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Coping with environmental change

The importance of intraspecific physiological flexibility in Malagasy bats

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Summary

Maintaining a proper balance between energy expenditure and energy acquisition in the long term is crucial for all animal species. Especially endotherms are in constant interaction with the prevailing environmental conditions of the habitats they live in through their high thermoregulatory costs. Changes in these conditions can therefore challenge or even disrupt their physiological functioning. While organisms usually have developed means to cope with regular seasonal fluctuations, unpredictable and rapid changes such as extreme weather events or severe habitat alteration may push them to the limits of their capacities. Physiological variation can aid endotherms to mitigate certain environmental fluctuation but our understanding on how species function on broad environmental scales and the extent to which they may flexibly adjust physiological traits to address changing ecological pressures is limited.

In the scope of this thesis, I looked into the adaptive potential of the tropical Commerson's roundleaf bat *Macronycteris commersoni*. Throughout its wide distribution in Madagascar, it experiences a whole range of different environmental conditions. In a first step, I examined intraspecific physiological variation over seasonal and geographic scales. For that, I studied how two distinct populations in both the dry and the wet season: one population roosts in large colonies in well-insulated caves and the second population roosts in the open vegetation, unprotected from external environmental extremes. To trace their physiological responses, metabolic rate and skin temperature were measured directly in their natural microhabitat, i.e. cave and forest. In a second step, free-ranging individuals of each population were exposed to the respective opposite conditions and thus to novel microclimate within an ecologically realistic scope to identify *M. commersoni*'s potential for physiological flexibility when confronted with acute short-term fluctuations.

Torpor was a central component of the specific physiological responses of both populations and we quantified considerable intraspecific variation. Torpor timing, frequency and duration were tailored to the respective roosting conditions. The cave in particular offered thermoneutral conditions and access to water, which even negated the need for long-term hypometabolism in better-conditioned individuals during the lean dry season, when the bats did not leave the cave for months. The exposed foliage roost, by contrast, induced extensive use of torpor, with patterns never observed before. During "hot" torpor, the bats tolerated hyperthermia and body temperature increased at the onset of a torpor bout. Withstanding acute heat stress through torpor lowers endogenous heat production and conserves both energy and water, which otherwise would be expended to lower body temperature. Repetitive micro-torpor bouts on the other hand allow for substantial energy savings combined with an increased number of euthermic periods. Both newly discovered patterns broaden our general idea of hypometabolism and demand for a redefinition of torpor that does not fundamentally include a decrease in body temperature. Interestingly, we found comparable body conditions across populations, suggesting that the fine adjustment of energy expenditure through various torpor patterns mitigated the contrasting environmental pressures. This supports the need to study species across their broad ecological reality to avoid underestimating a species' full natural potential and unfounded conclusions.

In the second part of the project, I focused on how the two populations coped with immediate alterations in their microclimate and found limited scope for rapid and especially sustainable responses. Cave bats exposed to forest setting had limited capacity to maintain euthermia to the point that two individuals became hypothermic when ambient temperature dropped below their commonly experienced cave temperature. The forest population by contrast, had difficulties to dissipate heat in the humid and hot cave replica. Interestingly though, the response to heat was surprisingly homogeneous and all bats entered torpor combined with hyperthermia at the latest at temperatures exceeding their respective thermoneutral zones. Thus, *M. commersoni* could flexibly compensate for heat through "hot" torpor. However, both populations also showed patterns indicating limited potential to cope with conditions outside their typically occupied microclimates, presumably due to local adaptation and/or acclimatisation.

The results of my thesis highlight the need to study particularly widespread species on broad environmental scales. Individual populations only provide a snapshot of the species as a whole and thus reflect a biased and limited picture of their potential physiological capacity and thus their ecological tolerance. Furthermore, it is of major importance to consider the mechanism driving intraspecific variation. Populations may be locally adapted and/or acclimatised, which may ultimately hamper their flexibility when local and rapid compensatory responses are required. Comparative studies across species' distributions, combining field research with more experimentally-driven approaches to examine what species and populations currently do and potentially could do when they are pushed out of their typically experienced environment, point towards their flexibility as well as limits in it. Such understanding is vital to make accurate predictions about species' chances for persisting in ever rapidly and drastically changing habitats and climates.

Zusammenfassung

Ein ausgewogenes Verhältnis zwischen Energieaufnahme und -verbrauch aufrechtzuerhalten, ist für alle Tierarten von grundlegender Bedeutung. Insbesondere Endotherme befinden sich durch ihren hohen thermoregulatorischen Aufwand in ständigem Austausch mit den vorherrschenden Umweltbedingungen der von ihnen bewohnten Lebensräume. Veränderungen in diesen Bedingungen können daher ihre physiologische Funktionsfähigkeit beeinträchtigen oder sogar ganz aushebeln. Während die meisten Lebewesen Mechanismen entwickelt haben, um mit regelmäßigen saisonalen Schwankungen zurechtzukommen, können unvorhersehbare und schnelle Änderungen wie etwa durch erhebliche Lebensraumzerstörung oder extreme Wetterereignisse sie an die Grenzen ihrer Belastbarkeit bringen. Physiologische Variation kann Endotherme unterstützen, bestimmte Umweltfluktuationen abzumildern. Unser grundlegendes Verständnis darüber, wie Arten auf breiten Umweltskalen funktionieren und inwieweit sie physiologische Merkmale flexibel justieren können, um veränderten ökologischen Belastungen gerecht zu werden, ist jedoch begrenzt.

Im Rahmen dieser Arbeit untersuchte ich die Anpassungsfähigkeit der tropischen Commersons Blattnasenfledermaus *Macronycteris commersoni*, die in ihrem großen Verbreitungsgebiet in Madagaskar einer ganzen Reihe unterschiedlicher Umweltbedingungen ausgesetzt ist. In einem ersten Schritt betrachtete ich die intraspezifische physiologische Variation über saisonale und geografische Skalen hinweg. Dafür habe ich zwei verschiedene Populationen sowohl in der Trocken- als auch in der Regenzeit erforscht: eine Population ruhte in einer großen Kolonie in einer gut isolierten Höhle und die Zweite in offener Vegetation, ungeschützt vov externen Umweltextremen. Um ihre physiologischen Reaktionen zu erfassen, wurden ihre Stoffwechselrate und die Hauttemperatur direkt in ihrem natürlichen Mikrohabitat aufgezeichnet. In einem zweiten Schritt wurden freilebende Individuen jeder Population den jeweils gegensätzlichen Umweltbedingungen und damit in einem ökologisch realistischen Rahmen einem ungewohnten Mikroklima ausgesetzt, um die physiologische Flexibilität von *M. commersoni* bei akuten Umweltschwankungen zu bestimmen.

Torpor war eine zentrale Komponente der jeweiligen physiologischen Reaktionen beider Populationen und wir konnten eine beträchtliche intraspezifische Variation feststellen. Torpordauer, -frequenz und -timing waren an die jeweiligen Bedingungen der Tagesquartiere angepasst. Insbesondere die Höhle bot stabile thermoneutrale Bedingungen und Zugang zu Wasser, was sogar langfristigen Hypometabolismus in besser-konditionierten Individuen während der kargen Trockenzeit negierte, in der die Fledermäuse die Höhle monatelang nicht verließen. Das exponiertere Waldquartier hingegen erforderte intensiven Gebrauch von Torpor, mit Mustern, die so noch nie beobachtet wurden. Im "heißen" Torpor tolerierten die Fledermäuse Hyperthermie und die Körpertemperatur stieg zu Beginn einer Torporperiode sogar an. Die Überwindung von akutem Hitzestress durch Torpor senkt die körpereigene Wärmeproduktion und spart sowohl Energie als auch Wasser, was

normalerweise zur Absenkung der Körpertemperatur benötigt würde. Wiederholte Mikro-Torpor-Phasen ermöglichen dagegen eine erhebliche Energieeinsparung in Kombination mit einer erhöhten Anzahl euthermischer Perioden. Beide neu entdeckten Muster erweitern unser allgemeines Verständnis von Torpor und fordern eine Neudefinition, die nicht grundsätzlich eine Absenkung der Körpertemperatur beinhaltet. Interessanterweise fanden wir bei allen Populationen eine vergleichsweise Körperkondition, was darauf hindeutet, dass die feine Justierung des Energieverbrauchs durch verschiedene Torpor-Muster die unterschiedlichen Umweltbelastungen abmildert. Dies unterstreicht wie wichtig es ist, Arten über ihre gesamte ökologische Realität hinweg zu untersuchen, um eine Fehleinschätzung ihres vollen physiologischen Potenzials sowie unfundierte Schlussfolgerungen zu vermeiden.

Im zweiten Teil des Projekts konzentrierte ich mich darauf, wie die einzelnen Populationen mit unmittelbaren Veränderungen in ihrem Mikroklima zurechtkommen und fand bei beiden nur begrenzte Kapazität für schnelle und vor allem nachhaltige Reaktionen. Höhlenfledermäuse, konnten ihre eutherme Körpertemperatur nur begrenzt aufrechterhalten und zwei Individuen unterkühlten sogar, als die Umgebungstemperatur unter die üblicherweise erlebte Höhlentemperatur fiel. Die Waldpopulation hingegen hatte Schwierigkeiten, Körperwärme bei hoher Luftfeuchtigkeit abzugeben. Interessanterweise war jedoch die Reaktion auf Hitze erstaunlich homogen und alle Fledermäuse machten spätestens bei Temperaturen, die ihre jeweilige thermoneutrale Zone überstiegen, Torpor in Kombination mit Hyperthermie. *M. commersoni* konnte also Hitze flexibel kompensieren. Allerdings zeigten beide Populationen auch Muster, die auf eingeschränkte Kompensationsmöglichkeiten von Bedingungen außerhalb ihrer typischerweise besetzten Mikroklimata hinweisen, vermutlich aufgrund lokaler Anpassung und/oder Akklimatisierung.

Die Ergebnisse meiner Arbeit unterstreichen die Notwendigkeit, insbesondere weitverbreitete Arten auf breiten Umweltskalen zu untersuchen. Einzelne Populationen liefern nur eine Momentaufnahme der Art als Ganzes und geben daher nur ein verzerrtes Bild ihrer potenziellen physiologischen Kapazität und somit ihrer ökologischen Toleranz wider. Darüber hinaus ist es wichtig, den Mechanismus hinter intraspezifischer Variation zu verstehen. Populationen können lokal angepasst und/oder akklimatisiert sein, was letztendlich ihre Flexibilität einschränken kann, wenn schnelle Reaktionen gefragt sind. Vergleichende Studien quer über Verbreitungsgebiete von Arten hinweg, die Feldforschung mit eher experimentell-basierten Ansätzen kombinieren, um zu untersuchen, was Arten und Populationen derzeit tun und potenziell tun könnten, wenn sie aus ihrer angestammten Umgebung herausgedrängt werden, lassen Rückschlüsse auf ihre Flexibilität und mögliche Grenzen zu. Ein solcher Einblick ist entscheidend, um genaue Vorhersagen über die Überlebenschancen von Arten in sich immer schneller und drastischer verändernden Lebensräumen und Klimazonen treffen zu können.

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General introduction

An endothermic lifestyle in a changing world

Successful energy management is critical for all animal species. It is closely linked to their ecology because the balance between energy expenditure (e.g. for maintenance, growth, locomotion, and reproduction) and energy intake (feeding) is crucial for survival (Kronfeld-Schor and Dayan 2013). Within the scope of their physiological characteristics, animals constantly interact with the abiotic and biotic conditions of the habitats they live in. Changes in these conditions can therefore be a major challenge, whether these are natural such as seasonal fluctuations or unpredictable weather events, or anthropogenically induced such as large-scale habitat alteration or global warming and its concomitants. For many animals though, we are lacking basic understanding of how they generally balance their energy budget in a given environment and to what extent they may flexibly adjust certain behavioural and particularly physiological traits to address changing ecological pressures.

Environmental changes are particularly challenging for mammals, above all when ambient temperature (T_a), water availability, resource availability or the interaction of these factors are affected. They all have a direct and immediate effect on a mammal's physiological functioning and may increase thermoregulatory costs (Schmidt-Nielsen 1997; Tattersall et al. 2012). Mammals are endotherms that regulate their body temperature (T_b) within a set range through endogenous heat production, evaporative cooling and behaviour (Bartholomew 1972; Lyman 1982; Mitchell et al. 2018). While this allows them to shield their inner milieu from the ambient environment and to inhabit various habitats relatively independent of the surrounding conditions (McNab 1978), it comes with high costs related to defending a fairly stable T_b (Geiser 2004; Heldmaier et al. 2004). Active thermoregulation sets in as soon as T_a is above (upper critical temperature) or below (lower critical temperature) the thermal neutral zone, i.e. the temperature range where no additional energy or water expenditure above basal levels to sustain high endothermic T_b is needed (TNZ; Scholander et al. 1950). This range however, is narrow and "in the real world" only temporarily encountered. Moreover, the surface-area-to-volume ratio increases with decreasing size (Heldmaier et al. 2013; Hill et al. 2016; Clarke 2017), thus lowering thermal inertia and promoting heat flux at both ends, heat loss at low T_a and heat accumulation at high T_a. Especially small mammals consequently face high basal thermoregulatory costs and constantly have to fuel their high energy demands and, in warm environments, water requirements.

Coping with energy-demanding times

Given the high cost associated with endotherm thermoregulation, efficient mechanisms are key to survival and indeed, especially mammals living in seasonal or generally unreliable environments have developed considerable variation in traits associated with thermal maintenance. At low T_a, mammals have to reduce heat lost to the environments and adopt a more frugal lifestyle to conserve energy needed to counteract the increased cost of thermoregulation. Morphological or behavioural adaptations such as growing denser fur or moving to a better-insulated resting site during the colder season, for example, are pathways observed commonly (e.g. Terrien et al. 2011). Some mammals benefit from behavioural thermoregulation by trapping heat through huddling (e.g. bent-winged bats, grey mouse lemurs, Figure 0.1B, C), or basking (e.g. ring-tailed lemurs, Figure 0.1A; Kelley et al. 2016). If these mechanisms are not sufficient, shivering thermogenesis through contraction of skeletal muscles can increase heat production four to five fold within minutes can go on for days or even weeks (Hohtola 2004). Shivering is induced by acute cold-exposure and represents a relatively universal pathway to produce heat (Hohtola 2004). Non-shivering thermogenesis (NST) can further aid upholding T_b, whereby either skeletal muscles or brown adipose tissue (BAT) is involved. In both cases, membrane pumps are uncoupled from their regular function and instead, heat is produced. In skeletal muscle, the transport of Ca^{2+} ions is suppressed and the energy from ATP hydrolysis is converted to heat, whereas in BAT, ATP synthesis is bypassed by a proton leak and fat is metabolised to produce heat in a futile cycle (Heldmaier et al. 2013; Withers et al. 2016; Nowack et al. 2017). NST in BAT is limited to mammals that possess this specialised, highly efficient tissue, i.e. placental mammals and possibly marsupials, although for the latter the functionality of BAT is controversial (Gaudry et al. 2017; Hill et al. 2016; Withers et al. 2016). Capacity for muscular NST has been demonstrated in birds (Dumonteil et al. 1995; Bicudo et al. 2001), fish (Block 1994; Jastroch et al. 2005) and mammals (de Meis et al. 2005; Anderson et al. 2015), but its importance for thermoregulation has only been described recently (Bal et al. 2012, 2016).

Coping with heat is more problematic. High T_a can have disastrous physiological impacts on mammals because their safety margin between euthermia and upper lethal temperatures is narrow (41-44°C; Schmidt-Nielsen 1997; Lepock 2003). Simultaneously, there are few mechanisms to control or even downregulate T_b: passive mechanisms of heat loss such as radiation, convection or conduction are effective only at T_as lower than the desired T_b (Hill et al. 2016). Especially when T_a exceeds T_b, evaporative cooling is the only means to dissipate heat, whereby the water budget ultimately dictates the extent of heat compensation (Mitchell et al. 2018). This entails that in arid regions, where water availability may be limited and/or unpredictable, endotherms have to trade off the risk of dehydration from extensive evaporation with the risk of potentially fatal hyperthermia (Cunningham et al. 2013; Conradie et al. 2019; McKechnie and Wolf 2019). Small mammals, for example, cannot necessarily afford the high amount of water turnover that is required to maintain body temperature below T_a for longer than several hours (Maloney et al. 1999). Maintaining a stable T_b at high T_a is therefore a balancing act and already small increases in daytime temperature extremes, as are predicted for the current century, may push some species near or even beyond the edges of their thermal and physiological capacities.

A second option to cope with heat is to desist from maintaining a T_b within the desired range and to tolerate short-term hyperthermia. By accumulating heat instead of dumping it, and thereby tolerating a controlled increase in body temperature, the onset of evaporative cooling is postponed or even offset, and substantial amounts of water are conserved (Schmidt-Nielsen et al. 1956; Licht and Leitner 1967; Maloney et al. 1999). Thereby, a positive T_b-T_a differential ($T_b > T_a$) is maintained and the aforementioned passive mechanisms radiation, convection and conduction can additionally counteract overheating (Tattersall et al. 2012; Mitchell et al. 2018; Turner 2020). The most prominent example for adaptive hyperthermia comes from dromedary camels: when dehydrated, they regularly cycle between 41°C at daytime and 34-35°C at night (*Camelus dromedaries*; Schmidt-Nielsen et al. 1956). Other endotherms also routinely allow hyperthermia, including llamas (Riek et al. 2017), elephants (Weissenböck et al. 2012), large treeshrews (Levesque et al. 2018), ringtail possums (Turner 2020), bats (Maloney et al. 1999; Bondarenco et al. 2016; Czenze et al. 2020) and several bird species, for which this phenomenon is often termed facultative hyperthermia (Tieleman and Williams 1999).

Avoiding stressful times

Instead of riding out the hardships of survival during stressful periods, “escaping” them either spatially by migration or temporarily by torpor are more radical strategies to ultimately maintain homeostasis and effective at both low and high T_a. A temporary shift in location can improve environmental, energetic and/or reproductive conditions (Fleming and Eby 2003; Avgar et al. 2014) but might be impaired by life history, general mobility, species interaction or high levels of habitat fragmentation (Fleming and Eby 2003; Urban 2018). Migrants also require suitable corridors, stopover sites and/or destinations that provide considerably more favourable conditions than their regular habitat for them to pay off (Popa-Lisseanu and Voigt 2009; Avgar et al. 2014). This is a serious problem on islands, where suitable habitat is finite and animals may be stuck.

Tropical torpor

Abandoning a euthermic lifestyle and entering torpor (hypometabolism), by contrast, is the most efficient physiological mechanism to save energy and water (Geiser 2004; Heldmaier et al. 2004). Torpor is a temporary, controlled downregulation of virtually all metabolic processes whereby T_b is shifted to a new hypothalamic setpoint temperature and passively approximates ambient temperature within this range. Bodily functions are decreased to the minimum necessary to sustain survival, allowing animals to conserve considerable amounts of energy (Geiser 2004; Heldmaier et al. 2004). Torpor has long been believed to be an adaptive response of endotherms to cold stress or food limitation during seasonal periods of scarcity. However, the last two decades have shown that torpor is also common in the tropics (see Nowack et al. 2020 for review) and some species, such as fat-tailed dwarf lemur (*Cheirogaleus medius*; Figure 0.1D) even hibernate there (Dausmann et al. 2004). A reduction of metabolic rate (MR) is accompanied by reduced water loss through respiration, defecation, urine formation and metabolic heat dissipation (Herreid and Schmidt-Nielsen 1966; Cooper et al. 2005; Levin et al. 2015). This might play an even more important role for torpor use in the tropics during extended dry periods or high T_a (e.g., Schmid and Speakman 2000; Bondarenco et al. 2014) and indeed, torpor has been proposed to be an excellent response to heat (Lovegrove et al. 2014; Welman 2018). Reduced metabolic heat production could permit torpid animals to tolerate greater heat storage from the environment than euthermic ones, negate the need for evaporative cooling and thus increase heat tolerance. However, torpor at T_a exceeding T_b has never been observed so far. In general it remains poorly understood how mammals perform and thermoregulate when facing high T_a (Huey et al. 2012; Lovegrove et al. 2014; Levesque et al. 2016; Welman et al. 2017; Mitchell et al. 2018). Their options appear to be much more limited at the upper than at the lower end of the thermal spectrum, which is alarming considering that not only is global warming topping natural environmental changes but also increasing frequencies in heatwaves.

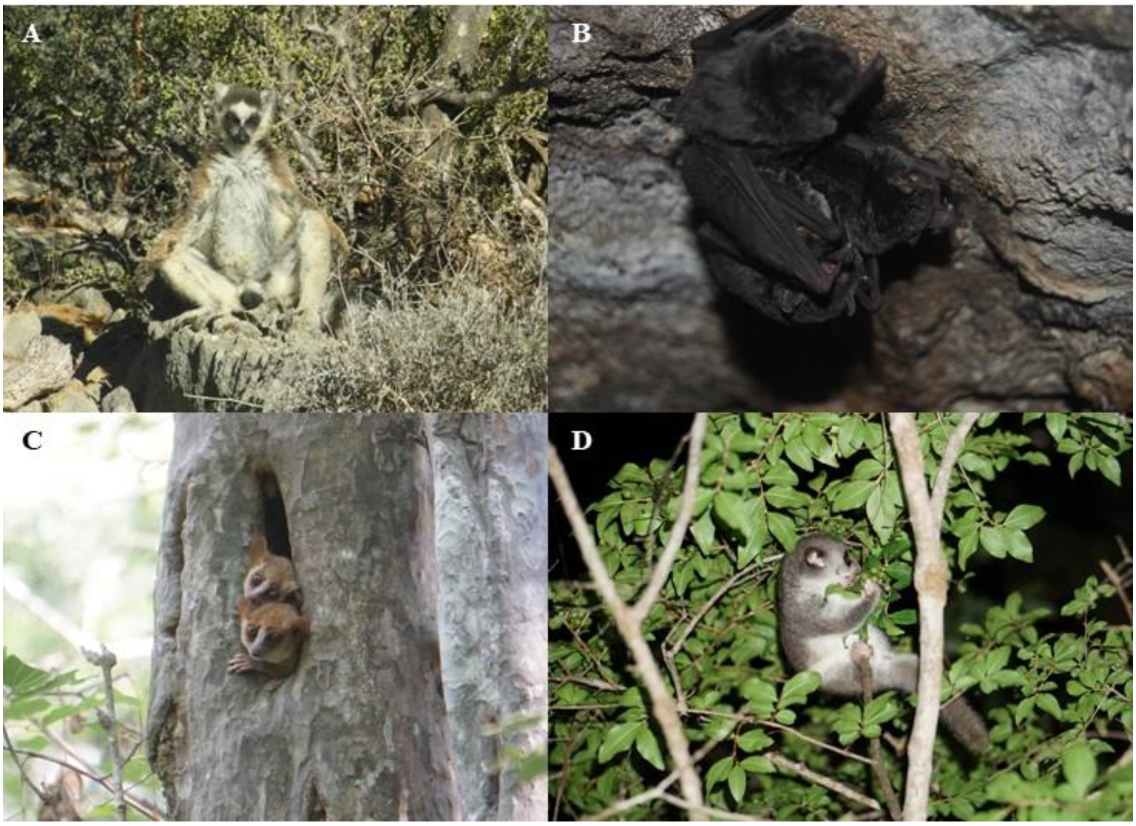

Figure 0.1. Different thermoregulatory mechanisms in tropical mammals. Basking in the early morning sun to take advantage of solar radiation during the cool, dry season in A, a ring-tailed lemur (*Lemur catta*); trapping heat through social thermoregulation in B, bent-winged bats (*Miniopterus mahafaliensis*) and C, grey mouse lemurs (*Microcebus murinus*); and storing fat as preparation for hibernation in D, a busy eating fat-tailed dwarf lemur (*Cheirogaleus medius*). Photo credit: Stephanie Reher.

Intraspecific physiological flexibility can mitigate environmental changes

Even though we have a general idea how endotherms thermoregulate, these are often only snapshots into species' potential capacities. We are lacking a mechanistic understanding of how organisms function on a broader environmental scale or when environments change over time (Boyles et al. 2011; Bozinovic et al. 2011). Especially widely distributed species experience a diverse array of climatic conditions and contrasting environmental pressures within their range, and these may additionally vary seasonally. A species as a whole may consequently be facing a broader ecological reality than we generally assume, and, accordingly, may have a broader adaptive scope than we generally assume.

Distant populations of the same species could seek out comparable microhabitats and display a relatively low potential for intraspecific variation, while others may adapt locally or be highly flexible (Kobbe et al. 2011; Hume et al. 2020; Noakes and McKechnie 2020; van Jaarsveld et al. 2021). Since physiological capacity ultimately determines species' tolerance limits to abiotic factors (Canale and Henry 2010; Bozinovic et al. 2011), certain environmental changes can be compensated for by physiological variation. Brushtail possums from arid habitats, for example, have a generally lower energy and water turnover and can dissipate heat more efficiently than their conspecifics from mesic areas (Cooper et al. 2018) and more northern populations of big brown bats have lower energetic costs at cooler ambient temperatures during hibernation than their southern conspecifics (Dunbar and Brigham 2010). Such differences on population-level therefore reflect species' capacity for phenotypic variation and/or local adaptation (Violle et al. 2012; Richardson et al. 2014) and ultimately their resilience to changes in their environment, as single populations may be better equipped for coping with disturbances/changes than others.

Intraspecific physiological variation may arise from genetic changes across generations within a given population (Violle et al. 2012; Richardson et al. 2014). Consequently, variations observed on population-level do not necessarily entail that each individual can generally take advantage of the entire flexibility seen in a species to respond flexibly to rapid, unpredictable vicissitudes. Phenotypic flexibility by contrast allows fast and reversible adjustments to a changing environment (West-Eberhart 1989; Piersma and Drent 2003). It is an organism's ability to produce more than one phenotype of a single genotype (West-Eberhard 1989), thus extends the compensatory capacity not only of a species but also of individuals and ultimately their environmental tolerance range (Ghalambor et al. 2007; Bozinovic et al. 2011; Huey et al. 2012). A seasonal shift in the TNZ towards more frequently experienced T_a, for example, saves energy that otherwise would be required for regulating T_b (Kobbe et al. 2014).

Physiological data restricted to only one season and/or location does not accurately represent a species as a whole. While seasonal physiological variation is better studied (e.g. Kobbe et al. 2014;

Bethge et al. 2017; Czenze et al. 2017), we lack information on how separate populations cope with different environmental pressures and how flexible these responses are on individual-level (Dunbar and Brigham 2010; Stawski 2012; Klüg-Baerwald and Brigham 2017; Cooper et al. 2018; Gearhart et al. 2020). This can have far-reaching consequences: physiological traits are often included as fixed for an entire species in predictive models (Atkins and Travis 2010; Chevin et al. 2010; Chown et al. 2010) and conclusions based on such models are likely to be more than tenuous, which is especially critical in the face of ongoing human disruptive interference. The results of predictive models are often used as a base to define structurally and functionally suitable habitats that might serve as refugia to ensure long-term viability of populations or for translocation programmes (Cooke et al. 2013; Tarszisz et al. 2014; Rezende and Bacigalupe 2015; Cooper et al. 2018). Consequently, it becomes increasingly important to understand and consider local adaptation within populations, intraspecific variation in physiological traits and the possibility of within-individual acclimatisation to a changing environment (e.g. Boyles et al. 2011), to ultimately assess general adaptive scopes.

Madagascar’s bats: an ideal system for studying the physiological spectrum

A highly variable island

Madagascar’s unique evolutionary history and long separation from major landmasses have driven an extraordinary development of distinct biomes and rich biodiversity. The island separated from continental Africa 165 million years ago (Mya), before becoming completely isolated 88 Mya when India broke off (Samonds et al. 2012). Drastic climatic shifts then shaped today's Madagascar, an island with steep environmental gradients and numerous microhabitats with associated microclimates in relatively small space (Goodman et al. 2018). Combined with high endemism in all taxa (e.g. 87% for terrestrial vertebrates; IUCN 2021), Madagascar is one of the most diverse biomes of the globally identified "biodiversity hotspots" (Myers et al. 2000; Ganzhorn et al. 2001; Vences et al. 2009). Simultaneously, rates of habitat destruction are high, with more than 90% of the island’s natural habitat modified already (Green and Sussman 1990; Ganzhorn et al. 2001). This dramatic degree of habitat loss and fragmentation is coupled with extensive and often unsustainable exploitation of wildlife for bushmeat and trade (Goodman 2006; Jenkins et al. 2011), and both are topped by global warming (Huey et al. 2012; IPCC 2014). Particularly the highly seasonal southern and western regions are predicted to face further warming with an increase in already ongoing droughts, heatwaves and cyclones (Ganzhorn 1995; Elmqvist et al. 2007; Hannah et al. 2008; Tadross et al. 2008), leading to a situation in which practically all organisms resident to Madagascar are threatened by the concomitants of environmental alteration.

The effects of environmental changes are expected to be particularly severe in tropical and subtropical regions, where small mammals are already closer to their upper thermal limits and generally considered to have a narrower adaptive scope than their temperate counterparts (Janzen 1967; Huey et al. 2012; IPCC 2014; Lovegrove et al. 2014). Madagascar's fauna, however, might have a pre-adaptive advantage. Due to the island's geographical location, it is regularly hit by climatic and weather events like sea current fluctuations, droughts, heatwaves and cyclones, all of which may affect the island's ecosystems differently in any given year (Dewar and Richard 2007). In addition, the western dry portions of the island are highly seasonal and in the south, rainfall may fail for entire years, all together creating a "hypervariable" environment (Dewar and Richard 2007; Ratovonamana et al. 2013). These highly variable environmental conditions may have exerted a selective pressure on the Malagasy fauna to have a broader ecological and physiological tolerance than other tropical organisms. Indeed, a comparatively high proportion of endothermic species in Madagascar has already been identified to use torpor to counter unfavourable seasons (Dausmann and Warnecke 2016). Whether this reflects concentrated research efforts on this topic, or whether Madagascar's unpredictable environment may have favoured physiological flexibility in its evolutionary past, remains unclear, yet is potentially a valuable adaptive advantage considering on-going habitat and climatic changes.

Neglected super-performers

In Madagascar, we currently recognize 46 bat species and 80% of them are endemic to the island (Goodman 2011; Lebarbenchon et al. 2017). However, they are severely understudied and we are lacking basic information documenting Malagasy bats' ecological and particularly physiological scopes (Goodman 2011). From many species, we do not know more than that they exist at all, which is alarming not only from an eco-physiological point of view. Bats generally provide important ecosystem services such as insect pest consumption, seed dispersal and pollination, which are vital to the preservation of natural ecosystems. In tropical regions, they are even considered the most important force ensuring ecosystem integrity (Welman et al. 2017).

Bats are ideal to study intraspecific physiological variation as well as adaptive thermoregulatory flexibility. They are small-bodied, have large uninsulated flight membranes accelerating water as well as heat energy exchange and often rely on temporally variable food resources that cannot be cached (Speakman and Thomas 2003; Jonasson and Willis 2012). At the same time, they are metabolic super-performers at night when fuelling flapping flight, the most costly mode of locomotion per unit time (Schmidt-Nielsen 1997; Winter and von Helversen 1998; Speakman and Thomas 2003). Bats therefore have to save energy efficiently during diurnal resting and already little changes in their local environment may disrupt their tight overall energy balance. However, compensatory measures such as intensified thermoregulation, prolonged foraging flights, or more elaborate searching for suitable roosts are related to increased energetic costs, missed opportunity costs and/or potentially predation risk through increased exposure in endotherms (Clarke 2017; Cunningham et al. 2021; Glass et al. 2021). Efficient thermoregulatory responses through physiological adjustments are consequently vital for bats. Given that some bats are widely distributed on this particularly variable island, they experience a diverse array of environmental conditions within their range. Distinct environmental pressures, especially through different diurnal roosting options, may have driven variation in thermoregulatory responses between and within species. Malagasy bats are therefore ideal for studying the significance of a species' natural ecological and physiological capacity as well as potential tolerance limits in the context of environmental variation over seasonal and geographic scales.

Study set-up and study overview

I worked with the endemic insectivorous bat *Macronycteris commersoni* (Hipposideridae). It is among the widest distributed bats in Madagascar, occurring in the dry southern and western parts of the island as well as humid rainforest and only absent in the central highlands (Goodman 2011). Throughout its distribution, *M. commersoni* uses contrasting diurnal roosts: it rests in large colonies in well-insulated caves (as in south-western dry spiny forest, figure 0.2B), but also in the open vegetation exposed to the effects of external environmental extremes (as in western dry deciduous forest, figure 0.2A; Raharinantenaina et al. 2008; Goodman 2011). Both regions are located in the driest zones of the island and are highly seasonal. During the harsh dry season night-time T_a, precipitation and food availability are reduced for up to nine months. Resources are more abundant during the wet season, but daytime temperature extremes may exceed 40°C regularly. Interestingly, *M. commersoni* is known to build up fat deposits by the end of the wet season and appears to "disappear" during the austral winter, suggesting that it stays in its roosting sites and hibernates (Rakotoarivelo et al. 2007). Together with the flexible roost selection within this seasonal environment, the behaviour and ecology seen in this species imply a certain intraspecific variation and raise intriguing questions on potential physiological plasticity. I therefore studied the flexibility of *M. commersoni* in its physiological responses to cope with different roost types and seasons. I aimed to unravel whether this potential flexibility reflects local acclimatisation of single populations or whether different responses can be accessed individually when the prevailing conditions change rapidly to get an insight into *M. commersoni*'s adaptive scope.

To answer this rather broad objective, I divided the project into several sub-topics, in which I focused on:

- defining the "baseline" physiological response, particularly the role of torpor, of different populations with different roosting types in the cool dry season as well as in the hot wet season,
- identifying *M. commersoni*'s capacity for intraspecific physiological variation displayed by distinct responses between seasons and sites,

- determining the species’ potential for physiological flexibility with regard to thermoregulation when the environmental conditions rapidly change.

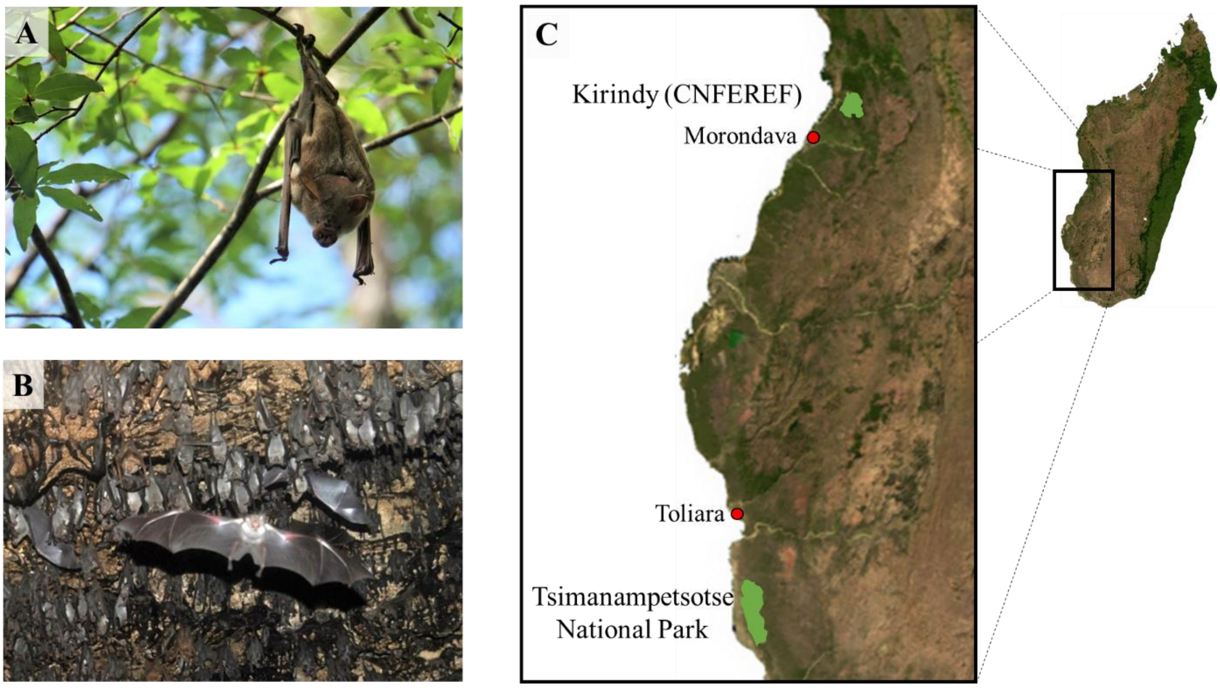

Figure 0.2. Locations of the two study sites in Madagascar (C) and respective roosting habits of *M.commersoni*: A) foliage roost in deciduous dry forest without caves in Kirindy (CNFEREF); B) cave roost in dry spiny forest in National Park Tsimanampetsotse. Photo credit: Stephanie Reher.

These topics are covered in the following six Chapters:

Chapter 1: Seasonal movements of insectivorous bat species in south-western Madagascar

Published as: Reher S., Rabarison H., Schoroth M. and Dausmann K.H. 2019. Seasonal movements of insectivorous bat species in southwestern Madagascar. Malagasy Nature 13: 117-124.

Seasonal effects can be particularly harsh in south-western Madagascar and alter habitat on various levels such as ambient temperature, food availability, water availability, and even habitat structure. These changes could render activity unfavourable for the local bat fauna, or encourage local shifts in roosts to adjust the microclimate to the prevailing seasonal conditions. However, there is a general paucity of information on Malagasy bats and their (micro-) habitat requirements and preferences, let alone on potential seasonal variations in their needs. The first step of the whole PhD- project and a prerequisite for implementing the following chapters was therefore to get an idea of the general availability of potential resting sites[1], the environmental conditions these are offering, and the respective bat occurrence. In this chapter, we describe potential ecological and physiological drivers on variation in bat species occurrence/activity, refine known species distributions, and finally, complement information on seasonal differences in the ecological requirements of Malagasy bats.

Highlights:

- first record of *Miniopterus griffithsi* and *Mops leucostigma* in Tsimanampetsotse National Park
- temporal and spatial variation in five further bat species occurrence
- unexpected preference of a hot cave for overwintering in four bat species

Chapter 2: Short and hyperthermic torpor responses in the Malagasy bat *Macronycteris commersoni* reveal a broader hypometabolic scope in heterotherms

Published as: Reher S., Ehlers J., Rabarison H. and Dausmann K.H. 2018. Short and hyperthermic torpor responses in the Malagasy bat Macronycteris commersoni reveal a broader hypometabolic scope in heterotherms. Journal of Comparative Physiology B 188: 1015-1027.

