawson 1976).

Bennett and Dawson (1976) defined general equations for the mass-specific metabolic rates of lizards, snakes, and one for turtles. However, not all species conform to these generalized equations, at all temperatures and under all conditions (Waldeschmidt et al. 1987). It is generally assumed that metabolic rate increases with increasing temperature, however, several species of reptiles are characterised by rate-temperature curves that have distinct plateaus where metabolic rate is independent of temperature (for e.g. Waldeschmidt et al. 1987; Al-Sadoon 1991; Kirsch and Vivien-Roels 1994; Litzgus and Hopkins 2003).

Resting metabolic rate is usually measured in the laboratory in reptiles that have been fasted for at least 48 h (longer in snakes and chelonians) and is used for comparison against the basal metabolic rate of other animal groups. However, these rates underestimate the energetic costs and maintenance metabolism of animals in the field, because free-ranging animals would not typically be in a fasted condition (Waldeschmidt et al. 1987). Indeed, the metabolic rates of reptiles that have food in their stomachs are usually elevated to varying degrees, known as specific dynamic action (SDA) (Cragg 1978; Hailey and Davies 1987; Waldeschmidt et al. 1987; Hailey 1998; Secor and Diamond 2000) and, recognising the importance of SDA, several studies have attempted to account for it by measuring the metabolic rates of animals straight out of the field (e,g. Karasov and Anderson 1984) or from

animals in a non-fasted condition (Nagy et al. 1984). As such, tortoises in this study were also measured in a non-fasted condition.

Daily variation in metabolic rate has been widely reported (reviewed by Bennett and Dawson 1976; Waldeschmidt et al. 1987). Maximum metabolic rates tend to occur just prior to or during the time that peak activity of free-ranging animals would occur, being either unimodal or bimodal in nature (Songdahl and Hutchison 1972; Gratz and Hutchison 1977; Cragg 1978; Heusner and Jameson 1981).

Seasonal cycles and changing environmental temperatures play a vital role in influencing the VO_2 of reptiles (Bartholomew and Tucker 1964; reviewed by Bennett and Dawson 1976; Kirsch and Vivien-Roels 1984; Cartland and Grimmond 1994, Hailey and Loveridge 1997). The reduction in metabolic rates during long periods of adverse weather (whether for extended periods of drought or cold) is widely documented (reviewed by Waldeschmidt et al. 1987) and thought to be an adaptation reducing the energetic cost of hibernation (Hailey and Loveridge 1997) or a mechanism that facilitates the storage of lipids prior to hibernation (Heusner and Jameson 1981).

The measurement of metabolism in chelonians is limited to only a few studies. The effect of temperature and seasonal variation on metabolic rate was reported in both *K. spekii* and *T. hermanni* with low temperatures and changing diel cycles causing a reduction in metabolic rate prior to winter hibernation (Kuchling 1981, Kirsch and Vivien-Roels 1984; Hailey and Loveridge 1997). In addition, the specific dynamic action was tested under different feeding regimes in *K. spekii*, and found to be elevated after feeding, especially when fed on infrequent single meals (Hailey 1998).

Southern African ectotherms survive wide fluctuations in temperature, food and water availability (Boycott and Bourquin 2000; Kruger, 2004). Leopard tortoises (*Geochelone pardalis*) are distributed over southern African in a variety of habitats, including the semi-

arid Nama-Karoo, where fluctuations in temperature and resources are further aggravated through El Nino Southern Oscillation (ENSO) events (Kruger, 2004). The effect of these fluctuations on the metabolic mechanisms and thermoregulation of reptiles are poorly studied, especially in southern Africa, with most focus being on the study of metabolic rates of lizards (reviews by Bennett and Dawson 1976; Waldeschmidt et al. 1987).

Therefore, changes in the metabolic rate of leopard tortoises with changes in T_a, time of day and season were investigated. Oxygen consumption was measured in juvenile and hatchling Leopard tortoises (*Geochelone pardalis*) with the hypothesis that metabolic rate would differ seasonally. It was predicted that oxygen consumption would increase with increasing T_a. In addition, slow growth rates in tortoises result in a broad range in body mass between age classes and as such a lower specific metabolic rate was predicted for juvenile tortoises compared to hatchlings.

Methods

Maintenance of captive tortoises

Three juvenile (mass: 0.4, 0.5 and 0.9 kg, respectively), and six hatchling (mass ranging from 0.03 to 0.06 kg) leopard tortoises were loaned to us for the purposes of this study (see Appendix II for morphometric details). These tortoises had been confiscated from illegal pet traders and as such could not be released back in to the wild. All juveniles had been in captivity for more than a year and the hatchlings were born in captivity. For practical purposes, this study had to focus on the smaller size classes of captive leopard tortoises, as the flow through respirometry system used for this study could not accommodate larger adult tortoises.

Tortoises were housed in outdoor enclosures measuring 20 m by 12 m at the Animal House of the School of Biological and Conservation Sciences, University of KwaZulu-Natal,

Pietermaritzburg campus. The enclosures were predominantly covered with kikuyu grass, but had patches of veld grass and weeds in them. In addition, tortoises were given supplementary food three times a week consisting of a variety of forbs, succulents and vegetables. Water was always available in shallow trays set into the ground.

Measurement of metabolic rate

Oxygen consumption (VO_2) was measured using a flow through respirometery system with the equipment and methods outlined in Boix-Hinzen and Lovegrove (1998). Individual tortoises were placed in 1800 ml plexiglass respirometers under a 12:12 light:dark photoperiod and flow rates set at 0.5 $l.min^{-1}$. VO_2 readings were taken every ten minutes, giving six readings per animal per hour. Animals were not starved prior to measurements but were not fed while in the chambers as their gut transit times are very long. Metabolic measurements were made at constant T_a's of 10, 15, 20, 25, and 30 $^{\circ}$C. Trials run at constant temperatures of 15, 20 and 25 $^{\circ}$C were conducted for 24 h each, while trails at 10 and 30 $^{\circ}$C were run for 18 h each, extending from the late afternoon, overnight and until midday the following day. These trials were conducted over a one-month period in summer and winter, with average minimum and maximum environmental T_a's of 12.7 to 26.8 and 5.2 to 23.4 $^{\circ}$C, respectively. Tortoises were collected from their outdoor enclosures, weighed and had their cloacal body temperatures (T_b) measured using a fine-gauge thermocouple thermometer (Cole-Palmer Digi-Sense®). The thermocouple thermometer was calibrated against an Ever Ready electronic reference thermometer by the comparison method using thermally stabilised liquid baths (following Nicholas and White, 2001). After the trial, tortoises were again weighed, had their cloacal T_b remeasured and were returned to their outdoor enclosures. Tortoises were allowed at least 5 days rest between each trial.

VO$_2$ data collected during the first two hours that tortoises were in respirometers, were ignored. Mean hourly oxygen consumption (ml O$_2$ h^{-1}) for each individual was calculated for the remainder of the period in the respirometer, at each experimental temperature and for summer and winter respectively.

Repeated Measures ANOVA (RMANOVA) was used to compare changes in body mass, and body temperature (T$_b$) of tortoises both within and between seasons using STATISTICA © ('98 Edition, Statsoft Inc.). RMANOVA was used to compare hourly VO$_2$ of individuals between day and night, and between seasons. Data were analysed separately for hatchlings and juveniles

Results

Body mass

Body mass of leopard tortoise hatchlings ranged from 0.028 to 0.057 kg and in juveniles from 0.380 to 0.850 kg. There was no significant difference in the individual body mass of each leopard tortoise between seasons or between different temperature trials (RMANOVA, $F_{(12, 4)} = 2.61$, $P > 0.05$).

Body temperature

The initial T$_b$ of hatchling and juvenile leopard tortoises collected from outside enclosures was significantly dependent on the season (Fig. 1a) (RMANOVA, Rao's R $_{(1, 5)} = 437.58$, $P < 0.05$). At the time of tortoise collection, T$_a$'s were consistently high in summer, but varied in winter. Consequently, initial T$_b$'s of individual tortoises showed no significant differences between experimental trials in summer (RMANOVA, $F_{(1,4)} = 0.53$, $P > 0.05$), whereas in winter, significantly greater variation in T$_b$'s between trials were found (RMANOVA, $F_{(1,4)} = 19.59$, $P < 0.05$) (Fig. 1a).

Final T_b's of hatchling and juvenile tortoises after experimental trials were tested using Chi-squared tests and no significant differences were found between any tortoises' final T_b and their respective experimental temperatures in summer or winter at $P > 0.05$ (Fig. 1b).

Oxygen consumption

Tortoise VO_2 (ml O_2 h^{-1}) was not expressed as a function of body mass for each individual because of the large variation in body mass, and hatchlings and juveniles were compared separately.

Metabolic rate was lower in hatchlings at lower T_a in summer, but variable in winter (Figures 2 and 3), while juveniles had lower metabolic rates at lower experimental T_a's in both seasons (Figures 4 and 5). At lower temperatures (10 and 15 ^{o}C), hatchling tortoises showed lower VO_2 over time in winter than in summer (Figures 2 and 3). There were no distinct daily cycles in VO_2 of hatchlings at any of the experimental temperatures in either of the seasons (Figures 2 and 3). Furthermore, VO_2 was not consistently low at the end of the night (04h00-06h00). In juvenile tortoises, VO_2 in winter was similar at lower temperatures (10 and 15 ^{o}C) compared with summer, but was elevated at 30 ^{o}C in summer (Figures 4 and 5). Similarly to hatchlings, there were no distinct daily cycles in VO_2 of juvenile leopard tortoises at any of the temperatures in either of the seasons except for occasional increases during the daylight period (05h00-08h00) (Figures 4 and 5). Juvenile VO_2 was also not consistently low at the end of the night (05h00-06h00) (Figures 4 and 5).

To explore the change in VO_2 with change in T_a, VO_2 was plotted against mass (both log transformed) in winter (Figure 6) and summer (Figure 7) for the time 21h00. It is clear that the correlation between VO_2 and body mass shows a positive slope only above 20 ^{o}C in winter (Figure 6) and at 10, 25 and 30 °C in summer (Figure 7).

