Thermoregulation in three southern African bat species inhabiting a hot, semi-arid environment.

By:

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Declaration

I, Dawn Cory Toussaint, declare that the following thesis/dissertation, which I hereby submit for the degree MSc Zoology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: DCoryToussaint Date: 17/09/2012

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Summary

Thermoregulation in three southern African bat species inhabiting a hot, semi-arid environment.

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Bats inhabiting arid, subtropical environments face diverse challenges related to energy and water balance. First, they may have to conserve water and energy during cool, dry winters when water is scarce and insect availability reduced. Second, during hot summers when air temperature may routinely exceed body temperature, bats may need to avoid both hyperthermia and dehydration. A common response to the energetically challenging winter period in temperate, subtropical and tropical species is heterothermy (i.e. torpor and hibernation). Despite evidence suggesting that heterothermy is of major significance in the energy balance of tropical and subtropical bats, its occurrence in southern African species especially those in semi-arid subtropical regions have received relatively little attention. Moreover, the physiological and thermoregulatory responses of bats to high air temperatures (T_a) are relatively poorly known. The goal of my project was to investigate various seasonal physiological challenges imposed on bats in an arid, sub-tropical climate.

I investigated the occurrence of winter heterothermy in *Nycteris thebaica* (Nycteridae) in the Limpopo Valley. Skin temperatures (T_{skin}) were measured using temperature-sensitive transmitters, and roost temperatures (T_{roost}) were recorded using miniature temperature loggers. *N. thebaica* used multiple roosting sites, including a hollow baobab tree (*Adansonia digitata*) and several caves, and exhibited only moderate heterothermy. T_{skin} was maintained around normothermic levels, with differences of 3-9°C (7.5±1.7°C) between overall maximum and minimum T_bs. A minimum T_{skin} of 28.4°C occurred at T_{roost} = 23.8°C, and patterns of thermoregulation did not appear to be influenced by prevailing weather conditions. Roost temperatures did not decrease below 10°C, and averaged 21.2±2.8°C and 23.3±2.9°C respectively. The lack of pronounced heterothermy in *N. thebaica* is surprising, particularly in view of the daily torpor cycles observed in many insectivorous bat species.

I also investigated the physiological responses of three sympatric bat species during summer using an open-flow respirometry system to measure resting metabolic rates (RMR) and evaporative water loss (EWL) over a range of T_as ~ 10-42°C, with body temperatures (T_b) simultaneously recorded via temperature-sensitive passive integrated transponder (PIT) tags. Basal metabolic rates for *Nycteris thebaica* and *Taphozous mauritianus* were 8.9±2.7mW.g^{-1} and 6.6±2.2mW.g^{-1} respectively, falling within the 95% prediction intervals for bat BMR, whereas the value for *Sauromys petrophilus* (3.4±0.6mW.g^{-1}) fell below the lower 95% prediction interval. Maximum EWL for *N. thebaica*, *T. mauritianus* and *S. petrophilus* were 18.6±2.1mg.g^{-1}.h^{-1} (T_a=39.4°C), 14.7±3.1mg.g^{-1}.h^{-1} (T_a=41.9°C) and 23.7±7.4mg.g^{-1}.h^{-1} (T_a=41.7°C) respectively. Maximum individual T_bs recorded were 46.5°C in *N. thebaica* (T_a=39.3°C), 44.9°C in *T. mauritianus* (T_a=41.8°C) and 46.5°C in *S. petrophilus* (T_a=41.7°C). Overall, I found that *N. thebaica* was the least heat tolerant species, with *T. mauritianus* and *S. petrophilus* being more heat tolerant. In the face of climate change, heat tolerance together with specific thermal properties of a given roost may play a major role in the ability of a species to persist in a hot, arid environment.

CONTENTS

General Introduction

Background Information

Most animals experience seasonal variation in energy and water demands, and respond through a variety of behavioural and physiological pathways. The highly seasonal climatic conditions of temperate latitudes, characterised by harsh, cold winters and the associated constraints on food availability, have been the focus of the majority of research on thermoregulation in bats and many other endotherm taxa. A large body of work on thermoregulation in mammals and birds from temperate latitudes has revealed that heterothermy, comprised of facultative, reversible decreases in body temperature below normothermic levels, is widespread, particularly during the winter months. Traditionally, heterothermy is divided into daily torpor and hibernation, on the basis of bout length, depth and associated physiological variables such as reductions in body temperature (T_b), metabolic rate (MR), heart rate and ventilation (Geiser & Ruf 1995, Heldmaier *et al.* 2004, Prothero & Jürgens 1986). Ambient temperature (T_a) and associated food resources have been the major factors shown to influence the use of torpor and hibernation in temperate regions (Park *et al.* 2007). Classic examples are rufous hummingbirds, *Selasphorus rufus* (Hiebert 1993), Alpine marmots, *Marmota marmota* (Heldmaier *et al.* 2004), Columbian ground squirrels, *Spermophilus columbianus* (Young 1990) and numerous temperate region bat species such as big brown bats, *Eptisicus fuscus* (Beer & Richards 1956) and little brown bats, *Myotis lucifugus* (Thomas *et al.* 1990). Although thermoregulation in temperate regions has been widely studied (Beer & Richards 1956, Hoskens & Withers 1999, Humphries *et al*. 2002), thermoregulation in tropical and subtropical regions is receiving increased attention. The numbers of studies are increasing documenting the use of torpor and hibernation in tropical and subtropical climates during the long, dry winter season when food availability is reduced, as well as daily torpor during adverse weather conditions during summer (Schmid 2000, Turbill *et al.* 2003, Dausmann *et al.* 2005). Torpor has also been investigated during events such as El Ninõ which causes unpredictable climatic fluctuations resulting in major disruptions in rainfall regimes in tropical climates where droughts are experienced in productive areas and torrential rain events in otherwise arid regions (Rasmusson 1985). Thus these unpredictable climatic events impact food availability, often negatively, resulting in food shortages (Giroud *et al.* 2008, Canale & Henry 2010). Tropical and subtropical mammals, particularly those from Madagascar such as the lemurs *Microcebus murinus* (Schmid 2000) and *Cheirogalues medius* (Dausmann *et al.* 2005) and the dormouse, *Glis glis* (Stawski & Geiser 2010b) have been shown to use torpor, and the lesser hedgehog tenrec *Echinops telfairi* uses daily torpor and hibernates (Lovegrove & Génin 2008).

A link between heterothermy and the occupation of arid, unpredictable habitats has also emerged from recent work. For instance, Superina & Boiley (2007) found that *Zaedyus pichiy,* in arid and semi-arid environments of southern Argentina and Chile, used daily torpor and hibernation during the dry winter period when food was scarce *Z. pichiy* was found to hibernate for 75±20 h with skin temperatures (T_{skin}) of 14±2.1°C (Superina & Boiley 2007). In specifically hot, arid environments, animals not only have to deal with cold winter temperatures but also high summer T_as and the associated risk of desiccation.

Bats are one of the most understudied groups in terms of seasonal variation in physiological demands. However, they may experience significant physiological challenges associated with seasonal cycles in many subtropical regions. Even when T_as are warm during winter, food availability is greatly reduced during the dry winters, and since most microchiropterans are exclusively insectivorous, it is not surprising that heterothermy similar to that observed in temperate species is widespread in Australian and apparently southern African and other tropical species such as; *Tadarida aegyptiaca* (Cory Toussaint *et al.* 2010), *Scotophilus dinganii* (Jacobs *et al.* 2007), *Mops condylurus* (Vivier & van der Merwe 2007), *Nyctophilus gouldi* (Turbill & Geiser 2008), *N. bifax* (Stawski *et al.* 2008), *N. geoffroyi* (Turbill *et al.* 2003), *Syconycteris australis* (Coburn & Geiser 1998), *Molossus molossus* (Dechmann *et al.* 2011), *Hipposderos terasensis* (Lui & Karasov 2010) and the Neotropical bats *Glossaphaga sorcinia, Artibeus lituratus, Micronycteris megalotis, Myotis nigricans* and *Saccropteryx bilineata* (Studier & Wilson 1970). Although the influence of environmental conditions and food availability have been viewed as major factors influencing the expression of torpor, Stawski & Geiser 2010(b) showed that bats with a high body condition index had torpor bouts which were more frequent, longer and deeper that individuals with a lower body condition index and thus suggested that the use of torpor was perhaps not in response to low food availability and low energy stores but aerial predator avoidance when bats do not need to feed. Similarly, many other bat species use torpor when T_as are warm (Jacobs *et al.* 2007, Lui & Karasov 2010).

In tropical and subtropical regions, not only do mammals have to deal with reductions in T_a and food availability during winter and occasionally during summer (as observed in Australia; Turbill *et al.* 2003, Stawski & Geiser 2010b), but high T_as during summer can pose massive thermoregulatory challenges on animals with regards to both overheating and cooling (Scholander 1950). When hot, many animals offload heat by sweating, panting, being inactive during the heat of the day, avoid the sun and seek shade or resort to water for cooling (Scholander 1950). Many desert mammals have been shown to have physiological traits that favour water and energy conservation under very hot conditions such as low water requirements, low resting and basal metabolic rates, low evaporative water loss and water intake predominantly acquired from the diet as observed in many desert-dwelling rodents (Haim & Izhaki 2005), Wagner’s gerbil, *Gerbillus dasyurus* (Haim 1987), Mountain vizcachas, *Lagidium viscacia* (Tirado *et al.* 2007), Desert-mountain mice, *Peromyscus spp* (McNab & Morrison 1963), Antelope ground squirrel, *Ammospermophilus leucurus*, Merriam’s kangaroo rat, *Dipodomys merriami*, Black-tailed jackrabbit, *Lepus californicus* and Springbok antelope, *Antidorcus marsupialis* (Nagy 1994).

Many bats have been shown to either tolerate or behaviourally avoid extremely hot roosts, e.g., *M. condylurus* (Vivier & van der Merwe 2007, Bronner *et al.* 1999), *Vespadelus troughtoni* (Law & Chidel 2007), *Antrozous pallidus*, *Myotis yumanensis*, *Tadarida brasiliensis* and *M. coibensis* (Herreid 1967, Licht & Leitner 1967). *Lavia frons* from central Africa behaviourally thermoregulates by basking in full sun in the morning and on cool days, and moves into shade during the heat of the day allowing their wing membranes to dissipate heat, and does not use torpor (Vaughan 1987). However, there is a host of species that are heat intolerant and exposure to heat can be fatal, for instance, roost temperatures (T_{roost}) above 40˚C have been found to be lethal to many temperate-zone and tropical microchiropterans, including *Myotis velifer, Macrotis californicus,* (but see Licht & Leitner 1967), *Macroderma gigas, Artibeus hirsutus* and *Desmodus rotundus* (Reeder & Cowles 1951, Lyman &Wimsatt 1966, Carpenter & Graham 1967, Leitner & Nelson 1967, Maloney *et al.* 1999).

Considering the above mentioned challenges faced by many animals in tropical, subtropical and those particularly in hot, arid environments, global climate change, could increase the severity of already challenging environmental conditions which can cause range shifts and contractions, mass mortality events associated with extreme heat waves, or local extinctions (Peck *et al.* 2009, Welbergen *et al.* 2008, McKechnie & Wolf 2010, Cameron & Scheel 2001, William *et al.* 2003, Root *et al.* 2003). We already have an understanding that climate change will and currently is affecting species distributions (Peck *et al.* 2009). However, which individuals and species are likely to survive and why is currently poorly understood therefore we need to understand community or assemblage level effects and the balance of mechanisms that are important over different time-scales (Peck *et al.* 2009). The ecophysiological response of a given species is affected by ecological processes that are in turn affected by changes in temperature (Peck *et al.* 2009). Even though great emphasis has been placed on the physiological limits of species, and this is an important factor, physiological limits are only one of several mechanisms dictating ecological balance (Peck *et al.* 2009).

The ecological and phylogenetic determinants of heterothermy in tropical bat taxa and those species inhabiting hot, semi-arid tropical environments are poorly understood and with particular reference to the widespread bat family Nycteridae, no data are available on thermoregulation within this group. Thus, the goal of this study was to examine these physiological demands in a bat community inhabiting a warm, seasonal habitat focusing on thermoregulation at two key times of the year; mid-winter and mid-summer. First, I investigated heterothermy patterns in *Nycteris thebaica* roosting during the cool, dry austral winter in a tropical semi-arid environment, the Limpopo River Valley in northern South Africa. Second, I investigated the thermoregulation of sympatric *Nycteris thebaica, Taphozous mauritianus* and *Sauromys petrophilus* that use roosts that differ in thermal properties, and used these species as a model system to investigate suggested potential responses of these species to climate change.

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Chapter 1: Patterns of winter heterothermy in *Nycteris thebaica* (Nycteridae) in the Limpopo River Valley, northern South Africa.

(prepared for submission to *Journal of Mammalogy*)

Nycteris thebaica – Egyptian slit-faced bat © Dawn Cory Toussaint

Introduction

Heterothermy is a widespread physiological response in endotherms, and is traditionally categorized into two patterns of thermoregulation, namely daily torpor and hibernation (Geiser & Ruf 1995, Barclay *et al.* 2001, Heldmaier *et al.* 2004). Heterothermy is defined as the controlled lowering of the body temperature (T_b) set point, with an associated reduction in metabolic rate (MR) and thus substantial energy and water savings (Chruszcz & Barclay 2002, Geiser 2004, Geiser & Turbill 2009, Bartels *et al.* 1998). Minimum T_b and MRs are typically lower during hibernation compared to daily torpor (Geiser & Ruf 1995,), although recent data from free-ranging tropical mammals such as mouse lemurs and tenrecs reveal that this distinction is blurred (Dausmann *et al.* 2005, Lovegrove & Génin 2008). Recent work has shown that heterothermy is, in fact, just as common in tropical and subtropical taxa as in temperate-zone groups, and occurs in Monotremata, Xenarthra, Rodentia, Primates, Carnivora, Macroscelidea, Insectivora and several marsupial taxa (Geiser & Turbill 2009).

Bats are one of the most heterothermic mammalian taxa (Turbill & Geiser 2008, Bartels *et al.* 1998, Herreid 1963). As small endotherms with high mass-specific rates of heat loss coupled with comparatively high energy requirements associated with flight, bats are highly vulnerable to mismatches between energy supply and demand (Chruszcz & Barclay 2002, Jacobs *et al.* 2007, Brown 1999). Moreover, many species feed on food resources such as aerial insects or nectar, whose availability is strongly dependent on air temperature (Turbill & Geiser 2008). Thus, it is not surprising that bats make extensive use of heterothermy. In temperate latitudes with long, cold winters, many non-migratory bats such as *Eptesicus fuscus*, *Rhinolophus ferrumequinum*, *Myotis lucifugus* and *M. evotis* hibernate for much of the year, usually in thermally stable hibernacula such as caves (Park *et al.* 2000, Beer & Richards 1956, Chruszcz & Barclay 2002).

Many tropical and subtropical species, on the other hand, use daily torpor and/or hibernation bouts lasting days to weeks during winter, as they too experience seasonal decreases in food availability and T_a (Turbill *et al.* 2003a, Stawski & Geiser 2010b). It has previously been thought that bat species occupying tropical and subtropical regions were unable to use hibernation, as it was assumed that cool stable roost temperatures are a prerequisite (Liu & Karasov 2011). However, in the last decade several examples of hibernation in tropical and subtropical species have been documented. The subtropical Australian tree-roosting species *Nyctophilus bifax*, for example, has been shown to hibernate continuously for up to 119 hr (Stawski *et al.* 2008). Lui and Karasov (2010) reported hibernation in the Taiwanese species, *Hipposideros terasensis*, and in South Africa free-ranging *Tadarida aegyptiaca* responds to cooler weather by switching from daily torpor to hibernation lasting several days (Cory Toussaint *et al.* 2010). In addition to these hibernators, a host of tropical and subtropical species are known to use daily torpor, including *Mops condylurus* (Vivier & van der Merwe 2007), *Scotophilus dinganii* (Jacobs *et al.* 2007), *N. bifax* (Stawski & Geiser 2010a, Stawski & Geiser 2010b), *N. geoffroyi* (Turbill *et al.* 2003), *Syconycteris australis* (Coburn & Geiser 1998) and *Molossus molossus* (Dechmann *et al.* 2011). Moreover, the use of torpor is not only restricted to the winter period but has been reported for several subtropical Australian species during the summer period, namely *Nyctophilus bifax* (Stawski *et al.* 2008, Stawski & Geiser 2010a), *N. geoffroyi*, *N. gouldi* (Turbill & Geiser 2008, Turbill *et al.* 2003).

There remain many gaps in our knowledge regarding the phylogenetic and ecological determinants of heterothermy in tropical bat taxa. For instance, the family Nycteridae is widespread in Africa and Asia (Thomas *et al.* 1994), but there are no data available on thermoregulation within this group. There is also a lack of data for species from hot, semi-arid tropical environments; most of the previous work focused on species inhabiting rainforest or woodland. I investigated patterns of heterothermy in *Nycteris thebaica* roosting during the austral winter in a tropical semi-arid environment, the Limpopo River Valley in northern South Africa. I hypothesised that, as is the case for many Australian species, *N. thebaica* uses pronounced heterothermy during the cool, dry winter months.