Heterothermy is generally a powerful response to energy bottlenecks and recent research has revealed that many tropical and subtropical species are heterothermic, even displaying torpor with patterns unlike those of “classical” heterotherms from temperate and arctic regions. Only a handful of studies investigating torpor in bats’ natural environment exist, but based on the results of the previous chapter, in Chapter 2 we tried to unravel the physiological response of the species *Macronycteris commersoni* resting and even overwintering in a hot cave with high humidity. We identified a variety of different torpor patters, including short bouts, daily bouts, prolonged bouts and even hibernation, all occurring at the same time within the same population. Some of our findings suggest that the width of hypometabolism within a species is broader than often assumed, expanding the general idea of torpor that we have had so far.

Highlights:

- extreme variability in torpor patterns within a species regardless of season

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[1] *Finding the caves monitored in this remote region was a crucial point of the whole PhD project and complete team effort: we worked together with agents from the association Analasoa, the local national park service MNP, the WWF and, most importantly, local villagers willing to share their knowledge on cave locations. In this part of the island, some caves are sacred places used for traditional ceremonies while others are used for bat hunting or guano extraction, which is prohibited at least within the borders of the National Park. Consequently, I am still very grateful for meeting us with such great trust and guiding us to suitable caves (no matter how far and accessible).*

- remarkably low metabolic rate during torpor despite high body temperature owing to high temperature in the hot cave
- rapid alternation between torpid and euthermic metabolic rate

Chapter 3: Tropical bats counter heat by combining torpor with adaptive hyperthermia

Published as: Reher S. and Dausmann K.H. 2021. Tropical bats counter heat by combining torpor with adaptive hyperthermia. Proceedings of the Royal Society B 288: 20202059.

High environmental temperatures can have disastrous physiological impacts on tropical mammals because their water budget dictates the extent of heat compensation. While a cave, even a hot one, buffers tropical environmental extremes such as heatwaves, storms or regular daytime fluctuations, bats living in regions lacking insulating resting sites are facing fatal mismatches between environmental conditions and their upper thermal restrictions. In Chapter 3, I present data of a population of *M. commersoni* resting among branches during the day without further protection. Interestingly, the bats entered torpor not despite but rather because of the heat, with T_b increasing at the beginning of the torpor bout and thereby even tolerating hyperthermia. These findings challenge a fundamental concept in thermal physiology, i.e. a drop in body temperature is clearly not a mandatory prerequisite of hypometabolism. Hot torpor is a novel and elegant mechanism to cope with heat and aridity, because it also saves water besides energy and extends the tolerable (thermal) niche. However, we also discuss the downsides and limitations of entering such a deep state of inactivity at the upper thermal limit in the lights of global warming.

Highlights:

- micro-torpor bouts lasting on average only 12 minutes on regular warm days
- extended “hot” torpor bouts coupled with adaptive hyperthermia up to 42.9°C on hot days

Chapter 4: Disparate roost sites drive intraspecific physiological variation in a Malagasy bat

Submitted to Oecologia as: Reher S., Rabarison H., Montero B.K., Turner, J.M. and Dausmann K.H. Disparate roost sites drive intraspecific physiological variation in a Malagasy bat.

Separate populations of widely distributed species might experience different environmental conditions within their range and these may additionally vary within and between seasons. The complete ecological reality of a species can therefore be broad and data restricted to only one point in time and/or space may not accurately represent it as a whole. To this end, in Chapter 4 we compared the physiological responses of the already mentioned *M. commersoni* populations: one population roosting in a well-buffered hot cave (Chapter 2) and one roosting in trees, unprotected from the local weather (Chapter 3). Indeed, torpor frequency, duration and timing were tailored to the respective environmental conditions while metabolic rate during torpor and resting was stable. Interestingly, the exposed forest roost required extensive use of torpor, which even exceeded torpor use in overwintering bats not leaving the cave for months and amounted to comparable low daytime resting energy expenditure. The very fine adjustment of metabolism consequently mitigated different ambient pressures, supporting the need to study species on a broad environmental scale to avoid underestimating a species' full natural potential and unfounded conclusions.

Highlights:

- the stable and thermal neutral cave microclimate negated the need for long-term hypometabolism in better-conditioned individuals
- the exposed foliage roost imposed extensive use of torpor
- metabolic adjustment at fine scale compensated contrasting environmental pressures: we found little variation in overall body condition

Chapter 5: Local acclimatisation hampers physiological compensation of rapid environmental changes in a Malagasy bat

In preparation as: Reher S., Rabarison H., Nowack, J. and Dausmann K.H. Local acclimatisation hampers physiological compensation of rapid environmental changes in a Malagasy bat.

Differences among populations as discussed in Chapter 4 reflect a species' capacity for phenotypic variation and/or local adaptation, and may even hint at incipient speciation. Intraspecific variation observed in a species as a whole does therefore not necessarily entail that all individuals can take advantage of it when rapid responses are required. To examine the effect of acute microclimate change on the thermal physiology of *M. commersoni*, we exposed individuals of each population to the respective opposite conditions and thus to novel microclimate within an ecologically realistic scope. While we observed potential for flexible compensation of heat through "hot" torpor, both populations showed patterns suggestive of limited potential to cope with conditions outside their typically occupied microclimates. Consequently, intraspecific variation among populations could be misleading when assessing species' adaptive scopes, as variation may arise from local adaptation or acclimatisation, ultimately hampering flexible compensatory responses. Identifying the basis of variation is therefore vital to make accurate predictions of species' chances for persisting in ever rapidly changing habitats and climates.

Highlights:

- cave bats had limited capacity to maintain euthermia to the point that two individuals became hypothermic
- forest bats had difficulties to dissipate heat in the humid cave set-up
- unexpected uniform response to heat in all bats: “hot” torpor

Chapter 6: Heterothermy in Malagasy mammals

In press as: Dausmann K.H., Reher S. and Bethge J. Heterothermy in Malagasy Mammals. In: The new natural history of Madagascar (ed. Goodman S.M.). Princeton, USA: Princeton University Press.

Madagascar is a very diverse island, ranging from the dry and unpredictable conditions in the dry spiny thickets of the south to the moist evergreen rainforests of the east and extreme north. The entire island is frequently struck by weather extremes and especially the west is additionally highly seasonal, which altogether forms a “hypervariable” environment. Accordingly, Malagasy animals developed many strategies to cope with these challenging conditions. Chapter 6 is a book chapter addressing physiological adaptation, especially heterothermy, in tenrecs, bats and lemurs, with the whole section on bats arising from this doctoral thesis itself or affiliated (sub-) projects. It provides a nice wrap up of physiological and ecological responses of several bat species and/or populations roosting at similar locations but coping quite differently with their respective environment. We also discuss overlaps and marked discrepancies among the three mammalian groups and provide a perspective on which further Malagasy endotherms may take advantage of physiological adaptations.

Projects affiliated with this thesis

After the initial feasibility field trip of this study, some topics have been expanded and are now being addressed as separate projects, which are not part of the core chapters of this thesis and are therefore only briefly mentioned or not included at all in the abovementioned chapters.

For example, other bat species roost in the same cave as my focal species *M. commersoni*: e.g. *Triaenops menamena* and *Paratriaenops furculus*. They all have to cope with the same harsh dry season conditions with limited food as well as water availability and the unpredictable wet season. Nevertheless, *T. menamena* can be trapped regularly in both seasons, *P. furculus* is trapped less frequently in the dry season and *M. commersoni* completely disappears in the dry season (Raharinantenaina et al. 2008; Goodman 2011), implying different ecological and physiological adaptations. To better understand these inter-specific variations, we combined some ecological and physiological approaches. We monitored the thermal patterns of all three species in both seasons to track whether some bat species, particularly the ones that are less frequently trapped during the dry season, enter torpor to overcome this period of limited food availability. In addition, to get an impression how the thermal patterns of the species relate to their dietary niches, we collected hair samples for stable isotope analyses and matched these with insect remains from faeces samples to identify their consumed prey. These data were compared to the results of a seasonal insect survey. Another PhD student, Hajatiana Rabarison, is currently spearheading this part of the project, but I was involved in study design, data collection and data analyses.

To assess the general transferability of our physiological data on *M. commersoni*, especially as there are no further ecophysiological data on Malagasy bats available, we included *T. menamena* into our project. Both species are widely distributed throughout Madagascar and occur in the western forest habitat as well as the southern cave habitat, where they even share at least one cave (see above). However, *T. menamena* also roosts in a cave with a strikingly different T_a profile and, interestingly, remains active year-round in both habitats. We therefore collected thermo-physiological data of *T. menamena* at differently insulated roosts (i.e. a stable hot cave, a less-buffered cave and a forest, where they roost in tree holes) in both the dry and wet season and related those to the respective roost's ambient conditions. These data do not only add important information on microhabitat demands to a generally poorly studied mammal group in Madagascar. They also allows us to study the whole spectrum of potential factors influencing bat energetics (T_a, humidity, roost structure [cave/tree roost]) in two species that clearly differ in physiological and ecological traits, which ultimately permits conclusions on both, inter- and intraspecific physiological variation. Two Master students are working on this particular part of the project, Sina Remmers and Marie Schoroth. I designed the study, supervised these theses and was involved in data collection and data analyses.

Given the major differences in roosting conditions we observed (well-buffered vs. no buffer, large colony vs. small group or even roosting solitarily, high T_a and humidity vs. fluctuating environmental conditions), we further studied the role of ectoparasites in roost selection and energetics. Especially the warm and humid cave offers ideal conditions for parasites to thrive and the large colony promotes horizontal inter- and intraspecific transmission (Hofstede and Fenton 2005). Parasites have a substantial influence on their hosts' ecology and physiology (Hart 1994; Giorgi et al. 2001). High parasite loads increase grooming activity and simultaneously reduce resting bout duration (Giorgi et al. 2001), affecting energy budgets. At the same time, such a microclimate might be more favourable and regular roost changes can be costly (Reckardt and Kerth 2007). Thus, over the course of the whole project, we have been collecting ectoparasites of bats in cave and forest habitats to quantitatively characterise the ectoparasite community and its role in both, bat energetics and roost selection. Hajatiana Rabarison and Master student Anja Biesdorf conduct this sub-project with my support.

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Chapter 1

Seasonal movements of insectivorous bat species in southwestern Madagascar

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Abstract

Seasonal environmental changes can be challenging for animals because they are usually characterized by fluctuations in temperature, considerable decreases in food resources and, in tropical and subtropical regions, water availability. One response to less favorable conditions is migration, which is energetically costly and potentially dangerous. This aspect raises the question of whether there is a critical tipping point where environmental characteristics render residency unfavorable. There is a paucity of information on Malagasy bat habitat requirements and few details are available on migratory species. Here, we aimed to elucidate drivers of seasonal differences in bat species occurrence in the southwest of Madagascar and determine which species are potentially migratory. This region encompasses dry spiny forest with pronounced seasonal fluctuations in ambient temperature and rainfall, conditions that can prompt habitat shifting, among other strategies. During trapping phases in the wet summer and dry winter, we recorded the temporal and spatial occurrence of seven different insectivorous bat species in Tsimanampetsotse National Park. While *Macronycteris commersoni, Triaenops menamena*, *Paratriaenops furculus*, and *Miniopterus mahafaliensis* were present year-round, *M. griffithsi*, *Mormopterus jugularis*, and *Mops leucostigma* were only trapped in the rainy season, suggesting site relocation. Furthermore, this study provides the first record of *Miniopterus griffithsi* and *Mops leucostigma* in Tsimanampetsotse National Park. Although we have no information on where the migrating species reside during the dry season, this study provides data for refining known species distributions and augments information on seasonal variation in the ecological requirements of bats.

Keywords

Habitat shift, seasonality, bats, roosting sites, Madagascar

Résumé détaillé

Les changements de conditions environnementales peuvent rendre un habitat difficile à vivre pour les animaux. Les dégradations de l'habitat se produisent par des variations du climat naturel et de l'interaction humaine qui se traduisent par des changements annuel ou saisonnière de l'environnement. Ces variations saisonnières représentent une contrainte environnementale importante pour de nombreux vertébrés et s'accompagnent en général d'un changement de la disponibilité des ressources alimentaires et de la disponibilité de l'eau dans les régions (sub)tropicales. Pour cela, les animaux ont développé alors des adaptations comportementales et physiologiques pour améliorer sa survie. La migration est une approche comportementale adaptative pour faire face aux changements des conditions ambiantes. Une réponse assez complexe qui nécessite une aptitude physique et même physiologique pour bénéficier la sélection de la qualité d'une destination appropriée. Ainsi, cette étude vise à déterminer quelles espèces sont potentiellement migratrices dans le Sud-ouest de Madagascar et de savoir quelles espèces ont préféré quel microhabitat sous différents changements saisonniers favorables à la migration ainsi que la survie de ces espèces migratrices.

Cette étude a été menée dans le Parc National de Tsimanampetsotse à Madagascar. Ce parc, localisé dans la région du Sud-ouest de la Grande île englobe une forêt épineuse sèche comprenant des variations des conditions ambiantes prononcées, favorisant un changement flexible de l'habitat. Entre autres, les informations sur la préférence en habitat des chauves-souris sont encore incomplètes à Madagascar. Il en est de même pour les informations sur les espèces potentiellement migratrices. Lors de cette étude, les chauves-souris ont été capturées dans deux sites différents du parc : la grotte d'Andranolovy et la grotte de Vintany. Les deux sites possèdent des caractéristiques de microhabitats très différentes. La température et l'humidité restent stables et élevées toutes l'année dans la grotte d'Andranolovy mais par contre fluctuent suivant les saisons dans la grotte de Vintany. La saison sèche et la saison de pluies ont été choisies pour deux sessions de capture d'individus pour chaque site en mai/juin 2016 et février 2017. Une session de capture a consisté à cinq nuits de capture dans un site. Le piège harpe a été utilisé pour capturer les chauves-souris. Ce matériel a été placé à l'entrée pour la grotte d'Andranolovy, tandis qu'il a été placé sur le couloir principal menant à la grotte pour la grotte de Vintany. Au total, 572 individus de *Macronycteris commersoni*, 246 individus de *Triaenops menamena*, 90 individus de *Paratriaenops furculus*, 87 individus de *Miniopterus mahafaliensis*, 2 individus de *M. griffithsi*, 3 individus de *Mops leucostigma* et 3 individus de *Mormopterus jugularis* ont été capturés. Au maximum, 20 individus par espèces ont été choisis pour les mensurations de l'avant-bras et la prise des poids. Ces individus ont été marqués sur leur aile membranaire d'un tatouage à trois chiffres avec une encre non toxique, puis relâchés dans leur milieu d'origine.

Dans la grotte d'Andranolovy, *T. menamena*, *P. furculus* et *Miniopterus mahafaliensis* ont été

capturés pendant les deux saisons tandis que *Macronycteris commersoni* n'a été capturé que pendant la saison des pluies avec un nombre important de juvéniles. Dans la grotte de Vintany, *T. menamena* et *Miniopterus mahafaliensis* ont été capturés pendant les deux saisons, tandis que *Macronycteris commersoni*, *Miniopterus griffithsi*, *Mops leucostigma* et *Mormopterus jugularis* ont été capturés pendant la saison des pluies. Cette étude a permis de recenser pour la première fois *Miniopterus griffithsi* et *Mops leucostigma* dans le Parc National de Tsimanampetsotse. *Macronycteris commersoni* accumulait des réserves de graisses nécessaires pour le déplacement de longues distances. Cette espèce a reposé dans la grotte d'Andranolovy et a entré probablement en période de torpeur pour faire face à la saison sèche, ce qui n'a pas permis sa capture pendant la saison sèche. Certaines espèces de chauves-souris reposent régulièrement dans les deux grottes pendant les deux saisons : c'est le cas de *T. menamena* et *Miniopterus mahafaliensis*. Ces deux espèces élargissent leur site de repos à un grand nombre de grottes pendant la saison des pluies mais préfèrent la grotte d'Andranolovy pendant la saison sèche, probablement en raison de conditions microclimatiques chaudes et humides de la grotte. Bien que nous ne disposions d'aucune information sur la provenance de ces espèces, cette étude fournit des données importantes pour étendre et affiner la distribution géographique des espèces, y compris les variations saisonnières des besoins écologiques des chauves-souris.

Mots clés : déplacement d'habitat, saisonnalité, chauves-souris, perchoirs, Madagascar

Introduction

Changes in environmental conditions can be challenging for animals because they have the potential to render a habitat unsuitable or more difficult to occupy (Heldmaier et al. 2013; Hill et al. 2016). Habitat modification is caused by natural weather variation and human interference, resulting in environments that can change on an annual, seasonal, daily or completely unpredictable basis. The effects of season can be particularly severe as they are relatively long (several months) and accompanied by changes in food availability and, particularly in tropical and subtropical regions, water availability (Janzen and Schoener 1968; Pinheiro et al. 2002). However, unlike other environmental changes such as daily fluctuations and weather extremes, seasonality is predictable and animals have evolved different responses spanning morphological, physiological, and behavioral adaptations.

An adaptive behavioral approach for coping with spatio-temporal shifts in ambient conditions is migration. This is a complex response and requires certain cognitive, physical, and physiological abilities, as well as knowledge (Avgar et al. 2014) of the existence, location, and quality of a suitable destination. Awareness of stopover points for fueling the energetic requirements of long-distance movement is also essential (Popa-Lisseanu and Voigt 2009). Additionally, migration needs preparation such as fat storage and muscle gain (Fleming and Eby 2003), and is costly in terms of time, invested energy, and management of risks, which include increased mortality during long-term movement owing to predators, sudden food shortages or unpredictable inclement weather (Fleming and Eby 2003; Newton 2007; Avgar et al. 2014). In markedly seasonal environments, the benefits likely exceed these costs and migratory species profit from travelling to more favorable microclimatic conditions, food that is higher in quantity and/or quality, and, putatively, reduced exposure to parasites and disease (Fryxell and Sinclair 1988; Fleming and Eby 2003; Avgar et al. 2014).

For bats, migration is particularly advantageous because they are small-bodied and lose considerable body heat from their flight membranes when temperatures are low (McNab, 1969), rendering them vulnerable to energetic imbalance in seasonally fluctuating environments. Additionally, they usually live in large aggregations, which can rapidly deplete local resources (Avgar et al. 2014), making the relocation to preferred habitat highly beneficial. The main advantage of migration for temperate bats is primarily related to the utilization of roosts with thermal characteristics that benefit metabolism, whereas fluctuations in resource availability and rainfall are the main drivers of bat migration in the tropics (Moreno-Valdez et al. 2000; Fleming and Eby 2003; Rodrigues and Palmeirim 2008).

Madagascar’s highly seasonal dry spiny forest is a challenging environment incorporating the driest and least climatically predictable area of the island (Génin 2008; Kobbe et al. 2014). Such variable environments often encourage flexible habitat shifting or nomadism (Mueller and Fagan 2008), especially for insectivorous bats, whose prey availability decreases with nighttime temperature in the austral winter (Janzen and Schoener 1968; Pinheiro et al. 2002). However, information on the habitat preferences of Malagasy bats and their general distribution patterns are still incomplete (Goodman and Ramasindrazana 2013). Because at least one bat species occurring in the spiny forest of southwestern Madagascar is reported to “disappear” during the lean, dry season (Goodman 2006; Rakotoarivelo et al. 2007), we aimed to determine whether there are seasonal differences in insectivorous bat species occurrence and which species are migratory.

Methods

Study site

This study was conducted in Tsimanampetsotse National Park in the extreme southwest. The park is located in the driest and most climatically unpredictable area of the island and receives between 300 and 600 mm of rain each year (Rasoloariniaina et al. 2015). Precipitation, ambient temperature, and food resource availability are influenced by season. Rainfall is mainly restricted to the hot rainy season between November and March (austral summer), but the exact timing and amount are highly unpredictable. The dry season, from May to September (austral winter), receives almost no rainfall and is characterized by colder nights (minimum of 6°C in the dry season vs. 15°C in the rainy season; Kobbe et al. 2014) and limited resource availability, i.e. plant material and insects. Ambient temperature varies over the course of the year between 6°C and 45°C (Kobbe et al. 2014). The region is located on a calcareous plateau covered in dense spiny forest, with numerous different microhabitat types including complex underground cave and stream systems.

We trapped bats at two different sites in the park; Andranolovy Cave (24.04585° S / 043.75396° E) and Vintany Cave (24.04383° S / 043.75519° E). The caves are only 270 m apart but differ in environmental characteristics. Andranolovy Cave is a large, buffered underground system consisting of several connected chambers and a water body covering approximately 25% of the cave floor. Temperature and relative humidity (RH) are stable and high year-round with daily fluctuations less than 1°C and 1% RH, respectively, and were never lower than 29.4°C and 94.8% RH, respectively (Figure 1.1a, b; recorded for 101 consecutive days per season using Hygrochron iButtons placed at 1.5 m height, Maxim integrated, San Jose, USA). Vintany Cave is smaller, adjoins a former sinkhole and is influenced more by ambient conditions and weather extremes, with daily fluctuations of 7.3 ± 1.5°C (daily mean min – daily mean max: 19.9 – 27.2°C) and 24.3 ± 5.9% RH (55.2 – 79.5%) in the dry season, and 4.7 ± 1.0°C (25.8 – 30.5°C) and 20.3 ± 5.1% RH (63.9 – 84.2%) in the rainy season (Figure 1.1c, d).

Trapping and handling

Each trapping period consisted of five consecutive nights per site in May/June 2016 (dry season) and February 2017 (rainy season). A harp trap was placed at the entrance of Andranolovy Cave and the main approaching corridor at Vintany Cave. To capture bats emerging from their roosts for their first evening foraging flight, the trap was opened approximately half an hour before sunset at 18:00 (dry season) and 19:00 (rainy season) and closed at 21:30, and checked every 15-20 min. At each site, a maximum of 20 adult individuals of each species were randomly chosen for the measurements of body mass and forearm length, and for sex determination. Individuals were aged based on wing bone and joint ossification (Brunet-Rossinni & Wilkinson, 2009). To avoid pseudoreplication, these bats were individually marked with a 3-digit wing membrane tattoo using non-toxic ink (Hauptner-Herberholz, Solingen, Germany) after local anesthesia (EMLA, AstraZeneca, Wedel, Germany).

Analysis

We only compared forearm length and body mass of adult *Triaenops menamena* and *Miniopterus mahafaliensis* between seasons and caves, owing to the small sample sizes of the other species (Table 1.1). After testing for normality using the Shapiro-Wilk test, data were further analyzed using unpaired samples t-tests or Mann-Whitney-U tests. IBM SPSS v24 was used for all analyses.

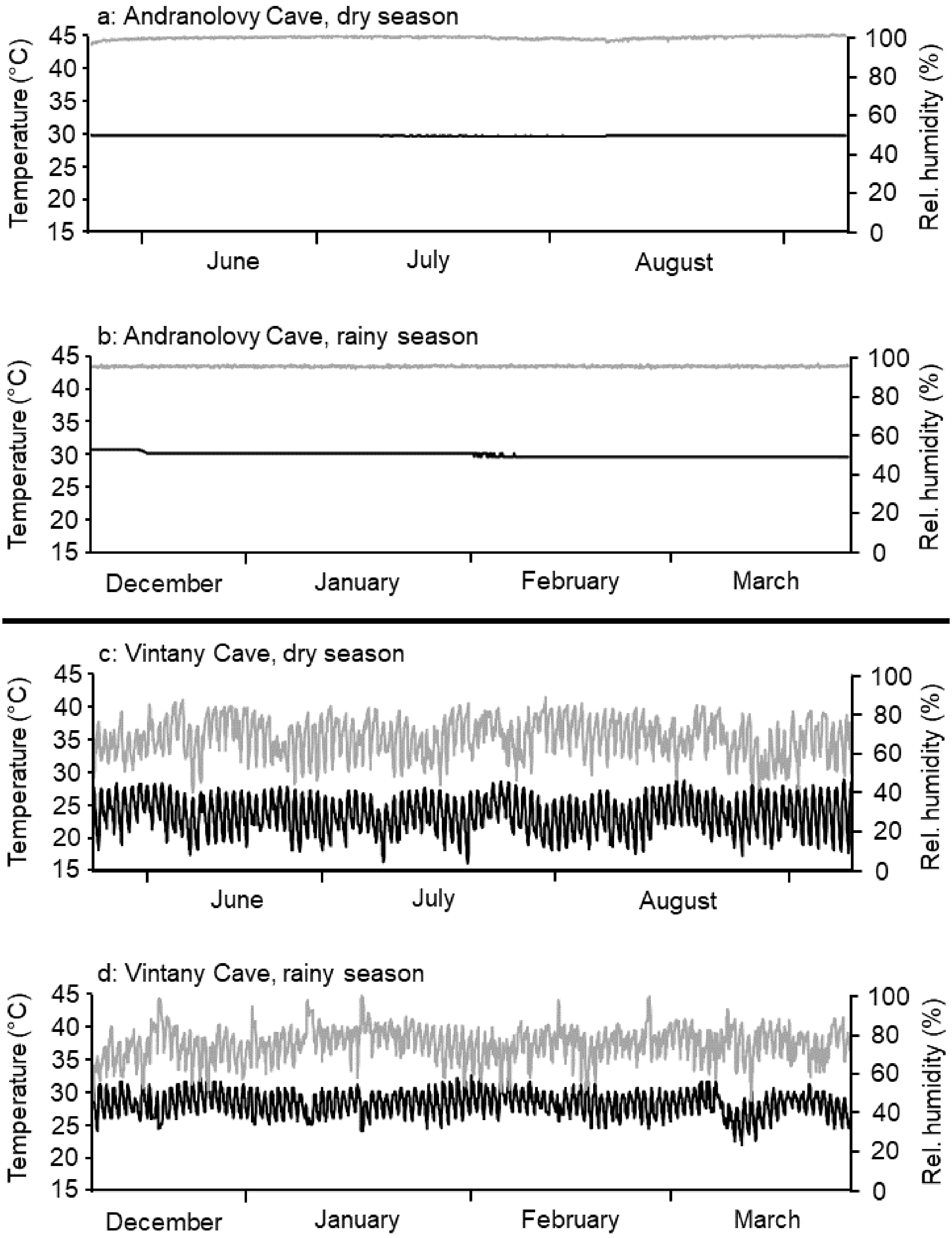

Figure 1.1. Ambient temperature (black line) and relative humidity (grey line) in two different caves, Andranolovy (a, b) and Vintany (c, d), in Tsimanampetsotse National Park in the dry (a, c) and rainy (b, d) seasons. Data were recorded once per hour for 101 consecutive days.

Results

Species composition differed temporally and spatially between the two cave systems (Table 1.1). At Andranolovy Cave we captured four different bat species. The most frequent species was *Macronycteris commersoni* with 566 individuals; however, it was only trapped in the rainy season, and 92% of individuals were juveniles. *Paratriaenops furculus* was also trapped more often in the rainy season than the dry season. *Triaenops menamena* and *Miniopterus mahafaliensis* were trapped in both seasons, each with about twice as many individuals in the dry season as the rainy season. *Triaenops menamena* trapped in the dry season had smaller forearm lengths than those in the rainy season (Z = -3.078, P = 0.002, N = 21; Table 1.1) and *M. mahafaliensis* were heavier in the rainy season (Z = -2.188, P = 0.029, N = 20; Table 1.1). *Paratriaenops furculus* was only trapped at Andranolovy Cave.

At Vintany Cave, six species were captured. Similar to at Andranolovy Cave, *T. menamena* and *M. mahafaliensis* were trapped in both seasons while *Macronycteris commersoni*, *Miniopterus griffithsi*, *Mops leucostigma,* and *Mormopterus jugularis* were trapped exclusively in the rainy season and with few individuals (Table 1.1; N = 2-6). In contrast to the trapping success at Andranolovy Cave, the number of trapped *T. menamena* and *Miniopterus mahafaliensis* was higher in the rainy season than in the dry season and we did not find any difference in forearm length (*T. menamena*: t_{18} = -0.399, P = 0.695, N = 20; *M. mahafaliensis*: Z = -1.845, P = 0.065, N = 15) or body mass (*T. menamena*: t_{18} = -0.257, P = 0.800, N = 20; *M. mahafaliensis*: t_{13} = -0.511, P = 0.618, N = 15) between seasons. *Miniopterus griffithsi*, *Mops leucostigma*, and *Mormopterus jugularis* were only trapped at Vintany Cave.

A direct comparison of the two roosting sites revealed that only individuals from *T. menamena* were smaller at Vintany Cave than Andranolovy Cave and only in the rainy season (Z = -2.360, P = 0.018, N = 21; Table 1.1).

Discussion

We found seasonal differences in bat species occurrence in Tsimanampetsotse National Park that imply spatial relocation or torpor as strategies to cope with the extreme seasonal environment. Additionally, this study provides the first record of *Miniopterus griffithsi* and *Mops leucostigma* in this protected area.

Miniopterus griffithsi was only described about a decade ago (Goodman et al. 2009) and little information on its distribution and ecology are known (Goodman and Ramasindrazana 2013). Our observation at Vintany Cave is the most northern record for this species, but this is in a location similar to those of previous studies in the extreme southeast near Ranopiso, and the southwest near Itampolo (Goodman et al. 2009; ~70.3 km from our record). The vegetation at these previous trapping sites ranged from spiny forest and coastal bushland to disturbed gallery forest and all locations had caves or rock shelters (Goodman et al. 2009). Interestingly, both trapped individuals were about twice as heavy (25.3 ± 1.1 g, N = 2) as previously captured *M. griffithsi* (13.6 ± 1.2 g, N = 6; Goodman et al. 2009), which were trapped at the end of the rainy season (late February) and in May. The two individuals trapped in May had accumulated considerable amounts of subcutaneous fat (body mass = 15.5 and 16.5 g; Goodman et al. 2009) but still weighed much less than the bats we trapped. Thus, the new listing in Tsimanampetsotse National Park may either provide information on the species' morphological variation (i.e. expanding the range in body mass) and distribution.

Table 1.1. Total number (#) as well as body mass (BM, g) and forearm length (FA, mm) of a subsample of individuals (N) of trapped bat species at two different caves, Andranolovy and Vintany, in Tsimanampetsotse National Park in the dry (DS) and rainy season (RS). Each trapping period consisted of five consecutive nights per site. Only adults were included in BM and FL measurements. For statistical details, see text. * including 92% juveniles, Δ including 51% juveniles, † this species also uses a second entrance.

		Andranolovy				**Vintany**			
	Season	**#**	**BM [g]**	**FA [mm]**	**N**	**#**	**BM [g]**	**FA [mm]**	**N**
Hipposideridae									
Macronycteris commersoni	DS	0				0			
	RS	566*	47.5 ± 11.7	84.4 ± 4.7	20	6	41.3 ± 3.8	81.5 ± 2.3	3
Rhinonycteridae									
Triaenops menamena	DS	121	8.9 ± 1.1	48.1 ± 1.1	10	34	9.3 ± 0.9	48.8 ± 1.5	10
	RS	51	9.5 ± 1.3	50.5 ± 2.8	11	40	9.1 ± 1.5	48.6 ± 1.8	10
Paratriaenops furculus	DS	5	6.3 ± 0.6	44.7 ± 1.4	4	0			
	RS	35Δ	6.8 ± 1.1	45.2 ± 1.6	12	0			
Miniopteridae									
Miniopterus mahafaliensis	DS	28†	4.7 ± 0.5	37.5 ± 0.8	10	17	4.7 ± 0.4	38.1 ± 0.8	10
	RS	13†	5.0 ± 0.6	36.8 ± 1.3	10	29	4.9 ± 1.0	37.4 ± 0.6	5
Miniopterus griffithsi	DS	0				0			
	RS	0				2	25.3 ± 1.1	46.8 ± 0.7	2
Molossidae									
Mops leucostigma	DS	0				0			
	RS	0				3	22.5 ± 0.7	43.6 ± 0.4	3
Mormopterus jugularis	DS	0				0			
	RS	0				3	12.7 ± 1.5	36.4 ± 0.7	3

Mops leucostigma is widely distributed across different habitats on Madagascar but had not been reported for Tsimanampetsotse National Park (Ramasindrazana and Goodman 2012; Ramasindrazana et al. 2012). Current distribution estimations include southwestern spiny bush, western dry deciduous forest, and eastern humid forest (Ratrimomanarivo et al. 2008). Furthermore, this is the first record of this species roosting in a cave (Goodman 2011). *Mormopterus jugularis* is also known to have a broad distribution throughout varying bioclimatic zones, ranging from the driest to the most humid regions in Madagascar and up to 1750 m in altitude (Goodman et al. 2005; Ratrimomanarivo et al. 2009). Along with *Mops leucostigma*, *Mormopterus jugularis* has been found to share synanthropic day roosts (Goodman and Cardiff 2004; Ratrimomanarivo et al. 2008). Both species are hunted for food in Madagascar (Goodman 2006; Monadjem et al. 2017). Although no reliable human consumption rates are available for either species, studies on *Macronycteris commersoni* revealed that exploitation of these bats is unsustainable (Goodman 2006). This makes information on exact distribution ranges and suitable habitat types of both species crucially for assessing the availability of possible refugia.

Interestingly, during the dry season *M. commersoni* was never trapped, and *P. furculus* rarely trapped, even though both species were found in large numbers roosting in Andranolovy Cave during both seasons. Individuals observed in the dry season were generally less responsive than in the rainy season, suggesting that these species entered torpor to cope with the lean dry season (Reher et al. 2018). *Triaenops menamena* and *Miniopterus mahafaliensis* still foraged regularly during this season. Both species and *P. furculus* have a diet primary consisting of Lepidoptera, which, especially in the dry season, is supplemented by Coleoptera and, in the case of *M. mahafaliensis*, Hymenoptera (Ramasindrazana et al. 2012). *Macronycteris commersoni* however prefers Coleoptera year-round (Ramasindrazana et al. 2012). Thus, the habitat offers sufficient insect abundance for *T. menamena* and *Miniopterus mahafaliensis* to allow activity in both seasons, while *Macronycteris commersoni* and *P. furculus* reduced activity to cope with the leaner dry season. Nevertheless, all four species are residents in Tsimanampetsotse National Park, indicating that the region offers them suitable environmental conditions year-round. *Triaenops menamena* and *M. mahafaliensis* were the only two species that regularly roosted in both caves during the two seasons. Interestingly, we trapped more individuals from each of these two species at Andranolovy Cave in the dry season than in the rainy season, but the opposite at Vintany Cave. Consequently, both species appeared to have broadened their roosting preferences to a wider range of caves in the rainy season, but to prefer Andranolovy Cave for the harsh dry season, probably owing to stable humid and warm microclimatic conditions (Figure 1.1). Because seasonal fluctuations in ambient conditions in tropical areas are often small, negating the need for long distance movement, a local shift in microhabitat preference may be enough to ensure survival (Popa-Lisseanu and Voigt 2009). Therefore, a slight relocation to a more constant roosting environment probably allows *T. menamena* and *M. mahafaliensis* to remain in the park. Indeed, our data indicate that more bats use Andranolovy Cave, with stable conditions, than the fluctuating microclimate of Vintany Cave. These results are in particular interesting as bats are captured by villagers in Andranolovy Cave. We found several traps from villagers in each chamber of the cave but no signs of hunting in Vintany Cave. Thus, our results suggest that the stable microclimatic conditions in Andranolovy Cave were more favorable regardless of hunting pressure, probably because the high temperature makes energetically costly active thermoregulation unnecessary (Reher et al. 2018).

The complete absence of *M. griffithsi*, *Mormopterus jugularis*, and *Mops leucostigma* during the dry season suggests a seasonal habitat shift in response to the highly fluctuating environment of Vintany Cave. All three species were trapped in small numbers and only late in the rainy season (late February), indicating that they may have been passing through Tsimanampetsotse National Park. Bats inhabiting tropical and subtropical regions that undergo seasonal pressures and bottlenecks in resource availability often migrate (Fleming and Eby 2003), although they tend to move shorter distances than temperate migrants (Popa-Lisseanu and Voigt 2009). This habitat shifting, or regional migration, is more likely to be found in forest dwelling than cave roosting bats, and more likely in nectar and fruit feeding bats than in insectivorous species (Bonaccorso 1979; Fleming and Eby 2003; Popa-Lisseanu and Voigt 2009). However, in regions like Madagascar’s southwest, where seasonality strongly influences ambient temperature and precipitation, the advantage of shifting habitat also increases for insectivorous bats, because insect activity and availability decreases sharply during the dry season (Janzen and Schoener 1968; Pinheiro et al. 2002). This likely also affected the three potentially migratory species in our study (*Miniopterus griffithsi*, *Mormopterus jugularis*, and *Mops leucostigma*), which presumably tracked their food resources rather than roosting opportunities.

We acknowledge the limitations of our presence/absence data. Nevertheless, the information presented herein improves knowledge of habitat preferences for certain bats species under varying seasonal and environmental pressures, and for better understanding the critical tipping point at which habitat becomes unsuitable, making spatial shifts necessary. We found clear seasonal spatial and temporal dynamics in bat species composition in Tsimanampetsotse National Park. While some species remained in the region year-round, others clearly had a lower tolerance for seasonal environmental pressures. Although we have no data as to where the migrating species relocated to, or the scale of their movements, this study provides important data for correcting and refining what is known of the distribution of certain taxa and improves awareness of the seasonal variation in their ecological requirements.

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Author contribution

I hereby confirm that Stephanie Reher (SR) and Kathrin H. Dausmann (KHD) conceived and designed the study, SR conducted the measurements in the field with support of Hajatiana Rabarison and Marie Schoroth, SR analysed the data and took the lead in writing the manuscript with input from KHD. All authors discussed and interpreted the results, revised the manuscript and approved the final version of it.

Prof. Dr. Kathrin Dausmann

Chapter 2

Short and hyperthermic torpor responses in the Malagasy bat *Macronycteris commersoni* reveal a broader hypometabolic scope in heterotherms

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Abstract

The energy budgets of animal species are closely linked to their ecology, and balancing energy expenditure with energy acquisition is key for survival. Changes in animals’ environments can be challenging, particularly for bats, which are small endotherms with large uninsulated flight membranes. Heterothermy is a powerful response used to cope with changing environmental conditions. Recent research has revealed that many tropical and subtropical species are heterothermic and display torpor with patterns unlike those of “classical” heterotherms from temperate and arctic regions. However, only a handful of studies investigating torpor in bats in their natural environment exist. Therefore, we investigated whether the Malagasy bat *Macronycteris commersoni* enters torpor in the driest and least predictable region in Madagascar. We examined the energy balance and thermal biology of *M. commersoni* in the field by relating metabolic rate (MR) and skin temperature (T_{skin}) measurements to local environmental characteristics in the dry and rainy seasons. *Macronycteris commersoni* entered torpor and showed extreme variability in torpor patterns, including surprisingly short torpor bouts, lasting on average 20 min, interrupted by MR peaks. Torpid MR was remarkably low (0.13 ml O_2 h^{-1} g^{-1}), even when T_{skin} exceeded that of normothermia (41 °C). *Macronycteris commersoni* is thus physiologically capable of 1) entering torpor at high ambient temperature and T_{skin} and 2) rapidly alternating between torpid and normothermic MR, resulting in very short bouts. This suggests that the scope of hypometabolism amongst heterothermic animals is broader than previously assumed and underlines the importance of further investigation into the torpor continuum.