Discussion

Body mass of each tortoise in this study did not differ significantly over time, thus allowing for the comparison of seasonal and trial temperature effects on the metabolic rates of individual tortoises. Although initial T_b's were consistently higher than after trial temperatures in summer, and somewhat variable in winter, the tortoises were allowed two hours to equilibrate at the start of each trial and had final trial T_b's not significantly different from trial temperatures. Equilibration periods vary widely in chelonian studies of metabolic rate, ranging up to 10 h long (Hailey and Davies 1987; Hailey and Loveridge 1997; Hailey 1998). Two hour equilibration periods were used here following Litzgus and Hopkins (2003) and were deemed sufficiently long to allow hatchling and juvenile leopard tortoise T_b to attain that of experimental temperature.

As previously stated, metabolism was measured in tortoises straight from maintenance enclosures where they had constant access to food. Tortoises were therefore not in a starved condition during measurement. Waldeschmidt et al. (1987) considered that animals in the field are not typically in a fasted condition and as such, metabolic rates of reptiles measured straight out of the field would be more ecologically relevant. Other studies have also attempted to record realistic metabolic rates with reptiles freshly collected from the field (Anderson and Karasov 1981; Karasov and Anderson 1984; Merker and Nagy 1984) or in a non fasted condition (Nagy et al. 1984), as was followed in this study. Tortoises have very long gut transit times that are dependent on food intake and the quality of the diet itself, and standardising a particular length of time for starvation could be difficult and lead to erroneous measurements. As such, metabolic measurements in tortoises that are not starved are biologically more relevant.

Oxyigen consumption of leopard tortoises were broadly similar to values reported for other species of chelonians, but were only loosely comparable due to the variation in method and experimental temperatures that were used in different studies. At experimental temperatures of 20 and 30 ^{o}C, VO_2 of leopard tortoises were higher than values obtained for various freshwater turtle and terrapin species (Gatten 1974; Steyermark and Spotila 2000; Litzgus and Hopkins 2003), and for the tortoise *Kinixys spekii* and *Testudo hermanni* (Kirsch and Vivien-Roels 1984; Hailey and Loveridge 1997; Hailey 1998). Leopard tortoise VO_2 in winter was also much higher than the dormant winter VO_2 of other tortoise species (Kuchling 1981; Kirsch and Vivien-Roels 1984; Hailey and Loveridge 1997).

Hatchling and juvenile leopard tortoises had lower oxygen consumption at lower T_a's. Oxygen consumption rates generally increase with T_a (Bennett and Dawson 1976; Waldeschmidt et al.1987; Beaupre 1993, 1996; Karasov and Anderson 1998; Withers et al. 2000; Angilletta 2001), and have been reported to do so in turtles (Gatten 1974; Steyermark and Spotila 2000; Litzgus and Hopkins 2003) and the tortoise *K. spekii* (Hailey and Loveridge 1997). However, Waldeschmidt et al. 1987 discussed that some reptile species have plateaus of temperature independent metabolism, for example in some snake species (Al-Sadoon 1991) and the tortoise *T. hermanni* (Kirsch and Vivien-Roels 1994). In this study, the increase in metabolic rate with temperature showed some seasonal variation and occurred primarily in summer and at high temperatures in winter, with a state of temperature-independent metabolism at lower temperatures during the winter.

Oxygen consumption of leopard tortoises showed large seasonal variation with VO_2 being lower winter, particularly at low T_a and elevated in summer at high T_a. The influence of season on metabolic rate has been reported in chelonians, especially in those species that hibernate or aestivate (Kuchling 1981; Kirsch and Vivien-Roels 1984; Hailey and Loveridge 1997). Metabolic depression is usually associated with chelonians going into very cold or

very dry seasons and, in the case of *T. hermanni*, was independent of temperature effects, but variable in response to an intrinsic annual cycle and the time of year (Kuchling 1981). Although free-ranging Leopard tortoises do not hibernate during winter and remain active in response to T_a irrespective of season (Chapter 2), a lower metabolic rate during cold periods, particularly in juveniles and hatchlings, would be adaptively advantageous.

There were no distinct daily cycles in oxygen consumption of leopard tortoises in either summer or winter, with daily oxygen consumption showing little variation over 24 hours at lower experimental temperatures, but being more variable at 25 and 30 ^{o}C.

Distinct daily cycles in VO_2 reported in reptiles are usually affected by season, temperature and photoperiod (Songdahl and Hutchison 1972; Gratz and Hutchison 1977; Cragg 1978; Heusner and Jameson 1981; Kirsch and Vivien-Roels 1984). Data concerning the daily variations in VO_2 in chelonians are scarce (Kirsch and Vivien-Roels 1984; Waldeschmidt et al. 1987) with Hailey and Loveridge (1997) reporting random spontaneous activity during the day in *K. spekii* and a distinct diurnal rhythm in VO_2 found in *T. hermanni* at higher T_a (Kirsch and Vivien-Roels 1984).

Specific metabolic rates within leopard tortoises varied seasonally and under different temperature conditions, with juveniles only having a lower specific metabolic rate at lower temperatures and particularly in winter. The specific metabolic relationships were therefore not consistent as found in other studies (Dawson & Bartholomew 1956).

Despite the few studies on the metabolic rates of chelonians, it is evident from this and other studies on chelonia that oxygen consumption can vary between species and in response to temperature, season, photoperiod, and body mass. It is most likely that phenotypic plasticity in metabolism is a result of a combination of these factors at any given time. A more comprehensive exploration of Leopard tortoise metabolism across all size

classes would give a better idea of the primary and ecologically significant influences on metabolic rate, as has been achieved to certain extent in lizards and snakes.

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FIGURE LEGENDS

Figure 1. Comparison of mean (±SE) cloacal body temperatures of hatchling (square symbols) and juvenile (diamond symbols) leopard tortoises a. initially and b. finally at experimental ambient temperatures during summer (closed symbols) and winter (open symbols).

Figure 2. Comparison of mean (±SE) oxygen consumption (VO_2) of leopard tortoise hatchlings at experimental ambient temperatures during winter.

Figure 3. Comparison of mean (±SE) oxygen consumption (VO_2) of leopard tortoise hatchlings at experimental ambient temperatures during summer.

Figure 4. Comparison of mean (±SE) oxygen consumption (VO_2) of juvenile leopard tortoises at experimental ambient temperatures during winter.

Figure 5. Comparison of mean (±SE) oxygen consumption (VO_2) of juvenile leopard tortoises at experimental ambient temperatures during summer.

Figure 6. Comparison of oxygen consumption (VO_2) of leopard tortoises against body mass (both log transformed) at experimental ambient temperatures during winter.

Figure 7. Comparison of oxygen consumption (VO_2) of leopard tortoises versus body mass (both log transformed) at experimental ambient temperatures during summer.

A

B

Figure 1. Comparison of mean (±SE) cloacal body temperatures of hatchling (square symbols) and juvenile (diamond symbols) leopard tortoises a. initially and b. finally at experimental ambient temperatures during summer (closed symbols) and winter (open symbols).

Figure 2: Comparison of mean (±SE) oxygen consumption (VO$_2$) of leopard tortoise hatchlings at experimental ambient temperatures during winter.

Figure 3: Comparison of mean (±SE) oxygen consumption (VO_2) of leopard tortoise hatchlings at experimental ambient temperatures during summer.

Figure 4: Comparison of mean (±SE) oxygen consumption (VO$_2$) of juvenile leopard tortoises at experimental ambient temperatures during winter.

a.

10°C summer

b.

15°C summer

c.

20°C summer

d.

25°C summer

e.

30°C summer

Figure 5: Comparison of mean (±SE) oxygen consumption (VO$_2$) of juvenile leopard tortoises at experimental ambient temperatures during summer.

a.

b.

c.

d.

e.

Figure 6: Comparison of oxygen consumption (VO$_2$) of leopard tortoises against body mass (both log transformed) at experimental ambient temperatures during winter.

