METABOLIC RESPONSES TO HYPERTHERMIA IN TWO SMALL DESERT MAMMALS, THE PYGMY ROCK MOUSE, *PETROMYSCUS COLLINUS* AND THE NAMAQUA ROCK MOUSE, *AETHOMYS NAMAQUENSIS*

University of KwaZulu-Natal

By

Metoboroghene Oluwaseyi Mowoe

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Abstract

The negative consequence of recent climate change on the Earth's biodiversity has become more evident in recent years. Some animals, due to insularity or habitat fragmentation, are unable to shift their ranges altitudinally and latitudinally. Vulnerable species need to rely on behavioural and, more importantly, physiological responses in order to persist through present climatic changes. It has therefore become more obvious that physiological responses of individuals need to be incorporated into predictive models of the responses of mammals to accelerated climate change.

The primary purpose of this study was to test the 'Hyperthermic Daily Torpor' hypothesis proposed recently by Lovegrove et al., (in press). The hypothesis suggests that, based on albeit limited evidence, some small mammals may be capable of hyperthermia-induced hypometabolism equivalent to that experienced during torpor and hibernation in response to cold temperatures. These authors argue that such hyperthermic hypometabolism should reduce the risk of entry into pathological hyperthermia and also reduce the rate of water loss driven by heat-induced evaporative cooling. The reaction norms of desert mammals have been selected to be adaptive over a wide range of climatic conditions due to the unpredictability of their habitat. Thus, they are good models for testing the reaction norms that may be expressed in response to accelerated climate change. We therefore tested our hypothesis using two presumably heat-adapted desert rodents; the Namaqua rock mouse, *Aethomys namaquensis,* and the pygmy rock mouse, *Petromyscus collinus,* as model species.

We used indirect respirometry to measure metabolic rate at high ambient temperatures. We progressively exposed the animals to high temperatures to induce thermal

tolerance and thus minimize the risks of lethal hyperthermia. We also measured subcutaneous and core temperatures, using temperature-sensitive PIT tags (BioTherm Identipet) and modified iButtons (Maxim Integrated), respectively.

A. namaquensis displayed the capacity for hyperthermia-induced hypometabolism (Q_{10} = 1.27 ± 1.61) whereas the *P. collinus* did not (Q_{10} = 2.45 ± 1.41).

The implications of such a physiological response in *A. namaquensis* are crucial in terms of its capacity to minimize the risks of lethal, pathological hyperthermia. Recent models of endothermic responses to global warming based on ectothermic models predict a dichotomy in the thermoregulatory responses of mammals to high temperatures. This study, to our knowledge, provides some of the first data on these interspecific variations in the thermoregulatory responses of mammals to high temperatures. However, the different physiological responses to hyperthermia between these two species cannot be meaningfully interpreted without phylogenetically independent comparisons with other species, that is, a more expansive interspecific analysis. Nonetheless, we provide some autecological sketches to assist in future multivariate interspecific analyses.

Physiological differences between captive or captive-bred and free-ranging mammals preclude the extrapolation of our findings to free-ranging mammals. It is almost impossible to collect MR data in the field, although a few authors have successfully done so, and it is often not feasible to collect T_b data in small free-ranging mammals. Most studies have therefore made use of externally-mounted temperature-sensitive data loggers in order to collect T_{skin} data as a proxy for T_{core} data in free-ranging mammals. However, misleading gradients between T_{skin} and T_{core} can occur if data loggers are placed too close to major-heat producing tissues and the effects of the external environment on these data loggers may result in large T_{skin} – T_{core} gradients.

The second objective of this thesis therefore was to test the validity of using subcutaneous temperatures (T_{sub}) from subcutaneously injected temperature-sensitive PIT tags as a proxy for T_{core} using the Namaqua rock mouse, *Aethomys namaquensis*.

We found that the difference between T_{core} and T_{sub} was minimal (~ 0.34˚C) within the thermoneutral zone (TNZ) with slight, non-significant, differences outside the TNZ. There was a tendency for T_{sub} to underestimate T_{core} below thermoneutrality and overestimate it above thermoneutrality. We attributed these differences to the various heat loss and heat gain mechanisms activated in response to heat and cold stress in order to maintain a setpoint T_b. Nevertheless, we found that the T_{core} – T_{skin} differential never exceeded 1.59˚C above the wide range of T_as (5˚ – 41˚C) measured. Thus, we can conclude that subcutaneous temperatures provide a reasonably reliable proxy for core temperature in small mammals.

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Thesis structure

This thesis consists of five chapters. Chapter 1 provides a general introduction to climate change and species responses to climate change and high ambient temperatures in particular. It also introduces the novel concept of hyperthermic hypometabolism which is the main focus of the thesis.

Chapter 2 provides a short description of the model mammals *Aethomys namaquensis* and *Petromyscus collinus,* chosen following Krogh's principle (Bennett, 2003). Chapter 3 and 4 are written as individual papers formatted for the *Journal of Comparative Physiology B*. Chapter 3 highlights the common problem faced by physiologists when measuring the body temperature of small free-ranging mammals. It tests the validity of using subcutaneous temperatures as proxies for core temperatures in free-ranging mammals as it is often not possible to measure core temperatures of small, free-ranging mammals in the field. *A. namaquensis* alone is used as a model species in this study. Chapter 4 reports the test of the Hyperthermic Daily Torpor hypothesis in both study mammals. Chapter 5 outlines the general conclusions reached in both studies and discusses the overall significance and contribution of the thesis research. Possible future research directions are also discussed. Because chapters 2 and 3 are written in the form of individual papers, there is some text that is repetitive in Chapter 1 and Chapter 4.

Table of Contents

List of Figures

Figure 1.1 Time course graphs of ambient T_a of one individual taken over a range of 3 hours for the lowest temperature to 1.5 hours for the highest temperatures. Closed circles represent core temperatures whereas open circles represent subcutaneous temperatures.

Figure 1.2 The linear relationship (solid line) between T_{sub} as a function of T_{core} of *A. namaquensis* (n =6) over a range of T_as. The dotted line represents thermal equilibrium between T_{sub} and T_{core}, i.e. $T_{sub} = T_{core}$

Figure 1.3 The mean ± SD of T_{core} and T_{skin} at different ambient temperatures in *Aethomys namaquensis.* Closed symbols represent T_{core} and open symbols represent T_{skin}

Figure 1.4 Differences between the treatment group T_{core} and individual T_{skin} for six *Aethomys namaquensis*. The data were divided into the different T_a repeated measure groups: 5°, 15°, 25°, 29°, 33°, 37°, 38°, 39°, 40°, and 41°C. Values > 0 (dashed line) indicated an over estimation of T_{core} by T_{skin} and values < 0 indicated an underestimation of T_{core} by T_{skin}

Figure 1.5 Differences between the treatment group T_{core} and individual T_{skin} for four *Aethomys namaquensis*. The different T_a repeated measure groups were divided into three sections: below T_{lc} (< 33°C), TNZ ($33^{\circ} < T_a < 37^{\circ}$C) and above T_{uc} (> 37°C). Values > 0 (dashed line) indicate an over estimation of T_{core} by T_{skin} and values < 0 indicate an underestimation of T_{core} by T_{skin}

Figure 2.1 Body masses before and after the start of the body temperature measurements for a) the Namaqua rock mouse and b) the pygmy rock mouse

Figure 2.2 Mean ± SD body temperature and metabolic rate of the Namaqua rock mouse

Figure 2.3 Mean ± SD body temperature and metabolic rate of the pygmy rock mouse

Figure 2.4 The Arrhenius effect of body temperature on metabolic rate in a) the Namaqua rock rat and b) the pygmy rock mouse.

Chapter 1

Overview

Recent climate change has been attributed, primarily, to anthropogenic elevations of carbon dioxide and other greenhouse gases in the atmosphere (IPCC, 2007). The Earth is warming at a faster rate than formerly predicted by the 2001 Intergovernmental Panel on Climate Change models (IPCC, 2001, 2007; Rahmstorf et al., 2007). The 2001 models showed that the Earth had warmed up by 0.6˚C in the past century (IPCC, 2001). However, according to the 2007 models, average global temperatures have increased by 0.74˚C over the past century (1905-2005) and the most extreme climate change models predict a 4˚C increase by the end of the century (IPCC, 2007).

Climate change affects every habitat on the planet and is, therefore, one of the greatest threats to the Earths' biodiversity (IPCC, 2007). Increased desertification and drought (Houerou, 1996), as well as an increased frequency of catastrophes (IPCC, 2007), are some of its predicted consequences for the terrestrial ecosystem (Solomon et al., 2008; Thuiller, 2007). Extinctions have already occurred and are occurring in a wide variety of plants and animals from a broad range of regions (Franco et al., 2006; McKechnie et al., 2012; Parmesan and Yohe, 2003; Pounds et al., 1999; Thomas et al., 2006). Moreover, its effects to date are irreversible - even if CO_2 emissions were to cease immediately the effects of climate change will still remain until the end of the next millennium (Solomon et al., 2008).

Ecological responses to climate change

Habitat suitability and bioclimatic envelopes are a common approach to predicting species responses to climate change (Austin and Rehfisch, 2005; Hickling et al., 2006; Humphries et al., 2002; Jeschke and Strayer, 2008; Thomas and Lennon, 1999). By using present day species-climate relationships these models are able to determine species distributions and range shifts under predicted climatic conditions in the future (Pearson and Dawson, 2003). Vertical and latitudinal range shifts have already been documented in some species (Chen et al., 2011; Rowe et al., 2010; Thomas and Lennon, 1999; Thuiller, 2007). Range shifts are correlated with simultaneous shifts in ambient temperature (T_a) and humidity patterns that define species boundaries. In order to maintain their boundaries or find a location better suited to their physiological capabilities, species need to be able to track these climatic changes (Perry, 2005; Thuiller, 2007).

However, for insular mammals and those in habitats that have been fragmented by anthropogenic activities (e.g. deforestation), migration is problematic (Honnay et al., 2002, Sekercioglu et al., 2012). For such individuals, two outcomes are likely; species will persist through reliance upon behavioural (Huey and Tewksbury, 2009; Kearney et al., 2009) and physiological (Dillion et al., 2011; Hofmann and Todgham, 2010) responses, or they will go extinct (Thuiller, 2007).

Life history traits are usually defined as a set of adaptations whose phenotypic responses to environmental stresses are constrained by an individual's physiology but which determine success and survival rates (Ricklefs and Wikelski, 2002; Sinervo and Svensson, 1998). Understanding the physiological functions, such as metabolic rate (MR) and body temperature

(T_b), which drive organisms, may therefore help us to better understand and more accurately predict individual responses to climate change (Chown et al., 2010; Fuller et al., 2010; Ricklefs and Wikelski, 2002; Sinervo and Svensson, 1998; Williams et al., 2008).

As the T_b of ectotherms is almost completely dependent on T_a they are believed to be more vulnerable than endotherms to the effects of climate change. Most climate studies have, as a result, focused more on the physiological responses of ectotherms to climate change (Deutsch et al., 2008; Dillion et al., 2011; Huey et al., 2009; Huey et al., 2012; Tewksbury et al., 2008). However, when T_as deviate from the 'thermoneutral zone' (TNZ) (a range of T_a's over which heat generated from metabolism in a post-absorptive, quiescent state adequately maintains a species-specific T_b), it is very energetically costly for endotherms to maintain their normothermic T_b (McNab, 2002) and therefore they too are vulnerable to the effects of recent global warming.

Krogh's Principle: Desert mammals as physiological models for climate change physiology (Bennett, 2003)

Low, highly variable precipitation resulting in low resource productivity and extreme temperature fluctuations characterize desert habitats (Noy-Meir, 1973). Survival in deserts therefore requires a sparing use of energy (Bradshaw, 2003) and the ability to adapt to very unstable environmental conditions.

Despite such hostile conditions, desert mammals often have a fairly high mammalian diversity, the majority of which are small rodents (<100 g) (Walsberg, 2000). This is surprising as their large surface area: volume (SA/V ratio) (a result of their small size) means they are prone to higher rates of heat, energy and water flux than their larger counterparts (Schmidt-Nielsen, 1990; Withers, 1992). Moroever, dispersal is, to a certain extent, limited for small mammals living in deserts (Haim and Izhaki, 1994).

Mammals are characterized by their ability to maintain a certain species-specific core T_b within narrow (upper and lower) limits and over a wide range of T_as, regardless of habitat (Schmidt-Nielsen, 1990; Withers, 1992). However, desert T_as however, often exceed the normothermic T_bs (normal body temperatures) of the inhabiting rodents. The thermoregulatory stresses this places on xeric mammals is somewhat ameliorated by behavioural specializations namely; nocturnality (MacMillen, 1972), fossoriality (McMillen, 1972) and granivory (Brown et al., 1979).

Most xeric small mammals with only a limited tolerance for extreme diurnal T_as, often confine their activities to the cooler part of the day and are, therefore, often nocturnal (MacMillen, 1972). Nocturnal temperatures are about 15^o - 20^oC lower than the diurnal maxima (Walsberg 2000). Nocturnal activity allows desert small mammals to avoid very high temperatures and thereby effectively maintain their T_b (MacMillen, 1972).

Fossoriality is another common behavioural mechanism utilised my small, xeric mammals to avoid unfavourable desert conditions (MacMillen, 1972). During the inactive phase most small mammals retreat into subterranean burrows in order to escape the harsh conditions of the external environment (MacMillen, 1972). In fact, during the active phase, the activity of these nocturnal rodents is often limited to a small area around a burrow system (Eisenberg 1963).

As well as being nocturnal and fossorial, the majority of small desert rodents are granivorous (Brown et al., 1979). A granivorous animal can loosely be defined as an individual whose diet is largely composed (> 50%) of seeds (Murray and Dickman, 1994) as the diet of desert rodents is rarely composed solely of seeds due to its dependence on precipitation usually unpredictable in desert systems (Brown et al., 1979, Lovegrove, 2000). Granivory is an advantageous dietary specialization for xeric species because it is an abundant food resource in a resource scarce habitat and is often easily available (Brown et al., 1979; Murray and Dickman, 1994)

However, these behavioural adaptations alone are unable to sufficiently insulate xeric species from the extreme thermal fluctuations of their unpredictable environment (Schmidt-Nielsen 1964). In order to maintain a normothermic core T_b heat needs to be released via one or more of several avenues such as vasodilation, urine and faeces, respiration, salivation, sweating and evaporative water loss against the $T_b – T_a$ gradient (Schmidt-Nielsen, 1990). The rate of evaporative water loss is driven by the difference between the water vapour pressure at the animal’s surface and that of the atmosphere (Withers, 1992), a problem in a water deficient habitat such as deserts (Walsberg, 2000). Xeric species have also developed, in addition to their behavioural specialization, certain physiological modifications that aid their survival in their harsh desert environment (Schwimmer and Haim, 2009).

