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An energy budget for the lizard *Pseudocordylus melanotus melanotus*, an extreme sit-and-wait forager

Suzanne McConnachie

A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

JOHANNESBURG

February 2006

DECLARATION

I declare that this thesis is my own, unaided work unless specifically acknowledged in the text. It has not been submitted before for any degree or examination in any other university, nor has it been prepared under the aegis or with the assistance of any other body or organisation or person outside the University of the Witwatersrand, Johannesburg.

_____

_____ day of February 2006

“For the animal shall not be measured by man. In a world older and more complete than ours they moved finished and complete, gifted with the extensions of the senses that we have lost or never attained, living by voices we shall never hear. They are not brethren, they are not underlings; they are other nations, caught with ourselves in the net of life and time, fellow prisoners of the splendor and travail of the earth.”

HENRY BESTON, THE OUTERMOST HOUSE



ABSTRACT

Characteristics comprising an energy budget of *Pseudocordylus melanotus melanotus* were investigated in terms of limiting factors for the lizard's distribution, and included measures of thermal biology, metabolism and digestive physiology. *Pseudocordylus m. melanotus* is rupicolous, is limited to the temperate parts of southern Africa and exhibits adaptations to these environments. The distribution of the lizard was modelled using two different climate envelope modelling techniques, which are discussed in terms of the energy budget and factors limiting the distribution of the lizard.

Pseudocordylus m. melanotus exhibits wide thermal tolerances. It can withstand body temperatures (T_b) below freezing, but freezing of body water, which occurs at ca. -5 ºC, is lethal. The lower critical minimum temperature was a surprisingly high measure for an apparently cold-adapted lizard. In the laboratory, the selected body temperature (T_{sel}) was ca. 30 ºC. In the wild, lizards thermoregulate by shuttling between hot and cold microclimates, modifying body postures and regulating activity times, and select T_bs of ca. 29 ºC in summer and ca. 26 ºC in winter.

Energy expenditure was measured over a range of temperatures. MR increases significantly with increasing temperature. Body mass and metabolic rate (MR) were significantly related at 30 ºC only. The lack of significance at other temperatures can be ascribed to the small range of body mass over which measures were made.

Energy gain in *P. m. melanotus* was quantified in terms of prey capture rates and by measuring aspects of digestive physiology. Prey capture attempts were 35 % successful and occurred ca. every 2 h 30 min in the field. Lizards consumed a variety of arthropods, but mainly ate beetles. Digestive rate and appetite increased significantly with increasing temperature. Apparent digestive efficiency (ca. 94 %) and apparent assimilation efficiency (ca. 87.2 %) were not affected by temperature.

Two energy budgets were calculated; one based mainly on laboratory measures and the other based on the thermal profile of lizard body temperature measured in the field. The laboratory energy budget was approximately half that of the field energy budget. The field energy budget, however, provided a more realistic view of energy expenditure since it covered almost the entire thermal range experienced by lizards. Between 592.53 kJ (field energy budget) and 940.06 kJ (laboratory energy budget) per year is required for a standard 30 g *P. m. melanotus* to remain in energy balance. This means that lizards need to consume between 73.55 and 116.69 g of mealworms per year, which equates to ca. 740 to 1200 average mealworms. Any energy gained over and above these requirements can be allocated to growth, reproduction and storage.

In terms of the lizard's distribution, it will be excluded from areas where the available thermal environment and prey abundance do not allow the lizard to maintain a positive energy balance. The lizard is apparently at the cold limit of its range, so predicted climate change in southern Africa is unlikely to have a significant effect on the distribution of the lizard. Suitable crevices are essential to the lizard's survival, particularly during periods of extreme cold when lizards may experience T_bs where they are incapacitated, or effectively 'comatose', while in retreats.

ACKNOWLEDGEMENTS

I gratefully thank Professors Graham Alexander and Martin Whiting for their advice and supervision. Graham – thank you for your continual, tolerant advice and many laughs along the way. Martin – thank you for your steady, unwavering help, support and understanding. You have both given me so much, including your passion for reptiles, and I am very grateful to you.

I thank the following people for their invaluable field support, be it catching lizards, or just the company: Lorren Haywood, Kerry Nel, Jenny Lancaster, Bryan Maritz, Gavin Masterson, Toby Hibbits, Trevor McIntyre, Johan Marais, Stephen Secor, Julie Coetzee and Hans Wagener. I would especially like to thank Andrew and Ross McConnachie who spent many hours at Suikerbosrand with me, without them I would not have been able to catch a single lizard. Thanks also to Nico Douths, Jenny Lancaster and the staff of the Milner Park Animal Unit, who helped look after the lizards, particularly when I was out of action.
I also thank Barend Erasmus and Bryan Maritz for their extraordinarily patient assistance with the multivariate climate envelope model and Barry Lovegrove for his help with analysis of metabolic data.

Special thanks go to Prof. Mike Perrin in the School of Biological and Conservation Sciences at the University of KwaZulu-Natal for his encouragement and generosity while I was finishing the project. It was greatly appreciated.

Gauteng Nature Conservation and Neville Green granted permission to work at Suikerbosrand Nature Reserve (permit numbers: 1143, 021, 0476, and 0756).

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are not necessarily to be attributed to the NRF.

All experimental procedures used were cleared by the Animal Ethics Screening Committee of the University of the Witwatersrand under the following numbers: 2001/27/2A, 2001/28/2A, 2001/30/2A, 2001/31/2A, 2001/74/2A, 2002/84/2A, 2002/85/2A, 2003/56/6, and 2003/57/3.

Particular thanks go to the Moon and McConnachie families for their support and encouragement. Mum and Dad – thank you for giving me the opportunity to achieve so much. Mum, thank you for many long conversations about life, the universe and everything. Dad, thanks for teaching me to write "proper English" and for giving me the dream and courage for a PhD. To my twin, Heath, thank you for always being there and I wish you could have joined me on this journey. To my brother, Jon, even though you were far away, somehow you always made me laugh through the difficult times – thanks.

Finally, extra special thanks go to my extraordinary husband, Andrew. Thank you for your support, love, understanding, encouragement, patience, hope, courage and belief in me. I could never have done all of this without you.

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CHAPTER 1

INTRODUCTION

1.1) General

The underlying principles of physiology arise in of evolutionary theory and involve understanding mechanisms that operate at all levels (subcellular to integrated whole organisms) in chemical and physical terms. The physiology of an organism is generally very well matched to its environment (Randall *et al.*, 1997), which also means that an organism will be limited to the environments for which it is best suited. The distribution of a particular organism can thus be inferred from physiological attributes and the environmental effects on those attributes can point towards factors limiting the organism's distribution. In understanding the physiology, knowledge can therefore be gained on the limiting environmental factors, which can then be used in management or conservation decisions.

Environmental temperature is one of the main abiotic factors that limit the distribution of animals and is also an important determinant of their activity and metabolic reactions (Bozinovic and Rosenmann, 1989; Prosser and Heath, 1991). For example, body temperature (T_b) in garter snakes is significantly positively correlated with substrate and air temperature (Gregory, 1984) while birds from mesic areas have higher basal and field metabolic rates than those from deserts (Tieleman and Williams, 2000). In birds though, it was generally thought that passerines have higher basal metabolic rates than other avian groups (Rezende *et al.*, 2002). This is, however, not the case when body mass and phylogeny are taken into account (Reynolds and Lee, 1996; Rezende *et al.*, 2002; McKechnie and Wolf, 2004).

Scale is an important consideration when investigating distribution-shaping factors because broad effects by abiotic factors may not be evident on a local level. On small scales, biotic interactions overshadow environmental influences (Root, 1988). At the

largest scale, distribution is limited predominantly by climatic factors, whereas, at a regional level, landscape structure might be important (Bellamy *et al.*, 1998). Landscape structure may exceed the critical level of habitat fragmentation at which the viability of a regional population would be decreased (Bellamy *et al.*, 1998). The level of fragmentation is important because species tend to be increasingly habitat specific in areas of high habitat diversity (Pagel *et al.*, 1991). Some species may be limited because the thermal environment may impose high-energy requirements for regulating temperature that cannot be compensated by food availability (Bozonovic and Rosenmann, 1989). Weathers (1979) suggests that knowledge of the energetics of a species can help to explain its distribution and abundance. Bioenergetics is generally presented in the form of an energy budget.

1.2) Energy budgets

There have been few studies that investigate energy budgets as a whole, but many that involve thorough investigation of various aspects of energy allocation (e.g., energy gain: Pough, 1973; Huey *et al.*, 2001; Cruz-Neto and Bozinovic, 2004; metabolism: Roberts, 1968; Beaupre *et al.*, 1993a; Angilletta, 2001a; storage and reproduction: Derickson, 1976; Vitt, 1978; Vitt and Cooper, 1985; tail regeneration: Vitt *et al.*, 1977) and occasionally, some studies have been combined to form a comprehensive energy budget (e.g., Niewiarowski, 2001). In studies of complete energy budgets, there is also a degree of inconsistency as to how the energy budget is related. For example, Smith (1976) relates the energy balance of Southern Toads (*Bufo terrestris*), Eastern Hognose Snakes (*Heterodon platyrhinos*) and Corn Snakes (*Elaphe guttata*) to reproductive costs while Niewiarowski (2001) relates the energy balance of Eastern Fence Lizards (*Sceloporus undulatus*) to growth. This may be expected because it is sometimes difficult to partition physiological processes into the separate sections of an energy budget (e.g., resting metabolic rate is likely to include the costs of protein synthesis, which is necessary for maintenance; Owen, 2001). Generally, an energy budget is partitioned into energy that is assimilated, used in maintenance metabolism, stored, used for growth and used for reproduction. The balance of energy allocation within these different compartments is dynamic and includes the integration of physiological processes and behaviour (Owen, 2001).

Measuring an energy budget involves the quantification of the energy resources of an individual and includes energy ingested, stored, used in metabolism and egested. This partitioning is greatly affected by temperature, but may also be affected by other factors such as gender and body size. An energy budget can be divided into two main parts – a temperature energy budget (how temperature affects energy partitioning) and a food energy budget (how much energy is assimilated from ingested food). These two factors interact through their effects on T_b and metabolism (Spotila and Standora, 1985). Body temperature is maintained within a particular range of tolerance through thermoregulation. Body temperature affects behaviour, which, in turn, affects thermoregulation, and therefore T_b. Body temperature also affects energy gain (Alexander *et al.*, 2001), which affects energy expenditure, which is itself dependent on the effects of T_b. Energy gain is also affected by behaviour (Figure 1.1). For ectotherms, temperature is therefore central to a series of interactions that affect an organism's energetics and life history.

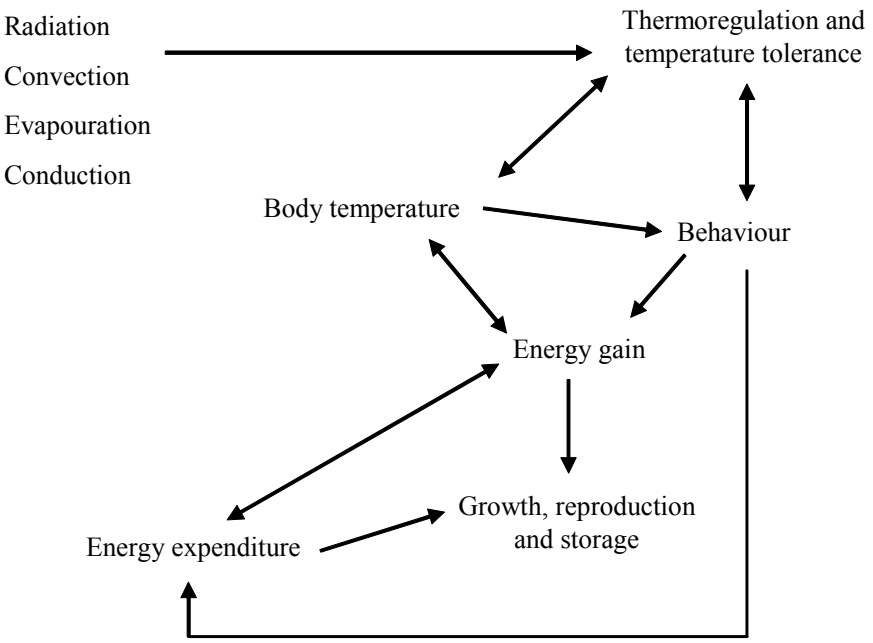


Figure 1.1: Schematic diagram showing the relationship between temperature energy budget and food energy budget of a vertebrate ectotherm. Arrows represent causal effects.

1.3) Study animal

The Drakensberg Crag Lizard (*Pseudocordylus melanotus*) is a member of the Cordylidae, a family endemic to Africa. *Pseudocordylus melanotus* is divided into two subspecies: *Pseudocordylus melanotus melanotus* and *P. m. subviridis*. The distribution of *P. melanotus* (cumulative distribution of both subspecies) extends across the escarpment of South Africa from the Amatola Mountains to Gauteng Province, Mpumalanga Province, the north-eastern Free State Province highveld and northern KwaZulu-Natal Province (Branch, 1998). In addition to the distribution indicated by Branch (1998), there is also an isolated population of *P. m. melanotus* on the Gauteng highveld, which is the population I used in this study.

Pseudocordylus m. melanotus is a rupicolous, extreme sit-and-wait forager (Cooper *et al.*, 1997). These lizards occur in dense colonies on rocky outcrops (Branch, 1998) and are conspicuous when perching on rocks (McConnachie and Whiting, 2003). The species exhibits well-developed sexual dimorphism such that males are larger and more colourful than females (Mouton and van Wyk, 1993). Snout-vent length (SVL) of adults ranges between 80 and 120 mm, but may reach a maximum of 143 mm in males (Branch, 1998). The tail is usually longer than SVL and is typically 53 to 61 % of the total length (Jacobsen, 1989). During the breeding season, males respond aggressively toward other males (Moon, 2001). The male and female reproductive cycles are asynchronous. Males are reported to store sperm in the epidydimi until late winter when ovulation occurs in females (Flemming, 1993a). These lizards reproduce annually and one to six live young are born in late summer (Flemming, 1993b). Although Branch (1998) records this species as being omnivorous, De Waal (1978) reports a diet of insects and spiders and McConnachie and Alexander (2004) found only invertebrate prey in the stomachs of 36 preserved museum specimens (see also Chapter 5).

All lizards used in my study originated from the Suikerbosrand Nature Reserve (SNR), approximately 40 km southeast of Johannesburg, South Africa (26º 27′ – 26º 34′ S, 28º 09′ – 28º 21′ E; 1800 m a. s. l.). The habitat in this area is typically

Highveld Grassland (Rutherford and Westfall, 1986) and is dominated by *Eragrostis*, *Hyparrhenia*, *Themeda* and *Setaria* species (Panagos, 1999). There are numerous rocky outcrops, especially in the higher lying parts of the reserve. Rainfall is less than 500 mm per year and is summer seasonal. The climate is typically temperate; winters are cold with frequent frosts and very occasional snow, while summer temperatures frequently exceed 30 ºC.

Lizards were acclimated in captivity for a minimum of two weeks prior to any experimentation. Lizard body mass increased by approximately 15 % after six months in captivity. Individuals of this species have previously been maintained in captivity in excess of three years (pers. obs.; lizards captured as adults) and generally appear to adapt well to captive conditions. Unless specified, all lizards were housed individually in 300 x 300 x 500 mm glass terraria with a tile shelter. Food (mealworm larvae and beetles; *Tenebrio* sp.) and water were supplied *ad libitum*. Lizards were maintained in a temperature controlled room (28 ± 1ºC) with a light:dark cycle of 12:12 h.

1.4) Factors affecting the distribution of *Pseudocordylus melanotus*?

Factors that affect distribution can be investigated by modelling the distribution of the species (or groups of species at any particular taxonomic level). By including a variety of variables in a distribution model, hypotheses can be generated as to the particular factors affecting the species' distribution, and possibly the intensity of the effect. Distribution modelling based on selected variables can therefore help to direct research towards an organism's tolerances that are likely to affect its distribution. Potentially limiting factors are often manifested in the organism's physiology and ecology, so testing hypotheses can be simple and generate useful measures of limitation.

There are two main requirements in distribution modelling. The first, obviously, is an accurate definition of the current distribution of the species. Potential limiting factors are usually correlated with the current distribution and careful selection of variables in distribution models can generate more realistic hypotheses. The second important

requirement is the phylogenetic relationships of the species. Often a particular taxonomic level (e.g., genus) may be favoured when using any particular model. This is because some clades have been fragmented and have diverged into different species as a result of historic allopatry (see Ridley, 1993). Populations remain isolated because they have limited dispersal, particularly in clades which are, for example, substrate limited. Under these conditions, the factors affecting the distribution generally act at a higher taxonomic level than species and the clade is likely to have the same or similar distributional limitations.

1.5) Distribution modelling

Climate envelope modelling is a commonly used method in distribution modelling since it can be expected that climate is the most important factor limiting species distributions. This method can incorporate all, or most, aspects of climate that can provide information about the factors limiting distribution, or that can be manipulated to produce a desired modelled distribution. In addition, climate envelope modelling can also be used to show how distributions respond to changing climate. This is particularly important in light of current predictions of climate change.

There are a variety of modelling computer programmes, which inevitably focus on one or other potentially limiting factor, and will often yield different results for the same distribution. I used two different climate models to generate hypotheses regarding the factors affecting the distribution of *Pseudocordylus melanotus melanotus*. The first was CLIMEX (© CSIRO, 1999) and the second, a multivariate model similar to that used by Erasmus *et al.* (2000) and Robertson *et al.* (2001; see below). My analysis in both the models used matches the pattern (i.e., distribution) and postulates a process; the hypothesis used is that the variables used in the analysis are causing limits to the distribution.

CLIMEX is a simulation model which enables the estimation of a plant or animal's geographic distribution and relative abundance as determined by climate. It is applied to different species by selecting the values of a series of parameters, which

describe a species' physiological responses to temperature and moisture. Four stress indices (cold, hot, wet and dry) describe the probability of a population surviving and relate to factors limiting geographical distribution (Sutherst *et al.*, 1999). Each stress function has a threshold parameter and a rate parameter. The threshold parameter determines the level above or below which the stress accumulates and the rate parameter determines the rate at which the stress accumulates. The stress indices, together with the growth index determined by the programme, are combined into an 'Ecoclimatic Index' (EI), with values between 0 and 100. This provides an overall measure of the favourability for permanent occupation by the species for a particular location. An EI of 100 indicates a climate for optimal survival of the species.

The programme contains a database of meteorological data from over 2500 locations worldwide and matches the climate profile of a species with the database. CLIMEX then selects the locations, which parameters indicate as suitable for the species, and plots them on a map of the selected area. CLIMEX assesses what locations have a favourable environment and quantifies the stresses, which limit the species distribution. Since the spread of locations with meteorological data over southern Africa is diffuse (CLIMEX only uses locations where there are records from weather stations), a model could not be generated for *P. m. melanotus*. CLIMEX was thus used to generate a climate model for the monophyletic clade including *P. m. melanotus* (see below).

I used CLIMEX to 'reverse' model the distribution of *P. m. melanotus* and the most closely related monophyletic *Pseudocordylus* species from the phylogeny by Frost *et al.* (2001; Figure 1.2) in order to generate hypotheses as to which factors might be limiting the distribution of the '*melanotus* clade'. First, a distribution map for the monophyletic group that included *P. melanotus*, as defined by Frost *et al.* (2001), was compiled from the distribution maps presented in Branch (1998; Figure 1.3). With no data on climatic requirements for *Pseudocordylus*, I began modelling with the temperate template parameters supplied by CLIMEX. The parameters were altered individually until the distribution produced by CLIMEX was approximately the same as the distribution map for the '*melanotus* clade'. Limiting temperatures

were estimated from monthly maximum (December) and minimum (July) temperature maps (Schulze, 1997). I assumed that distribution was not limited by moisture because these lizards are viviparous and do not require a moist environment for embryo development in eggs. Desiccation is an important source of embryo mortality in oviparous species and Shine (1985) has argued that the evolution of viviparity may be in response to desiccation stress. It is, however, possible that moisture may limit other physiological processes. The parameters (and values for the predicted distribution) are presented in Table 1.1, and the resulting map in Figure 1.4.

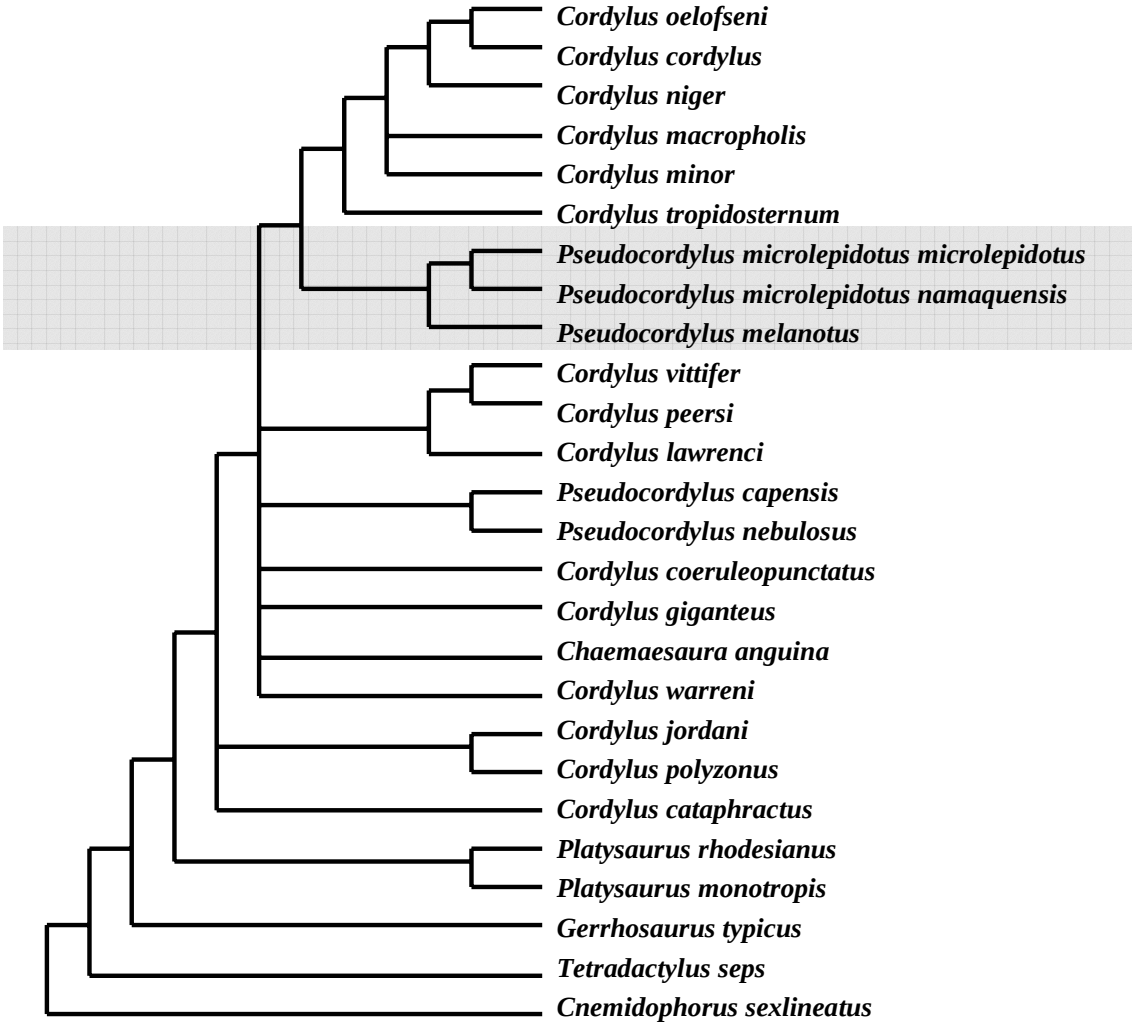


Figure 1.2: Phylogenetic tree showing the relationships between cordylid genera, including outgroups from the Gerrhosauridae and Teiidae (after Frost *et al.*, 2001). Grey area indicates the monophyletic clade including *Pseudocordylus melanotus* and the most closely related *Pseudocordylus* species use to generate the distribution map for climate envelope modelling in CLIMEX.

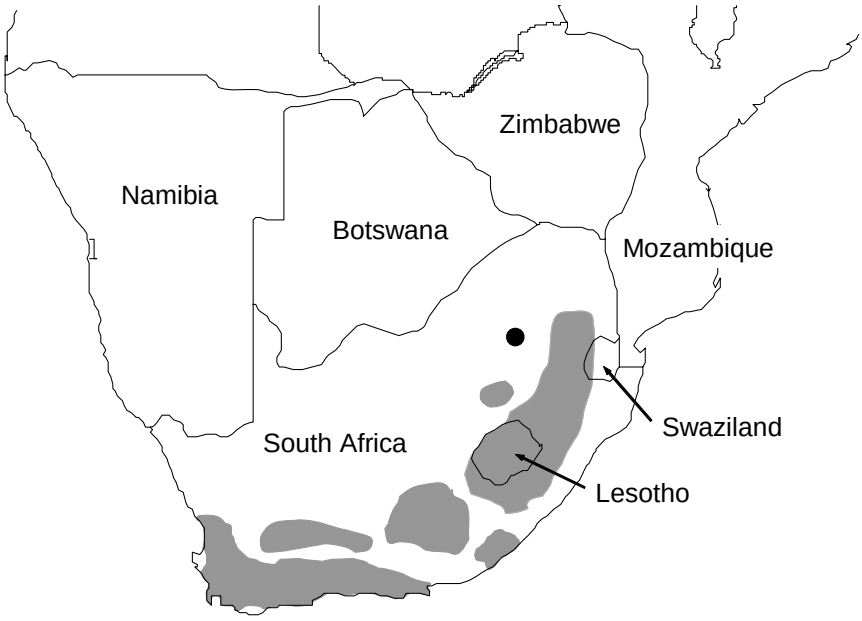


Figure 1.3: Map of southern Africa showing the distribution of the *Pseudocordylus* clade as defined by Frost *et al.* (2001). The distribution map was compiled using Branch (1998). The black circle indicates the population of *P. m. melanotus* used in this study and which is not indicated on Branch's (1998) map.

Table 1.1: CLIMEX (© CSIRO, 1999) parameter values used for generating the map to model the distribution of the monophyletic group of *Pseudocordylus* species from Frost *et al.* (2001).

Parameter	**Value**	**Parameter**	**Value**
Temperature indices (°C)		*Stress indices continued…*	
Limiting low temperature	-2	Dry stress threshold	0.08
Lower optimal temperature	11	Dry stress rate	0.075
Upper optimal temperature	30	Wet stress threshold	1.5
Limiting high temperature	40	Wet stress rate	1
		Cold-dry degree-day threshold	11
Moisture indices (% soil moisture)		Cold-dry moisture threshold	0.35
Limiting low moisture	0	Cold-dry stress rate	1
Lower optimal moisture	0.35	Hot dry temperature threshold (°C)	30
Upper optimal moisture	9.999	Hot-dry moisture threshold	0.25
Limiting high moisture	10	Hot-dry stress rate	0.75
		Hot-wet temperature threshold (°C)	27.5
Stress indices		Hot-wet moisture threshold	0.2
Hot stress temperature threshold (°C)	32	Hot-wet stress rate	1
Hot stress temperature rate	0.05		

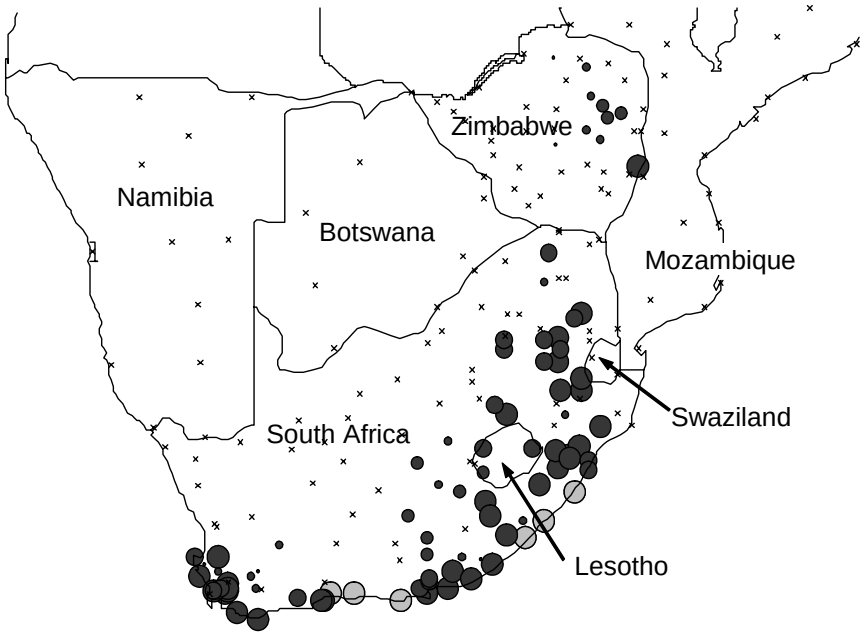


Figure 1.4: Map of southern Africa showing the distribution of the *Pseudocordylus* clade from Frost *et al.* (2001) as predicted by CLIMEX. Crosses indicate where the species would be unable to survive, grey circles indicate an Ecoclimatic index of 100, and size of black circles indicates the probability of survival.

CLIMEX parameters for distribution in southern Africa were also plotted on a map of Africa. The modelled distribution is limited to the southern Africa, east Africa and the north African coast (Figure 1.5). The southern African distribution produced by CLIMEX was considered plausible since the Africa map shows that lizards in this clade will be able to survive in east Africa, in accordance with the typical relic pattern exhibited by numerous plant and animal species (Poynton, 1964). Also, the lizards will be able to survive in a typical Mediterranean temperate climate on the north African coast. Although the map created in the programme is not an exact match, it is possible that inappropriate substrata are excluding the lizard from certain areas, for example, along the south coast.

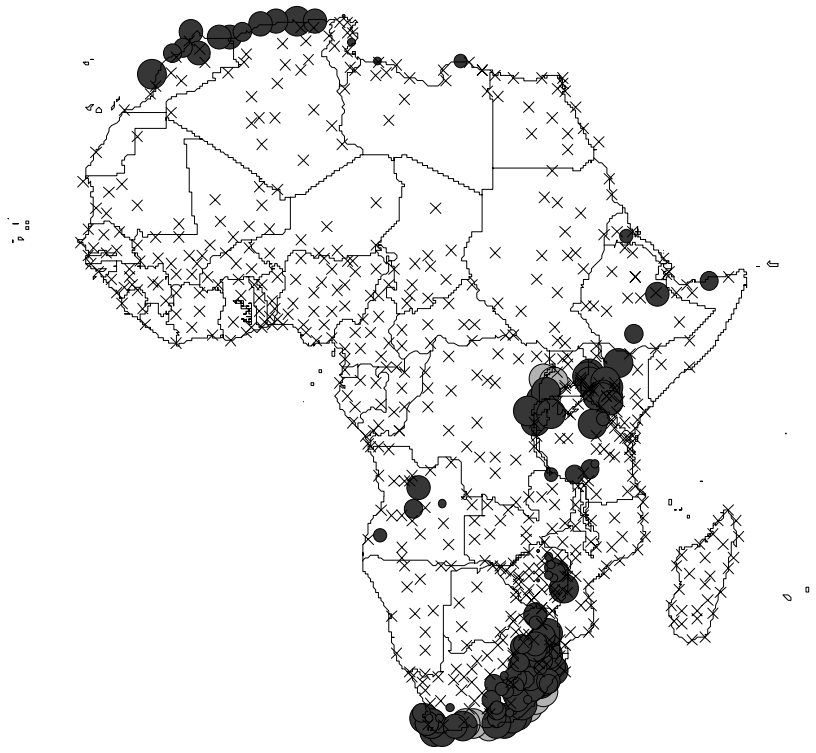


Figure 1.5: Map of Africa showing a modelled distribution of the *Pseudocordylus* clade from Frost *et al.* (2001) as predicted by CLIMEX. Crosses indicate the species would be unable to survive, grey circles indicate an Ecoclimatic index of 100, and size of black circles indicates the probability of survival.

The climate envelope for *P. melanotus* was also modelled using a multivariate model with principal components analysis (PCA) using the method in Erasmus *et al.* (2000), which is similar to that of Robertson *et al.* (2001). In this model, climate data and the current distribution of the lizard were used to derive a climate envelope, which was also applied to predicted climate for 2050. This created values of probability of climatic suitability for each quarter degree square (QDS; approximately 25 x 25 km) in South Africa, Lesotho and Swaziland.

The current distribution of *P. m. melanotus* and *P. m. subviridis* was first defined at QDS scale. Records were collected from museum specimens at the Transvaal Museum in Pretoria and the National Museum in Bloemfontein, as well as from De Waal (1978) and Jacobsen (1989). These records were inserted into ARCGIS (ver. 8.3) where a distribution map at a QDS scale was produced for *P. m. melanotus* and *P. m. subviridis* (Figure 1.6).

