THERMOREGULATION IN FREE-RANGING AFRICAN-ENDEMIC SMALL MAMMALS: THE ROCK ELEPHANT SHREW, *Elephantulus myurus* AND THE LESSER BUSHBABY, *Galago moholi*

by

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PREFACE

The fieldwork described in this thesis was carried out at Weenen Game Reserve, KwaZulu Natal and Nylsvlei Nature Reserve, Limpopo Province, South Africa, between May 2001 – October 2003. All experimental work was carried out in the School of Botany and Zoology, University of KwaZulu-Natal, Pietermaritzburg. The study was under the Supervision of Prof. Barry G. Lovegrove and was co-supervised by Dr Judith C. Masters.

These studies present original work by the author and have not been submitted in any form for any degree or diploma to any other University. Where use has been made of the work of others it is duly acknowledged in the text.

Outside the University of KwaZulu-Natal, I collaborated with Professor David O. Ribble, Department of Biology, Trinity University, San Antonio, TX, USA (Chapter 2). Professor Ribble determined the elephant shrew territories and captured animals for a preliminary study to test the feasibility of the methods used.

Published work included in this thesis is referred to by publication (chapters 2, 3 and 7) whereas papers in review (chapters 4 and 6) and unpublished work (chapter 5) are referred to by chapter.

All procedures used in these studies complied with the "Principles of animals care" publication no. 86 – 23, revised 1986 (National Institute of Health) and the

"Code of ethics for animal experimentation" manual adopted by the University of KwaZulu-Natal.

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Abstract

Endothermy in birds and mammals is invariably associated with homeothermy. However, homeothermy can only be maintained if sufficient energy is obtained to meet the animals' maintenance budget. In mammals, daily torpor and hibernation have evolved to conserve energy when energy inputs from the environment are insufficient to meet maintenance requirements. Several studies have suggested that daily torpor and hibernation do not represent distinct physiological responses but are components of a continuum of heterothermy. Under laboratory conditions, even within phylogenetically ancient eutherian mammals, such as elephant shrews, it is unclear whether daily torpor or hibernation is used. Furthermore, an interpretation of the torpor patterns observed under laboratory conditions is complicated by the fact that torpor patterns often differ between laboratory and free-ranging conditions.

Within the more recent mammal lineages, torpor has been observed in strepsirrhine primates. The occurrence of torpor in strepsirrhines is interesting as it pertains to arguments relating to the colonization of Madagascar by strepsirrhine primates as well as implications for human physiology.

The first aim of this study was to investigate and quantify parameters that characterize torpor in a phylogenetically ancient eutherian mammal (Macroscelidae: *Elephantulus myurus*) under free-ranging conditions. This was done mainly to resolve whether daily torpor and hibernation were physiologically discrete responses. The second aim was to investigate torpor occurrence in a more recently evolved eutherian mammal (Primates: *Galago moholi*). The objective was to gain insights into the phylogenetic distribution of torpor and to provide a physiological verification of torpor occurrence in a mainland strepsirrhine relative to arguments about the colonization of Madagascar.

I measured body temperatures in three monthly cycles between May 2001 – May 2002 in 22 free-ranging *E. myurus*. I recorded a total of 467 torpor bouts throughout the study period. The elephant shrews were capable of daily torpor throughout the year, with torpor most prevalent during winter and correlated with ambient temperature, photoperiod and invertebrate abundance. Only two torpor bouts were observed during summer. I suggest that although torpor use was most prevalent during winter, summer torpor might also be important for energy conservation in this species during drought years. This highlights the need for long-term physiological data in free-ranging animals.

The mean torpor $T_b$min and the mean bout length for the whole year were in the range expected for daily heterotherms. However, there was some marginal overlap with hibernation characteristics; a few torpor bouts were longer than 24 hrs in duration indicating that the animals were capable of opportunistically extending torpor bouts longer than 24 hours in response to unpredictable energetic shortfalls. $T_b$min also decreased below 10°C. However, a consideration of behavioural and ecological factors argues against hibernation in *E. myurus*. Instead, these results support the idea of a physiological continuum for heterothermy.

A return to normothermic body temperatures requires considerable energy expenditure, and this is perceived to be one of the major disadvantages of torpor.

E. myurus offset the high cost of arousal from low body temperatures by using exogenous passive heating. This is achieved by coupling of the timing of arousal with ambient temperature cycles. Laboratory studies that quantify torpor energetics are usually conducted under constant temperature conditions and are likely to underestimate the energetic benefits accrued through the use of ambient temperature cycles during arousal.

Torpor is often displayed during the animal's rest phase. However, nocturnal small mammals that utilize passive heating to assist arousal from torpor may enter torpor during the nighttime, thus effectively advancing the onset of the rest phase. I investigated the functional significance of daily and seasonal rhythms of body temperature in normothermic and torpid free-ranging *E. myurus*. Daily patterns of T_b in normothermic *E. myurus* suggested polyphasic T_b patterns that nevertheless indicated a rest phase coincident with the daytime. I suggested that the principal benefit of a flexible daily rhythm of T_b is that it facilitated torpor use during the nighttime and arousal by passive exogenous heating using ambient temperature cycles.

It has been suggested that the evolution of endothermy precluded the need for homeothermic mammals to be sensitive to T_a cycles because they could maintain physiological function despite fluctuations in the ambient temperature. Elephant shrews utilize passive heating and provide excellent models with which to investigate whether mammals can entrain their body temperature rhythms to ambient temperature cycles. I experimentally tested whether food restricted

E. myurus can entrain torpor cycles to shifts in the T_a cycle while holding the light-dark cycle constant. Food restriction and short photoperiod were only sufficient to induce torpor in *E.myurus* if photoperiod and T_a cycles are in phase with each other. Shifting the cold T_a into the photophase prevented the expression of torpor. I concluded that the body temperature rhythm is most probably tightly coupled with the photoperiod cycle and that although T_a and photoperiod usually act synergistically in nature, photoperiod is probably the stronger zeitgeber.

The evolution of endothermy is thought to have been facilitated by the advent of endothermic energy sources such as brown adipose tissue (BAT), the principal site of nonshivering thermogenesis (NST). Rock elephant shrews are amongst the smallest members of the Afrotheria, the most basal of the eutherian lineage. I determined whether the phylogenetic placement of *E. myurus* and reliance on passive heating might result in a decreased capacity for NST relative to other eutherians. I investigated the capacity for NST in winter acclimated *E. myurus* by measuring the thermogenic response to noradrenalin (NA) injection. I used phylogenetically independent analyses to compare *E. myurus* NST capacity with other eutherians. *E. myurus* had an NST capacity that was no different from other eutherian mammals. Although they displayed a NST capacity that was 74% of that expected on the basis of body mass, this value was not significantly different from phylogenetically independent allometric predictions.

Although heterothermy is almost always considered in the context of how the environment affects function, its use may offer insights into topics such as

island biogeography and species dispersal. For example, there have been suggestions that heterothermy might have played an important role in the successful colonization of Madagascar by strepsirrhine primates. To my knowledge no studies exist as yet that provide a physiological verification of this suggestion. Currently no data exist on thermoregulation and heterothermy in any free-ranging African strepsirrhines. The lesser bushbaby, *Galago moholi,* is a small nocturnal strepsirrhine primate that experiences severe winters and drastic food reduction during winter and is a candidate employer of torpor. I measured body temperatures of 11 free-ranging lesser bushbabies, *Galago moholi*, captured at different times between February 2002 – September 2003. I did not record any incidents of heterothermy throughout the study period. Why does *G. moholi* not employ heterothermy? I consider several alternatives; phylogenetic placement, physiological and ecological factors that might preclude the use of torpor in this species. I suggest that the breeding pattern observed in *G. moholi* obviates torpor use whilst increasing fecundity, which would be adaptive if the animals are confronted with high predation risks.

Much is currently known about the advantages of torpor use. This study highlights the need to consider and investigate those physiological, ecological and phylogenetic factors that might constrain species from utilizing heterothermy. Furthermore, this study highlights the potential for thermoregulatory studies to offer insights into topics as widely separated as evolution of endothermy to species dispersal and island biogeography.

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A very special thanks to my family for their patience, encouragement, emotional and financial support throughout the past 22 years of my studying. And finally, a heartfelt thanks to my "famous" High School Biology teacher, Manz'andonga Vabaza, whose enthusiasm started it all.

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Chapter 1

Introduction

Endothermy, which involves metabolic heat production to maintain an elevated body temperature, has evolved in birds and mammals as well as some reptiles, fish and insects (McNab 1978; Hayes and Garland 1995). The principal difference between these groups is that endothermy in birds and mammals results primarily from metabolic heat production in the visceral organs and the brain, whereas that in insects, fish and reptiles results mainly from myogenic heat production (Pough et al. 1996). Endothermy in birds and mammals is invariably associated with homeothermy; the maintenance of a stable body temperature within narrow limits. Advantages of homeothermy are thought to include thermal niche expansion and increased enzymatic and physiological efficiency (review in Hayes and Garland 1995).

Homeothermy is attainable if sufficient energy is obtained from the environment to meet the animals' maintenance budget, which has a basal, thermoregulatory and locomotory component (Withers 1992). A variety of environmental factors may have different effects on various components of an animal's maintenance budget. For example, small mammals inhabiting environments characterized by low seasonal fluctuations in daylength, ambient temperature and low/unpredictable rainfall are likely to have low basal metabolic rates, resulting in low overall maintenance costs (Lovegrove 2000a). In contrast, small mammals that experience pronounced seasonal changes tend to have high BMRs and the resultant maintenance costs may be high. In highly seasonal environments winters are predictably cold, food availability is reduced and this is coincident with increased thermoregulatory requirements (Heldmaier 1989). Because small mammals have limited capacity for energy storage, they may not be able to meet their thermoregulatory and locomotory requirements (associated with foraging) during winter. Heterothermy (daily torpor and hibernation) is a proximate physiological response that has evolved to conserve energy in small mammals when energy inputs from the environment into the animal are insufficient to meet maintenance costs (Lyman et al. 1982; Körtner and Geiser 1998).

Daily torpor and hibernation are generally understood to be responses to predictably cold and harsh winters such as those experienced in the Holarctic and Palaearctic environments. However daily torpor (including summer torpor) seems to be more common in Australasian and Afrotropical species, inhabiting generally warmer environments (Geiser and Baudinette 1987; Brown and Bernard 1994; Ortmann et al. 1996; Lovegrove and Raman 1998; Coburn and Geiser 1998; Lovegrove 2000b; Lovegrove et al. 2001b; Geiser and Drury 2003). In addition, Dausmann et al. (2004) recently reported on the first account of hibernation in a tropical primate, highlighting the importance of daily torpor and hibernation for energy conservation in milder climates.

Daily torpor and hibernation occur in all three mammalian subclasses. Within the placentals, heterothermy has been recorded in the Macroscelidea, Tenrecidae, Chrysochloridae, Gliridae, Sciuridae, Heteromyidae, Muridae, Dipodidae, Primates, Eulipotyphla and Chiroptera (Geiser 1998; Lovegrove 2004). Heterothermy in the mammals tends to be more common in the older phylogenetic lineages and appears to have been lost in those taxa that can survive without using heterothermy for energy conservation, particularly those with larger body sizes. The retention of daily torpor is most likely determined by diet and small body size (Geiser 1998).

Molecular data suggest that the African-endemic clade, Paenungulata, (including elephant shrews, golden moles, tenrecs, hyraxes, dugongs, the aardvark and elephants), may be ancestral to other mammalian orders (Porter et al. 1996; Stanhope et al. 1996; Springer et al. 1997). The occurrence of torpor in the smallest members of this basal mammalian lineage (elephant shrews, golden moles and tenrecs) lends support to the plesiomorphic origin of torpor (Lovegrove et al. 1999). Recent studies have suggested that daily torpor and hibernation do not represent discrete and distinct physiological responses but they are extremes of a physiological continuum of heterothermy (Wilz and Heldmaier 2000; Zosky 2002). Even within the phylogenetically ancient elephant shrews, laboratory studies have failed to resolve whether daily torpor or hibernation is used. For example, the values for torpor bout length were consistent with those expected for daily heterotherms, whereas others such as minimum torpor body temperature (T_b) and oxygen consumption (VO_2), as well as the magnitude of basal metabolic rate (BMR) reduction, were consistent with those of hibernators (Lovegrove et al. 2001a). Heterothermy parameters measured under laboratory conditions often differ markedly from those observed under field conditions, with torpor often more frequent, deeper and longer in free-ranging animals (Geiser et al. 2000). Thus, although useful inferences may be made regarding animal energetics from laboratory studies, only further studies in free-ranging animals can resolve some of these questions.

Malan (1996) suggested that the wide-amplitude circadian cycling of body temperature in heterothermic animals is reminiscent of the primitive reptilian tendency. He further suggested that the evolution of endothermy in mammals was likely to have been facilitated by the advent of endothermic heat sources such as brown adipose tissue (BAT). Brown adipose tissue appears to be absent in monotremes and marsupials (Rose et al. 1999). The next logical group in which to investigate the occurrence of BAT are the basal placental mammals (for example, elephant shrews). Recently, Geiser et al. (2002) proposed that exogenous passive heating may have been important during the evolution of mammalian endothermy. Because elephant shrews are known to use torpor and exogenous passive heating during arousal from torpor (Mzilikazi et al. 2002), and because of their phylogenetic placement, they are likely to provide valuable insights into the evolution of endothermic thermoregulation.

In the more recent mammalian lineages, the Malagasy cheirogaleids are the only primates known to use daily torpor and hibernation (Ortmann et al. 1996; Schmid 1998; 2000; Dausmann et al. 2004). The cheirogaleids are related to the African and Asian Lorisidae and the African Galagidae. If torpor is a plesiomorphic trait we would expect that there should be evidence of torpor in mainland strepsirrhines (or at least some of them), otherwise the presence of heterothermy in the cheirogaleids would present the only known example of independent evolution of heterothermy in mammals. Furthermore, although heterothermy is almost always considered in the context of how the environment affects function, its use may offer insights into topics such as island biogeography and species dispersal. For example, there have been suggestions that heterothermy played an important role in the successful colonization of Madagascar by strepsirrhine primates (Kappeler 2000). To my knowledge no studies exist as yet that provide a physiological verification of this assertion. Currently no data exist on thermoregulation and heterothermy in any free-ranging African strepsirrhines, the closest sister group to the Malagasy cheiorogaleids.

The lesser bushbaby, *Galago moholi* is an excellent model to investigate heterothermy in an African mainland strepsirrhine for several reasons. The distribution of lesser bushbabies is restricted to sub-Saharan Africa, a region known to be inherently unpredictable climatically (Philander 1983). They weigh ca. 160g, with body mass ranging from 120 to 290g (Skinner and Smithers 1990), which falls within the range of known daily heterotherms (Geiser and Ruf 1995). They are closely related to the lorises and the cheirogaleids, both groups known to use heterothermy (Whittow et al. 1977; Müller et al. 1985; Schmid 2000; Dausmann, et al. 2004). In mid-winter, the ambient temperature in the lesser bushbaby habitat often decreases to below freezing, although ambient temperatures often reach the mid-twenties during the daytime (Scholes and Walker 1993). Lesser bushbabies subsist mainly on gum from *Acacia* trees and this diet is often by complemented with arthropods (Bearder and Martin 1980). Although changes in gum availability at different times of the year aren't well documented, it is well known that insect availability decreases during winter, with potential consequences for energy availability for lesser bushbabies.

Thus, in summary the principal aim of this study was to investigate torpor in free-ranging Afrotropical small mammals. I chose two model species, the rock elephant shrew, *Elephantulus myurus* and the lesser bushbaby, *Galago moholi*. Elephant shrews are members of the Afrotheria, and occur at the base of the eutherian mammalian lineage. Because of their phylogenetic placement, elephant shrews are likely to offer insights into the evolution of both heterothermy and endothermy in mammals. I chose *Galago moholi*, a member of the African Galagidae because of its phylogenetic relationship with the Malagasy lemuriforms. The use of heterothermy in primates is important in terms of the phylogenetic distribution of torpor in mammals and the concomitant implications for human physiology. Furthermore, verification of torpor in a mainland African strepsirrhine is important because of its implications for arguments relating to the colonization of Madagascar by strepsirrhine primates.

Thesis structure

Chapter 1 provides an overall introduction as well as some background information on the model species used in this study.

In Chapter 2 I investigated the use of torpor during winter and quantified parameters that characterize torpor in free-ranging *E. myurus*. I also compared torpor parameters measured in free-ranging *E. myurus*, with those measured in the laboratory and investigated the role of exogenous passive heating during arousal from torpor.

In Chapter 3 I investigated the influence of environmental factors such as ambient temperature, photoperiod and food availability on torpor frequency in free-ranging rock elephant shrews. I also investigated torpor incidence during the different seasons and attempted to identify environmental factors that might account for the occurrence of torpor during summer.

In Chapter 4 I investigated daily rhythms of body temperature and the functional significance of polyphasic body temperature and activity patterns in free-ranging rock elephant shrews.

In Chapter 5 I investigated the effect of ambient temperature and photoperiod cycles on body temperature cycles and torpor frequency in winter-acclimated *E. myurus*. Specifically, I determined whether *E. myurus* were capable of entraining their body temperature cycles to those of ambient temperature.

In Chapter 6 I investigated whether winter-acclimated rock elephant shrews possessed the capacity for non-shivering thermogenesis (NST). I also investigated whether subtropical and tropical small mammals have lower NST capacities relative to those from higher latitudes.

In Chapter 7 I investigated the occurrence of torpor in free-ranging lesser bushbabies and discussed the lack of torpor in the context of the evolution of heterothermy, island biogeography and colonization of Madagascar by strepsirrhine primates. The ecological determinants of torpor as well as the possible constraints and costs of torpor in lesser bushbabies are also discussed.

Chapter 8 provides general conclusions reached in this study.

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Chapter 2

Exogenous passive heating during torpor arousal in free-ranging rock elephant shrews, *Elephantulus myurus*

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Abstract

In previous laboratory studies rock elephant shrews *(Elephantulus myurus ;* mean body mass 56,6g) displayed the lowest torpor $T_b$min yet recorded (*ca.* 5°C) in a placental daily heterotherm. It was unknown whether these low T_bs were characteristic of daily heterothermy in free-ranging animals. It was also unclear how cost effective these low T_bs were since considerable energy is required to arouse from low T_bs on a daily basis. We continuously measured body temperature once every hour for 85 days in 13 free-ranging *E. myurus* from May – August 2001 (winter) in Weenen Game Reserve, KwaZulu-Natal, South Africa. We recorded a total of 412 torpor bouts. Free-ranging *E. myurus* had a high propensity for torpor with females displaying higher torpor frequency than males. The lowest T_b recorded was 7.5°C at T_a = 3.7°C and the minimum torpor T_b was strongly correlated with ambient temperature. Torpor arousal was tightly coupled with ambient temperature cycles. Low torpor $T_b$min at low T_as was

therefore cost-effective because the animals offset the high cost of arousal through exogenous passive heating. Laboratory studies under constant ambient temperatures may therefore underestimate the energetic benefits of torpor in free-ranging small mammals that inhabit regions where seasonality is moderate.

Keywords: heterothermy, torpor setpoint, unpredictable environments

Introduction

The proximate expression of heterothermy in endotherms represents a physiological response to energy shortfall (Lyman et al. 1982 ; Ruf and Heldmaier 1992 ; Lovegrove 1996). This may be due to either seasonal cold and low food availability (Lyman et al. 1982), or to aseasonal episodes of spatial and temporal flushes of resources (Lovegrove 2000).

Elephant shrews belong to the order Macroscelidea and are endemic to Africa (Corbet and Hill 1991) where they occupy a range of habitats from desert to forest (Skinner and Smithers 1990). They are members of the Paenungulata clade that includes hyraxes, elephants, dugongs and the aardvark (Springer et al. 1997). Several species of elephant shrews are known to employ heterothermy in the laboratory when confronted with energy stress (Seguignes 1983 ; Lovegrove et al. 1999a ; Lovegrove et al. 2001a,b). Although the rock elephant shrew, *Elephantulus myurus* may be classified as a daily heterotherm, it displays several intriguing patterns of heterothermy. Lovegrove et al (2001a) measured several torpor variables in the laboratory in *E. myurus* that were difficult to characterize either as daily torpor or hibernation. For example, the values for torpor bout length were consistent with those expected for daily heterotherms (< 24 hrs), whereas others such as minimum torpor body temperature (T_b) and oxygen consumption (VO_2), as well as the magnitude of basal metabolic rate (BMR) reduction, were consistent with those of hibernators (Geiser and Ruf 1995). Moreover, torpor body temperatures were the lowest yet recorded (*ca.* 5°C) in a placental daily heterotherm (Lovegrove et al. 2001a). At the lowest measured ambient temperature (T_a), the animals also did not appear to defend a setpoint torpor T_b. Thus, it is possible that the setpoint T_b for this species is below 5°C or that a setpoint does not exist, in which case minimum torpor T_b is determined by ambient temperature.

It remains unclear how cost effective the low body temperatures recorded in *E. myurus* are, since theoretically considerable energy is required to arouse from these low T_bs. It is also unclear whether the same minimum T_bs are characteristic of free-ranging animals. To date, the majority of studies have measured the energetics of torpor in laboratory acclimated animals (Lyman et al. 1982 ; Ruf and Heldmaier 1992 ; Geiser and Ruf 1995). However, a recent study by Geiser et al. (2000) revealed that heterothermic responses measured in mammals and birds in the laboratory differ markedly from those in the field. In addition, they found that torpor in free-ranging animals was often more frequent, deeper and longer than in captive animals. Thus, although useful inferences may be made regarding animal energetics from laboratory studies, it is possible that torpor may be more widely used than is currently appreciated. Only further studies in the field will reveal the importance of torpor in terms of the survival and energy utilization of free-ranging animals.

The aim of this study was therefore to investigate patterns of daily torpor and possibly hibernation in free-ranging *Elephantulus myurus*. We also aimed to quantify parameters that characterize torpor in free-ranging *E. myurus* and to compare torpor parameters measured in free-ranging animals with those previously measured in the laboratory. We hypothesized that free-ranging *E. myurus* would exhibit daily torpor rather than hibernation and that torpor $T_b$min values would be similar to those previously measured in the laboratory. We further hypothesized that a torpor setpoint would be absent with torpor $T_b$min simply determined by T_a.

Materials and methods

Rock elephant shrews, *Elephantulus myurus,* are usually found in close association with rocky habitats that provide shelter and resting sites (Skinner and Smithers 1990). They are not known to build nests either in the laboratory (pers. obs) or in the wild. They are reportedly diurnal although there have been suggestions of polyphasic activity (Woodal 1989).

The study was conducted at Weenen Nature Reserve, KwaZulu Natal, South Africa (28° 52.534′ S; 30° 00. 264′E) during winter 2001 (May – August). The reserve is situated in a summer rainfall area and its vegetation is classified as valley bushveld (Acocks 1993) or valley thicket (Low and Rebelo 1996). It is dominated by *Acacia* spp. and perennial grasses. The reserve is very hilly with large rocks, the preferred habitat of the rock elephant shrew (Skinner and Smithers 1990).

Eighteen adult *Elephantulus myurus* were captured using Sherman traps baited with peanut butter rolled in oats. Pre-calibrated Thermochron iButtons (Dallas Semiconductor Corp.) were surgically implanted into the intraperitoneal cavities of the elephant shrews under inhalation anaesthesia (Isoflorane in oxygen; induction and maintenance, 2%; flow rate, *ca* 0.5 $l.\text{min}^{-1}$). The iButtons (ca. 3g) did not exceed 5.3% of the animals' body mass. The animals were released at the exact capture locations < 24hrs after capture.

iButtons are miniature dataloggers armoured in stainless steel caps. They integrate a thermometer, a clock and a calendar, and hence are suitable for continuous recording of body temperatures over specified periods. They can log up to 2048 consecutive temperature measurements in read-only nonvolatile memory. iButtons measure temperatures from – 40°C to 85°C in 0.5°C increments and have a temperature conversion accuracy of 1°C from –20° to 70°C. The real-time clock accuracy is ± 2 minutes per month from 0 - 45°C. We programmed iButtons to measure body temperature once every hour, resulting in a total of 2040 data points per animal, over a period of 85 days.

An MC Systems 120-01E datalogger was used to measure ambient temperatures, with probes in the shade (under a large rock) and exposed directly to sunlight. We also measured the temperature of a blackbody (T_{black}) exposed to the sun at the same site by inserting an iButton within a black tin cylinder. Because rock elephant shrews are not known to build nests, the shade T_a aimed to measure diel fluctuations of T_a within the typical resting sites of elephant shrews (i.e. under rocks). Moreover, because animals' bodies act as blackbodies in the visible electromagnetic spectrum (Withers 1992) our blackbody temperatures provided a reference for potential passive heating in elephant shrews during sun basking. We were not able to measure T_a for all animals captured so we sited the measurement equipment on the crest of a small hillock at the centre of the capture sites, within 400m of any capture site. Depending on the aspect of each elephant shrew capture site relative to the position of the measuring equipment T_a could therefore vary slightly from the main site.

Data and statistical analyses

We recaptured and obtained data from a total of 13 animals (8 females ; 5 males) in August 2001. Following damage by a rhino to the MCS datalogger, ambient temperature data were unavailable between 15 May – 05 June 2001. All calculations involving T_a therefore exclude this period. However, data not including T_a, e.g. minimum T_b, torpor bout length and torpor frequency were available for the whole duration of data measurement (85 days) and were used in the respective descriptive frequency distributions.

The body temperature below which the animals were deemed to be torpid was 32°C. This value is slightly more than 2°C lower than the lower limit of the normal distribution of rest-phase normothermic T_bs in both sexes measured in the laboratory at $T_a = 25°C$ by Lovegrove et al. (2001b). The torpor bout length was therefore determined as the total time during which T_b was maintained below

32°C. The T_b min for both torpid and non-torpid animals was taken as the lowest T_b measured during a 24-hour period. Within each torpor bout the local time of entry into and arousal from torpor were determined. Heating rates were approximated with slopes of linear models fitted to the data from the onset of arousal until T_b attained steady-state normothermia. Blackbody heating rates were approximated the same way over the same time intervals as those used for body temperature heating.

Torpor frequency was calculated for individual animals as the proportion of days during which the animals displayed torpor. Torpor frequency data were arcsine transformed to allow for parametric tests.

Animals did not enter torpor on the same days and also displayed torpor at different ambient temperatures. This resulted in incomplete balanced designs which made it difficult to compute repeated measures analyses of variance that assess variance of dependent variables associated with time effects (repeated measures) and factorial variance. We observed significant time effects on $T_b$min in both sexes ($T_b$min progressively decreased over time). Because ambient temperature progressively decreased throughout the entire period of data measurement, T_a could potentially explain the time effects on $T_b$min. Indeed there was a significant positive correlation between $T_b$min and T_a (see Results). Residuals computed from this correlation showed no correlation with time, suggesting that the time effect could be explained by T_a effects. We therefore used single factor ANOVA to test for sex differences in minimum T_b, torpor bout length and torpor frequency. All correlations were calculated using Pearson product-moment coefficients (Zar 1984). All mean values are reported ± SD. Throughout the text N = the number of animals (N = 5 and 8, for males and females, respectively) and n = number of observations.

Results

Body mass

The mean species body mass was 56.6 ± 2.9g. At the start of data measurement there were no significant mass differences between sexes ($F_{1,11} = 2.22$; $p > 0.05$). The mean body mass was 57.4 ± 2.3g (N = 8) and 55.0 ± 3.5g (N = 5) for females and males, respectively. At the end of data measurement the females weighed 53.6 ± 3.7g and males 53.0 ± 4.0g, representing a mass loss of 6.6 % and 3.6 % for females and males, respectively. Mass loss was not significantly different between sexes ($F_{1,11} = 0.07$; $p > 0.05$).

Minimum body temperature

The mean normothermic $T_b$min was 34.4 ± 0.5°C (N = 8, n = 350) and 33.9 ± 0.4°C (N = 5, n = 300) for females and males, respectively (Fig. 1A). The overall mean normothermic T_b min was 34.2 ± 0.5°C. There were no significant differences in normothermic $T_b$min between sexes ($F_{1,11} = 3.66$; $P = 0.08$).

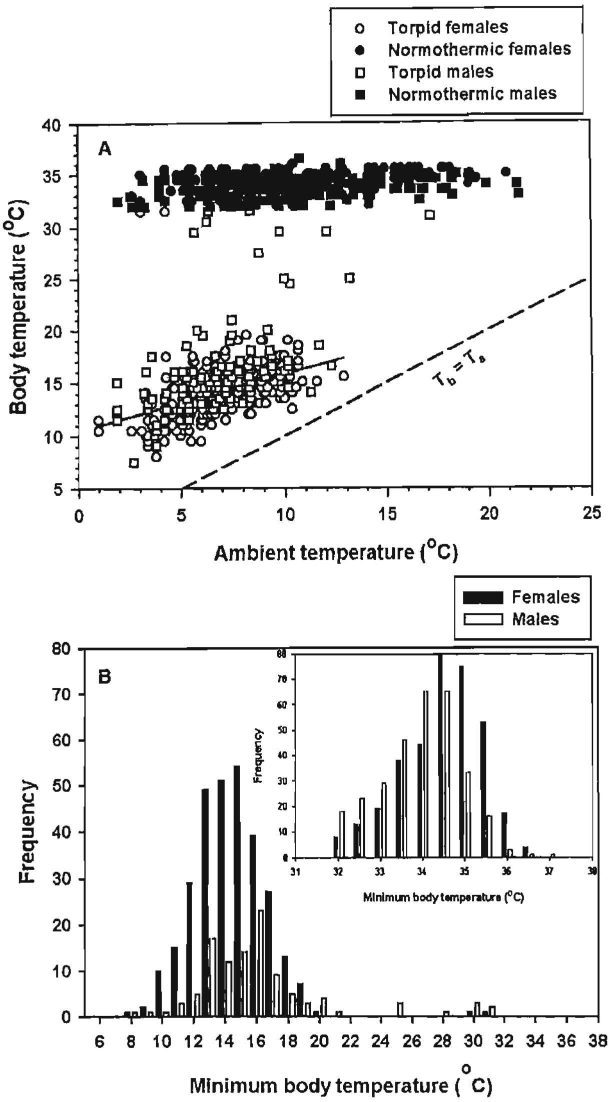


Fig.1A The minimum body temperatures in normothermic and torpid free-ranging female (circles) and male (squares) *E. myurus*. **B** Frequency distributions of minimum body temperature for females (filled bins) and male (clear bins) *E. myurus* for torpid animals and normothermic animals (inset). The bin intervals were 0.5°C.

There was no easily discernible inflection of torpor $T_b$min that typically indicates the regulation of T_b with respect to a torpor setpoint at the lowest T_as (Geiser and Ruf 1995). Instead, T_b continued to decrease with decreasing T_a (Fig. 1A). There was a significant linear correlation between torpor $T_b$min and T_a ($r = 0.448$; $t = 9.87$; $p < 0.01$) represented by the equation $T_b\text{min} = 9.8 + 0.7 \times T_a$

(Fig. 1A). Furthermore, the mean gradient between $T_b$min and T_a ($T_b$min – T_a) was not constant over the range of T_as at which torpor occurred. The mean ($T_b$min – T_a) was 7.3 ± 2.6°C and 9.4 ± 4.1°C for females and males, respectively. There was a significant negative correlation between ($T_b$min – T_a) and T_a ($r = -0.198$; $t = -3.99$; $p < 0.01$). The smallest ($T_b$min – T_a) was 2.2°C at T_a = 10.34°C for females and 2.8°C at T_a = 11.24°C for males (Fig. 2).