Material and Methods

Study site

The study was conducted on the game farm Makulu Makete (28˚ 51,78’ & 28˚ 52,29’S; 22˚ 34,52’ & 22˚ 35,09’ E) in the Alldays area, situated in the subtropical Limpopo River Valley, Limpopo Province, South Africa. The Limpopo River Valley is situated in northern Limpopo Province and is separated from the central Bushveld plateau and the Lowveld by the Soutpansberg and Blouberg mountain range (Reyers 2004). The general vegetation type of the Limpopo River Valley is northern savanna comprising open grasslands with shrubs and a discontinuous tree canopy of assorted tree species (Beyer 2002) or *Adansonia digitata-Acacia nigrescens* Soutpansberg Arid Northern Bushveld (Mostert *et al.* 2008). It is one of the hottest regions of South Africa, with air temperatures known to exceed 45˚C, and rainfall of approximately 400-500 mm/year (Thomas *et al.* 2007, Twine *et al.* 2003). The reserve is 3703ha and characterised by hot summers and cold winters. It is semiarid with a varying annual rainfall ranging between 200-700mm. The topography of the area is predominantly flat lying with two raised areas namely Madiapala Mountain and Kremetartkop-hill which comprise 208ha of the reserve. The vegetation type is classified as Transvaal sweet Bushveld which includes Mopane veld and a scattering of baobab trees (*Adansonia digitata*) (de Klerk 2002).

Study Species

Nycteris thebaica, has a widespread distribution across the African continent that falls into the northern Limpopo province and have been recorded roosting in baobab trees as well as a variety of other roost types (Taylor 2000). It roosts in colonies that vary from a few individuals to several hundred (Taylor 2000).

A *N. thebaica* roost site in a baobab tree (*Adansonia digitata*) at 22˚35.738’ S 28˚52.372’ E was located during a survey conducted in 2008 by the Gauteng and Northern Regions Bat Interest Group. *N. thebaica* were captured using mist nets as they exited from this roost to forage. Due to the locality and structure of the occupied tree cavity, bats were captured from the roost using a mist net as they exited the roost to forage in the evening. Eighteen *N. thebaica* (females n=12, males n=6), were captured on 19/07/2010, 03/08/2010, 11/08/2010 and 21/08/2010. Each bat was sexed, mass and forearm measured and placed in its own clean bag.

Skin and roost temperature measurements

To measure T_{skin}, temperature sensitive transmitters with mass of 0.5-0.8 g (BD-2NT, Holohil Systems, Carp, Ontario) were used, calibrated against a mercury in glass thermometer (accuracy traceable to National Institute of Standards and Technology) in a water bath ranging over temperatures of 5 - 45˚C prior to being attached to the bats. The temperature-sensitive transmitters were attached to the bat by clipping away a small patch of fur between the scapulae and using a latex-based adhesive (Osto-Bond, M.O.C., Vaudreuil, Qc. Canada) to secure the transmitter in place (Turbill & Geiser 2008, O'Donnell & Sedgeley 1999, Jacobs *et al.* 2007, Vivier & van der Merwe 2006, Cory Toussaint *et al.* 2009).

Skin temperature data were collected manually using a broad-band communications receiver (IC-R10, Icom, Bellevue, WA) (McKechnie *et al.* 2007). Data were collected hourly during the day and every 30 minutes from 1600 h until the bats left the day roost to forage and upon arrival at the day roost in the morning for two hours. For each datum, pulse intervals were estimated by timing 21 pulses using a stopwatch (McKechnie *et al.* 2007). T_{skin} values were estimated from these pulse intervals using the respective calibration curves for each transmitter. Of the 18 individuals with transmitters attached, reliable T_{skin} data were obtained from seven individuals, six females and a single male. The remaining individuals either disappeared after one or two days, did not return to any of the roosting sites or had premature transmitter failure. T_{skin} is a good indicator of core body temperature (T_b). Studies assessing the relationship between core T_b and T_{skin} of torpid and active nightjars and bats have found that T_{skin} is within 3-4°C of T_b when T_b and T_{skin} are measured simultaneously over a range of T_as. Simultaneous measurements in these studies were achieved using skin sensitive transmitters to measure T_{skin} and a thermocouple inserted rectally to measure core T_b (McKechnie *et al.* 2007, Stawski & Geiser 2010a, Cory Toussaint *et al.* 2010). Roost temperatures (T_{roost}) were recorded using miniature data loggers (iButtons Thermochron, Dallas Semiconductor, Dallas, USA, resolution=0.06°C) placed directly in the day roosts as close to the roosting bats as possible, suspended so as to measure air rather than surface temperature. Ambient temperature (T_a) was recorded using Thermochron iButtons placed 1m above the ground, suspended in ventilated white polystyrene cups, in a shaded area in the vicinity of the day roosts. The iButtons were calibrated in a water bath against a mercury thermometer (resolution=0.2°C) with an accuracy traceable to the US National Bureau of standard before data collection over the range of 10-40°C according to the range of temperatures each iButton would potentially experience.

Data analysis

To describe the variation in body temperature of an individual bat during the daytime roosting period, the new comparative metric called the heterothermy index, was used (Boyles *et al.* 2011). The equation is as follows:

$$\mathrm{HI} = \sqrt{\frac{\sum(\mathrm{T_{b-mod}} - \mathrm{T_{b-i}})^2}{n-1}}$$

where $T_{b\text{-mod}}$ is the modal T_b determined as the mode of all T_b recorded if the T_b distribution is unimodal or if the T_b distribution is bi-modal or multi-modal the mode of the peak of the highest T_b is used, T_b-*i* is the body temperature measured at a given time *i* and *n* is the number of times body temperature is sampled (Boyles *et al.* 2011). In addition, I used traditional T_b cut-off values to examine whether N. thebaica used torpor/hibernation comparable to other species. A bat would be considered torpid if T_{skin} was recorded at 25°C (see Hollis & Barclay 2008, Cory Toussaint *et al.* 2010, Jacobs *et al.* 2007). Data are represented as means ± one standard deviation for n=sample size (number of individuals). To test for a significant difference between T_{skin}s and T_{roost}s an ANOVA statistical test was used and a t-test (assuming equal variances) was used to compare T_{roost} and T_a.

Results

Roost localities and microclimates

All individuals were initially captured from the known hollow baobab tree day roost and released in the vicinity of the roosting. Subsequently two additional cave roosting sites were identified on two separate mountains namely Kremetartkop and Madiapala Mountain (22˚35.168'S & 28˚52.978'E; 22˚34.694'S & 28˚51.194'E respectively), by tracking individuals with transmitters attached using triangulation to locate the various day roosts from individuals initially captured from the baobab tree day roost. Movement between the various roosts appeared to be random with the Madiapala Mountain cave only being used towards the end of the study (23 August 2010), but maybe related to climatic cues or social interactions that require further investigation. Each individual used two of the three identified day roosts with exception of one individual that roosted only in the Kremetartkop cave during the data collection period. The roosts used when the bats were absent from the three identified roosts were unknown.

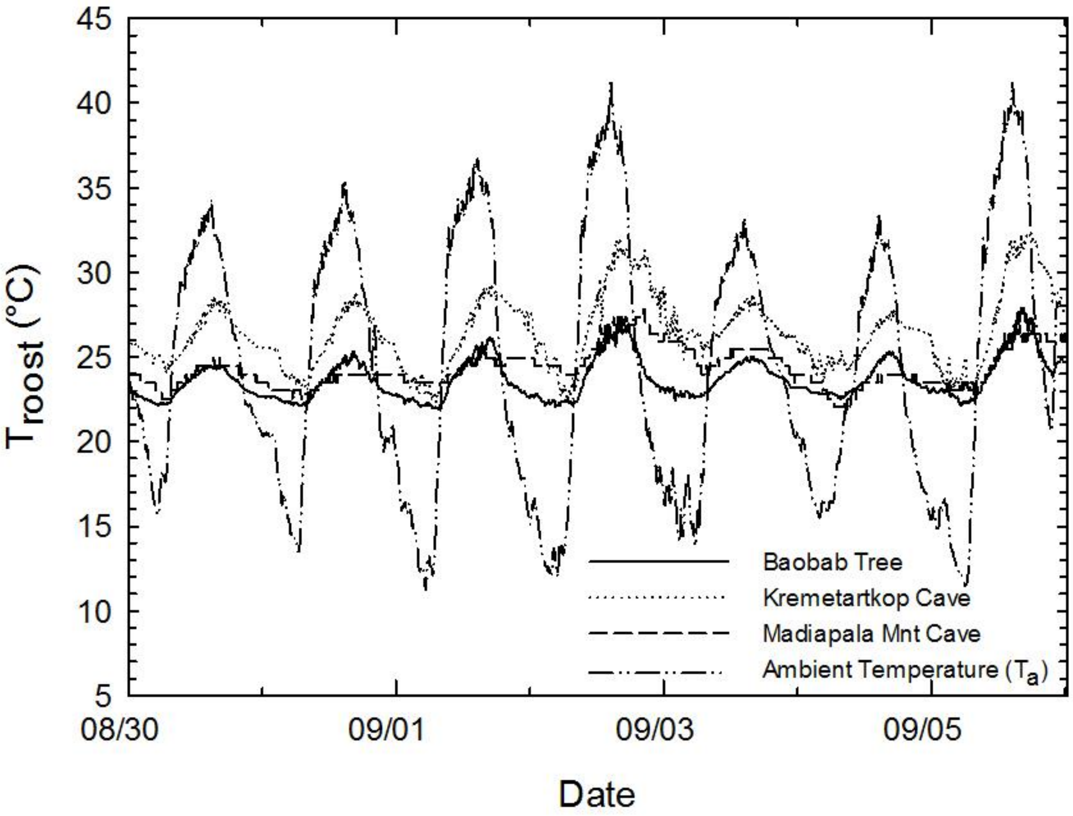


Figure 1. The temperature profiles of the three different day roost types used by *Nycteris thebaica* in relation to ambient temperature (T_a) over a portion of the study period when all three roosting sites were occupied.

There was a significant difference between all three roosting sites and T_a ($F_{3, 28051}$=360.1, P<0.0001). Over the range of T_as and environmental conditions there was a significant difference between all three roosting sites ($F_{2, 39341}$=2808.8, P<0.0001) with the hollow baobab tree and Madiapala Mountain cave roosts being consistently warm and having lower daily temperature fluctuations than Kremetartkop cave (Figure 1). During the hottest time of the day, the baobab tree, Kremetartkop cave and Madiapala Mountain cave were significantly cooler than T_a (n=25 days, P<0.0001 for all roosting sites). Similarly, during the coldest time of day, all three roosts were significantly warmer than T_a (n=25 days, P<0.0001 for all three roosting sites). As mentioned above, the iButtons, particularly those placed to record Madiapala Mountain and Kremetartkop T_{roost}s, could not be placed in the vicinity of the roosting bats and these results for these specific roost are only broad indications of potential roost microclimates experienced by the bats whereas the iButtons placed in the hollow baobab tree roost were closer to the roosting bats and may present a more accurate estimation of T_{roost}s.

Skin Temperatures and Heterothermy Index

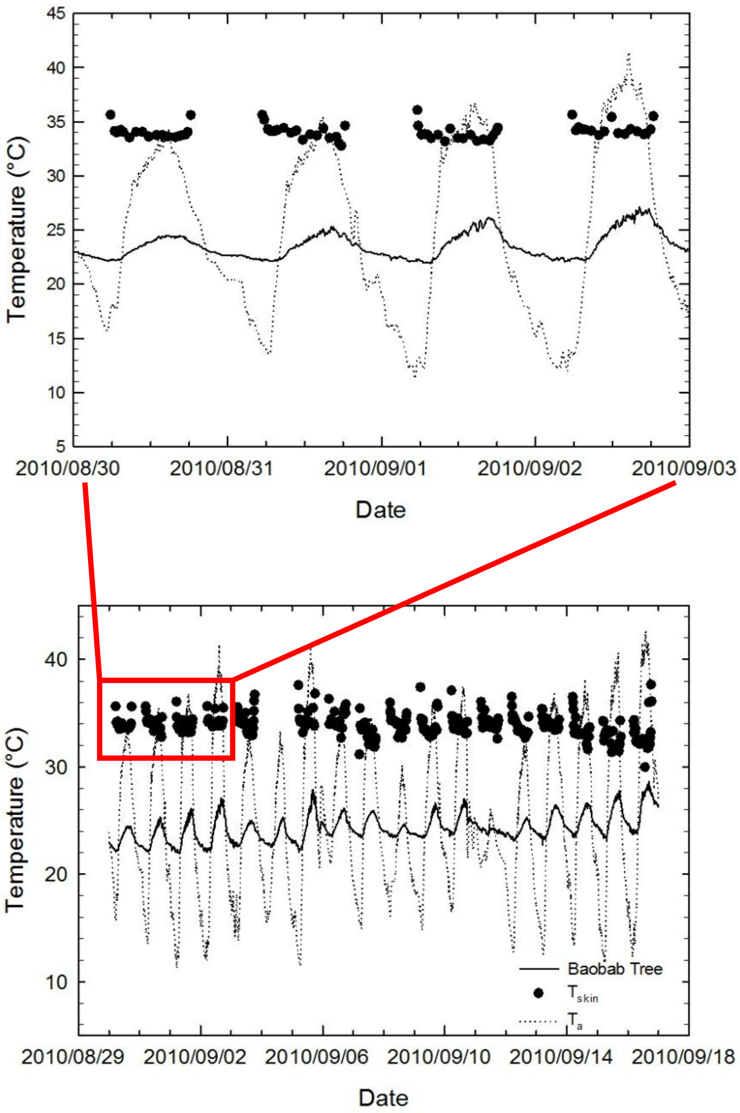


Figure 2. Skin temperature (T_{skin}) (dotted line) of a female *Nycteris thebaica* in relation to roost temperature (T_{roost}) (solid line) showing the representative pattern of heterothermy that was observed in all individuals in the study. The highlighted section shows a detailed view of T_{skin} fluctuations over four days.

N. thebaica did not use typical daily torpor as all T_{skin}s were maintained >28°C with the lowest minimum T_{skin}=28.4°C at T_{roost}=24.6°C recorded once from a single individual (Figure 3, 4). The heterothermy index values for the seven individuals ranged from 4.5-5.8°C, indicating that the temporal variation in T_{skin} of each individual during the daytime sampling period were fairly shallow. At a low T_{skin} (e.g. 29.2°C in Figure 2) the bat did not maintain its T_{skin} at this level but promptly increased T_{skin} up to 32.5°C within an hour (Figure 2). This pattern was observed across all individuals (Figure 4). Thus at no stage was there a 10°C difference between minimum T_{skin} and normothermic T_{skin}s and these patterns could not be classified as classic torpor and is thus broadly referred to as heterothermy as relatively small fluctuations in T_{skin} were observed (Figure 2 & 4). At the minimum T_{roost} of 16.5°C, the corresponding T_{skin} was 30.8°C (Figure 3). Regardless of T_a and weather conditions (rain and high wind conditions), bats always left the day roost to forage and roost at a various night roosts. For example, the study site experienced a thunderstorm on the 10/09/2010, and it was still raining when the bats exited their various day roosts to forage (Figure 2).