Figure 7: Comparison of oxygen consumption (VO_2) of leopard tortoises versus body mass (both log transformed) at experimental ambient temperatures during summer.
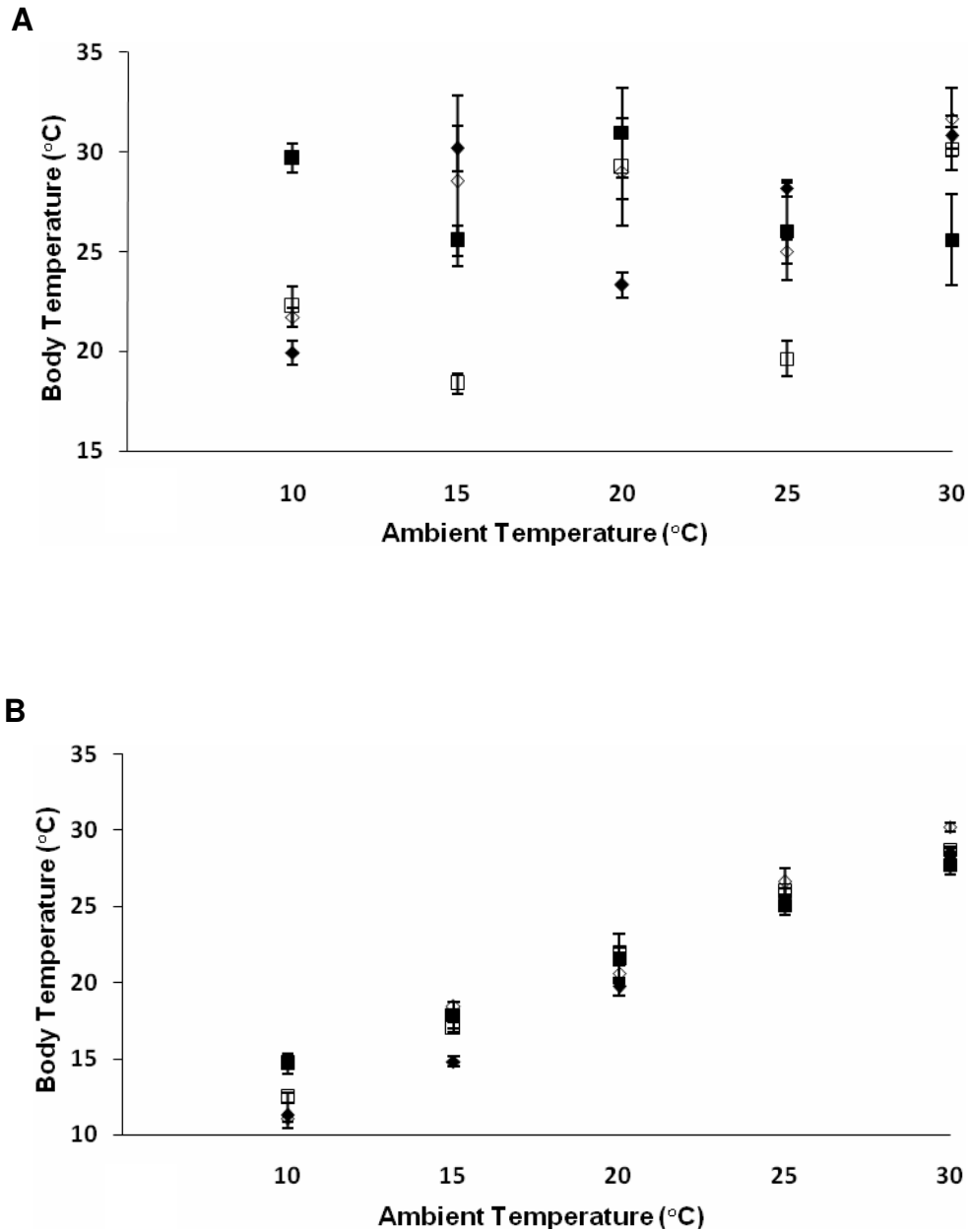
CHAPTER 6

Digestive Parameters and Water Turnover of the Leopard Tortoise (*Geochelone pardalis*).

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Comparative Biochemistry and Physiology Part A

ABSTRACT

Leopard tortoises (*Geochelone pardalis*) experience wide fluctuations in environmental conditions and unpredictable availability of food and water within the Nama-Karoo biome. Various digestive parameters of captive leopard tortoises fed two diets differing in amount of preformed water and fibre content were investigated. It was hypothesised that tortoises fed two diets differing in preformed water and fibre content would have differing food intake, gut transit rate, assimilation efficiency, faecal and urinary water loss, and urine concentrations. It was predicted that tortoises fed these contrasting diets would attempt to maintain energy and water balance by altering their digestive parameters. Leopard tortoises fed lucerne had a low food intake coupled with long gut transit times, which resulted in the lowest amount of faecal energy and faecal water lost. Tortoises fed tomatoes had higher food intake and faster gut transit times, but more energy and water was lost in the faeces. However, daily energy assimilated and assimilation efficiency were comparable between tortoises fed the two diets. Urine osmolality was significantly different between tortoises on the two diets. The results indicate that leopard tortoises can adjust parameters such as transit rate, food intake, water loss and urine osmolality to maintain body mass, water and energy balance in response to a high fibre, low water content and a low fibre, high water content

diet. This study suggests that this digestive flexibility allows leopard tortoises in the wild to take advantage of unpredictable food and water resources.

Keywords: digestion, assimilation efficiency, energy balance, water balance, diet, gut transit rate, urine concentration, osmolality, Leopard tortoise, *Geochelone pardalis*.

1. Introduction

Animals living in arid regions or in unpredictable environments rely on the fluctuating availability of resources and are able to adjust their diet seasonally or opportunistically according to food and water availability (Waldschmidt *et al*., 1987; Murphy, 1996). They may also possess a variety of morphological, physiological and behavioural characteristics that enable them to survive in an unpredictable environment (Murphy, 1996; Henen, 1997; Henen *et al*., 1998).

Chelonian species have been shown to relinquish maintenance of internal homeostasis on a daily basis year round, tolerating imbalances in water, energy, and salt and enabling them to cope with fluctuating resources in a fluctuating environment (Nagy and Medica, 1986; Peterson, 1996a, b; Henen, 1997). Tortoises are predominantly herbivorous and employ various mechanisms to meet their energy and nutrient demands from their herbivorous diet (Waldschmidt *et al*., 1987). It has been reported in various tortoise species that the digestive parameters of food intake, gut transit time and water management differed greatly when tortoises were fed on different diets (Cloudsley-Thompson, 1970; Bjorndal, 1989; Meienberger *et al*., 1993; Hailey *et al*., 1998; Spencer *et al*., 1998; Hatt *et al*., 2002). Some digestive parameters directly influence others, for example, increased food intake

shortens transit time (Meienberger *et al.*, 1993) and increases urinary water loss (Cloudsley-Thompson, 1970).

In the wild, seasonal rainfall affects tortoise digestive parameters and the osmotic balance both directly, because of the availability of free-standing water, and indirectly, by enabling tortoises to eat annuals and other plants with higher water contents (Peterson, 1996a; Hailey *et al.*, 1998; Henen *et al.*, 1998). In the desert tortoise (*Gopherus agassizii*), energy acquisition and expenditure were strongly constrained by the contingencies of rainfall and seasonal temperature fluctuations (Peterson, 1996a).

Leopard tortoises (*Geochelone pardalis*) occur throughout the savannah regions of Africa from the southern Cape up into Sudan, experiencing wide geographic variation in environmental conditions across their range, including unpredictable availability of food and water in some regions such as the Nama-Karoo (Dean and Milton, 1999; Boycott and Bourquin, 2000; Kruger, 2004). Therefore, leopard tortoises living in these areas have to adapt to fluctuations in available energy and water in their environment. Leopard tortoises are considered to be generalist herbivores, feeding extensively on grass, but also utilizing a variety of forbs, succulents, fruit, and legumes in certain habitats or according to the seasonal availability of certain species (Milton, 1992; Rall and Fairall, 1993; Hailey, 1997; Mason *et al.*, 1999; Kabigumila, 2001).

While leopard tortoises in the Kalahari were found to use plant foods according to seasonal abundance (Rall and Fairall, 1993), no seasonal differences were reported in the diets of leopard tortoises in the southern Karoo (Milton, 1992) or Valley Bushveld in the eastern Cape (Mason *et al.*, 1999). As a result, leopard tortoises may compromise transit times and assimilation efficiency depending on the type of food eaten.

In this study, two diets differing in amount of metabolic and preformed water, with one low and one high, were fed to captive leopard tortoises. It was hypothesised that tortoises

fed two diets differing in preformed water and fibre content would have differing food intake, gut transit rate, assimilation efficiency, and faecal and urinary water loss in an attempt to maintain energy and water balance. Greater food and preformed water intake, assimilation efficiency, and faecal and urinary water loss were predicted in tortoises that were fed the diet high in preformed water content. In addition the urine concentrating ability of leopard tortoises was examined. Urine osmolality was used as an indication of water stress and compared to seasonal variation in urine osmolality of wild tortoises.

The high fibre diet which was low in water content consisted of lucerne, while the low fibre diet which was high in water content consisted of tomatoes. All of the tortoises involved in the trials had been in captivity for at least a year, as such their dietary preferences were known. All of the individuals readily ate tomatoes, a low fibre, high water content item which was ideally suited to the purpose of the study. Some of the individuals, probably because of the length of time in captivity, refused to eat grass. It was also difficult to obtain grass in sufficient quantities and of sufficient quality to support the trials. In addition, it was known that all of the individuals would eat lucerne, a high fibre, low water content food item. This therefore was chosen as the comparative diet to tomatoes.

2. Materials and Methods

2.1 Captive Tortoises

2.1.1. Maintenance of captive tortoises

Twelve captive leopard tortoises of various body masses were loaned for the duration of 2003. The population comprised nine adults and three juvenile tortoises (see Appendix II for morphometric details). These tortoises had been confiscated from illegal pet traders and as such could not be released back in to the wild. All had been in captivity for more than a year.

Tortoises were housed in outdoor enclosures measuring 20 m by 12 m at the Animal House of the School of Biological and Conservation Sciences, University of KwaZulu-Natal (UKZN). The enclosures were predominantly covered with kikuyu grass, but had patches of wild indigenous grass and weeds in them. Water was always available in shallow trays set into the ground, and shade was provided in the form of open-sided roofed shelters.

2.1.2. Digestion Experiments

Two trials were conducted to examine the digestive efficiency of leopard tortoises. Both trials allowed for the controlled feeding of individual tortoises and collection of faeces, while Trial 1 additionally allowed for urine collection. Tortoises were weighed daily to the nearest gram for the duration of the trials.

Trials 1 and 2

For Trials 1 and 2, adult leopard tortoises were kept in a constant-temperature room at 25 °C on a 12-hour photoperiod from 06h00 - 18h00. Tortoises were held individually in a suspended wire cage (1 x 1 x 1m^3) with a mesh floor (2 x 2 cm^2). A removable frame with a finer mesh size (1 x 1 cm^2) was placed under the floor of the cage to allow for collection of faeces. A removable 10cm deep tray was placed below this mesh frame to collect urine, and was filled to a depth of 2 cm with paraffin oil to prevent evaporative water loss from urine.

Tortoises did not have access to drinking water for the duration of the trials. Two diets were fed to the tortoises in separate trials. Tortoises were fed the experimental diet and kept for three days in the cages to acclimate before the trials were started. Trials then lasted a further five (Trial 1) or seven (Trial 2) days. The experimental diet consisted of 200 g of chopped lucerne (Trial 1) or 800 g of quartered tomatoes (Trial 2) daily. At 06h00 the food was placed in food trays and placed in the cages with the tortoises. At 12h00, any remaining food was collected and weighed. This was then replaced with fresh food and reintroduced

into the tortoise cages. At 18h00, any remaining food items were collected and weighed. No tortoises had access to food overnight. As a control, on each day, the experimental diet was placed in a food tray, left in the CE room and the daily evaporative moisture loss calculated.