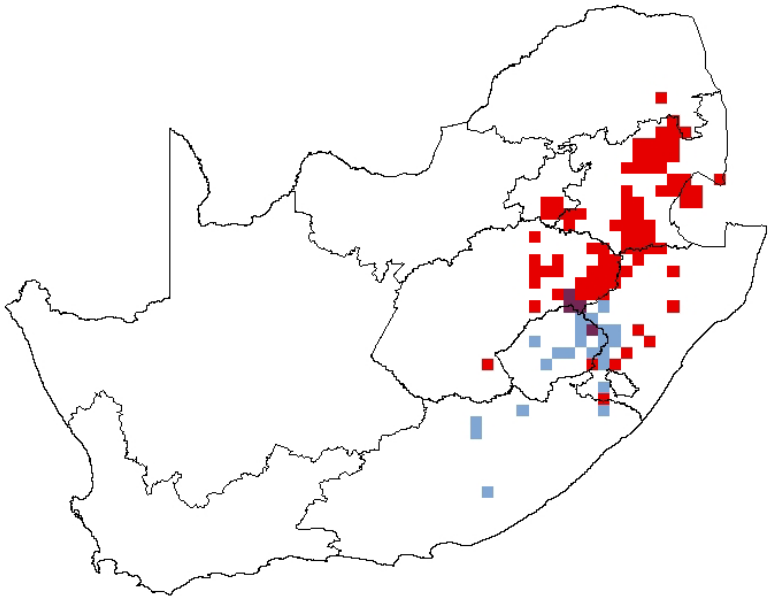


Figure 1.6: Map of South Africa showing the distribution of *Pseudocordylus melanotus* at quarter degree square (QDS) scale. The red QDSs show the distribution of the subspecies *P. m. melanotus*, while the blue QDSs show the distribution of the subspecies *P. m. subviridis*. The purple QDSs indicate both subspecies occur in the same QDS. Map generated from museum records and from De Waal (1978) and Jacobsen (1989).

The climate data selected (also at a QDS scale) were the mean annual minimum and maximum temperatures, mean summer minimum and maximum temperatures, mean winter minimum and maximum temperatures, thus providing the summer and winter limits of temperature, as well as overall average temperatures. Mean minimum and maximum precipitation was also included in the analyses because of the seasonal patterns of rainfall in southern Africa; the strong east to west gradient in rainfall has a significant influence on plants and animals (Harrison *et al.*, 1997).

Probability of climate suitability was calculated based on the climate in the QDSs where the species is known to occur (i.e., QDS records used to generate the distribution map in Figure 1.6; presence records). Data were analysed for current climate and climate predicted for 2050 using the Hadley unified model (HadCM3 model) A2 scenario, which the worst case scenario for southern Africa (www.metoffice.com/research/hadleycentre/models). Climate change predicted for South Africa in this climate model includes a temperature increase of approximately 2.5 ºC and a decrease in annual rainfall of approximately 4.5 mm. The results of the

multivariate analysis were plotted on a map of South Africa, Lesotho and Swaziland using ARCGIS. This climate envelope model was produced for *P. melanotus* (both subspecies; Figure 1.7) and *P. m. melanotus* (Figure 1.8). The predicted climate envelope shows very low probability of climate suitability for the lizards in 2050 ($P < 0.1$). This means that the lizards will experience climates that they have not experienced before and suggests that the species may become extinct if the climatic changes cannot be tolerated.

The distribution of the presence records for *P. melanotus* and both subspecies over the probability of occurrence categories (0.1 intervals; based on current climate variables) was examined to investigate the plausibility of the climate model (Figure 1.9). The greater the number of presence records in the higher probability categories, the more likely the predicted distribution and thus useful the model. At the 50 % level of probability of climate suitability, there are equal chances for presence and absence, therefore, the 60 % level of probability of climate suitability was taken as an arbitrary measure since there is greater chance of presence than there is for absence.

For *P. melanotus*, 60 % probability of climate suitability contained 56 % of the presence records, and for *P. m. melanotus*, 60 % probability of climate suitability contained 59 % of the presence records. Since the majority of the presence records fell within 60 % probability of climate suitability, the models were thus a likely match for the current climate envelope. Should the presence records have been more widely distributed through the probability categories, the model would be less likely. Since only 56 % of the population's current occurrence (i.e., presence records) is predicted by only 60 % of the climate envelope, I suggest that there are additional factors associated with this species' distribution. This model may be similar to the CLIMEX model in that the predicted distribution (based on the climate envelope) may be an overestimate owing to substrate limitations, especially because *P. melanotus* is a rupicolous lizard. Also, since the model is based entirely on climate, the thermal tolerances of the lizard may exceed the temperatures currently

experienced in the field and *P. m. melanotus* may not, in fact, be affected by a 2.5 ºC increase in environmental temperatures.

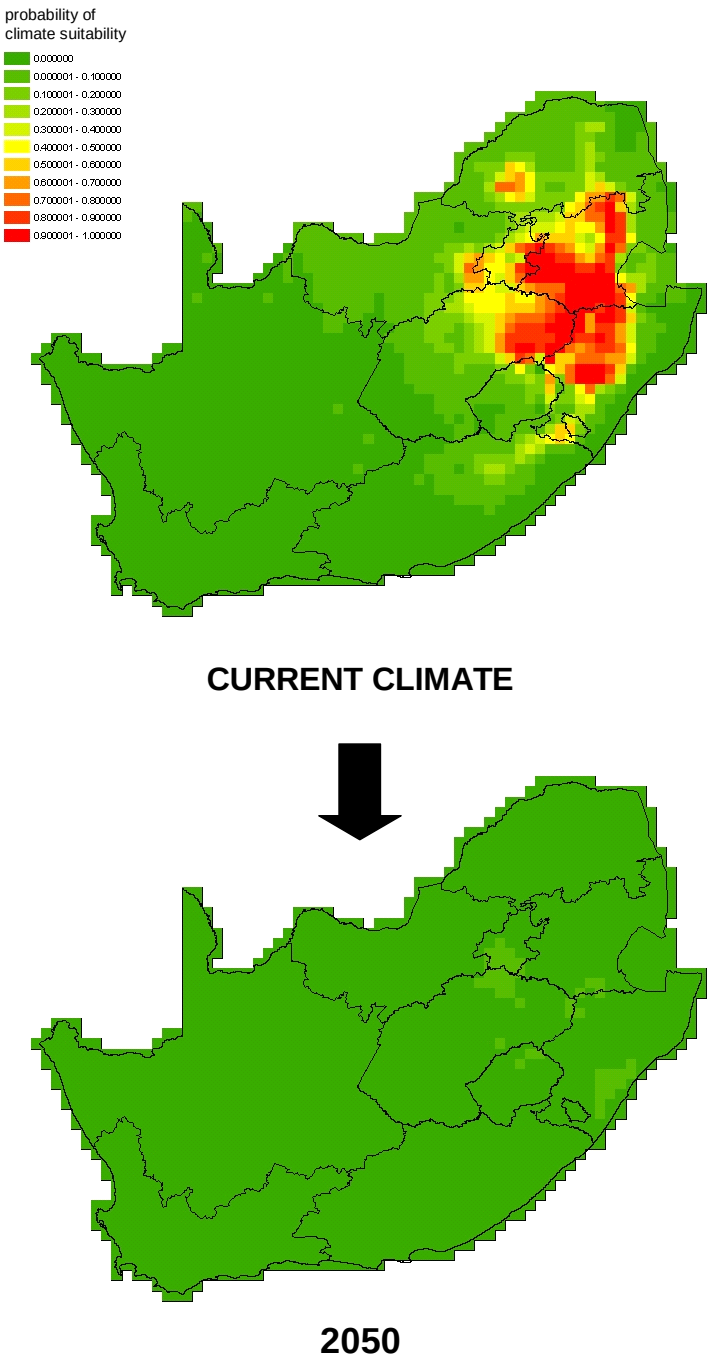


Figure 1.7: Climate envelope model for the species *Pseudocordylus melanotus* modelled for current climate and climate predicted for 2050 showing the probability of climate suitability based on current presence records for the species.

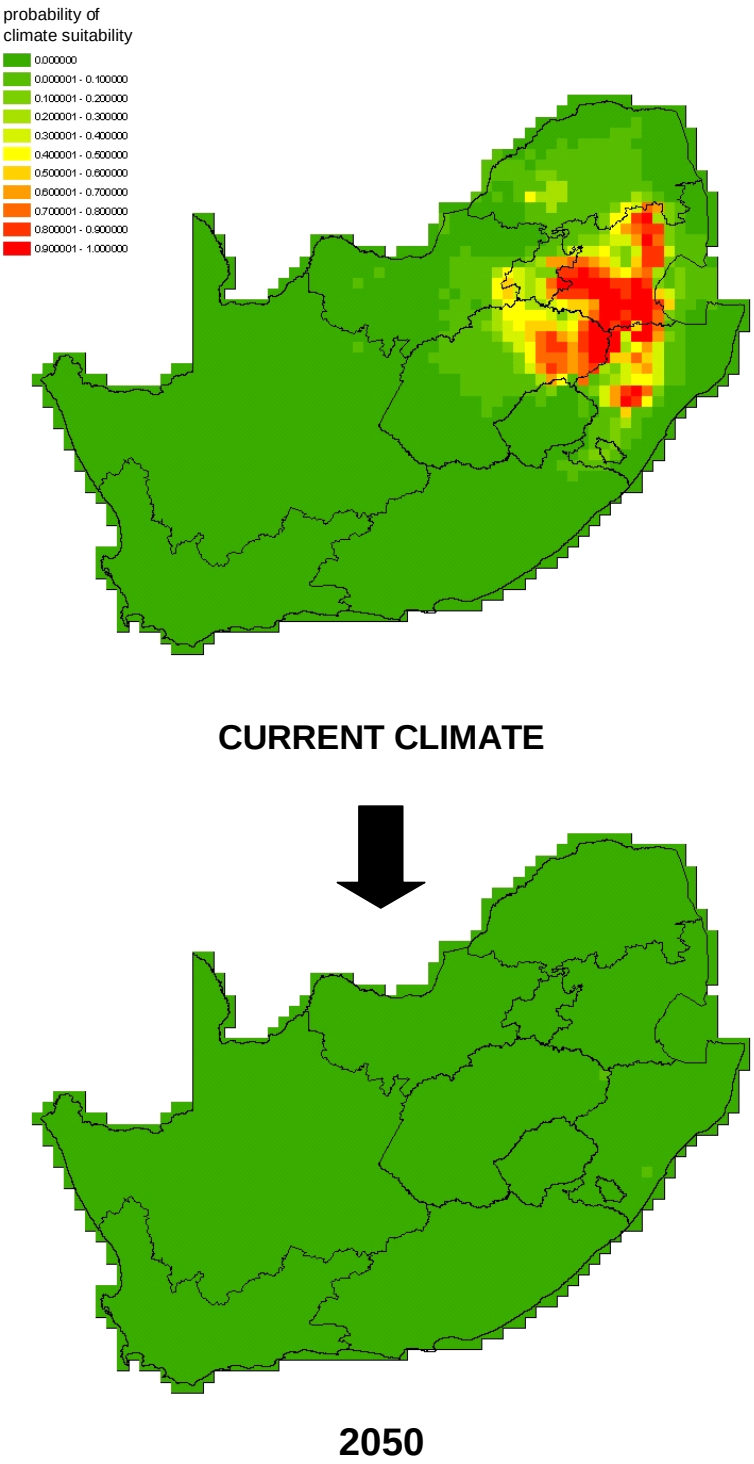


Figure 1.8: Climate envelope model for the subspecies *Pseudocordylus melanotus melanotus* modelled for the current climate and climate predicted for 2050 showing the probability of climate suitability based on current presence records for the subspecies.

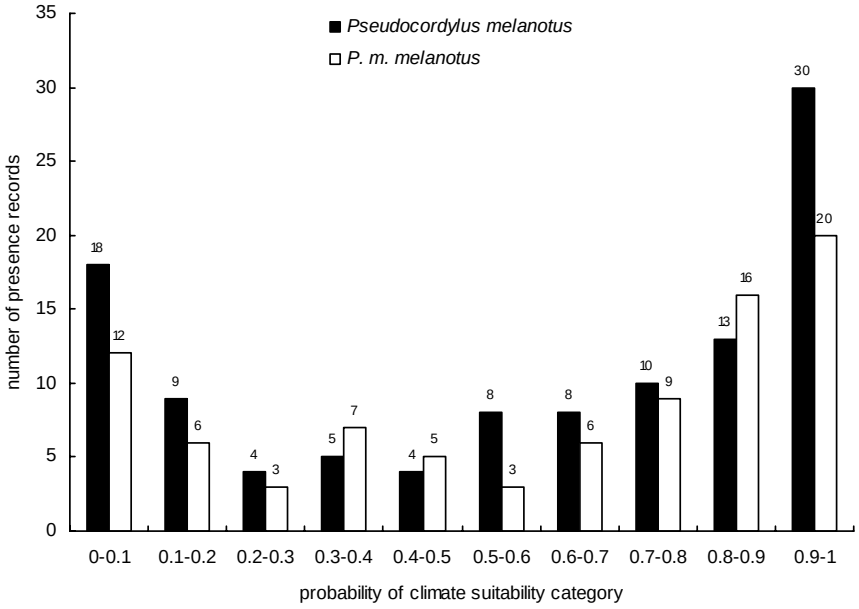


Figure 1.9: Distribution of presence data for *Pseudocordylus melanotus* (both subspecies, n = 109) and *P. m. melanotus* (n = 87) over the probability of climate suitability categories.

1.6) Aims and objectives

The climate envelope models suggest that temperature is the factor limiting the lizard's distribution. In terms of *P. m. melanotus*' thermal biology, it is hypothesized that:

1. The limiting low temperature for *P. m. melanotus* is -2 °C (lower lethal temperature).
2. The lower optimal temperature for *P. m. melanotus* is 11 °C (lower critical limiting temperature).
3. The upper optimal temperature for *P. m. melanotus* is 30 °C (selected body temperature).
4. The limiting high temperature for *P. m. melanotus* is 40 °C (upper lethal temperature).

Temperature is central to energetics in that it affects a series of physiological processes that influence energy gain and energy expenditure, and the thermal limits of these processes give an indication as to thermal environment, and thus climate,

required for the lizard to maintain a positive energy balance. The main aim of my study was to calculate an energy budget for *P. m. melanotus* (i.e., the relationships between body temperature, metabolism and food intake) and the effects of temperature on energy balance. The thermal biology of the lizard is also important, not only because of thermal effects on energy gain and energy expenditure, but also to give an indication of the absolute upper and lower thermal tolerances and optimal thermal requirements of the lizard, which may affect the lizard's distributional limits.

The objectives of this study, therefore, were:

1. to determine the thermal requirements of *Pseudocordylus melanotus melanotus* in terms of selected and operative temperatures, and limiting temperatures;
2. to measure the digestive and assimilation efficiency of *P. m. melanotus*, including gut passage time and appetite (energy gain);
3. to measure the metabolic rate of *P. m. melanotus* at various temperatures (energy expenditure); and
4. to measure the field active body temperatures, activity time and feeding frequency of *P. m. melanotus* (field measures of thermal requirements and energy gain).

There have been few studies of these relationships in lizards in general.

1.7) Overview

In this thesis, an energy budget for the lizard *Pseudocordylus melanotus melanotus*, how its energy requirements are influenced by temperature and how this may influence its distribution relative to climate is considered. Since there are many aspects to an energy budget, which can make discussion cumbersome and confusing, I have divided this study into the following:

In Chapter two, I consider the critical limiting and lower lethal temperatures, and essentially deals with the absolute limits of the lizards' thermal range and therefore the expected absolute limits of its distribution.

In Chapter three, I consider selected and operative temperatures and thermoregulation, and comprises the target thermal range of the lizards, and how it is

achieved and maintained. In terms of distribution, this would indicate the optimal area of the distribution should the lizards be operating within their optimal thermal range.

Chapter four includes metabolic expenditure in terms of oxygen consumption. As part of the energy budget, this is, in effect, the lowest required amount of energy to survive. If the environment in a particular area does not allow for the lizards to obtain sufficient energy for maintenance metabolism, it would be excluded from that area.

Chapter five comprises aspects of the lizards' digestive physiology and feeding, covering diet, how much it eats and how much energy the lizards get from their food.

In Chapter six, aspects from all the chapters to construct an energy budget for *P. m. melanotus* and relate the energy requirements back to its distribution are considered.

CHAPTER 2

TEMPERATURE TOLERANCE

2.1) Abstract

Reptilian ecology and distribution are significantly impacted by environmental temperature. The upper and lower thermal tolerances offer a measure of an organism's thermal tolerance to the environment and therefore may indicate a thermal limit of geographical distribution. I measured the lower critical minimum (CTMin) and the lower physiological temperature tolerance (LPTT) for *Pseudocordylus melanotus melanotus*. The end point for CTMin was taken as the body temperature at which the righting response was lost. LPTT was taken as the average temperature at which lizards died from cold exposure. The mean CTMin was 10.17 ± 0.48 °C and was not significantly different between males and females. The mean LPTT was –5.15 ± 0.31 °C and ranged between –3.38 and –5.67 °C. All lizards that froze, i.e., exhibited an exotherm spike in body temperature evident when latent heat is released during freezing, did not survive the cold exposure. The lethal temperature for *P. m. melanotus* is thus ca. 15 °C below the CTMin, raising the possibility that these lizards can allow their body temperatures to drop well below CTMin without any ill effect. CTMin was also surprisingly high for an apparently cold-adapted lizard, and appears to be limited by their supercooling point. They are thus able to tolerate short periods of subzero temperatures, but not freezing, and are therefore restricted to areas where the microhabitat allows them to avoid freezing.

2.2) Introduction

Temperature has a significant impact on reptilian ecology and distribution (Cowles and Bogert, 1944). Liebig's "law of the minimum" states that the factor for which a species has the narrowest range of tolerance, or the least adaptability, governs the distribution (Lutterschmidt and Hutchison, 1997a). Environmental temperature directly influences an ectotherm's body temperature (T_b), which, in turn, has a significant effect on performance, fitness (Gvoždík and Castilla, 2001) and metabolic expenses. Most lizards maintain their T_b within a selected range (T_{sel}; Patterson and Davies, 1978) when allowed to thermoregulate. The selected or preferred T_b is generally considered to be the optimal temperature, or temperature range, for optimal physiological processes and behaviour. Relative performance (physiological, reproductive and ecological) tends to increase with increasing temperature until

optimal levels are reached, and decline again as temperatures increase above optimal levels (Figure 2.1). The upper and lower critical temperatures (X and Y in Figure 2.1) offer a measure of thermal tolerance to the environment (Doughty, 1994) and this measure can be used in evaluating the thermal requirements and physiology of the organism (Lutterschmidt and Hutchison, 1997a). The critical thermal tolerances also give an indication of the range of climatic conditions that can be tolerated and, therefore, distributional limits based on physiological tolerances because the risk of exposure to thermal tolerances is greatest at the edge of a geographical distribution (Cossins and Bowler, 1987).

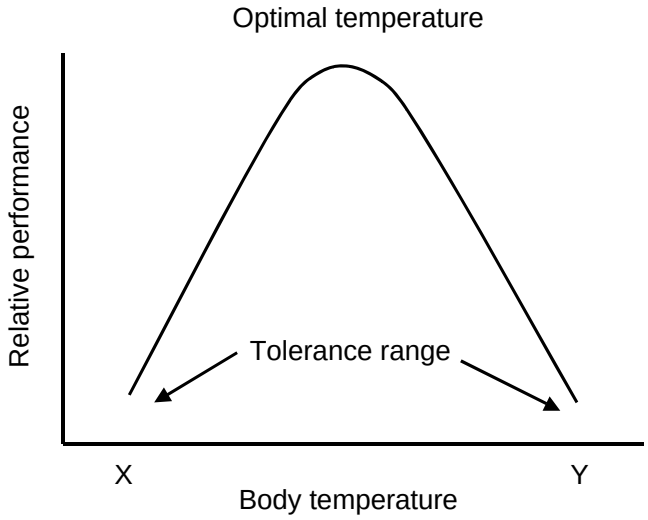


Figure 2.1 The relationship between body temperature and performance for an ectotherm. Performance is highest at optimal temperature and entirely constrained at the lower (X) and upper (Y) critical temperatures (adapted from Huey, 1982).

Cowles and Bogert (1944) originally defined the limit to thermal tolerance as the "thermal point at which locomotor activity becomes disorganised and the animal loses its ability to escape from conditions that will promptly lead to its death". The end points for the upper and lower critical thermal limits (CTMax and CTMin, respectively) have subsequently been defined as the temperature at the upper/lower extreme of tolerance that is indicated by the inability of the animal to right itself

when placed on its back, i.e., the loss of righting response (LRR; Doughty, 1994; Lutterschmidt and Hutchison, 1997a). Subsequently, the endpoint for CTMax has been redefined as the onset of spasms (OS; Lutterschmidt and Hutchison, 1997a). Alexander *et al.* (1999), however, argue that LRR still satisfies Cowles and Bogert's original definition since locomotor activity is disorganised and the animal is unable to escape. In other ectotherms such as fish, the cessation of spiracle contraction and the loss of equilibrium are sometimes used as endpoints in studies on critical limiting temperatures (Fangue and Bennett, 2003).

Exposure to the critical temperature is not itself lethal, only continued exposure, or temperatures exceeding the critical temperature will lead to death (Hutchison, 1979). Loss of righting is generally considered as ecologically lethal, rather than physiologically lethal (Hutchison, 1979), because the animal would be incapable of escaping predators, or conditions that would subsequently lead to its death. Seasonal and daily patterns of activity are also limited by environmental temperature (Witz, 2001), and, where environmental temperatures are likely to exceed CTMin or CTMax, lizards are likely to seek thermally-buffered shelters. Species active during warms periods are generally found in shelters at low T_bs (Brown, 1996) and may be avoiding low environmental temperatures by making use of warm microclimates. Changes in the tolerance range, i.e. the range between CTMin and CTMax, will determine the range of T_bs where locomotion is possible, and could lead to an increase, or decrease, in the time when a reptile would be active (Brown, 1996), or seek shelter.

Many animals rarely encounter extreme critical temperatures in the wild (Witz, 2001). This does not, however, mean that some animals do not encounter, and tolerate, extreme temperatures. Adaptations to exposure to extremely low temperatures are generally directed toward controlling or preventing the formation of ice (Ramlov, 2000). Some species of reptiles and amphibians regularly experience freezing of body water under natural conditions (freeze tolerance; Storey and Storey, 1996), whereas other species can tolerate temperatures below freezing, but do not tolerate actual freezing (freeze avoidance; e.g., Claussen *et al.*, 1990; Costanzo *et al.*,

1995). The depression of freezing point can occur under wet or dry conditions and occurs as a result of antifreezes or cryoprotectants (e.g., glycerol and glucose) found in the body fluids. The concentration of antifreezes increases in the body fluids to prevent (freeze avoidance), or delay (freeze tolerance), ice nucleation. Supercooling occurs where the animal is cooled to temperatures below that which a seed ice crystal will grow because there is no spontaneous nucleation.

Nucleation is essential in ice crystal formation in body fluids and can result from the surrounding environment or gut contents freezing (see Cossins and Bowler, 1987, Packard *et al.*, 2001). Supercooling has been recorded in lizards (Claussen *et al.*, 1990; Costanzo *et al.*, 1995; Burke *et al.*, 2002), snakes (Churchill and Storey, 1992a), turtles (Packard *et al.*, 2001; Packard and Packard 2002) and frogs (Layne and Kefauver, 1997). Supercooling may occur in both freeze avoiding and freeze tolerant species. Freeze tolerant species are those that can tolerate a relatively large proportion of their body water freezing. Freeze tolerance has been recorded in lizards (Claussen *et al.*, 1990; Costanzo *et al.*, 1995), snakes (Churchill and Storey, 1992a), turtles (Churchill and Storey, 1992b; Packard *et al.*, 1999; Costanzo *et al.*, 2001) and frogs (Costanzo *et al.*, 1992; Layne and Kefauver, 1997; Layne, 1999; Croes and Thomas, 2000). In some studies (e.g., Costanzo *et al.*, 1995; Layne and Kefauver, 1997; Burke *et al.*, 2002), nucleation is initiated by inoculation with a frozen probe, aerosol coolant or the addition of ice to the experimental chamber.

CTMin, CTMax and upper/lower lethal tolerances are usually measured in the laboratory. There are two major methods for determining thermal tolerances, 1) the dynamic method, and 2) the static or lethal method. CTMin and CTMax are measured using the dynamic method, where the animal is cooled or heated at a slow rate (usually about 1 °C per minute) to prevent thermal acclimation, until LRR, or OS in some cases (Lutterschmidt and Hutchison, 1997a, b). Lethal temperatures are measured by placing an animal at a single test temperature, and the exposure time to that temperature until death occurs is measured. Animals are not able to escape and exhibit heat stress, panting, LRR, OS, heat rigor, coma and death (Heatwole and

Firth, 1982; Lutterschmidt and Hutchison, 1997a, b). The lethal limit does not usually lie much above the voluntary maximum (Heatwole and Firth, 1982).

At low body temperatures, reactions are slowed down and are therefore generally less damaging (in terms of biochemical integrity) than high temperatures, which can cause irreversible damage to membranes and proteins (Sherwood *et al.*, 2005), and in a normal, active state, many animals' body temperatures are only a few degrees below permanently damaging high temperatures. The upper limit of the range of temperatures that can be tolerated is near the temperature at which proteins begin to denature, which is usually between 45 and 50 °C (Bartholomew, 1977), and most animals will die at 50 ºC, although many die at lower temperatures (Schmidt-Nielsen, 1997). The CTMax for lizards and snakes is generally close to 42 °C (e.g., Spellerberg, 1972a; Greer, 1980; Heatwole and Firth, 1982; Brown, 1996; Ji *et al.*, 1996; Alexander *et al.*, 1999; Gvoždík and Castilla, 2001; Witz, 2001), appears to show little potential for adaptation as it is likely to reflect biochemical constraints.

Pseudocordylus m. melanotus and other Cordylid species are restricted to the cooler regions of southern Africa (temperate distribution) and can thus be expected to be cold adapted, and also exhibit signs of heat stress at body temperatures over 35 °C (see Chapter 5). The Animal Ethics Screening Committee also does not allow experiments on the upper critical thermal limits because of the ill effects of heat exposure (e.g., Alexander *et al.*, 1999). I did not, therefore, measure the upper thermal tolerance in this study. I measured the lower limiting temperatures of *P. m. melanotus* in terms of CTMin and the lower physiological temperature tolerance (LPTT). Since CTMin is a recognised ecological limit, one would expect the CTMin to be the same, or close to, the LPTT.

2.3) Materials and methods

2.3.1) Lower critical limiting temperature

Lower critical limiting temperatures were measured for male (n = 10) and female (n = 10) lizards. Lizards were placed individually in an environmental chamber set at a temperature (-20 °C) that allowed the lizards to cool at a rate of 1 ºC/min. A slower

cooling rate may allow for partial thermal acclimation (Lutterschmidt and Hutchison, 1997a). Body temperature was monitored throughout the experiment using a thermocouple inserted approximately 10 mm into the lizard's cloaca and taped to the tail using Micropore™ tape. CTMin was taken as the temperature at which the lizards lost the ability to right themselves once placed on their backs (i.e., LRR). CTMin for males and females was compared using a two-sample t-test and the effect of body mass on CTMin was examined using regression analysis.

2.3.2) Lower physiological temperature tolerance

The LPTT of *P. m. melanotus* was measured in the laboratory. Seventeen lizards (8 females, 9 males) were acclimated at 25 ºC for 14 days. The LPTT was measured by placing lizards individually in an environmental chamber. Moisture and contents of the stomach and gut may act as ice nucleation sites (see Cossins and Bowler, 1987, Packard *et al.*, 2001). A small packet of desiccant (Drierite) was therefore also placed in the chamber and the 14-day acclimation period also allowed for gut clearance. The environmental chamber was connected to a HAAKE C25 water bath with a HAAKE F8 programmable circulator (Figure 2.2). Since most of the test temperatures were below zero (see below), the water bath was filled with alcohol. The water bath was programmed to cool, until reaching the required minimum temperature, and warm again at rates similar to those in the field. Cooling and warming rates are important to survival because rapid cooling at subzero temperatures results in the formation of intracellular rather than intercellular ice. In situations where intracellular ice has formed, rapid warming results in less damage than slow warming (Cossins and Bowler, 1987). Slow cooling also enables the full potential for freeze tolerance to be realised leading to the depression of freezing and supercooling points of intracellular fluids (Cossins and Bowler, 1987).

Each trial lasted for 24 hours. The water bath was programmed so that the chamber was cooled for 11 h 30 min until reaching the minimum test temperature which was maintained for one hour, and subsequently warmed for another 11 h 30 min. The start and end temperatures in all cases was 25 ºC. A single lizard was used for each trial and the minimum temperature of the first trial was 5 ºC. Thereafter, the

minimum temperature of each trial was 5 ºC lower until the first death was recorded. Lizards in subsequent trials were cooled to minimum temperatures at 1 ºC increments within the 5 ºC range above which the first lizard died, i.e., the first four lizards were used as pilot testers to determine the range of temperatures to be used in subsequent trials. If lizards did not immediately die from cold exposure during the trial, they were transferred to terraria and allowed an additional 24 h recovery period. All surviving lizards were euthanased after this period. Lizards that died all exhibited an exotherm (a rapid increase in body temperature). A similar response was recorded by Claussen *et al.* (1990), Churchill and Storey (1992a) and Packard *et al.* (2001), and the exotherm is probably the result of the lizard freezing. Thus the T_b at the start of the exotherm was taken as the temperature at which death occurred. The LPTT was taken as the mean of the temperatures at which the majority of the lizards died (i.e., the mean T_b at the initiation of the exotherm from each lizard). Lizard T_b was monitored using a thermocouple inserted approximately 10 mm into the lizard's cloaca and taped to the tail. The thermocouple was attached to a MC Systems data logger, which logged temperature every minute over the 24 h test period.

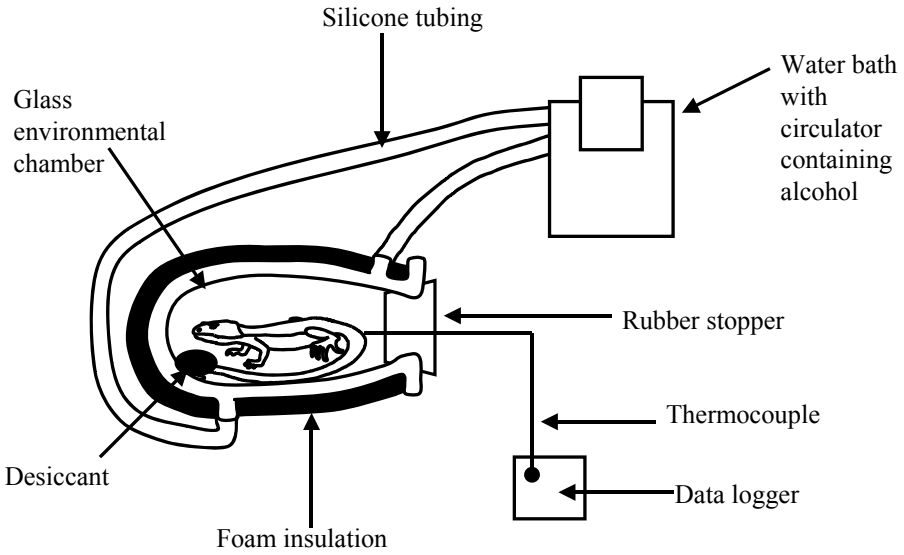


Figure 2.2: Diagram showing the environmental chamber and associated equipment used to measure the lower physiological temperature tolerance in *Pseudocordylus melanotus melanotus*.

2.4) Results

2.4.1) Lower critical limiting temperature

In *P. m. melanotus*, the CTMin for males was not significantly different to that of females (t_{18} = 0.54, P = 0.59; ♂ 10.43 ± 0.67 ºC, ♀ 9.90 ± 0.71 ºC) The CTMin pooled for males and females was 10.17 ± 0.48 ºC. Body size had no effect on CTMin (Figure 2.3).

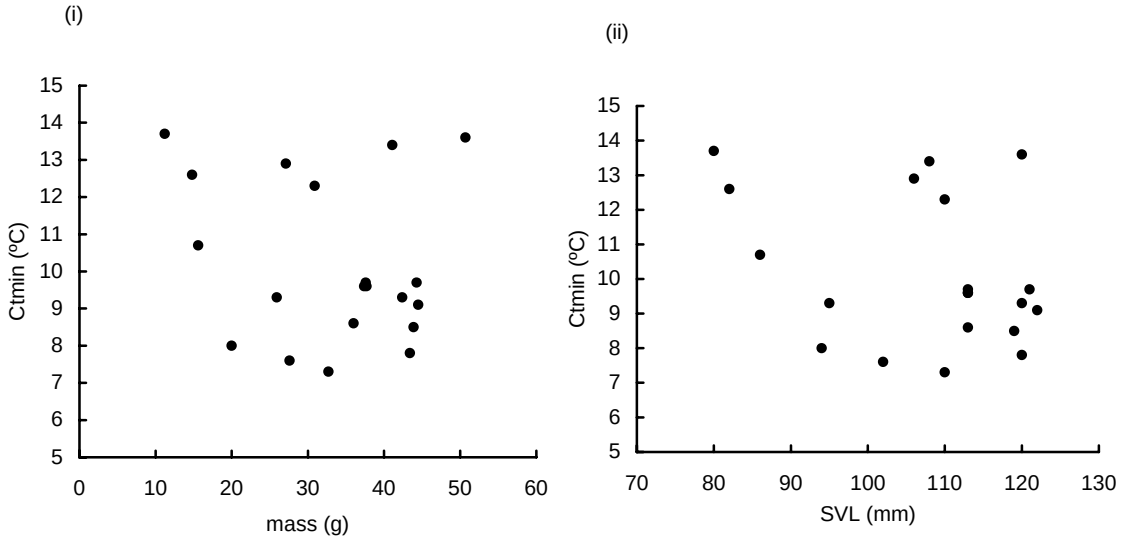


Figure 2.3: Relationship between the critical limiting temperature (CTMin) and (i) mass; and (ii) snout-vent length (SVL) for all lizards.