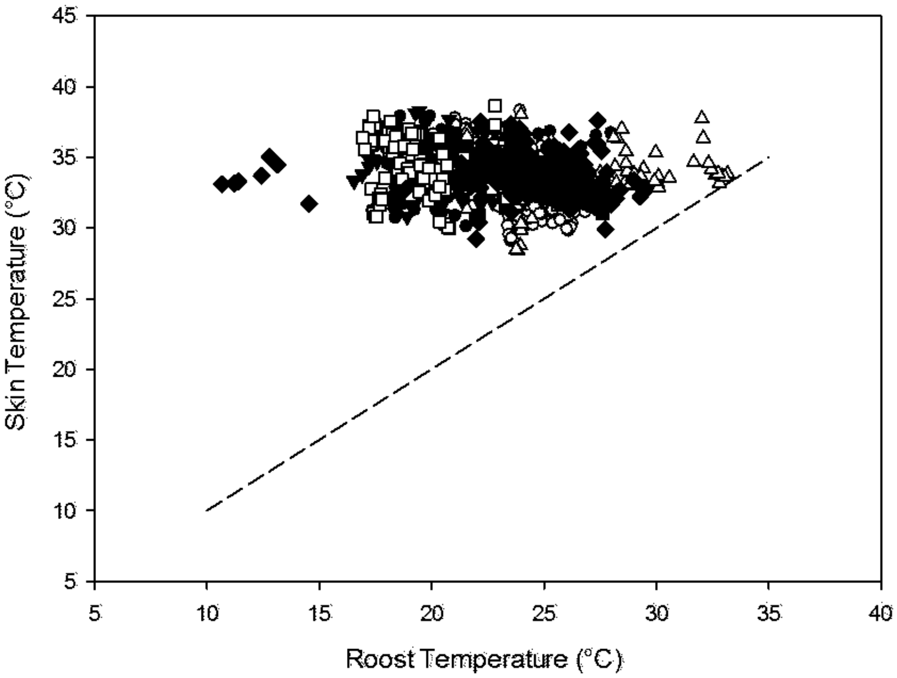
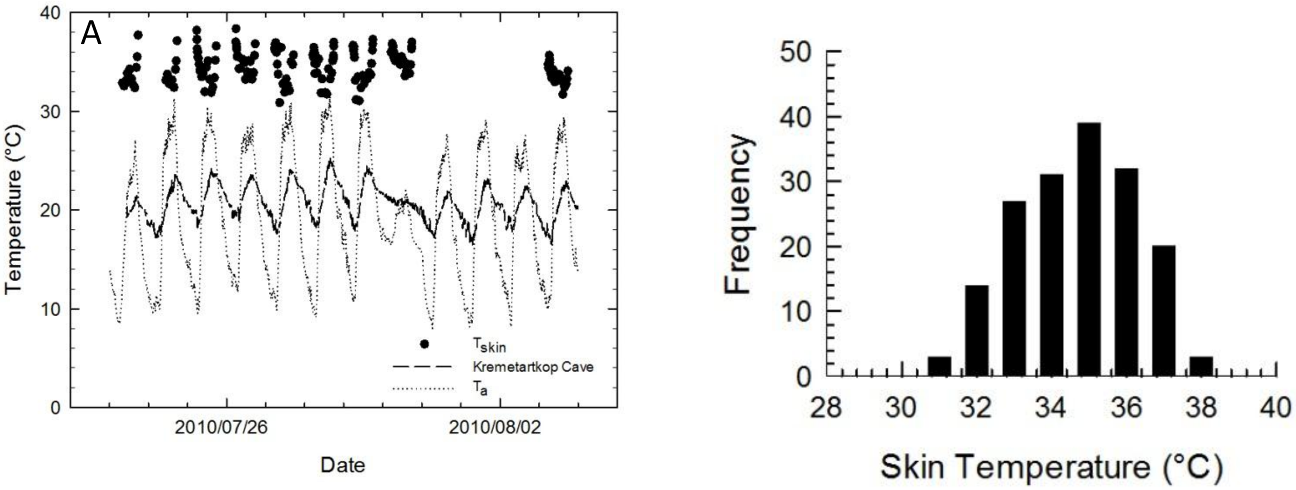
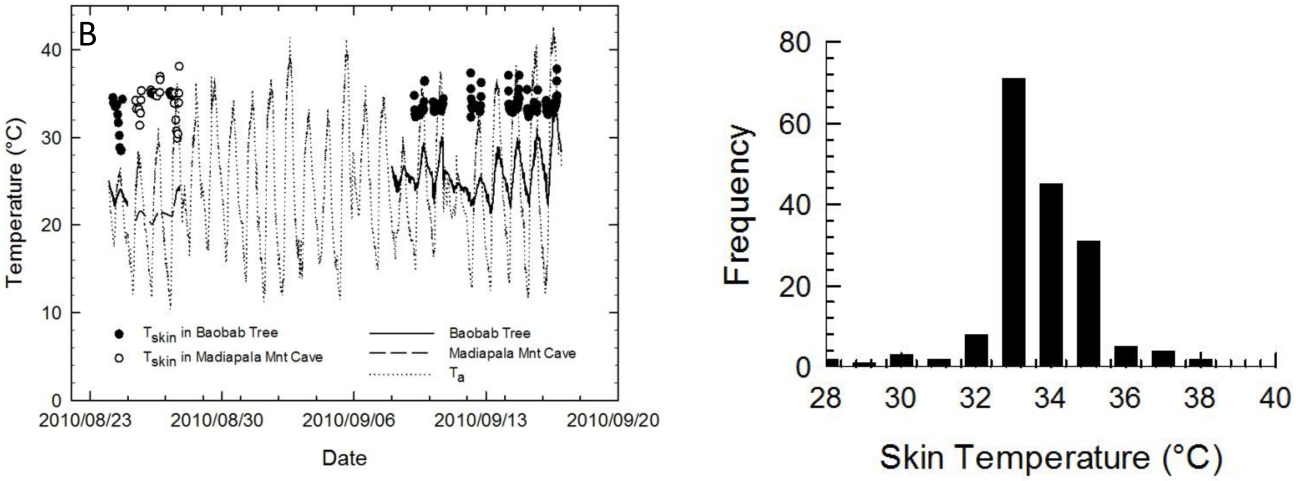
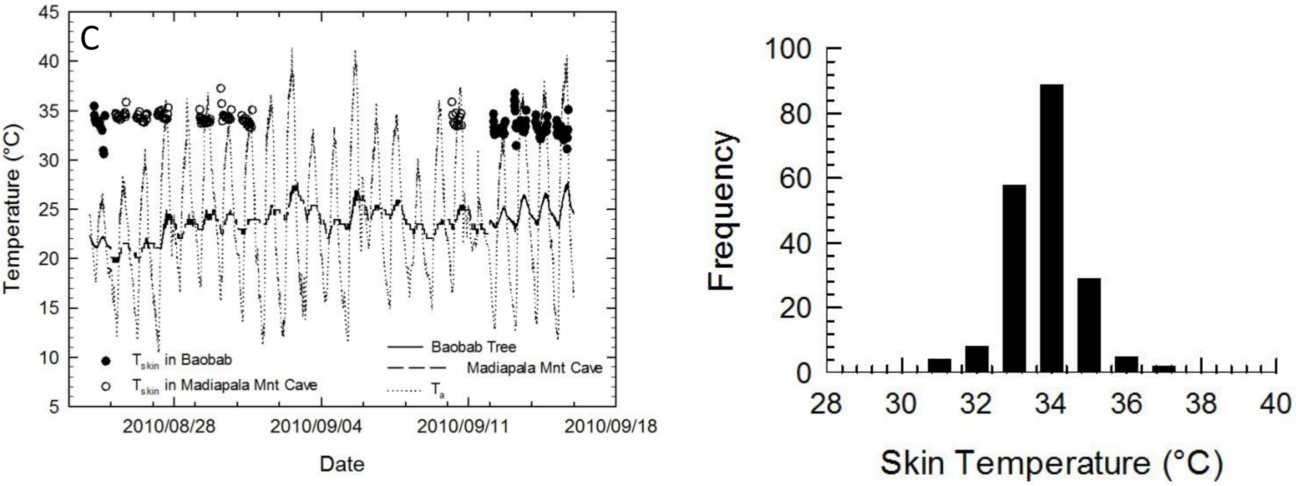
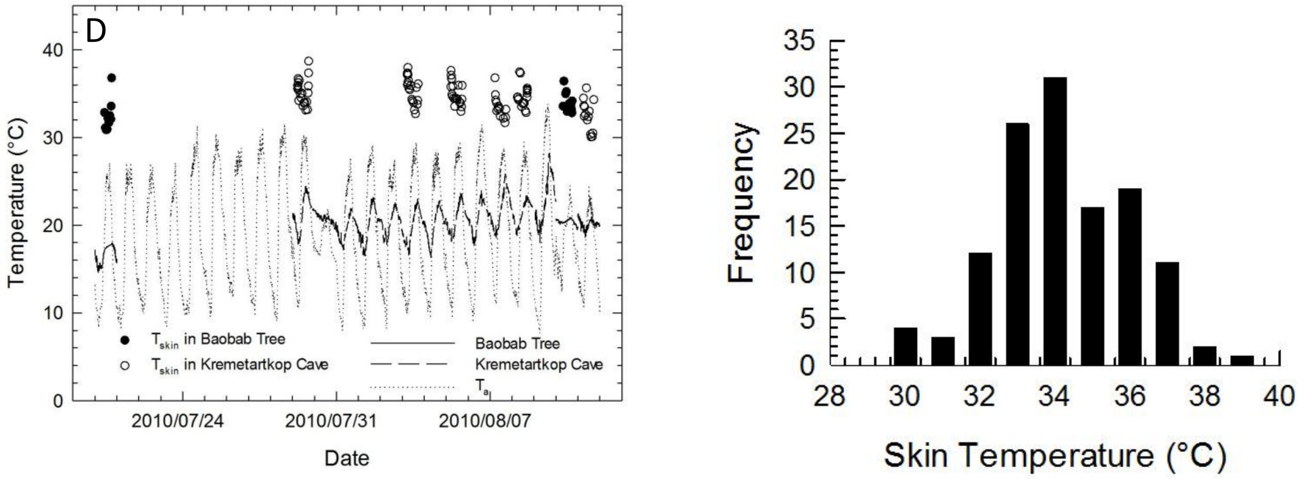


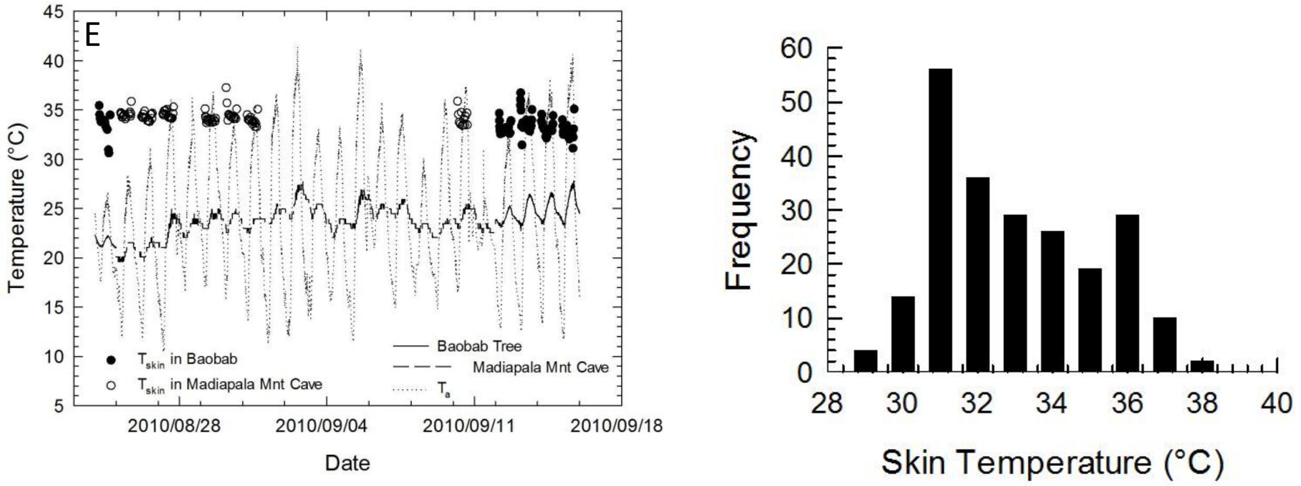
Figure 3. The relationship between skin temperature (T_{skin}) and roost temperature (T_{roost}) of seven *Nycteris thebaica* individuals denoted by different symbols. The dashed line represents the line of equality.

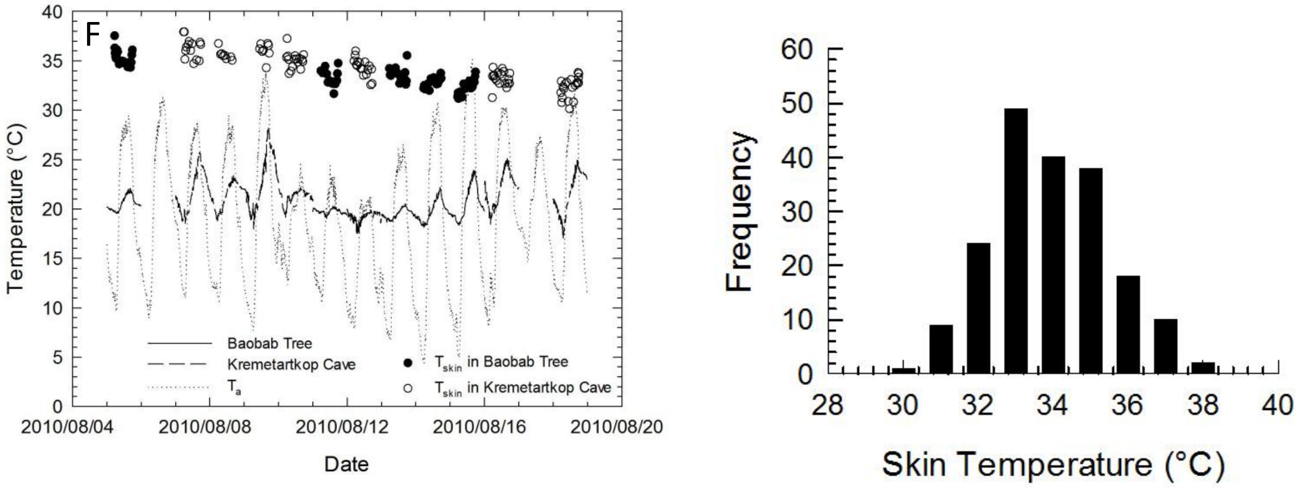

A
Temperature (°C)
40
30
20
10
0
2010/07/26
2010/08/02
Date
T_{skin}
Kremetartkop Cave
T_a
Frequency
50
40
30
20
10
0
28
30
32
34
36
38
40
Skin Temperature (°C)


B
Temperature (°C)
40
30
20
10
0
2010/08/23
2010/08/30
2010/09/06
2010/09/13
2010/09/20
Date
T_{skin} in Baobab Tree
T_{skin} in Madiapala Mnt Cave
Baobab Tree
Madiapala Mnt Cave
T_a
Frequency
80
60
40
20
0
28
30
32
34
36
38
40
Skin Temperature (°C)


C
Temperature (°C)
45
40
35
30
25
20
15
10
5
2010/08/28
2010/09/04
2010/09/11
2010/09/18
Date
T_{skin} in Baobab
T_{skin} in Madiapala Mnt Cave
Baobab Tree
Madiapala Mnt Cave
T_a
Frequency
100
80
60
40
20
0
28
30
32
34
36
38
40
Skin Temperature (°C)


D
Temperature (°C)
0
10
20
30
40
T_{skin} in Baobab Tree
T_{skin} in Kremetartkop Cave
Baobab Tree
Kremetartkop Cave
T_a
2010/07/24
2010/07/31
2010/08/07
Date
Frequency
0
5
10
15
20
25
30
35
28
30
32
34
36
38
40
Skin Temperature (°C)


E
Temperature (°C)
5
10
15
20
25
30
35
40
45
T_{skin} in Baobab
T_{skin} in Madiapala Mnt Cave
Baobab Tree
Madiapala Mnt Cave
T_a
2010/08/28
2010/09/04
2010/09/11
2010/09/18
Date
Frequency
0
10
20
30
40
50
60
28
30
32
34
36
38
40
Skin Temperature (°C)


F
Temperature (°C)
0
5
10
15
20
25
30
35
40
Baobab Tree
Kremetartkop Cave
T_a
T_{skin} in Baobab Tree
T_{skin} in Kremetartkop Cave
2010/08/04
2010/08/08
2010/08/12
2010/08/16
2010/08/20
Date
Frequency
0
10
20
30
40
50
60
28
30
32
34
36
38
40
Skin Temperature (°C)

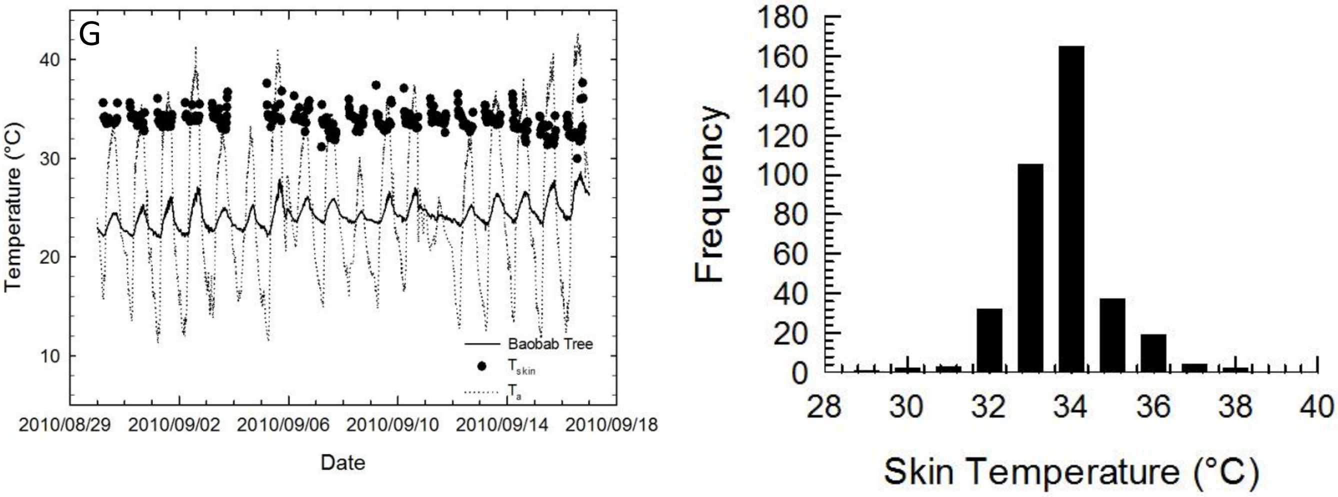


Figure 4. Skin temperature (T_{skin}) traces and associated T_{skin} distribution of seven free-ranging *Nycteris thebaica* individuals showing the range and frequency distribution pattern of T_{skin} experienced during winter by each bat in various day roosts.

The T_{skin} frequencies show a similar unimodal pattern across all individuals with the majority of values between 33 and 35°C (Figure 4).