Xeric species lose water via their faeces, urine and various thermoregulatory mechanisms (Schmidt-Nielsen, 1990; Withers, 1992). However, during prolonged periods of paucity not uncommon in desert habitats, animals need to consume more water whilst at the same time possessing some resistance to water loss (Schwimmer and Haim, 2009). All rodents are capable of excreting hypersomatic urine in order to regulate and decrease water loss. Only desert rodents however, have the capacity to utilize this ability to maintain a positive water balance in the water scarce deserts in order to survive (MacMillen, 1972; Palgi and Haim, 2003). In this way water is conserved as less water is lost in urine and small desert rodents are able to deal somewhat with the scarcity of water typical of their natural habitats.

Desert rodents often have lower BMRs than their mesic counterparts (Lovegrove, 2000, 2003). Lovegrove (2000) found that the BMR of mesic species, on average, exceeded that of xeric species by 24.31%. Similarly, McNab and Morrison (1963) found that the mesic subspecies of exhibited in some cases a 13% increase and in other cases a 24% increase in BMR in comparison to their xeric counterparts. Moreover, Shkolnik and Schmidt-Nielsen (1975) found that the desert hedgehog *Paraechinus aethuopicus* had a lower BMR than both the semi-arid and temperate hedgehogs *Hemiechinus auritus* and *Erineceus europaeus.* The low BMR is probably an evolutionary response to the unpredictability and low productivity of the desert environment (Lovegrove, 2000, 2003). It is an advantageous adaptation as it offsets the energetic costs of maintaining homeostasis in a constantly fluctuating thermal environment and reduces dependence on evaporative cooling for heat loss (Lovegrove, 2003; McNab and Morrison, 1963).

In spite of a low BMR in xeric species they have been found to maintain an efficient mechanism of heat production resulting in an increased capacity for NST (Schwimmer and Haim, 2009). In a study on the ecological significance of RMR and NST in 21 species of rodents, Haim and Izhaki (1993) found that the arid species which exhibited lower RMRs also had significantly higher NST values than the mesic species. Non-shivering thermogenesis is an important mechanism in desert species because despite very high daily T_as, nocturnal T_as may be much lower than the diurnal maxima (Walsberg 2000) and winters can be very cold (Desmet and Cowling 1999). Moreover, it is ecologically economical as metabolism can be kept low during normothermy thus conserving energy. However during periods when low T_as are experienced heat production can be increased in a relatively short period of time (Haim and Izhaki 1993; Scantlebury et al., 2002).

The reaction norms of these desert mammals have probably been selected to be adaptive over a wide climatic range due to the unpredictability of their habitat and this is evident from their aforementioned array of behavioural and physiological specializations (Canale and Henry 2010). Thus, according to Krogh’s principle (Bennett, 2003) these desert rodents may be the best models for the discovery of the mechanism of phenotypic plasticity likely to be expressed in the face of recent accelerated climate change (Canale and Henry 2010)

Climate change and Arrhenius effects

Tropical mammals being basoendothermic (Lovegrove 2000, 2012) may be the most vulnerable to the effects of global warming despite experiencing the least elevations in T_a (Dillion et al., 2010). Their vulnerability is increased by the fact that most of these tropical mammals are insular or live in fragmented habitats where migration is inhibited (Sekercioglu et al., 2012). Recently a more precise concept of hyperthermic daily torpor (HDT), in the form of hyperthermia-induced hypometabolism, has been proposed (Lovegrove et al., in press). These authors provide some, albeit limited, evidence to show that hypometabolism, equivalent to that which occurs during daily torpor and hibernation, can also occur during hyperthermia to offset the high energetic costs of Arrhenius effects in small tropical mammals. They argue that, hyperthermia-induced metabolic downregulation is a putative mechanism of reducing the T_b – T_a gradient thus retarding entry into pathological heat stress.

The ecological implications of hyperthermia-induced hypometabolism are critical in terms of the capacity of mammals to survive accelerated climate change. It potentially not only reduces the risk of pathological hyperthermia, it also reduces the energetic costs of the Arrhenius effect of body temperature on metabolic rate. Moroever, by down-regulating metabolism, the rate of respiration is reduced and, since it is proportional to evaporative water loss (Cooper and Withers, 2008), hyperthermia-induced hypometabolism may also be a mechanism for water conservation.

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Chapter 2

Study animals

The desert of Namaqualand is characterised by low winter-rainfall (± 150 mm per anmum) and very hot, dry summers (Cowling et al., 1998; Rutherford and Westfall, 1986). These deserts experience cloudless days for 70% of the year (Rutherford and Westfall, 1986) with a diurnal maxima and nocturnal minima of 41^{o}C and 10^{o}C in summer and 26^{o}C and -3^{o}C in winter (http://uk.weather.com/climate/annualClimo). Therefore, we assumed that these species would be heat-adapted thus informing our decision to use them as model species.

Petromyscus collinus

The pygmy rock mice, (~17 g) are nocturnal desert rodents that inhabit the rocky habitats of the arid zones of Southern Angola, Namibia, and South Africa (Nowak, 1999; Smithers, 1983). They occur in, rocky outcrops or kopjies, preferably with an abundance of loose boulders (Nowak, 1999; Skinner and Chimimba, 2005). These mice have been observed to survive for weeks in arid, dry conditions on a diet of air-dried seeds alone (Withers et al., 1980). The adaptive traits exhibited by the pygmy mouse may be associated with the environmental characteristics and selective pressures typical of desert habitats from which it is unable to migrate (Garland and Carter, 1994; Lovegrove, 1999).

Aethomys namaquensis

The Namaqua rock mouse (~ 60 g) inhabits rocky outcrops or kopjies of the deserts of Angola, southern parts of Zambia, Mozambique, north of the Zambezi River, Malawi and South Africa (Coetzee, 1969). In some habitats, the rock mouse has been observed to use rock crevices as a shelter from predation and intense solar radiation (Roberts 1951). The Namaqua rock mouse is believed to be well-adapted to desert habitats and displays the characteristics of a low BMR (Lovegrove, 2003), low rates of evaporative water loss, and a narrow thermoneutral zone, typical of desert mammals (Buffenstein, 1984; Lovegrove et al., 1991, Lovegrove, 2000).

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Chapter 3

Subcutaneous temperature as a proxy for body temperature in a small rodent

M.O Mowoe, Lovegrove, BG

Abstract

The physiological patterns in free-ranging and captive or captive-bred mammals differ; data from laboratory studies therefore cannot easily be extrapolated to field situations. In this respect it has become increasingly important to obtain physiological data, such as body temperature, from free-ranging animals. However, small body sizes pose real logistical challenges. Many free-ranging studies make use of external temperature-sensitive devices to measure T_{skin} as a proxy for T_{core}. In some studies misleading gradients between T_{core} and T_{skin} can occur if dataloggers are situated too close to the major heat-producing tissues, such as brown adipose tissue. Moreover, due to the effect of the external environment on the transmitters, differences of up to 6˚C between T_{core} and T_{skin} have been measured.

In this study, we quantified core-to-subcutaneous gradients using intraperitoneal and subcutaneous temperature-sensitive devices. We validated the use of T_{sub} as a proxy for T_{core} by surgically implanting iButtons into the peritoneal cavity of *Aethomys namaquensis* (~48 g) to measure $T_{core,}$ and subcutaneously injecting temperature-sensitive PIT tags into the nape region to measure T_{sub}.

At least during the rest-phase, there were no significant differences between T_{core} and T_{sub} at all T_as. T_{sub} was the most accurate proxy for T_{core} within the TNZ. We attributed this to the fact that the rate of heat loss and heat gain is more or less equal within the TNZ as the metabolic heat produced by the individuals within this range of T_as is sufficient to maintain the T_b at setpoint. Below and above the TNZ however, heat loss and heat gain mechanisms brought about by the maintenance of the T_b setpoint resulted in slight under- and overestimation of T_{core} by T_{sub}, respectively, but these differences were not significant. Thus, subcutaneous temperatures provide reasonably reliable proxies for core temperature in small mammals.

Keywords Skin temperature · Core temperature · Thermoneutral zone · Namaqua rock mouse

Abbreviations

MR	Metabolic rate
T_b	Body temperature (˚C)
T_{core}	Core temperature (˚C)
T_{skin}	Skin temperature (˚C)
T_{sub}	Subcutaneous temperature (˚C)
T_{uc}	Upper critical limit of thermoneutrality
T_{lc}	Lower critical limit of thermoneutrality
TNZ	Thermoneutral zone

Introduction

Physiological patterns may differ significantly between free-ranging and captive or captive-bred individuals (Geiser and Ferguson, 2001; Geiser et al., 2000). Consequently, animal physiology has increasingly taken to the field over the past decade in order to obtain measurements from free-ranging individuals that can be incorporated in physiologically-based climate change models. Typically, core body temperature (T_{core}) has been the easiest trait to measure, whereas metabolic rates (MR) are almost impossible to measure (although see Dausmann et al., 2000; Ortmann et al., 1997; Schmid and Heldemaier, 2000).

Ideally, T_b should reflect core temperatures (T_{core}) and is therefore often based on rectal temperatures, measured by inserting a thermocouple up the anus of the individual, or T_bs recorded using temperature data loggers or temperature-sensitive transmitters surgically implanted into the peritoneal cavities of animals (e.g. Canale et al., 2011; Geiser and Drury, 2003; Levesque and Tattersall, 2009; Lovegrove et al., 1999). For relatively large mammals, dataloggers or temperature transmitters can be implanted easily to obtain T_{core} measurements in the field (Arnold et al., 2006; Arnold et al., 2004, Barnes 1989). However, the collection of these data becomes less feasible for mammals smaller than 30 g because device weights can exceed a critical percentage (~ 5%) of the animal's body mass. Studies of small mammals (≤50 g) in the field often have to rely on a measure of skin temperature (T_{skin}) as a proxy for T_{core} (Audet and Thomas, 1996; Barclay et al., 1996; Brigham et al., 2000; Dausmann, 2005; Kobbe et al., 2011; Willis and Brigham, 2003).

In free-ranging mammals and birds, T_{skin} is measured using external temperature-sensitive devices which are either collar-mounted or glued dorsally between the scapulae (e.g. Audet and Thomas, 1996; Barclay et al., 1996; Dausmann, 2005; Willis and Brigham, 2003). The skin surface represents the thermal boundary which protects the body and the internal environment from the external, ambient environment (Lovegrove et al., 1991). Moreover, a mammal's thermoregulatory response is determined by inputs from peripheral thermoreceptors (Lovegrove et al., 1991).

A high correlation between T_{skin} and T_{core} is often assumed in small mammals as their high thermal conductance (Aschoff, 1981; Herreid and Kessel, 1967) and the short distance between the core and the skin prevents the formation of a steep $T_{core} – T_{skin}$ gradient (Audet and Thomas, 1996). However, misleading gradients between T_{core} and T_{skin} can occur if data loggers are situated too close to the major heat-producing tissues, for example above the brown adipose tissue deposits between the scapulae. Willis and Brigham (2003) found T_{skin} to be influenced by heat production from brown adipose tissue located in the nape region of bats.

Very few comparative studies have closely examined the use and accuracy of T_{skin} as a proxy for T_{core} in small mammals (Audet and Thomas, 1996; Barclay et al., 1996; Dausmann, 2005; Willis and Brigham, 2003). Audet and Thomas (1996) found no difference between T_{skin} and T_{core} (measured rectally), but found that ambient temperature (T_a) significantly influenced the T_{core} - T_{skin} relationship; large differences occurred at high temperatures (> 21^0C). Similarly, Barclay et al. (1996) found a correlation between T_{skin} and T_{core} (measured rectally) of big brown bats, *Eptesicus fuscus,* that was not significantly different and could be described by the relationship T_{skin} = O.51 + $0.98T_{rectum}$ (r^2 = 0.98). Brigham et al. (2000) also found a strong correlation between T_{skin} and T_{core} (T_{core} explained 85% of the variation in T_{skin}) but with instances where T_b differed from T_{skin} by up to 6˚C. Such a large thermal gradient was attributed to cooling effects of the external environment on the data loggers. Thus, a device such as a subcutaneously injected thermally-sensitive transponders data logger may provide a closer approximation of T_{core} than externally attached devices.

The aim of this study was to validate the use of subcutaneous temperatures (T_{sub}) as a potential proxy for T_{core}, especially in small mammals. We used passive integrated transponder (PIT) tags injected subcutaneously to measure T_{sub} and Themocron data loggers (iButtons) implanted into the intraperitoneal cavity to measure $T_{core.}$ As a model small mammal we used the Namaqua rock mouse (*Aethomys namaquensis*), a small (~ 60 g), nocturnal, granivorous desert mammal that has been the subject of several thermoregulatory studies (Buffenstein, 1984; Lovegrove et al., 1991). We expect to find no differences between the core and skin temperatures in *Aethomys namaquensis*

Materials and Methods

Study animals

The Namaqua rock mice, *A. namaquensis* (47.86 ± 6.65 g, n = 14), were live-trapped using Sherman traps baited with peanut butter and oats on the farms Roopersfontein at Keimoes (28˚69'S, 21˚17'E) and Norriseep at Onseekpans, (28˚76'S, 22˚42'E), Northern Cape, South Africa, during March/April 2012. Each individual was sexed and weighed immediately after capture and maintained in individual cages. The rodents were transported by road to the animal house in the University of KwaZulu-Natal, Pietermaritzburg.

The mice were housed individually, indoors, in sawdust-lined cages (380 x 220 x180 mm) and were provided with toilet paper rolls for shelter. The mice were summer maintained at a T_a of 25˚C at a 16:8 LD photoperiod (lights off CAT 21:00h) and fed a diet of sunflower seeds supplemented with rodent pellets, with water *ad libitum*.

