Energy expenditure and strategies to cope with high seasonality in the African lesser bushbaby, *Galago moholi* (Galagidae)

DISSERTATION

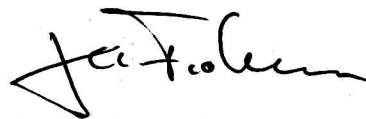submitted for the degree of
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To Whom It May Concern:

As a native speaker I hereby confirm that the PhD thesis of Julia Nowack, entitled *Energy expenditure and strategies to cope with high seasonality in the African lesser bushbaby, Galago moholi (Galagidae)*, is written in good English grammar and comprehensive style.

Yours sincerely,

Andrew E. McKechnie
Associate Professor

Für meine Mutter
†2013

Zusammenfassung

Das afrikanische Bushbaby *Galago moholi* ist ein kleiner, nachtaktiver Primat, der in den trockenen Baumsavannen im südlichen Afrika vorkommt. Seine Nahrung besteht vor allem aus kleinen Arthropoden und Baumharzen einiger Akazienarten. Die geringe Körpermasse sowie die schwierigen Habitatbedingungen während des südlichen Winters lassen vermuten, dass *G. moholi* bemerkenswerte Anpassungen (in Ernährung, Metabolismus oder Verhalten) entwickelt hat, die es der Art ermöglichen, den kalten, trockenen Winter zu überleben. Da diese Anpassungen bisher noch kaum untersucht wurden, hat sich diese Arbeit mit der Frage beschäftigt, wie *G. moholi* die Habitatsaisonalität bewältigt.

Die vorliegende Studie wurde zwischen März 2009 und August 2011 im Nylsvley Naturreservat in Südafrika durchgeführt. Ziel war es einen umfassenden Überblick über die physiologischen Anpassungen und Verhaltensstrategien zu ermitteln, mit denen *G. moholi* die nahrungsarme und kalte Jahreszeit übersteht. Um generelle metabolische oder mögliche heterotherme Strategien zu identifizieren, wurden Messungen der Körpertemperatur sowie Messungen des Energieverbrauchs durchgeführt. Außerdem wurde untersucht, ob *G. moholi* die Fähigkeit zur zitterfreien Wärmebildung besitzt, um weitere Informationen über physiologische Anpassungen der Art zu gewinnen. Zudem wurden Verhaltensbeobachtungen durchgeführt und einigen Tieren mittels Radiotelemetrie gefolgt, um Informationen über zusätzliche Verhaltensanpassungen zu erlangen. Im selben Zeitraum wurden zudem Nahrungsverfügbarkeit (Arthropoden und Baumharze von *Acacia karroo*) und Umweltbedingungen (Niederschlag und Temperatur) des Habitats dokumentiert.

Die Ergebnisse dieser Arbeit zeigen, dass *G. moholi* die Fähigkeit besitzt, Energie durch Tagestorpor zu sparen. Dies ist der erste Nachweis von Heterothermie in einem Primaten außerhalb Madagaskars. Torpor konnte in allen drei Studienjahren beobachtet werden, allerdings nur jeweils während der kalten Trockenperiode zwischen April und Ende August. Nur 27 % der in dieser Zeit untersuchten Tiere haben Tagestorpor gezeigt: Sechs Tiere gingen im Zuge von Nahrungsentzug während Messungen der metabolischen Rate im Labor in einen Tagestorpor, weitere drei Tiere haben in einem kurzen Zeitrahmen wiederholte Torporereignisse im Freiland gezeigt. Die offensichtlich unregelmäßige Verwendung dieser Strategie legt nahe, dass *G. moholi*, im Gegensatz zu den nah verwandten madagassischen Lemuren, Torpor nur als eine Art Notfallstrategie nutzt und nicht als reguläre saisonale Strategie, um Energie zu sparen.

Die Torporparameter, wie die Länge und die Tageszeit des Torporeintritts und –austritts, unterscheiden sich nicht von denen anderer nachtaktiver, heterothermer Tiere. Die Erwärmungsraten während der Aufwachphasen aus dem Torpor lagen hingegen rund 50 % unterhalb des anhand der Körpermasse errechneten Wertes. Dies ist wahrscheinlich auf die

niedrigen Energiereserven der Tiere zurückzuführen: Die Tiere gingen dann in den Torpor, wenn sie massivem Nahrungsstress ausgesetzt waren (z.B. Nahrungsentzug im Labor) und Torporereignisse haben an ungewöhnlich kalten Tagen, an denen die Tiere kein passives Aufwärmen anhand der Außentemperatur nutzen konnten, z.T. zu lebensbedrohlichen Situationen geführt. Eine andere mögliche Erklärung für die niedrigen Erwärmungsraten könnte das Fehlen von zitterfreier Wärmebildung sein. Dieser Mechanismus befähigt kleine Säugetiere zur Wärmeproduktion im Winter und zur schnellen Erwärmung aus dem Torpor oder Winterschlaf. Die Untersuchung der Kapazität der zitterfreien Wärmebildung hat allerdings darauf hingewiesen, dass *G. moholi* die Möglichkeit zur zitterfreien Wärmebildung besitzt.

Es erscheint ungewöhnlich, dass *G. moholi* Heterothermie nur selten nutzt, da gerade Winterschlaf und Tagestorpor generell als sehr profitable Strategien angesehen werden, um Wasser und Energie zu sparen. Heterothermie ist, neben allen Vorteilen, aber auch mit selektiven Kosten verbunden. Einige Studien bei Nagetieren kamen zu dem Ergebnis, dass Heterothermie die Reproduktion negativ beeinflusst. *G. moholi* hat zwei Paarungsperioden pro Jahr und eine relative lange Tragedauer, so dass wahrscheinlich eine „Trade-off"-Situation zwischen der Möglichkeit zur Reproduktion und der Möglichkeit der Torpornutzung entsteht.

Die Verfügbarkeit von Baumharzen und Insekten ist während der Wintermonate deutlich reduziert. Nahrungsanalysen haben gezeigt, dass Baumharze im Winter zwar in niedrigerer Quantität vorliegen, jedoch einen höheren Energiegehalt als im Sommer aufweisen. Dies befähigt *G. moholi* wahrscheinlich dazu, während des Winters normotherm zu bleiben und sich auf die Reproduktion zu konzentrieren. Statt in einen Torporzustand zu verfallen, passt *G. moholi* sein Verhalten an, um mit den kalten Wintertemperaturen umzugehen: Diese Arbeit konnte veranschaulichen, dass die Tiere im Winter vermehrt in Gruppen in ihren Schlafplätzen anzufinden sind, häufiger isolierte und geschlossene Schlafstätten nutzen und ihre nächtliche Aktivität reduzieren.

Zusammenfassend weisen die Ergebnisse dieser Arbeit darauf hin, dass *G. moholi* ökologische Anpassungen und saisonale Verhaltensänderungen während des Winters der Nutzung von Torpor vorzieht, da diese ausreichend sind, um ihren Energiebedarf zu decken und die Vorteile der Normothermie die Energieeinsparung durch Heterothermie übertreffen.

Abstract

The African lesser bushbaby, *G. moholi*, is a small, nocturnal primate that lives in dry woodlands of Southern Africa, with a diet mainly restricted to arthropods and exudates from certain species of *Acacia* trees. Its small size, as well as unfavourable conditions in its habitat during the austral winter suggests that this species shows remarkable adjustments (e.g. nutritional, metabolic, and behavioural) to cope with its highly seasonal habitat. As these adaptations remain elusive so far, this project addressed how *G. moholi* manages to cope with the pronounced seasonality of its habitat.

The study was conducted between March 2009 and August 2011 in the Nylsvley Nature Reserve in South Africa and aimed for a comprehensive overview of behavioural and physiological mechanisms of *G. moholi* for coping with food scarcity and low ambient temperatures. The methodology of this study has been diverse. In particular, body temperature and oxygen consumption measurements were used to identify general metabolic and possible heterothermic responses to the cold, dry period and non-shivering thermogenesis (NST) was determined to get further information about physiological adaptations. In addition, behavioural observations and tracking of animals were conducted with the aid of radio telemetry to gain information about (additional) behavioural strategies. Data on food availability (arthropods and gum of *Acacia karroo*) and ambient conditions (precipitation, ambient temperature) were obtained in parallel.

The results of this study showed that *G. moholi* does have the ability to employ torpor and therefore present the first confirmation of heterothermy in a primate outside Madagascar. Torpor was observed in all three study years, but only during the cold, dry season from April to the end of August. Only 27 % of individuals examined during the cold, dry period underwent daily torpor: six individuals after food and water deprivation in the laboratory and three individuals showed repeated torpid states within a close time frame under free-ranging conditions. This obvious reluctance of *G. moholi* to undergo torpor suggests that, in contrast to its close relatives the Malagasy lemurs, torpor in *G. moholi* is utilized in response to especially adverse conditions and not as a routine strategy to save energy during unfavourable seasons.

The torpor parameters of *G. moholi* (torpor bout length, entry and arousal times) did not vary markedly from the parameters described for torpor bouts of other daily heterotherms. Rewarming rates during arousal from torpor bouts, however, differed significantly. The average peak rewarming rates were found to be almost 50 % lower than expected for *G. moholi* on the basis of body mass, a condition probably due to depleted energy reserves of individuals. Animals obviously entered torpor when they were food stressed (e.g. food restriction in the laboratory) and torpor resulted in life-threatening

situations when energy reserves of the individuals were low and ambient temperature was not high enough to exploit effective passive rewarming. The low heating rates of *G. moholi* during arousal from torpor could also be due to a lack of NST capacity. This mechanism allows small mammals to produce heat during long term cold-exposure and to quickly rewarm from torpid states. However, the determination of seasonal NST capacity revealed that *G. moholi* possesses the capacity for NST.

The occasional use of heterothermy by *G. moholi* seems puzzling as hibernation and torpor are generally seen as beneficial strategies to save energy and water during unfavourable periods. However, even if heterothermy may enhance fitness via energy savings it also results in selective costs. Several earlier studies revealed that reproduction is, at least in rodents, seriously impaired by the use of torpor or hibernation. This trade-off between reproduction and torpor use might also be the case for *G. moholi,* which has a long gestation period and two mating periods a year.

Behavioural observations and food analyses have revealed that availability of low quantities of gum with increased energy content presumably enables galagos to stay normothermic throughout the winter and to focus on reproduction activities: Instead of utilizing torpor, *G. moholi* copes with the low winter temperatures through behavioural adaptations. This study found a significant increase in huddling behaviour and a significant increase in the use of enclosed and insulated sleeping sites during winter, as well as a reduction in nightly activity. Furthermore, *G. moholi* showed a seasonal shift of feeding behaviour, and increased gum intake in winter, when gums showed a rise in energy content.

In summary, the results of this study suggest that *G. moholi* favours ecological and behavioural adjustments over torpor, because these suffice to meet energy requirements of this species, and the advantages of normothermy outweigh the energetic benefits of heterothermy.

Contents

PUBLISHED CONFERENCE PRESENTATIONS

Abbreviations

BAT – brown adipose tissue

BMR – basal metabolic rate

MR – metabolic rate

mya – million years ago

NA – noradrenaline

NST – non-shivering thermogenesis

SA – saline

T_a – ambient temperature

T_b – body temperature

T_{bcore} – body core temperature

T_{skin} – skin temperature

UCP1 – uncoupling protein 1

SUMMARY

Introduction

ENDOTHERMY

Endothermic animals are able to maintain a constant body temperature (T_b) regardless of ambient conditions, enabling them to inhabit a variety of habitats and stay active during unfavourable conditions (McNab 1978). However, the energetic costs of endotherms are high, especially as they have to increase their metabolic rate (MR) during cold, unproductive seasons by means of thermoregulatory heat production (Lyman 1982, Carey et al. 2003). This is especially challenging for small endotherms as they have a high mass specific energy expenditure, due to their high surface-to-volume ratios. Small mammals have evolved a variety of strategies to minimize heat loss: insulation of endotherm species is higher compared with ectothermic species and fur density is increased during cold periods. The amount of fat, fur or feathers to increase insulation, however, is limited by the body size of the animal (Kleiber 1947).

Therefore, small mammals respond to cold exposure with a variety of behavioural and physiological adaptations. Physiological adaptations allow the animal to reduce its energetic costs through reduction of MR. Behavioural strategies to conserve energy can be applied simultaneously with reduction of MR (leading to further reduction of energy costs) or as an alternative strategy to provide winter survival. Behavioural thermoregulation includes reduced activity (Ostner 2002), selection of suitable microhabitats (e.g. good isolated nesting site; Entwistle et al. 1997), changes in body posture (reduction of surface area; Øritsland 1970), aggregation with conspecifics (Genoud et al. 1997, Perret 1998, Gilbert et al. 2010) or generating muscle heat through locomotion (Zerba and Walsberg 1992).

SEASONAL CHANGES IN THERMOGENIC CAPACITY

Behavioural modifications are sufficient to respond to moderate cold exposure. However, during acute cold exposure endotherms have to increase their MR to maintain a constant T_b (Lyman 1982, Carey et al. 2003). As a reaction to short-term cold exposure an animal will increase heat production through shivering thermogenesis. This strategy is costly as heat is produced through muscle contractions. Animals that possess brown adipose tissue (BAT) adapt to long-term cold exposure with only minimal muscular activity and primarily through non-shivering thermogenesis (NST). NST occurs in the BAT of mammals: the uncoupling protein 1 (UCP1) alters proton conductance in the inner mitochondrial membrane and leads to heat generation instead of ATP production (reviewed in Cannon and Nedergaard 2004). Cold-adaptation through increased basal MR (BMR) would lead to a constant increase in metabolic costs. Those metabolic costs can be minimized, when an animal maintains a

constant low level of BMR and reacts to cold periods only through the modification of NST (Degen 1997). In this case metabolism is only increased in periods of acute cold stimuli, but otherwise maintained on a low level.

Another response of small mammals to cold exposure is to abandon maintenance of a high body temperature and to undergo heterothermic states such as torpor and hibernation. In heterothermic, eutherian mammals, NST has also been described as an important mechanism of heat production during arousal from hibernation and from torpid states (Cannon and Nedergaard 2004).

HIBERNATION AND DAILY TORPOR

Endothermic species of all three mammalian subclasses have been found to undergo heterothermic states as a strategy to deal with seasonal food shortage and low ambient temperature (T_a): they either enter daily torpor (heterothermic phases <24 h; slightly lowered T_b) or hibernation (heterothermic phases >24 h; T_b close to T_a) (Lyman 1982, Wang 1989). These states are characterized by profound changes in physiology. Among other physiological changes, heart rate during torpor is reduced and respiration depressed (reviewed in Carey et al. 2003). Reduction of MR and a decrease in T_b reduces the energetic costs of the animal to a fraction of normothermic values (Lyman 1982, Wang 1989).

It is often debated whether torpor in endotherms is ancestral and plesiomorphic, or a derived trait (Twente and Twente 1964, Malan 1996, Geiser 1998, Lovegrove et al. 1999, Grigg et al. 2004, Geiser 2008). At present most authors see the occurrence of torpor and hibernation in such a wide diversity of mammalian groups as evidence of the plesiomorphic origin of heterothermy (reviewed in Grigg et al. 2004, Lovegrove 2012a, Lovegrove 2012b). According to this view, homeothermy is a derived trait that evolved from heterothermy (Lovegrove 2012a).

HYPOMETABOLISM IN PRIMATES

In the last decade heterothermy has been discovered in a multitude of mammalian species ranging from less than 100 g (e.g. small rodents) to more than 100 kg (e.g. black bear, *Ursus americanus*; Tøien et al. 2011) in body mass and inhabiting a variety of habitats. Hibernation and torpor are traditionally known as adaptations to temperate and arctic climates. However, heterothermy is not primarily an adaptation to cold periods, but rather to resource scarcity in periods of high energy requirements (Lovegrove 2000). As long as an animal has enough food to compensate for increased energy demands it can theoretically stay normothermic, throughout pronounced cold periods (e.g. Abert´s squirrels, *Sciurus aberti*; Golightly and Ohmart 1978). Therefore, it is not surprising that heterothermy has also been found in

mammals living in warm, but seasonal food scarce periods as the tropics (e.g. Geiser 1994, McKechnie and Mzilikazi 2011), including the order Primates. Among the primates, heterothermy is most common in one family of the Malagasy lemurs, the Cheirogaleidae (incidence is confirmed in *Microcebus murinus, M. myoxinus*, *M. berthae, M. ravelobensis*, *M. rufus*, *M. griseorufus*, *Mirza coquereli*, *Cheirogaleus medius*, *C. major* and *C. crossleyi*) (Ortmann et al. 1997, Schmid 2000, Schmid et al. 2000, Dausmann et al. 2004, Lahann 2007, Dausmann 2008, Kobbe and Dausmann 2009, Blanco and Rahalinarivo 2010, Lahann and Dausmann 2011). The capacity of these lemurs to utilize heterothermy is presented as evidence that this trait was a prerequisite for the successful colonization of Madagascar by the lemurs from mainland Africa by means of drifting vegetation mats.

<div align="center">THE RAFTING THEORY</div>

Madagascar broke away from the African mainland (165 - 121 mya, Rabinowitz et al. 1983; separation from India ~88 mya, Storey et al. 1995) before the estimated origin of the primates (~90 mya, Martin 2000), raising the question when and how lemurs arrived on Madagascar. Fossil records on Madagascar are scarce, starting only a few thousand years back (reviewed in Krause 2010) and therefore cannot shed light on this question. African and Asian lorises and galagos (Lorisiformes) are the closest relatives of the Malagasy lemurs (Lemuriformes). The invasion of Madagascar must have occurred between the separation of the Strepsirrhini into Lemuriformes and Lorisiformes at around 75 mya and the earliest lemuriform divergence at around 66 mya (between *Daubentonia* and all other lemurs; Horvath et al. 2008) and phylogenetic results indicate a single, African origin of Malagasy primates (Yoder et al. 1996). Three possible explanations about the colonization of Madagascar have been suggested: (1) a temporary reduction in sea level (2) the presence of a temporary land bridge and (3) rafting on drifting vegetation (reviewed in Kappeler 2000). Kappeler (2000) noted that the rafting theory is the only theory explaining the comparatively small number of terrestrial mammalian taxa (Rodents, Carnivores, Tenrecs, Lemurs) that successfully colonised Madagascar. The other theories should have led to a greater diversity of African mammals. To date, science has failed to provide geological evidence for the first two scenarios and it has also long been argued that it was impossible for mammals to have rafted from Africa to Madagascar because the currents could not have facilitated west-to-east transport across the Mozambique Channel (Stankiewicz et al. 2006). A recent model by Ali and Huber (2010), however, suggests that during the Eocene currents did indeed flow towards Northern Madagascar. Furthermore, these currents seemed to be episodically strong enough to have transported large rafts of matted vegetation torn from the banks of East African rivers to Madagascar in a mere 25 – 30 days (reviewed in Krause 2010).

Even after this finding, the rafting theory is still controversial, and Masters et al. (2007) argued that based on fossil records the estimated size of the lemuriformes and lemuroid ancestor (~2 kg) is well outside the range of most mammals employing heterothermy. However, as the main argument against this theory, all authors cite the lack of heterothermy in mainland relatives of the lemuriformes. The animals would not have survived a journey of several weeks without adaptations enabling survival when no or only limited food and fresh water was accessible. If torpor indeed enabled the mainland ancestor of today's lemurs to colonize Madagascar, heterothermy would presumably be a plesiomorphic character and should also be found in mainland relatives (Lorisiformes: Lorisidae & Galagidae), unless it was secondarily lost (reviewed in Kappeler 2000; Masters et al. 2007; Schülke and Ostner 2007). This topic has only been studied in two species so far. Müller et al. (1985) found that slender loris, *Loris tardigradus* (Southeast Asian Lorisidae), cool large parts of the body during cold exposure but still keep the body core at a high temperature when tested under laboratory conditions. Anecdotal observations suggest that the African lesser bushbaby, *Galago moholi* (Southeast African Galagidae), when disturbed from 'sleep' during the day, appear lethargic and take a while before becoming active (Skinner and Smithers 1990). However, there have been three earlier studies on thermoregulatory behaviour of *G. moholi* that did not find any incidence of heterothermic responses: Dobler (1978) studied temperature regulation and metabolic adjustments in summer-acclimatized *G. moholi*. His work revealed that *G. moholi* has a thermoneutral zone reaching from 28 °C to 35 °C and a MR of 0.71 ml $g^{-1}h^{-1}$. All individuals had been kept in captivity for some years or had even been born in captivity. Knox and Wright (1989) conducted a similar study with captive individuals reporting a minimal MR of 0.7 ml $g^{-1}h^{-1}$ in adult males and 0.9 ml $g^{-1}h^{-1}$ in adult females during the active period of the species. In a study that concentrated only on core T_b, Mzilikazi et al. (2006) investigated the possibility of *G. moholi* employing torpor or hibernation under free-ranging conditions, but did not find any incidence of heterothermy.

Aim of this study

G. moholi is a small, nocturnal primate that lives in dry woodlands of Southern Africa, with a diet mainly restricted to arthropods and exudates from certain species of *Acacia* trees (Bearder and Martin 1980). Its small size, as well as unfavourable conditions in its habitat during the austral winter, makes it a suitable candidate for heterothermy, leading to the intriguing question of whether *G. moholi* indeed never uses torpor or hibernation as found in the study by Mzilikazi et al. (2006).

In the last few years newly developed methods and equipment available for measuring T_b and MR *in situ* under natural conditions led to the discovery of more and more species utilizing heterothermic adaptations. Early studies did for example discover that the grey mouse lemur, *M. murinus,* enters daily torpor, but the use of hibernation in this species was only found in later studies in the field (Schmid and Ganzhorn 2009). The lack of occurrence of torpor in older studies does therefore not necessarily exclude the possibility that a species could use heterothermy under different conditions, e.g. in other years, or in other populations. Recent studies have also shown that physiological traits are not as fixed as previously thought and physiological parameters can be highly variable, differing not only between closely related species, but also between different populations of a single species, between animals within a population (e.g. between sexes) or even within an individual under different conditions (reviewed in Dausmann 2008).

On the other hand, the surprising result of the absence of heterothermy in *G. moholi* in previous studies could indicate that this species indeed never uses heterothermy. This would either mean that galagos never possessed this trait or that it was secondarily lost in *G. moholi*, raising the question of how *G. moholi* manages to survive the cool period of food scarcity. Nevertheless, *G. moholi* would be expected to show adjustments (e.g. nutritional, metabolic, and behavioural) to cope with its highly seasonal habitat. As these adaptations remain elusive, this project addressed the following questions on how *G. moholi* (as an example of a small Afrotropical mammal) manages to cope with the pronounced seasonality of its habitat:

Energy expenditure (MR) and thermoregulation (T_b):

Does *G. moholi* reduce T_b, BMR and resting MR during the austral winter, reducing energy expenditure to deal with low food abundance and a cold climate?

Capacity of NST:

Does *G. moholi* show changes in NST between seasons?

Diet (food analyses):

Are there differences in the diet (e.g. composition, contents and energy value) between different seasons that allow *G. moholi* to meet its energy demands during periods of food limitation?

Behaviour (telemetry):

Does *G. moholi* show behavioural adjustments in addition to or alternatively to metabolic adjustments? As a strategy to save energy, do they show different group composition during the cold season, choose sleeping sites with different insulation parameters, or change their ranging patterns?

Material & Methods

STUDY AREA & STUDY PERIOD

The field work of this study was conducted at the Nylsvley Nature Reserve (Limpopo, South Africa, 24° 38.802'S 28° 40.095'E), which is a semi-arid, mixed bushveld habitat. The hot, wet season lasts from October to March, and the cool, dry season from April to September. Unless otherwise stated the following meteorological definitions of season are used throughout this work: summer (December-February), autumn transition (March-May), winter (June-August) and spring transition (September-November). All fieldwork was done between March 2009 and August 2011. During this time ambient conditions were measured using humidity and temperature loggers (Hygrochron IButton/DS1923, Dallas Semiconductor, USA) every 30 min (resolution temperature: 0.0625 °C, resolution humidity: 0.04 %). Temperature data (only minimum and maximum) for time spans between field seasons as well as data on rainfall were provided by the local weather station at Nylsvley Nature Reserve. Sunset and sunrise data were taken from http://www.timeanddate.com/worldclock/sunrise.htm (01.06.11; location Johannesburg). In the three years of the study the annual rainfall occurred predominantly during the hot, wet season and varied between 788 and 1057 mm. Coetzee et al. (1976) reported a mean annual precipitation of 630 mm. The monthly mean of daily minimum temperature within the study period ranged from –1 °C to 5 °C (winter) and from 15 °C to 17 °C (summer, Fig. 1). Overall minimum daily temperatures were measured in June or July and dropped as low as -5 °C.
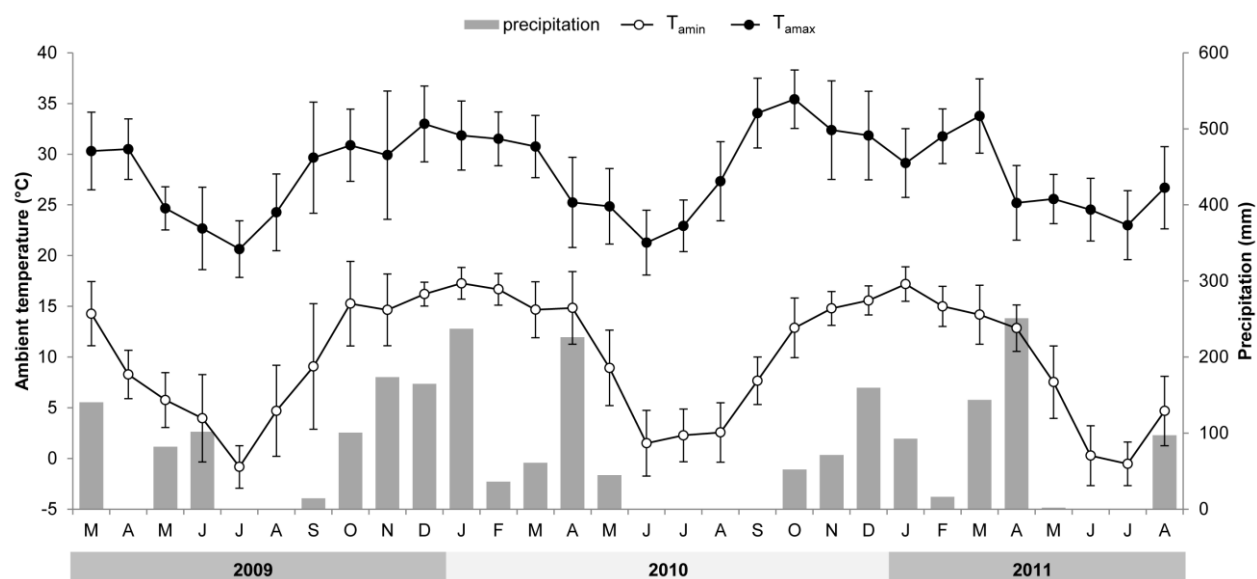

Figure 1: Ambient temperatures during the study period between March 2009 and August 2011. *Black dots* indicate monthly mean maximum temperature (T_{amax}), *open dots* show monthly mean minimum temperature (T_{amin}). *Grey bars* show monthly sum of precipitation.

Nylsvley Nature Reserve has been a proclaimed Ramsar site since 1998 and therefore classified as a wetland area of international importance. The Nylsvley Nature Reserve covers an area of 3975 ha, and includes a part of the Nyl river floodplain. The Nyl river floodplain is the largest inland wetland in South Africa (Noble and Hemens 1978) at 70 km in length, 7 km at widest and, when wholly inundated, about 16000 ha in extent (www.nylsvley.co.za/; 03.09.2012). The area is characterized by infrequent flooding events that usually occur in December and January as a response to summer rainfall and are highly variable in extent, depth and duration. The floodplain and surrounding wetland usually dry out completely by early winter (reviewed in Tooth et al. 2002).

<div align="center">STUDY ANIMAL</div>

The African lesser bushbaby, *G. moholi*, is a small (~200 g), nocturnal primate with a wide distribution on the African continent, ranging from northern Namibia and Angola, eastwards through south-eastern Democratic Republic of the Congo, Zambia, Zimbabwe and northern Botswana to western Tanzania, Malawi, eastern Mozambique and the northern and north-eastern parts of South Africa (Fig. 2; www.iucnredlist.org/details/8788/0; 04.09.2012).
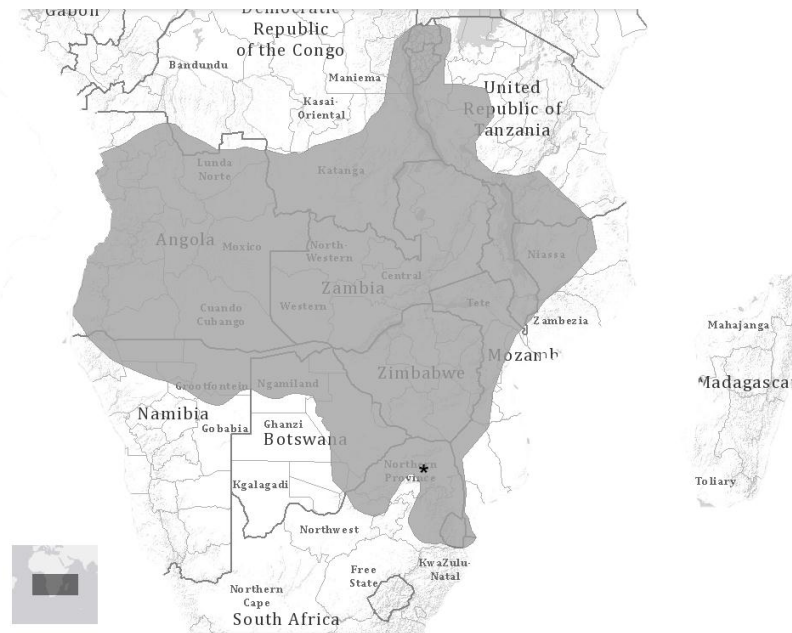
Figure 2: Distribution map of *Galago moholi*.
The *star* marks the study site (Nylsvley Nature Reserve)
in the North-East part of South Africa (Graphic: IUCN red list, modified).

Galagos belong to the primate suborder Strepsirrhini, which includes Asian and African Lorisidae and Galagidae and the Malagasy lemurs (Cheirogaleidae, Lemuridae, Lepilemuridae, Indriidae, Daubentoniidae). The distribution of *G. moholi* is often associated with the occurrence of *Acacia* trees that are often used as nesting sites (Skinner and

Smithers 1990). Its specialised diet consists of arthropods and exudates from certain species of *Acacia* trees, which are available throughout the year (Bearder and Martin 1980). *G. moholi* does not only have one main mating season in May, but also a post-partum mating period between September and October. Females of *G. moholi* give birth to twins once or twice a year (January-February and September-November) after a mean gestation length of 123 days (Pullen et al. 2000). Thus females have to adapt to two different sets of environmental conditions during pregnancy. Most females are pregnant during the cool austral winter when food availability is low and additional energy is needed to maintain a constant T_b, but some females are additionally pregnant during summer (pers. obs. J.N., Pullen et al. 2000).