2.4.2) Lower physiological temperature tolerance

Of the pilot tester lizards, those exposed to 5, 0 and –5 °C survived. The lizard exposed to –10 °C died. All lizards that died at subsequent test temperatures (i.e., between –5 °C and –9 °C) experienced some degree of freezing. This was evident in an exotherm spike that occurs when latent heat is released during freezing (Figure 2.4).

The point of death was taken as the T_b at which the lizard froze since no lizards that experienced freezing recovered and those that did not freeze all survived. The mean T_b at death was –5.15 ± 0.31 °C. The range of body temperatures at which death may occur was –3.38 to –5.67 °C (Figure 2.5).

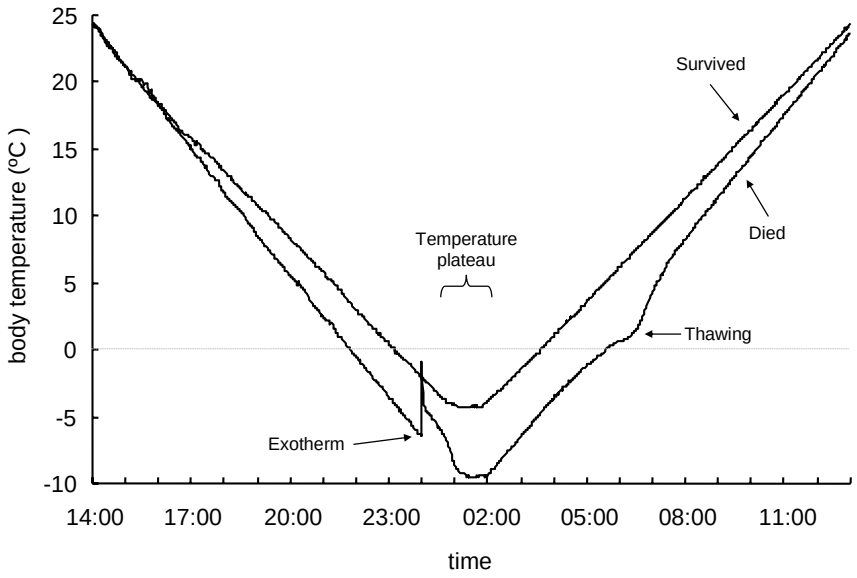


Figure 2.4: Lizard body temperature for two individual lizards during cooling experiments over 24 hours. Lizards cooled to test temperature, which was maintained for 1 hour, and warmed again. Upper line shows body temperature profile of a lizard that survived. Lower line shows body temperature profile of a lizard that died and illustrates exotherm spike indicating freezing and a lag in increasing body temperature near 0 °C when the lizard thaws. The body temperature at death was taken as the body temperature of the lizard at the start of the exotherm; in this case body temperature at death was -6.46 °C.

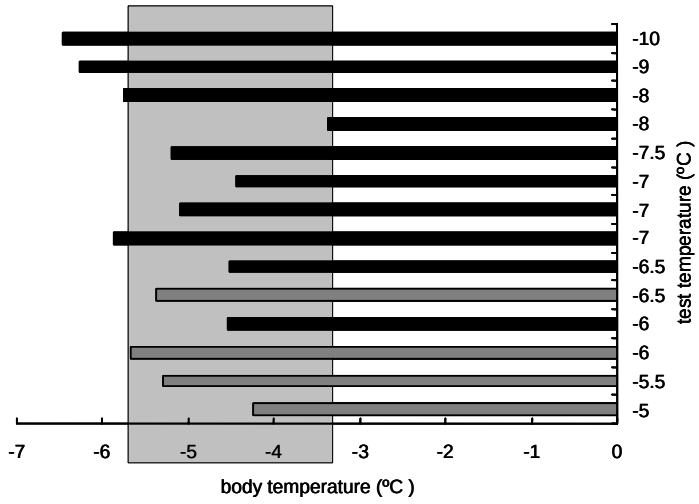


Figure 2.5: Body temperatures of lizards during lower physiological temperature tolerance trials. Black bars indicate lizard body temperature at death (i.e., the body temperature at the start of the exotherm). Grey bars indicate body temperatures of lizard that did not die, and is the minimum body temperature experienced by lizards during the trial (not always the same as the minimum chamber temperature due to thermal inertia etc.). The minimum plateau temperatures for trials are given on the y axis. Shaded area indicates the range of body temperatures at which death might occur and is bounded at the lower edge by the lowest temperature of survival (12th bar from the top), and at the upper edge by the highest temperature at death (4th bar from the top).

2.5) Discussion

The lethal temperature measured for *P. m. melanotus* was approximately 15 °C below the CTMin and lizards were unable to tolerate freezing. This difference is considerable and raises questions as to the rationale of CTMin as an adequate measure of thermal tolerance.

Lillywhite (1987) reports that CTMins for squamates generally range between 1 and 9 ºC. The CTMin measured for *P. m. melanotus* can thus be considered relatively high when compared to other squamates (Table 2.1). There is otherwise little published information available on CTMin in lizards and lower physiological temperature tolerances are rarely investigated. The measured CTMin for *P. m. melanotus* was approximately 10 ºC, a surprisingly high CTMin for an apparently cold-adapted ambush foraging lizard. The large range between CTMin and LPPT in *P. m. melanotus* suggests that, in this species at least, CTMin is not a good measure of the lower thermal limit.

Table 2.1 Lower critical limiting temperatures (CTMin) reported in lizards and a snake occurring sympatrically with *Pseudocordylus melanotus melanotus*.

Species	CTMin (°C)	Reference
Tarentola boettgeri	7.3-9.3	Brown, 1996
Sphenomorphus indicus	♂ 3.9, ♀ 5.9	Ji *et al.*, 1996
Eumeces chinensis	6.9	Ji *et al.*, 1996
Eumeces elegans	9.3	Du *et al.*, 2000
Elgaria multicarinata	9.4*	Kingsbury, 1994
Amphibolurus muricatus	3.0	Heatwole and Firth, 1982
Zootoca vivipara	1.9-2.8	Gvoždík and Castilla, 2001
Hemachatus haemachatus	3.7	Alexander *et al.*, 1999
Pseudocordylus m. melanotus	10.17	This study

* field active body temperature interpreted as CTMin.

There have been few direct studies on lethal limiting temperatures in vertebrates. Most studies deal with cold-hardiness or freeze tolerance and usually incorporate some aspect involving survival after exposure to a range of potentially limiting temperatures. Freeze tolerance among squamates is particularly uncommon (Costanzo *et al.*, 1995).,Dependence on supercooling is, however, important for species that cannot withstand freezing (Voituron *et al.*, 2002). *Zootoca vivipara* is

one lizard species that is known to exhibit both freeze avoidance and freeze tolerance depending on environmental conditions (Costanzo *et al.*, 1995). The European Wall Lizard (*Podarcis muralis*) can tolerate temperatures as low as –5.9 °C, and can also tolerate freezing at –1.05 °C, but with only 30 % survival (Claussen *et al.*, 1990). The percentage of body water that freezes appears to be an important factor in freeze tolerance. In *Podarcis muralis*, the maximum ice content tolerated was 28 % (Claussen *et al.*, 1990), and *Podarcis sicula* can survive 20 % ice content, but not 30 % (Burke *et al.*, 2002). The ice content of the frozen *P. m. melanotus* is unknown in my study, but it appears that this species cannot tolerate freezing to any degree since, regardless of the intensity of the exotherm, all lizards that froze, died.

Organisms occurring in temperate, or arctic, climates have developed mechanisms that aid in increasing the supercooling capabilities and reducing the associated stress (Voituron *et al.*, 2002). For animals that avoid freezing, the supercooling point (SCP) is important. *Pseudocordylus m. melanotus* cannot tolerate freezing, but can supercool to approximately -5 °C, and can therefore survive below freezing temperatures, even if they are unable to move or are unconscious (because they would be at body temperatures below their CTMin). The lizards *Podarcis muralis* and *Podarcis sicula* can supercool and survive exposures to –4 or –5 °C (Claussen *et al.*, 1990; Burke *et al.*, 2002). Invertebrates are known to survive months in a supercooled or frozen state, however, vertebrates may only survive weeks (Voituron *et al.*, 2002). Costanzo *et al.* (1995) report that *Zootoca vivipara* can survive periods of supercooling to –3.5 °C for several weeks. Torpor may also affect the degree to which an animal can supercool. Nedvěd (2000) reported that the beetle *Stenotarsus rotundus* can supercool to –7 °C under normal conditions, but can supercool to –13 °C during its diapause stage. *Pseudocordylus m. melanotus* can supercool to approximately -5 °C when acclimated to 25 °C and can potentially tolerate colder temperatures if acclimatized to lower environmental temperatures. It is thus likely that these Crag Lizards can withstand below freezing temperatures for extended periods and be able to survive periods of extreme cold without freezing.

It has also been shown that geographically separated populations may exhibit different cold tolerance abilities (Kalushkov and Nedvěd, 2000). Kalushkov and Nedvěd (2000) showed a clear difference in the SCP between two isolated populations of the bug *Pyrrhocoris apterus*. Spellerberg (1972a) indicated that cold adapted lizards in the genus *Sphenomorphus* had very different tolerance limits to other *Sphenomorphus* that are warm adapted. A within-species investigation of CTMin in warm vs. cold adapted populations of *Trachylepis* (= *Mabuya*) *striata* showed that cold acclimated individuals had a significantly lower CTMin than warm acclimated individuals (Patterson, 1991). (The taxonomy of *Mabuya*, however, has recently been revised (Bauer, 2003), and the species in Patterson's (1991) study may in fact be different species.) *Pseudocordylus m. melanotus* were acclimated at 25 °C for 14 days, which may have resulted in higher than expected CTMin and LPPT values. Many studies on thermal tolerances in reptiles do not indicate the acclimation temperature or period, but longer periods are generally used with lower acclimation temperatures where they are reported (periods of between 10 days and 3 months have been reported). While a lower acclimation temperature for *P. m. melanotus* may have resulted in lower CTMin and LPPT values, 25 °C can be considered an acceptable acclimation temperature since lizards are able to achieve and maintain body temperatures of approximately 26 °C during winter (Chapter 3). A cooler acclimation temperature may be more suitable for lizards which do not emerge from retreats, but in *P. m. melanotus*, this would have to be evaluated on an individual basis.

Species in warm environments where the environmental temperatures do not reach the limiting temperatures may not exhibit variation between geographically isolated populations, even when the thermal environments are very different (Brown, 1996). The Suikerbosrand population of *P. m. melanotus* is generally considered to be isolated from other populations of the species since the nearest recorded population is near Ermelo, approximately 100 km from Suikerbosrand. Neither Brown (1996) nor Gvoždík and Castilla (2001), however, found significant differences between the CTMin and CTMax between geographically isolated populations of *Tarentola boettgeri* and *Zootoca vivipara*, respectively. It may be expected, therefore, that other

populations of *P. m. melanotus* might have the same, or similar, thermal tolerance limits.

Freeze risk is affected mainly by climate, altitude and latitude, and it may be expected that animals living in cooler regions, or high altitudes or latitudes, may have the physiology to survive lower temperatures (Doughty, 1994). On a smaller scale though, the risk of freezing can also be influenced by local topography, microenvironment (e.g., ambient temperature, moisture) and the efficacy of any insulation that may be available (Costanzo *et al.*, 1995). Freeze avoidance can therefore also be achieved by the selection of suitable hibernacula (Churchill and Storey, 1992a). Rupicolous species such as *P. m. melanotus* can be expected to select crevices that would remain dry, and may be deep enough to maintain some warmth or buffering from the thermal inertia of the rocks in which they shelter.
During overwintering, many animals are aphagic and must therefore rely on stored energy (Voituron *et al.*, 2002). An empty gut has been shown to be a necessary attribute for supercooling (Nedvěd and Windsor, 1994) because nucleation is the single most important event in ice-crystal formation (Cossins and Bowler, 1987). The freezing of the contents of the stomach and intestine seed the formation of ice in the body water, usually extracellular fluids (Packard *et al.*, 2001). In my study, *P. m. melanotus* were subjected to an acclimation period of 14 days prior to LPTT experiments at 25 °C. At this temperature, gut passage time was less than half this period (see Chapter 5), so it was assumed that all lizards had clear guts during measurement of LPTT and therefore removing the possibility that there was spontaneous nucleation through the gut.

The period of exposure to sub-zero temperatures during LPTT measurements in *P. m. melanotus* is less than 12 h (at least half the trial period), and only one hour at the test temperature. This suggests that cold tolerance in this species is limited to the SCP and can therefore survive environmental temperatures of approximately –5 °C. If the environmental temperature decreases sufficiently for the lizards to freeze, they will not survive, and a single extreme event could result in large-scale death within a population. It is clear that *P. m. melanotus* will easily survive short periods of

subzero temperatures (e.g., overnight frosts) by supercooling, especially if suitable, dry hibernacula are found and they are in a fasted state. The lizards may also be restricted to areas of summer rainfall since, in areas receiving winter rainfall, refugia may become too moist and ice nucleation may occur as a result of increased exposure to environmental ice. The difference between CTMin and LPPT suggests that CTMin is not a relevant measure of thermal tolerance since the actual physiological tolerance is considerably lower than the expected tolerance predicted by CTMin.

CHAPTER 3

THERMOREGULATION

3.1) Abstract

The maintenance and regulation of body temperature (T_b) is essential because most biochemical and physiological processes are temperature dependent and usually operate at an optimal temperature. In the wild, lizards thermoregulate by shuttling between hot and cold microclimates, modifying body postures, or by regulating activity times. Here I investigate the thermoregulatory abilities and behaviour of *Pseudocordylus melanotus melanotus* in terms of operative temperature (T_e), selected temperature (T_{sel}), exposure to low temperature, body postures and activity. They maintain their T_b at approximately 30 ºC in a laboratory thermal gradient, where there are no constraints of the thermal environment. I assumed this to be the mid-point of the set point range (after Hertz *et al.*, 1993). In the field, however, lizards achieved significantly lower T_bs, which suggests that the thermal environment limits the T_bs that lizards are able to achieve. Lizards are active for significantly longer and select significantly higher T_bs in summer, and, during winter, lizards spend a significant portion of their time at T_bs below their lower critical limiting temperature. It is clear that *P. m. melanotus* can thermoregulate efficiently, but the T_b maintained may be constrained by the environment. It appears that *P. m. melanotus* is at the lower end of its thermal range and thus any climate warming may alleviate thermal constraints and improve the overall energy balance.

3.2) Introduction

The regulation of body temperature (T_b) in reptiles is essential because the rates of most biochemical and physiological processes are determined by temperature (Huey, 1982; Angilletta *et al.*, 1999; Martín and López, 2000; DeNardo *et al.*, 2002; Seebacher and Shine, 2004, Sherwood *et al.*, 2005). Reptiles of any size will eventually equilibrate with their thermal environment given sufficient time, but the environment is constantly changing through time and animals move within the thermally-heterogeneous environment (Andrews and Kenney, 1990; Seebacher and Shine, 2004). Although physiological adjustments may be important, most lizards' ability to thermoregulate is primarily through behavioural adjustments (Bauwens *et al.*, 1996).

Careful regulation of T_b reduces the chances of being exposed to extreme temperatures that may be lethal, and also increases periods spent at physiologically favourable T_bs (Huey *et al.*, 1989). Physiological benefits are maximised in an ideal environment where there are few or no environmental constraints and the reptile is always at its optimal T_b when active (Huey and Slatkin, 1976). In the wild, however, the thermal environment is varied and lizards thermoregulate by shuttling between hot and cold microclimates (or between sun and shade), through posture modifications (such that the surface area exposed to heat sources is altered) and by regulating activity times (Huey, 1974). The T_bs of lizards are thus dependent on both the variation in environmental temperature and on the ability of the lizard to regulate heat exchange (De Witt, 1967; Peterson, 1987; Carrascal *et al.*, 1992; Tosini *et al.*, 1992; Beck, 1996).

The thermal environment affects an organism through the net addition or removal of heat, which results in changes in T_b, evaporation or metabolic heat production (Bakken *et al.*, 1985). The range of temperatures available to a lizard, as determined by the thermal environment, can be used to establish the extent to which T_bs are constrained by physical factors (Peterson, 1987). Operative temperature (T_e) can be used to map the thermal environment (Bakken, 1992) and takes into account mechanisms of heat transfer including solar radiation, thermal radiation, convection and conduction (Bartholomew, 1977; O'Connor and Spotila, 1992). Operative temperature can also be used to infer potential activity times and thermoregulatory efficacy (Shine and Kearney, 2001).

Many lizards regulate T_b within a narrow range (Huey, 1974) and this level is high if they bask in the sun during the day (Bennett, 1980). This selected body temperature (T_{sel}) is generally considered the T_b at which a the lizard can most effectively capture prey, escape predators, dig nest holes, engage in social behaviour or undertake any energetically demanding activity (Bartholomew, 1977). Selected body temperature has also been shown to be affected by numerous factors (Huey, 1982) including reproductive condition (Beuchat, 1986; Gibbons and Semlitsch, 1987; Andrews *et al.*, 1997; Rock *et al.*, 2000; Rock *et al.*, 2002), gender (Gibbons and Semlitsch,

1987; Rock *et al.*, 2000; Rock *et al.*, 2002), digestive state (Gibbons and Semlitsch, 1987; Beck, 1996) and season (Pentecost, 1974; Gibbons and Semlitsch, 1987; Christian and Bedford, 1995). However, the selected temperature is often placed in the middle of the activity range (i.e., the optimal temperature range delimited by upper and lower set points, or set point range; Hertz *et al.*, 1993), and during the day, different behavioural patterns are used to keep the T_b within the activity range (Dreisig, 1984). Both heliotherms (bask and directly absorb solar radiation; Rismiller and Heldmaier, 1988; Willmer *et al.*, 2005) and thigmotherms (gain heat through conduction and convection from hot air and substrata; Rismiller and Heldmaier, 1988; Willmer *et al.*, 2005) make behavioural adjustments to maintain their T_b at the selected level (Bartholomew, 1977; Sievert and Hutchison, 1989; Tosini *et al.*, 1992; Rummery *et al.*, 1994).

Body temperature is behaviourally maintained through the modification of basking frequency and microhabitat use, regulating activity times and changes in body posture (Huey and Slatkin, 1976; Huey and Pianka, 1977; Muth, 1977a; Waldschmidt, 1980; Hertz and Huey, 1981; Carrascal *et al.*, 1992; Hertz, 1992; Bauwens *et al.*, 1996; Melville and Swain, 1997; Willmer *et al.*, 2005). Generally, when the environment is cool, lizards will be in the sunshine, but in the shade when the environment is warm (Cowles and Bogert, 1944; Huey and Pianka, 1977; Sherwood *et al.*, 2005), and shuttling pattern is adapted to the use of particular basking sites, non-basking retreats and vegetation forms (Spellerberg, 1972b).

Postural changes alter the body's orientation to the sun (Bartholomew, 1977; Walsberg, 1992) and also alter body position such that only part of the body is exposed to sunshine (Peterson, 1987). Changes in body posture have the greatest effect on T_b when the difference between air and substrate temperature is greatest, usually during the hottest time of day (Roberts *et al.*, 1993). A lizard with a finite range of thermal preferences, or tolerances, will utilize the available microclimates which fall within their preferred thermal range and may only use a certain proportion of the available microclimates (Grant and Dunham, 1988; Angert *et al.*, 2002). The

time spent thermoregulating depends on the thermal properties and available microclimates of its environment (Gvoždík, 2002).

Activity in lizards is primarily determined by the environment and is generally a behavioural response to changes in solar radiation and ambient temperatures (Foà and Bertolucci, 2001). Thermoregulation is one of the benefits of activity (Huey, 1982); however, during periods of inactivity in retreats, T_b is largely determined by air and substrate temperature, which limits thermoregulatory options (Adolph and Porter, 1993). Lizards tend to be more active during times when environmental temperatures are optimal (Martín and Salvador, 1995) and daily activity is typically longer (Adolph and Porter, 1996) and is usually bimodal in summer (Gannon and Secoy, 1984; Firth and Belan, 1998; Foà and Bertolucci, 2001). In winter, activity is sporadic (Foà *et al.*, 1992) and in spring and autumn, activity is usually unimodal (Gannon and Secoy, 1984; Foà and Bertolucci, 2001), especially in temperate species.

Pseudocordylus m. melanotus is a strictly rupicolous lizard. Since most rocky outcrops are spatially heterogeneous in almost all their characteristics, it is likely that they are also a highly heterogeneous environment from a thermal perspective. Drakensberg Crag Lizards are also sit-and-wait foragers, suggesting either distinct thermoregulatory behaviour in terms of activity time, postures and positions, or wide thermal preferences because it spends a large portion of its day in exposed positions. Here, I (1) measured the range T_es available to lizards in order to quantify the thermal environment; (2) measured the T_{sel} of the lizard in the laboratory where there are few thermoregulatory constraints; (3) measured the field achieved T_bs with respect to T_{sel} and evaluate the lizards ability to thermoregulate; (4) assessed the lizards' exposure to low temperatures in the field in light of the surprisingly high CTMin (Chapter 2); and (5) investigated the lizard's thermoregulatory behaviour in terms of activity and body posturing.

3.3) Materials and methods

3.3.1) Operative temperature

Two painted, copper tube models were empirically calibrated against live lizards in the laboratory. The models were placed in a 300 x 200 x 250 mm terrarium with a lizard. Thermocouple wires were inserted approximately 10 mm into the models and the lizard's cloaca and attached to a datalogger (MC Systems, Cape Town), which recorded lizard body temperature and the temperature of the models every minute for one hour. A 250 W infrared lamp was placed approximately one metre above the tank to warm the lizard and models. The lizard was monitored throughout to ensure that it was not experiencing discomfort and behaved normally. The lamp was removed after half an hour so that the lizard and models could cool at room temperature. The copper tube models were considered good lizards models based on the R^2 value when model temperature was regressed against the lizard's body temperature, i.e., the higher the R^2, the better the model. The model with the closest representative thermal properties for *P. m. melanotus* was a 100 mm length of 28 mm diameter copper pipe, painted black and filled with water (Figure 3.1).

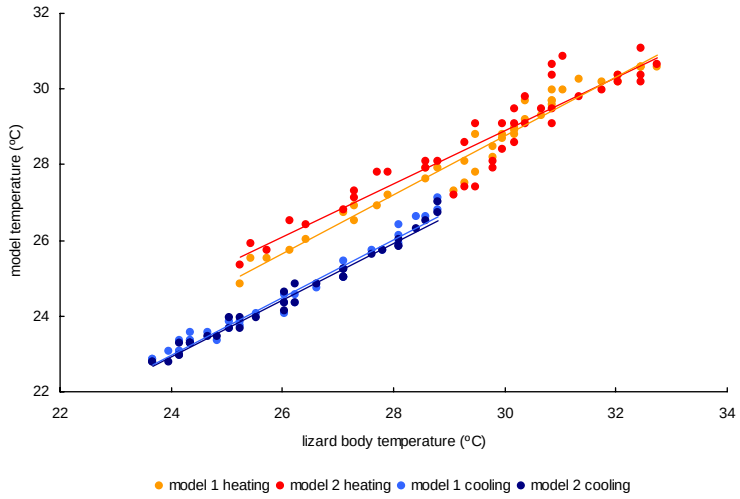


Figure 3.1: Relationship between lizard body temperature and temperature in two copper tube models with the closest representative thermal properties of *Pseudocordylus melanotus melanotus* (100 mm of 28 mm diameter copper pipe, painted black and filled with water). model 1: heating – $R^2 = 0.97$, $y = 0.78x + 5.43$, cooling – $R^2 = 0.97$, $y = 0.76x + 4.62$; model 2: heating – $R^2 = 0.88$, $y = 0.70x + 7.91$, cooling – $R^2 = 0.98$, $y = 0.75x + 4.82$; $P << 0.001$ in all cases.

Lizard T_e was measured in the field at Suikerbosrand Nature Reserve between summer 2002 and winter 2004. Three models were placed in the field and interfaced with a datalogger (MC Systems, Cape Town), which was programmed to log temperatures of the models every 20 min. Models were placed individually in locations in which lizards would be expected to experience the highest and lowest environmental temperatures: In a crevice and on the rock surface such that one model received morning sunshine and the other afternoon sunshine. Air temperature and the temperature of a small black bulb were also logged. The small black bulb was used to indicate when the sun was shining as it heats and cools very quickly in response to incoming levels of solar radiation. I used records from the models as measures of the highest and lowest temperatures available to the lizards.

3.3.2) Selected body temperature

3.3.2.1) Laboratory study

The T_{sel} of lizards was measured in a thermal gradient in the laboratory in winter (n = 32 lizards, 2 categories) and summer (n = 43 lizards, 3 categories). Each lizard was measured over a two-day period. Lizards were separated into three categories based on the length of time they had been in captivity. One group of lizards had been in captivity for 12 to 18 months when measured, while the other group had only been acclimated in captivity for between two weeks and six months. The third group were captured for the summer study only and were therefore only acclimated for two weeks before measures.

The thermal gradient consisted of a 1 x 1.5 x 0.5 m wooden enclosure. The gradient was divided lengthways into three separate compartments. Heat was provided at one end of each compartment by a 250 W infrared lamp. Cooling was achieved with a copper cooling plate connected to a water bath at the opposite end of the enclosure. A single lizard was placed in each compartment. Body temperature was recorded not more than once per hour to avoid effects of stress. Body temperature measures were made by catching lizards by hand and inserting a thermocouple probe approximately 10 mm into the cloaca. Each lizard's T_b was sampled for seven hours per day (from 09h00 to 16h00), for two days. Lizards were place in the thermal gradient overnight

before the experiment began and the lamps were turned on 30 min before the first reading was taken. All lizards spent the same amount of time in the gradient and measurements were made at the same time of day to control for any influences of time of day on temperature selection (after Sievert and Hutchison, 1989).

3.3.2.2) Field study

Lizards were collected at Suikerbosrand Nature Reserve (SNR) during March-April (n = 18; summer) and May-June 2004 (n = 20; winter). All lizards were individually marked using colour coded plastic ties and toe-clipping, and all were measured (SVL – nearest 1 mm, head length, head width – nearest 0.01 mm) and weighed (nearest 1 g). In order to measure body temperature in the field, small temperature dataloggers (thermocron iButtons®; Dallas semiconductor, Texas, USA) were surgically implanted into each lizard. Lizards were anesthetised using 2 % isoflourane gas and a small incision was made into the peritoneal cavity. The dataloggers, which were pre-programmed to record body temperature every 20 min, were coated with wax and sterilised with hibicol. Data logger assemblies did not exceed 10 % of the recipients' body mass. The wax-coated datalogger was inserted into the body cavity through the incision, which was sutured, cleaned and covered with Tegaderm™. Lizards were released at the point of capture at SNR within four days of capture; all lizards had a recovery period – at least 36 h – after surgery prior to being released.

Lizards were recaptured after a minimum of three weeks and data loggers were removed using similar procedures to those used in implantation. All lizards were returned to the point of capture at SNR after removal of the data loggers. Identification collars were also removed prior to releasing the lizards. The wax coating on the data loggers was removed and data was downloaded and analysed in terms of T_{sel} and exposure to low temperatures. Operative temperatures were measured concurrently for comparison with recorded body temperatures.

Selected body temperature was calculated for each lizard over the period recorded on the implanted data loggers (approximately 28 days). T_{sel} was taken as the mean T_b between the highest T_b reached in the morning and the highest T_b before a three hour

decrease in T_b in the afternoon or evening, for example, see Figure 3.2. The three hour decrease allowed the inclusion of shorter periods of reduced T_b during the day when lizards may have retreated into crevices.

The first three days of data were excluded to remove any effects of surgery or disruption. Days when the lizards were not obviously thermoregulating (i.e., when T_b was dependent on T_e) were also excluded from the analysis because, if a lizard was not actively thermoregulating, it would not be able to achieve and maintain its T_{sel}.

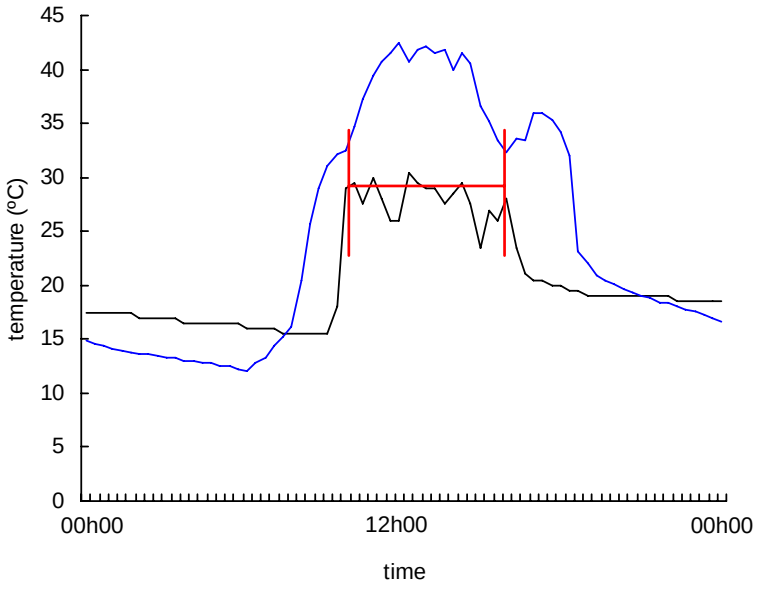


Figure 3.2: Example of measured lizard body temperature (black line) and operative temperature (blue line; outside of crevice) over 24 hours. Red lines indicate the range over which the mean selected body temperature was calculated.

3.3.3) Exposure to low temperatures

Exposure to low temperatures was calculated as the number of days in an average 30-day period that the lizards experienced periods where their T_b was at or below 10 ºC. This is the critical minimum temperature (CTMin) measured in the laboratory (Chapter 2), so any period spent at or below 10 °C means that the lizard would be severely compromised in terms of locomotor ability. The total number of 20 min intervals at or below 10 °C was also determined as a measure of the time spent at or

below 10 °C. The period each lizard spent at or below 10 ºC may also have been an underestimation since there were occasions when the lizard's T_b was at or below 10 ºC for a single measured interval. In such cases, the lizards T_b is likely to have been at or below 10 ºC for more than the single point in time when the lizards body temperature was logged. During summer, no lizards experienced periods where their T_bs were below 10 ºC. Exposure to low temperatures in summer, therefore, was taken as exposure to T_bs at or below 15 ºC, which is an arbitrary measure to obtain some understanding as to the lower T_bs experienced by lizards in the field.

3.3.4) Thermoregulatory behaviour

Behavioural thermoregulation in *P. m. melanotus* was qualified in terms of the period when lizards were active and the body postures and positions assumed by lizards during that period. Activity time was investigated by inferring activity from elevated T_bs measured using thermocron iButtons. Activity time was taken as the time from the first obvious increase in T_b until the time of the highest temperature before a three hour decrease in T_b. Increase in T_b in the morning was always obvious as there was a sudden and extreme increase in T_b when the lizard first emerged. There were also numerous decreases and increases during the day; the decrease in T_b for three hours excluded the decreases in T_b for predator avoidance and thermoregulation which occurred over shorter periods.

Body posture and position was measured during focal animal sampling in the field at Suikerbosrand Nature Reserve. Individual lizards were observed continuously for 30 min when they were encountered in the field. Owing to their strict site fidelity, some individuals were observed on more than one occasion. If there was any uncertainty as to whether or not a lizard had been previously observed (variation in colour allowed for easy identification of individuals), it was not included in any further focal sampling. Body posture and position are difficult to quantify because of individual variation in the structure of home ranges and selected body temperatures. The postures and positions assumed by the lizards were thus identified and described in terms of their effects on temperature regulation. Body postures were categorised into the following (see Figure 3.3):

a) Prostrate (head and body flat on the rock surface).
b) Prostrate with head raised.
c) Upper body raised (front of body and head raised, forelegs partially or fully extended, abdomen and tail flat on rock surface).
d) Body raised (whole body raised off rock surface, all legs partially of fully extended).
e) Legs raised (body and head flat, hind legs and feet raised off rock surface).
f) Side of rock (on side of rock, head extended above top of rock).
g) Side of rock extended (on side of rock with head and upper body extended above top of rock, forelegs partially or fully extended, sometimes body raised off rock surface).
h) Other (any other body posture or position assumed).