Surgical procedure

Modified temperature sensitive dataloggers (DS1922L Thermochron iButtons, Dallas semiconductor, resolution 0.625˚C, storage capacity 104832 values <2.5 g) modified after Lovegrove (2009) were surgically implanted into each mouse under inhalation anaesthesia. Prior to implantation, each iButton was individually calibrated and programmed to record temperature every 10 minutes 24 hours a day. Waxed iButtons were then surgically implanted into the peritoneal cavity of the rock rats to measure $T_{core.}$ Passive integrative Bio-Thermo Microchip transponder (PIT) tags (Destron Fearing) were also injected dorsally and subcutaneously into themice to provide measures of $T_{sub.}$

Prior to the date of surgery, all surgical equipment wasautoclaved (120˚C, 45 minutes) and telemeters were sterilized in 70% ethanol. Inhalation anaesthesia was induced at 3% Isoflurane (Safeline Pharmaceuitcals (PTY) LTD in oxygen and maintained for the duration of the surgery at 1.5% Isoflurane in oxygen.

To avoid hypothermia, the surgical procedure was conducted on a hot water bottle, previously heated to keep the mice warm. The surgical area was prepared by shaving a small area of the stomach and cleaning it with 70% alcohol and Betadine antiseptic (10% Povidone-Iodine), after which the animal was covered with a surgical drape. A small mid-ventral incision was made in the skin and peritoneum and the iButton was then inserted into the peritoneal cavity. iButtons, previously sterilized in 70% alcohol were also soaked in betadine prior to implantation. The peritoneum and skin were sutured separately using coated Vicryl rapide (Ethicon, polyglactin 910) absorbable sutures and coated Silk (Perma-hand) non-absorbable sutures respectively. The Bio-Therm PIT tags (Identipet) (± 0.2 g) were injected subcutaneously into the nape region of the mice.

Following surgery, animals were kept in clean cages lined with paper towels and placed in a temperature-controlled cabinet at 27ºC for a few hours before being returned to maintenance housing. In accordance with Leon et al. (2004), animals were given a week to recover before temperature trials began. Paper towels lining the cages were replaced with sawdust after the recovery period.

Core and skin temperatures

The mice were placed in cube-shaped Perspex chambers (476 mℓ) within a temperature-controlled cabinet. Air was pushed through the respirometers with a pump and mass-flow controller (Sable Sytems) and regulated at a constant rate of ~ 500 mℓ.min^{-1}. The rats were measured for 1.5 – 3 h at each T_a during their rest phase (day) between 05h00 and 18h00, and individuals were measured at not more than two high T_as (> 25˚C) daily. The animals were measured individually at each T_a (5˚, 15˚, 25˚, 29˚, 33˚, 37˚, 38˚, 39˚, 40˚ and 41˚C) sequentially in order to induce 'chronic thermotolerance' i.e. thermal resistance following exposure to temperatures greater than normal body temperature mammals (Lepock, 2003). Metabolic rates at the lower temperatures (<33˚) were measured in order to determine the TNZ and T_{lc} of the species.

Thermal signals from the PIT tags were detected using an FS2001ISO portable reader attached to a racquet antenna (Destron Fearing) which was placed adjacent to the respirometers in the temperature-controlled cabinet. T_{core} was recorded by the modified iButtons at 10 minute intervals.

Chamber temperatures

DS1922LThermochron iButtons were taped to the floor of each respirometer in order to measure chamber temperatures every 10 mins during each temperature trial. These values were taken as the T_a at which the metabolic rates were measured.

Statistical analysis

A repeated measures analysis would have been the most appropriate statistical analysis for the data (Zar 1984). However, the premature failure of a number of data loggers reduced the sample size from n = 14 to n = 6 and precluded this analysis. Time courses of T_{core} and T_{sub} at the various T_as for each individual were graphed (Fig 5). Where possible, the point where T_{sub}

reached thermal equilibrium was determined as the point of inflection where T_{sub} attained an asymptote. The point of inflection was determined using a piecewise regression analysis (Macro in Excel written by BGL and following Yeager and Gordon (1989)). The T_{sub} and T_{core} data at each T_a were calculated as the mean of all values measured after the point of inflection.

We removed inter-individual autocorrelation effects (i.e. the relationship between the body temperatures of individuals at different points in time) by calculating the mean T_{core} of all individuals for each temperature (T_a) treatment. Each individual T_{sub} at each T_a was then subtracted from the mean T_{core} value to obtain a measure of the gradient between T_{core} and T_{sub}. We used a Bonferroni-adjusted t-test to determine if the difference between the group mean T_{core} and $T_{sub,}$ differed from zero. To test for individual and T_a effects on the dependent variables (T_{core} and T_{sub}), we ran a 2-factor ANCOVA with T_a as the covariate and individuals as the dependent variable to determine if there were individual differences in T_{core} and T_{sub} and to determine the influence of T_a on T_{core} and T_{sub}. Paired samples t-tests were used to determine if there were differences between T_{core} and T_{sub} at the various T_a's. Values were reported as mean ± SD and all analyses were performed using IBM SPSS Statistics version 19 with significance for all tests assessed at $p < 0.05$.

Results

Observations of mice in the respirometers indicated that they rested after the first half hour to an hour in the chamber and remained so for most of the time except for occasional short periods of activity. At low temperatures (< 24˚C) they were observed to curl up with piloerected fur. At high temperatures (> 37˚C) they were observed to lick their fur until it was matted and they became completely inactive and lay on their sides in a stretched out position on the respirometer floor or pressed up against the sides of the respirometers in a position that presumably increased surface area for heat loss.

The average T_{core} of *Aethomys namaquensis* (n = 6) ranged from 36.1 ± 2.30˚C at T_a = 5˚C to 40.21 ± 0.80˚C at T_a = 41˚C. Similarly, average T_{sub} ranged from 34.65 ± 2.16˚C at T_a = 5˚C to 40.77 ± 0.48˚C at T_a = 41˚C. There was a significant, positive linear relationship between T_{core} and T_{sub} for *A. namaquensis* ($F_{1, 6}$ = 223.06, p < 0.001; Fig 1) which can be described by the equation $T_{sub} = 1.17T_{core} – 6.85$ ($r^2 = 0.73$). The difference between T_{core} and T_{sub} was relatively low and was not significantly different from zero ($t_{56, 6}$ = 0.33, p = 0.76; Fig 3).

There were no individual differences between T_{core} and T_{sub} (T_{core}: $F_{5, 6}$ = 2.36, p = 0.05; T_{sub}: $F_{5, 6}$ = 1.48, p = 0.21). There was, however, a significant influence of T_a on T_{core} and T_{sub} (T_{core}: $F_{1, 6}$ = 48.91, p < 0.001; T_{sub}: $F_{1, 4}$ = 120.63, p < 0.001). We found that, at temperatures below the lower thermal critical limit (T_{lc}) $T_a < 29^{\circ}$C (See chapter 4), T_{sub} underestimated (i.e. was lower than) T_{core} and at temperatures above the upper critical limit of thermoneutrality (T_{uc}) ($T_a > 33^{\circ}$C), T_{sub} overestimated (i.e. was higher than) T_{core}. The $T_{core} – T_{sub}$ difference was minimal within the thermoneutral zone (TNZ) ($29^{\circ} \leqq T_a \leq 33^{\circ}$C) ($T_{core} - T_{sub} = - 0.34 \pm 1.61^{\circ}$C; Fig 3, 4Despite these temperature differences however, we found no significant differences between T_{core} and T_{sub} at the different ambient temperatures ($t_{56, 4}$ = 0.16, p = 0.88).

Discussion

We found no significant difference between T_{core} and T_{sub} over the entire range of T_as measured (5˚C - 41˚C). Similarly, Brown and Bernard (1991) found subcutaneous temperature to be an accurate measure of T_{core} in Schreiber's long-fingered bats, *Miniopterus schreibersii* and Cape horseshoe bats, *Rhinolophus capensis*. These findings are similar to those previous studies testing T_{skin} as a proxy for T_{core} (Audet and Thomas 1996; Barclay et al., 2000, Willis and Brigham 2003). This study therefore shows that T_{sub} for *Aethomys namaquensis* can be used as a proxy for T_{core} at certain T_as, at least during the rest-phase.

Similar to the T_{skin} studies (Audet and Thomas 1996; Barclay et al., 2000; Willis and Brigham 2003), we found that the relationship between T_{core} and T_{sub} was dependent on T_a. In contrast to previous bat studies, however, where T_{skin} and T_{core} differed by as much as 6˚C (Audet and Thomas, 1996; Barclay et al., 1996; Willis and Brigham, 2003), we found that the average difference between T_{sub} and T_{core} never exceeded 1.59˚C ± 2.69˚C, whichoccurred at T_a = 5˚C

Within the TNZ (29˚C $\leq T_a \leq$ 33˚C), the $T_{core} – T_{sub}$ differential was at its minimum (mean: -0.35˚ $\pm$ 0.16˚C), probably because at these T_as heat generated from the metabolism of the mice adequately maintains the T_b setpoint (Canon and Nedergaard, 2010; Schmidt-Nielsen, 1990; Withers, 1992). As a result, the rate of heat loss and heat gain is equal and T_{sub} and T_{core} values are indistinguishable. Moreover, similar to Audet and Thomas, (1996), we found that the T_{core} - T_{sub} differences were much higher at the lower temperatures (range: 1.59 ± 2.69˚C at T_a = 5˚C to 0.38 ± 0.68˚C at T_a = 24˚C) than at the higher temperatures (range: -0.81 ± 1.18˚C at T_a = 37˚C to -0.74 ± 0.48˚C at T_a = 41˚C).

Our findings that T_{sub}, despite not being significantly different from T_{core}, underestimated T_{core} at the lower temperatures (below T_{lc}), was similar to those of studies that measured T_{skin} and T_{core} (Audet and Thomas, 1996; Barclay et al., 1996; Kobbe et al., 2011; Willis and Brigham, 2003). This thermal gradient may reflect the mechanisms of heat production used to defend the setpoint T_b at low temperatures.

Blood carries heat from the core of the body to the surface via arterio-venous shunt vessels to skin capillaries on the surface of the body (Rowoand, 1992). At low temperatures, the temperature control centre in the hypothalamus is triggered by the lowered temperatures of the blood passing through it. To maintain the T_b setpoint, therefore, nerve impulses from the skin receptors travel to the heat gain centres of the hypothalamus which results in the vasoconstriction of the peripheral blood vessels. Vasoconstriction subsequently results in the reduction of the rate of blood flow to the surface (Rowoand, 1992). At the same time, nerve signals travel to the heat gain centre to increase the rate of heat production by increasing metabolism (Rowoand, 1992; Schmidt-Nielsen, 1990; Withers, 1992). The combination of these two responses may explain the low subcutaneous temperatures observed in *A. namaquensis* at the low temperatures. At the lowest temperatures ($T_a \leq 10^{\circ}C$), the $T_{core} - T_{sub}$ gradient was probably augmented by the pilomotor response which would have reduced the rate of dry heat loss via conduction and convection thereby further lowering T_{sub} (Withers, 1992). Simultaneously, shivering thermogenesis which involves the generation of heat via the hydrolyzation of ATP due to involuntary muscle contractions (Canon and Nedergaard, 2010) would have increased heat production raising T_{core}.

Again, despite a non-significant difference between T_{core} and T_{sub}, there was an overestimation of T_{core} by T_{sub} at the higher T_as ($\geq$ 37˚C). This thermal gradient at the higher T_as may also be a reflection of the mechanisms of heat loss, activated by heat stress, that are used to defend a T_b setpoint and prevent entry into pathological hyperthermia. In response to high temperatures, nerve impulses from the heat loss centres in the hypothalamus travel to the skin resulting in the augmentation of blood circulation to the skin and the dissipation of excess stored heat (Rowoand, 1992; Schmidt-Nielsen, 1990). Stored heat can be lost via conductance, convection, radiation or evaporative cooling. However, evaporative cooling can only occur when T_a is high enough to result in the effective evaporation of sweat from the surface of the skin. As reflected in the trace of T_{core} which crosses the line of thermal equilibrium ($T_{core} = T_a$) (Fig 2), *A. namaquensis* effectively employed evaporative cooling at the highest T_as ($\geq$ 40˚C) to maintain $T_{core} < T_a$ at sublethal levels. The peripheral cooling of the blood that re-enters the core will lower core temperatures probably resulting in lower T_{core} values than T_{sub}.

It is important to note that T_{sub} did not reach a state of equilibrium at the high temperatures. Thus, these measures of T_{sub} may be slightly underestimated. We found that the thermal gradient between T_{core} and T_{skin} was much higher at the lower temperatures ($\leq$ 25˚C) that at the high temperatures ($\geq$ 37˚C). Similar to Audet and Thomas (1996) we ascribe this to the high normothermic T_bs maintained by mammals.

Conclusion

Surgically-implantable, temperature-logging devices cannot be used on small (< 20 g) free-ranging mammals because of the weight and physical dimensions of the devices. As an alternative, T_{skin} is often measured as a proxy for T_b using small, external collar- or shoulder-mounted devices. In this study we have shown that T_{sub} can be used as a proxy for T_{core} in the laboratory. Although average T_{sub} explained 74% of the variation in average T_{core}, the T_{sub} – T_{core} differential does show increases, albeit not significant, outside the TNZ. T_{sub} tends to slightly underestimate T_{core} at $T_a < T_{lc}$, and overestimate T_{core} at $T_a > T_{uc}$. Nevertheless, the T_{sub} - T_{core} differential was never significantly different from zero at T_as between 5˚C and 41˚C, suggesting that the subcutaneous transmitters may represent reasonable valid measures of $T_{core.}$

This study suggests that T_{sub} may be a better representative of T_{core} than T_{skin}. Whereas, some studies have shown T_{core} - T_{skin} differences of up to 6˚C, our study showed that the T_{core} – T_{skin} differential never exceeded 1.59˚C, over a wide range of temperatures (5˚C – 41˚C). Moreover, some degree of error is inevitable in the T_{skin} measurements of externally placed data loggers due to the effects of the external environment on the data loggers (Willis and Brigham, 2003). However, this problem is avoided with subcutaneously injected temperature sensitive devices. There may therefore be a promising future for the use of T_{sub} as a proxy for T_b in free-ranging mammals.

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Figure legends

Figure 1 Time course graphs of ambient T_a of one individual taken over a range of 3 hours for the lowest temperature to 1.5 hours for the highest temperatures. Closed circles represent core temperatures whereas open circles represent subcutaneous temperatures.

