

**THERMOREGULATION IN THE AFRICAN SIDE-NECKED
TERRAPIN, *PELOMEDUSA GALEATA***

By

Stephanie M. Lyle

Submitted in fulfilment of the academic requirements for the degree of

Master of Science

in the discipline of ecology,

School of Life Sciences,

College of Agriculture, Engineering and Science,

University of KwaZulu-Natal,

Pietermaritzburg, South Africa.

November 2014

ABSTRACT

Ectotherm body temperature (T_b) is influenced by the thermal environment, thus maintaining stable T_b requires adjustments to physiological and behavioural thermoregulation. There is an abundance of literature on thermoregulation in reptiles, however, little is known about the thermoregulatory behaviour of the recently taxonomically resurrected semi-aquatic, African side-necked terrapin, *Pelomedusa galeata*. Adult ($n = 17$) and hatchling ($n = 16$) *P. galeata* were evaluated for preferred body temperature (T_{pref}), set point range temperature (T_{set}) and associated thermoregulatory behaviours. Wild adult terrapins were captured and implanted with temperature data loggers (Thermochron iButton®, Dallas Semiconductor, Texas, USA), and observed for basking behaviour in a semi-natural environment. Captive bred hatchlings (neonates and yearlings) were evaluated for T_{pref} , T_{set} and thermophilic responses to an aquatic thermal gradient. All experiments were carried out in the Animal House, at the University of KwaZulu-Natal, Pietermaritzburg, South Africa. Adult T_b was weakly correlated with mass and ambient air temperature (T_a) in winter and only T_a in summer. Adults basked most frequently in summer and basking behaviours differed between seasons. Basking at the water surface in full sun was observed most often during summer and out the water in full sun during winter. Hatchling T_b was highly correlated with aquatic thermal gradient temperature (T_{ag}), and was higher than acclimation temperature. Neonate and yearling T_{pref} were not significantly different and mass affected relocation between T_{ag} sites. Adult *P. galeata* displayed ‘cooling’ behaviours during hot periods in summer and ‘warming’ behaviours during cold periods in winter, and T_a and T_b were good predictors for *P. galeata* thermoregulatory behaviour. Hatchling *P. galeata* are highly sensitive to the thermal environment. They selected for high T_b although this may not be possible in natural environments, owing to increased predation risk while basking at exposed sites. In light of increasing global temperature, *P. galeata* will be relatively unaffected by higher temperatures by making adjustments to basking behaviour to maintain T_{pref} . General climate change, however, may change the landscape and water bodies *P. galeata* depend on, thus affecting distribution and survival. Empirical data from species-specific studies contribute to the knowledge required for implementing conservation measures.

DECLARATION

I, Stephanie Margaret Lyle, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
 - a. Their words have been re-written but the general information attributed to them has been referenced
 - b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Stephanie Margaret Lyle
November 2014.

As the candidate's supervisors we have approved this thesis for submission,

Sue McConnachie
Supervisor

Mike Perrin
Supervisor

University of KwaZulu-Natal, Pietermaritzburg, South Africa,
November 2014.

PREFACE

The data described in this thesis were collected in the Republic of South Africa, from January 2012 to November 2014. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg Campus under the supervision of Doctor Sue McConnachie and Professor Mike Perrin. This thesis consists of four chapters, a single reference section and one appendix (A1). Chapter one is the introductory literature review, chapter two is the methods and materials section, chapter three is the results section, and chapter four is the discussion and conclusions section. Figure and table lists are provided (p: viii-xi), and figures and tables are in text. References are compiled into one section, after chapter four, and appendix A1 follows the reference section. Referencing has been styled as per the journal, *Ecology*. Terms that are abbreviated are given in full at first appearance in each chapter, and abbreviations follow. Due to temperature data logger failures, operative temperatures could not be determined. These data have been filed under the appendix section. Comparisons for seasonal body temperature was limited by temperature data logger failures, however, data from a few individuals were assessed.

Stephanie Margaret Lyle
November 2014.

We certify that the above statement is correct,

Sue McConnachie
Supervisor

Mike Perrin
Supervisor

University of KwaZulu-Natal, Pietermaritzburg, South Africa,
November 2014.

TABLE OF CONTENTS

LIST OF FIGURES	vi
LIST OF TABLES	x
ACKNOWLEDGEMENTS	xi
CHAPTER ONE: INTRODUCTION	1
Thermal biology of reptiles	2
Thermoregulation, thermoconforming and acclimation	3
Factors affecting thermoregulation	4
Thermal ecology of terrestrial, aquatic and semi-aquatic reptiles	8
Advantages of basking for turtles and terrapins	9
Aims and objectives	11
CHAPTER TWO: METHODS AND MATERIALS	12
Study animal	12
Adult terrapins	13
Hatchling terrapins	21
CHAPTER THREE: RESULTS	27
Adult terrapins	27
Hatchling terrapins	35
CHAPTER FOUR: DISCUSSION AND CONCLUSIONS	40
Discussion	40
Adult terrapins	40
Hatchling terrapins	51
Conclusions	54
REFERENCES	57

APPENDIX

68

A1: Operative temperatures

68

LIST OF FIGURES

	Page
<i>Figure 1</i>	Dip net (a), funnel trap (b) and bait rigger (c). All were used to capture terrapins; the bait rigger was used to attach bait to a fishing line, the dip net was used to catch <i>Pelomedusa galeata</i> within reach and the funnel trap was set up and left for 24 hour periods. 13
<i>Figure 2</i>	Straight measurements of <i>Pelomedusa galeata</i> carapace length and width (a) and plastron length (b) taken with a rigid ruler. 14
<i>Figure 3</i>	Colour coded numbers painted on <i>Pelomedusa galeata</i> carapaces for easy identification. 15
<i>Figure 4</i>	One notch mark on the first left anterior marginal scute (a) and the first right posterior marginal scute (b). Respective codes are AL1-1 (Anterior Left, Scute 1, Notch 1) and PR1-1 (Posterior Right, Scute 1, Notch 1). Notches (3-4mm) were small enough for two notch marks per marginal scutes. 15
<i>Figure 5</i>	Semi-natural pond and viewing station. Trees provided shade and the wooden pallets provided refuge areas as well as sloping basking platforms. 16
<i>Figure 6</i>	Mean maximum and mean minimum monthly temperatures and mean monthly precipitation for the moist Midlands mistbelt region of Pietermaritzburg, KwaZulu-Natal, South Africa. Data and graph are adapted and based on records collected by the Cedara Agricultural College weather station, Hilton, Pietermaritzburg, KwaZulu-Natal, South Africa. 18
	19

- Figure 7* Water surface (a), partially submerged (b) and entirely out of water (c) were the basking positions recorded for *Pelomedusa galeata*. These three positions were further categorised as (i) in full sun and (ii) in the shade, such that; water surface in full sun, water surface in the shade, partially submerged in full sun, partially submerged in the shade, entirely out the water and in full sun, and entirely out the water in the shade were the six positions observed.
- Figure 8* A temperature gradient in the water was created by allowing estimated ratios of hot ($\approx 47^{\circ}\text{C}$) and cold ($\approx 18^{\circ}\text{C}$) water, through holes drilled out of irrigation piping, into the runs. Water flow rates were similar for all six runs ($\approx 1.2\text{L}\cdot\text{min}^{-1}$). **22**
- Figure 9* Heated (47°C ; a) and cooled (18°C ; b) water was pumped out of hot and cold water baths, respectively, into two separate sections of irrigation pipe. Water mixing occurred at the beginning of each run (c), flowed towards the ‘free area’ where terrapins (*Pelomedusa galeata*) could access all runs, and back into the water baths via outlets (e and f). The outlets directed the warmest and coolest water to the hot and cold baths, respectively. **23**
- Figure 10* Reference points marked out on the aquatic thermal gradient to compare site choices in the control gradient (24°C throughout) and preferred body temperatures in alternated temperature gradients ($22\text{-}33^{\circ}\text{C}$, in both) for neonate and yearling *Pelomedusa galeata*. **24**
- Figure 11* Adult *Pelomedusa galeata* mass was correlated with girth ($R^2 = 0.92$), plastron length ($R^2 = 0.91$), and carapace length ($R^2 = 0.91$) and width ($R^2 = 0.91$). All variables were highly significantly correlated with mass, $df = 17$; $p < 0.001$. **28**
- Figure 12* *Pelomedusa galeata* winter body temperature (mean = $15.24 \pm 0.11^{\circ}\text{C}$ SE and set point range = $13.30\text{-}16.50^{\circ}\text{C}$) correlated with mass (mean = $1331 \pm 165\text{g}$ SE and range = $550\text{-}2700\text{g}$; $R^2 = 0.02$; $df = 14$; $p < 0.001$) and with ambient air temperature (mean = $19.94 \pm 0.13^{\circ}\text{C}$ SE and range = $16.50\text{-}23.40^{\circ}\text{C}$ and; $R^2 = 0.27$, $df = 14$; $p < 0.001$). **29**

- Figure 13** Total frequencies of five thermoregulatory behaviours displayed by *Pelomedusa galeata* (out the water in full sun, out the water in the shade, partially submerged in the sun, partially submerged in the shade and water surface basking), mean daily ambient air temperatures (T_a , solid line) and mean daily body temperatures (T_b , dotted line) for five periods (early morning, mid-morning, midday, mid-afternoon and early evening) during the day. Numbers above bars represent total frequencies of basking positions. Data were derived from observations and temperature data logger information for 11 days during winter, 2013. **31**
- Figure 14** *Pelomedusa galeata* summer body temperature (mean = $21.68 \pm 0.06^\circ\text{C}$ SE and set point range = $19.50\text{-}23.00^\circ\text{C}$) correlated with ambient air temperature (mean = $25.75 \pm 0.10^\circ\text{C}$ SE and range = $22.40\text{-}28.00^\circ\text{C}$; $R^2 = 0.15$, $df = 1$; $p < 0.001$). **32**
- Figure 15** Total frequencies of five thermoregulatory behaviours displayed by *Pelomedusa galeata* (out the water in full sun, out the water in the shade, partially submerged in the sun, partially submerged in the shade and water surface basking), mean daily ambient air temperatures (T_a , solid line) and mean daily body temperatures (T_b , dotted line) for five periods (early morning, mid-morning, midday, mid-afternoon and early evening) during the day. Numbers above bars represent total frequencies of behaviours. Data were derived from observations and temperature data logger information for 14 days during summer, 2013. **34**
- Figure 16** Mass was significantly different between age groups of hatchling *Pelomedusa galeata*, $t_{(15)} = 6.50$, $r_{(15)} = 0.87$, $p < 0.001$. Neonates ($9.86 \pm 0.22\text{g}$ SE) were significantly smaller than yearlings ($27.48 \pm 2.71\text{g}$ SE). **35**
- Figure 17** Hatchling *Pelomedusa galeata* mass was significantly correlated with carapace length ($R^2 = 0.92$), but not with plastron length ($R^2 = 0.98$), and carapace width ($R^2 = 0.99$). Only carapace length explained for a significant proportion of variation in mass, $df = 16$; $p < 0.001$. **36**

- Figure 18* Proportion of observations of *Pelomedusa galeata* hatchlings at 24 reference points in a control treatment (24°C) aquatic thermal gradient. Blue highlighted bars represent the ‘free area’ reference points at the end of six runs. **37**
- Figure 19* *Pelomedusa galeata* neonate (n = 8) and yearling (n = 8) body temperatures (T_b) were strongly related to temperatures of the aquatic thermal gradient (T_{ag} ; $R^2 = 0.99$, $p < 0.001$). Acclimation tests were run for the control gradient (24°C; T_b is clustered at ≈ 24 -25°C of T_{ag}), and forward and backward temperature gradients (22-33°C and 33-22°C, respectively; T_b is clustered at ≈ 28 -30°C; described in methods and materials). **38**
- Figure 20* *Pelomedusa galeata* neonates (n = 8) and yearlings (n = 8) mean relocation between temperature sites in the thermal aquatic gradient was negatively correlated with mass ($\beta = -0.10$, $R^2 = 0.34$, $p < 0.05$). **39**
- Figure 21* Mean preferred body temperature (T_{pref}) of *Pelomedusa galeata*, over 24 hours. Means were derived from T_{pref} of terrapins in a semi-natural environment at the UKZN Animal House, Pietermaritzburg for 14 days during summer (n = 17) and 11 days during winter (n = 16), 2013. **46**
- Figure 22* Design of a physical model to predict operative temperature for the *Pelomedusa galeata*. **68**
- Figure 23* Heating ($y = 0.83x + 7.06$; $R^2 = 0.98$) and cooling ($y = 1.40x - 10.97$; $R^2 = 0.91$) rates of operative temperature models compared with a terrapin of approximately mean mass (*Pelomedusa galeata*; 1.70kg). **69**
- Figure 24* iButtons were attached to cable ties (left) and were threaded through 3mm holes, 30mm apart. They were attached to the third and fourth posterior marginal scutes on the right side of the carapace of *Pelomedusa galeata*. **71**

LIST OF TABLES

	Page
<i>Table 1</i> Summary of mean body temperature and set point range of <i>Pelomedusa galeata</i> and mean and range of ambient air temperatures, including standard error for mean temperatures, during winter 2013 in Pietermaritzburg, South Africa.	29
<i>Table 2</i> Summary of mean body temperature and set point range of <i>Pelomedusa galeata</i> and mean and range of ambient air temperatures, including standard error for mean temperatures, during summer 2013 in Pietermaritzburg, South Africa.	32

ACKNOWLEDGEMENTS

Foremost, I thank my supervisor, Doctor Sue McConnachie, for all her time and effort spent on this project, for financial support and equipment, and her continual encouragement and advice when needed. Her enthusiasm and interest in this project never failed and I am grateful that she made herself available to me whenever I needed guidance. With many years of academic experience and wisdom, Professor Mike Perrin, my second supervisor, has provided me with advice, expertise and funding – for which I am very grateful. I thank the University Ethics Committee for granting ethical clearance for the use of *Pelomedusa galeata* in this project. I thank the National Research Foundation and the College of Agriculture, Engineering and Science for provision of funds. Thanks also to Doctor Oliver Tatham for performing surgical procedures, and Ebrahim Ally and team for assisting me in care of the terrapins, set up of equipment and advice on procedure. The administrative staff in the School of Life Sciences have been so helpful to me, special mention must be made of Jane Flockheart, Tanya Karalic, Natalie Jones, Pat Joubert and Renny Noble. Lastly, I thank my friends and family for support throughout the project, especially to Mark Summers for assistance in experimental trials and editing.

CHAPTER ONE: INTRODUCTION

It is important to understand the thermal biology of ectotherms to explain their physiology and ecological interactions (Huey and Slatkin 1976). Unlike endotherms, ectotherms may only attain body heat from their immediate surroundings (Seebacher 2005). Thermal environments, which are highly variable both spatially and temporally, affect the physiology and behaviour of ectotherms, because of its direct effect on ectotherm body temperature (T_b ; Huey and Slatkin 1976, Hertz et al. 1993, Tucker et al. 1995, Angilletta et al. 2002, Seebacher and Shine 2004). Some ectotherms can regulate their T_b by altering their behaviour and physiology, but this can only be achieved within the thermal limits of the ectotherm (Huey and Slatkin 1976, Smith et al. 1981, Smith et al. 1986, Bakken 1992, do Amaral et al. 2002). For these reasons, climate change, and particularly increasing global temperatures may alter ectotherm fitness and distributions (Gibbons et al. 2000, Buckley et al. 2008, Huey and Tewksbury 2009, Kearney et al. 2009).

Performance function of ectotherms may be positively influenced by increasing environmental temperatures and ectotherms in temperate zones will have greater opportunity to bask and absorb heat (Angilletta et al. 2002, Buckley et al. 2008, Huey and Tewksbury 2009, Kearney et al. 2009). However, in tropical and desert areas, activity time will be negatively influenced and ectotherms will have reduced performance, because higher temperatures will force diurnal ectotherms to seek shade and shelter more frequently (Angilletta et al. 2002, Huey and Tewksbury 2009, Kearney et al. 2009). There will be some species that are exceptions to these projected generalisations of future ectotherm behaviour and performance. Some temperate species, specialised for cooler temperatures, will fail to cope with higher environmental temperatures, while some tropical and dessert species will benefit because they are able to maintain stable and high T_b with very little thermoregulatory effort (Luiselli and Akani 2002).

Environmental temperature is a major determinant for reptile performance and function, hence understanding the thermal biology of reptiles is imperative for conserving species diversity (Margules and Usher 1981). Examining the thermal biology of individual species of reptiles will broaden our understanding of the effects environmental temperature has on fitness, life histories, distributions and behaviour of reptiles (Angilletta et al. 2002, Buckley et al. 2008, Huey and Tewksbury 2009, Kearney et al. 2009). Thermal biology is of fundamental importance to reptiles.

Thermal biology of reptiles

Body temperature is one of the most important and limiting factors of physiological performance in reptiles, where most rely entirely on their environment for thermal resources and are bound by species-specific temperature ranges or thermal thresholds (Magnuson et al. 1979, Reynolds and Casterlin 1979, Ben-Ezra et al. 2008, Dubois et al. 2009). Reptile performance is measured in the fitness of the organism in terms of locomotive ability, immune system function, searching for food and mates, and reproductive and growth potential. Critical thermal maximum (CT_{max}) and minimum (CT_{min}) limits are determined by a reptiles' potential to perform these functions, and when performance ceases, it has reached a T_b that exceeds its critical thermal limits (Cowles and Bogert 1944, Hutchison et al. 1966, Huey and Stevenson 1979, Angilletta et al. 2002).

Preferred body temperature (T_{pref}), set-point range (T_{set}) and operative body temperature (T_e) are measures used to describe and explain reptile ecophysiology (Hertz et al. 1993, Seebacher and Shine 2004). These measures are species-specific and are mostly achieved by changing behaviours in response to thermal fluctuations of the environment (Huey and Slatkin 1976, Smith et al. 1981, Smith et al. 1986, Hertz et al. 1993, Ivanov 1999, do Amaral et al. 2002, Seebacher and Shine 2004, 2005, Dubois et al. 2009). Most reptiles shuttle in and out of warmer and cooler areas to regulate their T_b , which may boost physical performance, survivorship and metabolism (Hertz et al. 1993, do Amaral et al. 2002, McConnachie et al. 2007, Dubois et al. 2009). Conversely, basking in open areas might increase the risk of predation and antagonistic encounters with other animals (McConnachie et al. 2007, Storey et al. 2008).

Counteracting the effects of thermal fluctuations in the environment by means of T_b regulation (thermoregulation) involves altering physiological processes and behaviour, which may be costly in terms of energy and predation risk (Huey and Slatkin 1976, Smith et al. 1981, Smith et al. 1986, do Amaral et al. 2002). However, reducing these costs may involve allowing T_b to parallel the thermal fluctuations of the environment (thermoconforming), rendering a reptile vulnerable to T_b that may exceed critical thermal limits. For this reason, thermoregulation should take precedence over thermoconforming because it results in immediate survival or death of a reptile (Wills and Beaupre 2000, McConnachie et al. 2007). However, genetic variability in a changing environment can be preserved through adaptive acclimation, thus ultimately increasing the chance of the species survival (Lagerspetz 2006).

Thermoregulation, thermoconforming and acclimation

Behaviour and physiology of reptiles co-vary so that they may achieve optimal functional ability within a given thermal range (Seebacher 2005, Glanville and Seebacher 2006). Thermoregulation, thermoconformation and acclimation are responses demonstrated by reptiles when dealing with hetero- and homogenous thermal environments. These responses are species-specific, depend on resource availability and predation risk, and result when there is a change in environmental temperature (Bligh and Johnson 1973, Patterson and Davies 1978, Pough and Gans 1982, Lagerspetz 2006).

Thermoregulation is achieved by making behavioural and/or physiological changes for the purpose of maintaining T_{pref} or optimal body temperature (T_o ; Huey and Slatkin 1976, Ivanov 1999, Angilletta et al. 2002, Seebacher 2005). Thermoregulation contributes to homeostasis and assists in more effective digestion of food and assimilation of energy, which in turn increases metabolic expense, but also increases chances for survival and searching for food and mates (Bligh and Johnson 1973, Pough and Gans 1982, Ivanov 1999, Aragón et al. 2001, Angilletta et al. 2002, Seebacher 2005, Glanville and Seebacher 2006). Aside from metabolic expense, thermoregulation may also be costly in terms of predation risk or antagonistic encounters with other individuals for resources or mates, in which case inactivity may be the better option (Huey and Slatkin 1976, Patterson 1991, Bury et al. 2000, Aragón et al. 2001, Tamplin 2006, Harlow et al. 2010).

Less active reptiles tend to be considered thermal generalists and they do not regulate their T_b as precisely as thermal specialists (Huey and Slatkin 1976). These are the thermoconformers, and they have wider T_{set} than thermoregulators. Generalist thermoregulation is widely considered less advantageous because narrower T_{set} ranges provide better conditions for optimal performance (Blouin-Demers and Nadeau 2005). By thermoconforming, a reptiles' T_b parallels that of the ambient/substrate temperature, which may reduce metabolic activity and energy requirements (Bligh and Johnson 1973, Huey and Slatkin 1976, Pough and Gans 1982, Blouin-Demers and Nadeau 2005, McConnachie et al. 2007). This may be beneficial at times, but may also reduce growth and reproductive potential and render reptiles more vulnerable to predation or death caused by extreme environmental conditions (Huey and Slatkin 1976, Blouin-Demers and Nadeau 2005, McConnachie et al. 2007).

To avoid these adverse effects of extreme thermal conditions, some reptiles will enter

a kind of 'torpor' or hibernation, allowing their T_b to parallel that of the environmental temperature (Seebacher 2005). Thermoconforming is, in some ways, similar to acclimation, whereby reptiles have the ability to alter their physiology so that they may cope with the surrounding thermal environment (Bligh and Johnson 1973).

When environmental temperature restricts a reptile's ability to reach T_{pref} , it may become habituated to an environmental thermal range that reaches to the extremes of its T_b range (Patterson and Davies 1978). This is known as acclimation and it allows the individual to function as optimally as possible, even if it is not at T_{pref} (Patterson and Davies 1978). In doing so, reptiles are able to decrease energy expenditure, alter their physiology and survive at temperatures outside of T_b range specific to their species and distribution (Patterson and Davies 1978, Reynolds and Casterlin 1979, Seebacher 2005, Glanville and Seebacher 2006). There may even be variation within species that have broad T_b and altitudinal ranges, where those living at higher altitudes are acclimated to cooler temperatures and those at lower altitudes, warmer temperatures (Patterson 1991). By changing physiological and behavioural traits, to achieve T_{pref} , acclimation results in varying "final preferendums" of reptiles (Reynolds and Casterlin 1979). Reptile T_{pref} can be attained within two hours of exposure to temperatures that reach beyond acclimation temperature, and individuals will continue to select for these temperatures even if there are environmental temperatures suitable for improved performance (Reynolds and Casterlin 1979).