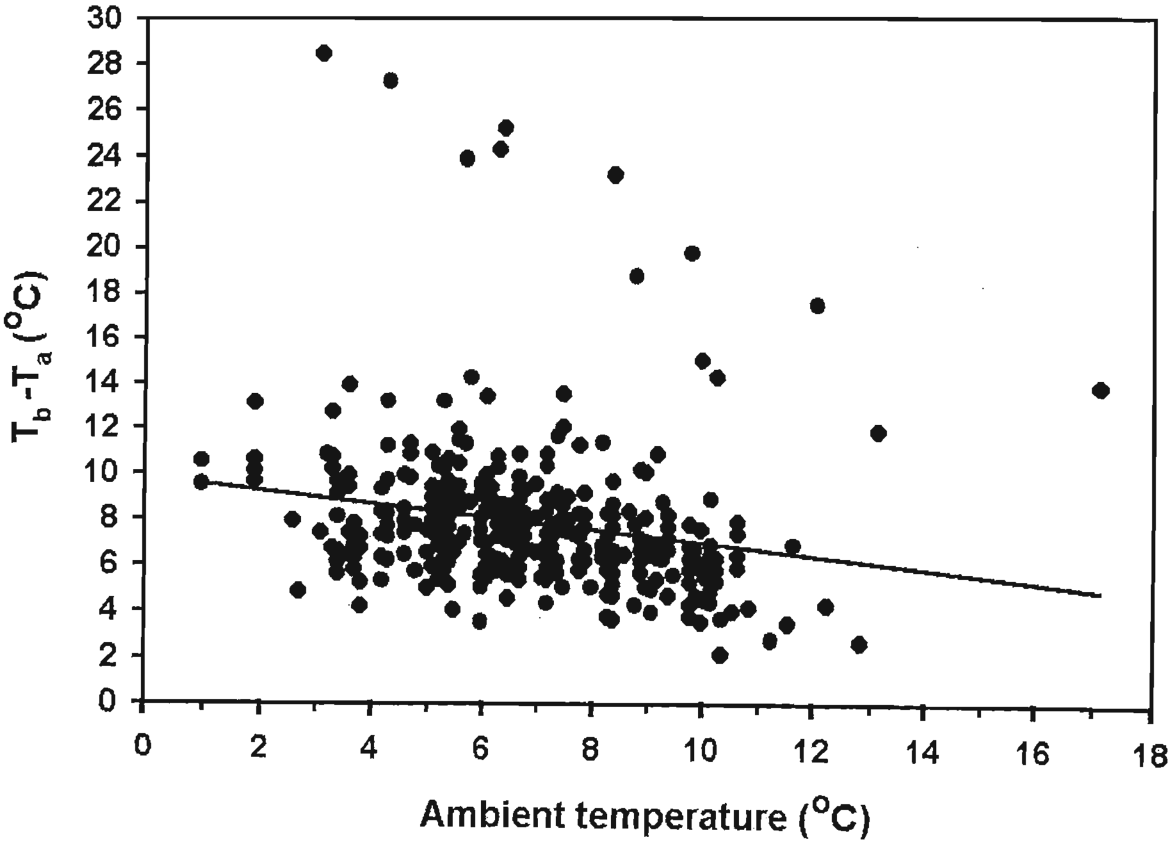


Fig. 2 The relationship between the ($T_b$min – T_a) gradient and ambient temperature. The elevated outliers are data from short duration (1h) test drops

Torpor bout length

Generally, the females spent longer in torpor than the males ($F_{1,11} = 11.29$; $P < 0.01$). The mean bout lengths (excluding test drops) were 8.9 ± 0.7h (N = 8, n = 297) and 7.2 ± 1.2h (N = 5, n = 102) for females and males, respectively. All torpor bouts where $29°C < T_b min < 31°C$ never exceeded 1 hour in duration and were taken to represent shallow "test drops" (Fig. 3A). Torpor bouts ranged from 1 – 27 hrs for females and 1 – 15 hrs for males. Only one female exhibited a torpor bout longer than 24 hrs (bout length = 27 hrs Fig. 3A). The torpor $T_b$min recorded during this bout was 9.5°C at $T_a = 3.78°C$. Both males and females displayed a significant negative correlation between bout length and ambient temperature so data were pooled for both sexes ($r = -0.188$; $t = -3.75$; $p < 0.05$; Fig. 3B).

Torpor frequency

We recorded a total of 412 torpor bouts (females, N = 8, n = 302; males, N = 5, n = 110). The lowest number of torpor bouts observed per animal for the entire duration of data measurement was 16 torpor bouts. The females entered torpor on 19 – 64 % of the total number of days whereas males were torpid on 20 – 32% of all days. Thus torpor frequency was significantly higher in females than in males ($F_{1,11} = 6.53$; $p < 0.05$). For both sexes no significant correlation was observed between body mass and torpor frequency ($r = -0.95$; $t = -0.32$; $p > 0.05$). The highest incidence of torpor occurred during July 2001 for all animals.

The number of consecutive daily torpor bouts ranged from 2 – 16 days in females and 2 – 7 days in males.

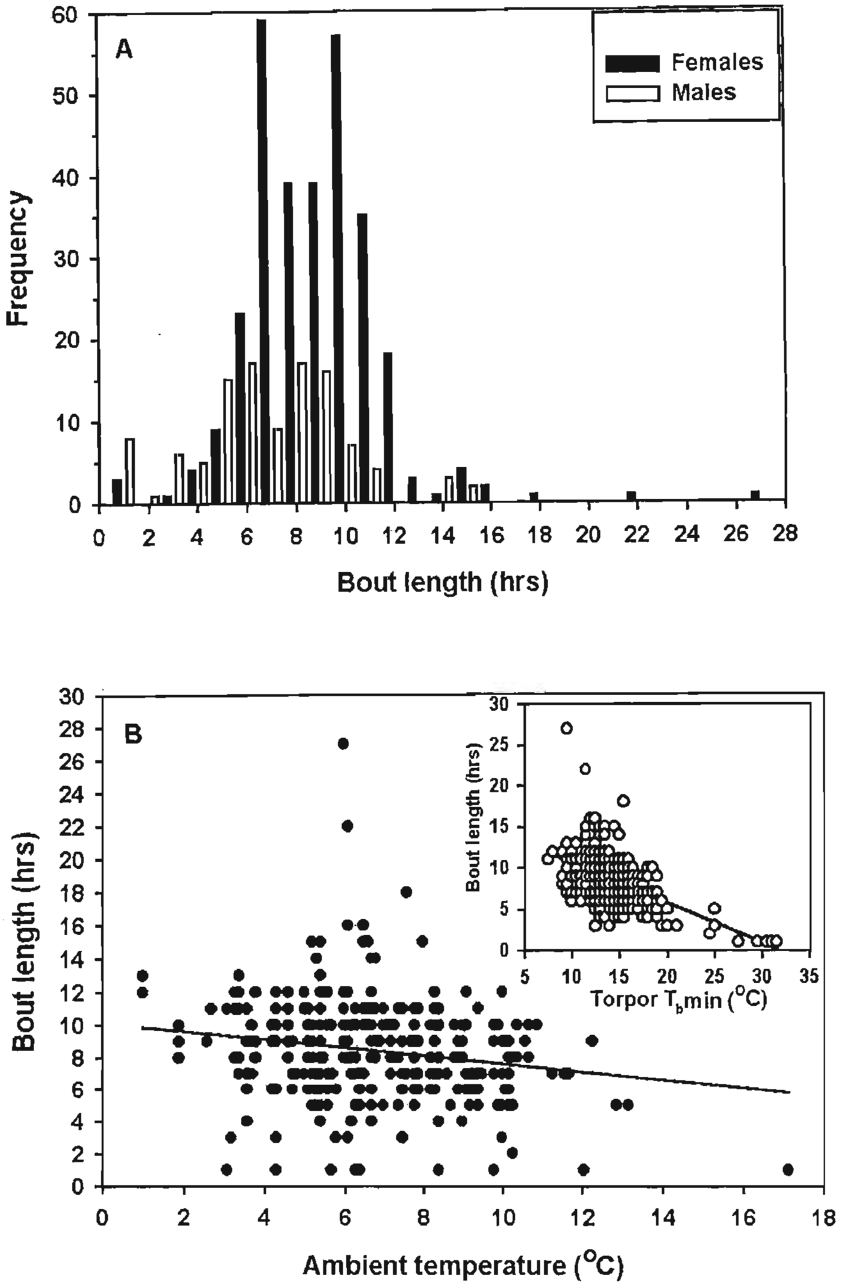


Fig 3. A Frequency distributions of torpor bout length in free-ranging female (filled bins) and male (clear bins) *E. myurus*. **B** The relationship between torpor bout length and ambient temperature. The inset graph shows the relationship between torpor $T_b$min and bout length

Torpor entry, arousal and heating rates

During entrance into torpor T_b followed the nighttime decrease in T_a similar to that of blackbody temperature (T_{black}) by *ca.* 5 hours (Figs. 4A,B). The torpor $T_b$min was reached immediately prior to sunrise, a time that also coincided with the lowest T_a as well as the lowest blackbody temperature.

Torpor entry occurred almost exclusively during the night although there was a high variability in the timing of entry into torpor. Torpor entry times ranged from 20h00 to 04h00 (Fig. 5A). The highest number of entries occurred between 22h00 and 02h00 although the latest entries occurred around 06h00, shortly before sunrise. The earliest times of torpor entry were associated with the longest torpor bouts. Very few animals entered torpor during the day. During the maintenance phase T_b stabilised at a fairly constant ($T_b$min – T_a) gradient that tracked the continuous decrease in T_a towards sunrise (Figs. 4A,B).

Most torpor bouts (79%) were terminated within a 2-hour period following sunrise (Fig. 5B). For most animals the T_b pattern of the arousal coincided with that shown by the heating of the blackbody, i.e. most arousals were tightly coupled to T_{black} and therefore the sun's heating cycle (Fig. 4B). The elephant shrews were frequently observed basking on the rocks in the sun during the early morning (Ribble, personal observation), although it is not known for certain whether those particular individuals had spent the night torpid.

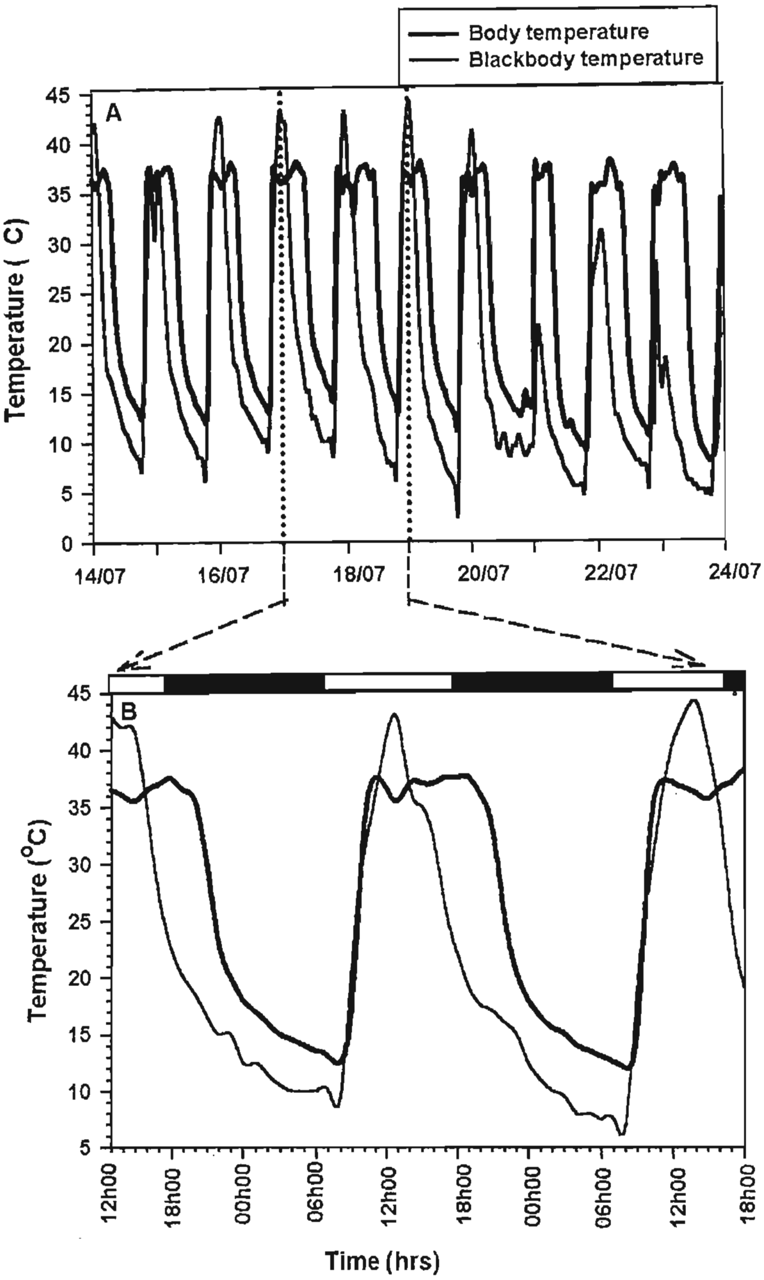


Fig. 4A, B Fluctuations in body temperature of a female *E. myurus* and a blackbody over a 10-day period during July 2001. Torpor commenced at night and was usually terminated shortly after sunrise. The dark bars on the top of the expanded lower 2-day figure indicate the dark phase.

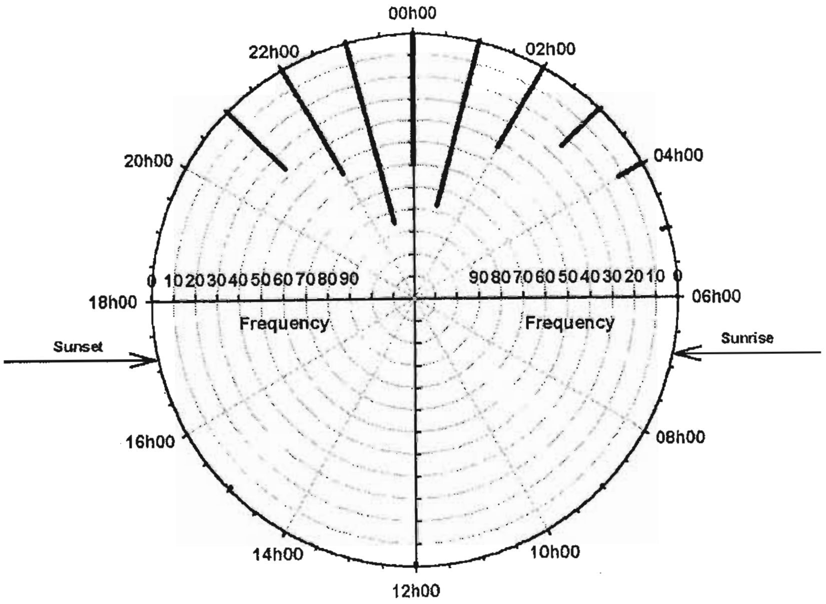
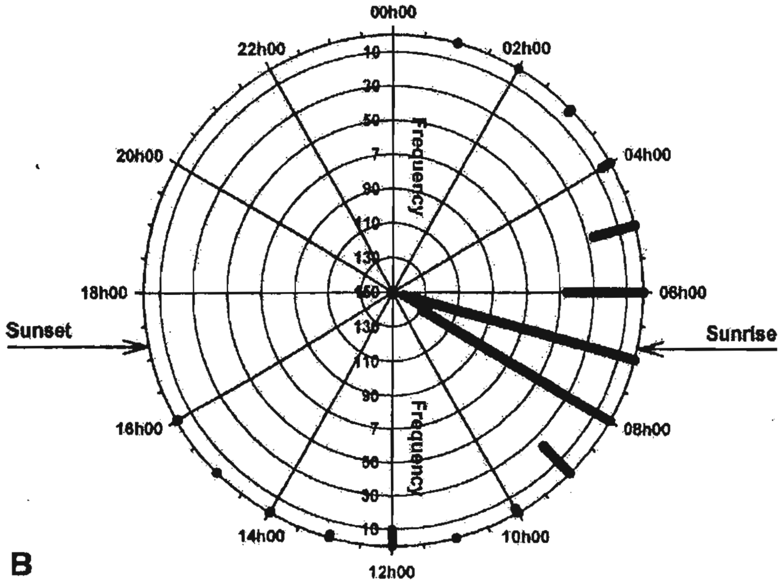


Fig. 5 A The timing of entrance into torpor in free ranging *E. myurus*. The inner axis represents the frequency of entries. The bin interval was 1h.



B The timing of arousal from torpor in free-ranging *E. myurus*. The inner axis represents the frequency of arousal from torpor. The bin interval was 1h

We identified two T_b heating patterns that we classified as assisted passive heating and endogenous heating. Assisted passive heating constituted 82% of observations where T_b heating rate (HR) was not more than double T_{black} HR. Observations where T_b HR < T_{black} HR presumably represent either situations in which the animals remained under rocks and crevices and did not bask in the sun, or the true T_{black} was less than that measured at our single measurement site (Figs. 6A,B). The endogenous heat production component accounts for the right skewed distribution of T_b HR – T_{black} HR, i.e. those values larger than ca. 0.10°C/min (Fig. 6B). Endogenous heating constituted 18% of observations (0.258 – 0.350°C/min; Figs. 6A, B) and was consistent with values previously measured in the laboratory (Lovegrove et al. 2001b) although slightly lower than that predicted by body mass (0.426° C/min; Geiser and Baudinette 1990). During endogenous heating T_b HR was always much higher than T_{black} HR (2 – 40 times higher; Figs. 6A,B; 7). One female (WWF06) accounted for 65 % of the endogenous self-arousals. It is not clear why this female used endogenous heating to this extent. Of all the endogenous self-arousals, 75% occurred prior to the onset of blackbody heating, i.e. before sunrise and the rest on days when T_a did not increase above 20°C. Furthermore, endogenous arousals were always completed within 1-hour, whereas assisted passive arousal ranged from 2 – 4 hrs in duration.

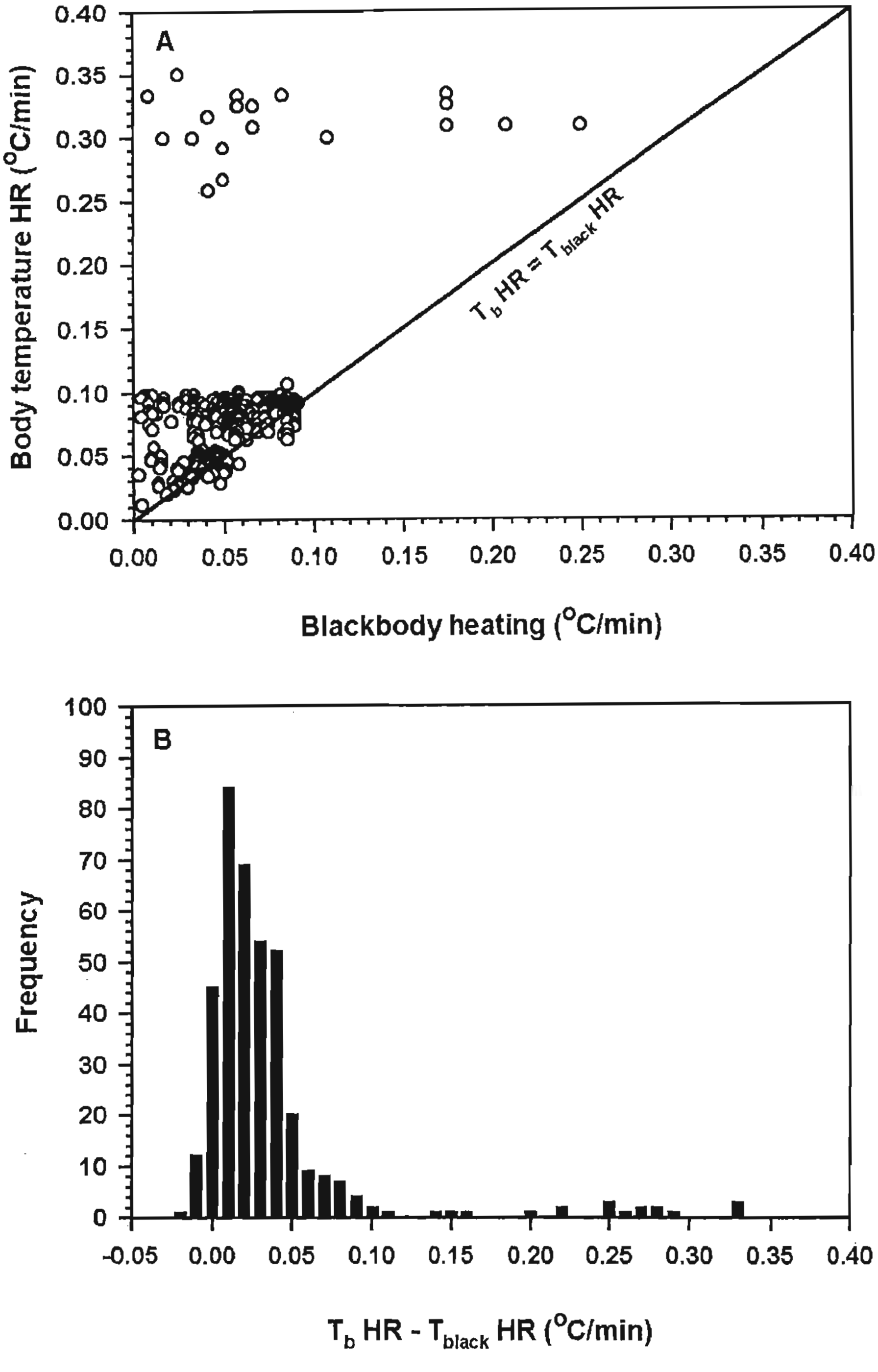


Fig. 6 A Body temperature heating rates as a function of blackbody heating in 387 torpor bouts recorded in free-ranging *E. myurus*. **B** Frequency distribution showing differences between T_b HR and T_{black} HR

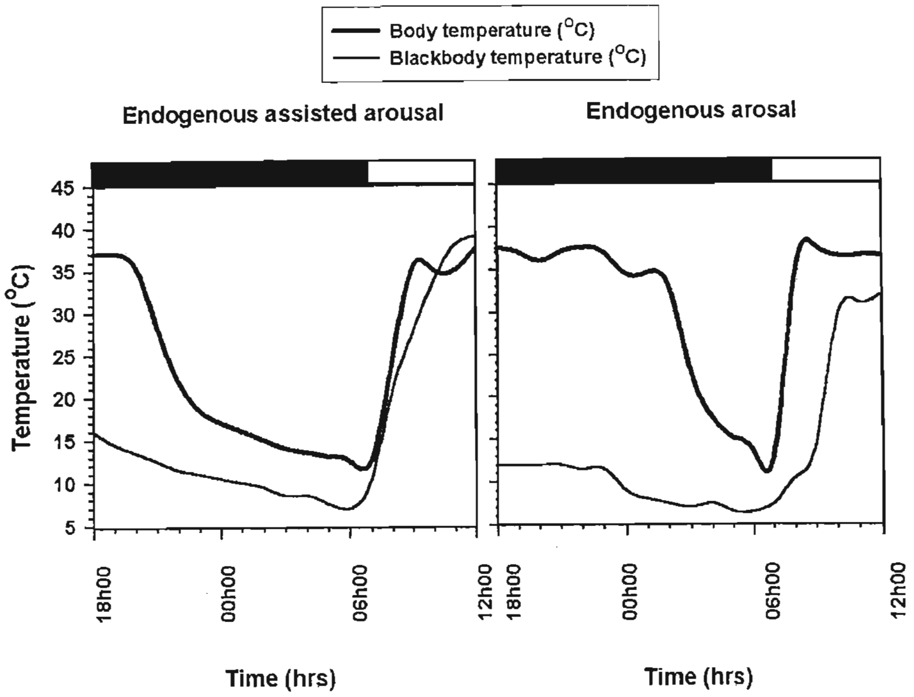


Fig 7 The fluctuation of body temperature and blackbody temperature for a female *E. myurus* (no. WWF05) showing assisted passive heating (T_b HR = 1.7X T_{black} HR) and endogenous heating (T_b HR = 17X T_{black} HR). The dark bars on top indicate the dark phase

Discussion

To our knowledge this is the first record of continuous daily torpor measurements in a free ranging small placental mammal. Similar recording of hibernating patterns have however, been made in fat-tailed dwarf lemurs, *Cheirogaleus medius* (Dausmann et al. 2000). Free-ranging rock elephant shrews displayed a high propensity for heterothermy during winter. The mean torpor $T_b$min in free-ranging *E. myurus* (14.6 ± 1.8°C) was *ca.* 5°C lower than that measured in the laboratory under short photoperiods by Lovegrove et al. (2001a) (19.8 ± 0.04°C) whereas the lowest torpor $T_b$min (7.5°C) was close to that measured in the laboratory (5.5°C). A significant amount of energy is allocated to arousal from torpor (Ruf and Heldmaier 1992). Generally, the deeper the bout, i.e. the lower the minimum torpor T_b, the more the energy that is required for arousal. Thus, the energetic significance of the low torpor $T_b$min measured in the laboratory in *E. myurus* was unclear. In contrast to constant temperature conditions in the laboratory, elephant shrews in the field experience daily T_a fluctuations (typically, 4 - 26°C). These T_a cycles may potentially decrease the cost of arousal from torpor through passive heating (Ortmann et al. 1996 ; Lovegrove et al. 1999a). For most *E. myurus* arousal from torpor coincided with the onset of blackbody heating. This tight coupling of T_b increase with T_{black} (Figs. 4A,B) suggests that arousals were synchronized with diel T_a cycles. Although torpor arousal and blackbody heating were clearly synchronized it is not easy to ascertain whether termination of torpor was triggered by sunrise i.e. light (79% of arousals occurred within a 2-h period surrounding sunrise) or an increase in T_a. Only further laboratory studies can clearly distinguish between these light-dark and T_a effects.

Our data imply that hypothermic animals are somehow able to emerge into the sun from under the rocks to sun bask while in a torpid state. Although we presently do not have any behavioral data to substantiate this assertion, elephant shrews have been observed to bask at sunrise during mid-winter (Ribble, pers obs). We are currently investigating the activity (if any) of free-ranging elephant shrews during arousal from torpor. These behavioral data will be coupled with simultaneous measurements of T_b and should provide insights into the extent of passive heating and hypothermic mobility.

We have no simultaneous energy expenditure (metabolic rate) measurements to correspond with the T_b records. Our inferences about the cost-effectiveness of passive heating are therefore based on the assumption that the ability to heat passively translates into energetic benefits for the animal as less endogenous thermogenesis is required to attain normothermic T_bs. All animals were capable of endogenous heat production in the dark when necessary but presumably at a much higher cost (75% of all endogenous self-arousals occurred before sunrise).

We suggest that our observations of passive heating are similar to those described for the grey mouse lemur, *Microcebus murinus* (Ortmann et al. 1996), and the striped-faced dunnart, *Sminthopsis macroura* (Lovegrove et al. 1999a). In these studies T_b was elevated passively through exogenous heating to *ca.* 25°C before endogenous heat production commenced. Thus the total HR during assisted passive heating involved passive heating initially, followed by endogenous thermogenesis. Lovegrove et al. (1999a) have suggested that during assisted passive heating, the onset of endogenous heat production may be triggered by a specific T_b and that there may exist a threshold T_b at which heat production occurs.

The principal aim of this study was to determine the frequency and depth of torpor in free-ranging *E. myurus* during winter. We therefore programmed the iButtons to measure T_b once every hour, resulting in continuous data for three months during mid-winter. The 1-hour sampling interval was sufficient to determine torpor $T_b$min and frequency but insufficient within each arousal to objectively discern inflection points that may identify the onset of endogenous thermogenesis. A shorter sampling interval (for example, 10 minutes) combined with behavioral data would most probably provide more information about these free-ranging heating patterns.

The lowest ($T_b$min – T_a) measured in free-ranging *E. myurus* (2.2°C) was close to the mean measured in the laboratory by Lovegrove et al. 2001a (2.84°C). Although there was a correlation between torpor $T_b$min and T_a, the animals were capable of maintaining fairly large ($T_b$min - T_a) gradients (mean = 7.3°C and 9.4°C for females and males, respectively), suggesting a certain capability to defend a torpor T_b in the event of further decreases in T_a. We therefore reject our original hypothesis that a torpor setpoint is absent in this species. The slightly higher $T_b$min – T_a gradient observed in males may account for the 1.9°C difference observed in torpor $T_b$min between sexes.

The mean bout length measured in the laboratory (9.9h) was *ca.* 1-h longer than that measured in free-ranging females (8.8h). Long torpor bouts were negatively correlated with torpor $T_b$min and resulted in larger energetic savings (Ruf and Heldmaier 1992 ; Geiser and Ruf 1995). Although Lovegrove et al. (2001a) suggested that *E. myurus* may be capable of hibernation, they did not record torpor bout lengths >24hrs. Furthermore, several behavioural, ecological and physiological characteristics render elephant shrews unlikely candidates for seasonal hibernation (Lovegrove et al. 2001a). However, of a total of 412 torpor bouts, we did record one torpor bout that exceeded 24 hrs (27hrs; $T_b$min = 9.5°C). Although limited conclusions may be drawn on the basis of one bout, this observation does not preclude the use of hibernation by free-ranging *E. myurus*. Furthermore, this observation lends support to the suggestion of a physiological gradient of heterothermy, whereby ecological and energetic criteria favour two regions of the gradient that currently characterize daily torpor and hibernation (Wilz and Heldmaier 2000). This is important in the context of differentiating between hibernation and daily torpor because bout length remains the one parameter in which there is no overlap between the two heterothermic states (Geiser and Ruf 1995 ; Wilz and Heldmaier 2000).

Male *E. myurus* entered torpor less frequently than females. Sexual disparity in the use of torpor has previously been observed in the Afrotropical pouched mouse, *Saccostomus campestris* (Lovegrove and Raman 1998 ; Mzilikazi and Lovegrove 2002) . The differential propensity for daily torpor between sexes in pouched mice was a consequence of incompatibility between year-round maintenance of gonadal activity and heterothermy. The literature on the breeding biology of *E. myurus* is scanty. Furthermore, male *E. myurus* have internal testes, which made it difficult to ascertain whether the males were breeding or had functional gonads during winter. However, the females showed no signs of breeding during winter. According to Skinner and Smithers (1990), reproduction is confined to the summer months between September and March. Hence, it is unlikely that the males were constrained in their use of torpor by maintenance of functional gonads.

Our study was carried out during winter. The diet of *E. myurus* consists mainly of insects, with high proportions of formicids and isopterans (Churchfield

1987). The availability of these food items decreases markedly during winter. Hence, in addition to low T_as the high propensity for torpor during winter may be associated with decreased food supplies. Accounts of invertebrate abundance throughout the year are required to clarify these questions. As yet, it remains unknown whether free-ranging rock elephant shrews use torpor throughout the year. Under laboratory conditions they utilize torpor both under winter and summer acclimation conditions (Lovegrove et al. 2001a,b). Furthermore, free-ranging elephant shrews are expected to display aseasonal torpor because of the climatic variability characteristic of the Afrotropical region, resulting in unpredictable temporal and spatial reliability of resource availability (Lovegrove 2000).

This study demonstrated that the elephant shrews benefit energetically from the remarkably low torpor $T_b$min because they can offset the high cost of arousal through almost always-reliable passive heating. Thus although useful inferences may be made from laboratory studies regarding the energetics of torpor, they underestimate the energetic benefits that may be accrued by use of diel ambient temperature cycles during arousal from torpor, particularly in those environments where seasonality is moderate.

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Chapter 3

Daily torpor in free-ranging rock elephant shrews, *Elephantulus myurus*: A year-long study

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Abstract

Under laboratory conditions rock elephant shrews, *E. myurus* utilize daily torpor under both short and long photoperiod acclimation. However, use of heterothermy often differs under field and laboratory conditions. We investigated the use of torpor in free-ranging elephant shrews from May 2001 – May 2002. The elephant shrews were capable of daily torpor throughout the year, with torpor most prevalent during winter. We recorded two torpor bouts during early summer (November). We recorded a total of 467 torpor bouts during the year. The mean torpor $T_b$min for the whole year was 15.3 ± 4.4°C and the mean bout length was 8.6 ± 3.5hrs. These values were in the range expected for daily heterotherms. However, there was some marginal overlap with hibernation characteristics; a few torpor bouts were longer than 24 hrs in duration and $T_b$min decreased below 10°C. Torpor was highly correlated with low ambient temperature and photoperiod. Torpor was also correlated with invertebrate abundance after controlling for photoperiod effects. During the year in which this study was

conducted the rainfall was 14% below long-term average. Historical rainfall records show that summer rainfall during strong El Niño years is up to 40 % below the long-term average. Perhaps during these drought years the frequency of summer torpor may be higher, highlighting the need for long-term physiological data in free-ranging animals.

Introduction

Mammalian hibernation and daily torpor are proximate physiological responses that conserve energy following environmentally induced or inclement energy stresses (Lyman et al. 1982; Ruf and Heldmaier 1992; Lovegrove 1996; Grigg and Beard 2000). Daily torpor is particularly common in the Afrotropical and Australasian mammals (Geiser 1994; Lovegrove 2000a), regions characterized by high climatic variability, resulting in unpredictable temporal and spatial reliability of resources (Tyson 1986; Lovegrove 2000a,b). Small mammals inhabiting these regions display conservative physiological traits (for example, low basal metabolic rates and use of daily torpor) that aid in the conservation of energy (Lovegrove 1996, 2003; Lovegrove and Raman 1998).

Elephant shrews (Macroscelidea) are African endemics (Corbet and Hill 1991) that occupy a range of habitats from desert to forest (Skinner and Smithers 1990). They belong to a phylogenetically ancient African clade (Afrotheria) that includes elephants, dugongs, hyraxes, tenrecs, golden moles and the aardvark (Stanhope et al.1996; Springer et al. 1997). Several species of elephant shrews are known to utilize torpor in the laboratory both under winter and summer acclimation conditions (Seguignes 1983; Lovegrove et al. 1999a; Lovegrove et al. 2001a,b). Year-round use of daily torpor is consistent with models predicting that animals inhabiting unpredictable environments should display a high propensity for heterothermy, regardless of season (Geiser and Baudinette 1988; Lovegrove 2000a). However, because heterothermic responses often differ between field and laboratory conditions (Geiser et al. 2000), it is unknown whether free-ranging rock elephant shrews utilize heterothermy throughout the year. It is also unclear whether heterothermic responses in the field are photoperiod or cold-mediated or whether torpor may be induced by the temporal reliability of food resources.

We previously recorded daily torpor in free-ranging *E. myurus* during winter (Mzilikazi et al. 2002). Although daily heterothermy was clearly correlated with low ambient temperatures, we did not investigate whether the employment of torpor was associated with potential food availability. Invertebrate prey constitute the major component of the diet of *E. myurus*, with ants, termites and beetles the most common food items (Churchfield 1987). In addition however, *ca*. 10% of the diet consists of plant material. Typically, the availability of potential food items, i.e. invertebrates, decreases markedly in winter (Samways et al. 1996; Kotze and Samways 2001). Hence, although elephant shrews employ daily torpor in response to cold, the potential influence of invertebrate abundance also requires investigation.

In this study we investigated whether free-ranging *E. myurus* utilized heterothermy during all seasons and determined which parameters characterized heterothermy during the different months of the year. Furthermore we investigated the correlation between torpor incidence, photoperiod, ambient temperature and invertebrate abundance.