Keywords

Energy budgets, thermoregulation, torpor, seasonality, Chiroptera, Madagascar

Introduction

Tropical heterotherms promise to reshape standard theories describing thermoregulation and torpor patterns. Traditionally, heterothermy was thought to be restricted to temperate and arctic regions and subdivided into two categories: hibernation as the classical, seasonal expression of torpor (torpor bouts longer than 24 h in duration) and daily torpor as a shorter, more plastic response (torpor bouts less than 24 h). Both hibernation and daily torpor were defined by a dramatic drop in metabolic rate (MR) and body temperature (T_b) in response to low ambient temperature (T_a) (Geiser 2004; Heldmaier et al. 2004). Hibernation is described as a series of multi-day torpor bouts with extremely low metabolic rate (torpid metabolic rate; TMR) interrupted by spontaneous arousals to normothermia. It is also associated with an intense preparation period, i.e. extensive fattening and/or the establishment of a food cache, to support energetic requirements (Geiser 2004; Heldmaier et al. 2004; Kronfeld-Schor and Dayan 2013). In addition, circadian rhythms are not expressed while torpid (Williams et al. 2012). Daily torpor, in contrast, is a shorter, often a seasonal hypometabolic state with a comparatively higher TMR. It usually follows a circadian rhythm, which allows animals to become active and obtain food energy between torpor bouts, thus negating the need for energy stores (Geiser 2004; Heldmaier et al. 2004; Kronfeld-Schor and Dayan 2013).

In recent years, however, field research in tropical and arid regions revealed that these traditional assumptions are not the rule. Many species inhabiting tropical and subtropical regions are heterothermic, despite the generally milder climate, and these species often show a striking variety and flexibility in torpor patterns that do not fit into the classical dichotomy (e.g. fat-tailed dwarf lemurs *Cheirogaleus medius*, Dausmann et al. 2009; common tenrecs *Tenrec ecaudatus*, Lovegrove et al. 2014; greater and lesser mouse-tailed bats *Rhinopoma microphyllum* and *R. cystops*, Levin et al. 2015; rufous hummingbirds *Selasphorus rufus*, Carpenter and Hixon 1988). Because patterns of metabolic suppression appear intriguingly similar among these different expressions of heterothermy, it is a matter of lively debate whether daily torpor and hibernation are actually distinct forms of torpor or simply two points along a continuum (Canale et al. 2012; Boyles et al. 2013; Ruf and Geiser 2015; van Breukelen and Martin 2015). The recently identified forms of torpor in tropical regions that are not strictly related to low T_a and do not necessarily include low T_b may be the key to resolving these debates and better understanding the physiological underpinnings of torpid states. For example, the reddish-gray mouse lemur *Microcebus griseorufus*, which inhabits the driest and most unpredictable habitat of Madagascar, copes with this harsh environment by flexibly employing a range of different torpor responses, even at T_a, and thus torpid T_b, of up to 37 °C. Individuals in a single population may use occasional daily torpor, regular daily torpor or prolonged torpor (1–3 days), or continuous hibernation; some lemurs even switched strategies within a season (Kobbe et al. 2011). Other tropical heterotherms also enter torpor at relatively high T_a without drastically reducing T_b. Golden spiny mice *Acomys russatus*, for example, show a pronounced decrease in metabolic rate within their thermoneutral zone without an associated drop in T_b (Grimpo et al. 2013). Sometimes torpid T_b even exceeds normothermic T_b ("hyperthermic daily torpor", Lovegrove et al. 2014; Toussaint and McKechnie 2012; Bondarenco et al. 2013). The Australian desert bat *Mormopterus petersi* can tolerate extreme heat and has the largest known skin temperature (T_{skin}) range for any mammal: T_{skin} increased to 45.8 °C during a heat wave and dropped to 3.3 °C in winter (Bondarenco et al. 2014).

While torpor is traditionally linked to the leanest season with lowest food availability, making energy savings most profitable (Geiser 2004; Heldmaier et al. 2004), in warmer regions torpor also appears to be a powerful response for enduring weather extremes and other short-term environmental events like storms, heat waves, fires and drought (Doucette et al. 2012, Bondarenco et al. 2014, Nowack et al. 2015, Stawski et al. 2015). Indeed, water conservation through lowered metabolism might play a more important role for torpor use in the tropics during extended dry periods or high T_a (e.g. Schmid and Speakman 2000; Bondarenco et al. 2014) than season or food availability (Kronfeld-Schor and Dayan 2013). Owing to reduced metabolic activity, and because additional water-depleting processes such as respiration, urine production and defecation are reduced or even absent when torpid, water is more effectively retained by, for example, closing the nostrils during apnoea (Levin et al. 2015). Another efficient water-preserving mechanism is to thermoconform at high T_a and become hyperthermic (Geiser and Brigham 2012; Heldmaier et al. 2013; Hill et al. 2016). By accumulating heat during the hottest time of day instead of trying to dump it, water is not wasted via evaporative cooling, which is the only option for actively regulating T_b when $T_a > T_b$ (Heldmaier et al. 2013; Hill et al. 2016). Dromedary camels *Camelus dromedarius* are a classic example: when water-stressed, they cycle between a T_b of 41 °C during the daytime and 34-35 °C at night, thereby sparing water and dissipating heat aggregated during the day at night when conditions are cooler (Schmidt-Nielsen et al. 1956). Comparable strategies are used by llamas (*Lama glama*, Riek et al. 2017), elephants (e.g. *Elephas maximus*, Weissenböck et al. 2011), treeshrews (*Tupaia tana*, Levesque et al. 2018) and desert birds ("facultative hyperthermia", Tieleman and Williams 1999).

These different functions and broad range of varying expressions make torpor a very efficient tool, because temporarily avoiding environmental pressures and constraints via decreased metabolism is a major advantage that can enable survival in arid, hot and unpredictable environments. Madagascar is comprised of many such demanding habitats. Irregular events such as sea current fluctuations (e.g. El Niño and La Niña), droughts and cyclones can affect the island at any given time and, coupled with variable and unpredictable rainfall, create a "hypervariable" environment (Dewar and Richards 2007). Indeed, a fairly high number of endothermic species in Madagascar have already been identified as being heterothermic and the unpredictable environment may have facilitated this occurrence by favoring physiological flexibility in the evolutionary past (Dausmann and Warnecke 2016). Insectivorous bats are particularly affected by environmental fluctuations because they are small-bodied, have large uninsulated flight membranes that readily lose water and heat energy and often rely on temporally variable food resources that cannot easily be cached (Speakman and Thomas 2003; Jonasson and Willis 2012). In Madagascar, no information documenting bats' physiological demands or responses exists, even though 46 species occur on the island (Lebarbenchon et al. 2017), including in the most challenging habitats (Goodman 2011). Moreover, owing to the tropical and subtropical climates of Madagascar, torpor may not necessarily be restricted by low T_a or season. Thus, this group likely offers new insights into the physiological underpinnings of torpid states and thermoregulation, including the relationship between metabolic rate and T_b.

We investigated whether Commerson's leaf-nosed bat *Macronycteris commersoni* (formerly *Hipposideros commersoni*; Foley et al. 2017) enters torpor to cope with the dry and unpredictable environmental conditions in the dry spiny forest of south-western Madagascar. We aimed to compare their torpor patterns to those of bats in temperate and arctic regions and to the other heterothermic species of Madagascar. Because *M. commersoni* builds up fat deposits before the dry, cooler season and appears to "disappear" during the austral winter (Goodman 2006, Rakotoarivelo et al. 2007), we predicted that it would stay in its cave roosting sites and hibernate. Additionally, we aimed to determine how tightly bat populations are adapted to prevailing environmental conditions and how flexibly they can react if these conditions change on a short- to mid-term basis. Since *M. commersoni* is widely distributed across different habitats in Madagascar, including areas without caves where individuals must rely on poorly buffered tree hollows, we hypothesized that the species could easily respond to a change in roosting conditions (i.e. stable vs. fluctuating ambient conditions). We expected the bats to enter deep and stable torpor regularly in their preferred and natural cave roost, particularly in the lean dry season. On the other hand, we assumed they would show more variable and shallower torpor patterns in an experimental set-up where they were exposed to daily ambient extremes without a thermal buffer.

Methods

Study site

The study was conducted in and near Andranolovy cave (S 24.04585° / E 043.75396°, 26 m above sea-level) in the Tsimanampetsotsa National Park in south-western Madagascar. The area receives only between 300 and 600 mm of rainfall each year (Rasoloariniaina et al. 2015) and rainfall events are spatially and temporally unpredictable. The climate is characterized by a warmer rainy season (austral summer) between November and March, and a cooler dry season (austral winter) with virtually no precipitation, colder nights and lower food availability, from May to September. The park is situated on a calcareous plateau covered by dense spiny forest with various microhabitats potentially useful to bats, including underground cave and stream

Study species

Macronycteris commersoni is the largest insectivorous bat endemic to Madagascar and has a mean body mass of 46/61 g (female/male) and a mean forearm length of 79/86 mm (female/male). It roosts in large colonies in caves and individually on the peripheral branches of large trees (Raharinantenaina et al. 2008). The species is widespread across the island and occurs in a variety of habitat types, including spiny, dry deciduous, littoral and gallery forests from sea level up to 1325 m (Goodman et al. 2005; Raharinantenaina et al. 2008; Goodman 2011). The species is an ideal model organism for investigating the patterns and flexibility of thermoregulation and potential torpor use of Malagasy bats because all these habitats are subjected to drastic seasonal changes in T_a, relative humidity (RH), and resource availability. Moreover, *M. commersoni* deposits fat before the austral winter and is said to "disappear" during this time (Goodman 2006, Rakotoarivelo et al. 2007), suggesting that it either remains in its roosts and hibernates or migrates.

M. commersoni coexists with the cryptic species *M. cryptovalorona* at the southern extent of Tsimanampetsotse (Rakotoarivelo et al. 2015; Goodman et al. 2016). However, the two species were only separated recently and species determination in the *Macronycteris* complex is still being debated (Rakotoarivelo et al. 2015; Goodman et al. 2016; Foley et al. 2017). Because the species are not distinguishable by morphological characteristics and coexist in the same microhabitat (i.e. within the same cave), we could not discriminate between the two species in the field; genetic analysis revealed that less than 3 % of our study individuals were *M. cryptovolarona*. Moreover, as individual responses were highly variable, regardless of species identity (pers. obs. SR), we did not expect any physiological differences and thus analyzed all study individuals together as *Macronycteris commersoni*.

Skin and ambient temperature measurements

Bats were trapped using a hand net in 2016 (dry season) and a harp trap in 2017 (rainy season), owing to differing seasonal activity patterns. The harp trap was erected in front of the main cave entrance. Two adult bats per trapping event were transferred to individual cloth bags while juveniles and additional adults were released immediately at the point of capture. Females did not have dependent young at the time of capture.

Captive bats were sexed, weighed and the forearm length was measured. A small patch of fur was removed between the shoulder blades using a razor blade and shaving cream. The area was then dried with a sterile swab and a temperature-sensitive radio transmitter (~0.9 g, Biotrack, Wareham, UK) was attached to the skin using a medical latex adhesive (Osto-Bond, Canada) to record T_{skin}. Transmitters were less than 2.6 % of bat body mass and thus well below all recommended maxima (e.g., Aldridge and Brigham 1988). All transmitters were calibrated in a water bath at 3 - 45 °C against a thermometer traceable to the national standard. External transmitters provided a non-invasive but reliable index for T_b because the T_{skin} of small mammals does not differ significantly from their core T_b (Audet and Thomas 1996; Dausmann 2005; Langer and Fietz 2014; but see Willis and Brigham 2003). Transmitter signals were recorded every 5 min during each respirometry run (see below) using a remote receiver/logger (DataSika SRX-800-D, Biotrack, Wareham, UK). T_{skin} was then calculated using equations derived from the calibration of the transmitters ($R^2 > 0.99$). Temperature (T) and RH were recorded once per hour for 47 consecutive days in both seasons using temperature/humidity loggers (Hygrochron iButtons, Maxim integrated, San Jose, USA); one installed at 1.5 m height within the preferred roosting chamber of Andranolovy to record the cave temperature (T_{cave}) and RH_{cave} and one placed at 1.5 m height in the shade outside the cave to record environmental temperature (T_{envir}) and RH_{envir}. Andranolovy consists of several chambers, so iButtons were also installed in the two adjoining rooms and seven other caves in the park.

All studied bats were individually marked with a three-digit wing membrane tattoo using non-toxic ink (Hauptner-Herberholz, Solingen, Germany) after local anesthesia (EMLA, AstraZeneca, Wedel, Germany).

Tissue sampling

A small tissue sample was obtained from the uropatagium with a 2 mm biopsy punch for genetic analyses (stored and preserved in 95 % ethanol). Biopsy punches are commonly used for bats; healing of 3 mm punches is rapid and complete closure of the wound takes only two weeks (Faure et al. 2009; Weaver et al. 2009). Complete animal handling lasted about 10 min and never exceeded 15 min (including transmitter attachment and tattooing).

Respirometry measurements

The bats' energy expenditure was determined by measuring oxygen consumption with an open-flow respirometry system in pull mode. After processing (see above), a bat was directly transferred to a 2-l plastic metabolic chamber equipped with a net for the bat to hang on to and an iButton programmed to record T_a and RH at five-minute intervals. Chambers were connected via gas-tight tubing (Tygon, Saint-Gobain, Courbevoie, France) to portable oxygen analyzers (OxBox; T. Ruf and T. Paumann, University of Veterinary Medicine Vienna, Austria) with electrochemical fuel-cell O_2 sensors (7OX-V CiTicel, Bieler + Lang, Achern, Germany), run on standard 12 V car batteries. Sample air, dried and filtered with silica gel before entering the gas analyzer, passed through the metabolic chamber at a constant flow of 50 l/h. Oxygen content of the sample air was measured every 10 s for 55 min and data was stored on a SD memory card. To control for any drift of the oxygen sensor, reference air from outside the metabolic chamber was analyzed for 5 min every hour and used to correct animal values with Clampfit v10.3.1.4 (Molecular Devices, Sunnyvale, USA). The oxygen sensors were calibrated before and after each field trip in the laboratory using calibration gases generated by a gas mixing pump (2KM300/a; 0, 3 and 5 % N_2 in air, Wösthoff Messtechnik GmbH, Bochum, Germany).

Measurements started immediately after capture (0830 – 1230 h in the dry season and 2000 – 0000 h in the rainy season) and lasted for 18 – 126 h depending on individual bats' behavior and torpor state; very active and continuously normothermic individuals were released earlier and torpid bats were measured for longer. Bats were provided with fresh water and food (living beetles, squashed beetles and/or squashed cockroaches; their preferred prey according to Ramasindrazana et al. 2012) every 24 h after the first 24 h, after sunset at the beginning of their usual active phase. Before and after each respirometry run, bats were weighed to calculate mean body mass (BM).

Because *M. commersoni* occurs in different habitats, including regions without caves where individuals must rely on poorly buffered tree hollows, we tested the physiological flexibility of *M. commersoni* by measuring the bats' oxygen consumption in two different set-ups. In both seasons, half of the respirometry runs were conducted in Andranolovy cave in a chamber next to the bats roosting site under the usually experienced, near-stable ambient environmental conditions. The other half were conducted in a shaded enclosure outside the cave under naturally fluctuating conditions. However, all measured bats were obtained from the same colony in Andranolovy cave. To avoid unnecessary disturbance of the colony, all experiments in Andranolovy cave were performed in an adjoining cave room. Although this chamber of the cave was not the main roosting room and was slightly colder (mean temperature difference dry season = ~ 3.1 °C; ΔT_{cave} (mean temperature difference rainy season = ~ 1.1 °C), it was frequently visited by individual *M. commersoni*.

We alternated the set-up (constant vs. fluctuating environmental conditions) every 12-14 days to avoid a potential timing bias from the progressing season.

Processing and analysis

The rate of oxygen consumption ($\dot{V}O_2$) was calculated as ml O_2 h^{-1} following Lighton (2008): $\dot{V}O_2 = FR_e(F_iO_2 – F'_eO_2) / [1 – F_iO_2(1\text{-}RQ)]$ where FR_e is the excurrent flow rate and F_iO_2 - F'_eO_2 is the difference in fractional concentration of oxygen entering and leaving the respirometry chamber; assuming an average respiratory quotient (RQ) of 0.85 (oxidation of 50 % fat and 50 % carbohydrate; Dausmann et al. 2009). To calculate mass-specific MR (ml O_2 h^{-1} g^{-1}), we included mean BM, assuming a constant rate of mass loss during measurements. MR was individually variable, particularly in the measurements conducted under fluctuating ambient conditions. Consequently, it was not possible to derive a general

MR or T_{skin} threshold for torpor from frequency distributions as usually applied. Instead, we defined torpor via the amplitude of MR fluctuations because these were smaller for torpid than normothermic individuals (Figure 1). Data per minute were used for this analysis. A torpor interval of at least 20 min was chosen from each individual that entered torpor to calculate 1) mean TMR and, within the interval, 2) the distance of each measurement point from the respective mean (residuals; Figure 2.1). These residuals were calculated for each individual and used as a threshold to distinguish between TMR and normothermic MR. We then calculated the distances between each point for the whole measurement run and a stepwise 10-minute moving average, which were finally compared to the threshold residuals to identify torpor phases (Figure 2.1). To classify torpor bout duration, we followed Kobbe et al. (2011): bouts < 24 h = daily torpor; bouts between 1-3 days = prolonged torpor and bouts > 3 days = hibernation.

Data were combined and processed using Cran R (R Core Team 2016) and the packages “RStudio” (RStudio Team 2016), “plyr” (Wickham 2011), “readxl” (Wickham and Bryan 2017), “lubridate” (Grolemund and Wickham 2011) and “zoo” (Zeileis and Grothendieck 2005). IBM SPSS 24 was used for further analysis: Daily MR rhythm was analyzed with paired Wilcoxon signed rank tests using sunset and sunrise as limit for daytime.TMR and T_{skin} data were compared between seasons using unpaired samples t-tests. Normal data distribution was checked using Shapiro-Wilk tests prior to analyses.

Data are presented either as mean ± standard deviation or, when N < 6, as median and range; N represents the number of individuals, n the number of included data points.

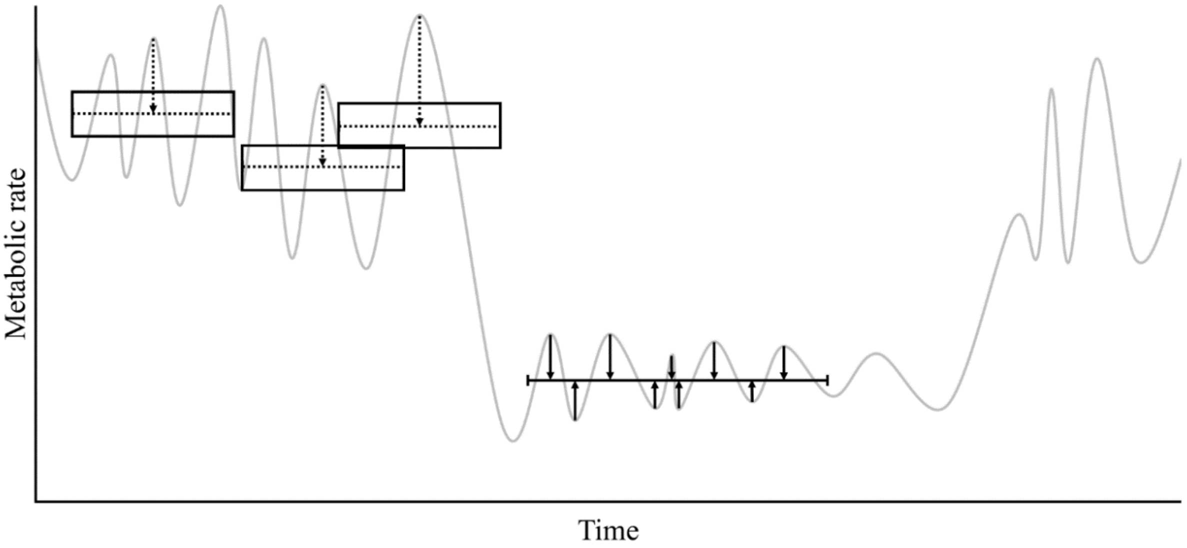

Figure 2.1. Torpor was defined based on metabolic rate (MR, grey line) fluctuations. Mean torpid MR (TMR, black horizontal line), and the distance of each measurement point from the respective mean, were calculated within a definite torpor interval of at least 20 min (residuals, black arrows). This typical fluctuation in MR during torpor was used as the threshold to distinguish between TMR and normothermic MR. The distances between each measurement point and 10-minute moving MR averages (dotted arrows and line, respectively) were compared to the threshold residuals (black boxes) in a stepwise approach over the course of the whole measurement to identify torpor phases.

Results

In total, we recorded oxygen consumption and T_{skin} of 49 bats; 20 bats were measured in the dry season (10 under stable cave conditions [6 females, 4 males], 10 under fluctuating environmental conditions [3 females, 7 males]) and 29 bats in the rainy season (15 stable [9 females, 6 males], 14 fluctuating [5 females, 9 males]).

Metabolic rate and skin temperature patterns: stable conditions

Andranolovy cave was the hottest and most thermally stable cave in the area: T_{cave} at 1.5 m height in the main roosting chamber was 29.4 ± 0.0 °C in the dry season and 30.3 ± 0.6 °C in the rainy season (Figure 2.2). The floor of the roosting chamber was completely flooded year-round ensuring a constantly high RH of 98.36 ± 1.03 % in the dry season and 95.18 ± 0.40 % in the rainy season. Bats roosted at 4-6 m height at 32.1 °C ± 0.3 °C. In the chamber where MR measurements were conducted, T_{cave} at 1.5 m was 26.3 ± 0.6 °C in the dry season and 30.3 ± 1.1 °C in the rainy season with a RH_{cave} of 65.1 ± 5.3 % and 74.8 ± 4.3 %, respectively.

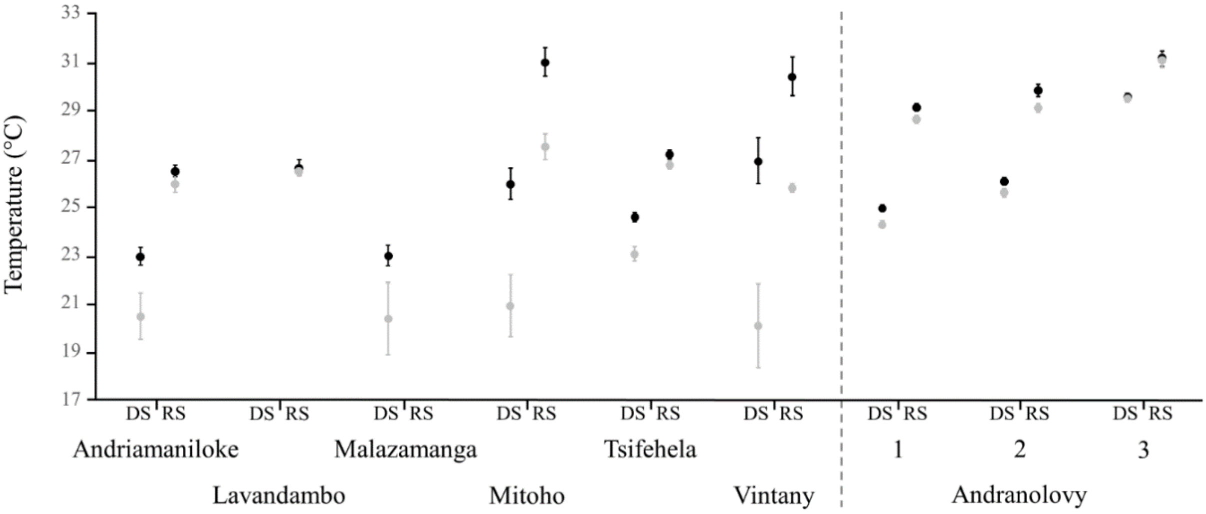

Figure 2.2. Mean daily maximum (black dots) and mean daily minimum (grey dots) temperature in all three chambers of Andranolovy cave and six other caves in Tsimanampetsotsa National Park in the dry (DS) and rainy season (RS). Data was obtained from the coldest and hottest month (July and January, respectively) to highlight the temperature range within alternative roosting spots. The bats preferred roosting room was chamber 3; measurements were conducted in chamber 2. For Malazamanga cave only dry season data was available, for Lavandambo cave only rainy season data owing to logger failure.

Inside the cave, i.e. in the natural, preferred roosting site of this population, 70 % of all captured bats (N = 10) entered torpor in the dry season and 33 % in the rainy season (N = 15), while the rest remained normothermic. In the dry season, they entered torpor either in the morning after their usual active phase (between 0700 and 0900 h; N = 3, n = 9), at night (between 2300 and 0300 h; N = 3, n = 5), or immediately after the beginning of the respirometry measurement (1130 h; N = 1, n = 1). In the rainy season all bats entered torpor in the morning (between 0900 and 1100 h; N = 5, n = 6). Torpid individuals showed extreme flexibility in their thermoregulatory patterns. They entered "classical" daily torpor or hibernation, but also expressed torpor bouts of varying and intermediate lengths, such as short bouts (16 minutes, range: 8 - 51 min, N = 3, n = 45) with regular MR peaks (Figure 2.3), often uncoupled from their daily rhythm in MR. Apart from these short torpor bouts, the alternation between torpid and normothermic MR was extremely fast: torpor entry took 4.1 ± 1.4 minutes (N = 3, n = 45) and arousal took 12.3 ± 3.3 minutes (N = 3, n = 45). Some individuals were inconsistent in their torpor expression and remained normothermic for several days before entering torpor (Figure 2.3), or vice versa, remained normothermic during daytime after, for example, daily torpor bouts.

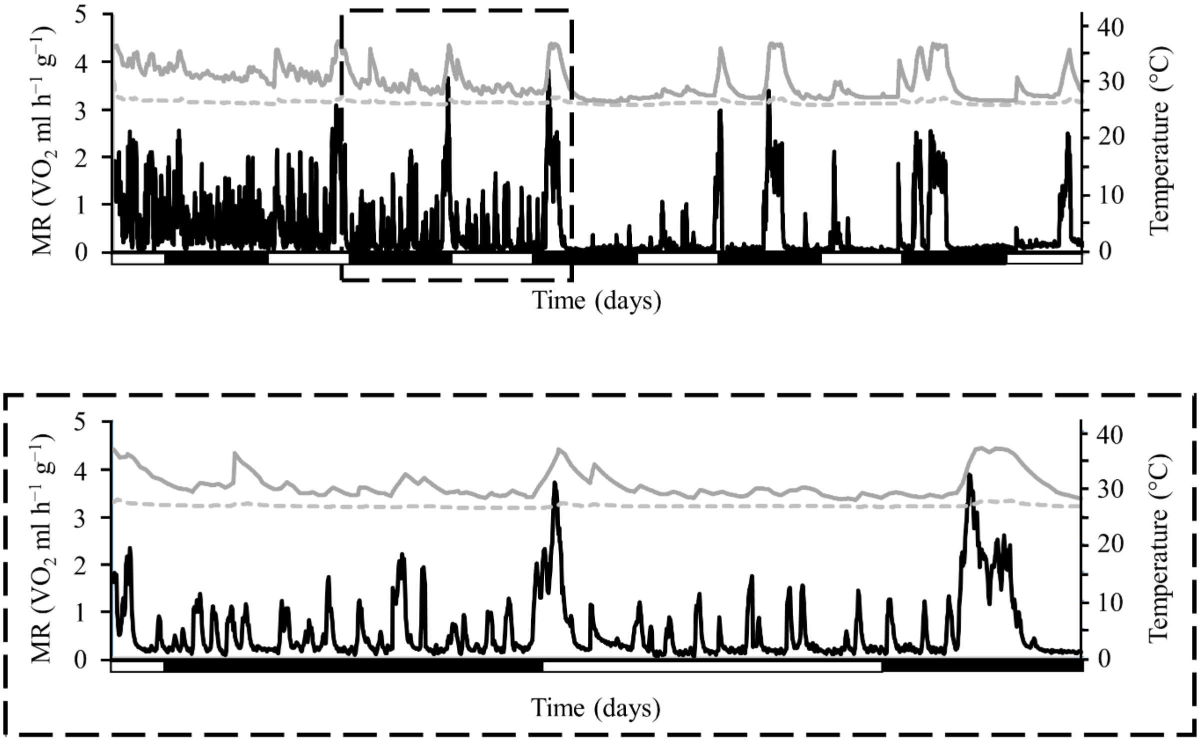

Figure 2.3. Skin temperature (°C, grey line), metabolic rate (MR, ml O_2 h^{-1} g^{-1}, black line) and ambient temperature (°C, dashed grey line) of a male *M. commersoni* over a period of five days during a cave measurement in the dry season. Enlarged section (black dashed box) illustrates short torpor bouts interrupted by MR peaks before this individual entered a longer torpor bout. Black horizontal bars below the x-axis indicate dark phases.

Neither T_{skin} nor TMR differed between seasons (T_{skin}: dry season = 30.5, 27.8 – 34.4 °C, N = 5, rainy season = 31.8 ± 0.8 °C, N = 7, t_{10} = -0.962, p = 0.359; TMR: dry season = 0.165 ± 0.090 ml O_2 g^{-1} h^{-1}, N = 7; rainy season = 0.280, 0.093 – 0.470 ml O_2 g^{-1} h^{-1}, N = 5; t_{10} = -1.802, p = 0.102). In the dry season, the highest torpid T_{skin} measured was 35.3 °C with a TMR of 0.034 ± 0.033 ml O_2 $h^{-1}g^{-1}$. The lowest T_{skin} was 27.2 °C with a TMR of 0.034 ± 0.025 ml O_2 $h^{-1}g^{-1}$. In the rainy season, highest recorded T_{skin} was

34.7 °C with a TMR of 0.086 ± 0.061 ml O_2 h^{-1} g^{-1} while the lowest was 30.6 °C with a TMR of 0.095 ± 0.031 ml O_2 h^{-1} g^{-1}.

Excluding the torpor phases, individuals showed distinct rhythms in MR in both seasons (Figure 2.4); MR was higher at night (1801 - 0600 h; 1.678 ± 0.839 ml O_2 h^{-1} g^{-1}, N = 18) and lower during the day (0601-1800 h; 1.030 ± 0.407 ml O_2 h^{-1} g^{-1}, N = 18; $Z = -3.636$, $p < 0.001$).

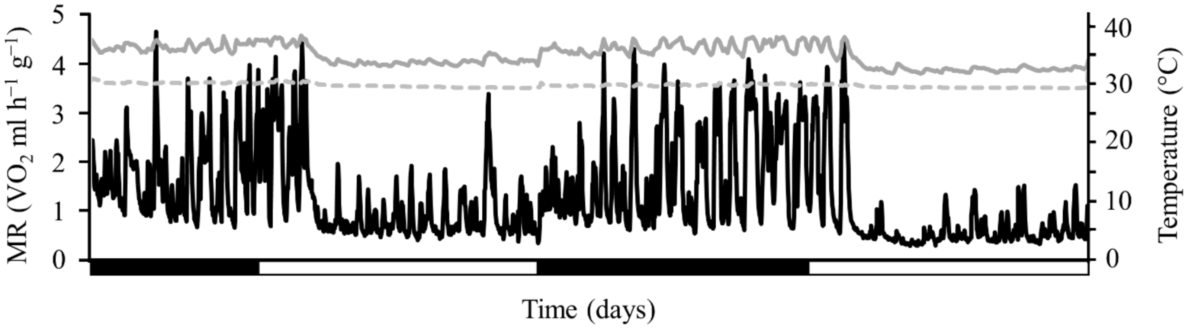

Figure 2.4. Skin temperature (T_{skin}, °C, grey line) and metabolic rate (MR, ml O_2 h^{-1} g^{-1}, black line) of a male *M. commersoni* over a period of two days during a cave measurement in the rainy season with ambient temperature (°C, dashed grey line). This individual did not enter torpor but showed a clear rhythm with elevated metabolic rate and T_{skin} at night (the bats' usual active phase) and lower MR and T_{skin} during the day (resting phase). Black horizontal bars below the x-axis indicate dark phases.

Metabolic rate and skin temperature patterns: fluctuating conditions

Outside the cave, i.e. in the fluctuating environment, temperature and humidity changed daily and seasonally. In the dry season, T_{envir} fluctuated on average 15.7 ± 2.8 °C (mean minimum T_{envir} – mean maximum T_{envir}) and RH 53.9 ± 7.7 % (mean minimum RH_{envir} – mean maximum RH_{envir}) each day (15.2 ± 2.5 °C – 30.9 ± 2.2 °C; 33.9 ± 8.1 % – 87.8 ± 7.1 %). In the rainy season, temperatures were generally elevated but daily fluctuations were comparable to those in the dry season (14.3 ± 2.1 °C; 24.3 ± 1.6 °C – 38.7 ± 2.8 °C). RH_{envir} fluctuated on average 49.3 ± 7.1 % (36.4 ± 7.3 % – 85.7 ± 7.2 %). Compared to the cave conditions, mean daily T_{envir} maxima were higher in both seasons, particularly in the rainy season (Δ maximum T_{envir} dry vs. rainy season: 2.2 ± 1.5 °C vs. 8.8 ± 2.8 °C). Mean daily T_{envir} minima were lower than those of T_{cave}, particularly in the dry season (daily temperature minima difference: dry season = 14.2 ± 2.5 °C; rainy season = 5.5 ± 1.5 °C). Mean daily RH was always higher inside the cave compared to outside but differences in both daily mean maximum and minimum were smaller in the rainy season than in the dry season, implying that outside the cave it was slightly more humid in the rainy season (daily RH maxima difference: dry season = 11.5 ± 6.9 %; rainy season = 10.6 ± 6.7 %; daily RH maxima difference: dry season = 64.21 ± 7.9 %; rainy season = 58.0 ± 7.2 %).

All individuals measured under fluctuating, and thus not their naturally accustomed conditions, entered torpor every day and none remained normothermic, independent of season. Bats entered daily torpor and prolonged torpor, and also showed patterns of very short torpor bouts (Figure

2.5) that did not strictly follow the standard classifications and should be included into definitions for torpor. Most individuals remained normothermic during their usual active phase at night and entered torpor during the day. However, torpor patterns varied seasonally and individually with more prolonged torpor bouts over two days in duration occurring in the dry season and shorter daily and "hot" bouts (i.e. with unexpectedly high T_{skin}; Figure 2.6c) in the rainy season.

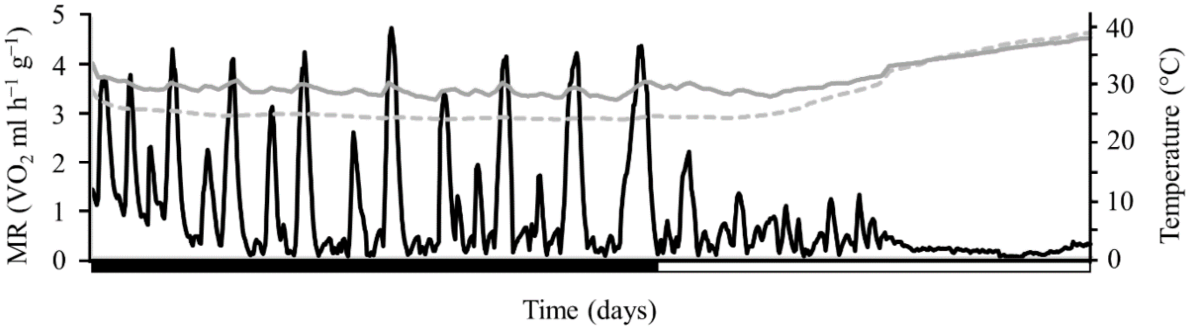

Figure 2.5. Skin temperature (°C, grey line) and metabolic rate (MR, ml O_2 h^{-1} g^{-1}, black line) of a male *M. commersoni* over a period of 13 hours during a measurement under fluctuating conditions in the rainy season, with ambient temperature (°C, dashed grey line). This individual showed short, shallower torpor bouts (duration 5 – 30 min) interrupted by MR peaks before entering a daily torpor bout of 8 h in the morning, where T_{skin} thermoconformed with T_{envir}. Black horizontal bars below the x-axis indicate dark phases.

Patterns of entry into torpor differed between seasons. In the dry season, one bat entered torpor immediately after the beginning of the measurement and remained torpid until shortly before it was released again. The other bats tried to rewarm against decreasing T_a in the afternoon. While 44 % entered torpor in the night/early morning as T_a decreased (between 2300 and 0400 h; N = 4, n = 8), 56 % did not enter torpor before morning when T_a started to increase (between 0900 and 1100 h; N = 6, n = 10; Figure 2.6a). However, most of the individuals (N = 7) that attempted to increase MR and T_{skin} against decreasing T_a were not successful over several hours and T_{skin} decreased constantly despite the bats increasing MR (Figure 6a, b). In the rainy season, all bats entered torpor in the early morning when T_a started to rapidly increase, usually between 0800 and 1000 h (N = 14, n = 17; Figure 2.6c), and some bats expressed several short torpor bouts with normothermic peaks before entering longer torpor bouts in the afternoon.