Discussion

Roost site preference and fidelity in N. thebaica

Frequent roost switching behaviour was observed by *N. thebaica* in this study with frequent switching between the three day roosting sites, however, a single male bat that was trapped and tagged at the baobab roost tree roosted only in the Kremetartkop cave and did not switch between the various roosts (Figure 4). Aldridge *et al.* (1990) reported considerable variation in day roost use patterns where some bats occupied the same roosts during the study and others occasionally used the same roosts as observed in this study. *N. thebaica* is a long-lived bat (recorded up to 8 years of age) with relatively high rates of adult survival (Monadjem 2006). They are strong fliers, capable of moving large distances (9 km) from initial sites of capture (Monadjem 2006) and cover large distances whilst foraging (1.1 km) based on their low wing-loading (Monadjem *et al.* 2009). They are known to use different day and night roosts ranging from natural semi-permanent roosts such as Aardvark (*Orycteropus afer*) burrows and hollow trees to permanent structures such as road culverts, caves and abandoned buildings with day roosts being associated with rest and night roosts associated with foraging (Aldridge *et al.* 1990, Monadjem 2006, Monadjem *et al.* 2009, Taylor 2000, Monadjem *et al.* 2010). Selected day roosts by *N. thebaica* have been shown to be 2 to 12°C cooler than T_a during a hot summer in Mana Pools National Park, Zimbabwe (Aldridge *et al.* 1990). Similarly, during the heat of the day my data showed the day roosts were consistently and significantly cooler than the T_a during the winter study period (Figure 1). *N. thebaica* numbers were reported to vary greatly on a seasonal basis, thus suggesting some movement between roosting sites as similarly observed in my study area however the observed seasonal movement may be irregular and random or migration (Monadjem 2006, Monadjem *et al.* 2009). *N. thebaica* in my study area were present in very small numbers for the first two weeks of July 2010 as observed from the night roost and lack of individuals from their day roost in the baobab tree. From the 20 July 2010 numbers began to increase and by mid September colony numbers were high. When they left the area, where they moved to and why, remain a mystery.

Roost site preference and fidelity in many bat species has been shown to vary according to season, gender and reproductive status (Sedgeley 2001, Monadjem 2006, Monadjem *et al.* 2009, Fenton 1997, Fenton *et al.*1985, Fenton 1983, Brigham *et al.* 1997). Roost site fidelity differs among species and individuals with some species with high roost fidelity (e.g. *Chalinolobus tuberculatus* (Sedgeley 2001), *Carollia perspicillata*, *Phyllostomus hastatus* (Fenton *et al.* 1985)) and others switching roosting sites regularly, even on a daily basis (Fenton 1997 e.g. *Vampyrodes caraccioli*, *Artibeus lituratus* and *Scotophilus leucogaster* (Fenton *et al.* 1985)). Hutchinson & Lacki (2000) showed that Red bats (*Lasiurus borealis*) roosting in mixed mesophytic forests in eastern Kentucky used 44 different day roosts. Frequent roost switching of bats reduces predation rates, lowers their exposure to ectoparasites, allows them to choose alternative microclimates and is thought to lower the costs of commuting to foraging areas, however, there are few data to support this idea (Fenton 1997, Hutchinson & Lacki 2000, Lewis 1995). *N. thebaica* did not appear to have a preference or high fidelity for a particular roost in the study, and to speculate as to why this may be the case relating to roost microclimate, social interactions, potential predation events or distance to foraging areas would be challenging as it was impossible to acquire detailed roost microclimate data and to observe social interactions or predation events in the day roosts based on the nature of the roosts which were impossible to enter and due to the fact that foraging areas were not investigated in the study. No predators were observed near the roosts during the study.

Skin temperatures and roost microclimates

Most insectivorous bat species investigated so far use pronounced torpor bouts with T_bs lowered to within several degrees of T_a (Cory Toussaint *et al.* 2010, Heldmaier *et al.* 2004, Park *et al.* 2000). However, patterns of heterothermy typical of bats were not observed in *N. thebaica*. Instead, *N. thebaica* maintained high T_{skin}s throughout the study of 33.8±1.6°C across all individuals and only exhibited shallow decreases of 6-9°C in T_{skin} (Figure 3, 4). Prevailing weather conditions appeared to have no effect on T_{skin}s, and the bats left their respective roosts to forage every night during the study period, despite occasional light rain and heavy winds (personal observation). All individuals showed similar qualitative patterns of heterothermy, with little variation between individuals (Figure 2, 4). The slight decreases in T_{skin} observed may in fact be indicative of the bats cooling down from an active to a resting T_b (Figure 2, 4).

The absence of typical daily torpor in *N. thebaica* contrasts with the general microchiropteran pattern. Studies on the use of torpor at warm T_as are scarce but have been investigated in several bat species (Jacobs *et al.* 2007, Maloney *et al.* 1999, Bronner *et al.* 1999, Turbill *et al.* 2003a, b, Stawski & Geiser 2010b, Bartels *et al.* 1998). Thermoregulation patterns in *Scotophilus dinganii* in subtropical South Africa were shown to be determined by roost type and microclimate, with a single torpor bout per day used while roosting in buildings, tree foliage and tree cavities during wet and mild T_a conditions with associated low insect abundance and availability (Jacobs *et al.* 2007). When T_a and T_{roost} were at their maximum these bats were normothermic and passively aroused as T_{roost} increased in the afternoon (Jacobs *et al.* 2007). *Mops condylurus* is known to use very hot roosts, often selecting microclimates with T_a often exceeding 40°C to maintain high MRs and remain alert for potential predators (Bronner *et al.* 1999, Maloney *et al.* 1999, Vivier & van der Merwe 2007). *M. condylurus* is cold sensitive and has been shown to only enter torpor when they are exposed to $T_a < 29°C$, which is below their TNZ during both summer and winter and thus allows them to conserve energy during unfavourable conditions that increase energy requirements as forage availability potentially decreases (Maloney *et al.* 1999, Bronner *et al.* 1999, Vivier & van der Merwe 2007). Thus, both *S. dinganii* and *M. condylurus* have been shown to enter torpor at warm T_{roost}s< 20°C (Maloney *et al.* 1999, Bronner *et al.* 1999, Vivier & van der Merwe 2004, Jacobs *et al.* 2007). Similarly, in subtropical Australia *Nyctophilus bifax*, *N. geoffroyi* and *Vespadelus pumilus* have been shown to use torpor during both winter and summer with *V. pumilus* using torpor during mild and warm T_as even when food was abundant (Stawski & Geiser 2010a, Stawski & Geiser 2010b, Turbill & Geiser 2008, Stawski *et al.* 2008, Geiser 2004, Turbill *et al.* 2003). It has been shown that during winter, T_a fluctuations are larger than during summer, and since bats are more energetically constrained, torpor bouts have been shown to be longer than those used in summer (Stawski & Geiser 2010a). These three species roost under the exfoliating bark of trees and as such are exposed to the daily fluctuations in T_a with periodic arousals from torpor coinciding with the maximum T_{roost} which lower the costs of rewarming and which in turn could largely reduce energy expenditure during winter hibernation (Turbill & Geiser 2008, Stawski *et al.* 2008, Turbill *et al.* 2003).

The tropical Australian megachiropteran, *Macroglassus minimus* was previously thought to be strictly homeothermic with no need for torpor since they are fairly large (14g) and restricted to regions with readily available food and seasonal changes are less pronounced but along with several other species of small fruit and blossom bats (<20g) such as *Syconycteris australis*, they were shown to use torpor with minimum T_bs of 20°C resulting in a significant reduction in MR (Bartels *et al.* 1998). In subtropical Taiwan, Liu & Karasov (2011) found that *Hipposideros terasensis* had reduced activity during winter and recorded individuals hibernating at T_{roost} >22°C for up to 72 days. Hibernating adult bats lost an average of 13-14% of their body mass, suggesting that over the hibernating period their torpid MR must have been remarkably low, allowing them to hibernate for more than two months. *H. terasensis* differs from temperate region hibernators in that adults do not increase their body mass during the autumn leading up to their hibernation, they hibernate at T_{roost}s and T_{skin}s greater than 20°C, and even at these warm T_{roost}s they do not have a high arousal frequency as would be expected and finally, they are the largest bat species (60g) recorded to hibernate (Liu & Karasov 2011). In light of the above evidence of bat species roosting at warm T_{roost}s and T_as, the lack of torpor use in *N. thebaica* is particularly surprising.

Although the absence of pronounced heterothermy in *N. thebaica* reveals that this species does not use torpor to conserve energy under the conditions prevailing during my study, however several studies have shown that even small decreases in T_b result in significant energy savings (Barclay *et al.* 2001, Chruszez & Barclay 2002, Jacobs *et al.* 2007, Studier 1981, Webb *et al.* 1996). An important selective force in the ecophysiological adaptation of bats is roost microclimate, since bats spend more than half their lives in roosts and such microclimates have a profound effect on energy, water turnover rates and MRs (Chruszez & Barclay 2002, Buffenstein *et al.* 1999, Bronner *et al.* 1999). Potentially, the small decreases in T_{skin} observed in *N. thebaica* may allow them to conserve energy during the day, but this requires further investigation. Rock crevices, mines and caves provide suitable thermally stable roosts that shelter bats from climatic extremes resulting in T_{roost}s that are warmer than T_as during the night and cooler than T_as during the day (Lawson & Barclay 2006, Monadjem *et al.* 2009). This T_{roost} pattern was observed in the baobab tree and Kremetartkop cave roost used by *N. thebaica* as day roosts (Figure 1). The hollow baobab tree and Madiapala Mountain cave day roosts of *N. thebaica* were consistently warm and T_{roost}s were fairly constant whereas the Kremetartkop cave day roost had greater fluctuations in the daily temperature cycle (Figure 1). Perhaps the observed heterothermy pattern in *N. thebaica* is based on T_{roost}s potentially being too warm for torpor use and the small decreases in T_{skin} could simply be the bats cooling down from an active to resting phase.

Potentially, the reproductive status of *N.* thebaica could have influenced the heterothermy pattern, as warm T_{roost}s have been shown to be energetically advantageous for reproductive bats as the costs associated with maintaining high T_bs of females and young are lowered, gestation periods are shorter, milk production is higher and growth rates of young increase once they are able to regulate their T_bs (Sedgeley 2001, Lausen & Barclay 2006). Copulation and fertilization occurs in June in *N. thebaica*, therefore females would have been in early stages of pregnancy in August (Bernard 1982) and the lack of use of torpor could be expected. However, if this was the reason behind the pattern of heterothermy observed in *N. thebaica*, different heterothermic patterns should have been observed in males and some degree of torpor use would have been expected; however this was not the case. A larger sample size of males and females would allow for a more vigorous comparison of heterothermic patterns between sexes.

Other possible reasons for a lack of typical daily torpor in *N. thebaica* are predator avoidance, foraging success and meal size (Matheson *et al.* 2010), with the latter two factors potentially bearing the greatest influence on heterothermy in *N. thebaica*. Foraging success can vary substantially between individuals on a daily basis and may be very important for animals that need to budget energy on a day-to-day basis (short time scales) therefore energy expenditure needs to be closely balanced by energy intake (Matheson *et al.* 2010). Unlike most bat species that are clutter edge and high aerial foragers which use torpor and are highly dependent on the availability and abundance of aerial insects in order to forage successfully (Dechmann *et al.* 2011), *N. thebaica* is a clutter forager and gleans stationary or slow moving prey from vegetation or the ground (Aldridge *et al.* 1990, Monadjem *et al.* 2010, Taylor 2000). Many open-air forager species such as the Neotropical bat *Molossus molossus* which rely heavily on an unpredictable food source in both time and space, use torpor to balance the high energetic cost associated with flight and acquiring food during the short one to two hour foraging period per day (Dechmann *et al.* 2011). Matheson *et al.* 2010, investigated the role of diet (meal size) and T_a on the expression of torpor. This was investigated in *Myotis lucifugus* and recent feeding on a high protein diet of crickets resulted in a delayed entry into torpor, largely independent of T_a, and that normothermic T_bs were maintained longer (Matheson *et al.* 2010). A similar response has been shown in chipmunks (*Tamias striatus*) which used less torpor when supplied with larger amounts of food hoards during winter (Matheson *et al.* 2010).

Recent successful foraging may also influence torpor use by increasing metabolic heat production or the heat increment of feeding (HIF) which is associated with digestion (Matheson *et al.* 2010). The HIF is the sum of all heat producing actions required to digest a meal, and the magnitude and effect of such is generally proportional to the meal size and diet composition (Matheson *et al.* 2010). Large, protein rich diets result in a larger postprandial metabolic response and thus if an animal draws on the HIF for thermoregulation, normothermy could be maintained for a longer period of time or the depth and or duration of torpor could be reduced following feeding and in turn mitigate the potential ecological and physiological costs associated with the use of torpor (Matheson *et al.* 2010). Similarly, in temperate regions, bats have been shown to meet increased energetic demands during cooler weather by increasing the frequency and duration of foraging bouts or by entering torpor (Sedgeley 2001). Thus, a potential reason why *N. thebaica* did not use the classic torpor pattern that so many other Vespertilioniformes bat species exhibit could be due to the fact that during the winter period, these bats have a foraging period ranging from 9.97-12.42hrs (11.45±0.65hrs, n=60 nights) during which separate night roosts from the day roosts are used. These night roosts, which can be a specific bush or man-made structure, are used by *N. thebaica* to consume large bodied arthropod prey items from large Lepidoptera and Neuroptera to Arachida, in particular, scorpions are consumed during winter (Pers. Obs. Monadjem *et al.* 2010, Aldridge *et al.* 1990, Fenton & Thomas 1980, LaVal & LaVal 1980, Seamark & Bogdanowicz 2002). Regardless of weather conditions *N. thebaica,* was observed to leave their various day roosts to forage. Therefore, considering the above mentioned characteristics of foraging observed in *N. thebaica* particularly the approximately 12 hr foraging period and the large bodied prey items, they may be drawing on the HIF of their large, protein rich diet and only allowing their T_bs to decrease by several degrees during the day roosting period independent of T_{roost}s even when T_{roost} fell below 25°C. However, the HIF factor will only affect heterothermy for several hours after feeding and not for the duration of the day roosting period, therefore, the predominant factor or reason as to why *N. thebaica* does not use typical torpor is unclear.

In summary, *N thebaica* does not use typical daily torpor observed in many other animal groups and insectivorous bats. The thermoregulatory pattern of this species may be dependent on roost microclimate selection, diet and foraging ecology and or potential predation risk. The slight decreases in T_{skin} that has been observed in *N. thebaica* likely conserve energy during the resting period, but this requires further investigation.

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Chapter 2: Interspecific variation in thermoregulation in three sympatric southern African bats in a hot, arid environment, the Limpopo River Valley.

(prepared for submission to*Functional Ecology*)

Nycteris thebaica – Egyptian slit-faced bat

Taphozous mauritianus – Mauritian tomb bat

Sauromys petrophilus – Roberts’ free-tailed bat © Dawn Cory Toussaint

Introduction

Life originated in aquatic environments, and terrestrial habitats, particularly those that are hot and/or arid, pose significant physiological challenges related to the risks of hyperthermia and desiccation (Walsberg 2000). Many endotherms in hot environments regularly encounter air temperatures (T_a) that exceed body temperature (T_b) and under these conditions evaporative heat loss is the only mechanism whereby animals can maintain T_b below T_a (Walsberg 2000, Brown & Lee 1969). Evaporative water loss (EWL) via cutaneous and/or respiratory pathways thus represents a crucial thermoregulatory mechanism in hot environments for heat transfer against the temperature gradient (Walsberg 2000, Brown & Lee 1969). Body mass is a variable of considerable physiological significance for animals inhabiting hot environments, as rates of heat exchange between an organism and the environment are proportional to the ratio of body surface area to volume, and an organism's capacity to store heat (thermal inertia) is inversely proportional to its mass (Lindstedt 1980, McNab 1974). Thus, under very hot environmental conditions, the smallest endotherms are the most vulnerable to hyperthermia and desiccation due to their high surface area-volume ratio, limited insulation of short fur and feathers and very high mass specific metabolic rates (Lindstedt 1980).

Many small mammals avoid very high environmental temperatures by being nocturnal and using thermally-buffered microsites such as burrows during the day, but one taxon that represents a notable exception is the Chiroptera. In tropical and subtropical latitudes, bats often occupy day roost sites that are not thermally-buffered and where they are potentially exposed to very high environmental temperatures. High roost temperatures (T_{roost}) pose significant challenges in terms of water and energy balance since small bats have large surface area-volume ratios with associated high mass-specific evaporative water loss rates, but generally experience zero drinking or dietary water intake during the hottest part of the day (Walsberg 2000, Lovegrove 1993, Chew & White 1960, Carpenter 1969). Some species, including *Myotis yumanensis, Antrozous pallidus* and *Tadarida brasiliensis*, are frequently exposed to T_{roost} values of 40 - 50˚C, whereas *Molossus coibensis* individuals experienced environmental temperatures up to 54˚C whilst hanging in contact with a galvanized metal roof (Herreid 1967, Licht & Leitner 1967). The southern African molossid *Mops condylurus* regularly roosts at T_{roost} > 40°C, and has been shown to actively select regions with T_{roost} between 35-42°C (Bronner *et al.* 1999).

Although the use of hot roosts by bats is well documented, considerably less is known about thermoregulation at high environmental temperatures, and even less about dehydration tolerances (Licht & Leitner 1967, Bronner *et al.* 1999, Maloney *et al.* 1999, Marom *et al.* 2006). When exposed to hot conditions, bats from tropical or subtropical latitudes appear to either tolerate high T_{roost} or behaviourally avoid them by means of postural changes, moving to cooler microclimates or by flying within the roost which allows for heat dissipation as more surface area of the bat is exposed (Herreid 1967, Licht & Leitner 1967, Marom *et al.* 2009, Carpenter 1969). Bats that roost in hot, dry environments may lose substantial fractions of their body water pool via evaporative cooling on a daily basis (Studier *et al.* 1970). The latter authors examined dehydration tolerance in several *Myotis* species by measuring diurnal body weight loss, and found that diurnal mass loss in *Myotis spp.* ranged from 6.4-21.9% of body mass, while EWL rates varied between 44 and 77mg hr^{-1} in *M. yumanensis, T. brasiliensis* and *A. pallidus*.