Figure2 The linear relationship (solid line) between T_{sub} as a function of T_{core} of *A. namaquensis* (n =6) over a range of T_as. The dotted line represents thermal equilibrium between T_{sub} and T_{core}, i.e. $T_{sub} = T_{core}$

Figure 3 The mean $\underline{+}$ SD of T_{core} and T_{skin} at different ambient temperatures in *Aethomys namaquensis.* Closed symbols represent T_{core} and open symbols represent T_{skin}

Figure 4 Differences between the treatment group T_{core} and individual T_{skin} for six *Aethomys namaquensis*. The data were divided into the different T_a repeated measure groups: 5°, 15°, 25°, 29°, 33°, 37°, 38°, 39°, 40°, and 41^{o}C. Values > 0 (dashed line) indicated an over estimation of T_{core} by T_{skin} and values < 0 indicated an underestimation of T_{core} by T_{skin}

Figure 5 Differences between the treatment group T_{core} and individual T_{skin} for four *Aethomys namaquensis*. The different T_a repeated measure groups were divided into three sections: below T_{lc} (< 33°C), TNZ ($33^{\circ} < T_a < 37^{\circ}$C) and above T_{uc} (> 37°C). Values > 0 (dashed line) indicate an over estimation of T_{core} by T_{skin} and values < 0 indicate an underestimation of T_{core} by T_{skin}

Figure 1

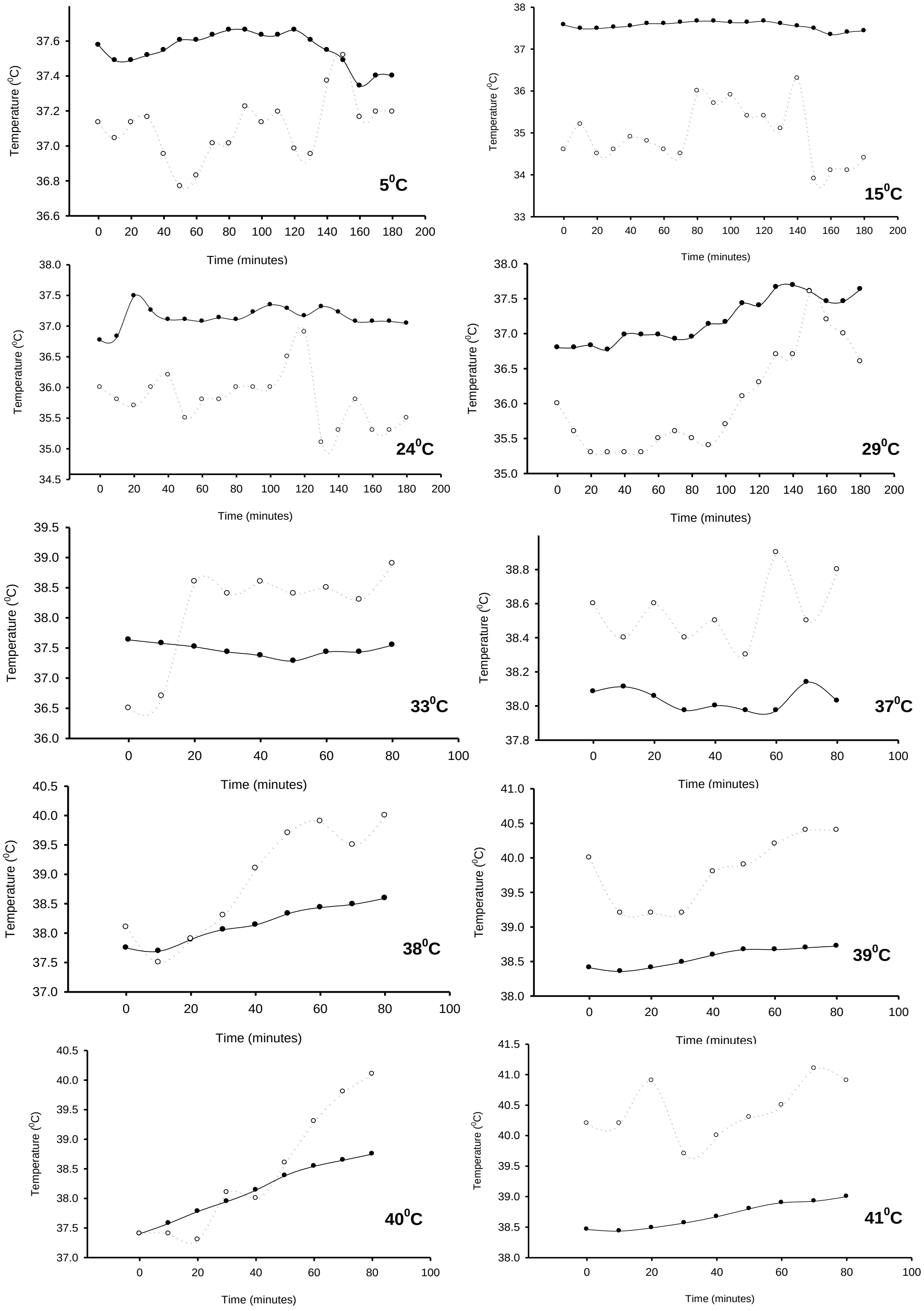
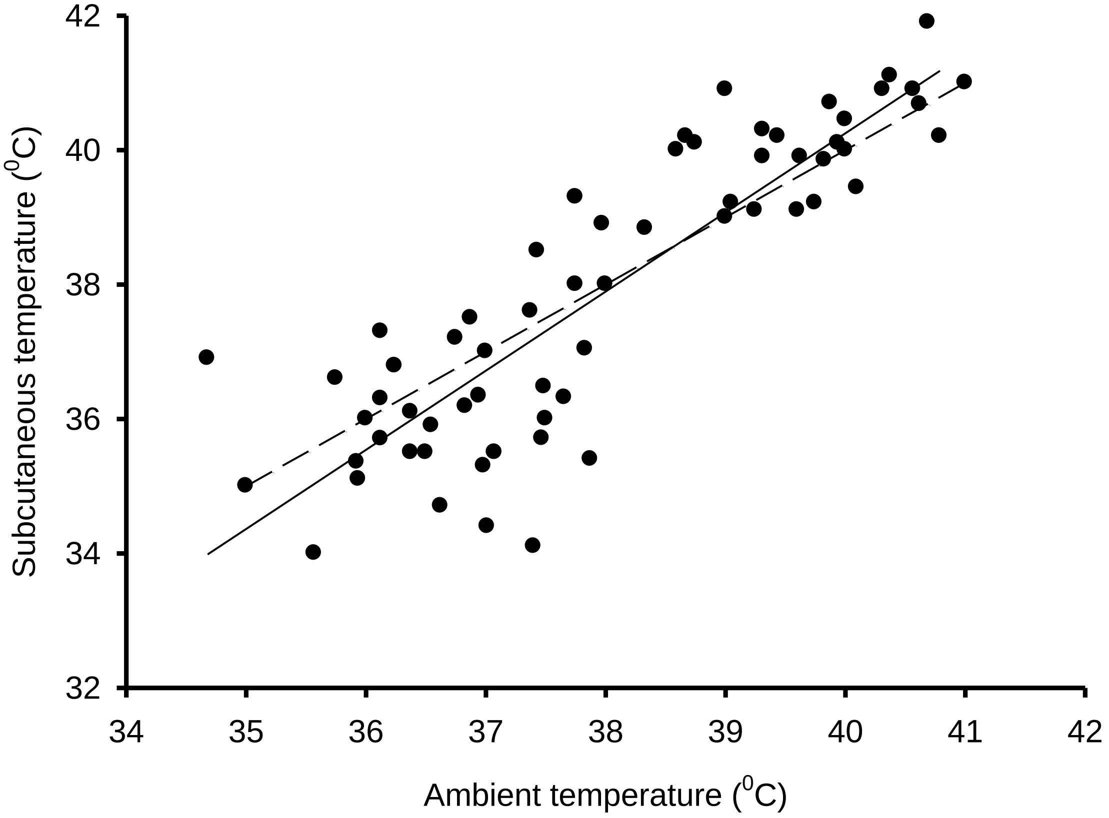

5⁰C
15⁰C
24⁰C
29⁰C
33⁰C
37⁰C
38⁰C
39⁰C
40⁰C
41⁰C
Temperature (⁰C)
Time (minutes)
0
20
40
60
80
100
120
140
160
180
200

Figure 2



Subcutaneous temperature (^{0}C)
Ambient temperature (^{0}C)
42
40
38
36
34
32
34
35
36
37
38
39
40
41
42

Figure 3

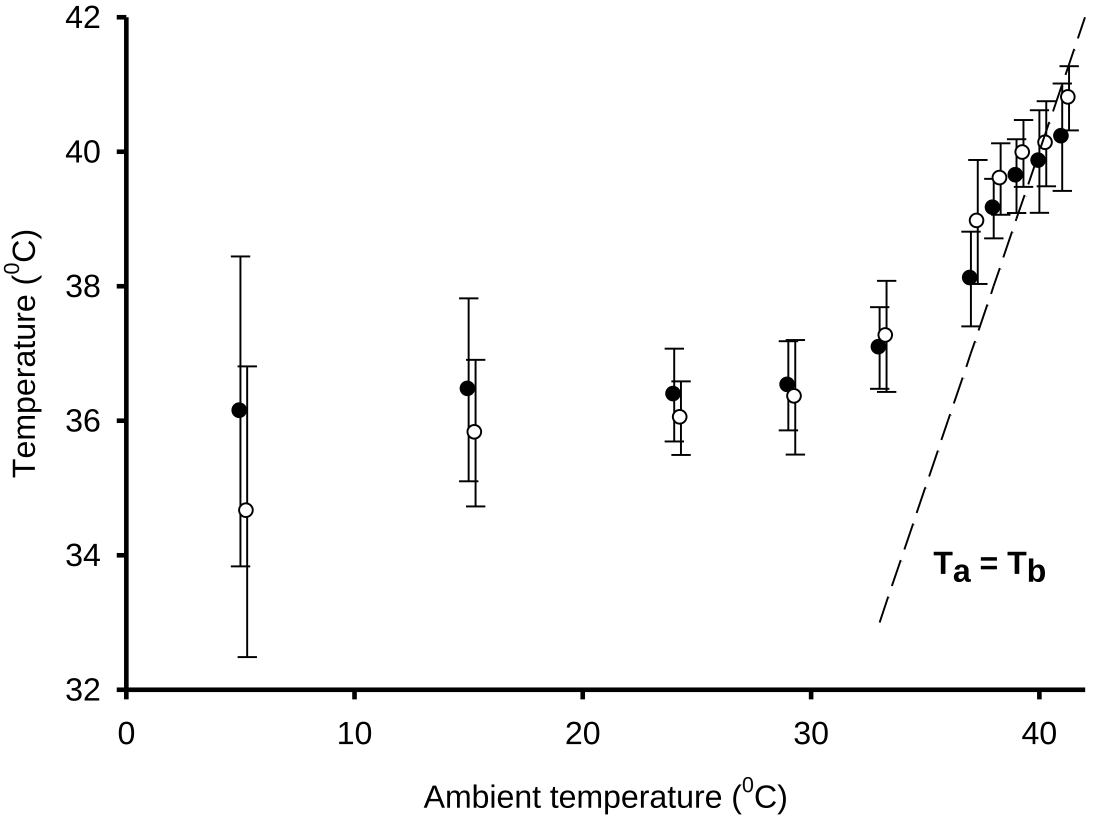

Temperature (^{0}C)
42
40
38
36
34
32
0
10
20
30
40
Ambient temperature (^{0}C)
$T_a = T_b$

Figure 4

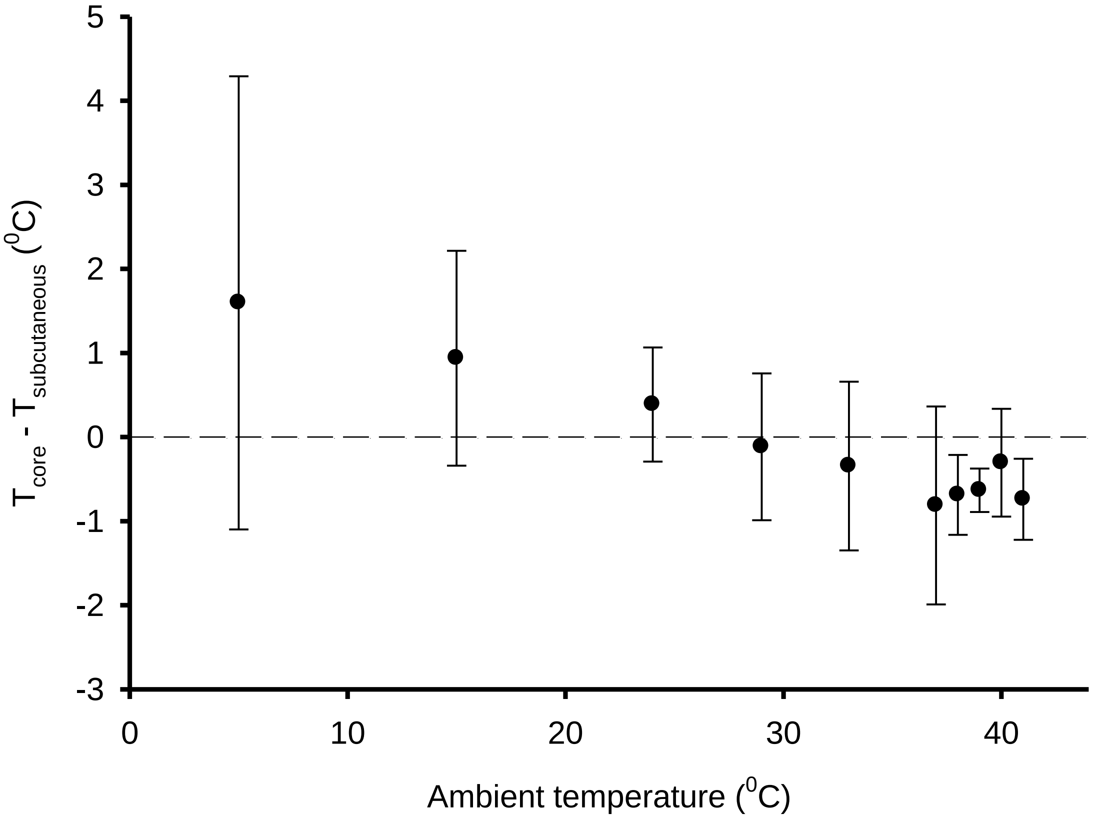
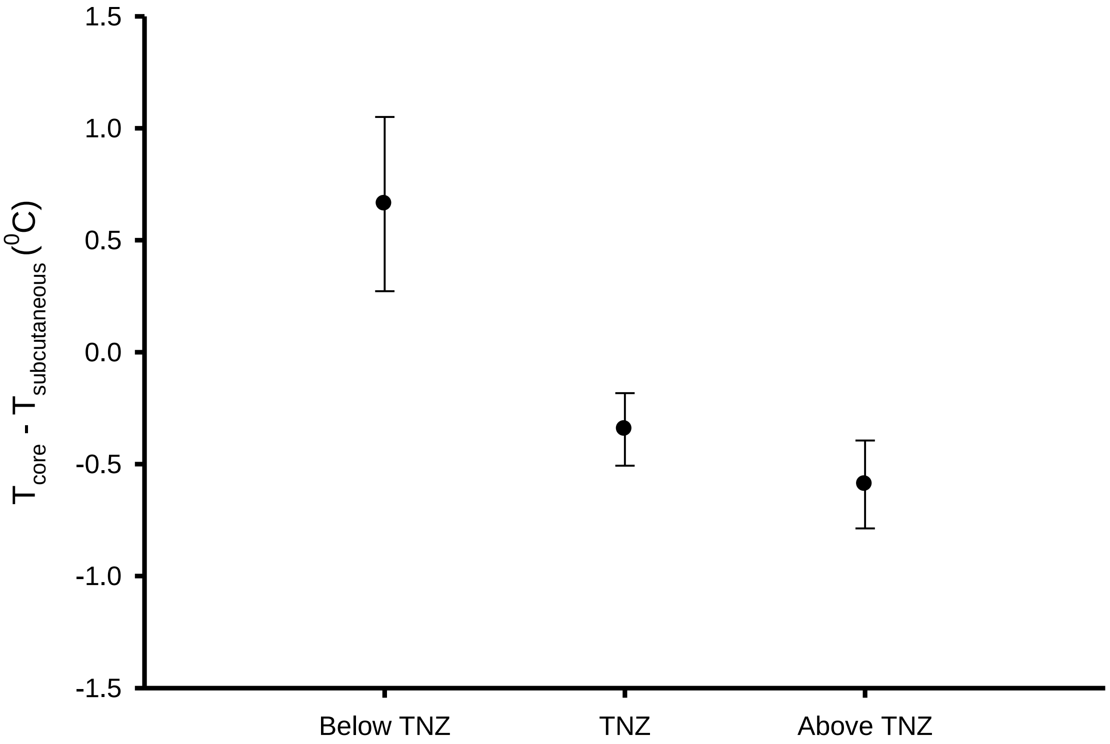