Factors affecting thermoregulation

It was previously thought that certain reptiles may only be able to occupy a zone that falls within their thermal range – known as the thermal specialisation theory (Seebacher 2005). However, more recent studies have shown that reptiles are capable of selecting microhabitats that are less thermally variable than the general surrounding habitat (Smith et al. 1981, Seebacher 2005, Fitzgerald and Nelson 2011). Although reptile T_b are constrained by the thermal environment, some are able to maintain stable T_b through thermoregulation; however, the thermal environment may not be the only factor affecting temperature management in reptiles.

Some reptiles may be exposed to highly variable temperatures between seasons, and may thermoregulate (and thermoconform) within broader T_{set} than those exposed to less

variable seasonal temperatures (Seebacher 2005). Mass and reproductive potential may also influence ability to thermoregulate, because size influences ability to retain heat and gravity may affect temperature requirements. Increases in T_b have also been noted in response to stress, and may well be a physiological adaptation for readying the animal for an escape, as higher T_b can result in better muscle contraction, thus the animal can make a faster escape (Cabanac and Bernieri 2000). Environmental temperature, season, mass, growth and reproductive potential, and resource availability are factors affecting physiological and behavioural thermoregulation, which are outlined below.

Environmental temperature

Solar radiation directly influences thermal habitats of reptiles (aerial, terrestrial and aquatic environments). Additionally, there is heat transfer between substrates, mediums and reptiles, which directly affect reptile T_b . To avoid continual warming or cooling alongside these substrates and mediums (thermoconforming), reptiles change their body posture and orientation to the sun (Cowles and Bogert 1944, Huey 1974, Huey and Pianka 1977). Some mediums, like water, require less thermoregulatory effort because of its homogeneous nature. At times, mean substrate or medium temperature is similar to T_{pref} and remaining at a single site may be beneficial (Law and Bradley 1990). Terrestrial environments are more thermally heterogeneous, and require greater thermoregulatory effort. Thus, by shuttling between warmer and cooler sites, active reptiles narrow the environmental thermal range (Manning and Grigg 1997, Ben-Ezra et al. 2008, Dubois et al. 2009). In doing so, they can also avoid temperature extremes and reach their T_{pref} (Manning and Grigg 1997, Dubois et al. 2009).

Reptiles may make use of behaviours, other than shuttling between warm and cool temperatures, to thermoregulate in heterogeneous environments, because moving around to find a suitable thermal environment may not always be possible. For example, reptiles that live in extreme hot and dry environments may be forced to make use of evaporative thermoregulation, by means of salivation over the head and front legs and/or urination over the back legs, to cool themselves when necessary (Riedesel et al. 1971).

Season

Behavioural and physiological thermoregulation assists in maintenance during periods of extreme climatic conditions. Between seasons, temperate zones experience higher temperature variation than tropical zones and reptiles respond to these environmental conditions with various behavioural and physiological mechanisms. Heat-hardening is associated with high environmental temperatures and is a response more common during summer in temperate regions, as well as in tropical regions (Hutchison and Maness 1979, Lagerspetz 2006); while thermoconforming or periodic acclimation are strategies associated with cold, winter temperatures (Hutchison et al. 1966, Lagerspetz 2006).

Reptiles subjected to low temperatures or very dry conditions may enter periods of hypoxia (or hibernation) and slow their metabolism or reduce muscle movement and energy expenditure (Aleksiuk 1976, Glass et al. 1978, Adolph and Porter 1993, Hailey and Loveridge 1997, Storey and Storey 2001, Seebacher 2005). Similarly, the effect of aestivation during extreme hot periods coupled with the inability to forage while taking refuge in cooler retreat sites, may be energetically costly (Norris 1953, Hailey and Loveridge 1997).

Mass

The effect of mass on a reptile's ability to thermoregulate is largely as a result of surface-to-mass ratios increasing with decreasing body mass. Small reptiles (<0.1kg) will gain and lose heat more readily than large reptiles (>10kg; Stevenson 1985a), and in response to this, they tend to conform to environmental temperatures more often than regulate their T_b (Bury et al. 2000, Harlow et al. 2010). Large reptiles have the advantage of greater thermal inertia than small reptiles, allowing them to retain heat over longer periods of time (Cowles 1945, Fitzgerald and Nelson 2011). Moderately sized reptiles (0.1-10kg) have greater opportunity to regulate T_b because they are an intermediate between large, heat retaining and small, highly thermally conductive reptiles (Stevenson 1985a, b).

Greater heat retention and lower thermal sensitivity in large reptiles enables them to have better overall fitness than smaller, more thermally sensitive reptiles (Dubois et al. 2009). This is because potential to thermoregulate more precisely increases with increasing body size (Smith et al. 1986, Tamplin 2006). Adult leatherback turtles (*Dermochelys coriacea*) are

large enough to retain heat produced via movement and metabolism (Bostrom and Jones 2007, Bostrom et al. 2010). Living in homogeneous environments, as these turtles do, requires less emphasis on behavioural or physiological changes to thermoregulate, than in heterogeneous environments.

Growth and reproductive potential

Thermoregulation is innately linked to well-maintained T_b , because it will have positive effects on both growth and reproductive potential. Reptiles bask to increase their metabolism, with the intention of improving digestive and assimilative efficiencies for gaining energy so that they may search for food, grow, find mates and produce viable offspring (Gatten 1974, Jarošík et al. 2004, Carrière et al. 2008). Females may thermoregulate differently to males when gravid because basking for greater lengths of time may be necessary for energy assimilation (Schwarzkopf and Shine 1992, Carrière et al. 2008). Males may focus less on the energetic benefits of thermoregulation and spend more time in search of mates (Carrière et al. 2008).

Resource availability

Metabolic activity in reptiles is highly dependent on the thermal environment, whereby metabolic rate (rate of converting energy) increases with increasing environmental temperature and T_b (Gillooly et al. 2001). In turn, energy demands of reptiles are increased with higher metabolic rates (Gillooly et al. 2001). They must therefore have access to or be capable of foraging for food when environmental temperatures are high. Sometimes reptiles are incapable of feeding when temperatures are too low, in which case it is energetically more efficient to acclimate to the environmental temperature (Patterson and Davies 1978).

Attaining environmental temperatures that are physiologically beneficial to a reptile may not always coincide with what is ecologically possible because of competition for thermal resources (Huey and Slatkin 1976, Magnuson et al. 1979, Cadi and Joly 2003). Cost-benefit trade-offs associated with reaching optimal function at risk of predation or antagonistic encounters may affect thermoregulatory opportunity for a reptile (Huey 1974,

Huey and Slatkin 1976). For example, hierarchy in social dominance may affect T_b , whereby dominant individuals will occupy the preferred environmental temperature range more often than subordinate individuals (Magnuson et al. 1979).

Thermal ecology of terrestrial, aquatic and semi-aquatic reptiles

Terrestrial reptiles are better known for shuttling between shaded and sunny areas (Cowles and Bogert 1944, Huey and Slatkin 1976), while aquatic and semi-aquatic reptiles bask at the surface of the water (in the case of fully-aquatic and marine reptiles; Spotila and Standora 1985, Paladino et al. 1990) or shuttle between aerial basking and submersion in water (as in semi-aquatic reptiles; Boyer 1965, Spellerberg 1972, Gatten 1974, Peterman and Ryan 2009). Rarely, green sea turtles bask on the shoreline, which is particularly unusual for marine turtles (Balazs and Ross 1974, Whittow and Balazs 1982, Spotila and Standora 1985). Aquatic and semi-aquatic reptiles are usually exposed to a greater range in the thermal environment than terrestrial reptiles, and therefore make use of a number of different behavioural and physiological manipulations for thermoregulation (Seebacher and Franklin 2005). There are many studies on thermoregulation and associated thermoregulatory behaviours of lizards and snakes that describe the costs and benefits involved in T_b maintenance (Boyer 1965, Meek and Avery 1988, Shine and Madsen 1996, Dubois et al. 2009, McConnachie et al. 2011), whilst there have been few studies on aquatic and semi-aquatic reptiles in Africa, and even fewer that relate to terrapin thermoregulatory behaviour (Boyer 1965, Meek and Avery 1988, Dubois et al. 2009). It is important to understand the species-specific thermal biology of reptiles, as well as the associated costs and benefits of their thermoregulatory habits, to better understand the ecology of reptiles (Seebacher and Franklin 2005).

Heterogeneous terrestrial environments are more energetically costly to live in than homogenous aquatic environments, because reptiles are subjected to a wider range of environmental temperatures than in aquatic environments. The thermal inertia of water protects reptiles in aquatic environments from extreme temperature changes, while highly variable ambient air temperatures (T_a) fluctuate more rapidly (Spotila and Standora 1985, McConnachie et al. 2011). Loggerhead (*Carretta carretta*), green (*Chelonia mydas*) and olive ridley sea turtles (*Lepidochelys olivacea*) T_b equilibrate to sea water temperature readily

because they lose heat very quickly in water (Spotila and Standora 1985). As a result, they may not require as much energy to regulate their T_b and may acclimate to thermal environments more often than terrestrial reptiles (Luiselli and Akani 2002, Seebacher and Franklin 2005, McConnachie et al. 2011).

Semi-aquatic reptiles benefit from exploiting both terrestrial and aquatic environments (Meek and Avery 1988, Storey et al. 2008, McConnachie et al. 2011). In terrestrial environments semi-aquatic reptiles can enjoy the benefits of basking and relocating to more suitable areas, and in aquatic environments they benefit from better predator avoidance, decreased thermoregulatory and metabolic expense, feeding and mating (Seebacher and Franklin 2005, Storey et al. 2008, McConnachie et al. 2011). The ability to make use of both these environments gives reptiles, like terrapins, an additional dimension to their thermoregulatory performance (Meek and Avery 1988). Basking outside the aquatic environment is particularly interesting in terrapins and it is important for thermoregulation in most species, but not all (Manning and Grigg 1997, Bury et al. 2000, Seebacher 2005). Examples of semi-aquatic reptiles that do not tend to thermoregulate are Australian water pythons (*Liasis fuscus*; Shine and Madsen 1996) and some species of colubrid water snakes (*Natriciteres fuliginoides*, *N. variegata*, *Afronatrix anoscopus*, and *Grayia smythii*; Luiselli and Akani 2002), which are able to maintain stable T_b with little thermoregulatory effort. Boyer (1965) has done one of the first comprehensive works on thermoregulation in turtles and terrapins, and from it has stemmed many studies. Turtles and terrapins are interesting for their thermoregulatory habits because of their ability to exploit both terrestrial and aquatic environments for T_b maintenance.

Advantages of basking for turtles and terrapins

Basking behaviour in all reptiles is defined by the physical environment and physiology of reptiles (Boyer 1965). Light intensity and incidence angle, water temperature and T_a , and wind and cloud cover are important predictors for operative temperature (T_e) of reptiles, and behaviour, shape, mass, sex and species determine their thermoregulatory traits (Boyer 1965, Crawford et al. 1983). In places where extreme cold conditions occur during winter, there may be very little or no opportunity for basking, but overwintering in water bodies might improve chances for survival in terrapins and thermoconforming behaviour may be the only T_b management option (Greaves and Litzgus 2007, Rollinson et al. 2008). Water not only

acts as a buffer against extreme and constantly changing thermal environments, but can be more effective for heat transfer than ambient air (Smith et al. 1981, Fitzgerald and Nelson 2011). Many terrapins are known to thermoregulate by basking at the water's surface, and is the preferred method of basking in some species when the cost of aerial basking is high (Tamplin 2006, Storey et al. 2008). Not only does water assist in T_b maintenance, terrapins also rely on water for feeding, reproduction and predator avoidance, and if T_b is already at environmental temperature they are less likely to bask (Crawford et al. 1983, Chessman 1984). Terrapins must however, leave the water periodically for other activities including; aestivation, nesting and migration between water bodies (Chessman 1984).

Studies have suggested that basking is also an immune system booster, and when afflicted with infection and/or parasites, turtles and terrapins will bask more frequently and show preference for aerial basking (Manning and Grigg 1997, Ryan and Lambert 2005, Swimmer 2006). Green turtles (*Chelonia mydas*) with fibropapillomatosis basked more often to increase T_b and enhance immune response to resist infection (Swimmer 2006). Map (*Graptemys geographica*) and common musk turtles (*Sternotherus odoratus*) showed similar responses to infestations of parasite carrying leeches, and exhibited aerial basking more frequently to reduce the number of leeches (Ryan and Lambert 2005).

Generally, it is accepted that the main reason behind basking in turtles and terrapins is for thermoregulation, and that the benefit of basking for all other activities is a secondary effect (Boyer 1965). However, some species (particularly those in tropical climates) have shown little dependence on basking for thermoregulation. Turtles that have not been shown to thermoregulate include *Emydura signata*, which thermoconforms to water temperature (Manning and Grigg 1997), and *Chelydra serpentina* hatchlings which has low T_{pref} (relative to *Trachemys scripta* hatchlings) and infrequently leaves water (Bury et al. 2000). This demonstrates the need for species-specific studies on thermoregulation of turtles and terrapins. Given the rarity of some turtle and terrapins species, it is better to focus experimental work on species that are not critically endangered. For this reason, the African side-necked terrapin (*Pelomedusa subrufa*) was selected for this study.

Pelomedusa subrufa was previously considered a pan-African species and at no risk of extinction. It has since been reassessed and molecular analyses have revealed that the phylogeny of *P. subrufa* is more divided than originally thought. Petzold et al. (2014) have distinguished between these species through mitochondrial and nuclear DNA fragments. One species is common in KwaZulu-Natal, South Africa – *Pelomedusa galeata*. This species has recently been resurrected in a revision of the taxonomic classification of the African side-

necked terrapin (Petzold et al. 2014), therefore this study is one a few on *P. galeata*, and perhaps the first detailed work on T_b and basking behaviour of this species. I hypothesised that *P. galeata* would make adjustments in behaviour to achieve a T_{pref} , and that there would be differences in behaviour, T_{pref} and T_{set} among seasons and mass.

Aims and objectives

The main aim of this study was to investigate the thermoregulation of *P. galeata*. My objectives were to:

- To quantify field T_{pref} and T_{set} in adult terrapins
- To observe basking behaviour of adult terrapins in a semi-natural environment
- To quantify T_{pref} and T_{set} of hatchling terrapins in an aquatic thermal gradient

CHAPTER TWO: METHODS AND MATERIALS

Study animal

Pelomedusa galeata (Petzold et al. 2014) was first described by Johann David Schoepff in 1792. It was then grouped under the name of *Pelomedusa subrufa*, which was considered a pan-African species. Its wide-spread distribution occupied 16.2 million km² of sub Saharan Africa (except in mountainous and severely dry areas), and as far north as the coastline of the Mediterranean region (Branch 1998, Wong et al. 2000, Alexander and Marais 2007, Branch 2008, Buhlmann et al. 2009, Vargas-Ramírez et al. 2010, Bates et al. 2014). Although *P. subrufa* was thought to be a single species divided into three sub-species (southern, western and eastern), nine deeply divergent lineages were identified in literature (Branch 1998, Wong et al. 2000, Alexander and Marais 2007, Branch 2008, Vargas-Ramírez et al. 2010, Fritz et al. 2011, Bates et al. 2014).

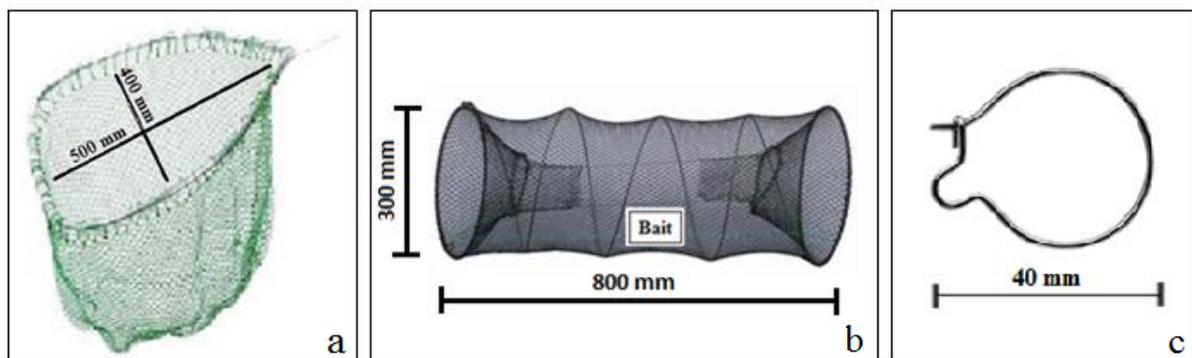
Pelomedusa galeata (as it has been re-established; Fritz et al. 2011, Petzold et al. 2014) is known to occupy most of South Africa (KwaZulu-Natal, Eastern Cape, Western Cape, North West, Northern Cape, Free State and Gauteng provinces). Currently, there appears to be two deeply divergent clades that suggest species divergence, however, it has been suggested they remain as one species (*P. galeata*) until further analyses are complete (Petzold et al. 2014). *Pelomedusa galeata* is relatively large (carapace length up to 325mm), and males are larger than females (Boycott et al. 2008). They are diurnal and mainly aquatic, preferring slow moving rivers and streams, still water, dams and ponds (Alexander and Marais 2007, Branch 2008). They are commonly found in temporary wetlands (Cowles 1936, Branch 1998, Alexander and Marais 2007, Branch 2008, Boycott 2014).

Adult terrapins, greater the 500 grams, were collected from areas in and around Pietermaritzburg, KwaZulu-Natal, South Africa (29.62°S, 30.38°E). Sites were between 760-1400m above sea level and in the Midlands mistbelt grassland, which is characterised by a temperate climate (Scott-Shaw and Escott 2011). The area is frequently misty in summer and frosty in winter, with summer annual rainfall between 900-1150mm (Cedara Agricultural College weather station). Adult terrapins were maintained in a semi-natural pond and hatchlings were born and raised in captivity. Hatchlings were maintained in a controlled environment, in 1m tanks.

Adult terrapins

Capture and marking

Twenty African side-necked terrapins (*Pelomedusa galeata*; 3 male, 17 female) were collected from the Midlands area of KwaZulu-Natal, South Africa. Terrapins were collected by hand, dip net, funnel trap and caught on a line using a fishing rod and a bait rigger (to avoid piercing the mouth of the animal; figure 1c). I baited funnel traps and bait riggers with fresh sardines, hake or chicken liver. A dip net was used to catch terrapins within reach from the perimeter of the ponds/dams. Funnel traps were set up for 24 hour periods, and monitored and re-baited each day. The funnel traps were anchored, in the shallows, to the shoreline of the ponds and dams using tent pegs. Approximately 5-8cm portions of the traps remained above the water surface to allow terrapins to breathe. The bait rigger was used to attach bait to a fishing line. Once a terrapin was “hooked”, it was drawn in slowly by line and when within reach was netted with a dip net. Terrapins were measured on straight carapace length and width, and plastron length using a rigid cm ruler to the nearest mm (figure 2), terrapins were weighed using an electronic balance to the nearest 0.1g, and terrapin sex was recorded. Only individuals larger than 500 grams were collected as they were considered large enough for implanting temperature data loggers (Thermochron iButton®, Dallas Semiconductor, Texas, USA; see below).



*Figure 1: Dip net (a), funnel trap (b) and bait rigger (c). All were used to capture terrapins; the bait rigger was used to attach bait to a fishing line, the dip net was used to catch *Pelomedusa galeata* within reach and the funnel trap was set up and left for 24 hour periods.*

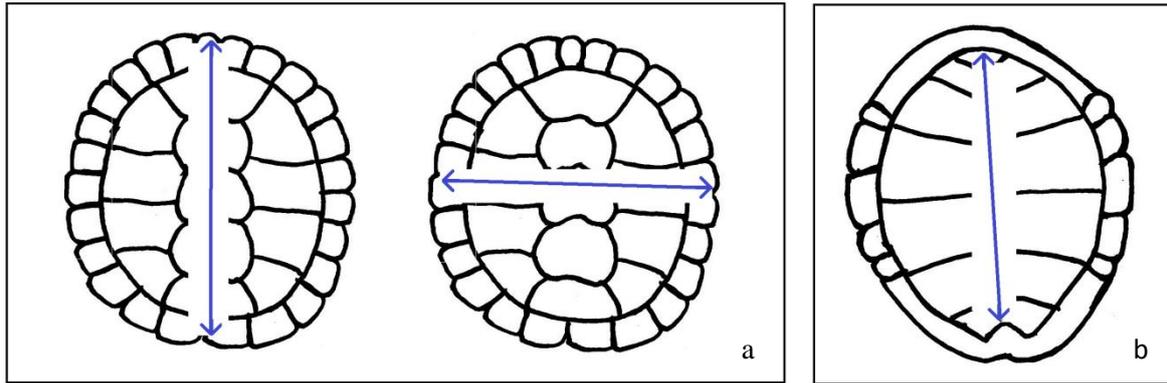


Figure 2: Straight measurements of *Pelomedusa galeata* carapace length and width (a) and plastron length (b) taken with a rigid ruler.

All terrapins were assigned and marked with a colour coded number for identification purposes. They were marked with brightly coloured non-toxic paint so that they were easily identifiable from a distance (figure 3). Terrapins were also permanently marked by notch marking the marginal posterior and anterior scutes (figure 4) using a rotary tool (Tork Craft TC 08720, 230V ~ 50Hz 135W, no.: 8000-32500min⁻¹) with a 3mm engraving bit (methods similar to Cagle 1939). Notch marks were round, 3-4mm in diameter and smaller than those described by Cagle (1939) and plastron plates were not notch marked. Notch marking terrapins ensured that if paint marking faded, they were still identifiable (figure 4).



Figure 3: Colour coded numbers painted on *Pelomedusa galeata* carapaces for easy identification.

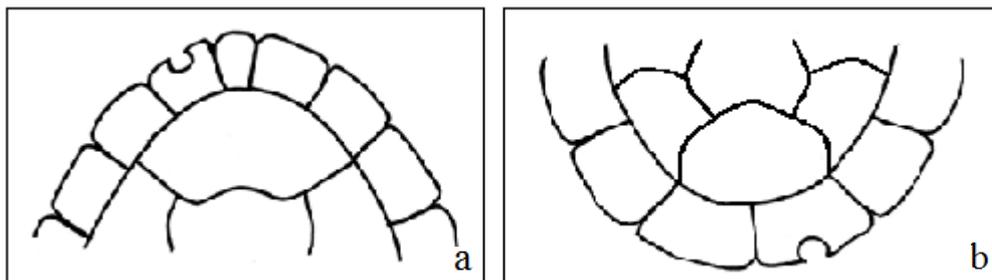


Figure 4: One notch mark on the first left anterior marginal scute (a) and the first right posterior marginal scute (b). Respective codes are AL1-1 (Anterior Left, Scute 1, Notch 1) and PR1-1 (Posterior Right, Scute 1, Notch 1). Notches (3-4mm) were small enough for two notch marks per marginal scutes.