CAPTURE, HANDLING & HOUSING

Between March 2009 and August 2011 55 animals were captured using self-made walk-in live traps (Fig. 3) and commercial Sherman traps baited with bananas, honey and peanut butter. The traps were set late in the afternoon and checked early in the morning. Animals were classified according to teeth abrasion and reproductive status into one of three age categories: adult (strong abrasion of teeth, testes or nipples visible, males with prominent penis spines), subadult (teeth sharp, testes visible, but no nipples visible in females) or juvenile (babyface, weight <150 g, no testes or nipples visible). Furthermore, animals were sexed, measured, weighed, individually marked with subcutaneously injected passive identification transponders (ID100 Trovan, EURO I.D. Usling GmbH, Weilerswist, Germany) and equipped with collars for temperature measurements.

Figure 3: Self-made walk-in life trap with captured bushbaby (left) and captured bushbaby in capture bag (right).

Some of the animals were briefly anaesthetized with Ketaminhydrochloride (Ketanest® 1 mg/100 g, Parke-Davis, Berlin) for handling. Animals were either released the same day or held in outdoor enclosures for a few days. Outdoor cages (180.5x61.5x193.5 cm) were each equipped with a wooden nest box (0.4 mm plywood, 25x20x20 cm, 10 l), branches and leaves. During the night the animals were provided with water *ad libitum*, banana, gum of *Acacia* trees (if available) and mealworms. All animals were released at the exact capture locations. It was aimed to recapture animals in regular intervals.

METHODOLOGY

The methodology of this study has been diverse, aiming for a comprehensive overview of behavioural and physiological mechanisms of *G. moholi* to cope with food scarcity and low T_as. T_b and oxygen consumption measurements were used to identify general metabolic and possible heterothermic strategies. NST was determined to obtain further information about physiological adaptations. Behavioural observation and tracking of animals were conducted with the aid of radio telemetry to gain information about additional behavioural strategies. Data on food availability and ambient conditions (precipitation, T_a) were obtained simultaneously.

BODY TEMPERATURE MEASUREMENTS

Skin temperature

Skin temperature (T_{skin}) of 31 free-ranging animals was measured using collar temperature loggers (Weetags, 2.6 g; Alpha Mach Inc., Mont St-Hilaire, Canada) and temperature-sensitive collar transmitters (TW-4 button cell tags, 4 g; Biotrack, Wareham, UK). Collar loggers were programmed to measure and store T_{skin} automatically in a defined time interval (see below). For improved precision, calibration curves provided by the manufacturer were used (calibration from 5 °C to 30 °C). Temperature-sensitive collar transmitters vary their pulse rate according to temperature and were calibrated in a water bath using a digital thermometer as a standard (accuracy ± 0.4 °C; testo 700, Lenzkirch, Germany). The temperature range for calibration was chosen due to the expected T_b of the animals (4 °C to 40 °C, increments of 4 °C). Transmitter signals were detected using a TR-4 receiver (Telonics, Inc., Mesa, Arizona, USA) with a flexible two-element yagi antenna. Data from transmitter-collared animals were collected as and whenever possible. Radio-collared animals were checked for torpor occurrence in the early morning and T_{skin} was recorded every 15 min for the duration of a torpor bout. As *G. moholi* is a small animal that shows the typical curled-up body posture during the resting phases, with a collar logger or transmitter positioned inside and firmly pressed against the ventral surface, T_{skin} during sleeping phases is a reasonable approximation of core T_b (Dausmann 2005). The T_{skin} data for *G. moholi* were

found to be a good proxy for T_b when the animals were curled up in a sleeping position (as during a torpor bout). An exemplary calculation of the mean normothermic T_{skin} of curled up animals during rest revealed a mean T_{skin} 35.3 °C (N= 18) and was therefore well within the range of mean core T_b reported in literature (34.8 to 38.6 °C at daytime; Mzilikazi et al. 2006). However, during activity the temperature measurement was not as accurate and T_{skin} data of active animals have not been considered for further analyses. To differentiate between these artificial fluctuations and true decreases of T_{skin} due to the initiation of torpor bouts, animals were defined to have entered torpor when T_{skin} showed a distinct and stable decrease (<30 °C) for longer time periods (>2 hours). Termination of a torpor bout was defined as the moment T_{skin} increased above 30 °C. This cut-off point between normothermy and torpor was chosen since Mzilikazi et al. (2006) never measured body core temperature (T_{bcore}) below 33 °C.

Settings of collar loggers:
- *Laboratory*

  During all measurements of MR T_{skin} was measured simultaneously with collar loggers which were programmed to log between once a minute and every 10 min (resolution: 0.0625 °C).

- *Free-ranging*

  Sample frequency of collar loggers used on free-ranging individuals was programmed to log once an hour for a period of 85 days (resolution: 0.0625 °C).

Core body temperature

The use of collar loggers and transmitters avoids the need for surgical implantations, but does have drawbacks. In small animals, the high thermal conductivity and small distance between the surface and the core precludes the establishment of a steep and variable temperature gradient between the skin and the body core. In larger animals, these distances can be substantial and the resulting temperature gradient can therefore be considerable, limiting the use of external collars. As stated above our data were a reasonable approximation when the animals were curled up in a sleeping position. However, the collars are of limited use when the animals are active as they do not stay fixed on the animal´s skin and thus give inaccurate measurements. Therefore, implanted temperature loggers (thermochron iButtons, Maxim, USA) in the peritoneal cavity of the animals were used to additionally gain data on T_{bcore}. Temperature loggers (iButton, resolution: 0.5 °C) were programmed to measure and store T_{bcore} automatically every thirty minutes, so that these

data loggers could log T_{skin} for about five and a half months. For improved precision, temperature loggers were calibrated in a water bath using a digital thermometer as a standard (accuracy ± 0.4 °C; testo 700, Lenzkirch, Germany). The temperature range for calibration was chosen due to the expected T_b of the animals (4 °C to 40 °C, increments of 4 °C). All surgical processes were done under inhalation anesthesia (isoflorane in oxygen; induction and maintenance, 2 % flow rate, ca. 0.5 l min^{-1}). Animals typically recovered within 24 h of initial surgery and were closely monitored during this time. No pregnant females and no juvenile individuals under a body mass of 100 g were used for implantations. Animals were used for implantations only once each. Implantations were done in three adult and three subadult individuals (5 males, 1 female) and data loggers of three adult individuals were retrieved.

METABOLIC MEASUREMENTS

Energy expenditure was determined by measuring the rate of VO_2 with open flow-through respirometry using a portable oxygen analyser (FoxBoxC, Sable Systems International, USA). The metabolic chamber was connected to the oxygen analyser with airtight tubes (pull mode). Flow rates were adjusted to maintain <1 % oxygen depletion between incurrent and excurrent air. CO_2 and water vapour were scrubbed prior to analysis of VO_2 using soda lime and silica gel. Sample air was measured alternating with reference air (baseline) to account for any drift of the oxygen sensor (sampling frequency every 10 sec). MR was calculated using the data acquisition program Expedata (Sable Systems International, USA) and the equation of Withers (1977).

For each sampling period, the mean value of MR was calculated from all values which represented the most stable readings in the cycle (33 % in laboratory measurements with smaller metabolic chamber; 90 % in enclosure measurements and during measurements of NST). Resting MR and torpid MR were calculated as mean values from the three lowest consecutive values under rest or torpid conditions. Active MR was correspondingly calculated as mean values from the three highest consecutive values (assuming that they reflect the animal at activity).

Animals were placed in the metabolic chamber in the morning of the capture day and were left undisturbed for at least one hour before the beginning of the measurements.

Metabolic measurements in the laboratory

Captured animals were placed in a plastic metabolic chamber (30x15x18 cm, 8 l) for 24 h in a laboratory at the study site. The animals did not have any access to food or water during the measurements. 14 animals were measured (9 females, 4 males, 1 uncertain sex) during the cold, dry period and 18 (6 females, 12 males) animals during the hot, wet period. To reflect prevailing T_a at the study site, windows were kept open in the laboratory during measurements. The flow rate during measurements varied between 900 ml min^{-1} and 1400 ml min^{-1}.

Metabolic measurements in the enclosure (semi-natural conditions)

MR of animals kept in outdoor enclosures (180.5x61.5x193.5 cm) was measured for one to four consecutive days, using a nest box (0.4 mm plywood, 25x20x20 cm, 10 l) as the metabolic chamber. For housing details see paragraph "Capture, handling & housing". 22 animals (11 females, 11 males) were measured during the cold, dry period and ten (6 males, 4 females) animals within the hot, wet period. The flow rate was set to 1200 ml min^{-1}.

Non-shivering thermogenesis

The NST capacity of *G. moholi* was obtained through measurement of the increase in MR following noradrenaline (NA) injection (Jansky 1973, Wunder and Gettinger 1996). The reactions to NA injection in seven summer acclimatized and ten winter acclimatized male individuals (>160 g) captured during summer 2010/11 and winter 2011 were measured for seasonal comparison. No female individuals were used in experiments due to high numbers of pregnant individuals throughout the year. Captured animals were held in outside enclosures (under natural climatic condition and photoperiod) for a few days (<1 week; see "Capture, handling & housing"). All measurements were conducted in a temporary laboratory.

Animals were individually placed in the 8 l respirometry chamber located in a portable thermal cabinet (35x66x44 cm) with a transparent section in the lid for behavioural observations. T_a inside the thermal cabinet was maintained using a self-built water bath, driven by an aquarium heater, pump and processor fan (Fig. 4). The water was pumped from the water bath through copper pipes inside the chamber, thereby ensuring that T_a during measurements was within the range of the thermoneutral zone of the species (28-35 °C, Dobler 1978) to guarantee basal energy expenditure. T_a inside the thermal cabinet was monitored with a thermometer inserted into the box and a temperature logger (iButton). Due to high weight loss in captivity (~10 % in 4 days), no dose response curve was performed to calculate the optimal dose of NA. Instead 0.5 mg kg^{-1} NA was used.
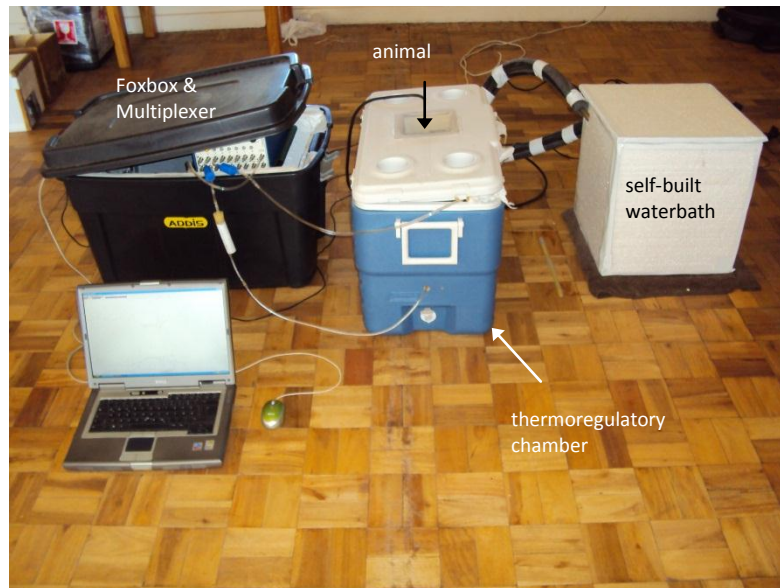
Figure 4: Setup of thermoregulatory chamber for determination of non-shivering thermogenesis. The temperature inside the thermal cabinet was maintained using a self-built water bath, driven by an aquarium heater, pump and processor fan. The water was pumped from the water bath through copper pipes inside the chamber.

Energy expenditure of individuals was determined by measuring the VO_2 with open flow-through respirometry using a portable oxygen analyzer (detailed description of metabolic measurements above). On each measurement day food was removed from the animal cages at least 3 h prior to commencement of data measurement to ensure that the animals were post-absorptive. All measurements were conducted during the rest-phase during daytime between 10am and 3pm. VO_2 was measured for 2 h before NA or saline solution (0.9 % NaCl; control) was injected subcutaneously. VO_2 measurements were continued for 2 h after each NA injection and for 1 h after each saline injection. Each animal was measured with NA and saline solution. Both experimental procedures were conducted on consecutive days.

BEHAVIOUR AND FEEDING ECOLOGY

Focal animal observations were performed at night with the aid of the thermosensitive radio transmitters and telemetry equipment. The data obtained in this way were used to quantify activity budgets, as well as to determine whether home ranges, feeding behaviour, social behaviour and/or group composition change between seasons.

During focal animal observations the exact position of the animals was recorded every 15 min using a hand-held GPS device (Garmin, etrexH, Garmin Ltd., Europe). Animals were never out of visual range for more than 10 min (instantaneous sampling, Altmann 1974) and distance to focal animals could always be reduced to about 1 m without obvious changes in behaviour (e.g. alertness, escape). Seven animals (3 females, 4 males) were equipped with collar transmitters during winter (July 2009 and August 2010), and each was followed for two nights respectively, beginning with emergence from its resting site until return to a resting site. During summer, six different animals (2 females, 4 males; February 2010/2011, November 2010) were observed. In total, individuals were followed for about 136 h during winter and 109 h during summer. Data from different years were pooled for each season. Data analysis on home range size was done with the Animal Movement Software for ArcView GIS 3.2a (ESRI) using the Minimum Convex Polygon method.

Radio-collared animals were further located during daytime to characterize resting site use. Seven individuals were regularly tracked during winter 2009 and 2010 and six individuals during summer 2009/10 and 2010/11. During transition times seven individuals (March - May 2010/2011) and four individuals (September - November 2010) were tracked and their resting sites documented.

Food availability

To determine the temporal changes in food accessibility in the study area the seasonal changes in the two food sources of *G. moholi*, namely small arthropods and *Acacia* gum, were examined between June 2010 and May 2011.

Invertebrate Sampling

As *G. moholi* has been primarily observed catching flying insects, but has also been seen eating invertebrates crawling on the ground surface on some occasions, seasonal availability of arthropods were determined for

a) active arthropods on the ground surface and

b) active insects flying between 1 m and 2 m above the ground.

Abundance of crawling arthropods was determined using pitfall traps (50 ml, diameter 3 mm), a standard method for trapping epigaeic invertebrates (e.g. Kotze and Samways 1999, Mzilikazi and Lovegrove 2004). A total of 32 pitfall traps, filled with 70 % alcohol mixed with glycerol, were placed in two sites within the study area (16 traps/site were installed one meter apart in a 16 m^2 grid). The pitfall traps were checked at 2-week intervals due to high rates of destruction by animals.

Abundance of flying insects was determined with the aid of an insect light (Wasco, insect killer, 1.1 cm gap size), which attracts the insects by emitting blue light and kills them on contact by a high voltage cord. Dead insects can later be removed directly from the cords. This standard method is suitable for quantifying gross differences in insect abundances between seasons (e.g. Black 1974, Churchill 1994). The light was placed on a tree within the study area at about 1.5 m from the ground for one night each week.

Availability and chemical analyses of gum of Acacia karroo

The studied populations of *G. moholi* have been primarily seen feeding on gum of *A. karroo*, therefore, the gum production of 98 *A. karroo* trees within the home range of the studied animals was examined. Trees were checked monthly for gum availability up to a height of 2 m. Additional characteristics as gum color (light/dark) and softness were documented (Fig. 5).

Figure 5: *Acacia karroo* with light (left) and dark gum (right).

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Between June 2009 and April 2011 39 gum samples were collected from *A. karroo* within the study site. Chemical analyses of gum samples were examined by a bachelor student (Marta Wippich) at the laboratory of the University of Hamburg. Energy content was analysed with an oxygen bomb calorimeter (6100 Parr Instrument GmbH, Frankfurt, Germany). 0.2 g of each gum sample were pressed into a tablet and analysed with a semi-micro oxygen bomb. Additional concentrations of soluble sugars were determined as the equivalent of galactose after acid hydrolization of the 50 % methanol extract (Kates 1972).

STATISTICAL ANALYSES

Statistical analyses were performed with SPSS (PASW Statistics 18, 2009). All values are means ± SD. All performed analyses were non-parametrical tests.

Results and Discussion

TORPOR IN A MAINLAND PRIMATE

The results of this study show that *G. moholi* does have the ability to employ torpor and therefore present the first confirmation of heterothermy in a primate outside Madagascar. Torpor was observed in all three study years, but only during the cold, dry season from April to the end of August. Only 27 % of all individuals examined during the cold, dry period underwent daily torpor: six individuals after food and water deprivation in the laboratory and three individuals showed repeated torpid states within a close time frame (five to eleven days) under free-ranging conditions. This obvious reluctance of *G. moholi* to undergo torpor suggests that, in contrast to its close relatives the Malagasy lemurs, torpor in *G. moholi* is utilized as a strategy in response to especially adverse conditions and not as a routine strategy to save energy during unfavourable seasons.

TORPOR PATTERN DOES NOT VARY IN COMPARISON TO OTHER DAILY HETEROTHERMS

Interestingly, the torpor parameters of *G. moholi* did not vary markedly from the parameters described for other nocturnal daily heterotherms. Torpor entry occurred in the second half of the night or during the early morning hours, as summarized for nocturnal daily heterotherms by Geiser (2004). Animals had torpor bout durations between two and eleven hours, arousing from torpid states around midday, which is also consistent with data from other nocturnal daily heterotherms (Geiser 2004). Whenever possible exogenous passive heating was exploited by all individuals before active rewarming commenced. This two-phase rewarming process is known from tropical hibernators and is assumed to lead to marked energy savings (reviewed in Geiser et al. 2004). However, average minimal T_{skin} of torpid *G. moholi* was higher than expected. Due to elevated T_{skin}s, energy expenditure during torpor was more than twice as high as found for the two closely related cheirogaleid species *M. murinus* and *C. medius*. The ratio of reduction in energy expenditure and T_b did not vary from that found in other daily heterotherms of various species (Fig. 6; Geiser 2004). Taken together these results provide further evidence of heterothermy as a basal trait with general characteristics shared in all daily heterotherms and hibernators.
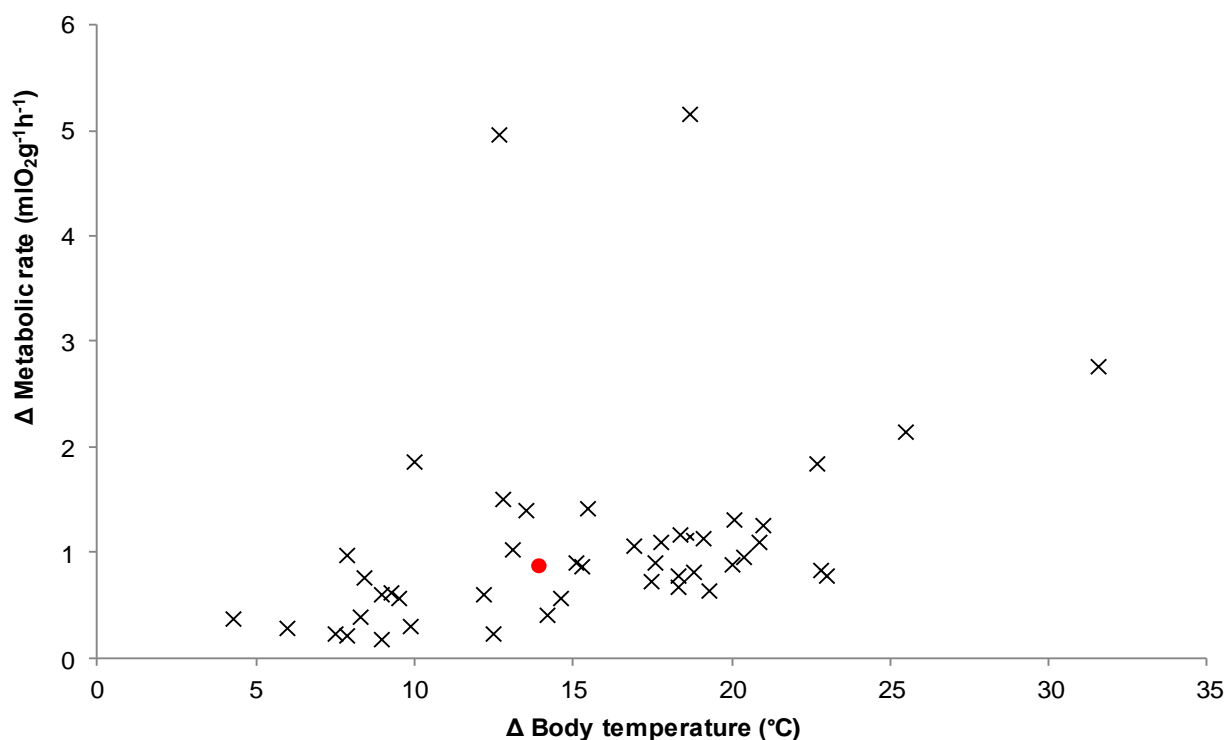
Figure 6: Reduction of metabolic rate as a function of reduction in body temperature during torpor of daily heterotherms. Differences in metabolic rate and body temperature between normothermic and torpid individuals are used according to data reviewed in Geiser (2004). *Black crosses* represent data taken from Geiser (2004); *red dot* represents the mean value for the six individuals of *G. moholi*.

DEPLETION OF ENERGY RESERVES LEADS TO LOW HEATING RATES FROM TORPOR

General torpor characteristics found in *G. moholi* did not vary markedly from those of other daily heterotherms. However, rewarming rates during arousal from torpor bouts observed in this study differed significantly. The average peak rewarming rates were found to be almost 50 % lower than expected for *G. moholi* on basis of body mass (using the equation of Geiser and Baudinette 1990) and individuals with T_{skin}s ≤19 °C had substantial difficulties returning to normothermic values. Nicol and Andersen (2008) suggested that food deprivation depletes energy reserves and leads to reduced heating rates in starved short-beaked echidnas (*Tachyglossus aculeatus)*. Torpor in *G. moholi* was induced in young animals by 24 h-food and water deprivation during the food-scarce period, and individuals lost about 10 g of body mass during measurements (12.3 ± 6.1 g, N= 13; no significant difference between age classes; Kruskal-Wallis-test, N= 4/4/5, X^2= 0.318, df= 2, p= 0.853), suggesting that low rewarming rates could have been indeed due to low energy reserves. Similarly, three free-ranging animals used this strategy on repeated occasions within a close time frame, equally

suggesting a trigger event (e.g. low body condition, illness, or temporarily low energy availability). These incidences suggest torpor as an acute survival strategy in *G. moholi.* Animals obviously enter torpor when they are severely food stressed and torpor can result in life-threatening situations when energy reserves of the individual are low and T_a is not high enough to exploit effective passive rewarming.

REWARMING DIFFICULTIES ARE NOT DUE TO LACK OF NST CAPACITY

The low heating rates of *G. moholi* during arousal from torpor could also be due to a lack of NST capacity, as a study comparing rewarming abilities of a marsupial lacking classical NST and a similar-sized eutherian possessing this mechanism found that rewarming ability was also about 50 % reduced in the species without NST capacity (Nicol et al. 2009). Determination of seasonal NST capacity, however, revealed that *G. moholi* exhibits seasonal changes in NST, indicating a seasonal increase of UCP1-mediated thermogenesis in BAT. *G. moholi* maintains a steady BMR throughout the year and alters NST capacity according to ambient temperature. Cold adaptation through increased BMR would lead to a constant increase in metabolic costs. These costs can be reduced by maintaining a constant low level of BMR and a cold response through rapid modification of NST (Jansky 1973, Wunder 1985). Winter acclimatized *G. moholi* showed a 3.5-fold increase of NST in comparison to BMR and NST capacity was consistent with predicted capacity. However, a difference in summer and winter NST capacities was only found after correction for acclimatization temperatures (removal of days with uncharacteristically low or high temperature) and was, even after correction, with only 25 % increase, relatively low. This indicates a low adaptive increase in NST in response to T_a and is of particular importance as the adaptive increase in NST capacity during cold periods is generally used to differentiate between classical NST and an unspecific increase of VO_2 due to activation of NST-unrelated receptors (Cannon and Nedergaard 2004).

Potential reasons for the comparatively low seasonal difference in NST capacity are varied. The NST capacity during winter corresponds with the predicted value based on body mass and therefore one can assume that the NST capacity in summer is comparably high. *G. moholi* was found to use torpor as an emergency solution in response to acute food shortage only. So far torpid states have only been observed during the cold, dry period, when food availability is naturally low, but it is conceivable that emergency torpor bouts can be entered at any time. As NST is needed for rewarming from torpor (Cannon and Nedergaard 2004, Jefimow et al. 2004) this could explain the elevated NST (and the subsequent low seasonal difference) throughout the year and further explains why *G. moholi* only needs to alter NST capacity in especially cold phases. Alternatively, small seasonal differences in NST

may also be accounted for by low seasonal differences in T_a combined with a high degree of social nesting behavior. Within the three study years ambient conditions changed markedly between seasons within the habitat of *G. moholi*, however, daily maximum temperatures during the winter generally were between 20 °C and 25 °C and a severe decrease of nightly temperatures only occurred during a short period of time during mid-winter. Minimum temperatures were reached during early winter mornings and increased fur insulation and huddling might prevent the need for highly elevated NST.

FOOD ABUNDANCE AND BEHAVIOURAL ADAPTATIONS REDUCE THE NEED FOR HETEROTHERMY

It seems puzzling that a small species as *G. moholi,* living in a challenging and seasonal habitat with food scarce periods only uses heterothermy rarely, leading to the question of how *G. moholi* copes with the unfavourable season, instead. An important factor influencing thermal patterns of mammals is diet. Within the Malagasy Cheirogaleidae, the only genus without any evidence of heterothermy is *Phaner*. These lemurs have the peculiarity of feeding almost exclusively on tree exudates. It might therefore be plausible, that this diet does not necessitate (e.g. feeding places can be self-sustained and are therefore independent of the season) or does not allow for the use of torpor (Dausmann 2008). An argument against the latter is that mouse lemurs are also known to feed on gum during the cold winter and can be regularly found to utilise heterothermic adaptations (e.g. *M. griseorufus*; Génin 2008). It might, however, well be possible that a gum diet generally does not necessitate for the use of heterothermy. The diet of *G. moholi* is mainly restricted to arthropods and exudates from certain species of *Acacia* trees (Bearder and Martin 1980) and the amount of gum feeding is significantly increased during winter. Arthropods are on average twice as high in energy content than gum (Wippich 2012), but are subject to higher variations in seasonal abundance as tree exudates. Analyses of the energy content of gum sampled in the study area showed that energy content varied with color of gum. Light gum had a significantly higher energy content than dark gum. Seasonal comparison further revealed a winter increase in energy content of dark gum (light gum was not tested for all four seasons due to low sample size), making this resource even more attractive during time of food scarcity. Water availability is low during winter and trees presumably reduce water content of gum, resulting in a higher concentration of soluble particles. Additionally, microbial fermentation in the gastro-intestinal tract of *G. moholi* (Caton et al. 2000) probably even increases the energy content of gum. Even though energy content of the analysed gum was still lower than that of arthropods, the availability of gum with increased energy content in low quantities presumably enables galagos to stay normothermic throughout the winter and to

focus on reproduction activities. Indeed, telemetric surveys of certain individuals during summer and winter season showed a shift in feeding behaviour, from small arthropods available throughout the hot, wet period to energy-rich exudates during mid-winter. Nevertheless, *G. moholi* has to cope with the low winter temperatures through behavioural adaptations. Correspondingly, this study further found significant changes in sleeping site use and group composition between the seasons paired with a shift of activity times. Heterothermy may enhance fitness via energy savings but is also thought to result in selective costs. This study suggests that *G. moholi* favours ecological and behavioural adjustments over torpor, because these suffice to meet energy requirements of this species, and the advantages of normothermy outweigh the energetic benefits of heterothermy.

REASON TO STAY NORMOTHERMIC

The advantages associated with the use of torpor, mainly the conservation of energy and water, are well understood (Heldmaier et al. 2004), whereas the potential ecological and evolutionary forces constraining the use of torpor remain unclear. In *G. moholi* the avoidance to enter torpor may be associated with its breeding pattern (Mzilikazi et al. 2006). *G. moholi* has a markedly longer breeding period than most Malagasy lemurs that are comparable in size and known to undergo torpor or hibernation, but lives in a less seasonal habitat and can successfully raise young throughout the year and thus reproduce more often. *G. moholi* give birth to twins once or twice a year shortly before and shortly after winter time. One can assume that the physiological potential of entering energy-saving heterothermic states is only tapped by individuals that are unable to participate in reproduction at that point, e.g. young males without a territory. Females can already become pregnant at the age of six months, and due to their long gestation period, they are reproductive active most of the year. Since both reproduction and lactation are seriously impaired by heterothermy (e.g. Wilde et al. 1999, Farmer 2000), this would explain why torpid females are rarely encountered. Arrest of foetal development would conflict with two mating periods a year: a shift of the first birth season would cause a shift of the second mating season to later in the year, which may be too late for the second litter to develop enough before the next winter to survive the cold and food restricted period. Adult males, on the other hand, presumably cannot afford to spend time in a "suspended" state at any time of the year, neglecting territory defence. As territoriality is known to be correlated with high testosterone levels in rodents (e.g. Darrow et al. 1988) it is also likely that torpor use during winter is precluded by a high testosterone concentration in the blood of the males, as shown in rodents (e.g. Mzilikazi and Lovegrove 2002). Furthermore, there is evidence that low T_bs during heterothermy impair the maturation of testes (e.g. Barnes et al. 1986). Fietz et al. (2004) found that males of the edible

dormouse (*Glis glis*) that were sexually active during summer stayed normothermic during most of the previous winter. Sexual quiescent males, on the other hand, were seldom found with T_b above 30 °C during winter. Behavioural observations have indeed shown that male *G. moholi* have overlapping home ranges that are defended throughout the night during winter and testes were still prominent during winter, a time where heterothermic Malagasy lemurs show a pronounced reduction of testes size (*C. medius*: Fietz and Dausmann 2003, *M. ravelobensis* and *M. rufus*: Randrianambinina et al. 2003).

IMPLICATIONS FOR THE RAFTING THEORY

The lack of heterothermy in mainland relatives of the lemuriformes has long been mentioned as a main argument against primate colonization of Madagascar by means of rafting vegetation (e.g. Stankiewicz et al. 2006, Masters et al. 2007). The ancestor(s) of today's lemurs would not have survived a journey of several weeks without adaptations enabling survival when no or only limited food and fresh water was accessible. The finding of heterothermy in a mainland primate sheds new light on the rafting theory. The results of this study clearly show that at least one species of the lorisiformes shares the heterothermic trait with the Malagasy lemuriformes and also imply that torpor or heterothermy might be found in other species of galagos or lorises. The finding of torpor in *G. moholi* does further support the rafting theory, but there are still various counter arguments and puzzle pieces that do not fit within this theory. To my knowledge, there is no evidence of any species that successfully colonized an island through rafting, even though, oceanic dispersal is assumed for a number of taxa, including platyrrhine monkeys in South America (Schrago and Russo 2003), carnivores on Madagascar (Yoder et al. 2003) and a number of amphibians (e.g. Censky et al. 1998, Measey et al. 2007).