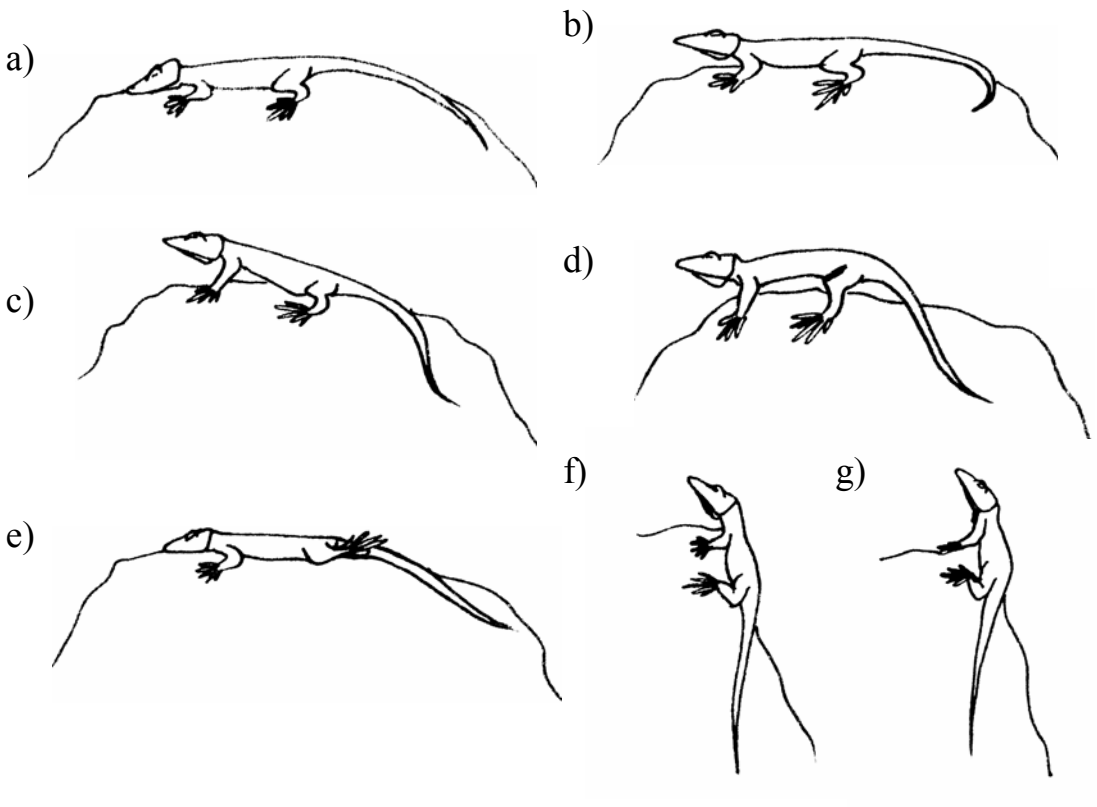


Figure 3.3: Categories of body postures and positions assumed by *Pseudocordylus melanotus melanotus*. a) prostrate; b) prostrate with head raised; c) upper body raised; d) body raised; e) legs raised; f) side of rock; g) on side of rock extended.

In total, 122 focals were recorded for 92 individual lizards (53 male and 39 female). Not all lizards were observed for the whole 30 min focal duration since lizards were occasionally obscured from view by rocks or vegetation and one to four focals were conducted per lizard. The observation time per lizard ranged between two and 120 minutes and the total, overall observation time was 51 h 55 min. Focal sampling started at 07h00 and continued through the day until no more lizards were observed; this was usually between 17h00 and 18h00 (Figure 3.4).

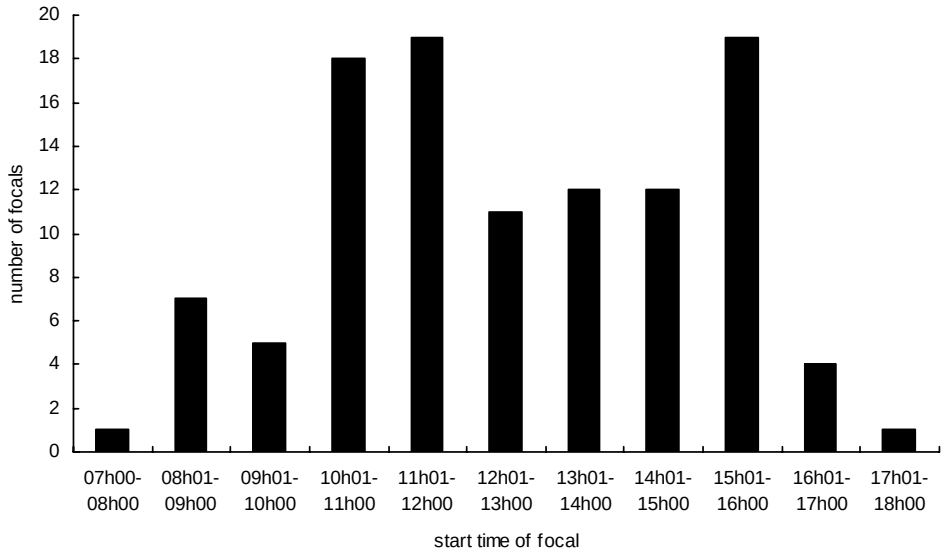


Figure 3.4: Temporal spread of focal starting times from when the first lizard was observed in the morning (07h00) until the last lizard was observed in the afternoon (between17h00 and 18h00).

3.4) Results

3.4.1) Operative temperature

Figures 3.5 shows examples of operative temperature relative to ambient air temperature for average four-day summer and winter periods. During winter, crevice temperature rarely exceeded 10 ºC, and the lowest recorded operative temperature in a crevice was –4 ºC, recorded during July 2002. For 2004, during the period when lizards carried implanted thermocron iButtons and were active in the field, operative

temperatures varied between –3.20 ºC and 54.94 ºC (in crevice and outside in the sunshine; Table 3.1). The overall range of operative temperature (over summer and winter) was therefore 58.14 ºC.

Table 3.1: Minimum and maximum operative temperatures (ºC) measured in a crevice and outside in the sunshine for summer and winter during 2004 at Suikerbosrand Nature Reserve.

		Summer	Winter
Crevice	Min	21.8	-0.7
	Max	29.4	18.3
Outside	Min	10.6	-3.2
	Max	54.9	42.3

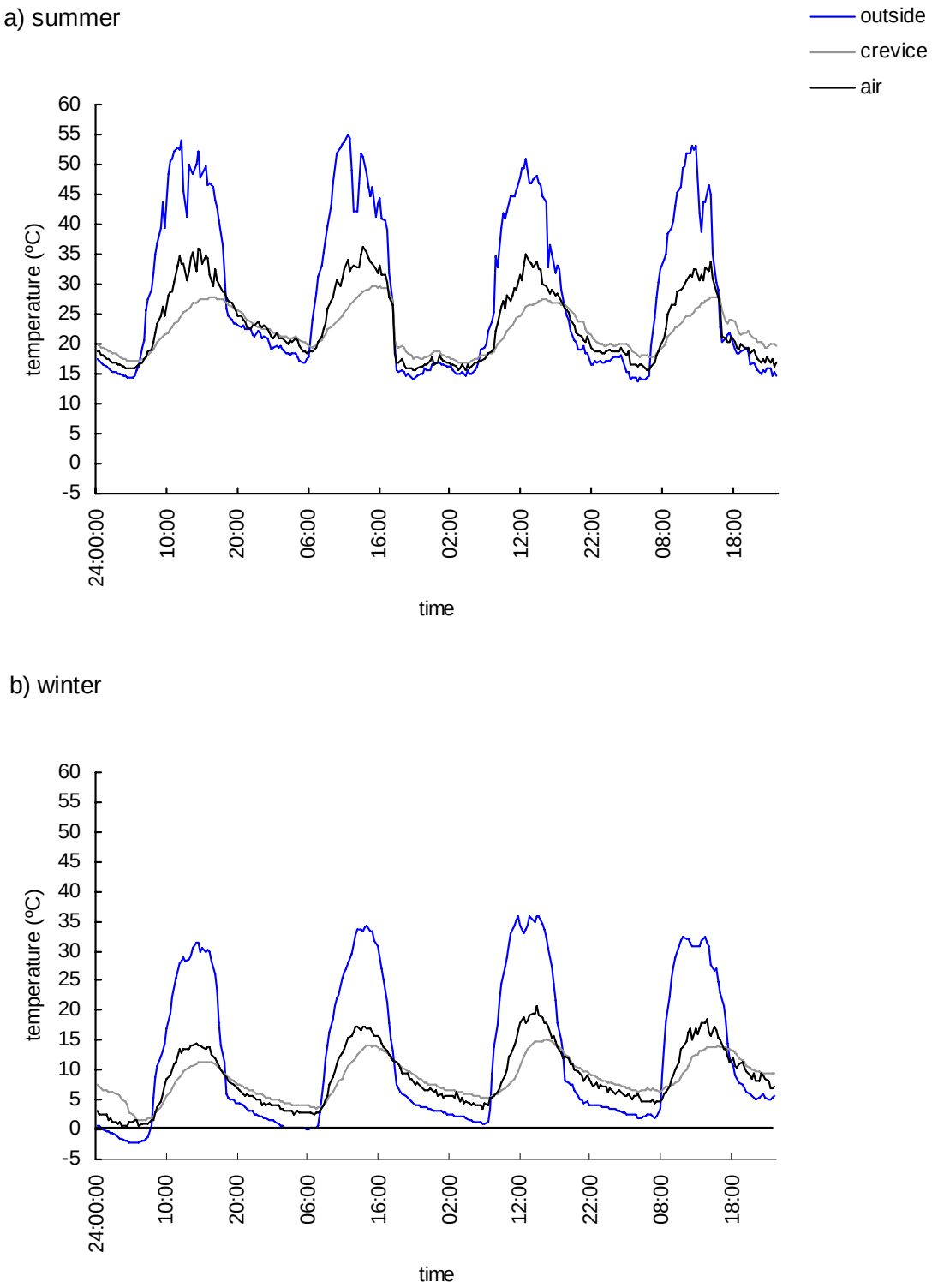


Figure 3.5: Example of operative temperatures measured in a crevice and outside in the sunshine relative to ambient air temperature for an average four-day period (each peak indicates a day, four days shown) in a) summer, and b) winter at Suikerbosrand Nature Reserve.

3.4.2) Selected body temperature

3.4.2.1) Laboratory study

Selected body temperature was approximately 30 °C for both males and females. Body sizes and T_{sel} of males, females and the different categories are summarised in Table 3.2. The mean T_{sel} for all lizards from all categories was 30.08 ± 0.14 °C and 30.99 ± 0.11 °C for winter and summer, respectively. Lizards selected significantly higher temperatures in summer (t_{73} = -4.27, P = 0.001). In both summer and winter, body mass and T_{sel} were not significantly related (summer: mass: P = 0.07, R^2 = 0.09; SVL: P = 0.06, R^2 = 0.1; winter: mass: P = 0.67, R^2 = 0.0006; SVL: P = 0.84, R^2 = 0.001). Lizards selected significantly higher temperatures on the first day of measure (winter: $F_{1,59}$ = 3.93, P = 0.05; summer: $F_{1,66}$ = 8.11, P = 0.006). No rhythm of selected temperature was evident during photophase in either winter or summer, however, lizards are maintaining a setpoint through the day (Figure 3.6).

Table 3.2: Summary of mean body size (mass [g] and snout-vent length [SVL, mm]) and selected body temperature (T_{sel}) for winter (W) and summer (S) for lizard groups. Means presented ± standard error.

		Category 1 (n = 9♂, 3♀)		**Category 2** (n = 10♂, 10♀)		**Category 3** (n = 5♂, 6♀)		**All**	
	Season	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**
Mass	**Males**	54.01 ±3.81	56.14 ± 1.75	37.89 ± 3.50	44.24 ± 3.41	-	50.70 ± 4.39	45.53 ± 3.14	50.02 ± 2.11
	Females	37.37 ± 2.55	41.33 ± 2.12	28.59 ± 3.14	34.97 ± 2.98	-	32.28 ± 2.07	30.62 ± 2.66	35.13 ± 1.81
	All	49.85 ± 3.60	51.70 ± 2.62	33.24 ± 2.52	39.09 ± 2.45	-	40.65 ± 3.62	39.47 ± 2.50	42.76 ± 1.83
SVL	**Males**	123 ± 1.73	123 ± 1.57	111.70 ± 4.00	114.75 ± 3.06	-	121.60 ± 3.71	117.05 ± 2.57	119.35 ± 1.77
	Females	109 ± 2.65	113.67 ± 1.76	103 ± 4.15	104.90 ± 3.22	-	109.33 ± 2.88	104.38 ± 3.28	107.68 ± 2.03
	All	119.50 ± 2.30	120.20 ± 1.84	107.35 ± 2.98	109.28 ± 2.49	-	114.91 ± 2.92	111.91 ± 2.28	113.67 ± 1.63
T_{sel}	**Males**	30.16 ± 0.25	29.99 ± 0.24	29.90 ± 0.28	30.84 ± 0.24	-	31.22 ± 0.30	30.23 ± 0.17	30.68 ± 0.15
	Females	29.94 ± 0.49	30.15 ± 0.56	29.84 ± 0.21	31.84 ± 0.15	-	30.77 ± 0.33	29.86 ± 0.22	31.30 ± 0.16
	All	30.44 ± 0.22	30.16 ±0.24	29.86 ± 0.17	31.40 ± 0.14	-	30.97 ± 0.22	30.08 ± 0.14	30.99 ± 0.11

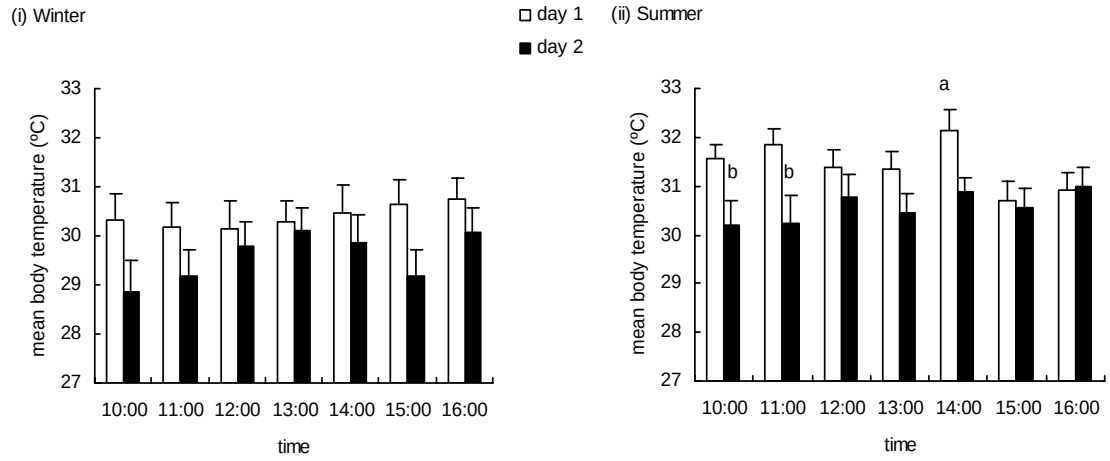
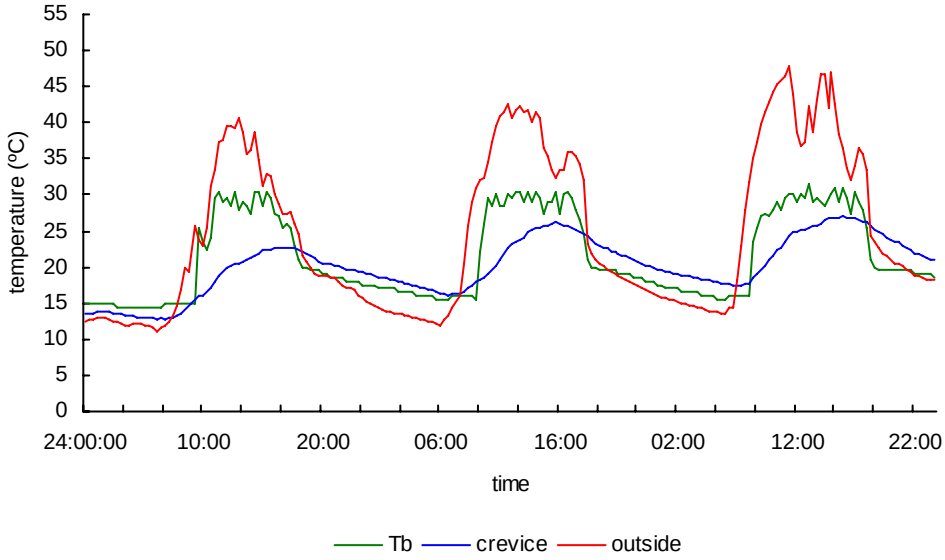


Figure 3.6: Mean selected body temperature for each interval between 10h00 and 16h00 during (i) winter (n = 32) and (ii) summer (n = 43) for the first and second days measurement (day 1 and day 2). Letters indicate significant differences between days and time intervals.

3.4.2.2) Field study

Of the 38 lizards with implanted data loggers (18 summer and 20 winter), 22 lizards were recaptured (11 each for summer and winter), resulting in an overall recapture success of 58 %. On days where lizards were actively thermoregulating, there was a clear difference between the lizard's T_b and the measured environmental temperature (Figure 3.7). Selected body temperature in the field during summer was 28.91 ± 0.26 ºC, and during winter was 26.30 ± 0.47 ºC. Lizards selected significantly higher temperatures in summer (t_{20} = 4.38, P = 0.0001). In summer larger individuals selected significantly lower body temperatures in terms of both mass (R^2 = 0.57, P = 0.007; Figure 3.8) and SVL (R^2 = 0.58, P = 0.008; Figure 3.9). Selected temperature and body size in winter was not significantly related (mass: P = 0.63, R^2 = 0.03; SVL: P = 0.77, R^2 = 0.01). Body size of lizards in the summer and winter groups, however, were not significantly different (mass: t_{20} = 0.76, P = 0.46; SVL: t_{20} = -0.42, P = 0.68).

a) summer



b) winter

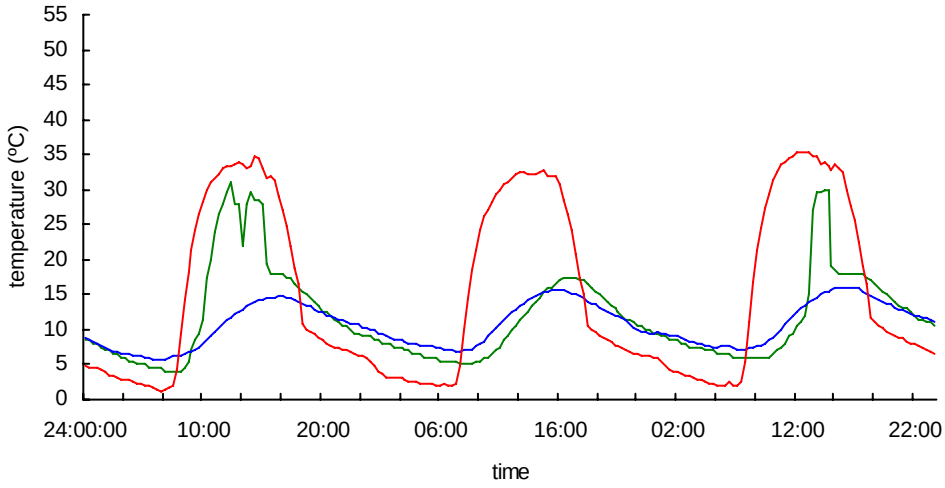


Figure 3.7: Example of field active body temperature (T_b) of a lizard relative to operative temperatures (T_e) over an average three-day period (each peak indicates a day) during a) summer and b) winter at Suikerbosrand Nature Reserve. Active thermoregulation is evident in the difference between the measured T_b and operative temperatures inside a crevice and outside in the sunshine. During the night lizard T_b is buffered by rock and therefore do not drop to the T_e outside its retreat. During the day (peaks), lizards actively thermoregulate such that T_b does not match the T_e. In winter (b), lizards are active for short periods only (indicated by narrower peaks in T_b) and, in this case, the lizard does not leave its retreat (indicated by the peak in T_b mirroring that of the peak in crevice temperature).

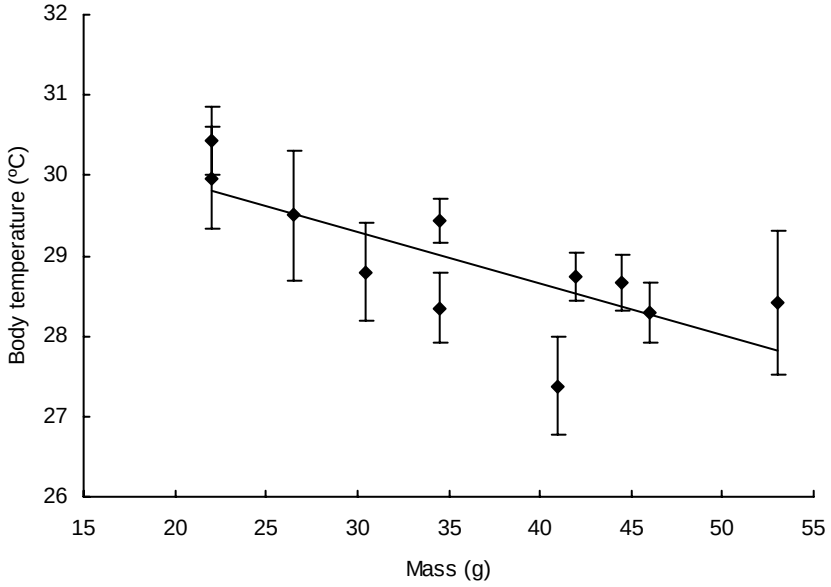


Figure 3.8: The relationship between selected body temperature in the field and body mass during summer for *Pseudocordylus melanotus melanotus* ($R^2 = 0.57$, P = 0.007, y = -0.06x + 31.21).

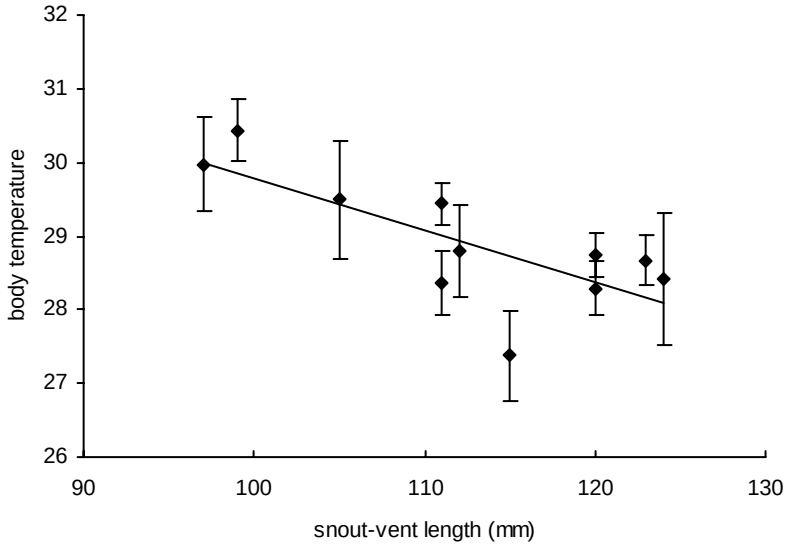


Figure 3.9: The relationship between selected body temperature in the field and snout-vent length during summer for *Pseudocordylus melanotus melanotus* ($R^2 = 0.58$, P = 0.008, y = -0.07x + 36.79).

3.4.3) Exposure to low temperatures

In an average 30-day period in summer, lizards experienced 13.15 ± 2.48 days with periods of 3 h 43.2 min ± 21 min (11.08 ± 1.03 20 min intervals) spent at or below 15 ºC. This equates to approximately 44 % of a lizards time when it experiences T_bs at or below 15 ºC. The lowest T_b experienced by lizards during summer was 14.15 ± 0.15 ºC.

In an average 30 day period in winter, lizards experienced 27.91 ± 1.24 days with periods of 11 h 1.8 min ± 1 h 46.2 min (33.51 ± 5.41 20 min intervals) spent at or below 10 ºC. This means that lizards are spending approximately 47 % of their time at or below their CTMin and are therefore incapacitated, or effectively 'comatose', for a significant portion of the day. The mean lowest T_b experienced by lizards during winter was 7.27 ± 0.38 ºC. The lowest T_b experienced by any lizard in this study was -4.25 ºC, which was recorded in a crevice in July 2002.

3.4.4) Thermoregulatory behaviour

In terms of activity, lizards were active for significantly longer during summer than in winter (t_{20} = 9.96, P << 0.001). In summer, lizards emerged at 08h16 ± 0h11 and were active for 8 h 51.84 min ± 20.80 min, returning to their retreats at 17h07 ± 0h10. Summer activity time between males and females was not significantly different (t_9 = 0.05, P = 0.96). In winter, lizards emerged at 10h48 ± 0h41 and were active for 4 h 20.61 min ± 17.59 min, returning to their retreats at 14h31 ± 0h13. Winter activity time between males and females was not significantly different (t_9 = 0.43, P = 0.68). Lizards were active for significantly more days in summer than in winter (t_{20} = -4.05, P = 0.001), and were active every day in summer and on 21.71 ± 2.05 days out of an average 30-day period in winter (i.e., they were active 72.36 % ± 0.68 of winter days).

Changes in position and posture were evident during focal animal sampling. Males and females exhibited the same postures and positions. Lizards shuttled between rock crevices, full sunshine and areas in the shadows of rocks, or vegetation adjacent to the rock on which the lizard perched. Lizards tended to either face the sun directly,

face directly away from the sun thus receiving full sunshine on their backs, or turned side-on to the sun. The most frequently assumed postures were b) prostrate with head raised and c) upper body raised, and d) legs raised and other postures and positions were only observed during focal animal sampling in the afternoon (Figure 3.10).

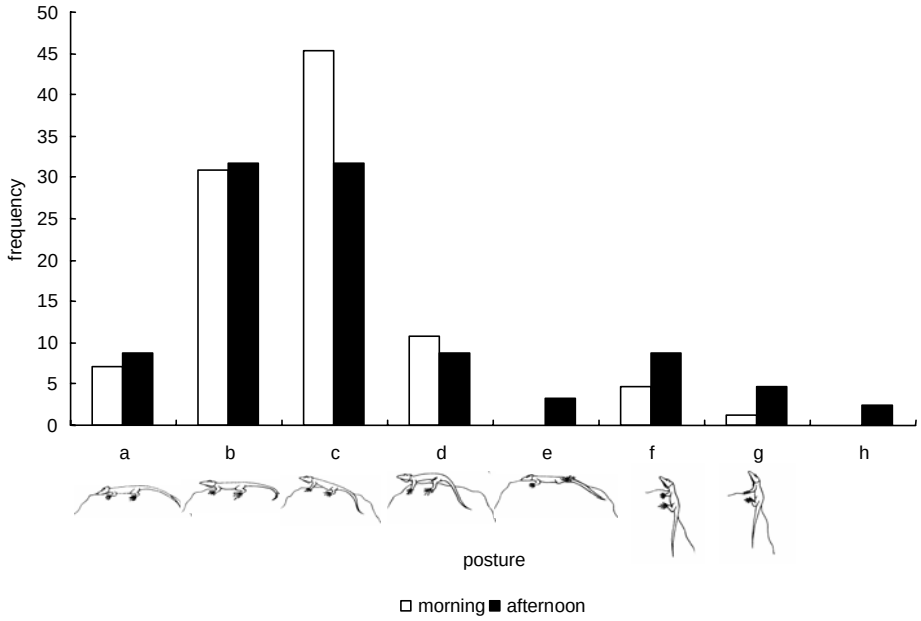


Figure 3.10: Frequency of postures and positions assumed by *Pseudocordylus melanotus melanotus* during the morning and afternoon (a - prostrate, b – prostrate with head raised, c – upper body raised, d – body raised, e – legs raised, f – side of rock, g – side of rock extended, h – other).

Postural changes were not dependent on the direction that the lizard was facing (i.e., postures were assumed whether facing towards or away from the sun, or were turned side-on to the sun; see Muth, 1977b for descriptions of lizard orientations relative to the angle of the sun). In the afternoon, lizards tended to shuttle between shaded areas and sunny areas, and predominantly assumed postures with raised heads and facing the sun. They also tended to alternate postures between facing the sun and turning side-on to the sun.

3.5) Discussion

The measured range of T_e for *P. m. melanotus* was -3.20 ºC to 54.94 ºC in the coldest and hottest microclimates, respectively. This means that lizards had a wide range of environmental thermal opportunities over the seasons. The T_{sel} measured in the laboratory thermal gradient (ca. 30 ºC) was higher than the T_{sel} in the field (summer ca. 29 ºC; winter ca. 26 ºC). The difference between laboratory and field measures of T_{sel} suggests that there may be limitations to the lizards' thermal environment.

During summer, no lizards experienced T_bs below 10 ºC, but experienced periods where their T_b was below 15 ºC approximately 13 days out of 30. The lowest T_b experienced during summer was approximately 14 ºC. During winter, lizards experienced periods where their T_b was below 10 ºC approximately 28 days out of 30, and the lowest T_b experienced was approximately 7 ºC. This suggests that, during the summer, lizards will rarely, if ever, experience T_bs below their lower critical limiting temperature (CTMin), whereas, in winter, lizards spend a significant amount of time (93 %) unable to move.

Lizards were active for significantly shorter periods during winter than during summer and activity time in winter was around half that of activity time in summer. There was therefore greater thermoregulatory opportunity for the lizards in summer. Changes in position and posture, and shuttling between sunshine and shade were obvious thermoregulatory behaviours. These behaviours are consistent with those associated with both heliotherms and thigmotherms, thus suggesting a mixed thermoregulatory strategy in *P. m. melanotus*.

Selected body temperatures in lizards generally approach 30 ºC (for example, see Arad *et al.*, 1989; Andrews and Kenney, 1990; Rocha and Vrcibradic, 1996; Angilletta *et al.*, 1999; Grbac and Bauwens, 2001). The T_{sel} measured for *P. m. melanotus* is thus within the range expected for lizards, as well as for other cordylid lizards. Selected T_bs measured in thermal gradients for *Cordylus vittifer* and *C. jonesi* were 32.1 ºC (Skinner, 1991) and 33.5 ºC (Wheeler, 1986), respectively. The field T_bs of *P. m. melanotus*, however, are much lower than expected. Bauwens *et al.*

(1999) reported the range of field T_bs for *Cordylus cataphractus*, *C. macropholis*, *C. niger*, *C. polyzonus* and *P. capensis* to be between 29 and 32 ºC. The field T_b of *C. macropholis* sheltering in *Euphorbia caput-medusae* plants was 28.4 ºC (Bauwens *et al.*, 1999), but this is possibly the coolest microclimate available to these lizards as they occur in the coastal dunes on the west coast of South Africa (Branch, 1998). The mean summer field T_b in *P. m. melanotus* was 28.9 ºC. Compared with *C. macropholis*, this is reasonable because *P. m. melanotus* are temperate species and occur in more temperate areas. The winter field T_b of *P. m. melanotus*, which is lower than the summer field T_{sel} and ambient temperatures, and the time available at suitable ambient temperatures, may not allow the lizards to maintain optimal body temperatures for a significant period during winter. The field T_bs in *P. m. melanotus* are the lowest recorded for any cordylid species (see Bauwens *et al.*, 1999).

The T_{sel} of male and female *P. m. melanotus* was not significantly different for either summer or winter measures. The lack of gender differences in selected temperature has also been noted in other lizards including *Trachylepis (= Mabuya) agilis*, *Trachylepis macrorhyncha* (Rocha and Vrbcibradic, 1996) and *Sceloporus siniferus* (Lemos-Espinal *et al.*, 2001). The difference between T_{sel} in males and females is usually explained by reproductive condition where reproductive females either select higher or lower T_bs than non-reproductive females or males. For example, reproductive female *Sceloporus jarrovi* (Beauchat, 1986) and *S. grammicus* (Andrews *et al.*, 1997) have lower T_{sel}s than non-reproductive females and males, whereas, pregnant female *Haplodactylus maculatus* selects higher T_bs than post-parturient/non-pregnant females and males (Rock *et al.*, 2000; Rock *et al.*, 2002). Young of *P. m. melanotus* are born during late summer (Flemming, 1993a; i.e., after the field study). Some females observed during the field study may therefore have been gravid. The lack of difference between T_{sel} in males and females of *P. m. melanotus* suggests that these lizards are have similar thermoregulatory behaviour and use similar microhabitats, or are active at the same time. The direct effects of reproductive status on thermoregulation in this species, however, remain to be investigated.

Pseudocordylus m. melanotus selected higher T_bs in summer than in winter. Christian and Bedford (1995) suggest that seasonal shifts in preferred T_b could be due to acclimatization in response to environmental temperatures, photoperiod, reduced food availability and hormonal cycles. This has been noted in *Lacerta viridus*, where seasonal changes in T_b are not solely dependent on the availability of thermal resources, but also photoperiod, especially where food availability and thermal regimes remain constant (Rismiller and Heldmaier, 1982, 1988). In *P. m. melanotus*, the thermal environment may not be limiting because most lizards are active during winter, even if only for a few days in a given month. Food availability could be a limiting factor since some reptiles are known to select higher T_bs after eating (e.g., Gibson *et al.*, 1989; Beck, 1996; Brown and Griffin, 2005).

If food availability is low, a shift in T_{sel} could improve the balance of energy (Brown and Griffin, 2005). Lower T_{sel}s will result in lower metabolic costs and energy requirements for maintenance (thus coinciding with reduced prey availability). Photoperiod is also likely to have an effect on T_{sel} in *P. m. melanotus*, particularly since it is a temperate lizard. This may, however, be an indirect effect as shorter photoperiods limit the environmental temperatures available because the insolation period is shorter. The availability of food, and photoperiod effects, on T_{sel} deserve further investigation not only in *P. m. melanotus*, but also other temperate reptiles.

The lowest temperature in the coldest microclimate (i.e., in a crevice) was -4 ºC in winter 2002. This is 1.15 ºC above the measured lower physiological temperature tolerance (LPTT = -5.15 ºC; Chapter 2), and 14 ºC below the measured lower critical limiting temperature (CTMin ca. 10 ºC; Chapter 2). In addition, crevice temperature rarely exceeds 10 ºC during winter and T_bs of lizards measured in the field are below CTMin for approximately 47 % of the time. This suggests that lizards are significantly affected by the thermal environment in winter and are totally incapacitated, or effectively 'comatose', during most winter nights. Although a colder acclimation temperature may have resulted in lower CTMin values (see Chapter 2), free-ranging lizards, although they experience lower night time temperatures, are selecting high T_bs when they are active during winter.