Urine was collected from under the layer of paraffin oil using syringes and the total volume of urine measured to estimate urinary water loss (UWL). Samples of urine were placed in sterile, airtight containers and frozen until analysed. Duplicate 50-ųL samples were analysed for total osmolality using a Wescor vapour pressure osmometer (Electron Microscope Centre, UKZN).

Trial 3

In order to replicate a more natural environment, in Trial 3, adult and juvenile leopard tortoises were housed individually in concreted outdoor enclosures measuring 5 x 1.5 m^2. Shade cloth covered half the roof of the enclosures so that tortoises could seek shade as necessary. Tortoises were kept on the experimental diet in the enclosures for three days to acclimate, before the trial. Trial 3 lasting a further 10 days was then started. Tortoises did not have access to drinking water for the duration of the trial. Daily weighed amounts of quartered tomatoes (typically 800 g for adult tortoises and 100 g for juveniles) were fed to the tortoises and maintained as for Trial 2. Controls of quartered tomatoes were used on a daily basis to estimate evaporative moisture loss from food.

2.1.3. Digestive Parameters

Minimum faecal transit times of food ingested were determined as the time from first digestion to the first appearance of food items in excreta. Mean faecal transit times were determined when over 50% of the faecal sample consisted of the experimental dietary item.

No markers were used because tomato skins and seeds were clearly identifiable in the faeces of tortoises. Similarly, lucerne was identifiable from its flowers and stalks.

For all trials, faeces were collected daily, however faecal water loss and faecal energy loss were calculated from faecal samples that were excreted over the same number of days as the trial, delayed by the mean gut transit time. Faeces were weighed and oven-dried at 60 °C for five days to determine faecal water loss (FWL). Total water loss (TWL) was then determined for tortoises in Trial 1 and 2, as the sum of UWL and FWL (excluding evaporative water loss).

Samples of the different trial diets were oven-dried at 60 °C for five days to determine moisture content and, together with all faecal samples, were analyzed for Gross Energy (GE) using bomb calorimetry with a DDS Isothermal CP500 bomb calorimeter (Department of Animal Science, UKZN).

In addition, diets were further analysed for fat (Soxhlet procedure using a Buchi 810 Soxhlet Fat Extractor), crude protein (Dumas Combustion Method using LECO FP2000 Nitrogen Analyser) and fibre content (fitted glass crucible method) following Helrich (1990).

Food intake (g gBM^{-1}) of Leopard tortoises was quantified as the daily difference between total mass of food provided and total mass of food remaining, per gram body mass (BM). Gross daily energy intake (GEI) of tortoises was determined by multiplying food intake by gross energy values. Water intake (ml gBM^{-1}) was calculated as the product of food intake (g) and the percentage of preformed water in the diet. Daily faecal energy loss (FE) was subtracted from gross daily energy intake (GEI) to determine the daily energy assimilated (DEA). Apparent assimilation efficiency (%) (AE) was calculated using the following equation:

$$AE(\%) = \frac{GEI - FE}{GEI} X100$$

2.2 Free-ranging Tortoises

Fieldwork was conducted on a 5500 ha area of a 26000 ha mixed commercial sheep and game farm in the De Aar District, Nama-Karoo biome, South Africa (31°04'S, 23°41'E). Leopard tortoises were located opportunistically when riding transects on horseback through the study area. While taking cloacal temperatures in the field, tortoises would sometimes urinate. Samples of this urine were collected and frozen on site to -4 °C. Gender of the individual and time and season in which urine was collected were recorded. Osmolality of these urine samples was determined using the same methods as described above for urine collected in the laboratory trials. Osmolality for field urine samples was compared with those found for captive tortoises.

2.3 Statistics

All descriptive statistical tests were calculated using STATISTICA software (Statsoft, USA). Kruskal-Wallis tests for non-parametric datasets were used to compare the total osmolality between captive and free-ranging tortoises. Analysis of variance (ANOVA) and repeated-measures analysis of variance (RMANOVA) were used to test comparisons between the diets and trials, and between days. The results from the first three days of all trials were ignored while the tortoises were habituated.

3. Results

3.1 Comparison of laboratory diets

Lucerne had less preformed water, less protein, but more fat, sodium, potassium and carbon fibre than tomatoes, although the gross energy of the diets was roughly the same (Table 1).

3.2. Body Mass of Captive Tortoises

Mean initial body mass of captive Leopard Tortoises was adult males 12.8 ±2.7 kg, adult females 10.5 ±1.8 kg and juveniles 542.5 ±203.6 g. Tortoises in all trials showed slight variations in body mass during the trials, but no significant differences between initial body mass and final body mass were found (Trial 1: $t = 0.26$, $df = 10$; Trial 2: $t = 0.59$, $df = 6$; Trial 3: $t = 0.192$, $df = 22$; $P > 0.05$).

3.3. Gut Transit Rates

Gut transit rates differed widely between individuals and were shorter in those individuals that had a higher food intake. Minimum transit time of lucerne through the gut was 3.0 - 5.5 days (mean ± SE = 4.25 ± 0.5), with 50% of the faeces consisting of lucerne (mean transit time) after a mean time of 8.75 days.

Minimum transit times of the experimental diet of pure tomato ranged from 0.75 - 1.86 days, with a mean ± S.E across individuals of 1.19 ± 0.31 days. Mean transit time (50% of the faeces consisting of tomatoes) was much faster than in tortoises on a lucerne diet, being 1.5 - 3.75 days; mean ± SE of 2.25 ± 0.3.

3.4. Preformed Water Intake

Leopard tortoises did not have access to water for the duration of trials, and therefore, their main source of water was from their food. Percentage preformed water in lucerne and tomato was 12.22% and 95.48% respectively (Table 1). Mean (± S.E) daily intake of preformed water for leopard tortoises fed lucerne was 0.098 ± 0.0015 ml kgBM^{-1}. This was significantly different to the daily preformed water intake of tortoises fed tomatoes (ANOVA, $F_{(6,17)} = 2.52$, $P < 0.05$) where the mean daily intake was 2.869 ± 0.204 ml kgBM^{-1}.

3.5. Food Intake

Daily food intake (mean ± S.E) of leopard tortoises fed lucerne was 0.807 ± 0.091 g kgBM^{-1}, with no significant difference in food intake for the duration of the trial (RMANOVA, $F_{(8,2)} = 0.37$). There was great individual variation with some individuals sometimes not eating.

Tortoises fed tomatoes had a significantly higher daily food intake (mean ± SE) of 31.817 ± 2.062 g kgBM^{-1} (ANOVA, $F_{(6,17)} = 2.32$, $P < 0.05$). There was no significant decrease in daily food intake over the length of the trial (RMANOVA, $F_{(5,55)} = 0.53$).

3.6. Gross Energy Intake

Corresponding to amount of food eaten, daily gross energy intake (GEI) (mean ± S.E) for the diet trials was 20.921 ± 2.305 KJ kgBM^{-1} for tortoises fed lucerne (trial 1), a higher 2922.419 ± 12.206 KJ kgBM^{-1} for tortoises fed tomatoes in trial 2, and a relatively high 3621.692 ± 12.588 KJ kgBM^{-1} for tortoises fed tomatoes in trial 3.

There was a significant difference in the gross energy intake between tortoises fed lucerne and tortoises fed tomatoes (ANOVA, $F_{(6,17)} = 2.61$, $P < 0.05$). However, there was no significant difference in the daily GEI for tortoises fed on lucerne (RMANOVA, $F_{(3,12)} = 0.0.57$) (Figure 1a), or tomatoes in trial 2 (RMANOVA, $F_{(4,20)} = 2.62$) (Figure 1b) or trial 3 (RMANOVA, $F_{(6,60)} = 2.1$) (Figure 1c).

3.7. Faecal Energy Loss

Faecal energy loss was significantly different between tortoises fed on the two diets (ANOVA, $F_{(6,17)} = 1.72$, $P < 0.05$). Leopard tortoises did not defecate every day in trial 1, so repeated measures test of changes in faecal energy loss could not be calculated. However, the mean ± SE FE value obtained for trial 1 was 9031.76 ± 82.67 KJ kgBM^{-1}. Daily FE loss

decreased for the first few days of Trial 2, and then increased sharply again (Figure 2a), with a mean ± SE of 10040.72 ± 27.15 KJ kgBM^{-1}, and no significant change over the days (RMANOVA, $F_{(5,5)}$ = 4.087).

In Trial 3, daily FE loss had a mean ± SE of 10561.48 ± 54.89 KJ kgBM^{-1}. Daily FE loss was fairly constant for the first three days of the trial, and then increased from the fourth day (Figure 2b), but was not significantly different across the days (RMANOVA, $F_{(4,4)}$ = 0.991).

3.8. Daily Energy Assimilated

Tortoises fed lucerne had the lowest daily energy assimilation (DEA) (mean ± SE of 3847.14 ± 254.73 KJ kgBM^{-1}) that decreased over the days of the trial (Figure 3a) (RMANOVA, $F_{(3,12)}$ = 0.814, P > 0.05). DEA decreased significantly for the first three days of trial 2, and then showed a significant increase on the last day (Figure 3b) (RMANOVA, $F_{(4,20)}$ = 3.280, P = 0.032), with a mean ± SE of 5481.31 ± 248.69 KJ kgBM^{-1}). Tortoises fed tomatoes in trial 3 had mean ± SE DEA of 6784.85 ± 196.93 KJ kgBM^{-1}), with DEA increasing over the first 5 days of the trial and then decreasing for the last two (Figure 3c), but with no significant change over the days of the trial (RMANOVA, $F_{(6,60)}$ = 1.038). DEA was significantly higher in tortoises fed tomatoes than in those fed lucerne (ANOVA, $F_{(6,17)}$ = 2.15, P < 0.05).