A further argument against the rafting theory was presented by Masters et al. (2007), who argued that the estimated size of the lemuriformes and lemuroid ancestor (~2 kg) is well outside the range of most mammals employing heterothermy. Heterothermy is indeed mostly employed by small sized mammals, but examples, as hibernation in the black bear (*U. americanus,* females ~80 kg, males ~120 kg; Tøien et al. 2011), the American badger (*Taxidea taxus,* ~9 kg; Harlow 1981), the short-beaked echidna (*T. aculeatus,* ~28 kg; Grigg et al. 1989) or various marmot species (2.5 – 4 kg; reviewed in Geiser 2004), show that high body mass does not necessarily exclude this possibility.

In 2001 Marivaux et al. reported the finding of the primate fossil *Bugtilemur mathesonigen* in India and assigned this fossil to the Malagasy Cheirogaleidae. The affiliation of this fossil to the Malagasy lemurs led to new questions rather than new insights and is not generally accepted (e.g. Godinot 2006). If this fossil indeed belongs to the

Malagasy lemurs, this finding could indicate a colonization of Madagascar from India or, given that Yoder et al. (1996) found evidence of an African origin of Malagasy lemurs, a second rafting event from Madagascar to India. The timing of the origin of primates and strepsirrhines is still under discussion and was only recently suggested to have occurred much earlier than previously thought (Martin 2000). It is imaginable that strepsirrhine primates originated even before Madagascar split from India and such a scenario could well explain the fossil record. At this point in time, however, the rafting theory is the most likely explanation for the lemur invasion in Madagascar and only further paleontological evidence will be able to solve the mystery of Madagascar's colonization.

Conclusion

The results of this study present the first confirmation of heterothermy in *G. moholi*. Our finding is yet another example of the potential flexibility of physiological parameters within animal populations, as earlier studies on the physiological strategies of *G. moholi* did not find any evidence of torpid states (Dobler 1978, Knox and Wright 1989, Mzilikazi et al. 2006). Earlier studies on Malagasy lemurs suggested that physiological traits are not as fixed as previously thought and physiological parameters can be highly variable. Those studies have found differences in physiology between closely related species, between different populations of one species, within populations (e.g. between sexes), and even for one individual under different conditions (Dausmann 2008, Kobbe and Dausmann 2009, Lahann and Dausmann 2011).

Up to now, heterothermy within primates was thought to be restricted to Malagasy lemurs (Ortmann et al. 1997, Schmid 2000, Schmid et al. 2000, Dausmann et al. 2004, Lahann 2007, Dausmann 2008, Kobbe and Dausmann 2009, Blanco and Rahalinarivo 2010, Lahann and Dausmann 2011), raising the question if this trait may have evolved in this group to cope with the especially challenging and unpredictable habitat conditions of Madagascar (Dewar and Richard 2007) or if it is a plesiomorphic character that might have enabled mainland strepsirrhines to colonize Madagascar (Kappeler 2000). The results of this study suggest that the heterothermic pattern shown by *G. moholi* does not vary markedly in comparison to other heterotherms in general or to Malagasy lemurs in particular. As it is unlikely that this trait evolved more than once in the evolution of strepsirrhine primates, this suggests that heterothermy may have at least evolved in the primates before the division of African and Malagasy strepsirrhines. This also has the possible implication that more primate species than previously thought have the potential to use heterothermy to reduce energy and water expenditure. Together with the widespread distribution of heterothermy within the mammals this finding further supports the idea that adaptive heterothermy might even be a plesiomorphic characteristic shared in all mammal species (e.g. Lovegrove 2012a).

The reluctance of *G. moholi* to enter torpid states suggests that the underlying trigger of heterothermy might vary from that in, for example Malagasy Cheirogaleidae. This species undergoes torpor as a preventive strategy to save energy during the cold, dry and food scarce period and all individuals of a population have been found to undergo a certain heterothermic strategy during times (e.g. Dausmann et al. 2005, Kobbe et al. 2011). In contrast, the torpor use of *G. moholi* suggests that this strategy is only used under especially

challenging conditions as an emergency strategy, when all other strategies to facilitate survival have failed.

Even though researchers gained various new insights regarding species using heterothermy over recent decades, the evolution of heterothermy (single or separate evolution in mammalian history) or the question of how evolution acted on the manifestation of heterothermy in the different species still remains unclear and will need to be the focus of future research.

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PUBLICATIONS
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MANUSCRIPTS

Torpor on Demand: Heterothermy in the Non-Lemur Primate *Galago moholi*

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Abstract

Background: Hibernation and daily torpor are energy- and water-saving adaptations employed to survive unfavourable periods mostly in temperate and arctic environments, but also in tropical and arid climates. Heterothermy has been found in a number of mammalian orders, but within the primates so far it seems to be restricted to one family of Malagasy lemurs. As currently there is no evidence of heterothermy of a primate outside of Madagascar, the aim of our study was to investigate whether small primates from mainland Africa are indeed always homeothermic despite pronounced seasonal changes in weather and food availability.

Methodology/Principal Findings: One of the nearest relatives of Malagasy lemurs, the African lesser bushbaby, *Galago moholi*, which inhabits a highly seasonal habitat with a hot wet-season and a cold dry-season with lower food abundance, was investigated to determine whether it is capable of heterothermy. We measured skin temperature of free-ranging individuals throughout the cool dry season using temperature-sensitive collars as well as metabolic rate in captured individuals. Torpor was employed by 15% of 20 animals. Only one of these animals displayed heterothermy in response to natural availability of food and water, whereas the other animals became torpid without access to food and water.

Conclusions/Significance: Our results show that *G. moholi* are physiologically capable of employing torpor. However they do not use it as a routine behaviour, but only under adverse conditions. This reluctance is presumably a result of conflicting selective pressures for energy savings versus other ecological and evolutionary forces, such as reproduction or territory defence. Our results support the view that heterothermy in primates evolved before the division of African and Malagasy Strepsirhini, with the possible implication that more primate species than previously thought might still have the potential to call upon this possibility, if the situation necessitates it.

Introduction

Hibernation and daily torpor are traditionally seen as energy-saving adaptations to survive unfavourable periods in temperate and arctic climates. However, heterothermy is also known to occur in mammals living in tropical and arid habitats and has been observed in Australasian, Neotropical and Afrotropical animals [1,2,3,4,5,6,7,8,9,10,11,12]. Heterothermy has been found in a number of mammalian orders. Whereas in some groups a large number of species show heterothermy [13], within the primates it seems to be restricted to one family of small (30–500 g), nocturnal Malagasy lemurs, the Cheirogaleidae (incidence is confirmed in *Microcebus murinus*, *M. berthae*, *M. ravelobensis*, *M. rufus*, *Mirza coquereli* and *Cheirogaleus medius*, and suspected in most others) [4,5,7,8,11,14]. Since the Cheirogaleidae are the most primitive of the lemur families, and heterothermy is so widespread within this family, it seems likely that the first primate inhabitants of Madagascar also exhibited this trait. Furthermore the capacity of lemurs to use heterothermy is believed to be a prerequisite for the

successful colonization of Madagascar by lemurs from mainland Africa via rafting [15,16]. Accordingly, torpor should be a plesiomorphic character and heterothermy should also be found in mainland relatives (Lorisiformes: Lorisidae & Galagidae), if it was not secondarily lost. However, there is no published evidence of heterothermy of a primate outside Madagascar.

At present, there are only three thermoregulatory studies on the closest mainland relatives of lemuriforms, which could not confirm the use of heterothermy: Müller et al. (1985) found that slender loris, *Loris tardigradus* (Southeast Asian Lorisidae) cool large parts of the body during cold exposure but still keep the body core at a high temperature under laboratory conditions [17]. A study on captive-bread African lesser bushbabies, *Galago moholi* (Galagidae) by Knox and Wright (1989) found a high degree of homeothermy when the animals were exposed to temperatures ranging from 6°C to 35°C in the laboratory for less than three hours [18]. Similarly Mzilikazi et al. (2006) did not find any incidence of heterothermy in *Galago moholi* in the wild [19]. This is especially surprising, as *Galago moholi* is one of the closest relatives of Malagasy lemurs and

one of the most likely candidates within the primate group to show hypometabolic states. It is a small (~200 g), nocturnal primate, that lives in dry woodlands of South Africa, as well as in the region from Angola to Tanzania in a highly seasonal habitat with a hot wet-season and a cold dry-season with lower food abundance [19]. The results from the study by Mzilikazi et al. (2006) raise the question of how *G. moholi* manages to cope with the high seasonality of its habitat, especially as females of *G. moholi* give birth to twins once or twice a year (January–February and September–November) and gestation mainly takes place during the most energy demanding winter period. However the Mzilikazi at al. (2006) study only measured body temperature (T_b) and no metabolic rate (MR) or other ecological, ethological or physiological parameters were measured to determine possible seasonal adjustments. Recent studies also indicate that physiological traits are not as fixed as previously thought and physiological parameters can be highly variable and can differ not only between closely related species, but also between different populations of one species, within populations (e.g. between sexes), and even for one individual under different conditions [20,21,22].

The aim of our study was to investigate whether *G. moholi* does indeed never employ heterothermy as a strategy to survive unfavourable periods. Our results show that *G. moholi* is physiologically capable of employing torpor, but does use it only under especially adverse conditions.

Results

Ambient conditions

During the study period, climatic conditions were typical for a semi-arid seasonal habitat during the austral winter. Ambient temperature (T_a) was low during the night, but fairly high during the day. The lowest T_a during the study period was $-4.1°C$ in July

(mean: $5.2\pm5°C$) and the warmest day was recorded in March with a maximum temperature of $40.8°C$ (mean: $25.2\pm5.1°C$). The amplitude of daily variations in T_a varied between a minimum value of $2.8°C$ and a maximum value of $29.9°C$ (mean: $20.0\pm4.7°C$). The coldest month was July with the lowest minimum and maximum T_a of the study period. During the whole study period it rained on twelve out of 148 days. The average rainfall was 15.5 mm (total: 201.5 mm). Most rainy days occurred in March; however it also rained on two days during midwinter.

Incidences of heterothermy

Torpor bouts were observed in autumn (April) and in the middle of winter (July). Torpor occurred in one free-ranging animal, as well as in two animals in the laboratory (during metabolic measurement). All three animals went into torpor in the early morning hours (6am–7am) and stayed torpid for four to six hours. Minimal skin temperature (T_{skin}) varied between $21.8°C$ and $25.9°C$ (mean: $24\pm1.7°C$).

Only one animal entered torpor under free ranging conditions multiple times. Torpor in this sub adult (sb) male (165 g) was recorded on six out of nine monitored days under unrestricted free-ranging conditions during the coldest month (between the 4th and 19th of July 2009, after which transmitter signal could no longer be detected). In all cases the animal went into torpor in the early morning hours and was normothermic again between 10am and 12am. The minimum T_{skin} recorded for this animal was $24.2°C$ (T_a $9.2°C$) during a four hour torpor bout (6am until 10am) (fig. 1).

Two out of twelve animals (female, sb; juvenile with uncertain sex) entered torpor during MR measurement in the laboratory, presumably as a reaction to food and water restriction. Both

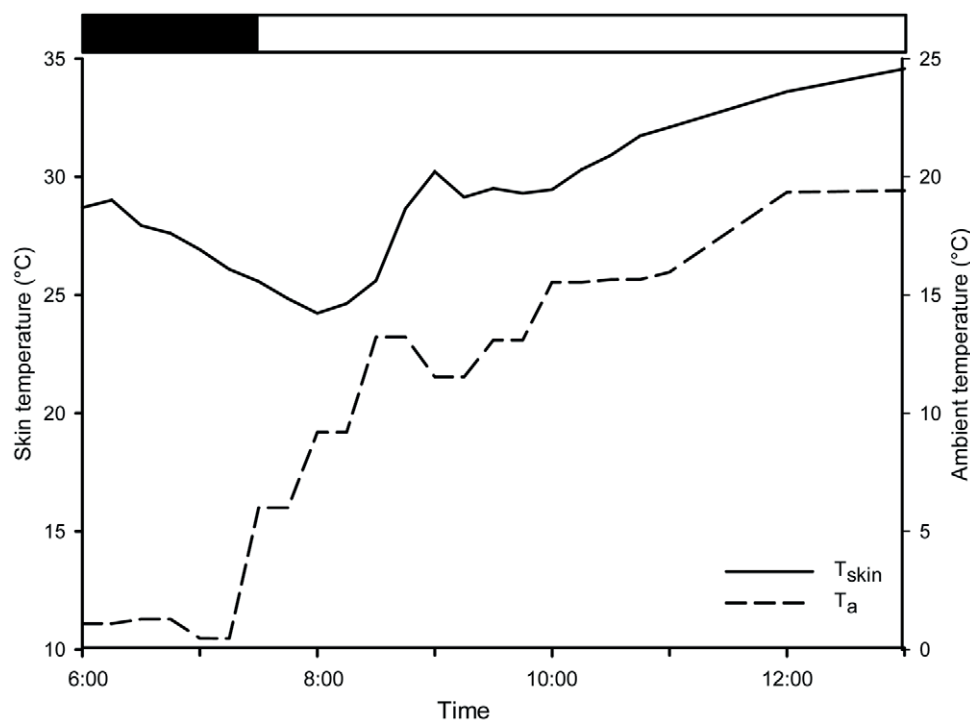**Figure 1. Skin temperature profile of a sub adult male *G. moholi* in winter including a torpor bout.** Skin temperature (T_{skin}) started dropping in the morning at 6am. The male was torpid for about four hours until 10am. *Black line* shows T_{skin}, *dashed line* shows ambient temperature (T_a). *Black bar* indicates the dark phase, *white bar* indicates the daytime.
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incidences occurred between the 12th and 16th of April 2009. The minimum T_{skin} recorded was 21.8°C (female, 180 g) closely reflecting T_a and 25.9°C (juvenile, 95 g). MR started decreasing about 60 min before T_{skin}. For the female, the minimal oxygen consumption ($\dot{V}O_2$) during torpor was only 1/10th (0.09 ml g^{-1} h^{-1}, T_{skin} 22.8±1.8°C, T_a 22.2±0.7°C) of minimal $\dot{V}O_2$ during normothermic resting conditions (0.99 ml g^{-1} h^{-1}, T_{skin} 35.3±0.7°C, T_a 28±0.2°C) (fig. 2). The torpor bout was initiated around 5:30am and was terminated by the animal around 12pm. The energy savings of the juvenile during torpor were not as pronounced, with $\dot{V}O_2$ decreasing down to a value of 0.4 ml g^{-1} h^{-1} (T_{skin} 26.4°C±0.5°C, T_a 23.3±1.5°C), which was a 32% reduction of energy expenditure (1.23 ml g^{-1} h^{-1} under normothermic resting conditions, T_{skin} 34.4±0.3°C, T_a 27.3±0.4°C). This animal was disturbed before it spontaneously terminated its torpor bout after about 4.5 hours at 11pm with a T_{skin} of 27°C.

All other animals remained normothermic during metabolic rate measurements as well as under free-ranging conditions. Mean $\dot{V}O_2$ of normothermic animals (N = 10) under resting conditions in the laboratory was 0.96±0.25 ml g^{-1} h^{-1} (T_{skin} 33.9±1,5°C, T_a 23.6±3.3°C) and 2.79±0.52 ml g^{-1} h^{-1} (T_a 19.4±2.3°C) during the activity phase.

In contrast to the metabolic measurements under laboratory conditions no incidence of torpor was recorded during metabolic measurements inside the enclosure.

Discussion

Our study presents the first confirmation of heterothermy in a primate outside Madagascar, showing that *G. moholi* does indeed have the ability to employ torpor and thereby decrease energy expenditure by up to 90%. In contrast to the Malagasy lemurs where most individuals enter torpor or hibernation at some point during winter only a very small proportion of the Galagos became torpid. In fact, only one animal was torpid under undisturbed, free-ranging conditions. The other animals became torpid in the metabolic chamber, presumably in response to food and water deprivation over a 24-hr period. Evidently, *G. moholi* has the potential to enter torpid states, but probably only does so under especially adverse conditions, so torpor bouts are relatively rare. These results, together with earlier studies on free living *G. moholi* that did not find any incidence of torpor [19], demonstrate the potential flexibility of physiological parameters within one population. Physiological plasticity with regard to thermoregulatory adaptations was found to depend on T_a and body weight which might also be the case for *G. moholi* [4,20,23]. All animals found to use heterothermy in this study were either sub adults or even born within the study year. Therefore, torpor seems to be restricted to or at least more common in younger *G. moholi*. Adult *G. moholi* might either be better adapted to cope with unfavorable conditions such as cold climate and food scarcity (e.g. because of their greater body weight), or they can simply not afford to become torpid, e.g. not to compromise reproduction or territory defense.

The advantages associated with the use of torpor, mainly the conservation of energy and water, are well understood [13] whereas the potential ecological and evolutionary forces constraining the use of torpor remain unclear. In *G. moholi* the widespread torpor avoidance may be associated with its breeding pattern [19]. For females it might be disadvantageous to enter torpor during their first pregnancy in winter because of a delay of foetal development as found in bats [24,25,26]: a shift of the first births would cause a shift of the second mating season to later in the year, which may be too late for the second litter to develop
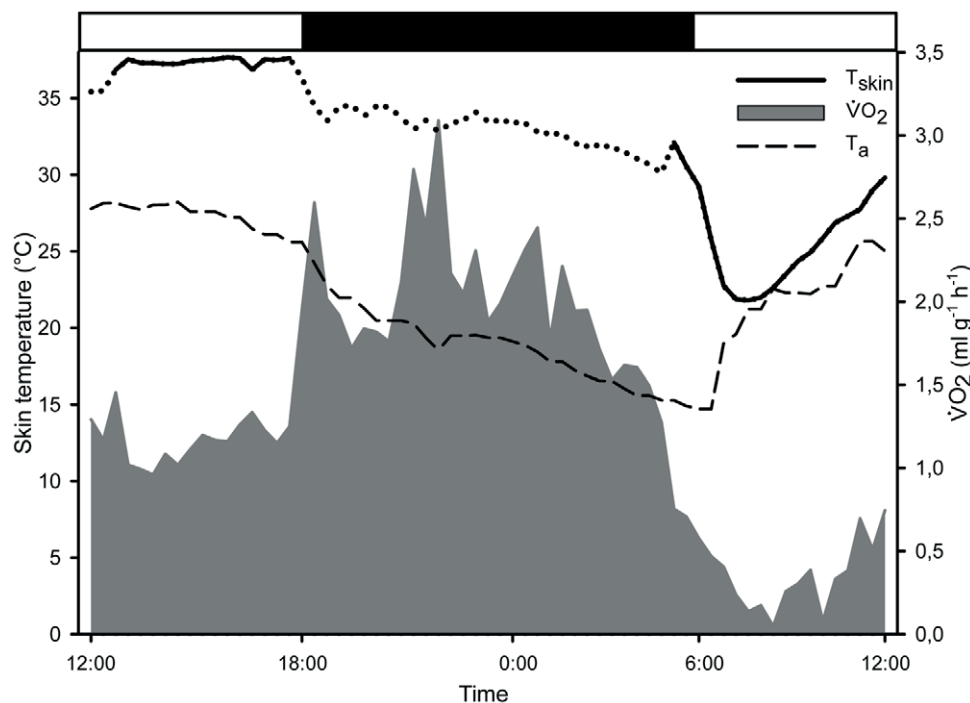
Figure 2. Metabolic rate and skin temperature of a sub adult female *G.moholi* including a torpor bout. Skin temperature (T_{skin}) started dropping below 30°C in the morning at 5:30am; Metabolic rate ($\dot{V}O_2$) started decreasing earlier at 4am. The female was torpid for about six hours. *Grey area* illustrates $\dot{V}O_2$, *black line* shows T_{skin}, *dotted line* indicates the period of artificial variations in T_{skin} measurements due to movements of the animal, *dashed line* shows ambient temperature in the laboratory room (T_a); *black bar* indicates the dark phase, *white bars* indicate the daytime.
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enough before the next winter to survive the cold and food restricted period. We therefore suggest a trade-off situation in the possibility to save energy and water by entering torpor versus the possibility of producing a second litter.

Males, on the other hand, might struggle to defend their territories during winter when undergoing torpor and therefore will have a lower mating success during the subsequent mating season in October. As territoriality is known to be correlated with high testosterone levels it is likely that torpor use during winter is precluded by a high testosterone concentration in the blood of the males. Behavioural observations have indeed shown that male *G. moholi* have overlapping home ranges that are defended throughout the night during winter (pers. obs. JN).

Up to now, heterothermy within primates was thought to be restricted to Malagasy lemurs, [4,5,7,8,20,27] raising the question if this trait may have evolved in this group to cope with the especially challenging and unpredictable habitat conditions of Madagascar [28] or if it is a plesiomorphic character that enabled mainland Strepsirhini to colonize Madagascar [15]. Our study provides the first evidence of heterothermy in a primate outside Madagascar, thus suggesting that this trait may have evolved in this group before the division of African and Malagasy Strepsirhini, with the possible implication that more primate species than previously thought have the potential to use heterothermy to reduce energy and water expenditure. This finding supports the idea that adaptive heterothermy is a plesiomorphic characteristic in mammals and birds [6,29,30,31].

Materials and Methods

Ethics statement

All procedures in this study complied with the "Principles for animal care", publication no. 86–23, revised 1986 (National Institute of Health) and the "Code of ethics for animal experimentation" manual adopted by the Nelson Mandela Metropolitan University (animal ethics clearance no. A09-SCI-ZOO-001) and all experiments comply with the current laws of the country where they were performed. Animals were captured under permit no. CPM-002-00003, issued by the Department of Economic Development, Environment and Tourism.

No injured animals, lactating females or juvenile animals under the weight of 95g were used for metabolic measurements. Animals used in metabolic measurements were monitored at regular intervals to ensure animal welfare. Most animals were released after 24 hours, but no animal was kept longer than 84 hours (metabolic measurements in the enclosure) in captivity.

Study site

The field work described in this study was conducted at the Nylsvley Nature Reserve (South Africa, Limpopo, 24°38.802′ 28°40.095′, altitude: 1100m), which is a semi-arid, mixed bushveld habitat. The hot wet season lasts from October to March, and the cool, dry season from April to September. The reserve receives a mean annual precipitation of 630 mm. Monthly mean winter temperatures range between −0 and 3.5°C [32]. The annual mean temperature is 19°C and monthly mean temperature ranges between −3.2 and 23°C [33].

We captured 23 animals from March to August 2009 using self-made walk-in live traps baited with bananas, honey and peanut butter. The traps were set late in the afternoon and checked early in the morning. Animals were classified in age according to teeth abrasion and reproductive status into one of three categories: adult (ad) (teeth blunt, testes or nipples visible), sb (teeth sharp, testes visible, but no nipples visible in females) or juvenile

(weight <150 g, no testes or nipples visible). Animals were sexed, measured, weighed, individually marked with subcutaneously injected passive identification transponders (ID100 Trovan, EURO I.D. Usling GmbH, Weilerswist, Germany) and equipped with collars for temperature measurements (see below). Some of the animals were briefly anaesthetized with Ketaminhydrochloride (Ketanest® 1 mg/100 g, Parke-Davis, Berlin) for handling. All animals were released at exact capture locations.

Skin temperature

T_{skin} of free-ranging animals was measured using collar temperature loggers (Weetags, 2.6g; Alpha Mach Inc., Mont St-Hilaire, Canada) and temperature-sensitive collar transmitters (TW-4 button cell tags, 4g; Biotrack, Wareham, UK). We equipped 20 of the 23 animals with Weetag loggers (resolution: 0.0625°C) which were programmed to measure and store T_{skin} automatically once an hour, so that these data loggers could log T_{skin} for about three months. We recaptured and obtained data from thirteen animals from March to July 2009 and time spans of data collection varied between ten days and 100 days. For improved precision, calibration curves provided by the manufacturer were used (calibration from 5°C to 30°C). On six animals, we used temperature-sensitive collar transmitters (three of them were fitted with Weetag loggers prior to the collar transmitters). These change their pulse rate according to temperature. The temperature-sensitive transmitters were calibrated in a water bath using a digital thermometer as a standard (testo 700, Lenzkirch, Germany). The temperature range for calibration was chosen due to the expected T_b of the animals (4°C to 40°C, steps of 4°C). Transmitter signals were detected using a TR-4 receiver (Telonics, Inc., Mesa, Arizona, USA) with a flexible two-element yagi antenna. Data from transmitter collared animals were collected as and whenever possible. The use of collar loggers and transmitters avoided implantations. As *G. moholi* is a small animal that shows the typical curled-up body posture during the resting phases, with a collar logger or transmitter positioned inside and firmly pressed against the ventral surface, T_{skin} during sleeping phases is a reasonable approximation of core T_b [32,33,34]. Our data were accurate when the animals were curled up in a sleeping position (as during a torpor bout). The mean normothermic T_{skin} of curled up animals during rest was 35.2 ± 2.3°C (N = 18) and therefore well within the range of core T_b reported in literature (34.8°C to 38.6°C, [19]). However during activity the temperature measurement was not as accurate and T_{skin} data of active animals have not been considered for further analyses. To differentiate between these artificial fluctuations and true decreases of T_{skin} due to the initiation of torpor bouts, animals were defined to have entered torpor when T_{skin} showed a distinct and stable decrease (<30°C) for longer time periods (>2hours) (e.g. see fig. 2). Termination of a torpor bout was defined as the moment T_{skin} increased above 30°C. This cutoff point between normothermy and torpor was chosen, because Mzilikazi et al. [19] never measured core T_b below 33°C.

Metabolic rate

Measurements of MR were conducted in a laboratory as well as within an outside enclosure. In both cases the animals were placed in the enclosure in the morning of the capture day and were left undisturbed for at least one hour before the beginning of the measurements.

Energy expenditure was determined by measuring rate of $\dot{V}O_2$ with open flow-through respirometry using a portable oxygen analyzer (FoxBoxC, Sable Systems International, USA). The metabolic chamber was connected to the FoxBox with airtight

tubes and air which was dried with silica gel prior to analysis, was pumped through the system (pull mode). Flow rates were adjusted so as to maintain <1% oxygen depletion between incurrent and excurrent air (about 500 ml min^{-1} in the enclosure and between 900 ml min^{-1} (rest) and 1400 ml min^{-1} (activity) in the laboratory). CO_2 was scrubbed prior to analysis of $\dot{V}O_2$ using soda lime and silica gel. $\dot{V}O_2$ was calculated using the data acquisition program Expedata (Sable Systems International, USA) and the equation by Withers, 1977 [35]. The use of a multiplexer (TR-RM8, Sable Systems International, USA) allowed for automatic switching between an animal channel (15 min and 30 min in the laboratory and enclosure, respectively) and a reference channel (7 min and 15 min in laboratory and enclosure, respectively). For each 15 min or 30 min measurement, the mean value was calculated from 33% of the values (sampling frequency every 10 sec) which represented the most stable readings in the cycle. $\dot{V}O_2$ values for the resting MR were computed by using the mean of the three lowest consecutive values recorded during the entire rest phase. Similarly, active MR was calculated from the mean of the three highest values recorded during the active phase. The resting phase was defined as the time frame from 6am to 6pm due to behavioural observation (pers. obs. JN).

During all measurements of MR T_{skin} was measured simultaneously with Weetag collars in the laboratory that where programmed to measure T_{skin} every minute, and temperature-sensitive radio collars in the enclosure for real time monitoring. Ambient conditions were measured with the help of humidity and temperature loggers (thermochron iButtons, Maxim, USA) every 30min (resolution temperature: 0.0625°C, resolution humidity: 0.04%).

Metabolic measurements in the laboratory

Measurements of MR in the laboratory were performed from end of March until end of April. To reflect the prevailing temperatures at the study site windows were kept open during measurements. MR was measured from a plastic metabolic chamber (30×15×18 cm, 8 l) for 24 hours in twelve wild *G. moholi* (6 males (4ad, 2sb), 5 females (2ad, 3sb), 1 juvenile animal, sex uncertain). The animals did not have any access to food or water throughout the measurements.

Metabolic measurements in the enclosure

Measurements in the enclosure (180,5×61,5×193,5 cm) were performed from end of April until end of June under natural ambient conditions. MR was measured in nine wild *G. moholi* (4 males (2ad, 2sb), 5 females (2ad, 3sb)) during the resting phases (about 12 hours) of the animals on up to four consecutive days using a wooden nestbox (0.4 mm Plywood, 25×20×20 cm, 10l) as the metabolic chamber. During the night the animals were provided with water *ad libitum*, banana, gum of *Acacia* trees (if available) and mealworms.

Ambient temperature

T_a was recorded within the animals' home ranges of both populations with thermochron humidity and temperature loggers every 30min. Data on rainfall was provided from the local weather station at Nyslvley Nature Reserve.

Author Contributions

Conceived and designed the experiments: JN KHD NM. Performed the experiments: JN. Analyzed the data: JN. Contributed reagents/materials/analysis tools: KHD NM. Wrote the paper: JN KHD NM.

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Surviving the cold, dry period in Africa: behavioural adjustments as an alternative to heterothermy in *Galago moholi*

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Abstract

Behavioural and physiological adaptations are common and successful strategies used by small endothermic species to adjust to unfavourable seasons. Physiological adaptations, such as heterothermy (e.g., torpor), are usually thought to be more effective energy-saving strategies than behavioural adjustments. The African lesser bushbaby, *Galago moholi,* is physiologically capable of torpor but expresses heterothermy only under conditions of extreme energy limitation, suggesting that it has evolved alternative strategies to compensate energetic bottlenecks. We hypothesized that *G. moholi* survives the unfavourable winter period, without -or only rarely- employing torpid phases to save energy, by using behavioural thermoregulation. We compared the ecology and behaviour of *G. moholi* in summer and winter by telemetric tracking and examined food availability by determination of arthropod and gum availability. We found a significant increase in huddling behaviour and a significant increase in the use of enclosed and insulated sleeping sites during winter, as well as a reduction in nightly activity. *G moholi* hunted for insects significantly more in winter than in summer, and increased gum intake in winter, when gum showed an increase in energy content. The availability of high quality food, albeit in low quantities, presumably enables *G. moholi* to stay normothermic throughout the cold, dry period and to focus on reproduction activities. We propose that *G. moholi* favours ecological and behavioural adjustments over torpor because these are sufficient to meet energy requirements of this species, and their advantages (flexibility, unrestricted activity and reproduction) outweigh the energetic benefits of heterothermy.