Like other small endotherms, bats dramatically increase rates of EWL at high T_a. For example, an increase in T_a of 25°C to 40°C was associated with a 3-4 fold increment in EWL in *Rhinonycteris aurantius, Miniopterus schreibersii* (Baudinette *et al.* 2000), *Artibeus hirsutus, Leptonycteris sanborni* (Carpenter & Graham 1967), *Eptesicus fuscus, T. brasiliensis* (Heirred & Schmidt-Nielsen 1966) and *M, condylurus* (Maloney *et al.* 2006), and a 5-8 fold increase in *Chalinolobus gouldii* (Hosken & Withers 1997), *Dobsonia minor* (Bartholomew *et al.* 1970), *Macroderma gigas* (Baudinette *et al.* 2000), *Otonycteris hemprichii* and *T. teniotis* (Marom *et al.* 2006). An increase in T_a from 25 to 45°C was associated with a 12-fold increase in EWL in *M. condylurus* (Maloney *et al.* 1996). In one of the few detailed examinations of how bats cope with very high T_{roost}, *M. condylurus* roosting under a corrugated iron roof in eastern South Africa exhibited T_bs as high as 43˚C, and dissipated up to 130% of metabolic heat production via EWL (Maloney *et al.* 1999). This study reveals how “close to the edge” bats roosting in hot roosts can be; very few endotherms can tolerate T_bs higher than 43-45˚C (Cossins & Bowler 1987, Maloney *et al.* 1999). Furthermore, observed rates of evaporative heat loss at T_a of 45˚C in *M. condylurus* represent water loss equivalent to approximately 2% of body mass per hour (Maloney *et al.* 1999). Data on dehydration tolerance for bats are scarce, but in similarly sized birds, acute dehydration tolerance are thought to be between 12 and 20 % of body mass (McKechnie & Wolf 2010). The body mass loss that is considered lethal for

Vespertilionid bats lies between 23-32% of the initial body mass (Studier *et al.* 1970), but these values are based on chronic dehydration at moderate T_a and thus likely represent both water and tissue mass losses.

The issues outlined above with regards to bats roosting in hot climates are particularly pertinent in the face of the increases in maximum T_a and the frequency of heat waves associated with climate change (IPCC 2007, IPCC 2011). It has been shown that $T_{roost} > 40$˚C is intolerable to many temperate-zone and tropical microchiropterans, including *M. velifer, Macrotis californicus* (but see Licht & Leitner 1967), *M. gigas, A. hirsutus* and *Desmodus rotundus* (Reeder & Cowles 1951, Lyman &Wimsatt 1966, Carpenter & Graham 1967, Leitner & Nelson 1967, Maloney *et al.* 1999). There is evidence that Australian megachiropterans roosting in exposed sites are experiencing large scale mortality as a result of more frequent and intense heat waves, with at least 18 die-off events involving the death of more than 33 000 individual flying foxes of *Pteropus alecto* and *P. poliocephalus* recorded during heat waves since 1994 (Welbergen *et al.* 2008). A related observation is that the frequency of catastrophic die-offs among bird communities in hot deserts is expected to increase dramatically during heat waves in coming decades (McKechnie & Wolf 2010). These observations are highly relevant for bat species that may experience exceptionally high T_{roost} and are unable to select cooler microsites within a given roost.

The aims of this study were to investigate thermoregulation at high T_as in three southern African bat species that occur sympatrically in hot, semi-arid environments, namely *Nycteris thebaica, Taphozous mauritianus* and *Sauromys petrophilus*. These species use roosts that differ in thermal properties, particularly in terms of buffering bats from maximum T_as (D. Cory Toussaint, *pers. ob.*). Thus, these three sympatric species represent a model system suitable for addressing questions related to interspecific variation in heat tolerance, and thermoregulatory abilities at high environmental temperatures. The Limpopo River Valley is one of the hottest parts of southern Africa, with T_as sometimes exceeding 45˚C, and is generally arid with mean annual rainfall of 400-500 mm/year (Thomas *et al.* 2007). Increases in T_a maxima of 2-5˚C are predicted for many hot regions of the tropics and subtropics, including the Limpopo River Valley (IPCC 2007, Norström *et al.* 2005). In addition, the area is expected to experience more frequent droughts, with a delayed start to the wet season (Thomas *et al.* 2007). These higher T_as and associated reductions in surface water availability will potentially have major effects on water and energy balance of small endotherms in this region. In particular, evaporative water demands increase rapidly at environmental temperatures above T_b, and even small increases in maximum T_as have the potential to severely decrease survival times of small endotherms during very hot weather (McKechnie & Wolf 2010). As outlined above, bats are likely to be particularly susceptible to dehydration and/or hyperthermia during heat events, on account of their nocturnal activity and lack of drinking and foraging during the day. I hypothesised that interspecific variation in heat tolerance and evaporative cooling capacity between the three bat species is correlated with roost thermal properties, and varies among sympatric species that roost in microsites that differ in the extent to which they are thermally buffered from high outside T_a.

Materials and Methods

Study site

The study was conducted on the private game farm Makulu Makete (28˚ 52’ S 22˚ 35,09’ E) near the town of Alldays in the subtropical Limpopo River Valley, Limpopo Province, South Africa. The reserve is 3703 ha in extent and characterised by very hot summers and cool winters. It is semi-arid with annual rainfall averaging 388 mm (De Klerk 2002). The topography of the area is predominantly flat, with two elevated topographical features, Madiapala Mountain and Kremetartkop, which together comprise 208 ha of the reserve. The vegetation is classified as Transvaal sweet bushveld, which includes mopane veld and scattered baobab trees (*Adansonia digitata*) (de Klerk 2002).

Study species and roost sites

Evaporative water loss (EWL), metabolic rate (MR) and body temperature (T_b) was measured during the austral summer of 2010-2011 in three insectivorous bat species, namely *Nycteris thebaica* (Egyptian slit-faced bat), *Taphozous mauritianus* (Mauritian tomb bat) and *Sauromys petrophilus* (Roberts’ flat-headed bat). The mean body masses (M_b) of *N. thebaica, T. mauritianus* and *S. petrophilus* individuals used in the study were 11.65±0.97g (n=32), 26.17±2.70g (n=15) and 10.97±1.40g (n=37) respectively.

Each species was caught using different strategies based on their ecology and roosting habits. *N. thebaica* roosted in a hollow baobab tree during the day and used an old hunter’s hut as a night roost. They were actively captured with a hand net from their night roost between 2.30 and 3.00am, just before they returned to their day roost. The captured bats were placed in cloth bags and set in a quiet room to be placed in the respirometry system later in the morning from 6 am onwards once they had settled down. *T. mauritianus* are exceptionally vigilant and were captured on the morning of data collection from two day roost sites on the sides of two buildings using a mist net extended in front of the roost. The bats were placed in bags and left to calm down for about an hour and then placed in the respirometry system. Lastly, *S. petrophilus* roosted in rock crevices during the day and were captured the night before experimentation at a drinking hole in the vicinity of their day roosts. Bats were captured in a free-standing mist net extended over a waterhole. These bats were placed into bags and held overnight and placed in the respirometry system in the morning. Each species was captured on alternating days so as to minimise stress imposed at the night and day roosting sites and at relevant drinking holes.

Roost, air and body temperatures

Air temperatures within the three species’ roost sites (T_{roost}) were recorded using miniature data loggers (iButton Thermochron, Dallas Semiconductor, Dallas, TX, USA) placed as close to the roosting bats as possible and suspended in shaded locations so as to measure air rather than surface temperature. Outside air temperature (T_a) was recorded using an iButton placed in the shade on the side of a building that was also used by *T. mauritianus* as a day roost, suspended in a ventilated white polystyrene cup.

Body temperatures (T_b) were measured using temperature sensitive passive integrated transponder (PIT) tags (Destron Fearing, St. Paul, MN 5507) injected subcutaneously between the scapulae. T_bs were measured using a Pocket Reader *EX* handheld reader (Destron Fearing, St. Paul, MN 5507, product number RE 6100), modified to energise and receive signals from PIT tags via external antennae. An external antenna, housed in a rectangular plastic box, was attached in a vertical position to one side of each metabolism chamber.

All iButtons and a representative sample of 10 PIT tags were calibrated before use. iButtons and PIT tags were calibrated using a mercury thermometer in a waterbath over a range of temperatures from 15-45°C and 25-47.5°C respectively. The calibration equation for the PIT tags, determined using a linear regression, was y =

1.0384x + 0.1055 ($R^2 = 0.9995$).

Evaporative Water Loss and Metabolic Rates

Respiratory gas exchange [oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$)] and EWL were measured at T_as of 10-42°C in a field laboratory using an open flow-through respirometry system. Bats were placed individually in 1.3 L metabolic chambers constructed from Lock-tight™ storage containers. A small rectangle of shade cloth was secured to the inside of the chamber lid and the vertical wall of the chamber nearest the PIT tag antenna, in order to encourage bats to cling within range of the antenna. To prevent the EWL measurements being confounded by evaporation from urine and faeces, mineral oil was placed in the bottom of each chamber to a depth of 1 cm, with a plastic mesh platform (large enough mesh to allow faeces to fall through) positioned approximately 10 cm above the oil.

Atmospheric air supplied by a pump (model DOA-P13-BN, Gast Air Pumps, Benton Harbour, Michigan, USA) passed through a filter (model F3000-8G, CKD corporation, Shanghai, China), before being partially dried using two glass columns containing silica gel connected in series. The air supply was then split and passed through needle valves, which regulated the flow rate to each chamber (maximum of three chambers used simultaneously). Flow rates were measured before and after each run using the mass flow meter of an SS-3 Subsampler (Sable Systems, Las Vegas NV, USA), regularly calibrated using a 1-L soap bubble flow meter (Baker & Pouchot 1983) as before and after flow rates often varied between experiments. Flow rates of 500-700 ml min^{-1} were used, which ensured that O_2 concentrations within the chambers remained above 20.4 %, and relative humidities remained low, with a maximum water vapour partial pressure of 1.7 kPa. The 99 % equilibrium times for these chambers calculated following Lasiewski *et al.* (1966) ranged from 8.5 – 11.9 min. To enhance mixing of air within the chambers, the air inlet was positioned near the bottom of the chamber and the outlet near the top. Air temperatures in the chambers were measured using Cu-Cn thermocouples inserted into the chambers through small holes in the lids and secured in place. Chamber air temperatures were measured using a TC-1000 Thermocouple Meter (Sable Systems).

Excurrent air from each chamber and a baseline channel of incurrent air, were sequentially subsampled using a TR-RM8 Respirometry Multiplexer and SS-3

Subsampler (Sable Systems). At the start of each run, baseline air was first subsampled for 3 min, then air from each chamber was subsampled for 15 min, followed by another 3-min baseline. Subsampled air was first pulled through a RH-300 water vapour analyser (Sable Systems), which was regularly zeroed using nitrogen and spanned by calculating the water vapour pressure of saturated air at a known temperature, generated by bubbling atmospheric air through water at room temperature and then through water 3-4 °C cooler than room temperature. Subsampled air then passed through a CA-10a CO_2 analyser (Sable Systems) and FC-10a oxygen analyser (Sable Systems) to determine fractional CO_2 and O_2 concentrations respectively. The CA-10a was regularly zeroed using nitrogen gas and spanned against an analytically certified gas with a known CO_2 concentration of 2002 ppm (AFROX, Johannesburg, South Africa). The FC-10a was regularly spanned to a fractional O_2 concentration of 20.95% using dry CO_2-free air that was generated by passing atmospheric air through soda lime (Merck Chemicals, Wadeville, South Africa) and then magnesium perchlorate (Merck Chemicals, Wadeville, South Africa) through the analyser, following Lighton (2008). All tubing used in the system was Bev-A-Line tubing (Thermoplastic Processes Inc., Warren, NJ, USA). Baseline water vapour, O_2 and CO_2 concentrations were recorded for 3 min at the beginning of each subsampling cycle. Voltage outputs from all analysers were digitised using a Universal Interface II (Sable Systems). Each channel was analysed for 15min with a sampling interval of 1 second. Data were recorded using a personal computer with Expedata software (Sable Systems).

Bats were exposed to T_as of 10-42°C in increments of 5°C, except at $T_a > 40$ °C. At T_a values between 10 and 25°C, 1-3 individuals per species were exposed to three different T_a values selected randomly on each day of experiments. At higher T_as between 30-40°C, however, only two temperatures were used per run and bats were exposed to each T_a for a maximum of 3 hr. At the highest T_a of approximately 42°C, individuals were only exposed for 45-60 min and removed immediately thereafter and given water to drink. If T_b spiked to levels suggestive of uncontrolled hyperthermia (T_b>43°C), the bats were immediately removed from the environmental chamber and similarly, when T_b begin to rapidly increase, particularly in *T. mauritianus*, rapid, shallow breathing was visually observed while the animals were in the chambers (personal observation).

The dry heat transfer coefficient (dry thermal conductance) was calculated as

C_{dry} = (MR - EHL)/(T_b - T_a)A_b following Marom *et al.* (2006) where MR is the rate of heat production, EHL is the rate evaporative heat loss and A_b is surface area.

Data analysis

$\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated using equation 9.4 and equation 9.5 respectively, $\dot{V}_{H_2O}$ was calculated using equation 9.6 and excurrent flow rate was calculated using equation 9.3 in Lighton (2008).

Resting metabolic rates and EWL rates were calculated from steady-state traces of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ in ExpeData, with the lowest 1-min mean values considered to be indicative of resting values. Respiratory exchange ratios (RER) were determined as $\dot{V}_{CO_2} / \dot{V}_{O_2}$. RER values averaged 0.77 ± 0.17, with a value of 0.71 indicative of lipid metabolism, and thus indicating a post absorptive state (Walsberg & Wolf 1995). On several occasions, bats exhibited RER values outside the range of 0.71-1.00. Although the latter range is considered typical for animals metabolising carbohydrates and lipids values less than 0.71 and greater than 1.00 are normal for birds and not just a product of experimental error (Walsberg & Wolf 1995). Values below 0.71 may be due to the incomplete oxidation of fat and loss of CO_2 by non-pulmonary routes, such as bicarbonate ion excretion and storage, whereas values > 1.00 are generally associated with fat synthesis (Walsberg & Wolf 1995). I am not aware of reliable thermal equivalence data for RER values beyond the 0.71-1.00 range; thus, I used the relevant values for RER = 0.71 for lower values and RER = 1.00 for higher values. Metabolic rates (W) were determined by converting the gas exchange measurements using the thermal equivalence data in table 4.2 in Withers (1992). Using this approach within the 0.71-1.00 range involves the assumption that only carbohydrates and lipids are metabolized, and a maximum error of 6% is associated with protein catabolism (Walsberg & Wolf 1995).

The thermoneutral zone of each species was determined using the Broken Stick procedure in R (R 2.13.0, R Development Core Team) to identify the lower critical limit of thermoneutrality (T_{LC}). The upper critical limit of thermoneutrality (T_{UC}) was determined by calculating the intercept of two regressions fitted to the most level part of the metabolic rate data and through the increase in metabolic rate thereafter, as there were usually too few data points above the T_{UC} to use the Broken

Stick procedure.

For interspecific comparisons, body mass (M_b) and BMR (W) data were $\log_{10}$ transformed before the analysis. In order to compare BMR in the three species I investigated to those of other bats, I first tested for phylogenetic signals in M_b and BMR using randomization tests for the mean-squared error and by calculating the *K*-statistic (Blomberg *et al.* 2003, MatLab program PHYSIG_LL.m). I constructed a tree for the 91 species in my data set (Table 3) based on the Bininda-Emonds *et al.* (2007) supertree using the program Mesquite. Since M_b and BMR both exhibited significant phylogenetic signal, I next fitted conventional ordinary least squares (OLS) and phylogenetically informed least squares (PGLS) regressions to $\log_{10}$BMR (W) and $\log_{10}M_b$ (g) data using the MatLab program REGRESSIONv2.m (Lavin *et al.* 2008). In order to determine the model that provided the best fit to the data I applied the various branch length transformations available in REGRESSIONv2.m, namely Brownian motion (no transformation), Ornstein–Uhlenbeck (OU), Grafen's ρ, and Pagel's λ, and then compared original Akaike Information Criterion (AIC) and corrected AIC (AICc) values to identify the model that provided the best fit (Lavin *et al.* 2008; Swanson & Garland 2008). I used a similar approach for EWL and heat tolerance, although the smaller sample sizes compared with BMR means that tests for phylogenetic signal are less reliable (Blomberg et al 2003).

Results

Body temperature

Most *Nycteris thebaica* and all *Taphozous mauritianus* individuals remained normothermic during the measurements. The T_b of several *N. thebaica* decreased below 35°C at T_a values between 10-20°C, with one individual decreasing T_b to 26.8°C at T_a = 9.9°C (Figure 1). Normothermic T_bs of *N. thebaica* ranged from 35.1-40.8°C, with the highest T_b of 45.8°C recorded for a single individual at T_a=39.3 °C (Table 7). *T. mauritianus* exhibited T_bs ranging from 35.5-43.8°C over the range of T_as that they were exposed to. In contrast, *Sauromys petrophilus* individuals were highly heterothermic, with their T_b closely tied to T_a between 10-30°C and the vast majority of T_b values falling below 25°C, and therefore not measurable by the PIT tags I used (Figure 1). This species exhibited T_b values similar to normothermic values in *N. thebaica* and *T. mauritianus* at T_as between 35-42°C (Figure 1, 2).