The lowest recorded temperature in the coldest microclimate during summer was 1.09 ºC (00h20, October 2004), suggesting that lizards do occasionally experience environmental temperatures below CTMin during summer, but for relatively short periods. The hottest recorded temperature was 54.94 ºC outside in full sunshine. Consequently lizards would be able to attain significantly higher body temperatures than they are actually selecting. The discrepancy between the laboratory and field measures of T_{sel} may therefore be explained by variations in individuals and territory structure; for example, some lizards may hold territories which have fewer thermal resources, or restricted available microclimates, thus restricting the T_es available in any particular area.

Since measures of T_{sel} in thermal gradients are indicative of T_bs selected and maintained in an environment with few or no thermoregulatory constraints, this suggests that there may be environmental constraints on the thermoregulatory ability of *P. m. melanotus*. The lizards were, however, actively thermoregulating in the field. This is evident in the discrepancy between the T_b and T_e measures (see Figure 3.6). Also, the highest T_e recorded (54.94 ºC) is obviously too high for the lizards to withstand (see also Chapter 2), so lizards would have to avoid places where their T_bs might exceed their critical limiting maximum. Body temperatures are thus maintained at a level which is determined primarily by the environment and the lizards ability to behaviourally regulate its T_b through activity, shuttling or postural changes.

Pseudocordylus m. melanotus selected higher T_bs on the first day of measure of T_{sel} in the thermal gradient. This could be attributed to the recent thermal history of the lizards. All lizards were maintained in captivity at 28 ± 1 ºC (approximately 2 ºC below T_{sel}) and did not have the opportunity to thermoregulate. When lizards were given the opportunity to thermoregulate when placed in the thermal gradient (temperature range between 5 and 50 ºC), they may have compensated for the lower temperature by selecting slightly higher than normal body temperatures. The effects

of recent thermal environment in captivity thus deserve further investigation in terms of thermoregulatory opportunity.

Activity has been shown to increase energy expenditure (Paladino, 1985) and the costs of locomotion itself comprise a significant portion of daily energy expenditure (Christian *et al.*, 1997). As a sit-and-wait forager, *P. m. melanotus* could be expected to have a relatively low energetic expenditure in terms of the costs of activity. *Pseudocordylus m. melanotus* were active for significantly longer periods, and significantly more often, in summer than during winter indicating that this species also exhibits altered behavioural thermoregulation, in terms of activity, between seasons. The lizard *Eumeces laticeps* also exhibits considerable behavioural thermoregulation in marked seasonal changes in activity patterns (Pentecost, 1974).

Generally, activity in reptiles during cool periods is more sporadic, shorter and unimodal (Foà *et al.*, 1992), while, in summer, activity is regular, longer and bimodal. For example, the Prairie Rattlesnake, *Crotalus viridus viridus*, shows unimodal activity with a peak during midday and bimodal activity in summer with a peak in the morning and again in the afternoon (Gannon and Secoy, 1984); the lizard *Tiliqua rugosa* exhibits bimodal activity during summer also with peaks in the morning and afternoon (Firth and Belan, 1998). On days when *P. m. melanotus* are active in winter, T_b measured in the field suggests that activity is unimodal. This is likely because lizards maintain high T_bs for short periods only once during the day. In summer, high T_bs are maintained throughout the day, which also suggests a unimodal activity pattern. During focal animal sampling, however, the number of lizards active during the heat of midday were obviously lower, which points towards a degree of bimodality of activity.

Even when thermal conditions are suitable, not all lizards within a population are simultaneously active (Martín and Salvador, 1995), but basking lizards are exposed, thus increasing the probability that they will be observed (Foà *et al.*, 1992). So, although *P. m. melanotus* appear to be active, they may, in fact, seek out suitable retreat sites during the hottest times of the day. It is clear that *P. m. melanotus* are

thermoregulating throughout the day (i.e., differences between T_b and T_e), which implies that, although they may not be active, as such, they are selecting retreats where they can still maintain T_b at a selected level. Huey *et al.* (1989) suggest that retreats offer equivalent, or sometimes superior, thermoregulatory opportunities to those available in more exposed environments. These periods of inactivity may be important in avoiding environmental extremes (Kearney, 2002) and conserving energy or water (Martín and López, 2000).

In heliothermic reptiles, body posture and shuttling are important for maintaining T_b at activity levels (Spellerberg, 1972b) because the position of the sun and the amount of radiation change diurnally (Sievert and Hutchison, 1989). Lizards that use solar radiation as a main source of body heat make behavioural adjustments to maintain constant T_bs by constantly monitoring the position of the sun and the available heat (Sievert and Hutchison, 1989). *Pseudocordylus m. melanotus* exhibits distinct shuttling behaviour and postural adjustment during thermoregulation.

Four strategies of heat regulation in lizards have been identified. These include 1) gaining external heat or avoiding heat loss to the environment, 2) retaining internal heat, 3) generating internal heat, and 4) losing excess heat or avoiding heat gain from hot environments (Sherwood *et al.*, 2005). Generating and retaining internal heat (2 and 3) generally constitute physiological measures of heat regulation, while losing and gaining external heat (1 and 4) include behavioural thermoregulation. Sherwood *et al.*'s (2005) descriptions of external heat loss and heat gain are clearly evident in the behaviour of *P. m. melanotus*. In gaining external heat, lizards bask in sunlight on cold mornings and gain heat from radiation and conduction from warmed surfaces. Once an optimal level has been reached, the lizards become active, seeking food, while using behavioural and physiological methods of T_b maintenance.

As a sit-and-wait forager, *P. m. melanotus* does not actively seek food, and can be expected to use postural adjustments to maintain T_b while exposed on perch sites. In losing excess heat, lizards will generally seek shady environments, usually in crevices or in the shade of adjacent vegetation. *Pseudocordylus m. melanotus* thus

exhibits typical shuttling behaviour, but uses a combined thigmothermic-heliothermic strategy because it uses both solar radiation and conduction/convection of heat from the environment to maintain its T_b at a selected level.

Posture and orientation affect the surface area exposed to radiation (Walsberg, 1992), and hence the potential T_b that can be achieved and maintained. Muth (1977a) showed that T_b in the Zebra-tailed Lizard, *Callisaurus draconoides*, was correlated with body posture. For example, when lizards were prostrate and had maximum contact with the ground, T_b was lower (33.9 ºC) than when lizards were elevated and had least contact with the ground (42.7 ºC; Muth 1977 a). Muth (1977a) also indicates that the rate of heat gain was highest when lizards were prostrate, and lowest when elevated. This could explain why *P. m. melanotus* assume particular postures during certain times of the day.

The most frequently assumed postures, throughout the day, were b) prostrate with head raised and c) upper body raised (see Figure 3.3). This suggests that lizards are effectively using both solar radiation and conduction from the rock surface (i.e., a combination of heliothermic and thigmothermic thermoregulation). Generally, lizards assumed more and/or different postures during the afternoon. During the hottest periods, if lizards were exposed, lizards assumed more elevated postures, with either more of the body or legs raised off the rock surface. This means that the lizards are potentially avoiding direct contact with the rock surface and thus minimising heat gained from it, as well as increasing potential heat loss by exposing a greater surface area to the air, which may be especially important when it is windy. Also, lizards also assumed more prostrate postures when it was overcast (pers. obs.) thus maximising heat gain from the rock.

Orientation to the sun also has a significant effect on T_b, although to a lesser degree in smaller lizards (Muth, 1977b). In *P. m. melanotus*, there was an obvious shift of orientation to the sun over the course of the day. Since posture affects the surface area exposed to radiation (Walsberg, 1992), particular postures were assumed with different orientations during the day; usually the largest area was oriented towards

the sun during early morning and late afternoon. There is obviously a great variety of body postures in *P. m. melanotus*, which can be assumed at various orientations to the sun. This suggests that *P. m. melanotus* should be able to maintain T_b within a narrow range through postural adjustments.

It is clear that *P. m. melanotus* can regulate and maintain its T_b at a selected level. In summer, T_{sel} may be limited environmentally in such a way that the lizards are unable to maintain *optimal* T_{sel} (as measured in a thermal gradient), although they are able to achieve T_bs greater than T_{sel}. In winter, it is likely that there are limitations to the thermal environment, which do not allow lizards to achieve and maintain T_{sel}.

CHAPTER 4

ENERGY EXPENDITURE

4.1) Abstract

In reptiles, metabolic rate (MR) is affected by many different factors, including temperature, sex, body size and digestive state. Standard MR was measured for the lizard *Pseudocordylus melanotus melanotus* over a range of temperatures (20, 22, 25, 30, and 32 °C). I measured oxygen consumption using a flow-through system and tested for the effects of body mass and temperature. Body mass and MR were significantly related at 30 ºC only probably because of the range of body mass of the lizards was small. MR at 20 ºC was significantly different to MR at 30 ºC (total MR), and 32 ºC (total and mass specific MR). MR was calculated for each temperature for a standard 30 g lizard to investigate the effects of temperature on MR. MR increases significantly with increasing temperature. The temperature quotients (Q_{10}) between 22 and 32 °C were close to one suggesting that this was the lizards preferred temperature for activity. Both mass exponents and Q_{10} values were similar to those reported for other lizards and snakes. At 30 ºC, the MR in a standard 30 g *P. m. melanotus* equates to a minimum energy requirement for maintenance metabolism of 2943.24 J/day. A 30 g lizard, therefore, requires the equivalent of 0.315 g of mealworms per day, or 115.15 g per year, to cover the costs maintenance metabolism. Any additional energy assimilated can be allocated to growth, reproduction and storage.

4.2) Introduction

Metabolic rate (MR), very simply, is the metabolism of energy per unit time (Schmidt-Nielsen, 1997). The MR of reptiles is affected by many intrinsic and extrinsic factors, including temperature, sex (Niewiarowski and Waldschmidt, 1992), body size (Bennett and Dawson, 1976) and digestive state (Secor and Diamond, 1998). Reptiles that inhabit temperate areas are subjected to a wide range of environmental temperatures (Buikema and Armitage, 1969) and exhibit seasonal changes in MR. Metabolic rate for any given T_b in winter is lower, which may act in conserving energy during inactive, winter periods, when the maintenance metabolism is expected to be lower (Bennett and Dawson, 1976).

High thermal dependence of metabolism at temperatures above or below the range at which a reptile is normally active could result in significant energy conservation

during periods of inactivity (Bennett and Dawson, 1976). Dorcas *et al.* (2004) suggest that understanding the effects of temperature on MR is essential to understanding an ectotherm's energy expenditure and ecology. Also, knowledge of the relationships between MR, body size and temperature can be used in developing models to explain energy requirements (Dorcas *et al.*, 2004). The total available energy and its allocation to maintenance, growth, reproduction and storage may even vary geographically among populations owing to differences in the general physical environment (Beaupre, 1996).

Gender is known to affect MR in numerous reptiles, which may occur as a result of higher activity in males or hormonal differences (Bennett and Dawson, 1976). Generally, females tend to have lower metabolic rates than males, and gravid females tend to have higher mass specific MRs (MSMR) than non-gravid females (Bennett and Dawson, 1976, Beaupre *et al.*, 1993a). Gravid females may even have higher MRs than males. In amphibians, this has been found in gravid Small-mouthed Salamanders, which had distinctly higher oxygen consumption than post-gravid females, as well as males (Finkler and Cullum, 2002). Finkler and Cullum (2002), however, suggest that this could be due to the inclusion of the egg mass in the whole mass of gravid female salamanders, thus affecting the MSMR, and also assuming that the eggs have the same metabolism as the female salamander's tissue.

There is an inverse relationship between the MSMR and the total mass of an organism, where the standard MR (SMR) per gram decreases with increasing body size (Schmidt-Nielsen, 1997). This relationship is represented by $MR = aM^b$, where a is the mass co-efficient and is the intercept in a log-log regression, M is body mass and b is the mass exponent, which expresses the rate of change of SMR with a change in body mass. For many taxonomic groups, the value of b is usually 0.75 (Randall *et al.*, 1997). Andrews and Pough (1985), however, suggest that, based on metabolic analysis of 34 squamate families, a mass exponent of 0.80 would be more appropriate for reptiles. When log transforming the axes, this relationship becomes linear and is described by the equation $y = mx + c$, where m becomes the mass exponent and c is the y-intercept. This relationship, in either form, is occasionally

difficult to demonstrate at an intraspecific level since the range of body mass may be relatively small (Stephen Secor, pers. comm.). Other factors, such as gender, nutritional state and season may also have additional effects (for example, see Bennett and Dawson, 1976; Finkler and Cullum, 1976; Niewiaroski and Waldschmidt, 1992; Secor and Diamond, 1998).

For ectotherms, MR is usually measured as the standard MR (SMR). Although standard metabolic energy requirements account for only a small proportion of the total energy expenditure (McCue and Lillywhite, 2002; for example see Bennett and Nagy, 1977; Mautz and Nagy, 1987; van Marken Lichtenbelt *et al.*, 1993), it allows for comparison at the population and species level and is the rate of metabolic free energy production when the ectothermic organism is resting, fasting, awake and in a thermoneutral environment (Bligh and Johnson, 1973). Standard MR is fundamentally determined by an animal's body temperature and body size (Bennett and Dawson, 1976). Measurement is made either in a closed, or flow-through, system, where the animal is placed in a sealed chamber and the concentration of oxygen is measured before and after (closed system) or during (flow-through system) a set period (Vleck, 1987). Standard MR does not, however, necessarily yield an ecologically important measure since free-ranging lizards usually have food in their stomachs (Niewiarowski and Waldschmidt, 1992).

Digestion of food in the stomach leads to specific dynamic action (SDA) on MR. SDA includes the energy required for the initiation and upregulation of the physiological and metabolic responses associated with the ingestion of food, including the secretion of digestive fluids into the intestine and stomach, absorption of digestive products and hypertrophy of the gastrointestinal system (Secor and Diamond, 1995; Wang *et al.*, 2001). These processes are associated with an elevation in the MR, and Secor and Faulkner (2002) suggest that the total cost of digestion could be a function of the total chemical and mechanical breakdown requirements of the meal. The amount, type or composition of food and the frequency of meals can therefore influence the amount of energy required to break a meal down (Wang *et al.*, 2001; Overgaard *et al.*, 2002; Secor and Faulkner, 2002). Sit-and-wait foragers,

however, experience long periods of fasting, followed by meals and a large increase in MR compared to pre-feeding values (Robert and Thompson, 2000). The lowest level of maintenance metabolism in sit-and-wait foragers, therefore, can be taken as the SMR without the effects of SDA since sit-and-wait foragers experience extended periods in a post absorptive state.

This study deals with the thermal dependence of SMR in *Pseudocordylus melanotus melanotus*. I measured the effects of temperature on oxygen consumption as an indirect measure of SMR. This forms an integral part of the energy budget (Chapter 6) since these measures give an indication of the lowest required maintenance metabolism at any particular temperature, and, generally, the minimum food requirement can be estimated by calculating the amount of food required for the minimum metabolic energy requirement (Nagy, 2001). Here I show that MR increases with increasing temperature, which is indicative of increased maintenance energy requirements at higher temperatures and therefore a greater energy requirement to maintain a positive energy balance.

4.3) Materials and methods

Nine lizards (four female, five male) were collected at Suikerbosrand Nature Reserve. They were housed and maintained under standard conditions (Chapter 1, Section 1.3). Standard metabolic rate (SMR) was measured using the definition and conditions described by Bligh and Johnson (1973), where SMR is the oxygen consumption of the animal in a rested, awake, fasting and thermoneutral state. All lizards were post absorptive during measures of metabolic rate to comply with conditions required to measure SMR and also because digestive processes are known to increase MR (Secor and Diamond, 1998). Lizards were maintained at each test temperature for at least three days prior to measuring oxygen consumption and were therefore considered to be in thermal equilibrium. Also, since activity is known to increase MR (Dmi'el, 1972), the system was designed to reduce the activity of the lizards: The metabolic chambers were glass cylinders with a diameter of 75 mm, 300 mm long and were closed on each end with rubber stoppers. The chambers were airtight and allowed the lizards to stand/lie normally, but restricted any excess

activity. In addition, the chamber was covered during trials to reduce stress during metabolic measures. Lizards were also placed in the chamber at least 30 min prior to measurements.

Oxygen consumption was measured in a flow-through respirometry system (Figure 4.1). Air was pulled through the system at approximately 100 ml/min using a pump (R-2 Flow Control Ametek) and flow was measured using a Brookes model 5860 mass flow meter (nearest 0.01 ml). Water vapour was removed from air entering the flow meter using anhydrous calcium sulphate (indicating Drierite). Carbon dioxide was removed from air entering the chamber and entering the mass flow meter using indicating soda lime (Associated Chemical Enterprises (PTY) LTD). Oxygen concentration was measured down-stream of the pump (i.e., air was pushed through the oxygen analyser) using a S102 oxygen sensor (Qubit systems). A pressure release system ensured that air pushed through the oxygen analyser was at atmospheric pressure, which was measured at least once during measurements using a handheld barometer (YCM). A three-way tap was used to control flow through either the experimental chamber or the bypass chamber, which was used to measure a baseline. A small bypass pump (Type NMP 02L/U) was used to maintain airflow through the experimental chamber during baselining, thus preventing the build up of CO_2 in the experimental chamber. The bypass pump pumped air out of the chamber at approximately the same rate as that pumped through the system thereby reducing the time required for the system to reach equilibrium after baselining. Temperature in the experimental chamber was monitored using a thermocron iButton (± 1 °C accuracy; Dallas Semiconductor, Texas, USA), which was placed inside the chamber. I assumed that lizard T_b was identical to the chamber temperature. Chamber temperature was logged at one-minute intervals during metabolic measures. The chamber temperature did not deviate more than 1 °C for each temperature trial.

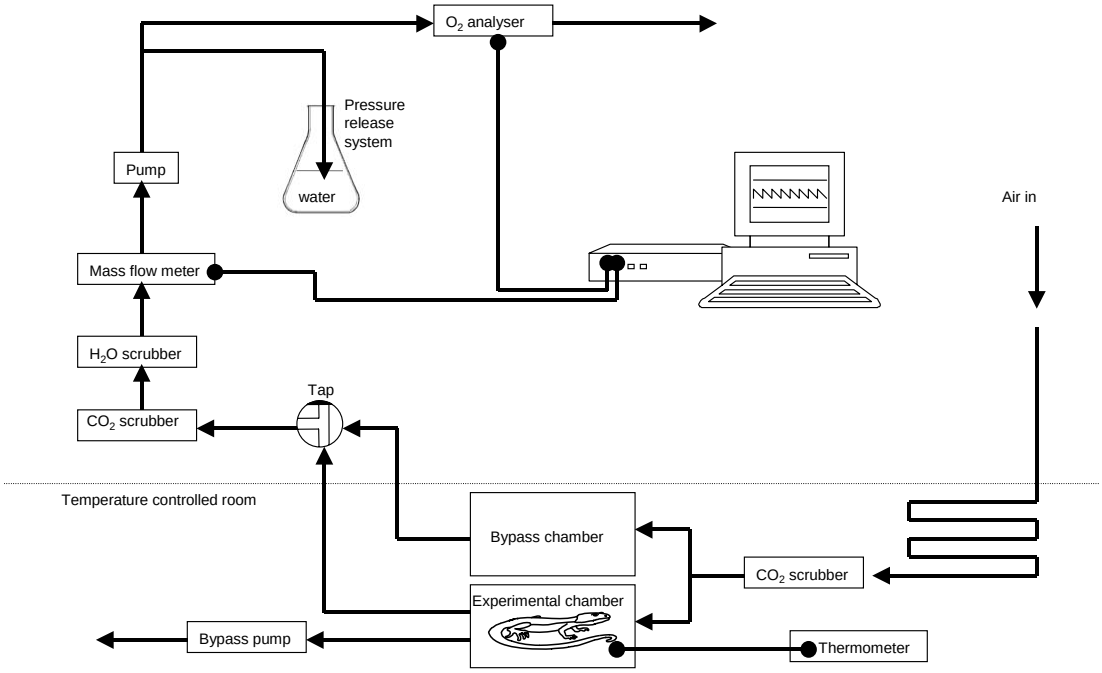


Figure 4.1: Equipment configuration for oxygen consumption measurement of metabolic rate. Arrows indicate the direction of the flow of air.

The oxygen analyser and flow meter were linked to a computer through a Prolab/800 system interface and analysed using Prolab software, CHART v 4.2.3 (ADInstruments). The percentage oxygen in the air flowing through the system was measured at 30 s intervals. Baseline measures were taken for 20 min before oxygen consumption of the lizard was measured. Oxygen consumption was measured for one hour and 20 minutes for each lizard, followed by an additional 20 min baseline period. The percentage oxygen used was taken as the mean oxygen concentration over one hour (after 20 min equilibration period) minus the mean oxygen concentration for both 20 min baseline periods averaged. The percentage oxygen used was corrected for flow rate, pressure and time, to determine the total MR in mlO_2/h. In addition, this value was corrected for body mass of individual lizards to determine the mass specific MR (MSMR) in $mlO_2/g/h$ only after the mass specific relationship was tested. Lizards were weighed before each trial. Total MR and MSMR were calculated for individual lizards for each temperature using the following equations:

Total MR = (% O_2 exp - % O_2 bl)/100 x FR x (P/1000) x 60
MSMR = (% O_2 exp - % O_2 bl)/100 x FR x (P/1000) x 60 x 1/M,

where % O_2 exp is the mean percentage oxygen measured for one hour with air flow through the experimental chamber containing a lizard,
% O_2 bl is the mean percentage oxygen measured during baselining before and after % O_2 exp was measured (mean difference between baseline measures before and after was 0.08 ± 0.009 % O_2),
FR is flow rate,
P is atmospheric pressure, and
M is the body mass of individual lizards.

The top 25 % of measurements were removed for each lizard to account for any effects of increased MR due to activity. For each temperature, the total MR and MSMR were regressed against lizard body mass to determine the effects of body mass on MR. The effect of temperature on MR was investigated using a repeated measures ANOVA, with individual lizards as the repeated measure since oxygen consumption was measured for the same lizards at each of the test temperatures. The mean MR for each lizard at each temperature was also converted to a MR for a lizard of 30 g to remove any potential effects of body mass, and regressed against temperature to determine the effects of temperature on MR. This transformed MR (TMR) was calculated for each temperature using the equation:

TMR = (total MR x $(30/mass)^b$),

where b is the mass exponent determined by calculating the exponent in a power relationship between MR and body mass for each temperature. Where there was no significant effect of body mass on MR at any particular temperature, the TMR was calculated by multiplying the MSMR by 30, thus providing a total MR for a 30 g lizard.

4.4) Results

The total O_2 consumption ranged between 2.89 ± 0.70 mlO_2/h at 20 °C and 6.70 ± 1.04 mlO_2/h at 30 °C (Figure 4.2). Mass specific O_2 consumption ranged between 0.10 ± 0.02 $mlO_2/g/h$ at 20 °C and 0.21 ± 0.03 $mlO_2/g/h$ at 30 °C (Figure 4.2). Total O_2 consumption and mass specific O_2 consumption were not significantly different among the different temperatures (repeated measures ANOVA using individuals as the repeated measure; total MR: $F_{4, 28} = 0.87$, P = 0.49; MSMR: $F_{4, 28} = 1.07$, P = 0.39).

There was a significant effect of mass on total O_2 consumption at 30 °C only, where O_2 consumption increased with increasing body mass (log transformed; P = 0.02, R^2 = 0.55; Figure 4.3), but this effect was not significant for the mass specific O_2 consumption relationship (Figures 4.4 and 4.5).

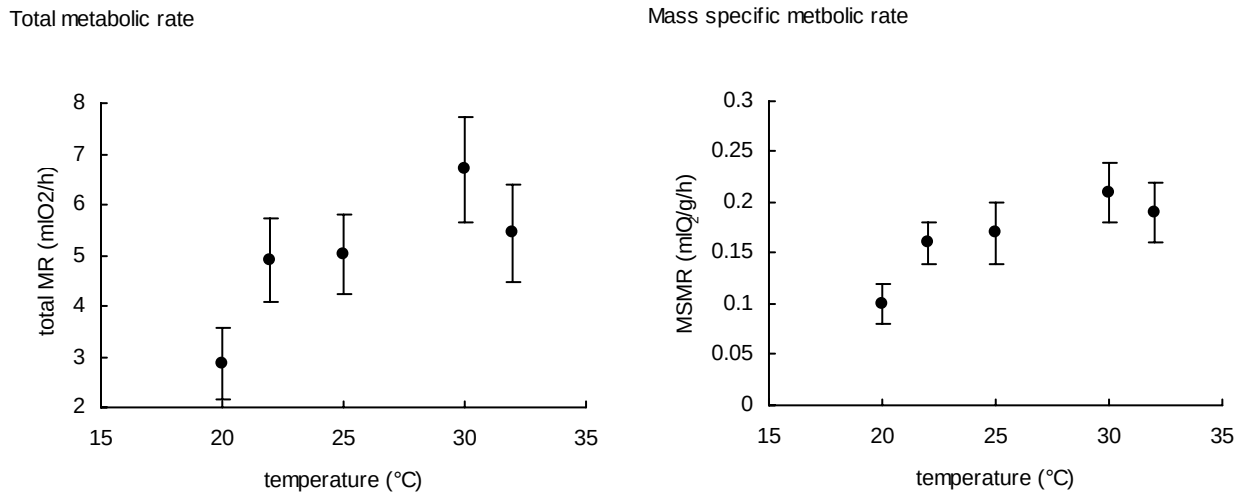


Figure 4.2: The effects of temperature on total metabolic rate (total MR) and mass specific metabolic rate (MSMR) in terms of oxygen consumption in the lizard *Pseudocordylus melanotus melanotus*. Means presented ± SE.

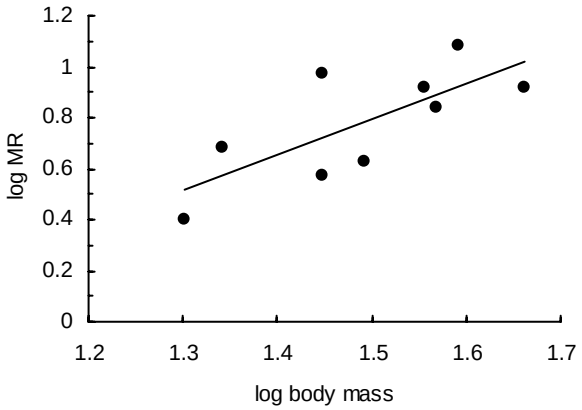


Figure 4.3: The effect of body mass on total metabolic rate (MR) at 30 °C. Axes have been log transformed to show a linear relationship (regression analysis; $y = 1.39x - 1.28$, $R^2 = 0.55$, $P = 0.02$). This was the only temperature where body mass had a significant effect on metabolic rate.

Mass exponents were not calculated for temperatures where there was no significant mass relationship. Since there was only a significant relationship between MR and mass at 30 ºC, MR was transformed to the equivalent of a 30 g lizard by multiplying MSMR at the other test temperatures by 30. At 30 ºC, MR was transformed using the equation given (see above). Total MR for 30 g lizards, i.e., TMR, increases with increasing temperature (Figure 4.6; $R^2 = 0.77$, $P = 0.048$).

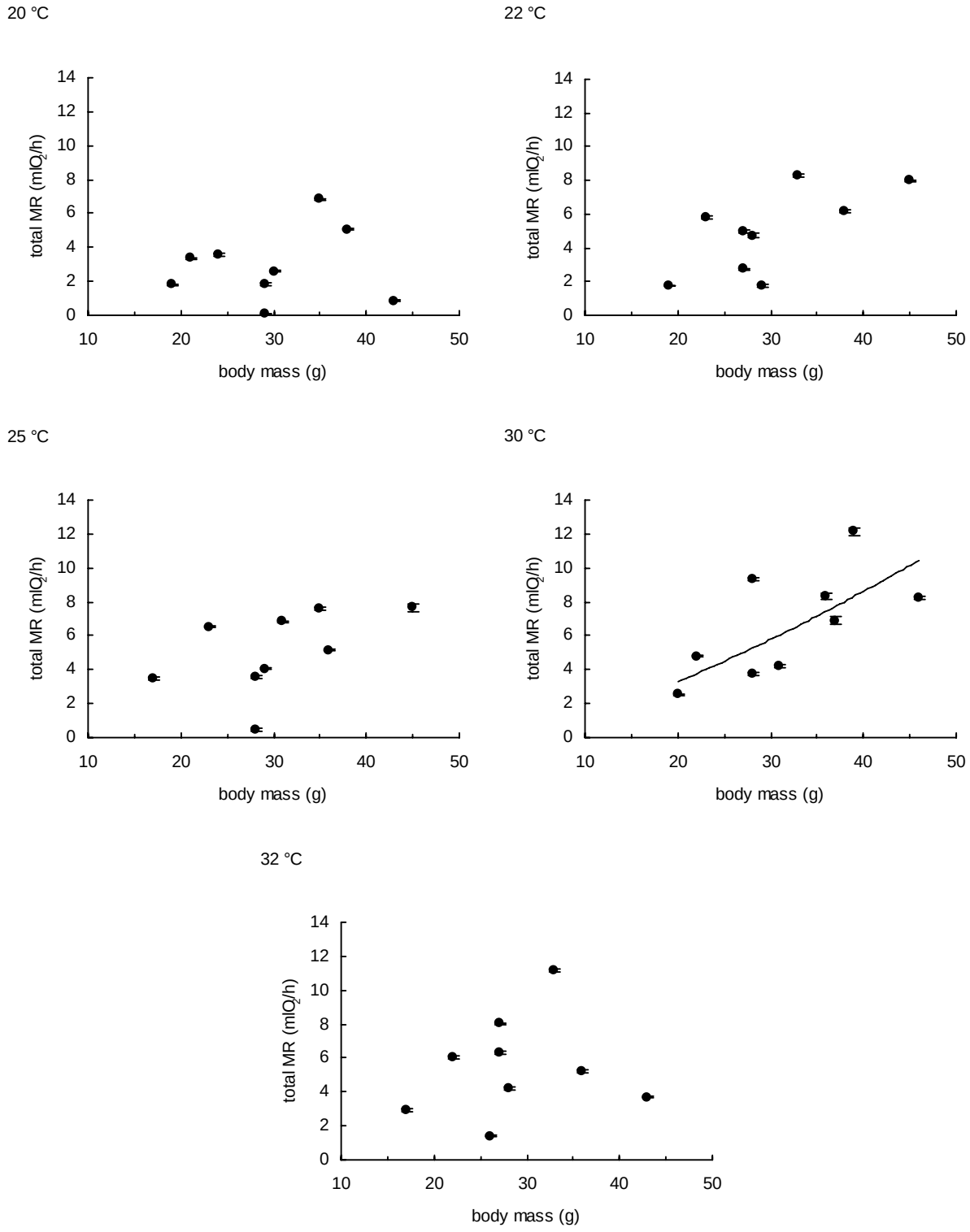


Figure 4.4: The effects of body mass on total metabolic rate (total MR) at different temperatures for the lizard *Pseudocordylus melanotus melanotus*. The relationship between body mass and total MR was significant at 30 ºC only (see Figure 4.3; power relationship: $y = 0.05x^{1.39}$, $R^2 = 0.55$).

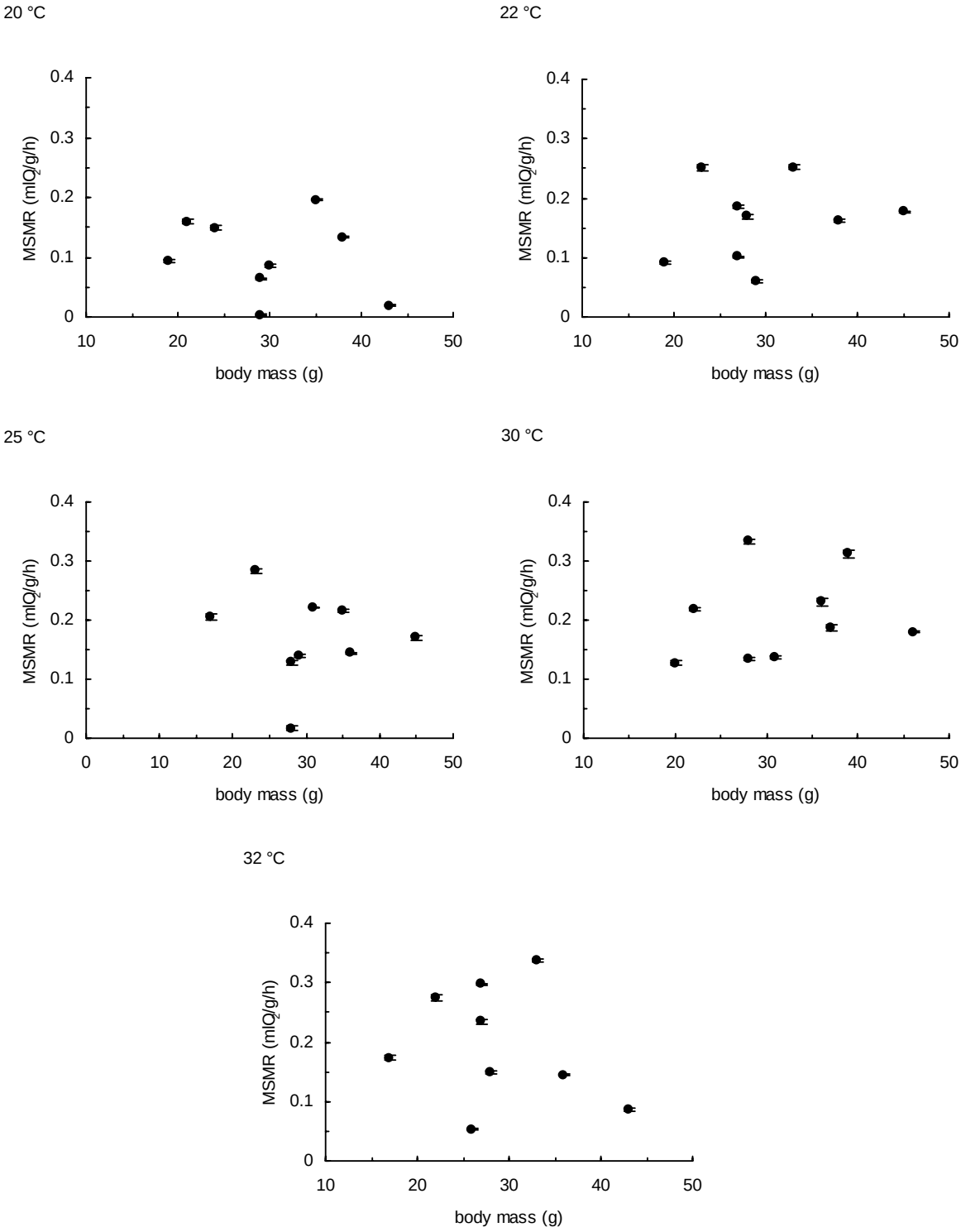


Figure 4.5: The effects of body mass on mass specific metabolic rate (MSMR) at different temperatures for the lizard *Pseudocordylus melanotus melanotus*.