Introduction

Cold periods pose an energetic challenge to small endotherms, which respond to cold exposure with a variety of behavioural and physiological adaptations. Behavioural thermoregulation strategies include reduced activity (Ostner 2002; Donati et al. 2011; Morland 1993), selection of suitable microhabitats (e.g., well isolated nesting site, Entwistle et al. 1997), changes in body posture (reduction of surface area, Øritsland 1970; Dasilva 1993) and aggregation with conspecifics (Genoud et al. 1997; Perret 1998; Gilbert et al. 2010). Moreover, the intake of energy can also be adjusted, in addition to expenditure. Since thermoregulatory challenges often co-occur with resource scarcity, changes in feeding habits may also be needed. Possible strategies include a change in group size (to decrease food competition or increase food detection probability; Furuichi et al. 2001), longer travel distances in search of food (Harris et al. 2010), and change in diet composition (e.g., switch to low-quality food) that may be accompanied by an increase in feeding time (Hill and Dunbar 2002) during the winter. Behavioural strategies to conserve energy and ecological adaptations can be applied as autonomous strategies to provide winter survival or simultaneously with a reduction in metabolic rate (leading to further reduction of energy costs).

Physiological adaptations such as hibernation or daily torpor allow an animal to reduce its energetic costs through reduction of metabolic rate. These strategies are also very efficient solutions to cope with energetic challenging situations (for review see Geiser 2004a; Heldmaier et al. 2004) and both strategies can occur in mammals inhabiting non-temperate habitats (e.g., Geiser 2004b; McKechnie and Mzilikazi 2011), most notably in the Cheirogaleidae, a family of small, nocturnal lemurs in Madagascar (reviewed in Dausmann 2008). Recently, it has been shown that the closely-related bushbaby, *Galago moholi*, is also physiologically capable of becoming torpid, but uses this ability only under adverse conditions (e.g., when food deprived) and not as a routine or seasonal behaviour (Nowack et al. 2010). *G. moholi* is a small (~200 g), nocturnal primate, that lives in dry woodlands of Southern Africa, with a diet mainly restricted to arthropods and exudates from certain species of *Acacia* trees (Bearder and Martin 1980). Its small size, as well as unfavourable conditions in its habitat during the austral winter, makes it a suitable candidate for heterothermy. It therefore seems puzzling that a fairly small animal species, which is physiologically capable of entering heterothermic states, and lives in a seasonal habitat with seasonally low temperatures and reduced food availability, does not habitually exploit this possibility. This lack of use of torpor or hibernation suggests that *G. moholi* relies on other adaptations to survive the austral winter. However, these adaptations are poorly understood.

We hypothesized that *G. moholi* survives the unfavourable winter period, without, or only rarely, employing torpid phases to save energy, by using behavioural thermoregulation. We tested the following predictions using detailed data on food availability and behavioural observations:

(A) *G. moholi* is able to adjust to seasonal food shortage via a shift in its food spectrum.

(B) *G. moholi* reduces its activity and increases its home range size to compensate for reduced food availability during the cold, dry period.

(C) *G. moholi* buffers temperature extremes, saving energetic costs, by modification of nesting behaviour from solitary to group nesting, and shifts sleeping sites to more insulated sites during the cold period.

Material and methods

Study site

We conducted our study at Nylsvley Nature Reserve (South Africa, 24° 38.802'S 28° 40.095'E, altitude: 1100 m), a semi-arid, mixed bushveld habitat with a hot, wet season from October to March, and a cold, dry season from April to September. Unless otherwise stated, we use the meteorological definition of season: summer (December-February), autumn transition (March-May), winter (June-August) and spring transition (September-November). We acquired data between March 2009 and April 2011.

Ambient conditions

We recorded ambient temperature (T_a) and humidity every 30 min during the field seasons with data-loggers placed in the shade within the animals' home ranges (resolution 0.0625 °C; Hygrochron IButton/DS1923, Dallas Semiconductor, USA). We obtained temperature data (only minimum and maximum T_a) for the time between field seasons and data on rainfall from the local weather station at Nylsvley Nature Reserve. We took sunset and sunrise data from http://www.timeanddate.com/worldclock/sunrise.html (01.06.11; location Johannesburg).

Food availability

To assess temporal changes in food availability, we compared the abundance of the two main food sources of *G. moholi* (small arthropods and *Acacia* gum) in summer and winter.

Arthropod Sampling

As we primarily observed *G. moholi* catching aerial insects, but also saw it eating arthropods crawling on the ground surface on some occasions, we determined the relative abundance for both groups. We obtained arthropod samples between July 2010 and April 2011. We

obtained no data for flying insects during April 2011, due to technical failure of the insect light. We determined the number of the arthropods caught, and classified all arthropods to the level of order.

We determined the abundance of crawling arthropods using pitfall traps (50 ml, diameter 3 mm), a standard method for trapping epigaeic invertebrates (e.g., Kotze and Samways 1999; Mzilikazi and Lovegrove 2004). We placed a total of 32 pitfall traps, filled with 70 % alcohol mixed with glycerol, in two sites in the study area (16 traps/site installed one meter apart in a 16 m^2 grid). Due to high rates of destruction by wildlife we checked the pitfall traps at 2 week intervals, resulting in two datasets per plot per month. For statistical analyses, we pooled these two datasets together (mean values from both data sets) every month to compensate for the loss of traps. We included only traps that were effective throughout the sampling period in statistical analyses (N = 13/plot). We caught significantly fewer arthropods in plot 2 compared to plot 1 (Chi^2 goodness of fit test, X^2 = 307.51, df = 9, P < 0.05). However, as the distribution over the course of the year followed the same pattern for both plots (Spearman's rank correlation coefficient: 0.927, N = 10, P = 0.01), we pooled the data for further analyses.

We determined the abundance of flying insects with the aid of an insect light (Wasco, insect killer, 1.1 cm gap size), which attracts the insects by emitting blue light and kills them on contact by a high voltage cord. Dead insects can later be removed directly from the cords. This standard method is suitable for quantifying gross differences in insect abundances between seasons (e.g., Black 1974; Churchill 1994). We placed the light on a tree within the study area at about 1.5 m from the ground for one night each week.

Availability of Acacia karroo gum
We primarily observed *G. moholi* taking gum from *Acacia karroo* trees, and saw them feeding on gum of other trees (e.g., *A. tortilis* and *Terminalia sericea*) only occasionally. Therefore, we concentrated on availability of the species' main gum source. We monitored gum production of 89 *A. karroo* trees distributed over four plots in the study area. Four trees died during our study period in March/April 2011, reducing the sample size to 85 trees. We checked the trees once a month for the occurrence of gum up to a height of 2 m. We also documented the colour and softness of gum. We excluded spots smaller than 1x1 cm from the analyses, assuming that these do not constitute a crucial food resource for *G. moholi*.

Energy content of A. karroo gum
We determined the energy content of 39 gum samples (N_{summer} = 9, $N_{transition\ to\ winter}$ = 7, N_{winter} = 12, $N_{transition\ to\ summer}$ = 11) collected between June 2009 and April 2011 with an oxygen bomb

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calorimeter (6100 Parr Instrument (Germany) GmbH, Frankfurt). In total, we pressed 0.2 g of each gum sample into a tablet and analysed it with a semi-micro oxygen bomb. We determined additional concentrations of soluble sugars as the equivalent of galactose after acid hydrolization of the 50 % methanol extract (Kates 1972).

Radiotelemetry

We captured the animals using home-made, walk-in live traps baited with bananas, honey and peanut butter. We sexed, measured and weighed animals, classified them by age and reproductive state and marked them individually with subcutaneously injected passive identification transponders (ID100 Trovan, EURO I.D. Usling GmbH, Weilerswist, Germany). We released all animals at the exact capture locations and kept no animal longer than four days (for details see Nowack et al. 2010).

To investigate the thermoregulatory behaviour of *G. moholi* we equipped individuals (>160 g, >6 month, no juveniles) with temperature-sensitive collar transmitters (TW-4 button cell tags, 4 g, Biotrack, Wareham, UK). We detected transmitter signals using a TR-4 receiver (Telonics, Inc., Mesa, Arizona, USA) with a flexible two-element yagi antenna. We used the collar transmitters to follow individuals at night and to find resting sites during the daytime. We calibrated temperature-sensitive transmitters in a water bath prior to each field season. We noted the T_{skin} of radio-collared animals at hourly intervals in the early morning to test for normothermy.

Nightly follows

We equipped seven animals (3 females, 4 males) with collar transmitters during winter (July 2009 and August 2010), and followed each from emergence from its resting site until return to a resting site for two non-consecutive nights. During summer we observed six different animals (2 females, 4 males; February 2010/2011, November 2010). One individual lost its collar and could only be followed for one night. Due to a thunderstorm we had to interrupt one night in the early morning before the animal had returned to its resting site.

We never lost sight of an animal for more than 10 min and were able to approach animals as close as 1 m without notable changes in behaviour (e.g., alertness, escape). In total, we followed individuals for 136 h during winter and 109 h during summer. We pooled data from different years for each season. We only included data from individuals followed for two nights in pooled analyses. We calculated the nightly activity of individuals as the total time an animal spent active excluding the time spent resting.

Feeding behaviour

We recorded the feeding behaviour of focal individuals during nightly observation sessions. We considered an individual to be "feeding on gum" when we observed it biting or licking on a gum spot on a tree. We counted each visit to a gum tree once, regardless of whether the animal fed on one or more spots on that tree. We considered an individual to be "hunting" when it was following an insect actively, approaching it (often notable as a fast goal-oriented movement), and trying to catch it. We did not distinguish between successful and unsuccessful hunting events. We calculated individual mean values of feeding observations per night for all activities before statistical analyses and report mean values for all individuals in the results.

Home Range Size

We recorded the focal animal's location every 15 min using a hand-held GPS (Garmin, etrexH, Garmin Ltd., Europe). We calculated home range sizes for each sex and season with Animal Movement Software for Arcview GIS 3.2a (ESRI) using the Minimum Convex Polygon method.

Resting site use

We located radio-collared animals during the day to characterize resting site use (Table 1) for seven individuals during winter 2009 and 2010 and six individuals during summer 2009/10 and 2010/11. During transition times we tracked seven individuals in March - May 2010/2011 and four individuals between September - November 2010. Individuals used the following types of resting sites: open sites (branch of tree/bush), enclosed tree holes (also includes nest boxes), and insulated nests. We calculated the percentage of days each individual used each type of resting site. We also recorded group size during resting (solitary, group) and summarised this as the percentage of observations during which the individual was found resting solitary or in a group.

Data analyses

Unless stated otherwise, we present data as mean ± SD. We performed all statistical analyses with SPSS (PASW Statistics 18, 2009) and used non-parametric tests to compare winter and summer in each case. We tested for differences in insect availability (crawling and aerial) (winter 2010: July/August 2010; summer 2010/11: December 2010-February 2011) using Mann-Whitney U-tests. We used Chi^2-tests to test for seasonal differences in gum availability and colour and Kruskal-Wallis analyses with Mann-Whitney U-tests as post-hoc analyses to test for seasonal differences in sugar and energy content. We used Mann-

Whitney U-tests to test for seasonal differences in feeding. We could not test for seasonal differences in home range size due to low sample sizes. We used Mann-Whitney U-tests to test for seasonal differences in activity pattern. We examined both the total time the animals spent active and the percentage of the total night that animals were active. We tested for a seasonal difference in variability of nightly activity times using a Siegel-Tukey test and tested the onset of activity and variability in the start of activity with a Mann-Whitney U-test and a Siegel-Tukey test.

Ethical note

All procedures in this study complied with the "Principles for animal care", publication no. 86–23, revised 1986 (National Institute of Health) and the "Code of ethics for animal experimentation" manual adopted by the Nelson Mandela Metropolitan University (animal ethics clearance no. A09-SCI-ZOO-001). We captured animals under permit no. CPM-002-00003, issued by the Department of Economic Development, Environment and Tourism.

Results

Temperature data

Seasonal changes in rainfall and T_a were typical for an austral savannah habitat (Figure 1): During summer minimum T_a (T_{amin}) seldom decreased below 15 °C at night (mean for both years: 16.3 ± SD 0.8 °C). The coldest months were July (2009 and 2011; mean T_{amin}: -0.8 ± SD 2.3 °C/-0.5 ± SD 2.1 °C) and June (2010; mean T_{amin}: 1.5 ± SD 3.2 °C) with a T_{amin} of -5 °C in June 2010. Continuous cold phases (T_a < 1 °C) concentrated on three phases at the end of June and July 2009 (between 5 and 17 days) and occurred from end of July until mid-August in 2010 (between 3 - 12 days). The amplitude of daily variation in T_a varied from 2 °C (April) to 35 °C (October). Rainfall concentrated in the hot, wet season with a total of 1057 mm (Sept 2009-May 2010) and 788 mm (Oct 2010-May 2011).

Food availability

Arthropod abundance showed pronounced seasonal variation. The number of crawling arthropods captured per month varied from 439 (July) to 2454 (February). We caught significantly fewer crawling arthropods during winter (mean 459 ± SD 29) than summer (1665 ± SD 711; U-test; N = 3/2, Z = -2.558, P < 0.01; Figure 2a). We found representatives of 21 orders with Hymenoptera constituting the major proportion in all samples. The number of orders present in the traps was lowest during winter (11 orders) and highest during summer (18 orders). We caught *G. moholi* prey items (such as termites or grasshopper) in all seasons.

The seasonal distribution of flying insects was more irregular than that of crawling arthropods (Figure 2b). We found significantly lower numbers of flying insects during winter (mean 175 ± SD 206) than summer (788 ± SD 801; U-test: N = 12/9, Z = -2.160, P < 0.05). We captured representatives of 12 orders of flying insects, with Diptera constituting the major proportion in all samples. We captured Lepidoptera (the main prey of *G. moholi*) in all seasons. The mean number of insects captured per month varied throughout the year from 16 (June) to 1543 (September) insects. The number of orders was lowest during winter (5 orders) and highest during spring transition (11 orders).

Gum availability

Gum was present throughout the year, but the proportion of trees with old, fresh or no gum at all varied significantly between summer and winter (Chi^2 Test, N = 85 trees, X^2 = 40.1, df = 2, P < 0.001; Figure 2c). Old gum constituted the major gum source during winter (10-19 % of trees) when the proportion of trees with fresh gum decreased to about 5 %. The maximum numbers of trees with fresh gum occurred at the beginning of summer (December: ~50 %).

The number of gum spots/tree was highly variable between trees and the mean number of spots/tree was lowest between August and October (Aug: 1.7 ± SD 0.8; Sept: 1.9 ± SD 2.0; Oct: 1.5 ± SD 0.8) and highest for March (4.2 ± SD 4 spots/tree). During summer the mean number was 3 spots/tree (Nov: 3.0 ± SD 3.2; Jan: 3.0 ± SD 2.0; Feb: 2.8 ± SD 2.5). Only five trees produced no gum during the course of the study.

Trees produced two distinct forms of gum: light (yellowish or transparent) and dark (brown). The monthly proportion of dark gum was always lower than that of light gum, but increased significantly during winter (Chi^2 test, N = 85 trees, X^2 = 5.11, df = 1, P < 0.05). We found significantly higher sugar content in light gum (N = 16) than in dark gum samples (N = 23; U-test: Z = -2.214, P = 0.034). Analyses of dark samples showed no significant seasonal difference in sugar content (Kruskal-Wallis test, N = 6/5/6/6, X^2 = 2.019, df = 3, P = 0.569). However, energy content in these samples was significantly higher during winter (Kruskal-Wallis test, N = 6/5/6/6, X^2 = 7.970, df = 3, P = 0.047; Table 2).

Behavioural observations

Feeding behaviour

G. moholi fed primarily on aerial insects and gum. The diet changed significantly across the year. Pooled analyses of nightly food intake revealed that *G. moholi* hunted for insects significantly more often during summer than in winter (summer 65 % vs. winter 29 % of feeding observations; U-test, N = 6/7, Z = -2,510, P = 0.008) and increased gum intake significantly during winter. Data for individual animals showed that all focal animals hunted

more often for insects during summer and only one female consumed more insects than gum during winter.

Torpor use

Only one individual entered torpor during the time of this study. The animal underwent six torpor bouts within nine days within the coldest month (July 2009), and then disappeared (Nowack et al. 2010). All other animals remained normothermic throughout the time of the study.

Home Range Sizes

Our data show a clear tendency for females to almost double their home range from 1.8 ha in summer (N = 2, both 1.8 ha) to 3.1 ha during winter (N = 3, 2.1 - 7.7 ha; no statistics due to low sample sizes). Males had a mean home range size of 5.2 ha during both summer (N = 3, 3.3 - 7.3 ha) and winter (N = 4, 2.9 - 7.7 ha).

Seasonal differences in choice of resting sites

Sleeping site use showed a clear seasonal pattern (Figure 3a). Individuals used tree holes throughout the year, but most frequently in winter, when all animals rested in tree holes on 18-100 % of observation days (N = 7). In contrast, all individuals rested frequently on trees or bush branches without cover during summer (25-91 % of observation days, N = 6) and during both transition times, but not during winter, when only three of seven individuals rested on trees or bush branches on 8-25 % of observation days. We found that the difference in the use of enclosed/insulated sites was significantly different between summer and winter (U-test, N = 6/7, Z = -2.978, P = 0.001). Animals used nests primarily during the winter months, and only occasionally during the rest of the year.

G*roup composition during resting*

Females rested with their current offspring after birth periods (January/February and September/October) and were sometimes joined by offspring from the previous year. Mother-offspring groups were occasionally accompanied by an adult male but males usually rested solitarily during this time. We excluded data points for mothers and new-born offspring from analyses.

Group composition differed significantly between winter and summer (U-test, N = 6/7, Z = -2.289, P < 0.05). Six of seven individuals occasionally rested alone in winter, but only two did so in more than 50 % of the observed days. Resting alone was much more frequent during summer, when all animals rested alone and five did so in more than 50 % of

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observations (females until parturition) (Figure 3b). During summer, we only found four of six individuals (2 females, 2 males) grouping occasionally with one or more conspecifics (for 9-52 % of observational days), whereas all individuals rested in groups of two or more at least occasionally during winter, and only two individuals did on less than 50 % of occasions.

Activity

G. moholi showed a distinct seasonal shift in its nightly active phase. In summer individuals were active for 10:05 ± SD 0:14 h per night (excluding resting periods from activity time; N = 6) and started their activity shortly before or after sunset. Night length was 10:59 ± SD 0:12 h. Animals sometimes rested in a tree for up to an hour during the night (between 10pm and 1:30am), but never entered one of their resting sites during this time. T_a never decreased below 13 °C during nights of animal observation. We observed no diurnal activity during summer months.

During winter activity was highly variable. Activity times during winter showed significantly higher variability than during summer (Siegel-Tukey test: N = 10/14, U = 24, P = 0.02) and varied from less than six to more than eleven hours (mean activity: 8:51 ± SD 2:00 h; night length: 13:05 ± SD 0:16 h). The nightly duration of activity did not differ significantly between seasons (U-test: N = 10/14, Z = -0.937, P = 0.371). However, animals were active for a smaller percentage of the night in winter than summer (U-test: N = 10/14, Z = -3.689, P < 0.001). Neither the timing of the onset of activity, nor the variability in the start of activity differed between summer and winter (U-test: N = 11/14, Z = -0.796, P > 0.05; Siegel-Tukey test: N = 11/14, U = 51.5, P > 0.05). Mean temperature during animal observations was 6.8 ± SD 3.9 °C (T_{amin} -4 °C). Individuals rested (were inactive) for between 11 min and more than 3.5 h (coldest night) during eleven of the 14 nights. We observed no resting behaviour on the other three nights. On four of the occasions when animals rested they retreated into a better insulated resting site (nest, tree hole). Females showed a tendency to decrease in activity from summer (557 ± SD 35 min, N = 3) to winter (419 ± SD 20 min, N = 2), whereas male activity remained constant (615 ± SD 26 min, N = 4 vs. 589 ± SD 92 min, N = 4; no statistical tests due to low sample sizes). During July 2009 and 2010 we saw individuals of *G. moholi* outside their resting site during the day, between 12pm and 2pm on four opportunistic occasions.

Discussion

This study demonstrates how ecological and behavioural adaptations enable *G. moholi* to survive the cold and dry period without relying on regular, obligatory energy savings through heterothermy. Individuals reduced heat loss during winter by using enclosed or insulated

sleeping sites and switching from solitary to more social resting groups, modified their feeding behaviour and changed their activity pattern. We did not find a significant seasonal change in home range size. However, all other findings correspond with our predictions.

During summer, most individuals rested on a tree or bush directly exposed to T_a and the sun, whereas they used enclosed tree holes and insulated nests during winter. This winter behaviour reduces energetic costs by buffering temperature extremes, and establishing a microclimate closer to the thermoneutral zone of the species (reviewed in Gilbert et al. 2010). Lovegrove et al. (1991) found that the nests of black tailed tree rats (*Thallomys paedulcus)* increase T_{amin} by 2.7 °C and decrease daily T_a fluctuations by around 9 °C in the Southern Kalahari Desert. However, in contrast to the night, buffering by the use of tree holes or nests during the midday hours in winter months may actually be detrimental to energy budgets. T_a in our study area rarely rises above 25 °C and individuals resting inside a tree hole cannot use passive heating as much as when they rest on a tree. This may explain why some individuals chose to rest at the top of a tree with direct exposure to sun during winter.

Animals can also reduce energy expenditure through huddling which decreases the surface area of an animal exposed to the surroundings, reducing heat loss. Additionally, the T_a directly surrounding animals can increase when more animals share a confined space for resting, reducing the temperature gradient between the individual and the (cooler) environment (reviewed in Gilbert et al. 2010). During winter, *G. moholi* seldom slept alone, but rested in groups of two or more individuals, instead. Several studies of birds and mammals have shown that aggregation influences thermoregulatory costs (e.g., birds: Boix-Hinzen and Lovegrove 1998, primates: Perret 1998, rodents: Scantlebury et al. 2006). Mouse lemurs (*Microcebus murinus*) can reduce their individual energy expenditure between 20 % and 40 % when sharing a nest with two or more individuals (Perret 1998) and we can expect comparable savings for *G. moholi*.

Reduced costs of thermoregulation also lower the need for food intake and can therefore enhance survival during times of food scarcity. Food availability in the study area showed a marked decrease during winter months. Our results revealed highly seasonal variation in the occurrence of crawling arthropods and flying insects, with least availability during the cold, dry period. Nightly T_{amin} is reached during the early morning hours and insect activity is assumed to diminish during this time (e.g., Taylor and O`Neill 1988). Accordingly, we found *G. moholi* to be most active during the first (warmer) half of the coldest nights, showing longer resting periods or even an early end to the active period. Due to low sample sizes our data are not tested statistically, but they suggest that females reduced their nocturnal activity during winter, while at the same time increasing their home range sizes.

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Males, however, showed stable and consistently bigger home ranges than females. Male territories overlap several smaller female territories (reviewed in Harcourt and Bearder 1989) and high territorial defence in males (Nowack et al. 2010) could explain the need to be active throughout the whole night. An increase in home range sizes of females during winter would overlap with pregnancy in most females (Pullen et al. 2000). Females not only have to sustain their own bodies during the cold and dry period, but must also ensure the successful development of their unborn offspring (birth occurs in September/November, Pullen et al. 2000). The main food source we identified during the winter months is gum, and females are likely to increase travel distances to gain access to a larger number of gum trees, a pattern often described in other primates (e.g., Harris et al. 2010).

Gum availability also showed seasonal variation with more and larger spots of fresh gum available during the wet summer months. However, old gum spots were present throughout the year and were visited regularly by *G. moholi*, which scrape off the hard gum with the aid of their tooth combs. *G. moholi* increased its gum intake during the cold, dry period, presumably to meet energetic needs when arthropod availability is low. Earlier studies of *G. moholi* also indicate similar seasonal changes in food intake depending on winter conditions (Bearder and Martin 1980; Harcourt 1986). Chemical analyses revealed a winter increase in the energy content of dark gum, making this resource even more attractive during time of food scarcity. Water availability is low during winter and trees presumably reduce the water content of gum, resulting in a higher concentration of soluble particles. Increased gummivory during scarce periods has also been found in Malagasy lemurs (e.g., mouse lemurs, Dammhahn and Kappeler 2008; Joly-Radko and Zimmermann 2010) as well as in birds, rodents and marsupials (reviewed in Nash 1986). Gummivory has been generally viewed as a fall-back diet when other food resources are limited or completely lacking (e.g., Hladik et al. 1980), but has recently been discussed as an adaptation to hypervariable environments (Génin et al. 2010) and some highly specialized species as the Masoala fork-marked lemur (*Phaner furcifer*) rely exclusively on this food resource throughout the year (Schülke 2003). Relying on this food source requires special adaptations. Gum contains significant amounts of indigestible carbohydrates that need to be processed by microbial fermentation in the gastro-intestinal tract before the energy content is accessible by the individual (Power 2010). Fork-marked lemurs are also the only species of Cheirogaleidae without any indication of undergoing torpid states (Dausmann 2008). This, together with data for *G. moholi*, raises the question of whether gum feeding excludes the possibility of torpor, perhaps due to long digestion times, or eliminates the need for it. The availability of high quality food is known to reduce torpor frequency (Eastern chipmunks (*Tamias striatus*): Humphries et al. 2003; brush-tailed mulgara (*Dasycercus blythi*): Pavey et al. 2009). Gum

may not be a high quality food *per se*, but its availability, in addition to an increase in energy content of gum during winter, presumably enables *G. moholi* to stay normothermic throughout the winter and to focus on reproductive activities (Nowack et al. 2010; Dausmann et al. 2012). *G. moholi* is physiologically capable of torpor but only one of the seven focus animals that we monitored extensively in this study underwent torpid states. Torpor use occurred within such a short time-frame in this individual that it was obviously triggered by either extraordinarily low availability of food or illness, rather than as a general response to season (Nowack et al. 2010). We suggest that *G. moholi* usually remains normothermic, but can respond to phases of unusual severely food shortage (caused by especially harsh winters or individual emergency situations) with spontaneous torpor when other survival strategies fail.

Conclusion

Hibernation and daily torpor are common and effective physiological strategies used by small mammals to save energy during periods of scarcity as well as cold and dry periods. It therefore might seem puzzling that a fairly small animal species, which is physiologically capable of entering heterothermic states, and lives in a seasonal habitat with seasonally low temperatures and reduced food availability, does not habitually exploit this possibility. However, as beneficial as these strategies might be from an energetic point of view, they appear to have their drawbacks and conflict with other activities such as reproduction, growth, or territory defence (reviewed in Gilbert et al. 2010; Nowack et al. 2010). We propose that *G. moholi* favours ecological and behavioural adjustments over torpor, as, together with sufficient food availability, they serve to meet energy requirements of this species, and their advantages (flexibility, unrestricted activity and reproduction) outweigh the energetic benefits of heterothermy.

Acknowledgements

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Tables

Table 1: Animals observed for resting site use by season and sex. We collared animals at different times during the field season resulting in variable numbers of observations per animal.

	Winter	Transition	Summer	Transition
Males	5	2	4	5
Females	2	2	2	2
Total observations	154	50	81	53
Observations per animal	9 - 46	4 – 24	2 - 23	3 - 17

Table 2: Seasonal energy content of dark gum samples. Rows with different letters are significantly different from each other (post-hoc U-tests).

	Energy content (MJ/kg)
Winter (N = 6)	$16.2 \pm SD\ 0.5^b$
Transition to summer (N = 6)	$15.8 \pm SD\ 0.4^{ab}$
Summer (N = 6)	$14.6 \pm SD\ 1.3^a$
Transition to winter (N = 5)	$14.9 \pm SD\ 1.4^{ab}$

Figure legends

Figure 1: Monthly mean maximum (T_{amax}) and minimum temperature (T_{amin}) and rainfall at Nylsvley Nature Reserve, Limpopo, South Africa from March 2009 to April 2011.

Figure 2: Distribution of food availability

a) Total number of crawling arthropods per month.

b) Monthly mean and SD of the number of flying insects caught during weekly trapping events each month.

c) Monthly percentage of *A. karroo* trees with fresh gum (*light grey*) or old gum (*dark grey*) available.

*** $P < 0.001$. Statistical tests are reported in the text.

Figure 3: Resting behaviour.

a) Percentage of individuals found in different types of resting sites during summer (*white bars*) and winter (*black bars*).

b) Percentage of individuals resting in groups vs. solitary during summer (*white bars*) and winter (*black bars*).