Corresponding to T_{envir}, T_{skin} during torpor differed significantly by almost 10 °C between the seasons (dry season: 28.3 ± 6.3 °C, N = 10; rainy season: 37.2 ± 2.7 °C, N = 14; $Z = -3.806$, $p < 0.001$) and TMR in the dry season (0.115 ± 0.054 ml O_2 h^{-1} g^{-1}) was significantly lower than TMR in the rainy season (0.409 ± 0.264 ml O_2 h^{-1} g^{-1}; $t_{-14.505} = -4.052$, $p = 0.001$). In the dry season, T_{envir} dropped lower than in their natural roost in the cave, particularly at night and during the early morning hours. The highest torpid T_{skin} recorded in the dry season was 38.9 °C with a TMR for this period of 0.104 ± 0.020 ml O_2 h^{-1} g^{-1} and the lowest T_{skin} was 7.8 °C with a corresponding TMR of 0.084 ± 0.013 ml O_2 h^{-1} g^{-1}. In the rainy season, T_{envir} was generally elevated and bats outside the cave were exposed to higher T_a than they faced within their natural roost. Under these conditions, bats entered hot torpor with comparably high T_{skin} and TMR. The highest torpid T_{skin} was 41.5 °C with a TMR of 0.113 ± 0.006 ml O_2 h^{-1} g^{-1}. The lowest T_{skin} recorded during torpor was 22.7 °C with a TMR of 0.084 ± 0.010 ml O_2 h^{-1} g^{-1}.

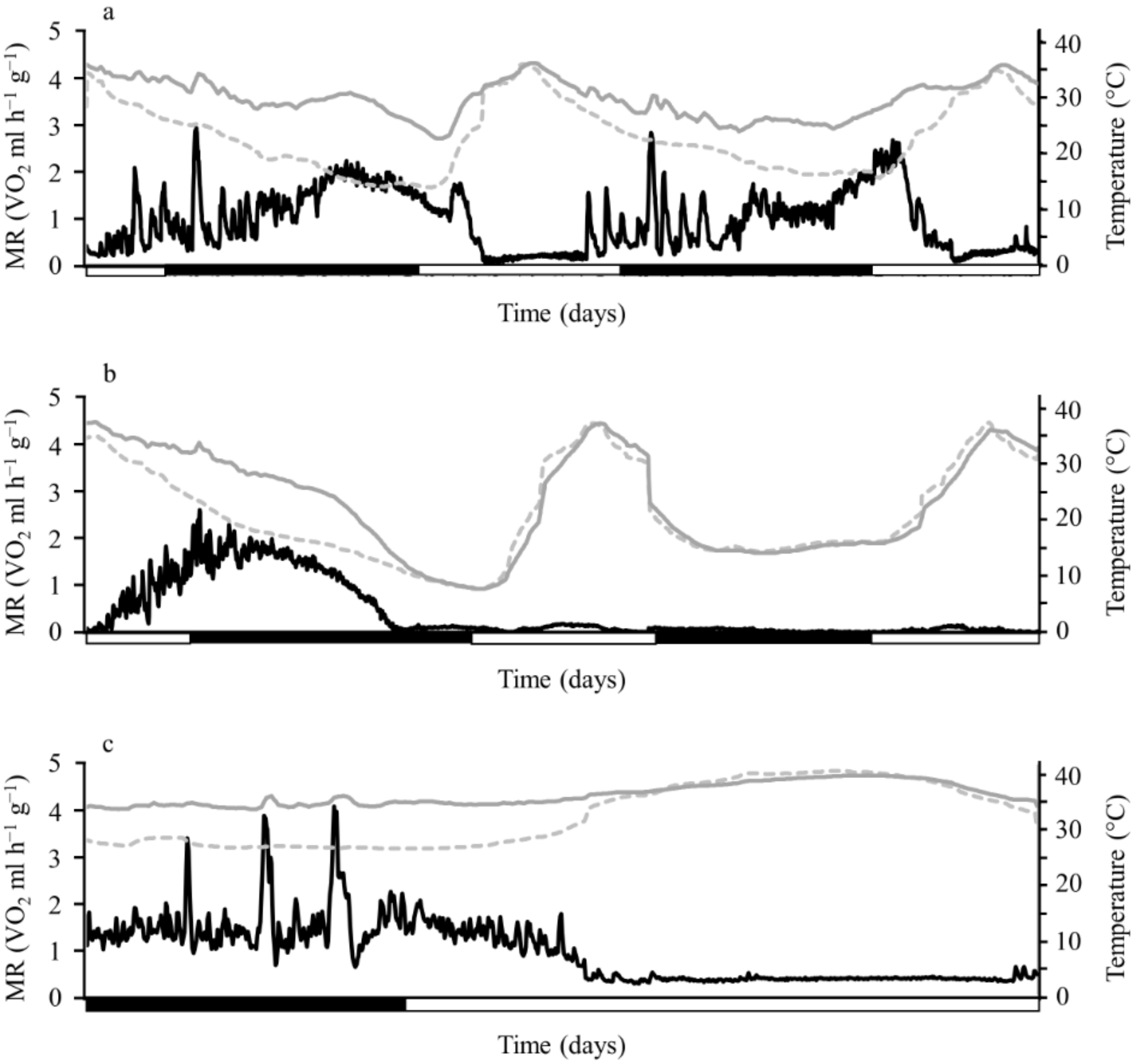

Figure 2.6. Skin temperature (T_{skin}, °C, grey line) and metabolic rate (MR, ml O_2 h^{-1} g^{-1}, black line) of female (b) and male (a, c) *M. commersoni* over a period of one (c) to two (a, b) days during measurement in the fluctuating environment in the dry (a, b) and rainy season (c), with ambient temperature (T_a, °C, dashed grey line). We show different heterothermic strategies including daily torpor (a, c) and prolonged torpor (b) to illustrate differing torpor entries, lengths and depths that this species is physiologically capable of expressing. Individual (a) entered daily torpor when T_a started increasing in the morning and allowed T_{skin} to thermoconform. Individual (b) entered prolonged torpor at night/early morning after several hours of attempting to maintain a high and stable T_{skin}. For both individuals (a) and (b), T_{skin} decreased despite increasing MR over a period of hours. Individual (a) and (c) entered hyperthermic daily torpor with a relatively high torpid MR and T_{skin}. Black horizontal bars below the x-axis indicate dark phases.

Discussion

Our study demonstrates for the first time that torpor occurs in Malagasy bats. We provide the first ecological and physiological data from *M. commersoni* and show that 73 % of individuals entered torpor during our measurements. However, compared to heterothermic Malagasy lemur or tenrec species, or even temperate bat species, torpor expression was highly flexible among individuals, revealing hitherto unknown torpor patterns.

In addition to daily torpor, prolonged torpor and hibernation, bats entered very short torpor bouts lasting only about 20 minutes. They also used "hotter and shallower" torpor bouts with relatively high TMR and T_{skin}. In the cave, their natural resting site, less than 50 % of the bats entered torpor while the rest remained normothermic. Under fluctuating conditions, however, bats seemed to unsuccessfully attempt to maintain a high T_{skin} for up to several hours, while T_{envir} slowly decreased. In the dry season in particular we observed several instances of bats with decreasing T_{skin} despite increasing MR (Figure 6a, b). Considering the small size of the bats, with their relatively high thermal conductance and low thermal inertia, we expected a clear drop in T_{skin} to from normothermia to torpid temperatures slightly above T_a, similar to *M. griseorufus* (Kobbe et al. 2014). Because their natural resting site offers highly stable and most likely thermoneutral conditions year-round, this population is probably never exposed to fluctuating conditions while roosting. Additionally, bats generally generate enough heat during flight to maintain normothermic T_b outside the cave. Together, this suggests that *M. commersoni* rarely needs to actively thermoregulate during the rest phase and consequently the slowly decreasing T_{skin} with increasing MR indicates that active thermoregulation is challenging for this species.

Unexpectedly, many individuals remained normothermic or only entered relatively short torpor bouts during the measurements made in their preferred cave roost. No bats were trapped outside the cave during the 10 weeks of our study and rarely during other studies in the austral winter (Razakarivony et al. 2005, Goodman 2006, Kofoky et al. 2007, Rakotoarivelo et al. 2007). Churchill et al. (1997) and Brosset (1962) made similar observations in the closely related African *Macronycteris vittatus* (formerly *Hipposideros commersoni s. l.*; Foley et al. 2017) and Indian *H. lankadica*. For several consecutive days, these species were inactive without signs of torpor or the restless behavior typically seen in bats preparing to leave the cave, and both authors assumed that these bats may even overwinter by relying only on large fat deposits and not hypometabolism. In *M. commersoni*, half of the measured individuals also appeared to rely on inactive phases without a pronounced reduction in MR, despite possessing the ability to enter torpor. The other half entered torpor in various durations, depths and patterns, so torpor use in this species seems to be related to body mass or condition (Kelm and Helversen 2007; Kobbe et al. 2014). Although related to bout duration and depth, and thus highly variable in this population, energy savings from torpor can be substantial in the tropics. Fat-tailed dwarf lemurs (*Cheirogaleus medius*), for example, hibernate for several months and save about 70 % of the energy compared to normothermia (Dausmann et al. 2009), and the reddish-gray mouse lemur (*M. griseorufus*) saves up to 21 % by entering daily torpor with bouts only lasting a few hours (Kobbe et al. 2014). Additionally, O'Mara et al. (2017) have recently shown that tropical frugivorous bats can reduce their daily energy expenditure by 10 % by lowering their heart rate for only 5–7 min per day. The short torpor bouts measured in our study resemble a similar short-term strategy and likely reflect a trade-off between the benefits and costs of hypometabolism. Reduced responsiveness to the environment, diminished immune function and memory loss can occur during torpor (Millesi et al. 2001; Humphries et al. 2003; Bouma et al. 2010), making it an unfavorable response when energy conservation is not vital.

Our population of *M. commersoni* chose the hottest and most humid cave in the region for roosting and overwintering even though there were colder caves available that 1) are frequently used by other bat species and 2) would possibly allow higher energy savings during torpor. These colder caves, however, are less buffered and greater fluctuations in T_a and RH occur (Figure 2). In Andranolovy, T_{cave} (30 °C at 1.5 m height) most likely lies within the bats' thermoneutral zone and thus no additional energy is needed to maintain normothermic T_b. The cave offers a consistently high RH that never dropped below 94.8 % and a stable water source year-round. *Hipposideros caffer*, a close relative of *M. commersoni*, used the warmest and most humid location available inside another cave (25.8 ± 1.0 °C, 93.9 ± 6.9 % RH) and only roosted in caves that were less humid (40 % RH) when permanent waterholes were in close proximity (Churchill et al. 1997). This seems to be the strategy also used by *M. commersoni*. Our studied population was most likely never water-stressed: it managed to find the most thermally stable and humid roost available within the driest and least predictable region of Madagascar. A stable, high RH minimizes the deficit in water vapor saturation between air and individual, thereby limiting evaporative water loss. Consequently, the thermoneutral and humid cave conditions enabled the bats to conserve considerable amounts of both energy and water compared to individuals roosting outside in the fluctuating environment.

All bats entered torpor when exposed to fluctuating conditions, even though they were acclimatized to their stable cave roost, indicating physiological flexibility. This clear response suggests that fluctuating conditions are unfavorable and demand such an emergency response to sudden environmental change. Fluctuating environments with high T_a peaks and limited water availability are generally challenging for small endotherms, particularly bats. Their large wing membranes expedite cutaneous water loss (Hatting 1972) and evaporative cooling results in high pulmonary water loss, especially when T_a exceeds T_b (Heldmaier et al. 2013). Temperate bats combat thermal constraints by choosing buffered roosts (Speakman and Thomas 2003). They are rarely exposed to daytime extremes approaching normothermic T_b and at low T_a they cluster to trap body heat (Roverud and Chappell

1991). In the tropics, however, tree-roosting bats appear to select roosts primarily to avoid overheating (Speakman and Thomas 2003) and respond to fluctuating conditions via either local migration to regions with higher food and water availability (Vaughan 1977; McWilliam 1982), or by hibernating (Geiser and Stawski 2011; Liu and Karasov 2011). Water savings during torpor can be substantial, even at high T_a. The Australian bat *Chalinolobus gouldii,* for example, can reduce evaporative water loss by more than 86 % by entering torpor at T_a of up to 25 °C (Hosken and Withers 1997) and the Angolan free-tailed bat *M. condylurus* reduced evaporation by up to 46 % at T_a = 45 °C (Maloney et al. 1999). The hyperthermic torpor we found in *M. commersoni* in fluctuating conditions most likely saves a considerable amount of water. While MR and T_b are not directly affected by varying RH, evaporative water loss increases with decreasing RH (Ben-Hamo et al. 2013). Bats are often reported to have an impressive capacity for tolerating high T_a (e.g. Licht and Leitner 1967, Bronrier et al. 1999, Maloney et al. 1999, Toussaint and McKechnie 2012, Bondarenco et al. 2014) and, indeed, our bats become hypometabolic when T_{envir} increased and their T_{skin} sometimes even exceeded normothermic T_{skin} at noon. Lovegrove et al. (2014) described such a state as heat-induced hypometabolism ("hyperthermic daily torpor"), during which animals could reduce the high energetic costs of hyperthermia by abandoning MR regulation. We measured remarkably low bat TMR (0.13 ml O_2 h^{-1} g^{-1}), even during a T_{envir} peak where T_{skin} remained above 41 °C for 53 min. Similar TMRs were measured, for example, in hibernating bats *Rhinopoma microphyllum* and *R. cystops* (0.14 and 0.16 ml O_2 h^{-1} g^{-1}, respectively) and the reddish-gray mouse lemur (0.15 ml O_2 h^{-1} g^{-1}) at T_a, and thus T_b, 21 °C cooler (Kobbe et al. 2014, Levin et al. 2015). Thus, it appears that *M. commersoni* is able to down-regulate MR during hyperthermia to a TMR comparable to those of hypothermic individuals of other species.

Our study has expanded current knowledge on the use of torpor under natural conditions. The ecological factors driving torpor entry, duration and depth in *M. commersoni*, and the physiological mechanisms facilitating the fast alternation between high and low MR, remain unclear. However, we found that *M. commersoni* has a quiver of torpor responses that can be employed flexibly in response to prevailing ambient conditions. We acknowledge that this population inhabits a single cave that may not represent the average roosting environment of *M. commersoni* living elsewhere on the island. The cave is characterized by a stable and likely thermoneutral T_a, with a high RH and reliable water source that would prevent this population from becoming water-stressed. We suggest that these exact conditions, and the species' ability to enter torpor despite high T_a and T_{skin}, are key requirements for the species to successfully survive and overwinter in this extreme environment. Although the flexibility of tropical heterotherms, particularly bats, has been hypothesized for decades, the breadth of variation in torpor patterns that this species displays is remarkable and, to our knowledge, new for endotherms. The classical dichotomous approach of classifying hypometabolism into daily torpor and hibernation does not hold for *M. commersoni* and, for this species, torpor is uncoupled from low T_a

and T_b. Thus, to better understand the underlying mechanisms enabling such a scope of hypometabolic states, appreciate torpor as a true continuum, and identify physiological states masked by high T_b, research into the intermediate torpid states of tropical heterotherms is vital.

Acknowledgments

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Author contribution

I hereby confirm that Stephanie Reher (SR) and Kathrin H. Dausmann (KHD) conceived and designed the study, SR conducted the measurements in the field with support of Hajatiana Rabarison, SR analysed the data, did the genetic analysis with substantial support of Julian Ehlers and took the lead in writing the manuscript with input from KHD. SR and KHD discussed and interpreted the results; all authors revised the manuscript and approved the final version of it.

Prof. Dr. Kathrin Dausmann

Chapter 3

Tropical bats counter heat by combining torpor with adaptive hyperthermia

Stephanie Reher and Kathrin H. Dausmann

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Abstract

Many tropical mammals are vulnerable to heat because their water budget limits the use of evaporative cooling for heat compensation. Further increasing temperatures and aridity might consequently exceed their thermoregulatory capacities. Here, we describe two novel modes of torpor, a response usually associated with cold or resource bottlenecks, as efficient mechanisms to counter heat. We conducted a field study on the Malagasy bat *Macronycteris commersoni* resting in foliage during the hot season, unprotected from environmental extremes. On warm days, the bats alternated between remarkably short micro-torpor-bouts and normal resting metabolism within few minutes. On hot days, the bats extended their torpor bouts over the hottest time of the day while tolerating body temperatures up to 42.9°C. Adaptive hyperthermia combined with lowered metabolic heat production from torpor allows higher heat storage from the environment, negates the need for evaporative cooling and thus increases heat tolerance. However, it is a high-risk response as the torpid bats cannot defend body temperature if ambient temperature increases above a critical/lethal threshold. Torpor coupled with hyperthermia and micro-torpor-bouts broaden our understanding of the basic principles of thermal physiology and demonstrate how mammals can perform near their upper thermal limits in an increasingly warmer world.

Keywords

Heat tolerance, heterothermy, hyperthermia, thermal limits, bats, tropics

Background

The safety margin between euthermia and lethally high body temperatures in mammals is very narrow and even minor changes can be life threatening, particularly in the tropics. Mammals obtain their body heat mainly from metabolic activities and usually regulate their body temperature within a set range of a few degrees [1,2]. They reach their thermal limits when body temperature approaches 41-44°C due to overheating or dehydration [3,4]. The maximum temperatures experienced by mammals are increasing because of extensive habitat modification [5] or more frequent and more intense heatwaves associated with global climate change [6], and these maxima can be fatal [7–11]. Indeed, heatwaves have led to several recent mass mortalities of flying foxes (*Pteropus* spp) in Australia [10,12] with ambient temperatures (T_a) of 42°C or higher representing a critical threshold [8].

Efficient thermoregulation is key for survival, but there are few options for down-regulating body temperature near the upper limit. Radiation, convection and conduction are passive mechanisms of heat loss only used when T_a is lower than the desired body temperature [13]. When T_a exceeds body temperature, the only options are evaporative cooling or tolerating short-term hyperthermia [14]. In warm and dry environments with unpredictable or limited water availability, evaporation might be constrained by the risk of dehydration. By accumulating heat instead of dumping it and thereby tolerating an increase in body temperature, the need for evaporative cooling can be postponed or even avoided, and considerable amounts of water can be conserved [15,16]. The classic example of this adaptive hyperthermia comes from dromedary camels (*Camelus dromedarius*), which regularly cycle between 41°C in daytime and 34-35°C at night when dehydrated [17], but other mammals also allow hyperthermia during hot phases (llamas [18], elephants [19], large treeshrews [20], ringtail possums [11], bats [7,16,21]).

The most dramatic thermoregulatory response of mammals is the controlled down-regulation of virtually all metabolic processes during torpor (hypometabolism) [22,23]. This is widely believed to be an adaptive response of endotherms to cold stress or food limitation during seasonal periods of scarcity. However, the last two decades have shown that torpor is also common in the tropics [24] and could negate some effects of heat [25]. A reduction of metabolic rate (MR) is accompanied by reduced water consumption through respiration, defecation, urine formation, and metabolic heat dissipation [26–28], which could permit torpid animals to tolerate greater heat loads than euthermic ones. Nonetheless, how mammals perform and thermoregulate at high temperatures remains poorly understood [14,25,29–31], yet is essential to predict their responses to rising temperatures. Global warming confronts many small mammals more regularly with fatal mismatches between environmental conditions and their physiological limitations. To this end, we studied how a small tropical bat withstands heat in its natural environment and found that novel modes of torpor were a critical part of their response.

Short methods

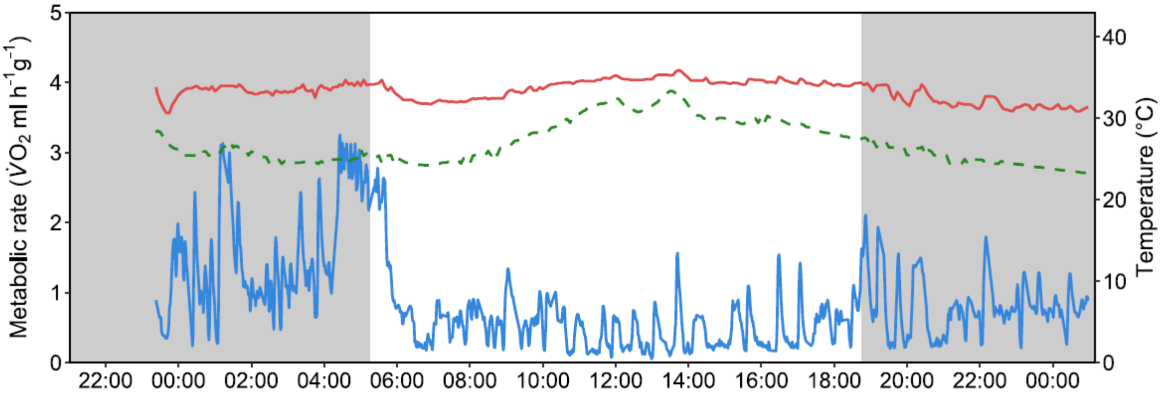
We worked with the insectivorous bat *Macronycteris commersoni* (mean body mass: females = 46.3 ± 6.8g; males = 79.6 ± 8.0g) in a tropical dry forest in western Madagascar (Kirindy Forest/CNFEREF; S 20.06714°/E 44.65745°, 40m) during the hottest season of the year. The bats rest alone in vegetation during the day exposed to temperature extremes and cannot necessarily expend the water required to cool their bodies below ambient temperatures. On sunny days, maximum T_a can reach over 41°C in Kirindy Forest/CNFEREF whereas relative humidity is comparatively low at 44.2 ± 6.6% (29.0 – 53.6%). We monitored the physiological responses to regular daytime heat (73% sunny days) by measuring mass-specific MR as rate of oxygen consumption ($\dot{V}O_2$ ml h^{-1} g^{-1}) and skin temperature (T_{skin}) in 16 adult bats in their natural environment (9 females, 7 males; see supplements for a detailed description of procedures). Our methods complied with the current ethical regulations and laws of Madagascar and were approved by the Directeur du Système des Aires Protégées, Ministère de l'Environnement, de l'Ecologie et des Forêts, Antananarivo (permit no. 296/17/MEEF/SG/DGF/DSAP/SCB.Re).

Two novel modes of torpor

Unexpectedly, all bats entered torpor on all days and we observed two unique, novel modes of torpor, regardless of sex. On warm, rainy or cloudy, days (mean max T_a = 33.9 ± 1.98°C), most bats (67%) alternated between short micro-torpor bouts lasting between 3 and 53 min (mean = 12.4 ± 10.5 min; n = 462 bouts) and resting MR (figure 3.1A, S1A). This pattern occurred throughout their resting phase from sunrise at ~05:30 to sunset at ~18:30 hours before nightly activity. Interestingly, micro-torpor did not lead to notable changes in T_{skin} compared to euthermia (figure 3.1A, S1A). The pattern of recurring micro-torpor bouts is likely a fine balance: Individuals can save energy [32] and water [27] on the one hand, but simultaneously are still alert and responsive to any threats given the exposed roosting conditions.

On hotter, days (mean max T_a=37.8 ± 1.85°C), the bats used micro-torpor bouts only during the cooler, early morning hours (figure 3.1B, S1B). However, when T_a exceeded normal body temperature, most bats (94%) stopped switching between micro-torpor and resting MR and extended their torpor bouts. This usually led to a significant increase in T_{skin} because active regulation of body temperature was suspended and thus T_{skin} approximated T_a. This pattern is contrary to the traditional view of torpor as cold response and we refer to it as "hot torpor". Therewith we want to stress that the physiological underpinnings seem to be the same as in arctic species entering torpor, only the environmental conditions lead to different patterns.

A: Micro-torpor bouts on warm days



B: Extended torpor bouts on hot days

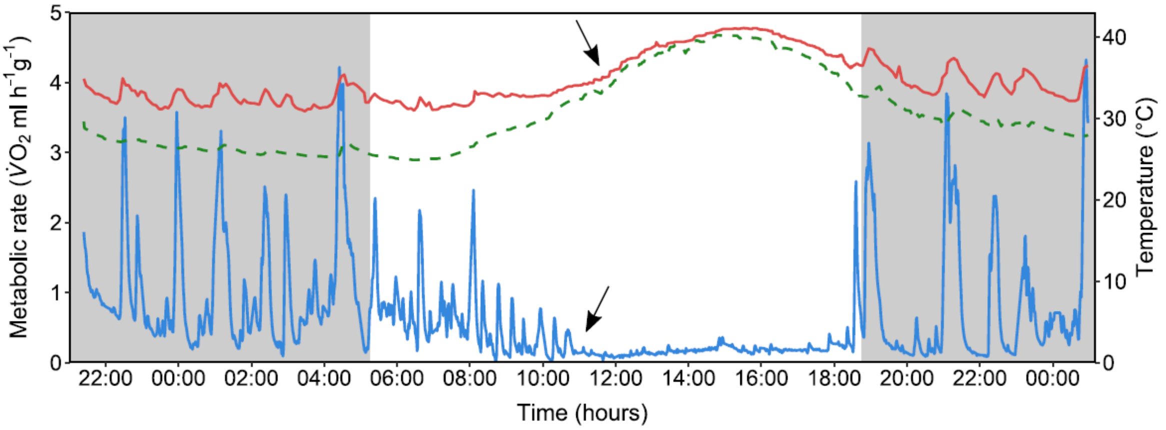


Figure 3.1. Two new modes of torpor in a tropical bat species. Metabolic rate as $\dot{V}O_2$ (MR, ml h^{-1} g^{-1}; blue) and skin temperature (T_{skin}, °C; red) of two male individuals of the bat *Macronycteris commersoni* and the bats' ambient temperature (T_a, °C; yellow). Grey shaded blocks indicate the dark phase. Panel A shows the typical response on regular warm (rainy or cloudy) days. Under these conditions, bats alternated between regular resting MR and micro-torpor bouts (12.4 ± 10.5min) over the course of their usual inactive phase (~ 05:30 – 18:30 hours). Panel B shows the response of bats on hot days. When T_a exceeded euthermic body temperature, the bats extended their torpor bouts, which lasted until the beginning of their active phase in the late afternoon when T_a had decreased again (293.6 ± 101.2min). The arrows highlight the decline in MR and almost simultaneous increase in T_{skin}, which passively followed T_a, during extended torpor up to 42.9°C indicating that bats tolerated hyperthermia while torpid (see supplementary figure S1 for two examples of female bats showing the same patterns).

Individuals were torpid over the hottest time of the day and aroused at the beginning of their active phase when T_a and T_{skin} had decreased again to 33.5 ± 2.3°C and 36.0 ± 1.8°C, respectively. The extended torpor bouts lasted between 78 and 436 min (mean = 293.6 ± 101.2min; n = 27 bouts) and T_{skin} passively increased with T_a up to a maximum value of 42.9°C. Thus, the bats applied a well-known response to cold conditions, i.e. entering torpor, while tolerating hyperthermia (figure 3.1B, S1B). In contrast to adaptive hyperthermia used by e.g. camels, the bats actively depressed metabolism and bodily functions to a minimum during hottest body temperatures. This allows for negligible internal heat production and thus substantial water savings, making hot torpor an efficient option to withstand tropical heat. When small mammals are euthermic and confronted with T_a near the upper critical temperature, excess heat generated by metabolism or activity and the absorption of heat from the environment can quickly exceed their thermoregulatory capacities. Other tropical bats enter extended torpor, but only in the cooler morning hours and always accompanied by a reduction in body temperature, probably to save water in anticipation of responding to afternoon heat [33,34]. In contrast, *M. commersoni* entered hot torpor only under heat stress and maintained micro-bouts during cooler daytimes. This flexible response to high T_a might allow *M. commersoni* to thrive in the dry tropics in areas where buffered diurnal roosts, such as caves, are absent.

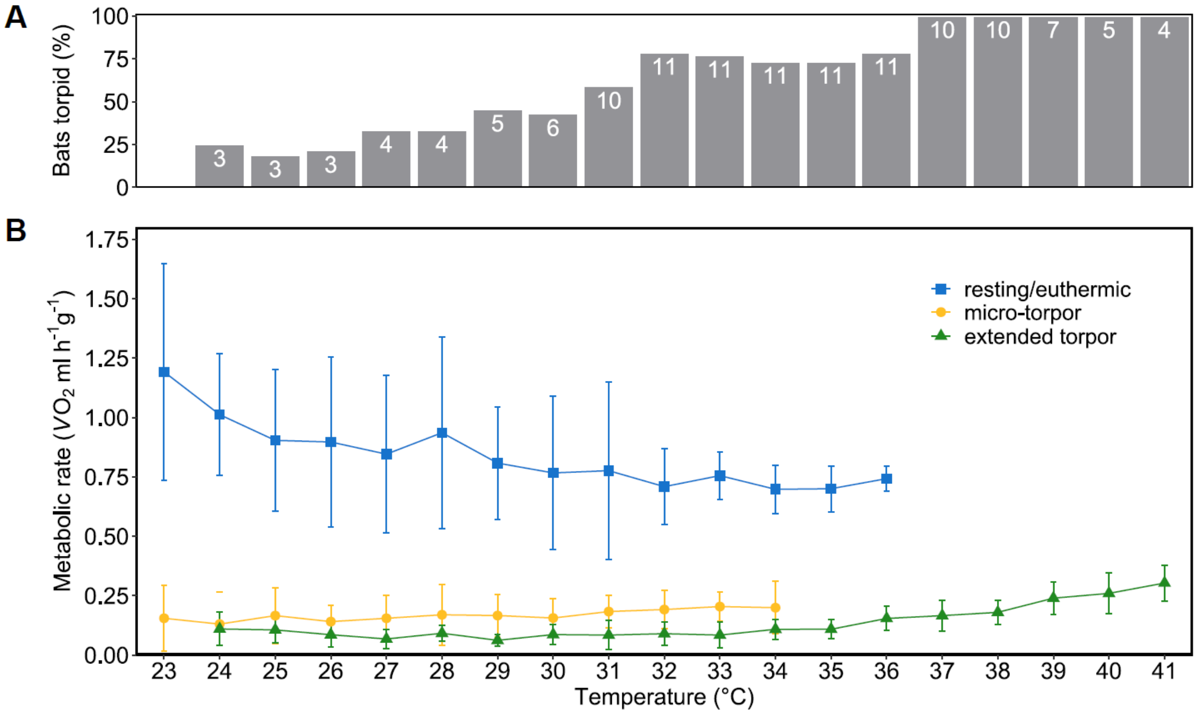


Figure 3.2. More bats enter torpor as ambient temperature rises. (A) The proportion of torpid bats at different ambient temperatures (T_a; integers indicate number of bats measured per interval). When T_a increased, more bats entered torpor and all bats were torpid above 36°C. (B) The mass-specific metabolic as $\dot{V}O_2$ (ml h^{-1} g^{-1}) of bats in the three different physiological states; resting (blue; squares), micro-torpor bouts (yellow; circles) and extended torpor bouts (green; triangles) at each temperature interval. Error bars represent standard deviation.

Over the inactive phase, we found that resting MR decreased with increasing T_a and plateaued between 31.9°C and 36°C (figure 3.2B). We could not determine the upper end of this plateau, because all individuals entered extended torpor above 36°C, but it very likely represents the thermal neutral zone of this population during the hot season, i.e. the range in which heat production and loss to the environment are balanced and no energy is needed to actively thermoregulate. In general, the warmer it became, the more individuals entered torpor (figure 3.2A). Above 36°C, thermoregulation at euthermia required excessive water consumption (see supplementary video 1 for a thermal imaging video of a bat salivating its forearms extensively for evaporative cooling at $T_a = 36.9$°C) and we found bats to be torpid even at T_a of 41°C (figure 3.2B). Clearly, torpor was more beneficial than defending euthermia, suggesting the existence of an upper limit of tolerable euthermia.

High body temperature masks torpor

The magnitude of metabolic depression during torpor was high and the MR for both modes of torpor was significantly lower than resting MR, despite similar daytime temperatures (resting MR vs. extended torpor: $t_{11} = 7.737$, $P < 0.001$; resting MR vs. micro-torpor: $t_{11} = 7.434$, $P < 0.001$; table 3.1, figure 3.3A). Although MR during extended torpor bouts was significantly lower than during micro-torpor bouts ($t_{11} = -3.427$, $P = 0.006$; table 3.1, figure 3.3A), both modes of torpor resulted in a similar level of metabolic depression of 82.2 and 77.6%, respectively. This is close to the highest metabolic reductions seen during more continuous torpor in warm environments (25-84% [35–38]). In contrast, variation in corresponding T_{skin} values of these three different physiological states was less clear-cut (maximum mean difference 1.9°C; figure 3.3B). T_{skin} during micro-torpor bouts was similar to that of euthermia ($t_{11} = 1.052$, $P = 0.316$), but both were significantly lower than T_{skin} during extended hot torpor bouts (extended vs. micro-torpor: $t_{11} = 2.511$, $P = 0.029$; extended torpor vs. euthermia: $t_{11} = -2.425$, $P = 0.034$; table 3.1, figure 3.3B). Consequently, although the reduction in metabolism during torpor was substantial, the magnitude of the MR decline did not dictate T_{skin}, regardless of torpor length.

Table 3.1. Physiological key variables of the metabolic states. Mass-specific metabolic rate as $\dot{V}O_2$ (ml h^{-1} g^{-1}) and mean maximum skin temperature (°C) of bats (N = 12) when resting and euthermic, during micro-torpor and during extended hot torpor.

	Euthermia	**Micro-torpor**	**Extended torpor**
Mean metabolic rate [ml O_2 h^{-1} g^{-1}]	1.07 ± 0.41	0.24 ± 0.08	0.19 ± 0.06
Mean skin temperature [°C]	36.7 ± 1.9	36.2 ± 2.1	38.1 ± 2.6

Both modes of torpor could not have been detected with the classical temperature recordings often used in eco-physiological research because their impact on body temperature was marginal; the mean deviation of T_{skin} from euthermia was less than 2°C. Our study has identified two hitherto unknown modes of torpor, which suggest that the traditional concept of torpor needs to be re-considered. Torpor was originally defined as a substantial decline in MR and body temperature, mainly in response to low T_a [22,23]. However, a decline in body temperature is clearly not a necessity and we have shown that it may even increase when an animal is entering torpor. Thus, while a controlled depression in MR has always been a central part of torpor, a more universal definition should also specify that body temperature during torpor can be variable: it may approximate T_a in either direction (as per the traditional definition and as seen during hot torpor [24,37,39–41] or may remain stable as seen during micro-torpor bouts. Different environmental conditions can result in different patterns of torpor and study methods should recognize this. Researchers should not rely solely on traditional indicators of torpor such as body temperature and strive to include other proxies for torpor, such as heart rate or oxygen consumption, particularly when studying endotherms in hot environments.

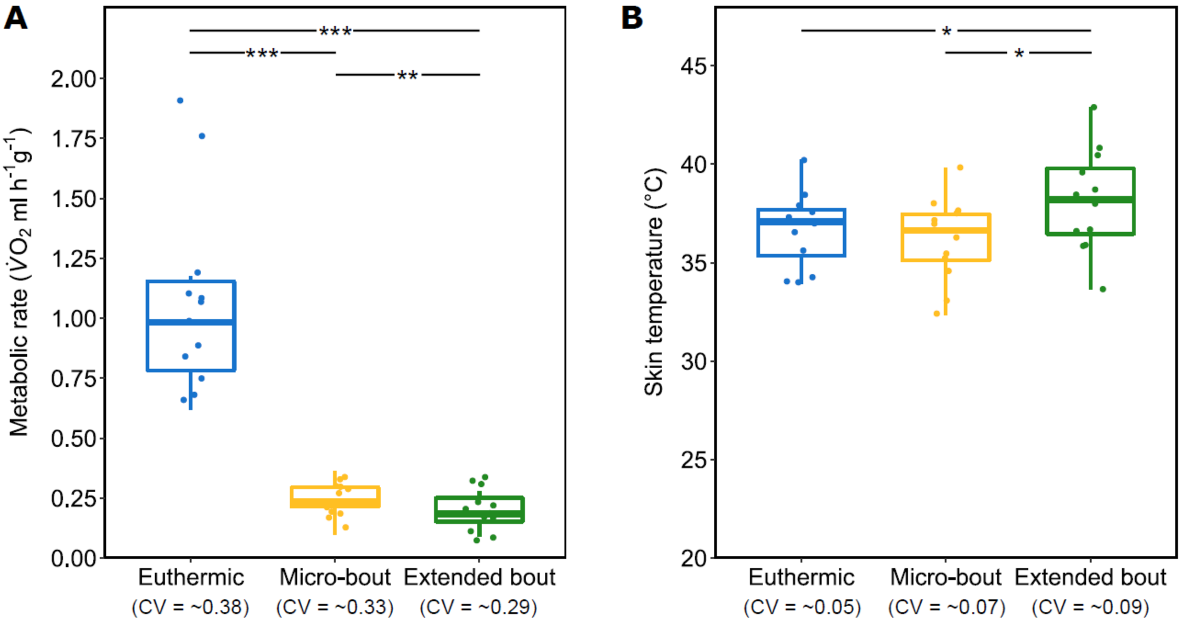


Figure 3.3. Depressed metabolism does not dictate skin temperature. Mass-specific metabolic rate as $\dot{V}O_2$ (MR, ml h^{-1} g^{-1}; panel A; N = 12) and skin temperature (T_{skin}, °C; panel B; N = 12) of animals in three different physiological states; resting metabolism (blue), micro-torpor bouts (yellow), and extended torpor bouts (green; centre line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, all data included). The coefficient of variation (CV) is given in parentheses; significant differences are marked with asterisks (*$P \leq 0.05$; **$P < 0.01$; ***$P < 0.001$).

Conclusions

Our study dramatically broadens our knowledge on the fundamental concepts of thermal physiology and describes newly-discovered options for mammals to cope with heat based on data from a free-living endotherm. We found that a tropical bat coped with daytime heat by using torpor (a response classically still associated with cold conditions), in novel ways: micro-torpor bouts at regular warm temperatures and hot torpor coupled with body temperature above euthermia during hot afternoon hours. This demonstrates the capacity of small mammals to survive rising ambient temperatures. Hot torpor allows *M. commersoni* to exist in habitats lacking well-buffered diurnal roosts and in regions that are even more arid. However, this response is not without risk: these bats could face lethal temperatures while in a torpid state if temperature increases too much, which would necessitate a premature re-arousal including evaporative cooling as a last resort. The inevitable up-regulation of metabolic heat production during emergency arousals could easily push the individual beyond tolerable temperature maxima. Hot torpor is thus a high-risk response that relies on ambient temperature to not exceed certain maxima and cool again. Whether hot torpor occurs more widely in the dry tropics remains to be determined. Our discovery of variations of the classical pattern of torpor should spur studies of the responses of other species living near their thermal limits in an era of global warming and is also relevant for the potential use of induced torpor for medical purposes and possible future space travel near human euthermic levels.