5
4
3
2
1
0
-1
-2
-3
T_{core} - $T_{subcutaneous}$ (^{0}C)
0
10
20
30
40
Ambient temperature (^{0}C)

Figure 5


1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
T_{core} - $T_{subcutaneous}$ (^{0}C)
Below TNZ
TNZ
Above TNZ

Chapter 4

Hyperthermic torpor: Temperature-induced hypometabolism in desert rodents, the Namaqua Rock Mouse, *Aethomys namaquensis* and the pygmy rock mouse, *Petromyscus collinus*

M.O Mowoe, Lovegrove, BG, Levesque D

Abstract

Individual responses of organisms to accelerated climate change may be better understood by further studying their physiology. Mechanisms of phenotypic plasticity may offset the effects of climate change and are therefore central to the formulation of predictive climate change models. Employing Krogh's principle, desert mammals which have reaction norms adapted to a wide range of climatic conditions are good models for determining the mechanisms of phenotypic plasticity that may be expressed in response to accelerated climate change. This study tested the 'Hyperthermic Daily Torpor' hypothesis proposed recently by Lovegrove et al., (in press) that some mammals are capable of hypometabolism in response to high temperatures which can offset the energetic costs of the Arrhenius effect of temperature on metabolism. Two presumably heat-adapted desert rodents; the Namaqua rock mouse, *Aethomys namaquensis* (~47.86 g) and the pygmy rock mouse, *Petromyscus collinus* (~17.33 g) were used as models species. *Aethomys namaquensis* showed hyperthermia-induced of hypometabolism (Q_{10} = 1.27 ± 1.61) whereas the pygmy rock mouse did not (Q_{10} = 2.45 ± 1.41). Such a physiological response in *A. namaquensis* is crucial in terms of its capacity to minimize the risks of lethal, pathological hyperthermia. The disparity of the physiological responses of the two species cannot be interpreted meaningfully without phylogenetically independent

multi-species analyses. Nevertheless, we propose a few behavioural and autecological sketches that may assist in future interspecific multivariate analyses.

Keywords Arrhenius (Q_{10}) effect ·climate change· hyperthermia· hypometabolism · Metabolic down-regulation· physiological response

Abbreviations

MR:	Metabolic rate (mℓ O_2 g^{-1} h^{-1})
T_b:	Body temperature (˚C)
T_a:	Ambient temperature (˚C)
TNZ:	Thermoneutral zone
HDT:	Hyperthermic daily torpor
Q_{10}:	A simplified temperature coefficient derived from the Arrhenius equation of the effects of temperature on metabolic rates
BMR:	Basal metabolic rate (mℓ O_2 g^{-1} h^{-1})
RER:	Respiratory exchange ratio
REWL:	Respiratory evaporative water loss

Introduction

Species responses to recent climate change have become a topic of increasing interest for evolutionary physiologists worldwide (Fuller et al., 2010). It has become clear that individual responses to climate change can only be understood better by further, more in-depth studies of physiological functions, such as metabolic rate (MR) and body temperature (T_b), which drive organisms (Chown et al., 2010; Fuller et al., 2010; Ricklefs and Wikelski, 2002; Sinervo and Svensson, 1998; Williams et al., 2008). For example, a common question is; to what extent will organisms be able to adjust physiologically to accelerated, global warming?

Phenotypic plasticity is defined as the ability of a genotype to express a variety of phenotypes in response to environmental variation and is adaptive when it maintains the fitness of the individual in spite of rapidly changing environments (Price et al., 2003; Visser 2008). Phenotypic plasticity involving phenotypic flexibility (Piersma and Drent, 2003) may offset the effects of increased unpredictability brought about by climate changes. The measurements of phenotypic plasticity are thus central to the formulation of predictive climate change models.

Physiological responses to unpredictable climates, and the resource availability consequences thereof, are particularly well understood in desert-dwelling mammals (Schwimmer and Haim, 2009). Desert habitats are defined by extreme fluctuations in ambient temperature (T_a) and a low and highly variable precipitation resulting in a scarcity of resources (food and water) (Noy-Meir, 1973). The reaction norms of desert mammals are assumed to reflect adaptation to a wide range of climatic extremes associated with the unpredictability of desert environments. Adaptations are evident in the various behavioural (Brown et al., 1979; McMillen 1972; Murray and Dickman, 1994) and physiological (Schwimmer and Haim, 2009) specializations utilized by desert mammals. Employing the Krogh principle (Bennett 2003), desert mammals should therefore be good models for the quantification and appreciation of reaction norms that are essential in terms of our understanding of the mechanisms and scale of phenotypic plasticity likely to be expressed with recent accelerated climate change (Canale and Henry 2010).

However, reliance upon reaction norms in response to climate change is insufficient to prevent extinctions in certain species in particularly susceptible habitats. Indeed, extinctions due to elevated T_as brought about by recent climate change are already occurring (Franco et al., 2006; McKechnie et al., 2012; Parmesan and Yohe, 2003; Pounds et al., 1999; Thomas et al., 2006). Particularly vulnerable species include desert, insular, and high-latitude specialists, and those inhabiting anthropogenically-fragmented areas from which migration options are limited or not possible (Haim and Izhaki, 1994; Honnay et al., 2002, Sekercioglu et al., 2012).

Theoretical working models that attempt to conceptualize endotherm responses to climate change are in the formative stages and rely at present on modified performance curves originally conceived to model ectotherm responses to climate change (Angilletta et al., 2010). Endotherm performance curves predict contrasting generalist and specialist responses to climate change (Angilletta et al., 2010; Buckley 2008). Some endotherms may, however be able to deviate from the reaction norm and adjust their thermal performance allowing for some degree of thermal tolerance at high temperatures (Boyles et al., 2011; Mkechnie et al., 2006, 2007).

In addition to the incorporation of phenotypic flexibility into predictive climate change models, it is also important to verify our understanding of the kinetic responses of endotherms to high T_as. The temperature dependence of physiological variables is particularly well understood in ectotherms, simply because their T_bs are influenced directly by T_a. For example, in a sample of 309 species, rate increases in a diversity of traits varied by multiples of 1.31 and

5.13 over a 10^{o}C increase in T_a (Dell et al., 2011). In endotherms, though, the understanding of temperature dependence is generally ignored, because endotherms theoretically maintain a T_b independent of T_a. The T_b of endotherms is influence by T_a during adaptive hypothermia (torpor and hibernation) (Geiser and Ruf, 1995), during pathological hypothermia, or at T_as above the upper critical limit of thermoneutrality. It is the latter potentiality which is the focus of this study.

In a typical ectotherm, as T_a increases MR should increase according to the Arrhenius (Q_{10}) effect of temperature on biochemical processes (Withers, 1992). The Arrhenius effect is quantified with the following equation:

$$\ln\left(\frac{MR_1}{MR_2}\right) = -\frac{Ea}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$ Equation 1 (Withers, 1992)

where MR_1 and MR_2 are metabolic rates at body temperature (T_bs) of T_1 and T_2, respectively. Ea is the apparent enthalpy of activation, and R the gas-constant (8.314 J K^{-1} mol^{-1}). The formula for the temperature coefficient, Q_{10}, is widely used as a measure of the Arrhenius effect on rate processes:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\overline{\frac{10}{(T_2 - T_1)}}}$$ Equation 2 (Withers, 1992)

where R_1 and R_2 are rate processes at T_1 and T_2, respectively. The Q_{10} value has been found to be between 2 - 3 for every 10^{o}C increment in endotherms (Schmidt-Nielsen, 1990; Withers, 1992), although as mentioned earlier, the maximum range is between 1.31 and 5.13 for all organisms (Dell et al., 2011).

In a hyperthermic state, there is no reason to expect that an endotherm should not display $Q_{10} > 2$, provided that no pathological, cellular heat damage has occurred. However, exceptions to this rule have been found (Barthelomew and Rainy, 1971; Weathers and Schoenblacher 1976). In a study on the body temperature regulation of the rock hyrax *Heterohyrax brucei,* Bartholomew and Rainey (1971) found an anomalous, albeit slight, reduction in MR in conjunction with a hyperthermic response at high T_as ($35^{\circ}C \leq T_b \leq 42.5^{\circ}C$). They concluded that the hyperthermia-induced metabolic downregulation enhanced the rate of evaporative heat loss thereby offsetting the Arrhenius. Weathers and Schoenblaechler (1976) found similar MR reductions within the thermoneutral zone (TNZ) of budgerigars*, Melopsittacus undulates,* despite a ~ $4^{\circ}C$ increase in T_b. They associated the MR dependent changes in T_b with adaptations to a small body size rather than to a hot climate as the phenomena was found only in birds weighing < 150 g.

Lovegrove et al., (in press) recently proposed a concept of hyperthermic daily torpor (HDT), in the form of temperature-induced hypometabolism. These authors provide some, albeit limited, evidence to show that hypometabolism equivalent to that which occurs during daily torpor and hibernation, may occur during hyperthermia to offset the high energetic consequences of Arrhenius effects. They argued that, by reducing MR, the $T_b - T_a$ gradient is minimized thus reducing entry into pathological heat stress.

In this study, we tested the HDT model using two desert mammals from Namaqualand, the Namaqua rock mouse, *Aethomys namaquensis,* and the pygmy rock mouse, *Petromyscus collinus* (Chapter 2). Specifically, we tested the hypothesis that the hyperthermic responses in these mammals should involve a concomitant metabolic down-regulation resulting in values of $Q_{10} < 2$.

Materials and Methods

Animals

Aethomys namaquensis (47.86 ± 6.65 g, n = 14) and *Petromyscus collinus* (17.33 ± 1.67 g, n = 12) were trapped using Sherman live traps baited with peanut butter and oat balls on the farms Roopersfontein at Keimoes (28˚69'S, 21˚17'E) and Norriseep at Onseekpans, (28˚76'S, 22˚42'E), Northern Cape, South Africa, during March/April 2012. Permission for the trapping, transporting and holding of the rodents had previously been received from Ezemvelo Wildlife and the Northern Cape Department of Environment and nature conservation. Each rodent was weighed and sexed immediately after capture and housed individually in cages (380 x 220 x180 mm) under a 16:8 LD photoperiod (lights off CAT 21h00). They were maintained on a diet of sunflower seeds supplemented with rodent pellets with water *ad libitum*.

At the conclusion of trapping, the rodents were transported, by road, to the UKZN animal house in Pietermaritzburg. The rodents were maintained, individually, in sawdust lined cages in a control room at a constant temperature of ±25⁰C. Cages were cleaned and sawdust was changed once a week.

Surgical procedure

All animals were implanted with temperature sensitive data loggers (DS1922L Thermochron iButtons, Dallas semiconductor, resolution 0.625°C, Maxim Integrated) modified according to Lovegrove (2009) and weighing no more than 2.5 g. Each temperature data logger was individually calibrated and programmed to record temperature every 10 mins, 24 h a day. Waxed iButtons were surgically implanted into the peritoneal cavity of the rock rats to measure $T_{b.}$

Prior to the date of surgery, all surgical equipment and disposables were autoclaved (120°C, 45 minutes) and telemeters were sterilized in 70% ethanol. Inhalation anaesthesia was induced at 3% isoflurane (Safeline Pharmaceuticals (PTY) LTD) in oxygen and maintained throughout the duration of the surgery at 1.5% isoflurane in oxygen.

The animals were placed on a hot water bottle for the duration of surgery to avoid hypothermia. The surgical area was prepared by shaving a small area of the stomach and preparing it with 70% alcohol and Betadine antiseptic (10% Povidone-Iodine), after which the animal was covered with a surgical drape. Incisions were made in the skin and peritoneum and the iButton inserted. The iButtons were sterilized in 70% alcohol and in betadine before implantation. The peritoneum and skin were sutured separately using coated Johnson and Johnson Vicryl rapide (Ethicon, polyglactin 910) absorbable sutures and coated Johnson and Johnson Silk (Perma-hand) non-absorbable sutures, respectively. The Bio-Therm PIT tags (± 0.2 g) were injected subcutaneously into the nape region of the rats.