Housing and husbandry

Terrapins were kept in the Animal House at the University of KwaZulu-Natal, Pietermaritzburg, South Africa, for the duration of the study. Terrapins were maintained in an open paddock, where they had access to a pond (5.5m x 5.5m; 50cm at the deepest point; figure 5). The area allowed for opportunity to access shade and full sunshine. Wooden pallets were placed in the water near the edge of the pond to provide sloping basking sites (figure 5).

The terrapins were maintained on a diet of fish, fruit, vegetables and a specially prepared “turtle pudding” – meat, fish, vegetables and fruit in a gelatine base. Food was supplied once a week so there was no surplus food debris to foul the water and live Mozambique tilapia (*Oreochromis mossambicus*) were introduced to the pond to assist in maintaining water quality.

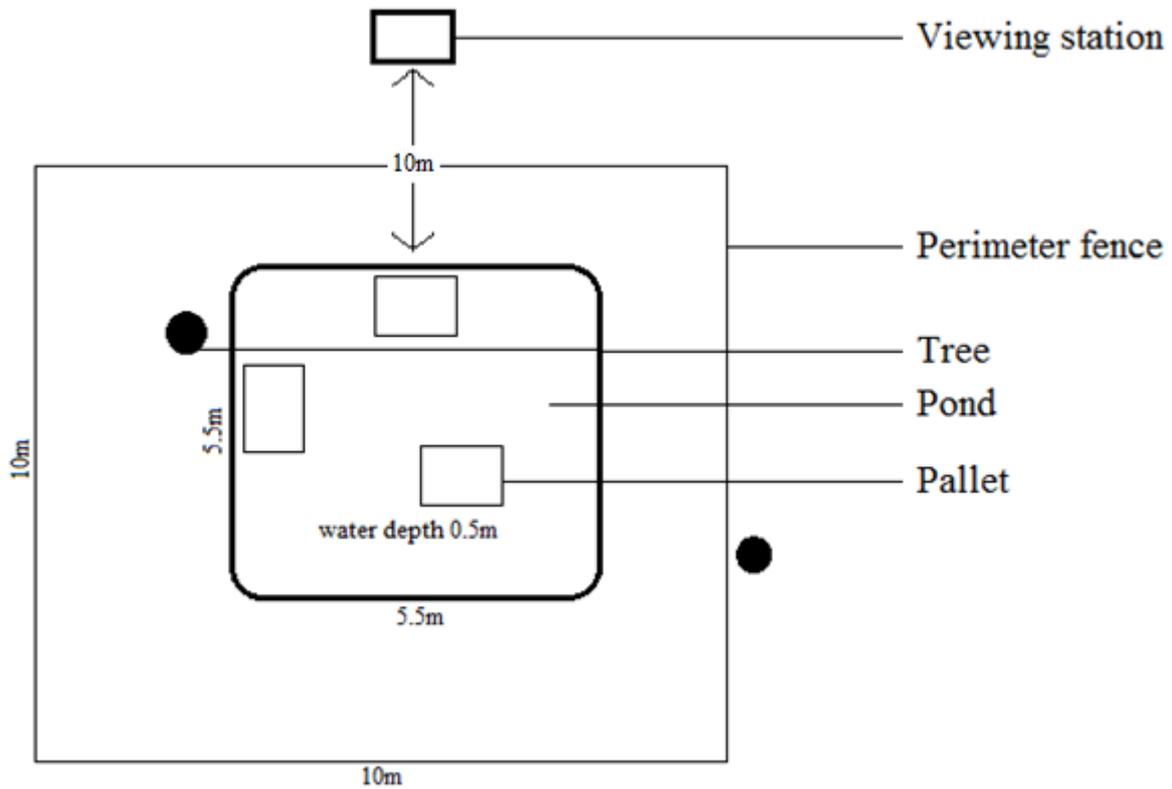


Figure 5: Semi-natural pond and viewing station. Trees provided shade and the wooden pallets provided refuge areas as well as sloping basking platforms.

Preferred body temperature

To measure preferred body temperature (T_{pref}), temperature data loggers (iButtons; Thermochron iButton®, Dallas Semiconductor, Texas, USA) were surgically implanted into terrapins under anaesthetic. A combination of three different anaesthetics was used for surgery: Medetomidine (1mg/ml; 0.05ml per kg), Ketamine (100mg/ml; 0.05ml per kg) and Butorphenol (10mg/ml; 0.05ml per kg). All iButtons were placed in the centre of the

terrapiin's coelomic cavity. The inner muscle layer was stitched together with two sutures and the skin was stitched together with three to four sutures. For stitching, a 19mm reverse cut $\frac{3}{8}$ circle needle and monofilament nylon 4/0 sized (NY974-1) thread was used. Two iButtons were set to record body temperature (T_b) every 30 minutes and an additional two iButtons recorded T_b every 90 minutes. The iButtons were programmed using Cold Chain (v. 4.9).

Operative temperatures

See appendix A1 for details on methods

Thermal environment description

Mean monthly precipitation, and mean maximum and minimum temperature data were retrieved from the Cedara Agricultural College weather station. Cedara Agricultural College is situated in the Midlands mistbelt grassland region of Pietermaritzburg, KwaZulu-Natal, South Africa. The data were a collation of records for the area between 2000 and 2012, and are graphed below (figure 6).

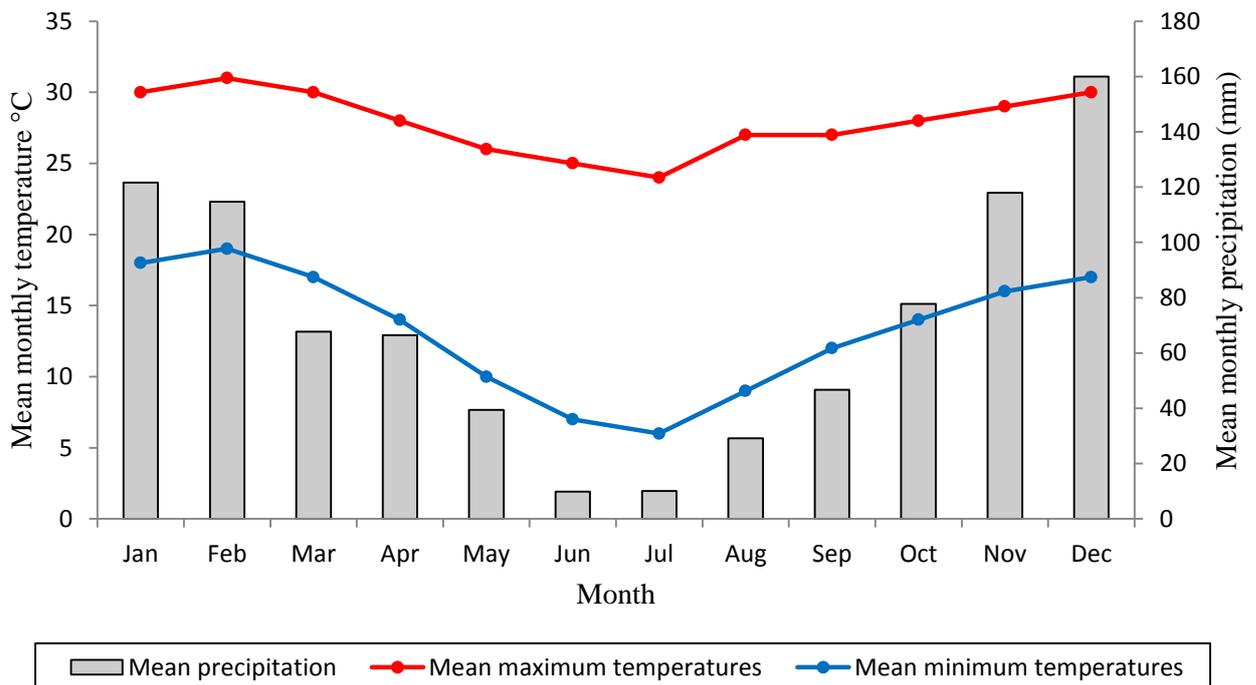


Figure 6: Mean maximum and mean minimum monthly temperatures and mean monthly precipitation for the moist Midlands mistbelt region of Pietermaritzburg, KwaZulu-Natal, South Africa. Data and graph are adapted and based on records collected by the Cedara Agricultural College weather station, Hilton, Pietermaritzburg, KwaZulu-Natal, South Africa.

Basking behaviour

For 14 days in summer and 11 days in winter, 2013, terrapins were observed over the same period of time (see below), from a vantage point 2m high and 5m away from the pond (figure 5). Feeding, mating and basking were behaviours recorded. Figure 7 outlines basking postures observed and includes the following:

1. Water surface basking, with only the upper part of the carapace exposed (a);
2. Partially submerged (b);
3. Out the water entirely (c).

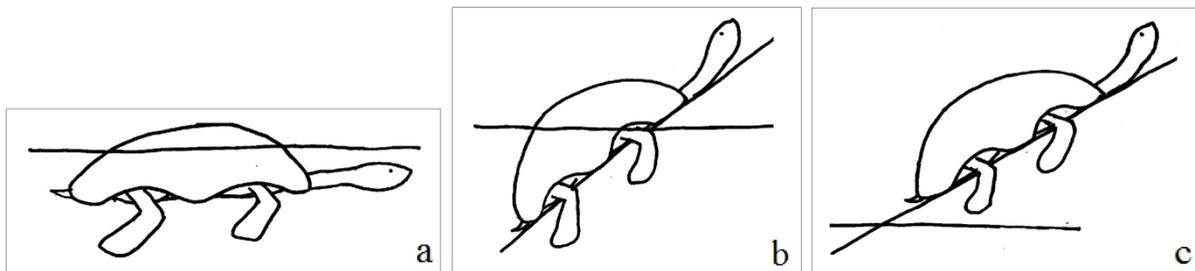


Figure 7: Water surface (a), partially submerged (b) and entirely out of water (c) were the basking positions recorded for *Pelomedusa galeata*. These three positions were further categorised as (i) in full sun and (ii) in the shade, such that; water surface in full sun, water surface in the shade, partially submerged in full sun, partially submerged in the shade, entirely out the water and in full sun, and entirely out the water in the shade were the six positions observed.

Terrapins were observed over 2 hour periods, for five periods of the day, between 07:00 and 18:00 (period 1 = 07:00-09:00; period 2 = 09:30-11:30; period 3 = 11:30-13:30; period 4 = 14:00-16:00; period 5 = 16:00-18:00). Point observations of terrapin behaviour were recorded every 5 minutes during each period and all visible terrapins were noted for their position, as well as their position relative to direct sunlight (whether in full sun or shade), and relative to the water (whether in, out or partially submerged in the water; figure 7). Ambient air temperature (T_a) was recorded at the beginning of each observation period.

After observations were completed, adult terrapins were euthanized because iButtons could not be removed without causing severe internal damage. X-rays were of little use because of the nature of the invasive surgery required for retrieval of iButtons. Additionally, I

was advised by the veterinarian that euthanasia would be more appropriate (O Tatham, 2014, pers. comm.¹). Terrapins were euthanized using Euthapent (intra-coelomic, pentobarbitone sodium 200 mg/ml; 2ml per kg). When blink reflexes ceased, and the terrapins were unconscious, their spinal cord was severed using a scalpel (decapitation) to ensure death (American Veterinary Medical Association, 2013; O Tatham, 2014, pers. comm.¹). Both shell bridges were cut using a hacksaw and the pelvic girdle separated from the plastron so that the coelomic cavity was accessible and iButtons could be removed.

The iButtons were removed and temperature data were extracted using Cold Chain (v. 4.9). Data were transferred into SPSS (v. 21) and mean T_b per period per season was calculated to obtain T_{pref} . Body temperature data were compared to observation and environmental data. Only active T_b was considered for analysis, and was calculated as T_b from first peak to T_b preceding a three hour decline. Mean daily T_b (considered T_{pref}), and set point range (T_{set} ; the first and third T_b quartiles) were calculated (after Hertz et al. 1993).

Statistical Analyses

All statistical analyses were conducted with SPSS v. 21. Pearson correlation analyses were performed between mass, girth, plastron length, and carapace length and width, to determine which body measurements should be included for further analysis. Mean T_b , T_a and total frequencies of the six basking positions (1. water surface, in the sun; 2. water surface, in the shade; 3. partially submerged, in the sun; 4. partially submerged, in the shade; 5. out the water, in the sun; and 6. out the water, in the shade; figure 7) were calculated per two hour period over all days during both summer and winter.

Body temperature, T_a and basking frequency data were tested for normality, which could not be satisfied through data transformations, thus loglinear models were conducted to assess the data. I analysed summer and winter data separately to simplify the interactions between predictors (basking position and period of the day). Frequencies of basking positions for all terrapins were combined for each period over all the days in each season. The aggregated behaviour data were tested for interaction and main effects of basking behaviour and period, with mass and mean T_a as covariates. Mean T_b , basking position frequency, and mean T_a per two hour periods were graphically presented for both summer and winter to

¹ Dr. O Tatham, Veterinary House Hospital, Prince Alfred Street, Pietermaritzburg, South Africa.

provide a simple visual analysis of the thermoregulatory trends exhibited by *P. galeata*.

Hatchling terrapins

Marking and Husbandry

Thirty two captive bred hatchling African side-necked terrapins were notch marked (using a 2mm needle file), weighed on a digital balance (to the nearest 0.1g) and straight measurements of plastron length, and carapace length and width were made using an electronic calliper (to the nearest 0.01mm). Sixteen individuals were approximately one year old (clutches of March/April 2013), and sixteen neonates (clutches of March/April 2014) were maintained in the Animal House, at the University of KwaZulu-Natal, Pietermaritzburg, South Africa. All hatchlings were housed in 1m tanks, fed *ad libitum* on water plants, Tubifex Worms (freeze dried worms, Far East Freeze and Drying Mfg Co, Ltd), Nutrafin Max Turtle Pellets with Gammarus Shrimp (Hagen, Rolf C. Hagen (U.S.A) Corp., Mansfield, MA, 02048) and supplemented with the same diet as the adults. All tanks were equipped with tiles for basking platforms retreat sites, and a reptile UV light, with lights on a 12h light: 12h dark cycle. Ambient room temperature ($26^{\circ}\text{C} \pm 1$) and water temperature ($24^{\circ}\text{C} \pm 1$) were kept constant throughout the year.

Aquatic Thermal Gradient

An aquatic thermal gradient was constructed using a cardboard box as the outer frame (1.26m x 1.07m x 0.30m). Standard packaging Expanded Polystyrene (EPS; 25mm, thermal conductivity 0.03 W/mK) was glued to the inner side of the base (1.26m x 1.07m) and walls (0.3m depth) of the box. Extruded Polystyrene (XPS; polyisocyanurate (PIR) IsoBoard, 25mm, R value of R-0.299 to R-0.314 per mm) was glued in for the run walls (0.8m x 0.3m). This high density, moulded foam board was rigid, non-permeable and had high insulation properties (thermal conductivity 0.023 W/mK). The outer frame was divided into six runs (0.18m x 0.80m x 0.20m) and a 'free area' (1.21m x 0.27m x 0.30m) was available to the terrapins, which allowed them access to all runs. Figure 9 (below) depicts the layout described above.

All interior and exterior surfaces were covered in a layer of roof waterproofing membrane (Powa Fix wall mesh, Wandsbeck, South Africa) and paint (dark grey Acry Proof™ Multi-purpose waterproofing, ProLong Quality Products, Dura Paints, Edenvale, South Africa). Drying time between coats of paint was 24 hours and the gradient was considered safe for animal use seven days after the final coat of paint was applied (as per pond maintenance instructions, Acry Proof™ Multi-purpose waterproofing, ProLong Quality Products, Dura Paints, Edenvale, South Africa).

Hot (47°C) and cold (18°C) water baths were attached to opposite sides of two separate pieces of irrigation pipe (150cm in length, 20mm diameter); both were closed off at one end. To create a gradient in water temperature, holes of different sizes (3, 5, 6, 7, and 8mm) were drilled in the pipe, such that the first run had only hot water running into it and the last run had only cold water running into it (8mm hole, no mixing of water). From left to right of the gradient, inlet size combinations were as follows: 8 and 0mm, 7 and 3mm, 6 and 5mm, 5mm, 6mm, 3mm, 7mm, 8mm (Figure 8).

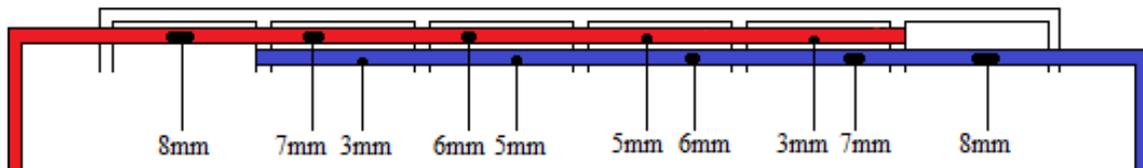


Figure 8: A temperature gradient in the water was created by allowing estimated ratios of hot ($\approx 47^\circ\text{C}$) and cold ($\approx 18^\circ\text{C}$) water, through holes drilled out of irrigation piping, into the runs. Water flow rates were similar for all six runs ($\approx 1.2\text{L}\cdot\text{min}^{-1}$).

A temperature gradient (22°C to 33°C) was created as the water mixed in each run, aided by an aquarium aeration stone ($10\text{cm} \times 2\text{cm} \times 2\text{cm}$) that was placed 20cm from the inlet/s (figure 8). The aeration stone helped to avoid water stratification so that each run would maintain a moderately consistent temperature throughout the length of the run. The flow of the water was regulated by water pumps, tube clamps and degree of incline of the structure, such that the volumetric flow rate (approximately 1.2 litres per minute) in all runs was similar.

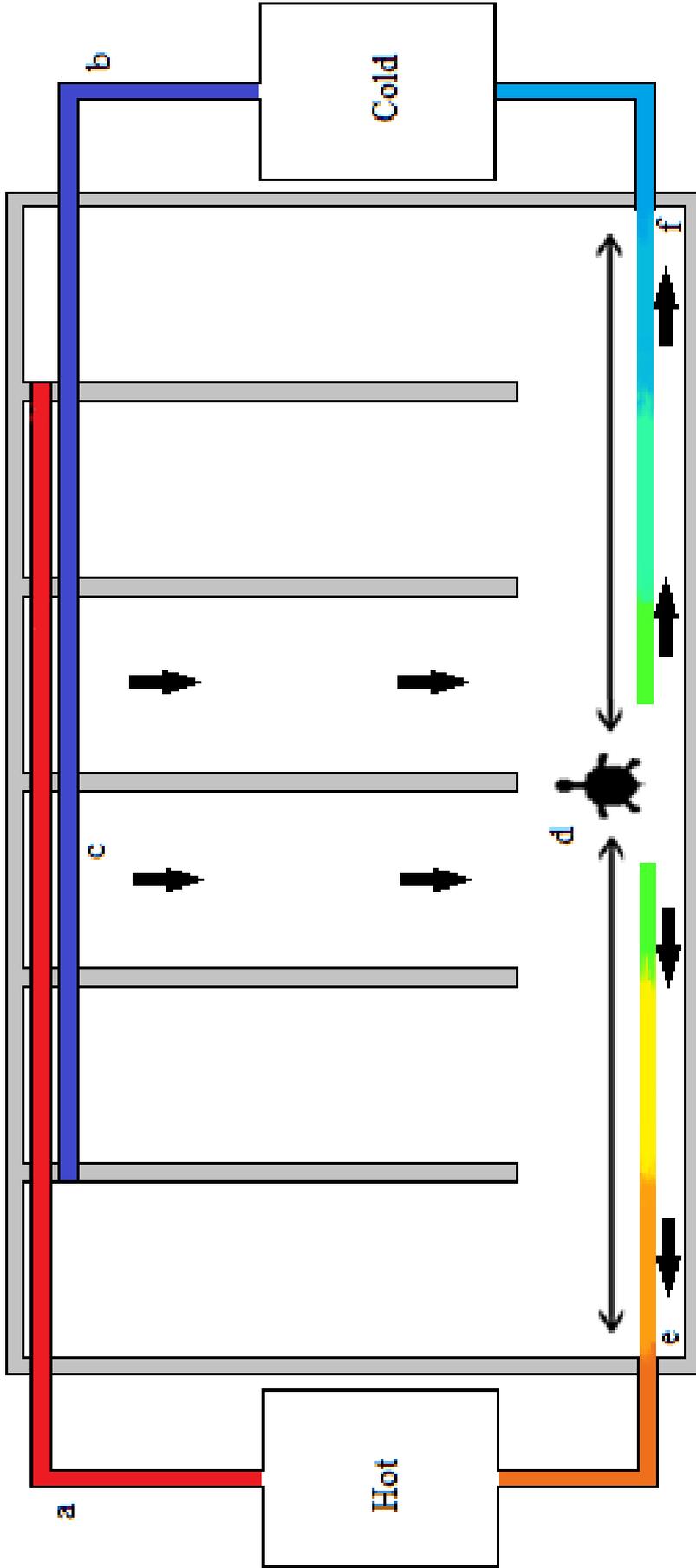


Figure 9: Heated (47°C; a) and cooled (18°C; b) water was pumped out of hot and cold water baths, respectively, into two separate sections of irrigation pipe. Water mixing occurred at the beginning of each run (c), flowed towards the 'free area' where terrapins (*Pelomedusa galeata*) could access all runs, and back into the water baths via outlets (e and f). The outlets directed the warmest and coolest water to the hot and cold baths, respectively.

For each run, aquatic gradient temperature (T_{ag}) was recorded and marked at 0cm, 20cm, 40cm, 60cm and 80cm down the length of the run. Water temperature in the ‘free area’ was also recorded and marked. These markings were used as reference points for terrapin T_b (Tamplin 2006, 2009). To avoid preference for certain areas and readings for aerial T_b , no platforms, basking sites or food were provided; and the gradient was run forward and in reverse (cold at point 1 and hot at point 1 respectively, figure 10). Plastic aquarium plants were anchored to the bottom of each run to shelter hatchlings when searching for desirable T_{ag} (after Tamplin 2006).