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Ethics

This study has been conducted under the "Accord de Collaboration" between the Université d'Antananarivo (Département de Biologie Animale), Madagascar National Parks and the Universität Hamburg. We thank these authorities and the Ministère de l'Environnement, de l'Ecologie et des Forêts for support and project authorization. The research was approved by the Directeur du Système des Aires Protégées, Ministère de l'Environnement, Antananarivo (permit no. 296/17/MEEF/SG/DGF/DSAP/SCB.Re) and all described procedures comply with the current ethical regulations and laws of Madagascar.

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Supplementary material

Extended Methods

Study site and species

We worked in a dry deciduous forest, Kirindy Forest (Centre National de Formation, d'Etude et de Recherche en Environment et Foresterie, CNFEREF, S 20.06714°/E 44.65745°) in western Madagascar. The forest is located ~20km from the Mozambique Channel and influenced by the western dry climate [42]. The whole region is characterized by a hot wet season from November to March during which ~96% of the annual rain falls (~900mm) and a cold dry season that can last up to 9 months with little to no precipitation [42,43]. Mean ambient temperature (T_a) in the wet season is ~30°C with maximum temperatures reaching over 40°C occasionally. During the cool dry season, mean T_a is usually ~20°C but can drop to ~3°C on single nights [42,43]. The core of the forest covers about 12,500ha [43] and has a mean canopy height of ~18m with individual tree species overtopping the canopy up to 25m. The Kirindy River is the main water source, but flows only intermittently during the peak of the wet season. No caves are known to be nearby Kirindy forest and our focal species was roosting among understory branches.

We studied Commerson's leaf-nosed bat, *Macronycteris commersoni* (Hipposideridae, mean body mass: females = 46.3 ± 6.8g; males = 79.6 ± 8.0g), the biggest insectivorous bat in and endemic to Madagascar [44]. This species hibernates and generally often enters torpor in a buffered, hot cave in south-western Madagascar [45], but has a wide distribution across the island, including forest habitat lacking cave systems [44].

Trapping and handling

Bats were trapped in February and March 2018 (wet season). We installed two mist nets (3m height x 6m length, mesh size 19mm; Ecotone, Sopot, Poland) blocking the main flight corridors in one part of the forest. The nets remained open for the first three hours after sunset and were checked every 10min. We changed the trapping sites every night to prevent trap avoidance owing to habituation. At each trapping event, we transferred two adult bats into cloth bags for the respirometry measurements while additional adults, juveniles, and individuals of other bat species were released immediately at the capture sites. Neither females nor males were reproductively active during data collection as assessed by carefully controlling the nipples and testes, respectively.

All captive bats were processed at the point of capture within 30min. They were weighed, sexed, and forearm length was measured. We shaved a patch of fur between their shoulder blades to attach a temperature-sensitive radio transmitter (~0.9g, Biotrack, Wareham, United Kingdom) using a medical skin glue (Manfred Sauer GmbH, Lobbach, Germany). The thermal sensor of the transmitter was placed directly on the skin and the transmitter itself was framed and partially covered by the bat's fur. The proportion of transmitter weight to the lightest bat's body mass was 2.6%, which is well below recommended maxima [46]. All transmitters were calibrated in a water bath at seven different temperatures covering a range of 3–45°C against a precision thermometer traceable to a national standard. Although skin temperature (T_{skin}) obtained via external transmitters might not reflect body temperature precisely, it provides a non-invasive and reliable proxy, particularly in small mammals [47–50].

To avoid pseudo-replication, we marked all bats with an individual, three-digit wing tattoo using non-toxic ink (Hauptner-Herberholz, Solingen, Germany) after the membrane had been locally anaesthetized (EMLA, AstraZeneca, Wedel, Germany). Animal handling was usually complete in 10min and never exceeded 15min.

Respirometry experiments

We measured metabolism as rate of oxygen consumption ($\dot{V}O_2$) of 16 adult bats (9 females, 7 males) using an open-flow respirometry system in pull mode. Directly after preparing the bats at their capture site, we transferred them individually into 2L plastic metabolic chambers, which were equipped with netting for the bat to grip onto and a logger (Hygrochron iButton, Maxim integrated, San Jose, USA) to record ambient temperature (T_a) and ambient relative humidity (RH_a) every 5min. These chambers were then connected to portable gas analysers (OxBox; T. Ruf and T. Paumann, University of Veterinary Medicine, Vienna, Austria) running on standard 12V car batteries. During the measurement, sample air was drawn from the metabolic chambers at a constant flow of 50L.h^{-1}, dried and filtered with silica gel, and an aliquot thereof was drawn through the gas analysers via the OxBox's integrated mass flowmeter. The oxygen content of the air was measured every 10s for 55min. For the remaining 5min per hour, reference air from outside the metabolic chambers was analysed to control for drifts in the oxygen sensors and used to correct the sample air values performing zero-checks with Clampfit v10.3.1.4 (Molecular Devices, Sunnyvale, USA). The oxygen sensors were calibrated in the laboratory before and after the field trip using calibration gases generated by a gas mixing pump (0, 3, and 5% N_2 in the air; 2KM300/a, Wösthoff Messtechnik GmbH, Bochum, Germany). To determine the bats' physiological responses under natural conditions, we placed the whole setup consisting of chamber, OxBox, pump and car battery in a shady spot within the forest (within enclosures, to protect the bats from predators during measurements).

To record T_{skin} during each respirometry experiment, a remote receiver/logger (DataSika Telemetry Receiver, Biotrack, Wareham, United Kingdom) was placed next to the setup, which recorded T_{skin} at 5min intervals.

We started measurements immediately after capture and processing, usually between 21:00 and 23:00 hours. Three bats were trapped via hand net in the morning and these measurements started between 09:00 and 10:00 hours. The first estimates of metabolic rate (MR) immediately following the introduction of a bat to the metabolic chamber were used as indicators of individual stress. If this initial MR did not decrease within the next 30 or 60min (depending on inactive and active phase, respectively), bats were released at point of capture. After the first 24h, all individuals were provided with fresh water and squeezed as well as living beetles [45], but only one individual consumed the food. One respirometry experiment typically lasted ~45h and ended at the start of the normal active phase between 17:30 and 18:30 hours to ensure animals could feed immediately after release. Before the bats were released at their capture site, they were weighed again to estimate mean body mass (BM) during the experiments.

Analyses

We analysed mass-specific metabolic rate (ml O_2 h^{-1} g^{-1}) per individual. Therefore, we first calculated the rate of oxygen consumption ($\dot{V}O_2$) as ml O_2 h^{-1} following equation 11.2 from Lighton [51], whereby an average respiratory quotient of 0.85 was included, assuming the oxidation of 50% fat and 50% carbohydrate [37]. We then added mean body mass ($\dot{V}O_2$ x BM^{-1}).

Different physiological states were defined via visual inspection of MR data. We differentiated among resting metabolic rate (RMR), torpid metabolic rate (TMR), arousal phases, and torpor entry phases. The high T_a range prevented us using classical approaches such as MR or T_{skin} thresholds, frequency distributions, or general percentages of RMR to determine torpor because MR was variable with fluctuating T_a (figure 2B). Therefore, we calculated one-minute means of MR to reduce the noise in the data, plotted mass-specific metabolic rate against time, and visually identified the different states. TMR was reduced by 77-82% compared to RMR, which lies in the normal range of metabolic depression during shorter or daily torpor bouts at lower T_a (depression of 70-80% compared to BMR/RMR [22,23]) and at the upper range of the reduction seen in warm environments (reduction by 25-84% of BMR/RMR [35–38]). Since the magnitude of metabolic depression was high in *M. commersoni*, we are confident that our approach neither missed torpor bouts nor assigned them incorrectly.

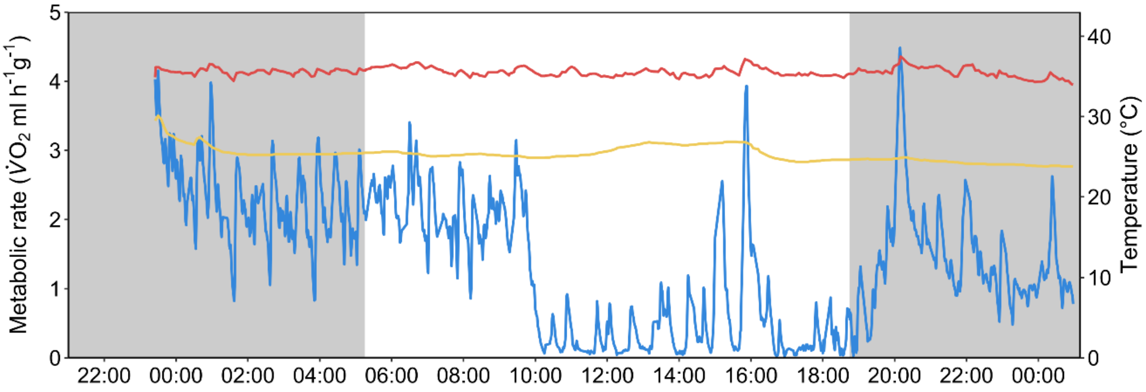
For further analysis, we only used the mean per individual of the lowest 50% of RMR of bats' usual resting phase, i.e. from sunrise to 30 min before sunset (~05:30 – ~18:15 hours, considering date-dependent sunset/sunrise times), to ensure that data from any non-resting phases (owing to disturbance or minor activity) were excluded [52]. For TMR, we included the mean per individual of the lowest 65% of previously assigned torpor phases since we had already carefully excluded arousal and entry phases before. We differentiated between micro-torpor bouts and more extended torpor bouts. Micro-torpor bouts usually occurred in an alternating manner, in which the bats constantly alternated between rest and torpor without noticeable arousal (over-shoot) and entry phases. More extended torpor bouts in contrast had characteristic entry and arousal segments, and lacked the alternating component. To obtain T_{skin}, we converted the transmitter signals recorded during the respirometry experiments from beats per minutes into temperature values using second-order polynomial regressions obtained from the calibration curves (all $R^2 \geq 0.99$). We are aware of the limitations of T_{skin} measurements. Therefore, these data were only used as estimates of body temperature to complement already defined torpor states with T_{skin}.

We compared RMR and TMR of both torpor states as well as corresponding T_{skin} values using paired sample t-tests adjusted for unequal variances where necessary. We analysed maximum T_{skin} values as these are most relevant in the context of high T_a. For the t-tests, we included data from only 12 bats for which MR values of all three different physiological states were available. We then allocated RMR and TMR to different bins of T_a since T_a fluctuated over the course of the day. We rounded T_a to the nearest integer and assigned individual means of RMR and TMR to the respective step in T_a to avoid pseudo-replication. Means per individual were then plotted against each T_a step to show the relationship between MR and T_a. We estimated the lower limit of the thermal neutral zone using broken-stick regression, i.e. iterative fitting of linear models combined with a bootstrap restarting approach, which makes the algorithm less sensitive to the estimated starting value, to determine whether the observed RMR distribution could be explained by multiple linear segments [53,54]. Lastly, we counted the number of torpid bats to euthermic bats for each respective T_a step. Data are shown as mean ± standard deviation and range if appropriate, N represents the number of individuals, n the number of included data points.

We processed and analysed the data and plotted the figures using R [55] and RStudio [56] with support of standard code and the following packages: lubridate [57], plyr [58], readxl [59], segmented [60], car [61], ggplot2 [62], ggpubr [63], cowplot [64] and scales [65].

Supplementary figure S1 and video S1

A: Micro-torpor bouts on warm days



B: Extended torpor bouts on hot days

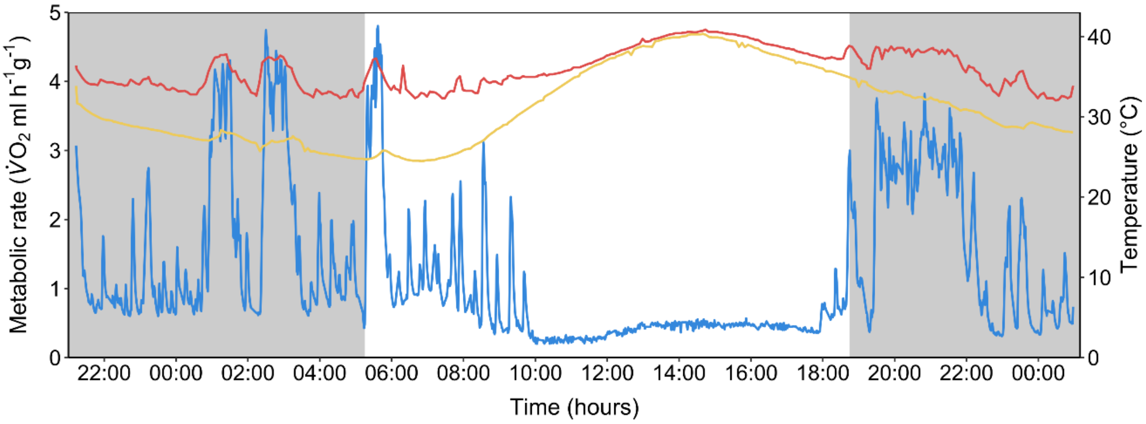


Figure S1. Two new modes of torpor in a tropical bat species (examples of female bats). Metabolic rate as $\dot{V}O_2$ (MR, ml h^{-1} g^{-1}; blue) and skin temperature (T_{skin}, °C; red) of two female individuals of the bat *Macronycteris commersoni* and the bats' ambient temperature (T_a, °C; yellow). Grey shaded blocks indicate the dark phase. Panel A shows the typical response on regular warm (rainy or cloudy) days. Under these conditions, bats alternated between regular resting MR and micro-torpor bouts (12.4 ± 10.5min) over the course of their usual inactive phase (~05:30 – 18:30 hours). Panel B shows the response of bats on hot days. When T_a exceeded euthermic body temperature, the bats extended their torpor bouts, which lasted until the beginning of their active phase in the late afternoon when T_a had decreased again (293.6 ± 101.2min). Please note the decline in MR and simultaneous increase in T_{skin}, which passively followed T_a, during extended torpor up to 42.9°C indicating that bats tolerated hyperthermia while torpid.

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Video S1. Costly evaporative cooling in *M. commersoni*. Thermal imaging video (FLIR T540, FLIR systems, Wilsonville, USA) of a male bat resting under a hot roof next to a temperature logger (Hygrochron iButton, Maxim integrated, San Jose, USA) at 36.9°C. This individual extensively licks its forearms for evaporative cooling. For scale, please note that the bat's length is ~13.6cm from head to toe.

Author contribution

I hereby confirm that Stephanie Reher (SR) and Kathrin H. Dausmann (KHD) conceived and designed the study, SR conducted the measurements in the field, analysed the data and took the lead in writing the manuscript with input from KHD. Both discussed and interpreted the results, revised the manuscript and approved the final version of it.

Prof. Dr. Kathrin Dausmann

Chapter 4

Disparate roost sites drive intraspecific physiological variation in a Malagasy bat

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Abstract

Many species are widely distributed and individual populations can experience vastly different environmental conditions over seasonal and geographic scales. With such a broad ecological reality, datasets with limited spatial and temporal resolution may not accurately represent a species and could lead to poorly informed management decisions. Because physiological flexibility can help species tolerate environmental variation, we studied the physiological responses of two separate populations of *Macronycteris commersoni*, a bat widespread across Madagascar, in contrasting seasons. The populations roost under dissimilar conditions: either a hot, well-buffered cave or within open foliage, unprotected from the local weather. We found that flexible torpor patterns, used in response to prevailing ambient temperature and relative humidity, were central to keeping energy budgets balanced in both populations. While bats' metabolic rate during torpor and rest did not differ between roosts, adjusting torpor frequency, duration and timing helped bats maintain body condition. Interestingly, the exposed forest roost induced extensive use of torpor, which exceeded the torpor frequency of overwintering bats that stayed in the cave for months and consequently minimised daytime resting energy expenditure. Our current understanding of intraspecific physiological variation is limited and physiological traits are often considered to be fixed. The results of our study therefore highlight the need for examining species at broad environmental scales to avoid underestimating a species' full capacity for withstanding environmental variation, especially in the face of ongoing, disruptive human interference in natural habitats.

Keywords

Torpor, tropics, physiological flexibility, adaptive/facultative hyperthermia, season

Introduction

Discrete populations of widely distributed species may inhabit areas with different climatic conditions and therefore face contrasting environmental pressures. Prevailing conditions may also vary within and between seasons, making the complete ecological sphere of a species considerably broad. Among regions, individuals may seek out similar microhabitats and consequently display relatively low potential for withstanding environmental variation, while others can adapt locally or possess highly flexible physiological traits (Kobbe et al. 2011; Noakes and McKechnie 2020; Hume et al. 2020; van Jaarsveld et al. 2021). Differences on a population level therefore reflect a species' capacity for phenotypic variation and/or local adaptation, and may even hint at incipient speciation (Violle et al. 2012; Richardson et al. 2014). Since physiological capacity ultimately determines species' tolerance limits to abiotic factors, and their resilience to changes in their environment (Canale and Henry 2010; Bozinovic et al. 2011), climatic variation can be tolerated via individuals' physiological flexibility. Brushtail possums from arid habitat, for example, have a lower energy and water turnover and can dissipate heat more efficiently than conspecifics from mesic areas (Cooper et al. 2018). Similarly, big brown bat populations from higher latitudes have lower energetic costs at cooler ambient temperatures (T_a) during torpor than their conspecifics closer to the equator (Dunbar and Brigham 2010). As a consequence, conclusions drawn from physiological data collected during only one season and/or from a single location may under- or overestimate a species' full natural capabilities. This is especially critical in the face of ongoing human disruptive interference, when accurate research and reporting can help define suitable habitats that might serve as refugia and ensure the long-term viability of both populations and species (Irwin et al. 2010; Cooke et al. 2013; Rezende and Bacigalupe 2015; Cooper et al. 2018).

Bats are an ideal group for studying intraspecific physiological variation over broad environmental scales because many species are widely distributed, in some cases populating entire continents and thus a variety of habitats and environmental conditions. They are highly efficient at minimising energy expenditure and species from arid habitats can finely balance body water (e.g., Geiser and Stawski 2011; Klüg-Baerwald and Brigham 2017; Gearhart et al. 2020). Diurnal roost selection can limit exposure to unfavourable environmental conditions, such as weather extremes, help avoid predators (Fenton et al. 1994; Solick and Barclay 2006) and facilitate social interactions and reproduction (Kunz 1982; Willis and Brigham 2004). Moreover, both bat abundance and species distributions are correlated with roost availability (Humphrey 1975). Roosting sites are therefore a critical resource for many bats and can be an important determinant of extinction risk (Sagot and Chaverri 2015). Roosts range from well-buffered caves or crevices and tree holes, to other animals' nests, constructed leaf tents and completely exposed roosts in foliage (Kunz and Lumsden 2003). Some species or individuals rest strictly in only one type of roost, while others change depending on seasonal and/or life history requirements (e.g., overwintering, reproduction or rearing offspring; Kunz and Lumsden 2003). Understanding both the seasonal preferences of different bat populations for a specific roost type and the significance of roost type variation are therefore important steps for determining the full scope of a species' ecology and physiology.

The endemic insectivorous Commerson's leaf-nosed bat *Macronycteris commersoni* uses contrasting types of diurnal roost throughout its wide distribution in Madagascar (Goodman 2011). In dry spiny forest in south-western Madagascar, *M. commersoni* roosts in a large colony in a hot cave with a highly stable microclimate (32 ± 0.1°C at bat height, 98 ± 0.5% relative humidity; Reher et al. 2018) throughout the year that is buffered from external weather (Reher et al. 2019). In western dry deciduous forest, on the other hand, the bats roost solitarily in the open vegetation among branches (Reher and Dausmann 2021). These tree roosts provide no insulation, leaving bats vulnerable to predators and the effects of external environmental extremes. Both habitats are located in the western formations in the driest zones of the island and are highly seasonal. During the harsh dry season night-time T_a, precipitation and food availability are reduced for up to 9 months, whereas during the milder wet season food resources are more abundant. Dry season daytime T_a extremes regularly exceed 40°C and are coupled with low relative humidity (Kappeler and Fichtel 2012; Ratovonamana et al. 2013), challenging the bats' thermoregulatory systems.

To conserve water and energy, both populations use torpor (Reher et al. 2018; Reher and Dausmann 2021), which is a controlled state of metabolic depression (Geiser 2004; Heldmaier et al. 2004). Torpor is highly beneficial but the extent that torpor patterns expressed by *M. commersoni* vary with roost type or season and are thus modified to cope with prevailing conditions is unknown. There is evidence that torpid metabolic rate (TMR) might not vary among bat populations resting at different humidity levels, even though evaporative water loss does (Klüg-Baerwald and Brigham 2017; Gearhart et al. 2020). However, torpor duration, frequency and general patterns, as well as resting metabolic rate (RMR), may differ at different resting sites and among seasons (Stawski and Geiser 2011; Kobbe et al. 2014; Bethge et al. 2017; Boyles et al. 2020) to support the maintenance of homeostasis.

In our study, we investigate the energetic costs and benefits of physiological strategies used by *M. commersoni* to cope with different roosting conditions, i.e. roost type and season, and quantify intraspecific physiological variation therein. We compare torpor occurrence and timing in two habitats with differing roost types: a cave with near-constant environmental conditions and an open forest with fluctuating environmental conditions. We hypothesise that bats use torpor more often, and torpor bouts are longer, in the protected cave roost, especially during the lean dry season. Given that variations in TMR in thermoconforming animals are mainly a function of ambient temperature (Geiser 2004), we predict a higher TMR in the forest bats when torpor is used in response to heat stress (Reher and Dausmann 2021), but no differences in TMR between habitats at the same T_a. We also evaluate the energetic costs associated with the different roosting conditions by examining body condition and daytime resting energy expenditure (DREE).

Methods

Study sites

To study the effects of roosting conditions on bat metabolism, we collected data at two sites in Madagascar separated by 379.8 km that differ in roost availability: a cave habitat in south-western spiny forest of Tsimanampetsotse National Park and a forest habitat in western dry forest in the Kirindy Forest/Centre National de Formation, d'Etudes et de Recherche en Environnement et Forestier (CNFEREF) (figure S1).

Tsimanampetsotse National Park encompasses a variety of different ecosystems including dry plains around a salt lake, dry spiny forest and a calcareous plateau with underground cave and stream systems (Reher et al. 2018; Reher et al. 2019; figure S1B,C). Although it experiences a cool dry season (April - October) and a hot wet season (November – March), this region is generally very dry year-round with only sporadic, unpredictable rainfall. Within the cave system, Andranolovy Cave (24.04585°S, 43.75396°E) is used by *M. commersoni* and several other bat species as a diurnal roost in the wet season and for overwintering during the dry season (Reher et al. 2019; figure S1E). It is the hottest and most humid cave in the region and cave conditions are highly stable year-round (table 4.1) (Reher et al. 2018; Reher et al. 2019). The bats roost at a height of ~6 m, where cave temperature is 32.4 ± 0.1°C and relative humidity is 98.3 ± 0.5%.

Kirindy Forest (CNFEREF) is a dry deciduous forest and is located further north (20.06714°S, 44.65745°E) (Kappeler and Fichtel 2012; figure S1A,C). The climate is also characterised by a hot wet season, in which most of the annual rainfall occurs, and a cold dry season that can last up to nine months with little to no precipitation (Kappeler and Fichtel 2012). Compared to the southern spiny forest, Kirindy Forest is denser and has a closed canopy at 15-18 m (figure S1A,C). Importantly, no caves are known to be nearby and *M. commersoni* roosts solitarily in trees, exposed to highly variable environmental conditions (Reher and Dausmann 2021; figure S1D). Ambient conditions fluctuate greatly on a daily basis (table 4.1), with highest T_a and lowest relative humidities (RH_a) occurring in the early afternoon and lowest T_a and highest RH_a in the early morning. We did not trap any *M. commersoni* during the dry season, presumably because they were hibernating in tree hollows or had migrated to caves further away. Therefore, we are only able to present data from the wet season for Kirindy Forest. To highlight the main structural differences between the two habitats that strongly affect the bats' roost characteristics, we will henceforth refer to Tsimanampetsotse National Park as the "cave habitat" or "cave roost" and to Kirindy Forest as the "forest habitat" or "forest roost".

Table 4.1. Environmental conditions (ambient temperature and relative humidity) during the wet and dry seasons at each of the sites used by *M. commersoni*, measured at a height of 1.5 m above the ground, in Tsimanampetsotse National Park and Kirindy Forest (CNFEREF).

Region	Season	Variable	Mean	Range	Daily variation
Tsimanampetsotse National Park	Dry	Cave temperature (°C)	29.4 ± 0.1	29.3 – 29.6	0.1 ± 0.1
		Cave relative humidity (%)	98.8 ± 0.1	96.4 – 100.5	1.1 ± 0.3
	Wet	Cave temperature (°C)	30.6 ± 0.1	30.3 – 30.7	0.2 ± 0.1
		Cave relative humidity (%)	95.2 ± 0.1	93.8 – 96.6	1.6 ± 0.3
Kirindy Forest (CNFEREF)	Wet	Forest temperature (°C)	27.5 ± 1.2	21.2 – 41.7	13.8 ± 2.3
		Forest relative humidity (%)	80.1 ± 6.5	29.0 – 101.2	50.8 ± 9.8

Trapping and handling

Cave bats were trapped in June/July 2016 and February/March 2017 (dry and wet seasons, respectively; Reher et al. 2018) and forest bat trapping took place in February/March and July/August 2018 (wet and dry seasons, respectively; Reher and Dausmann 2021). We used different trapping methods owing to the differences in habitat structures and bats' roosting behaviours. During the dry season, we hand-caught adult cave-dwelling bats in the early morning between 07:00 and 09:00 h because this species is generally inactive at this time of year. During the wet season we erected a two-bank 4.2 m^2 harp trap (Faunatech~Austbat, Bairnsdale, Australia) in front of the same cave's entrance. In the forest habitat, two to three mist nets (3 m height x 6 m length, 19 mm mesh; Ecotone, Sopot, Poland) were opened each night in major flight corridors. The harp trap and mist nets remained open for the first three hours after sunset and were checked every 10 to 20 min, depending on season. Per trapping event, we transferred the first two adult bats into a cloth bag while additional adults and juveniles were released immediately at the capture site. Only non-reproductive females were studied.

All captive bats were processed at the site of capture within 30 min. They were weighed, sexed and forearm length was measured. A patch of fur was removed from between the shoulder blades using a razor and shaving cream to allow the attachment of a temperature-sensitive radio transmitter (~0.9 g, Pip Ag376; Biotrack, Wareham, UK) using a medical skin glue (Osto-Bond, Montreal, Canada or Manfred Sauer GmbH, Lobbach, Germany). The thermal sensor was placed on the skin and after attachment the transmitter itself was partially covered by the bat's fur. Transmitters weighed ≤ 2.6% of a bat's body mass (mean = 1.8 ± 0.43%) and thus well below recommended maxima (e.g., Aldridge and Brigham 1988). Prior to attachment transmitters were calibrated in a water bath from 3–45°C against a precision thermometer traceable to a national standard. Skin temperature (T_{skin}) obtained via external transmitters provides a non-invasive and reliable proxy of T_b, particularly in small mammals (Audet and Thomas 1996; Dausmann 2005; Langer and Fietz 2014; but see Willis and Brigham 2003). We marked all bats with an individual, three-digit wing tattoo using non-toxic ink (Hauptner-Herberholz, Solingen, Germany) after the membrane was locally anaesthetised (EMLA, AstraZeneca, Wedel, Germany). Animal handling lasted about 10 min and never exceeded 15 min.

Respirometry

We measured metabolism as the rate of oxygen consumption ($\dot{V}O_2$) using an open-flow respirometry system in pull mode. Directly after processing, bats were transferred into individual 2 L plastic metabolic chambers, which were equipped with a net for roosting. Each chamber was connected to a portable gas analyser (OxBox; T. Ruf and T. Paumann, University of Veterinary Medicine Vienna, Austria) powered by a standard 12 V car battery. During measurements, sample air was drawn from the metabolic chambers at a constant flow of 50 L h^{-1} using a diaphragm pump, then dried with silica gel and filtered before passing through the OxBox's integrated mass flowmeter, and an aliquot thereof was drawn through the gas analyser. The oxygen content of the air was measured every 10 s for 55 min. For the remaining 5 min per hour, reference air from outside the metabolic chambers (also dried with silica gel and filtered) was analysed for oxygen content to control for drift in the electrochemical fuel-cell oxygen sensors (7OX-V CiTicel; City Technology, Portsmouth, UK). This was used to correct the sample air values with Clampfit v10.3.1.4 (Molecular Devices, Sunnyvale, USA). The oxygen sensors were calibrated in the laboratory before and after measurements using calibration gases mixed by a gas mixing pump (0, 3, and 5% N2 mixed with fresh air; 2KM300/a, Wösthoff Messtechnik GmbH, Bochum, Germany).

The whole setup consisting of chamber, OxBox, pump and car battery was placed within the cave or forest to measure the bats' metabolism under natural ambient conditions. Within the cave, we placed the measurement set-up in an adjoining chamber next to the bats' roosting chamber to avoid unnecessary disturbance of the whole colony. Although this chamber was slightly cooler by ~3.1°C in the dry season and ~1.1°C in the wet season, it was frequently visited by individual *M. commersoni*. Within the forest habitat, we placed the measurement set-up in a shaded enclosure near the camp in the centre of the forest to avoid abundant local predators such as fossas.

During each respirometry run we recorded T_a and RH_a using a data logger placed within each respirometry chamber (Hygrochron iButtons, Maxim integrated, San Jose, USA) and T_{skin} using a remote receiver/logger placed next to the setup (DataSika SRX-800-D; Biotrack, Wareham, United Kingdom). All temperature data were recorded at 5 min intervals.

We started measurements immediately after capture and processing, usually between 21:00 and 23:00 h in the wet season and 09:00 and 11:00 h in the dry season owing to different trapping times.

We used the initial metabolic rate values after a bat was introduced to the metabolic chamber as indicators of individual stress levels. If there was no clear reduction in these first values within the next 30 or 60 min (depending on inactive and active phase, respectively), bats were released at their point of capture to avoid continuous stress. After the first 24 h, all individuals were provided with fresh water and food (see Reher et al. 2018). One respirometry run typically lasted ~45 h, thus included the activity and rest phase, and ended with the beginning of the bats' usual active phase between 17:30 and 18:30 h to ensure immediate foraging opportunities. Before the bats were released at their capture site, they were weighed and offered food and water. All applicable institutional and national guidelines for the care and use of animals were followed.

Data processing and analysis

We analysed all data using Cran R (R Core Team 2018) in "RStudio" (RStudio Team 2016) and the packages "plyr" (Wickham 2011), "dplyr" (Wickham et al. 2020), "readxl" (Wickham and Bryan 2017), "lubridate" (Grolemund and Wickham 2011), "ggplot2" (Wickham 2016), "ggpubr" (Kassambara 2020a), "cowplot" (Wilke 2020). Data are shown as mean ± standard deviation and range if appropriate; *N* represents the number of individuals, *n* the number of included data points.

We first calculated the rate of oxygen consumption ($\dot{V}O_2$) as ml O_2 h^{-1} corrected to standard temperature and pressure dry with Equation 11.2 in Lighton (2008) before dividing by average body mass (BM) during a measurement to determine mass-specific metabolic rate (MR, ml $\dot{V}O_2$ g^{-1} h^{-1}). We assumed an average respiratory quotient of 0.85 (oxidation of 50% fat and 50% carbohydrate, Dausmann et al. 2009). We determined different physiological states via visual inspection of MR patterns following Reher and Dausmann (2021). We defined torpor as a decrease in MR by at least 50% compared to resting MR (RMR), which lies within the range of the highest metabolic reductions seen during torpor in warm environments (25–84%; Song et al. 1997; Dausmann et al. 2009; Grimpo et al. 2013; Kobbe et al. 2014). For downstream analysis, we removed arousal and entry phases and continued with a subset of MR data. We only used the lowest 50% of resting metabolic rate (RMR) values per hour per individual during the bats' usual resting phase, i.e. from sunrise to 30 min before sunset, to ensure that data from any active or disturbed animals were excluded (Bethge et al. 2017; Reher and Dausmann 2021; Rodgers and Franklin 2021). For TMR, we included the lowest 70% of data per hour per individual. We differentiated between micro-torpor bouts, the mean duration of which were as short as 17 ± 8 min (range: 5 – 36 min, n=857; mTMR), and extended torpor bouts lasting 4.9 h ± 58 min (range: 1.7 – 8.3 h, n=44; eTMR) after Reher and Dausmann (2021). Briefly, micro-torpor bouts had a similar reduction in MR to extended torpor bouts but bats alternated rapidly between torpid and euthermic states, whereas extended bouts had characteristically slower entry and arousal curves. T_{skin} during the respirometry runs was calculated using second-order polynomial regressions obtained from the calibration curves (all $R^2 \geq 0.99$) and used as a proxy for body temperature (T_b).

Bat morphology

To compare body mass, forearm length, body condition (standardized mass index, SMI, following Peig and Green 2009, 2010) and TMR among the different roosting conditions we used t-tests adjusted for unequal variances if necessary or Wilcoxon signed-rank tests. In all cases, p-values were adjusted with Bonferroni-Holm corrections to account for multiple comparisons (package "rstatix"; Kassambara 2020b).

Torpor bout timing

We used Rayleigh's tests and Watson two-tailed tests to determine whether the timing of torpor entry and arousal differed significantly from a random distribution and to identify differences in timing between sites and seasons (package "circular," Jammalamadaka and Sengupta 2001). Time is given as circular mean ± standard deviation.

Metabolic rates

To analyse MR, we allocated RMR and TMR to different T_a bins by rounding T_a to the nearest integer and assigned individual means of the different metabolic states to each T_a step to avoid pseudo-replication. We then explored the influence of roost site and season (as site-season: cave dry season, cave wet season, forest wet season), sex, body condition and roost temperature on metabolic rate using either linear mixed models (LMEs) or generalized linear mixed models (GLMMs; package "lme4", Bates et al. 2015). We did not include RH_a in the models because T_a and RH_a were strongly negatively correlated but we report RH_a mean and range during the measurements. Only T_a steps at which we measured at least three bats per roosting site were included. We fitted separate models for extended torpor MR (eTMR), micro-torpor MR (mTMR) and RMR, in which site-season, T_a, the interaction of site-season and T_a, sex and body condition were defined as fixed factors and bat ID was a random effect to account for repeated measures. While the environmental conditions in the cave were almost constant, T_a and RH_a fluctuated considerably in the forest. We therefore also modelled RMR and mTMR as a function of the roosting site at only overlapping temperature bins (28-30°C and 31-34°C) with bat ID as random effect. For analysing the subset of eTMR, we used t-tests owing to the small sample size of bats measured at overlapping T_a steps (N=3 bats per site and T_a) and non-replicated data. We excluded data from one bat that entered a single multi-day torpor bout (> 3 days) for the analysis of eTMR and the subsequent analysis of torpor bout duration (see below). Please note that we could not directly compare the cave-dwelling bats' MR between seasons because of no common T_a steps (figure 4A,B,C, left panel).

Data exploration and validation was achieved following Zuur et al. (2010) and Zuur and Ieno (2016). Significance was calculated using Satterthwaite's method (package "lmerTest"; Kuznetsova et al. 2017).

Torpor bout duration and frequency

We analysed the effect of sex, body condition and site-season on micro-torpor bout frequency, micro-torpor bout duration and extended torpor bout duration using separate GLMMs (package "glmmTMB"; Brooks et al. 2017). Micro-torpor bouts occurred multiple times each day so an average bout frequency per day was used in analyses. We did not model extended torpor bout frequency because there was rarely more than one bout per day and instead report proportions of bats torpid within the populations. In all models, we included site-season, sex and body condition as fixed factors and bat ID as a random effect. For eTMR, we defined an additional model including the interaction of site-season and body condition, and compared both using likelihood ratio testing (Zuur et al. 2009). No environmental predictor (i.e., T_a, RH_a) was included because the site-season term encompassed per day differences in environmental variation.

Resting energy expenditure

To evaluate whether there were differences in bats' energetic costs among roosting conditions, we calculated individuals' daytime resting energy expenditure (DREE, in kJ) between sunrise and sunset from per-minute metabolic rate values using an oxycalorific equivalent of 20.37 kJ/L O_2 (Schmidt-Nielsen 1997). This approach takes into account different daytime torpor strategies, e.g., remaining euthermic (n=5), entering only micro-torpor (n=42), entering only extended torpor (6.9 h ± 59 min N=10) or entering a combination of micro-torpor with a shorter extended torpor bout (4.4 h ± 54 min; N=35). We analysed the effect of sites and seasons on DREE using a GLMM; site-season, sex, body condition and torpor strategy were included as fixed factors and bat ID as a random effect.

Results

We measured the physiological responses of 41 individual *M. commersoni*; 25 from the cave habitat and 16 from the forest habitat (table 4.2). We studied cave bats during the wet and dry season but because we did not trap any bats in the forest during the dry season, despite 261 trapping hours, we only present their data from the wet season (table 4.2). Bats from the forest population were larger but not heavier than their conspecifics from the cave habitat (table 4.2, table S1). Interestingly, body condition was similar in both roosting conditions and sexes, but the forest population's females had a significantly lower body condition than all other bats (t-test, $t_{27.7}$=-6.54, p<0.001; table 4.2).

Table 4.2. Overview of the different populations in the dry and wet seasons and their key physiological and morphological variables. The number of females and males studied are given in parentheses. Mean body mass, forearm length and body condition; the number of bats that entered either extended or micro-torpor; mean metabolic rate during extended torpor and micro-torpor and as a percentage reduction of resting metabolism; mean torpor bout duration for both modes of torpor; and daytime resting energy expenditure (DREE) are shown. We pooled sexes when calculating means of all physiological variables because sex was not included in the most parsimonious models. For simplicity, MR reduction was compared over the mean of the entire measured T_a range because testing among subset T_a ranges yielded the same trends.