Materials and methods

The study was conducted at Weenen Nature Reserve, KwaZulu Natal, South Africa (28° 52.534′ S; 30° 00. 264′E) from May 2001 – May 2002. The habitat and study site are described by Mzilikazi et al. (2002).

Adult rock elephant shrews, *Elephantulus myurus,* were captured (Permits no. 26048/2001 and 27765/2001; KZN Wildlife) using Sherman traps baited with peanut butter rolled in oats. Pre-calibrated Thermochron iButtons (Dallas Semiconductor) were surgically implanted into the peritoneal cavities of the animals under inhalation anaesthesia (Isoflorane in oxygen; induction and maintenance, 2%; flow rate, *ca* 0.5 $l.\mathrm{min}^{-1}$). The animals were released at the exact capture locations < 24h after capture.

iButtons are miniature dataloggers (*ca.* 3g) armoured in stainless steel caps. They integrate a thermometer, a clock and a calendar, and hence are suitable for continuous recording of body temperatures (T_b) over specified periods. They can record up to 2048 consecutive temperature measurements in read-only nonvolatile memory. iButtons measure temperatures from – 40°C to 85°C in 0.5°C increments and have a temperature conversion accuracy of 1°C from –20° to 70°C. The real-time clock accuracy is ± 2 minutes per month from

0 - 45°C. We programmed iButtons to measure body temperature once every hour, resulting in a total of 2, 040 data points per animal, over a period of 85 consecutive days.

To measure ambient temperature (T_a), we placed an iButton in the shade (under a rock) and also measured the temperature of a blackbody (T_{black}) by inserting an iButton into a sealed black tin cylinder and exposed it to the sun at the same site. Because rock elephant shrews are not known to build nests (Skinner and Smithers 1990), the shade T_a aimed to measure diel fluctuations of T_a within the typical resting sites of the elephant shrews (i.e. under rocks). We used blackbody temperatures as a reference for potential passive heating in elephant shrews during sun basking. We were not able to measure T_a at all animal capture sites. Therefore, depending on the aspect of each elephant shrew capture site relative to the position of the measuring iButton, T_a could vary slightly from the measurement site. Sunrise and sunset times at the fieldsite were obtained using a GPS (eTrex Vista: Garmin) and from these, mean monthly photoperiod was calculated.

Invertebrate sampling

To quantify potential food availability during the different seasons, the relative abundance of invertebrates active on the ground surface was determined using pitfall traps. A major criticism of pitfall trapping is that sometimes there may be no direct correlation between estimates of invertebrate abundance and intake by potential invertebrate predators (Churchfield 1982). The diet of rock elephant shrews consists of termites, ants and beetles. We used pitfall trapping as it is a standard method for trapping these epigaeic invertebrates (Kotze and Samways 1999a). Furthermore, the method provides a fairly accurate measure of temporal changes in insect abundance and is more objective and consistent than searching techniques (Samways et al. 1996; Kotze and Samways 1999b; Kotze and Samways 2001). We placed a total of 48 pitfall traps in three sites from which elephant shrews had been captured (16 traps in each site). Each site was $16m^2$ and the pitfall traps were filled with 70% alcohol mixed with glycerol. The pitfall traps were checked at four-week intervals. We obtained pitfall samples from May 2001 – March 2002, and no invertebrate samples were obtained in April and May 2002. For the invertebrate analysis we identified all samples to order level. Before parametric tests could be performed invertebrate abundance data were log-transformed to better approximate a normal distribution.

Data and statistical analyses

In winter (May – August 2001) we implanted 18 elephant shrews with iButtons, 13 of which were recaptured. During spring (August – November 2001) we implanted a further 18 animals. Before recapture of the spring animals, the study site was burnt as part of the grassland savanna maintenance programme in the reserve (September 2001). After the fire our recapture success was reduced and we only recaptured two of the spring animals. In summer (November 2001 – February 2002) we implanted 12 animals and recaptured four animals and in autumn (March – May 2002) we implanted 15 animals, of which three were recaptured. Although the fire might explain low recapture rates during spring, it is unclear why the recapture rates were so low during autumn and summer. Each season consisted of 85 consecutive data measurement days. However, because our sample sizes for the different seasons varied, we were unable to perform statistical tests to investigate seasonal effects on torpor parameters such as bout length, minimum torpor T_b and $T_b - T_a$. Our results are therefore of a descriptive nature, depicting torpor parameters in free-ranging rock elephant shrews.

Apart from torpor frequency, all torpor parameters do not vary significantly between sexes during winter (Mzilikazi et al. 2002). We therefore pooled all data and did not test for sex differences in torpor parameters at the different times of the year.

The body temperature ($T_b$min) below which the animals were deemed to be torpid was 32°C. This value is slightly more than 2°C lower than the lower limit of the normal distribution of rest phase normothermic T_bs in both sexes measured in the laboratory at 25°C by Lovegrove et al (2001b). The torpor bout length was determined as the total time during which T_b was maintained below 32°C. T_b min for both torpid and non-torpid animals was taken as the lowest T_b measured during a 24-h period. Within each torpor bout the local time of entry into and arousal from torpor were determined. We also recorded the T_a at which entry into torpor commenced as well as the T_a at the time that $T_b$min was attained. Due to equipment damage between May 15 – 05 June 2002 we were unable to obtain T_a in the shade. Hence, all analyses involving T_a have been excluded for this period. However, bouts measured during this period have been included in respective frequency distributions.

Torpor frequency was calculated for individual animals as the proportion of days during which the animal displayed torpor (number of bouts/individual/time). Torpor frequency data were arcsine transformed to allow for parametric tests. Normality of the data was confirmed using the Kolmogorov – Smirnov procedure (Zar 1984). We investigated the potential effects of T_a, invertebrate abundance and photoperiod on torpor frequency using a multiple regression model. However, inter-correlation between variables influences the choice of independent variables that can be included in multiple regression models. We therefore calculated a correlation matrix of all variables to identify multi-collinenearity between T_a, invertebrate abundance and photoperiod (Table 1). There was no significant correlation between invertebrate abundance and T_a ($r = -0.33$; $p > 0.05$) or photoperiod ($r = -0.80$; $p > 0.05$). However, T_a and photoperiod were strongly correlated ($r = 0.96$; $p < 0.05$). We present two separate multiple regression models to test the correlation between torpor frequency, invertebrate abundance and T_a (Model 1) or photoperiod (Model 2). In multiple regression *beta* is the standardized partial regression coefficient used to compare the relative strength of each significant independent variable on the dependent variable (Zar 1984).

Throughout the text n refers to the number of observations and N refers to the number of animals. All mean values are reported ± SD.

Table 1 Correlation matrix showing Pearson product-moment correlation coefficients (r) for torpor frequency, T_a, photoperiod and invertebrate abundance. Correlations marked with an asterisk are significant at $p < 0.05$

Variable	Torpor frequency	Invertebrate abundance	T_a	Photoperiod
Torpor frequency	1.00	0.56	-0.88*	-0.80*
Invertebrate abundance	0.56	1.00	-0.33	-0.08
T_a	-0.88*	-0.33	1.00	0.96*
Photoperiod	-0.80*	-0.08	0.96*	1.00

Results

Rainfall, photoperiod and ambient temperature

Because our study area is situated in a summer rainfall area, we calculated the total annual rainfall (TAR) between October – September so as to include the entire summer rainfall season. The total annual rainfall at our study site was 634mm in 2001. The shortest day at the study site was 10hrs, 20 mins and the longest 14 hrs, representing a maximum difference of 3hrs, 40 mins between the shortest and longest day of the year. Ambient temperatures measured in the shade ranged from 0.9 - 23.4°C in winter, 3.0 – 33.5°C in spring, 11.0 – 34.5°C in summer and 2.5 – 31.0°C in autumn.

Invertebrate abundance

We captured invertebrates representative of 19 orders and the number of animals captured per month varied throughout the year from 372 – 2733 animals/month (Figure 1). The mean was 1412 ± 741 animals/month. Hymenopterans constituted the major proportion in all the samples (Figure 1). The lowest proportion of hymenopterans was observed in December. Except during May, invertebrate numbers were unexpectedly high during the winter months, compared with the summer months. The marked decrease in invertebrate numbers in October coincided with heavy rains, which flooded the pitfall traps. Hence the low numbers in October represent a sampling artifact. Only during November and December were invertebrate numbers above the average measured for the whole year.

Body mass

The mean body mass at the beginning of winter was 56.6 ± 2.9g (N = 13). The animals weighed 58.2 ±9.5°C (N = 2) and 60.5 ± 0.6g (N = 4) at the beginning of spring and summer, respectively. At the beginning of autumn the body mass decreased to 53.3 ± 4.2g (N = 3).

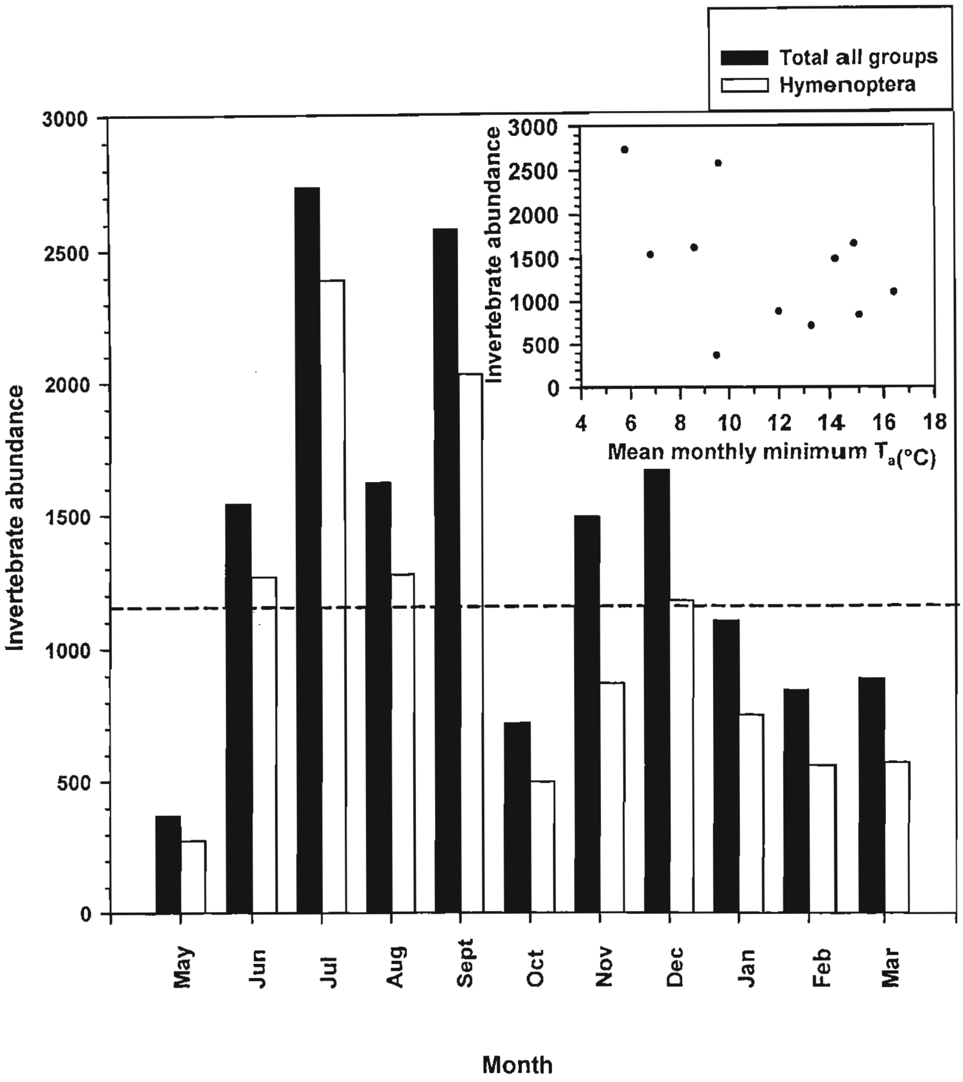

Figure 1. Relative invertebrate abundance from May 2001 to March 2002. The open bars show the total number of hymenopterans in the sample during each month. The inset graph shows the relationship between invertebrate abundance and monthly minimum ambient temperature.

Torpor frequency

For the whole year we recorded a total of 467 torpor bouts. Free-ranging *E. myurus* displayed the highest propensity for torpor during winter (Table 2). Of the 467 bouts recorded 412 (88.2%) were recorded in the period 17 May – 06 August 2001 (N = 13). Torpor frequency was highest during June/July 2001 (Figure 2), coinciding with the coldest months of the year (Figure 2 inset). By September torpor incidence had decreased to a total of 15 bouts (N = 2) between 21 August – 30 September 2001. In October no torpor bouts were observed and in November only two torpor bouts were recorded. Between December 2001 and February 2002 no torpor was observed in any of the elephant shrews. From 25 March 2002 onwards, the animals again displayed torpor (Figure 2). We recorded a total of four bouts in March, six bouts in April and 28 bouts in May 002 (N = 3; Table 2). In the T_a model (Model1), torpor frequency was not correlated with invertebrate abundance (*beta* = 0.30; p = 0.067) but was significantly correlated with T_a (*beta* = -0.78; $p < 0.005$; Table 3). However, torpor frequency was also highly correlated with photoperiod (*beta* = -0.75; $p < 0.005$; Model 2). In the photoperiod model, invertebrate abundance also had a significant effect on torpor frequency (*beta* = 0.5; $p < 0.005$).

Table 2 Physiological parameters of torpor and normothermia in free-ranging *Elephantulus myurus* from May 2001 – May 2002.

	Winter		Spring		Summer		Autumn	
	(N = 13)	*n*	(N = 2)	*n*	(N = 4)	*n*	(N = 3)	*n*
T_b normothermia (°C)	34.2 ± 0.5	416	34.4 ± 0.5	149	35.2 ± 0.8	330	35.2 ± 0.9	213
T_b torpor (°C)	14.6 ± 2.1	412	16.4 ± 6.4	15	24.3 ± 1.1	2	18.5 ± 4.4	38
Range torpor T_b (°C)	7.5 - 31		9.5 - 30		23.5 - 25		12.5 – 31.5	
Bout length range (h)	1 - 27		4 - 39		4 - 5		1 - 15	
Bouts > 24h	1		2		0		0	
Mean bout length (h)	8.3 ± 1.3	412	13.6 ± 9.4	15	4.5 ± 0.7	2	9.7 ± 2.6	38
Mean $T_b – T_a$ (°C)	7.8 ± 1.5	389	10.0 ± 5.5	15	9.3 ± 0.4	2	7.7 ± 3.5	38

Note: N refers to the number of animals and *n* to the number of observations

Table 3 Statistics of a multiple regression model investigating the influence of invertebrate abundance and mean monthly minimum T_a on torpor frequency in free-ranging *Elephantulus myurus.*

	Invertebrate abundance	Mean monthly T_b min	Photoperiod	Model $F_{2,8}$
Model 1 (mean monthly T_a)				23.798
β	0.30	- 0.78	…	
P	0.067 (NS)	< 0.005	…	< 0.005
Model 2 (photoperiod)				28.93
β	0.50	…	- 0.75	
P	< 0.05	…	< 0.005	< 0.05

Note: In multiple regression *beta* (β) is the standardized partial regression coefficient used to compare the relative strength of each significant independent variable on the dependent variable

Figure 2

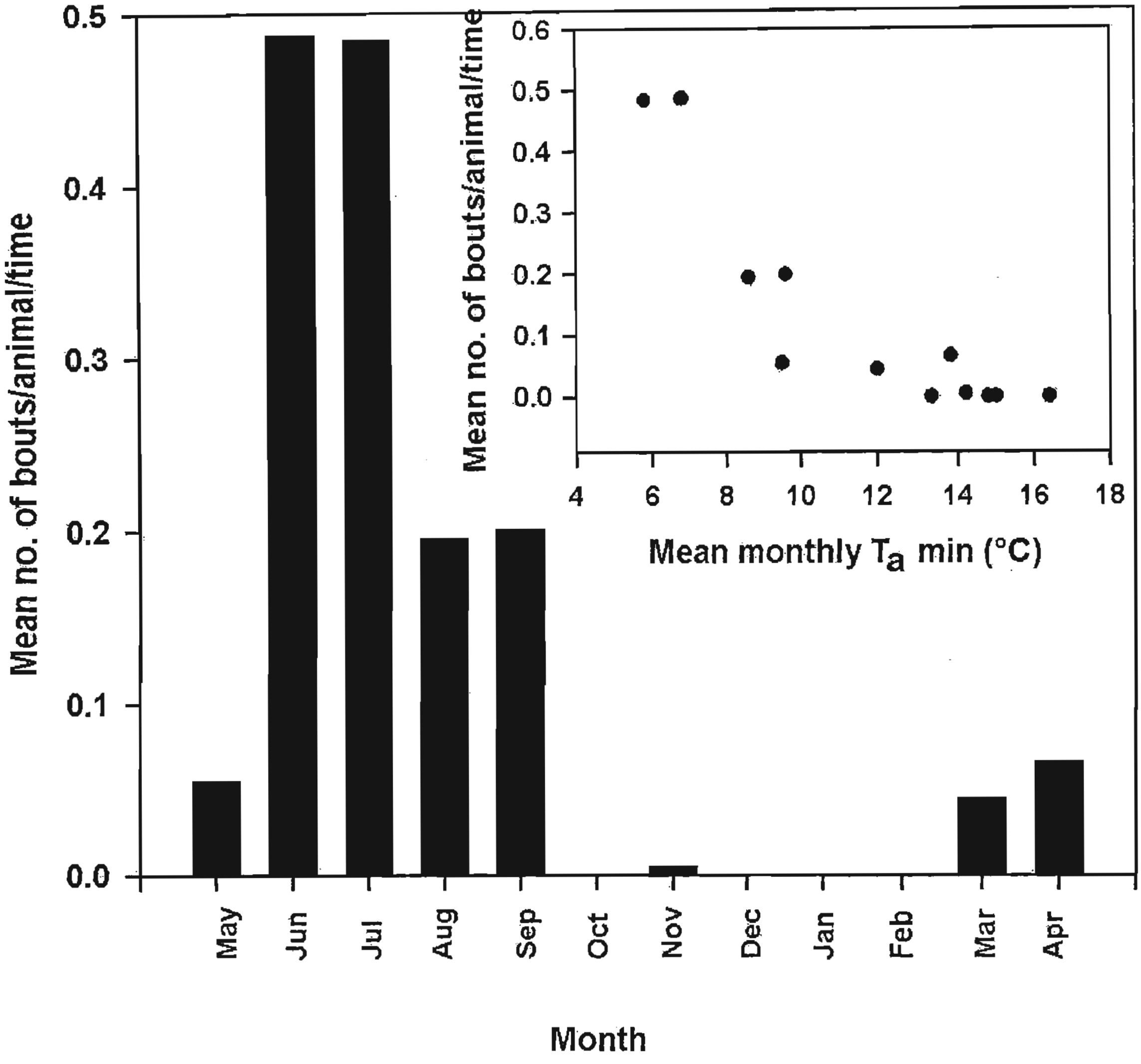

Figure 2. Torpor incidence in free-ranging *Elephantulus myurus* from May 2001 – April 2002. The highest number of torpor bouts was recorded during the coldest months of the year. The inset graph shows the relationship between torpor incidence and mean monthly minimum ambient temperature. In both graphs the unit time refers to the number of days for which data were available because for some animals we did not obtain data for a full month.

Minimum body temperature

The T_a at which animals entered torpor ranged from 2.9°C to 19.7°C (Figure 3) and the mean T_a at torpor entry was 9.9° ± 3.1°C for the whole year. The lowest torpor T_bs were recorded in winter and showed a gradual increase through spring and summer (Figure 3). The mean torpor $T_b$min for the whole year was 15.3 ± 4.4°C (n = 467) and ranged from 7.5 – 31°C (Figure 4). Throughout the year normothermic T_b's ranged from 32 – 37°C and the mean $T_b$norm for the whole year was 34.6± 1.0°C (Figure 4). The lowest body temperature measured in winter was 7.5°C at T_a = 2.7°C. That measured in spring was 2°C higher (9.5°C). In summer the lowest minimum torpor T_b was 23.5°C and in autumn 12.5°C. (Figure 5). The range of $T_b - T_a$ throughout the year was 7.7 – 10.0°C (Figure 5; Table 2).

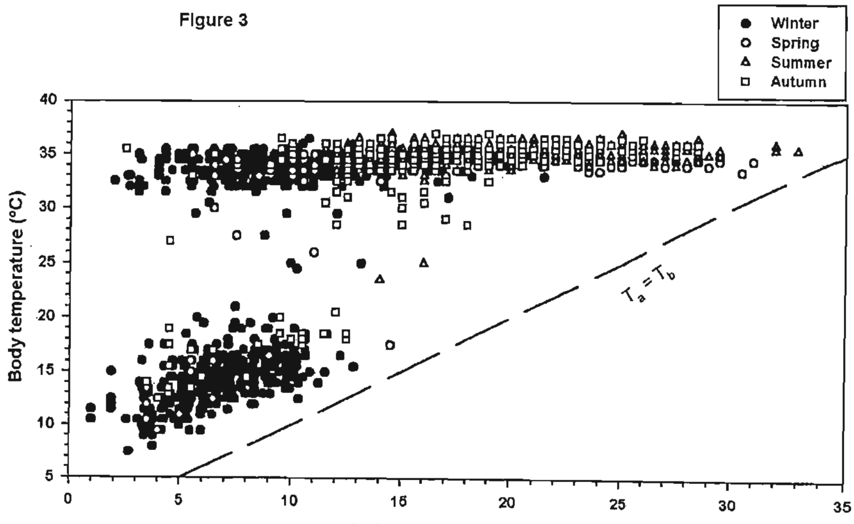

Figure 3. The relationship between body temperature and ambient temperature at which T_b was measured, during the different seasons. Minimum torpor body temperature was correlated with ambient temperature.

Figure 4

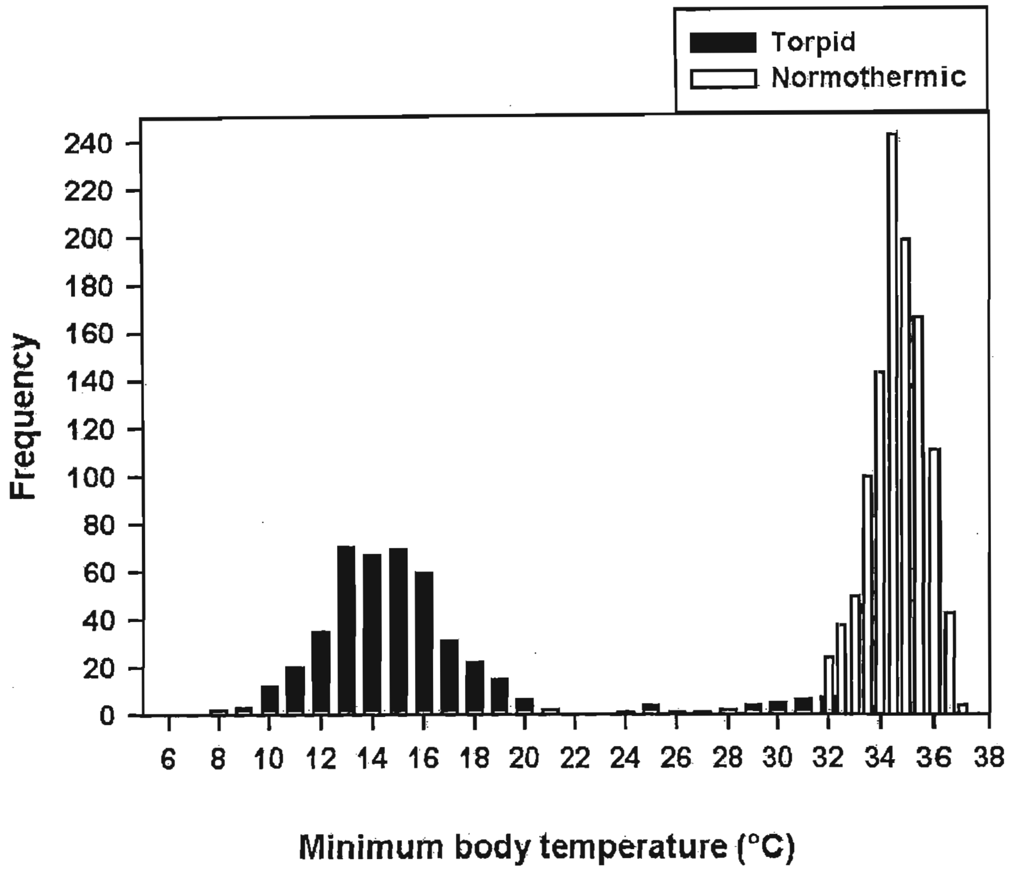

Figure 4. The frequency distribution of the minimum daily body temperature (T_b) in torpid (solid bars) and normothermic (open bars) free-ranging *Elephantulus myurus* measured between May 2001 – May 2002.

Figure 5

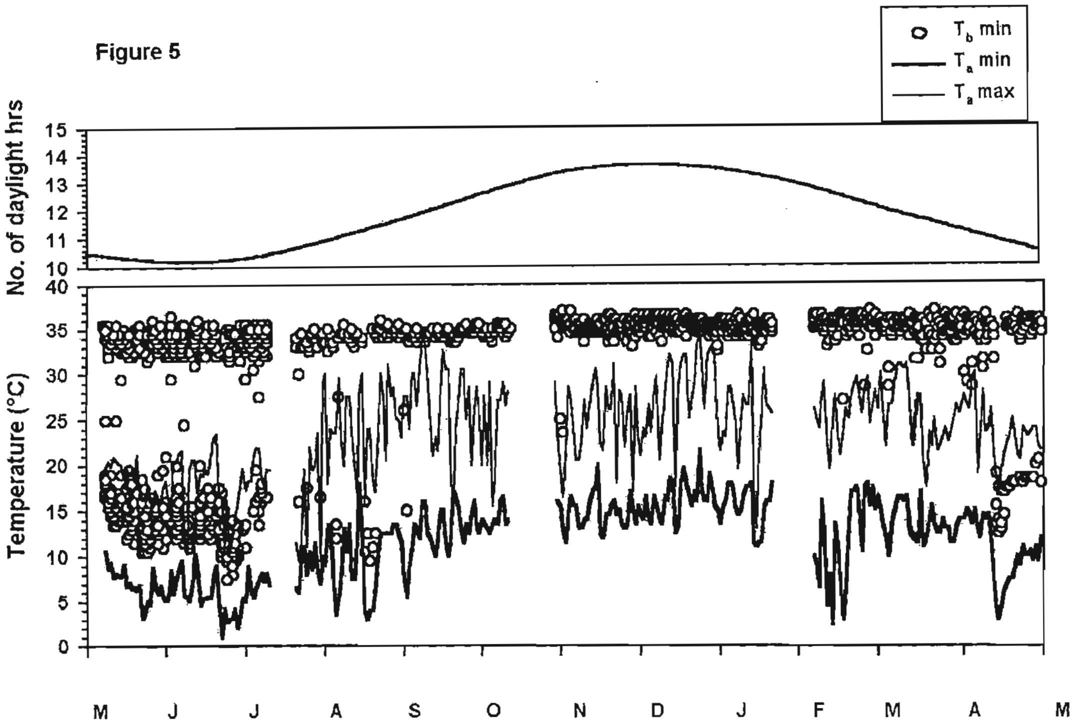

Figure 5. Minimum body temperature measured in free-ranging *Elephantulus myurus* between May 2001 – May 2002 (bottom plot). Top clusters show normothermic T_b. Animals were classified as torpid if body temperature (T_b) decreased below 32°C. The thick line shows daily T_a minima and the thin line shows daily T_a maxima. The solid line on the top plot shows changes in photoperiod throughout the year.

Torpor bout length

Overall, torpor lasted from 1 – 39 hrs (Figure 6). The mean bout length for all torpor bouts > 1hr was 8.8 ± 3.3 hrs. In winter, torpor bout duration ranged from 1 – 27 hrs. All animals did not enter torpor on the same night, or at the same ambient temperature. Using the individual displaying the highest number of torpor bouts as the baseline, we therefore calculated a multiple regression with ambient temperature and individual animals as independent variables and bout length as the dependent variable. Torpor bout length was negatively correlated with ambient temperature (*beta* = -0.28 ; t = -6.18; $p < 0.05$), with the longest torpor bouts associated with the lowest ambient temperatures (Figure 6 inset). The relationship between ambient temperature and bout length could be described by the equation, bout length = $13.2 - 0.28\ T_a +$ individual effect.

A typical torpor bout usually commenced *ca.* 5hrs after sunset, following the nighttime decrease in ambient temperature. Torpor bouts were usually terminated within a 2-hr period following sunrise. Most arousals were tightly coupled with the sun's heating cycle (Figure 7A).

In spring we measured bouts ranging from 4 – 39 hrs and in summer torpor bouts were relatively short and were recorded on the 24th and 25th November 2001 and lasted four and five hrs, respectively (Figure 7B). In autumn bout lengths ranged from 1 – 15 hrs. During the entire study period we measured three bouts that lasted longer than 24 hrs (27, 28 and 39 hrs). These were recorded on the 22nd July, 12th and 13th September 2001, respectively. The latter two bouts were recorded during a severe cold spell that lasted four days.

Figure 6

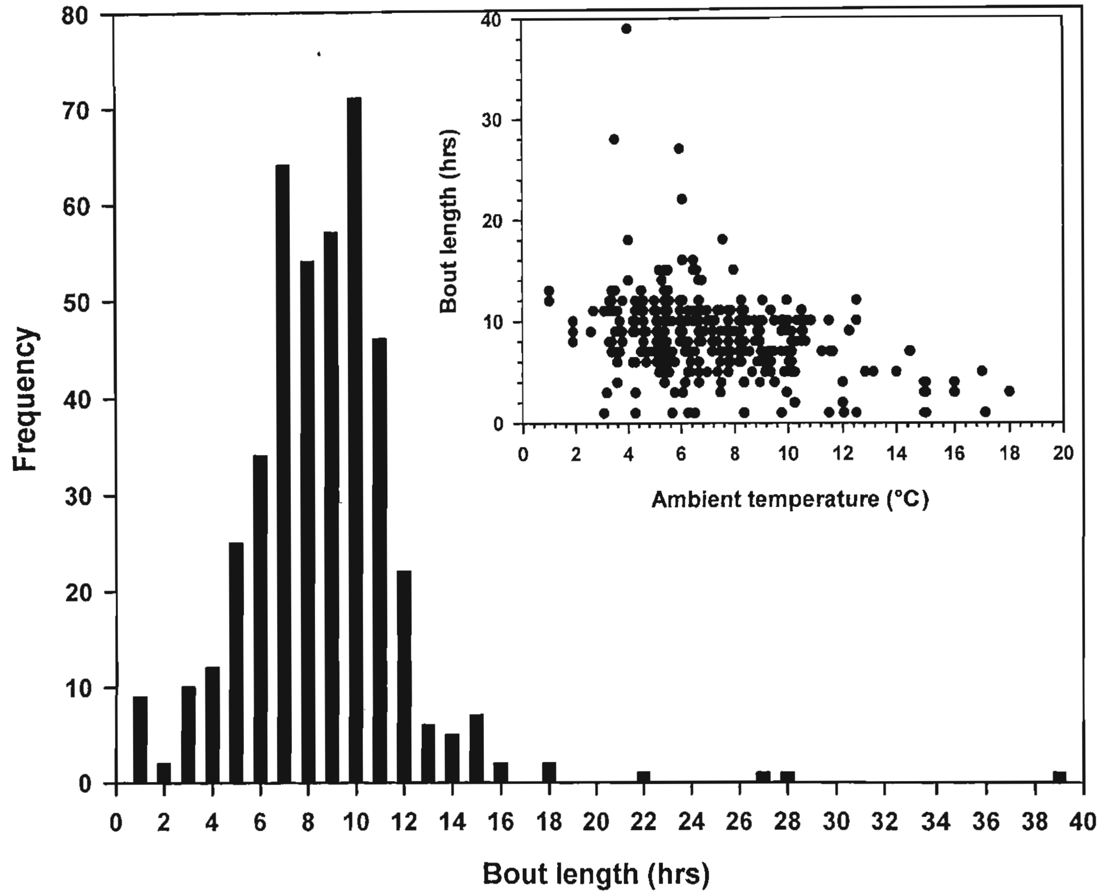

Figure 6. The frequency distribution of all bout lengths measured between May 2001 – May 2002 in free-ranging *E. myurus*. The inset graph shows the relationship between torpor bout length and ambient temperature

Figure 7

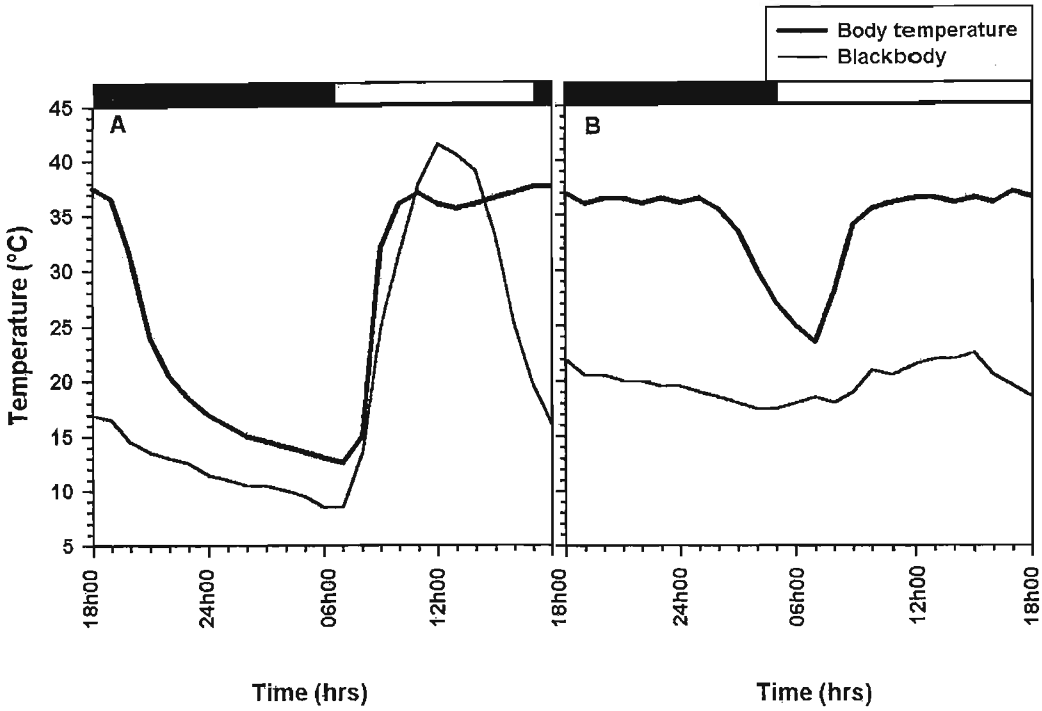

Figure 7A. Temperature profile of a female *Elephantulus myurus* (thick line) during a torpor bout measured in winter 2001. The thin line represents temperature of a blackbody measured over the same time interval. Arousal from torpor was clearly synchronized with the sun's heating cycle. The dark bars at the top indicate the night. B. A much shorter torpor bout measured in summer 2001 in a free-ranging male *Elephantulus myurus*. The dark bars at the top indicate the night

Discussion

This study is the first to investigate daily torpor for a whole year in a free-ranging small eutherian mammal. Free- ranging *E. myurus* displayed torpor during all seasons of the year, although torpor incidence was low during summer. Only two torpor bouts were measured between October and February. We recorded the highest number of torpor bouts during June, the coldest month of the year. The high torpor incidence during winter was significantly correlated with both photoperiod and ambient temperature, suggesting that both cues were important in the expression of torpor in free ranging rock elephant shrews. In the laboratory, rock elephant shrews are capable of entering torpor under both winter and summer acclimation conditions, provided they are in negative energy balance, a response also observed in dasyurid marsupials (Geiser and Baudinette 1987; Geiser et al. 1996; Holloway and Geiser 1996). Similarly, honey possums, *Tarsipes rostratus*, are also capable of torpor during most months of the year when captured in the field, with the highest torpor incidence observed during the winter months (Withers et al. 1990). The ability of some species to enter torpor under short and long photoperiods suggests that, at different times of the year, other factors may have an overriding influence over photoperiod. Torpor is known to be influenced by a multitude of factors including food and water availability, T_a, photoperiod, reproductive state, prevailing weather conditions and innate timing mechanisms (Heldmaier and Steinlechner 1981; Geiser 1986; Kenagy 1986; Song and Geiser 1997; Geiser and Körtner 2000; Lehmer et al. 2003) . However, the trigger that exerted the primary influence on torpor frequency in free- ranging rock elephant shrews could not be discerned from our data.