3.9. Apparent Assimilation Efficiency

Apparent assimilation efficiency (taking body mass into account and expressed as a percentage) of leopard tortoises were significantly higher in those fed tomatoes to those fed lucerne (ANOVA, $F_{(6,17)}$ = 1.72, P = 0.017), although it was variable over the days of the trials and between individuals (Figure 4). Tortoises in trial 1 had a mean ± SE AE of 1.35 ± 0.19 %, compared with 1.43 ± 0.11 in trial 2 and 3.63 ± 0.31 % in trial 3.

3.10. Water Loss

3.10.1. Faecal Water Loss

Faecal water loss was relatively low on the first two days of trial 1, decreased further on the last two days (Figure 5a), but showed no significant decrease over the trial (RMANOVA, $F_{(3,9)} = 0.99$). The largest amount of faecal water was lost from leopard tortoises in trial 2, with the amount decreasing over the days of the trial (Figure 5b), but the decrease was not significant (RMANOVA, $F_{(4,20)} = 0.90$). Daily faecal water loss of tortoises in trial 3 increased initially, and then decreased for the last three days of the trial (Figure 5c), with the change of faecal water loss being significant over the time of the trial (RMANOVA, $F_{(6,77)} = 2.38$, $P = 0.02$). Faecal water loss was significantly lower in tortoises fed lucerne (ANOVA: $F_{(6,17)} = 2.18$, $P = 0.003$), with a mean $\pm$ S.E of 4.472 ± 0.023 ml kgBM^{-1} compared with 8.126 ± 0.67 ml gBM^{-1} and 6.263 ± 0.31 ml gBM^{-1} for trial 2 and 3 respectively.

3.10.2. Urinary Water Loss

Leopard tortoises showed a steady decrease in the daily volume of water lost through urine in trials 1 and 2 (Figure 6), with urinary water loss being greater in tortoises fed tomatoes. The mean $\pm$ S.E. daily urinary water loss for tortoises fed lucerne was lower at 16.2 ± 0.08 ml kgBM^{-1} than the mean of 22.10 ± 0.01 ml kgBM^{-1} for tortoises fed tomatoes, but not significantly so (ANOVA, $F_{(4,9)} = 2.63$).

3.10.3. Total Water Loss

Total amount of daily water lost by leopard tortoises (excluding evaporative water loss) in both trials decreased steadily (Figure 7), but not significantly so for tortoises fed tomatoes

(RMANOVA, $F_{(5,10)}$ = 1.84). There was no significant difference between the volume of total water lost by tortoises fed lucerne or tomatoes (ANOVA, $F_{(4,9)}$ = 2.63), although the mean S.E. 12.01 ± 0.16 ml $kgBM^{-1}$ of tortoises fed lucerne was lower compared with 26.21 ± 0.15 ml $kgBM^{-1}$ for tortoises fed tomatoes.

3.11. Osmolality of Urine

Osmolality of urine from leopard tortoises fed lucerne showed an initial daily increase and then showed a sharp decrease from the third day (Figure 8a), however there was no significant difference in urine osmolality over the days of the trial (RMANOVA, $F_{(4,12)}$ = 0.96). In contrast, urine osmolality of urine from tortoises fed tomatoes decreased slightly for the first five days, but then sharply increased (Figure 8b), with a significant difference in urine osmolality of urine over the days of the trial (RMANOVA, $F_{(7,14)}$ = 3.31, P = 0.03). The osmolality of urine from tortoises fed tomatoes had a higher mean ± SE of 0.821 ± 0.318 Osmol compared with 0.376 ± 0.120 Osmol for tortoises fed lucerne (Figure 8), and were significantly different (ANOVA, $F_{(4,9)}$ = 2.81, P = 0.01).

3.11.2. Free-ranging Tortoises

Urine samples from free-ranging Leopard tortoises were obtained on 26 occasions from 11 adult tortoises, 7 females and 4 males. Females weighed a mean ± S.E. of 13.5 ± 0.6 kg and males 12.6 ± 0.2 kg. Osmolality (mean ± S.E) of urine from free-ranging tortoises in the Nama-Karoo was 0.47 ± 0.05 Osmol, with a minimum osmolality of 0.16 Osmol, and a maximum of 1.23 Osmol. There was no significant difference between urine osmolality of females and males (Kruskal-Wallis ANOVA, $H_{(1,26)}$ = 0.026), nor in relation to body mass (Kruskal-Wallis ANOVA, $H_{(9,25)}$ = 5.40). Osmolality of urine samples from free-ranging leopard tortoises was compared with that of captive held tortoises and no significant

difference was found (Kruskal-Wallis ANOVA, $H_{(1,10)} = 0.906$), however urine osmolality of the urine of captive tortoises was higher than that of free-ranging tortoises.

4. Discussion

Over the duration of these trials, leopard tortoises showed no significant difference in loss or gain of body mass. This would indicate that the species has sufficient dietary plasticity to cope with a range of diet quality. In this study the diet quality ranged from the high water, low fibre diet consisting of tomatoes to the low water, high fibre diet of lucerne. This latter should have a longer gut transit time as the amount of fibre and preformed water in a diet influences the gut transit times, (Bjorndal, 1989). In this study, gut transit times were variable between individuals, however the mean transit time was much shorter in tortoises fed tomatoes. The gut transit time for the lucerne diet was in agreement with studies done by Taylor *et al*. (1996), who obtained a transit rate of 8 days for leopard tortoises, compared to this study where the mean transit time was 8.75 days in the lucerne trial. The gut transit time for the tomato diet was considerably shorter (mean transit time = 2.25 days). Hailey (1997) found minimal dietary differences in the gut transit rates of leopard tortoises, but obtained mean gut transit rates of 3.8 when leopard tortoises were fed on kale.

The ingested food and gross energy intake of tortoises on tomato diets were significantly greater than for those tortoises on a lucerne diet. Faecal energy loss was also significantly higher in tortoises fed tomatoes. However, the daily energy assimilated and the assimilation efficiency between the two diets were comparable, although slightly higher in tortoises fed tomatoes. As has already been pointed out, the gut transit time of the tortoises eating lucerne were significantly longer. Other generalist herbivores use lower food intake and increased gut transit time as a method of maximising nutrient extraction (Bjorndal,

1989). The increase in gut transit time results in greater digestive efficiencies and reduction of the amount of faecal energy lost (Bjorndal, 1987; Meienberger *et al*., 1993). Therefore, leopard tortoises being fed lucerne were able to assimilate comparable amounts of energy from their high fibre diet by having lower food intake and increasing gut transit time.

The faecal water lost by tortoises on the tomato diet and the amount of preformed water ingested by the tortoises on the same diet, was significantly higher than that of tortoises being fed lucerne. Urine concentration was also significantly higher in tortoises fed tomatoes, however, urinary and total water loss were not significantly different between individuals on the experimental diets. Tortoises on a high fibre, low preformed water diet that consequently have longer gut transit times (Bjorndal, 1989; Hailey, 1997), can use the retention time to absorb preformed water and lose significantly less faecal water, thus maintaining urine concentration and preventing dehydration (Nagy and Medica, 1986). Tortoises on a low fibre, high preformed water content diet had higher faecal water loss associated with rapid gut transit times which resulted in high urine concentrations (Nagy and Medica, 1986; Peterson, 1996a,b).

The results show that tortoises fed two diets differing in preformed water and fibre content have significant differences in food intake, gut transit rate, assimilation efficiency, faecal and urinary water loss and urine concentration. The results further show that leopard tortoises have variable digestive parameters that can maximise water conservation depending on water availability and diet composition. This allows these tortoises to inhabit arid areas like the Nama-Karoo where food quality and water availability are unpredictable. Henen *et al*. (1998) reported similar physiological and digestive flexibililty in desert tortoises (*G. agassizii*) in response to unpredictable climatic variation as central to the ability of desert tortoises to survive droughts and take advantage of periods of resource abundance.

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Table 1: Nutrional composition of dietary items fed to leopard tortoises in the laboratory trials. Protein, fat and carbon fibre percentages are given as the percentage of dry weight.

Dietary Item	Protein (%)	Fat (%)	Carbon Fibre (%)	Sodium (%)	Potassium (%)	Gross Energy (MJ/Kg)	Preformed Water (%)
Lucerne	13.62	4.33	19.36	0.43	0.60	16.61	12.22
Tomato	17.87	2.11	9.55	0.03	0.02	16.14	95.48

FIGURE LEGENDS

Figure 1: Change in gross energy intake (KJ g^{-1}) by leopard tortoises over the days of a. Trial 1, b. Trial 2 and c. Trial 3.

Figure 2: Change in faecal energy (KJ g^{-1}) excreted by leopard tortoises over the days of a. Trial 2 and b. Trial 3.

Figure 3: Change in daily energy assimilation (KJ g^{-1}) of leopard tortoises over the days of a. Trial 1, b. Trial 2 and c. Trial 3.

Figure 4: Assimilation efficiency (%) of leopard tortoises over the days of a. Trial 1, b. Trial 2 and c. Trial 3.

Figure 5: Change in the volume of faecal water lost per gram of body mass (ml gBM^{-1}) by leopard tortoises over the days of the trials where a. Trial 1, b. Trial 2 and c. Trial 3.

Figure 6: Change in the volume of urinary water lost per gram of body mass (ml gBM^{-1}) by leopard tortoises over the days of a. Trial 1, and b. Trial 2.

Figure 7: Change in the total volume of water lost per gram of body mass (ml gBM^{-1}) by leopard tortoises over the days of a. lucerne diet trial 1, and b. tomato diet trial.

Figure 8: Change in urine concentration (Osmol) from leopard tortoises over the days of the a. lucerne diet trial and b. tomato diet trial.

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a.

b.