	Cave habitat				**Forest habitat**	
	Dry season		**Wet season**		**Wet season**	
	Female (6)	Male (4)	Female (9)	Male (6)	Female (9)	Male (7)
Body mass (g)	45.2 ± 6.8	55.5 ± 12.7	45.1 ± 7.9	53.8 ± 10.4	46.3 ± 6.8	79.6 ± 8.0
Forearm length (mm)	79.2 ± 1.8	85.5 ± 1.9	79.5 ± 1.0	85.8 ± 2.1	88.0 ± 1.4	94.4 ± 2.8
Body condition (SMI)	60.9 ± 10.7	54.9 ± 9.6	59.6 ± 12.2	53.1 ± 10.8	41.0 ± 4.9	53.8 ± 4.6
Bats that used extended torpor	N = 3	N = 3	N = 1	N = 5	N = 8	N = 7
Bats that used micro-torpor	N = 5	N = 3	N = 8	N = 6	N = 9	N = 7
MR during rest (ml h^{-1} g^{-1})	1.04 ± 0.19		1.09 ± 0.36		1.05 ± 0.42	
MR during micro-torpor (ml h^{-1} g^{-1}) *(reduction [%])*	0.24 ± 0.05 *(75.4 ± 4.8)*		0.31 ± 0.07 *(68.6 ± 11.8)*		0.27 ± 0.07 *(73.3 ± 7.4)*	
MR during extended torpor (ml h^{-1} g^{-1}) *(reduction [%])*	0.10 ± 0.02 *(89.4 ± 0.8)*		0.17 ± 0.04 *(82.4 ± 7.1)*		0.20 ± 0.05 *(80.2 ± 5.3)*	
T_{skin} during euthermia (°C)	36.4 ± 1.7		36.8 ± 1.5		36.6 ± 2.0	
T_{skin} during micro-torpor (°C)	36.1 ± 1.4		35.9 ± 1.2		36.1 ± 1.9	
T_{skin} during extended torpor (°C)	33.5 ± 2.9		33.0 ± 1.6		38.5 ±2.5	
Extended torpor bout duration (min)	345.2 ± 190.4		176.0 ± 83.6		312.5 ± 157.1	
Micro-torpor bout duration (min)	21.1 ± 16.9		12.8 ± 8.9		13.3 ± 6.8	
DREE (kJ day^{-1} g^{-1})	0.10 ± 0.04		0.18 ± 0.08		0.11 ± 0.05	

Occurrence of torpor and deviations from euthermia

Most bats (38 out of 41) entered torpor (n=44 extended bouts, n=857 micro-torpor bouts). Only three cave-roosting individuals remained normothermic over the whole measurement period (one female in the wet season and one female and one male in the dry season). During the wet season the forest bats made extensive use of both modes of torpor (94% used extended torpor and 100% used micro-torpor), compared to the cave population, which opted for micro-torpor (94%) rather than extended torpor (40%). During the dry season fewer cave bats entered torpor (60% used extended torpor and 80% used micro-torpor). TMR was similar across sites and seasons despite the high T_a, differing roosting conditions (table 4.2, figure 4.2A) and sometimes very short micro-torpor bouts. mTMR was reduced by 72% compared to RMR, without differences between sites or seasons (table 4.2). eTMR was reduced by ~85%, with the greatest reduction in the cave during the dry season (89.4 ± 0.8%) and lowest in the forest during the wet season (80.2 ± 5.3%, figure 4.1A), where extended torpor was most commonly associated with high T_a (Reher and Dausmann 2021). Although the drop in MR during torpor was considerable, changes in T_{skin} were less evident (table 4.2, figure 4.1A,B). While it was not possible to detect micro-torpor bouts through a clear T_{skin} signal under any roosting conditions studied, T_{skin} approaching T_a often indicated an extended torpor bout. In the cave, T_{skin} dropped by 2.9 ± 2.0°C during torpor but increased by 1.9 ± 2.8°C in the forest (table 4.2, figure 4.1B).

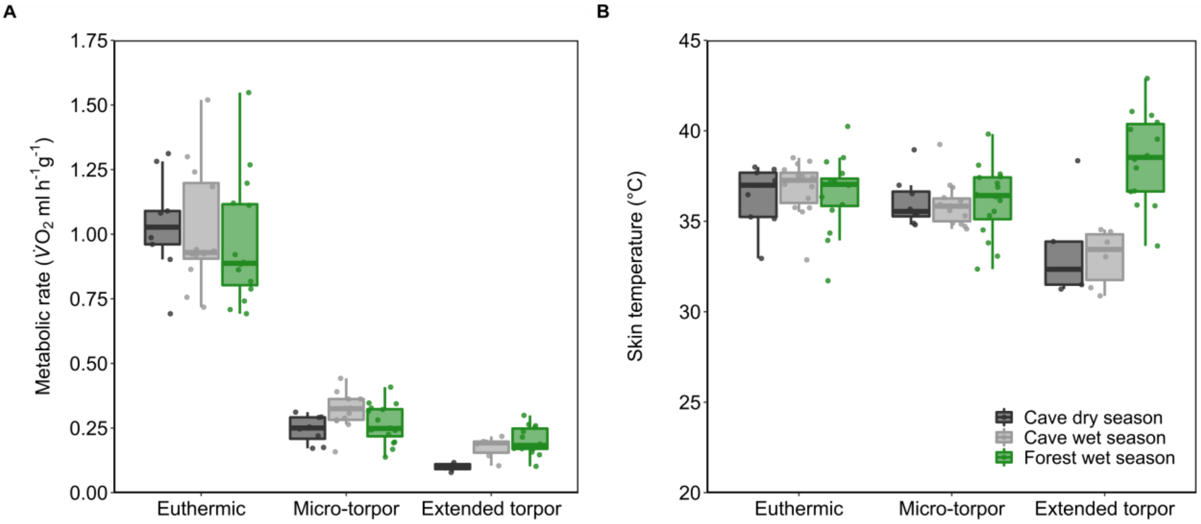

Figure 4.1. (A) Mean mass-specific metabolic rate ($\dot{V}O_2$ (MR, ml h^1 g^1) and (B) skin temperature (T_{skin}, °C) of animals at rest (euthermic), in a micro-torpor bout and in an extended torpor bout. Bats roosting in a cave during the dry (dark grey) and wet season (light grey) and forest-dwelling bats during wet season (green). The centre line represents the median, box limits indicate upper and lower quartiles, whiskers show 1.5× interquartile range and points are all included data.

Temporal synchronization of torpor bouts

We found seasonal and site-specific timing in entry into torpor and arousal from torpor. The entry and arousal times of both extended and micro-torpor bouts were randomly distributed in the cave during the dry season (table S2, figure 4.2), when the bats did not leave the cave for months. In the wet season, extended torpor and micro-torpor entry and arousal followed a clear pattern at both sites (table S2, figure 4.2), and the timing of extended torpor entry and arousal was more synchronized than for micro-torpor (i.e., extended torpor bout times had higher Rayleigh's r-values). The bats in the cave entered into and aroused from extended torpor earlier than in the forest (entry: $x = 0.24$, $p < 0.05$, cave 09:21 ± 40 min, forest 10:23 ± 12 min; arousal: $x = 0.1872$, $p < 0.05$ cave 14:54 ± 29 min, forest 16:56 ± 17 min; table S2; figure 4.2). Micro-torpor bouts appeared to occur around the clock in the wet season but entry and arousal times differed from a random distribution (table S2, figure 4.2). In the cave, both were more frequent during the day (entry: 12:58 ± 5 min, arousal: 13:18 ± 7 min), whereas in the forest entries and arousals occurred predominantly in the morning (entry: 09:28 ± 9 min, arousal: 09:43 ± 11 min; table S2), often before extended torpor bouts at high T_a.

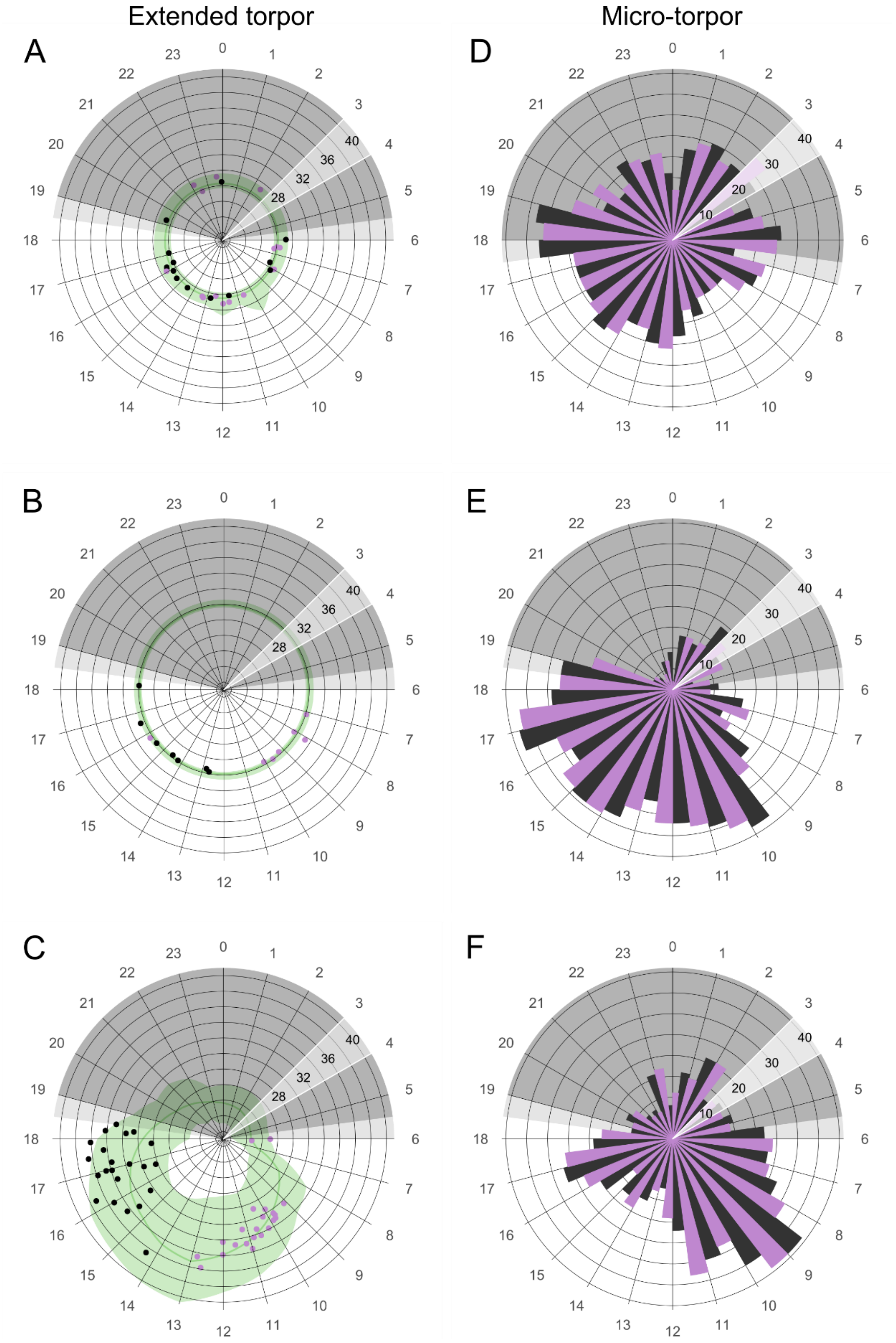

Figure 4.2. Timing of entry into (mauve), and arousal from (black), extended and micro-torpor bouts under three different roosting conditions: in a cave during the dry and wet seasons (A, D and B, E, respectively) and in a forest during the wet season (C, F). For extended torpor (A, B, C), the data shows the time of the day (circular axis) and skin temperature (°C; radial axis distance). Dark grey shaded areas indicate scotophase and light grey areas indicate twilight. The green line illustrates hourly mean ambient temperature with hourly minima and maxima bounding the green shaded areas. For micro-torpor (D, E, F), the data shows the occurrence of micro-bout entries (mauve) and arousals (black; radial distance) over the course of the day at hourly intervals.

The effect of roosting environment on MR

We analysed the RMR of 39 individuals. The interaction of site-season and T_a step and body condition had significant effects on RMR. RMR generally decreased with increasing T_a (GLMM; t=-3.30, p<0.001), but the slope was significantly flatter in the forest (GLMM, t=2.64; p=0.008; figure 4.3A, left panel) than in the cave, where the slope did not differ between seasons (GLMM, t=0.92; p=0.355; figure 4.3A, left panel). There was no significant variation in a subset of RMR at similar temperature ranges (28-30°C: GLMM, t=-0.50, p=0.613, N=26; 31-34°C: GLMM, t=-0.45, p=0.650, N=25; figure 4.3A, right panel). Higher body condition was correlated with lower RMR (GLMM, t=-2.17; p=0.030) but sex was no significant predictor (GLMM, t=0.82; p=0.412).

mTMR decreased slightly with increasing T_a (LME; t=-4.52, p=0.034, N= 38; figure 4.3B, left panel). Neither site-season (LME; cave wet season t=0.92, p=0.362; forest wet season t=0.42, p=0.664), body condition (LME; t=-0.97, p=0.340), nor sex significantly affected mTMR (LME; t=0.17, p=0.870). Accordingly, site-season was not a significant predictor of a subset of mTMR at only similar temperature ranges (28-30°C: LME, t=0.807, p=0.43, N=23; 31-34°C: LME, t=-0.97, p=0.340, N=26; figure 4.3B, right panel).

We analysed eTMR for 25 individuals. In the forest population, eTMR increased with increasing T_a (LME, t=8.87; p<0.001; figure 4.3C, left panel) while sex (LME, t=-0.06; p=0.951) and body condition had no significant effect (LME, t=0.11; p=0.912). We found no differences in a subset of eTMR at similar T_a (29°C: t-test, $t_{2.48}$=-0.08, p=0.946, N=6; 32°C: t-test, $t_{9.28}$=-0.12; p=0.909; N=15; figure 4.3C, right panel).

Torpor bout frequency and duration

Site-season affected micro-torpor bout frequency: the cave-dwelling bats in the wet season entered micro-torpor bouts more frequently than their conspecifics while we found no such effect in number of micro-torpor bouts per day between cave bats in the dry season and forest-dwelling bats in the wet season (table 3; figure 4.4A). Micro-torpor bout frequency was influenced by sex, with females entering micro-torpor more frequently than males and there was a tendency for individuals with higher body condition to enter micro-torpor bouts more often (table 3). For micro-torpor bout duration, site-season was the single significant predictor; cave-dwelling bats entered longer bouts in the dry season than bats in the wet season, regardless of roost (table 3; figure 4.4B). Neither sex nor body condition influenced micro-torpor bout duration significantly.

Extended torpor bout duration was better explained when the interaction of site-season and body condition was included (Chi^2=3.97; p=0.036). In cave conditions, extended torpor bout duration was negatively related to body condition but the slope of this relationship was steepest during the dry season (table 3, figure S3). The duration of extended torpor bouts was shortest in wet season cave bats and similar between wet season forest bats and dry season cave bats (figure 4.4C). We did not analyse the frequency of extended torpor bouts because the bats usually only entered one extended bout per day. Under all three different environmental conditions, the ratio of males entering extended torpor was always higher than females (cave dry season: females 50%, males 75%; cave wet season: females 11%, males 83%; forest wet season: females 89%, males 100%; table 4.2).

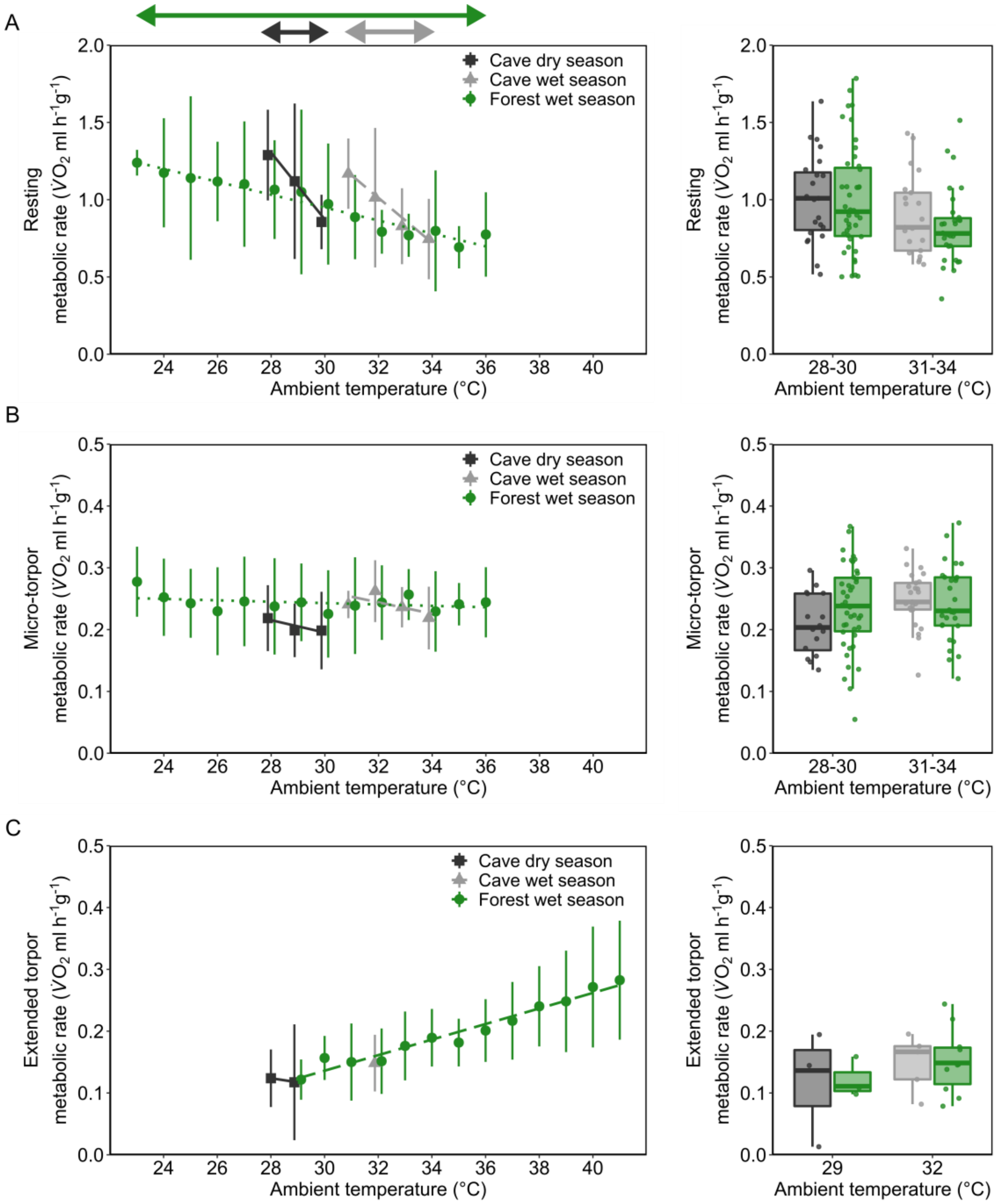

Figure 4.3. The mass-specific metabolic rate (MR, ml $\dot{V}O2$ h^{-1} g^{-1}) of bats when resting (A), in micro-torpor (B) and in extended torpor (C) in a cave in the dry season (dark grey squares), the same cave in the wet season (light grey triangles) and a tree roost in the wet season (green circles). The left panel shows MR as a function of ambient temperature; error bars represent standard deviation. The arrows above the left panel indicate the T_a range and colours correspond with roost/season. The right panel shows MR at only overlapping ambient temperature (centre line, median; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range; dots, data included). Please note the different y-axis scale of B and C.

Daytime resting energy expenditure (DREE)

Important predictors for total energy expenditure during daytime rest (DREE) were site-season and the physiological strategy used by the bats, i.e. either remaining euthermic (0.26 ± 0.10 kJ day^{-1} g^{-1}), entering micro-torpor bouts (0.14 ± 0.06 kJ day^{-1} g^{-1}), entering micro-torpor bouts together with an extended torpor bout (0.09 ± 0.05 kJ day^{-1} g^{-1}) or only entering extended torpor (0.05 ± 0.03 kJ day^{-1} g^{-1}; figure 4.4D). In this order, each response saved approximately 40, 44, 64 and 79% of the energy expended during a day remaining euthermic (figure 4.4D). Site-season had an effect, whereby the cave population in the wet season had a higher DREE than the other bats (table 4.2, table 3).

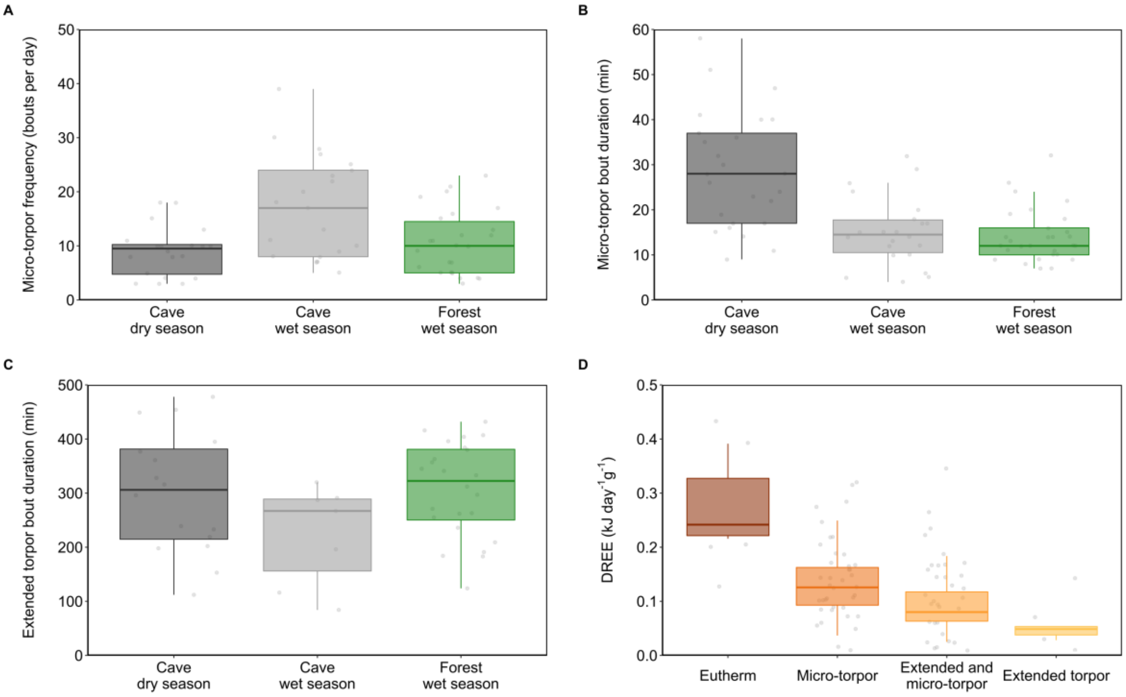

Figure 4.4. Micro-torpor bout frequency (A), micro-torpor bout duration (B) and extended torpor bout duration (C) of individuals roosting in cave habitat in the dry season (dark grey) or wet season (light grey) and in forest habitat in the wet season (green). (D) shows daytime resting energy expenditure according to metabolic strategy (remaining euthermic, entering micro-torpor bouts, entering micro-torpor bouts together with a more extended torpor bout, entering extended torpor; graduated orange shades). In all plots, the centre line represents the median, box limits indicate upper and lower quartiles, whiskers show 1.5× interquartile range and grey points are all data included.

Table 4.3. Estimated regression parameters, standard errors (SE), 95% confidence intervals (CI), test-statistics and alpha-levels for the best-fitting generalized linear mixed effects models (GLMMs), as well as the number of included individuals per model and the number of observations.

Estimates of GLMM for micro-torpor bout duration

Predictors	***Estimates***	***SE***	***CI***	***Statistic***	***p***
(Intercept)	27.32	3.44	21.34 – 34.96	26.26	**< 0.001**
Cave, wet season	0.52	0.08	0.38 – 0.71	-4.18	**< 0.001**
Forest, wet season	0.50	0.08	0.36 – 0.69	-4.26	**< 0.001**
Sex [males]	1.02	0.12	0.81 – 1.29	0.16	0.875
Body condition	0.97	0.07	0.85 – 1.10	-0.50	0.617

Number of bats: 37 | Number of observations: 76

Estimates of GLMM for micro-torpor bout frequency

Predictors	***Estimates***	***SE***	***CI***	***Statistic***	***p***
(Intercept)	9.33	1.38	6.97 – 12.48	15.04	**< 0.001**
Cave, wet season	1.88	0.32	1.34 – 2.63	3.68	**< 0.001**
Forest, wet season	1.28	0.24	0.89 – 1.84	1.33	0.182
Sex [males]	0.75	0.10	0.58 – 0.96	-2.27	**0.023**
Body condition	1.13	0.08	0.99 – 1.29	1.77	*0.077*

Number of bats: 37 | Number of observations: 76

Estimates of GLMM for extended torpor bout duration

Predictors	***Estimates***	***SE***	***CI***	***Statistic***	***p***
(Intercept)	385.37	30.36	325.86 – 444.87	12.69	**< 0.001**
Cave, wet season	-138.44	47.23	-231.01 – -45.88	-2.93	**0.003**
Forest, wet season	-94.73	40.80	-174.70 – -14.77	-2.32	**0.020**
Body condition	-121.33	32.45	-184.93 – -57.74	-3.74	**< 0.001**
Cave, wet season * body condition	84.52	40.81	4.53 – 164.52	2.07	**0.038**
Forest, wet season * body condition	97.76	46.35	1.91 – 188.61	2.11	*0.056*

Number of bats: 24 | Number of observations: 48

Estimates of GLMM for daytime resting energy expenditure

Predictors	***Estimates***	***SE***	***CI***	***Statistic***	***p***
(Intercept)	0.22	0.04	0.14 – 0.31	5.14	**< 0.001**
Cave, wet season	0.05	0.02	0.00 – 0.09	2.12	**0.034**
Forest, wet season	0.01	0.02	-0.03 – 0.06	0.55	0.581
Sex [males]	0.01	0.02	-0.02 – 0.04	0.73	0.468
Body condition	-0.01	0.01	-0.03 – 0.01	-1.05	0.294
Micro-torpor bouts	-0.11	0.04	-0.18 – -0.03	-2.62	**0.009**
Micro-torpor + extended torpor	-0.16	0.04	-0.24 – -0.08	-3.83	**< 0.001**
Extended torpor bouts	-0.17	0.04	-0.25 – -0.08	-4.01	**< 0.001**

Number of bats: 39 | Number of observations: 83

Discussion

Two populations of *M. commersoni* showed variation in their physiological responses to environmental conditions experienced while roosting in contrasting habitat types. For both populations, torpor was a key response used to finely balance energy expenditure. While metabolic rate during rest and during torpor were both similar across roosts, torpor timing, duration and frequency were flexibly adjusted to prevailing T_a and RH_a.

Surprisingly, cave-dwelling bats used relatively low rates of torpor in the leaner and cooler dry season. During this time of the year, the bats rarely leave their cave for months and food availability is drastically reduced (Razakarivony et al. 2005; Goodman 2006; Rakotoarivelo et al. 2007; Reher et al. 2019). Hence, we hypothesised that bats roosting in the cave would hibernate and show prolonged torpor (i.e., torpor bouts >24 h in duration) most often. Instead, only 60% of these individuals entered extended torpor and 80% used repetitive micro-torpor bouts, which was less than during the warmer, more abundant wet season (93%) or site (100%). Only one measured individual entered a multi-day torpor bout and it appears to be common that several strategies, i.e. hibernation (multi-day torpor), extended torpor, micro-torpor and no torpor, are used by different individuals within the same population in this species (Reher et al. 2018; Dausmann et al. *in press*). This was also observed in the Malagasy mouse lemur *Microcebus griseorufus* (Kobbe et al. 2011). Interestingly, the timing of both extended and micro-torpor occurred randomly throughout the day unlike during the wet season, when bats left the cave at dusk. Photoperiod is often a stimulus for torpor timing but in the absence of light T_a can also act as an important trigger (Heldmaier et al. 1982; Körtner and Geiser 2000). In Andranolovy Cave, however, T_a and RH_a were relatively constant. Animals living in a constant environment like this can experience shifts in their circadian rhythm. For instance, patterns of activity become arrhythmic in arctic mammals when they are confronted with continuous dark or light conditions (van Oort et al. 2005; Appenroth et al. 2021) and circadian patterns in the timing of arousal from torpor are lost in some hibernators (e.g., Körtner and Geiser 2000; Revel et al. 2007; Williams et al. 2017). The loss of circadian rhythmicity in torpor timing therefore indicates that these bats cease foraging during this time of year, even though it is likely that not all were hibernating. We suggest that a stable microclimate within the bat's thermal neutral zone (32-36°C; Reher and Dausmann 2021; this study), i.e. the T_a-range at which no active thermoregulatory support is needed to maintain euthermic T_b, permits an overwintering strategy fuelled by large fat deposits without the need for long-term hypometabolism.

Ample food resources are available in the wet season, making longer torpor bouts unnecessary. Longer active periods in a large colony also benefit social interaction (Kunz and Lumsden 2003). The cave-dwelling bats we studied used fewer extended torpor bouts in the wet season (40% of individuals) and these bouts were shorter than during the dry season or in the forest. Three out of six torpid animals in the cave were measured when the outer fringes of a cyclone crossed the region and the colony did not leave the cave for several days. Torpor is a powerful response for enduring extreme weather events such as droughts, heatwaves, storms and fires (Doucette et al. 2012; Bondarenco et al. 2014; Nowack et al. 2015; Stawski et al. 2015). It can also be beneficial in the non-lean season (Heldmaier et al. 2004; Geiser and Brigham 2012); for example, short torpor bouts can compensate for unsuccessful foraging (Heldmaier et al. 2004) or speed up pre-hibernation fattening (Giroud et al. 2012). Because *M. commersoni* accumulates fat at the end of the wet season (Goodman 2006; Jenkins and Racey 2008), extended torpor may be used flexibly on an individual basis or to get through environmental bottlenecks.

In contrast, forest-roosting bats made extensive use of torpor in the wet season (94% of individuals), which was more than the cave bats regardless of season (60% of individuals in the dry season and 40% in the wet season). Furthermore, extended torpor bouts used by forest bats in the wet season were as long as those used by dry season cave bats. This was unexpected because the foliage used for roosting did not offer protection from predators or daytime extremes of high T_a or low

RH_a. Water loss is presumably high under these conditions, as insensible water loss increases as ambient water vapour pressure decreases (Mitchell et al. 2018) and the maximum difference in RH_a between the cave and forest was 65% (cave: never below 94% RH_a, foliage: down to 29% RH_a at noon). During torpor water loss can be reduced by over 90% compared to euthermia, because water-consuming processes such as respiration, urine production and defecation are downregulated or even stopped (Morris et al. 1994; Webb et al. 1995; Muñoz-Garcia et al. 2012; Levin et al. 2015; Hill et al. 2016). Indeed, the timing of longer torpor bouts in *M. commersoni* is related to times when T_a exceeds euthermic T_b (Reher and Dausmann 2021). Above this threshold, only evaporative cooling can regulate T_b, which is unfavourable in a dry region if water reserves are unable to be replenished (Mitchell et al. 2018). Entering torpor at high T_a reduces metabolic heat and water production, allowing higher rates of heat from the environment to be stored in the body via facultative hyperthermia (Lovegrove et al. 2014; Welman et al. 2017; Reher and Dausmann 2021). Compared to the bats roosting in the cave at near stable conditions, it appears the forest bats in our study used this strategy to mitigate heat and water stress.

In addition to modulating torpor frequency and duration, heterotherms can adjust the level of metabolic reduction to reduce the costs of torpor while maximising the benefits (Boyles et al. 2020). The mean reduction in eTMR from euthermia (80-89%) and mTMR (69-75%) was relatively similar among the different roosting conditions. Given that T_{skin} during torpor ranged between 33.0°C in the cave and 38.5°C in the forest (total range: 24.6–42.9°C), this reduction in MR is remarkable and among the highest reported for warm environments (25–84%; Song et al. 1997; Dausmann et al. 2009; Grimpo et al. 2013; Kobbe et al. 2014). The observed variability in metabolic depression reflects the T_a fluctuations at each roost. Bats were torpid at higher a T_a, and therefore T_b, in the forest than in the cave. The forest and cave bats had a similar TMR at the same T_a, comparable to Kuhl's pipistrelle and big brown bats that also display a TMR that varies very little between populations roosting under different climatic conditions (Klüg-Baerwald and Brigham 2017; Gearhart et al. 2020). Therefore, the level of metabolic depression at a given T_a appears to be less plastic than torpor pattern variation in these bats (but see Dunbar and Brigham 2010). It is worth noting, however, that we excluded one individual that entered a multi-day torpor bout from analyses. The body condition of this individual was among the poorest 20% of bats in this study and it reduced MR by 94% at a T_{skin} of 33°C. TMR during multi-day torpor is lower than during daily torpor (Geiser 2004) and it is possible that this individual entered a multi-day torpor bout to maximise energetic savings and slow the depletion of its meagre fat reserves (Humphries et al. 2003; Jonasson and Willis 2011).

Macronycteris commersoni appeared to avoid longer torpor bouts when energy or water conservation were not vital. Instead, they entered the shortest torpor bouts of any heterotherm studied so far. All but three cave-dwelling individuals used micro-torpor bouts with patterns reflecting prevailing environmental conditions, appearing to be the preferred mode of torpor. Micro-torpor bouts were less frequent but longer during the dry season than the wet season. They were especially frequent in cave bats during the wet season and in the forest, they were used in combination with an extended torpor bout on hot days (Reher and Dausmann 2021). Micro-torpor bouts combine an increased number of active phases, and thus higher vigilance, with energy and water savings. This could enable bats in exposed roosts in foliage to react quickly to threats and be beneficial in caves for maintaining social and territorial activities (Kunz and Lumsden 2003). However, micro-torpor usually occurred in a repetitive manner (12.0 ± 6.9 consecutive bouts), increasing not only the number of active periods but also the number of arousal phases. Arousing from torpor is potentially harmful, especially at low T_a, because it increases oxidative stress and can cause cellular damage (Carey et al. 2003; Brown and Staples 2011; Nowack et al. 2019). Interestingly, Australian desert bats can arouse from torpor passively without active thermogenesis when T_a is near euthermic T_b (Bondarenco et al. 2013) and the energetic costs of rewarming from torpor for long-eared bats are reduced at higher T_a (Currie et al. 2014). Since *M. commersoni* MR during an arousal period rarely exceeded RMR, we assume that the costs of arousing at thermoneutrality are negligible, making micro-torpor an effective strategy.

Flexible torpor expression helped *M. commersoni* cope with different roosting conditions but its use comes with potential costs. These include reduced responsiveness to the environment, missed opportunities for reproduction, reduced territory defence (Choi et al. 1998; Mzilikazi and Lovegrove 2002), diminished immune function and memory loss during hibernation (Millesi et al. 2001; Bouma et al. 2010; Landes et al. 2020). Energy and water can also be conserved by adjusting RMR; many mammals shift their TNZ in response to seasonal changes in T_a to reduce energy expenditure during euthermia (Lovegrove 2005). We did not find any differences between roosts or seasons in overall RMR or RMR at overlapping T_a. This is unsurprising within the cave roost because it offers stable ambient conditions year-round. However, the lack of difference in RMR between the cave and forest roosts is unexpected, given the large fluctuations in T_a in the forest. The forest population has a TNZ between ~32-36°C in the wet season (Reher and Dausmann 2021); while we were unable to determine the cave population's TNZ due to very stable conditions, we would suspect their TNZ to be close to the cave’s 32°C. Thus, our measurements were probably below their thermal neutral conditions, suggested by the steeper slope in RMR. A more experimentally driven approach examining the bats’ responses under controlled conditions may consequently uncover geographical and seasonal variation in RMR.

Despite marked environmental differences between seasons and sites, bat body condition was similar among groups. Only forest-dwelling females had lower body condition than other bats, but sex was only a predictor for micro-torpor bout frequency and body condition only a predictor of extended torpor bout duration in the models. Since little is known about *M. commersoni* in general, we speculate that possibly younger, leaner females were present in the study or that reproduction is much more costly in the forest. We conducted the wet season measurements between mid-February and the end of March to keep seasonal life-history differences low, but it is likely that some females were still recuperating the costs of recent reproductive activities (in the cave, females wean their offspring in January; pers. obs.). In the wet season, DREE in the forest was ~1.6 times lower than in the cave and as low as in the cave in the dry season. This was largely due to the use of extended torpor as a response to heat; flexible torpor use dictated energy expenditure and not roosting conditions *per se*. For example, using several repeated micro-torpor bouts throughout the day reduced DREE by 45% compared to remaining euthermic; several micro-bouts combined with extended torpor bouts reduced DREE by 70% and entering a single, even longer extended torpor bout reduced DREE by 84%. Individual bats could balance DREE and body condition by adjusting bout duration and frequency. This strategy, combined with the insignificant costs of arousal from torpor at high T_b, equips *M. commersoni* with a versatile physiological toolbox. Its broad repertoire allows the regulation of energy consumption and water depletion in direct response to prevailing conditions at a fine scale. This enables the species to effectively compensate for variable environmental pressures, and roost under contrasting ambient conditions, with little to no variation in body condition.

Our findings stress that physiological traits are not fixed within a species over seasonal and geographic scales. While there are many published studies of seasonal physiological variation (e.g. (Brigham et al. 2000; Stawski and Geiser 2010; Czenze et al. 2017a), fewer examples exist investigating how separate populations of the same species cope with different environmental pressures (e.g., Dunbar and Brigham 2010; Stawski 2012; Noakes et al. 2016; Czenze et al. 2017b; van Jaarsveld et al. 2021). In a region like Madagascar, where almost 20% of the endemic vertebrate fauna are endangered or facing extinction (IUCN 2021), it is of utmost importance to understand the entire range of variability within species' ecophysiological traits. The variation in physiological traits that we observed for *M. commersoni*, for example, allows it to roost under vastly different environmental conditions, which has likely contributed to its successful colonisation of a range of habitat types distributed across almost all of Madagascar. Whether the flexibility uncovered in our study is related to phenotypic flexibility and can be expressed by individual *M. commersoni*, or whether these are local adaptations of each population, remains unclear (Geiser and Ferguson 2001; Dunbar and Brigham 2010). Nonetheless, our results demonstrate that conclusions drawn from limited datasets may not accurately represent a species as a whole. Studying more than one population and at different times of the year is logistically and financially challenging. However, this can illuminate intraspecific physiological, behavioural or morphological variation and ultimately give a clearer picture of a species' potential for enduring a range of environmental pressures. Such insight is vital for predicting the consequences of disturbance events or rapid climatic changes and ensuring that conservation and species management actions do not fall short of their targets in regions with high environmental variability.

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Ethics statement

This study was conducted under the "Accord de Collaboration" between Université d'Antananarivo (Département de Biologie Animale), Madagascar National Parks and Universität Hamburg. We thank these authorities and the Ministère de l'Environnement, de l'Ecologie et des Forêts for support and project authorisation. The research was approved by the Directeur du Système des Aires Protégées, Ministère de l'Environnement, Antananarivo (Autorisation de recherche no. 90/16/MEEMF/SG/DGF/DAPT/SCBT.Re, 003 & 296/17/MEEF/SG/DGF/DSAP/SCB.Re) and all described procedures comply with the current ethical regulations and laws of Madagascar for the care and use of animals.