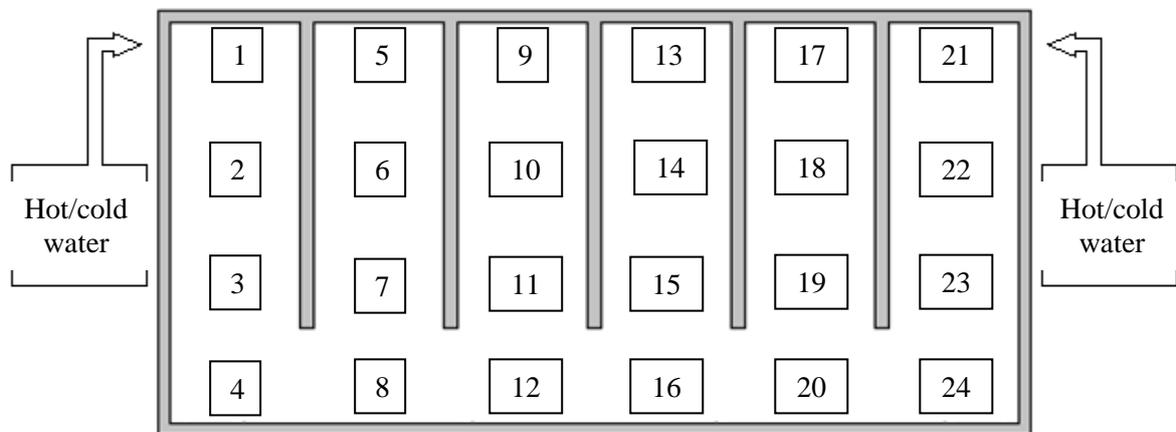


Figure 10: Reference points marked out on the aquatic thermal gradient to compare site choices in the control gradient (24°C throughout) and preferred body temperatures in alternated temperature gradients (22-33°C, in both) for neonate and yearling *Pelomedusa galeata*.

Acclimation

Yearlings were tested in three treatments; hottest (33°C) at point 1, coldest (22°C) at point 1 and control (24°C throughout); for their ability to acclimate to T_{ag} (figures 9 and 10). The control treatment was chosen on the basis that all terrapins were habituated under similarly controlled conditions (ambient room temperature of $26 \pm 1^\circ\text{C}$; tank water temperature $24 \pm 1^\circ\text{C}$) for more than 2 weeks. Only yearlings were used in the acclimation trial because they were considered large enough for T_b measurements to be made with a thermocouple probe (Fluke thermometer, 2mm copper probe; inserted $\pm 2\text{mm}$ into the cloaca).

To investigate whether terrapin T_b conformed to T_{ag} , 8 yearlings (randomly selected, with the same individuals re-examined in the temperature treatments) were placed between

points 12 and 16 (at the centre of the “free area”) for the control treatment (figure 10). For the temperature treatments, yearlings were placed at a site where T_{ag} was similar to that of the temperature they had been habituated (24°C ; point 8 in treatment ‘cold at point 1’ and point 20 in treatment ‘hot at point 1’; figure 10). Hatchling T_b was recorded using the Fluke thermometer every 5 minutes over a 30 minute period, and mean T_b was compared to mean T_{ag} (after Tamplin, 2006).

Site Preference

Neonates ($n= 8$) and yearlings ($n = 8$) were assessed for site preference in the control treatment gradient. Terrapins were tested individually, placed at the same starting point (between position 12 and 16; figure 10), and allowed 30 minutes to become familiar with the environment. For a further 90 minutes, point observations of position in the gradient were recorded every 5 minutes, for a total of 18 observations (figure 10). Hatchlings and yearlings were tested for differences in site selection, and for significantly higher or lower selection for particular sites in the gradient.

Preferred body temperature

Hatchlings (8 neonates and 8 yearlings, randomly selected and repeatedly tested) were placed individually in the gradient at points 8 and 20, as previously described above (figure 10). Positions and relative T_{ag} were recorded, in the same manner as in the site preference test, every 5 minutes over 90 minutes for a total of 18 observations. The number of times each hatchling relocated between temperatures in the aquatic thermal gradient was recorded. Following acclimation assessments, T_{ag} was considered equal to T_b .

Hatchling T_{pref} was calculated as:

$$\frac{T_{ag} \times \text{No. of times observed at } T_{ag}}{18} = T_{pref}$$

Where T_{ag} is temperature of the water in the aquatic thermal gradient, 18 is the total number of observations made per hatchling test, and T_{pref} is preferred body temperature of each

hatchling. Preferred T_b and frequency of relocation between temperatures in the aquatic thermal gradients were assessed for effects of mass and age.

Statistical analyses

All statistical analyses were conducted with SPSS vs. 21. Pearson correlation analyses were performed between age, mass, plastron length, and carapace length and width, to determine which body measurements should be included for further analysis. The controlled environment in which trials took place eliminated all other variables so that only mass and age were considered predictor variables for T_{pref} and number of times relocated between temperatures in the aquatic thermal gradient. Using ANOVA, I tested for significant differences in mass, T_{pref} , and relocation frequencies between neonates ($n = 8$) and yearlings ($n = 8$).

CHAPTER THREE: RESULTS

Adult terrapins

Mass was tested for correlation with measurements of girth, plastron length, and carapace length and width because it is influenced by the size and dimensions of the terrapin (Iverson 1984). All assumptions for the regression analysis were met, and mass was statistically significantly predicted by girth ($\beta = 123.89$, $t = -9.544$, $p < 0.001$), plastron length ($\beta = 240.67$, $t = -8.183$, $p < 0.001$), and carapace length ($\beta = 158.66$, $t = -7.552$, $p < 0.001$) and width; ($\beta = 182.40$, $t = -7.797$, $p < 0.001$; figure 11). A significant proportion of variance in mass was explained by girth, plastron length, and carapace length and width; $R^2 = 0.974$, $F_{(4, 12)} = 111.270$, $p < 0.001$.

Mass was considered most important of these variables, and I found that males (2133.30 ± 384.4 g SE) were heavier than females (1121.43 ± 136.75 g SE). Independent samples t -test showed mass was not significantly different between the 3 males and 14 females, $t_{(15)} = 2.974$; $p < 0.05$. There was overlap in mass between the sexes (males: 1400-2700g, females: 550-2000g) and owing to the small sample size of males, the data were pooled. Mass was retained as a covariate, however, sex was removed from the model. Hereafter, reference will be made to “terrapins” to describe pooled data.

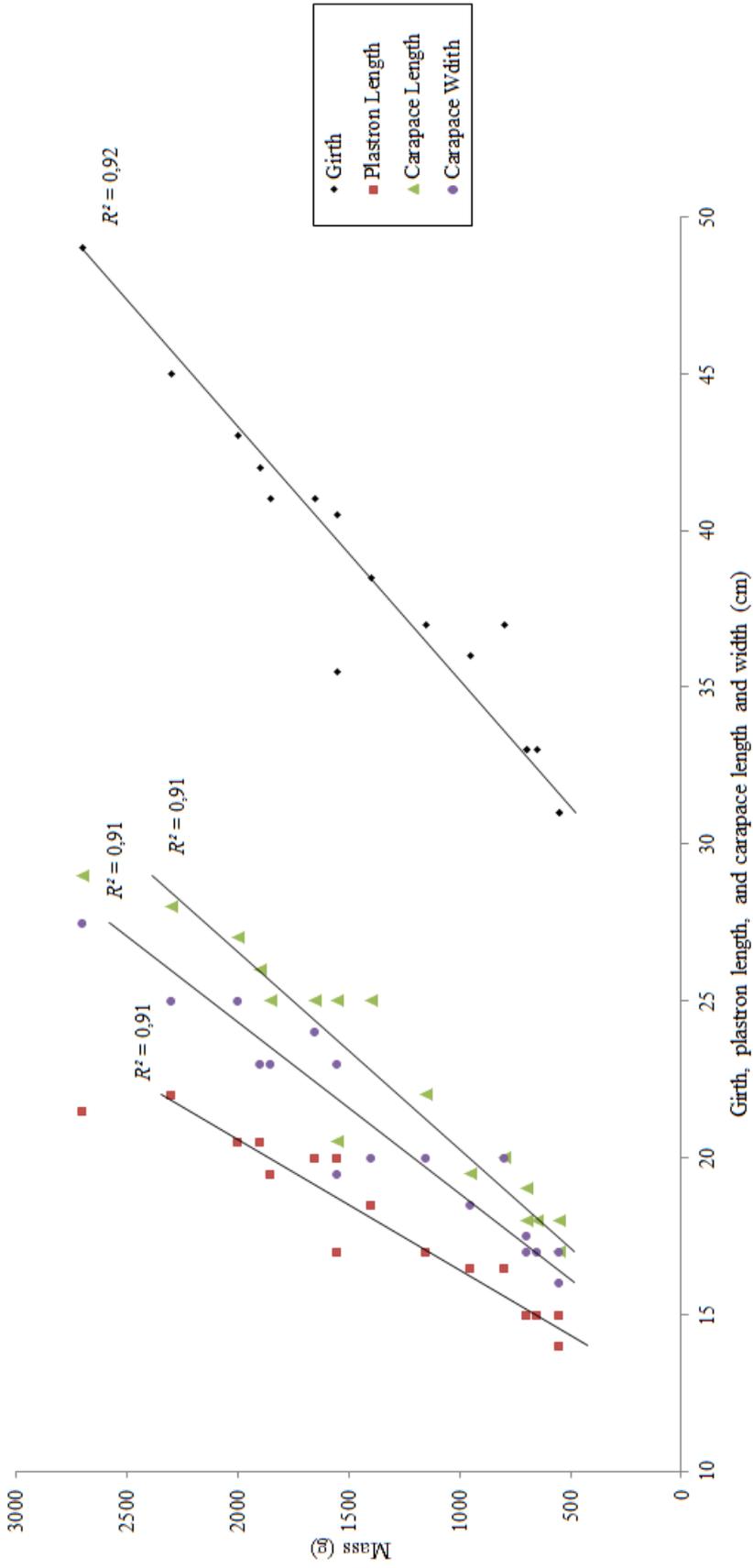


Figure 11: Adult *Pelomedusa galeata* mass was correlated with girth ($R^2 = 0.92$), plastron length ($R^2 = 0.91$), and carapace length ($R^2 = 0.91$) and width ($R^2 = 0.91$). All variables were highly significantly correlated with mass, $df = 17$; $p < 0.001$.

Winter body temperature

Mean terrapin body temperature (T_b) data were evaluated for effects of season (summer and winter) and period (periods 1-5; as per descriptions in methods and materials). Winter T_b was significantly but weakly correlated with mass, $r_{(14)} = 0.07$, $p < 0.001$, and strongly correlated with ambient air temperature (T_a), $r_{(14)} = 0.50$, $p < 0.001$ (table 1 and figure 12 below).

Table 1. Summary of mean body temperature and set point range of *Pelomedusa galeata* and mean and range of ambient air temperatures, including standard error for mean temperatures, during winter 2013 in Pietermaritzburg, South Africa.

	Temperature range			
	Mean	\pm SE	Q1	Q3
Body temperature	15.24	0.11	13.30	16.50
Ambient air temperature	19.94	0.13	16.50	23.40

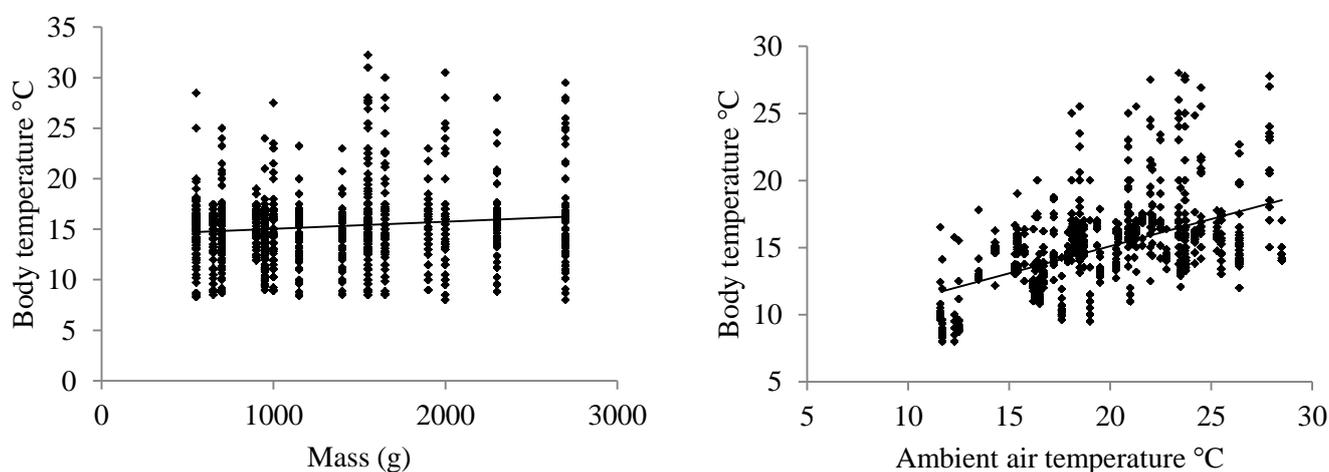


Figure 12: *Pelomedusa galeata* winter body temperature (mean = $15.24 \pm 0.11^\circ\text{C}$ SE and set point range = $13.30\text{-}16.50^\circ\text{C}$) correlated with mass (mean = $1331 \pm 165\text{g}$ SE and range = $550\text{-}2700\text{g}$; $R^2 = 0.02$; $df = 14$; $p < 0.001$) and with ambient air temperature (mean = $19.94 \pm 0.13^\circ\text{C}$ SE and range = $16.50\text{-}23.40^\circ\text{C}$ and; $R^2 = 0.28$, $df = 14$; $p < 0.001$).

Winter basking behaviour

Out of water in full sun (N = 708) and water surface in full sun (N = 600) basking positions were most commonly observed during winter (figure 13 below). Basking activity was infrequent during the early morning (period 1; N = 27; figure 13), peaked during mid-morning and midday (periods 2 and 3; N = 705 and 692, respectively; figure 14) and decreased during the mid-afternoon and early evening (periods 4 and 5; N = 251 and 87, respectively; figure 13).

The log-linear model (Poisson distribution) was statistically significant and interactions between all periods and all basking positions were significant, except three positions by period associations (likelihood ratio $\chi^2(14) = 334.60, p < 0.001$). There were no significant interactions in position 1 by period 4 ($z = 0.59, p = 0.55$); position 2 by period 4 ($z = 0.33, p = 0.74$) and position 3 by period 3 ($z = 1.96, p = 0.05$).

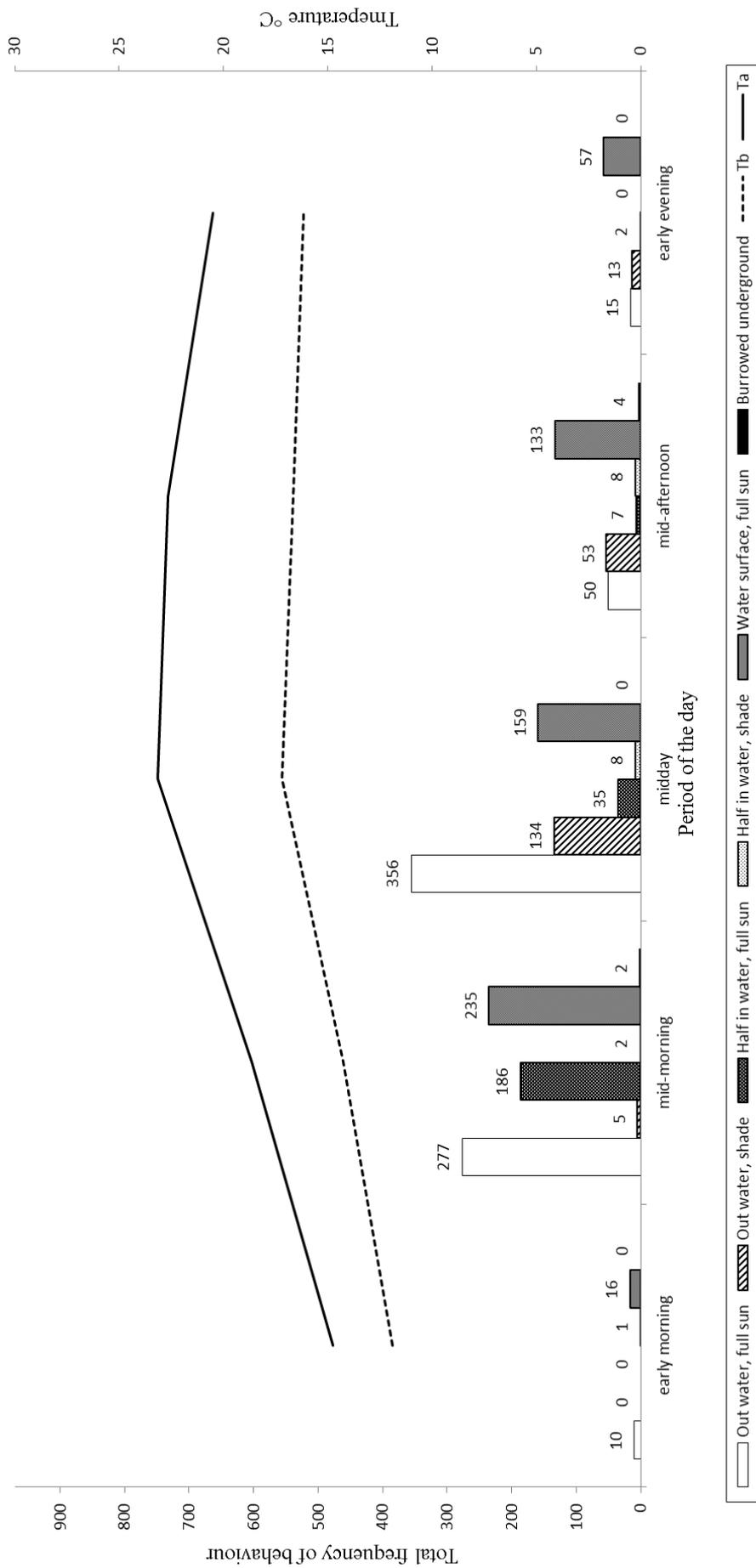


Figure 13: Total frequencies of five thermoregulatory behaviours displayed by *Pelomedusa galeata* (out the water in full sun, out the water in the shade, partially submerged in the sun, partially submerged in the shade and water surface basking), mean daily ambient air temperatures (T_a , solid line) and mean daily body temperatures (T_b , dotted line) for five periods (early morning, mid-morning, midday, mid-afternoon and early evening) during the day. Numbers above bars represent total frequencies of basking positions. Data were derived from observations and temperature data logger information for 11 days during winter, 2013.

Summer body temperature

Body temperatures for only 3 females were used to calculate mean T_b (table 2), because of high iButton failure. Summer T_b was significantly correlated with T_a , $r_{s(2)} = 0.48$, $p < 0.001$ (figure 14), but not correlated with mass, $r_{s(2)} = 0.003$, $p = 0.894$.

Table 2. Summary of mean body temperature and set point range of *Pelomedusa galeata* and mean and range of ambient air temperatures, including standard error for mean temperatures, during summer 2013 in Pietermaritzburg, South Africa.

	Temperature range			
	Mean	\pm SE	Q1	Q3
Body temperature	21.68	0.06	19.50	23.00
Ambient air temperature	25.75	0.10	22.40	28.00

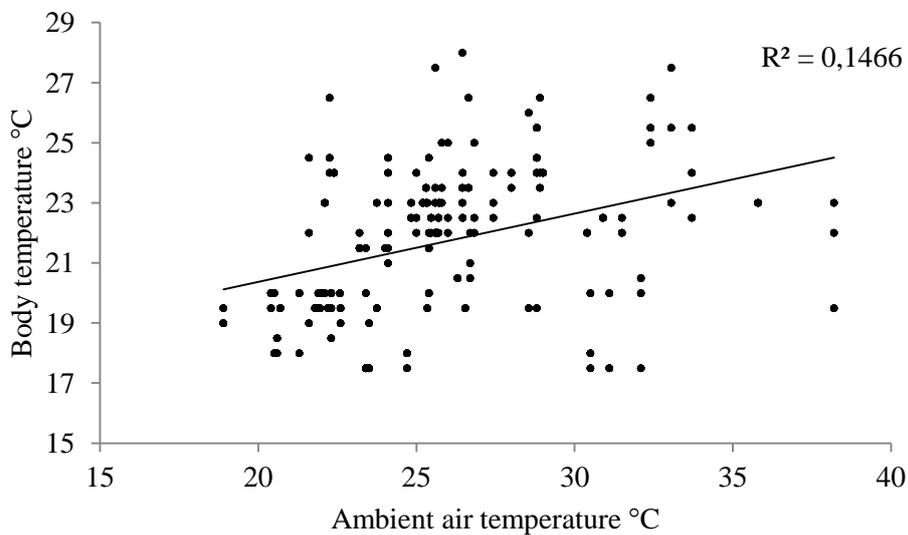


Figure 14: *Pelomedusa galeata* summer body temperature (mean = $21.68 \pm 0.06^\circ\text{C}$ SE and set point range = $19.50\text{-}23.00^\circ\text{C}$) correlated with ambient air temperature (mean = $25.75 \pm 0.10^\circ\text{C}$ SE and range = $22.40\text{-}28.00^\circ\text{C}$; $R^2 = 0.15$, $df = 1$; $p < 0.001$).

Terrapins showed varying frequencies of types of basking positions throughout the day. The most distinct position over all periods was at water surface in full sun basking (N = 2154; figure 15). Basking frequency was higher during the early morning, mid-morning and midday (periods 1, 2 and 3; N = 774, 1030 and 1075, respectively; figure 15) than during the mid-afternoon and early evening (periods 4 and 5; N = 563 and 354 respectively; figure 15).

The log-linear model (Poisson distribution) was statistically significant and interactions between all periods and all basking positions were significant, except two position by period associations (likelihood ratio $\chi^2_{(16)} = 162.831, p < 0.001$). There were no significant interactions in position 1 by period 3 ($z = 1.784, p = 0.074$) and position 1 by period 4 ($z = 1.619, p = 0.105$).