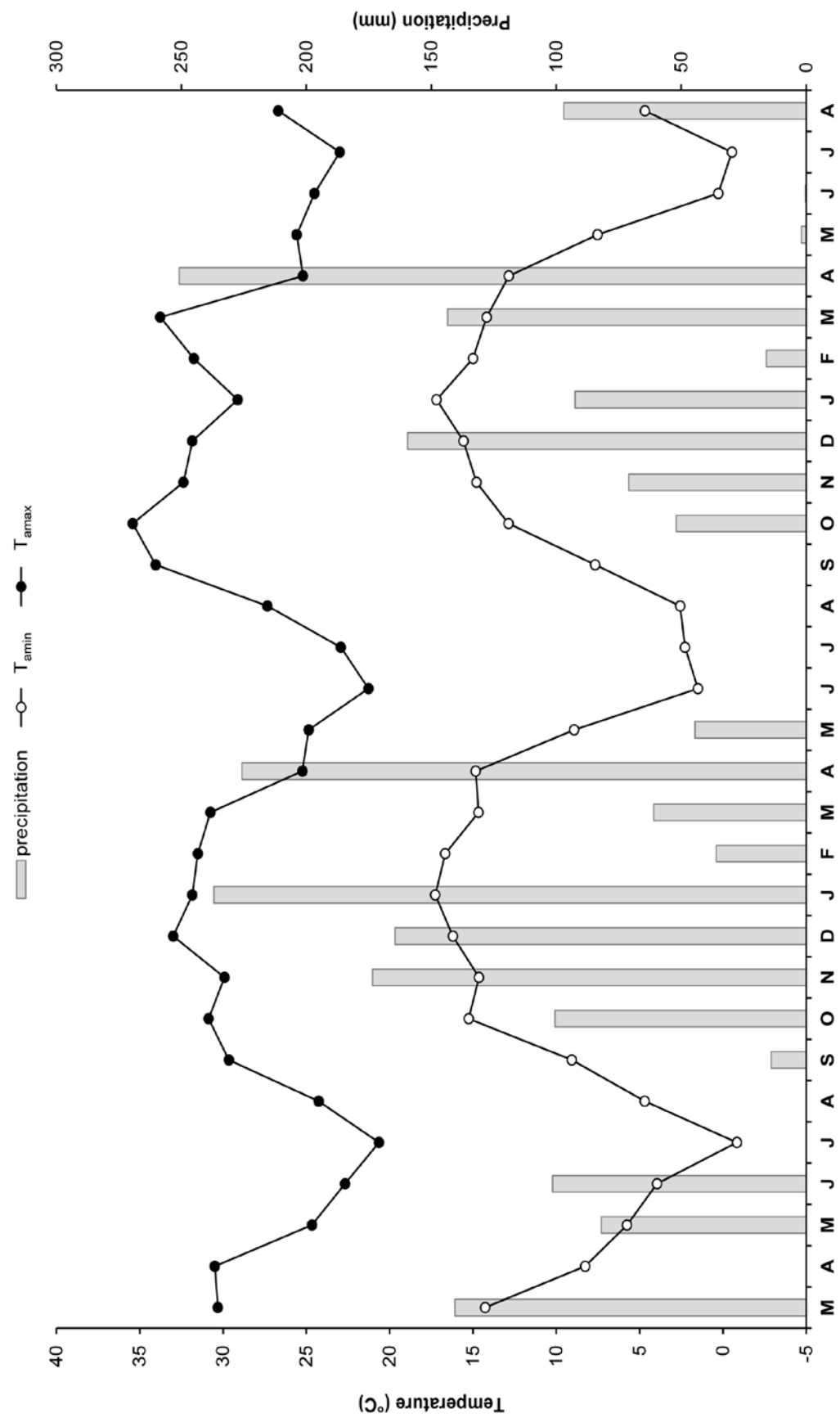* $P < 0.05$; *** $P < 0.001$. Statistical tests are reported in the text.
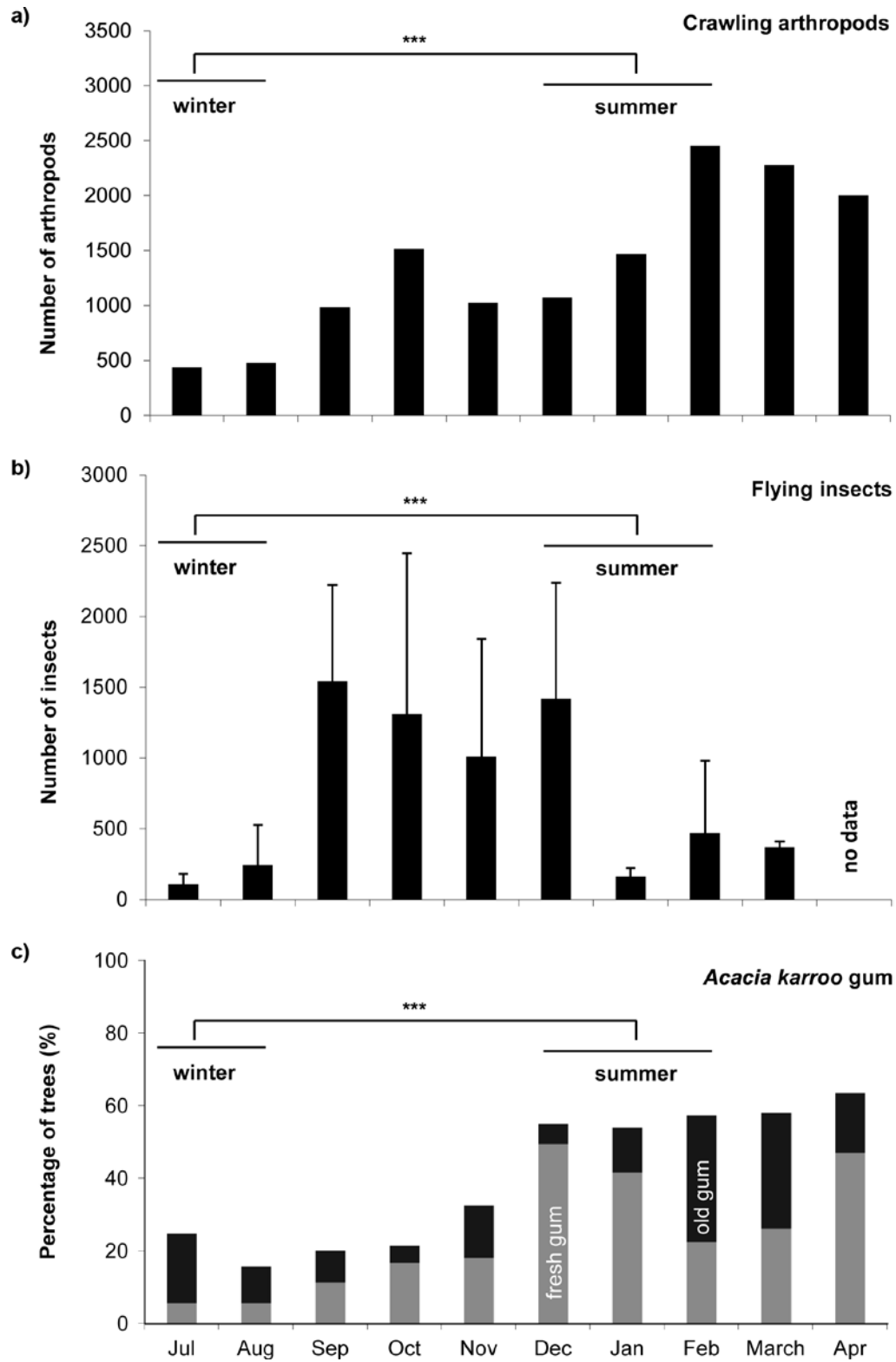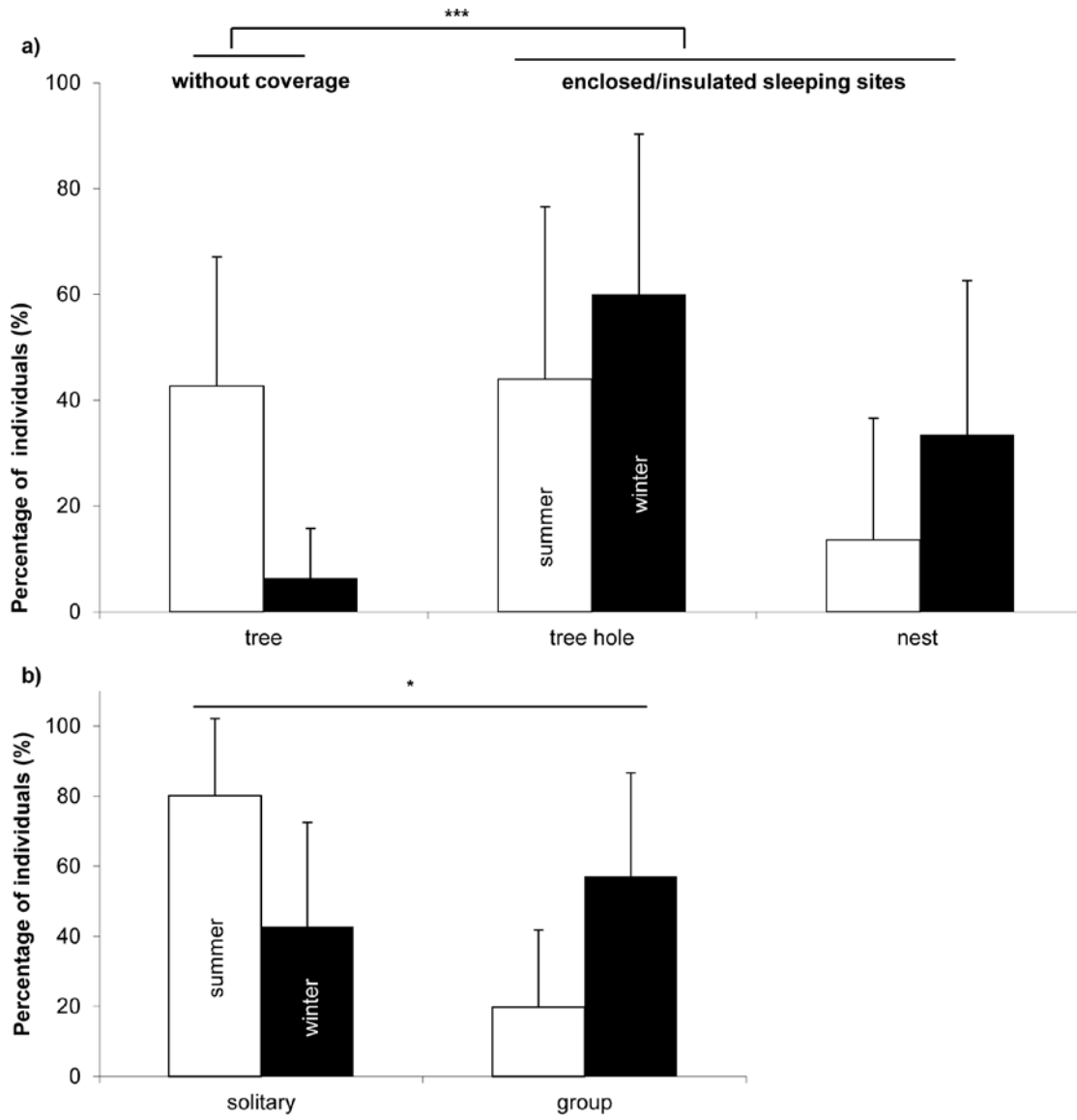
Figure 1

Figure 2

Figure 3

Torpor as an emergency solution in *Galago moholi*: heterothermy is triggered by different constraints

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Abstract

The expression of heterothermy in the Africa lesser bushbaby, *Galago moholi*, seems to be strikingly different to most other heterotherms: *G. moholi* uses its ability to enter torpor only rarely and torpor is only used by a small fraction of the population. The aim of this study was therefore to summarize the parameters of torpor use in *G. moholi* to conclude general patterns and discuss them in comparison to other heterotherms to elucidate possible causes and constraints that underlie these differences in deployment of heterothermy. Our study was carried out on wild animals using temperature loggers and open-flow respirometry for measurements of body temperature and metabolic rate, respectively. *G. moholi* uses torpor only as a last resort and not as a routine, seasonal behavior. Nevertheless, we found that the general physiological patterns of torpor, e.g., torpor bout duration or entry and arousal times from torpor, were mainly consistent with those described for other nocturnal daily heterotherms. The greatest difference found was the unusual low rewarming rates during arousal from torpor, probably due to already depleted internal energy stores and thus inability to mobilize sufficient energy for endogenous heating. We therefore conclude that while general physiological parameters of heterothermy seem to have remained conserved in heterotherms, the underlying causes which elicit this physiological response, and thus the extent of expression and timing of heterothermy, have evolved very differently in different groups, depending on body mass and the specific habitat and lifestyle of the species.

Introduction

Heterothermy, the depression of body temperature by the active reduction of metabolic processes, occurs in all mammalian subclasses (e.g., Geiser 1998) and is traditionally seen as a strategy for energy and water conservation during lean or demanding seasons (Heldmaier et al. 2004). Most heterotherms known so far use this strategy for prevention of physiological shortfalls and all individuals of a population capable of becoming heterothermic are generally found torpid at some time of the year (e.g., Dausmann et al. 2005; Kobbe et al. 2011).

The exception to this rule represents the African lesser bushbaby, *Galago moholi*: it uses its ability to enter torpor only rarely and not as a routine seasonal response. Preliminary results show that only a small proportion of the population ever enters torpor and usually only on single occasions (Nowack et al. 2010). *G. moholi* is a small (~200g), nocturnal primate, that lives in dry woodlands of Southern Africa, with a diet mainly restricted to arthropods and exudates from certain species of *Acacia* trees (Bearder and Martin 1980). Gestation time of *G. moholi* is unusually long for a strepsirhine primate of this size and *G. moholi* does not only have one main mating season in May, but also a post-partum mating period between September and October (Pullen et al. 2000). Thus, females that reproduce twice a year are reproductively active during almost eleven of twelve months and this reproductive pattern is thought to account for the rare use of torpor in *G. moholi* (Mzilikazi et al. 2006; Nowack et al. 2010).

Until recently, heterothermy within the primates was only known from one family of Malagasy lemurs, the Cheirogaleidae (Blanco and Rahalinarivo 2010; Dausmann 2008; Dausmann et al. 2004; Kobbe and Dausmann 2009; Lahann 2007; Lahann and Dausmann 2011; Ortmann et al. 1997; Schmid 2000; Schmid et al. 2000). Its discovery in a closely related, mainland primate supports the view that heterothermy is a plesiomorphic trait that evolved at least before the separation of African and Malagasy strepsirhine primates. However, the expression of this physiological possibility is strikingly different between the Cheirogaleidae and *G. moholi* (regular seasonal response vs. emergency solution). The aim of this study was therefore to summarize the parameters of torpor use in *G. moholi* to conclude general patterns and discuss them in comparison to other daily heterotherms to elucidate possible causes and constraints that underlie these differences in deployment of heterothermy to overcome lean seasons.

Material and Methods

Study site & ambient conditions

The field work of this study was conducted at the Nylsvley Nature Reserve (South Africa, Limpopo, 24° 38.802'S 28° 40.095'E, altitude: 1100m), a semi-arid, mixed bushveld habitat with a hot-wet season from October to March, and a cold-dry season from April to September. Monthly mean minimum ambient temperature (T_a) ranges from –1°C (July) to 17°C (January) and mean annual rainfall is about 700mm (Limpopo parks data for Nylsvley). Throughout this paper we use the terms winter and summer according to the meteorological definition (winter: June- August, summer: December- February) and the terms cold-dry/ hot-wet period to refer to the rainy (October- March) or to the dry season (April- September).

T_a during the field seasons was recorded every 30min within the animals' home ranges with humidity and temperature loggers (resolution 0.0625°C; Hygrochron IButton/DS1923, Dallas Semiconductor, USA). Temperature data (only minimum and maximum T_a) for time spans between field seasons as well as data on rainfall were provided by the local weather station at Nylsvley Nature Reserve.

Capture and Handling

Animals were captured between March 2009 and August 2011 using self-made walk-in live traps baited with bananas, honey and peanut butter. Animals were sexed, aged (for details see Nowack et al. 2010), measured, weighed, individually marked with subcutaneously injected passive identification transponders (ID100 Trovan, EURO I.D. Usling GmbH, Weilerswist, Germany) and equipped with temperature sensitive collars (see below). For animals that were recaptured within one season, only the highest measure of weight/testes size was included in the later analyses. Some of the animals were briefly anaesthetized with Ketaminhydrochloride (Ketanest® 1 mg/100 g, Parke-Davis, Berlin) for handling. All animals were released at exact capture locations.

Measurement of body temperature of free-ranging G. moholi

Skin Temperature (T_{skin}) of free-ranging animals was measured using temperature-sensitive collar loggers (Weetag; 2.6g; Alpha Mach Inc., Mont St-Hilaire, Canada; calibration curves provided by manufacturer) and temperature-sensitive collar transmitters (radio collar; 4g; TW-4 button cell tags, Biotrack Ltd., Wareham, Dorset, UK) in a total of 31 animals (17 males, 12 females, 2 with uncertain sex; 5 of them were measured in two age classes (subadult and adult)).

Temperature-sensitive transmitters change their pulse rate according to temperature and were calibrated in a water bath prior to each field season (testo 700, Lenzkirch, Germany,

accuracy ± 0.4°C). Transmitter signals were detected using a TR-4 receiver (Telonics, Inc., Mesa, Arizona, USA) with a flexible two-element yagi antenna. T_{skin} of radio-collared animals was checked at hourly intervals in the early morning and every 15min for the duration of a torpor bout. Sample frequency of collar loggers was programmed to once an hour for a period of 85 days (resolution 0.0625°C).

Six animals (5 males, 1 female) were implanted with temperature loggers (3g, thermochron iButtons/DS1922, Dallas Semiconductor, USA; coated in Elvax; sample frequency: every 30min for 170days; Maxim, Dallas Semiconductor, USA; calibrated in water bath) into the peritoneal cavity under inhalation anesthesia (isoflorane in oxygen; induction and maintenance 2%; flow rate ca. 0.5l min^{-1}; for detailed description see Mzilikazi et al. 2006). Analgesia was administered intramuscular during surgery (Metacam®, 0.4ml kg^{-1}). The coated temperature loggers were inserted through a ~1.5cm long cut in the lower middle of the abdomen. Skin and lower layer were sewed with self-absorbable suture (chromic catgut suture, absorbable; needle: 19mm, reverse cutting, 3/8 circle; CliniGut, Port Elizabeth, South Africa).

To ensure that the animals had fully recovered and that surgery did not affect measurements, we programmed the loggers to start recording at least one week after surgery. Only three animals (adult males) could be recaptured and time spans of data acquisition ranged between twelve and 21 weeks.

In total we were able to obtain body temperature (T_b) data (T_{skin} and core T_b) of 31 animals.

Measurement of metabolic rate

Metabolic rate (MR) was measured with one of two setups:

1) Laboratory setup: Animals were captured in the mornings and placed in a plastic metabolic chamber (30x15x18cm, 8l) for 24h in a laboratory situated within the study site. The animals were left undisturbed for at least one hour before the beginning of the measurements and did not have any access to food or water during the measurements. We measured 14 (9 females, 4 males, 1 uncertain sex) animals during the cold-dry period and 18 (6 females, 12 males) animals during the hot-wet period. To reflect prevailing T_a at the study site, windows were kept open in the laboratory during measurements.

2) Enclosure setup (semi-natural conditions): We measured MR of animals kept in outdoor cages (180.5x61.5x193.5cm) for one to four consecutive days, using a nest box (0.4mm plywood, 25x20x20cm, 10l) as the metabolic chamber. Outdoor cages were equipped with branches, and animals were provided with water, banana, gum of

Acacia trees (if available) and mealworms ad libitum. We measured 22 (11 females, 11 males) animals during the cold-dry period and 10 (6 males, 4 females) animals within the hot-wet period.

Energy expenditure was determined by measuring the rate of VO_2 (MR) with open flow-through respirometry using a portable oxygen analyser (FoxBoxC, Sable Systems International, USA). The metabolic chamber was connected to the oxygen analyser with airtight tubes (pull mode). Flow rates were adjusted to maintain <1% oxygen depletion between incurrent and excurrent air (between 900ml min^{-1} and 1400ml min^{-1}). CO_2 and water vapour were scrubbed prior to analysis of VO_2 using soda lime and silica gel. We measured sample air for 15min or 25min alternating with 5min reference air (baseline) to account for any drift of the oxygen sensor (sampling frequency every 10sec). MR was calculated using the data acquisition program Expedata (Sable Systems International, USA; for detailed description see Nowack et al. 2010). For each sampling period, the mean value of MR was calculated from those 33% of all values which represented the most stable readings in the cycle. Resting MR and torpid MR (TMR) were calculated as mean values from the three lowest consecutive values under rest or torpid conditions.

During all measurements of MR T_{skin} was measured simultaneously with collar loggers which were programmed to log between once a minute and every 10min (resolution 0.0625°C). Humidity and T_a was recorded with data loggers inside and next to the metabolic chamber (Hygrochron iButton/DS1923, Dallas Semiconductor, USA).

Data analyses

Animals were defined to have entered torpor when T_{skin} showed a distinct and stable decrease (<30°C during the rest phase) for prolonged time spans (>2hours). A torpor bout was defined to be terminated when T_{skin} increased again above 30°C (for more details see Nowack et al. 2010). During rewarming from torpor we differentiated between passive exogenous rewarming and active endogenous heating. Passive arousal was characterized by an increase of T_b with T_a during which VO_2 remained approximately constant at the torpid level, whereas active heating was defined as T_b rising due to a marked increase in VO_2 (see Schmid et al. 2000).

Statistical analyses

Unless stated otherwise, we present data as mean ± SD. We performed all statistical analyses with SPSS (PASW Statistics 18, 2009) and analysed the data with non-parametrical tests. To evaluate differences between ambient conditions within the three study years we performed Kruskal-Wallis analyses. Differences in body mass for females

were only tested for non-reproductive individuals between summer and winter with Mann-Whitney-U-tests. Differences in body mass and testes size for male individuals were tested for three out of four seasons (sample size for Sept-Nov to small) with Kruskal-Wallis analyses and independent Mann-Whitney-U-tests as post-hoc analyses. We tested for correlation between torpor entry/arousal and T_a and correlation between minimal TMR and minimal T_{skin} with Spearman rank analyses. A possible link between torpor use and body mass was tested by Mann-Whitney-U-test.

Results

Ambient conditions during winter

Within all three study years, mean of minimal daily T_a was always lowest in June or July (Tab. 1). The warmest winter was 2010, with mean minimal T_a not dropping below zero. Moreover, unlike 2009 and 2011, the lowest temperatures in 2010 were not restricted to one month, but were distributed over all three winter months (June-August), with an unusually cold August and no rainfall at all during mid-winter. However, differences in minimal T_a, maximal T_a and precipitation were not statistically significant between years (Kruskal-Wallis-tests, N= 10/12/8, df= 2, p> 0.05).

Occurrence of torpor: season, age and sex

Torpor was observed in all three study years (2009-2011), but only during the cold-dry season from April to end of August (laboratory) or in mid-winter (July; free-ranging). Only 27% (9/33) of all individuals examined during the cold-dry period entered torpor episodes: six individuals under food and water deprivation in the laboratory and three individuals under free-ranging conditions (Tab. 2).

Torpor was primarily found in subadult or juvenile individuals and only in one adult, but subdominant male (living within the territory of a dominant male). Five of the nine individuals were males; the sex of one juvenile individual could not be determined.

Frequency of torpor

In the laboratory *G. moholi* entered torpor only on single occasions (Tab. 2). We were able to gain further data on two of the laboratory individuals, but none of them re-entered torpor in a later measurement or under free-ranging conditions.

All three free-ranging individuals which entered torpor did so repeatedly (Tab. 2), albeit during a narrow time span. One radio-collared subadult male was found torpid on six out of nine monitored days (within July) between the 4th and the 19th of July 2009 (see

Nowack et al. 2010), before he disappeared. His absolute minimal T_{skin} was 24.2°C (range: 24.2 - 28.7°C, N= 6) and torpor bouts lasted between about two and four hours.

During the same month T_{skin} of another free-ranging individual, a subadult female, was obtained for 21 days (collar logger). This female entered torpor on six consecutive days between the 14th and the 19th of July 2009, but never during the 16 days before. This individual failed to rewarm from the last torpor bout and died during the next morning. The cause of death is not known. The last torpor bout showed the lowest minimal T_{skin} (19°C) and this temperature was only reached at midday (before T_{skin} increased again to a maximum of 28°C). During all other bouts T_{skin} remained well above 20°C (range: 21.6 - 28.2°C, N= 5) and the torpor bout duration (TBD) was between two and more than seven hours.

During July 2010 torpor was also displayed on three occasions within 11 days by one adult male (sampling period with collar logger: 38 days). TBD ranged from two to eleven hours and absolute minimal T_{skin} was 19.5°C during the longest torpor bout. Minimal T_{skin} for the other two bouts were 24.1°C and 24.6°C and were dependent on TBD.

For calculations of mean values of torpor parameters we only used parameters of the torpor bouts with the lowest T_{skin}s for each individual.

Body condition

Body mass of adult *G. moholi* did not statistically differ between summer and winter for males or non-reproductive females. Males increased their body mass non-significantly for a short period before winter, however, this increase coincided with the main mating period (May) and thereafter body mass decrease significantly during winter (Kruskal-Wallis-test: N= 8/7/15, X^2= 6.621, df= 2, p= 0.037, Mann-Whitney-U test: main-mating period - winter: N= 7/15, Z= -2.374, p= 0.017; Fig. 1a). Seasonal comparison of testes size (Kruskal-Wallis-test: N= 8/7/15, X^2= 8.689, df= 2, p= 0.013, pairwise Mann-Whitney-U tests for post hoc analyses) revealed that testes size increased significantly during the same time (Mann-Whitney-U test: main-mating period – summer: N= 7/8, Z= -2.382, p= 0.016; main- mating period - winter: N= 7/17, Z= -2.222, p= 0.027; Fig. 1b). Increase of testes size or body mass during the period of post-partum mating could not be tested for significance due to small sample size (N= 2).

Body mass of torpid individuals

Food and water deprivation during metabolic measurement always induced torpor in juveniles (N= 4) and in two out of five subadult individuals between April and end of August (cold-dry period); the same treatment never led to torpor in any individual (N= 18, including 4 juveniles) during the hot-wet period. Despite being older, the juveniles displaying torpor during the cold-dry period were about 10g lighter (112.5 ± 16.5g, N= 4) than the juveniles

measured within the hot-wet period (123.8 ± 4.8g, N= 4). However, we cannot differentiate between effects of age and body mass in this age group. We only gained data on one juvenile, free-ranging individual that did not enter torpor during time of data acquisition. Differences in mean body mass of adult and subadult individuals before metabolic measurements differed only slightly between seasons (3.6g and 1.8g respectively). Torpor use was not correlated with body mass in subadult or adult individuals (164 ± 12.9g vs. 178.6 ± 19.9g; Mann-Whitney-U-test, N= 5/37, Z= -1.655, p> 0.05).

Torpor pattern

During entrance into torpor bouts T_{skin} started declining from normothermic values between 2300 hours and 0700 hours and decreased below 30°C between 30min and 3h later (Fig. 2). T_a at the time of entry varied between 3°C and 17°C under laboratory conditions and between -1°C and 6°C under free ranging conditions and was strongly associated with minimum T_a of that day (Spearman rank coefficient: 0.872, N= 17, p< 0.001). T_{skin} was generally lowest between 0800 hours and 0900 hours with the exception of two free-ranging individuals, that showed minimal T_{skin} at 1030 hours (adult male) and 1200 hours (incomplete torpor bout of subadult female; see above). Arousal from torpor bouts started at T_as between 13°C and 29°C in laboratory animals and between 0°C and 19°C in free-ranging individuals and T_a at arousals was dependent on minimum T_a (Spearman rank coefficient: 0.592, N= 18, p= 0.01). Arousal from torpor bouts (>30°C) were completed between 1015 hours and 1300 hours for all but three individuals which had substantial difficulties to rewarm themselves (see section *Difficulties in torpor arousal*). Individuals returned to normothermic values of T_{skin} between 1100 hours and 1700 hours. Average TBD (T_{skin} <30°C) was 348 ± 183min (N= 6, animals with arousal difficulties excluded; Tab. 3).

Minimal T_{skin} and heating rates

Minimal T_{skin} during torpor bouts was on average 21 ± 4.7°C (N= 9; Tab. 3). All but three individuals maintained a minimal T_{skin} above 19°C (Fig. 3). Maximum heating rates (HR_{max}) during endogenous (active) heat production varied between 0.01°C min^{-1} and 0.33°C min^{-1} for individuals rewarming from torpor without apparent difficulties (Tab. 3). HR_{max} values were calculated by taking the difference between consecutive readings. Mean active HR_{max} (0.16 ± 0.09°C min^{-1}) was only about half (51.6%) of the predicted value for daily heterotherms on the basis of body mass (Geiser and Baudinette 1990). Before the start of endogenous heat production (Fig. 4ab) individuals used passive rewarming, the extent being dependent on surrounding T_a. Average passive rewarming rates were lower than active rewarming rates (Tab.3). We were able to compare average MR during passive and active heating for two of

the laboratory individuals and energy expenditure during passive heating was in both cases only half of that during the endogenous arousal phase.

Energy saving

Normothermic animals had an average MR (0600-1800 hours) of $1.5 \pm 0.2 mlO_2g^{-1}h^{-1}$. Minimal TMR varied strongly between individuals (N= 6; Tab. 3) with an average minimal TMR of $0.3 \pm 0.2 mlO_2 g^{-1}h^{-1}$. Minimal TMR was related to the minimal T_{skin} reached during that particular torpor bout (Spearman's rank correlation coefficient: 0.829, N= 6, p= 0.042) and to TBD (Spearman's rank correlation coefficient: -0.929, N= 6, p= 0.003). Mean average TMR for all laboratory animals without rewarming difficulties was $0.6 \pm 0.2 mlO_2g^{-1}h^{-1}$ (N= 4; Tab. 3). Comparing average TMR with average MR during normothermia (time span before torpor) for every individual, mean energy savings between 59% and 76% per hour became apparent.

Difficulties in torpor arousal

Individuals where T_{skin} decreased below ≤19°C (N= 3) showed substantial difficulties rewarming: One subadult female did not manage to rewarm herself from a T_{skin} of 19°C (T_a= 16.1°C) after repeated daily use of torpor for six days. She showed a last unsuccessful attempt to rewarm herself actively (HR_{max} 0.11°C min^{-1}) after an extended period of slow passive heating, but died later (see above). In two juvenile individuals T_{skin} decreased to 13.7°C (T_a= 12.6°C) and 14.9°C (T_a= 13.8°C), during laboratory measurements, levels that impeded the return to normothermic T_b. One of these individuals (105g) was taken out of the measurement at around 5pm with a T_{skin} just above 24°C and externally rewarmed. The other one (110g) did not manage to rewarm itself above 22.6°C (4:30pm) and died 4 hours later despite our attempts to rewarm it exogenously.

Rewarming difficulties did not occur on especially cold days (minimal T_a: -1.9°C, 5.9°C, 10.9°C; average minimal T_a on all days when torpor occurred: 3 ± 5.2°C, N= 20): Maximal T_a on those days was 21.4 ± 0.2°C (N= 3) and therefore only slightly lower than average maximal T_a (23.4 ± 2.8°C, N= 20).

All three individuals with difficulties returning to normothermic T_b had rewarmed themselves passively to some extent (e.g., Fig. 4b): They used passive heating as long as possible and the switch to active heating occurred only when T_a started to decrease again from its daily maximum to evening temperatures. However, unlike the other individuals (Fig. 4a), animals with rewarming difficulties were unable to switch to effective endogenous heating (average active HR_{max} 0.06 ± 0.04°C min^{-1}, Tab. 3). Active HR_{max} could either not be maintained over a long time period, did not essentially exceed passive HR_{max} (0.04°C min^{-1}

vs. 0.03°Cmin^{-1}) or even decrease markedly in comparison to active HR$_{max}$ (0.03°C min^{-1} vs. 0.1°C min^{-1}).

Discussion

Our study shows that although general physiological parameters of torpid phases are mainly consistent between *G. moholi* and other heterothermic species, heterothermy seems to be used fundamentally different. Whereas torpor is traditionally seen as an adaptation to counter – usually seasonal – bottlenecks, largely preventing energy or water emergencies, torpor in *G. moholi* seems to be a last–resort strategy. Torpor entry in *G. moholi* occurred in the second half of the night or during the early morning, and minimal T$_b$ was reached during the coldest morning hours, as reviewed for nocturnal daily heterotherms by Geiser (2004). *G. moholi* had a TBD between two and eleven hours, arousing from torpid states around midday. Whenever possible exogenous passive rewarming was exploited before active endogenous heating commenced. This two-phase heating process is known for other heterotherms in warmer climates and is known to significantly enhance energy savings (Dausmann et al. 2009; Geiser and Drury 2003; Schmid et al. 2000). However, torpor only occurred on single occasions or -if repeatedly- during a narrow time span and minimal T$_{skin}$ of torpid *G. moholi* was higher than found for most other daily heterotherms (reviewed in Geiser 2004). Individuals with T$_{skin}$s ≤19°C even had serious difficulties returning to normothermic values. Due to the higher T$_{skin}$ levels, energy expenditure during torpor was more than twice as high as found for the two closely related cheirogaleid species *Microcebus murinus* and *Cheirogaleus medius* (Dausmann et al. 2012).