Following surgery, animals were housed individually in clean cages lined with paper towels and allowed to recover at 27°C in a temperature-controlled cabinet for a few hours before being returned to their maintenance housing housing. In accordance with Leon et al. (2004), animals were given a week to recover before temperature trials began.

Respirometry

Metabolic rate (oxygen consumption, $\dot{V}O_2$; carbon dioxide, $\dot{V}CO_2$) was measured using flow-through respirometry for individuals that had been fasted for at least 4 h prior to the commencement of data measurement. The mice were measured for 1.5 – 3 h at each T_a during their rest phase (day) between 05h00 and 18h00 and individuals were measured at not more than two high T_as (> 25°C) daily. Each temperature was measured no less than 30 mins apart from each other in order to enable recovery.

Outside air was scrubbed of water and CO_2 using a Sable Systems PC-4 Peltier effect condensing air dryer and a soda lime scrubber (Merck (PTY) LTD, respectively, and any excess water vapour was scrubbed using a silica gel column. Mass-flow controllers (Sable Systems) regulated the flow of incurrent air at a constant rate of 500 $mℓ.min^{-1}$ for *A. namaquensis* and 400 $mℓ.min^{-1}$ for *P .collinus,* through cube-shaped Perspex respirometry chambers (476 mℓ), located in a temperature-controlled cabinet. Individuals were measured at each T_a (T_a = 5°, 14°, 24°, 29°, 33°, 37°, 38°, 39°, 40° and 41°C) sequentially in order to induce ‘chronic thermotolerance’ i.e. thermal resistance following exposure to temperatures greater than normal body temperature (Lepock, 2003). Metabolic rates at the low temperatures (< 33°) were measured in order to determine T_{lc} and TNZ. To compensate for the baseline drift of the analyzers, the O_2 and CO_2 of an empty respirometer were measured every 15 min and these values were subtracted on-line from the subsequent samples. Air leaving the chambers was subsampled (200 mℓ min^{-1}) before being passed through a Sable Systems relative humidity meter (RH-300) and subsequently dried using a dessicant (Drierite anhydrous Na_2SO_4) and passed through a carbon dioxide (CA-10a) and oxygen (FC-10) analyzer (Sable Systems). Oxygen and carbon dioxide concentrations were measured continuously and average values were stored every 5 s. $\dot{V}O2$ was quantified as the oxygen consumption over the lowest 48 consecutive values (4 min). $\dot{V}O2$ was calculated using the following equation:

$$\dot{V}O_2 = [FR * [F_I O_2 - [F_E O_2 * [1 - [F_I O_2 - F_I CO_2 - F_I H_2 0]/[1 - [F_E O_2 - F_E CO_2 - F_E H_2 O]]$$

(Withers, 2001)

The data were recorded by a personal computer (PC) throughout the experimental period using Expedata version PRO release 1.4.8 (Lighton, 2001), a 16-bit data acquisition software. Body mass was measured before and after each metabolic measurement and the mean value was used in further analysis. The respiratory exchange ratio (RER) was calculated as $\dot{V}CO_2/\dot{V}O_2$.

Data analysis

A paired samples t-test was used to determine if there was a significant mass change over the course of all the temperature measurements. The mean of the lowest 48 $\dot{V}O_2$ measurements (4 minutes) and the corresponding $\dot{V}C0_2$ and T_b were calculated for each animal for the last 60 minutes of measurement at each T_a. Statistical analyses were conducted with IBM SPSS Statistics (v. 19). Values were presented as mean ± standard deviation.

The effect of T_a on each physiological variable (MR and Tb) was examined using a repeated measures analysis of variance (RMANOVA), with each individual as a replicate and T_a as the repeated measure (Rencher, 2002). The premature failure of ibuttons reduced our sample size in some cases resulting in insufficient residual degrees of freedom for the RMANOVA. Instead, a covariate analysis of variance (ANCOVA) was used with T_a as the covariate, to determine T_a effects on MR and T_b. In the absence of a *post hoc* tests for a RMANOVA, polynomial *a priori* contrasts were used in order to examine linear changes of MR and T_b (where possible) with T_a. Contrasts were calculated in an Excel spread sheet using a custom-written *a priori* contrast macro based on Rencher (2002) and provided by Withers and Cooper (2011).

Results

Body mass during experimentation

Aethomys namaquensis

The mean body mass of *A. namaquensis* at the start of the metabolic trials was 57.00 ± 9.39 g (n = 11) and at the end it was 53.87 ± 9.35 g . There were mass differences between individuals (range 77.46 – 48.25 g; $F_{10, 11} = 7.21$, p = 0.002). There was however, no significant change in the mass of the individuals over the nine temperature measurements ($F_{10, 11} = 2.52$, p = 0.14) (Fig. 1a).

Petromyscus collinus

The mean body mass of *P. collinus* at the start and end of the metabolic trials was 16.51 ± 0.88 g and at the end it was 16.98 ± 1.03 g (n = 6). There were mass differences between individuals (range 17.40 – 15.35 g; $F_{5, 6}$ = 7.11, p = 0.03). There was, however no significant change in the mass of the individuals over the nine temperature measurements ($F_{5, 6}$ = 0.99, p = 0.37) (Fig. 1b).

Behavioural observations

In captivity, *P. collinus* were observed to hardly ever drink water whereas *A. namaquensis* was observed on several occasions to drink. We also found that, although both mice were fed a diet of sunflower seeds and rodent pellets, the pygmy rock mice only ate the sunflower seeds. Moreover, whereas *A.namaquensis* was observed to deplete the entire daily ration of food provided, the *P. collinus* was never observed to completely deplete its food supply. Weights from capture to the start of experimentation for the two species ranged from 47.07 ± 8.23 g to 57.00 ± 9.39 g (n = 11) for *A. namaquensis* and from 17.50 ± 1.38 g to 16.98 ± 1.03 g (n = 6) for *P.collinus.* These data suggest that *P.collinus* is capable of controlling energy intake whereas *A. namaquensis* seems to be more opportunistic in terms of energy intake.

Thermoregulatory observations

Aethomys namaquensis

Observations of *A. namaquensis* in the respirometers indicated that they rested after the first half an hour to an hour in the chambers and remained so for most of the time except for occasional short periods of activity that corresponded to slight increases in $\dot{V}O_2$ and $\dot{V}CO_2$. At low temperatures (< 24°C) they were observed to curl up and display piloerection. At high temperatures (> 37°C) they were observed to lick their fur until it was matted and they became almost completely inactive and lay on their sides in a stretched out position on the respirometer floor or pressed up against the sides of the respirometers in a position that increased surface area for heat loss.

Petromyscus collinus

Observations of *P. collinus* showed that they calmed down within half an hour of being placed in the respirometers and rested and sometimes appeared to sleep for most of the time except for a few short bouts of activity indicated by elevations of $\dot{V}O_2$ and $\dot{V}CO_2$. At low temperatures ($T_a \leq 20$°C), animals also assumed a curled up position and showed piloerection. Shivering thermogenesis commenced at $T_a = 15$°C. At $T_a \geq 33$°C the mice were observed to stretch out on the chamber floor or on the sides of the chamber and their fur was more depressed. At the highest temperatures ($T_a \geq 35$°C) the mice were observed to assume an inactive/ lethargic state and fur was matted from licking of their fur however.

Body temperature

Aethomys namaquensis

T_bs ranged from 35.82 ± 1.02°C at T_a = 5°C to 39.99 ± 0.65°C at T_a = 41°C (n = 6) (Fig 2). At our closest estimate of T_{lc} (29.04°C), T_b = 36.99°C, more than 1°C higher than that (T_b = 35.80°C) measured by Lovegrove et al. (1991). Individuals did not differ with respect to T_b ($F_{5, 6}$ = 1.06, P = 0.39) at the different temperatures but there was a significant effect of T_a ($F_{1, 6}$ = 103.34, P < 0.001). The pattern of T_b versus T_a (Fig. 2) suggested that the onset of a hyperthermic response occurred at T_a = 37°C in *A. namaquensis*. In support of this observation we found that when T_as $\geq$ 37°C were removed from the statistical analyses, there was no longer an effect of T_a on T_b ($F_{5, 6}$ = 2.35, p = 0.09). Moroever, at $T_a \geq$ 40°C, the trace of T_{core} crossed the line of thermal equilibrium ($T_{core} = T_a$) (Fig. 2) and at this point T_{core} was lower than T_a.

Petromyscus collinus

T_bs ranged from 35. 61 ± 1.28°C at T_a = 14°C to 39.51 ± 1.31°C at T_a = 39°C (Fig. 3). At our closest estimate of T_{lc} (T_a = 28.62°C), T_b = 34.11°C. We found no individual differences in T_b ($F_{4, 5}$ = 0.62, p = 0.65). However, there was a significant effect of T_a on T_b ($F_{1, 5}$ = 22.29, p < 0.001). The pattern of T_b versus T_a suggested that there was a significant increase in T_b commencing at T_as $\geq$ 35°C. In support of this observation we found that when T_as $\geq$ 35°C were removed from the statistical analyses there was no longer an effect of T_a on T_b ($F_{3, 5}$ = 1.90, p = 0.18). Thus *P.collinus* began to exhibit hyperthermic T_bs from T_a = 35°C. However, unlike *A. namaquensis*, the T_b of *P. collinus* tracked but did not cross the $T_a = T_b$ line of thermal equilibrium.

Although we attempted to avoid cases of pathological hyperthermia, one mouse died when its T_{core} attained a value of 42.68°C at $T_a = 40$°C. We discontinued all measurements at this T_a.

Metabolic rate

Aethomys namaquensis

A RMANOVA ($F_{2.23, 11} = 57.12$, $p < 0.001$) confirmed that there was a significant influence of T_a on $\dot{V}O_2$ across the range of all T_as. The $\dot{V}O_2$ of *A. namaquensis* decreased from 3.13 ± 0.70 mℓ O_2 g^{-1} h^{-1} at $T_a = 5$°C to a minimum of 0.98 ± 0.19 mℓ O_2 g^{-1} h^{-1} ($n = 11$) at $T_a = 33$°C (Fig. 2). This minimal value at $T_a = 33$°C could be considered to be the BMR. However, the calculation and estimate of BMR should theoretically include all $\dot{V}O_2$ values calculated at T_as > 33°C if these values are not significantly different from that at $T_a = 33$°C. This latter mean BMR estimate is 1.23 ± 0.15 mℓ O_2 g^{-1} h^{-1}. We estimated T_{lc} (29.04 °C) to occur at the intercept between the latter BMR estimate and the linear regression fitted to the data below $T_a = 33$°C ($\dot{V}O_2 = -0.08T_a + 3.64$; $r^2 = 0.98$, $p < 0.001$). Linear ($p = 0.05$), quadratic ($p = 0.17$) and cubic ($p = 0.28$) polynomial *a priori* contrasts confirmed that there was no significant increase in $\dot{V}O_2$ at T_as $\geq$ 33°C. The pattern of $\dot{V}CO_2$ was similar to that of $\dot{V}O_2$ and was not analysed separately. The RER was 0.83 $\pm$ 0.06 (mean for all 10 T_as) and, curiously, was dependent on T_a ($F_{9, 11} = 3.23$, $p = 0.02$) and the individual ($F_{9, 11} = 3.04$, $p = 0.02$).

Petromyscus collinus

An ANCOVA ($F_{1,6}$ = 63.58, $p < 0.001$) confirmed that there was a significant influence of T_a on $\dot{V}O_2$ across the range of all T_as. The $\dot{V}O_2$ of *P. collinus* decreased from 5.57 mℓ O_2 g^{-1} h^{-1} at T_a = 15°C to 1.15 mℓ O_2 g^{-1} h^{-1} at T_a = 33°C (n = 6) (Fig. 3). We considered this minimal value at T_a = 33°C to be BMR. However, again, the calculation and estimate of BMR should theoretically include all $\dot{V}O_2$ values calculated at T_as > 33°C if these values are not significantly different from that at T_a = 33°C. This latter mean BMR estimate was 1.40 ± 0.21 mℓ O_2 g^{-1} h^{-1}. We estimated T_{lc} (28.62°C) to occur at the intercept between the latter BMR estimate and the linear regression fitted to the data below T_a = 33°C ($\dot{V}O_2 = -0.28T_a + 9.49$; $r^2 = 0.99$, $p = 0.02$). There were no differences in $\dot{V}O_2$ between individuals ($F_{5,6}$ = 1.62, p = 0.23). The pattern of $\dot{V}CO_2$ was similar to that of $\dot{V}O_2$ and was is not presented separately. The RER was 0.84 ± 0.08 (mean for all 10 T_as) and was dependent on the individual ($F_{5,6}$ = 3.87, p = 0.007) but not on T_a ($F_{5,6}$ = 0.67, p = 0.71).

Effects of body temperature on metabolic rates

We visually determined the onset of hyperthermia at $T_a \geq 37$°C in *A. namaquensis* and at $T_a \geq 35$°C in *P. collinus*. The effect of body temperature on metabolic rate at all T_as at which hyperthermia was exhibited was calculated using the temperature coefficient Q_{10} equation (Schmidt-Nielsen, 1990; Withers, 1992).

Aethomys namaquensis

Aethomys namaquensis had a mean Q_{10} = 1.27 ± 1.61 (n = 6) between T_a = 37°C and T_a =41°C.

Petromyscus collinus

Between T_as = 35°C and T_a = 39°C, *P. collinus* displayed much higher elevations in metabolic rate (Fig. 4b) than *A.namaquensis* (Fig. 4a). The mean Q_{10} was 2.45 ± 1.41 (n = 5).

Discussion

Typically, hyperthermia generates large T_b – T_a gradients which increase the rate of dry heat loss via convective and or conductive means (Calder and King, 1974; Schmidt-Nielsen, 1990; Tieleman and Williams, 1999). A large T_b – T_a gradient reduces the need for heat loss via evaporative cooling which is a more costly mechanism of heat loss and can only be activated at temperatures that are high enough to permit evaporative water loss from the skin surface (Tieleman and Williams, 1999; Weathers and Schoenblaechler, 1976). However, hypometabolism should theoretically reduce this thermal gradient and thereby reduce the potential rate of dry heat loss. Hyperthermia-induced hypometabolism may seem counterintuitive but by downregulating metabolism the risk of entry into pathological hyperthermia is minimized. Moreover, elevations in respiratory evaporative water loss rates (REWL) due to increased metabolic rates with hyperthermia are reduced (Cooper and Withers, 2008; Withers et al., 2010). Thus, hyperthermic daily torpor as defined by Lovegrove et al. (in press) is also a mechanism of conserving water at high ambient temperatures.