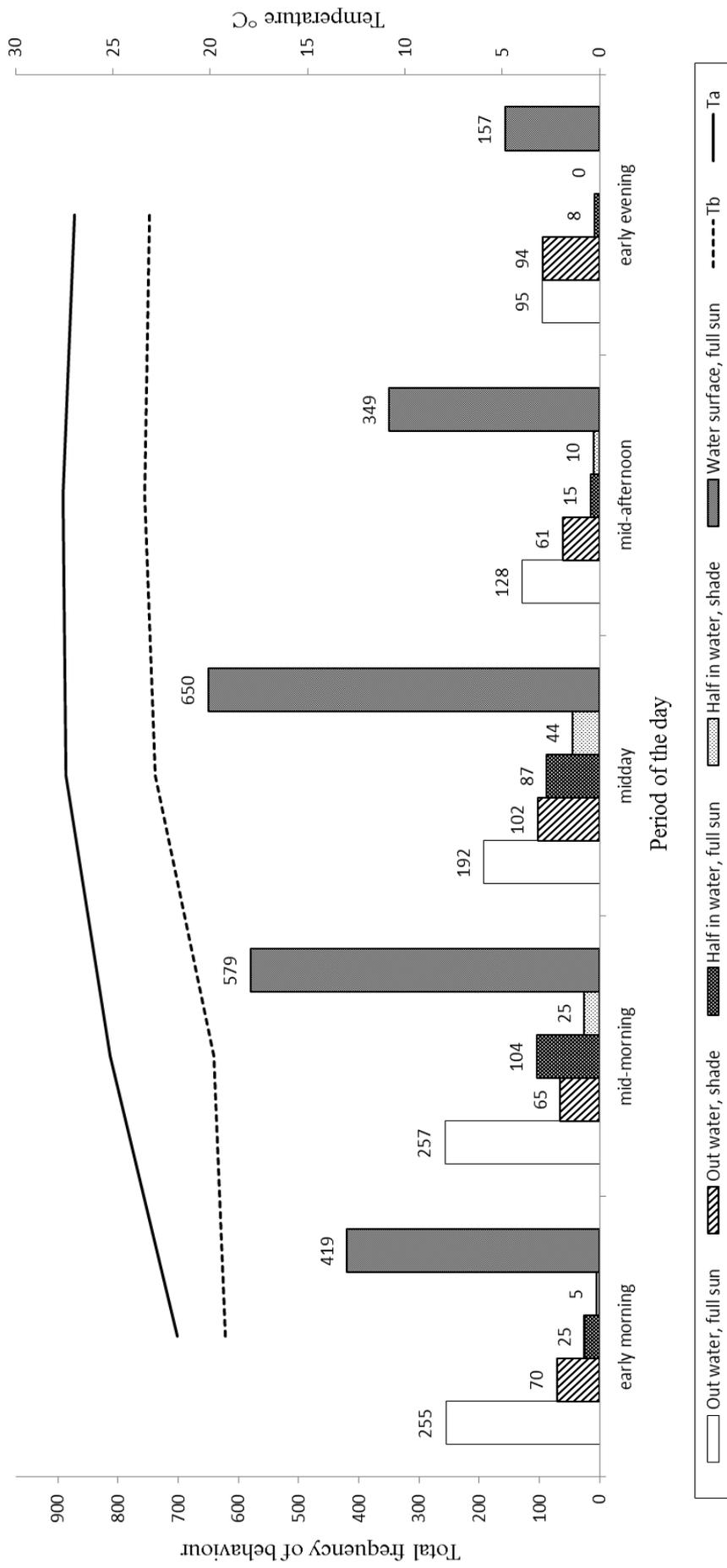


Figure 15: Total frequencies of five thermoregulatory behaviours displayed by *Pelomedusa galeata* (out the water in full sun, out the water in the shade, partially submerged in the sun, partially submerged in the shade and water surface basking), mean daily ambient air temperatures (T_a , solid line) and mean daily body temperatures (T_b , dotted line) for five periods (early morning, mid-morning, midday, mid-afternoon and early evening) during the day. Numbers above bars represent total frequencies of basking positions. Data were derived from observations and temperature data logger information for 14 days during summer, 2013.

Hatchling terrapins

Similar to analyses on adult terrapins, mass was tested for correlations with measurements of plastron length, and carapace length and width (Iverson 1984). All assumptions for the regression analysis were met, and mass was statistically significantly predicted by carapace length ($\beta = 0.86$, $t_{(15)} = 3.17$, $p < 0.05$), but not by plastron length ($\beta = 0.01$, $t_{(15)} = 0.18$, $p = 0.86$) and carapace width; ($\beta = 0.28$, $t_{(15)} = 0.82$, $p = 0.43$; figure 17). A significant proportion of variance in mass is explained by carapace length; $R^2 = 0.99$, $F_{(1, 14)} = 955.08$, $p < 0.001$ (figure 17). Yearling mass (27.48 ± 2.71 g SE) was significantly greater than neonate mass (9.86 ± 0.22 g SE), $t_{(15)} = 6.50$, $r_{(15)} = 0.87$, $p < 0.001$ (figure 16).

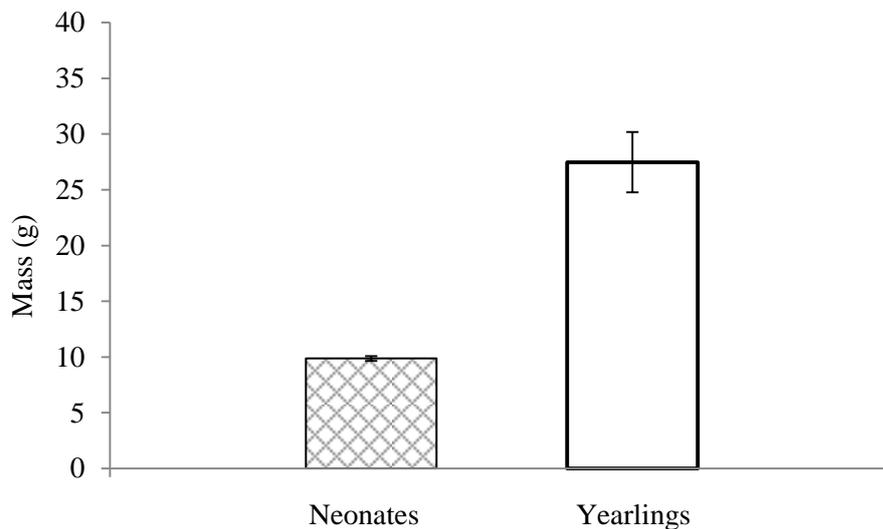


Figure 16: Mass was significantly different between age groups of hatchling *Pelomedusa galeata*, $t_{(15)} = 6.50$, $r_{(15)} = 0.87$, $p < 0.001$. Neonates (9.86 ± 0.22 g SE) were significantly smaller than yearlings (27.48 ± 2.71 g SE).

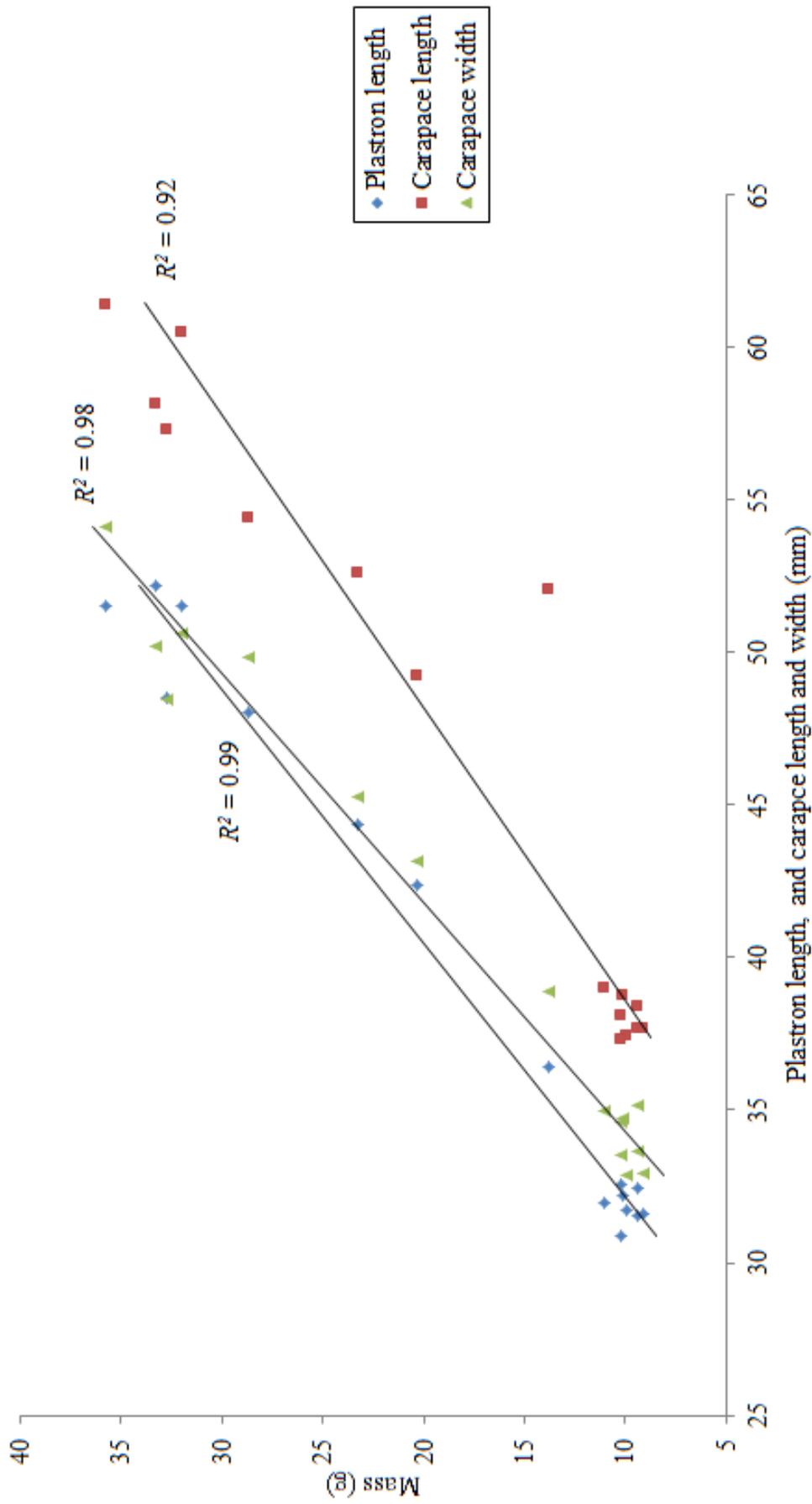


Figure 17: Hatchling *Pelomedusa galeata* mass was significantly correlated with carapace length ($R^2 = 0.92$), but not with plastron length ($R^2 = 0.98$), and carapace width ($R^2 = 0.99$). Only carapace length explained for a significant proportion of variation in mass, $df = 16$; $p < 0.001$.

Site preference and acclimation

There was no significant difference in site preference between neonates and yearlings, thus data were pooled. Hatchlings showed slight preference for the ‘free area’ (figure 10) in the aquatic thermal gradient (‘free area’ reference points are highlighted in blue, figure 18). There was a strongly significant relationship between aquatic thermal gradients temperature (T_{ag}) and hatchling T_b , $r_{s(7)} = 0.92$, $p < 0.001$ (figure 19). Hatchling T_b therefore followed T_{ag} and was regarded as equivalent.

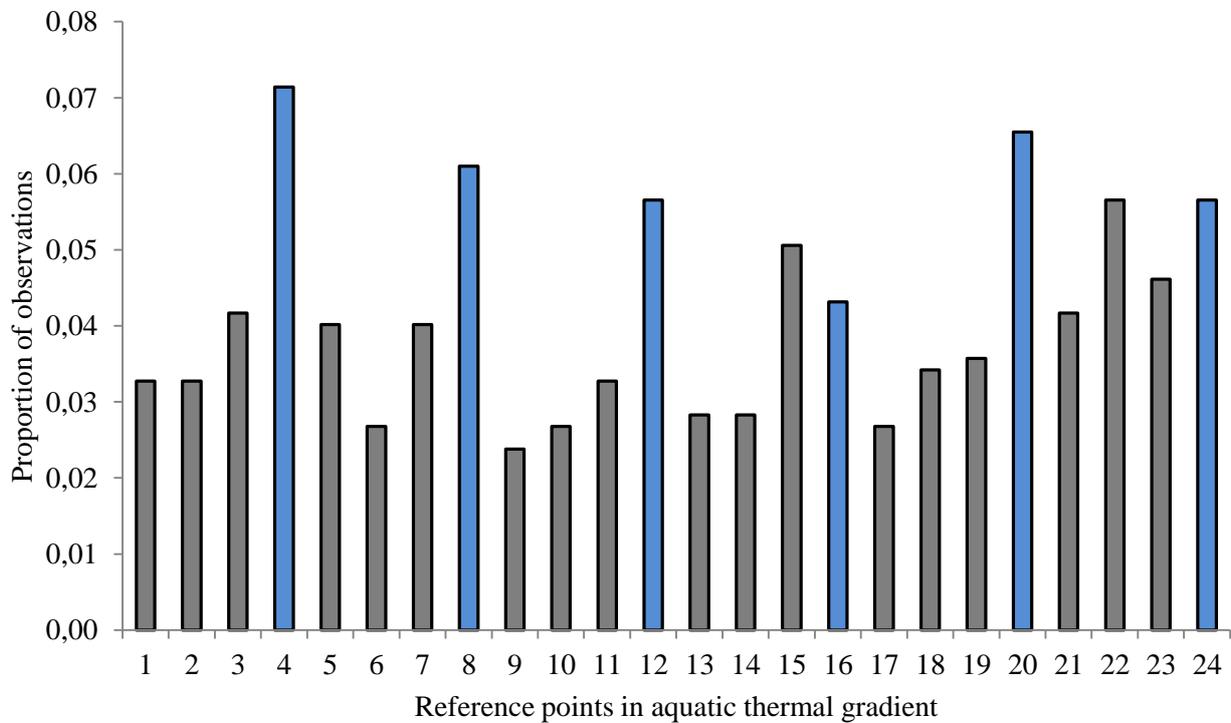


Figure 18: Proportion of observations of *Pelomedusa galeata* hatchlings at 24 reference points in a control treatment (24°C) aquatic thermal gradient. Blue highlighted bars represent the ‘free area’ reference points at the end of six runs.

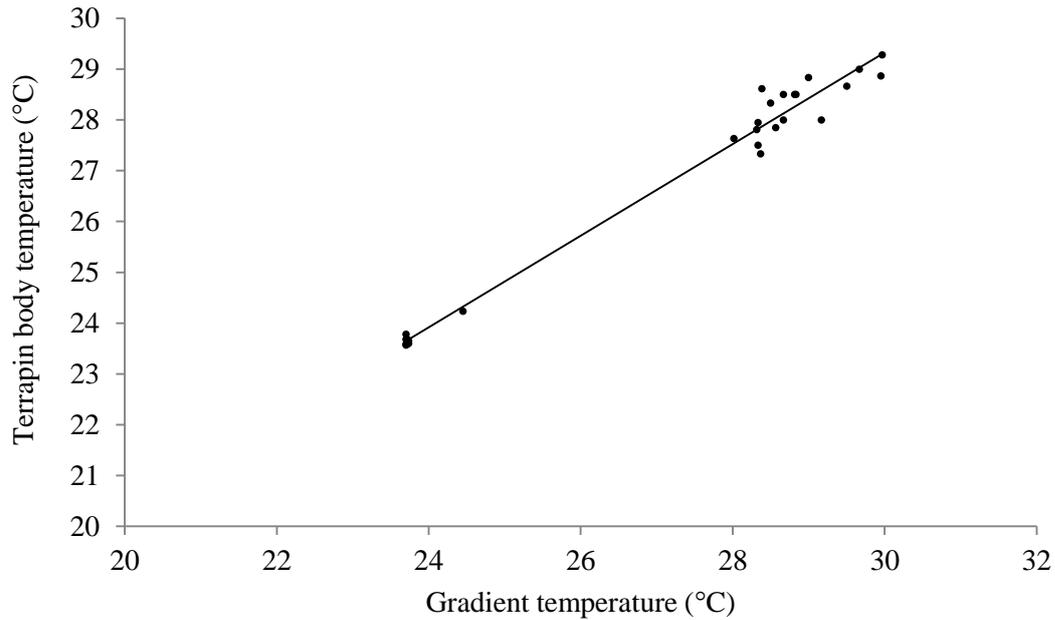


Figure 19: *Pelomedusa galeata* neonate (n = 8) and yearling (n = 8) body temperatures (T_b) were strongly related to temperatures of the aquatic thermal gradient (T_{ag} ; $R^2 = 0.99$, $p < 0.001$). Acclimation tests were run for the control gradient (24°C; T_b is clustered at ≈ 24 -25°C of T_{ag}), and forward and backward temperature gradients (22-33°C and 33-22°C, respectively; T_b is clustered at ≈ 28 -30°C; described in methods and materials).

Preferred body temperature

Mass and age stage had no effect on temperature preference, therefore the data were pooled. A mean of all hatchling T_{pref} was calculated to determine hatchling T_{pref} (28.86 ± 0.26 °C SE) and set point range (T_{set} ; 27.83 - 29.93°C). Aquatic thermal gradient temperature was significantly correlated with T_b ($r_{s(7)} = 0.92$, $p < 0.001$; figure 19).

Relocation between gradient temperatures

Mass significantly affected the mean number of times hatchlings relocated between T_{ag} (mean relocation for two temperature treatments; $\beta = -0.10$, $t_{(15)} = 2.66$, $p < 0.05$), and explained for a significant proportion of variance in relocation ($F_{(1, 14)} = 7.05$; $p < 0.05$). Small hatchlings relocated between T_{ag} more frequently than large hatchlings.

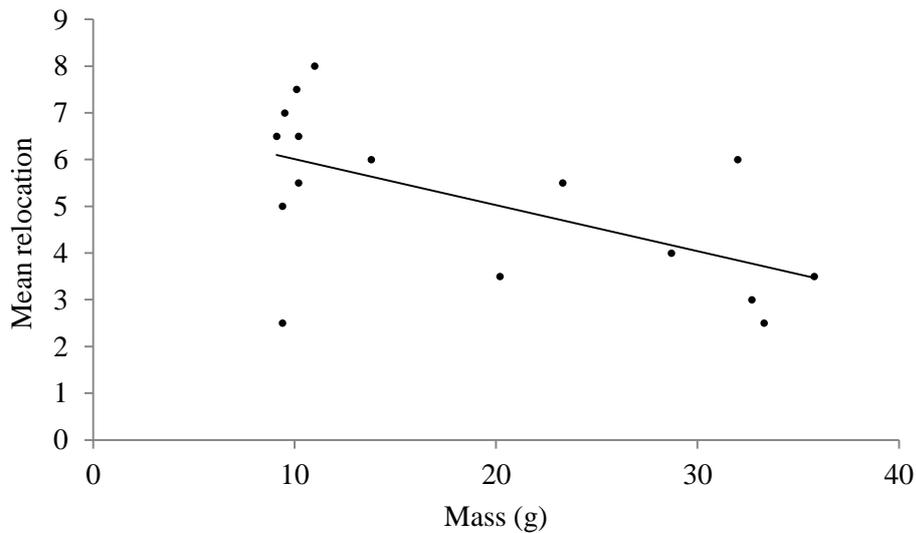


Figure 20: Pelomedusa galeata neonates ($n = 8$) and yearlings ($n = 8$) mean relocation between temperature sites in the thermal aquatic gradient was negatively correlated with mass ($\beta = -0.10$, $R^2 = 0.34$, $p < 0.05$).

CHAPTER FOUR: DISCUSSION AND CONCLUSIONS

Discussion

Adult terrapins

Capture and marking

Pelomedusa galeata were more easily caught in ponds and dams where terrapin population concentrations were high. The presence of an organic substrate and lack of herbaceous shrubs on the perimeter of these ponds and dams may have contributed to higher densities of terrapins at these sites (Marchand and Litvaitis 2004). Using a hand net was the most successful method for capture of terrapins and was particularly successful when used in conjunction with a fishing rod. Funnel traps were also successful, however the netting encasing the trap was too fine for the terrapin's claws and were often torn, allowing them to escape. Funnel traps were only useful for catching small terrapins. Laying bait in the shallows on the pond or dam edge and in the traps and nets aided in attracting the terrapins. Frozen or fresh sardine fish were the best attractants because of its firm texture and strong smell that spread easily over the water surface in an oily film.

Notch marking the marginal scutes was successful and showed minimal change in the notch mark over time (Cagle 1939). This was an effective and reliable method for permanent marking and identification purposes. The brightly coloured numbers painted on the shells faded within 8 weeks and were repainted for each season's observations. For each observation period the painted markings served as good indicators of individuals, making the terrapins easily distinguishable from each other. These markings did not appear to have adverse effects on overall terrapin behaviour; however this observation is purely subjective and not based on any empirical data.

Sex and mass

Male *P. galeata* were on average heavier than females, although these results are likely unreliable because of the small sample size of males. There is some supporting evidence for this sexual dimorphism, based on *P. subrufa*, but size difference between the sexes is usually

categorized as “females about the same size or smaller” (Ewert and Nelson 1991, Fritz et al. 2011). One might interpret these size differences between sexes as negligible in relation to other species such as roofed (*Kachuga* sp.) and map (*Graptemys* sp.) terrapins, which show much larger differences (females two to three times longer than males) in sexual dimorphism and mass (Cox et al. 2007).

In terms of the *P. galeata* species as a whole, the possibility of reasonably indistinguishable size differences between the sexes might explain the weakly, although significantly, positive correlation of body temperature (T_b) with body mass in my sample population of terrapins. This indicates there may be a sex or season effects (or both) on T_b , with males selecting for and achieving higher T_b than females in winter. Unfortunately the female-biased sample population would not have rendered a fair comparison for differences in T_b between sexes and deserves further investigation.

Female-biased sex ratios in natural populations in the KwaZulu-Natal Midlands

Bias for male or female sex ratios have been reported in some studies on turtles and terrapins, with most showing that egg incubation at warm temperatures yield mostly females and cool temperatures mostly males (Seigel 1984, Ewert and Nelson 1991). Although empirical data for temperature-dependent sex determination (TSD) in *P. galeata* have not yet appeared in literature, phylogenetic analyses show that *P. galeata* are genetically divergent from *P. subrufa* but remain morphologically very similar (Fritz et al. 2014). It is thus tempting to assume that incubation temperatures affect both species' sex ratios similarly. In *P. subrufa*, low incubation temperatures (24-27°C) results in female-biased sex ratios, at 29°C sex ratios are approximately even and temperatures over 29°C show male-biased sex ratios (Ewert and Nelson 1991). Surrounding landscape features and climate may have contributed to lower nest-site temperatures and may have resulted in female biased sex ratios of *P. galeata* at capture sites (Ewert and Nelson 1991, Marchand and Litvaitis 2004).

At the capture sites there were few herbaceous shrubs and trees near the pond/dam edges to provide shaded, cooler nesting sites, while the majority of the areas surrounding the ponds were exposed to direct sunlight and possibly predators (pers. obs.). Both factors may support explanations for female-biased sex ratio (Ewert and Nelson 1991, Marchand and Litvaitis 2004). Explanation 1: Females may have selected for cooler nesting sites because these sites were more cryptic and sheltered from predators and extreme ambient

temperatures, thus resulting in higher numbers of female hatchlings than male hatchlings. Explanation 2: Nest sites that were exposed and warmer produced male-biased sex ratios, but were more heavily affected by predation than cryptic cool nests, thereby increasing the impact female-biased cool nests, on the overall population.

Nesting and egg incubation periods coincide with warmer months in the midlands mistbelt capture area (pers. obs.). Mean maximum air temperatures in this area range between 29 and 31°C (Cedara Agricultural College weather station; figure 6, chapter 2), indicating there will be short incubation periods and male-biased sex ratios in hatchlings (Yntema 1978, Ewert and Nelson 1991, Marchand and Litvaitis 2004). Soil temperatures of cool nests may, however, be low enough to influence sex ratios so they are skewed towards greater numbers of female than male hatchlings, and this might account for the female-biased sex ratio of the sample population captured.