Torpid phases in *G. moholi* appear to be only adopted when other possibilities (e.g., behavioral thermoregulation) to cope with the unfavorable season fail and *G. moholi* usually remain normothermic throughout the year, exploiting the advantages of year-round reproduction (and all corresponding factors, e.g., territoriality; Nowack et al. 2010). Even when conditions are challenging during winter, *G. moholi* still has access to low quantities of food with sufficient energy content (Nowack et al. in press), allowing- at least adult-individuals to remain normothermic and continue reproductive efforts. Moreover, *G. moholi* engage in social thermoregulation to minimize heat loss, and appear to adjust their diet during winter to prevailing supply (Nowack et al. in press), facilitating survival under unfavorable conditions. Torpor in *G. moholi* was expressed primarily by young (non-reproductive) individuals, that were born shortly before the winter season and did not have much time to increase body mass and improve body condition before ambient conditions became stressful. This is consistent with other studies that primarily found torpor in juvenile individuals of insectivorous species as a response to unpredictable fluctuations in prey

availability (Geiser and Kenagy 1990). Poor body condition appears to require the use of torpor when experiencing additional food shortage and since these young *G. moholi* are not yet engaged in reproductive efforts, the need to remain active is obviously reduced.

Peak rewarming rates from torpor in *G. moholi* were found to be almost 50% lower than expected on the basis of their body mass, as is the case in short-beaked echidna (*Tachyglossus aculeatus),* a Monotreme which is known to lack classical non-shivering thermogenesis (in comparison to the same sized marmot (*Mormota marmota*), possessing this mechanism; Nicol et al. 2009). This raises the question of whether *G. moholi* possesses the ability of non-shivering thermogenesis which usually is responsible for fast rewarming from torpor or hibernation bouts (Cannon and Nedergaard 2004). However, other studies also found reduced rewarming rates when torpor was induced by starvation (e.g. *Ningaui yvonneae*, Marsupialia: Geiser and Baudinette 1988; *Tachyglossus aculeatus*, Monotremata: Nicol and Andersen 2008). Accordingly, Nicol and Andersen (2008) suggested that sufficient active heat production is not possible when food deprivation depletes energy reserves. In the laboratory setup, torpor in *G. moholi* was induced by food and water deprivation. Since our study animals were wild animals, caught during periods with low food availability, already low internal energy reserves seem plausible. Thus, the low rewarming rates in *G. moholi* might indeed be a consequence of critically exhausted energy reserves. The individuals we observed entering torpor under free-ranging conditions did so on repeated occasions, but only within a close time frame and one subadult individual even failed to rewarm in the wild. This also suggests a trigger event, such as extraordinarily low availability of food or illness, rather than a general response to seasonal changes. Interestingly, torpor occurrence in *G. moholi* seems not to be linked with the days of coldest minimum T_a.

The apparent failure of *G. moholi* to regain normothermy from T_{skin} below 19°C might be also linked to low energy reserves in animals of especially low body condition (two juveniles, one subadult individual). At least three other studies that induced torpor in heterotherms by starvation also described rewarming difficulties of individuals with especially low T_bs during torpor (*Ningaui yvonneae*: Geiser and Baudinette 1988; *Sminthopsis crassicaudata*: Geiser et al. 1986; *Mus musculus*: Tomlinson et al. 2007) and Geiser et al. (1986) reported a link between the lowest successfully regulated T_b and temperature changes in mitochondrial respiration in the fat-tailed dunnart, *Sminthopsis crassicaudata.* Individuals that are undergoing torpor seem to be forced into hypothermia when T_a and/or energy reserves are too low. In these cases energy reserves of the individuals are obviously not sufficient to defend the lower critical T_b threshold of the species. Enzymatic activity is dependent on T_b and consequently Geiser et al. (1986) found that the lowest regulated T_b in *S. crassicaudata* during torpor was just above the temperature where changes in

mitochondrial respiration were detected. Thus rewarming difficulties might generally be linked to temperature-impaired enzymatic activity in mitochondria.

What does torpor in Galago moholi tell us about the evolution of heterothermy?

Recent studies discuss heterothermy as a plesiomorphic trait that evolved only once in mammalian evolution (e.g., Grigg et al. 2004; Lovegrove 2012; Nowack et al. 2010), i.e., the "ancestral mammalian species" already possessed this trait. Indeed, heterothermy in mammals usually occurs in phylogenetically older groups (Geiser 1998). Moreover, heterothermy is energetically most beneficial for small mammals, and most Late Cretaceous mammals were small (Luo 2007). Several conclusions arise from this assumption. On the one hand, it implies that in those orders that do not express heterothermy today, this trait has been secondarily lost, or not used. On the other hand, if heterothermy was an old trait, one would expect evolution to act on it. This leads to the intriguing question of whether heterothermy in recent groups is a conserved trait, similar between species, or underwent special adaptations in response to the particular ambient conditions of a species. If the later was the case one should expect varying expressions of heterothermic parameters or use of this trait. The finding of torpor in *G. moholi* and the fact that general torpor characteristics do not vary markedly from that of other (nocturnal) daily heterotherms supports the view of heterothermy as a plesiomorphic trait. However, in contrast to most other heterotherms torpor use of *G. moholi* implies that individuals of this species usually stay normothermic, exploiting the advantages of year-round reproduction, and torpor is only used in emergency situations.

The manifestation of heterothermy is diverse within mammalian species. An opposite extreme of heterothermy (in comparison to *G. moholi*) is shown by the Malagasy lesser hedgehog tenrec, *Echinops telfairi*. This species displays torpor every day during the hot-wet and the cold-dry season and normothermic phases seldom last more than 24h (Wein 2010). This extensive heterothermy is only abandoned during breeding (Lovegrove and Génin 2008) where phases of torpor are known to be disadvantageous (e.g., Fietz et al. 2004; Racey and Swift 1981; Wilde et al. 1999). Considering the position of *E. telfairi* within the basal Afrotherian clade, this pattern is regarded as the primitive expression of heterothermy (Lovegrove and Génin 2008). A similar pattern of heterothermy is found in the edible dormouse (*Glis glis)* that extends its hibernation phase by a period of summer dormancy in years with high predator density, coinciding with a skip of reproduction (Bieber and Ruf 2009). Most heterothermic species, however, show a more intermediate expression of heterothermy, displaying regular heterothermic phases as a seasonal adaptation. The extent of heterothermy can vary from species to species, within species or even for individuals of

one population, and for individuals during different years. Even within one family, differences in extent of and underlying causes for heterothermy can be remarkable, e.g., in the Malagasy Cheirogaleidae: In the fat-tailed dwarf lemur, *C. medius*, the length of the hibernation period in different populations was found to depend on habitat seasonality (Lahann and Dausmann 2011), whereas individuals of one population of the reddish-grey mouse lemurs, *M. griseorufus*, exhibit different heterothermic patterns (irregular short torpor bouts, regular use of daily torpor, prolonged torpor or hibernation) depending on individual body condition (Kobbe et al. 2011). For the grey mouse lemur, *M. murinus,* living in the dry forests along the west coast reduction in water turnover seems to be the driving force to enter torpor, rather than energetic bottlenecks (Schmid and Speakman 2009; Schmid and Speakman 2000). In the eastern rain forests, water is obviously not a scarce resource. Nevertheless, *M. murinus* also enter torpor and even hibernation in these habitats.

The consistent general characteristics of heterothermy found in distant species together with its deep embedding in the mammalian phylogeny, suggest a common origin of heterothermy within the mammal group with largely conserved traits. However, the underlying causes (routine seasonal strategy or response to acute energy emergencies) that elicit this physiological response, and thus the extent of expression and timing of heterothermy, seem to have evolved very differently in different groups, depending on the specific habitat and lifestyle of the species.

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J Comp Physiol (submitted)

Tables

Table 1: Ambient conditions for all three winter seasons. For each year mean ± SD of monthly minimum temperature (T_{amin}), monthly maximum temperature (T_{amax}) and sum of precipitation are given.

		2009	**2010**	**2011**
T_{amin} (°C)	June	4.0 ± 4.3	1.5 ±3.2	0.3 ± 2.9
	July	-0.8 ± 2.1	2.3 ± 2.6	-0.5 ± 2.1
	August	4.7 ± 4.5	2.6 ± 2.9	4.0 ± 3.4
T_{amax} (°C)	June	22.7 ± 4.1	21.3 ±3.2	24.5 ± 3.1
	July	20.6 ± 2.8	22.9 ± 2.5	23.0 ± 3.4
	August	24.3 ± 3.8	27.3 ±3.9	26.7 ± 4.0
Precipitation (mm)		101.5	0.0	97.3

J Comp Physiol (submitted)

Table 2: Detailed description of torpid animals found in this study. For each animal age, sex, body mass, torpor bout duration (TBD) and minimum skin temperature (T_{skin}) are stated. Source of data, month and frequency of occurrence are specified.

Ad, adult; MR, metabolic rate; juv, juvenile; r.d., rewarming difficulties; sb, subadult.

Animal			Measuring condition	Data source	Month	Frequency	Mean body mass (g)	TBD (min)	Minimum T_{skin} (°C)
M1	sb	Male	Free ranging	Radio collar	July	Repeated	165	225	24.2
M2	ad	Male	Free ranging	Collar logger	July	Repeated	170	660	19.5
F1	sb	Female	Free ranging	Collar logger	July	Repeated	160	r.d./died	19
J1	juv	n.a.	Laboratory	Collar logger & MR	April	Once	95	240	25
J2	juv	Male	Laboratory	Collarl ogger & MR	July	Once	105	r.d.	13.7
J3	juv	Female	Laboratory	Collar logger & MR	June	Once	113	r.d./died	14.9
J4	juv	Male	Laboratory	Collar logger & MR	July	Once	128	160	27.9
M3	sb	Male	Laboratory	Collar logger & MR	Aug	Once	140	360	23.4
F1	sb	Female	Laboratory	Collar logger & MR	April	Once	173	440	21.2

J Comp Physiol (submitted)

Table 3: Torpor parameters of *G. moholi*. Total sample size is 9 (3 free ranging individuals (only T_{skin}) and 6 individuals used in laboratory measurements (T_{skin} and MR)). All torpor incidences occurred between April and end of August.

	Mean values	Range	N
Mean minimal T_a (°C)	6.0 ± 6.5	-1.9 - 14.7	9
Mean body mass (g)	139 ± 30	95 - 170	9
Mean T_{skin} normothermic (°C)	34.9 ± 1.1	33.3 - 36.7	9
Mean diurnal MR normothermic ($mlO_2g^{-1}h^{-1}$) Measured between 0600-1800 hours	1.5 ± 0.2	1.2 - 1.9	6^b
Mean minimal T_{skin} (°C) For individuals with more than one torpor bout absolute minimal T_{skin} was used.	21 ± 4.7	13.7 - 27.9	9
Mean TBD (min)	348 ± 183	160 - 660	6^a
Mean minimal TMR ($mlO_2g^{-1}h^{-1}$)	0.3 ± 0.2	0.1 - 0.6	6^b
Mean average TMR ($mlO_2g^{-1}h^{-1}$)	0.6 ± 0.2	0.4- 0.9	4^c
Mean energy saving (%) comparison of average TMR to average MR (night + day) during normothermia	66 ± 9	59 - 76	4^c
Mean maximum heating rate (°C min^{-1})			
Active (complete torpor bout)	0.16 ± 0.09	0.10 - 0.33	6^a
Active (rewarming difficulties)	0.06 ± 0.04	0.03 - 0.11	3
Passive	0.06 ± 0.03	0.03 - 0.10	4

[a] N= 3 individuals had substantial difficulties rewarming from torpor and did not show complete rewarming.

[b] N= 6: laboratory measurements (T_{skin} & MR)

[c] 2 individuals had substantial difficulties rewarming from torpor during laboratory measurements.

Figure Legends

Figure 1: Seasonal variation in a) body mass and b) testes size of adult male *G. moholi.* Sample sizes are given in brackets. Significant differences are indicated with stars: * = p< 0.05.

Figure 2: Occurrence of torpor entry (*black bars*) and arousal (*white bars*) from torpor in relation to ambient temperature (T$_a$; *dotted line;* exemplary day in July 2011).

Figure 3: Minimal skin temperatures of torpid individuals. Individuals with skin temperatures ≤19°C had substantial difficulties to rewarm from torpor.

Figure 4: Comparison of phases of passive and active heating for a) a complete torpor bout and b) an incomplete torpor bout of an animal that had substantial troubles to rewarm itself from torpor. *Grey area* VO_2, *black line* skin temperature (T$_{skin}$), *dashed line* surrounding ambient temperature (T$_a$), *dashed area* phase of passive heating, *arrows* example of active heating.
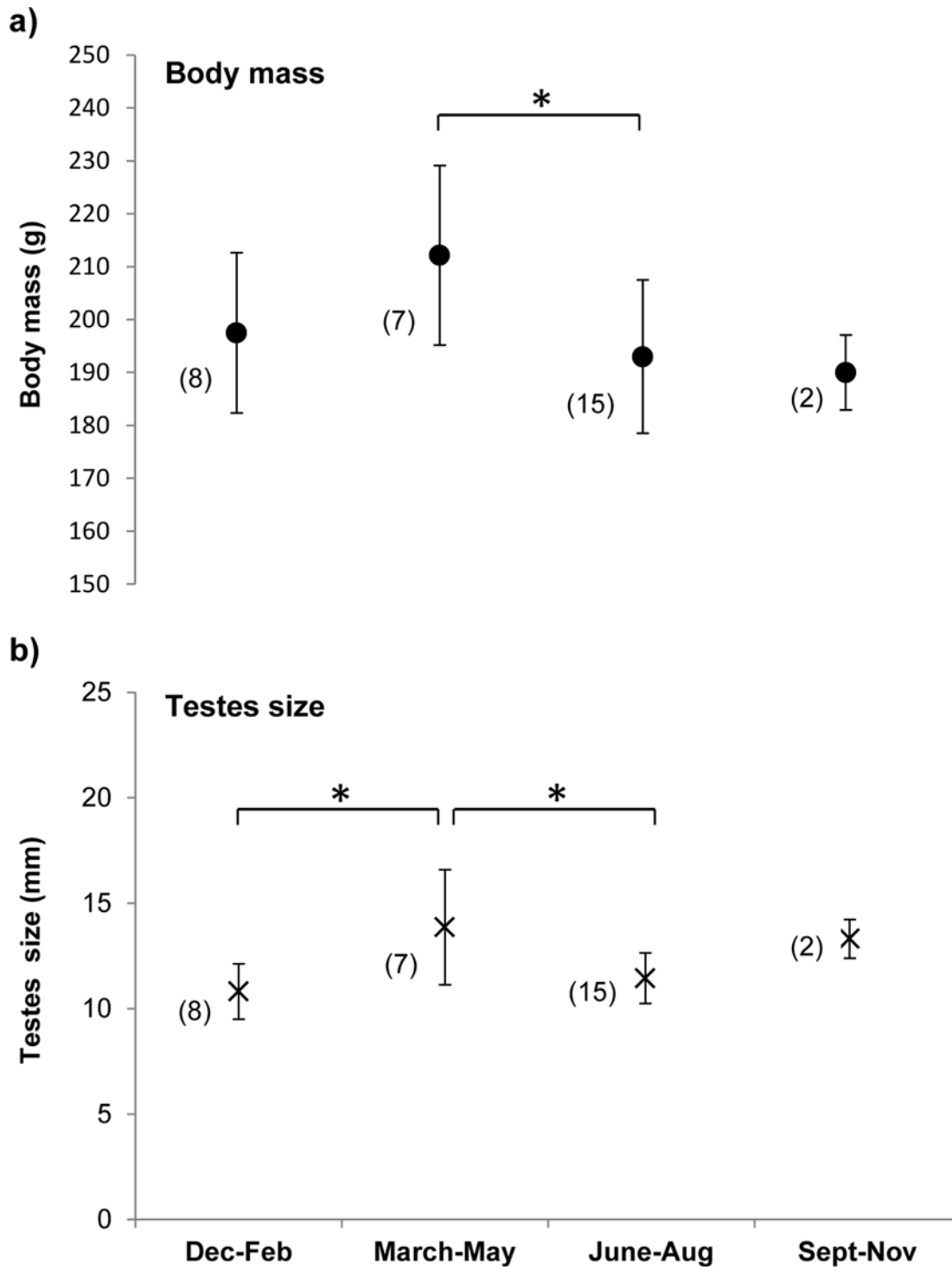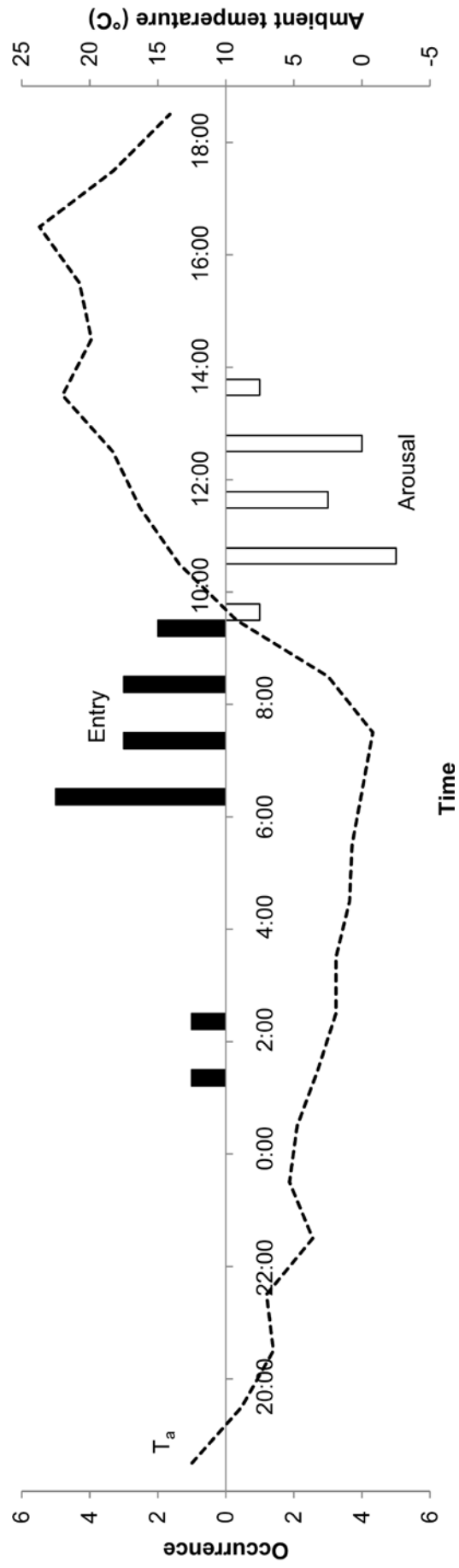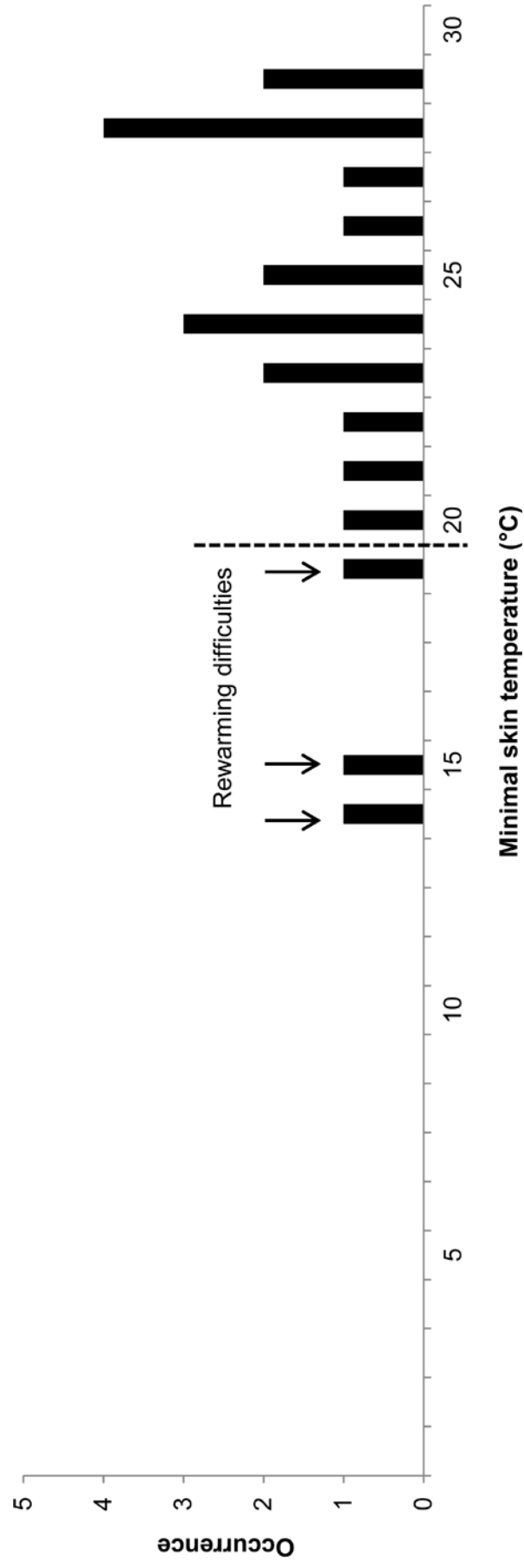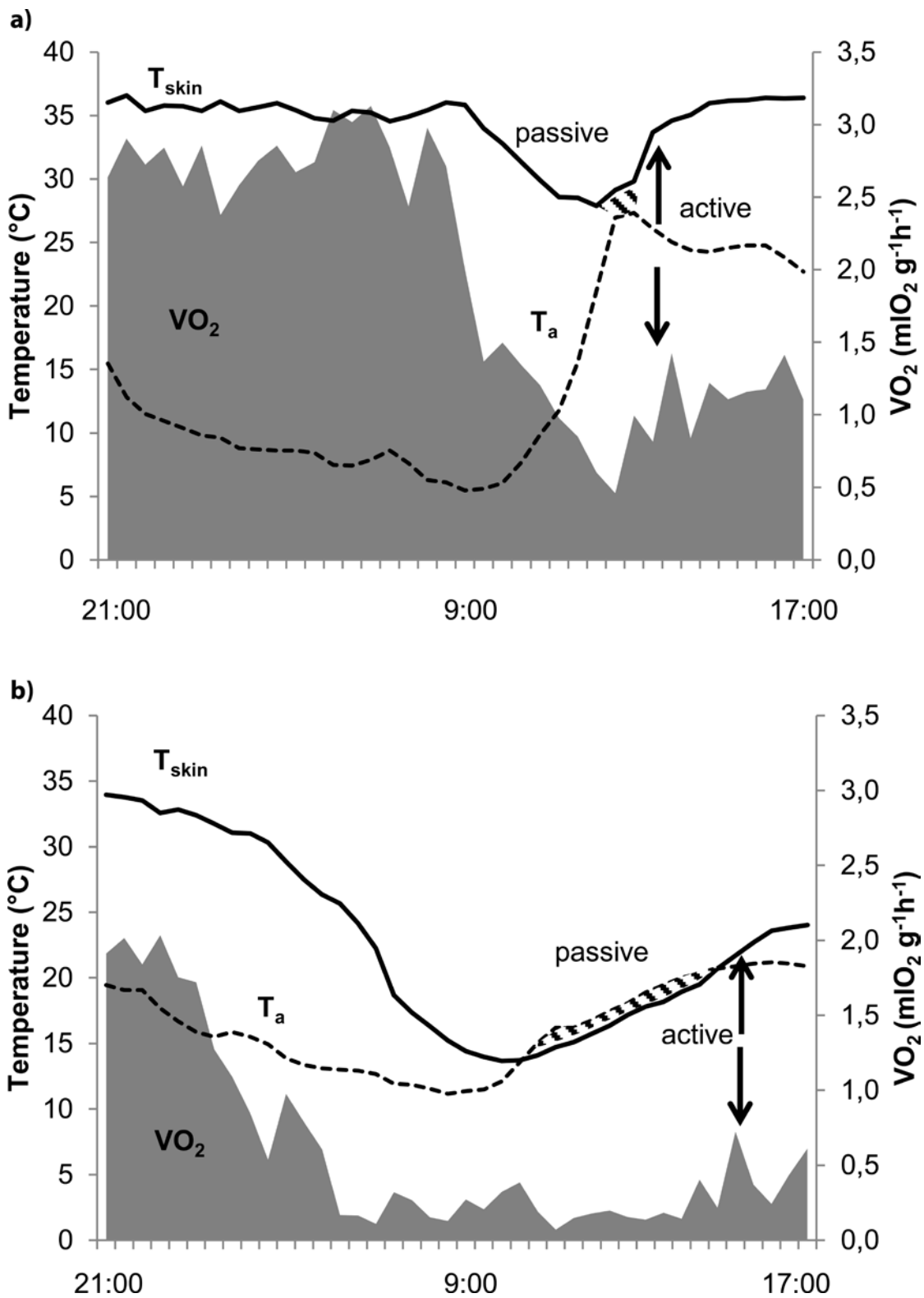
Figure 1

Figure 2

Figure 3

Figure 4

Non-shivering thermogenesis in the African lesser bushbaby *Galago moholi*

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Abstract

During long-term cold exposure small endothermic mammals either abandon homeothermy and enter torpor or hibernation, or need to increase their heat production via non-shivering thermogenesis (NST) to maintain a constant body temperature. The African lesser bushbaby, G*alago moholi*, enters torpid states as an emergency response only, but otherwise stays normothermic throughout the cold and dry winter season. Since this species shows unusual rewarming difficulties during arousal from torpor on days with cold ambient temperature, we examined the seasonal adjustments of the capacity of NST of naturally acclimatized *G. moholi* by stimulation with noradrenaline (NA) injection. Our study revealed that *G. moholi* exhibits seasonal changes in NST capacity, indicating a seasonal increase of UCP1-mediated thermogenesis in brown adipose tissue. NA treatment (0.5 mg/kg, s.c.) induced an elevation in oxygen consumption compared to control (saline) injection. However, the increase in oxygen consumption following injection of NA was only 1.3-fold higher in winter compared to summer and therefore seasonal difference was comparatively low, and the increase in capacity occurred only on especially cold days. Our results show that the ability to produce heat via NST seems to be available throughout the year and that *G. moholi* is able to increase NST capacity within a very short time frame in response to unpredictable cold spells. Together with results from studies on other tropical heterotherms which also indicate low seasonal difference in NST capacity, this raises the question of whether the definition of NST needs to be refined.

Abbrevations

BAT – brown adipose tissue

BMR – basal metabolic rate

NA - noradrenaline

NST – non-shivering thermogenesis

SA - saline

T_a – ambient temperature

T_{amin} – minimum ambient temperature

T_{skin} – skin temperature

UCP1 - uncoupling protein 1

Introduction

Endothermic animals are able to maintain a constant body temperature almost regardless of ambient conditions which is presumed to enable them to inhabit a variety of habitats and stay active during changing conditions (McNab, 1978). Behavioral adaptations such as change of resting site (Entwistle et al., 1997) or body posture (Øritsland, 1970), huddling in groups (Gilbert et al., 2010) or generating muscle heat through locomotion (Zerba and Walsberg, 1992) help endothermic animals to deal with moderate cold. However, during acute cold exposure endotherms have to increase their metabolic rate to defend their constant body temperature (Carey et al., 2003; Lyman et al., 1982). As a reaction to short-term cold exposure an endothermic animal will increase heat production through shivering thermogenesis (reviewed in Cannon and Nedergaard, 2011). Asides from that, animals which possess brown adipose tissue (BAT) adapt to long-term cold exposure with non-shivering thermogenesis (NST) and only minimal muscular activity. NST occurs in BAT of mammals where the uncoupling protein 1 (UCP1) alters proton conductance in the inner mitochondrial membrane and leads to heat generation instead of ATP production (reviewed in Cannon and Nedergaard, 2004; 2011). Producing heat through NST is of special importance in the thermoregulation of small mammals and has also been described as an important mechanism during arousal from hibernation and torpid states (Cannon and Nedergaard, 2004).

The NST capacity of an animal can be established through measurement of the increase of metabolic rate (oxygen consumption; VO_2) following noradrenaline (NA) injection, which stimulates UCP1-mediated thermogenesis in the BAT (Jansky, 1973; Wunder and Gettinger, 1996). To differentiate between classical NST and an unspecific increase of VO_2 due to activation of NST-unrelated receptors, true NST is generally defined by significant changes in seasonal NST capacity (warm vs. cold acclimatization in the laboratory) as suggested by Cannon and Nedergaard (2004). This definition has worked well for temperate mammals, but might have its limitations when working with tropical heterotherms that are exposed to somewhat different seasonal temperature differences (high daily fluctuations instead of constant temperatures). The NST capacity of several South African rodent species (e.g., Haim and Izhaki, 1993; Hislop and Buffenstein, 1994; Lovegrove et al., 1991; Richter et al., 1997) as well as NST capacity of the smaller members of the Afrotheria (Mzilikazi et al., 2007; Mzilikazi and Lovegrove, 2006; Oelkrug et al., 2012; Scantlebury et al., 2008) has been intensively studied. However, there is virtually nothing known about the NST capacity of any of the primate species. The only studies available so far have found classical NST in captive grey mouse lemurs, *Microcebus murinus* (Génin et al., 2003), and in captive common marmosets, *Callithrix jacchus* (Rothwell and Stock, 1985). However, laboratory studies of

NST conducted under constant cold conditions might not be indicative of the natural physiological response in species inhabiting tropical habitats.

The African lesser bushbaby (*Galago moholi)* is a small (~200 g) nocturnal primate that inhabits highly seasonal habitats in southern Africa and only enters torpid states as an emergency response to acute food shortage during pronounced cold periods (Nowack et al., 2010). In this species, heating rates during arousal from torpor were found to be almost 50 % lower than expected on the basis of body mass (Nowack, Mzilikazi and Dausmann, unpub. data) and animals with body temperatures below a certain threshold during torpor showed substantial difficulties rewarming. This finding raises the question whether *G. moholi* possesses the capacity for NST. So far NST was found in all small-sized, non-marsupial heterotherms and the finding of a tropical heterothermic mammal without the capacity for NST would be contrary all expectations. We therefore examined NST capacity of naturally acclimatized *G. moholi* by stimulation with NA injection and tested for significant seasonal differences between summer and winter NST to answer the question if rewarming difficulties could be due to a lack of NST.

Material and Methods

Study site & ambient conditions

We conducted our fieldwork at the Nylsvley Nature Reserve (South Africa, Limpopo, 24° 38.802'S 28° 40.095'E, altitude: 1100 m), which is a semi-arid (mean annual rainfall: 700 mm; Limpopo Parks data for Nylsvley), mixed bushveld habitat with the hot-wet season from October to March, and the cool-dry season from April to September. Monthly mean of daily minimum temperature (T_{amin}) ranges from –1 °C (July) to 17 °C (January; pers. obs. J.N. in 2009-2011). We recorded ambient temperature (T_a) during the field seasons every 30 min within the known home ranges of *G. moholi* using humidity and temperature loggers (resolution 0.0625 °C; Hygrochron iButton/DS1923, Maxim, Dallas Semiconductor, USA).