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Figure 8: Change in urine concentration (osmol) from leopard tortoises over the days of the a. lucerne diet trial 1 and b. tomato diet trial.
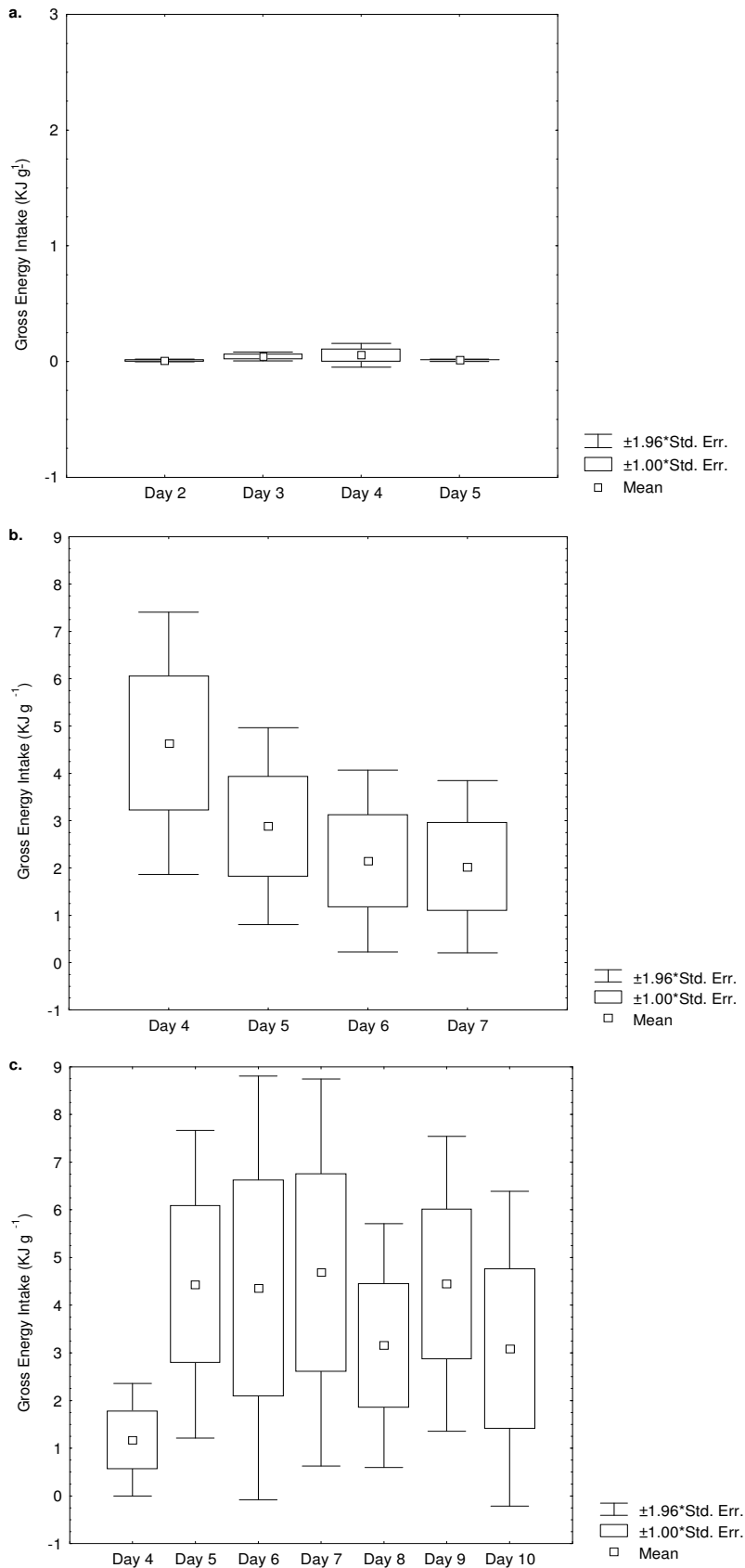
CHAPTER 7

CONCLUDING REMARKS

AND SO……

Leopard tortoises (*Geochelone pardalis*) in South Africa inhabit areas as diverse as the summer rainfall areas of the sub-tropical woodlands of northern KwaZulu-Natal in the east, the savannahs and woodlands of the Kruger National Park in the north, the valley bushveld thickets of the south and the winter rainfall semi-arid Nama-Karoo of central South Africa. As such throughout their geographic range they occur in areas that have different fluctuations in ambient temperature (T_a), water and energy availability. This is especially true in the Nama-Karoo, where daily and seasonal fluctuations are further perturbed by unpredictable climatic disturbances. The tortoises' ability to survive droughts and periods of low resource abundance reflect the physiological and behavioural plasticity of the species.

Little is known concerning the ecology of the leopard tortoise within the Nama-Karoo, however, the species' role as an herbivore has been studied in the Thicket biome of South Africa (Kerley *et al*., 1995; Kerley and Boshoff, 1997, Kerley *et al*., 1998). These studies have indicated that leopard tortoise biomass exceeds all individual herbivore species, including Black Rhinoceros (*Diceros bicornis*) and Cape Buffalo (*Syncerus caffer*), but excluding Elephant (*Loxodonta africana*). Kerley *et al*. (1998) also point out that leopard tortoises have a historically crucial role in seed dispersal and habitat maintenance within the Thicket biome, while Milton (1992) has also emphasised a similar role in the arid Little Karoo. Therefore, although no formal study exists of the relationship between leopard tortoises and habitat maintenance within the Nama-Karoo, it is likely that they play an integral and essential role in seed dispersal and faunal community structure and have, as a

result of their feeding behaviour, been enormously influential in determining how the Nama-Karoo functions today.

However, what the role of leopard tortoises will be in the future is under extreme doubt. At the moment only a single conservation area has been proclaimed within the Nama-Karoo (Branch *et al*., 1995), with the rest of the biome under pastoralism, sheep farming and game hunting. This is affecting leopard tortoise numbers in many ways. Many leopard tortoises are fatally injured by farm vehicles and killed by electric fencing. Game and sheep fences may adversely affect the social and breeding life of the tortoises, cutting through the home range of adults which might be as much as 500 ha (McMaster, 2002). Organised removal of tortoises for the pet trade and opportunistic poaching by farm workers, who pick up tortoises they encounter and sell them by the roadside, reduce the total population of the tortoises. Poaching becomes a more serious threat when it is the breeding adults, the more conspicuous individuals that are removed. How pastoralism and the introduction of alien herbivores (e.g. goats *Capra hircus*) will affect tortoises is simply not known. Uncontrolled, it can be expected to result in severe habitat degradation as it has elsewhere (Kerley *et al*., 1995) and ultimately habitat loss for the leopard tortoise.

An adult leopard tortoise is one of the largest modern-day native herbivores within the Nama-Karoo biome and although tortoises play and have played an intrinsically important role in the formation and maintenance of the biome, there is a lack of knowledge concerning that role and how that role will change as a result of present day agricultural practices. This study begins to address this paucity and examines the adaptations which have allowed the leopard tortoise to successfully colonize the Nama-Karoo. The persistence in a habitat of any species depends on its ability to survive and reproduce (Morrison *et al*. 1987). Intrinsic to this ability is the individual's capacity to adapt to the climatic conditions

prevalent in its habitat, therefore in Chapters 2-4, the thermoregulatory mechanisms employed by tortoises to cope with fluctuating temperatures are explored.

In chapter 2, it was hypothesised that there was no difference between the cloacal, non-cloacal and internal body temperatures (T_b) of individual tortoises. The results showed that cloacal temperatures were significantly different from all other T_b's. More importantly, the results showed a temperature gradient between the extremities and the core of individuals, leading to the conclusion that the core T_b's of tortoises are largely buffered from the greater range of T_a's experienced by the extremities. This is significant for the thermoregulation of the animal, for instance in winter the extremities of an individual may rapidly lose heat overnight through conductance, but the core T_b will remain relatively warm and any heat gained through basking can be shunted to the core through the vascular system and allow the individual to heat up to its preferred T_b faster.

Differences between the external and internal T_b of tortoises led to the hypothesis that leopard tortoises can maintain T_b within preferred limits in fluctuating daily and seasonal temperatures (chapter 3). The results show that tortoises were able to manipulate their T_b independently of T_a (chapter 3). They have evolved a number of behavioural activities such as retreat site selection, extended periods of basking and shading which helps individuals maintain T_b's within their preferred limit. However, an important adaptive feature to temperature maintenance was thermal inertia, augmented by physiological mechanisms such as vascular shunting, that allows the tortoises' to retain heat and cool down slower than T_a overnight. In summer and in winter individual tortoises were therefore able to be active earlier in the day and for longer periods, thus giving them more time to find, manipulate and utilise the resources required to complete their life cycle (Koplin 1972; Whitman 1979; Ligon *et al*. 1991).

Chapter 4 addressed whether behavioural activities alone were sufficient to allow leopard tortoises to manipulate their T_b. It was hypothesised that tortoises would differ in their rates of heating and cooling. Laboratory studies, where no behavioural activity was possible, showed differences in heating and cooling rates which could only be accounted for by physiological mechanisms. The differences in heating and cooling rates were exacerbated by the role played by surface area to volume ratio as individuals increase in size.

A physiological mechanism common amongst animals which cope with extreme temperatures and fluctuating resources is to reduce metabolic rate. It was hypothesised that an individual tortoise's metabolism would be affected by lower T_a and seasonal differences would exist (chapter 5). It was found that lower metabolic rates were recorded in tortoises at lower T_a in both winter and summer; additionally there was a significant reduction in absolute winter metabolic rate compared with summer (chapter 5). This would only occur if the tortoises were altering their metabolic rate as a thermoregulatory mechanism or if a lack of resources caused a lowering of the metabolic rate in winter.

The ability to adapt to differences in resource availability was further examined in the last chapter (chapter 6) which dealt with the relationship between physiological mechanisms for maximising energy and water retention, and differences in diet quality. Tortoises were able to utilise diets of different fibre and preformed water content by adjusting the food intake, gut transit rate and water loss in order to maximise the energy assimilated. The ability to withstand fluctuating nutrient, fibre and water intake and urine osmolality, while still being able to gain energy allows leopard tortoises to cope with the seasonal variation in diet and availability of water.