Mammalian hibernation and daily torpor in the wild are often assumed to be, in part, a consequence of cold and/or low food availability (Lyman et al. 1982), although data on food availability are lacking. At the $p = 0.05$ level, the correlation between invertebrate abundance and torpor frequency was not significant in the T_a model. However, in the photoperiod model, invertebrate abundance had a significant effect on torpor frequency suggesting that, outside the winter season, food availability may also be an important trigger for torpor use by free ranging rock elephant shrews. The diet of *E. myurus* consists mainly of ants, termites and beetles as well as ca. 10% plant material (Churchfield 1987). There were very low termite numbers in our samples. This pronounced absence of termites in our samples may have underestimated the influence of invertebrate abundance on torpor frequency in the T_a model. We have no immediate explanation for why invertebrate numbers were relatively higher during the winter months, compared to the summer months because invertebrate abundance is typically low during winter (Samways et al. 1996). It is possible that the winter insect abundance figures were over- inflated by the number of hymenopterans in the samples. In July, hymenopterans constituted 87% of the invertebrates in the samples. By contrast, during summer the invertebrates captured were fewer but larger in size (mainly coleopterans, orthopterans and lepidoptera larvae) and the hymenopterans were not as numerous in the summer samples as during winter. Furthermore, it is possible that the sampling intervals we used were insufficient to provide enough resolution to allow correlation between invertebrate abundance and particular torpor events.

High steroid levels associated with reproductive activity influence the use of torpor in small mammals (Hall and Goldman 1980; Darrow et al. 1987; Mzilikazi and Lovegrove 2002). We recorded the fewest torpor bouts during summer. We also captured juvenile elephant shrews during summer and autumn, suggesting that the animals were breeding during the summer months. Our observations thus confirm reports that female *E. myurus* breed from the end of July to the middle of January (Woodall 1987; Woodall and Skinner 1989; Skinner and Smithers 1990). It was difficult to ascertain the breeding status of the males in the field since the testes are internal in this species. *Elephantulus rupestris* females in the Namib have truly polyestrous cycles (Withers 1983) and seem to be capable of year-round reproduction. However, we do not have any evidence of all-year breeding in *E. myurus*. It is possible therefore, that reduced torpor display during summer may be partly a consequence of breeding activity.

In the laboratory *E. myurus* are physiologically capable of displaying torpor throughout the year when in energy shortfall (Lovegrove et al. 2001b). Thus, although we only measured two torpor bouts throughout the summer season, we cannot preclude the use of summer torpor by free ranging elephant shrews. Summer torpor is most likely to be used (and most energetically beneficial) when resource availability is decreased, usually as a result of unpredictable rainfall or during unseasonal cold spells. In the tropics and subtropics rainfall unpredictability is generated mostly as a consequence of summer rainfall anomalies related to the El Niño Southern Oscillations (ENSO) (Philander 1983; Stone et al. 1996). ENSO-associated rainfall anomalies result in decreased productivity (Tyson 1986), rendering resource availability highly unpredictable in time and space. ENSO is thus potentially an important force in the selection of physiological traits that conserve energy, such as use of daily torpor and low basal metabolic rates (BMR) (Lovegrove and Raman 1998; Nicol and Anderson 2000; Lovegrove 2003). In Afrotropical mammals, rainfall variability exerts the strongest influence (after body mass) on BMR (Lovegrove 2003).

There is a large variation in total annual rainfall in our study area (Figure 8; rainfall data were downloaded online from the National Climate Data Center; NCDC; in Asheville, N.C. from 1895 – 2000). We obtained a chronology of El Niño and La Niña events from Couper-Johnston (2000) and found that for most years, summer precipitation below the MAR in our study site was closely associated with El Niño events and that above mean annual rainfall (MAR) with La Niña events (Figure 8). The rainfall during some years in this time series was up to 40% below the summer rainfall average. During the year in which our study was conducted the TAR at the study site was 14% lower than the long-term average. We only recorded two torpor bouts for the entire summer season. The animals may have displayed a higher incidence of torpor had rainfall been much lower than the long-term average. For example, following the well-documented 1982 – 83 El Niño, the TAR remained 27% below average for a few consecutive years (Figure 8). During this extended El Niño drought, elephant shrews may have avoided mortality through the employment of summer torpor.

Figure 8

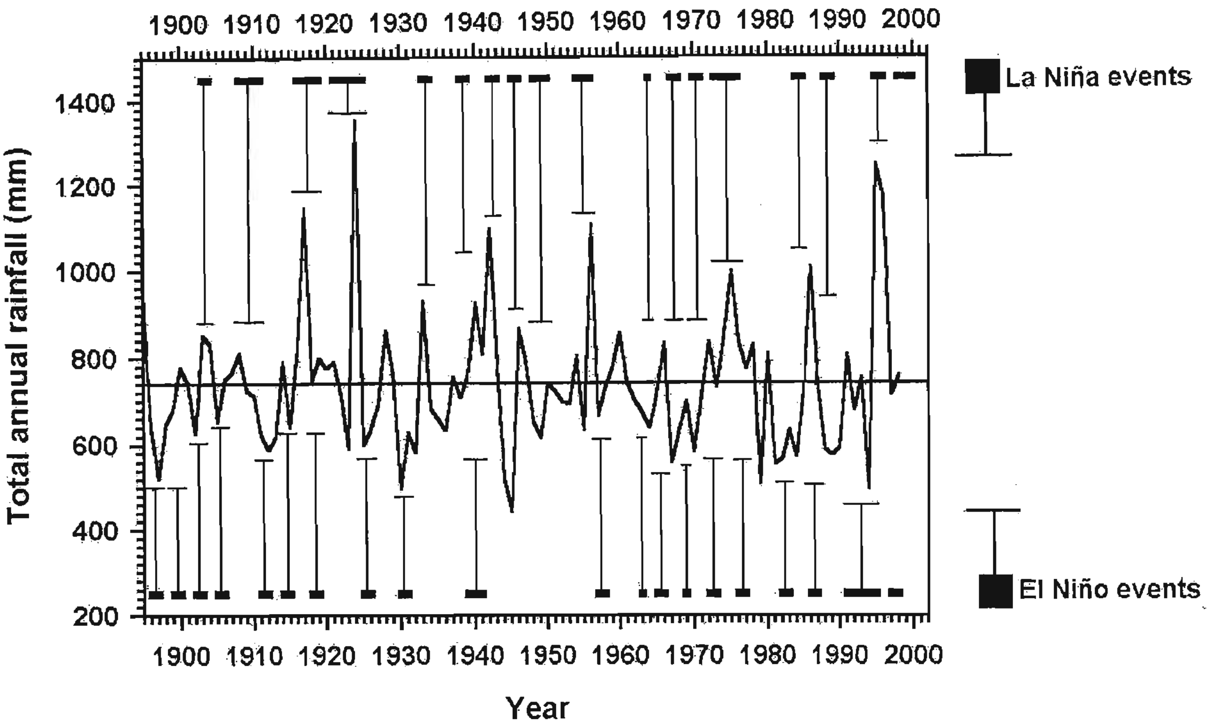

Figure 8. Total annual rainfall recorded from 1895 – 2000 at a weather station situated ca 30 km from the study site. Because our study area is situated in a summer rainfall area, we calculated the total annual rainfall (TAR) between October – September so as to include the entire summer season. Precipitation below and above the long-term average was associated with El Niño and La Niña events, respectively. The El Niño and La Niña chronology was obtained from Couper-Johnston (2000)

Unfortunately, data from a single field season are not adequate to verify such speculation and long-term physiological data from free-ranging animals are needed, especially when attempting to elucidate selection forces associated with the evolution of conservative physiological traits.

Summer is the warmest part of the year and our data suggest that summer torpor usually occurs when T_a decreases below ca. 15°C. Free-ranging subtropical microbats, *Vespadelus pumilus*, use summer torpor when roost temperatures decrease below the species' thermoneutral zone even when food resources are plentiful (Turbill et al. 2003). A number of studies have investigated the use of summer torpor under laboratory conditions mainly by manipulation of photoperiod and ambient temperature (Geiser and Baudinette 1985; Geiser and Baudinette 1987; Ellison and Skinner 1991; Webb and Skinner 1996; Lovegrove and Raman 1998; Lovegrove et al. 2001b). These studies confirm that although several southern hemisphere small mammals are indeed physiologically capable of summer torpor, its expression in the wild is rare because cold spells are also rare. In spring however, when late winter cold fronts penetrate the interior of southern Africa, the elephant shrews did enter torpor, in certain cases for longer than 24 hrs.

Arousal from torpor in rock elephant shrews is closely synchronized with diel ambient temperature cycles (Mzilikazi et al. 2002). The synchrony confers an energetic advantage as the animals can passively reheat and hence decrease the cost of arousal. The use of passive heating during arousal may be achieved through basking and has been observed in a number of avian and mammalian species (Schmid 1996, 2000; Ortmann et al. 1996; Brigham et al. 2000; Geiser et al. 2002; Turbill et al. 2003). In the laboratory, arousal costs are halved during passive heating in the striped-faced dunnart, *Sminthopsis macroura* (Lovegrove et al. 1999b; Geiser and Drury 2003). Moreover, Geiser and Drury (2003) have shown that during arousal, metabolic rates in radiant heat-assisted *S. macroura* increased 3.2-fold with a corresponding 15°C change in T_b, whereas there was an 11.6-fold increase in unassisted individuals. Because the high cost of arousal from torpor is the major cost of torpor, the decreased cost of arousal through the use of passive heating may explain common use of torpor in tropical and subtropical environments (Geiser and Drury 2003). Lack of arousal from torpor, resulting in the 39-hr bout (longest in this study), may have been a consequence of very low daytime temperatures (< 10°C) and therefore lack of opportunity for passive heating.

The mean torpor bout length during the whole year (8.6 ± 3.5 hrs) and mean torpor T_b min (15.3 ± 4.4°C) are within the known range for daily heterotherms (Geiser and Ruf 1995). Although we observed that *E. myurus* were capable of displaying torpor bouts longer than 24 hrs, these were rare ($n = 3$). We are reluctant to classify these instances as hibernation. Although thermoregulation in the elephant shrews was in some respects similar to hibernators (T_b min = 7.5°C; bout length > 24 hrs), they certainly did not show all of the characteristics typical of hibernation. The longest torpor bout was shorter than those usually observed in deep hibernators (Geiser and Ruf 1995). Furthermore, most hibernators show fattening prior to the hibernation season

(Lyman et al. 1982), or usually hoard food. Fattening prior to winter is clearly absent in *E. myurus* (Woodall 1987). As yet, no evidence exists that any species of elephant shrew hoards food. Also, these animals do not seasonally retreat into burrows or nests. Moreover, no *Elephantulus* species is known to build or use insulated nests (Rathbun 1979). However, although these behavioral and physiological considerations argue against hibernation in *E. myurus*, this species may be capable of opportunistically extending torpor bouts in response to unpredictable energetic shortfalls. Feathertail gliders are known to use prolonged torpor as an emergency response when foraging is inadequate to support endothermy (Jones and Geiser 1992). Under laboratory conditions bout lengths longer than 24 hrs were never measured for *E. myurus* (Lovegrove, et al. 2001a). This confirms the observation that torpor is often more frequent, deeper and lasts longer in free-ranging animals than in captive animals (Geiser et al. 2000).

A clear gap between the longest torpor bout and the shortest hibernation bout is always apparent even in species that employ both daily torpor and hibernation (Geiser and Ruf 1995; Wilz and Heldmaier 2000). Our records of bout lengths longer than 24 hours in a species that routinely enters daily torpor support the assertion that daily torpor and hibernation may not be two discrete physiological responses but simply components of a physiological continuum (Wilz and Heldmaier 2000). Furthermore, a study on cardio-respiratory function in mammalian heterotherms revealed that patterns of respiration during heterothermy were similar during daily torpor and hibernation (Zosky 2002). A consideration of behavioural, physiological and ecological factors determines which of the two responses will be employed.

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Chapter 4

Use of daily torpor during the active phase in free-ranging rock elephant shrews, *Elephantulus myurus*

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Abstract

In highly seasonal environments animals that utilise daily torpor usually do so during the rest phase. However, in subtropical and tropical environments daily torpor may not necessarily be confined to the animals' rest phase. Animals may display torpor during the active phase. We investigated the daily rhythms and seasonal rhythms of body temperature in normothermic and torpid free-ranging rock elephant shrews, *Elephantulus myurus* at Weenen Game Reserve, KwaZulu-Natal, South Africa. We recorded body temperatures using miniature dataloggers from May 2001 – May 2002. Daily patterns of T_b in normothermic *E. myurus* suggested polyphasic T_b patterns that nevertheless suggested a rest phase that was coincident with the daytime. When the animals utilised torpor, the lowest body temperatures were observed during the night time, such that torpor was utilised during the coldest part of the night and arousal was associated with the 3-hr period around sunrise. We suggest that the principal

benefit of a flexible daily rhythm of T_b was that it facilitated arousal by passive exogenous heating using ambient temperature cycles.

Keywords: *Elephantulus*, heterothermy, passive heating, polyphasic patterns, body temperature

Introduction

Most endotherms possess an endogenous circadian clock that synchronizes the physiology and activity of the animal with daily cycles of light, temperature and nutrient availability (Chew, Lindberg and Hayden, 1965; Refinetti and Menaker, 1992; Rutter, Reick and McKnight, 2002). The most important and widely reviewed circadian energetic rhythms are those of metabolic rate and of body temperature (Aschoff, 1983; Refinetti and Menaker, 1992). The endogenous circadian rhythm of body temperature (T_b) is generated by endogenous increments of metabolic rate which are thought to be further influenced by circadian rhythms of thermal conductance (Aschoff and Pohl, 1970; Aschoff, 1982). The circadian rhythm of T_b in a normothermic endotherm typically consists of elevated T_b during the active phase (α) and depressed values during the rest phase (ρ) (Aschoff and Pohl, 1970).

When confronted with energy stress, small endotherms are capable of offsetting high thermoregulatory costs by employing daily torpor. In highly seasonal environments daily torpor is usually confined to the animals' rest phase (Lyman et al., 1982; Geiser and Ruf, 1995; Lehmer, Bossenbroek and Van Horne, 2003), and a return to normothermic T_b during the active phase is achieved through endogenous heat production. However, there is a growing body of literature indicating that animals inhabiting subtropical and tropical environments synchronise arousal from torpor to ambient temperature cycles and thus use exogenous passive heating to decrease the cost of arousal from torpor (Schmid, 1996; Ortmann et al., 1996; Lovegrove, Körtner and Geiser, 1999a; Mzilikazi, Lovegrove and Ribble, 2002; Geiser, Goodship and Pavey, 2002; Geiser and Drury, 2003).

The majority of small mammals are nocturnal and diurnal small mammals are uncommon. However, in tropical and subtropical environments the use of torpor during the daytime may not necessarily be conducive to efficient employment of torpor, that is, it may not necessarily maximise energetic benefits because of the diel range of T_a. Low ambient temperatures during the night increase the potential energy savings that can be made through the use of torpor as the animals can attain low torpor T_bs. The diel range of T_a in tropical and especially subtropical environments is often large during winter when daily torpor is most likely to be employed. Thus the employment of torpor during the night would afford the animals an opportunity to decrease the cost of arousal through passive heating, using diel ambient temperature cycles (Lovegrove and Raman, 1998).

It might be asked therefore whether small nocturnal mammals employing heterothermy might display flexibility in the timing of T_b minima from the daytime into the nighttime when torpor is employed. This would maximise the energetic benefits of torpor through decreased energy expenditure during arousal from torpor by use of exogenous passive heating (Mzilikazi et al., 2002).

Previous studies in our laboratory have shown that under constant conditions, when in energy balance, the Afrotropical rock elephant shrews, *Elephantulus myurus,* (Corbet and Hill, 1991) display comparatively weak T_b daily rhythms that are nevertheless indicative of a rest phase coincident with the photophase (Lovegrove, Raman and Perrin, 2001). When confronted with energy stress, they utilize daily torpor and appear to be capable of shifting the timing of T_b min from the photophase into the scotophase (Lovegrove et al., 2001). A similar shift has also been recorded in another elephant shrew species, *Macroscelides proboscideus,* under food restriction conditions (Lovegrove, Lawes and Roxburgh, 1999b). Data from these two studies suggest that the timing of torpor in elephant shrews is under photoperiodic control.

In addition to photoperiod other factors such as sociality, nest building, food hoarding and availability as well as rainfall may also influence whether animals utilise torpor. Rock elephant shrews typically inhabit valley bushveld habitats that have large rocks, the preferred habitat of *E. myurus* (Skinner and Smithers 1990). *E. myurus* do not hoard food or seasonally retreat into burrows or nests. Moreover, no *Elephantulus* species is known to build or use insulated nests (Rathbun, 1979). We have previously shown that invertebrate abundance, ambient temperature and photoperiod influence torpor frequency in free-ranging *E. myurus* (Mzilikazi and Lovegrove, 2004).

There are conflicting accounts in the literature regarding the activity rhythms of elephant shrews in general. *E. myurus* have been classified as predominantly diurnal (Skinner and Smithers, 1990), mainly crepuscular (Woodall, 1989) or polyphasic (Rathbun, 1979 ; Du Toit, 1993). We have often observed elephant shrews moving at high speeds between rocks during the day at our study site (B. Lovegrove, N. Mzilikazi pers obs). In summer, rock elephant shrews extend their activity well into midnight (D. Ribble, pers comm). Thus the consensus is that *E. myurus* are capable of polyphasic activity. We suspect that such a flexible activity pattern, that is a lack of adherence to well-defined activity and rest phases, may facilitate shifting of the time at which T_b min is attained depending on the time of the day when daily torpor is used.

Apart from data on phase shifting of activity phases in microtine rodents (Hoogenboom et al., 1984; Halle and Lehman, 1987; Halle, 2000a,b), no data exist on the daily cycles of T_b in normothermic free-ranging small eutherian mammals. Whether the shifts in T_b patterns observed under controlled laboratory conditions in elephant shrews represent a real proximate physiological response in free-ranging animals remains to be investigated. The occurrence of changes in the daily cycles of T_b in free-ranging elephant shrews during the utilisation of heterothermy and the significance of such changes therefore remains unknown.

We predicted that free-ranging normothermic elephant shrews would display the lowest T_bs during the daytime but that when torpor was employed the lowest T_bs would be observed during the nighttime. We also predicted that elephant shrew body temperatures would lack a clearly defined normothermic daily amplitude of T_b, associated with specific times during the day-night cycle.

Materials and methods

The study was conducted at Weenen Nature Reserve, KwaZulu Natal, South Africa (28° 52.534′ S; 30° 00. 264′E) from May 2001 – May 2002. The habitat and study site are described by Mzilikazi et al. (2002).

Adult rock elephant shrews, *Elephantulus myurus,* were captured (Permits no. 26048/2001 and 27765/2001; KZN Wildlife) using Sherman traps baited with peanut butter rolled in oats. Pre-calibrated Thermochron iButtons (Dallas Semiconductor) were surgically implanted into the peritoneal cavities of the animals under inhalation anaesthesia (Isoflorane in oxygen; induction and maintenance, 2%; flow rate, *ca* 0.5 $l.\mathrm{min}^{-1}$). The animals were released at the exact capture locations < 24h after capture.

iButtons are miniature dataloggers (*ca.* 3g) armoured in stainless steel caps. They integrate a thermometer, a clock and a calendar, and hence are suitable for continuous recording of body temperatures (T_b) over specified periods. They can record up to 2048 consecutive temperature measurements in read-only non-volatile memory. iButtons measure temperatures from – 40°C to 85°C in 0.5°C increments and have a temperature conversion accuracy of 1°C from –20° to 70°C. The real-time clock accuracy is ± 2 minutes per month from

0 - 45°C. We programmed iButtons to measure body temperature once every hour, resulting in a total of 2040 data points per animal, over a period of 85 consecutive days.

To measure ambient temperature (T_a), we placed an iButton in the shade (under a rock). Because rock elephant shrews are not known to build nests (Skinner and Smithers, 1990), the shade T_a aimed to measure diel fluctuations of T_a within the typical resting sites of the elephant shrews (i.e. under rocks). We were not able to measure T_a at all animal capture sites. Therefore, depending on the aspect of each elephant shrew capture site relative to the position of the measuring iButton, T_a could vary slightly from the measurement site. Sunrise and sunset times at the fieldsite were obtained using a GPS (eTrex Vista: Garmin) and from these, mean monthly photoperiod was calculated.

Data and statistical analyses

The data presented in this study were obtained from the same animals as reported in the Mzilikazi and Lovegrove (2004) study. Due to different recapture success rates we obtained data for 13 animals in winter (May – August 2001; 8 females, 5 males), 2 animals in spring (August – November 2001; 1 female, 1 male), 4 animals in summer (November 2001 – February 2002; 2 females, 2 males) and 3 animals in autumn (March – May 2002; 1 female, 2 males).

The body temperature ($T_b$min) below which the animals were deemed to be torpid was 32°C. This value is slightly more than 2°C lower than the lower limit of the normal distribution of rest-phase normothermic T_bs in both sexes measured in the laboratory at T_a = 25°C by Lovegrove et al. (2001). For each animal data were obtained for 85 consecutive measurement days. If torpor was not observed during a 24-hr period, the animal was considered to have been normothermic during that day. There are conflicting accounts of the activity patterns of *E. myurus* in the literature, so we had no a priori knowledge of the rest and activity phases of the animals. We obtained data for different animals during the different seasons (our ethics permit precluded repetitive surgery on the animals), furthermore, the animals did not enter torpor on the same days, and this resulted in missing data in a consecutive time series (days). Our approach was therefore to calculate the daily T_b parameters during the day and night times. For each animal, within each season, we determined normothermic $T_b$min, $T_b$mean, and $T_b$max, both during the daytime and night time and used a Chi-squared test to discern differences in the frequency distributions of these T_b parameters.

To investigate the timing of T_b min during normothermy and torpor we calculated mean hourly T_b values, for both normothermic and torpid animals for the whole period of measurement and plotted these for easy visual inspection.

All mean values are presented ± SD. Throughout the text n refers to the number of observations and N refers to the number of animals. All statistical tests were performed using Statistica software (Statsoft, Tulsa).

Results

Body mass

The mean body mass at the beginning of winter was 56.6 ± 2.9g (N = 13). The animals weighed 58.2 ±9.5g (N = 2) and 60.5 ± 0.6g (N = 4) at the beginning of spring and summer, respectively. At the beginning of autumn the body mass decreased to 53.3 ± 4.2g (N = 3). The differences in body mass during the different seasons were not significantly different ($F_{1,3} = 2.62$; $p > 0.05$).

Daily patterns of body temperature in normothermic animals

During all seasons, individual plots of daily patterns of T_b during normothermic days revealed a flexible circadian pattern unlike those observed in strictly diurnal and nocturnal species. These individual plots revealed a lack of a clear T_b depression and elevation during a specific time of the day-night cycle. Instead, the T_b peaks and lows were distributed throughout the 24-hr period, making it difficult to discern rest and activity phases (Fig. 1).

In order to identify the rest and activity phases we considered the influence of the day-night cycle on three different T_b parameters, T_b min, T_b mean, and T_b max for all normothermic animals, during all seasons (Fig. 2). The T_b min, T_b mean and T_b max values during the different seasons are shown in Table 1. Because of the small sample sizes outside the winter season we analysed these parameters within seasons and did not analyse the data for any statistical differences in T_b parameters between seasons.

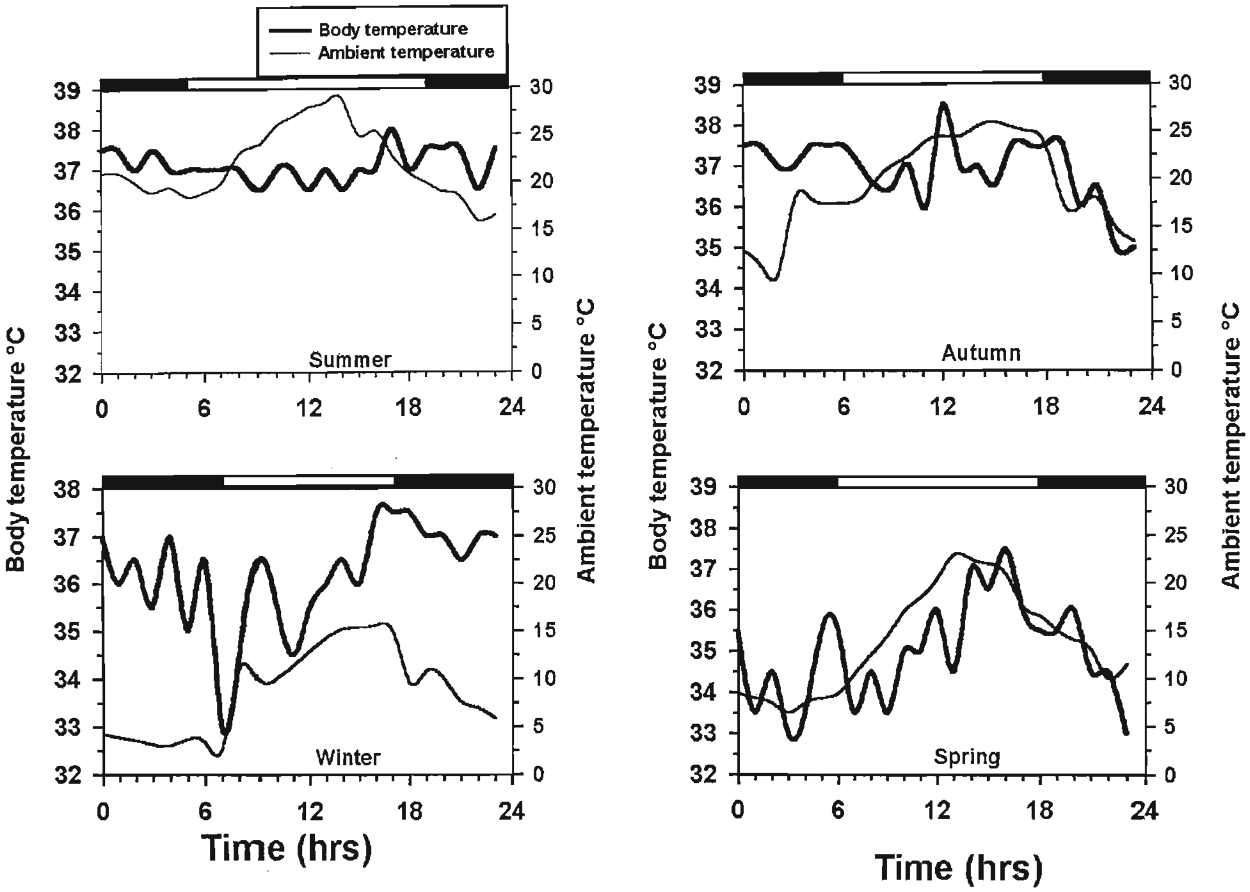

Figure 1. Body temperature plots of normothermic free-ranging *E. myurus* females showing the lack of clear activity and rest phases associated with the day-night cycles. Each plot was chosen randomly within each season and represents a single animal over a 24-hr period. The dark bars at the top indicate night time.

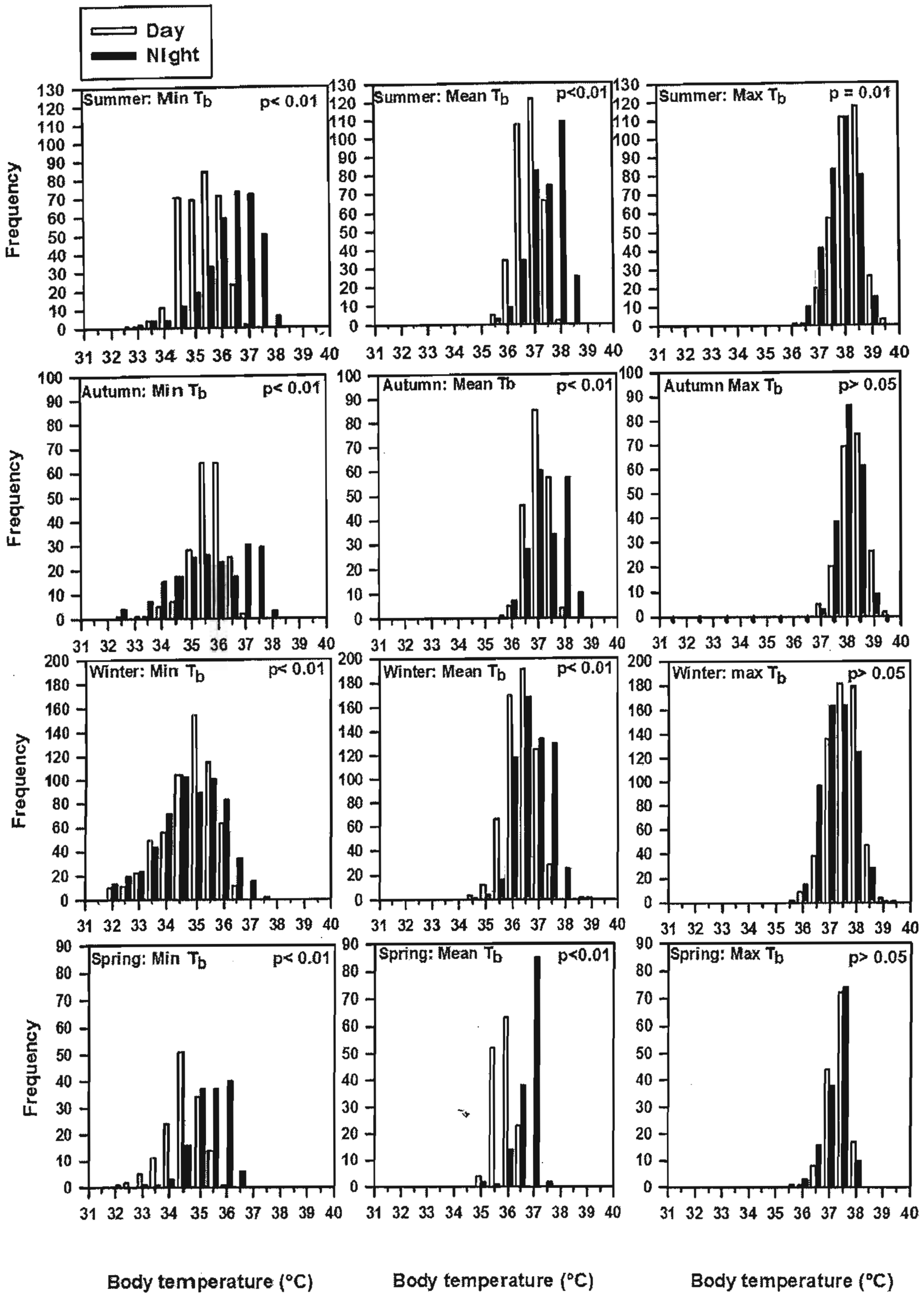

Figure 2. Frequency distributions (bin width = 1°C) of the daily minimum, mean and maximum T_b of normothermic free-ranging *E. myurus* during the day (clear bars) and night (filled bars) during the different seasons (* $p > 0.05$, not significantly different).