Mass and body temperature

Mass (or size) is a commonly used variable when assessing T_b and is particularly important for ectotherms (Boyer 1965, Stevenson 1985a). Reptiles rely on their thermal environment to provide them with heat, but the way in which they maintain, gain or lose heat depends, *inter alia*, on their mass. Those with low volume to surface ratios are more thermally sensitive and do not easily remain within the desired set point range (T_{set}) or at preferred body temperature (T_{pref}) – losing or gaining heat quickly (Boyer 1965, Stevenson 1985a). Those with greater volume to surface ratios, gain and lose heat more slowly, but are able to maintain more constant T_b (through thermal inertia; Boyer 1965, Seebacher et al. 1999). When discussing the effects of body size on T_b , defining ‘large’ (large volume to surface ratio) and ‘small’ (small volume to surface ratio) reptiles, is not simple.

Stevenson (1985a) has outlined body size effects on T_b ranges for terrestrial ectotherms. He showed that T_b of animals smaller than 0.1kg are closely linked to ambient air temperature (T_a), animals between 0.1 and 10kgs have better control over their T_b and make behavioural changes to do so, and animals larger than 10kgs are least affected by changes in the thermal environment (which decreases in effect with increasing mass). Thus body mass contributes to choices reptiles make in their thermoregulatory behaviours, where body mass of reptiles might result in trade-offs relating to metabolism, searching for resources and predator avoidance (Huey and Slatkin 1976).

Adult *P. galeata* fall within the 0.1-10kg category of ectotherms capable of implementing effective thermoregulation. My results showed only a slight positive correlation between T_b and mass, with males achieving higher T_b than females. This is supported by results in previous studies on ectotherms that have shown small or immature reptiles warm up faster than large reptiles, allowing them greater opportunity in searching for food and mates, escaping predators, and increased digestive and assimilative efficiencies and metabolic activity (which can potentially lead to growth; Gatten 1974, Huey and Slatkin 1976, Greenwald and Kanter 1979, Adolph and Porter 1993, Seebacher 2005, Webb and Whiting 2005, Carrière et al. 2008). The lag in heat gain and loss experienced by large or mature reptiles may reduce opportunity for these activities, but thermal inertia can save them the energetic expense of constantly moving to remain within a desired temperature range (which can potentially lead to reproduction; Derickson 1976, Stevenson 1985a,b, Adolph and Porter 1993).

Small (<0.1kg) reptiles are highly sensitive to the thermal environment, but on the other end of the mass continuum very large reptiles (*i.e.* leatherback turtles, >900kg; crocodiles, >1000kg) respond to the thermal environment with ‘gigantothermy’ – mass large enough to maintain relatively stable T_b with very little thermoregulatory effort (Stevenson 1985b, Paladino et al. 1990, Seebacher et al. 1999). Ranges in mass for *P. galeata* do not occur at such extremes, however T_b was positively correlated with body mass. Greater mass and better heat retention assists in buffering large *P. galeata* from extreme T_a , while small *P. galeata* are more vulnerable to thermal fluctuations in the environment.

For a more comprehensive understanding of the thermal biology of *P. galeata*, heat transfer between these terrapins and their environment can be assessed, taking mass (or their volume) into consideration. To do so, replicates of the same volume and dimensions of these animals were constructed to ‘map’ temperatures available to them in their thermal environment. These are operative temperature (T_e) models and they are important additions to modelling thermoregulation in ectotherms.

Operative temperature models

Operative models should resemble the animal’s colour, shape and mass, and should have similar heat conductance to make good predictors for temperatures available to the animal (Bakken 1992). These models are particularly good predictors for ectotherm T_b because they,

like ectotherms, do not generate heat from within and they represent temperatures that can be experienced by the animal on the same spatial scale. By placing these models at different sites, thermal environments experienced by the animal can be mapped (Bakken 1992). The complex thermal environments of semi-aquatic reptiles can be better understood by placing these models at sites they were commonly observed, thus taking into account their thermoregulatory behaviour and morphology.

My operative temperature models were designed based on the dimensions, mass, colour and heating and cooling rates of *P. galeata* (see appendix A1, operative temperatures). Through trial and error, the best operative model was chosen to represent an average sized terrapin, and to match its heating and cooling rates as closely as possible. Construction of the models was highly successful and they maintained their structural integrity throughout the study. These models would have made good predictors for temperatures available to terrapins; however the temperature data loggers inside the models failed (see appendix A1: operative temperatures). As such, the relationships between T_b and T_a were examined.

Ambient air temperature and body temperature

Studies on ectotherm T_b that predate T_e models were based on temperatures of the immediate environment without taking into consideration that ectotherm T_b may actually be above or below T_a (Cowles and Bogert 1944, Brattstrom 1965, Bakken 1992). While these methods have since been refined by including data from physical T_e models in statistical models, T_a information can still be useful for understanding fluctuations in T_b as well as the associated thermoregulatory behaviours, if any (Stevenson 1985b). The effect of T_a on T_b and thermoregulation of ectotherms is useful for future predictions relating to the impact global warming has on many reptilian species (Huey and Tewksbury 2009, Kearney et al. 2009, Clarke and Zani 2012).

Pelomedusa galeata T_b paralleled T_a during winter, indicating that the terrapins adopted thermoconforming responses to the thermal environment. However, this does not discount behavioural alterations to achieve this. Mean daily T_b remained within (and below) 6°C of mean daily T_a , with increasing T_b during the early morning (07:00-09:00), mid-morning (09:30-11:30) and midday (11:30-13:30) periods, and decreasing T_b during the mid-afternoon (14:00-16:00) and early evening (16:00-18:00) periods (figure 13). During winter, the highest mean daily T_a was 23.10°C (with an associated T_b of 17.20°C), but in summer

mean daily T_b reached 23.38°C – higher than available T_a in winter. These terrapins were capable of selecting higher T_b in summer than they did in winter, but may not have been able to because of the rate of heat exchange during the day. Body temperature changes were in response to the fluctuations of T_a and even though the rate of increasing T_b was higher than the rate of decreasing T_b , it did not increase to the point where T_b was equal to or higher than T_a .

Increase in winter T_b until midday may have been faster than the decrease during the afternoon because terrapins were seeking out warm T_a sites to warm up in the morning and then sites with better heat retention in the afternoon. Similar seasonal effects were noted in gila monsters (*Heloderma suspectum*), where in winter these lizards selected shelters with more favourable micro-environmental conditions (Beck and Jennings 2003). This would have been a necessity for the terrapins in winter, but not in summer because of warmer available T_a . Winter T_a was more variable than in summer, thus T_b of the terrapins responded differently between the seasons (figures 14 and 16, chapter 3).

During summer T_b also paralleled increases and decreases in T_a , but not to the same extent as in winter. Body temperature changed at a slower rate than T_a in the early morning, even though the rate of increase in T_a in the early morning was fastest (figure 16, chapter 3). This showed the overnight retreat sites were warm enough that the terrapins were not prompted to select warmer sites than their retreat sites. Similarly, Christian et al. (1983) showed that Galapagos land iguanas (*Conolophus pallidus*) selected for sites with desirable microclimates, and remained in those retreat sites when trying to maintain constant T_b . Additionally, mean daily summer T_b remained within (but below) 4°C of mean daily T_a – a smaller difference between T_b and T_a than in winter. Terrapins were able to maintain higher T_b in summer because T_a was higher throughout the day and thermal inertia assisted in maintaining these high T_b . Over mid-morning and midday periods, the rate of increase in T_b was greatest and then increased only slightly through to mid-afternoon.

Summer T_b indicated terrapins were thermoregulating more than thermoconforming to the thermal environment and where T_b plateaued (22.81 - 23.14°C) with T_a it was taken as T_{pref} for *P. galeata* (figure 16, chapter 3). Such differences in T_b responses to the thermal environment between seasons incited exploration of the T_b that preceded T_b during the early morning period. Figure 21 (below) shows similar trends in heat exchange through day and night periods during summer and winter, however T_{pref} in summer indicates that terrapins may have been trying to reach higher T_b in winter but were constrained by the thermal environment both through the night and during the day. One should, however, take caution

when interpreting these results because of the small sample size for summer. Further investigations into seasonal differences in T_b are necessary to yield more reliable results.

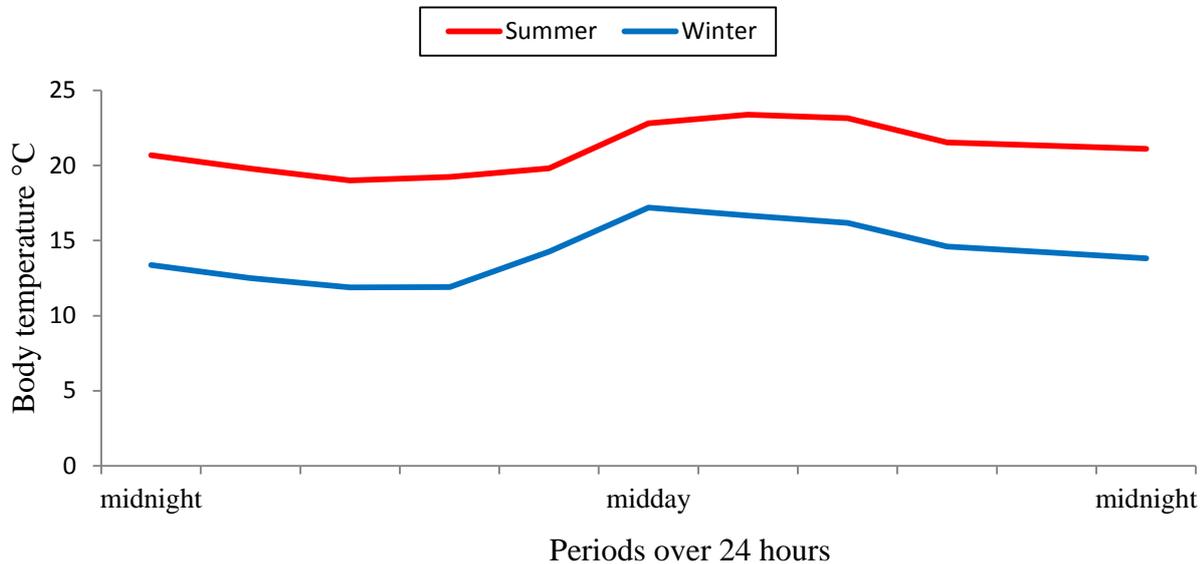


Figure 21: Mean preferred body temperature (T_{pref}) of *Pelomedusa galeata*, over 24 hours. Means were derived from T_{pref} of terrapins in a semi-natural environment at the UKZN Animal House, Pietermaritzburg for 14 days during summer ($n = 17$) and 11 days during winter ($n = 16$), 2013.

Summer T_b indicate that *P. galeata* were thermoregulating in some manner. The same is true for these terrapins in winter, however they may have been trying to achieve these T_b differently. By observing behaviours of *P. galeata* over the same period T_b data were collected, some conclusions can be drawn on how they were achieving these T_b . Environmental conditions may have cued change in position relative to the sun (basking in the sunshine or shade) and landscape (in or out the water), and the frequencies of these basking behaviours displayed affected T_b (Cowles and Bogert 1944, Brattstrom 1965, Huey and Pianka 1977, Hennemann 1979, Seebacher 2005). Aside from the ‘invisible’ physiological changes that take place within bodies of reptiles, ‘visible’ physical behaviours are adopted by reptiles to cope with heterogeneous thermal environments, which can be more complex in semi-aquatic reptiles such as terrapins. Shuttling between sunny and shaded sites is common in terrestrial reptiles (Huey 1974), but terrapins have the additional dimension of water for temperature regulation (Crawshaw 1979, Christian et al. 1983, Meek and Avery

1988, Beck and Jennings 2003). Behavioural movements between sunny, shady, aerial and water habitats were observed in *P. galeata*.

Thermoregulatory behaviour

Ectotherms generally respond to their thermal environment with behaviours that assist in heating and cooling their T_b and narrowing their T_{set} for optimal performance (Cowles and Bogert 1944, Magnuson et al. 1979, Stevenson 1985b, Seebacher 2005). *Pelomedusa galeata* were more active in summer than in winter, because in winter they were more limited by their thermal environment (figures 14, 16, chapter 3; figure 21). Warmer ambient environments in summer allowed for higher T_b , which are associated with increased metabolic activity. This results in positive feed-back whereby raised metabolic activity influences movement ability, allowing for foraging and greater energy gain – ultimately increasing metabolic activity (Crawshaw 1979). Lower T_a in winter do not allow for the same level of activity in terrapins. Not only were the terrapins observed basking more frequently in summer than in winter, but behaviour patterns between the seasons were also different. These behaviour patterns can be explained by variation in T_a and T_b as well as the terrapins' positions relative to the sunny and shady areas, and the landscape.

Overall, terrapins selected sunny areas more often than shady areas for aerial and water surface basking. Sunny areas were presumed to be warmer than shady areas, thus terrapins chose warmer sites more frequently than cooler sites for basking. Adult *P. galeata* fall into the category between ectotherms that follow T_a closely and ectotherms large enough to maintain stable T_b through thermal inertia (Stevenson 1985a). *Pelomedusa galeata* are within the body mass range (0.1-10.0kg) that permits them control over their T_b through behavioural thermoregulation (Stevenson 1985a). Preference for warmer basking sites showed that terrapins were raising their T_b more quickly than would have been possible in the shade. During winter, when mean daily T_a were low, terrapins were optimizing use of these warmer areas so that they may retain sufficient heat for when T_a decreases (particularly through the night; figure 14, chapter 3).

During summer, terrapins also preferred sunny areas to shady areas, but made use of shady areas more often than in winter (figures 14 and 16, chapter 3). Mean T_a was cooler during winter ($19.94 \pm 0.13^\circ\text{C}$) than in summer ($25.75 \pm 0.10^\circ\text{C}$), with only 1°C overlap between the ranges (winter = 16.50 - 23.40°C ; summer = 22.40 - 28.00°C). The T_a range available to the terrapins may have been warm enough in summer to allow for basking in

cooler shady areas. Not only could terrapins utilize sunshine and shade to heat and cool (respectively), they also shuttled between aerial and water basking sites.

During both seasons, entirely out the water and water surface basking were the most commonly observed thermoregulatory behaviours. These behaviours seem to be at opposite ends of the “basking continuum”, where being out of the water might be considered a T_b increaser (“warming behaviour”); and in the water a T_b reducer (“cooling behaviour”). Taking into account the body size range of these terrapins (0.55-2.70kg), behavioural cooling and heating was displayed for the purpose of regulating T_b (Stevenson 1985a). Being in or out of the water, however, may not simply be a method for raising or reducing T_b , but a choice based on which medium (air or water) is most suitable for heat gain, loss or retention during summer and winter months.

During winter, when T_a were low, terrapins tended to select for warming behaviours during the mid-morning and midday periods. Preference for aerial basking was clearly linked with an increase in T_b (figure 14, chapter 3), suggesting that terrapins altered their behaviour for the purpose of thermoregulation. Diamondback (*Malaclemys terrapin*; Grayson and Dorcas 2004, Harden et al. 2007) and painted terrapins (*C. picta*; Akins et al. 2014) have shown similar trends, by basking in exposed areas (aerial basking in the sunshine) more often during winter in order to raise carapace temperature (T_c ; to raise T_b). During the mid-afternoon and early evening periods, terrapins basked at the water surface in the sunshine more frequently than any other behaviour. It is possible that preference for water sites is not for the purpose of reducing T_b after becoming too warm out the water, but necessary for heat retention through the night (figure 19). As a result, T_b decreased at a slower rate than when it increased at the beginning of the day. The thermal inertia of the water may have conserved heat better than the night air, and provided a more desirable over-night environment. Scincid lizards (*Carlia rubrigularis*) showed similar behaviours and chose sheltered, more thermally stable refuge sites when escaping predation (Andersson et al. 2010).

High summer T_a were associated with water surface basking in *P. galeata* (through all periods of the day), which was selected more often than any other behaviour. High frequency of water surface basking during summer showed that, similar to pond sliders (*Pseudomys scripta*; Spotila et al. 1984), this behaviour may have been essential for maintaining high, stable T_b to achieve T_{pref} . Similarly, Grayson and Dorcas (2004; *M. terrapin*), Harden et al. (2007; *M. terrapin*) and Akins et al. (2014; *C. picta*), observed terrapins basking at the water surface more frequently than aerially during summer. Aerial basking for *P. galeata* only peaked in the early and mid-morning periods and then decreased throughout the day (figure

16, chapter 3). Peterman and Ryan (2009) showed terrapins (*C. picta*, *G. geographica*, and *Trachemys scripta*) in the Central Canal of Indianapolis, Indiana, optimized exposure to sunlight, to raise T_b by basking on western (morning sunlight) and eastern (afternoon sunlight) banks daily.

Pelomedusa galeata may have been aerially basking in the morning sun so that T_b were sufficiently raised to optimize locomotion (swimming) when searching for prey items in the water during the remainder of the day. Studies on diamondback terrapins (*M. terrapin*) suggest that foraging linked to prey availability, encouraged activity and movement in the water (Tucker et al. 1995, Roosenburg et al. 1999). With higher T_b as a result of higher T_a , activity levels in *P. galeata* were increased so they could optimize food intake during summer. This is further supported by a study on *C. picta*, where aerial basking occurred most often in the morning and they too proposed that morning warming assists in foraging capabilities, among other activities (Lefevre and Brooks 1995).

Position relative to sun, shade, aquatic or aerial basking sites may not simply be for heating or cooling in terrapins. In some instances, reptiles may perform these thermoregulatory behaviours for reasons beyond regulating T_b for physical maintenance (Huey and Slatkin 1976, Lefevre and Brooks 1995). Basking is primarily for raising T_b (Boyer 1965), which assists in food digestion, energy assimilation and general physiological processes (Cowles 1944, Greenwald and Kanter 1979, Crawford et al. 1983), and may only occur in winter months when assimilating energy from fat stores is essential for maintenance (Derickson 1976). This sample population was provided food throughout the study (food portions were reduced when terrapins were less active during cooler periods) and although locomotion was constrained by the thermal environment, basking during winter may still have been a viable option for food digestion and energy assimilation. As a result, basking behaviour during winter for these terrapins in a semi-natural environment may have shown comparatively unusual winter basking behaviours to fully-natural *P. galeata* populations. Based on the energy gain- T_b relationship in reptiles, observations for summer basking behaviour were unexpected, however activities required for maintenance other than energy gain may account for this.

Being in the water allows for cooling, regulating body fluids, drinking and soaking the skin of terrapins (Donaldson and Echternacht 2005), as well as searching for food and mates (Tucker et al. 1995, Boycott et al. 2008). These activities may take priority over others during summer because overall environmental temperatures are higher than during winter. Water temperature during summer may have been sufficiently high for digestion and energy

assimilation, thus aerial basking in summer may have been driven by vitamin D₃ photoregulation (Ferguson 2003), algal, and ecto- and endoparasite infestations and infection (Boyer 1965, Wikelski 1999, Swimmer 2006), anticipation of predation (Adams et al. 1989, Cooper 2000), gestation in females (Beuchat 1988, Braña 1993) and safeguarding territories in males (Auffenberg 1965, Brattstrom 1974).

Vitamin D₃ is an important hormone for body maintenance in many reptiles, and the panther chameleon (*Furcifer pardalis*) has demonstrated precise regulation of exposure to ultraviolet (UV) light to achieve optimal levels of this hormone (Ferguson et al. 2003, Karsten et al. 2009). Whether *P. galeata* terrapins were basking at the water surface or aerially, they were likely regulating their exposure to UV light so that they may synthesise vitamin D₃. Not only does basking expose terrapins to UV light, it also exposes them to ambient air, a much drier habitat.

Aerial basking may assist in reducing the number of ectoparasites (such as leeches, Annelida: Hirudinea) and transmitted endoparasites by drying the skin so that ectoparasites release their hold, as well as endoparasites succumbing to high T_b and reduction in infection (Boyer 1965, Swimmer 2006). Additionally, *P. galeata* bask gregariously (sometimes stacked), which may assist in reducing parasitism by predatory ticks (also carrying endoparasites) as in marine iguanas (*Amblyrhynchus cristatus*; Wikelski 1999). Raising T_b in reptiles not only helps to improve the individuals' immune system, it promotes other bodily functions and fitness of the species as well.

Adams et al. (1989) found that voluntary locomotion in the eastern box turtle (*Terrapin carolina carolina*) improved at higher T_b. The ability of an animal to make an escape from predation or antagonistic encounters is directly affected by its locomotory capacity (Cooper 2000). Escaping predators promotes chances of reproductive success, and gestation period might also be positively influenced by raising T_b through basking. Gestation period in the viviparous lizard *Sceloporus jarrovi* is affected by T_b, reaching optimal T_b yields more successful young (Beuchat 1988). There may however be trade-offs associated with gravidity and successfully raising T_b for more viable young. Pregnant female *Podarcis muralis* lizards had lower T_b in field experiments because they were thermally constrained by their refuge sites, which were a necessity for protection from predators (Braña 1993). Conversely, female southern water skinks (*Eulamprus tympanum*) increase basking while gravid, but remained cryptically motionless when approached by a predator, thus increasing both T_b and chances for survival (Schwarzkopf and Shine 1992). Territoriality is usually associated with male behaviour during mating seasons and may also influence basking in

terrapins.

Terrapins are generally only sociable when mating, basking or nesting (Brattstrom 1974). Some tortoises have been noted for their aggression toward each other. *Geochelone carbonaria* and *Geochelone denticulate* (two closely related South American land tortoises) display aggressive head movements during territorial and mate selection disputes (Auffenberg 1965). Territory establishment in terrapins is not as prominent as in some tortoises and diurnal basking lizards, however social hierarchy has been observed in box turtles (*Terrapene carolina*), with larger individuals obtaining higher rank and therefore prime choice of basking sites (Brattstrom 1974).

While basking is beneficial for many aspects of body maintenance and reproductive potential in adult or sexually mature terrapins, small or immature terrapins may trade precise thermoregulation for safety in less desirable microhabitats. Hatchling terrapins may behave similarly to juvenile broad-headed snakes (*Hoplocephalus bungaroid*) and trade heat gain to reduce predation risk (Webb and Whiting 2005). As a result, hatchling *P. galeata* may not achieve high T_b in the field, but when conditions are favourable (*i.e.* reduced predation risk) hatchlings may select for high environmental temperatures to increase metabolic rate for growth (Gatten 1974, Huey and Slatkin 1976, Greenwald and Kanter 1979, Adolph and Porter 1993, Seebacher 2005, Webb and Whiting 2005, Carrière et al. 2008).

Hatchling terrapins

Temperature gradient

Construction of the temperature gradient was fairly simple, and it maintained structural integrity throughout the duration of the study. The temperature range in the gradient was sufficiently broad (22-33°C) to expose terrapins to temperatures beyond their T_{set} . Water temperature within the gradient was well insulated by the polystyrene walls, and within runs it remained constant and was unaffected by adjacent runs.