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Supplementary material

Table S1. Detailed test-statistics of t-tests and Wilcoxon tests regarding the forearm length, body mass and body condition during torpid states of bats roosting in a cave during the dry and wet season as well as a forest in the wet season. Significance was adjusted through Bonferroni-Holm correction.

Variables tested	Test-statistics	*p*-value
Forearm length females		
Cave dry season vs. cave wet season	*W* = 21	0.529
Cave dry season vs. forest wet season	*W* = 0	**< 0.001**
Cave wet season vs. forest wet season	*W* = 0	**< 0.001**
Forearm length males		
Cave dry season vs. cave wet season	*W* = 11	0.914
Cave dry season vs. forest wet season	*W* = 0	**0.012**
Cave wet season vs. forest wet season	*W* = 0	**0.004**
Body mass females		
Cave dry season vs. cave wet season	*W* = 28	1
Cave dry season vs. forest wet season	*W* = 26.5	1
Cave wet season vs. forest wet season	*W* = 31	1
Body mass males		
Cave dry season vs. cave wet season	*W* = 15	0.61
Cave dry season vs. forest wet season	*W* = 2	*0.059*
Cave wet season vs. forest wet season	*W* = 10	*0.061*
Body condition females		
Cave dry season vs. cave wet season	*t* = 0.213	0.835
Cave dry season vs. forest wet season	*t* = 4.26	**0.009**
Cave wet season vs. forest wet season	*t* = 4.24	**0.005**
Body condition males		
Cave dry season vs. cave wet season	*t* = 0.273	1
Cave dry season vs. forest wet season	*t* = 0.200	1
Cave wet season vs. forest wet season	*t* = -0.160	1

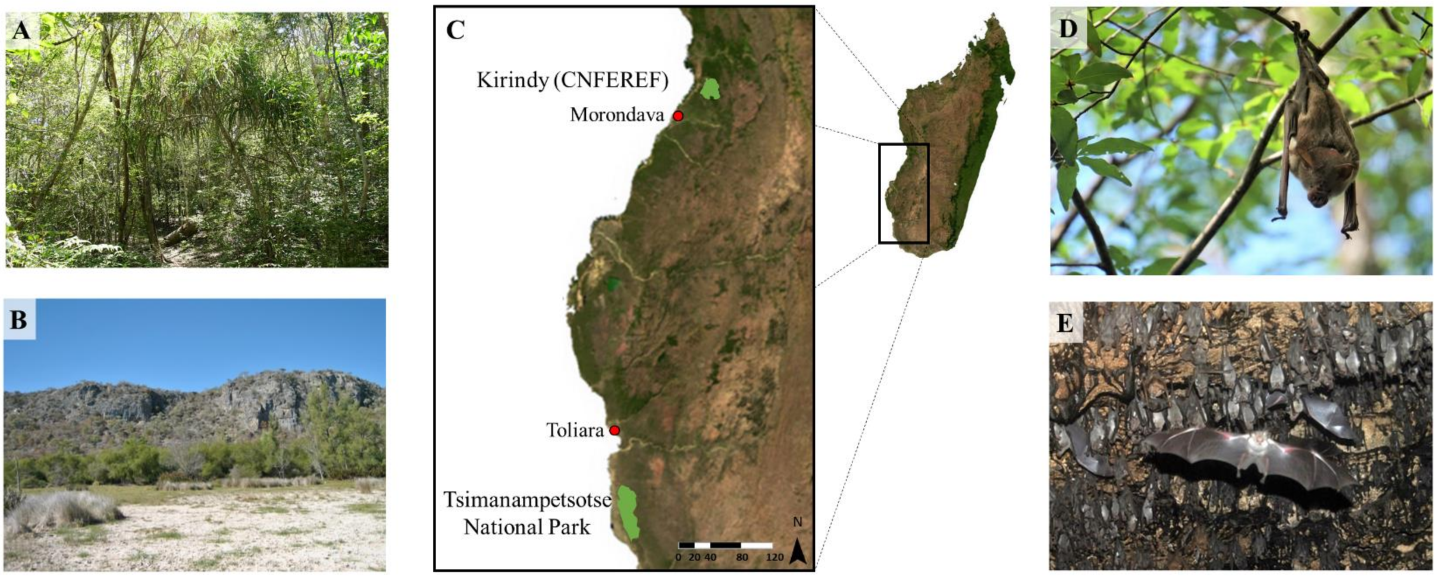

Figure S1. Typical habitat structure and roosting habits of *M. commersoni* in A) deciduous dry forest in Kirindy Forest (CNFEREF) and D) a tree roost; B) dry spiny forest in Tsimanampetsotsa National Park and E) a cave roost. C) shows the locations of the two study sites in Madagascar. Photo credi: Stephanie Reher.

Table S2. Rayleigh's test statistics, including sample sizes (N = individuals, n = pooled bouts across all individuals), describing the timing of entry into, and arousal from, extended and micro-torpor bouts in each season and roost site. ns indicates event timing that did not differ from a random distribution.

	Time of day	Test-statistics (*r*)	*p*-value	Sample size
Extended torpor				
Cave, dry season, entry	ns	0.25	0.3234	N = 6, n = 19
Cave, dry season, arousal	ns	0.33	0.1537	
Cave, wet season, entry	09:21 ± 00:40 h	0.80	**0.006**	N = 6, n = 7
Cave, wet season, arousal	14:54 ± 00:29 h	0.89	**0.001**	
Forest, wet season, entry	10:23 ± 00:12 h	0.91	**< 0.001**	N = 14, n = 22
Forest, wet season, arousal	16:56 ± 00:17 h	0.96	**< 0.001**	
Micro-torpor				
Cave, dry season, entry	ns	0.05	0.2787	N = 9, n = 538
Cave, dry season, arousal	ns	0.06	0.1403	
Cave, wet season, entry	12:58 ± 00:05 h	0.34	**< 0.001**	N = 14, n = 475
Cave, wet season, arousal	13:18 ± 00:07 h	0.34	**< 0.001**	
Forest, wet season, entry	09:28 ± 00:09 h	0.20	**< 0.001**	N = 16, n = 456
Forest, wet season, arousal	09:43 ± 00:11 h	0.20	**< 0.001**	

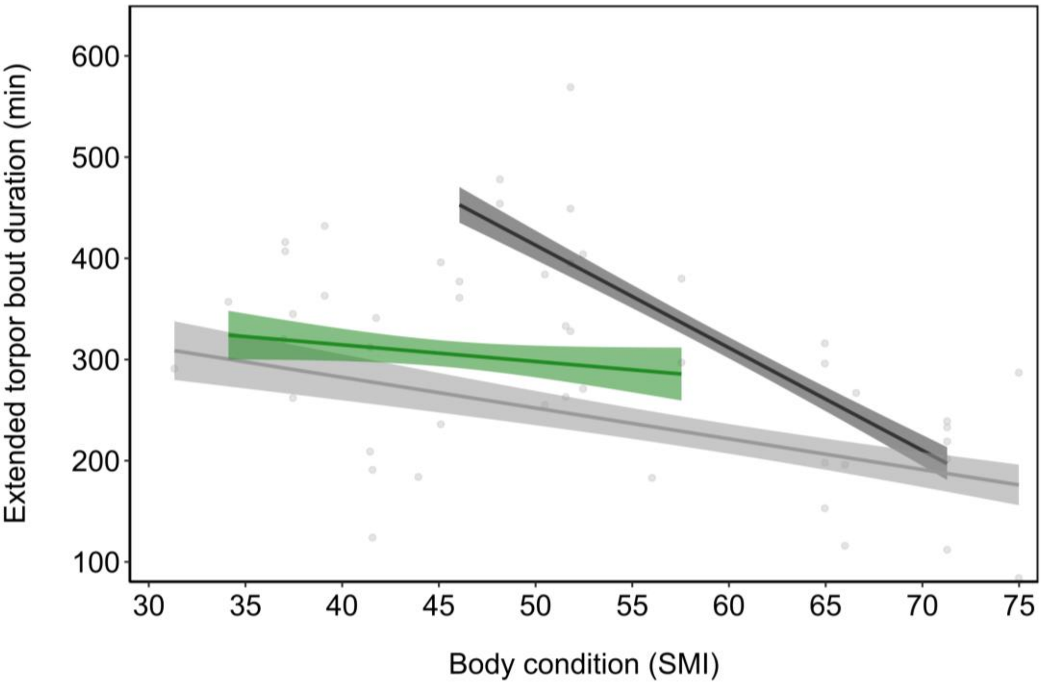

Figure S3. Estimated duration of extended torpor bouts (min) with 95% confidence interval (shaded area) as a function of body conditions (given as standardised mass index) of forest-dwelling bats (green) as well as cave-dwelling bats in the dry (dark grey) and wet season (light grey).

Author contribution

I hereby confirm that Stephanie Reher (SR) and Kathrin H. Dausmann (KHD) conceived and designed the study, SR conducted the measurements in the field with support of Hajatiana Rabarison, SR analysed the data with support of B. Karina Montero and took the lead in writing the manuscript with input from KHD. SR, KHD, BKM and JMT discussed and interpreted the results, revised the manuscript and all authors approved the final version of it.

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Chapter 5

Local acclimatisation hampers physiological compensation of rapid environmental changes in a Malagasy bat

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In preparation

Abstract

Rapid environmental changes are challenging for endothermic species because they have direct and immediate impacts on their physiology by affecting microclimate and fundamental resource availability. Physiological variation can compensate for certain ecological perturbations, but our basic understanding of how species function in a given habitat and the extent of their adaptive scope is limited. Here, we studied the effect of acute microclimate change on the thermal physiology of two populations of the widespread Malagasy bat *Macronycteris commersoni*. Bats of this species are found roosting under contrasting conditions, i.e. in a constant hot and humid cave or below foliage unprotected from ambient fluctuations. We exposed free-ranging individuals of each population to the respective opposite conditions and thus to novel microclimate within an ecologically realistic scope while measuring metabolic rate and skin temperature. Cave bats in forest settings had a limited capacity to maintain euthermia to the point that two individuals became hypothermic when ambient temperature dropped below their commonly experienced cave temperature. Forest bats on the other hand, had difficulties to dissipate heat in the humid cave set-up. The response to heat, however, was surprisingly uniform and all bats entered torpor combined with hyperthermia at temperatures exceeding their thermoneutral zones. Thus, while we observed potential for flexible compensation of heat through "hot" torpor, both populations showed patterns suggestive of limited potential to cope with conditions outside their typically occupied microclimates. Our study emphasises that intraspecific variation among populations could be misleading when assessing species' adaptive scopes, as variation may arise from genetic adaptation or phenotypic plasticity, and only the latter allows flexible compensatory responses. Disentangling these mechanisms and identifying the basis of variation is vital to make accurate predictions of species' chances for persisting in ever rapidly changing habitats and climates.

Keywords

Physiological flexibility, hyperthermia, hypothermia, torpor, tropics

Introduction

Rapid environmental alteration has an immediate impact on the ecological stability of habitats and their inhabitants. Interferences such as extreme weather events and habitat destruction affect the prevailing habitat structure, microclimatic conditions and/or resource availability and may thus disrupt species' physiological functioning (Schmidt-Nielsen 1999; Seebacher and Franklin 2012; Tattersall et al. 2012). Certain environmental variation, however, can be mitigated by physiological variation since the physiological scope determines species' tolerance limits to abiotic factors (Canale and Henry 2010; Bozinovic et al. 2011). Indeed, many endotherms living in unreliable or highly seasonal habitats or those that are widespread and face heterogeneous environmental conditions, have developed considerable variation in traits associated with thermal maintenance, and these may vary among and within populations (e.g., Stawski and Geiser 2010; Glanville 2012; Noakes et al. 2016; van Jaarsveld 2021). For example, in many species, populations inhabiting arid habitats have lower water turnover rates and mechanisms to retain water more efficiently than their conspecifics from more mesic habitats (e.g., Klüg-Baerwald and Brigham 2017; Cooper et al. 2018; Gearhart et al. 2020). Quantifying such intraspecific variation can aid our understanding of the physiological compensatory capacity of a species and ultimately its general ecological resilience.

Intraspecific physiological variation may arise from genetic changes across generations in response to different local selection pressures and may even imply incipient speciation (Angiletta et al. 2010; Violle et al. 2012; Richardson et al. 2014). Especially widespread species may face contrasting environmental conditions throughout their distribution and distinct populations may have locally adapted (Tracy and Walsberg 2000). Thus, variation observed among populations does not necessarily entail that each population can generally take advantage of the entire flexibility seen in a species. Phenotypic flexibility by contrast allows fast and reversible adjustments to a changing environment, e.g., through acclimatisation (Piersma and Drent 2003). For example, in zebra finches (*Taeniopygia guttata*) already short-term previous experience with high T_a conferred a more favourable physiological response during subsequent heat exposure (Cooper et al. 2020). Moreover, Djungarian hamsters (*Phodopus sungorus*) considerably increase the amount of brown adipose tissue and thus the capacity for non-shivering thermogenesis supporting thermoregulation at low T_a within weeks of cold exposure (Heldmaier and Hoffmann 1974; Heldmaier et al. 2013). Such flexible responses are considered key for species' survival in the long-term, as genetic-based traits are not necessarily adaptive in short term (Ghalambor et al. 2007; Canale and Henry 2010; Boyles et al. 2011; Huey et al. 2012). Disentangling these different mechanisms and identifying the basis of variation is therefore vital when assessing species' and populations' scopes for coping with rapid environmental perturbations but also when managing conservation action such as translocating wildlife to new habitat (Tarszisz et al. 2014; Madliger and Love 2015; Cooper et al. 2018).

Heterothermic species may have a pre-adaptive advantage for responding flexibly and efficiently to short-term changes through their ability to temporally abandon a euthermic life-style and enter energy- and water saving torpor (Geiser 2004; Heldmaier et al. 2004 Herreid and Schmidt-Nielsen 1966; Cooper et al. 2005; Levin et al. 2015). Torpor bout duration, frequency and/or level of metabolic depression may be finely adjusted depending on environmental pressures and individual constraints (Stawski and Geiser 2011; Turner et al. 2012; Lovegrove et al. 2014). Moreover, torpor has proven a powerful response for enduring acute disturbances caused by extreme weather events such as droughts, heatwaves, storms, fires and flooding (Doucette et al. 2012; Bondarenco et al. 2014; Nowack et al. 2015; Stawski et al. 2015; Nowack et al. 2016; Barak et al. 2019). The tropical Commerson's leaf-nosed bat *Macronycteris commersoni*, for example, makes extensive use of torpor in an exposed foliage roost, and torpor timing as well as duration are related to daytime heat (Reher and Dausmann 2021). This species is among the widest distributed bat species in Madagascar (Goodman 2011). In addition to exposed foliage, it also roosts in a large colony in a well-buffered hot cave that is offering a highly stable microclimate year-round (32°C at bat height, 98% relative humidity; Reher et al. 2018), with torpor patterns, i.e. frequency, duration and timing, varying depending on respective roost condition (Reher et al. submitted). Both populations are vulnerable to several threats: the cave population has already declined substantially due to ongoing unsustainable hunting and individuals may be forced to leave the cave when disturbance persists (Goodman 2006; Andriafidison et al. 2008). Simultaneously, the forest population is increasingly confronted with habitat destruction (Zinner et al. 2014) as well as global warming and its concomitants, i.e. warming of remaining fragments, more frequent heat waves and droughts (Tuff et al. 2016; Nematchoua et al. 2018). Thus, it is of major concern to understand to what extent these bats can potentially benefit from flexible compensatory mechanisms.

We aimed to examine the effects of rapid environmental change on the metabolic responses of a forest and a cave population of *M. commersoni*. We manipulated the bats' roosting microclimate to resemble the respective contrasting/opposite conditions (cave. vs foliage) and examined their thermo-physiology to determine the bats' physiological flexibility. Although these manipulated environmental conditions were outside the range normally experienced by the particular population, they were within the normal range faced by the species and thus within an ecologically realistic scope.

Methods

Study species and study sites

To study intraspecific flexibility on a population-level, we worked with the endemic insectivorous Commerson's leaf-nosed bat *Macronycteris commersoni* (Hipposideridae). It is widespread across various vegetation formations and climatic regimes in Madagascar (only absent in the central highlands) and uses different types of diurnal roosts within its range (Goodman 2011). For example, in dry spiny forest habitat, as in Tsimanampetsotse National Park in south-western Madagascar, a population of *M. commersoni* roosts in a humid hot cave year-round ("cave habitat"; Reher et al. 2019), while another population roosts in the open vegetation among branches in dry deciduous forest ~380 km further north, in Kirindy Forest/CNFEREF ("forest habitat"; Reher and Dausmann 2021). The hot cave is well buffered and offers constant conditions of 32°C and 98% relative humidity at bat height (Reher et al. 2018), whereas the bats roosting in exposed foliage without any insulation have to cope with daily ambient fluctuations and weather extremes (Reher and Dausmann 2021). Both habitats are situated in Madagascar's drier western formations and highly seasonal, with a pronounced dry season without any rainfall for up to 9 months and reduced resource availability. In dry spiny forest, rain may also be absent for several years (Ratovonamana et al. 2013). T_a fluctuates on a daily basis between 12.6°C and 32.5°C (mean daily minimum and maximum; cave habitat) as well as 13.3 and 34.0°C (forest habitat) in the dry season. During the wet season, resources are more abundant and particularly night-time T_a is higher, with daily fluctuations between 24.4°C and 38.5°C (cave habitat) as well as 23.6 and 36.0°C (forest habitat).

Trapping and handling

We trapped bats in June/July 2016 (dry season) and February/March 2017 (wet season) in the cave habitat and in February/March as well as July/August 2018 in the forest habitat (wet season and dry season, respectively). Depending on habitat structure and bats' roosting behaviour, we used different trapping methods. In the cave habitat during the dry season, we trapped the bats with a hand net in the early morning hours between 07:00 and 09:00 because this species is inactive during this time of the year. In the wet season, we erected a harp trap (two-bank 4.2m^2 harp trap; Faunatech~Austbat, Bairnsdale, Australia) in front of the same cave's entrance. In the forest habitat, two to three mist nets (3m height x 6m length, mesh size 19mm; Ecotone, Sopot, Poland) per trapping night were installed blocking main flight corridors in the local grid systems N5, CS5, CS6 and CS7 in both seasons. The trap and nets remained open for the first three hours after sunset and were checked every 10 to 20min depending on season. We transferred a maximum of two bats into separate cloth bags and released additional adults and juveniles immediately at the capture site. None of the females were reproductively active during data collection.

We manipulated the bats at the point of capture. They were weighed to the nearest of 1g, sexed and the forearm length was measured. We removed a patch of fur between the shoulder blades using a razor blade and attached a temperature-sensitive radio transmitter (~ 0.9g; Biotrack, Wareham, UK) with medical skin glue (Osto-Bond, Montreal, Canada; or Manfred Sauer GmbH, Lobbach, Germany), whereby the thermal sensor was placed directly on the skin. The proportion of transmitter mass to the lowest bat's body mass was 2.7% (mean = 1.8 ± 0.43%, N=70 bats) and thus well below recommended maxima of 5-10% (e.g., Aldridge and Brigham 1988). All transmitters were calibrated in a water bath at seven intervals over a temperature range of 3–45°C against a precision thermometer traceable to a national standard. T_{skin} obtained via external transmitters provide a non-invasive and reliable proxy of T_b, particularly in small mammals (Audet and Thomas 1996; Dausmann 2005; Langer and Fietz 2014; but see Willis and Brigham 2003). We marked all bats with an individual, three-digit wing tattoo using non-toxic ink (Hauptner-Herberholz, Solingen, Germany) after locally anaesthetising the membrane (EMLA, AstraZeneca, Wedel, Germany). Complete animal handling lasted about 10min and never exceeded 15min.

Study set-up

To study the effects of rapid environmental changes on the metabolic responses of the respective populations, we exposed some individuals of each population to the respective contrasting roosting conditions, i.e. cave bats had to cope with fluctuating conditions outside the cave and the forest population with constant hot and humid conditions. In the cave habitat, we ran the measurements (N=10 dry season, N=13 wet season) in a shaded enclosure near the camp without any further buffer to ambient extremes. In the forest habitat, two modified incubators (Exo Terra PT2445; HAGEN, Holm, Germany) served as climate chambers to mimic cave conditions and were calibrated to maintain constant T_a = 32.1 ± 0.3°C and RH_a = 88.9 ± 2.4%. RH_a was increased by passing the air entering the climate chamber through a humidified sponge and by adding a drawer with water on the floor of the climate chamber. Although 88.9% does not reflect cave relative humidity precisely (i.e. 98%), it never dropped below 82% and was as close as we could get under field conditions. We placed the respirometry chamber into the climate chamber and connected it to the oxygen analyser, whose pump drew humidified air from the climate chamber through the respirometry chamber and finally the excurrent dried air to the analyser. Since we did not trap any bats in the forest during the dry season, we only ran these measurements in the wet season (N=10). These data were then compared to the "original" physiological responses, parts of which are summarised in Reher et al. (submitted).

Respirometry run and temperature measurements

We used an open-flow respirometry system in pull mode to measure the rate of oxygen consumption ($\dot{V}O_2$). We transferred the bats individually into 2L plastic metabolic chambers, which were equipped with a net for the bats to hold onto, right after preparing them at their respective capture sites. The chambers were connected to portable gas analysers (OxBox; T. Ruf and T. Paumann, University of Veterinary Medicine Vienna, Austria) with integrated electro-chemical oxygen cells (Bieler & Lang, Achern, Germany). The oxygen sensors were calibrated in the laboratory before and after each field trip using room air and calibration gases generated by a gas mixing pump (0, 3, and 5% N_2 mixed with fresh air; 2KM300/a, Wösthoff Messtechnik GmbH, Bochum, Germany). During the measurements, ambient air was pulled through the respirometry chamber at a constant flow of 50L h^{-1} using a diaphragm pump, dried with silica gel and filtered before passing through a flowmeter integrated in the OxBoxes. An aliquot thereof was then drawn through the gas analysers. The oxygen content of the sample air was measured every 10s for 55min; reference air from outside the metabolic chambers was also dried with silica gel, filtered and its oxygen content was measured every 10s for the remaining 5min to control for drifts in the oxygen sensors. These data were used to correct the oxygen traces hourly (Clampfit v10.3.1.4, Molecular Devices, Sunnyvale, USA).

The measurements started after capture and processing between 21:00 and 23:00 hr in the wet season and 09:00 and 11:00 hr in the dry season owing to different trapping times. One respirometry run typically lasted between 20 and 45 hr and ended with the beginning of the bats' usual active phase between 17:30 and 18:30 hr to ensure immediate foraging possibilities. We used the first metabolic rate values right after introducing the bats to the metabolic chambers as indicators of individual stress levels. If there was no marked decrease in these initial values within 30 or 60min (depending on the inactive or active phase, respectively), the bats were released at the capture site. All individuals were provided with fresh water and food after 24 hr (see Reher et al. 2018). Before we released them at their capture site, they were weighed a second time to calculate mean body mass (BM) and again offered food and water. During each respirometry run, we recorded T_a and ambient relative humidity (RH_a) inside each respirometry chamber (Hygrochron iButtons, Maxim integrated, San Jose, USA), and T_{skin} using a remote receiver/logger placed next to the setup (DataSika SRX-800-D, Biotrack, Wareham, UK). All temperature data were recorded at 5-minute intervals.

Data processing and analyses

We processed and analysed all data using Cran R (R Core Team 2016) in the "RStudio" environment (RStudio Team 2016) with support of the packages "plyr" (Wickham 2011), "dplyr" (Wickham et al. 2020), "readxl" (Wickham and Bryan 2017), "lubridate" (Grolemund and Wickham 2011), "zoo" (Zeileis and Grothendieck 2005), “stringr” (Wickam 2019), ”tidyr” (Wickham 2020), “ggplot2” (Wickham 2016), “ggpubr” (Kassambara 2020a) and "cowplot" (Wilke 2020).

Of all data sets, including those collected under natural conditions (Reher et al. submitted), only the first complete day of a measurement (from sunset to sunset) was analysed because we had picked a shorter measurement duration when exposing cave bats to fluctuating conditions owing to the hot and dry climate outside the cave. Moreover, because the forest environment is somewhat milder and both warm (cloudy, rainy) and very hot days can occur in the rainy season, we only included data from forest bats measured on hot days (N=12 bats), which better resembled the southern regular weather. We calculated individual metabolic rate (MR, ml $\dot{V}O_2$ h^{-1}), using the rate of oxygen consumption ($\dot{V}O_2$) as ml O_2 h^{-1} corrected to STPD with equation 11.2 given by Lighton (2008) specifically for this set-up, assuming an average respiratory quotient of 0.85 (oxidation of 50% fat and 50% carbohydrate, Dausmann et al. 2009). Mean body mass (BM) was used for mass-specific MR calculations (ml $\dot{V}O_2$ h^{-1} g^{-1}). We defined different physiological states via visual inspection of MR patterns following Reher and Dausmann (2021). Thereby, a drop in MR by at least 50% compared to resting MR (RMR) was considered to constitute torpor, which lies within the range of highest metabolic reductions seen during torpor in warm environments (25–84%; Song et al. 1997; Dausmann et al. 2009; Grimpo et al. 2013; Kobbe et al. 2014). We removed torpor arousal and torpor entry phases and continued with a subset of MR data to determine RMR and torpid MR (TMR). For RMR, we only included the lowest 50% of resting data per hour and per individual within the bats’ usual resting phase, i.e. from sunrise to 30 min before sunset to ensure that variations owing to disturbance or minor activity were excluded (Bethge et al. 2017; Reher and Dausmann 2021; Rodgers and Franklin 2021). For TMR, we included the lowest 70% of data per hour and per individual. *Macronycteris commersoni* in known to enter torpor bouts of varying lengths, including micro-torpor bouts lasting 12-20 min on average (Reher and Dausmann 2021; Reher et al. submitted). However, the bats rarely entered micro-bouts in the manipulated conditions and we therefore only included torpor bouts lasting ≥1 hr for downstream analysis. We allocated RMR and TMR to different bins of T_a by rounding T_a to the nearest integer and assigning individual means of the different metabolic states to the respective T_a to avoid pseudo-replication.

To obtain skin temperature (T_{skin}), we converted the recorded transmitter signals from beat per minutes into temperature data using second-order polynomial regressions obtained from the calibration curves (all $R^2 \geq 0.99$). T_{skin} was used as a proxy for body temperature (T_b) to complement already defined torpor states through MR.

Forest-dwelling bats exposed to cave conditions (wet season)

The natural cave and artificially created cave conditions were highly constant in the wet season. We therefore used t-tests adjusted for unequal variances if necessary or Wilcoxon signed-rank tests (package "rstatix"; Kassambara 2020b) to compare RMR, TMR, T_{skin}, T_{skin}-T_a differential, time spent euthermic and the fraction of the measurement duration spent torpid between forest-dwelling bats exposed to cave conditions and cave-dwelling bats. To determine whether the timing of torpor entry differed between populations, we applied Watson two-tailed tests (library "circular," Jammalamadaka and Sengupta 2001). Time is given as circular mean ± standard deviation.

Cave-dwelling bats exposed to forest conditions (wet season)

To analyse the cave bats physiological responses in fluctuating forest conditions in the wet season, we explored the effect T_a, population as well as the interaction of T_a and population on different physiological variables. We fitted separate generalised linear mixed effect models (GLMMs; package "lme4", Bates et al. 2015) for TMR and T_{skin}, accounting for repeated measures by including bat ID as random effect. We fitted a similar GLMM for RMR below the TNZ to compare the respective slopes and used a Wilcoxon-test to identify potential differences in RMR within the TNZ between forest bats and cave bats exposed to forest conditions. For the forest population, the TNZ has already been calculated in Reher and Dausmann (2021) and we therefore followed the same approach for the cave population. We estimated the lower and upper limit using broken-stick regression, i.e. iterative fitting of linear models combined with a bootstrap restarting approach, making the algorithm less sensitive to the estimated starting value, to determine whether the observed RMR distribution could be explained by multiple linear segments (Wood 2001; Muggeo 2008). We did not fit separate models for torpid T_{skin} below the TNZ despite obvious variation between populations owing to the small sample size (N_{forest}=2 at $T_a \leq 31°C$). Furthermore, RH_a was excluded from the analysis because it was negatively correlated with T_a in the fluctuating environmental set-ups. We also did not include body conditions and pooled the data of females and males, as both were no important predictors when modelling RMR or TMR during longer torpor bouts in *M. commersoni* (Reher et al. submitted). Data exploration and model validation were done following Zuur et al. (2009; 2010) and Zuur and Ieno (2016).

Cave-dwelling bats exposed to forest conditions (dry season)

We did not trap any *M. commersoni* in the dry season in the forest habitat. Thus, we reported the cave-dwelling bats' physiological responses when exposed to fluctuating conditions outside their cave descriptively and occasionally analysed single variables to all other bats, i.e. torpor bout duration and TMR, using t-tests or Wilcoxon signed-rank tests. We compared energetic costs imposed by the unfamiliar roosting conditions compared to what the respective populations usually consume in their natural roosting environment. Energy expenditure was calculated as 20-hour energy expenditure (EE, in kJ) from 22:00 – 18:00 based on per-minute metabolic rate values using an oxycalorific equivalent of 20.37 kJ/L O_2 (Schmidt-Nielsen 1997). All data are shown as mean ± standard deviation and range if appropriate; N represents the number of individuals.

Results

In total, we worked with 70 bats (table 5.1). In the wet-season, we measured 12 individuals from the forest population under their natural conditions (BM=61.8 ± 13.1g) and 10 individuals from the forest-population under cave conditions (BM=63.9 ± 12.8g); further we exposed 15 cave-dwelling bats to their natural conditions (BM=49.6 ± 9.7g) and 13 cave-dwelling bats to fluctuating forest conditions in the wet season (BM=59.7 ± 12.5g). In the dry season, we measured 10 cave-dwelling bats under natural conditions (BM=49.3 ± 10.4g) and 10 individuals from the cave population were exposed to forest conditions (BM=62.4 ± 14.4g).

Table 5.1. Overview of the different populations in the dry and wet seasons exposed to either cave or forest conditions, and their key physiological variables. Shown are the number of bats included in the analysis, the number of individuals entering torpor, mean metabolic rate (MR) and skin temperature (T_{skin}) during torpor and euthermia, the mean duration spent in each respective state, the fraction of a day spent torpid, and daily energy expenditure (DEE).

	Cave conditions			**Forest condition**		
	Dry season	Wet season		Dry season	Wet season	
	Cave population	**Cave population**	**Forest population**	**Cave population**	**Cave population**	**Forest population**
Bats included in analysis (females | males)	N=10 (6 | 4)	N=15 (6 | 9)	N=10 (6 | 4)	N=10 (3 | 7)	N=13 (4 | 9)	N=12 (6 | 6)
Bats that used torpor (N, ratio [%])	N=6 (60%)	N=7 (47%)	N=10 (100%)	N=10 (100%)	N=13 (100%)	N=12 (100%)
MR during rest (ml O_2 h^{-1} g^{-1})	0.86 ± 0.15	1.01 ± 0.29	0.95 ± 0.17	0.91 ± 0.20	0.91 ± 0.19	0.94 ± 0.23
MR during torpor (ml O_2 h^{-1} g^{-1})	0.19 ± 0.08	0.30 ± 0.14	0.18 ± 0.06	0.11 ± 0.05	0.22 ± 0.09	0.19 ± 0.05
T_{skin} during euthermia (°C)	35.4 ± 1.8	35.1 ± 1.2	37.9 ± 0.7	27.6 ± 1.9	35.2 ± 2.0	35.4 ± 2.0
T_{skin} during torpor (°C)	31.8 ± 2.5	33.6 ± 1.3	36.2 ± 0.6	28.7 ± 6.6	37.4 ± 2.7	35.8 ± 2.6
Duration of euthermic periods (min)	165 ± 97	320 ± 143	19 ± 14	437 ± 253	188 ± 112	153 ± 74
Torpor bout duration (min)	436 ± 257	170 ± 114	263 ± 87	573 ± 226	472 ± 95	356 ± 144
Fraction spent torpid (%)	40 ± 27	26 ± 18	72 ± 6	44 ± 27	47 ± 18	41 ± 15
EE (kJ day^{-1} g^{-1})	0.25 ± 0.11	0.43 ± 0.17	0.14 ± 0.03	0.25 ± 0.10	0.24 ± 0.10	0.36 ± 0.11

Forest-dwelling bats exposed to cave conditions (wet season)

The forest population usually roosted in a fluctuating environment with daily variation in T_a between 23.6 ± 1.2°C and 36.0 ± 2.2°C as well as daily variation in RH_a between 47.6 ± 10.6% and 98.5 ± 2.2%. When exposing individuals from this population to constant 32.1 ± 0.3°C and 88.9 ± 2.4% RH (i.e artificial cave conditions), all bats entered torpor within the first hour after the measurement started. They were torpid for more than 70% of the total measurement duration (table 5.1) and while they aroused multiple times, they were euthermic for an average of only 19 ± 14 min at a time and never longer than a maximum of 57 min (figure 5.1A). This was in clear contrast to torpor pattern observed in the native cave population as only 60% of cave bats entered torpor and for a much smaller fraction of the total measurement duration (26%; W=1, P<0.001). Cave bats had longer euthermic phases (320 ± 143 min; W=144, P<0.001) and usually entered torpor in the late morning (figure 5.1C). We found no difference in RMR between the two populations (table 5.1; $t_{22.8}$=0.497, P=0.624) but TMR was lower in the forest population than the cave population (table 5.1; W=91, P=0.010). Despite lower TMR, forest bats had higher T_{skin} (table 5.1; t_{13}=-5.08, P<0.001) and maintained a bigger T_{skin}-T_a differential when torpid (2.3 ± 1.0°C vs. 4.2 ± 0.6°C; $t_{16.2}$=-5.21, P<0.001). Simultaneously, during the short euthermic periods T_{skin} increased to 37.9 ± 0.7°C in the forest population, which was significantly higher than euthermic T_{skin} in the cave population (35.1 ± 1.2°C; $t_{19.3}$=-4.65, P<0 .001), as was the T_{skin}-T_a differential (3.0 ± 1.0°C vs. 5.2 ± 0.6°C; $t_{22.3}$=-4.72, P<0.001).

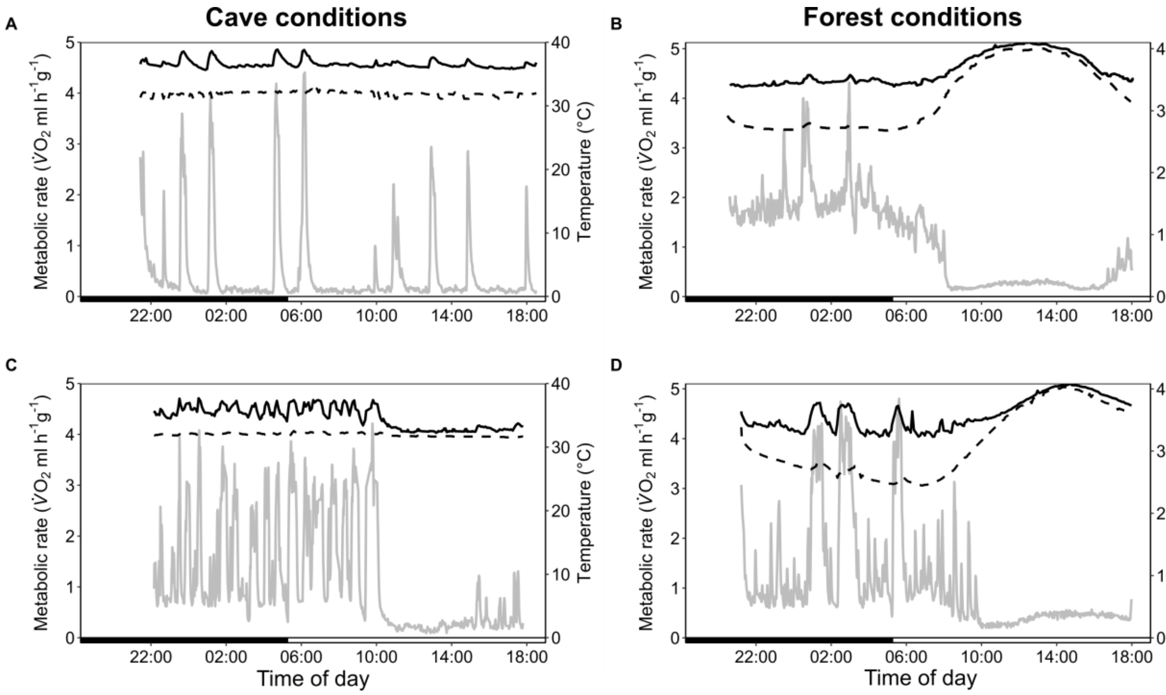
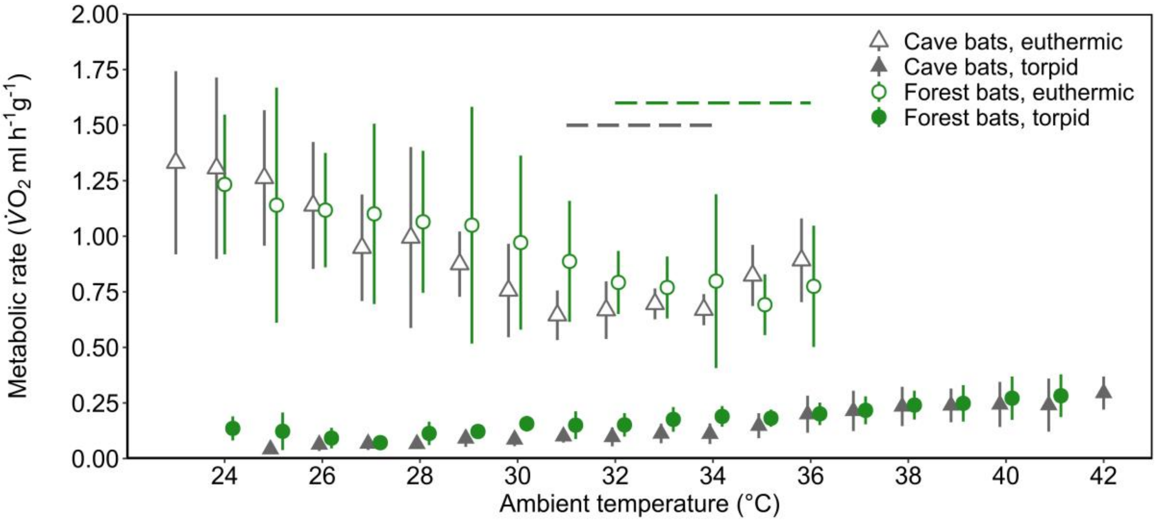


Figure 5.1. Variation in metabolic rate as $\dot{V}O_2$ (MR, ml h^{-1} g^{-1}; grey; solid line), skin temperature (T_{skin}, °C; black; solid line) and ambient temperature (T_a, °C; black; dashed line) over the course of the day. A, a forest-dwelling bat exposed to cave conditions; C, a cave-dwelling bat in its natural cave habitat; B, a cave-dwelling bat exposed to forest conditions and D, a forest-dwelling bat in its commonly experienced forest habitat.