Table 1 The body temperature parameters showing daytime and nighttime differences in normothermic free-ranging rock elephant shrews, *Elephantulus myurus* during the different seasons. N denotes the number of individuals and n denotes the number of observations. The differences between the daytime and night time differences were very small, suggesting very weak T_b amplitudes

Season	Minimum T_b (°C)		Mean T_b (°C)		Maximum T_b (°C)	
	Day	Night	Day	Night	Day	Night
Summer (N = 4, n = 336)	35.3± 0.7	36.3 ±1.0	36.6 ±0.5	37.3 ±0.6	38.1± 0.5	37.9 ±0.6
Autumn (N = 3, n = 197)	35.6 ±0.6	35.8 ±1.3	36.8± 0.4	37.2 ±0.7	38.3 ±0.5	38.1 ±0.4
Winter (N = 13, n = 596)	34.7 ±0.9	34.8 ±1.1	36.2 ± 0.6	36.6 ±0.6	37.5 ±0.6	37.5 ±0.6
Spring (N = 2, n = 143)	34.5 ±0.7	35.4 ±0.7	35.7± 0.4	36.6± 0.4	37.3 ±0.4	37. ±2 0.5

During all seasons there were significant differences in T_b min and T_b mean between the day and night. T_b max was only significantly different between day and night during summer. Overall (data for all seasons combined), the average day T_b min was 34.9 ± 0.5°C and that during the night was 35.3 ± 0.9°C. These differences were significant ($F_{1,21} = 4.74$; $p = 0.04$). The day T_b mean (36.3 ±0.5°C) was significantly lower than the night T_b mean (36.8 ± 0.6°C; $F_{1,21} = 33.8$ < 0.05). The day T_b max was 37.7 ± 0.5°C and that during the night was 37.6 ± 0.5°C and these values were not significantly different ($F_{1,21} = 1.81$; $p > 0.05$). Although the day-night differences in T_b min and T_b mean were significant (0.4 and 0.5°C, respectively) they were remarkably small, indicating very weak daily T_b amplitudes, presumably as a result of polyphasic activity patterns.

Normothermic T_b amplitude

The normothermic circadian T_b amplitude (R_t) is defined as the difference between the maximum T_b during the active phase (α) and the minimum T_b during the rest phase (ρ) (Aschoff ,1982). Calculating the R_t for polyphasic animals using this definition proved to be more difficult than for animals with clear diurnal or nocturnal patterns of activity. Visual examination of daily T_b plots (for example, Fig 1) revealed that T_b peaks and lows were distributed throughout the 24-hr period. We interpreted the lower $T_b$min values during the day to be indicative of a rest phase coincident with the day and calculated R_t as the difference between night $T_b$max and day $T_b$min. The resulting average R_t for all animals was 2.7°C. However, because peaks and lows in T_b are distributed throughout the 24-hr period, we would expect that a calculation of R_t assuming a ρ coincident with the night time would result in similar values as those calculated for ρ during the daytime. Indeed, R_t calculated from day $T_b$max – night $T_b$min = 2.5°C. These data thus question the significance of R_t values in animals that display polyphasic T_b patterns.

Influence of daily torpor on daily patterns of body temperature

We calculated hourly mean T_bs for normothermic and torpid animals to investigate whether the use of torpor would result in a change in the timing of T_b min from the daytime to the night time. During all seasons normothermic animals always displayed the lowest T_b minima during the daytime (Fig. 3). The average T_b patterns for all normothermic animals also shows two pronounced T_b peaks during the early morning and early evening (Fig. 3). These peaks were associated with the hour surrounding sunrise and sunset, respectively.

In summer, we recorded only two torpor bouts. The autumn animals started displaying torpor from March 25th onwards. The single female displayed a higher incidence of torpor than the males (24/85 days compared to 10/85 and 4/85 days spent torpid by males 1 and 2, respectively). Torpor use was most prevalent during winter, and was associated with low ambient temperature, short photoperiod and decreased invertebrate prey abundance (detailed analysis in Mzilikazi and Lovegrove, 2004). In spring, torpor use was confined to August and September. Towards the end of September the animals ceased to display torpor.

During all seasons the use of torpor resulted in a shift in the timing of the lowest T_b from the daytime into the night time (Fig. 3). Torpor entry was confined to the night time and entry times ranged between 20h00 – 04h00 and the majority of animals achieved complete arousal within 3 hrs after sunrise.

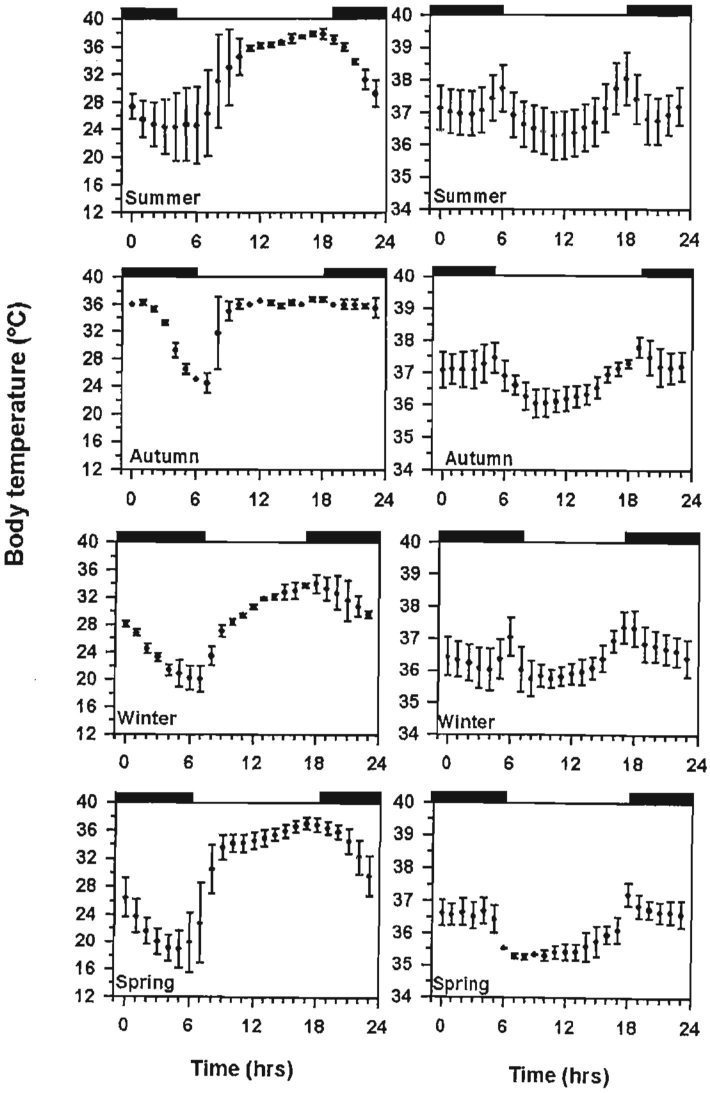

Figure 3. Average hourly body temperatures of torpid animals (left panel) and normothermic animals (right panel) measured during the different seasons. The dark bars at the top indicate the night time. In summer N = 4, autumn N = 3, winter N = 13, spring N = 2.

Although the average T_b patterns (Fig. 3) show that torpor use is associated with a shift in the timing of T_b min from the daytime into the night, the average normothermic T_b patterns suggest that T_b min occured during the daytime even during autumn and winter (when torpor is most frequently used). These average plots mask the extent of individual variation in the circadian rhythm of T_b of *E. myurus,* for example between the sexes. A closer examination of double circadian plots of T_b during the different seasons reveals the effect of torpor use on the rhythm of T_b (Fig. 4). In summer, both the female and male plots of T_b reveal that T_b min occurs during the daytime. During autumn the expression of the T_b min shifts into the night time in the female, although no torpor is yet observed (presumably in preparation for the winter season when torpor use is most common). In the male however, T_b min is maintained in the daytime (presumably because males use torpor less frequently). Both the female and the male use torpor in winter and T_b minima occur during the night time in both sexes. In spring, in both the female and the male T_b minima are observed during the daytime.

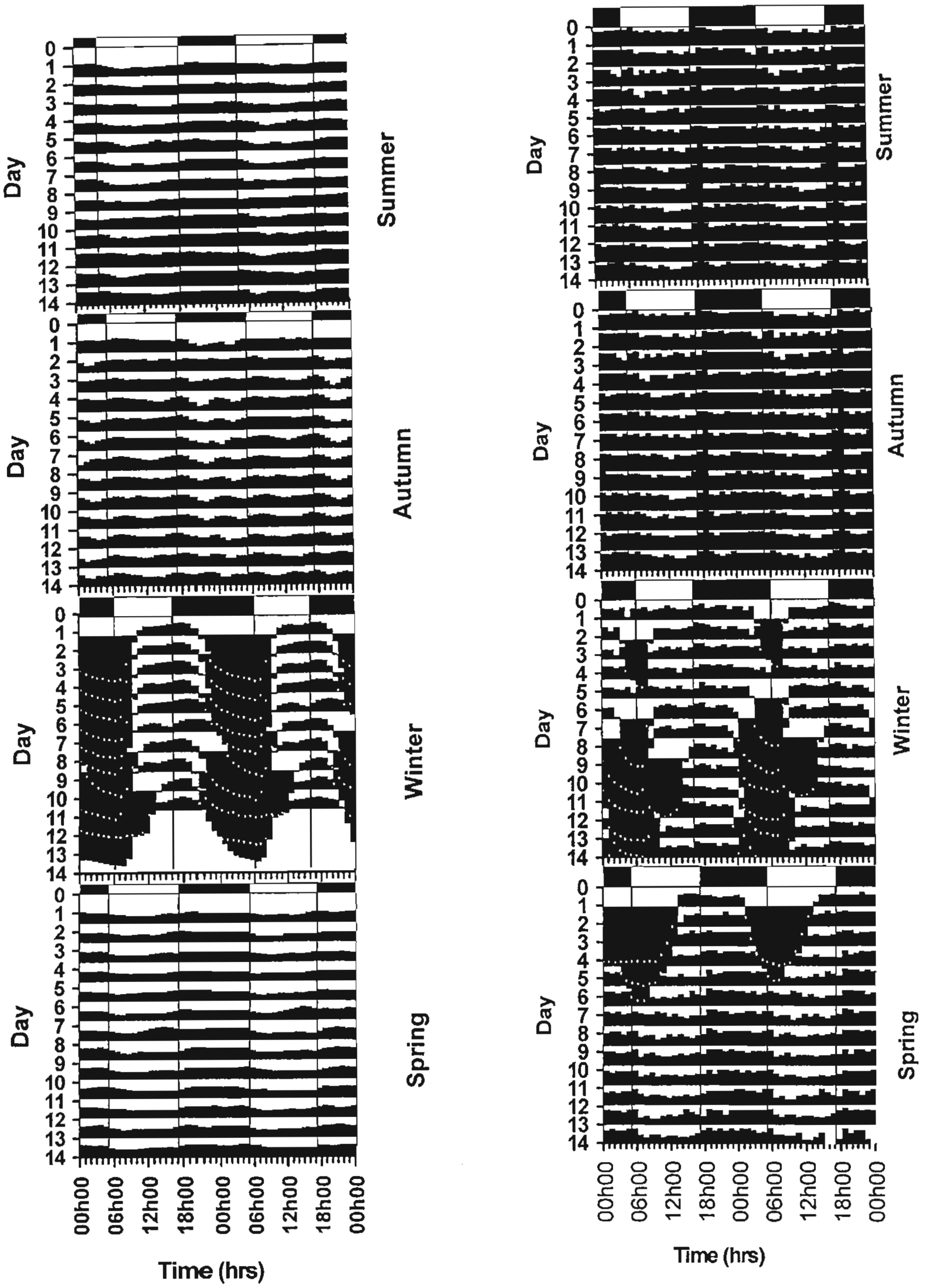

Figure 4. Double circadian plots of body temperature showing flexibility in the timing of T_b minimum (depending on whether torpor is used or not) during the different seasons. Each plot represents continuous measurements in a single female (left) and male (right) over a period of 14 days in the middle of each season. The dark bars at the top of each plot represent the night time. On each day the scale on the y-axis ranges between 32 – 40°C. The scalpel – shaped dark areas indicate torpor bouts.

Discussion

Most of the available data on daily and seasonal body temperature rhythms are derived from laboratory studies, under constant laboratory conditions and data on free-ranging small mammals are limited. Thus, although our sample sizes outside the winter season were small we believe that valuable insights can be gained from our data.

Individual plots revealed a polyphasic pattern (for example Fig. 1) with maximum and minimum T_bs distributed throughout the day and night. We interpreted these T_b profiles to be indicative of a polyphasic activity pattern. Because we do not have corresponding behavioural data, it is unclear whether the T_b peaks were generated by an endogenous heat production rhythm or whether they were associated with increased activity, locomotion and/or sun basking. Although T_b rhythms are not generated by rest-activity cycles, they are nevertheless affected by them and are usually closely correlated (Aschoff, 1983), hence our inferences about the activity patterns of the elephant shrews. Furthermore low T_bs are usually associated with ρ, whereas higher T_bs are associated with α (Aschoff,1982).

A consequence of polyphasic T_b patterns was that although statistically significant, the differences between daytime and night time T_b values were marginally low, resulting in very small daily T_b amplitudes. Nevertheless, the overall seasonal normothermic T_b suggested a rest phase that was coincident with the daytime and an active phase associated with the night time (Fig. 3). However, these overall plots masked the flexibility of the rhythm of T_b because in autumn and winter a shift in the timing of T_b min in female animals (and male in winter) was evident.

The expected T_b circadian amplitude for *E. myurus* (M_b = 56g) was 2.2°C (Aschoff, 1982). However, the largest difference between day and night T_b parameters in free ranging *E. myurus* was 0.5°C, observed for $T_b$mean. Similar small amplitudes have been observed in the laboratory in *E. myurus, E. rozeti* and *Macroscelides proboscideus*, maintained under long photoperiod conditions (Lovegrove et al., 1999b; Lovegrove et al., 2001). Such small daily T_b amplitudes are unusual for small mammals (Refinetti, 1996). Typically, animals that adhere either to strict diurnalism or nocturnalism display marked differences between ρ and α T_b values (Aschoff, 1970; Pittendrigh and Daan, 1976). Large differences between ρ and α T_b values translate into large R_t values that are often associated with energy conservation as they arise through a marked depression of RMR during the rest phase (Lovegrove and Heldmaier, 1994). Furthermore, large R_t values are considered adaptive in environments where productivity is low or where inputs of energy are highly variable in time and space (Boix-Hinzen and Lovegrove, 1998; McKechnie and Lovegrove, 1999). However for species such as *E. myurus* that show polyphasic patterns in T_b, R_t values and their significance need to be interpreted with caution.

Lovegrove and Heldmaier (1994) argued that, apart from ideas concerning the generation of endogenous circadian rhythms of T_b, there was a paucity of hypotheses regarding the significance of these rhythms in terms of an endotherm's fitness. We suggest that polyphasic activity patterns and hence the capacity for activity or rest at any time of the day resulted in the weak circadian rhythms observed in *E. myurus*. The resultant weak circadian rhythms are likely to facilitate a proximate response whereby the animals are capable of torpor entry at times other than the rest phase suggested by their T_b patterns.

In all animals torpor entry occurred exclusively during the night, between 20h00 – 04h00. Torpor was associated with a seasonal shift in the timing of the minimum T_b with respect to the day-night cycle. Most of the available literature deals primarily with the presence or absence of daily rhythms of T_b, the control of these rhythms and their entrainment to environmental stimuli (Aschoff, 1970; Steinlechner, Buchberger and Heldmaier, 1987; Refinetti and Menaker, 1992; Florant, Hill and Ogilvie, 2000; Rutter et al. 2002).

We are interested in the evolutionary significance of these flexible body temperature patterns. Firstly, the expression of torpor during the night allows torpor use to coincide with the lowest T_as. There is an obvious energetic advantage to entering torpor during the coldest part of the day-night cycle as low T_as translate to large $T_b - T_a$ gradients and therefore maximise the energetic benefits of torpor (Lovegrove and Raman, 1998). Secondly, if torpor entry occurs early at night, this presumably increases the time spent in torpor and hence increases energetic benefits. Thirdly, arousal from torpor in free-ranging rock elephant shrews is closely synchronised with diel T_a cycles (Mzilikazi et al., 2002). The synchrony confers an energetic advantage as the animals can passively reheat and hence decrease the cost of arousal. The use of passive heating during arousal is presumably achieved through basking and has been observed in a number of birds and mammal species (Schmid, 1996, Ortmann et al., 1996; Schmid, 2000; Brigham et al., 2000; Geiser et al., 2002; Turbill, Law and Geiser, 2003). Both laboratory studies (Geiser and Baudinette, 1985; Geiser, 1986; Lovegrove et al., 1999a) and studies under semi-natural and field studies (Schmid, 1996; 2000) reveal that, in nocturnal species that utilize passive heating during arousal, torpor entry is not confined to the rest phase but commences during the latter part of the active phase, usually during mid to late scotophase. The active phase therefore does not inhibit the mechanistic capacity for torpor.

Energetic gains accrued from the use of passive heating have been quantified for the striped-faced dunnart, *Sminthopsis macroura* (Lovegrove et al., 1999a) with radiant-heat assisted individuals displaying lower metabolic rates and decreasing costs of arousal by up to 85% (Geiser and Drury, 2003). Because the major energetic cost during torpor is the energy expended during arousal, we suggest that the principal benefit of a flexible T_b rhythm is that (a) it allows torpor to occur during the night and perhaps more significantly, (b) the synchrony of the arousal phase with rising early morning T_a reduces arousal costs through the use of exogenous passive heating (Mzilikazi et al., 2002). A rest phase associated with the daytime, as suggested by the normothermic T_b profiles in spring and summer, on the other hand, may be associated with heat stress avoidance and minimization of water loss and excessive heat gain (Roxburgh and Perrin, 1994; Downs and Perrin, 1995).

Another possible advantage for polyphasic activity and flexible T_b pattern may be associated with patterns of prey activity. The rock elephant shrews subsist primarily on invertebrates with isopterans and hymenpterans contributing a major component of their diet (Churchfield, 1987). We suggest that this capacity for a flexible polyphasic activity pattern may be adaptive for a small insectivorous mammal like *E. myurus*, which inhabits environments where rainfall is unpredictable and termite emergence is triggered by rain, which may occur randomly with respect to day-night cycles (Lovegrove et al., 1999b). Presumably the flexibility in T_b patterns has evolved in response to appreciable energetic advantages (Enright, 1970).

Although information on daily and seasonal changes in patterns of body temperature of free-ranging small mammals that employ daily torpor is scanty (but see (Chew et al., 1965; Perrigo, 1987), data on shifts in circadian patterns of activity are more common. Possible advantages of flexible activity patterns include competition avoidance (Rubal, Choshniak and Haim, 1992; Elvert et al., 1999) anti-predation mechanisms (Halle, 2000a;b) and direct responses to unfavourable conditions (Hoogenboom et al., 1984; Halle and Lehman, 1987). For example, in microtines changes in food quality result in polyphasic activity when the animals have to spend more time foraging in order to obtain adequate caloric requirements (Halle and Lehman, 1987). In shrews, polyphasic activity is a consequence of short-term hunger cycles due to small body size and high metabolic rates (Merritt and Vessey, 2000).

In summary, we suggest that polyphasic body temperature patterns resulted in weak daily T_b amplitudes in the rock elephant shrews. The resultant weak rhythms are likely to facilitate a proximate response whereby torpor entry is not confined to the rest phase. This would allow the animals to enter torpor during the coldest part of the day-night cycle and also result in energetic benefits as the animals decrease the cost of arousal through exogenous passive heating. From a fitness perspective, energy savings from torpor are potentially available for allocation to reproduction

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Chapter 5

The influence of photoperiod and ambient temperature on body temperature and torpor cycles in rock elephant shrews, *Elephantulus myurus*

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Abstract

It has been suggested that the evolution of endothermy precluded the need for homeothermic mammals to be sensitive to T_a cycles because they could maintain physiological function despite fluctuations in the ambient temperature. Elephant shrews utilize passive heating and provide excellent models with which to investigate whether mammals can entrain their body temperature rhythms to ambient temperature cycles. I experimentally tested whether food restricted *E. myurus* can entrain torpor cycles to shifts in the T_a cycle while holding the light-dark cycle constant. Food restriction and short photoperiod were only sufficient to induce torpor in *E.myurus* if photoperiod and T_a cycles are in phase with each other. Shifting the cold T_a into the photophase prevented the expression of torpor. I concluded that the body temperature rhythm is most probably tightly coupled with the photoperiod cycle and that although T_a and photoperiod usually act synergistically in nature, photoperiod is probably the stronger zeitgeber. It remains to be established whether normothermic animals in

positive energy balance might entrain their circadian rhythm of body temperature to T_a cycles.

Introduction

Mammals and birds maintain relatively stable and constant body temperatures when in positive energy balance. However, if maintenance requirements cannot be met, a number of birds and mammals abandon homeothermy and employ heterothermy during which metabolic rate and body temperature are decreased (Lyman et al. 1982; Geiser and Ruf 1995; Boyer and Barnes 1999). The use of heterothermy involves the regulation of body temperature at a lower setpoint and has attendant energy benefits (Ruf and Heldmaier 1992). A return to normothermic body temperature requires increased metabolic heat production, which may account for as much as two-thirds of the energy used during a torpor bout (Lovegrove et al. 1999). Hence, the cost of arousal is perceived to be one of the disadvantages of torpor, especially during daily torpor bouts that usually last for < 12 hours within a 24 hour cycle (Geiser and Ruf 1995).

Normothermic mammals maintain a circadian rhythm of body temperature (CRT) that is closely synchronized with light/dark (LD) cycles (Aschoff 1970; Refinetti and Menaker 1992). When LD cycles are shifted or altered the majority of mammals are capable of re-entraining their body temperature rhythms to the new photoperiod regimes. The CRT is retained even when animals employ heterothermy (Menaker 1959; Lyman et al. 1982; Vitale et al. 1985; Ruby 1992; Grahn et al. 1994; Körtner et al. 1998). For tropical and subtropical species, synchrony of the CRT (especially in heterothermic animals) with T_a cycles may have a significant bearing on torpor energetics, especially if animals employ exogenous passive heating during arousal (e.g Ortmann et al. 1996; Lovegrove et al. 1999).

Although a number of studies have investigated the effect of T_a on the circadian rhythm of activity in mammals (Tokura and Aschoff 1983; Francis and Coleman 1990; Goldman et al. 1997), similar studies on the CRT are not as common. However, Brown et al. (2002) showed that changes in the diel cycles of T_a were associated with a phase shift in the CRT of laboratory mice. They observed that whereas the laboratory mice normally displayed a T_b (and activity) peak during late afternoon and early evening, mice subjected to warm nights (inverted temperature cycles with respect to photoperiod) shifted peaks in T_b to late evening and early morning. The changes in phase of the CRT rhythms occurred without affecting the light-controlled central clock in the suprachiasmatic nuclei (SCN) (Brown et al. 2002). In addition, heat pulses shifted the phase of the circadian neuronal rhythm of the rat SCN, in vitro (Ruby et al. 1999). Nevertheless, data on the entrainment of the mammalian CRT to T_a cycles remain relatively scanty. However, data obtained in free-ranging animals suggest that some tropical and subtropical species may significantly decrease the cost of arousal through the use of exogenous passive heating (Ortmann et al. 1996; Mzilikazi et al. 2002; Turbill et al. 2003; Dausmann et al. 2004; Geiser et al. 2004). The use of passive heating necessitates that torpor entry should occur during the nighttime, and that arousal from torpor is closely synchronized with T_a cycles.

This raises the interesting possibility that those daily heterotherms that rely on passive heating for arousal may adjust their body temperature rhythms seasonally in order to entrain torpor to natural T_a cycles. In this chapter I experimentally tested whether daily heterotherms that use exogenous passive heating can entrain torpor cycles to shifts in the T_a cycle while holding the light-dark cycle constant. I used the rock elephant shrew, *Elephantulus myurus* which belongs to a phylogenetically ancient order (Macroscelidea) (Corbet and Hill 1991). In the laboratory, normothermic *E. myurus* display flexible and polyphasic T_b rhythms that are nevertheless indicative of a rest phase coincident with the daytime. Their polyphasic T_b patterns are thought to facilitate entry into torpor during the nighttime (although their rest phase is during the daytime; Chapter 4). I hypothesized that rock elephant shrews would entrain their T_b rhythm to the T_a cycle and not the photoperiod cycle. I expected the animals to enter daily torpor during the scotophase and arousal to commence in the morning when the photoperiod was in phase with normal T_a cycle (i.e warm days and cold nights). When the T_a cycle was reversed (cold days and warm nights), I expected the animals to shift the timing of $T_b$minimum (and hence torpor entry) and $T_b$maximum to correspond with the coldest part of the day and warmest part of the night, respectively, irrespective of the light – dark cycle.

Materials and methods

Study animals and general maintenance

This study was conducted from June – August 2003. Adult rock elephant shrews, *Elephantulus myurus,* (n = 12), were captured using Sherman traps baited with peanut butter rolled in oats. They were captured on the MacKay farm, 17 km east of Estcourt (28°56.749'S 30°00.848'E), KwaZulu-Natal, South Africa, in June 2003 (Permit no. 32065/2002 KZN Wildlife). They were transported by road and were housed in the animal house at the University of KwaZulu Natal, Pietermaritzburg. The animals were housed in glass terraria with sawdust bedding and were provided with wooden nesting boxes. Before commencing data measurement, the animals were maintained at 25°C under a 10L:14D photoperiod (lights on at 07h00 and off at 17h00). This photoperiod was similar to that prevailing at the capture site during mid-winter. The animals were fed ProNutro, a commercial high protein cereal (22% protein, 59% carbohydrate and 6% fat) mixed with water. This diet was supplemented daily with fresh lettuce and twice weekly with live mealworms. The food was replenished once every 24 hrs.

Surgical procedures, experimental design and protocol

Following a one-week adjustment period at T_a = 25°C, pre-calibrated Thermochron iButtons (Dallas Semiconductor) were surgically implanted into the peritoneal cavities of the animals under inhalation anaesthesia (Isoflorane in oxygen; induction and maintenance, 2%; flow rate, *ca* 0.5 $l.\text{min}^{-1}$). iButtons are miniature dataloggers (*ca.* 3g) armoured in stainless steel caps. They integrate a thermometer, a clock and a calendar, and hence are suitable for continuous recording of body temperatures (T_b) over specified periods. They can record up to 2048 consecutive temperature measurements in read-only nonvolatile memory. iButtons measure temperatures from – 40°C to 85°C in 0.5°C increments and have a temperature conversion accuracy of 1°C from –20° to 70°C. The real-time clock accuracy is ± 2 minutes per month from 0 - 45°C. I programmed iButtons to measure T_b once every 30-minutes, resulting in a total of 2016 data points per animal, over a period of 42 consecutive days. iButtons were also used to measure T_a in the experimental rooms.

The animals were allowed to recover from surgery for two weeks at 20°C. During the recovery period, the average mass of food eaten per day for all animals was estimated for a week prior to commencement of data measurement and this amount was used to represent the 100% diet (Lovegrove and Raman 1998). The animals were then divided into two groups of six animals each, and were transferred to controlled-environment rooms (CE rooms). The relative humidity (RH) was *ca.* 70%, photoperiod was maintained at 10L:14D.

For the first three weeks of data measurement the animals were maintained on a 70% diet to induce torpor. During this time the temperature in both CE rooms was programmed to simulate a diel cycle (Figures 1A B & C). The CE rooms cycled between 5 – 26°C, daily. This temperature cycle is similar the average monthly minimum and maximum, respectively, at the capture site in mid-winter, the time during which torpor is most prevalent (Mzilikazi et al. 2002).

The temperature in the CE rooms was manually changed every four hours, in approximately 4°C steps such that the average heating rate of the CE rooms was 3.5°C/hour. The initial temperature increase coincided with the onset of the photophase (07h00). By 14h00, the CE rooms had attained a T_a of ca. 26°C. In the afternoon, the CE rooms were programmed to cool from 26°C to 5°C throughout the night. Minimal T_a values were usually attained around 23h00.

At the beginning of the fourth week, the T_a cycle in CE room 1 (experimental) was reversed by 12 hours, such that T_a decreased at the onset of the photophase and the scotophase coincided with increased T_a (Figure 1D). The T_a cycle in CE room 2 (control) was left unchanged, that is, warm T_a coincided with the photophase and cold T_a coincided with the scotophase (Figure 1C).

Although the CE rooms were programmed to measure T_as between 5 - 26°C, due to technical problems the T_a seldom decreased below 9°C. In addition, the profiles in the two rooms were not identical for the whole duration of data measurement. During the 3rd week the temperature in the experimental room reached lower T_as compared to those in the control room. During the 5th week the T_a in the experimental room failed to decrease below 16°C.

Data analyses

Animals were deemed torpid if T_b decreased below 32°C (Lovegrove et al. 2001). The total torpor bout length was determined as the total time during which T_b was maintained below 32°C. Within each torpor bout the time of entry into and arousal from torpor as well as the time at which normothermia was attained were determined. The time at which minimum and maximum T_b were observed was determined for each day, for both normothermic and torpid animals. To establish whether reversed T_a cycles had an influence on the time at which $T_b$min and $T_b$max were observed, I compared the frequency distributions of timing of $T_b$min and $T_b$max between treatments. Because there were periods in the day (and night) during which no animals attained $T_b$min and $T_b$max, I grouped the observations into 4-hour intervals i.e. I determined the number of observations of $T_b$min and $T_b$max from 00 – 04h00; 04 – 08h00; 08 – 12h00; 12 – 16h00; 16 – 20h00; 20 – 00h00. I used a Chi-squared test to discern differences in the timing of $T_b$min and $T_b$max between treatments (Zar 1984). All values are represented as mean ± SD. Throughout the text N refers to the number of animals and n refers to the number of observations.

Results

General torpor characteristics

Between the two groups of animals I measured a total of 247 torpor bouts (N = 12; 6 in each group). Torpor bout length ranged between 2 – 16 hours. The mean ± SD bout length was 9.0 ± 2.5 hours (Figure 2A). Torpor bout length was significantly correlated with the T_a at which $T_b$min was attained ($r = 0.262$; $p < 0.01$), with the longest bouts observed at the coldest ambient temperatures (Figure 2B). The longest bout lengths were also associated with the lowest body temperatures (Figure 2C).

Torpor T_b min ranged from 8.0 – 31°C. The mean torpor $T_b$min was 13.3 ± 4.1°C. The mean normothermic $T_b$min for all animals (n = 256) was 34.8 ± 1.0°C and $T_b$max for all animals (n = 503) was 37.4 ± 0.5°C (Figure 3). The temperature differential between torpor $T_b$min and the T_a at which $T_b$min was observed ranged from 0.5 – 17.5°C, with only three observations where $T_b - T_a$ was >10°C. The mean $T_b - T_a$ was 2.4 ± 2.0°C.

Figure 1

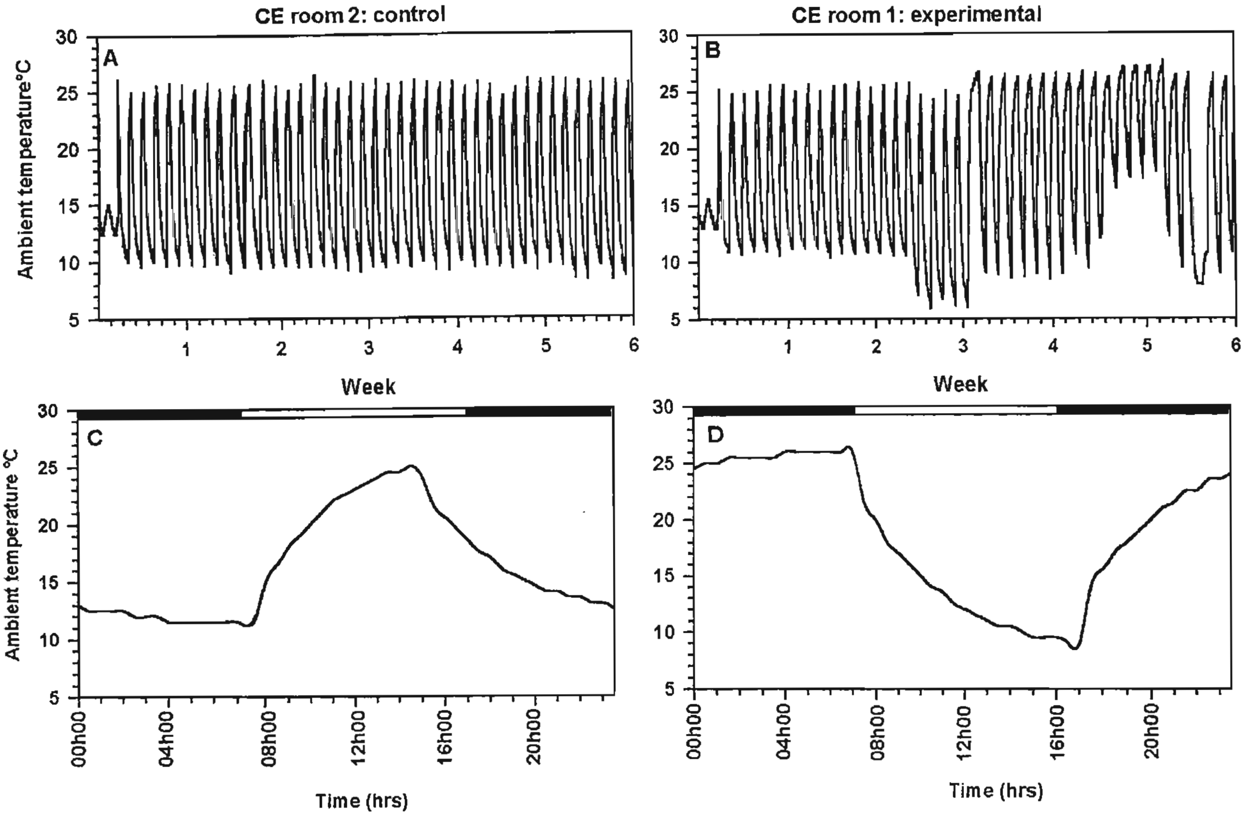


Figure 1. The ambient temperature profiles in the CE rooms (A = control room; B = experimental room). The profiles in the two rooms were not identical for the whole duration of data measurement. During the 3rd week the temperature in the experimental room reached lower T_as compared to those in the control room. During the 5th week the T_a in the experimental room failed to decrease below 16°C. C shows the temperature profile for a single day in the control room (normal T_a cycle). D shows the temperature profile in the experimental room, with reversed T_a cycle.