Temperature quotients (Q_{10}) were calculated, using the van't Hoff equation, for MSMR over the range of temperatures tested (Table 4.1). These values are in line with that expected for other squamates (Andrews and Pough, 1985). At the selected body temperature of *P. m. melanotus* (± 30°C; Chapter 3), a standard 30 g lizard would require 2943.24 J/day (ranging between 1452.02 and 2943.24 J/day for 20 and 30 ºC, respectively) for maintenance metabolism (assuming a respiratory quotient of 0.79, where, for every one litre of oxygen consumed, 20.1 kJ of heat energy is released; Randall *et al.*, 1997).

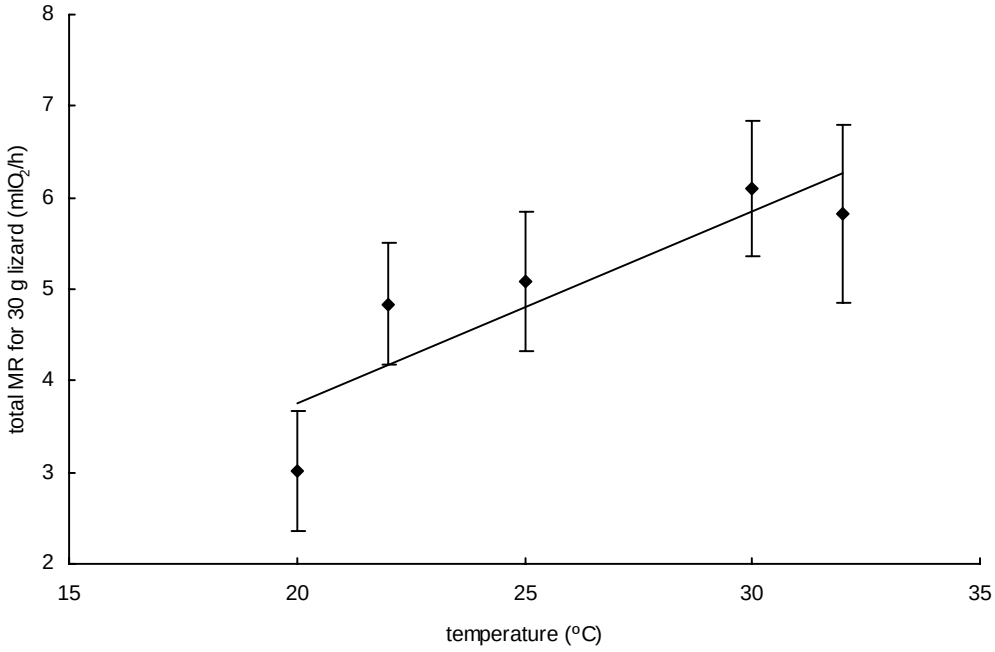


Figure 4.6: The effect of temperature on total metabolic rate (total MR) for a standard 30g lizard (*Pseudocordylus melanotus melanotus*). (Mean ± SE; regression analysis; $R^2 = 0.77$, P = 0.048, y = 0.21x – 0.41)

Table 4.1: Temperature quotients (Q_{10}) calculated using the van't Hoff equation over the range of temperatures at which oxygen consumption was measured. Measures calculated for a standard 30 g lizard.

Temperature range	Temperature quotient (Q_{10})
20 – 22 °C	10.71
22 – 25 °C	1.18
25 – 30 °C	1.44
30 – 32 °C	0.80
20 – 30 °C	2.03

4.5) Discussion

The metabolic rate of *P. m. melanotus* is temperature dependent such that MR increases with increasing temperature. This follows the trend found with the effects of increasing temperature on appetite (see Chapter 5). There was only a significant effect of mass on total MR at 30 °C, where MR increased with increasing body mass. Mass exponents were highly variable among the test temperatures; however, this may be attributed to the lack of significance in the relationship between MR and body mass. Q_{10} values were in line with that expected for other squamate reptiles, and, at selected body temperature (± 30 ºC), lizards require approximately 2940 J/day for maintenance metabolism.

The MSMR recorded for *P. m. melanotus* ranged between 0.10 ± 0.02 $mlO_2/g/h$ at 20 °C and 0.21 ± 0.03 $mlO_2/g/h$ at 30 °C. These values are comparable to metabolic measures for a variety of other squamates (e.g., see Bartholomew *et al.*, 1965; Bennett and Dawson, 1976; Al-Sadoon and Spellerberg, 1985; Niewiarowski and Waldschmidt, 1992; Thompson *et al.*, 1995; Robert and Thompson, 2000; Angilletta, 2001a). Rismiller and Heldmaier (1991) recorded MSMRs between 0.04 and 0.30 $mlO_2/g/h$ at 30 ºC for the lizard *Lacerta viridus*, which falls within a similar range of body sizes as *P. m. melanotus*; they used lizards between 30 and 46 g. They do not, however, report on any effects of body mass or present mass exponents.

The range of body masses of *P. m. melanotus* tested here was comparatively small. Secor and Faulkner (2002) found a significant relationship between MR and body mass in the Marine Toad (*Bufo marinus*) with 100-fold range in body mass. In my study, it was only possible to test a 2.7-fold range of body mass (17 to 46 g, range = 29 g), i.e., the largest individual was only 2.7 times as big as the smallest individual. Buikema and Armitage (1969) also suggest that different sized individuals are likely to be affected by different temperatures in different ways. If each lizard were affected in the same way, the same pattern could be expected for each of the test temperatures. It is important to note, however, that an energy budget does not depend on mass specific relationships with MR, but is dependent on the average MR and its response to temperature.

Pseudocordylus m. melanotus showed an increase in MR with increasing temperature. This trend has been noted in many other lizard and snake species (e.g., Al-Sadoon and Spellerberg, 1985; Al-Sadoon and Abdo, 1989; Al-Sadoon, 1991; Beaupre *et al.*, 1993a; McCue and Lillywhite, 2002). In *P. m. melanotus*, however, MR peaked at 30 °C and decreased again at 32 °C. Summer selected temperature of this species was approximately 31 °C (Chapter 3) and the Q_{10} value for 30 – 32 °C (0.80) was the lowest over the range of temperatures tested. Low Q_{10} values (i.e., Q_{10}s close to one) at or just below lizards' voluntary temperatures have been reported as a mechanism that aids in metabolic homeostasis (Al-Sadoon and Spellerberg, 1985). Also, where there is a metabolic plateau (i.e., $Q_{10} = 1$ or low thermal dependence), the range of temperatures should coincide with the body temperatures at which the animal is active (Angilletta, 2001a). Al-Sadoon and Abdo (1989) reported that the nocturnal geckos *Ptyodactylus hasselquistii* and *Bunopus tuberculatus* had low thermal dependence of MR between 15 and 25 °C – a range that may coincide with their preferred body temperatures. *Pseudocordylus m. melanotus* can be expected to be active at temperatures between 22 and 32 ºC since the Q_{10} values over this range are close to one, but is lowest between 30 and 32 ºC (near to their selected body temperature). The body temperatures recorded for active *P. m. melanotus*, however, are much lower (particularly) and higher than these values (see Chapter 3). It can thus only be said that, in *P. m. melanotus*, the Q_{10} values close to one only infer the range of temperatures over which the lizard may be *preferably* active, and that temperatures higher, or lower, than this predicted range cannot be discounted for periods of activity (e.g., see Chapter 3).

In terms of the energy required for maintenance metabolism, *P. m. melanotus* requires 2943.24 J/day (1074.28 kJ/year). The annual energy requirement, in terms of SMR only, could be considered conservatively high since lizards are likely to have lower energy requirements during winter when they are active for shorter periods. Given that daily energy expenditure in reptiles is two to three times the daily SMR (Stephen Secor, pers comm.), the calculated energy expenditure of these lizards is, however, an underestimation of the total daily energy requirement. There are few

metabolic studies that liken oxygen consumption to the metabolic expenditure in terms of energy. Metabolic expenditures of 2260, 3656 and 5799 kJ/year have, however, been reported for the snakes *Crotalus lepidus* (Beaupre, 1996), *Agkistrodon piscivorus conanti* (McCue and Lillywhite, 2002) and *Crotalus adamanteus* (Dorcas *et al.*, 2004), respectively. These snakes are significantly larger than *P. m. melanotus*, which is at least half the size of the smallest of these snakes (*Crotalus lepidus*; values reported for a snake of 100g). An annual energy requirement half that of *Crotalus lepidus* for *P. m. melanotus* is therefore plausible. It should be noted, however, that Beaupre (1996), McCue and Lillywhite (2002), Dorcas *et al.* (2004) and I have estimated yearly energy expenditure from laboratory measurements. Field metabolic rates, which take variable activity and environmental temperature into account, may be greater or smaller than laboratory measures of daily or annual energy expenditure. In *Sceloporus occidentalis*, field measures of MR were 2.5 times greater than laboratory measures (Bennett and Nagy, 1977), and in *S. virgatus*, field MR was 1.4 times greater and 1.1 times smaller than laboratory measures in spring and summer, respectively (Merker and Nagy, 1984). Additional measures of MR for *P. m. melanotus* in the field could thus provide additional information regarding daily metabolic energy expenditure in varying environmental temperatures and time-activity budgets.

CHAPTER 5

ENERGY GAIN

Parts of this chapter have been published in the Journal of Comparative Physiology B. Please see Appendix 1.

5.1) Abstract

Energy balance is critical to an animal's survival, maintenance, foraging, growth and reproduction. In order to remain in a positive energy balance, an animal needs to gain sufficient energy to supply its maintenance and activity requirements. After ingestion and metabolism, potential energy is available for activity, growth, reproduction and storage. In ectotherms, an increase in body temperature increases metabolic rate and may increase rates of digestive processes. Here, I measure the frequency of feeding, using focal animal sampling on free ranging lizards, and analysed stomach contents and faecal samples to assess the diet of *Pseudocordylus melanotus melanotus*. Thirty-five percent of prey capture attempts were successful and occurred at a frequency of one per 150 min. Prey included a variety of arthropods, predominantly coleopterans. I also measured the thermal dependence of the apparent digestive and apparent assimilation efficiencies (ADE and AAE), gut passage time (GP) and appetite. Trials were conducted at 20, 22, 25, 30, 32 and 35 ºC under controlled laboratory conditions. Each trial lasted 14 days, during which, lizards were fed approximately 1 g of mealworms per day. Faeces and urates were collected daily and oven dried at 50 ºC. The energy content of egested matter was then measured using bomb calorimetry. ADE and AAE were not affected by temperature for either males or females. The mean (± SE) ADE and AAE were 94.4 ± 0.3 % and 87.2 ± 0.6 % respectively. GP was not significantly different between males and females at any temperature, but decreased significantly with increasing temperature. Appetite was significantly different between the different temperatures measured. The decrease of gut passage time with increasing temperature was expected, since the digestive and assimilation efficiencies are similar over the range of temperatures tested. Lizards are thus assimilating a similar proportion of ingested energy, but at faster rates at higher temperatures.

5.2) Introduction

Energy balance is critical to an animal's survival, maintenance, foraging, growth and reproduction (Nagy, 1983; Secor, 2001). If an animal has gained energy such that it exceeds the costs of maintenance metabolism and activity, it will be in a positive energy balance (Pough, 1973; Huey *et al.*, 2001). After ingestion and metabolism of

food, potential energy is available for any immediate demands of the animal, including movement and growth; the rest is used later, after storage subcutaneously or in visceral fat bodies (Derickson, 1976), for maintenance or reproduction (Spotila and Standora, 1985).

Certain factors may affect how much energy can be gained, used and stored, and these vary seasonally or sporadically (Congdon *et al.*, 1982; Durtsche, 1995), but are generally manifested in foraging behaviour and prey characteristics. In optimal foraging theory, animals have a tendency to select prey sizes, or patches, that will result in the maximum energy gain for the energy expended in gaining that particular food item (Pough, 1973; Smith, 1990, Begon *et al.*, 1996). In other words, it is beneficial to eat less when the investment in obtaining the maximum amount of energy is too high (van Marken Lichtenbelt *et al.*, 1997). Generally, the optimal prey choice is dependent on the foraging time, energy value of the prey item and handling time (Krebs and Davies, 1996), and in low resource conditions, an animal should eat any food item encountered, even if it is of low energetic value (Durtsche, 1995). These expectations have led to the "expected energy budget rule" where an animal should be risk prone if the daily energy budget is negative, and risk-averse if the expected energy budget is positive (Caraco *et al.*, 1990; Smith, 1990; Orduña and Bouzas, 2004).

The success of foraging, or prey capture ability, is also of great importance (Pitt and Ritchie, 2002) and is often associated with foraging mode. Some lizards are known to alter their foraging mode based on the availability of food (Huey and Pianka, 1981). Chemosensory behaviour, reproduction and anti-predatory behaviour may also be affected by foraging mode (Cooper *et al.*, 1997, e.g., Vitt, 1983; du Toit *et al.*, 2002). Huey and Pianka (1981) suggested that the volumes of prey captured per day and prey type are also correlated to foraging mode. In dietary terms, active foragers and sit-and-wait foragers are expected to encounter different amounts of sedentary and mobile prey types. Although there is some overlap in the taxa consumed by the two foraging modes, sit-and-wait foragers consume relatively larger amounts (proportionately and volumetrically) of active prey (e.g., grasshoppers and

beetles) than active foragers, which consume relatively more sedentary prey (e.g., termites and larvae; McBrayer, 2004).
Optimal foraging theory also predicts that when food is more abundant, a more generalist diet may be expected (Miranda and Andrade, 2003).

Diet can be expected to broaden, or narrow, seasonally as food abundance changes with environmental factors (Durtsche, 1995; van Wyk, 2000). Availability, quality and predictability of food can also be inferred from factors such as latitude, temperature, rainfall, aridity and the productivity of the habitat type (Cruz-Neto and Bozinovic, 2004). Where environmental conditions are not stable, other factors that affect prey, such as vegetation, may also be more heterogeneous and thus affect the distribution of prey (e.g., Pitt and Ritchie, 2002). Eifler and Eifler (1999) found that the Grand Skink, *Oligosoma grande*, modified both its diet and foraging behaviour in response to changes in prey distribution. Pitt and Ritchie (2002) indicate that the distribution of prey can also affect the rate of food consumption, which, in turn, can be limited by the availability of food, the digestive capabilities of the animal, or appetite (Alexander and Brooks, 1999). Heterogeneity of the environment and seasonal variations in environmental factors can also affect the type of prey available. Prey type, mass, shape and firmness of the exoskeleton affect feeding behaviour and digestibility, which may, in turn, affect the expenditure of energy above resting levels (Cruz-Neto *et al.*, 2001).

Once food has been consumed, mechanical and chemical means make the products of digestion available for assimilation (Prosser and DeVillez, 1991). These nutrients are then channelled either into metabolic pathways, or into storage. Reliance on stored energy ultimately relates to the costs and benefits of different schedules of acquiring energy and energy expenditure (Doughty and Shine, 1998). For an ectotherm, energy flux is significantly impacted by body temperature (T_b; Stevenson *et al.*, 1985). Rates of both metabolism and digestion are temperature dependent; as T_b increases or decreases, metabolism and digestion respond accordingly (Greenwald and Kanter, 1979; Naulleau, 1983; Lillywhite, 1987). Two related components of

digestion that have been found to be influenced by T_b are gut passage time (GP) and efficiencies of energy acquisition.

Gut passage time is the time taken for any given meal to pass through the digestive system of an animal. Du *et al.* (2000) noted three general patterns of the thermal dependence of GP in lizards: 1) GP decreases with increasing temperature, 2) GP decreases with increasing temperatures at low temperatures and levels out at high temperatures, and 3) GP decreases with increasing temperatures at low temperatures and increases at high temperatures. Food type may also influence GP. *Klauberina riversiana*, an omnivorous lizard, was found to have a GP that was twice as fast when maintained on a diet of mealworms than when maintained on apple (Johnson and Lillywhite, 1979).

The efficiencies of energy intake are commonly expressed as digestive efficiency (DE) and assimilation efficiency (AE). Technically, DE and AE are calculated from the measured energy content of the ingested meal, faeces and excreted nitrogenous waste. DE represents the relative percent of ingested energy that is absorbed across the gut wall, quantified as food energy minus faecal energy divided by food energy. AE represents the percent of ingested energy that is absorbed and which is available for metabolism following the subtraction of absorbed energy that is lost through excretion of nitrogenous waste (ammonia, urea and/or uric acid) and it is quantified as food energy minus faecal and excreted energy divided by food energy (see Table 5.1 for equations). It should, however, be noted that it is not technically possible to measure actual DE and AE because faeces include other components that did not originate from the meal (Mitchell, 1964), including bacteria, sloughed intestinal cells, and bilirubin. The energy content of faeces therefore overestimates the energy of undigested food such that the calculated efficiencies are inherently an underestimation of the actual DE and AE. Thus, the measured DE and AE are thus better termed the “Apparent Digestive Efficiency” (ADE) and “Apparent Assimilation Efficiency” (AAE) (McKinon and Alexander, 1999).

Table 5.1: Processes, symbols and definitions used to calculate digestive and assimilation efficiencies (adapted from Johnson and Lillywhite, 1979).

Process	Symbol	Definition
Consumption	C	Energy consumed
Faeces	F	Energy of faecal waste
Urates	U	Energy of urinary waste
Egesta	F + U	Energy of both faecal and urinary wastes
Digestive energy	C – F	Energy absorbed through gut
Digestive efficiency	(C – F) / C * 100	Energy absorbed through gut (%)
Assimilation	C – (F + U)	Usable energy retained by the animal
Assimilation efficiency	(C – (F + U)) / C * 100	Usable energy retained by the animal (%)

Digestive efficiency of most lizards and snakes generally approaches or exceeds 90 % (Lillywhite, 1987), but may vary according to the type of food consumed (Slade *et al.*, 1994). The lizard *Klauberina riversiana* has an ADE approximately 5% greater when fed on mealworms, than when fed on apple (Johnson and Lillywhite, 1979). Bedford and Christian (2000), however, suggest that carnivorous reptiles usually have a higher efficiency than herbivores, but this difference may be due to the inability to chew thoroughly and the absence of cellulolytic intestinal microflora (Johnson and Lillywhite, 1979).

This study attempts to quantify energy gains in the *P. m. melanotus*. I have thus estimated the rate of energy acquisition by measuring rates of prey capture and average energy content of food. I have then measured the portion of the captured energy that the lizards are able to utilize by measuring the ADE and AAE of lizards consuming meals under typical temperature regimes. I also quantified the ability of the lizards to process food by measuring the GP and appetite of lizards across the temperature range they are likely to experience in the field. Previous studies (Cooper *et al.*, 1997; McConnachie and Whiting, 2003) demonstrate that *P. m. melanotus* is an extreme sit and wait forager, and study of the relative energy assimilated from meals by this lizard is thus useful in elucidating the relationship between energy assimilation and feeding strategy.

5.3) Materials and methods

5.3.1) Feeding frequency

Feeding frequency and prey capture rates were assessed during summer using focal animal sampling in the field at Suikerbosrand Nature Reserve. Individual lizards were observed continuously for 30 min periods after being randomly encountered in the field. Strict site fidelity, vivid colour patterns and significant colour variation between individuals allowed me to determine if any given lizards had previously been observed. If there was any uncertainty as to whether a lizard had been previously observed, it was not included in any further focal sampling. One to four focals were conducted per lizard encountered. The number of prey capture attempts (successful and unsuccessful) was recorded for each lizard during each focal period.

In total, 122 focals were recorded for 93 individual lizards (53 male and 39 female). Not all lizards were observed for the whole 30 min focal duration since lizards were occasionally obscured from view during a focal observation by rocks or grass tufts. The observation time per lizard ranged between two and 120 min and the total, overall observation time was 51 h 55 min with a mean time of 33.86 ± 2.30 min spent on each lizard. All focals were conducted during the normal activity period of the species, which ranged between 07h00 and 18h00.

5.3.2) Prey type

Prey type was examined in three ways. First, the type of prey consumed during focal animal sampling (see above) was recorded where possible, and, while in the field, if a lizard was observed feeding outside focal periods, the prey type was identified to order or, where possible, to family.

The second method involved the examination of museum specimens. Specimens previously collected from the Suikerbosrand Nature Reserve (n = 31) and from the area surrounding Ermelo (n = 10; the nearest population of *P. m. melanotus* to the Suikerbosrand population) were examined. Stomachs were removed from the preserved lizards and placed in individual vials of absolute alcohol. Each stomach was then cut open and the contents examined under a dissecting microscope. Any

identifiable prey item (whole or partial) found in the stomach was classified, at least, to order and, where possible, to family.

The third method involved the analysis of faeces collected in the field from Suikerbosrand Nature Reserve (n = 24 faecal pellets). While walking in the reserve during focal animal sampling, faeces were collected from basking sites on rocks where *P. m. melanotus* were known to occur. Often lizards were observed on rocks and faeces collected from in and around the individual lizards' retreat. Occasionally, lizards were observed defecating, and their faeces collected immediately. Since lizards are unable to digest chiton, faeces often contained almost complete exoskeletons. Faecal samples were individually broken up and any identifiable food item was classified to order, and, where possible, to family.

5.3.3) Digestive physiology

Methods used were similar to those used by Johnson and Lillywhite (1979). Twenty lizards (10 male, 10 female) were housed individually in 0.3 x 0.3 x 0.5 m glass terraria. Each was provided with a shelter as a retreat and water (supplied *ad libitum*). Lizards were maintained on a diet of mealworm larvae (*Tenebrio* sp.) in a temperature-controlled room (± 1 ºC) with a light dark cycle of 12:12. ADE and AAE, GP (used as an indirect measure digestive rate) and appetite were measured at 20, 22, 25, 30 32 and 35 ºC. The sequence of trials was randomised. There was a minimum period of seven days between trials during which lizards were maintained at room temperature (20 - 28 ºC) and given unlimited amounts of food and water. I assumed that the inter-trial period removed the effects of the previous feeding trial.

During each trial, lizards were offered approximately 1 g of mealworms per day for 14 days. Uneaten mealworms were collected, counted and weighed after 24 hours. Gut passage time was measured by force-feeding lizards small glass beads on days one and 14 of each trial, and recording the number of days taken for the beads to appear in the lizards' faeces. Blue and white beads were used at the start and end of trials, respectively, as these colours were easily visible in the faeces. Lizards were offered mealworms *ad libitum* after day 14 until white beads were passed. Gut

passage time was taken as the number of days between consumption and defecation of the beads. Two measures of GP were taken per lizard since beads were used to indicate the start and end of trials – lizards were fed *ad libitum* before and after trials, therefore beads marked faeces from meals consumed between bead markers. Appetite was measured in two ways: as the total mass and the number of mealworms consumed in the 14-day trial.

The trial at the highest temperature (35 ºC) was terminated after three days because the lizards showed obvious signs of distress and it was evident that their appetite was considerably reduced. Data collected during this trial were used only for appetite measures and were made comparable to the data collected during the other trials by increasing measures proportionately (i.e., measures were multiplied by 4.7).

Food consumption was determined by subtracting the mass of the uneaten mealworms from the total mass of mealworms offered to each lizard. The mass of the uneaten mealworms was adjusted to compensate for weight loss that the mealworms experienced due to dehydration during trials. Since this weight loss was dependent largely on the temperature regime, I kept a sample of 10 mealworms in an empty terrarium (conditions the same, only without a lizard) during each trial and reweighed these mealworms after 24 hr to estimate rates of weight loss at each experimental temperature.

All faeces and urates egested during the feeding period (14 days) were collected and separated daily. Urates were separated because it is considered metabolic waste rather than faeces. Faeces and urates were dried in an oven at 50 ºC until reaching constant mass. The faeces and urates were then milled for a minimum of 30 s in a high speed, water cooled mill (IKA® type A10; 20 000 revolutions per min) and the energetic content was measured by subjecting 0.5 – 0.7 g samples to bomb calorimetry (Digital Data Systems CP500 Calorimetry Systems, Johannesburg, South Africa). Energy content of mealworms was measured in the same manner. Mean ADE and AAE for each temperature was calculated for males and females using the equations shown in Table 5.1. Unfortunately, the small amounts of faeces and urates

produced by the lizards necessitated pooling of samples for measurements of energy content (minimum requirement for bombing if approximately 0.5 g per sample). ADE and AAE values were used to calculate the net energy absorbed through the gut and the net usable energy retained by the animal for each temperature respectively.

Gut passage time and appetite were compared between males and females, and among temperatures using a one way repeated measures ANOVA. Since GP and appetite were not significantly different between the sexes, data for males and females were pooled. The effects of body mass and temperature on GP and appetite, and the effects of temperature on ADE and AAE were investigated using regression analysis.

5.4) Results

5.4.1) Feeding frequency

Twenty prey capture attempts were observed during focal animal sampling. Seven were successful captures, five were unsuccessful and the success of eight attempts was indeterminate. Of the successful captures, two were grasshoppers, which the lizards did not consume. Assuming the lizards are active for 9 h during the day, this equates to a prey capture attempt every 2 h 33 min, of which at least 35 % are successful. This means that *P. m. melanotus*, on average, capture 1.34 prey items per day.

5.4.2) Prey type

Only three prey captures were observed outside focal sampling periods where the type of prey could be identified. In two cases, lizards had successfully captured and were consuming scarab beetles. In the third case, a lizard was observed with a large chilopod in its mouth, which it was attempting to eat.

The contents of five stomachs were excluded from the analysis as they were obviously from lizards that had been kept in captivity prior to being preserved, and contained fly larvae, *Tenebrio* beetles and canned dog food. Therefore, only 36 lizard stomachs were used in the analysis. Approximately half (52.8 %) of the stomachs

examined were empty and 44.4 % contained nematode parasites, which contained 7.38 ± 2.35 worms. Stomach content showed the lizards had a preference for beetles, bees, wasps and ants, but contained various other invertebrate prey items (Table 5.2).

Analysis of faecal samples also showed the lizards preference for beetles, in particular those of the family Scarabaeidae. Of the 24 faecal pellets collected from in and around lizard crevices, 100 % contained the remains of coleopterans. The faeces did, however, also contain the remains of various other invertebrate prey (Table 5.2). The absence of some groups of prey items could be due to the general body structure of animals in those groups, particularly those that are soft-bodied. The lizards may be better able to chew and/or digest inverts from these groups, thus making them less obvious, or unrecognisable in faecal samples.

Table 5.2: Frequency of prey items observed in stomach contents and faeces of *Pseudocordylus melanotus melanotus*.

Stomach contents (n = 36)		**Faecal analysis (n = 24)**		
Order	*%*	*Order*	*Family*	*%*
Blattodea	2.8			
Coleoptera	30.6	Coleoptera		100
Dermaptera	2.8		Scarabaeidae	70.8
Diptera	2.8		Curculionidae	16.7
Hemiptera	2.8		Tenebrionidae	12.5
Hymenoptera	13.9	Hymenoptera		4.2
Isoptera	5.6	Isoptera		4.2
Lepidoptera	5.6	Lepidoptera		16.7
Orthoptera	8.3	Orthoptera		4.2
Diplopoda	2.8	Diplodpoda		4.2
Nematoda	44.4			
Empty	52.8			

5.4.3) Digestive physiology

5.4.3.1) Gut passage time

Gut passage time was determined by the first appearance of blue and white beads at the beginning and end of trials. Defecation of the beads was usually staggered over two droppings. GP was not significantly different between males and females at different temperatures ($F_{9, 85} = 1.39$, $P = 0.21$), and decreased significantly with increasing temperature ($R^2 = 0.92$, $P = 0.01$; Figure 5.1). Mass effects on GP were

only evident at 22 ºC, where larger lizards showed shorter passage times than did smaller lizards ($R^2 = 0.27$, P = 0.02; Figure 5.2), but the low R^2 indicates that, even though the relationship is significant, only a small fraction of variation in GP is explained by body mass.

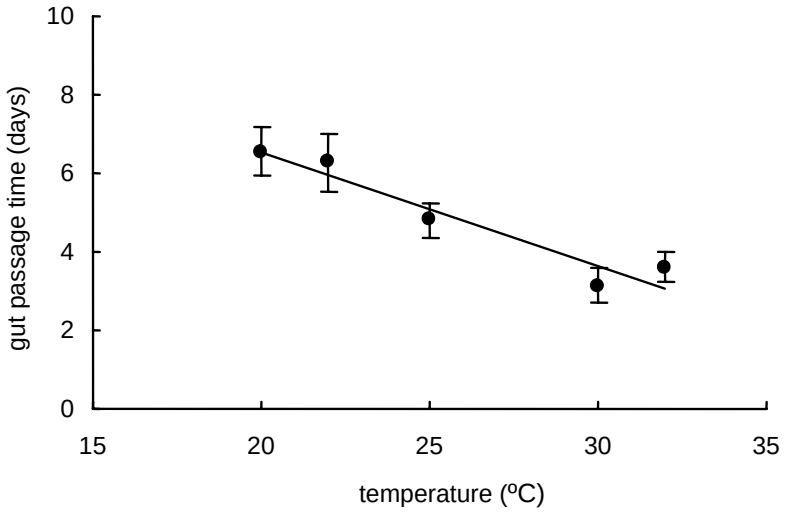


Figure 5.1: The effect of temperature on gut passage time for *Pseudocordylus melanotus melanotus* (Mean ± SE; N = 20, 10 ♂ 10♀ for all temperature treatments; regression analysis y = -0.29x + 12.29, $R^2 = 0.92$, P = 0.01).

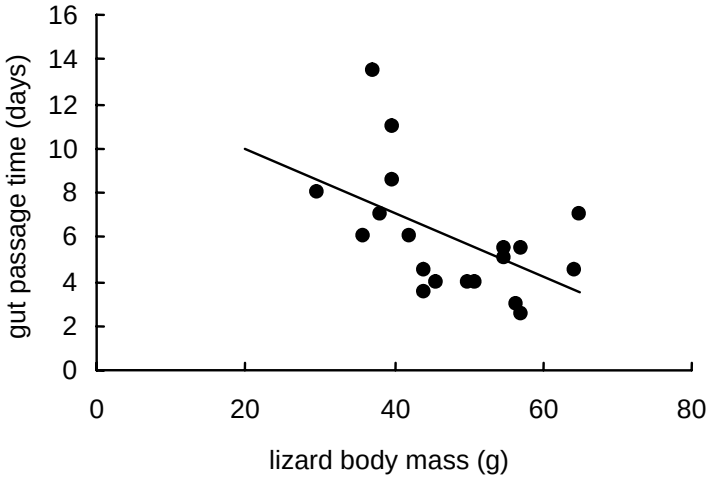


Figure 5.2: The effect of body mass on gut passage time for *Pseudocordylus melanotus melanotus* at 22 ºC (N = 20, 10 ♂ 10♀; regression analysis y = -0.14x + 12.79, $R^2 = 0.27$, P = 0.02). This was the only trial where body mass had a significant effect on gut passage time.

5.4.3.2) Appetite

There were significant differences in appetite at different temperatures, measured as either number or mass of mealworms eaten (number: $F_{9, 89} = 9.52$, $P << 0.001$; mass: $F_{9, 89} = 9.15$, $P << 0.001$). Regression analysis revealed that both number and mass of mealworms eaten increases significantly with increasing temperature (number: $R^2 = 0.91$, $P = 0.01$; mass: $R^2 = 0.89$, $P = 0.02$; Figure 5.3). Lizard body mass effects were evident for both number and mass of mealworms at 25 ºC, where larger lizards ate proportionally more than smaller lizards ($R^2 = 0.27$, $P = 0.02$, Figure 5. 4). The low R^2 value, however, suggests that although significant, the effect of body mass is small.