Normothermic and hyperthermic T_bs of *S. petrophilus* ranged from 35.0- 45.9°C at T_as between 35-42°C (Figure 1, 2).

Thermoregulation at moderate air temperatures

The minimum EWL of *T. mauritianus* was 0.8±0.4mg.$g^{-1}hr^{-1}$ (n=6) at T_a=10.3±0.2°C (Figure 1). At moderate air temperatures, EWL was low and approximately constant between 10-30°C with an increase of 1.8-fold between 30-35°C (Figure 1). A maximum RMR of 23.5±6.1mW.g^{-1} (n=6) was recorded at T_a=10.3±0.2°C and thereafter steadily decreased by 28.1% to the BMR at T_a=34.5°C (Figure 1, Table 2).

S. petrophilus were heterothermic at moderate T_as between 10 and 29°C (Figure 1). The minimum EWL of torpid individuals was 0.7±0.3mg.g^{-1}.hr^{-1} (n=11) at T_a=9.8±0.9°C (Figure 1). Maximum RMR was 12.0±14.0mW.g^{-1} (n=9) at T_a=9.9±0.9°C as similarly observed in *T. mauritianus* (Figure 1) and gradually decreased to a BMR equivalent to a quarter of maximum RMR (Figure 1). *S. petrophilus* had a slightly lower average EWL over moderate T_as than *T. mauritianus* (Figure 1).

Data from several *N. thebaica* individuals exhibiting T_bs<35°C were analysed separately from the data for individuals that clearly remained normothermic as the T_bs of normothermic (n=14) and heterothermic (n=10) individuals at 15< T_a <20°C were significantly different ($F_{1, 18}$=27.2, p<0.005). EWL was fairly constant between T_a=10 and 20 °C (Figure 1). The mean EWL of heterothermic individuals at T_a=14.7±2.0°C (2.08±0. mg.g^{-1}.hr^{-1}, n=5) was significantly lower than that of normothermic individuals ($F_{1, 7}$=6.76, p=0.04). There was no significant difference between normothermic bats' EWL of 3.5±1.3 mg.g^{-1}.hr^{-1} (n=6) and the evaporative water loss of heterothermic individuals of 3.8±1.4 mg.g^{-1}.hr^{-1} at T_a=19.5±0.1°C (n=9) ($F_{1,13}$=0.13, p=0.72). In comparison to *T. mauritianus* and *S. petrophilus*, *N. thebaica* had a EWL approximately four times higher over the range of moderate T_as with a gradual increase in EWL starting at approximately 20°C, whereas EWL began to increase at T_a= ~35°C in the other two species. Further, the maximum observed RMR in N. thebaica was 2.8 times higher than *S. petrophilus* and 1.4 times higher than *T. mauritianus* (Figure 1).

Table 1. Physiological variables of *Nycteris thebaica*, *Taphozous mauritianus* and *Sauromys petrophilus* determined from exposure to a range of T_as from 10-42°C in an open flow-through respirometry system. Sample sizes are reported in brackets.

Species	Body Mass (g)	TLC (°C)	TUC (°C)	T_b (°C)	BMR ($mW.g^{-1}$)	EWL at 25°C ($mg.g^{-1}.hr^{-1}$)	Thermal Conductance ($mW.cm^{-2}.°C^{-1}$)
N. thebaica	11.7±1.0 (32)	26.2	33.6	38.6±1.4 (19)	8.9±2.7 (12)	4.7±1.5 (9)	0.273
T. mauritianus	26.2±2.7 (15)	29.0	34.2	38.7±3.4 (11)	6.6±2.2 (7)	1.2±0.5 (8)	0.496
S. petrophilus	11.0±1.4 (37)	33.9	38.1	37.6±0.4 (7)	3.4±0.6 (9)	1.1±0.6 (10)	0.243

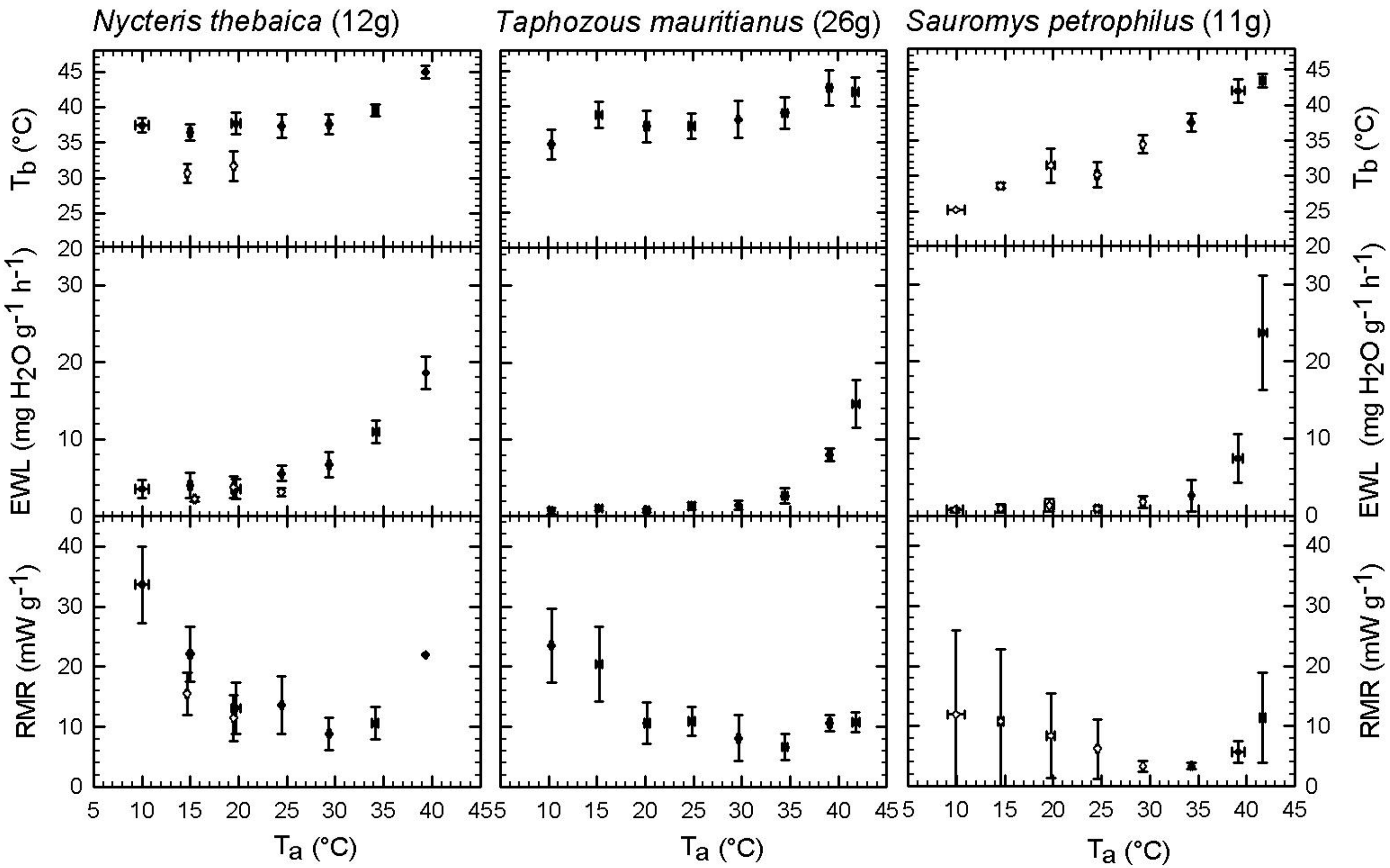

Figure 1. Body temperature (T_b), evaporative water loss (EWL) and resting metabolic rates (RMR) of *Nycteris thebaica*, *Taphozous mauritianus* and *Sauromys petrophilus* experimentally exposed to T_as ranging from ~10°C to 42°C. Sample sizes of each species varied at each T_a. T_b of *S. petrophilus* reported at T_a = 10-20°C do not represent the true average of core T_b as T_b that fell below 25°C could not be measured by the PIT tags.

Thermoregulation at high air temperatures

At high T_a, *T. mauritianus* showed a rapid 3-fold increase in EWL from 2.7±0.4 mg.g^{-1}.hr^{-1} (n=7) at T_a=34.5±0.3°C to 8.1±0.3 mg.g^{-1}.hr^{-1} (n=7) at T_a=39.1±0.2°C (Figure 1). *T. mauritianus* exhibited elevated T_bs of 42.9±1.6°C (n=14) between T_as of 39.1-41.9°C (Figure 1). Maximum EWL was 14.7±1.3 mg.g^{-1}.hr^{-1} (n=7) at T_a=41.9±0.4°C, a 1.8-fold increase from EWL at T_a=39.1± 0.2°C (Figure 1). *T. mauritianus* tolerated high T_as and breathed rapidly and shallowly at these high temperatures (Figure 1). Metabolic rates remained approximately constant at the highest T_as between 39.1-41.2°C (Figure 1). Between T_as=34.5±0.3°C and 39.1±0.2°C there was a 1.6-fold increase in MR to 10.6±1.3 mW.g^{-1} (n=6).

At T_as between 34.3±0.1°C and 41.7±0.2°C, mean T_b in *S. petrophilus* increased from 37.6±0.4 °C (n = 7) to 43.7±1.0°C (n = 6) (Figure 1). Maximum EWL was 23.70±7.39 mg.g^{-1}.hr^{-1} (n=8) at T_a=41.71±0.45°C, 3.2-fold higher than at T_a=39.1±0.5 °C (Figure 1). Similarly, between T_as=34.3-39.1°C EWL increased 2.9-fold to 7.4±0.5 mg.g^{-1}.hr^{-1} (n=11) (Figure 1). Unlike *T. mauritianus, S. petrophilus* showed a two-fold increase in MR at T_as=39.1-41.7°C to 11.4±7.5 mW.g^{-1} (n=6) and increased between T_as=34.3-39.1°C of 1.7-fold to 5.7±1.8 mW.g^{-1} (n=8) (Figure 1).

In *N. thebaica*, T_b increased from 39.6±0.9°C (n=10) at T_a=34.1±0.3°C to 45.0±0.9°C (n=3) at T_a=39.4±0.04°C (Figure 1). EWL in this species and *T. mauritianus* exhibited a more gradual increase at high T_a than *S. petrophilus*; EWL at T_a=34.1±0.3°C was equivalent to 197.5% of the value at T_a=~25°C whereas the corresponding value was 288.8% for *S. petrophilus* (Figure 1). Between T_a=34.1±0.3°C and T_a=39.4±0.04°C, EWL increased 1.7-fold to a maximum of 18.6±2.1 mg.g^{-1}.hr^{-1} (n=3). *N. thebaica* was the most heat intolerant of the three species, with several individuals dying at T_a=39.4±0.04°C (Figure 1). As a result, after the deaths of several individuals at T_a=39.4±0.04°C, data were only collected at T_as from 10-35°C. The maximum EWL of *N. thebaica* at T_a=34.2±0.3°C at which this species survived was 11.0±1.5mg.g^{-1}.hr^{-1} (n=12) (Figure 1). Only a single data point for MR at T_a=39.4±0.04°C was possible to record from the three individuals exposed to the high T_a (Figure 1).

The pattern of T_b changes during acute heat exposure could reveal the physiological ability of certain species to maintain T_bs below lethal limits and highlight potential heat sensitive species. At high T_as of ~35°C and 37.5°C, T_bs of *N. thebaica* fluctuated greatly with time once the bats were placed in the chambers and T_b continued to increase to lethal limits. *T. mauritianus* showed a different T_b pattern at high T_as of 35°C, 40°C and 42°C with T_bs stabilising around 39.1°C, 42.7°C and 42.1°C respectively (Table 7). The T_b of *T. mauritianus* appeared to stabilise quicker than both *N. thebaica* and *S. petrophilus*. Between T_a=34.3°C and 39.1°C *S. petrophilus* had less variation in T_b once T_b had stabilised around 37.6°C and 42.0°C respectively however at T_a=41.7°C, T_b progressively increased over time and was variable among individuals.

Interspecific variation in heat tolerance and thermal properties of roost sites

The three species I investigated in this study showed substantial variation in their tolerance of high T_a under laboratory conditions. To investigate the possibility that this variation is correlated with the thermal properties of their roosts, I measured T_a in the roost site used by each of the species. The day roost sites comprised of a baobab tree (*A. digitata*), exposed walls of two buildings and rock crevices for *N. thebaica, T. mauritianus* and *S. petrophilus* respectively. Of these sites, the baobab tree roost used by *N. thebaica* was the most buffered against daily temperature fluctuations and maintained an average T_{roost} = 27.6±0.6°C. However, the exposed wall and crevice roosts experienced greater fluctuations in temperature with the wall roost equalling that of T_a=28.8±3.1°C, and the crevice roost T_{roost}=29.2±3.3°C. The maximum T_{roost} of the hollow baobab tree roost and rock crevice used by *N. thebaica* and *S. petrophilus* respectively were significantly different to the exposed roosting site of *T. mauritianus* that was equal to T_a. When compared to each other the rock crevice was significantly hotter than the other two roosts and the hollow baobab was the coolest and most stable of the three roosts (Figure 2). For example, on the hottest day T_a=37.3°C, the rock crevice=37.6°C and the hollow baobab roost=29.9°C (Figure 2).

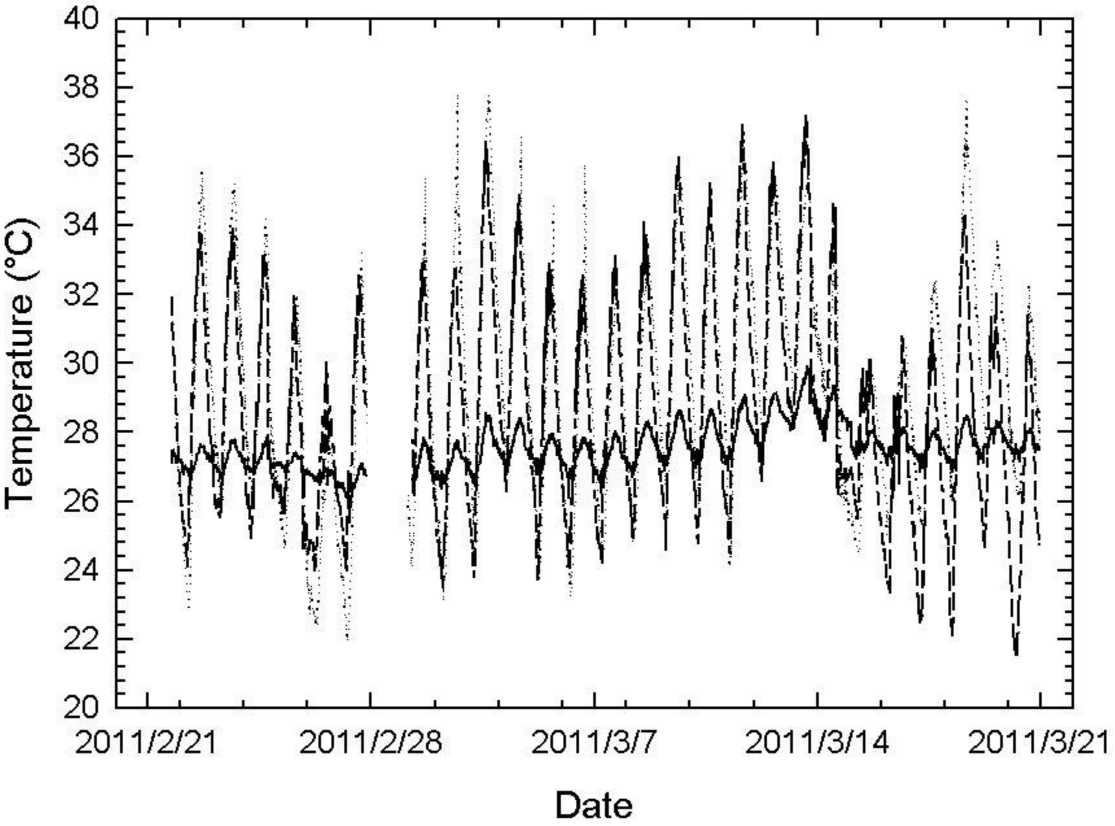

Figure 2. Temperature profiles of roosts used by *N. thebaica*, *T. mauritianus* and *S. petrophilus*. (Solid line = hollow baobab tree roost, dashed line = exposed wall roost and dotted line = rock crevice roost).