Figure 2

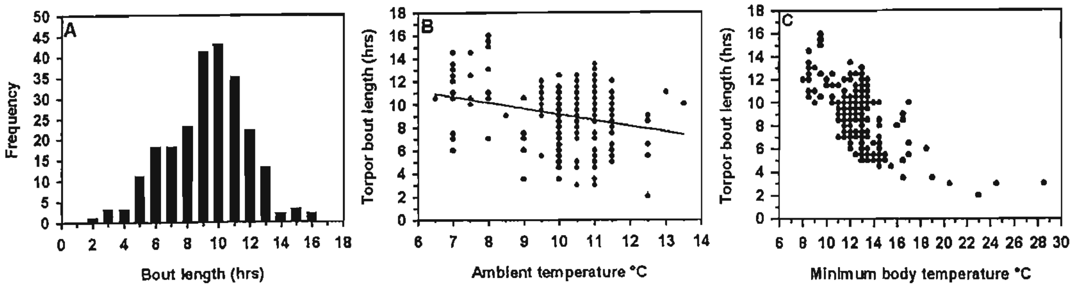


Figure 2. A. The frequency distribution showing bout lengths (n = 247) measured in winter-acclimated *E. myurus* maintained at 10L:14D and T_a between 9 - 26°C. B. The correlation between T_a at which $T_b$min was attained and bout length in winter-acclimated *E. myurus*. C. The relationship between minimum T_b and torpor bout length in winter-acclimated *E. myurus*.

Figure 3

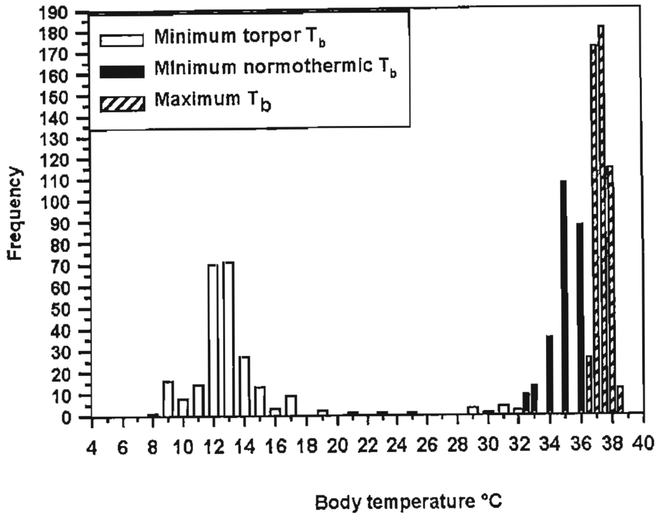


Figure 3. The frequency distribution of minimum torpor T_b, minimum normothermic T_b and maximum T_b measured in winter-acclimated *E. myurus*.

Influence of ambient temperature cycles and photoperiod on expression of daily torpor and the daily rhythm of body temperature

During the first three weeks of the experiment animals in both groups regularly entered daily torpor (Figures 4 A, B). In both groups torpor entry was restricted to the scotophase, coinciding with the lowest ambient temperatures (Figures 5 A, B). The majority of animals entered torpor between 20h00 – 05h00, with the highest number of entries occurring between 22h00 – 03h00.The minimum body temperatures in the majority of torpid and non-torpid animals in both groups were reached in the two-hour period surrounding the onset of the photophase (Figures

5 A, B). There was no significant difference in the timing of $T_b$min between the groups ($\chi^2 = 3.02$; df = 5, $p< 0.01$; Figures 6 A, B).

Figure 4A

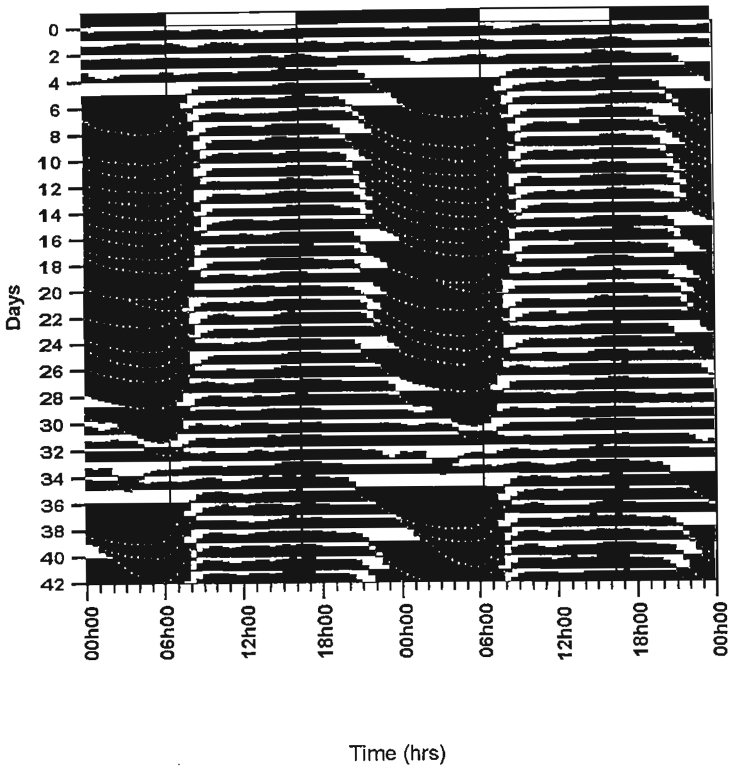
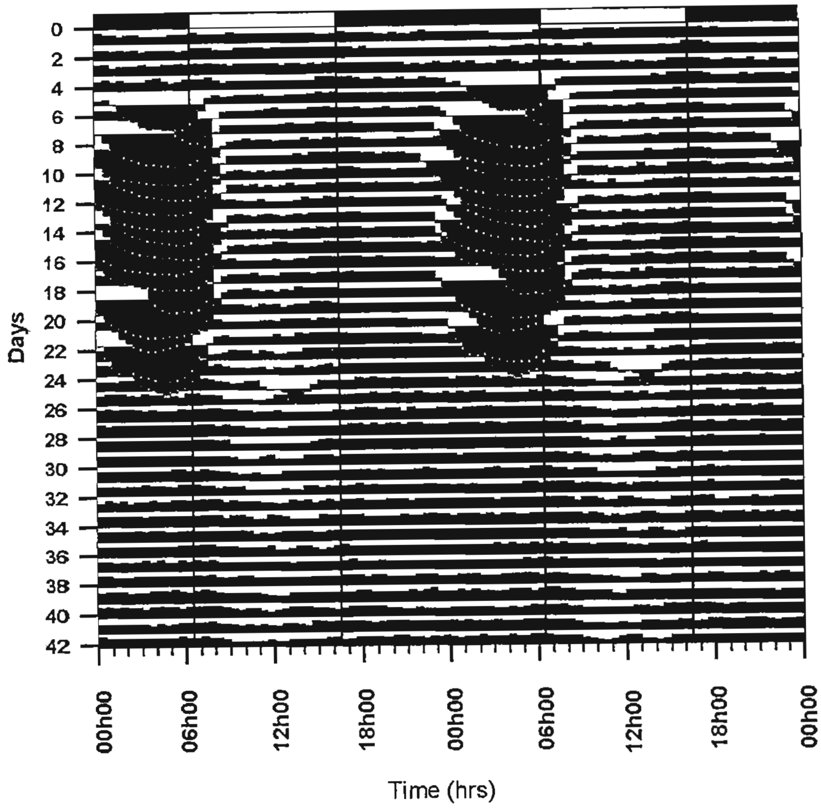


Figure 4 A.Double circadian plots of T_b representing continuous measurements in a single female *E. myurus* over a period of 42 days in the control room. Note that the animal employed torpor for almost the entire duration of data measurement. The dark bars at the top of each plot represent the nighttime. On each day the scale on the y-axis ranges between 32 – 40°C. The scalpel-shaped dark areas indicate torpor bouts.

Figure 4B



B. Double circadian plots of T_b representing continuous measurements in a single female *E. myurus* over a period of 42 days in the experimental room. Torpor display ceased when the diel cycle of T_a was inverted (day 22). The dark bars at the top of each plot represent the nighttime. On each day the scale on the y-axis ranges between 32 – 40°C. The scalpel-shaped dark areas indicate torpor bouts.

Figure 5

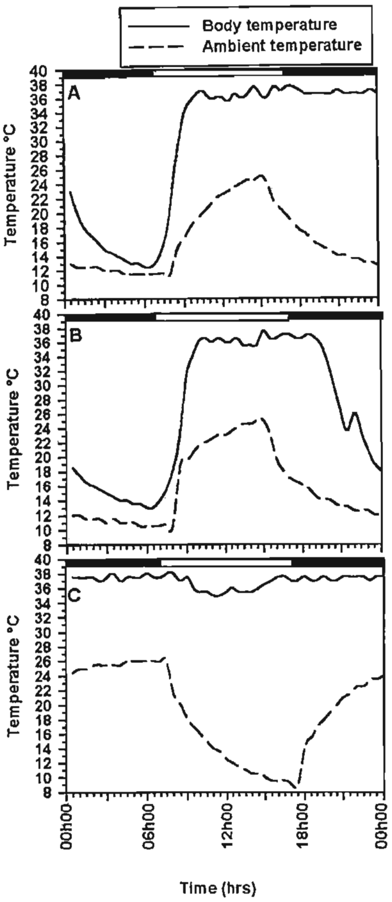


Figure 5. Plots showing synchrony between body temperature (solid line) and T_a (broken line) profiles measured in A. control female in week 2, with normal T_a cycle; B. experimental female in week 2 with normal T_a cycle; C. experimental female during week 5 with reversed T_a cycle.

Figure 6

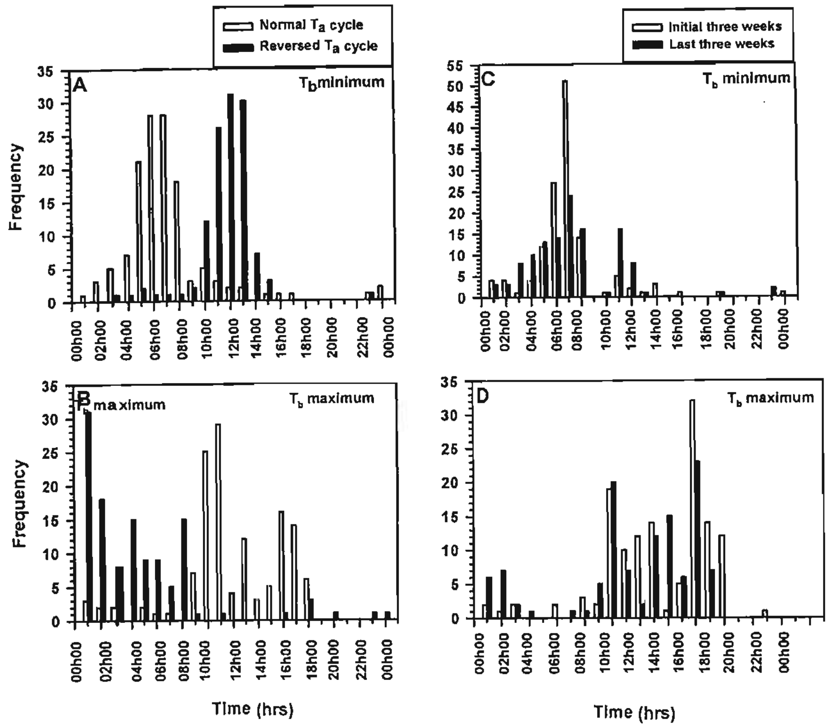


Figure 6. Frequency distributions showing the shifts in the timing of $T_b$minimum and $T_b$maximum in *E. myurus* under the different treatments. The plots on the left panel (A, B) represent the experimental group and those on the right panel (C, D) represent the control group.

Most animals (86.6% of all arousals) initiated arousal in the period between 07h00 – 08h00, coinciding with the onset of the photophase as well as an increase in T_a (Figures 5 A, B). 10.2% of the arousals were initiated between 06h00 – 07h00 and only 3.2% of the arousals were observed before the onset of the photophase. The time required to return to normothermic T_b ranged between 30 minutes – 3 hrs, although most animals required between 2 – 2.5 hrs to complete the arousal process. All arousals were completed by 10h00 by which time the T_a had reached 20°C.

The reversed T_a cycle had an effect on the T_b rhythm of the experimental group. None of the animals in the experimental group entered torpor in the latter three weeks of the experiment (Figure 4B). However, the animals shifted the time at which minimum T_b was attained such that $T_b$min was observed around midday, coinciding with the coldest part of the day (Figures 4B; 5C). The shift in the timing of the minimum T_b was significant ($\chi 2 = 133.22$; df = 5, $p < 0.01$; Figures 6 A, B).

A similar shift in the timing of $T_b$max was observed (Figures 6 C, D). During the first three weeks of the experiment, animals in both groups attained $T_b$max mostly during the photophase (Figures 6C,D). This pattern persisted in the control animals whereas in the experimental group, a shift was observed after the T_a cycle was uncoupled from the photoperiod. The frequency distribution of $T_b$max was significantly different between treatments ($\chi^2 = 152.32$; df = 5, $p< 0.01$). The expression of $T_b$max was shifted into the scotophase, such that $T_b$max coincided with the warmest T_a.

Discussion

This study showed that food restriction and short photoperiod are only sufficient to induce torpor in *E.myurus* if photoperiod and T_a cycles are in phase with each other. Reversing T_a cycles had a profound effect on torpor expression in rock elephant shrews. Whereas torpor was observed during the scotophase when T_a

cycles were in phase with photoperiod, shifting the cold T_a into the photophase prevented the expression of torpor and resulted in normothermic $T_b$min being expressed during the photophase.

Under the reversed T_a cycle I expected the elephant shrews in the experimental group to (a) shift timing of T_b min from the scotophase into the photophase and to express torpor during the photophase, coincident with the lowest T_as, and (b) to arouse from torpor during the scotophase when the T_a increased. It is not unreasonable to expect such a shift to occur because although rock elephant shrews display weak T_b circadian rhythms, the rhythms are nevertheless suggestive of an active phase that is coincident with the scotophase (Chapter 4). However, free-ranging rock elephant shrews that enter torpor during winter do so during the night. But, when rock elephant shrews remain normothermic, they maintain a rest phase that is coincident with the daytime even during the winter season (Chapter 4). It seems therefore that whilst rock elephant shrews are capable of opportunistically entering torpor during the active phase (scotophase) they are incapable of entering torpor during the photophase even when the T_as are low and they are food restricted.

The trigger for torpor is most probably tightly coupled with the LD cycle. Although T_a and photoperiod usually act synergistically in nature, photoperiod is probably the stronger zeitgeber. This is consistent with observations from other small mammals where torpor is under strong photoperiodic control and/or that both ambient temperature cycles and photoperiod are important in the mediation of torpor (Heldmaier and Steinlechner 1981; Geiser and Baudinette 1985;

Goldman et al. 1986; Pohl 1987; Canguilhem et al. 1988; Ruf et al. 1991; Aujard et al. 1998).

Limitations of the study

There were two potential sources of error in this study. Firstly, the T_a profiles between the experimental and control rooms were not similar throughout the entire period of data measurement. Between 22 – 27 July 2003, the T_a in the experimental room decreased to 6°C whereas that in the control room did not (Figure 1B). Furthermore, between 08 – 11 August 2003 the T_a in CE room 1 did not decrease below 16°C during the daytime due to a mechanical failure (Figure 1A). These differences make it difficult to make unbiased comparisons in the torpor patterns observed between treatments. Secondly, at the end of the third week of the experiment, some of the animals had lost ca. 6g in body mass. I therefore increased the food given to the animals from 15g to 20g for the remainder of the study. Rock elephant shrews have been shown to enter spontaneous torpor at 18°C (Lovegrove et al. 2001) so I reasoned that low T_a would probably be sufficient to induce torpor. In retrospect, this concern for the welfare of the animals was unjustified. Notwithstanding the unforeseen mechanical problems, the timing of the T_a cycles with respect to the light – dark cycle was not affected.

Concluding remarks

This study was conducted over only six weeks and was mainly aimed at investigating whether T_a cycles might influence the ability of rock elephant shrews to enter heterothermy and also whether the phase of the CRT might be affected. What remains to be established is whether normothermic animals in positive energy balance might entrain their CRT to T_a cycles. It has been suggested that the evolution of endothermy precluded the need for homeothermic mammals to be sensitive to T_a cycles because they could maintain physiological function despite fluctuations in the ambient temperature (Refinetti and Menaker 1992). Because of the phylogenetic placement of the elephant shrews, it would be worthwhile to examine the entrainment of the CRT of elephant shrews to T_a cycles more rigorously. Such a study would provide insights into whether sensitivity of the master circadian clock to temperature cycles has been lost in endotherms, and if so how early in the eutherian lineage this might have occurred.

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care" publication no. 86-23, revised 19986 (National Institute of Health) and the "Code of ethics for animal experimentation" manual adopted by the University of Natal. The KwaZulu-Natal Wildlife granted permission to work and capture animals at the MacKay farm. Mr and Mrs Bruce MacKay graciously allowed me to work on their farm and provided accommodation. Kirstern Coe-Mouton provided invaluable assistance in the field. Kelly and Mark Brown assisted with animal maintenance during my absence from the School. Stephen Burton assisted with surgery and helped maintain the temperature profiles in the CE rooms. Professor Norman. C. Ruby read an earlier draft of this chapter and offered invaluable insight.

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Chapter 6

The capacity for non-shivering thermogenesis in a phylogenetically ancient eutherian mammal, the rock elephant shrew, *Elephantulus myurus*

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Abstract

The evolution of endothermy is thought to have been facilitated by the advent of endothermic energy sources such as brown adipose tissue (BAT), the principal site of nonshivering thermogenesis (NST). In marsupials heat is primarily produced through shivering and NST in skeletal muscle because BAT is either absent or appears to be non-functional. The most basal group of the eutherian lineage are the Afrotheria. Rock elephant shrews, *Elephantulus myurus* are amongst the smallest members of the Afrotheria and are also known to use passive heating. The aim of this study was to determine whether the phylogenetic placement of *E. myurus* and its reliance on passive heating might result in a decreased capacity for NST relative to other eutherians. We investigated the capacity for NST in winter acclimated *E. myurus* by measuring the thermogenic response to noradrenalin (NA) injection. We used phylogenetically independent analyses to compare *E. myurus* NST capacity (1.58

ml $O_2.g^{-1}.hr^{-1}$) with other eutherians. *E. myurus* had NST capacity that was no different from other eutherian mammals. Although they displayed a NST capacity that was 74% of that expected on the basis of body mass, this value was not significantly different from phylogenetically independent allometric predictions.

Keywords: *Elephantulus*, Afrotheria, nonshivering thermogenesis, brown adipose tissue

Introduction

Homeothermy, which involves the maintenance of a stable body temperature within narrow limits through endogenous heat production, has evolved in birds and mammals (McNab 1978; Hayes and Garland 1995). It has been suggested that the evolution of endothermy was likely to have been facilitated by the advent of endothermic energy sources such as brown adipose tissue (BAT) (Malan 1996) and the success of early mammals is often attributed to the ability to produce heat endogenously (Cannon et al. 2000). In eutherian mammals, BAT is the principal site of non-shivering thermogenesis (NST) during which heat is produced through uncoupling respiration (Jansky 1973; Heldmaier et al. 1981; Wunder and Gettinger 1996). BAT appears to be either absent or non-functional in marsupials (Holloway and Geiser 2001). Shivering and thermogenesis in non-BAT tissues, such as skeletal muscle, are thought to be more important avenues for heat production (Holloway and Geiser 2001) and the mechanism for NST has yet to be resolved in marsupials (Nicol et al. 1997; Rose et al. 1999).

The most basal of the eutherian mammals are the Afrotheria (Springer et al. 1997) the next logical group above the marsupials in which to investigate the capacity for NST. The smallest members of the group would be expected to be most likely to display a capacity for NST because this is the most common avenue through which small mammals increase their ability to cope with cold, particularly during winter when proliferation of BAT becomes most apparent (Haim and Borut 1981; Heldmaier et al. 1990; Ruf et al. 1991). Thus far, no data exist on the capacity for NST in any of the members of the Afrotheria. Elephant shrews are amongst the smallest members of the Afrotheria and provide good candidates for investigation of NST capacity in an ancient eutherian lineage.

In addition to being small, elephant shrews are known to employ torpor, during which metabolism and body temperature are decreased as a means of energy conservation when confronted with energetic bottlenecks (Lovegrove et al. 1999a; Lovegrove et al. 2001a,b). A return to normothermic body temperatures usually requires endogenous heat production, a major component of which is NST (Heldmaier and Steinlechner 1981; Lyman et al. 1982; Nedergaard and Cannon 1984; Ruf and Heldmaier 1992). Rock elephant shrews, *Elephantulus myurus*, are also capable of using exogenous passive heating to arouse from torpor (Mzilikazi et al. 2002). It might be asked, therefore, whether this reliance on passive heating has resulted in a decreased capacity for NST in *E. myurus* relative to other eutherian small mammals. *E. myurus* might also be expected to have a lower than expected NST capacity because they are Afrotropical endemics (Corbet and Hill 1991). Small mammals inhabiting tropical, subtropical and warm-temperate environments have been shown to exhibit only about 50% of the NST capacity expected for their body mass (Saarela and Hissa 1993). For example, *Acomys cahirinus* and *A. russatus* occurring in the rocky deserts of Israel exhibit NST capacity that is 38% and 46%, respectively, of that expected on the basis of body mass (Kronfeld-Schor et al. 2000).

The aim of this study was to determine whether a phylogentically ancient eutherian mammal, *E. myurus*, possesses the capacity to produce heat through NST. We also aimed to determine whether the reliance of exogenous passive heating during arousal from torpor resulted in a lower than expected NST capacity relative to other eutherian mammals. The thermogenic capacity of an animal is most easily obtained through measurement of the increase in oxygen consumption (VO_2) following noradrenalin (NA) injection, which stimulates thermogenesis in the BAT (Jansky 1973; Wunder and Gettinger 1996).

Materials and methods

General maintenance

Adult *E. myurus* (n = 10) were captured on the MacKay farm, 17 km east of Estcourt (28°56.749'S 30°00.848'E), KwaZulu-Natal, South Africa, in July/August 2002. They were transported by road and were housed in the animal house at the University of KwaZulu Natal, Pietermaritzburg. The animals were housed in glass terraria provided with sawdust bedding and wooden nesting boxes. They were maintained at 20°C under a 10L: 14D photoperiod. This photoperiod was similar to that experienced at Estcourt during mid-winter. The animals were fed

ProNutro, a commercial high protein cereal (22% protein, 59% carbohydrate and 6% fat) mixed with water. This diet was supplemented daily with fresh lettuce and live mealworms. The food was replenished once every 24 hrs.

Metabolic measurements

Animals were placed in 2.3ℓ dome-shaped Perspex respirometers placed in a $1m^3$ constant environment cabinet. The light:dark cycle in the cabinet was matched to that in the animal house where the animals were maintained. Oxygen consumption was measured using an open-flow through system. Atmospheric air, acting as the control gas was pumped from outside the building into the cabinet at approximately $5ℓ.min^{-1}$ to maintain a positive pressure within the cabinet. Silica gel scrubbers partially dried incoming air (RH ≤ 60% in cabinet). This air was drawn through individual respirometers at flow rates chosen to maintain < 1% oxygen depletion between the incurrent and excurrent air. The flow rates were measured with a Brooks thermal mass flow meter (Model 5810) factory calibrated to STP at sea level. The use of solenoid relay valves and a pump for each respirometer allowed five respirometers as well as a control channel to be used simultaneously. The excurrent air from each respirometer was passed through CO_2 proof tubing and a dew point water condensor to remove water. After passing through the pumps, relay valves, filters and the mass flow meter, the excurrent air was sub-sampled with an Applied Electrochemistry oxygen analyzer (Ametek S-3A/1) and sensor (Ametek N-22 M) to determine the fractional concentration of O_2 in the dry air. Analogue signals from the mass flow meter and O_2 analyzer were digitized with an A/D converter and recorded every 6-min for each respirometer. Further details of the respirometry design are provided in Lovegrove et al. (2001b).

Experimental protocol

The experiments were conducted in November 2002. We divided the animals into a control and experimental group, with 5 animals in each group. On each measurement day food was removed from the animal cages at least three hours prior to commencement of data measurement to ensure that the animals were post-absorptive. Although elephant shrews display polyphasic patterns of activity, we have shown that when torpor is not employed, the rest phase is coincident with the daytime in *E. myurus* (Mzilikazi and Lovegrove, in press). We therefore conducted the experiments during the daytime, between 09h00 – 15h00.

To our knowledge there are no accounts of NST determination in elephant shrews or any other Afrotherian in the literature. Therefore, to establish the maximum VO_2 response to NA we determined dose responses to noradrenalin (NA) by injecting the animals with 25, 45, 65, 85 and 100% of the dosage calculated from Wunder and Gettinger (1996) (NA dose (mg/kg) = $2.53M^{-0.4}$ where M = mass in grams). The animals were allowed to recover for three days between successive doses. The resting metabolic rate (RMR) and NA-induced thermogenesis were determined at 25°C, ca. 5°C below the lower critical limit of thermoneutrality (T_{lc}) of this species (Lovegrove et al. 2001a) to avoid hyperthermia. RMR was measured for 2 hrs after which NA was injected subcutaneously. We continued VO_2 measurements for 2 hrs after each NA injection. Saline solution (0.9% NaCl) was used for control injections.

Data analyses

Elephantulus myurus

For each animal we determined RMR as the mean of the lowest three consecutive VO_2 values (at least 18 minutes) within the first 2hrs of measurement and the maximum VO_2 response as the mean of the three highest VO_2 values within the 1st hour of NA injection. The NST capacity was therefore calculated as the net increase in metabolic rate caused by the NA injection (VO_2 max after injection – RMR) (Kronfeld-Schor et al. 2000).

We used repeated measures ANOVA to determine if NA and saline injection resulted in a significant increase in VO_2, as well as to determine the effects of the different doses on VO_2. All mean values are reported ± SD.

Phylogenetically independent analyses

To investigate whether *E. myurus* display a lower capacity for NST relative to other eutherian mammals we obtained NST capacity data for eutherian small mammals from the literature (Figure 1 and Table 1). The NST capacity was calculated as the net increase in metabolic rate caused by the NA injection (VO_2 max after NA injection – RMR) for all species. In some studies, RMR values at which the measurements were made were not specified but the BMR was provided instead. In such cases, we calculated NST capacity as $NAVO_2max$ – BMR.

When examining correlated evolution between traits, inter-specific analyses need to control for the effects of phylogeny (inheritance) in order to render data independent and the variances homogenous (Felsenstein 1985). We therefore conducted phylogenetically independent analyses with the suite of Phenotypic Diversity Analysis Programs (PDAP), specifically the program PDTREE, which was used to a) construct a phylogeny, b) verify homogeneity of tip variances, and c) calculate independent linear contrasts of the data (Garland et al. 1992). The branch lengths were assigned using the arbitrary branch method of Pagel (1992). The contrast variances were found to be heterogenous so we used the method of Grafen (1989) to achieve homogeneity (rho value = 0.7).

Results

NST capacity in *Elephantulus myurus*

The mean body mass of all *E. myurus* (n = 10) at the beginning of measurements was 62.7 ± 4.2g and at the end of the experiment had significantly decreased to 59.1 ± 4.1g ($t = 7.71$, $p < 0.01$). The mean ± SD RMR at 25°C for all the animals was 2.48 ± 0.48 ml $O_2.g^{-1}.h^{-1}$. Oxygen consumption (VO_2) increased in response to both saline and NA injections (Figure 2A,B). For all animals, the maximum VO_2 was observed within 45 minutes of NA injection. However, the increase in VO_2 due to saline injection was not significant at any of the doses administered ($F_{1,8}$ =

1.54; $p > 0.05$). NA injection caused a significant increase in VO_2 ($F_{1,8} = 27.23$; $p < 0.01$). The dose of NA administered also had a significant effect on VO_2 ($F_{4,32} = 5.34$; $p < 0.01$). A significant increase in VO_2 in response to NA injection could be elicited from as low as 45% (Figures 2A,B) of the dose calculated from Wunder and Gettinger (1996), based on body size. However, the maximum response to NA injection was observed at 85% (Figure 2B). Post hoc comparisons revealed significant differences between the lower doses and that at 85%. At 100%, there was a slight decrease in maximum VO_2 compared to the dose at 85% (Figure 2B). The NST capacity for *E. myurus* was 1.58ml ± 0.39 $O_2.g^{-1}.\ hr^{1}$.

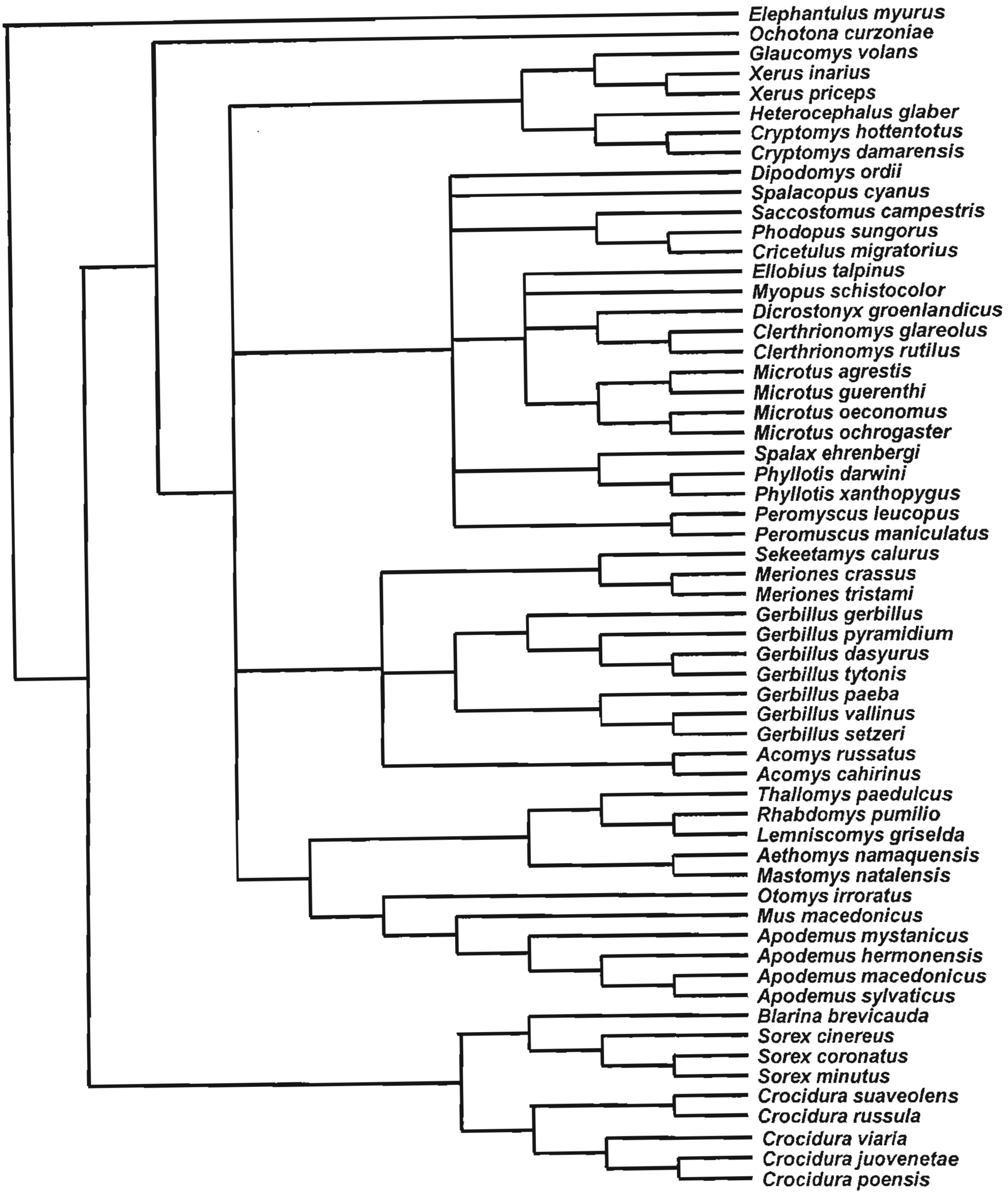

Figure 1. A phylogeny of the species used in the phylogenetically independent analyses. Taxonomic nomenclature follows Wilson and Reeder (1993). The inter-ordinal relationships followed those of Murphy et al. (2001). For relationships within the Insectivora we followed Ruedi (1998). For relationships among the Rodentia we followed Martin et al. (2000), Michaux and Catzeflis (2000), Catzeflis et al. (1995), Watts and Baverstock (1995), Huchon and Douzery (2001) and Filippucci et al. (2002) .