Site preference and acclimation

Hatchlings tended toward the open end of the runs and water exit points in the 'free area' of the aquatic thermal gradient (points e and f; see aquatic thermal gradient plan, figure 9, chapter 2). The walls of the gradient may have created an edge effect that drove the terrapins

towards the ‘free area,’ however site selectivity did not affect T_{pref} of hatchling *P. galeata*. The combined effect of accessibility to the ‘free area’ and mixing of water in the ‘free area’ to create a reasonably homogenous thermal environment may have made this area attractive to the hatchlings.

Yearlings were investigated for acclimation ability because cloacal openings of the neonates were too small for the thermocouple probe. They were highly sensitive to aquatic thermal gradient temperature (T_{ag}) and were within 0.3°C of the T_{ag} . Hatchlings weighed less than 0.1kg therefore T_{b} is strongly related to T_{a} (Stevenson 1985a), hence neonates were expected to have acclimated to the gradient temperature in the same manner as the yearlings because of their even smaller mass. The cloacal temperature measurements may not have been absolutely precise as core T_{b} , but were considered to be good estimates of it because aquatic reptile T_{b} is usually the same as water temperature (Brattstrom 1965).

Preferred body temperature and switching temperature zones

Hatchling *P. galeata* terrapins selected T_{b} of $28.9 \pm 0.3^{\circ}\text{C}$, which is surprisingly high compared with the acclimation temperature ($24 \pm 1^{\circ}\text{C}$ in a temperature controlled room of $26 \pm 1^{\circ}\text{C}$), however similar instances have been reported in other work on hatchling terrapins. Tamplin and Cyr (2011) tested hatchling western painted turtles (*Chrysemys picta bellii*) for effects of acclimation temperature on T_{pref} . They found that hatchlings acclimated to 20.0°C switched temperature zones less frequently and selected warmer temperatures than those acclimated to 25.0°C that did not show specific temperature selection (Tamplin and Cyr 2011). They reasoned that hatchlings acclimated to 20°C were seeking higher T_{b} to increase metabolic rate, as they had been maintained below their thermal optimum ($21\text{-}23^{\circ}\text{C}$, Tamplin and Cyr 2011). This may have been the case for hatchling *P. galeata*.

High temperature selection is an adaptive cost-benefit mechanism, adopted by small or juvenile ectotherms to increase growth rate (Jarošík et al. 2004), thus improving survival odds (Huey and Slatkin 1976, Avery et al. 1993, Angilletta et al. 2002). Growth in hatchling *P. galeata* is a priority (besides avoiding predation and adverse environmental conditions, resulting in immediate death), thus selection of higher T_{ag} was metabolically more beneficial than lower temperatures, which is particularly important for raising T_{b} so that they would have improved digestive and energy assimilative physiology.

As demonstrated by their ability to assume the temperatures of their habitat (the aquatic thermal gradient in this studies’ case), small terrapins warm readily, thus achieving

rapid food digestion and energy assimilation for accelerated growth through temperature selection (Cowles 1945, Hammond et al. 1988, Lefevre and Brooks 1995, O'Steen 1998). Red-eared (*Pseudemys scripta*) and ornate box (*Terrapene ornata*) turtles selected higher substrate temperatures in a thermal gradient after they were fed than when they were fasted (Gatten 1974). Avery et al. (1993) controlled for level of dietary protein, with higher levels resulting in higher growth rates, provided protein intake was coupled with high T_b .

A general rule is that, with higher temperatures comes increased growth rate (Kingsolver and Huey 2008). Basking in hatchling terrapins improved growth rates for hatchling and juvenile *T. scripta* and *C. picta* and *Sternotherus odoratus*, but at greater risk of predation (Janzen et al. 1992). Similarly, Slider turtles (*T. scripta*; Avery et al. 1993) and common snapping turtles (*Chelydra serpentina serpentina*; Brown et al. 1994) grew faster in a habitat with higher ambient temperature than those at cooler ambient temperatures. Increased growth rate is consistent with “bigger is better” (reviewed by Kingsolver and Huey 2008) and increased performance (or fitness and survival; Huey and Slatkin 1976).

Contrary to most experimental work on the effect of food intake on T_b , hatchling snapping turtles (*C. serpentina*) remained more sedentary when satiated than fasted, and did not seek warmer environmental temperatures for improved digestive and assimilative efficiencies (Knight et al. 1990). They tended to remain where they were placed in a thermal gradient rather than seek more a desirable thermal habitat. Knight et al. (1990) illuminated the point that this population of terrapins lived in a relatively low temperature, benthic environment, and had relatively higher digestive rates at lower temperatures than other hatchling species (*C. picta*, *T. scripta*, *S. odoratus*, *Sternotherus minor* and *C. serpentina*). Body temperature and associated physical performance was tested by Wu et al. (2013) in an experiment with acclimation temperature effect on swimming performance in hatchling soft-shelled turtles (*Pelodiscus sinensis*). They showed that “hotter is better” (Bennett, 1987; Kingsolver and Huey, 2008) and that hatchlings acclimated to a high temperature (30°C) outperformed hatchlings acclimated to a low temperature (10°C; Wu et al. 2013).

Additionally, ectotherms can be affected by ambient conditions at any life stage including incubation temperature of eggs. Tamplin and Cyr (2011) showed that western painted turtle (*C. picta bellii*) eggs that were incubated at different temperatures not only yielded different sex ratios, but also affected hatchling T_{pref} . Eggs incubated at 27.5°C (hatchlings were presumed to be a combination of males and females) selected warmest gradient temperatures and those incubated at 30.0°C (presumed to be females only) selected lower temperatures. Similar results have been found with snapping turtles (*C. serpentina*

O'Steen 1998, Rhen and Lang 1999). An exception to the general 'rules' of egg incubation temperature on T_{pref} is discussed in Spotila et al. (1994). They showed that incubation temperature does not affect T_{pref} of hatchling desert tortoises (*Gopherus agassizii*).

Although there are general trends of T_{pref} relative to size and life stage, species specific T_{pref} appear in literature (previously discussed) through individual experimentation, with some species displaying exceptions to these general 'rules'. *Pelomedusa galeata* nest sites temperature was not explored in this study, however, T_{pref} of hatchling *P. galeata* may be useful for understanding future *P. galeata* nesting studies.

Mass and preferred body temperature

Mass had no effect on T_{pref} and there was no significant difference between yearlings and neonates, but small hatchlings relocated between T_{ag} zones more frequently than large hatchlings. Not only does this reinforce that neonates acclimated to T_{ag} in the same manner as yearlings, but show a higher thermophilic response to the thermal environment and require greater thermoregulatory precision for maintaining constant T_{b} . Mean hatchling T_{set} was very narrow and although hatchlings showed slight site preference in the control treatment, results for T_{pref} were considered reliable based on the temperature sensitivity of hatchling *P. galeata*.

Conclusions

Basking behaviour in *P. galeata* was influenced by T_{a} and T_{b} . In the early morning in winter, when T_{a} were low, terrapins basked infrequently. As T_{a} increased, activities that allowed them to absorb heat became more prevalent. To retain this heat through the cold nights, terrapins then switched their position from out the water in full sun, for the more thermally stable environment of the water. In summer, the converse trend occurred and terrapins utilised the comparatively cooler water environment more frequently, especially during the hotter periods of the day.

Differences in T_{b} and basking behaviours between the seasons may have been influenced by low sample size of males during winter, and males and females during summer. Results in winter may have shown bias for female T_{b} and male T_{b} was not even represented in summer. Similarly, basking behaviour may have been over-represented by females in both seasons. Summer was a better predictor for T_{pref} , and winter showed that terrapin's

thermoconformed to T_a more often than thermoregulated.

Body temperature studies between hatchling and adult terrapins were carried out under different conditions and therefore not directly comparable. The study on hatchlings showed that terrapins of small mass are more sensitive to T_a than large terrapins. This might affect the manner in which they bask, tending to shuttle between water and terrestrial basking positions more frequently than adults. Smaller terrapins are not only more susceptible to T_b change through environmental fluctuations as well as predation. Hence a delicate balance between basking and retreat for protection must be struck when attaining higher T_b for better digestive and assimilative efficiencies for growth.

This study highlights the dependence of some ectotherms on the thermal environment, and apart from dangers such as; habitat loss and degradation, introduction of invasive species to non-native lands, pollution and unsustainable use of the environment, and disease; global climate change is a major threat to reptiles (Gibbons et al. 2000). Turtles are known to have specific, generic T_b (Brattstrom 1965), and I have outlined some aspects of the thermal biology of *P. galeata* in order to contribute to the collection of empirical data required for planning conservation measures for reptiles. Relatively slow growing and maturity rates associated with the life histories of turtle species may be an additional disadvantage for turtles through climate change (Burger and Garber 1995). It has however, been suggested by some, that the thermoregulatory ability of ectotherms may offer some resistance to climate change and global warming.

One critical aspect of climate change is not necessarily only that it will increase global T_a , but that it will change the physical environment (Kearney et al. 2009). This may result in many ectothermic species occupying areas different to the ones they occupy currently (Buckley et al. 2008, Huey and Tewksbury 2009), but population densities, abundance and evolution (Buckley et al. 2008, Huey and Tewksbury 2009) may not necessarily change because behavioural adjustments might buffer ectothermic species from environmental temperature change (Buckley et al. 2008, Huey and Tewksbury 2009, Kearney et al. 2009).

Ectotherms occupying temperate zones may be able to take advantage of warmer temperatures to achieve thermal optimums, while tropical ectotherms will need to make use of shady refuges more frequently to avoid more extreme environmental temperatures (Huey and Tewksbury 2009, Kearney et al. 2009, Clarke and Zani 2012). Temperate ectotherms will benefit from time spent basking, but tropical species may be impacted by the double-negative effect of higher energy expenditure and lack of ability to forage while retreating from high temperatures (Huey and Tewksbury 2009), Thermal constraints may also be amplified for

individuals at certain life stages. Eggs, hatchlings and juveniles are predicted to be most vulnerable because of their small size and higher sensitivity to the thermal environment, as well as inability to behaviourally thermoregulate (Huey and Tewksbury 2009).

Small scale studies such as mine on *P. galeata* demonstrate the effects of T_a on behaviour and can be applied to similar species constrained by similar environmental conditions. Summer behaviour reflected projected coping mechanisms of tropical ectotherms under higher temperature conditions, and winter behaviour reflected benefits available to ectotherms in temperate zones. Under these assumptions *P. galeata* are likely to benefit from global warming and climate change, provided water availability does not restrict their distributions and abundance. Further studies on *P. galeata* are necessary for contributing to species-based literature, so that conservation measures may include *P. galeata*.

REFERENCES

- Adams, N. A., D. L. Claussen and J. Skillings. 1989. Effects of temperature on voluntary locomotion of the Eastern box turtle, *Terrapene carolina carolina*. *Copeia* 905-915.
- Adolph, S. C. and W. P. Porter. 1993. Temperature, activity, and lizard life histories. *The American Naturalist* **142**:273-295.
- Akins, C. D., C. D. Ruder, S. J. Price, L. A. Harden, J. W. Gibbons and M. E. Dorcas. 2014. Factors affecting temperature variation and habitat use in free-ranging diamondback terrapins. *Journal of Thermal Biology* **44**:63-69.
- Aleksiuk, M. 1976. Reptilian hibernation: Evidence of adaptive strategies in *Thamnophis sirtalis parietalis*. *Copeia* 170-178.
- Alexander, G. and J. Marais. 2007. A Guide to the reptiles of southern Africa. Struik Publishers, Cape Town.
- Andersson, M., A. Krockenberger and L. I. N. Schwarzkopf. 2010. Experimental manipulation reveals the importance of refuge habitat temperature selected by lizards. *Austral Ecology* **35**:294-299.
- Angilletta, M. J., P. H. Niewiarowski and C. A. Navas. 2002. The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* **27**:249-268.
- Aragón, P., P. López and J. Martín. 2001. Seasonal changes in activity and spatial and social relationships of the Iberian rock lizard, *Lacerta monticola*. *Canadian Journal of Zoology* **79**:1965-1971.
- Auffenberg, W. 1965. Sex and species discrimination in two sympatric South American tortoises. *Copeia* 335-342.
- Avery, H. W., J. R. Spotila, J. D. Congdon, R. U. Fisher Jr, E. A. Standora and S. B. Avery. 1993. Roles of diet protein and temperature in the growth and nutritional energetics of juvenile slider turtles, *Trachemys scripta*. *Physiological Zoology* **66**:902-925.
- Bakken, G. S. 1992. Measurement and application of operative and standard operative temperatures in ecology. *American Zoologist* **32**:194-216.
- Balazs, G. H. and E. Ross. 1974. Observations on the basking habit in the captive juvenile Pacific green turtle. *Copeia* 542-544.
- Bates, M. F., W. R. Branch, A. M. Bauer, M. Burger, J. Marais, G. J. Alexander and M. S. d. Villiers. 2014. Atlas and red list of the reptiles of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria.

- Beck, D. D. and R. D. Jennings. 2003. Habitat use by Gila monsters: The importance of shelters. *Herpetological Monographs* **17**:111-129.
- Ben-Ezra, E., G. Bulté and G. Blouin-Demers. 2008. Are locomotor performances coadapted to preferred basking temperature in the Northern map turtle (*Graptemys geographica*)? *Journal of Herpetology* **42**:322-331.
- Bennett, A. F. 1987 Evolution of the control of body temperature: Is warmer better? *Comparative physiology: Life in Water and on Land* 421-431.
- Beuchat, C. A. 1988. Temperature effects during gestation in a viviparous lizard. *Journal of Thermal Biology* **13**:135-142.
- Bligh, J. and K. G. Johnson. 1973. Glossary of terms for thermal physiology. *Journal of Applied Physiology* **35**:941-961.
- Blouin-Demers, G. and P. Nadeau. 2005. The cost-benefit model of thermoregulation does not predict lizard thermoregulatory behaviour. *Ecology* **86**:560-566.
- Bostrom, B. L. and D. R. Jones. 2007. Exercise warms adult leatherback turtles. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **147**:323-331.
- Bostrom, B. L., T. T. Jones, M. Hastings and D. R. Jones. 2010. Behaviour and physiology: The thermal strategy of leatherback turtles. *PLoS ONE* **5**:e13925.
- Boycott, R., O. Bourquin, A. Rhodin, P. Pritchard, P. van Dijk, R. Saumure, K. Buhlmann and J. Iverson. 2008. *Pelomedusa subrufa* (Lacépède 1788), helmeted turtle, helmeted terrapin. *Conservation biology of freshwater turtles and tortoises: A compilation project of the IUCN/SSC tortoise and freshwater turtle specialist group. Chelonian Research Monographs*:007.001-007.006.
- Boycott, R. C. 2014. Atlas and Red List of the Reptiles of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria.
- Boyer, D. R. 1965. Ecology of the basking habit in turtles. *Ecology* **46**:99-118.
- Braña, F. 1993. Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. *Oikos* **66**:216-222.
- Branch, W. R. 1998. Field guide to snakes and other reptiles of southern Africa. Struik Publishers, Cape Town.
- Branch, W. R. 2008. Tortoises, terrapins and turtles of Africa. Struik Publishers, Cape Town.
- Brattstrom, B. H. 1965. Body temperatures of reptiles. *American Midland Naturalist* **73**:376-422.
- Brattstrom, B. H. 1974. The evolution of reptilian social behavior. *American Zoologist*

14:35-49.

- Brown, G. P., C. A. Bishop and R. J. Brooks. 1994. Growth rate, reproductive output, and temperature selection of snapping turtles in habitats of different productivities. *Journal of Herpetology* **28**:405-410.
- Buckley, L. B., G. H. Rodda and W. Jetz. 2008. Thermal and energetic constraints on ectotherm abundance: A global test using lizards. *Ecology* **89**:48-55.
- Buhlmann, K. A., T. S. B. Akre, J. B. Iverson, D. Karapatakis, R. A. Mittermeier, A. Georges, A. G. J. Rhodin, P. P. van Dijk and J. W. Gibbons. 2009. A global analysis of tortoise and freshwater turtle distributions with identification of priority conservation areas. *Chelonian Conservation and Biology* **8**:116-149.
- Burger, J. and S. D. Garber. 1995. Risk assessment, life history strategies, and turtles: Could declines be prevented or predicted? *Journal of Toxicology and Environmental Health* **46**:483-500.
- Bury, R. B., A. V. Nebeker and M. J. Adams. 2000. Response of hatchling and yearling turtles to thermal gradients: Comparison of *Chelydra serpentina* and *Trachemys scripta*. *Journal of Thermal Biology* **25**:221-225.
- Cabanac, M. and C. Bernieri. 2000. Behavioral rise in body temperature and tachycardia by handling of a turtle (*Clemmys insculpta*). *Behavioural Processes* **49**:61-68.
- Cadi, A. and P. Joly. 2003. Competition for basking places between the endangered European pond turtle (*Emys orbicularis galloitalica*) and the introduced red-eared slider (*Trachemys scripta elegans*). *Canadian Journal of Zoology* **81**:1392-1398.
- Cagle, F. R. 1939. A system of marking turtles for future identification. *Copeia* 170-173.
- Carrière, M.-A., N. Rollinson, A. N. Suley and R. J. Brooks. 2008. Thermoregulation when the growing season is short: Sex-biased basking patterns in a northern population of painted turtles (*Chrysemys picta*). *Journal of Herpetology* **42**:206-209.
- Chessman, B. C. 1984. Evaporative water loss from three south-eastern Australian species of water turtle. *Australian Journal of Zoology* **32**:649-655.
- Christian, K., C. R. Tracy and W. P. Porter. 1983. Seasonal shifts in body temperature and use of microhabitats by Galapagos land iguanas (*Conolophus pallidus*). *Ecology* **64**:463-468.
- Clarke, D. N. and P. A. Zani. 2012. Effects of night-time warming on temperate ectotherm reproduction: Potential fitness benefits of climate change for side-blotched lizards. *The Journal of Experimental Biology* **215**:1117-1127.
- Cooper, W. E. 2000. Effect of temperature on escape behaviour by an ectothermic vertebrate,

- the keeled earless lizard (*Holbrookia propinqua*). Behaviour **137**:1299-1315.
- Cowles, R. B. 1936. Casual notes on the poikilothermous vertebrates of the Umzumbe Valley, Natal, South Africa. Copeia 4-8.
- Cowles, R. B. 1945. Surface-mass ratio, paleoclimate and heat sterility. The American Naturalist **79**:561-567.
- Cowles, R. B. and C. M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. Bulletin of the American Museum of Natural History **85**:261-296.
- Cox, R. M., M. A. Butler, H. B. and John-Adler. 2007. The evolution of sexual size dimorphism in reptiles. Page 40 in D. J. Fairbairn, Blanckenhorn, W. U., Székely T., editor. Size, Sex and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism. Oxford University Press, Oxford.
- Crawford, K. M., J. R. Spotila and E. A. Standora. 1983. Operative environmental temperatures and basking behavior of the turtle *Pseudemys scripta*. Ecology **64**:989-999.
- Crawshaw, L. I. 1979. Responses to rapid temperature change in vertebrate ectotherms. American Zoologist **19**:225-237.
- Derickson, W. K. 1976. Lipid storage and utilization in reptiles. American Zoologist **16**:711-723.
- do Amaral, J. P. S., G. Marvin and V. H. Hutchison. 2002. Thermoregulation in the box turtles *Terrapene carolina* and *Terrapene ornata*. Canadian Journal of Zoology **80**:934-943.
- Donaldson, B. M. and A. C. Echternacht. 2005. Aquatic habitat use relative to home range and seasonal movement of Eastern box turtles (*Terrapene carolina carolina*: Emydidae) in eastern Tennessee. Journal of Herpetology **39**:278-284.
- Dubois, Y., G. Blouin-Demers, B. Shipley and D. Thomas. 2009. Thermoregulation and habitat selection in wood turtles *Glyptemys insculpta*: Chasing the sun slowly. Journal of Animal Ecology **78**:1023-1032.
- Ewert, M. A. and C. E. Nelson. 1991. Sex determination in turtles: Diverse patterns and some possible adaptive values. Copeia 50-69.
- Ferguson, G. W., W. H. Gehrmann, K. B. Karsten, S. H. Hammack, M. McRae, T. C. Chen, N. P. Lung and M. F. Holick. 2003. Do Panther chameleons bask to regulate endogenous Vitamin D3 production? Physiological and Biochemical Zoology **76**:52-59.
- Fitzgerald, L. A. and R. E. Nelson. 2011. Thermal biology and temperature-based habitat

- selection in a large aquatic ectotherm, the alligator snapping turtle, *Macrolemys temminckii*. *Journal of Thermal Biology* **36**:160-166.
- Fritz, U., W. R. Branch, M. D. Hofmeyr, J. Maran, H. Prokop, A. Schleicher, P. Široký, H. Stuckas, M. Vargas-Ramírez, M. Vences and A. K. Hundsdörfer. 2011. Molecular phylogeny of African hinged and helmeted terrapins (Testudines: Pelomedusidae: *Pelusios* and *Pelomedusa*). *Zoologica Scripta* **40**:115-125.
- Fritz, U., A. Petzold, C. Kehlmaier, C. Kindler, P. Cambell, M. D. Hofmeyr and W. R. Branch. 2014. Disentangling the *Pelomedusa* complex using type specimens and historical DNA (Testudines: Pelomedusidae). *Zootaxa* **3795**:501-522.
- Gatten, R. E. 1974. Effect of nutritional status on the preferred body temperature of the turtles *Pseudemys scripta* and *Terrapene ornata*. *Copeia* 912-917.
- Gibbons, J. W., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy and C. T. Winne. 2000. The global decline of reptiles, déjà vu amphibians. *Bioscience* **50**:653-666.
- Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. *Science* **293**:2248-2251.
- Glanville, E. J. and F. Seebacher. 2006. Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm. *Journal of Experimental Biology* **209**:4869-4877.
- Glass, M., W. W. Burggren and K. Johansen. 1978. Ventilation in an aquatic and a terrestrial chelonian reptile. *Journal of Experimental Biology* **72**:165-179.
- Grayson, K. L. and M. E. Dorcas. 2004. Seasonal temperature variation in the painted turtle (*Crysemys picta*). *Herpetologica* **60**:325-336.
- Greaves, W. F. and J. D. Litzgus. 2007. Overwintering ecology of wood turtles (*Glyptemys insculpta*) at the species' northern range limit. *Journal of Herpetology* **41**:32-40.
- Greenwald, O. E. and M. E. Kanter. 1979. The effects of temperature and behavioral thermoregulation on digestive efficiency and rate in corn snakes (*Elaphe guttata guttata*). *Physiological Zoology* **52**:398-408.
- Hailey, A. and J. P. Loveridge. 1997. Metabolic depression during dormancy in the African tortoise *Kinixys spekii*. *Canadian Journal of Zoology* **75**:1328-1335.
- Hammond, K. A., J. R. Spotila and E. A. Standora. 1988. Basking behaviour of the turtle *Pseudemys scripta*: Effects of digestive state, acclimation temperature, sex and season. *Physiological Zoology* **61**:69-77.
- Harden, L. A., N. A. Diluzio, J. W. Gibbons and M. E. Dorcas. 2007. Spatial and thermal

- ecology of diamondback terrapins (*Malaclemys terrapin*) in a South Carolina salt marsh. *Journal of the North Carolina Academy of Science* **123**:154-162.
- Harlow, H. J., D. Purwandana, T. S. Jessop and A. J. Phillips. 2010. Size-related differences in the thermoregulatory habits of free-ranging Komodo dragons. *International Journal of Zoology* 1-9.
- Hennemann, W. W. 1979. The influence of environmental cues and nutritional status on frequency of basking in juvenile Suwanee terrapins (*Chrysemys concinna*). *Herpetologica* **35**:129-131.
- Hertz, P. E., R. B. Huey and R. D. Stevenson. 1993. Evaluating temperature regulation by field-active ectotherms: The fallacy of the inappropriate question. *The American Naturalist* **142**:796-818.
- Huey, R. B. 1974. Behavioral thermoregulation in lizards: Importance of associated costs. *Science* **184**:1001-1003.
- Huey, R. B. and M. Slatkin. 1976. Cost and benefits of lizard thermoregulation. *The Quarterly Review of Biology* **51**:363-384.
- Huey, R. B. and E. R. Pianka. 1977. Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. *Ecology* **58**:1066-1075.
- Huey, R. B. and R. D. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *American Zoologist* **19**:357-366.
- Huey, R. B. and J. J. Tewksbury. 2009. Can behavior douse the fire of climate warming? *Proceedings of the National Academy of Sciences* **106**:3647-3648.
- Hutchison, V. H., A. Vinegar and R. J. Kosh. 1966. Critical thermal maxima in turtles. *Herpetologica* **22**:32-41.
- Hutchison, V. H. and J. D. Maness. 1979. The role of behavior in temperature acclimation and tolerance in ectotherms. *American Zoologist* **19**:367-384.
- Ivanov, K. P. 1999. Subject of temperature control and the main function of thermoregulation of an organism. *Journal of Thermal Biology* **24**:415-421.
- Iverson, J. 1984. Proportional skeletal mass in turtles. *Florida Scientist* **47**:1-11.
- Janzen, F. J., G. L. Paukstis and E. D. B. Iii. 1992. Observations on basking behavior of hatchling turtles in the wild. *Journal of Herpetology* **26**:217-219.
- Jarošík, V., L. Kratochvíl, A. Honěk and A. F. G. Dixon. 2004. A general rule for the dependence of developmental rate on temperature in ectothermic animals. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **271**:S219-S221.