Comparative BMR analysis

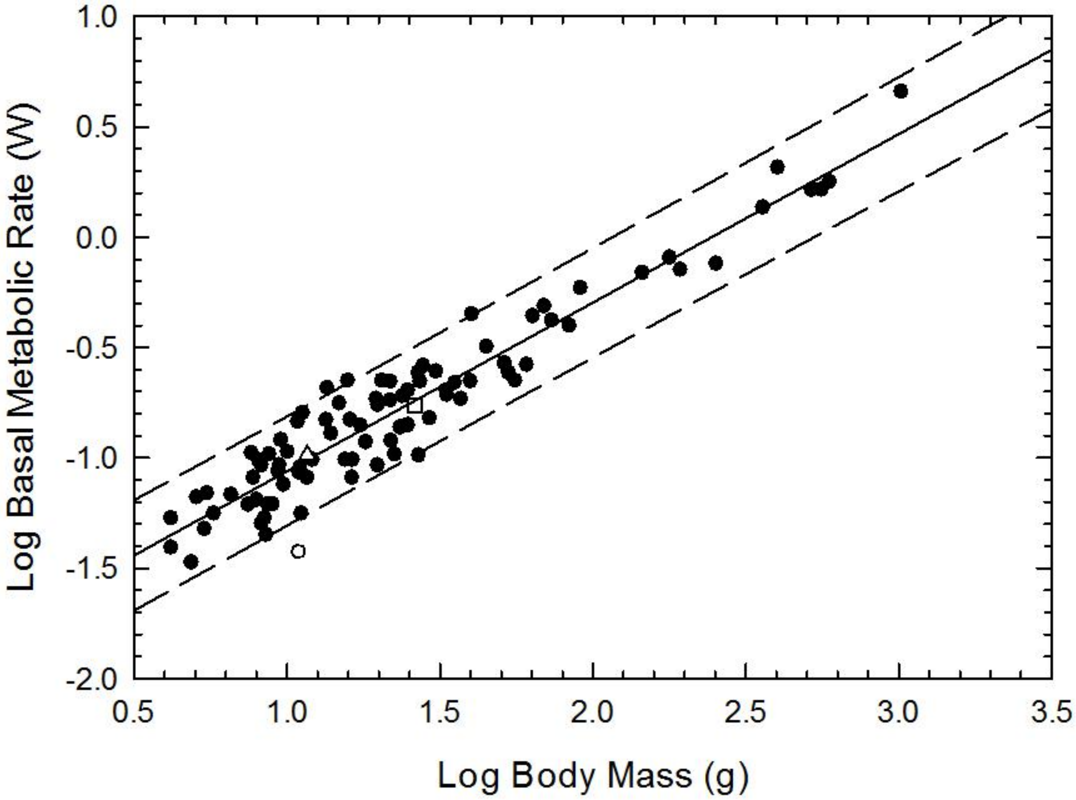

Figure 3. Allometric comparison of bat basal metabolic rates (BMR) with a phylogenetically informed regression (solid line, Grafen's ρ-transformation of branch lengths). The BMRs of *Nycteris thebaica*, *Taphozous mauritianus* and *Sauromys petrophilus* are represented by the open triangle, open square and open circle symbols respectively. The dashed lines are the 95% prediction intervals, calculated following Garland & Ives (2000).

Both M_b ($K = 0.689$, $P < 0.001$) and BMR ($K = 0.577$, $P < 0.001$) exhibited significant phylogenetic signals. My regression analysis indicated that a phylogenetically informed regression using Grafen's ρ-transformation provided the best fit to the data (Table 1). Thus, in order to compare the observed BMRs of *N. thebaica*, *T. mauritianus* and *S. petrophilus* to allometrically expected values, I calculated phylogenetically independent 95 % prediction intervals for each species, following Garland & Ives (2000). After branch lengths were transformed using Grafen's ρ-transformation, each species was sequentially pruned from the phylogeny, the tree re-rooted, and prediction intervals calculated from independent contrasts in the MS-DOS program PDTREE (Garland & Ives 2000). I also calculated a phylogenetically independent regression for the overall data set (Garland & Ives 2000) (Figure 3). There was an overall significant relationship between log BMR and log body mass ($p<0.001$) best described by the phylogenetically independent regression $\log BMR = -1.824+0.764M_b$ (Figure 3). The values for *T. mauritianus* and *N. thebaica* fell within the 95% prediction intervals, and are both virtually identical to the allometrically expected values (Figure 3). In contrast, the BMR of *S. petrophilus* lies below the phylogenetically corrected lower 95 % prediction interval, and is thus significantly lower than expected based on the phylogenetic position of this species. I could detect no significant scaling relationship between log EWL ($mg.h^{-1}$) and log M_b, most likely due to the narrow range of M_b in species so far investigated.

Table 2. Regression models fitted to $\log_{10}M_b$ (g) and $\log_{10}$BMR (W) data for 42 bat species (see Table 3) using either conventional ordinary least squares (OLS) or phylogenetically informed approaches. For phylogenetically informed regressions, I applied various branch length transformations available in the MatLab program REGRESSIONv2.m, namely PGLS (Brownian motion, i.e., no transformation), Ornstein–Uhlenbeck (Reg OU), Grafen's ρ (Reg ρ), and Pagel's λ (Reg λ) (Lavin et al., 2008). The model with the lowest AIC and AICc values, indicating best fit, is indicated in bold.

Model	**Intercept**	**SE**	**Slope**	**SE**	**Ln maximum likelihood**	**Transform parameter**	**R^{2*}**	**AIC**	**AICc**
OLS	-1.833	0.037	0.767	0.026	59.090	----	0.910	-112.179	-111.904
PGLS	-1.928	0.087	0. 829	0.033	60.291	----	0.874	-114.581	-114.305
Reg OU	-1.899	0.047	0. 799	0.033	68.091	$d = 0.385$	0.868	-128.183	-127.718
Reg ρ	**-1.882**	**0.059**	**0.790**	**0.035**	**68.329**	**$\rho = 0.428$**	**0.850**	**-128.659**	**-128.194**
Reg λ	-1.880	0.070	0. 790	0.036	67.575	$\lambda = 0.827$	0.843	-127.150	-126.685

*Not comparable between conventional and phylogenetically informed regressions (Lavin et al. 2008).

Discussion

My data reveal considerable variation in thermoregulatory responses to high T_a and heat tolerance under laboratory conditions among three sympatric bat species inhabiting a hot, semi-arid habitat in northern South Africa. Although all three species exhibited increases in T_b and EWL typical of endotherms experiencing environmental temperatures approaching or exceeding T_b, the shapes of EWL-T_a and T_b-T_a relationships varied markedly, with *N. thebaica* exhibiting a more gradual increase in EWL and a more rapid increase in T_b than either *T. mauritianus* or *S. petrophilus*. Moreover, the three species exhibited considerable variation in patterns of thermoregulation at moderate T_as, and in traits such as BMR, thermal conductance, and T_b within the thermoneutral zone.

Interspecific variation in basal metabolic rate and thermoregulation at moderate T_a

Metabolic rates of *Nycteris thebaica*, *Taphozous mauritianus* and *Sauromys petrophilus* increased with decreasing temperature below their T_{LC}, a pattern typical of endotherms (Scholander *et al.* 1950). The BMRs of *N. thebaica* and *T. mauritianus* were very close to allometric predictions, whereas t the BMR of *S. petrophilus* was only 44% of the predicted value and significantly lower than expected (Figure 3). The BMR measured in *S. petrophilus* represents the lowest BMR relative to M_b for any bat species investigated to date (Figure 3). I am confident that the BMR of *S. petrophilus* is a true BMR and not a torpid MR because a) there is a TNZ evident in the relationship between MR and T_a (Figure 1), and b) the T_bs of *S. petrophilus* in the TNZ are comparable with normothermic thermoneutral values in *N. thebaica*, *T. mauritianus* (Figure 1) and other species. In comparison with strictly desert-dwelling bat species, *S. petrophilus* has a mass specific BMR that is 9.6% lower than *E. perotis* (Leitner 1966), 14.6% lower than *O. hemprichii* (Marom *et al.* 2006), 57.4% lower than *N. geoffroyi* (Hosken & Withers 1999), 64.9% lower than *M. californicus* (Bell *et al.* 1986) and 72.8% lower than *M. yumanensis* (O'Farrell & Studier 1970) (Table 3).

Table 3. Literature summary of basal metabolic rates (BMR (mW.g^{-1})) of bat species from different families and desert/non-desert environments ranked from the lowest to highest BMR recorded. The current study species; *Nycteris thebaica*, *Taphozous mauritianus* and *Sauromys petrophilus* highlighted in bold font.

Species	***Desert/Non-desert**	****Family**	**Body mass (g)**	**BMR (mW.g^{-1})**	**Reference**
Sauromys petrophilus	**ND**	**Mol**	**11.0**	**3.4**	**Current study**
Otonycteris hemprichii	D	Ve	27.1	3.8	Marom *et al.* 2006
Eumops perotis	D	Mol	56.0	4.0	Leitner 1966
Noctilio leporinus	ND	No	61.0	4.3	McNab 1969
Phyllostomus hastatus	ND	Ph	84.2	4.7	McNab 1969
Histiotus velatus	ND	Ve	11.2	5.0	McNab 1969
Antrozous pallidus	D	Ve	22.0	5.4	Licht & Leitner 1967
Mormoops blainvilli	ND	Mo	8.9	5.5	Rodríguez-Durán 1995
Noctilio albiventris	ND	No	40.0	5.5	Chappell & Roverud 1990
Myotis myotis	ND	Ve	25.0	5.6	Kunz & Fenton 2003
Vespadelus vulturnus	ND	Ve	4.0	5.6	Willis *et al.* 2005
Phyllostomus discolor	ND	Ph	33.5	5.8	McNab 1969
Tadarida teniotis	ND	Mol	33.4	6.0	Marom *et al.* 2006
Phyllostomus elongatus	ND	Ph	35.6	6.1	McNab 1969

Hipposideros galeritus	ND	Hi	8.5	6.2	McNab 1989
Taphozous mauritianus	**ND**	**Em**	**26.2**	**6.6**	**Current study**
Molossus molossus	ND	Mol	16.5	6.8	McNab 1969
Pteronotus quadridens	ND	Mo	4.8	7.0	Rodríguez-Durán 1995
Tadarida brasiliensis	ND	Mol	11.0	7.8	Licht & Leitner 1967
Nyctophilus geoffroyi	D	Ve	8.0	8.0	Hosken & Withers 1999
Chalinolobus gouldii	ND	Ve	17.5	8.0	Hosken & Withers 1999
Plecotus auritus	ND	Ve	11.1	8.0	McLean & Speakman 2000
Myotis austroriparius	ND	Ve	7.5	8.1	McNab 1969
Nyctophilus major	ND	Ve	13.6	8.4	Hosken 1997
Natalus tumidirostris	ND	Na	5.4	8.6	Genoud *et al.* 1990
Nycteris thebaica	**ND**	**Ny**	**11.7**	**8.9**	**Current study**
Eptesicus serotinus	ND	Ve	27	8.9	Speakman *et al.* 2003
Pteronotus parnellii	ND	Mo	19.2	8.9	Bonaccorso *et al.* 1992
Lasiurus cinereus	ND	Ve	30.0	9.0	Cryan & Wolf 2003
Pteronotus davii	ND	Mo	9.4	9.1	Bonaccorso *et al.* 1992
Pteronotus personata	ND	Mo	14.0	9.2	Bonaccorso *et al.* 1992
Rhinolophus ferrumequinum	ND	Rh	28.0	9.3	Speakman *et al.* 2003
Macrotus californicus	D	Ph	13.0	9.7	Bell *et al.* 1986

Pipistrellus pipistrellus	ND	Ve	6.6	10.2	Speakman 1993
Saccopteryx bilineata	ND	Em	8.2	10.4	Genoud & Bonaccorso 1986
Rhinonycteris aurantius	ND	Hi	8.3	10.9	Baudinette *et al.* 2000
Myotis thysanodes	ND	Ve	8.1	12.0	O'Farrell & Studier 1970
Myotis yumanensis	D	Ve	5.5	12.5	O'Farrell & Studier 1970
Saccopteryx leptura	ND	Em	4.2	12.6	McNab 1982
Miniopterus schreibersii	ND	Ve	10.5	12.8	Brown 1999
Peropteryx macrotis	ND	Em	5.1	12.9	Genoud *et al.* 1990
Myotis lucifugus	ND	Ve	7.7	13.6	Kurta & Kunz 1988

*D-Desert, ND-Non-desert. **Mol-Molossidae, Ve-Vespertilionidae, No-Noctilionidae, Ph-Phyllostomidae, Hi-Hipposideridae, Em-Emballonuridae, Ny-Nycteridae, Rh-Rhinolophidae, Mo-Mormoopidae, Na-Natalidae..

T. mauritianus defended high T_bs at low and moderate T_as between 10 and 30°C by increasing RMR with decreasing T_a (Figure 1). However, a single large male allowed its T_b to decrease to a minimum temperature of 32.2°C at T_a=10.3°C. Excluding this individual, the mean T_b at T_a=10.3°C was 36.8°C which was 2°C lower than normothermic T_bs within the TNZ. These bats are known for being very active and vigilant at all times due to their exposed roosting habits (Monadjem *et al.* 2010), and the relatively shallow heterothermy exhibited by this individual was likely an energy saving mechanism as even shallow decreases in T_b have been shown to conserve energy (Barclay *et al.* 2001, Studier 1981). *N. thebaica* generally maintained high T_bs between T_as of 10 and 25°C, however 17 individuals allowed their T_bs to decrease several degrees below normothermic T_bs. This appears to be individual variation as all individuals were adults and may have potentially incurred an energy deficit as a result of a poor foraging bout prior to capture. However, during these incidences of “shallow torpor” the associated MRs were lower than those of normothermic individuals (Figure 1).

The T_b pattern in *S. petrophilus* differed from the classic thermoregulatory pattern observed in other animals that use typical daily torpor. This is evident at low to moderate T_as between 10 and 25°C as T_bs decreased with decreasing T_a, MR increased instead of remaining low and stable (Figure 1). This indicates that there was no clear T_b set point and that T_b was defended above a certain temperature which I could unfortunately not determine due to the temperature constraints of the PIT tags. However, the normothermic T_b of *S. petrophilus* was approximately 1°C lower than the normothermic T_b of *N. thebaica* and *T. mauritianus* in their respective TNZ (Table 2).

The EWL of *N. thebaica* at moderate temperatures between 20-25°C and on a typical roost day was substantially higher than *S. petrophilus* and almost double that of *T. mauritianus* (Figure 1, Table 4, 5). In comparison to other species *N. thebaica* had a EWL 21.4% higher than *M. californicus* which is the only other measured bat of similar mass (11.7g) (Bell *et al.* 1986). *T. mauritianus* had a EWL similar to that of *T. brasiliensis*, despite the M_b of the latter species being less than half of that of *T. mauritianus* (Table 4) (Herreid & Schmidt-Nielson 1966). Compared to *O. hemprichii*, a species of similar mass, *T. mauritianus* had a EWL 2.2 times higher (Table 4).

Table 4. Evaporative water loss (EWL) of 20 bat species at ambient temperatures (T_a) between 20-25°C ranked from the lowest to the highest EWL ($mg.min^{-1}$). The current study species; *Nycteris thebaica*, *Taphozous mauritianus* and *Sauromys petrophilus* highlighted in bold font.

Species	**Body mass (g)**	**EWL ($mg.min^{-1}$)**	**References**
Sauromys petrophilus	**11.0**	**0.2**	**Current study**
Nyctophilus gouldi	9.0	0.2	Morris *et al*. 1994
Otonycteris hemprichii	27.1	0.2	Marom *et al*. 2006
Tadarida teniotis	33.4	0.3	Marom *et al*. 2006
Mops condylurus	23	0.4	Maloney *et al*. 1999
Taphozous mauritianus	**26.2**	**0.5**	**Current study**
Tadarida brasiliensis	10.4	0.5	Herreid & Schmidt-Nielsen 1966
Myotis velifer	9.4	0.7	Studier *et al*. 1970
Chalinolobus gouldii	17.5	0.9	Hosken & Withers 1999
Eptesicus fuscus	16.9	0.9	Herreid & Schmidt-Nielsen 1966
Nycteris thebaica	**11.7**	**1.0**	**Current study**
Myotis lucifugus	7.9	1.9	Studier *et al*. 1970
Plecotus auritus	9.1	1.0	Webb *et al*. 1995
Myotis daubentonii	10	1.1	Webb *et al*. 1995
Macrotus californicus	11.7	1.2	Bell *et al*. 1986
Pipistrellus pipistrellus	6.2	1.4	Webb *et al*. 1995
Leptonycteris sanborn	22	1.5	Carpenter & Graham 1967
Myotis thysanodes	8.2	1.9	Studier *et al*. 1970
Noctilio albiventris	40.0	1.9	Chappell & Roverud 1990
Artibeus hirsutus	48.0	3.0	Carpenter & Graham 1967

S. petrophilus had the lowest EWL of 20 species investigated (Table 4). The use of torpor is known for water conservation and is particularly important in arid zone mammals (Stawski & Geiser 2010a, Bronner *et al*. 1999, Geiser 2004), therefore the low EWL recorded for *S. petrophilus* is more than likely due to heterothermy. *S.*

petrophilus has a EWL similar to that of the strictly desert-dwelling bat *O. hemprichii* which may have a lower EWL as an adaption to the aridity of the desert (Marom *et al.* 2006) whereas *T. mauritianus* and *N. thebaica* may have a higher EWL due to the subtropical climate environment that they were studied in. When compared with other molossid species such as *T. teniotis, M. condylurus* and *T. brasiliensis*, which all rank in the top seven species with the lowest evaporative water loss, *S. petrophilus* and *T. teniotis* have an evaporative water loss of 50% lower than the other species (Table 4). In comparison to *S. petrophilus,* at temperatures between 20-25°C, evaporative water loss of the South African molossid *Mops condylurus* is double (Table 4). When compared with species of similar size, *N. gouldii* (9g) is the only species with a similar EWL (Morris *et al.* 1994), whilst the other species of similar mass had EWL ranging between 2-9.5 fold higher than *S. petrophilus* (Table 4).

Interspecific variation in responses to high air temperatures

The T_b values I have reported here are the highest mean values reported so far for bats. However, very few studies have examined hyperthermic T_bs in response to heat exposure, with the exception of *Myotis lucifugus* tolerating T_bs of approximately 42°C (Henshaw & Folk 1966), *M. sodalis* surviving although stressed at T_bs between 41-42°C (Henshaw & Folk 1966) and *Mops condylurus* experiencing hyperthermic T_bs of 43°C (Maloney *et al.* 1999).