Leopard tortoises in this study were therefore shown to be able to cope with large variations in daily and seasonal T_a by changing their daily activity patterns, by performing certain behaviours at different preferred T_a and core T_b's depending on the season, by

allowing thermal gradients within the body that buffer core T_b and through the use of physiological mechanisms that further alter the rate of heat flux. In addition, leopard tortoises can adjust their metabolic rates either on a daily basis or during extreme weather conditions in order to minimise energy loss, and can adjust their digestive parameters in response to different diets such that body mass, energy and water balance are maintained.

Over 40 years ago, it was concluded that the major thermoregulatory mechanisms employed by ectotherms were physiological mechanisms (Bartholomew and Tucker, 1963). Indeed, Perrin and Campbell (1981) suggest that behavioural thermoregulation is time wasting and because of their limited mobility, tortoises would be unsuited to managing heat flux by behavioural means. However, further studies reduced the importance of physiological mechanisms in favour of behavioural activities (Hailey and Coulson, 1996). This study has shown, at least in terms of leopard tortoises, that both behavioural and physiological mechanisms are utilised to maintain T_b. In addition, thermal inertia allows the tortoise to retain body heat and cool down slower than environmental temperatures during the night. Thermal inertia, through larger body size, can therefore be thought of as an adaptation towards maximising an individual's daily time budget for foraging and breeding.

Physiological adjustment of heat flux was thought by Perrin and Campbell (1981) to be limited in leopard tortoises. However, in this study, leopard tortoises used physiological mechanisms to manipulate heat flux in the absence of behaviour. If T_a's fluctuate to extreme levels, as found in the wild at midday in summer and at night in winter, then tortoises must resort to behavioural methods or a combination of behavioural and physiological methods, in order to maintain T_b within preferred limits It is therefore behavioural adaptations that bestow on leopard tortoises the plasticity to inhabit areas where extreme climatic fluctuations occur.

Evidence of fluctuating climatic conditions and subsequently of food resource availability has been given by Rall and Fairall (1993) where in two successive years the difference in rainfall patterns was so great that leopard tortoises switched from a diet primarily of grass to one which constituted a greater variety of ephemeral species. A major event inducing climatic randomness is known as El Niño or the El Niño Southern Oscillation (ENSO). This event also affects the Mojave desert of California, where Henen *et al.* (1998) investigated the effects of two El Niño events on the desert tortoise (*Gopherus agassizzii*). Under normal climatic conditions, tortoises are extremely dependant on regular rainfall. Rain not only provides water for immediate drinking, but stimulates and sustains germination and the growth of plants that tortoises eat (Nagy and Medica, 1986). These plants also provide a considerable amount of preformed water from their tissues. In years of restricted rainfall, tortoises have very low reproductive rates following dry winters (Henen, 1997). Further effects of drought conditions are increased adult mortality (Peterson, 1994) and probable decrease in recruitment (Morafka, 1994). However, the effect of an El Nino event in the Mojave Desert was to generate higher than average winter rainfall. This was followed by high plant productivity the following spring. Desert tortoises reacted by greatly increasing their drinking and foraging intensity, their field metabolic rate and water influx rate also increased thus amplifying their behavioural and physiological response to the increased rainfall (Henen *et al.,* 1998). In other words, desert tortoises show an ability to adjust seasonal and annual field metabolic rates and food requirements to levels of food availability. And because females were able to increase their dry body matter composition in periods when food resources were high, they also increased their reproductive output (Turner *et al*., 1986). Therefore, behaviour and physiological plasticity is the key to survival during drought for the desert tortoise.

Similarly, the results of this study have shown that leopard tortoises show a behavioural and physiological plasticity in their thermoregulatory, metabolic and dietary response to temperature and resource fluctuations and are therefore adapted to survive in the unpredictable climate of the Nama-Karoo. Further research will be required to fully explore the plasticity of leopard tortoises in this biome. To my knowledge, no studies have concentrated on the behaviour and physiology of hatchlings and juveniles in the wild, yet a species' permanence is dependant on juvenile recruitment and survival. Hatchling and juvenile tortoises have been shown in this study to have very different rates of heat flux, but other aspects of their behavioural and physiological thermoregulation need to be investigated. Due to their small size, hatchlings and juveniles are more vulnerable to heat flux, to drought and to predators, and yet nothing is known of their ecology, behaviour or physiology in the wild. Such an investigation could also touch on subjects such as the recruitment and dispersal of young. These subjects alone would greatly add to our knowledge and understanding of tortoise biology and life history.

It has been shown that living in an arid region with an unpredictable climate can be energetically costly (Nagy and Medica, 1986; Henen, 1997), and that for the desert tortoise, the energetic costs of reproduction do not seem to be sustainable in drought years (Turner *et al*., 1986). Therefore the energy budgets of breeding leopard tortoise females must be very different to males. How they differ and what affects that difference would also prove to be a profitable area of research.

Recent developments in techniques of data collection now allow data to be collected in the field that was previously only able to be collected under controlled laboratory conditions. Future research into this species could therefore entail the investigation of previously lab based subjects in the field. This study has started trying to do just that by using implanted dataloggers and trying to compare data collected in the field with data in the

laboratory. However, future research will ideally be able to collect data solely from animals in the wild and therefore obtain data and results that are unbiased by the complications of captivity.

The majority of the tortoises used in this study were wild tortoises *in situ* in the Nama-Karoo. The importance of leopard tortoises in the Thicket biome has already been mentioned (Kerley *et al*., 1998). Throughout its distribution range, similar conclusions have been reached; that leopard tortoises are intermediate between generalist and specialist herbivores (Mason *et al*., 1999), eating some plant more frequently than their occurrence would merit (Broadley, 1989; Baard, 1994). In Tanzania it was found that leopard tortoises ate 47 plant species from 21 families, however 74.5% of their diet was forbs (Kabigumila, 2001). Similarly, leopard tortoises in the Addo district of South Africa consumed 28 species with 6 species comprising 75% of the diet (Mason *et al*., 1999). In the southern Karoo, 75 species of grass, succulents and forbs belonging to 26 plant families were eaten by leopard tortoises (Milton, 1992). Of the 356 plant items identified as being part of the tortoises' diet, seeds were an important component and these were largely vacated undigested (Milton, 1992). Most of these seeds were germinated from the faecal samples (Milton, 1992), suggesting that leopard tortoises play an important management role in their habitat as seed dispersers.

An area of less than 1% of the Nama-Karro biome is protected, the remaining majority is rangeland for livestock grazing by domestic sheep, goats and increasingly, game animals. Much of the vegetation and habitat has been altered through overgrazing. Lack of rainfall, poor soils and extreme temperatures result in low carrying capacities for domestic animals. This, in conjunction with drought events and fluctuating markets for agricultural products have increasingly marginalised the economic importance of domestic stock. Over the last decade, land owners have explored the economic viability of game animals, where

the product is now trophy hunting revenue and venison. Historically, many of these game species existed in the Nama-Karoo, however they were not enclosed and were able to migrate across the biome. Today's game animals are fenced, halting the migrations of the past and exerting intensive grazing pressure on the veld. It is therefore crucial that more emphasis is placed on management and conservation if the economy of the Nama-Karoo is to persist.

As large herbivores, it is probable that leopard tortoises play a crucial role in the management and persistence of the Nama-Karoo. Further investigation into the ecology, breeding biology, behaviour and physiology of leopard tortoises in the wild would allow researchers to make informative decisions on how to manage and conserve the species in the Nama-Karoo and therefore the Nama-Karoo itself.

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Appendix I: Morphological characteristics of free-ranging leopard tortoises over a two-year period in the Nama-Karoo. Leopard tortoises that had "internal" iButton^{TM}s surgically implanted in 2003 are indicated with an asterisk. Abbreviations are explained in Appendix IV.