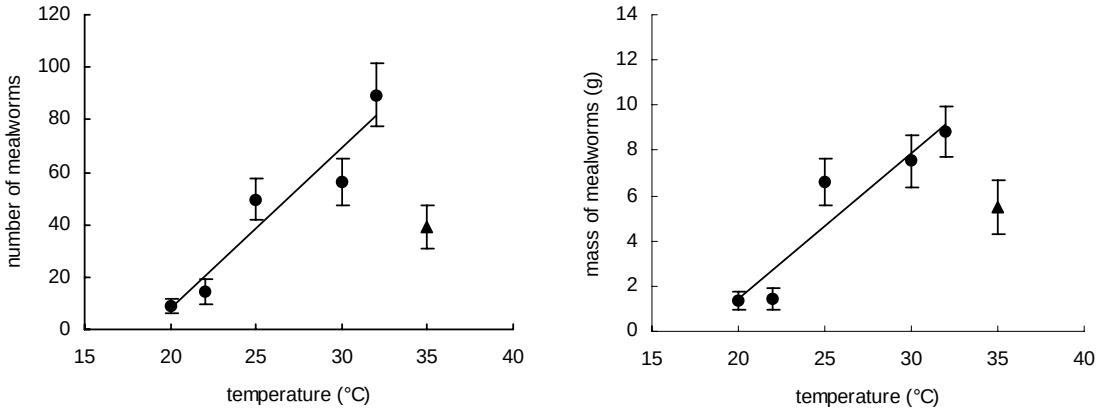


Figure 5.3: The effect of temperature on appetite in terms of the number of mealworms eaten (left), and of temperature on the mass of mealworms eaten (right) for *Pseudocordylus melanotus melanotus* (regression analysis; number: $y = 6.15x – 114.86$, $R^2 = 0.91$, $P = 0.01$; mass: $y = 0.65x - 11.55$, $R^2 = 0.89$, $P = 0.02$; Mean ± SE; N = 20, 10 ♂ 10♀ for all temperature treatments). Measurements at 35 ºC (▲) were not included in the regression analysis because lizards showed distress and the trial was aborted after only three days.

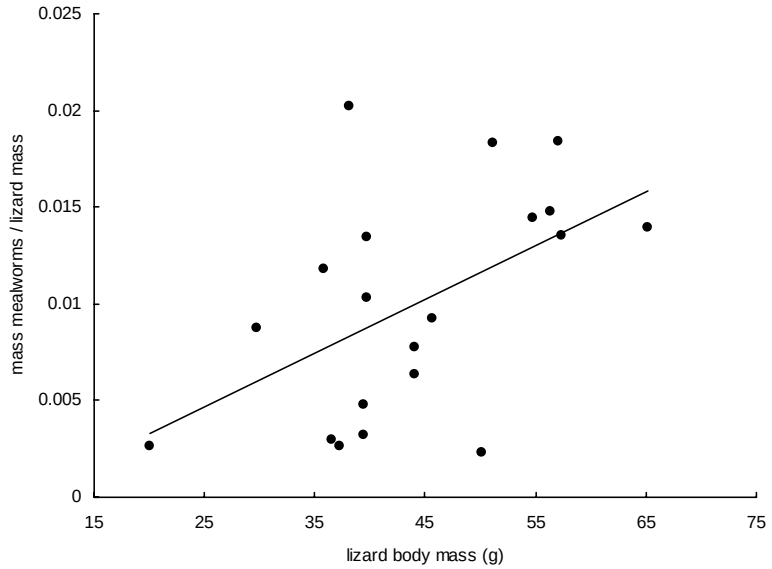


Figure 5.4: The effect of body mass on daily food consumption (proportional to lizard body mass) at 25 ºC (regression analysis y = 0.0003x – 0.0023, $R^2 = 0.27$, *P* = 0.02; N = 20, 10 ♂ 10♀). This was the only trial where body mass had a significant effect on food consumption.

5.4.3.3) Digestive and assimilation efficiency

The mean (± SE) energy contents, per gram dry mass, for mealworms, faeces and urates were 24.8 ± 0.05, 15.2 ± 0.20 and 10.7 ± 0.04 kJ/g respectively. The wet mass energy content of mealworms was 9.329 kJ/g; wet mass energy content of faeces and urates could not be calculated because they were collected at varying intervals after lizards had defecated and most were already partially dried out. I found no significant effect of temperature on either ADE or AAE (ADE: $R^2 = 0.05$, P = 0.72; AAE: $R^2 = 0.18$, P = 0.48; Figure 5.5). For lizards consuming mealworms, ADE averaged 94.4 ± 0.3 % and AAE averaged 87.2 ± 0.6 %.

5.4.3.4) Net energy gain

Both net energy absorbed (NEA) and retained (NER) increased significantly with increasing temperature (NEA: $R^2 = 0.89$, P = 0.02; NER: $R^2 = 0.90$, P = 0.01; Figure 5.6). This was expected because an increase in energy gain with increasing temperature is simply a function of lizards eating more at higher temperatures, since neither AAE nor ADE were affected by temperature.

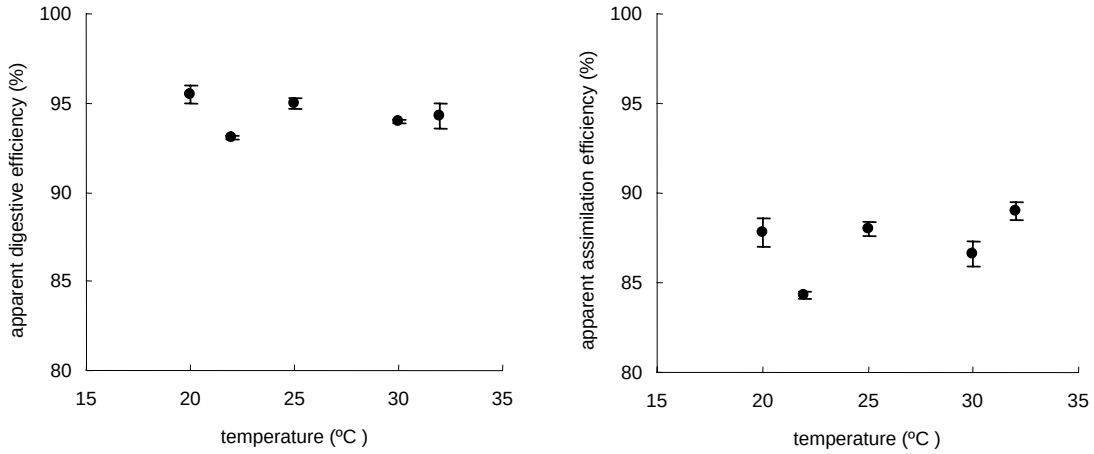


Figure 5.5: The effect of temperature on apparent digestive efficiency (left), and of temperature on apparent assimilation efficiency (right) for *Pseudocordylus melanotus melanotus* (Mean ± SE, n = 20, 10 ♂ 10♀ for all temperature treatments).

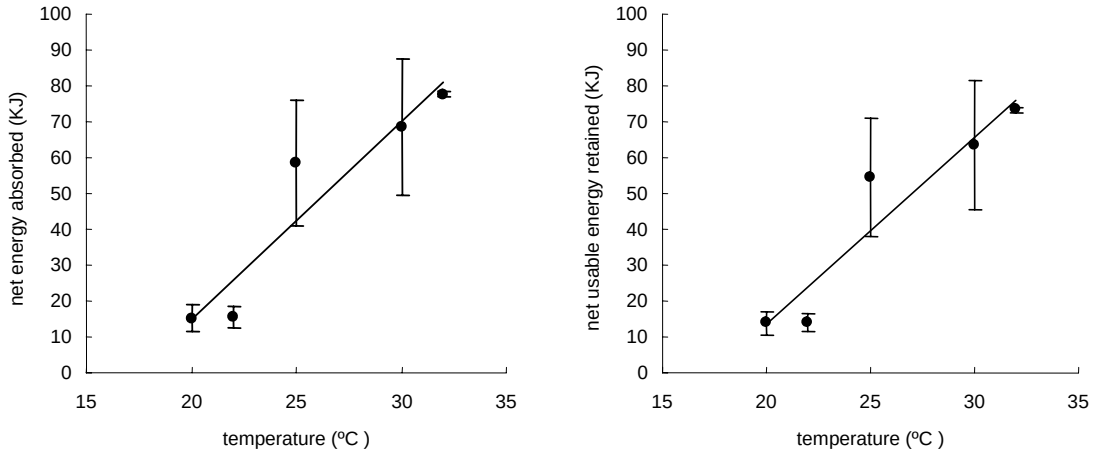


Figure 5.6: The effect of temperature on the net energy absorbed through the gut (NEA, calculated from apparent digestive efficiency) (left), and of temperature on the net usable energy retained (NER, calculated from apparent assimilation efficiency) for *Pseudocordylus melanotus melanotus* (regression analysis; NEA: y = 5.50x – 94.70, $R^2 = 0.89$, $P = 0.02$; NER: y = 5.19x – 90.02, $R^2 = 0.90$, $P = 0.01$; Mean ± SE; N = 20, 10 ♂ 10♀ for all temperature treatments).

5.5) Discussion

Under natural conditions, *P. m. melanotus* consume a wide variety of invertebrate prey. The digestive physiology of *P. m. melanotus* is highly temperature dependent in certain respects (i.e., rate of digestion and appetite), but is remarkably temperature independent in other respects (the general efficiency of energy extraction from a meal). I found that GP decreased with increasing temperature and was generally not

affected by lizard body mass (only one exception where there was a small mass effect at 22 ºC, where larger lizards had a marginally shorter GP). Neither ADE nor AAE were affected by temperature over the temperature range tested. Appetite increased significantly and dramatically with increasing temperature, although data collected during the first three days of an aborted trial at 35 °C indicates that appetite is also suppressed at high temperatures. I detected a small body mass effect on appetite for the 25 °C trial only, where larger lizards consumed a relatively larger amount of food. The thermal dependency of gut throughput rate results in the net energy absorbed through the gut and net usable energy retained also increasing with temperature under conditions where food availability is not limiting.

5.5.1) Feeding and prey

Foraging behaviour of *P. m. melanotus* was similar to that described by Cooper *et al.* (1997) for other cordylid species. The lizards usually sit motionless on top of rocks and rushed from their perch sites to capture prey before returning to the rock to resume visual search. Moving insects and other movements attracted the lizards' attention, which was indicated by the lizards tilting their heads and/or changing body position for a better view.

Finding, capturing and ingesting prey requires an expenditure of energy over and above that required for maintenance, and more food is required to achieve energy balance when an animal works harder to get food (Nagy *et al.*, 1984). The costs of prey searching in sit-and-wait foraging may be considered negligible, and prey handling and ingestion are least expensive in terms of feeding activity because of the cost of digestion (Nagy *et al.*, 1984; Cruz-Neto *et al.*, 2001). On this basis, a sit-and-wait foraging lizard should eat all potential prey encountered.

Pseudocordylus m. melanotus apparently attempt to capture any suitable prey item that is within a range which is energetically beneficial for the lizard (i.e., a prey item that results in energy gain which is greater than the energetic cost of capturing it). The low success rate in prey capture attempts may therefore not be costly in terms of the total energy expenditure. Nagy (2001) predicted that a typical 1 kg insectivorous

lizard required 18 g of food per day ("fresh matter intake"). This equates to approximately 0.5 g of food per day for a 30 g *P. m. melanotus*. A 30 g *P. m. melanotus* requires 2943.24 J/day for maintenance metabolism (Chapter 4), which equates to 0.36 g of mealworms per day (taking the measured AAE of 87.2 % into account). It should, however, be noted that this energy requirement is the lowest expected metabolic expenditure without taking the effects of activity into account. Field metabolic rates can be two to three times greater than standard metabolic rates measured in the laboratory. Bennett and Nagy (1977), for example, found that the lizard *Sceloporus occidentalis* had a field metabolic rate 2.5 times greater than that measured in the laboratory. *Pseudocordylus m. melanotus* would thus require the equivalent of approximately 1g of mealworms to meet the requirements of activity in the field. Given that adult crickets and mealworm beetles have energy equivalents between eight and nine KJ/g wet mass, 1.5 g of natural prey would likely cover their daily energy expenditure (Stephen Secor, pers. comm.). Anything over and above this can be used for activity, growth, reproduction and storage. A lizard capturing one or two medium sized beetles per day should therefore easily meet this small daily food requirement. A prey capture success rate of 35 % is therefore not as bad as it initially appears since a lizard is likely to attempt three or four prey captures when it is active for 9 hours.

Feeding frequency is also related to the availability of prey. If individuals alternate between environments of high and low prey availability, and therefore between feast and famine, there may be individuals in the population that have empty stomachs (Huey *et al.*, 2001). These individuals will inevitably be relying on stored energy resources, at least for their for maintenance requirements (Huey *et al.*, 2001). The proportion of lizards in a population with empty stomachs is highly variable (see Huey *et al.*, 2001; du Toit *et al.*, 2002; Clemann *et al.*, 2004; Pleguezuelos and Fahd, 2004; Van Sluys *et al.*, 2004; Greenville and Dickman, 2005), and in many studies is not reported. Huey *et al.* (2001) report that the association between the percentage of empty stomachs and foraging mode is inconsistent. Of the 41 *Pseudocordylus m. melanotus* stomachs that I examined, 52.8 % were empty. This is in line with that found by Mouton *et al.* (2000) for *C. cataphractus*, which is also a sit-and-wait

foraging cordylid lizard. Of 122 *Cordylus cataphractus* examined, 54.8 % had empty stomachs (Mouton *et al.*, 2000).

The prey consumed by *P. m. melanotus* are what I expected for a sit-and-wait forager; they feed predominantly on arthropods, especially Coleoptera, which included the families Scarabaeidae, Curculionidae and Tenebrionidae. These results are also comparable to those found for *Cordylus giganteus*, which also feeds predominantly on these coleopteran families (van Wyk, 2000). van Wyk (2000) reported a seasonal variation in the diet of *C. giganteus*, and that they fed on scarabs, curculionids and tenebrionids throughout the year. It can thus be expected that these would also be the predominant food source for *P. m. melanotus* year round, and that the remainder of the diet would fluctuate through the seasons with changes in abundance of other prey species.

An interesting inclusion in the diet of *P. m. melanotus* and a few other cordylid species (e.g., *Cordylus regius, C. giganteus, C. cataphractus*; Branch, 1998; Mouton *et al.*, 2000; van Wyk, 2000) are myriapods (millipedes and centipedes). This group of arthropods are known to produce toxins and other lizard species reportedly avoid them (Vitt and Cooper, 1986; Wapstra and Swain, 1996). Mouton *et al.* (2000) and van Wyk (2000) suggest that sit-and-wait foragers' lack of chemical prey discrimination may explain the presence of millipedes and centipedes in lizard diets. This is supported by Reaney and Whiting (2002) who reported millipedes in the guts of the Tree Agama, *Acanthocercus atricollis atricollis*, which is also a sit-and-wait forager. In addition, sit-and-wait foragers generally rush out at visually detected prey without preliminary chemical testing (du Toit *et al.*, 2002), which could lead to lizards capturing what could be viewed as unexpected or distasteful potential prey. I also observed two lizards capturing grasshoppers, and then rejecting them as food. The grasshoppers were later identified as belonging to the pyrgomorphidae, *Ochrophlebia ligneola*, which are known to feed on poisonous plants (Roger Price, pers. comm.). These brightly coloured grasshoppers, which were abundant at Suikerbosrand Nature Reserve during the field study (pers. obs.), are likely to be distasteful.

The presence of endoparasitic nematodes in the gut of lizards has rarely been reported. Whether this is because there are no nematodes, or that they are just not reported is unclear. It is apparent, however, that where diet is investigated, nematodes are not reported as they are not actually eaten by the species in question. In this study, 44.4 % of the stomachs examined contained nematode parasites, with 7-8 nematodes per stomach. Unfortunately, other studies on cordylid diets do not report on nematode presence, and neither De Waal (1978), nor Jacobsen (1989), reported the presence of nematodes in the guts of *P. m. melanotus*. This is an area of study of gut content and diet that deserves further attention because nematodes have already been implicated in affecting digestive processes and also reduce gut volume, thereby restricting appetite and potentially the amount of energy gained.

5.5.2) Passage rate and appetite

The reduction in GP, or increased digestive rate at higher temperatures, follows the pattern typical for most reptiles (Greenwald and Kanter, 1979), and has been reported for the lizards *Lacerta vivipara* (Van Damme *et al.*, 1991), *Sceloporus merriami* (Beaupre *et al.*, 1993b), *Eumeces elegans* (Du *et al.*, 2000), *Platysaurus intermedius wilhelmi* (Alexander *et al.,* 2001), and the snakes *Elaphe guttata* (Greenwald and Kanter, 1979), *Vipera aspis* (Naulleau, 1983) and various Australian pythons (Bedford and Christian, 2000). The shorter passage time may also contribute to the improved appetites at higher temperatures (Angilletta, 2001b), as the higher throughput at higher temperatures simply allows for more food to be processed. Passage time could thus be the proximal limiting factor of consumption at low temperatures (Angilletta, 2001b) and could affect consumption directly through appetite modulation under conditions where food availability is not limiting. Field measures for *P. m. melanotus*, however, indicate low rates of ingestion (McConnachie and Whiting, 2003) and suggest that food availability, rather than appetite, is likely to be the most important limitation on consumption under natural conditions.

The decrease in gut passage time with increasing temperature may explain the temperature independence of ADE and AAE in *P. m. melanotus*. At lower temperatures, digestion and absorption occurs at slower rates, and the passage of the meal is at a pace that matches its digestion and absorption. Thus, slower rates of extraction are compensated for by slower throughput, enabling the lizards to fully digest their meals at low temperatures. This digestive compensation at low temperatures is similar to digestive compensation to low food quality as reported by Hume (1989) for herbivores eating poor quality forage, and are also in agreement with Wang *et al*.'s (2003) and Secor and Faulkner's (2002) findings of the temperature independence of specific dynamic action.

Naulleau (1983) predicted that digestion would actually cease altogether at extreme temperatures, but that food would still be passed in order to prevent putrification in the gut. Digestion at these extreme temperatures, however, is not usually tested in most studies since appetite is greatly reduced at extreme temperatures and there are thus practical problems of inducing the animals to feed. Even so, the increased passage time at higher temperatures that has been reported in some studies supports the hypothesis. In the lizard *E. elegans*, passage time decreases with increasing temperature but then increases above 34 °C (Du *et al.*, 2000). Stevenson *et al.* (1985) noted that, in *T. e. vagrans*, passage time decreased, levelled, and then increased at 35 °C. In my study, the reduced appetite during the aborted 35 °C trial, suggests that passage time might also be decreased at this 'extreme' temperature. Further evidence also comes from the observation that some of the lizards in the 35 °C trial passed almost complete, undigested mealworms or regurgitated mealworms whole.

Various methods have been used to measure gut passage time, the most simple of which is fasting before trials (Alexander *et al.*, 2001), where the first appearance of faeces after starvation indicates the gut passage time. This is, however, not always a suitable method. A down-regulation of metabolic physiology may occur while fasting (Secor and Diamond, 2000) and once the animal is fed, it may take longer to pass through the gut because of extra time taken for up-regulation before digestion can begin. A number of recent studies (Overgaard *et al.*, 2002, Starck and Beese,

2001, 2002, Holmberg *et al.*, 2003), however, indicate that the reptilian gut may not be as down-regulated during fasting as originally proposed by Secor and Diamond (e.g. 1995, 2000). There is a distinct possibility that *P. m. melanotus* is a down regulator since observations of free ranging individuals suggest that it is an infrequent feeder; McConnachie and Whiting (2003) reported only two attempts at prey capture in 795 minutes of focal animal analysis. Cooper *et al.* (1997) also report ambush foraging to be the typical mode of foraging in this clade. Thus, fasting before trials would certainly be an inappropriate method for measuring GP in *Pseudocordylus* since the gut of a fasted lizard may require some start-up time.

Dyes, such as powdered florescent dyes, have also been used as markers, with or without small, indigestible objects such as short pieces of fishing gut (Beaupre *et al.*, 1993b, Walschmidt *et al.*, 1986). The dye is usually introduced into the food and in the case of insect prey, it is injected through the exoskeleton of the insect before it is offered as food (e.g., Beaupre *et al.*, 1993b). The use of dyes was not practical in my study, since this would have necessitated the injection of dye into 250 mealworms per day at the start and termination of each trial. Also, the rupture of the mealworms' exoskeleton resulting from administering the dye would likely have changed rates of dehydration of the mealworms during trials, ultimately resulting in inaccurate measures of consumption. It should be noted, however, that different techniques used in GP studies may yield different GP measures, depending on the digestibility of the material used as a marker.

It could also be argued that the use of beads as markers for GP might have affected my measures of GP and appetite since the indigestible beads occupy a portion of the volume of the stomach and the texture of the bead surface influence passage time in some way. The long duration of trials (14 days) is likely to reduce this overall effect, and since my comparisons were relative (same experimental setup, only temperature was changed), any effect would have little affect on my conclusions. The fact that the lizards also fed freely throughout the trial periods suggests that the effect of the beads was minimal.

5.5.3) Energy efficiencies

Previously measured ADEs in lizards range between 30 % (Ruppert, 1980) and 93 % (Johnson and Lillywhite, 1979). My measure of 94 % for ADE in *P. m. melanotus* thus slightly extends this range. ADEs of insectivores are expected to be in the higher part of the range. Johnson and Lillywhite (1979) recorded an ADE of 93% in *K. riversiana* fed entirely on mealworms (the same diet that I used for the lizards). Many other lizards have similar ADEs when maintained on an exclusively insectivorous diet: *Uta stansburiana* (Waldschmidt *et al.*, 1986); *S. merriami* (Beaupre *et al.*, 1993b) and *Hemidactylus turcicus* (Slade *et al.*, 1994).

Although I did not detect a temperature effect on ADE in my study, temperature is known to affect ADE in other species. ADE increases with increasing temperature in *E. elegans*, but asymptotes at 26 °C (Du *et al.*, 2000). Du *et al.* (2000), however, found no temperature effect on AAE in this species, which suggests that the lack of temperature effects on AAE are due to temperature effects on urate production (i.e., the loss of energy in the form of urates is included in AAE but not in ADE).

5.5.4) Ecological significance

The digestive physiology of *P. m. melanotus* is well-matched to its feeding strategy and naturally low rates of food intake. The lizard consumes a relatively wide variety of prey, most of which are generally considered active; this was as expected for a sit-and-wait foraging species. Temperature-independence of ADE results in the lizards extracting the maximum possible energy from any ingested meal, regardless of whether or not the lizard is able to maintain T_b in the selected range. Since *P. m. melanotus* has low rates of food consumption under natural conditions (McConnachie and Whiting, 2003), the longer time required for digestion at lower temperatures is unlikely to limit food intake under these conditions, as may well be the case for frequent feeders or for high consumption feeders. Thus, the digestive physiology *P. m. melanotus* appears to suit a food-scarce environment by maximising the energy gain from ingested food.

CHAPTER 6

ENERGY BUDGETS AND DISTRIBUTION LIMITATION

6.1) Introduction

Energy budgets can be used to explain limits to distributions of species because temperature is known to be an important determinant of animal distribution (Prosser and Heath, 1991), and temperature also has a significant effect on the energy requirements of organisms. Ambient temperature affects the level of energy required to regulate body temperature, maintain activity, grow and reproduce, and these requirements may not be met by the energy available in the environment, i.e., the abundance and availability of food (Bozinovic and Rosenmann, 1989), thus limiting the animal's distribution to areas where the energy budget is maintained in positive balance.

In this study I measured the effects of temperature on aspects of an energy budget for *Pseudocordylus melanotus melanotus* such that the minimum energy gains and expenditures could be calculated and the minimum requirements for energy balance could be determined over a range of temperatures. I also measured the thermal requirements of the lizard (in the laboratory and field) with respect to hypotheses generated from two climate envelope models (CLIMEX and a multivariate model). The energy budget and factors limiting distribution can be integrated through the thermal requirements of the lizard, which affect both distribution and the energy budget. In this chapter, I construct an energy budget for *P. m. melanotus* in terms of the requirements for energy balance, energy requirements in summer and winter and annual energy requirements. I also revisit the climate envelope models in light of the thermal biology and habitat requirements of the lizard and relate this to the lizards' requirements for maintaining a positive energy balance.

6.2) Energy budget

Two different approaches were taken to estimate energy balance because both laboratory and field measures of *P. m. melanotus*' thermal requirements were made, and energy expenditure was measured on the laboratory only. Two energy budgets were calculated: 1) a field energy budget, which was based on field measures of lizard body temperature (T_b) and prey capture rates, and using laboratory measures of energy expenditure, and 2) a laboratory energy budget, which was based on laboratory measures of energy expenditure (metabolism) and energy gain (from food consumed) over the range of temperatures used in the laboratory. All values presented in these energy budgets have been adjusted such that they are representative for a standard 30 g lizard (approximate average adult mass for the species). For rates of energy expenditure, the standard metabolic rate (SMR; measured as oxygen consumption; Chapter 4) was used and was assumed to be the minimum energy requirement for a lizard in a rested, awake, fasting and thermoneutral state (conditions laid out by Bligh and Johnson (1973) for measuring SMR). Although my measures may be an underestimation because free-ranging lizards are active and experience increased energy expenditure when feeding (Huey and Pianka, 1981), the energy budgets were calculated using a standardized method. Given the life history of this species (an extreme sit-and-wait forager; Cooper *et al.*, 1997), this increase is likely to be small. *Pseudocordylus m. melanotus* spend less than 0.001 % time moving and move less than 0.05 times per minute (McConnachie and Whiting, 2003), which suggests that these lizards are spending most of the time in a rested state, i.e., at resting MR.

Daily energy budgets were calculated for both summer and winter days based on activity and rates of prey capture in *P. m. melanotus* (Chapters 3 and 4). During focal animal sampling, prey capture attempts were noted approximately every 2.5 h, of which only 35 % were successful (Chapter 5). During summer, lizards were active for approximately 9 h per day, which means there were 3.6 prey capture attempts per day and 1.26 successful prey captures. In winter, lizards were active approximately every second day (72% of days) for about 4.5 h, which equates to 1.8 prey capture

attempts per day of which 0.63 are successful. In other words, lizards will successfully catch a prey item every 1.6 days in winter.

Annual energy budgets were calculated for *P. m. melanotus* based on the summer and winter daily energy budgets and the seasonal activity of the lizard. I assumed that lizards were active every day for half the year (summer) and only 72 % of days in winter for the other half (winter; this would take variable activity between 72 and 100 % during spring and autumn into account).

6.2.1) *Field energy budget*

This energy budget is based on field measures of lizard body temperature. First, a temperature profile was generated for a 'normal' day during summer and winter for the lizard. Body temperature profiles were created using the fourth day of body temperature measures at 20 min intervals for each lizard. A mean temperature profile was created by averaging the T_bs for each interval between 00h00 and 23h40 (Figure 6.1).

Based on the mean T_b profile, the energy expenditure was calculated for the T_b at each of the time intervals. First, the oxygen consumption was calculated for each of the T_bs at each interval using the regression equation for the relationship between T_b and metabolic rate (Chapter 4; $y = 0.21x - 0.41$). Since the lowest temperature at which metabolic rate was measured in the laboratory was 20 ºC, the oxygen consumption was approximated for lower temperatures by calculating the equation for a straight line between the oxygen consumption at 20 ºC and the temperature at which death occurs (i.e., the lower physiological temperature tolerance; -5.15 ºC; Chapter 2). Energy expenditure was thus calculated for T_bs below 20 ºC using the equation $y = 0.12x + 0.62$ (Figure 6.2). These measures were then converted to energy expenditure in Joules for each interval (Figure 6.3; assuming a respiratory quotient of 0.79, where 20.1 kJ of energy is released for every one litre of oxygen consumed; Randall *et al.*, 1997). The daily energy expenditure was calculated by summing the energy expenditure at each of the intervals over each profile to determine the average daily summer and winter energy expenditure.

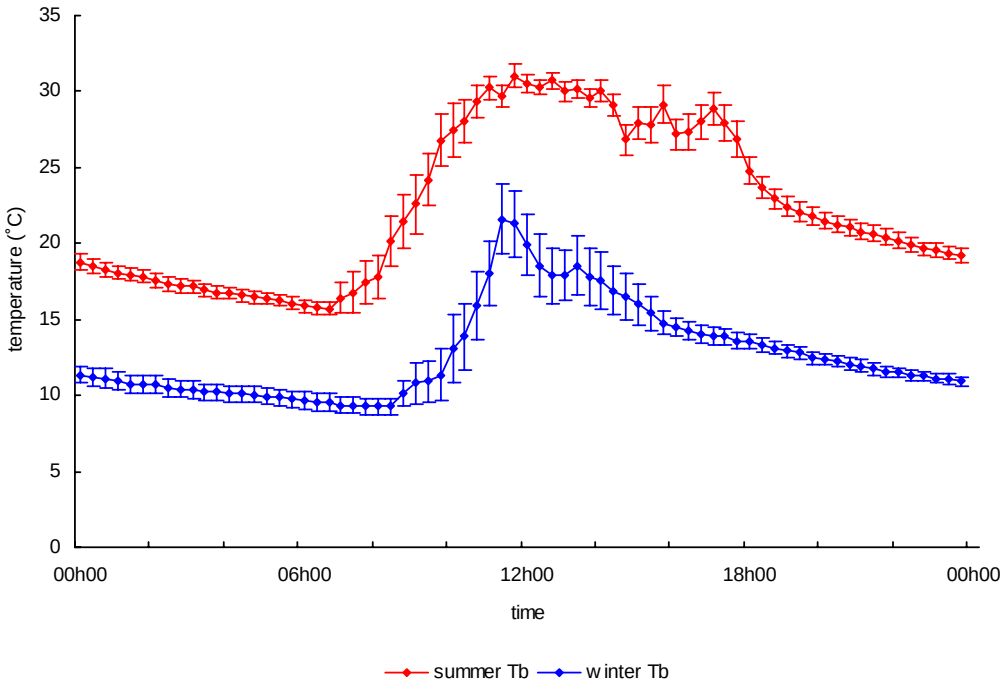


Figure 6.1: Body temperature profiles for a normal summer and winter day for the lizard *Pseudocordylus melanotus melanotus* (means presented ± SE for each 20 min interval).

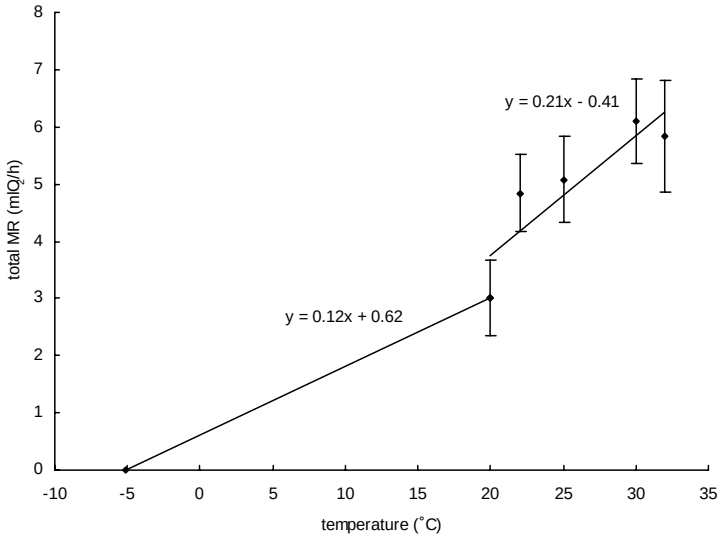


Figure 6.2: The effect of temperature on total metabolic rate (total MR) for a standard 30 g *Pseudocordylus melanotus melanotus* extrapolated to the lower physiological temperature tolerance (-5.15 ºC) from the lowest temperature measured in the laboratory (20 ºC). Lines used to estimate energy expenditure in daily field energy budget.

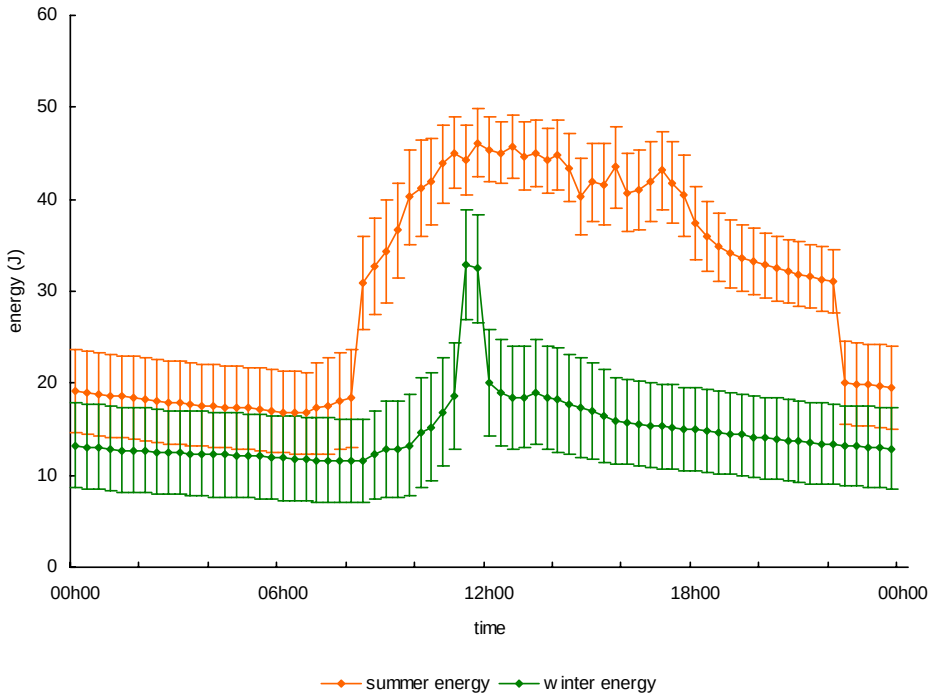


Figure 6.3: Energy expenditure profiles for a normal summer and winter day for the lizard *Pseudocordylus melanotus melanotus* (means presented ± SE for each 20 min interval).

To maintain energy balance in summer, a lizard requires 2188.78 J/day for maintenance metabolism. A lizard that is active for 9 h per day and successfully captures 1.26 prey items per day needs to consume 2510 J of energy per day to maintain a minimum energy expenditure of 2188.78 J, assuming an assimilation efficiency of 87.2 % (Chapter 5; i.e., of the energy consumed, only 87.2 % is assimilated by the lizard). This is equivalent to consumption of approximately 3 mealworms per day (average mealworm mass = 0.1 g; n = 230 weighing 22.78 g).