In all three study species, EWL increased rapidly at T_as above the TNZ in order to maintain T_bs below lethal limits (Figure 1). Even though some *S. petrophilus* individuals peaked at a maximum T_b=46.5°C, they were able to subsequently lower their T_bs and survive exposure to high T_as (Figure 1, Table 7). Survival at high T_as is crucial as this species will often experience T_{roost} greater than T_a (Figure 2). The family Molossidae is known for pronounced heat tolerance and occupation of hot roosts in which T_a often exceeds 40°C, conditions that prove lethal to most species of microchiropterans (Heirred & Schmidt-Nielson 1966, Maloney *et al.* 1999, Bronner *et al.* 1999, Buffenstein *et al.* 1999, Vivier & van der Merwe 2007). In South Africa the molossid *M. condylurus* has been shown to tolerate high rates of water loss between 23-32% of their body mass and when T_as exceed T_bs they could increase evaporative heat loss to over 130% of heat production to limit exogenous heat gain (Maloney *et al.* 1999). Compared with other Molossid species such as *T. brasiliensis* (Herreid & Schmidt-Nielson 1966), *T. teniotis* (Marom *et al.* 2006) and *M. condylurus* (Maloney *et al.* 1999), *S. petrophilus* exhibits the lowest EWL of 0.2 mg.min^{-1}. The physiological responses of *S. petrophilus* over the range of T_as are similar to those observed in the strictly desert-dwelling *O. hemprichii* (see Marom *et al.* 2006) with low overall EWL potentially due to low metabolic rates and heterothermy. Thus, water and energy are conserved in comparison with species that defend a high T_b for the duration of the roosting period.

T. mauritianus at high T_as similarly maintained T_bs below lethal limits by rapidly increasing EWL (Figure 1). *T. mauritianus* was the largest of the three species investigated and had the lowest mass-specific EWL. No emballonurid species have been investigated at high T_as and the only other bat of similar size is the strictly desert-dwelling bat *O. hemprichii* (27.1g) which has a EWL at T_a=~40°C approximately half of that of *T. mauritianus*. Since *T. mauritianus* roosts on exposed trees and man-made structures during the day, they are exposed to large fluctuations in daily T_a. This potential scenario of water loss in *T. mauritianus* could in fact be lower in reality since they can select cooler microsites by moving to alternative locations on a roost tree or building.

To estimate daily water losses experienced by free-ranging individuals on hot days, I calculated a daily T_{roost} profile for each species based on the hottest day recorded during the study period. I then estimated daily water losses based on the EWL-T_a curve for each species, by integrating EWL over a 14-hr roosting period. Estimated total water loss on a hot day was equivalent to 11.5% of M_b in *N. thebaica* at maximum T_{roost}=27.6°C, 5.5% of M_b in *S. petrophilus* at maximum T_{roost}=32.5°C and 6.4% of M_b in *T. mauritianus* at maximum T_{roost}=35.1°C (Table 5).

Table 5. The percentage body mass (M_b) loss due to evaporative water loss (EWL) in *Nycteris thebaica*, *Taphozous mauritianus* and *Sauromys petrophilus* over a 15hr roosting period on a hot day in Makulu Makete.

Species	**Average T_{roost} (°C)**	**Average EWL (g)**	**% M_b loss**
N. thebaica	27.7	1.3	10.7
T. mauritianus	31.3	1.2	6.4
S. petrophilus	31.3	0.6	5.0

Evidence supporting these estimates is provided by in observed changes in M_b

(i.e. difference in mean M_b between bats caught early in the morning and late in the evening) of free-ranging *N. thebaica* (n=46) roosting in a hollow baobab over five roosting days from the 13-25 February 2011. A M_b change of 11.1% was recorded on the hottest roost day (average of T_{roost} =27.2°C, maximum T_{roost}= 27.6°C) over 15.7 hr. I acknowledge that the mass loss of free-ranging individuals is inclusive of excretory water loss and additional EWL associated with activity in the roost during the day, and may therefore be an overestimate. *N. thebaica* is thus far the only nycterid investigated at high T_as and therefore no comparative data are currently available.

Heat tolerance and roost thermal properties

Among the three sympatric species, interspecific variation in heat tolerance was broadly correlated with respective roost thermal properties. *N. thebaica*, which was the least heat tolerant under laboratory conditions, used the coolest and most thermally stable roost, namely a baobab tree cavity (Figure 2) whereas *S. petrophilus* occupied the hottest roosting site, exhibited the most rapid increase in EWL and the smallest change in T_b as T_a increased from 35-40°C. However, the inferences possible from my data set are severely constrained by the fact that it involved just one roost site per species. To further explore the possibility of a correlation between the roost thermal properties and the thermoregulatory capacity at high T_as among bats in general, I surveyed the literature for studies that reported information on physiological responses to high T_as as well as roost site thermal properties. As an index of the capacity to defend T_b under extremely hot conditions, I used the change in T_b associated with an increase in T_a from 35°C (i.e., just below typical normothermic T_b) to 40°C (i.e., just above typical normothermic T_b) as an index of heat tolerance. I then asked whether this heat tolerance index is correlated with the extent to which roosts are buffered from high air temperatures, by plotting it against the difference between maximum T_{roost} and maximum T_a, an indication of the thermal buffering of a specific roost site (Table 6, Figure 2, 4). A conventional least-squares regression yielded a significant fit between the heat tolerance index and the thermal buffering of different roosts (Figure 4). The small number of species for which such data exists precludes reliably testing for a phylogenetic signal, and so I also fitted a phylogenetically-independent regression model using the approach outlined by Garland & Ives (2000).

Table 6. Heat tolerance index and thermal properties of various roosts used by several bat species.

Species	**Heat Index (Δ T_b at T_a 40-35°C)**	**Δ $T_{roost}max-T_amax$**	**References**
Taphozous mauritianus	3.8	0.00	Current study
Sauromys petrophilus	4.4	1.9	Current study
Nycteris thebaica	5.3	-5.3	Current study
Myotis yumanensis	3.2	13	Licht & Leitner 1967
Antrozous pallidus	3.4	13	Licht & Leitner 1967
Tadarida brasiliensis	4.0	13	Licht & Leitner 1967
Mops condylurus	3.8	4.2	Maloney *et al.* 1999

This analysis supports the notion that species occupying hotter (relative to outside air temperature) roosts have a better capacity to regulate T_b via evaporative heat loss (Figure 4). Species that occupy relatively cool roosts, such as *N. thebaica* exhibit larger increases in T_b as the temperature of their environment approaches and then exceeds normothermic T_b. In contrast, species occupying very hot roosts, such as *Myotis yumanensis* (Vespertilionidae), *Antrozous pallidus* (Vespertilionidae), *Tadarida brasiliensis* (Molossidae) and *Mops condylurus* (Molossidae) exhibit smaller, and presumably more tightly regulated increases in T_b, which I interpret as reflecting a higher tolerance of high T_as (Figure 4). However, this analysis is limited by the fact in all these studies, just one roost site per species was examined. Nevertheless, on the basis of this exploratory analysis, I hypothesise that there is a strong linkage between roost thermal properties and interspecific variation in thermoregulatory capacity at high environmental temperatures.

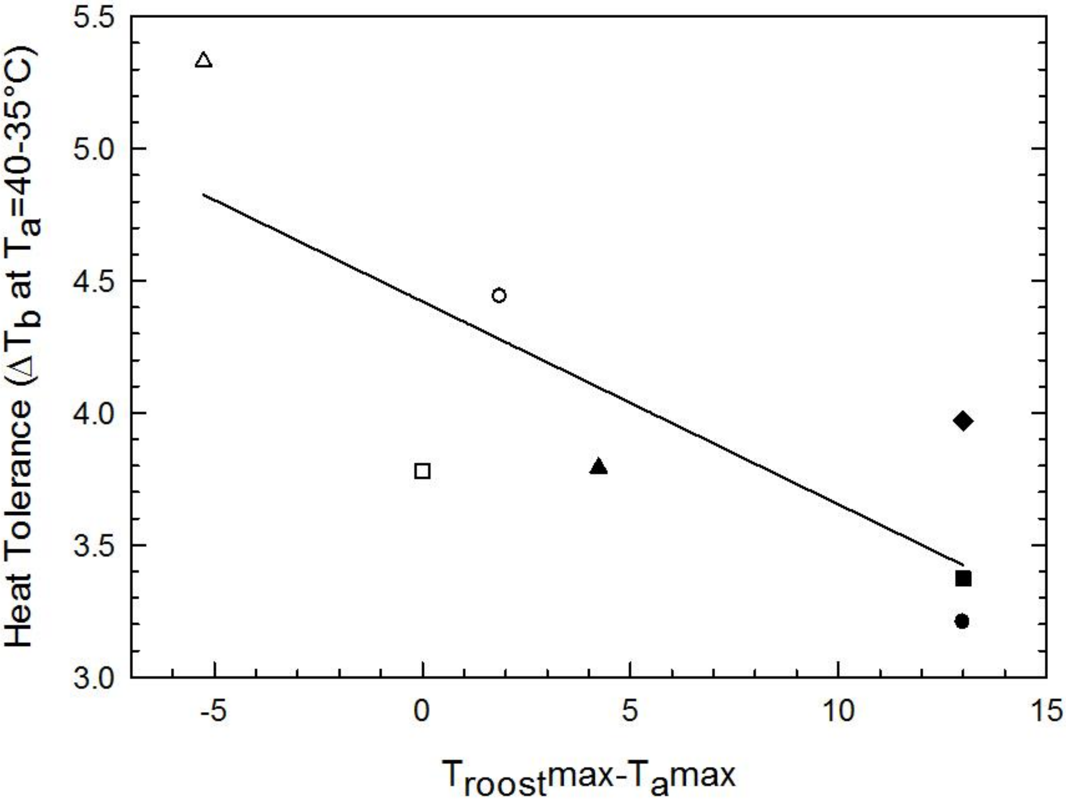

Figure 4. The index of the capacity of thermoregulation under hot conditions in *Nycteris thebaica* (Δ), *Taphozous mauritianus* (□), *Sauromys petrophilus* (○), *Mops condylurus* (▲), *Tadarida brasiliensis* (♦), *Antrozous pallidus* (■) and *Myotis yumanensis* (●) at air temperatures between 35-40°C. A least-squares regression showed a significant relationship between the heat tolerance index and the thermal buffering of different roosts (ΔT_b=-0.1(T_{roost}max-$T_a$max) + 4.5; R^2=0.64).

Table 7. The maximum body temperatures (T_bs) of *Nycteris thebaica*, *Taphozous mauritianus* and *Sauromys petrophilus* exposed to ambient temperatures (T_as) between 40-42°C

Species	**T_a**	**Maximum T_b**	**Mean Maximum T_b**
N. thebaica	39.3	46.5	45.0
T. mauritianus	39.1	44.0	42.7
T. mauritianus	41.8	44.9	42.1
S. petrophilus	39.1	42.7	42.0
S. petrophilus	41.7	46.5	43.5

Hot roosts impose different ecophysiological stresses on bats and high T_{roost} can be potentially stressful or lethal, particularly in exposed roosting sites or roosts that are heated by intense solar radiation (Licht & Leitner 1967, Maloney *et al.* 1999). The responses of insectivorous bat species to high temperatures are not well known, but behavioural responses such as wing-fanning, salivating and flying within the roost to select cooler microsites have been observed (Licht & Leitner 1967, Welbergen *et al.* 2008). At high T_{roost} between 40-50°C experienced by *Myotis yumanensis, Antrozous pallidus* and *Tadarida brasiliensis mexicana* roosting in a loft, they have been shown to avoid the full impact of heat stress by dispersing downward and increasing the distance between individuals when T_{roost}s approached 40°C (Licht & Leitner 1967). During the hottest roosting period only adult *Antrozous pallidus* were observed panting and salivating (Licht & Leitner 1967). On very hot days, they abandoned roosting positions near the ceiling and sought cooler microsites in the loft (Licht & Leitner 1967). *T. brasiliensis mexicana* was shown to be the most heat tolerant of the three, often remaining at T_as above 42°C, with the latter species and *Antrozous pallidus* facing higher temperatures than *Myotis yumanensis* on account of their greater reluctance to use cooler exposed roosting sites (Licht & Leitner 1967).

Interspecific variation in heat tolerance: Implications for predicting climate change

Over the past 100 years, a rise in approximately 0.6°C in mean global temperature has been recorded, but a more concerning issue is the rapid rate of current and predicted global climate change (Root *et al.* 2003, Williams *et al.* 2003). Past research has focused primarily on particular species, but interest is shifting towards predicting impacts on entire ecosystems and ecological communities (Albright *et al.* 2010, Scholze *et al.* 2006, Root *et al.* 2003, Williams *et al.* 2003). Changes in local and regional climate are predicted to drives changes in densities of species in specific regions, species ranges and distributions, phenological and biological behaviours, morphology and genetic frequencies, and ecological community composition (Root *et al.* 2003, Williams *et al.* 2003, Cameron & Scheel 2001, Scholze *et al.* 2006, Welbergen *et al.* 2008, Albright *et al.* 2010, van Jaarsveld *et al.* 2005).

A particularly concerning impact of global climate change in hot habitats involves extreme heat events and the associated more frequent droughts that have been predicted for many parts of the world (Albright *et al.* 2010, IPCC 2011). This is a major concern since such extreme events that exceed physiological and ecological tolerances of species may have a greater influence on the persistence of populations and communities than changes in long term average conditions (Albright *et al.* 2010). Heat waves are of particular importance since as a result of intense heat, animals are often pushed past their physiological heat tolerance limits, which can cause body temperatures to reach lethal limits resulting in death from hyperthermia. Catastrophic mortality events have been observed in Australia among flying-foxes, with massive dies-offs observed in the flying-foxes *Pteropus alecto* and *Pteropus poliocephalus* and 33 000 individuals perishing during 18 extreme heat events (Welbergen *et al.* 2008). Similar die-offs, but on much larger scales, have been reported in desert bird communities (McKechnie & Wolf 2010). Such mortality events not only directly impact individual species but community structure and ecosystem biodiversity (Albright *et al.* 2010).

As T_as are predicted to rise for the Limpopo River valley, effects on bat diversity could involve several non-mutually exclusive scenarios. First, if *N. thebaica, T. mauritianus* and *S. petrophilus* continue to use the same roosting sites, *N. thebaica* may be less affected by the anticipated increase in T_a and climate change associated extreme heat events. Even on the hottest days recorded during my study, the baobab tree roost remained comparatively cool, with $T_{roost} < 30°C$, whereas temperatures in the other two roost sites were equal to or higher than T_a (Figure 3), posing a greater physiological demand on individuals for evaporative heat loss. Second, competition may arise if bat species with similar roosting habits to *N. thebaica* occupying hotter roosts compete for cooler microclimates used by *N. thebaica. T. mauritianus* has the ability to select cooler microsites during the course of the day as they are active and roost on exposed objects. Conversely, *N. thebaica* and *S. petrophilus* are confined to their day roosts but could potentially select cooler microsites within the roost; however cooler microsites may be limited for *S. petrophilus* roosting in rock crevices. Third, the abundance, availability, roost specificity and cool microclimates of specific roosting sites in the landscape required by bats could affect bat biodiversity. Unlike *S. petrophilus, N. thebaica* and *T. mauritianus* use a great variety of roosts (Taylor 2000, Monadjem *et al.* 2010) and cooler roosting sites could potentially be selected as they are not restricted to specific roost types whereas *S. petrophilus* have been found to roost in rock crevices and under exfoliating granite (Monadjem *et al.* 2010) often with $T_{roost}>T_a$ (Figure 2) and may have to select deeper, cooler crevices. An issue may arise for *N. thebaica* regardless of roost type if T_{roost} exceeds 35°C since this species appears to be the most heat sensitive of the three species (Figure 4). The full impact of hot roosts used by bats can be avoided by selecting appropriate microsites (Licht & Leitner 1967). Provided that there are cooler roosting sites in the environment for general roosters, the impacts of climate change may not be as severe in generalist roosting bats or species that do not roost in confined roosting sites such as *N. thebaica* and *T. mauritianus* which may be able to avoid high T_{roost}s by moving within the given roost, but specialist roosters such as *S. petrophilus* may decrease in population numbers or become absent from roosting sites that have become too hot.

My data reiterate that an increase in T_a is not adequate when predicting the effects of climate change on specific bat species or populations, available roost microsites need to be taken into account and the correlation with roost thermal properties and interspecific variation in heat tolerance among species will have important implications for predicting the responses of bats to climate change.

Finally, my data highlight that when predicting the potential effect of a given increment of T_a, both T_b regulation and water requirements need to be considered. For instance, the risk of hyperthermia in *N. thebaica* will be severely increased if maximum T_a increases by 3°C and if they are exposed to temperatures >35°C during the day, but will only lead to a moderate increase in dehydration risk since EWL increases relatively slowly with increasing T_a. In stark contrast, *S. petrophilus* will face a greater risk of dehydration due to their steeper EWL-T_a relationship but an increase in T_a will not pose much of a hyperthermia risk. There are two key questions that need to be addressed, firstly dehydration tolerance limits need to be investigated by determining how much water bats can lose before they die and chronic vs. acute water loss by starving bats of water or exposing them to high T_as until they dehydrate. Secondly, phenotypic plasticity in heat tolerance needs to be addressed as it varies between species and individuals and that individuals that have physiological mechanisms that allow them to persist during extreme heat events associated with climate change may be selectively advantaged as climates become increasingly unstable (Canale & Henry 2010).

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