Table 1. Non-shivering thermogenesis (ml $O_2.g^{-1}.hr^{-1}$) data for the 61 mammal species used in the phylogenetically independent analyses

Species	**Zone**	**Mb (g)**	**Acclimation**	**NST**	**NST calculation**	**Reference**
Acomys cahirinus	Pal	51.2	8L:16D 28°C	1.90	NA VO2 – RMR	Kronfeld-Schor, et al. (2000)
Acomys russatus	Pal	74.2	8L:16D 28°C	1.40	NA VO2 – RMR	Kronfeld-Schor et al. (2000)
Aethomys namaquensis	Afr	62.2	W	1.52	NA VO2 – BMR	Lovegrove et al. (1991)
Apodemus flavicollis	Pal	35	W	8.80	NA VO2 – BMR	Klaus et al. (1988)
Apodemus hermonensis	Pal	20.5	12L:12D 24°C	4.28	NA VO2 – RMR	Haim and Izhaki (1995)
Apodemus mystacinus	Pal	45.4	8L: 16D 7°C	3.03	NA VO2 – RMR	Haim and Yahav (1982)
Apodemus sylvaticus	Pal	23.1	SP	5.20	NA VO2 – RMR	Haim et al. (1995)
Blarina brevicauda	Nea	18	W	5.97	NA VO2 – RMR	Merritt (1986)
Clethrionomys glareolus	Pal	21	W	10.10	NA VO2 – BMR	Klaus et al. (1988)
Clethrionomys rutilus	Nea	25.6	SP	5.45	NA VO2 – RMR	Feist and Feist (1986)
Cricetulus migratorius	Pal	29.6	8L:16D	4.40	NA VO2 – RMR	Haim et al. (1998)
Crocidura juovenetae	Afr	9.7	W	7.90	NA VO2 – BMR	Sparti (1992)
Crocidura poensis	Afr	17.2	W	4.40	NA VO2 – BMR	Sparti (1992)
Crocidura russula	Pal	11.7	W	8.60	NA VO2 – BMR	Sparti (1992)
Crocidura suaveolens	Pal	7	W	11.80	NA VO2 – BMR	Sparti (1992)
Crocidura viaria	Afr	19.2	W	7.10	NA VO2 – BMR	Sparti (1992)
Cryptomys damarensis	Afr	123	26°C	0.45	NA VO2 – RMR	Hislop and Buffenstein (1994)
Cryptomys hottentotus	Afr	102	25°C	2.73	NA VO2 – RMR	Haim and Fairall (1986)
Dicrostonyx groenlandicus	Nea	48.5	SP	5.23	NA VO2 – RMR	Maier and Feist (1991)
Dipodomys ordii	Nea	71	SP	2.94	NA VO2 – RMR	Gettinger and Ralph (1985)
Elephantulus myurus	Afr	62.7	W	1.58	NA VO2 –RMR	This study
Ellobius talpinus	Pal	42.6	April	4.10	NA VO2 – RMR	Moshkin et al. (2001)
Gerbillurus setzeri	Afr	43.8	12L:12D 25°C	1.23	NA VO2 – BMR	Downs and Perrin (1991)
Gerbillurus tytonis	Afr	33.6	12L:12D 25°C	1.28	NA VO2 – BMR	Downs and Perrin (1991)
Gerbillurus paeba	Afr	31.3	12L12D 25°C	1.18	NA VO2 – BMR	Downs and Perrin (1991)
Gerbillurus vallinus	Afr	44.9	12L:12D 25°C	1.04	NA VO2 – BMR	Downs and Perrin (1991)

Gerbillus allenbyi	Pal	30.7	12L:12D 28°C	0.91	NA VO2 – RMR	Haim (1984)
Gerbillus dasyurus	Pal	27.6	28°C	1.68	NA VO2 – RMR	Haim and Izhaki (1993)
Gerbillus gerbillus	Pal	29.7	28°C	2.73	NA VO2 – RMR	Haim and Izhaki (1993)
Gerbillus nanus	Pal	28.9	12L:12D 28°C	1.04	NA VO2 – RMR	Haim (1984)
Gerbillus pyramidium	Pal	53	28°C	1.98	NA VO2 – RMR	Haim and Izhaki (1993)
Glaucomys volans	Nea	76.7	W	3.39	NA VO2 – RMR	Merrit et al. (2001)
Heterocephalus glaber	Afr	32	30°C	0.60	NA VO2 – RMR	Hislop and Buffenstein (1994)
Lemniscomys griselda	Afr	47.5	25°C	1.96	NA VO2 – RMR	Haim and Izhaki (1995)
Mastomys natalensis	Afr	40	SP	4.00	NA VO2 – RMR	Haim and Fourie (1980)
Meriones crassus	Pal	120	SP	0.93	NA VO2 – RMR	Haim and Levi (1990)
Meriones tristrami	Pal	112	SD	1.41	NA VO2 – RMR	Haim and Izhaki (1993)
Microtus agrestis	Pal	20.3	W	6.23	NA VO2 – BMR	McDevitt and Speakman (1996
Microtus guentheri	Pal	60	SD	1.47	NA VO2 – RMR	Haim, Shabtay, Arad (1998)
Microtus ochrogaster	Nea	42 – 46	SP	3.30	NA VO2 – RMR	Wang et al. (1999)
Microtus oeconomus	Nea	24	SP	7.23	NA VO2 – RMR	(Wang et al. (1999)
Mus macedonicus	Pal	15	SD	1.36	NA VO2 – RMR	(Haim et al.(1998)
Myopus schistocolor	Pal	26.4	A	5.94	NA VO2 – BMR	Saarela and Hissa (1993)
Ochotona curzoniae	Pal	139	SP	1.38	NA VO2 – BMR	Wang et al. (1999)
Otomys irroratus	Afr	102	25°C	1.63	NA VO2 – RMR	Haim and Izhaki (1993)
Peromyscus leucopus	Nea	17.3	SP	4.50	NA VO2 – RMR	Lynch et al. (1978)
Peromyscus maniculatus	Nea	17	W	6.40	NA VO2 – RMR	Zegers and Merrit (1988)
Phodopus sungorus	Pal	26.5	W	6.44	NA VO2 – BMR	Böckler et al. (1982)
Phyllotis darwini	Neo	65.7	12L:12D 15°C	1.41	NA VO2 – BMR	Nespolo et al. (1999)
Phyllotis xanthopygus	Neo	65.1	12L:12D 15°C	3.83	NA VO2 –RMR	Nespolo et al. (1999)
Rhabdomys pumilio	Afr	39.6	SP	3.28	NA VO2 – RMR	Haim and Izhaki (1993)
Saccostomus campestris	Afr	85.2	W	3.43	NA VO2 – RMR	Haim et al. (1991)
Sekeetamys calurus	Pal	78.3	8L:16D	1.41	NA VO2 – RMR	Haim (1996)
Sorex cinereus	Nea	3.04	W	7.58	NA VO2 –RMR	Merritt (1995)
Sorex coronatus	Pal	11	W	5.40	NA VO2 – BMR	Sparti (1992)
Sorex minutus	Pal	4.9	W	8.10	NA VO2 – BMR	Sparti (1992)
Spalacopus cyanus	Neo	87.7	12L:12D 15°C	3.54	NA VO2 – BMR	Nespolo et al. (2001)

Spalax ehrenbergi	Pal	134	U	0.64	NA VO2 – RMR	Haim et al. (1984)
Thallomys paedulcus	Afr	142	W	1.59	NA VO2 - BMR	Lovegrove et al. (1991)
Xerus inauris	Afr	542	25°C	1.56	NA VO2 –RMR	Haim and Izhaki (1993)
Xerus priceps	Afr	602		1.29	NA VO2 –RMR	Haim and Izhaki (1993)

* SP = short photoperiod, W = winter, S = summer, U = unspecified, A = Autumn, SD = short day

Pal = Palaearctic, Afr = Afrotropical, Neo = Neotropical, Nea = Nearctic

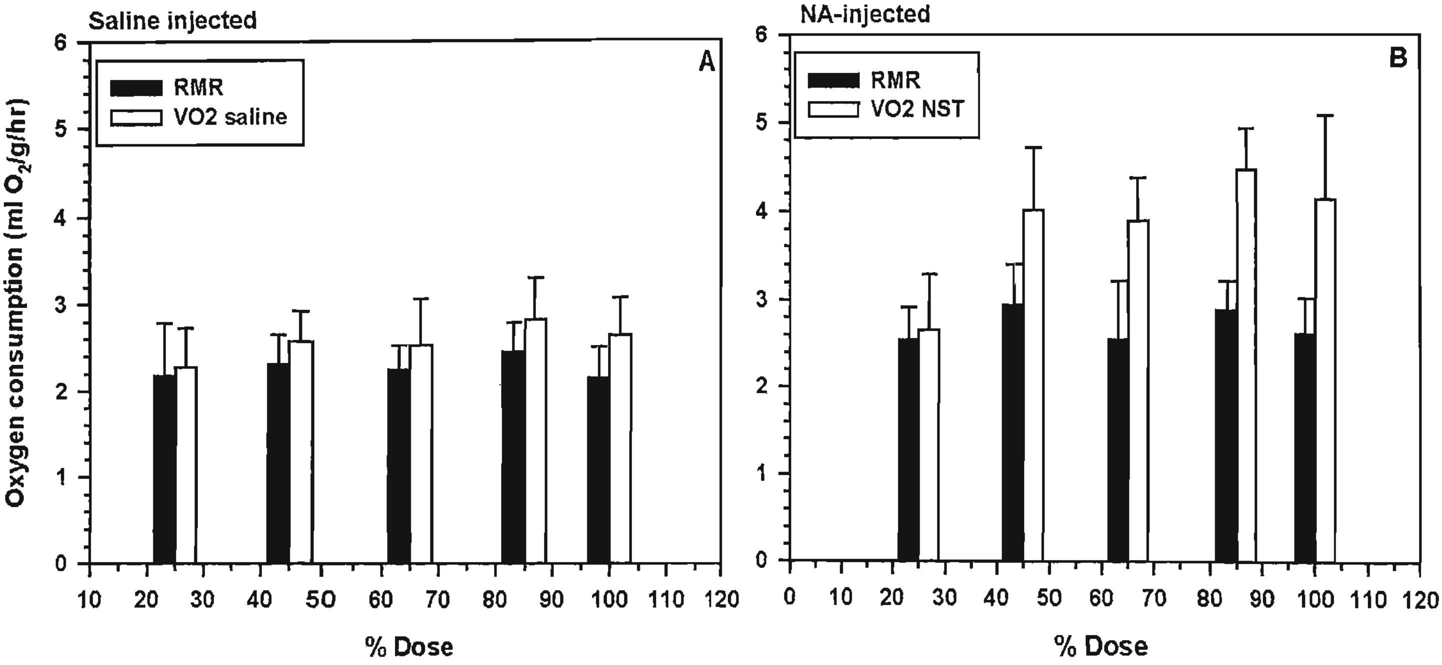

Figure 2A. Mean resting metabolic rates and saline-VO_2 (saline VO_2 = mean maximum oxygen consumption in response to saline injection) of *E. myurus* measured at 25°C (n = 5, mean ± SD). B. Mean resting metabolic rates and NA-VO_2 (NA-VO_2 = mean maximum oxygen consumption in response to noradrenalin injection) of *E. myurus* measured at 25°C C (n = 5, mean ± SD).

Inter-specific analysis

We fitted a regression line (through the origin; Figure 3) of standardized NST capacity contrasts on body mass contrasts for all the species and then fitted 95% prediction intervals to the data as recommended by Garland and Adolph (1994). The phylogenetically independent allometric relationship between NST and body mass was described by the equation: $\log NST = -0.442 \log M_b + 1.124$, where M_b = mass in grams. Although there was a wide variation around the regression, the *E. myurus* contrast fell comfortably within the 95% prediction intervals (Figure 3), suggesting that for its body size, *E. myurus* does not display NST capacity that is any different from other eutherian mammals. However, the NST capacity observed in *E. myurus* was 74% of that expected on the basis of body mass. There were three contrasts that lay outside the 95% prediction intervals. The contrast for *Cryptomys damarensis* (NST capacity 28% of that expected) fell below the interval, whereas those of *Microtus agrestis* and *Microtus oeconomus* lay above the prediction interval. The observed NST capacity was 177% and 221% for *M. agrestis* and *M. oeconomus*, respectively.

In the Afrotropical region, 50% of the species displayed lower-than-expected NST capacities (28 – 94% of expected capacity). Except for one species, data for the Afrotropical species that displayed higher-than-expected NST capacities were obtained from a single laboratory (60%) and the remaining species were all shrews (30%; Lipotyphla).

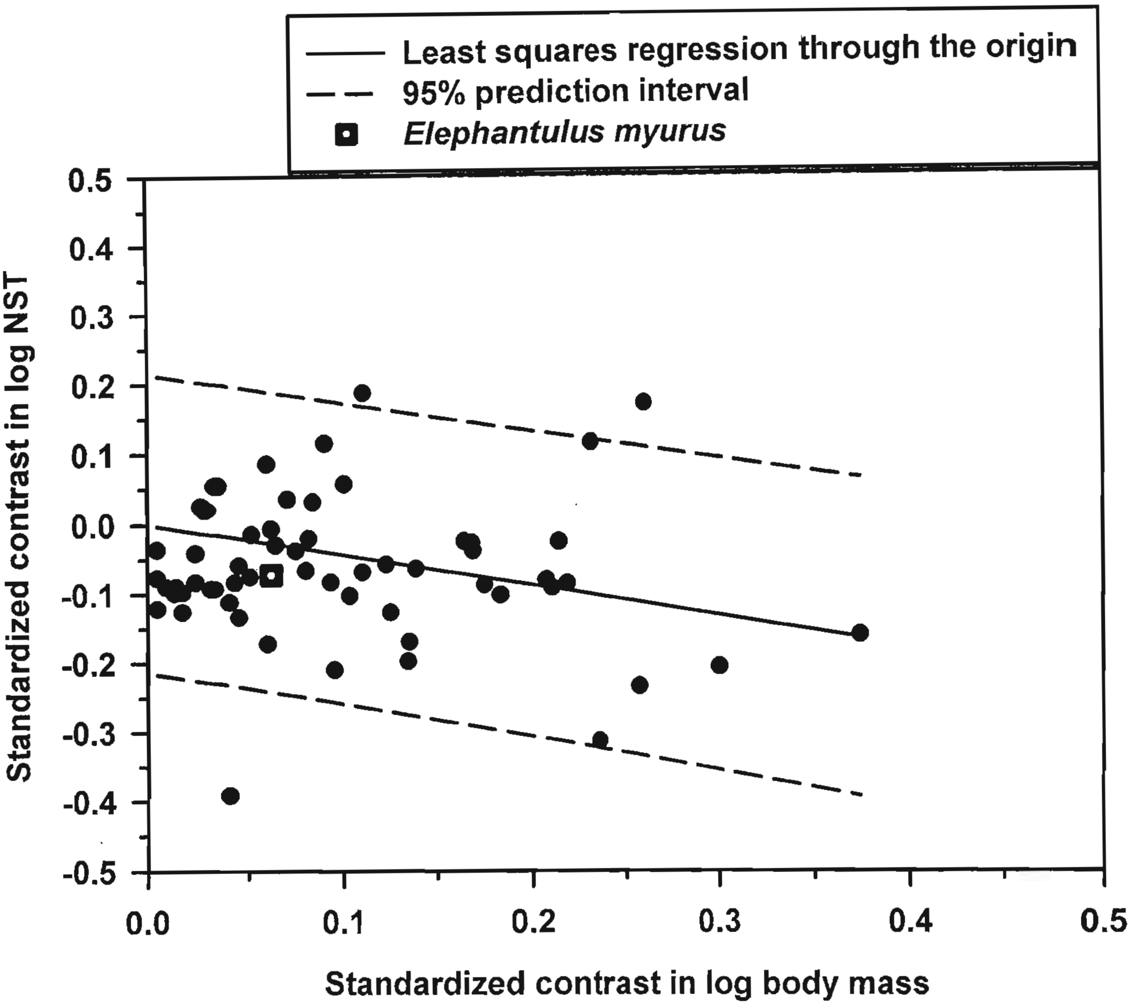

Figure 3. Use of phylogenetically independent contrasts to test whether NST in *E. myurus* differs from that of other eutherian mammals. We fitted a regression of log body mass and log NST contrasts as well as a 95% prediction interval. The regression was described by the line log NST = -0.442 log M_b + 1.12, where M_b = mass in grams.

All the Nearctic species had higher than expected NST capacity (119 – 218% of expected capacity), except for one shrew species, *Sorex cinereus*, (93% of expected capacity). Fifty percent of the Palaearctic species had higher than expected NST capacities (117 – 318% of expected capacity). The majority (71%) of the Palaearctic species that displayed lower than expected NST capacity were gerbils from the deserts of Israel. There were only three Neotropical species, insufficient to identify any general patterns.

Discussion

This study aimed to determine whether rock elephant shrews, *E. myurus* possess the capacity for NST. *E. myurus* showed a response to NA injection by significantly increasing oxygen consumption, which is usually interpreted as indicative of a capacity for NST through uncoupling respiration in BAT (Jansky 1973; Wunder and Gettinger 1996; Cannon and Nedergaard 2004). We showed that *Elephantulus myurus*, a member of the most basal eutherian mammal clade, had a capacity for NST that was similar to that of other eutherian mammals. This is the first account of NST capacity in a member of the Afrotheria.

Much of the NST in eutherian mammals is thought to occur in BAT where uncoupling proteins (UCPs) work as proton-conductance pathways to dissipate the proton gradient across the mitochondrial inner membrane to generate heat instead of ATP (Cannon and Nedergaard 2004). A response to NA is usually taken to imply the presence of BAT and UCPs, and the tissue specificity of UCP1 in BAT is usually used as a marker to identify whether BAT is present in a species. However, in the marsupials heat is produced primarily through shivering thermogenesis because BAT, the major source of NST is usually either absent or appears to be non-functional (Hope et al. 1997). Thermogenesis in non-BAT tissues such as skeletal muscle may be responsible for NST in marsupials (Holloway and Geiser 2001). Thus far, the mechanism for NST has not been resolved in marsupials (Nicol et al. 1997; Rose et al. 1999). For example in the Tasmanian bettong, *Bettongia gaimardi*, injection with NA increased NST, although the NST response was not attributable to BAT and UCP1 could not be detected even after the animals had been exposed to cold for two weeks (Rose et al. 1999). The presence of BAT in *E. myurus* remains to be verified. Because of the phylogenetic placement of elephant shrews the presence of BAT in *E. myurus* (and/or other elephant shrews) is of interest because functional BAT and UCP1 has yet to be detected in marsupials.

The dose required to elicit the maximum NST response in *E. myurus* was well predicted by the allometric equation of Wunder and Gettinger (1996). This equation was based on Holarctic species, but the *E. myurus* response suggests that the response of the Afrotropical species is similar to that of their Holarctic counterparts. The NST capacity measured in *E. myurus* in this study was 74% of that expected on the basis of an allometric relationship between body mass and NST. In general, small mammals inhabiting warm temperate, tropical and subtropical environments display lower-than-expected NST capacity (Sparti 1992; Saarela and Hissa 1993; Kronfeld-Schor et al. 2000). Lovegrove et al. (1991) also observed that although seasonal changes in NST capacity of small rodents from warm-temperate deserts do occur, they were considerably smaller than those recorded in similar-sized cold temperate rodents. Perhaps it is not unexpected that animals from warmer environments should display a lower capacity for NST compared to Nearctic and Palaearctic species. The Holarctic region has predictably harsh winters and, in preparation for the low temperatures, many small mammals increase their capacity for NST through increased deposition of BAT (Böckler et al. 1982; Heldmaier et al. 1982; Steinlechner and Heldmaier 1982; Dew et al. 1998). Because BAT is deposited subcutaneously, this also increases the insulation and thus decreases conductance during winter (Heldmaier et al. 1990). It is therefore not surprising that two of the outliers outside the 95% prediction interval (*M. oeconomus* and *M. agrestis*) were Holarctic species. The lower-than-expected NST capacity of *C. damarensis* may be explained by the fact that this species inhabits a fairly constant and thermally buffered environment as it lives underground and rarely ever ventures above ground (Lovegrove 1986; Lovegrove and Knight-Eloff 1988).

It is noteworthy that although 50% of the Palaearctic species had lower-than-expected NST capacities, 70% of those species inhabit the deserts of Israel. Lower than expected NST capacities in this region have previously been recorded and discussed quite extensively (Kronfeld-Schor et al. 2000). Similar to the Palaearctic region, 50% of the Afrotropical species had lower-than expected NST capacities. The shrews constituted 30% of the Afrotropical species whose NST capacities were higher-than-expected on the basis of body size. It is noteworthy that data for 60% of the remaining Afrotropical species that displayed higher-than-expected NST capacities were obtained from a single laboratory. Methodological biases resulting in high NST values therefore cannot be ruled out. If we exclude these data, the insectivores (shrews) were exceptions to the generalization that tropical and subtropical species have lower than expected capacities for NST. Sparti (1992) observed lower NST capacity in Afrotropical shrew species and argued that the lower capacity for NST in non-Holarctic shrews could be explained as a consequence of the fact that the animals experience milder winters resulting in a diminished need for increased NST capacity during winter. However, in our study, Afrotropical shrews did not display lower NST capacities relative to their Holarctic counterparts.

Lower-than-expected NST capacities in tropical, subtropical and warm-temperate species need to be interpreted with caution. In our calculation of NST capacity we subtracted $NAVO_2$ from RMR as it is the ability to produce heat below the T_{lc} when confronted with cold that best determines the capacity for NST. Therefore, heat production above the basal, obligatory component provides a good indication of the ability of the animal to produce heat endogenously. However, there were some notable differences in the methodology used to calculate NST capacity in the different studies. Firstly, whereas some studies subtracted BMR from the maximum NA induced VO_2, others only subtracted RMR at which the measurements were made. We subtracted BMR from NA VO_2 whenever possible, but where BMR was not specified, we used RMR values. Thus, for some species, NST capacity may have been underestimated. Secondly, some of the differences in NST capacity may have been a result of the different acclimation regimes used in the different studies. For example, in a number of studies animals were acclimated to a 12:12 LD cycle or in some cases, long photoperiod (Table 1). However, a combination of low ambient temperature and short photoperiod is known to enhance BAT development and hence NST capacity of most small mammals (Heldmaier et al. 1982;Heldmaier 1989; Haim et al. 1991;Steinlechner and Heldmaier 1982; Heldmaier et al. 1985). Therefore the NST capacities of some species may have been underestimated.

Sparti (1992) suggested that the ability to enter torpor should determine whether animals have a low or high NST capacity. He further suggested that those species that can enter torpor should display a higher capacity for NST compared to those that cannot enter torpor, as an increased capacity for heat production would be advantageous during arousal from torpor (Sparti 1992). However, BAT may not be as important for arousal from torpor as previously thought. Lyman and O'Brien (1986) showed that hibernating Turkish hamsters and ground squirrels were capable of rewarming from hibernation in the absence of as much as 70 and 81%, respectively, of their normal amount of BAT, suggesting a diminished role of BAT for arousal (Lyman and O'Brien 1986). They suggested that instead of supplying heat, BAT functioned as an endocrine gland for thermogenesis and that its presence in its entirety was not necessary for the maintenance of arousal ability. They further argued that it should not be unexpected that mammals with different lifestyles should have varied methods of arousal from torpor, depending on sources of heat available to them.

Recent studies suggest that tropical and subtropical species rely less on endogenous heat production during arousal from torpor (Ortmann et al. 1996; Lovegrove et al. 1999b; Schmid 2000; Mzilikazi et al. 2002; Geiser et al. 2002; Geiser and Drury 2003; Turbill et al. 2003; Mzilikazi and Lovegrove 2004; Dausmann et al. 2004; Geiser et al. 2004). A two-phase pattern of heating during arousal from torpor has been suggested (Schmid 1996; Ortmann et al. 1996; Lovegrove et al. 1999b). The first phase involves slow exogenous passive heating using diel cycles of ambient temperature. The second phase involves more rapid heating using endogenous heat production until normothermic T_bs are attained. The ability of rock elephant shrews to produce heat by NST as suggested by the NA response is therefore important with regards to the second phase of assisted arousal, which requires endogenous heat production. Furthermore, rock elephant shrews are capable of unassisted endogenous arousal (Mzilikazi et al. 2002). The ability to produce heat through NST therefore also explains the ability of the rock elephant shrews to arouse using endogenously produced heat, without relying on exogenous heat sources (Mzilikazi et al. 2002). However, the expected heating rate during arousal based on body mass is 0.426°C/min for *E. myurus* (Geiser and Baudinette, 1990). Free-ranging *E. myurus* have slightly lower heating rates (.0258 – 0.350°C; Mzilikazi et al. 2002). These lower-than-expected heating rates during endogenous arousal are probably a consequence of the fact that *E. myurus* display a NST capacity that is 74% of that expected on the basis of phylogenetically independent allometric predictions.

Concluding remarks

This study showed that *E. myurus*, a phylogenetically ancient mammal had a NST capacity that was no different from other eutherian mammals. Furthermore, the reliance of *E. myurus* on exogenous passive heating did not result in a low NST capacity compared to other species. This study also confirmed that tropical, subtropical and warm-temperate environment species had lower-than-expected NST capacities, although these capacities were not significantly different from phylogenetically independent allometric predictions. Furthermore, because of methodological differences when measuring NST, lower/higher-than-expected NST values need to be interpreted with caution.

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Chapter 7

No evidence for torpor in a small African mainland primate: the Lesser bushbaby, *Galago moholi.*

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Abstract. The investigation of heterothermy in the strepsirrhine primates has focussed largely on the Malagasy cheirogaleids. No data exist on the thermal biology of free-ranging African galagids. However, a physiological verification of torpor occurrence in mainland strepsirrhines is important with regard to arguments pertaining to the colonization of Madagascar by strepsirrhine primates. We measured body temperatures of 11 free-ranging *Galago moholi*, captured at different times between February 2002 – September 2003, for three consecutive months for each animal. We expected lesser bushbabies to employ daily heterothermy during winter. We did not record any incidents of heterothermy throughout the study period. Why does *G. moholi* not employ heterothermy? We consider several alternatives, including phylogenetic placement as well as physiological and ecological factors, i.e., predation pressure, food habits and breeding pattern. We suggest that the breeding

pattern observed in *G. moholi* obviates torpor use whilst increasing fecundity, which would be adaptive if the animals are confronted with high predation risks. Furthermore this study highlights the need for more data from free-ranging animals if convincing arguments are to be made regarding the possible role of heterothermy in species dispersal.

Introduction

Heterothermy, which includes daily torpor and hibernation, occurs in all three mammalian subclasses (Geiser and Ruf 1995; Geiser 1998; Grigg and Beard 2000). It has previously been suggested that daily torpor and hibernation reflect "alternative energy-conserving physiological solutions to different biotic and abiotic selection pressures", with daily torpor more common in unpredictable environments and hibernation associated with predictably cold seasons (Lovegrove 2000). However, Dausmann et al. (2004) recently recorded hibernation in free-ranging fat-tailed dwarf lemurs, *Cheirogaleus medius*, and provided the first record of body temperatures during hibernation in an Afrotropical primate. Their study suggests that both daily torpor and hibernation may be important for coping with energetic bottlenecks in tropical and subtropical species. More data from free-ranging Afrotropical species are therefore important to establish how widespread the use of hibernation is.

Within the primates, investigations of heterothermy have focussed largely on the strepsirrhines, particularly the Malagasy cheirogaleids (Schmid 1996; Ortmann et al. 1996; Schmid and Kappeler 1998; Aujard et al. 1998; Schmid

2001). Only one record of heterothermy has been reported in the South-east Asian Lorisidae, for the slender loris, *Loris tardigradus* (Müller et al. 1985). Thus, apart from detailed studies on the Malagasy cheirogaleids, there has been little investigation of heterothermy in other strepsirrhines. Yet, arguments on thermoregulation and how strepsirrhines cope with energetic bottlenecks are sometimes used in debates relating to species dispersal and island biogeography. For example, the expression of heterothermy by some Malagasy primates is important regarding the question of whether torpor is a plesiomorphic character that may have been inherited from an African mainland ancestor. It has been suggested that strepsirrhine primates utilized torpor to enable them to conserve energy whilst crossing the Mozambique channel to colonize Madagascar (Martin 1972; Kappeler 2000; Yoder et al. 2003). If torpor does occur in the mainland relatives of the cheirogaleids, this would lend support to the above argument. However, at present, there are no data from free-ranging mainland strepsirrhines to provide a physiological verification of this argument and information on the thermal biology of the African Galagidae remains conspicuously scanty.

The southern African lesser bushbaby, *Galago moholi* (ca. 200g) is a nocturnal savanna woodland species and in South Africa is usually associated with *Acacia* and mopane bushveld (Skinner and Smithers 1990). Lesser bushbabies subsist mainly on gum, which exudes from certain species of *Acacia,* a diet frequently augmented by insects (Skinner and Smithers 1990; Caton et al. 2000). Their feeding habits, phylogenetic placement, body size and zoogeographical location render them excellent candidate employers of heterothermy. Although torpor has neither been measured nor reported in lesser bushbabies, Skinner and Smithers (1990) report that when they are disturbed from "sleep" during the day, they appear lethargic and take a while before becoming active.

Except for a single study, using captive-bred animals (Knox and Wright 1989), the thermal biology of the lesser bushbabies has not been investigated. It is known that thermoregulation may differ markedly between captive and free-ranging animals (Geiser et al. 2000). The aim of this study was therefore to investigate the use of heterothermy (if any) by free-ranging southern lesser bushbabies, *Galago moholi*.

Study site

The study site was located in the Nylsvlei Nature Reserve, Limpopo Province, South Africa (24° 29S'; 28° 42'E) and is comprised of semi-arid, mixed bushveld habitat. The hot wet season lasts from November to March, and the cool, dry season from April to October. The reserve receives a mean annual precipitation of 630 mm. The annual mean temperature is 19°C and ranges between –3.2 – 23°C. Minimum temperatures below 0°C may be recorded between May and August (Scholes and Walker 1993).

Materials and methods

The study was conducted between February 2002 and September 2003. All the procedures in this study complied with the "Principles of animal care" publication no. 86 – 23, revised 1986 (National Institute of Health) and the "Code of ethics for animal experimentation" manual adopted by the University of KwaZulu-Natal. The animals were captured using walk-in live traps mounted on *Acacia* trees (Permit number 07877; Limpopo Province Department of Agriculture and Environment; South Africa). The traps were baited during the night with a mixture of peanut butter, bananas and honey and were checked immediately before sunrise. We measured the body temperatures of 11 free-ranging *Galago moholi*, captured at different times throughout the study period (overall recapture rate = 55%); February – May 2002 (2 males), June – August 2002 (1 female), October – December 2002 (4 males) and June – August 2003 (1 female; 3 males). Pre-calibrated Thermocron iButtons (temperature dataloggers; Dallas SemiConductor; accuracy 1°C from –20° to 70°C) were surgically implanted into the peritoneal cavities of the bushabies under inhalation anaesthesia (Isoflorane in oxygen; induction and maintenance, 2% flow rate, ca. 0.5ℓ min $^{-1}$). The surgical procedures did not have any adverse effects on the animals as they recovered within 60 minutes of surgery. They were released at the exact capture locations < 24 hrs after initial capture. We programmed iButtons to measure T_b once every hour, resulting in a total of 2 040 data points per animal, over a period of 85 consecutive days. To ensure that the animals had fully recovered and that surgery did not affect body T_b, we programmed the iButtons to start recording at least 15 days after surgery.

As tree holes are used for resting, we placed an iButton in a shallow tree hole to measure (T_a) in typical resting sites of the lesser bushbabies.

Data analyses

We determined the daily minimum, mean and maximum body temperature both during the daytime and the nighttime. We used analysis of variance (ANOVA) to test for statistical differences in body temperature between the daytime and the nighttime. The amplitude of the body temperature rhythm was calculated by subtracting the minimum T_b value measured during the daytime (rest phase) from the highest value measured during the nighttime (active phase) (Aschoff 1982). All mean values are presented ± SD. Throughout the text N = total number of animals, n = number of observations.

Results

Ambient temperature

The study site was characterized by wide variations in ambient temperature, with maximum temperatures often reaching 30°C during summer. In winter, ambient temperatures often decreased to below 0°C, although the temperature differences between nighttime and daytime values were sometimes as large as 30°C (Table 1).