During winter, the average energy expenditure is 1057.94 J/day, which is approximately half (48 %) that of energy expenditure in summer. A lizard that is active for 4.5 h per day and captures 0.63 prey items per day needs to consume 1213 J of energy per day to maintain a minimum energy expenditure of 1057.78 J, assuming and assimilation efficiency of 87.2 %. This equates to approximately 2 mealworms per day.

To maintain energy balance through the year, a standard 30 g *P. m. melanotus* requires 592.53 kJ. This means that the lizard must catch prey with a minimum

energy content of 679.50 kJ in a year to maintain energy balance. This is equivalent to 73.55 g of mealworms or 735.47 mealworms per year.

6.2.2) *Laboratory energy budget*

This energy budget is based on laboratory measures of energy expenditure and energy gain under constant, controlled temperature regimes. Energy gain was calculated using the mass of mealworms consumed in laboratory experiments (Chapter 4), which was converted to an equivalent mass consumed by a 30 g lizard by dividing the mass consumed by the lizard body mass and multiplying this value by 30. The mass consumed was converted to energy using the energy content of mealworms, where one gram of mealworms contains 9.239 kJ. The total energy consumed was also corrected for AAE (Chapter 5; Table 6.1).

Energy expenditure was calculated using the total metabolic rate for a standard 30 g lizard measured at different temperatures in Chapter 4, which was converted to a mean energetic consumption in Joules per day (Table 6.1; assuming a respiratory quotient of 0.79, where 20.1 kJ of energy is released for every one litre of oxygen consumed; Randall *et al.*, 1997). Energy expenditure and energy gain were regressed against temperature to investigate the effects of temperature on the energy budget and to determine the temperatures at which energy is balanced and where it is positive.

Both energy expenditure and energy gain were significantly affected by temperature such that both increase with increasing temperature (Figure 6.4; expenditure: R^2 = 0.77, P = 0.048; gain: R^2 = 0.90, P = 0.01). However, energy gain increases at a faster rate than does energy expenditure. The temperature at which energy expenditure and energy gain are equal (i.e., where energy is balanced) occurs where the regression lines intersect. In this case, energy balance is achieved at 28.82 ºC. This is in line with the measured selected body temperature (T_{sel}) in the field for *P. m. melanotus* (28.91 ± 0.26 ºC; Chapter 3).

Table 6.1: Variables used to construct the energy budget for the lizard *Pseudocordylus melanotus melanotus*. Total metabolic rate and the mass of mealworms consumed were calculated for a standard 30 g lizard, and were converted to daily energy expenditure and gain, respectively. Energy gain was also corrected for apparent assimilation efficiency. Energy balance indicated as negative (-), approximately neutral (≈) or positive (+).

Temperature (ºC)	Total metabolic rate (mlO_2/h)	Energy expenditure (J/day)*	Mass of mealworms consumed (g/day)	Energy gained (J/day)♦	Energy balance
20	3.01	1452.02	0.88	510.79	-
22	4.84	2333.21	0.93	538.34	-
25	5.08	2450.59	4.20	2439.01	≈
30	6.10	2943.24	4.99	2896.90	≈
32	5.83	2812.39	5.80	3367.60	+

* Values for energy expenditure calculated from total metabolic rate measured in Chapter 4.
♦ Values for energy gained calculated from the mass of mealworms consumed measured in Chapter 5.

In summer, a lizard requires 2702.37 J/day to maintain energy balance, i.e., where energy expenditure is equal to energy gain (at 28.82 ºC, which is approximately the T_{sel}) and assuming one successful prey capture attempt per day. Therefore the energy gained must exceed 2702.37 J/day if the lizard is to remain in positive energy balance. The energy content of the meal must therefore be at least 3099.05 J, which is equivalent to 0.33 g (approximately three) mealworms.

In winter, the T_{sel} of *P. m. melanotus* in the field was 26.30 ± 0.47 ºC (Chapter 3), so, if energy balance is maintained at this T_b, a lizard would require a minimum of 2448.67 J/day. The total energy content of the meal, therefore, should exceed 2808.11 J if the lizard is in positive energy balance. Since *P. m. melanotus* will only have one successful prey capture attempt every 1.6 days in winter, the daily energy requirement from a meal should be 1755.07 J, which equates to 0.19 g, or approximately two, mealworms per day. Any energy gained over and above this ensures a positive energy balance, which may allow the lizards to be more active in winter. Greater levels of activity in winter may also improve chances of encountering prey.

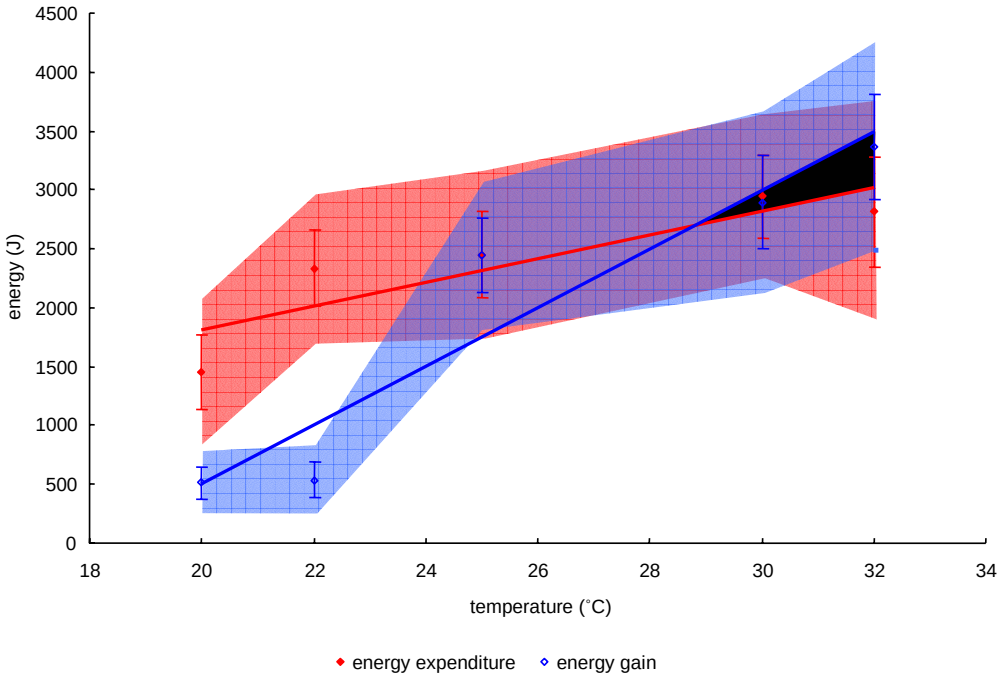


Figure 6.4: Daily energy expenditure and energy gain for a standard 30 g *Pseudocordylus melanotus melanotus* relative to temperature (means presented ± SE; regression analysis; expenditure: $R^2 = 0.77$, P = 0.048, y = 100.67x – 198.95; gain: $R^2 = 0.90$, P = 0.01, y = 248.92x – 4471.5). Shaded red and blue areas indicate 95 % confidence intervals for energy expenditure and energy gain, respectively; purple area indicates where confidence intervals overlap. Black area indicates where energy gain is greater than energy expenditure and the lizard would be in a positive energy balance.

To maintain energy balance through the year, a standard 30 g *P. m. melanotus* requires 940.06 kJ. This means that the lizard must catch prey with a minimum energy content of 1078.06 kJ in a year to maintain energy balance. This is equivalent to 116.69 g of mealworms or 1166.85 mealworms per year. Any energy consumed over and above this ensures a positive energy balance resulting in activity, growth, reproduction and storage of fat reserves.

6.3) Distribution

The hypotheses generated with respect to the thermal requirements of *Pseudocordylus m. melanotus* using the climate envelope model produced by 'reverse' modelling the distribution in CLIMEX (CSIRO ©, 1999; hereafter referred to as 'reverse model') were close to the measured values in this study. I used CLIMEX again to model the predicted distribution of the '*melanotus* clade' using the

measured temperature indices (Table 6.2) assuming that the clade has the same temperature tolerances and requirements as those measured for *P. m. melanotus* in this study (hereafter referred to as 'direct model'). I took the measured lower critical limiting temperature (CTMin = 10.17 ºC; Chapter 2) as the lower optimal temperature, and the lower physiological temperature tolerance (LPTT = -5.15 ºC; Chapter 2) as the limiting low temperature because lizards can be expected to die at temperatures below this (Figure 6.5). The selected body temperature (T_{sel} = 30.99 ºC; Chapter 3) was taken as the upper optimal temperature since this is essentially the target temperature for optimal performance and prolonged periods spent at temperatures above this are likely to be detrimental to the lizard (for example, see Chapter 5). The limiting high temperature was not measured for reasons given in Chapter 2, but would otherwise be taken as the upper critical limiting temperature (CTMax); in this case, the upper limiting temperature was maintained at 40 ºC. In addition, the predicted distributions do not change when this parameter is altered.

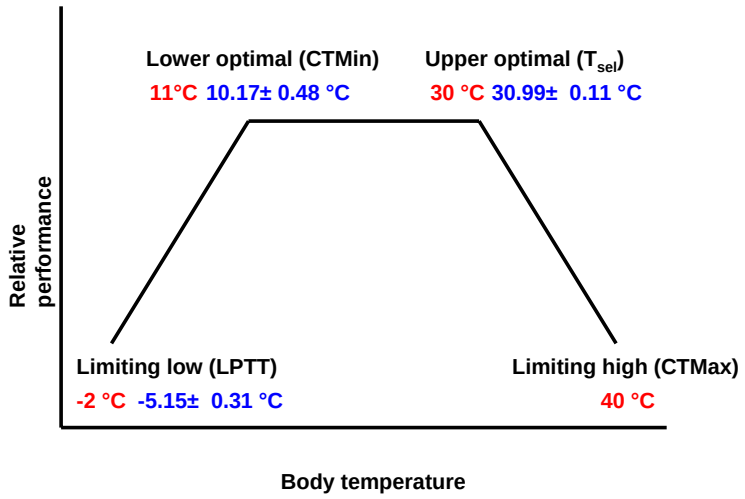


Figure 6.5: Temperature graph used by CLIMEX to predict distributions based on limiting and optimal temperatures. Values in red indicate those predicted by the CLIMEX model and values in blue indicate those measured in the laboratory. Lower physiological temperature tolerance (LPTT) was taken as the limiting low; lower critical limiting temperature (CTMin) was taken as the lower optimal temperature; selected body temperature (T_{sel}) was taken as the upper optimal temperature; and the upper critical limiting temperature (CTMax) was taken as the limiting high (CTMax was not measured due to ethical considerations).

The direct model of the predicted climate envelope of *Pseudocordylus* using measured values was identical to that produced by 'reverse' modelling the species' distributions (Figure 6.6), which suggests that the hypotheses regarding the lizards' thermal biology generated from the reverse model cannot be rejected. The direct and reverse models also show that there could be a number of alternative hypotheses generated regarding thermal preferences and limits , which would produce the same distribution. A model such as this should therefore be viewed with care, particularly when using it as a predictive tool. CLIMEX is nevertheless useful for generating alternative hypotheses with respect to limiting climatic factors. Another cause for concern is the high probabilities and ecoclimatic indices in coastal areas where the lizards do not occur. It is obvious that climate in these areas will not be limiting, but in predicting current distributions of the lizard, this is clearly inaccurate (Figure 6.7). There could be other components of the lizard's niche that are limiting in these areas. There may be no suitable rock outcrops, or if there are, the lizards have not been able to disperse into those areas because of geographical isolation.

Table 6.2: Temperature indices used in CLIMEX model as predicted by 'reverse' modelling the distribution of the monophyletic group of *Pseudocordylus* species from Frost *et al.* (2001), and thermal parameters measured in this study.

Temperature indices	CLIMEX predicted value (ºC)	Measured value (ºC)
Limiting low temperature	-2	-5.15
Lower optimal temperature	11	10.17
Upper optimal temperature	30	30.99
Limiting high temperature	40	*

*Limiting high temperature was not measured in this study.

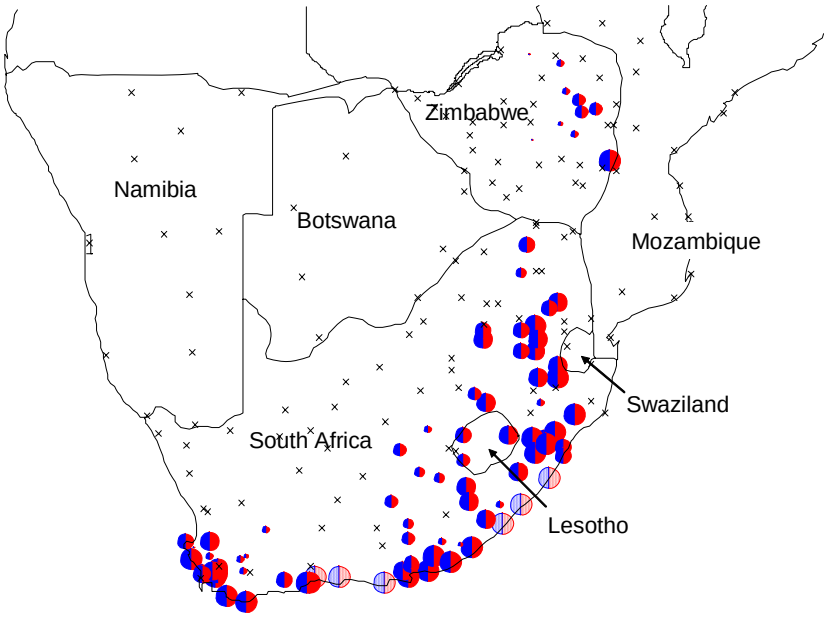


Figure 6.6: Map of southern Africa showing the distribution of the *melanotus* clade of *Pseudocordylus* as predicted by CLIMEX ('reverse' model; blue) and using temperatures indices measured during this study (direct model; red). Crosses indicate the species would be unable to survive, shaded semi-circles indicate a climate for optimal survival of the species, and size of filled semi-circles indicates the probability of survival. In this case, the CLIMEX predicted values and measured temperature indices produce identical probabilities and thus the distributions and probabilities are identical at each station.

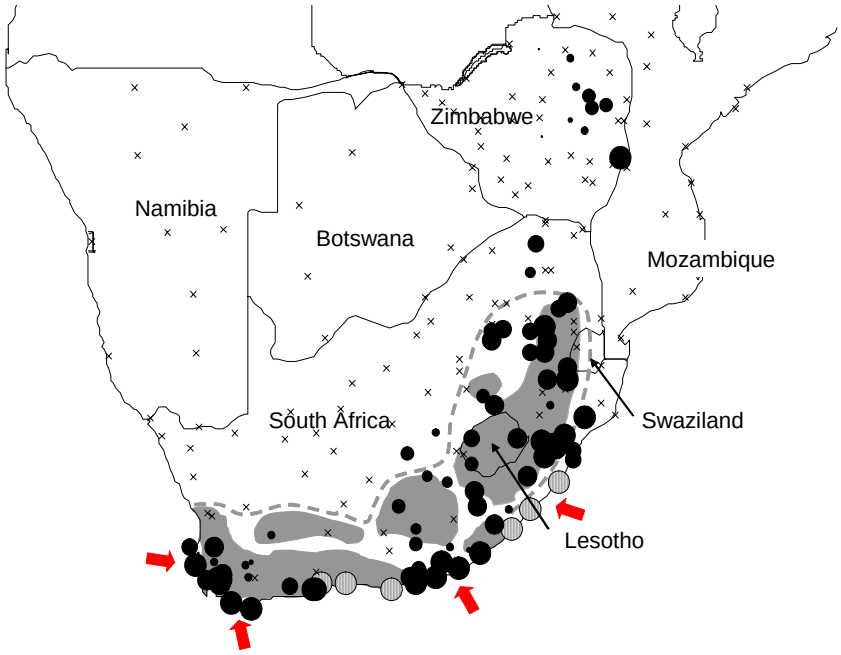


Figure 6.7: Map of southern Africa showing the distribution of the *melanotus* clade produced from the combination of maps from Branch (1998; grey area) and as predicted by CLIMEX using the measured temperature indices. Crosses indicate that the populations would be unable to survive, shaded circles indicate a climate for optimal survival of the species and the size of black circles indicates probability of survival. Red arrows indicate coastal areas where the lizards are currently not known to occur.

In the multivariate climate envelope model, the 60 % probability level accounted for only 56 % of the current distribution records. From a statistical perspective, this would be considered an unacceptably poor match, particularly since only 23 % of the current presence records fall within 90 % probability. There are several possible explanations for the poor match: First, it may be due to the inappropriate selection, or combination, of climatic variables. Second, *P. m. melanotus* may currently not be limited by climatic factors. Since the modelling approach implicitly assumes that current distribution is entirely limited by climate, breaches of this assumption can lead to inaccurate predictions. Since lizards appear to be challenged by low, rather than high, temperatures (Chapters 2 and 3), it is unlikely that predicted climate changes (particularly warming) will have any significant effect on the distribution of the lizard.

As mentioned, lizards may be restricted from certain areas by factors other than climate. Poynton and Broadley (1978) indicate that, in southern Africa, many lizards appear to be limited by substrate rather than climate. It can thus be expected that the distribution of substrata play an important role in limiting the distributions of these species (Yamawaki, 2001). Since *P. m. melanotus* are rupicolous they can be expected to be restricted to areas where there are exposed rocks and the size and shape of rock crevices provide different thermoregulatory opportunities and alternative microclimates. Even at Suikerbosrand Nature Reserve there was a noticeable difference in lizard abundance in different parts of the reserve with different rock types (Quartzite and Ventersdorp Lava, B. Moon, pers. comm.; pers. obs.). I explored the idea of substrate limitation and tested whether lizards use a subset of the available crevices.

I related characteristics of crevices at Suikerbosrand Nature Reserve with respect to the size of crevices preferred by the lizards and range of crevices available to lizards. Lizard preference for crevices was measured first by measuring the crevice entrance of crevices occupied by *P. m. melanotus* and, second, by measuring the head height of 24 lizards captured in the field for other parts of this study. To quantify the crevices available to lizards, I measured (with digital callipers to 0.01 mm) the width

of crevice entrances for all crevices in three 10 x 10 m quads and also noted which crevices were occupied by *P. m. melanotus*.

Lizards preferred crevices that were 22.45 ± 1.49 mm (range: 17.06 to 31.41 mm; n = 147) wide at the entrance and that usually narrowed deeper in the crevice (pers. obs.). The head height of lizards was 9.46 ± 0.33 mm, which means that adult lizards are generally excluded from using crevices, or parts of crevices, that are narrower than approximately 10 mm in width. The crevices available to lizards averaged 28.27 ± 1.80 mm wide at the entrance, but ranged from 3.72 to 129.04 mm. *Pseudocordylus m. melanotus* are therefore only using a subset of the available crevices (pers. obs.). Lizards were not found in crevices which were narrower than their heads, but it appears they do not use crevices with entrances bigger than 31 mm wide (Figure 6.8). Since the dimensions of the chosen crevices closely match the body proportions of the lizards, it is likely that crevices that deviate from these dimensions are unsuitable for the lizards. If there are rock outcrops without suitable crevices the lizards may be excluded from occurring there. If lizards use only the crevices within the measured range of preference, only 27.70 % of the available crevices are suitable for the lizards and of these suitable crevices, only 4.88 % are occupied by *P. m. melanotus*. This equates to one *P. m. melanotus* in 150 m^2.

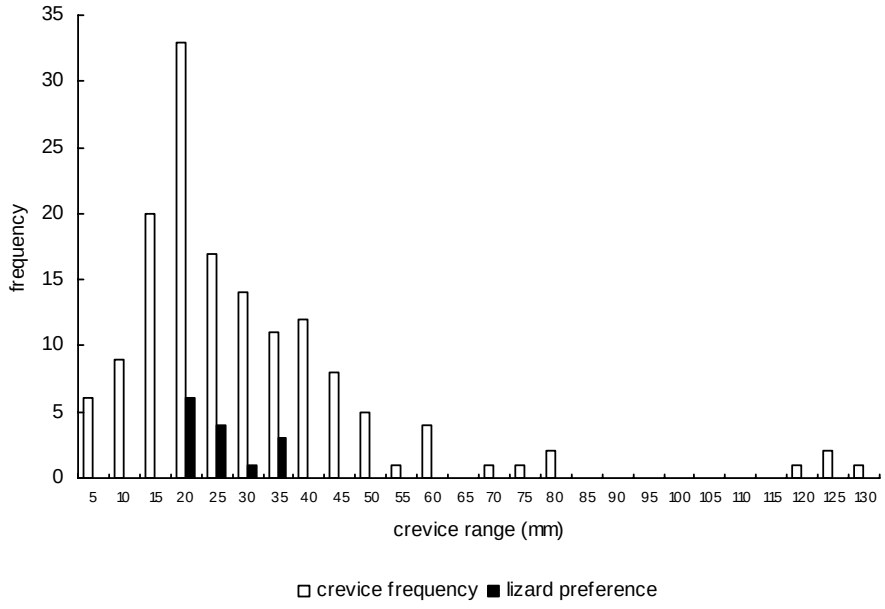


Figure 6.8: Frequency of crevices available to *Pseudocordylus melanotus melanotus* and the subset of crevices where lizards were found. This suggests that lizards choose only a small subset of the crevices in the environment.

Crevices clearly form an essential habitat component for the species, but may not limit the distribution at the species level. On a population basis, however, the presence of suitable rock types, which provide a particular crevice structure when it weathers, is important because some rock outcrops may not provide crevices that meet the lizard's specific requirements. Also, the lizard is unlikely to disperse if there are no suitable retreats between where it is and an area which has potentially suitable rock outcrops.

6.4) Discussion

In the field energy budget, a standard 30 g *P. m. melanotus* requires approximately three quarters (72 %) that in the laboratory energy budget. This can be attributed to the fact that, in the laboratory energy budget, measures of energy gain and energy expenditure were made at controlled, constant (24 h) temperature, while, in the field, there are daily and seasonal fluctuations in temperature. Lizards therefore experienced periods of lower T_b in the field, which implies lower energy expenditure, at least for some parts of the day. The laboratory energy budget does, however, cover a range of temperatures experienced by the lizards. Over similar temperature scales, it could be expected that both energy budgets would reveal similar results.

The laboratory energy budget could not be extrapolated accurately to predict measures of energy expenditure and energy gain over the entire range of T_bs experienced by lizards in the field. Due to time constraints and limitations imposed by the Animal Ethics Committee, measures of energy gain and expenditure could not be made over the entire thermal range, particularly at the high and low ends of the range (near the critical limiting temperatures and lower physiological temperature tolerances). It is clear that the lizards do experience T_bs very close to their lower thermal tolerances (see Chapter 3), but extrapolation of the laboratory energy budget (based on the linear relationship) at these low temperatures is highly inaccurate and predicts a zero metabolic expenditure at 1.98 ºC. In other words, the laboratory measures of energy expenditure predict that the lower lethal temperature tolerance is approximately 2 ºC. This is obviously not the case because the measure of lower

physiological temperature tolerance is approximately -5 ºC (Chapter 2). It is therefore likely that at the lower end of the energy budget, the relationships between energy gain, energy expenditure and temperature are not linear (although I used a linear relationship to estimate energy expenditure at temperatures below 20 ºC). Further investigation of metabolic rates and food consumption at low temperatures would help to clarify these relationships.

Selected temperatures of lizards in the field are maintained at temperatures at which lizards are in positive energy balance. *Pseudocordylus m. melanotus* have a T_{sel} of 28.91 ºC (Chapter 3), which is very close to the temperature required for energy balance (28.82 ºC; laboratory energy budget). Lizards are therefore restricting energy expenditure such that any energy gained in excess of that required for energy balance can be allocated to growth, reproduction and storage. If the 95 % confidence intervals are taken into account, it is likely that the lizards are able maintain energy balance at approximately 25 °C (where energy expenditure approximately matches energy gain) and will start making a large energy profit above approximately 28 °C (where energy gain exceeds energy expenditure).

In winter, the T_{sel} is approximately 2.5 ºC lower than that in summer. Many studies on other lizard species have shown that there is a seasonal rhythm in T_{sel}, which may be in response to limited thermoregulatory opportunities in a cooler environment or reduced prey availability. Regardless of the reason for lizards selecting lower T_bs in winter, a lower T_{sel} would reduce energy expenditure and thus reduce the energy required for maintenance metabolism, which is also beneficial in an environment where there is reduced prey abundance.

The field energy budget creates a more realistic view of the thermal regimes and thus energy expenditure in the field. In order to meet these requirements, *P. m. melanotus* requires approximately two average mealworms, or the equivalent thereof, per day. The laboratory energy budget shows that, at T_bs lower than T_{sel}, energy expenditure is greater than energy gain. This can be explained by the fact that lizards are diurnal and only catch prey while they are active during the day. Once the lizards become

active in the morning, their T_bs rise quickly to selected levels (see Chapter 3), which makes it easy for the lizards to remain in positive energy balance if they catch one or two large beetles during the day. I observed one lizard catch two large scarab beetles within one focal period and there were many large beetles and grasshoppers in and around the rock outcrops where lizards were observed (pers. obs.). It is therefore likely that *P. m. melanotus* are able to capture sufficient prey to, not only meet the requirements of metabolic energy expenditure, but also remain in positive energy balance.

Pseudocordylus m. melanotus have not been observed to capture prey in winter because of time constraints on this study, although, the field energy budget shows that the lizards require 0.63 prey items per day to meet maintenance energy requirements. Prey abundance in winter is likely to be much lower than in summer and may not be sufficient for the lizards to meet energetic requirements for maintenance metabolism. The lizards are, however, active during the winter (Chapter 3), but it is likely that they are unable to maintain energy balance owing to insufficient food resources. Even if there were prey available to the lizards during winter, they may not actually eat because they are not able to maintain adequate T_bs for the effective digestion of food. At very low temperatures, food in the gut may putrefy if it is not digested quickly enough and may have detrimental consequences for the lizard. This is supported by the results of the feeding trials in Chapter 5. *Pseudocordylus m. melanotus* did not eat all the mealworms presented to them at 20 or 22 ºC, but, at higher temperatures, ate all the mealworms, which provided more than sufficient energy to meet metabolic energy requirements. The lizards are thus consuming only small amounts of food at low T_bs even if there is more food available. At high T_bs, given the opportunity, the lizards will eat most, if not all, the food available. This was also evident in the 15 % increase in body mass of lizards kept in captivity for six months, which were maintained at constant temperature (28 ºC) and given an *ad libitum* food supply.

If the lizards do not feed during winter, they need ample fat reserves that will meet their energetic requirements for maintenance metabolism during the winter. It is thus

essential to remain in positive energy balance during summer when prey abundance is likely to be higher, and for lizards to allocate as much energy to fat reserves as they can. Where environmental temperatures are particularly low, it may be beneficial for lizards to remain in retreat sites even if T_b remains at or below the lower critical limiting temperature (CTMin).

On warmer days, it would be beneficial for lizards to be active; even though energy expenditure would be increased, the probability of increased energy gain (i.e., prey capture) increases. Lizards that are active for longer periods on more days in winter are likely to encounter, and catch, more prey, as well as maintain T_b at a level where food can be efficiently digested. These lizards will therefore have higher rates of energy gain than lizards that are less active. More active lizards would thus either require lower energy reserves in stored fat, or are likely to have higher reproductive success as they will be able to allocate a higher proportion of energy to reproduction.

Both climate envelope models predict a larger distribution for the lizard than its current known range. Since *P. m. melanotus* probably only uses a particular subset of crevices available, they are further restricted, not only within the current climate envelope, but also within their preferred habitat (rock outcrops). It is clear that *P. m. melanotus* have specific requirements with respect to the crevices they use as retreats, however, it could be expected that, if crevices were limiting the species' distribution, all suitable crevices would be occupied by a lizard. *Pseudocordylus m. melanotus* may be excluded from some suitable crevices by other rupicolous species occurring sympatrically with *P. m. melanotus* (e.g., *Cordylus vittifer* and *Agama* spp.), but less than 5 % of the suitable crevices were occupied by *P. m. melanotus*, which equates to one lizard every 150 m^2. This may be an underestimate of abundance because more than one *P. m. melanotus* may inhabit a single crevice, particularly during non-breeding season when males are less aggressive. I often observed male *P. m. melanotus* living in crevices with two or more females in the same cluster of rocks.

In winter, crevices may prove to be a limiting factor. My study on the lizards' preference for particular crevices was conducted during the summer when lizards were active and easily observed in the field. During winter, the lizards are obviously less active and absent from crevices where they were frequently observed during the summer (pers. obs.). Suitable winter retreats are likely to have different structures such that the lizards are able to avoid potentially lethal winter temperatures because of the different thermal properties of different size and shape crevices. Groups of lizards (sometimes inter-specific groups) may inhabit particularly deep and/or buffered retreat sites. On one occasion, while catching lizards, five individual *P. m. melanotus* (one male, two females and two juveniles) were found in one crevice with three juvenile *Agamas*. Further investigation of the thermal characteristics of the lizards' retreat sites would be beneficial in determining the level at which crevices are limiting to this and other rupicolous species.

Lizards T_bs are buffered from very hot or very cold environmental temperatures in crevices, and they are thus able to maintain relatively stable temperatures in this environment. If the climate change predicted for 2050 (2 to 5 ºC increase), it is likely that the lizards would be able to shelter in crevices to avoid periods of high environmental temperature. The highest crevice temperature measured during summer in this study was 29.4 ºC (for one 20 min interval). This is a conservatively high measure for crevice temperature since the size of the crevice used to measure operative temperature was limited by the size and shape of the copper tube model, i.e., the model was round and could thus not be placed in a crevice as deep as flattened lizards would be able to retreat. It is thus likely that the highest crevice temperature would be low enough for the lizards to easily avoid potentially harmful, high environmental temperatures while still being able to thermoregulate at preferred levels. *Pseudocordylus m. melanotus* are therefore unlikely to suffer any negative effects of predicted climate change because they will still be able to thermoregulate at preferred levels within cooler microclimates.

Some lizards can balance their energy budgets in order to persist in areas with low food resources, or even where the thermal environment is seemingly too hot or too

cold. *Xantusia* species living in food scarce environments process energy one third as fast as other reptiles of similar size, grow very slowly, have low reproductive rates and are reclusive (Mautz and Nagy, 2000). *Liolaemus* lizards are smaller than expected for cool climate species, many are herbivorous, but maintain high body temperatures, are active for longer periods and thermal tolerances do not appear to have any effect on distributional limits (see Carothers *et al.*, 1997, 1998, 2001; Espinoza *et al.*, 2004). In maintaining T_b at the same level, *P. m. melanotus* will easily be able to maintain energy balance in the predicted climate provided the climate change does not immediately significantly affect the abundance of prey.

6.5) Conclusion

Remaining in positive energy balance is essential for survival. Where energetic requirements are affected by temperature, the balance between expenditure and gain is particularly important. Since energy expenditure (metabolism) and energy gain (prey capture and energy assimilation) are dependent on temperature in ectotherms, environmental temperature is likely to affect the energy budget. In areas where environmental temperatures restrict energy gain at a level lower than energy expenditure (i.e., negative energy balance), lizards would not occur in those areas, and the distribution of the lizard species will be limited to areas where a positive energy balance can be maintained.

Pseudocordylus m. melanotus can generally be expected to have low energy requirements because they are extreme sit-and-wait foragers. Their movements are mainly restricted to postural adjustments and short, possibly anaerobic, bursts during prey capture during periods of activity. Energy gain from prey captured by *P. m. melanotus* exceeds metabolic energy expenditure at environmental temperatures where lizards are able to regulate and maintain body temperatures at or near preferred/selected levels. *Pseudocordylus m. melanotus* are efficient thermoregulators and restrict energy expenditure at minimal levels (with extremely low levels of movement during active periods) so that maximum energy can be gained and a positive energy balance sustained.

The population at Suikerbosrand Nature Reserve appear to be at the cold limit of the species' range; operative temperatures do occasionally come perilously close to the lizards' lower physiological temperature tolerance. Suitable retreat sites, however, may allow lizards to avoid potentially lethal environmental temperatures. Crevices provide a microclimate in which the lizards are able to thermoregulate, and which may be buffered from thermal extremes in the environment allowing lizards to escape extreme climatic conditions while maintaining thermoregulatory integrity. The presence of suitable crevices may restrict the lizard's distribution further. Although the lizard's climate envelope predicts a fairly wide distribution, *P. m. melanotus* is limited to areas where there are exposed rocks, which weather in such a way that crevices will provide suitable thermal environments for the lizard.

Future research should be directed towards forming comprehensive energy budgets in a more standard form. Many studies deal with various aspects of energy budgets and frequently by different research groups and institutions. Focus should be placed on combining studies on the different features of energy budgets, particularly with respect to temperature effects on energy balance. Understandably, complete energy budgets are time consuming and often expensive to measure. Cooperation and coordination of studies on different aspects of energy budgets will facilitate the development of structured and comprehensive energy budgets. Knowledge of the thermal requirements and effects on energy budgets can be used to evaluate factors affecting distribution limits. Also, life history characteristics and geographic variation in these characteristics can be further explored with respect to energy expenditure, energy gain and energy balance.



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APPENDIX 1

McConnachie, S. and Alexander, G.J. 2004. The effect of temperature on digestive and assimilation efficiency, gut passage time and appetite in an ambush foraging lizard, *Cordylus melanotus melanotus*. Journal of Comparative Physiology B 174: 99 – 105.