In this study we did indeed find evidence of hyperthermia-induced hypometabolism in the form of an unusually low Q_{10} in *A. namaquensis*. We also found that, at T_as $\geq 40^{\circ}$C, *A. namaquensis* was capable of maintaining T_{core} below T_a, as shown by the T_{core} trace crossing the line of thermal equilibrium ($T_{core} = T_a$) (Fig 4a). Thus at the highest ambient temperatures, *A. namaquensis* was in a hypometabolic state and showed an efficient capacity for evaporative cooling which maintained T_{core} at sublethal levels.

Petromyscus collinus, on the other hand, seemed incapable of hyperthermia-induced hypometabolism. The T_{core} increased linearly with increasing T_a and, unlike *A. namaquensis,* never decreased below Ta (Figure 3).

The literature has no examples, to our knowledge, where the approach of regressing metabolic rate with body temperature at high ambient temperature is intended specifically to test for hyperthermic Arrhenius effects. Weathers and Schoenblaechler (1976)'s study on budgerigars did indeed report such a regression, but not intentionally as a measure of Q_{10} under hyperthermia. Indeed, it is interesting that so few thermoregulatory studies on small mammals have provided a specific focus on hyperthermic responses. We presume that this lack of data indicate an understandable reluctance to expose study animals to potential lethal hyperthermia. We would argue that, given the urgency for the incorporation of physiological response data into predictive climate change models, our precautionary approach may require some reconsideration. For example, we attempted to minimize the risk of lethal hyperthermia by exposing our study animals to increasing experimental ambient temperatures over an extended period of time (several weeks). In this manner, resistance to high temperatures, otherwise known as "acquired thermal tolerance" was induced thereby minimizing the risk of entry into pathological hyperthermia (Lepock, 2003).

Models of endothermy based upon ectothermic models have predicted interspecific variation in thermoregulatory response mechanisms to hyperthermia (Angilletta et al., 2010; Boyles et al., 2011). This study provides some of the first data on these hyperthermic responses (also see Toussaint and McKechnie, 2012). However, despite an obvious contrast in the response of the two species that we studied, we cannot, at this stage, reach meaningful interspecific conclusions (Garland and Adolph, 1994). Nevertheless, we provide some basic autecological sketches to assist in future interspecific multivariate analyses.

Petromyscus collinus is a habitat specialist found only in rocky outcrops (Nowak, 1999; Smithers, 1983), whereas *A. namaquensis* is more cosmopolitan and, being a habitat generalist, is found in many types of desert habitat (Coetzee, 1969). *A. namaquensis* can be found sympatrically inhabiting rocky outcrops of the Northern Cape with *P. collinus*, but is also common throughout the sandy deserts of the Kalahari (Lovegrove et al., 1991). Thus, *A. namaquensis* is exposed to a wider range of T_as (see Lovegrove and Siegfried, 1993) than *P. collinus*. Moreover, whereas much is known about the microhabitat of *A. namaquensis* (Buffenstein, 1984), there are few data available for *P. collinus.*

Our observations of efficient evaporative water loss at high T_as (Fig 2) suggest that *A. namaquensis* has more access to water in the wild than *P. collinus*. We found that, unlike *A. namaquensis, P. collinus* reached pathological hyperthermia and the trace of T_{core} did not cross the line of thermal equilibrium ($T_a = T_b$). The thermoregulatory responses of *P. collinus* to high temperatures therefore may involve primarily water conservation considerations rather than the risks of potential pathological hyperthermia. In a study on the energetics and water relations of desert rodents, Withers et al. (1980) found that the pygmy rock mouse was able to survive for weeks on a diet of air dried seeds alone. We also observed a similar lack of water dependence in *P. collinus* in the laboratory.

Smaller animals have higher surface-to-volume ratios making them more susceptible to higher rates of heat, energy and more importantly water flux (Aschoff, 1981; Schmidt-Nielsen, 1990; Williams, 1996; Withers, 1992). *Petromyscus collinus* is one of the smallest (< 20 g) desert mammal in southern Africa. On a daily basis, it is unable to sustain the same rates of water loss that *A. namaquensis* experiences, and therefore avoids cooling via evaporative water loss. Despite being more than three times smaller than *A. namaquensis*, Withers et al. (1980) have measured an equitable water turnover rate (~ 0.4 mℓ g^{-1} day^{-1}) in *P. collinus*. Furthermore, these authors have shown that *P. collinus* has a kidney more specialized for urine concentration, and thus water conservation than *A. namaquensis*. They have also suggested, based upon their observations, that *P. collinus* “aestivates” during the hot summer months in order to conserve energy. Since evaporative water loss is proportional to the rate of respiration (Cooper and Withers, 2008), aestivation, or daily torpor, should also profoundly reduce daily water requirements.

The suggestion that *P. collinus* aestivates at high ambient temperatures during summer, conflicts with our observation of the lack of heat-induced hypometabolism. Although quite rare today when dealing with endothermy and deviations therefrom, the term aestivation is used sparingly, if at all, to describe what is generally considered as summer daily torpor (Geiser, 2010; Macmillen, 1965; Wilz and Heldmaier, 2000). *Petromyscus collinus* has been observed, at

T_a = 5°C, to become torpid for over 24 hours without attempting to arouse (Lovegrove, pers. obs.). This suggests a capacity for hypometabolism or heterothermy in response to cold temperatures (Lovegrove, 2012). However its capacity for hyperthermia-induced hypometabolism remains unknown.

We suggest that the disparity of potential hypometabolic responses to high temperatures may be quantitatively related to ecological conditions. For example, in the laboratory we have tested for, and found, a lack of hyperthermia-induced hypometabolism at T_as > 33°C. The question we need to ask is whether *P. collinus* are indeed ever exposed to such high ambient temperatures for extended periods of time. Being nocturnal, we presume that these small mice exploit thermally-buffered refugia deep within rock outcrops during the heat of the day. Sustained ambient temperatures in refugia may be sufficiently lower than the normothermic body temperatures of *P. collinus* (~ 34°C) (Lovegrove and Knight-Eloff, 1988) thus permitting torpor at high ambient temperatures. It may be that, in the wild, *P. collinus* are never exposed to T_as > 33°C in these thermally buffered refugia in kopjies (Lovegrove and Knight-Eloff, 1988). Thus selection for hyperthermia-induced hypometabolism may have been weak.

Conclusion

Mammals which are unable to shift their ranges altitudinally or latitudinally due to insularity or habitat fragmentation need to rely on physiological and behavioural responses to persist through current climate changes. In this study we show that a model heat-adapted small mammal, the Namaqua rock mouse, *A. namaquensis,* was capable of hyperthermia-induced hypometabolism in response to high T_as. Our observations of hyperthermic hypometabolism support the HDT Hypothesis proposed by (Lovegrove et al., in press). HDT reduces the risk of entry into pathological hyperthermia and also reduces the rate of water turnover driven by heat-induced evaporative cooling.

In contrast, the smallest desert mammal in southern Africa, the pygmy rock mouse *Petromyscus collinus,* displayed no evidence of HDT. We suspect that the lack of HDT in *P. collinus* may be associated with its thermoregulatory responses at high temperatures being adapted primarily for water conservation rather than minimizing the risks of pathological hyperthermia. Although our two-species data do not allow for meaningful interspecific comparisons, the dichotomy of responses of the two species to potential hyperthermia is marked, identifying putative contrasting phenotypic responses to hyperthermia. As we argue in the final Chapter, future studies need to focus on quantifying contrasting interspecific responses of metabolic rate to hyperthermia. In addition, associated biotic and abiotic data need to be obtained in order to conduct meaningful phylogenetically independent multi-species analyses.

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Figure and table legends

Figure 1 Body masses before and after the start of the body temperature measurements for a) the Namaqua rock mouse and b) the pygmy rock mouse

Figure 2 Mean ± SD body temperature and metabolic rate of the Namaqua rock mouse

Figure 3 Mean ± SD body temperature and metabolic rate of the pygmy rock mouse

Figure 4 The Arrhenius effect of body temperature on metabolic rate in a) the Namaqua rock rat and b) the pygmy rock mouse.

Figure 1

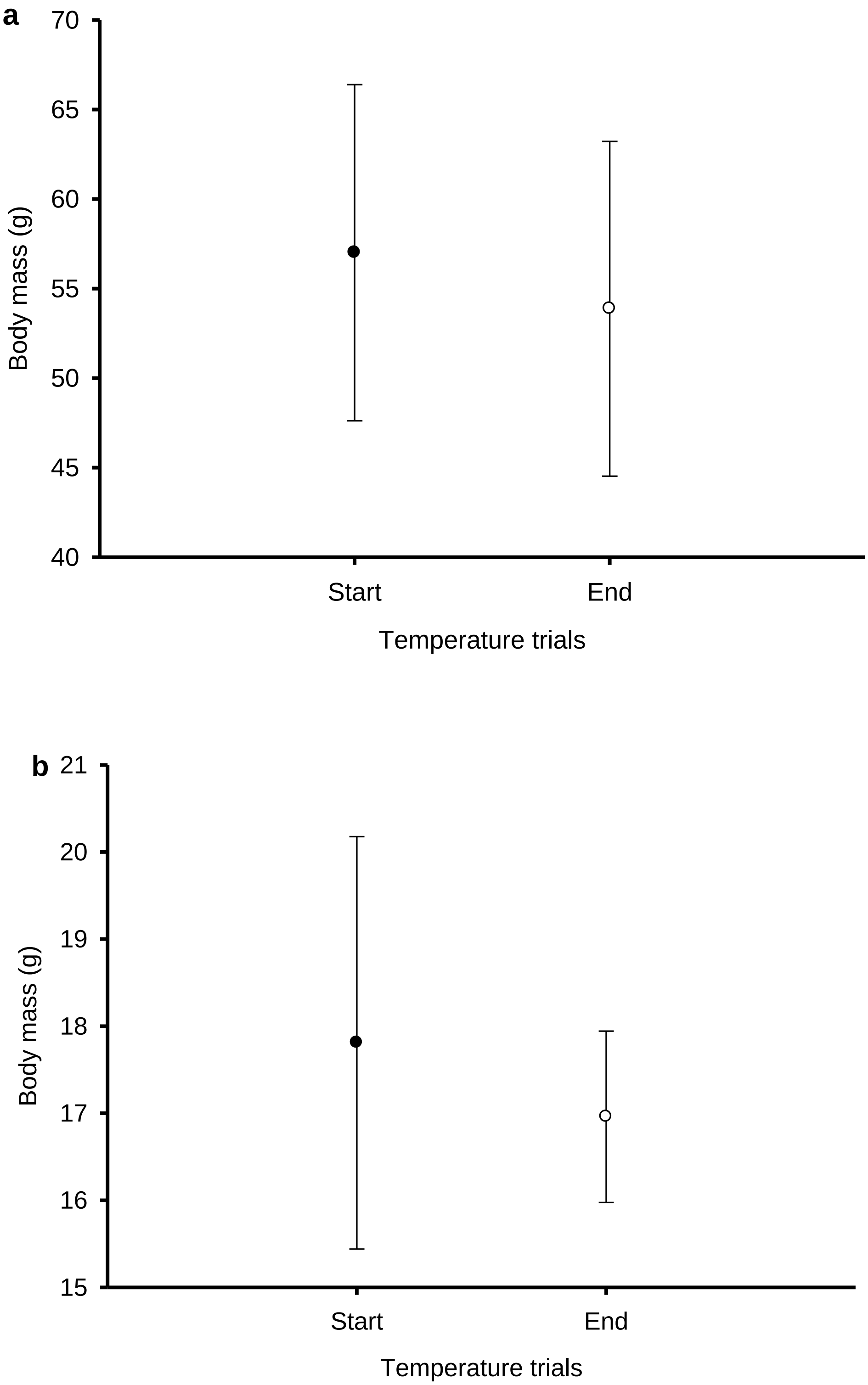

a
70
65
60
55
50
45
40
Body mass (g)
Start
End
Temperature trials
b
21
20
19
18
17
16
15
Body mass (g)
Start
End
Temperature trials

Figure 2

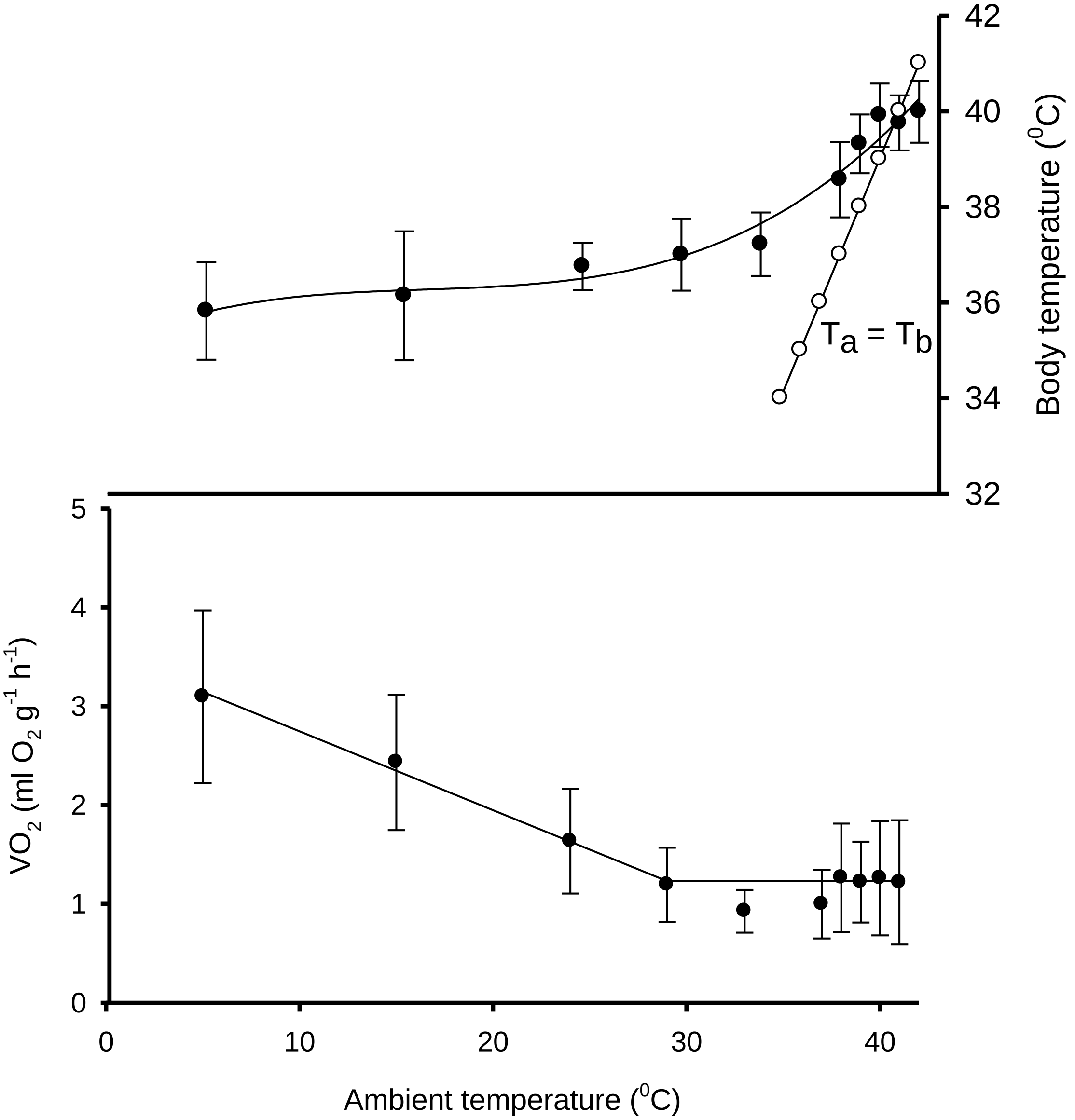

42
40
38
36
34
32
Body temperature (^{0}C)
$T_a = T_b$
5
4
3
2
1
0
VO$_2$ (ml O$_2$ g^{-1} h^{-1})
0
10
20
30
40
Ambient temperature (^{0}C)