Date	No	S	CCL	CCW	CCS	SCL	SCW	FMSG	SCH	PLL	PLW	Mass (kg)
Feb '02	9	M	52.0	50.0	53.0	42.0	27.0	7.3	20.0	37.0	28.0	12.0
Feb '02	17	M	58.0	53.0	59.0	48.0	30.0	11.0	21.0	39.0	29.0	14.5
Feb '02	40	M	46.0	52.0	53.0	42.0	28.0	9.0	19.0	33.0	26.0	9.0
Feb '02	64	M	56.0	53.0	57.0	46.0	29.0	12.0	21.0	38.0	29.0	12.6
Feb '02	11	F	61.0	62.0	59.0	48.0	32.0	10.0	22.0	37.0	34.0	15.0
Feb '02	15	F	64.0	61.0	63.0	51.0	34.0	11.0	21.0	41.0	35.0	19.0
Feb '02	22	F	57.0	57.0	56.0	45.0	30.0	9.0	20.0	39.0	31.0	14.5
Feb '02	57	F	59.0	56.0	60.0	46.0	31.0	8.0	21.0	38.0	30.0	15.5
Feb '02	111	F	51.0	50.0	52.0	39.0	26.0	9.0	16.0	32.0	25.0	10.0
Feb '02	112	F	52.0	55.0	55.0	43.0	29.0	9.0	19.0	34.0	30.0	13.8
July '02	9	M	56.0	53.0	59.0	47.0	32.0	10.0	21.0	39.0	30.0	11.9
July '02	17	M	60.0	57.0	64.0	50.0	32.0	11.0	21.0	40.0	31.0	12.4
July '02	40	M	54.0	51.0	56.0	41.0	28.0	9.0	19.0	34.5	27.0	9.2
July '02	64	M	58.0	53.0	58.0	47.0	30.0	12.0	21.0	38.0	29.0	13.1
July '02	22	F	65.0	59.0	58.0	52.0	32.0	10.5	20.0	39.0	30.0	13.4
July '02	11	F	64.0	62.0	64.0	52.0	35.0	10.0	22.0	37.0	32.0	13.6
July '02	15	F	66.0	65.0	70.0	51.0	36.0	11.5	21.0	42.0	34.0	16.8
July '02	57	F	58.0	56.0	63.0	43.0	30.0	8.0	21.0	39.0	33.0	12.6
July '02	111	F	52.0	52.0	52.0	38.0	26.0	9.0	16.0	32.5	25.0	8.8
July '02	112	F	55.0	55.0	59.0	45.0	30.0	8.0	19.0	35.0	29.0	11.4
Feb '03	9*	M	58.0	51.0	56.0	44.0	28.0	11.0	20.0	39.0	30.0	11.2
Feb '03	13	M	57.0	53.0	57.0	45.0	28.0	10.0	22.0	37.0	31.0	12.4
Feb '03	17	M	59.0	57.0	61.0	49.0	32.0	11.0	21.5	39.5	29.5	12.6
Feb '03	64*	M	59.0	54.0	61.0	52.0	29.0	12.0	21.0	38.5	28.0	13.4
Feb '03	113	M	40.0	39.0	39.0	32.0	22.0	7.0	17.0	27.0	20.0	4.8
Feb '03	11	F	61.0	61.0	64.0	49.0	34.0	10.0	22.0	37.0	32.0	14.2
Feb '03	15	F	64.0	65.0	67.0	48.0	36.0	11.0	25.0	42.0	35.0	19.8
Feb '03	22*	F	58.0	57.5	61.0	46.0	32.0	9.5	22.0	39.5	31.0	13.3
Feb '03	111*	F	53.0	51.0	50.0	41.0	27.0	9.0	19.0	33.0	27.0	9.4
July '03	9*	M	58.0	53.0	59.5	47.0	31.0	10.0	22.0	40.0	29.0	12.2
July '03	17*	M	60.0	54.0	63.0	53.0	53.0	12.0	25.0	42.0	32.0	13.8
July '03	64*	M	61.0	56.0	58.0	46.0	30.5	11.0	24.0	39.5	30.0	13.8
July '03	15	F	66.0	64.0	66.0	52.0	38.0	11.0	23.0	42.5	36.0	18.2
July '03	22*	F	58.5	57.5	61.5	45.0	33.0	10.0	23.0	40.0	32.0	12.6
July '03	57	F	60.5	56.0	61.0	46.0	34.0	9.0	22.0	39.0	30.0	13.8
July '03	62	F	56.0	58.0	59.0	45.0	31.5	10.0	23.0	37.0	30.0	12.8
July '03	111*	F	53.0	52.5	52.0	39.0	36.0	10.0	18.0	33.0	27.0	9.4
July '03	114	F	55.0	53.0	55.0	49.0	33.0	9.5	10.0	36.5	31.0	12.0

Appendix II: Morphological characteristics of captive leopard tortoises of different age classes over a two-year period. The individual tortoise number is preceded by a "T" designating adults and a "J" indicating juvenile Leopard tortoises. Abbreviations explained in Appendix IV.

Date	Tortoise	Sex	CCL	CCW	CCS	SCL	SCW	FMSG	SCH	PLL	PLW	Mass (kg)
Apr 2002	T1	female	57.0	56.0	57.0	42.0	32.0	11.0	18.0	36.0	31.0	13.6
Apr 2002	T2	female	68.0	64.0	70.0	48.0	34.0	12.0	23.0	42.0	33.0	19.8
Apr 2002	T3	female	53.0	50.0	52.0	38.0	28.0	9.0	17.0	33.0	29.0	11.5
Apr 2002	T4	male	55.0	52.0	56.0	42.0	28.5	9.0	20.0	35.0	38.0	12.5
Apr 2002	J5	male	19.8	19.5	21.2	15.3	11.7	1.3	7.7	13.2	11.1	0.7
Apr 2002	T6	female	36.0	35.0	34.5	26.0	18.5	5.0	14.0	23.5	17.0	3.6
Apr 2002	T7	male	44.0	46.0	43.0	34.0	25.0	7.0	16.0	27.5	23.0	7.4
Apr 2002	T8	female	54.0	49.0	51.0	40.0	26.0	8.0	21.0	35.0	26.0	9.4
Apr 2002	T9	female	42.0	43.0	42.0	32.0	23.0	6.5	17.0	26.0	22.0	6.0
Apr 2002	T10	male	64.0	64.0	66.0	53.0	33.0	12.0	21.0	42.0	32.0	23.2
Apr 2002	J11	male	12.5	12.0	13.5	8.9	7.5	2.5	4.0	8.0	7.0	0.2
Apr 2002	J12	female	15.0	15.6	17.5	11.5	8.5	2.0	5.0	10.4	8.2	0.3
Mar 2003	T1	female	57.0	58.0	58.0	44.0	32.0	11.0	18.0	36.0	32.0	13.4
Mar 2003	T2	female	69.0	65.0	70.0	50.0	35.0	12.0	22.0	42.0	35.0	22.6
Mar 2003	T3	female	53.0	51.0	53.0	40.0	29.0	9.0	17.0	33.5	30.0	12.6
Mar 2003	T4	male	55.0	54.0	57.0	43.0	30.0	10.0	20.0	35.0	39.0	11.9
Mar 2003	J5	male	22.1	21.6	24.3	16.2	11.9	1.9	8.0	14.7	11.8	0.8
Mar 2003	T6	female	36.0	35.0	35.0	27.0	18.5	6.0	14.0	24.0	17.0	3.9
Mar 2003	T7	male	45.0	47.0	44.0	34.5	26.0	8.0	16.0	28.0	23.0	7.6
Mar 2003	T8	female	54.0	51.0	52.0	41.0	27.0	8.0	22.0	35.0	26.0	9.2
Mar 2003	T9	female	42.0	43.0	43.0	33.0	23.0	7.0	17.0	27.0	22.0	6.5
Mar 2003	T10	male	67.0	67.0	70.0	55.0	34.0	12.0	22.0	45.0	33.0	23.8
Mar 2003	J11	male	13.0	13.0	14.0	9.3	8.1	3.0	5.0	9.0	8.0	0.2
Mar 2003	J12	female	18.0	17.5	19.0	14.0	9.0	3.0	5.0	11.2	9.4	0.4
Feb 2004	T1	female	58.0	59.5	59.0	45.0	32.0	11.0	19.0	36.0	32.0	14.0
Feb 2004	T2	female	70.0	66.0	70.0	51.0	36.0	13.0	21.0	42.5	35.5	23.1
Feb 2004	T3	female	53.5	52.0	55.0	42.0	29.0	9.5	17.0	33.5	30.0	13.5
Feb 2004	T4	male	55.5	55.0	58.0	43.0	31.0	10.0	20.0	35.0	39.0	11.2
Feb 2004	J5	male	23.5	23.0	26.0	17.5	12.5	2.5	8.0	15.5	12.0	0.9
Feb 2004	T6	female	37.0	35.0	35.0	27.5	19.0	6.5	14.0	24.5	18.0	4.0
Feb 2004	T7	male	45.0	47.0	45.0	35.0	26.0	8.5	16.0	28.5	23.0	8.0
Feb 2004	T8	female	54.0	52.0	53.0	41.0	28.0	8.0	22.0	36.0	27.0	9.8
Feb 2004	T9	female	43.0	44.0	43.0	33.0	24.0	7.0	18.0	27.0	22.0	7.0
Feb 2004	T10	male	69.0	68.0	72.0	57.0	35.0	12.5	22.0	46.0	34.0	24.0
Feb 2004	J11	male	13.0	13.5	15.0	10.7	8.5	3.0	5.0	9.5	8.5	0.4
Feb 2004	J12	female	19.0	19.5	20.0	15.0	10.5	3.5	8.0	12.5	10.5	0.5

Appendix III: Morphological characteristics of hatchling captive leopard tortoises born over a two-year period. Abbreviations are explained in Appendix IV.

Date	Number	Sex	CCL	CCW	CCS	SCL	SCW	FMSG	SCH	PLL	PLW	Mass (kg)
Apr 2002	Ha1	unknown	6.1	6.1	7.0	5.9	4.7	1.6	2.0	4.5	3.8	0.028
Apr 2002	Ha2	unknown	6.1	6.0	7.5	5.4	4.3	1.5	2.0	4.5	3.9	0.031
Mar 2003	Ha1	unknown	6.4	6.2	7.1	6.0	4.9	1.6	2.0	4.6	3.9	0.036
Mar 2003	Ha2	unknown	6.2	6.0	7.7	5.5	4.4	1.5	2.0	4.6	4.0	0.031
May 2003	Ha3	unknown	9.7	7.6	9.0	6.1	5.2	1.4	2.8	5.4	5.1	0.039
May 2003	Ha4	unknown	8.3	8.9	10.3	7.2	6.5	2.3	2.5	6.4	6.5	0.052
Aug 2003	Ha2	unknown	6.5	6.1	7.8	5.7	4.5	1.5	2.0	4.7	4.0	0.039
Aug 2003	Ha5	unknown	7.0	7.4	9.1	6.0	5.3	2.6	2.8	5.2	4.9	0.034
Aug 2003	Ha6	unknown	8.5	8.8	10.4	7.3	6.3	2.8	3.2	6.2	6.3	0.056
Feb 2004	Ha3	unknown	7.9	7.8	9.2	6.5	5.6	1.5	2.9	5.8	5.3	0.040
Feb 2004	Ha5	unknown	7.1	7.5	9.1	6.0	5.4	2.6	2.8	5.4	5.0	0.034
Feb 2004	Ha6	unknown	8.6	9.0	10.5	7.3	6.4	2.8	3.5	6.3	6.4	0.057
Feb 2004	Ha7	unknown	9.2	9.0	10.5	7.1	6.1	2.6	3.9	6.6	5.7	0.060

Appendix IV: Morphometric measurements taken from individual leopard tortoises. Abbreviations: **CCL** = Curved Carapace Length; **CCW** = Curved Carapace Width; **CS** = Carapace Side; **SCH** = Straight Carapace Height; **SCL** = Straight Carapace Length; **SCW** = Straight Carapace Width; **FMSG** = Front Marginal Shield Gap.