Capture and Handling

We captured *G. moholi* during summer 2010/11 and winter 2011 using self-made walk-in live traps, baited with banana, honey and peanut butter. All animals were sexed, measured, weighed, classified in age and reproductive status and individually marked with subcutaneously injected passive identification transponders (ID100 Trovan, EURO I.D. Usling GmbH, Weilerswist, Germany; for more details see Nowack et al., 2010). No female individuals were used in experiments (due to high numbers of pregnant females throughout the year) and we only worked with reproductively active (developed testes) males with a body mass of at least 160 g (summer: N= 7, winter: N= 10). Captured animals were held in

an outside enclosure (180.5x61.5x193.5 cm) for some days (<1 week; one at time). The enclosure was equipped with a wooden nest box and branches, and animals were fed with bananas, gum (when available) and water *ad libitum*. All measurements were conducted in a field laboratory. We released individuals at the exact capture location after measurements.

Determination of non-shivering thermogenesis

The NST capacity of *G. moholi* was obtained through measurement of the increase in VO_2 (as a proxy for metabolic rate) following NA injection (Jansky 1973, Wunder and Gettinger 1996). On each day of measurement, we removed food from the enclosure at least 3 h prior to commencement of data measurement to ensure that the animals were post-absorptive. All measurements were conducted during the rest-phase at daytime between 10am and 3pm. VO_2 was measured for 2 h before NA (1 mg ml^{-1}) or saline solution (0.9 % NaCl; control) was injected subcutaneously. Because of high weight loss in captivity (~10 % in four days, data from previous experiments), we did not establish a dose response curve to calculate the optimal dose of NA. Instead we used 0.5 mg kg^{-1} NA or control, respectively.

We continued VO_2 measurements for 2 h after each NA injection and for 1 h after each control injection. Each animal was measured with NA and control solution on two consecutive days.

Measurement of VO_2

Animals were individually placed in an 8 l respirometry chamber located in a portable thermal cabinet (35x66x44 cm) with a translucent part in the lid for behavioural observations. T_a inside the thermal cabinet was maintained using a self-built water bath, driven by an aquarium heater, pump and processor fan. The water was pumped from the water bath through copper pipes inside the chamber, thereby ensuring that T_a during measurements was within the range of the thermoneutral zone (28-35 °C; Dobler, 1978) to guarantee basal energy expenditure. T_a inside the thermal cabinet was monitored with a thermometer inserted into the box and a temperature logger (iButton). Energy expenditure was determined by measuring rate of VO_2 with open flow-through respirometry using a portable oxygen analyser (FoxBoxC, Sable Systems International, USA). The metabolic chamber was connected to the oxygen analyser with airtight tubes and air was pumped through the system (pull mode). CO_2 and water vapour were scrubbed prior to analysis of VO_2 using soda lime and silica gel. We measured sample air for 25 min alternating with 5 min reference air (baseline). For each measurement bout, the mean VO_2 was calculated from ~90 % of the values (sampling frequency every 10 sec) which represented the most stable readings in the cycle, using the data acquisition program Expedata (Sable Systems International, USA; for detailed

description see Nowack et al. 2010). For each individual, we determined average basal metabolic rate (BMR) as the mean of all consecutive VO_2 mean values within the first 2 h of both measurements (control and NA trial). Control and NA response were measured as the maximum mean VO_2 response (15 min around the highest peak) within the first hour of injection.

During all measurements of VO_2, skin temperature (T_{skin}) of animals was measured simultaneously with temperature loggers (Weetag collars, 2.6 g; Alpha Mach Inc., Mont St-Hilaire, Canada, resolution 0.0625 °C; for improved precision calibration curves provided by the manufacturer were used) that were programmed to measure T_{skin} every 10 min.

We compared the highest T_{skin} values after treatments (control/NA; timespan of 30 min) with the average T_{skin} values during BMR.

Data analyses

Statistical analyses were performed with SPSS (PASW Statistics 18, 2009). All values are means ± SD. Data were analysed with non-parametrical tests due to skewness of distributions. Seasonal differences in BMR, reaction to control injection and NST capacity were tested with Mann-Whitney-U test for independent variables. To compare the different measurements obtained for the same animals (VO_2 BMR, VO_2 NA, VO_2 control), we conducted Friedman tests for repeated measures. Multiple pairwise comparisons were made using Wilcoxon tests as post hoc analyses. Reaction to control as well as NST capacity was calculated as the net increase in metabolic rate caused by injection (VO_2 NA/control - VO_2 BMR). We further compared the increase of VO_2 after control and NA to levels of BMR. This comparison was done with absolute values including BMR (VO_2 NA/control + VO_2 BMR). Data are given as mass specific values ($mlO_2g^{-1}h^{-1}$).

Results

BMR and body mass

The mean body mass of all individuals during summer was 181 ± 18 g (N= 7) and did not differ from body mass during winter (N= 10; Tab. 1). Mean BMR was 0.6 $mlO_2g^{-1}h^{-1}$ and did not differ significantly between seasons (N= 7/10; Tab. 1).

Response to injections: T_{skin} & behavior

All individuals showed a behavioral response to NA injection, regardless of season. Individuals did not settle down into a resting position after injection, but showed a significantly increased activity. Maximum reaction to injection was reached after 20-40 min. For most individuals the following behaviors were noted: legs stretched out, lying down on

back, fast breathing, panting, sweating, salivation. All animals increased T_{skin} after NA injection by more than 1 °C in both seasons. After control injection animals curled up again and did not show any visible behavioral reaction to injection. T_{skin} after control injection was only marginally increased (mean increase <0.3 °C).

Capacity of NST

Controlling for ambient temperature

For determination of NST capacity in the laboratory, animals are usually acclimated at two distinct temperature regimes that differ by at least 10 °C (e.g., Nespolo et al., 2001). In this study, however, we examined the capacity of NST in naturally acclimatized animals. When we plotted the mean T_as of the three days before the NA injections (Fig. 1), we found that these acclimatization T_as were not clearly distinct between the summer and winter measurements, but showed an overlapping between T_as of 15 °C and 20 °C. NST is a highly plastic variable and is thought to change within days of acclimatization (e.g., Jansky 1973, Wunder 1985). To prevent confounding effects created by environmental temperature fluctuations we excluded the five data points that lay in an overlap temperature zone. After this, ambient conditions (mean T_as and T_{amin}s) for summer and winter measurements were markedly discrete (winter: T_{amin} <5 °C, T_a mean < 15 °C; summer T_{amin} >15 °C, T_a mean >20 °C; Fig. 1). We analysed the data for both approaches (all data points/ controlled data) and compared the results.

Analysis of all data points

Increase in VO_2 did not differ significantly between seasons for either control treatment or NST capacity, when using all data points for analyses (N= 7/10; Tab. 1). Since neither BMR (see above) nor any of the treatments (control/NA) showed any seasonal differences we pooled both seasons to test differences between BMR, NST and control. Friedman test revealed significant differences between these parameters (N= 17, X^2= 34.000, df= 2, p< 0.001). Pairwise Wilcoxon tests showed that handling/ injection had a significant effect on VO_2 as metabolic response to control injections differed significantly from BMR (Z= -3.622, p< 0.001). However, NA injections lead to significantly higher metabolic responses compared to BMR (Z= -3.622, p< 0.001) and control treatment (Z= -3.621, p< 0.001).

Analysis of controlled data

Analyses of the reduced data set (N= 6/6) did not reveal notably altered results for body mass, BMR or reaction to control measurement (Tab. 1). Body mass did not statistically differ between seasons, neither did the increase in VO_2 for BMR or control measurement (Tab. 1).

However, in contrast to the analyses of all data points, NST capacity was significantly higher for winter after temperature correction (N= 6/6; U-test: Z= -1.281, p= 0.041; Fig. 2). The controlled winter NST capacity of 1.34 $mlO_2g^{-1}h^{-1}$ was very close to that predicted on the basis of body mass of 1.32 $mlO_2g^{-1}h^{-1}$ (Mzilikazi and Lovegrove, 2006).

The metabolic response to control and NA injection in comparison to BMR was not affected by omission of data points when tested independently for summer and winter. As for the analyses of all data points, Friedman test revealed significant differences between these parameters (Friedman tests, winter/summer, both: N= 6, X^2= 12.000, df= 2, p< 0.001; results of Wilcoxon tests: Tab. 2). T_{skin} showed an increase of 0.1 °C (summer) and 0.4 °C (winter) after control injection in both seasons, but increased by 0.9 °C (summer) and 1.7 °C (winter) after NA injection.

Discussion

This study shows that *G. moholi* exhibits seasonal changes in NST capacity when T_a is sufficiently low, indicating a winter increase of UCP1-mediated thermogenesis in BAT. Seasonal alteration of NST capacity is accompanied by a maintainance of a basal level of BMR throughout the year that does not significantly change between seasons in *G. moholi*. Winter acclimatized animals showed a 3.5-fold increase of VO_2 in comparison to BMR, and NST capacity is consistent with predicted capacity using the equation by Mzilikazi and Lovegrove (2006).

However, seasonal adaptation of NST was found to be comparatively low (only 1.3-fold increase compared to summer NST) and seasonal changes in NST capacity could only be described after correcting for T_a during acclimatization. Studies on a temperate zone mammal, the broad-toothed field mouse (*Apodemus mystacinus*) found a threefold increase in winter NST compared to summer (Haim and Yahav, 1982), whereas the seasonal increase in NST in the Afrotherian western rock elephant shrew (*Elephantulus rupestris*) was also only 1.8-fold (Oelkrug et al., 2012).

Potential reasons for the comparatively low seasonal difference in NST capacity are varied. As the NST capacity during winter corresponds with the predicted value based on body mass, we can assume that the NST capacity in summer is comparably high. *G. moholi* was found to use torpor as an emergency solution in response to acute food shortage only (Nowack et al. 2010). So far torpid states have only been observed during the cold-dry period, when food availability is naturally low, but it is conceivable that emergency torpor bouts can be entered at any time. As NST is needed for rewarming from torpor (Cannon and Nedergaard, 2004; Jefimow et al., 2004) this could explain the elevated NST (and the subsequent low seasonal difference) throughout the year and further explains why *G. moholi*

only needs to alter NST capacity in especially cold phases. This result shows that *G. moholi* can increase the degree of NST capacity within a short time frame. In the habitat of *G. moholi* winter ambient conditions show only a very short pronounced cold period with high day to day variations in T_{amin} (pers. obs. J.N.) and various authors have stated the importance of short activation times for NST (Jansky, 1973; Wunder, 1985). This variability makes NST a highly plastic variable. Short activation times of NST were found in several studies comparing warm-acclimated with cold-acclimated individuals, e.g., Génin et al. (2003) found recruitment of UCP1 within 12 h in *Microcebus murinus* exposed to 7 °C in the laboratory. However, to our knowledge, the ecological significance of this finding was missing so far, as no study investigated variation of NST capacity within one season in animals exposed to their natural climate conditions.

Our results clearly show that *G. moholi* has the capacity for NST and rewarming difficulties from torpor found in this species are not due to a lack of this mechanism, but were suggested to be due to depletion of energy reserves as torpor was induced by food restriction (Nowack, Mzilikazi and Dausmann, unpub. data).

Generally, the expression of NST in tropical mammals seems to be equivocal and not all studies on tropical species found significant seasonal differences in NST capacity (e.g., not found in: *Aethomys namaquensis* (Rodentia): Lovegrove et al. 1991 and *Elephantulus myurus* (Afrotheria): Mzilikazi et al. 2007). However, the lack of seasonal differences in NST capacity are not necessarily equivalent to a lack of classical NST, as the study by Mzilikazi et al. (2007) did demonstrate the presence of functional BAT in *E. myurus.* Oelkrug et al. (2012) suggested that the lack of seasonal adaptive thermogenesis (indicating a fixed/constitutive NST capacity) in the basal Afrotheria *E. myurus* could be linked to adaptive heterothermy associated with unpredictable environments, whereas other studies on basal Afrotheria did find seasonal adjustments comparable to that known from Holoarctic rodents (Oelkrug et al., 2012; Scantlebury et al., 2008).

Alternatively, low seasonal difference in NST may also be accounted for by low seasonal differences in temperature combined with a high degree of social nesting behavior. Ambient conditions change markedly between seasons within the habitat of *G. moholi*, however, daily maximum temperatures during the winter generally lie between 20 °C and 25 °C (pers. obs. J.N.). For a short period of time nightly temperatures can decrease as low as -5 °C (Nowack et al., 2010) during early winter mornings, but an increased fur insulation (pers. obs. J.N.), reduced activity times and increased huddling (Nowack et al., in press; Bearder and Martin, 1980) might prevent the need for highly elevated NST. Haim et al. (1991) found a pronounced seasonal NST capacity for *Saccostomus campestris* living within the same temperature regime as *G. moholi*. However, this species regularly enters torpid

states, but is solitary without the possibility of staying warm through huddling. There are at least two studies on non-tropical species that found no significant difference between summer and winter NST [(*Discrostonyx groenlandicus* (Rodentia): Maier and Feist (1991); *Ochotona curzoniae* (Lagomorpha): Wang et al. (1999)]. Both studies were conducted on species living in groups in shallow burrows or deep burrow systems with reduced seasonal variation. Correspondingly, Merritt et al. (2001) found that squirrels nesting individually experienced a longer period of elevated winter NST and an overall increase in mean NST throughout all months in comparison to squirrels nesting socially. Interestingly, most species lacking seasonal adaptations in NST capacity (*G. moholi, D. groenlandicus, O. curzoniae, A. namaquensis)* live in groups (at least during the cold winter), except *E. myurus*.

The problem with defining classical NST

Injection of NA does not only lead to classical NST, but can also lead to activation of NST-unrelated receptors in species lacking BAT, like birds (reviewed in Cannon and Nedergaard, 2004). Studies in pigeons (*Columba livi*), for example, found an increased metabolic response to NA injection. Interestingly and in contrast to adaptive classical NST that should increase during cold periods, metabolic reaction to NA injection was significantly higher in warm acclimated birds than in cold acclimated birds (Hissa et al., 1975). Consistently, Cannon and Nedergaard (2004) argue that true NST can only be demonstrated by a comparably higher increase in thermogenesis in cold acclimated animals (i.e. seasonal adaptive thermogenesis), a method used in numerous studies.

However, a study on UCP1-ablated mice showed that individuals without functional UCP1 do not statistically differ in their reaction to control or NA treatment (Golozoubova et al., 2001), suggesting that NST capacity can also be defined on the basis of a comparison between reaction to control (or BMR) and NA injection. Furthermore, marsupials naturally lack functional UCP1 and are therefore an ideal group to investigate the validity of the aforementioned approach. Studies on NST capacity of marsupials generally failed to report any metabolic response to NA injection (e.g., Nicol et al., 1997; Opazo et al., 1999). However, UCP1 independent NST capacity as reaction to NA or β3-Adrenergic agonist was found in some Tasmanian species of the order Macropodoidae and one species of Dasyuridae (Kabat et al., 2003; Nicol, 1978; Rose et al., 1999). This reaction also differed from classical NST response. NA injections in mammals generally lead to a VO$_2$ increase in cold-acclimated mammals, but also to an increase in warm-acclimated mammals (even if significantly lower). In the Tasmanian species, which showed a reaction to NA injection, VO$_2$ was only significantly elevated in cold-acclimated but not in warm-acclimated animals (Kabat et al., 2003; Rose et al., 1999).

We therefore suggest refining the definition of classical NST. Cannon and Nedergaard (2004) recommended a comparison between the reactions to NA injection in warm- vs. cold- acclimated animals and defined classical NST through the occurrence of seasonal differences. However, comparison of the reaction to NA-injection in warm- as well as cold-acclimated animals does not only reveal information about classical NST, but also about seasonal adaptations, and these two compounds might not always be separable. Comparison of seasonal NST measured under natural temperature and light regime provides information about NST plasticity rather than defining classical NST. In the case of species living in areas without distinct seasonal temperature differences, the results obtained from naturally acclimatized animals may even lead to incorrect conclusions. On the other hand, strictly laboratory based studies performed under constant light and temperature regimes will most probably be able to reveal if a species is able to alter its NST capacity in response to temperature changes, but will not allow for the description of the natural seasonal plasticity of the species. Therefore, we suggest that NST capacity in mammals should rather be defined by comparing the reaction between control and NA injection for both warm and cold acclimatized animals in their natural habitat. Classical NST will elicit a significant increase in VO_2 in both acclimatized groups, whereas animals without classical NST will either not show any reaction at all, or only for one of the acclimations (as seen in Tasmanian marsupials).

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Tables

Table 1: Seasonal comparison of body mass, basal metabolic rate (BMR), VO$_2$ response to control (saline (SA) injection) and NST capacity for all measurements and controlled for acclimatization temperature. Results of Mann-Whitney-U tests for independent variables are stated with Z and p values.

		Summer	Winter	Statistic Z	p
All	N	7	10		
Measurements	Body mass (g)	181 ± 18	182 ± 17	-0.19	0.89
	BMR (mlO$_2$g^{-1}h^{-1})	0.59 ± 0.06	0.56 ± 0.06	-1.18	0.27
	SA (mlO$_2$g^{-1}h^{-1})	0.31 ± 0.15	0.40 ± 0.15	-0.98	0.36
	NST (mlO$_2$g^{-1}h^{-1})	1.12 ± 0.27	1.21 ± 0.22	-0.88	0.42
Controlled for	N	6	6		
acclimatization temperature	Body mass (g)	184 ± 16	182 ± 20	-1.45	0.18
	BMR (mlO$_2$g^{-1}h^{-1})	0.59 ± 0.06	0.55 ± 0.05	-0.96	0.39
	SA (mlO$_2$g^{-1}h^{-1})	0.30 ± 0.16	0.47 ± 0.16	-1.61	0.13
	NST (mlO$_2$g^{-1}h^{-1})	1.07 ± 0.25	1.34 ± 0.19	-1.28	0.04

Table 2: Significant levels for changes in O$_2$ consumption based on pairwise Wilcoxon tests for all three treatments (basal metabolic rate (BMR); saline (SA) injection as control; noradrenaline (NA) injection) after temperature correction.

		Summer	Winter
VO$_2$ BMR vs. VO$_2$ SA	Z=	-2.207	-2.201
	p=	0.031	0.031
VO$_2$ BMR vs. VO$_2$ NA	Z=	-2.207	-2.207
	p=	0.031	0.031
VO$_2$ SA vs. VO$_2$ NA	Z=	-2.201	-2.201
	p=	0.031	0.031

Figure legends

Figure 1: Controlling for acclimatization temperature.

NST capacity (VO_2) in correlation to mean acclimatization temperature (ambient temperature) during the three days before NST measurement. Mean ambient temperature during measurements showed an overlap area of about 5 °C between summer and winter (data points 1-5). These five data points were excluded in analysis of controlled data.

Figure 2: Oxygen consumption (VO_2) for BMR, response to control (saline) and noradrenaline (NA) injection during winter (*black bars*) and summer (*white bars*). All data are controlled for temperature: We excluded data point 1-5 and only included data points with clearly discrete ambient conditions for summer and winter measurements (winter: T_{amin} <5 °C, T_a mean <15 °C; summer T_{amin} >15 °C, T_a mean >20 °C) into analyses (summer N= 6; winter N= 6). VO_2 increase after saline and NA injection are statistically significant in comparison to BMR. NA reaction was significantly different between seasons. *= p< 0.05; ***= p< 0.001.
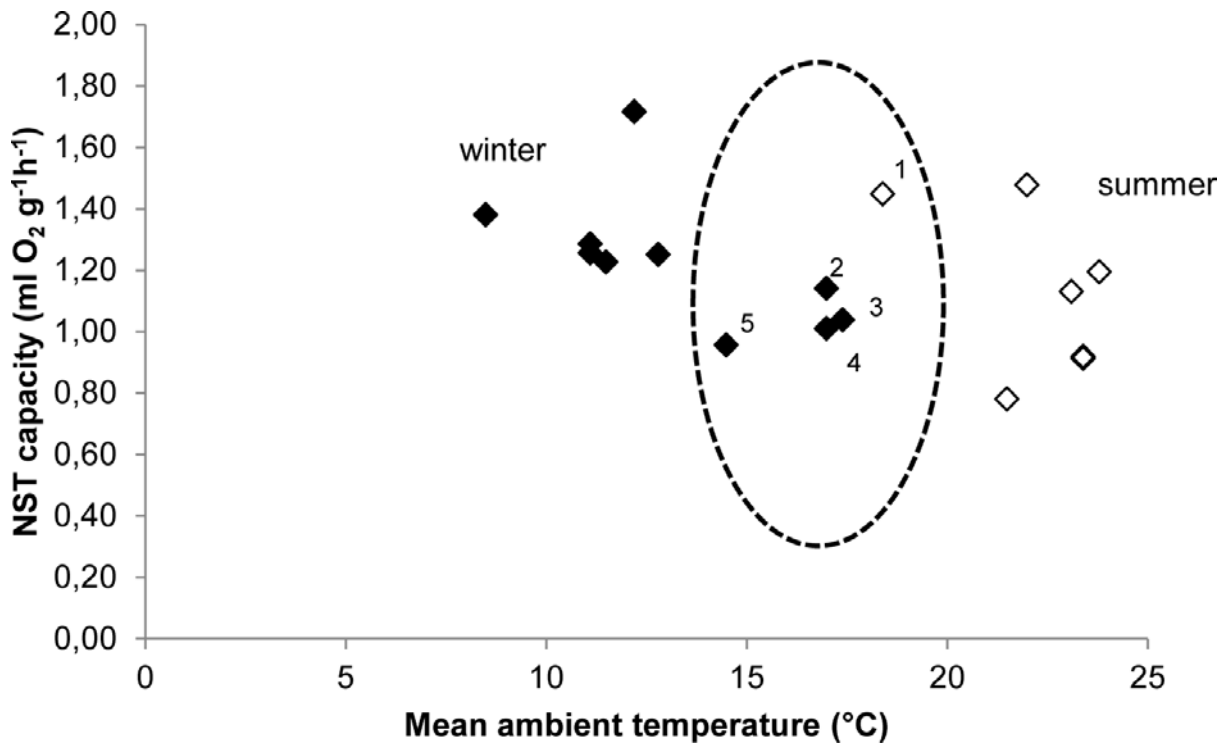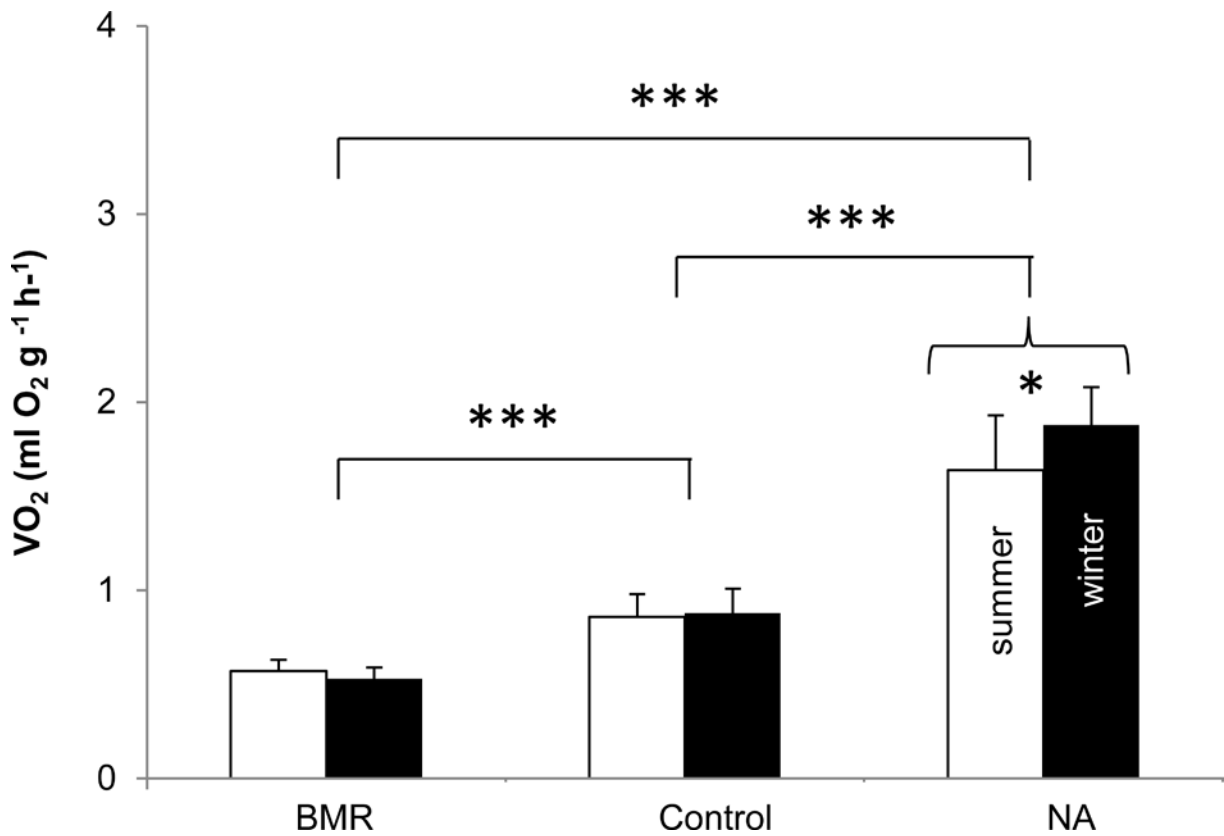
Figure 1

Figure 2

Chapter 2
Afrotropical Heterothermy: A Continuum of Possibilities

Kathrin H. Dausmann, Julia Nowack, Susanne Kobbe and Nomakwezi Mzilikazi

Abstract The three closely related primate species *Cheirogaleus medius, Microcebus griseorufus,* and *Galago moholi* employ a spectrum of thermoregulatory responses to environmental bottlenecks. *C. medius* is an obligate hibernator, *M. griseorufus* shows extreme flexibility in patterns of heterothermy, ranging from daily torpor to prolonged torpor and hibernation, whereas *G. moholi* becomes heterothermic under extremely adverse conditions only. To gain further insights into the proximate and ultimate factors that favour and constrain torpor use in small primates, we compared the characteristics of *M. griseorufus, C. medius* and *G. moholi* as well as the prevailing ambient conditions where each of the species are found. Our analyses did not reveal any fundamental differences in the physiological parameters of heterothermy between the three species that would indicate different underlying physiological mechanisms. Instead we propose that the different modes of reproduction, connected to climatic differences, are the key ultimate causes of the differences in thermoregulatory strategies between the three species. We suggest that the common ancestor of the strepsirrhine primates was a heterothermic endotherm and that the ability to express daily torpor/hibernation is closely linked to ecological and evolutionary forces that favour and prioritise activities such as reproduction.

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2.1 The Expression of Daily Torpor and Hibernation

The presence of heterothermy in unrelated mammalian groups has intrigued biologists, who have proposed two scenarios for the evolution of heterothermy: (1) the common ancestor of mammals was a heterothermic endotherm and subsequently many lineages lost the ability or propensity to express daily torpor/hibernation, or (2) heterothermy may have evolved repeatedly during the evolution of these lineages and are the result of convergent evolution. These opposing hypotheses are still under contention. For example, monotremes, the so-called primitive mammals, show evidence of 'proto-endothermy'—i.e., heterothermy is present and endothermy is facultative (e.g., Nicol and Andersen 1996), a pattern also observed in tenrecs (Lovegrove and Génin 2008; Wein 2010). This could be evidence that heterothermy is a plesiomorphic trait. More support for this theory comes from a model proposing that heterothermy was the ancestral condition from which homeothermy derived (Lovegrove 2011) and a maximum likelihood approach reconstructing the probability of heterothermy of late Mesozoic mammals, indicating a single origin of heterothermy in mammals (Lovegrove 2012). Furthermore, expression of heterothermy is flexible: within a single species, some individuals can display daily torpor, whereas others display hibernation (Kobbe et al. 2011). Studies on the ontogeny of torpor support the idea that heterothermy evolved more than once; Geiser (2008) suggested that heterothermy may be a derived trait in placental mammals because in some organisms, such as shrews and hamsters, poikilothermy (i.e., lack of effective endogenous temperature regulation) at birth is followed first by a homeothermic phase and the ability to employ torpor develops later. This ontogenetic sequence may signal the order of evolutionary change provided that 'ontogeny recapitulates phylogeny'. This argument, in fact, does not deny the possibility that heterothermy in endotherms has indeed evolved multiple times in several lineages (i.e., highly polyphyletic). Dietary habits and body mass correlate with the expression of torpor/hibernation in mammals (e.g., Geiser 1988). Although these factors cannot be viewed independently from phylogeny, these variables may provide a better explanation for the presence of heterothermy. Within the primates, most of what is known about heterothermic responses has been gained from studies on virtually a single family of Malagasy lemurs, the Cheirogaleidae (e.g., Dausmann et al. 2004; Kobbe and Dausmann 2009; Ortmann et al. 1997; Schmid et al. 2000) whose closest relatives (apart from Lorises) are the galagos from mainland Africa, to which they are comparable in terms of body size and life histories.

2.2 So Near Yet So Far

All habitats of the cheirogaleids in Madagascar, as well as of galagos in South Africa (Limpopo and Mpumalanga provinces), are characterised by seasonality and environmental unpredictability. Unpredictability results in periods of resource scarcity during the austral winter that are highly variable within and from year to year. Additionally, Madagascar forests have comparatively lower fruit production than

other tropical forests (Wright 1999). These circumstances pose great energetic constraints, especially to animals relying on highly seasonal high-energy food sources, e.g., small-bodied species with relatively high metabolic rates (MR). Given the relative geographical proximity of Madagascar and South Africa, as well the similarities relating to environmental unpredictability, it seems reasonable to expect comparable adaptations, such as the use of heterothermy, to similar environmental challenges. Admittedly, the geological history and biogeography of Madagascar, especially the long separation from the African mainland ($\sim$ 120 mya) may have led to a unique evolution of its mammals, creating an excellent natural experiment.

Currently, within the cheirogaleids heterothermy has been found in mouse lemurs (*Microcebus*), giant mouse lemurs (*Mirza*) and dwarf lemurs (*Cheirogaleus*) (Dausmann 2008; McKechnie and Mzilikazi 2011). A recent study has found torpor in a mainland primate, the African Lesser Bushbaby, *Galago moholi* (Nowack et al. 2010). Among these three closely related genera a spectrum of thermoregulatory responses is evident. *Cheirogaleus medius* is an obligate hibernator (Dausmann et al. 2004). *Microcebus griseorufus* shows extreme flexibility in patterns of heterothermy (Kobbe et al. 2011), whereas *G. moholi* utilises heterothermy under extreme conditions only (Nowack et al. 2010). Together these species provide an excellent opportunity for interrogating the proximate influences on the energy expenditure of small primates. It also raises the question of what the ultimate factors contributing to the reluctant use of torpor by *G. moholi* are. To gain further insights into the proximate and ultimate factors that favour and constrain torpor use in small primates, we compared the characteristics of *M. griseorufus, C. medius* and *G. moholi* as well as the prevailing ambient conditions where each of the species are found. The ultimate aim of this summary is to highlight similarities and the differences that have possibly led to the evolution of different thermoregulatory strategies in primates.