Table 1 Ambient temperature at Nylsvlei Nature Reserve during the study period

Temperature (°C)	February - May	June - August	October – December
Daily minimum	15.8 ± 2.6	4.2 ± 3.8	16.7 ± 2.8
Range daily minimum	11.0 – 20.5	-1.5 – 16.0	4.0 – 21.5
Daily mean	20.5 ± 1.6	13.1 ± 3.0	22.1 ± 3.2
Range daily mean	17.6 – 26.1	7.6 – 20.8	4.0 – 28.1
Daily maximum	28.8 ± 3.6	25.5 ± 4.0	28.7 ± 4.9
Range daily maximum	18.5 – 35.5	17.0 – 33.0	17.5 – 39.0

Body mass

The mean species body mass at initial capture was 182.5 ± 35.6g (N = 11) and the mean body mass at recapture was 193.4 ± 24.8g (without 3g iButton).

Body temperature

Year-round measurement of T_bs revealed no evidence of torpor in free-ranging *G. moholi.* Throughout the study period T_b did not decrease below 33°C in any of the animals (Fig 1). The minimum T_bs ranged from 33.1 – 38.2°C and 33.3 – 38.8°C during the day and night, respectively. The maximum T_bs during the day ranged from 35.9 – 39.7°C and from 36.2 – 39.5°C during the night. The mean T_b ranged from 34.8 – 38.6°C and 36.2 – 39.1°C, during the day and night, respectively (Fig. 1). On average, the daily minimum T_b, mean T_b and maximum T_b were all significantly higher during the night (Fig 1; minimum T_b $F_{1,10} = 6.12$,

$p < 0.05$; mean T_b $F_{1,10} = 20.88$, $p < 0.01$; maximum T_b $F_{1,10} = 7.18$, $p < 0.05$). The mean amplitude of the T_b rhythm of lesser bushbabies was 2.4 ± 0.7°C ($N = 11$; $n = 862$). The body temperature profiles were consistent with the nocturnal activity pattern that we observed (Fig 2).

We expected the animals to exhibit torpor during winter, the coldest part of the year. However, typical T_b plots (e.g. adult male Fig. 2) of all the animals during mid-winter showed no evidence of torpor. The minimum body temperatures were attained in the 2-hour period around sunrise whereas the maximum T_bs were attained in the 2-hr period around sunset (Figs 2,3).

Mzilikazi Fig 1

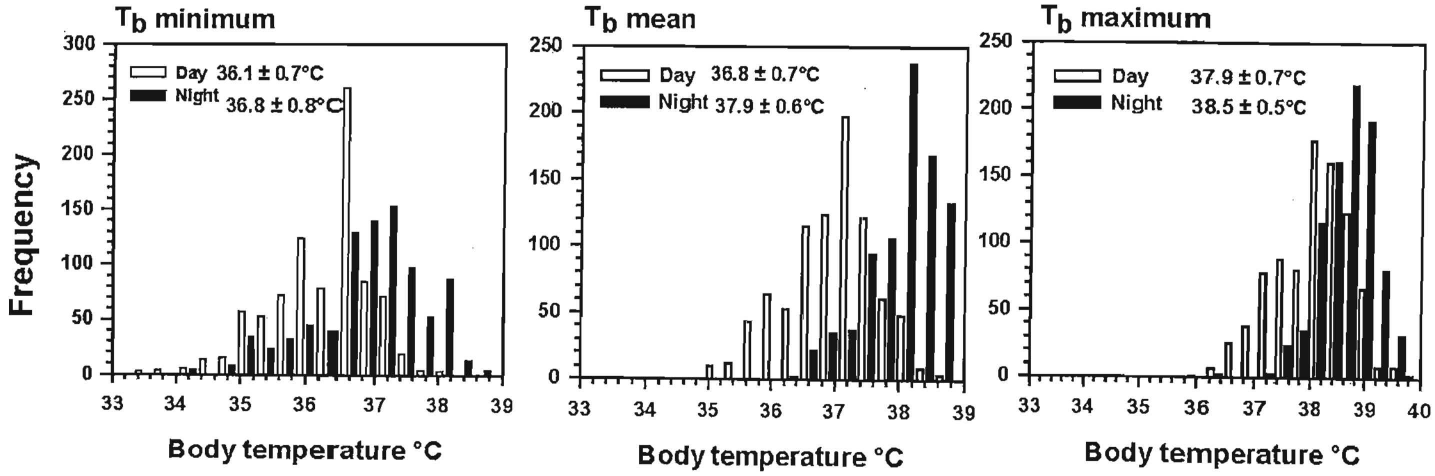

Figure 1. Frequency distributions of mimimum, average and maximum T_b in free-ranging *G. moholi* between February 2002 – August 2003.

Mzilikazi Fig 2

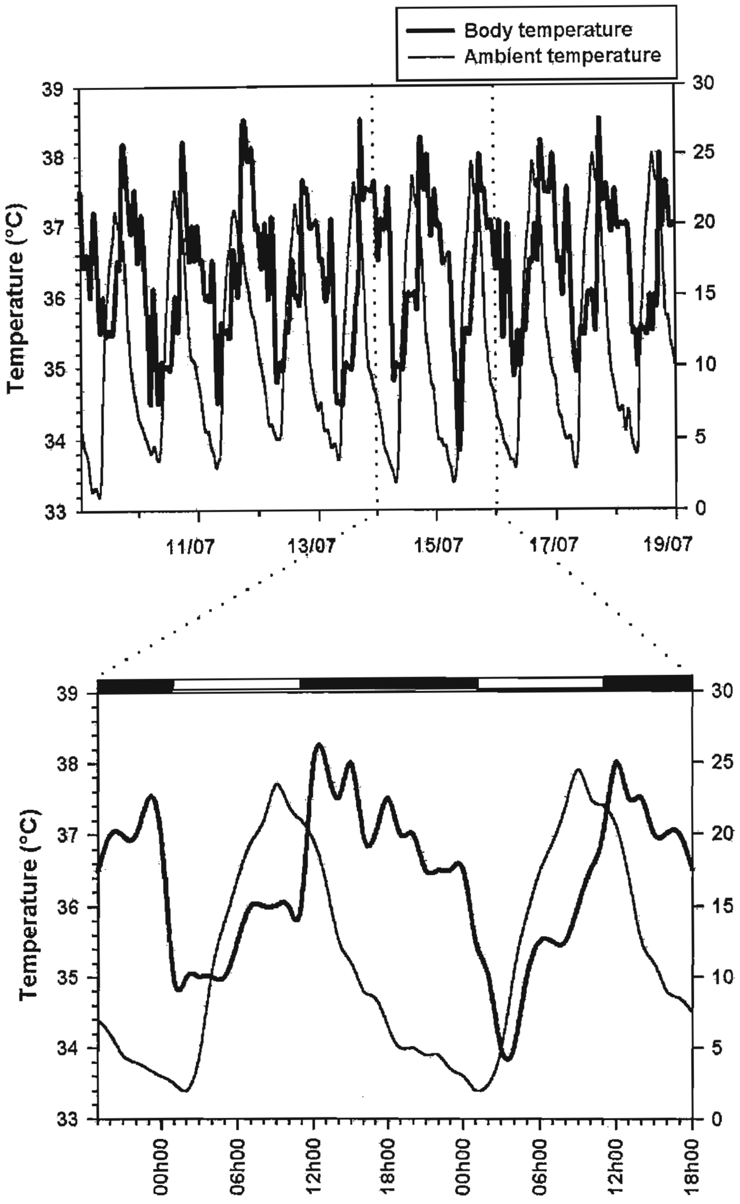

Figure 2. Daily patterns of body temperature (T_b) measured in the peritoneal cavity of a free-ranging male *G. moholi* over a 10-day period in July 2003. The ambient temperature (T_a) was measured in a nearby tree hole. The dark bars on the top of the expanded lower 2-day figure indicate the nighttime.

Mzilikazi Fig 3

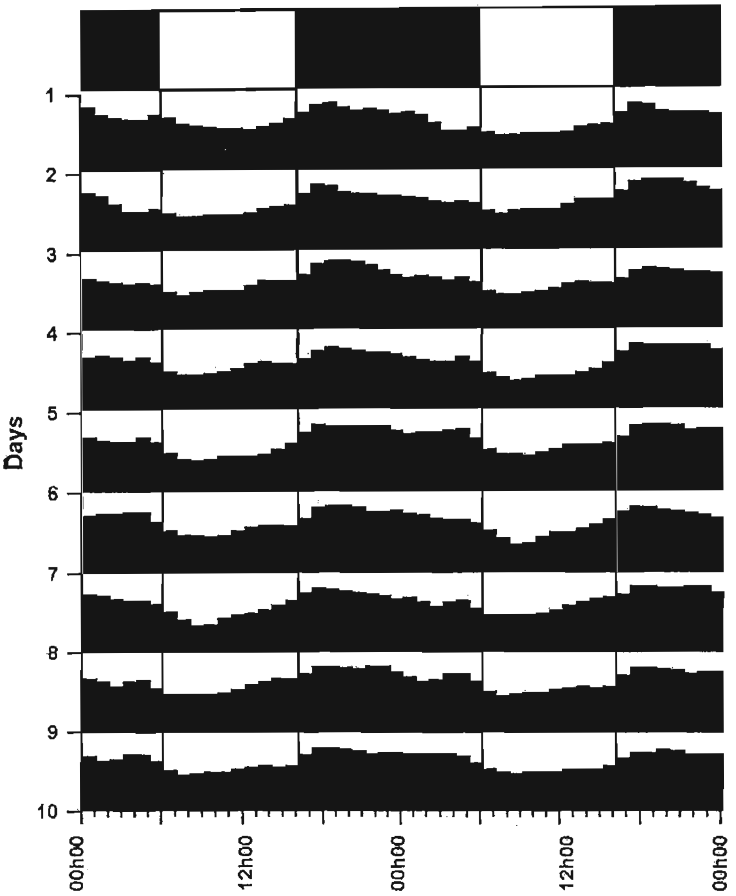

Figure 3. Circadian double plots of body temperature of an individual free-ranging male *G. moholi*. The T_b scale ranges from 32 – 39 °C in each plot of each day. The dark bars on top indicate the nighttime and the clear bars indicate the daytime.

Discussion

This study represents the first account of body temperatures in a free-ranging mainland African strepsirrhine primate. We did not record any incidence of torpor in any of the 11 animals recorded during different seasons in the yearly cycle (February 2002 – September 2003). Previous accounts of body temperature patterns in *Galago moholi* have been described under laboratory conditions, where lesser bushbabies maintained stable body temperatures throughout a range of ambient temperatures from 6 – 35°C (Knox and Wright 1989).

We expected lesser bushabies to employ daily heterothermy during winter based on their body size (Geiser 1998), food habits (Skinner and Smithers 1990), zoogeographical location (Lovegrove 1996; 2003) and behaviour (Bearder and Martin 1980). However, they adhered strictly to normothermy throughout the study period. Torpor use and frequency may differ between different years depending on the environmental conditions prevailing during a particular year (Mzilikazi and Lovegrove 2004). Therefore it might be argued that, the observation that free-ranging lesser bushbabies did not use torpor during our study period, does not necessarily imply that they are physiologically incapable of it. However, Kamau and Müller (1989) showed that food deprivation and dehydration did not elicit a metabolic depression response in *G. moholi*. They concluded that basal metabolism in lesser bushbabies had reached the lowest levels and no further adjustments could be made in response to energy stress.

The observed strict adherence to normothermy in *G. moholi* may be a consequence of phylogenetic, ecological, behavioural and physiological factors. We consider several alternatives.

Phylogenetic position

The lack of torpor in *G. moholi* raises some interesting questions regarding the evolution of torpor in primates. To show the known phylogenetic distribution of torpor within the strepsirrhine primates, we constructed a composite phylogeny based on phylogenetic studies (Adkins and Honeycutt 1994; Yoder 1994; Yoder et al. 1996; Delpero et al. 2000; Delpero et al. 2001; Pastorini et al. 2001; and Masters et al. in press) (Fig 4) . Although data on torpor in free-ranging strepsirrhines are scanty, the presence of torpor in *Loris tardigradus* (Fig 4) suggests that torpor must have been present in the strepsirrhine ancestor. It was retained in the Malagasy cheirogaleids (mouse and dwarf lemurs; Ortmann et al. 1996; Schmid and Kappeler 1998; Aujard et al. 1998; Schmid 1998; Schmid 2001) and these are the only primates for which detailed studies of the use of heterothermy are available. It is also possible that torpor may have evolved recently in the cheirogaleids and in *Loris*. However, this would represent the only known case of independent evolution of torpor in the mammals (Lovegrove 2004). It is likely that the reason why torpor is not evident in some of the cheirogaleids (*Phaner furcifer*, *Allocebus trichotis* and *Mirza coquereli*) is because it has not been investigated.

The cheirogaleids are the least specialized of the lemuroid families and have been interpreted by some to be closest to the ancestral strepsirrhine condition (Charles-Dominique and Martin 1970) although there remains a debate about their phylogenetic affinities (Yoder 1994). If it is true that the cheirogaleids closely resemble the ancestral condition, and since torpor appears to be a plesiomorphic character in mammals (Malan 1996), it is not unexpected that the cheirogaleids, all <600g, nocturnal and omnivorous (Yoder 1994), should retain the ancestral condition of adaptive heterothermy by virtue of small body size (Geiser 1998), phylogenetic placement and zoogeographical considerations (Lovegrove 1996; Lovegrove 2003).

Torpor seems to have been lost in the galagos. The extant lesser galago radiation appears to be fairly recent (Masters 1998). Because torpor in mammals occurs in the phylogenetically older taxa (Geiser 1998), phylogenetic placement of the lesser galago may explain the lack of torpor. However, the observation that heterothermy was not found in *G. moholi* does not preclude the use of heterothermy by other members of the Galagidae. For example, the smallest members of the family, *Galagoides demidoff* (Senegal to Uganda; M_b = 69g) and *Galagoides thomasi* (Zaire, Angola, Uganda and Cameroon; M_b = 99g) may well use torpor. Whilst these smaller species are found in the tropics, the mild climate is unlikely to prevent daily torpor and possibly hibernation from being used, as the first body temperature data on a tropical primate hibernator have recently been recorded (Dausmann et al. 2004). This suggests that in addition to low ambient temperatures, other factors may determine the expression of torpor.

Mzilikazi Fig4

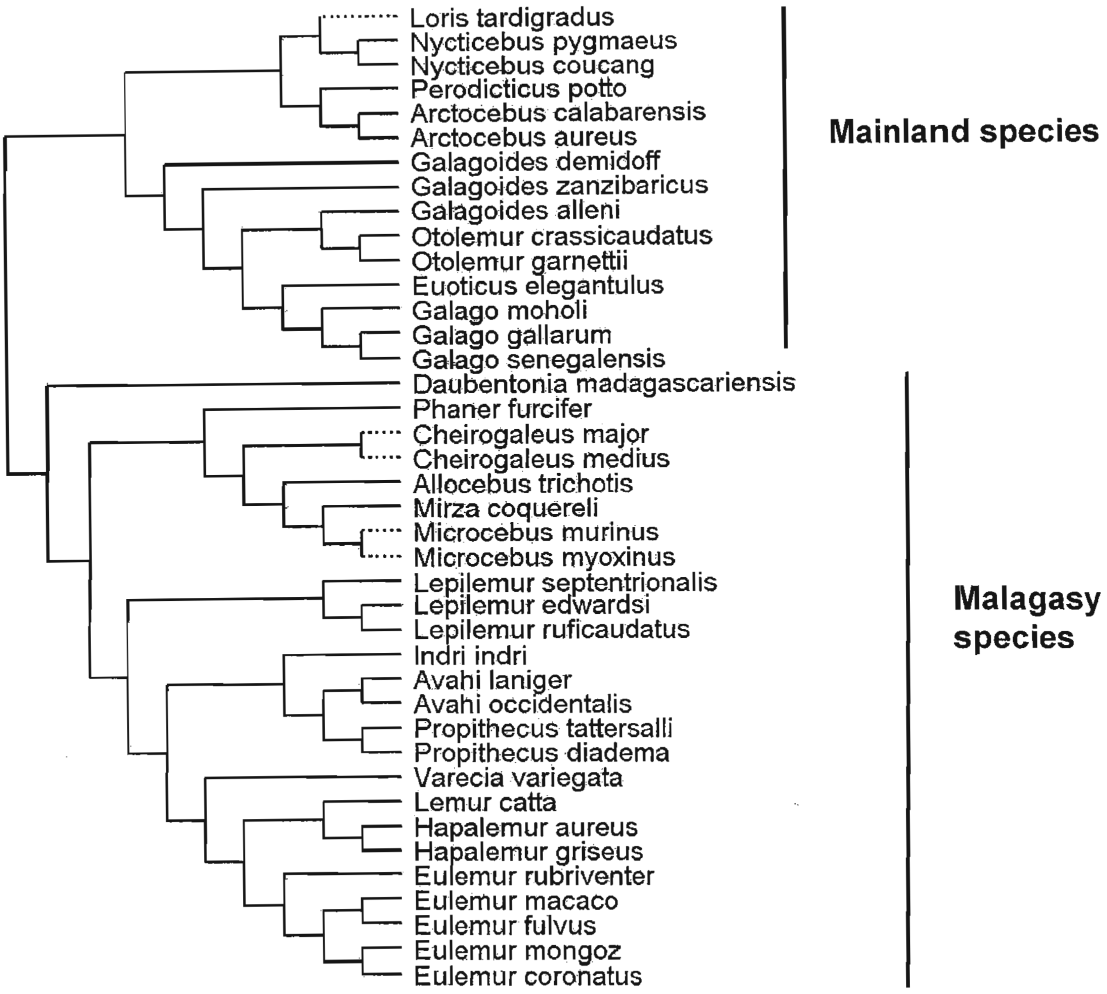

Figure 4. A phylogeny of the strepsirrhine primates showing taxa in which torpor has been observed (horizontal dotted lines). Note the relative paucity of heterothermy in other strepsirrhines other than the cheirogaleids.

Physiological factors

Geiser (1998) argued that the use of heterothermy is most likely to be expressed by those taxa the members of which are small and experience seasonal or aseasonal shortages in their food supply. The mean body mass for daily heterotherms is 253 ± 166g, with the majority of daily heterotherms being < 100g (Geiser and Ruf 1995). The lesser bushbaby is within this size range. It feeds mainly on *Acacia* exudate (gum), and arthropods (Bearder and Martin 1980). In our study site, insect availability decreases during winter, but gum is available throughout the year owing to the activities of wood-boring beetles and moths. The total activity budget spent on gum foraging increases from summer to winter in lesser galagos (Bearder and Martin 1980; Harcourt 1986). Increased gum foraging, coupled with curtailed overall activity, has been explained as a mechanism to enhance survival in *G. moholi* during the winter months (Bearder and Martin 1980). These authors suggested that it was doubtful whether survival would be possible without access to gum. However, both gum and arthropod exoskeletons contain polysaccharides, which can only be used as a source of energy through microbial fermentation (Caton et al. 2000). Fermentation takes place in the hindgut and is slower than catalytic digestion by enzymes, which can potentially limit energy availability, particularly for small mammals such as lesser bushbabies with high mass-specific metabolic rates. Caton et al. (2000) describe the digestive mechanism that enables lesser bushabies to survive on the apparently poor quality diet despite their small body sizes. Firstly, gum contains soluble polysaccharides, which travel in the fluid phase of the digesta, reaching the hindgut faster than particles from arthropod exoskeletons. The dissolved polysaccharides are fermented faster than particulate digesta because they have a greater surface area for microbial activity. Secondly, the digestion process is divided into two phases, with catalysis occurring in the small intestine and then followed by fermentation of undigested residues in the hindgut. However, Bearder and Martin (1980) reported incidents during a particularly harsh winter where lesser bushbabies experienced large weight loss, high mortality and loss of portions of the tails as a result of frostbite. Thus, the animals may not obtain adequate caloric intake from gum to meet their thermoregulatory requirements during severe winters. It therefore seems highly likely that because of the apparent lack of a physiological capacity for heterothermy, *G. moholi* may suffer high mortalities when their energy intake is insufficient to meet their thermoregulatory requirements.

A significant decrease in thermal conductance is a common and effective thermoregulatory adjustment that a majority of small mammals utilize during winter (B. Lovegrove in prep). Knox and Wright (1989) suggested that the ability of *G. moholi* to maintain stable body temperatures at low ambient temperatures could be a result of postural adjustments and thus thermal conductance adjustment. Although male bushbabies usually sleep alone, females and juveniles often sleep in groups during the rest phase (Skinner and Smithers 1990). Furthermore, on very cold nights free-ranging lesser bushbabies return to their sleeping sites unusually early, around midnight (Bearder and Martin 1980), thus decreasing total activity and effectively advancing the onset of the rest phase. Huddling and sleeping in family groups are likely to decrease thermal conductance and have consequences for energy spent on thermoregulation. In addition to postural and behavioral adjustments, lesser bushbabies decrease thermal conductance through an increase in fur density during winter. They display signs of heavy moulting during spring (S. Bearder, pers comm).

Predation pressure

G. moholi share similar morphological, behavioural and ecological characteristics with the cheirogaleids. One possible explanation for the absence of torpor in the lesser galago compared with the cheirogaleids may lie in the different predation pressures on the mainland and the Madagascan island. The heterothermic state decreases responsiveness to external stimuli and may render animals vulnerable to predation. The cheirogaleids are prey for a number of mammalian, reptilian and avian species including the ring-tailed and narrow-striped mongoose, the long-eared Madagascar owl, the Madagascar barn owl, Madagascar harrier-hawk, as well as tree and ground boas (Garbutt 1999). Although mouse and dwarf lemurs are nocturnal, during winter when they use torpor, they effectively advance their rest phase by curtailing their activity during the latter part of the night. We therefore assume that the greatest risk of predation would be during the night when the animals are torpid. We exclude reptiles and birds of prey from this argument because the reptiles presumably decrease activity during winter nights (when animals are torpid and most vulnerable) and the activity times for the predators (owls) and the prey do not overlap when torpor is used. We therefore only consider the mammalian Carnivora.

The primates are estimated to have colonized Madagascar between 66 – 62 mya while estimates for the carnivoran colonization are between 24 – 18 mya (Yoder et al. 2003), yielding an interval of 38 my separating the two colonization events. Thus, the expression of heterothermy may have been retained because of the lack of early predation by mammals. There is a longer association between the mainland carnivores and mainland strepsirrhines and this association may have obviated the use of heterothermy by *G. moholi*. Tree holes may offer relative safety during torpor. However, *G. moholi* do not use tree holes exclusively and, in our study site, typically slept in open-top nests that were about 4 – 6 m above ground in thorny trees (*Acacia nilotica* and *A.tortilis*) as well as in forked tree branches. At our study site lesser bushbabies are preyed upon by a number of species, including mongooses, genets, owls, eagles and snakes. We have occasionally found bushbabies attacked and killed by predators inside the traps. In predator rich environments, the decreased responsiveness during heterothermy would subject small daily heterotherms to high predation pressures. Thus, high risks of predation may also act to select against use of heterothermy in this species. We therefore suggest that the potential ecological costs outweighed the potential energetic benefits of torpor for lesser bushbabies.

Breeding pattern

The lack of heterothermic capacity may be associated with the breeding pattern observed in *G. moholi*. They breed during the winter months with mating observed during May. The gestation period is ca. four months, meaning that the females are pregnant throughout winter (Pullen et al. 2000). In addition, the females display post-partum oestrus, with a subsidiary mating season in October and subsequent births in February (Pullen et al. 2000). All the adult male animals we captured throughout the study period had scrotal testes, implying a lack of testicular regression even during the cold winter months. In the lesser bushbabies therefore the females are non-reproductively active for a short period only, between February and May, and the males seem to maintain a state of physiological readiness for reproduction throughout the year. The main difference between *G. moholi* and the cheirogaleids is that *G. moholi* breeds at the beginning of winter (May) whereas in the cheirogaleids, breeding is a summer event. Furthermore, the lesser bushbaby gestation period, ca. 4 months, is longer than in the cheirogaleids (ca. 2 months; Garbutt 1999). Low ambient temperatures, particularly in our study site where mid-winter temperatures may sometimes decrease below freezing, and decreased invertebrate availability in winter, are conditions that we expected to trigger the use of torpor in lesser bushbabies. Although some mammals such as monotremes, marsupials, and insectivores enter torpor whilst breeding, for a number of rodent species, reproductive activity with its concomitant high levels of reproductive hormones and use of heterothermy are mutually exclusive (Goldman et al. 1986; Barnes et al. 1986; Lee et al. 1990; Geiser 1996; Mzilikazi and Lovegrove 2002). It is possible therefore that the breeding pattern observed in *G. moholi* has precluded the use of heterothermy. It is noteworthy that although the breeding pattern observed in *G. moholi* obviates torpor use, it allows for two litters during the summer (high reproductive output), which would presumably be adaptive if the animals are confronted with high predation risk.

In summary, there were two aspects to this study. The first was to investigate the occurrence of heterothermy in a mainland African strepsirrhine. Although the lesser bushbaby, *G. moholi*, seemed an excellent candidate employer of heterothermy, we found no evidence of torpor in 11 free-ranging animals between February 2002 – September 2003. We suggest that the ecological cost of torpor surpassed the potential energetic benefits in this species. The second aspect of the study was to consider our results in the context of the role of heterothermy in biogeography and species dispersal. If it is true that a mainland strepsirrhine ancestor used heterothermy as a means of energy conservation whilst crossing the Mozambique channel, then this plesiomorphic character has been retained in the Malagasy cheirogaleids. It would appear that the capacity to use torpor was subsequently lost, at least in *Galago moholi*. However, that torpor does not occur in lesser bushbabies does not necessarily preclude use of torpor by other mainland species, particularly the smaller species. This study highlights the need for more data from free-ranging animals, if any convincing arguments regarding the role of heterothermy in species dispersal are to be made.

Ackowledgements

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Chapter 8

Conclusions

Model species 1: *Elephantulus myurus*

Do free-ranging elephant shrews utilize daily torpor or hibernation?

Laboratory studies have previously failed to resolve whether elephant shrews use daily torpor or hibernation when food restricted and exposed to low ambient temperatures. Torpor bout length and depth in free-ranging *E. myurus* were consistent with those measured in daily heterotherms, although there was some marginal overlap with hibernation characteristics (Chapters 2 and 3). For example, the lowest body temperature measured in this study was 7.5°C, and the animals were capable of opportunistically extending torpor bouts for longer than 24 hours in response to unpredictable energetic shortfalls. A consideration of behavioural and physiological characteristics argues against hibernation in *E. myurus*. Instead, torpor depth and length in this phylogenetically old eutherian supports the idea of a physiological continuum of heterothermy (Chapter 3).

Year-round use of torpor

Mammalian hibernation and daily torpor are often assumed, in part, to be a consequence of cold and/or low food availability. In free-ranging *E. myurus* the use of torpor during winter was associated with low ambient temperatures, photoperiod and invertebrate abundance. Torpor was also observed during early spring (September) and late autumn (March – April). The observation of two torpor bouts in summer suggests that this species is capable of entering torpor during the warmer parts of the year. I suggest that in years when summer rainfall decreases below average, as happens during El Niño years, primary productivity might be decreased and that summer torpor is most likely to be used then. However, this remains a speculation and highlights the need for long-term physiological data in free-ranging animals if evolutionary forces selecting for adaptive responses such as summer torpor are to be resolved (Chapter 3).

Low body temperatures and ambient temperature cycles

One often-cited disadvantage of daily torpor is that a considerable amount of energy is required to arouse to normothermic body temperatures on a daily basis. This study confirmed that the low body temperatures observed in *E. myurus* under laboratory conditions are similar to those recorded in free-ranging animals. Generally, daily heterotherms do not allow body temperature to decrease below 10°C, presumably because of the high cost of arousal from low body temperatures every 24 hours. However, the low body temperatures of *E. myurus* could be explained on the basis of the mechanism of arousal from torpor. *E. myurus* relies on assisted exogenous passive heating during arousal which is synchronized with the daily ambient temperature cycles. The elephant shrews could therefore attain low body temperatures during torpor because they offset the cost of arousal through exogenous passive heating (Chapter 2). This study emphasizes that laboratory studies usually conducted under constant ambient temperature conditions underestimate the energetic benefits of torpor under free-ranging conditions. Furthermore the ability to offset arousal costs through passive heating is likely to be an important facet of daily torpor in subtropical and tropical environments, as has been shown for several other small metatherians and eutherians.

Possible influence of passive heating on circadian rhythms of body temperature

It has been suggested that the evolution of endothermy precluded the need for homeothermic mammals to be sensitive to T_a cycles because they could maintain physiological function despite fluctuations in the ambient temperature. However, under both free-ranging and laboratory conditions heterothermic free-ranging rock elephant shrews have polyphasic body temperature cycles which allows them to adjust their body temperature rhythms in order to entrain torpor to T_a cycles (Chapters 4 and 5). It remains to be established whether normothermic animals in positive energy balance might entrain their circadian rhythm of body temperature to T_a cycles.

The capacity for nonshivering thermogenesis (NST), passive heating and implications for evolution of endothermy

The use of passive heating also raises the question of whether rock elephant shrews are capable of endothermic heat production through non-shivering thermogenesis. This study represents the first record of NST in a Macroscelid and Afrotherian mammal (Chapter 6). *E. myurus* were capable of producing heat through NST, presumably through uncoupling respiration in BAT. However, because some marsupials show an NST response to NA injection, even in the absence of BAT, the presence of BAT needs to be verified in elephant shrews.

Elephant shrews are capable of using both passive heating and presumably BAT-aided NST. A verification of the presence of BAT in elephant shrews is important because of the concomitant implications for the evolution of endothermy and homeothermy. It has been suggested that the advent of BAT was important during the evolution of endothermy and consequently homeothermy. However, so many other endotherms lack BAT that the presence of BAT is likely to be part of a suite of thermogenesis sites (Grigg and Beard 2000). Furthermore, it has been suggested that passive heating was important in the evolution of endothermy, and that it would have resulted in a small thermogenic capacity sufficient to attain efficient physiological function (Geiser et al. 2004). Because of their phylogenetic placement, determination of the occurrence of BAT in elephant shrews is important with respect to verification of these suggestions.

Model species 2: *Galago moholi*

Until now, the Malagasy cheirogaleids were the only strepsirrhine primates for which detailed data on thermoregulation under free-ranging conditions were available. They remain the only primates in which daily torpor and hibernation has been recorded. This is the first study to record body temperatures in a free-ranging mainland African strepsirrhine primate. I found no evidence of heterothermy in free-ranging animals. The lesser bushbabies experience high predation pressure, which I suggest may have obviated the use of daily torpor in bushbabies as their responsiveness to external stimuli would be reduced during torpor. However, the bushbabies are reproductively active for the major part of the year. Although this breeding pattern is likely to prevent torpor use, it allows for two litters to be born during the summer. This breeding pattern is therefore presumably adaptive if the animals are confronted with high predation pressure.

Future research

Resolution of the eutherian phylogeny

A number of studies are in agreement that the Afrotheria are the most basal eutherian radiation (Springer et al. 1997; Stanhope et al. 1998; Murphy et al. 2001). However, the same studies cannot statistically reject the possibility that the Xenathra may be basal to all other placentals. If BAT is uniquely eutherian as has been previously suggested (Hayward and Lisson 1992), then it must have evolved very early in the radiation of the subclass. This suggests that BAT should be present in the most basal group. Quantification and verification of the presence of BAT in the elephant shrews, the rest of the Afrotheria and Xenathra have the potential to yield a resolution of the eutherian phylogeny.

Hypothermic mobility and ecological costs of torpor

Much is known about the energetic benefits of using torpor. Another worthwhile research avenue will be to investigate more rigorously the ecological costs of torpor. An understanding of ecological costs of torpor is likely to provide insights into physiological, ecological and phylogenetic constraints and determinants of torpor expression. For example, during assisted exogenous passive heating it is not clear whether rock elephant shrews passively warm up through an increase in ambient temperature or through basking. If they bask, this implies that hypothermic *E. myurus* are somehow capable of emerging from their resting sites to bask in the sun. Hypothermic mobility, if any, in *E. myurus*, remains to be quantified. Resolving the question of hypothermic mobility is important in terms of whether a decrease in body temperature alone is sufficient to diagnose torpor. Furthermore, establishing the degree of responsiveness of torpid animals to external stimuli is likely to have more relevance ecologically (compared to body temperature alone), as decreased responsiveness during torpor is probably important, especially in environments where predation risks are high.

Evolution of heterothermy in strepsirrhine primates and implications for over-water species dispersal arguments

Comprehensive data on torpor in strepsirrhine primates are restricted to the Malagasy cheirogaleids and torpor has yet to be recorded in the mainalnd strepsirrhines. This raises the question of whether torpor was present in a strepsirrhine ancestor and was subsequently lost on the mainland whilst retained in the cheirogaleids or whether torpor evolved independently in Madagascar. However, the observation of torpor in a single slender loris placed in a refrigerator (Müller et al. 1985) suggests that torpor was present in a common strepsirrhine ancestor. It is highly likely that torpor has simply not been recorded in more mainland strepsirrhines because it has not been investigated. If torpor should be absent in the mainland species, this would imply that torpor has evolved independently in the cheirogaleids. Currently, insufficient data are available to resolve this question and it warrants extended research attention.

I also considered the results of this study in the context of colonization of Madagascar by strepsirrhine primates. If it is true that a mainland strepsirrhine ancestor used heterothermy as a means of energy conservation whilst crossing the Mozambique channel from mainland Africa, then this plesiomorphic character appears to have been lost in the mainland lineage, or at least in *Galago moholi*. This study highlights the need for more data from free-ranging animals if convincing arguments regarding the role of heterothermy in species dispersal are to be made.

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