- Karsten, K. B., G. W. Fergusin, T. C. Chen and M. F. Holick. 2009. Panther chameleons, *Furcifer pardalis*, behaviorally regulate optimal exposure to UV depending on dietary Vitamin D3 status. *Physiological and Biochemical Zoology* **82**:218-225.
- Kearney, M., R. Shine and W. P. Porter. 2009. The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. *Proceedings of the National Academy of Sciences* **106**:3835-3840.
- Kingsolver, J. G. and R. B. Huey. 2008. Size, temperature, and fitness: Three rules. *Evolutionary Ecology Research* **10**:251-269.
- Knight, T. W., J. A. Layfield and R. J. Brooks. 1990. Nutritional status and mean selected temperature of hatchling snapping turtles (*Chelydra serpentina*): Is there a thermophilic response to feeding? *Copeia* 1067-1072.
- Lagerspetz, K. Y. H. 2006. What is thermal acclimation? *Journal of Thermal Biology* **31**:332-336.
- Law, B. S. and R. A. Bradley. 1990. Habitat use and basking site selection in the Water skink, *Eulamprus quoyii*. *Journal of Herpetology* **24**:235-240.
- Lefevre, K. and R. J. Brooks. 1995. Effects of sex and body size on basking behaviour in a northern population of the painted turtle, *Chrysemys picta*. *Herpetologica* **51**:217-224.
- Luiselli, L. and G. C. Akani. 2002. Is thermoregulation really unimportant for tropical reptiles? Comparative study of four sympatric snake species from Africa. *Acta Oecologica* **23**:59-68.
- Magnuson, J. J., L. B. Crowder and P. A. Medvick. 1979. Temperature as an ecological resource. *American Zoologist* **19**:331-343.
- Manning, B. and G. C. Grigg. 1997. Basking is not of thermoregulatory significance in the "basking" freshwater turtle *Emydura signata*. *Copeia* 579-584.
- Marchand, M. N. and J. A. Litvaitis. 2004. Effects of habitat features and landscape composition on the population structure of a common aquatic turtle in a region undergoing rapid development. *Conservation Biology* **18**:758-767.
- Margules, C. and M. B. Usher. 1981. Criteria used in assessing wildlife conservation potential: A review. *Biological Conservation* **21**:79-109.
- McConnachie, S., G. J. Alexander and M. J. Whiting. 2007. Lower temperature tolerance in the temperate, ambush foraging lizard *Pseudocordylus melanotus melanotus*. *Journal of Thermal Biology* **32**:66-71.
- McConnachie, S., S. N. Greene and M. R. Perrin. 2011. Thermoregulation in the semi-aquatic yellow anaconda, *Eunectes notaeus*. *Journal of Thermal Biology* **36**:71-77.

- Meek, R. and R. A. Avery. 1988. Mini-review: Thermoregulation in chelonians. British Herpetological Society, London, ROYAUME-UNI.
- Norris, K. S. 1953. The ecology of the desert iguana *Dipsosaurus dorsalis*. *Ecology* **34**:265-287.
- O'Steen, S. 1998. Embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles *Chelydra serpentina*. *The Journal of Experimental Biology* **201**:439-449.
- Paladino, F. V., M. P. O Connor and J. R. Spotila. 1990. Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature* **344**:858.
- Patterson, J. W. 1991. Emergence, basking behaviour, mean selected temperature and critical thermal minimum in high and low altitude subspecies of the tropical lizard *Mabuya striata*. *African Journal of Ecology* **29**:330-339.
- Patterson, J. W. and P. M. C. Davies. 1978. Thermal acclimation in temperate lizards. *Nature* **275**:646-647.
- Peterman, W. E. and T. J. Ryan. 2009. Basking behavior of Emydid turtles (*Chysemys picta*, *Graptemys geographica*, and *Trachemys scripta*) in an urban landscape. *Northeastern Naturalist* **16**:629-636.
- Petzold, A. V.-R., M., C. V. Kehlmaier, M. Branch, W. R., L. H. Du Preez., M. D. Meyer, L., A. S. Schleicher., P. and U. Fritz. 2014. A revision of African helmeted terrapins (Testudines: Pelomedusidae: *Pelomdusa*), with descriptions of six new species. *Zootaxa* **3795**:523-548.
- Pough, F. H. and C. Gans. 1982. The vocabulary of reptilian thermoregulation. Academic Press New York, New York.
- Reynolds, W. W. and M. E. Casterlin. 1979. Behavioral thermoregulation and the "final preferendum" paradigm. *American Zoologist* **19**:211-224.
- Rhen, T. and J. W. Lang. 1999. Temperature during embryonic and juvenile development influences growth in hatchling snapping turtles, *Chelydra serpentina*. *Journal of Thermal Biology* **24**:33-41.
- Riedesel, M. L., J. L. Cloudsley-Thompson and J. A. Cloudsley-Thompson. 1971. Evaporative thermoregulation in turtles. *Physiological Zoology* **44**:28-32.
- Rollinson, N., G. J. Tattersall and R. J. Brooks. 2008. Overwintering habitats of a northern population of painted turtles (*Chrysemys picta*): Winter temperature selection and dissolved oxygen concentrations. *Journal of Herpetology* **42**:312-321.
- Roosenburg, W. M., Haley, K. L. and McGuire, S. 1999. Habitat selection and movements of

- diamondback terrapins, *Malaclemys terrapin*, in a Maryland estuary. *Chelonian Conservation and Biology* **3**:425-429.
- Ryan, T. J. and A. Lambert. 2005. Prevalence and colonization of *Placobdella* on two species of freshwater turtles (*Graptemys geographica* and *Sternotherus odoratus*). *Journal of Herpetology* **39**:284-287.
- Schwarzkopf, L. and R. Shine. 1992. Costs of reproduction in lizards: Escape tactics and susceptibility to predation. *Behavioral Ecology and Sociobiology* **31**:17-25.
- Scott-Shaw, C. R. and B. J. Escott. 2011. KwaZulu-Natal Provincial pre-transformation vegetation type map – 2011. Unpublished GIS Coverage [kznveg05v2_1_11_wll.zip] Biodiversity Conservation Planning Division, Ezemvelo KZN Wildlife, Pietermaritzburg.
- Seebacher, F. 2005. A review of thermoregulation and the physiological performance in reptiles: What is the role of phenotypic flexibility. *Journal of Comparative Physiology* **175**:453-461.
- Seebacher, F., G. C. Grigg and L. A. Beard. 1999. Crocodiles as dinosaurs: Behavioural thermoregulation in very large ectotherms leads to high and stable body temperatures. *The Journal of Experimental Biology* **202**:77-86.
- Seebacher, F. and R. Shine. 2004. Evaluating thermoregulation in reptiles: The fallacy of the inappropriately applied method. *Physiological and Biochemical Zoology* **77**:688-695.
- Seebacher, F. and C. Franklin. 2005. Physiological mechanisms of thermoregulation in reptiles: A review. *Journal of Comparative Physiology B* **175**:533-541.
- Seigel, R. A. 1984. Parameters of two populations of diamondback terrapins (*Malaclemys terrapin*) on the Atlantic coast of Florida. *Vertebrate Ecology and Systematics - a tribute to Henry S. Fitch*:77-87.
- Shine, R. and T. Madsen. 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiological Zoology* **69**:252-269.
- Smith, E. N., S. L. Robertson and S. R. Adams. 1981. Thermoregulation of the spiny soft-shelled turtle *Trionyx spinifer*. *Physiological Zoology* **54**:74-80.
- Smith, E. N., N. C. Long and J. Wood. 1986. Thermoregulation and evaporative water loss of green sea turtles, *Chelonia mydas*. *Journal of Herpetology* **20**:325-332.
- Spellerberg, I. F. 1972. Thermal ecology of allopatric lizards (*Sphenomorphus*) in southeast Australia. The environment and lizard critical temperatures. *Oecologia* **9**:371-383.
- Spotila, J. R., R. E. Foley, J. P. Schubauer, R. D. Semlitsch, K. M. Crawford, E. A. Standora

- and J. W. Gibbons. 1984. Opportunistic behavioral thermoregulation of turtles, *Pseudemys scripta*, in response to microclimatology of a nuclear reactor cooling reservoir. *Herpetologica* **40**:299-308.
- Spotila, J. R. and E. A. Standora. 1985. Environmental constraints on the thermal energetics of sea turtles. *Copeia* 694-702.
- Stevenson, R. D. 1985a. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *The American Naturalist* **125**:102-117.
- Stevenson, R. D. 1985b. The relative importance of behavioural and physiological adjustments controlling body temperature in terrestrial ectotherms. *The American Naturalist* **126**:362-386.
- Storey, K. B. and J. M. Storey. 2001. Hibernation: Poikilotherms. eLS. John Wiley & Sons, Ltd.
- Storey, E. M., S. M. Kayes, I. De Vries and C. E. Franklin. 2008. Effect of water depth, velocity and temperature on the surfacing frequency of the bimodally respiring turtle *Elseya albagula*. *Functional Ecology* **22**:840-846.
- Swimmer, J. Y. 2006. Relationship between basking and Fibropapillomatosis in captive green turtles (*Chelonia mydas*). *Chelonian Conservation and Biology* **5**:305-309.
- Tamplin, J. 2006. Response of hatchling wood turtles (*Glyptemys insculpta*) to an aquatic thermal gradient. *Journal of Thermal Biology* **31**:400-405.
- Tamplin, J. 2009. Effect of age and body size on selected temperature by juvenile wood turtles (*Glyptemys insculpta*). *Journal of Thermal Biology* **34**:41-48.
- Tamplin, J. W. and A. B. Cyr. 2011. Effects of acclimation and egg-incubation temperature on selected temperature by hatchling western painted turtles (*Chrysemys picta bellii*). *Journal of Thermal Biology* **36**:507-514.
- Tucker, A. D., N. N. FitzSimmons and J. W. Gibbons. 1995. Resource partitioning by the estuarine turtle *Malaclemys terrapin*: Trophic, spatial, and temporal foraging constraints. *Herpetologica* **51**:167-181.
- Vargas-Ramírez, M., M. Vences, W. R. Branch, S. R. Daniels, F. Glaw, M. D. Hofmeyr, G. Kuchling, J. Maran, T. J. Papenfuss, P. Široký, D. R. Vieites and U. Fritz. 2010. Deep genealogical lineages in the widely distributed African helmeted terrapin: Evidence from mitochondrial and nuclear DNA (Testudines: Pelomedusidae: *Pelomedusa subrufa*). *Molecular Phylogenetics and Evolution* **56**:428-440.
- Webb, J. K. and M. J. Whiting. 2005. Why don't small snakes bask? Juvenile broad-headed snakes trade thermal benefits for safety. *Oikos* **110**:515-522.

- Whittow, G. C. and G. H. Balazs. 1982. Basking behaviour of the Hawaiian green turtle (*Chelonia mydas*). *Pacific Science* **36**:129-139.
- Wikelski, M. 1999. Influences of parasites and thermoregulation on grouping tendencies in marine iguanas. *Behavioral Ecology* **10**:22-29.
- Wills, C. A. and S. J. Beaupre. 2000. An application of randomization for detecting evidence of thermoregulation in timber rattlesnakes (*Crotalus horridus*) from northwest Arkansas. *Physiological and Biochemical Zoology* **73**:325-334.
- Wong, R. A., J. J. Fong and T. J. Papenfuss. 2000. Phylogeography of the African helmeted terrapin, *Pelomedusa subrufa*: Genetic structure, dispersal, and human introduction. *Science* **61**:575-585.
- Wu, M. X., L. J. Hu, W. Dang, H. L. Lu and W. G. Du. 2013. Effect of thermal acclimation on thermal preference, resistance and locomotor performance of hatchling soft-shelled turtle. *Current Zoology* **59**:718-724.
- Yntema, C. L. 1978. Incubation times for eggs of the turtle *Chelydra serpentina* (Testudines: Chelydridae) at various temperatures. *Herpetologica* **34**:274-277.

APPENDIX

A1: Operative temperatures

Operative temperatures were measured using models that approximated the shape, size and weight of the terrapins. These were operative temperatures and were used to compare with terrapin body temperature (T_b). Models were constructed by binding two stainless steel plates (20cm diameter; 4cm depth) together with aquarium safe silicon, painting them black and filling them with water through a hole in the centre of the top of the model. Two temperature data loggers (iButtons; Thermochron iButton®, Dallas Semiconductor, Texas, USA; 30 minute and 90 minute intervals) were held in place with fencing wire, so that the iButtons were suspended in the centre of the model (figure 22, below). These iButtons recorded temperatures that were available to the terrapin in the water, air and sub-terrestrial areas. The model was sealed off with a lid that was glued in place with aquarium safe silicone.

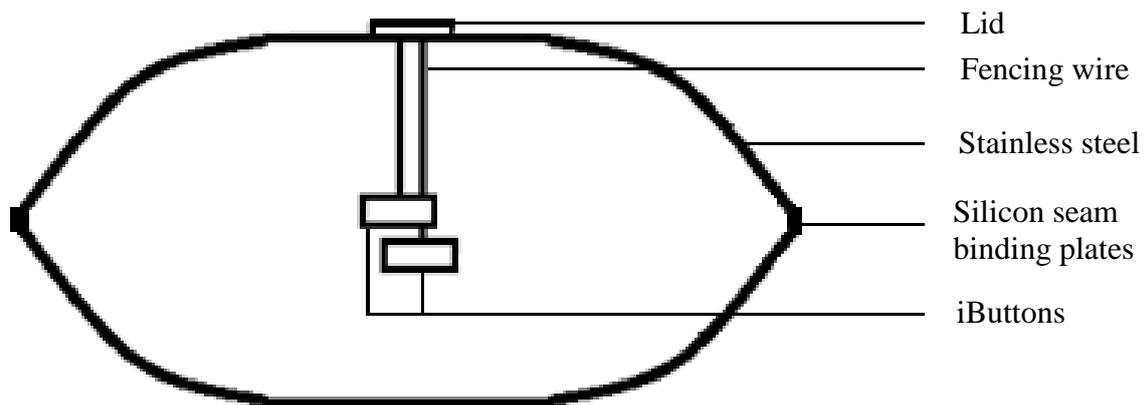


Figure 22: Design of a physical model to predict operative temperature for the *Pelomdusa galeata*.

The 'best fit' model was chosen on the basis that the model and an average sized terrapin (1.70kg) had similar cooling and heating rates. This was tested by heating the terrapin and the model at the same time with an infrared light for an hour, in a room at constant temperature (24 ± 1 °C). To determine heating rates, cloacal temperatures (taken as T_b) of the terrapin and water temperatures at the centre of the model were recorded every 10 minutes. For cooling rates, the light was switched off, both model and terrapin were allowed to cool for an hour and temperatures were recorded using the same methods, every 10 minutes. Figure 23 (below) shows a comparison of heating and cooling rates between the terrapin and model. There was 91% similarity in heating rate and 97% similarity in cooling rate.

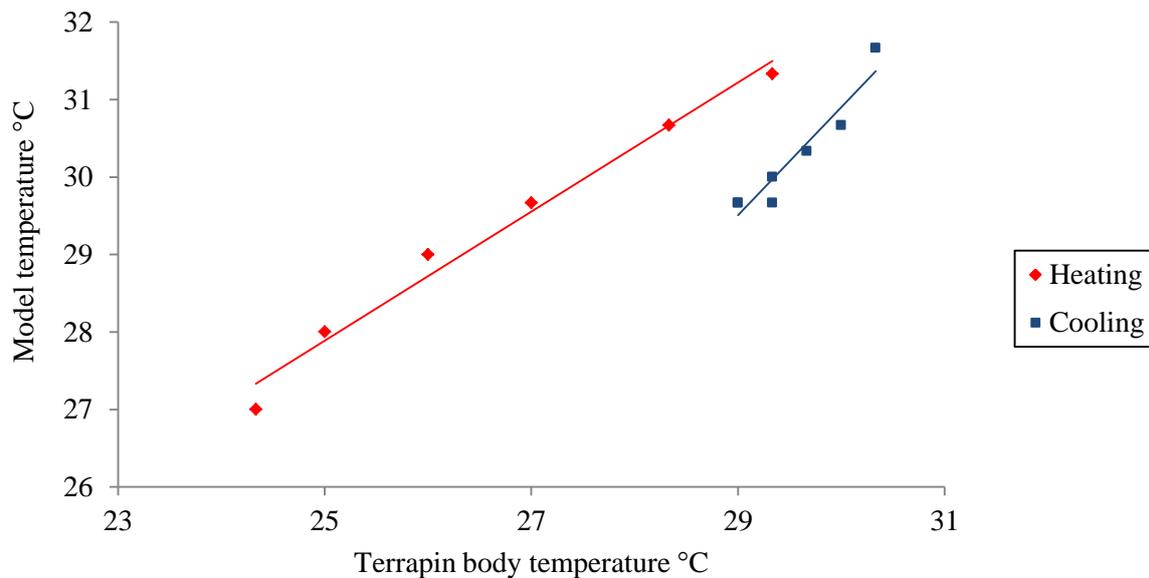


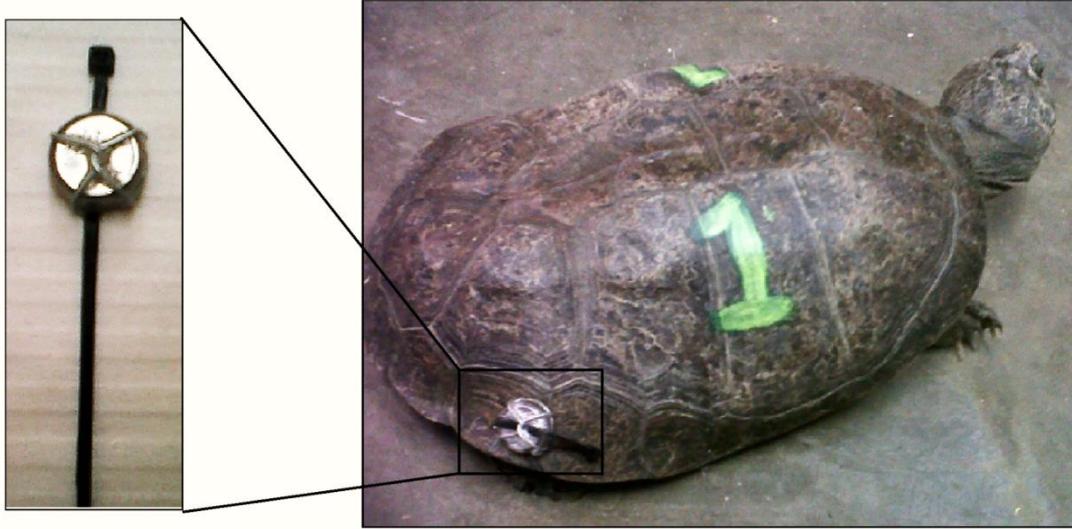
Figure 23: Heating ($y = 0.83x + 7.06$; $R^2 = 0.98$) and cooling ($y = 1.40x - 10.97$; $R^2 = 0.91$) rates of operative temperature models compared with a terrapin of approximately mean mass (*Pelomedusa galeata*; 1.70kg).

Models were placed in areas the terrapins were previously observed in both the field and the study pond:

1. Fully submerged, at the deepest point in the pond;
2. Partially submerged with top of model exposed, similar to water surface basking behaviour of terrapin;
3. On the pallet and out of the water entirely;
4. Buried in the ground; and
5. On land and in full sun

Temperatures recorded by the iButtons in the models were temperatures that were available to the terrapins. Two iButtons were wired into the models, one was set at 30 minute intervals, and the other at 180 minute intervals.

Micro-environment temperatures were recorded with an iButton that was fitted to the posterior marginal scutes of each terrapin. Each iButton was attached to a cable tie 2mm fencing wire and then covered in clear Plasti Dip multi-purpose rubber coating (Performix Brand®, Plasti Dip International, Blaine MN 55449, USA). The iButton was then covered in another coat of black spray-on Plasti Dip. This ensured the iButton remained attached to the cable tie and was protected from any abrasion caused by digging or physical contact with other terrapins. Two holes of 3mm in diameter were drilled through the third and fourth posterior scutes approximately 30mm apart. A cable tie was then threaded through the holes and the iButton fastened to the upper side of the carapace (figure 24, below). These iButtons were set to record temperatures every 30 minutes.



*Figure 24: iButtons were attached to cable ties (left) and were threaded through 3mm holes, 30mm apart. They were attached to the third and fourth posterior marginal scutes on the right side of the carapace of *Pelomedusa galeata*.*