Throughout this summary, data were obtained from the literature and from three studies carried out by us over a time span between 3 and 6 years each. *C. medius* was studied in the forest of Kirindy C.F.P.F./Madagascar, *M. griseorufus* within Tsimanampetsotsa National Park/Madagascar and *G. moholi* in the Nylsvley Nature Reserve/South Africa. In the course of these studies demographic data were obtained by capture–recapture and individual marking. Behavioural data and data on food intake were recorded during nightly tracking of radio-collared individuals. Physiological measurements were performed using temperature-sensitive radio collars and loggers (skin temperature [T_{skin}], as a proxy of body temperature [T_b]), and portable gas analysers (measurement of MR as rate of oxygen consumption). For more detail see Dausmann et al. (2009), Kobbe et al. (2011), and Nowack et al. (2010).

2.3 Habitat and Life Histories

2.3.1 Taxonomy and General Species Description

All three species [*Galago* (Galagidae), *Microcebus* (Cheirogaleidae) and *Cheirogaleus* (Cheirogaleidae)] are strepsirrhine primates and very similar in their

morphological appearance, but belong to two different taxa: the lemuriformes, endemic to Madagascar, and the lorisiformes, found in Africa and Asia.

M. griseorufus is the smallest of the three species and it occurs in Southern Madagascar (Table 2.1). *G. moholi* occurs in Southern Africa and is slightly heavier than *C. medius* (Western Madagascar). All three species are nocturnal; *M. griseorufus* and *G. moholi* are vertical clingers and leapers, while *C. medius* is an arboreal quadruped.

2.3.2 Similar Habitats, Similar Diets

The habitats of all three species are distributed at comparable latitudes (around the Tropic of Capricorn) and share general habitat characteristics (Table 2.1). All three habitats are characterised by a marked dry season during the austral winter. The habitat of *M. griseorufus* is the most unpredictable of the three areas with an annual average rainfall of less than 500 mm (November–March) and years with almost no rainfall (Ratovonamana et al. submitted). The habitats of *C. medius* and *G. moholi* are less arid (Fig. 2.1); however, annual rainfall also varies highly in amount (from 300/400 mm to over 1,000 mm per year) and timing. Annual average rainfall is 700 mm in the habitat of *G. moholi* [Limpopo parks data (Nylsvley)]. The dry period is often only restricted to 3 or 4 months during mid-winter (Scholes and Walker 1993). Annual rainfall is higher (about 800 mm) for the habitat of *C. medius* (Fietz and Dausmann 2006), but the dry period is extended to 6 or 7 months per year (April–October).

Although the pattern of annual precipitation is most comparable for the habitats of *G. moholi* and *C. medius,* the biomes of *G. moholi* and *M. griseorufus* are more similar. While *C. medius* occurs in tropical dry forests regions, *G. moholi* and *M. griseorufus* inhabit savannah areas with thorny bushes and trees. Ambient temperature (T_a) also varies between the three habitats (Fig. 2.1) and the lowest temperatures are found in the habitat of *G. moholi*. During the hot-wet season T_a is fairly high during the day and seldom decreases below 15°C during the night, whereas night temperature during winter can reach −5°C (Nowack et al. 2010). Temperatures in South and Western Madagascar are about 10°C warmer during summer nights, but never decrease below 5°C during winter nights (Fietz and Dausmann 2006). Daily amplitudes between day and night are high in all three habitat types (*G. moholi* and *C. medius:* 2–35°C, *M. griseorufus*: 6–35°C).

The pronounced seasonality of all three habitats is not only reflected in a decrease in T_a and precipitation, but also in a decline in food and water availability during the dry season. The diet of *G. moholi* and *M. griseorufus* consists mainly of gum and small arthropods. *M. griseorufus* extends its food repertoire to fruits and nectar. Both species increase gum feeding during the winter months: insect and fruit abundances decrease noticeably in winter, whereas gum is available throughout the year (Bearder and Martin 1980; Génin 2008; Nowack et al. submitted). The diet of *C. medius* does not contain any gum, but consists of fruits, flowers, nectar, and a varying proportion of insects. *C. medius* feeds on sugary fruits before hibernation to build a sufficient fat reservoir to fuel energy demands during hibernation (Fietz and Dausmann 2006).

Table 2.1 Life history parameters for *G. moholi*, *M. griseorufus* and *C. medius*

	G. moholi	*M. griseorufus*	*C. medius*
Distribution	Southern Africa	South-western Madagascar	Western Madagascar
Habitat	Thornveld savannah	Spiny forest	Deciduous dry forest
Weight	~180 g	~50 g[a]	~150 g[b]
Sexual dimorphism in weight	Males heavier	Females heavier (before winter)	None[b]
Seasonal fattening	No	+ (Hibernation: 49–73 g, prolonged torpor: 47–53 g, daily torpor: 43–48 g, short torpor: 44–46 g)	++ (130–250 g)[b]
Body length	19 cm	15.5 cm[c]	20 cm
Diet	Gum, small arthropods[d,e]	Fruits, nectar, insects, small vertebrates, gum[g]	Insects, fruits, flowers, nectar[b]
Seasonal change in diet	Increases gum feeding during winter[e]	Increases gum feeding in winter by 33% of total foraging activity[f]	Eat sugary fruits before hibernation, no food during winter[b]
Gestation time	123 days[g]	52–60 days[f]	60–65 days[b]
Litter size	Mostly twins[g]	1–3, mostly twins[f]	Mostly twins[b]
Birth periods	January/February and September–November[g]	December and February/March[f]	December–February (every second year)[h]
Predation	Mongooses, genets, birds of prey (owls, eagles), snakes[i]	Birds of prey (mainly owls), snakes, civets	Birds of prey (owls, hawks), snakes, civets[b]
Estimated average field life span	3–5 years	3–5 years	5–7 years (up to over 12)

[a] Kobbe et al. (2011)
[b] Fietz and Dausmann (2006)
[c] Rakotondranary et al. (2011)
[d] Bearder and Martin (1980)
[e] Nowack et al. submitted
[f] Génin (2008)
[g] Pullen et al. (2000)
[h] Fietz and Dausmann (2003)
[i] Mzilikazi et al. (2006)
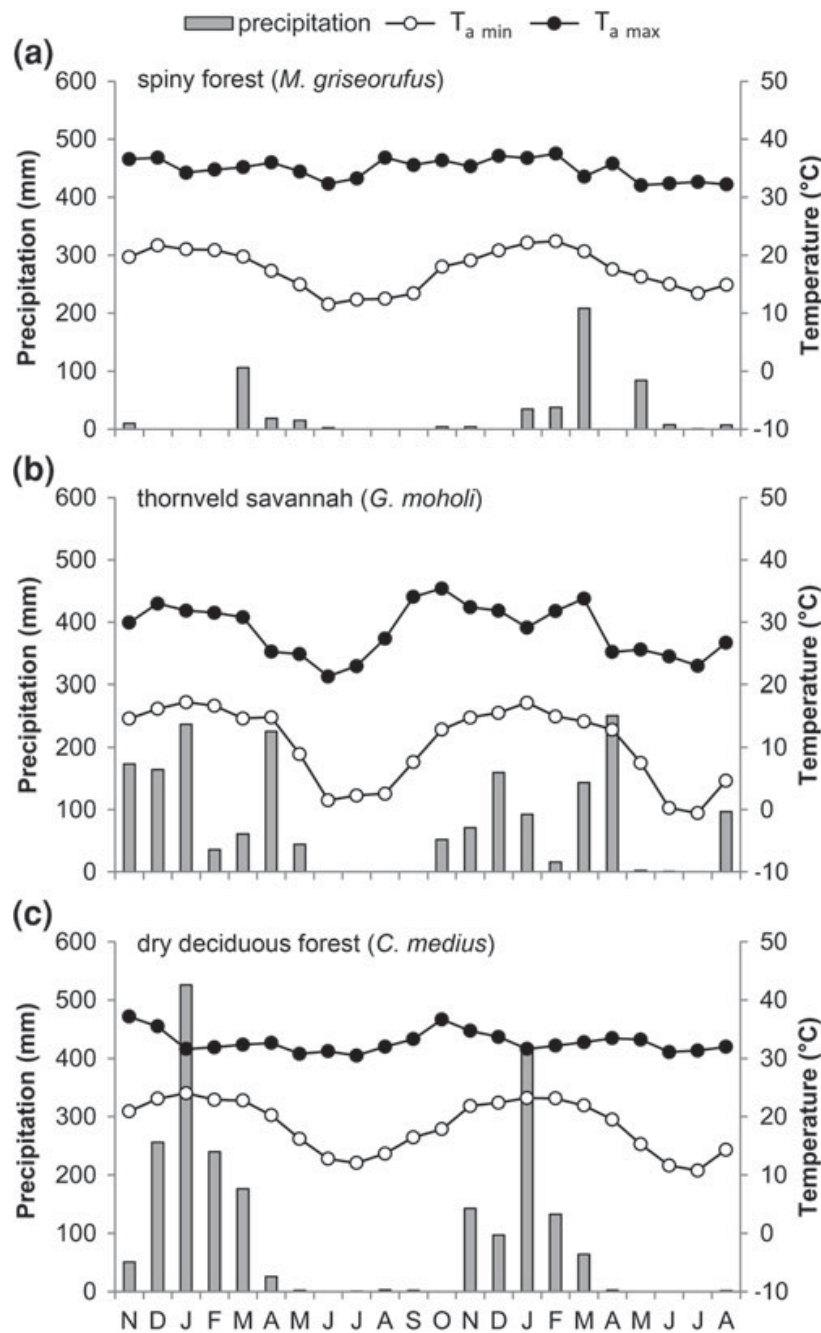Data not cited is unpublished data by the authors

Fig. 2.1 Seasonal variation of ambient temperature (T_a) and precipitation of **a** the spiny forest in South-Western Madagascar 2007–2009 (habitat of *M. griseorufus*), **b** the Thornveld Savannah in South Africa 2009–2011 (habitat of *G. moholi*) and **c** the deciduous dry forest in Western Madagascar 1999–2001 (habitat of *C. medius*) (modified from Fietz and Dausmann 2006)

2.3.3 Social System and Territoriality

G. moholi and *M. griseorufus* have a similar social organisation. They forage solitarily during the night, but females are seldom found nesting solitarily during daytime. They rest in family groups (one or more female and their current off-spring, in *G. moholi* sometimes accompanied by the assumptive father) or pairs

(Génin 2008; Nowack et al. submitted). However, in *G. moholi* some (probably subdominant) males are frequently found resting solitarily. In contrast, *C. medius* forms lifelong pair bonds (social monogamy). The pair defends a lifelong territory and usually shares a resting site during daytime; however, they are also solitary foragers and often hibernate separately (Dausmann et al. 2005).

All three species are territorial, but whereas *G. moholi* has been observed to maintain its territorial boundaries throughout the year (Nowack et al. 2010), territoriality of *C. medius* is restricted to the wet season and abandoned during the cold-dry season, when the animals become heterothermic. In *M. griseorufus* we can observe solitary males and females with overlapping home ranges as well as individuals sharing sleeping sites and home ranges (socio-territoriality; Génin 2010).

Reproductive patterns vary markedly between the three species (Table 2.1). *C. medius* usually reproduce only every second year (Fietz and Dausmann 2003) and give birth to twins after a gestation time of 60–65 days, which is only slightly longer than that of the almost three times smaller *M. griseorufus* (52–60 days) (Fietz and Dausmann 2006; Génin 2008). In the first 2 weeks after birth, both parents share babysitting duties; later, the parents guide the young during foraging (Fietz and Dausmann 2003). *M. griseorufus* and *G. moholi* have two reproductive periods per year, also usually giving birth to twins. Due to the relatively short gestation time, both birth periods of *M. griseorufus* occur during the hot-wet season. Parental care rests solely with the females, and young are independent after a few weeks. Within socio-territorial female pairs, alloparental care is a common phenomenon due to overlapping lactation periods (Génin 2008). Gestation time of *G. moholi* is about twice as long (123 days) and females give birth once shortly before the dry and cold winter time and once shortly after winter (Pullen et al. 2000). The young are weaned after few weeks and infants seldom have any contact with adult individuals other than the mother (Bearder 1969).

2.3.4 Predation and Mortality Rates

Various predators are described for each species (Table 2.1). Mortality rates of *C. medius* differ with habitat, but lie between 16 and 30% within the dry deciduous forest (Lahann and Dausmann 2011). Mortality rates are not known for *M. griseorufus* and *G. moholi*, but life spans in the wild are presumably considerably shorter than for *C. medius*.

2.4 Energy-Saving Responses: Three Species—Three Strategies

Heterothermy is utilised in all three species in order to cope with unfavourable conditions [during the southern winter or cold-dry season, respectively (i.e. between April and October)], however, in very different extent and variation (Table 2.2).

2.4.1 Differences in Energy-Saving Strategies

Most strikingly, there are great differences in the percentage of the population that becomes heterothermic. While 100% of *C. medius* and *M. griseorufus* employ heterothermy throughout (*C. medius*) or at some point (*M. griseorufus*) during winter, barely a fourth of the population of *G. moholi* becomes heterothermic at all (Nowack et al. 2010, unpublished data). Thus, between these three species we observe a continuum of different heterothermic responses: while *C. medius* is an obligate hibernator with all individuals hibernating throughout the dry season between mid-April and mid-October (Dausmann et al. 2004), *M. griseorufus* is physiologically more flexible (Kobbe et al. 2011). A part of the population of *M. griseorufus* stays normothermic most of the winter time and shows only spontaneous short torpor bouts whenever it seems necessary. However, most individuals use daily torpor and some individuals additionally enter prolonged torpor bouts for up to several days. The most extensive heterothermic behaviour we observe in this species is long-term hibernation. Similar to *C. medius,* hibernating individuals of *M. griseorufus* remain completely inactive up to 6 months during the dry season. *G. moholi* is also capable of heterothermy, however, in contrast to *M. griseorufus* and *C. medius* individuals exclusively use short torpor bouts on especially adverse conditions and not as a routine behaviour (Nowack et al. 2010). *M. griseorufus* and *C. medius* use tree holes for torpor and hibernation and the same is assumed, but yet needs to be ascertained, for *G. moholi*.

2.4.2 Influence of Body Mass, Sex and Age on Energy-Saving Strategies

In *C. medius* all individuals hibernate throughout the dry season regardless of their age, body mass or sex. Even juveniles display hibernation although they seem to be active longer than adults (Dausmann et al. 2005). Prior to hibernation *C. medius* accumulates large quantities of fat in the tail and on the body in order to survive many months without food intake (Fietz and Dausmann 2006). This seasonal body fattening also occurs in *M. griseorufus*, but in varying extent between individuals. Thermal behaviour utilised by *M. griseorufus* strongly depends on the individual's body condition before winter. Only the heaviest individuals hibernate up to several months. Individuals without sufficient fat stores occasionally use short torpor bouts and animals with intermediate body masses display daily torpor or prolonged torpor (Kobbe and Dausmann, unpublished data). In contrast to another mouse lemur species (*M. murinus*; Schmid 1999), *M. griseorufus* does not show any sex-specific differences in heterothermy (Kobbe et al. 2011). Compared to the cheirogaleids the thermal behaviour of *G. moholi* differs in so far as the exhibition of heterothermy does not seem to depend on body mass primarily but rather on the individuals' age and/or sex. Only subadults and juveniles, and mainly males use torpor during winter (Nowack et al. 2010, unpublished data).

Table 2.2 Heterothermic parameters for *G. moholi*, *M. griseorufus* and *C. medius* (N gives number of individuals, n number of days)

	G. moholi	*M. griseorufus*	*C. medius*
Heterothermy	Torpor under especially adverse conditions[a]	Short, daily or prolonged torpor hibernation[b]	Obligate hibernation[c]
% of heterothermic individuals during winter	26 (N = 8)	100 (N = 16)[b]	100 (N = 66)[d]
Sex-specific heterothermy?	Mostly males	No[b]	No[d]
Differences in age of heterothermic animals?	Subadult/juvenile[a]	Unknown, probably yes due to lower body mass in juveniles	Juveniles shortened hibernation period[e]
Occurrence of heterothermy	April–end of August	April–November[b]	Adults: Mid-April to Mid-October; juveniles might be active until end of May[e]
Absolute $T_{skin\ min}$ during torpor/hibernation (°C)	13.7	Short torpor: 10.5, daily torpor: 7.5, prolonged torpor: 7, hibernation: 6.5[b]	9.3[d]
Mean $T_{skin\ min}$ during torpor/hibernation (°C)	21.2 ± 5 (N = 8)	16.42 ± 1.63 (N = 16)[b]	18.2 ± 2.8 (N = 30)[e]
Mean TMR_{min} (ml O_2 h^{-1} g^{-1})	0.3 ± 0.2 (N = 4)	0.13 ± 0.05 (N = 4)	0.14 ± 0.058 (N = 11)[c]
Mean TMR (T_{skin} < 30°C)	0.6 ± 0.2 (N = 4)	0.28 ± 0.16 (N = 4)	0.29 ± 23.2 (N = 11)[c]

(continued)

Table 2.2 (continued)

	G. moholi	M. griseorufus	C. medius
Mean duration of torpor bouts/hibernation bout[g]	5.5 ± 3 h (N = 6, n = 6)	Short torpor: 5.6 ± 2.3 h (N = 4, n = 147)[f]; Daily torpor: 8.7 ± 1.5 h (N = 5, n = 504)[f]; Prolonged torpor: ~43.8 ± 19.9 h (N = 4, n = 6)[f]; Hibernation: 12.3 ± 1.3 days (N = 3, n = 17)[f]	6.7 ± 3.9 days (N = 5, n = 14)[e]
Frequency of torpor during winter (%)	Seldom[a]	Torpor: 29–80 (between May and October); Hibernation: 100[b]	Hibernation: 100[d]
Daily energy savings through heterothermy (T_{skin} < 30°C) (%)	~66	~80	~72[c]

[a] Nowack et al. (2010)
[b] Kobbe et al. (2011)
[c] Dausmann et al. (2009)
[d] Dausmann et al. (2004)
[e] Dausmann et al. (2005)
[f] Kobbe and Dausmann (2009)
[g] Between arousal
Data not cited in unpublished data by the authors

2.4.3 Physiological Characteristics of Heterothermy

2.4.3.1 Hibernation Bout Duration

The duration of torpor bouts are most extensive in those species and individuals that undergo hibernation. The longest hibernation bout (>70 days) without any active heat production was observed in *C. medius* (after that the logger stopped recording; Dausmann et al. 2005). The second longest bouts (45 days) were observed in hibernating individuals of *M. griseorufus* (Kobbe and Dausmann 2009). However, the average hibernation bout length of *M. griseorufus* was 12 days, and was thus twice as long as in *C. medius* with 6 days (Table 2.2). We attribute these differences to variations in insulation capacities of the tree holes used as hibernacula. While *M. griseorufus* uses only comparatively thin-walled poorly insulated tree holes, resulting in fluctuating T_a and T_b, and reducing the need of arousals, *C. medius* additionally uses thick-walled, well-insulated tree holes, necessitating regular arousals, thus decreasing the average value.

2.4.3.2 Torpor Bout Duration

The average duration of daily torpor bouts was longer in *M. griseorufus* than in *G. moholi*. However, in both species torpor bouts can range between 2 and 11 h (Table 2.2). As mentioned above the duration of torpor in *M. griseorufus* is strongly correlated to the body condition of the individual and T_a. *G. moholi* also undergoes torpid states only during the cold and dry season (from April to end of August) when food abundance and T_a are low, but heterothermy appears to be neither physiologically necessary nor possible for all individuals. Food-deprived juvenile and subadult individuals always entered torpor during the cold-dry season, but never during the hot-wet season. The length of torpor bouts seems to depend mainly on T_a (Nowack et al. 2010, unpublished data). *C. medius* only show daily torpor at the beginning of the hibernation season, so-called test drops (Dausmann et al. 2005). Prolonged torpor occurs only in *M. griseorufus* and only on occasions, where T_a decreases below a certain threshold (<9°C, Kobbe et al. 2011). Only individuals that have enough fat reserves to sustain the additional energetic demands before resuming delayed food intake can utilise this option.

2.4.3.3 General Patterns of Body Temperature

All three species show the regular pattern of T_b of small mammals during normothermia: T_b fluctuates around 37°C, with slightly lower values during the day, when the animals are resting (Dausmann et al. 2005; Kobbe et al. 2011; Mzilikazi et al. 2006).

General patterns of T_b in heterothermic individuals are also similar among the three species: T_b passively follows T_a for most of the heterothermic phase, and thus

T_b mainly depends on T_a and the insulation capacities of the resting place. Due to the differences in energy-saving strategies and environmental conditions (i.e. T_a) minimal T_{skin} ($T_{skin\ min}$) during heterothermy shows a significant variation among the species. In *M. griseorufus*, $T_{skin\ min}$ values seem to be strongly associated with the length of the heterothermic period. Both mean and absolute $T_{skin\ min}$ are lowest in hibernating *M. griseorufus* (6°C) and highest in individuals that enter short torpor bouts (absolute $T_{skin\ min}$ 10.5°C) (Kobbe and Dausmann 2009; Kobbe et al. 2011). $T_{skin\ min}$ of hibernating *C. medius* has been found to decrease down to 9.3°C (Dausmann et al. 2005; Table 2.2). The highest mean and absolute $T_{skin\ min}$ during heterothermy occur in *G. moholi*, where T_{skin} rarely decreases below 18°C (Nowack unpublished data). In cases where it did decrease below this value (two juveniles under laboratory conditions), the individuals had substantial difficulties rewarming from torpor.

2.4.3.4 Metabolic Rate

We were able to measure the MR of heterothermic individuals (torpid MR: TMR): of hibernating *C. medius* (Dausmann et al. 2009), torpid (but not hibernating) *M. griseorufus* and torpid *G. moholi*. The energy consumption of hibernating *C. medius* and torpid *M. griseorufus* are comparable (Table 2.2). This is due to the relatively low T_b during torpor bouts that occur in *M. griseorufus*. In contrast, *G. moholi* expends more than twice as much energy as the two cheirogaleid species during heterothermy, as anticipated from the higher minimal T_b during torpor.

In order to compare the potential of energy savings by heterothermy between all three species we compared average TMR to average MR during normothermia. In all three species heterothermic individuals utilise about 70% (between 66 and 80%) less energy per hour than normothermic animals (Table 2.2). Total energy savings depend not only on TMR but decisively also on the duration of the heterothermic period, i.e. on the particular thermal strategy. Thus we find a great variation of total energy savings by heterothermy among species and/or individuals with hibernating *C. medius* and *M. griseorufus* saving most and *M. griseorufus* and *G. moholi* with short torpor bout saving least.

2.5 Résumé

Our analyses did not reveal any fundamental differences in the physiological parameters of heterothermy between the three species that would indicate different basic physiological mechanism. Rather, patterns of T_b and MR during heterothermic phases, as well as length and depth of these, seem to follow general rules in all three species, depending on environmental variables, such as T_a, and individual variables, such as body condition, sex and age.

In our opinion, the finding that galagos and lemurs employ the same basic physiological mechanisms when using heterothermy suggests that the common

ancestor of these two groups was also heterothermic. Particularly since galagos only use this energy-saving mechanism sporadically it seems unlikely that they also evolved this capability convergently. Rather: they 'still' possess the ability, but only resort to it under exceptional circumstances. A plesiomorphic origin of heterothermy in mammals has also been suggested by Lovegrove (2011, 2012).

Environmental variables of the different habitats also did not show any substantial differences, the most important one being marked differences in the degree of unpredictability of rainfall. While the habitat of *C. medius* is highly seasonal, this seasonality, including food and water availability, is very predictable. The climate of the habitat of *M. griseorufus*, though showing the same seasonality, has much less precipitation, which additionally occurs very unpredictably within and between years, and can be totally skipped in some years. The habitat of *G. moholi* is less seasonal, rainfall being distributed more evenly over the year in amount and timing.

The most striking difference between *G. moholi*, *M. griseorufus* and *C. medius* is their differing reproductive strategies. Reproduction is a large component of energy budgets. In evolutionary terms, the ultimate goal of any animal should be the maximisation of its proportion of genes in the next generation. This can either be achieved by producing more offspring in a short period, or by reducing fecundity, but reproducing over a longer period. We propose that the different modes of reproduction, connected to climatic differences, are the key to the ultimate causes of the differences in thermoregulatory strategies between the three species. In the dry forest, rainfall is seasonal, but very predictable. Thus, *C. medius* can prepare for a defined period of scarcity when food is reliably abundant and hibernate during winter, reliably encountering food and water during the summer months, enabling itself and its offspring seasonal fattening before, and replenishment after hibernation. Frequent reproduction is not an option for *C. medius*, possibly because of the extensive and time-intensive parental care of both parents that is needed for the successful upbringing of offspring (Fietz and Dausmann 2003). Hibernating animals generally have a higher annual survival (Turbill et al. 2011) and *C. medius* may therefore use this energy-saving strategy during the lean dry season that cannot be used for reproduction anyway, to compensate less frequent reproduction events by a longer life. Other cheirogaleids in the dry forest are either smaller, and thus may not be able to store sufficient amounts of fat to make 7 months long hibernation an option (Dausmann 2008); or they are able to exploit a more exclusive or less seasonal spectrum of food: *Mirza coquereli* includes animal prey in its diet, and *Phaner furcifer* mainly feeds on tree exudates, both of which are available throughout the year.

G. moholi, on the other hand, lives in a less seasonal habitat and can successfully raise young throughout the year and thus reproduce more often, an option not available to *C. medius*. They, therefore, largely forgo the advantages of heterothermy in favour of reproduction possibilities, except in rare events. We assume that the physiological potential of entering energy-saving heterothermic states is only tapped by individuals that are unable to participate in reproduction at that point, e.g. young males without a territory. Females can already become pregnant at the age of 6 months, and due to their long gestation period, they are either

pregnant or lactating most of the year. Since both activities are seriously impaired by heterothermy (e.g., Farmer 2000; Wilde et al. 1999), this would explain why females are rarely encountered torpid. Adult males, on the other hand, presumably cannot afford to spend time in a 'suspended' state at any time of the year, neglecting territory defence (Nowack et al. 2010) and possibly mate guarding.

M. griseorufus inhabits the most unpredictable habitat and is the most flexible of the three species. Hibernation seems to be the preferred strategy for these animals, but can only be adopted by individuals whose body condition (fat stores) is sufficient to fuel long-term hibernation over several months with uncertain conditions when resuming activity. When hibernation is not possible, *M. griseorufus* can employ daily torpor or prolonged torpor, 'deciding' on a day-to-day basis, depending on their own current body condition and current ambient conditions. With this very flexible opportunistic strategy, they can brave the unpredictably of their habitat and are best equipped to adjust quickly to changing conditions.

In conclusion, the thermoregulatory physiology of *C. medius*, *M. griseorufus* and *G. moholi* is tightly adapted the environmental conditions of the habitats, where the species evolved, in combination with reproductive possibilities, highlighting how flexibly physiological traits can be expressed.

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PRESENTATIONS

Torpor on demand: heterothermy in the non-lemur primate *Galago moholi*

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Heterothermy occurs in nearly all mammalian orders, but within the primates so far seemed to be restricted to one family of Malagasy lemurs, the Cheirogaleidae. Here we present the first proof of torpor in a primate outside Madagascar, the African lesser bushbaby, *Galago moholi*. We measured skin temperature of free-ranging individuals throughout the cool dry season using temperature-sensitive collars as well as metabolic rate in captured individuals. Torpor was employed by 15% of 20 animals. Only one of these animals displayed heterothermy in response to natural availability of food and water, whereas the other animals became torpid in response to external stress exposure. Our results show that *G. moholi* are indeed physiologically capable of employing torpor. However, contrary to the Malagasy lemurs, they do not use it as a routine behaviour, but only under adverse conditions. This reluctance is presumably a result of conflicting selective pressures for energy savings versus other ecological and evolutionary forces, such as reproduction or territory defense. Our results prove the potential flexibility of physiological parameters within one population and support the view that heterothermy in primates evolved before the division of African and Malagasy Strepsirhini, with the possible implication that more primate species than previously thought might still have the potential to call upon this possibility, if the situation necessitates it.

Surviving the cold and dry period: behavioural and thermoregulation of the African lesser bushbaby (*Galago moholi*)

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Small mammals respond to cold exposure with a variety of behavioural and physiological adaptations. Behavioural strategies to conserve energy can be applied simultaneously with reduction of metabolic rate (leading to further reduction of energy costs) or as an alternative strategy to enhance winter survival, but are seldom favoured by a majority of the species over physiological adaptations. Here we present a rare exception: Unlike other small mammal species that employ torpor or hibernation in an obligatory manner during unfavourable periods, the African lesser bushbaby (*Galago moholi*) only employs torpor during strenuous events and relies heavily on seasonal variations of thermoregulatory behaviour to survive the cold and dry period. Heterothermic strategies might lead to higher energy savings, but can on the other hand conflict with other ecological and evolutionary forces. The results of a telemetric study of six individuals during summer 2009/10 and 2010/11 and seven individuals during winter 2009 and 2010 revealed significant changes in feeding behaviour and sleeping site use. Individuals reduced nocturnal activity during the winter months and showed additional diurnal activity instead. We propose that *G. moholi* employs seasonal variations in sleeping site use, food preference and activity pattern instead of regular heterothermic phases to survive the cold and dry period in order to allow unrestrained reproduction and territory defence throughout the year.

Staying warm during the cold African winter: alternatives to heterothermy in the African lesser bushbaby

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Hibernation and daily torpor are energy- and water-saving adaptations employed by small mammals to survive unfavourable periods. Recently it has been found, that the African lesser bushbaby, *Galago moholi,* is physiologically able to undergo torpor. However, unlike other species, *G. moholi* does not use this ability as routine behaviour, but only under adverse conditions. We studied behavioural and physiological thermoregulatory adaptations in a free ranging population of *G. moholi* for the course of two and a half years. During this time only about a fourth of the individuals entered torpor during the cold and dry period, most probably in response to natural or experimental food or water restriction. The main food source of *G. moholi* was significantly reduced during this period, however, a change in feeding behaviour and availability of high quality food in low quantities presumably enables galagos to stay normothermic. Telemetric tracking and behavioural observation showed that *G. moholi* relies on seasonal changes in sleeping site use and group composition paired with a shift of activity times to reduce energy expenditure during the cold. We propose that *G. moholi* favours ecological and behavioural adjustments over torpor, as they serve to meet energy requirements of this species, and their advantages (flexibility, unrestricted activity and reproduction) outweigh the energetic benefits of heterothermy. In contrast, torpor is only used as a last alternative in response to phases of unusual severely food shortage (caused by especially harsh winters or individual emergency situations) and not as a preventive strategy as found in other heterotherms.

Surviving the cold and dry period in Africa: behavioural thermoregulation as an alternative to heterothermy in *Galago moholi*

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