Figure 3

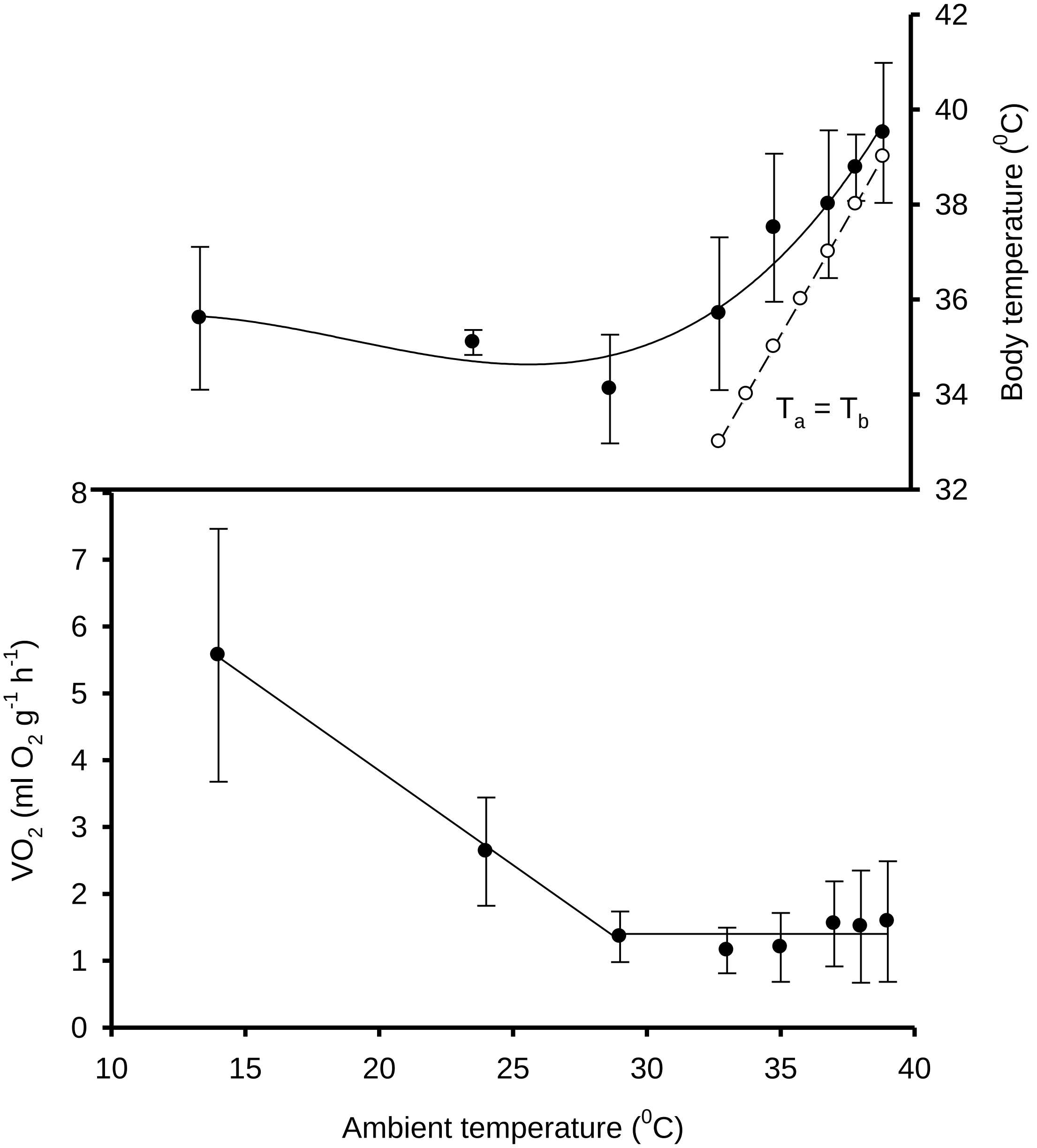
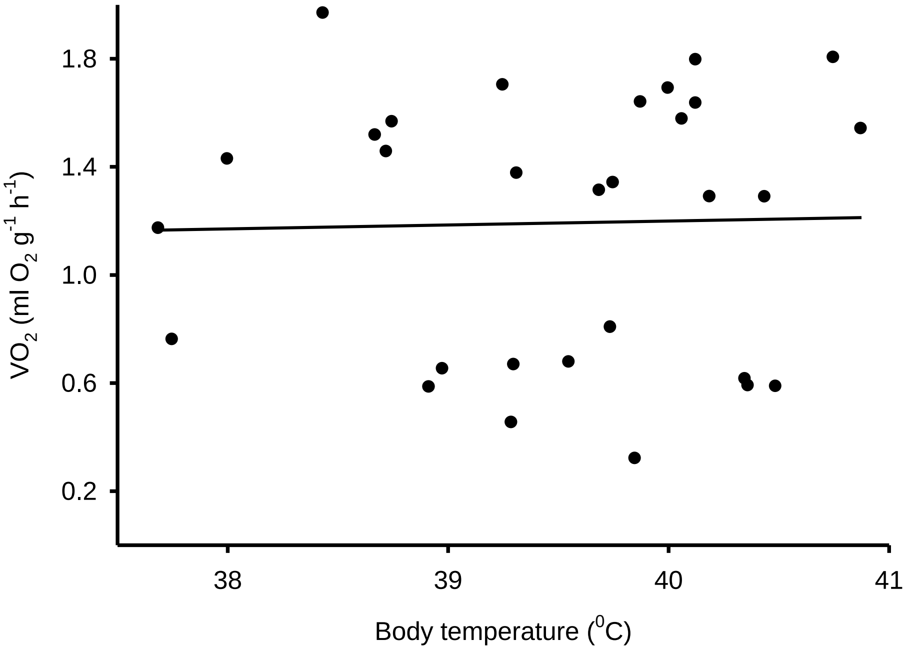
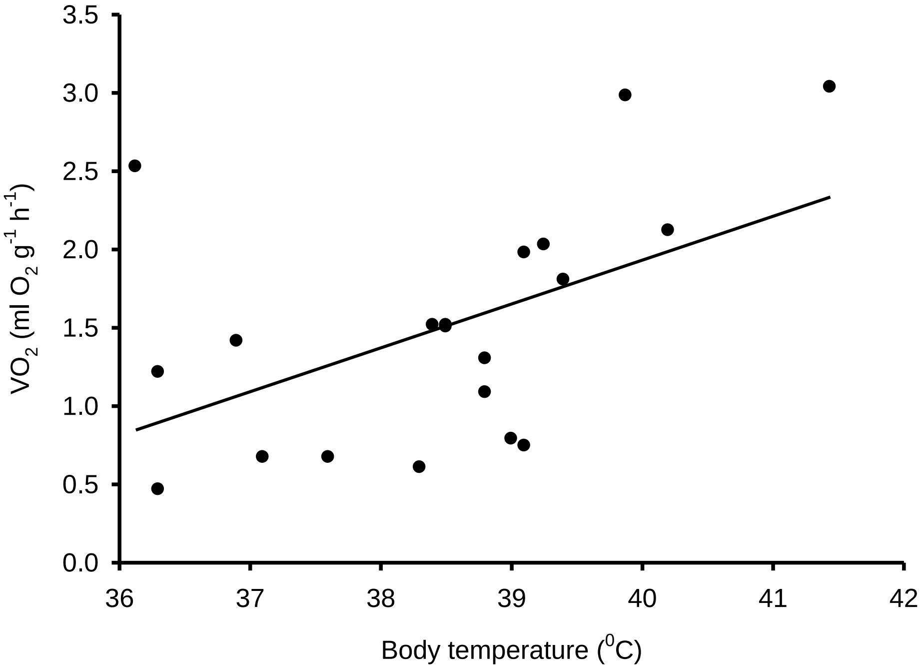

42
40
38
36
34
32
Body temperature (^{0}C)
$T_a = T_b$
8
7
6
5
4
3
2
1
0
VO$_2$ (ml O$_2$ g^{-1} h^{-1})
10
15
20
25
30
35
40
Ambient temperature (^{0}C)

Figure 4


1.8
1.4
1.0
0.6
0.2
VO$_2$ (ml O$_2$ g^{-1} h^{-1})
38
39
40
41
Body temperature (^{0}C)


3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
VO$_2$ (ml O$_2$ g^{-1} h^{-1})
36
37
38
39
40
41
42
Body temperature (^{0}C)

Chapter 5

General conclusions and future directions

This study has shown that some small mammals are capable of hyperthermia-induced hypometabolism recently proposed by Lovegrove et al., (in press). We found that *A. namaquensis* was able to downregulate its metabolism during hyperthermia whereas *P.* collinus was not. Such a physiological response will minimize the risk of entry into pathological hyperthermia and increase water conservation. The literature has no examples, to our knowledge, where the approach of regressing metabolic rate with body temperature at high ambient temperature is intended specifically to test for hyperthermic Arrhenius effects. Studies that have reported such regressions have not done so intentionally as a measure of Q_{10} under hyperthermia. These lack of data may indicate, understandably, a reluctance to expose study animals to potential lethal hyperthermia. Given the urgency for the incorporation of physiological response data into predictive climate change models, the precautionary approach of most mammalian physiologists may require some reconsideration. For example, we attempted to minimize the risk of lethal hyperthermia by exposing our study animals to increasing experimental ambient temperatures over an extended period of time thereby inducing thermal tolerance.

Endothermic models on thermal specificity based on ectothermic models have predicted differences in the thermoregulatory responses of mammals to high ambient temperatures predicted by climate change models. Our study provides some of the first data showing these thermoregulatory differences as shown by the incapacity of *P. collinus* for hyperthermia-induced hypometabolism. We also show that, despite slight over- and underestimations by subcutaneous temperatures outside the thermoneutral zone, there is no significant difference between the subcutaneous and core temperatures of *A. namaquensis* at least during the rest-phase. We attribute these slight thermal gradients between subcutaneous and core temperatures to the effect of the thermoregulatory mechanisms that are activated in order to maintain the body temperature setpoint when the ambient temperatures deviate from the thermoneutral zone. Nevertheless, we find that, over the wide range of ambient temperatures measured, this thermal gradient is much less than the maximum that has been measured by skin temperature dataloggers. Abiotic factors such as solar radiation or lowered winter temperatures may result in misleading thermal gradients being recorded by skin temperature dataloggers. We argue that a measure of subcutaneous temperature is a reliable proxy for core temperatures in free-ranging mammals.

Future directions

We have identified two seemingly opposing metabolic responses of small mammals to high ambient temperatures. This observation poses the important question about whether a continuum of physiological responses to hyperthermia exists in mammals. This is a topic that has received very little empirical attention, but has been formulated in theoretical models of endotherm responses to climate change. Angilletta et al., (2010) have attempted to model endotherm performance curves as a function of body temperature based upon ectotherm models. They have borrowed the concept of a continuum between “specialist” endotherms that have a high performance index but a narrow range of operative body temperatures, and "generalist" endotherms that have a much broader operative range of body temperatures but a lower modal performance index.

These endotherm models were formulated prior to the identification of three categories of endotherms, namely basoendotherms ($T_b < 35^{\circ}C$), mesoendotherms ($35^{\circ}C \leq T_b \leq 37.9^{\circ}C$) and supraendotherms ($T_b > 37.9^{\circ}C$) (Lovegrove 2012). Supraendotherms display a very narrow range of high body temperatures, and seldom if ever, relax this control in the form of heterothermy. Thus, in terms of the performance curves terminology, these mammals could be considered to be endothermic specialists. However, we doubt the prediction of Angilletta et al., (2010) and Boyles et al., (2011) for basoendotherms that meso- or basoendotherms may attain body temperatures higher than those of supraendothermsWe therefore suspect that the generalist body temperature ranges, especially the extremes, may be unrealistic for endotherms.

What we have identified in this study is a putative dichotomy of responses (specialist, generalist?) within small baso- and mesoendotherms, that is, within mammals with $T_b < 37.9^{o}C$. We would argue that the specialist-generalist performance curves models are well intended, but are too simplistic to describe the diversity of physiological responses in endotherms. Clearly there may be dietary, habitat, and water availability constraints which ectotherms do not face, but which may determine how small endotherms will respond to increasing ambient temperatures associated with climate change. The challenge in the future is to identify and quantity the variables associated with endothermy-related continua, and to obtain more physiological data on individual responses to hyperthermia. The quest will undoubtedly involve the measurement of new data, but large data bases can also be generated more rapidly by analysing published data.

References

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