Cave-dwelling bats exposed to forest conditions (wet season)

The cave population roosts at constant 32 ± 0.1°C and 98 ± 0.5% RH at bat height (Reher et al. submitted). When we exposed them to fluctuating condition in the wet season, i.e. T_a fluctuating on a daily basis between 24.4 ± 1.7°C and 38.5 ± 3.1°C as well as RH_a fluctuating between 37.0 ± 8.4% and 85.6 ± 7.0%, all individuals entered torpor. One individual was torpid for almost the complete measuring duration while the other twelve bats remained euthermic during their usual active phase and entered torpor in the morning (figure 5.1B). Similarly to the cave bats, all individuals from the native forest population entered torpor on hot days, i.e. days on which T_a exceeded its euthermic T_{skin} (table 5.1; figure 5.1D; Reher and Dausmann 2021). Individuals from the cave population as well from the native forest population entered torpor at high T_a, although individuals from the cave population did so earlier (cave bats entry: 09:02 ± 0:40hr vs. forest bats entry: 10:48 ± 1:06hr; Watson's test: x=0.272, P<0.01) and at lower T_a than individuals from the forest population, at 32.1 ± 1.8°C and 34.8 ± 1.4°C, respectively (t-test, $t_{19.4}=-3.98$, $P<0.001$). We determined a TNZ for the cave bats between 31 and 34°C, which was slightly lower and narrower than the TNZ in the forest population (32-36°C; Reher and Dausmann 2021; figure 5.2A). Within the TNZ, cave bats tended to have a lower RMR than forest bats (0.67 ± 0.09 ml O_2 h^{-1} g^{-1} vs. 0.77 ± 0.20 ml O_2 h^{-1} g^{-1}, respectively; $W=258$, $P=0.057$; figure 5.2A). Below the respective TNZ, RMR decreased with increasing T_a (GLMM, $t=-10.51$, $P<0.001$), whereby the slope was steeper in cave bats (GLMM, $t=2.41$, $P=0.016$; supplementary figure S1). In all bats, TMR increased with increasing T_a, which was especially apparent above the TNZ (GLMM, $t=11.53$, $P<0.001$); at $T_a \leq 35$°C, there was a tendency for cave bats to maintain a lower TMR than forest bats (GLMM, $t=1.77$, $P=0.076$) in addition to the T_a effect (GLMM, $t=2.73$, $P=0.006$, figure 5.2A). T_{skin} during both, torpor and euthermia increased with increasing T_a (torpor GLMM, $t=37.51$, $P<0.001$; euthermia GLMM, $t=12.74$, $P<0.001$; figure 5.2B), and the slope of this increase was steeper in the cave population (torpor GLMM, $t=-8.17$, $P<0.001$; euthermia GLMM, $t=-2.28$, $P=0.022$; figure S2). Below the respective TNZs, there was a noticeable difference in cooling rates in torpid individuals, with cave bats losing heat more quickly than their forest conspecifics (figure 5.2B).

A: Metabolic rate



B: Skin temperature

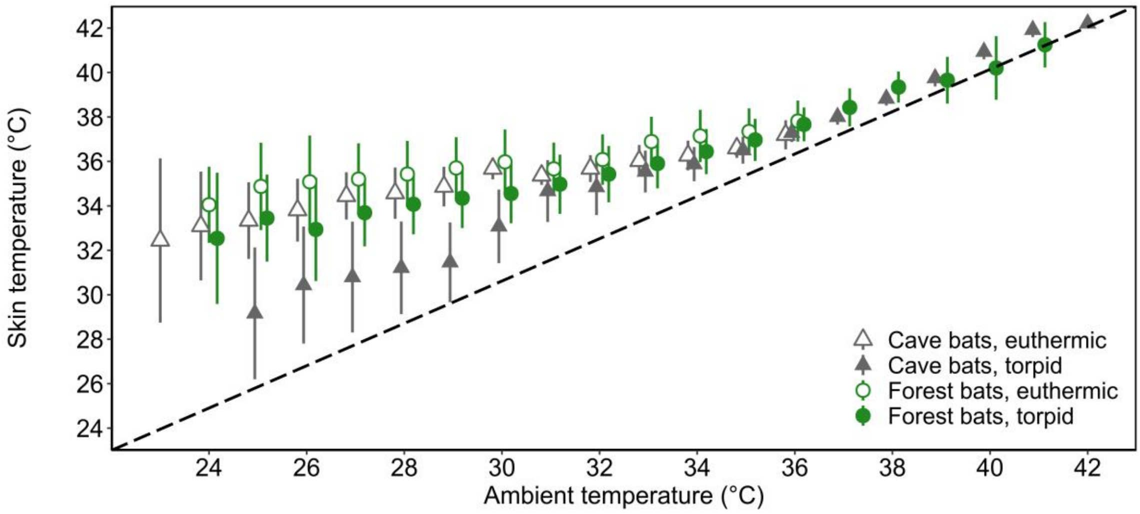


Figure 5.2. The mass-specific metabolic rate ([A]; MR, ml $\dot{V}$O2 h^{-1} g^{-1}) and skin temperature ([B]; T_{skin}, °C) as a function of ambient temperature (T_a;) of forest-dwelling bats (green circles) as well as cave-dwelling bats (grey triangles) during euthermia (open) and torpor (filled) when roosting in a forest environment in the wet season. The dashed lines in A indicate the thermoneutral zone for each population (colours correspond with population); the dashed line in B illustrates T_{skin}=T_a. Please note that at T_a≤29°C, $N_{forest\ bats}$=2 for torpid individuals. Error bars represent standard deviation; points are jittered for illustrative purposes.

Cave-dwelling bats exposed to forest conditions (dry season)

In the dry season, the cave population roosted at constant 31.4 ± 0.1°C and 98.1 ± 2.8% RH at bat height. All individuals entered torpor when we exposed cave bats to fluctuating T_a between 16.3 ± 1.9°C and 32.5 ± 2.6°C as well as RH_a fluctuating between 31.5 ± 8.4% and 86.9 ± 7.6%. One individual was torpid for the entire measurement duration and did not arouse before it was returned to the cave, while all other individuals entered torpor either at night (02:18 ± 1h 22min, 14.9 ± 3.4°C; N=4; figure

S3A) or in the morning when T_a had increased (11:13 ± 32min, 31.3 ± 1.0°C, N=5). Only three bats were able to defend a low but fairly stable T_{skin} when T_a decreased at night (T_{skin}: 29.3 ± 1.0°C; T_a: 17.5 ± 2.5°C; figure 5.3A). Mean euthermic T_{skin} in cave bats exposed to dry season forest conditions was lower than that of all other bats (*W*=2; *P*<0.001; table 5.1). In six bats, T_{skin} decreased with decreasing T_a (figure 5.4B) although they increased maximum MR up to 2x RMR between 20-30°C T_a (2.02 ± 0.60 ml O_2 h^{-1} g^{-1}) and up to 3x RMR below 20°C T_a (2.94 ± 0.15 ml O_2 h^{-1} g^{-1}; figure 5.4A), with an individual maximum of 6.46 ml O_2 h^{-1} g^{-1}, i.e. >6x RMR. Four of these bats entered torpor with a typical considerable drop in MR and reached a steady-state TMR within ~1 hour (55 min ± 17 min; figure 5.3B). The other two bats had torpor entry phases with slowly decreasing MR and T_{skin} over several hours (3 hr 38 min ± 49 min; figure 5.3C), a pattern resembling hypothermia and not torpor as suggested by reversed hysteresis of MR and T_{skin} (Geiser et al. 2014; figure S3). Nonetheless, mean MR during torpor and potential hypothermia were comparable (torpor 0.08 ± 0.05 ml O_2 h^{-1} g^{-1} vs. potential hypothermia 0.07 ± 0.04 ml O_2 h^{-1} g^{-1}). The cave bats exposed to forest dry season conditions entered the longest torpor bouts (*W*=282; *P*=0.039; table 5.1) with lowest mean TMR of all bats (*W*=80; *P*<0.001; table 5.1).

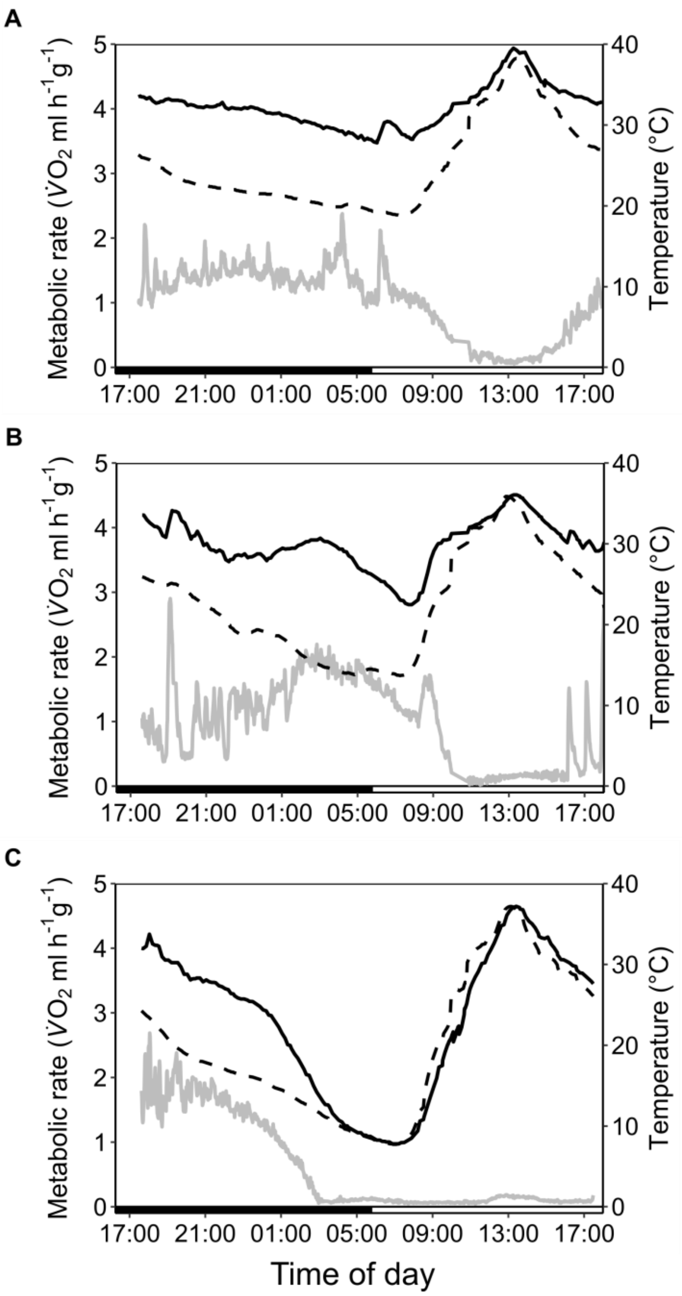


Figure 5.3. Variation in metabolic rate as $\dot{V}O_2$ (ml h^{-1} g^{-1}; grey; solid line), skin temperature (T_{skin}, °C; black; solid line) and ambient temperature (T_a, °C; black; dashed line) over the course of the day in cave-dwelling bats exposed to forest habitat in the cooler dry season. A, example of an individual defending a low euthermic T_{skin} at night and entering torpor in the morning when T_a increased (note the hyperthermic T_{skin}); B, example of an individual failing to defend a stable euthermic T_{skin} at night and entering torpor in the morning when T_a increased; C, example of an individual possibly becoming hypothermic (see figure S3).

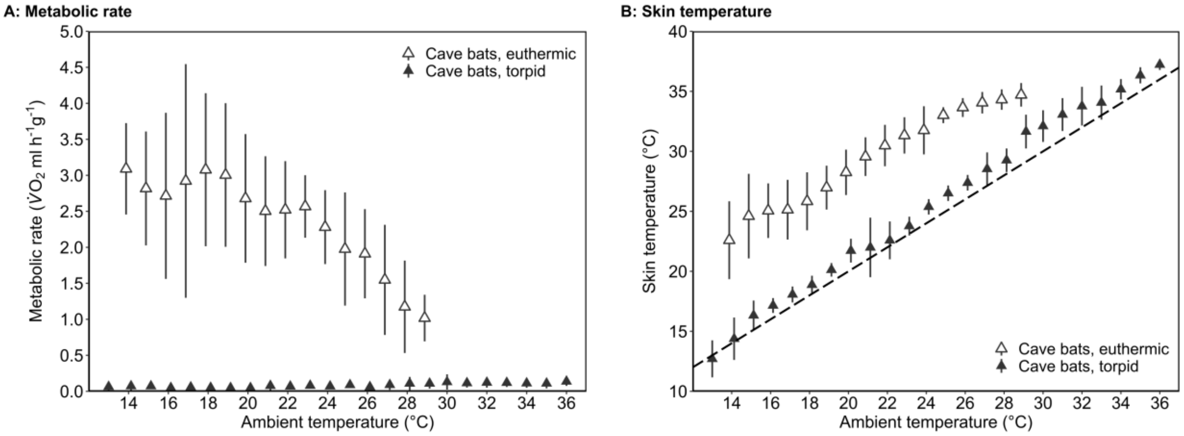


Figure 5.4. The mass-specific metabolic rate ([A]; MR, ml $\dot{V}O2$ h^{-1} g^{-1}) and skin temperature ([B]; T_{skin}, °C) as a function of ambient temperature (T_a;) of cave-dwelling bats when defending euthermia (i.e. maximum MR; open triangles) and when torpid (filled triangles) roosting in a forest environment in the dry season. The dashed line in B illustrates T_{skin}=T_a. Error bars represent standard deviation. Please note that the MR during euthermia shown here corresponds to the respective maximum MR.

Energetic costs of a change in microclimatic conditions

Interestingly, roosting in unfamiliar environmental conditions did not lead to increased energy expenditure under any of the conditions (table 5.1). In the wet season, both - cave bats exposed to the forest as well as forest bats exposed to cave conditions -consumed less energy than their conspecifics in their natural roost (cave bats in forest habitat: *W*=162, *P*=0.002; forest bats in cave habitat: *W*=1, *P*<0.001). In the dry season, the cave bats had a similar energy expenditure when roosting in the forest environment or in the cave (*W*=42, *P*=0.842) but spent 84% of their energy budget at night defending euthermia, much more than they usually do in the cave during this time of the year (55%; t_{17}=-5.276, *P*<0.001).

Discussion

We exposed individuals of two populations of Commerson's leaf-nosed bat *M. commersoni* living in contrasting habitats to the respective contrasting environmental conditions to examine the effects of rapid environmental changes on metabolic patterns and energy expenditure. Our data suggest limited ability of bats to to cope with conditions outside their typically occupied microclimates.

Bats usually roosting in constant cave conditions year-round could not defend high euthermic T_{skin} when T_a decreased below their TNZ under forest conditions. While some individuals attempted to maintain a stable, albeit low, T_{skin}, most bats failed to keep T_{skin} constant. This population roosts overwinters in a hot cave offering thermal neutral conditions year-round and does not leave the cave for the entire dry season (Rakotoarivelo et al. 2007; Raharinantenaina et al. 2008; Goodman 2011; Reher et al. 2019). While in the wet season T_a outside the cave may decrease to ~20°C, they experience this lower T_a while foraging. Flapping flight is costly and MR may increase up to 8-15 times, thereby generating substantial amounts of heat (Carpenter 1986; Speakman and Thomas 2003; Voigt and Lewanzik 2011). T_b regulation during the active phase is further supported by digestion (MacArthur and Campbell 1994; Beale et al. 2017), and thus, the cave individuals may rarely have to sustain T_b during resting, especially in cold conditions. Indeed, bats could either only defend a comparably low euthermic T_{skin} or resting T_{skin} decreased consistently despite maximal rates of MR of more than 6 times RMR, suggesting the bats had only limited capacity to produce sufficient amounts of heat inside the respirometry chamber without physical activity. Small mammals usually acclimatise to cold conditions by increasing their capacity for non-shivering thermogenesis (NST) in skeletal muscle or brown adipose tissue (Cannon and Nedergaard 2004; Bal et al. 2012, 2016; Nowack et al. 2017). Given the lack of cold exposure though, cave-dwelling bats likely have only limited amounts of brown adipose tissue and only limited capacity for muscle NST (Heldmaier and Hoffmann 1974; Heldmaier et al. 2013). Two individuals even showed hypothermia-like responses as they had noticeable slow torpor entries with reversed hysteresis and almost negligible T_{skin}-T_a differentials during torpor. Additionally, although they passively rewarmed to almost euthermic T_{skin}, they did not arouse spontaneously but only upon disturbance. These patterns resemble the descent into hypothermia rather than controlled torpor (Tomlinson et al. 2007; Geiser et al. 2014) and thus, reflect their limited capacity to generate sufficient heat. Simultaneously though, MR was substantially reduced and as low as TMR, which in turn is more indicative of torpor (Geiser et al. 2014). We therefore argue that these bats entered torpor involuntarily with a hypothermic descent and subsequent transition into torpor. While we cannot specify the exact mechanism, the bats evidently had difficulties to defend euthermia at low T_a, which also enforced a shift in the allocation of the energy budget. In the forest, they spent 84% of their budget at night, compared to 55% of their daily energy expenditure during the night under cave conditions. Interestingly, the cave-dwelling bats exposed to dry season forest conditions entered longer and deeper torpor bouts during the day than all other individuals, by which they compensated for the increased night-time energy expenditure and maintained a similar energy expenditure as under cave conditions. Although flexible torpor patterns in response to individual constraints, unpredictable resource availability or extreme weather events (e.g., Munn et al. 2010; Kobbe et al. 2011; Bieber et al. 2014; Nowack et al. 2015) can mitigate the energetic costs, the cave population struggled defending euthermic T_b when T_a dropped below their TNZ, suggesting acclimatisation or local adaption to high cave T_a.

All forest bats exposed to constant cave conditions remained torpid for a large fraction of the measurement duration and the occasional euthermic periods were the shortest that we recorded within this study. RMR during those short euthermic bouts was comparable to RMR of the native cave individuals, although resting T_{skin} was higher in forest bats than in cave-dwelling bats and increased to an individual, hyperthermic maximum of 39.4°C. The forest population usually experiences considerable daily fluctuations in both T_a and RH_a (total range: 21.2 – 41.7°C, 29.0 – 101.2%; Reher et al. submitted), but highest T_a always coincides with lowest RH_a. Many endotherms inhabiting warm and drier environments have developed adaptations to conserve water (Heldmaier et al. 2013; Fuller et al. 2016; Clarke et al. 2017). For example, different bird (Muñoz-Garcia and Williams 2005; Champagne et al. 2012) and bat species (Muñoz-Garcia et al. 2012; Ben-Hamo et al. 2016) from arid habitats have a modified skin lipid composition with higher proportions of waxy lipids compared to their conspecifics from mesic habitat, which reduces the skin's permeability and thus the rate of cutaneous water loss (Haugen et al. 2003). Such adaptations, however, may inhibit cooling capacities at high RH_a. High RH_a generally reduces the gradient driving evaporation and thus, the amount of heat an animal can dissipate (Gerson et al. 2014). However, cooling through evaporation is possible at high RH_a when the water vapour pressure e.g. on the skin surface is higher than that in the air (Schmidt-Nielsen 1997; Withers et al. 2016). Indeed, individuals may lick their forearms when heat stressed (Reher and Dausmann 2021). Combined with the high T_{skin} observed during short euthermic periods, we suspect that the bats tried to maintain cooling mechanisms by increasing both skin temperature and humidity. A higher T_{skin}-T_a differential additionally allows for passive pathways of heat loss through convection, conduction and radiation (Tattersall et al. 2012; Mitchell et al. 2018). Interestingly, torpid forest-bats also had a higher T_{skin} than their torpid cave-conspecifics despite lower rates of metabolic heat production. During torpor however, vasomotor regulation is considered less functional (Muñoz-Garcia et al. 2016), suggesting that the high T_{skin} was not necessarily actively upregulated by vasodilation but that the patterns reflect difficulties in efficient heat dissipation. The excessive use of torpor in forest bats measured under hot cave conditions was thus probably driven by heat stress, a response also observed in the same population in their natural environment but at higher T_a (Reher and Dausmann 2021). Although T_a of the imitated cave was within the forest bats' TNZ, we suggest that the combination of high T_a with high RH_a may have overwhelmed their thermoregulatory system (Gerson et al. 2014), indicating adaptation to more arid habitat.

Despite variation in patterns and evidence of local adaptation or acclimatisation of the respective populations, the bats' responses to heat were surprisingly homogenous, even during the dry season. When we exposed the cave bats to wet season forest conditions, and thus higher daytime T_a than usually experienced, all individuals responded by entering hyperthermic ("hot") torpor, thus showing the same adaptation than native forest bats (Reher and Dausmann 2021). Interestingly, bats from both populations did not use torpor in the early morning hours to benefit from lower T_a enhancing energetic savings like other tropical bats do (Geiser et al. 2000; Bondarenco et al. 2013), but entered torpor when T_a approached or exceeded their respective. Torpor at high T_a reduces metabolic heat production, allowing higher rates of heat from the environment to be stored in the body via facultative hyperthermia and reduces water lost during evaporative cooling when T_a exceeds T_b (Lovegrove et al. 2014; Welman et al. 2017; Mitchell et al. 2018; Reher and Dausmann 2021). Although the general patterns were intriguingly similar, cave bats entered torpor already at lower T_a, indicating that they were heat stressed at lower T_a than the native forest bats. We also found a trend for cave bats to have a slightly lower RMR in TNZ and a lower TMR at similar T_a as well as lower thermal inertia below the TNZ and higher cooling rates during torpor, all of which reflect their acclimatisation to hot cave conditions. A lower overall metabolic heat production may be more beneficial in hot and especially humid conditions (Rodríguez-Durán 1995; Cooper and Withers 2008; Gerson et al. 2014; Lovegrove et al. 2014) and lower thermal inertia is consistent with results discussed previously indicating that this population lacks traits for coping with T_a below their commonly experienced 32°C in the cave. Tropical bats experiencing high T_a in their diurnal roosts appear to have a great capacity for coping with heat (Cory Toussaint and McKechnie 2012; Bondarenco et al. 2013, 2014; Czenze et al. 2020; Noakes et al. 2021). Moreover, there are generally few options to downregulate T_b when T_a approaches the upper thermal limits (Heldmaier et al. 2013; Withers et al. 2016; Mitchell et al. 2018). Since the cave bats roost in a very specific microclimate of high T_a and high RH_a year-round, it is likely that they have acclimatised to their roosting conditions rather than the dry and hot climate of southwestern Madagascar as, for example, observed in Namaqua rock rats (*Micaelamys namaquensis*; van Jaarsveld et al. 2021). This would entail that they may not have developed means to effectively retain water in dry conditions, depleting their water budget rapidly and thus, entering torpor early may have been the only physiological option (Bondarenco et al. 2014; Reher and Dausmann 2021). However, torpor combined with hyperthermia never could have been observed at constant 32°C in the cave, which indicates that this respective population has a broader physiological repertoire than it commonly uses in its natural environment and thus, certain phenotypic flexibility in the context of heat exposure.

Bats of both populations dealt with the unfamiliar environmental conditions with increased use of torpor. Especially multi-hour torpor, however, is usually avoided when the populations roost in their natural conditions unless energy savings are vital (Reher and Dausmann 2021; Reher et al. submitted). Torpor is associated with certain costs (see Landes et al. for review) and especially the ecological and behavioural consequences may apply to *M. commersoni:* reduced sensory and motor capabilities and thus increased predation risk as well as missed social opportunities (Choi et al. 1998; Mzilikazi and Lovegrove 2002). While the observed responses may aid overcoming rapid short-term perturbations, they are probably not sustainable to cope with long-term environmental changes. However, endotherms may adapt to novel conditions within weeks or generations depending on their scope for phenotypic plasticity (Piersma and Drent 2003; Heldmaier et al. 2013; Noakes and McKechnie 2020), and torpor is likely beneficial to overcome these time spans at all. Torpor allows to drastically reduce internal heat production if the water budget does not allow for extensive evaporative cooling or to finely balance the energy budget if unanticipated perturbations have necessitated a short-term increase energy expenditure (Geiser 2004; Heldmaier et al. 2004 Herreid and Schmidt-Nielsen 1966; Cooper et al. 2005; Reher and Dausmann 2021). All of these are no options for homeotherms, which accordingly may face even greater challenges during rapid environmental perturbations.

In summary, our data showed that outside their commonly experienced environmental range, all bats showed patterns that may not necessarily be sustainable in the long-term. Forest bats had difficulties to dissipate heat at high RH_a to the point that they were hyperthermic during short euthermic periods, which may have severe physiological consequences when T_b exceeds certain thresholds during tropical cyclones or extreme rainfall events, adding humidity to high T_a (Lepock 2003; Schmidt-Nielsen 1997). The cave population exposed to T_a below their TNZ could not maintain a high and constant euthermic T_{skin} despite expending more than 80% of their energy budget and two individuals had intriguing torpor entries similar to hypothermia. Hypothermia is potentially fatal (Tomlinson et al. 2007; Geiser et al. 2014) and the high night-time energy consumption may become problematic in the long-term when torpor has to be traded off with social interactions like reproduction to avoid depleting energy reserves in the lean season. While the response to heat resembled the forest bats' response, cave bats were heat stressed already at lower T_a than forest bats. The narrow capacity of the cave population to cope with conditions unlike those specific to hot caves is of particular concern given that human-induced disturbance is already evident here (Goodman 2006), and no other hot caves are currently known to exist in this region (Reher et al. 2018). Based on our results, we argue that a wide distribution over a broad environmental ranges and intraspecific variation in physiological traits reflecting differences in local microhabitat as in *M. commersoni* may be misleading when assessing adaptive scopes of species. Distinct populations may be locally adapted or acclimatised (Violle et al. 2012; Richardson et al. 2014), ultimately hampering flexibility if rapid and sustainable responses are required. Quantifying the extent of intraspecific variation and understanding the mechanism behind this variation is therefore vital. Comparative studies over broad environmental scales combining field research with more experimentally-driven approaches to examine what species and populations currently do and potentially could do when they are pushed out of their typically experienced environment over different time scales may point towards their flexibility as well as limits in it. Such understanding is essential when managing conservation actions, including translocating or reintroducing species to new habitat, and when predicting wildlife's chances for persisting in ever rapidly changing environments and climates.

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Supplementary material

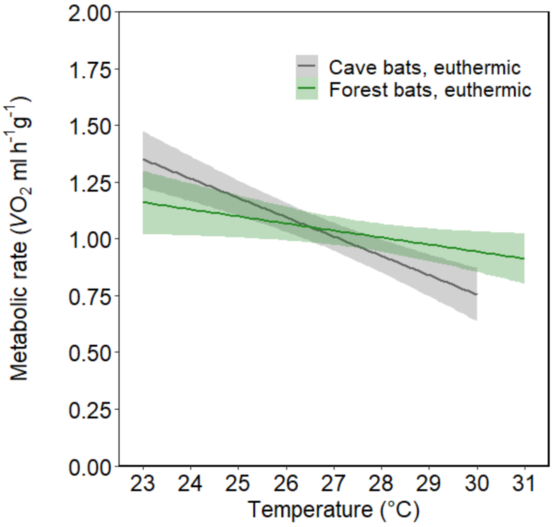

Figure S1. Estimated mass-specific metabolic rate (ml $\dot{V}O2$ h^{-1} g^{-1}) with 95% confidence interval (shaded area) as a function of ambient temperature below the respective thermoneutral zone of euthermic forest-dwelling bats (green) as well as euthermic cave-dwelling bats (grey) when roosting in a fluctuating forest environment in the wet season.

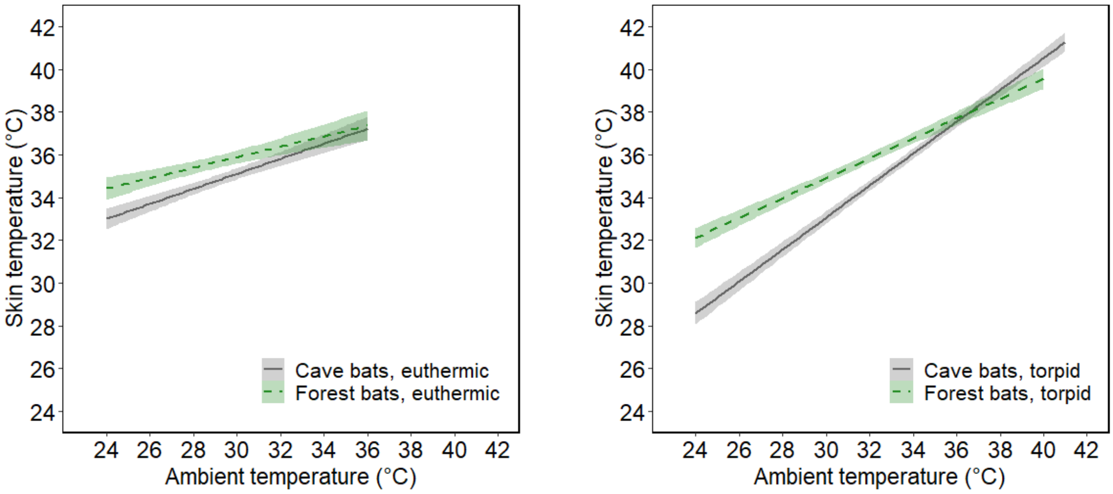

Figure S2. Estimated skin temperature (°C) with 95% confidence interval (shaded area) as a function of ambient temperature of euthermic (left panel) and torpid (right panel) forest-dwelling bats (green) as well as cave-dwelling bats (grey) when roosting in a fluctuating forest environment in the wet season.

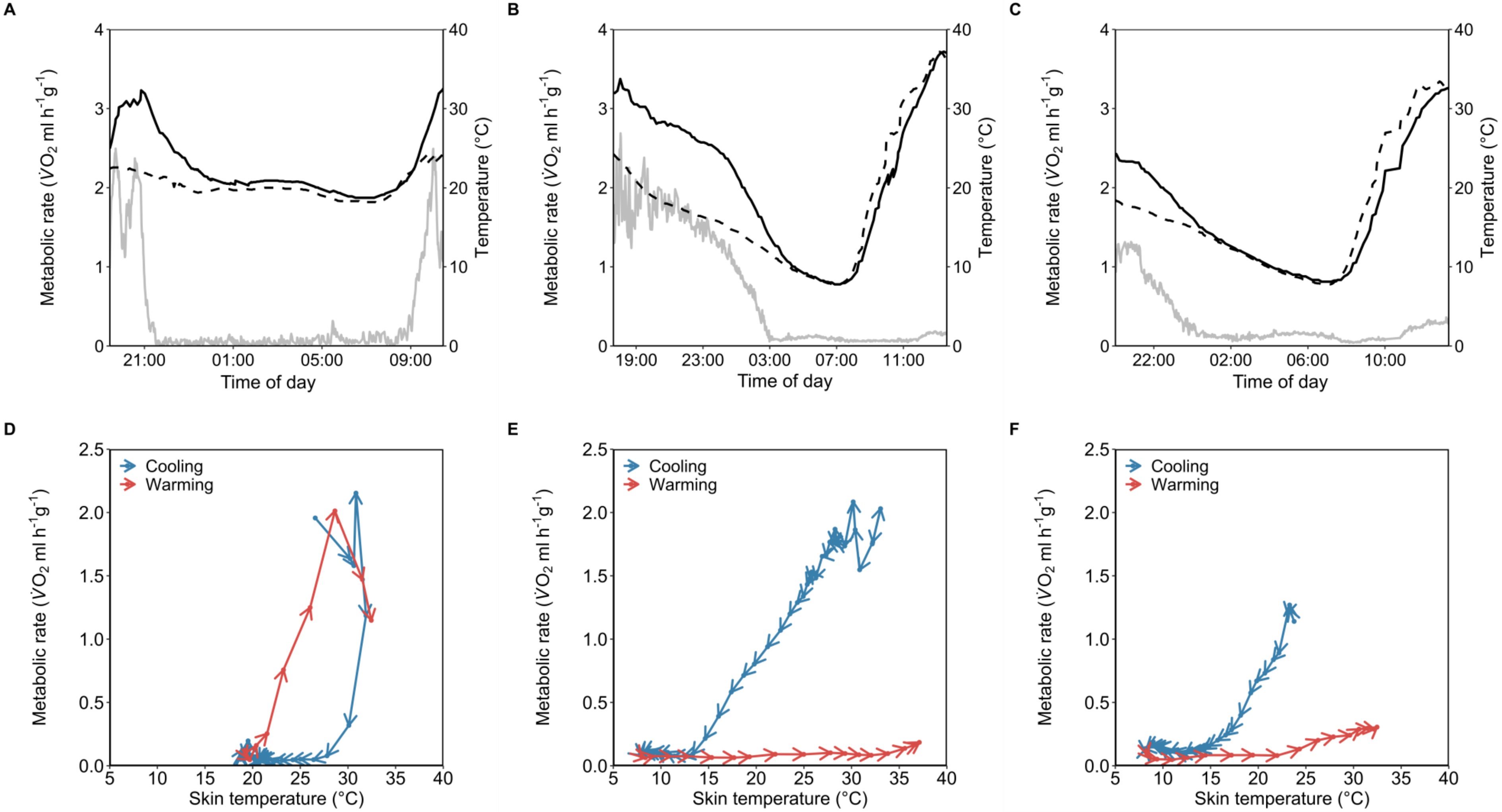

Figure S3. Metabolic rate as $\dot{V}O_2$ (MR, ml h^{-1} g^{-1}; grey; solid line), skin temperature (T_{skin},°C; black; solid line) and corresponding ambient temperatures (T_a, °C; black; dashed line) as a function of time (A, B, C) as well as MR as a function of T_{skin} (D, E, F). Please note the different y-axis scales in A, B, C vs. D, E, F. A typical torpor entry at low T_a with rapid reduction of MR and T_{skin} approaching T_a subsequently is shown in A and D. Two individuals deviated from this pattern (B, E as well as C, F); MR and T_{skin} decreased slowly with decreasing T_a and “reversed” hysteresis suggests that these bats became hypothermic (see Geiser et al. 2014). However, MR remained reduced over multiple hours despite increasing T_a. Both individuals aroused when we returned them to their cave roost right after the measurement.

Author contribution

I hereby confirm that Stephanie Reher (SR) and Kathrin H. Dausmann (KHD) conceived and designed the study, SR conducted the measurements in the field with support of Hajatiana Rabarison, SR analysed the data and took the lead in writing the manuscript, both with input from KHD and Julia Nowack (JN). SR, KHD and JN discussed and interpreted the results, revised the manuscript and all authors approved the current version of it.

Prof. Dr. Kathrin Dausmann

Chapter 6

Heterothermy in Malagasy Mammals

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Introduction

Madagascar is an island of multifaceted climates and associated vegetation formations, ranging from the dry, very unpredictable conditions in the dry spiny thickets of the south to the moist evergreen rainforests of the east and extreme north. All land species have to adapt to the varying conditions of their habitats, as the local environmental vicissitudes govern the performance and energy use and ultimately the abundance and distribution of the organism concerned (Buckley et al. 2018). Therefore, they have to cope with these differing conditions and changes, whether these are daily, seasonal or unpredictable (e.g., extreme weather events, fires, cyclones). The seasonal changes concomitant with the cool dry season are especially challenging for Malagasy animals as ambient temperatures are low, rainfall is virtually absent in some regions, and food availability (leaves, fruits, flowers, insects, and invertebrates) is markedly reduced (Donque 1972). For mammals in particular, this period is also energetically costly, as it requires an increased energy uptake to sustain high, endothermic body temperature (i.e., regulation via endogenous generation of heat). To cope with demanding times, Malagasy mammals have developed a variety of different responses, such as sunbathing in the cool mornings employed by certain diurnal lemurs (e.g., *Lemur catta*, see Sauther and Cuozzo, p. xx; *Propithecus* spp., see Lawlor and Richards, p. xx), social thermoregulation by huddling (e.g., *Hapalemur meridionalis*, Eppley et al. 2017), or choice of suitable microclimates by smaller nocturnal species (e.g., *Triaenops menamena*, Reher et al. 2019). Moreover, some species shift their food choices and activity patterns across seasons (Hemingway and Bynum 2005; Andriatsimietry et al. 2009; Farris et al. 2015). Beyond these more obvious adaptations, Malagasy mammals have mastered the art of responding flexibly to challenging conditions with exceptional physiological adaptations.

Most notably, members of at least three orders of Malagasy land mammals (Afrosoricidae, Chiroptera, Primates; Figure 1) abandon mammalian endothermy at least occasionally and become torpid in a for endotherms hitherto unknown extent of flexibility: from almost daily use of heterothermy, to very rare employment, from extremely short bouts of only a few minutes, to continuous hibernation over many months. Hibernation, prolonged and daily torpor (all three included in the term heterothermy) are physiological states of controlled interruption of normal maintenance of body temperature (normothermia) via an active depression of metabolic rate and virtually all bodily functions and an extension of the range of tolerated body temperature (Geiser 2004; Heldmaier et al. 2004). Daily torpor is limited to short bouts of less than 24 hours, prolonged torpor bouts last for several days, whereas hibernation consists of a series of approximately 1-2 week-long torpor bouts, usually totaling several months, punctuated by normothermic spontaneous arousals (i.e., phases with active heat production; Geiser 2004; Heldmaier et al. 2004). The function of these energy-demanding arousals remains enigmatic, but they seem to be necessary for the maintenance of vital bodily functions in hibernators with permanently low body temperatures (Humphries et al. 2003).

Body temperature during heterothermy often approximates ambient temperature, especially during deep torpor (Ruf and Geiser 2015). This suspension of active thermoregulation allows high savings in energy and water. Notably, for torpor use during periods of extended aridity or high ambient temperatures, water conservation might play the even more decisive role (e.g., Schmid and Speakman 2000; Bondarenco et al. 2013). When metabolic rate is reduced, water turnover is generally lowered and additional water-depleting processes such as respiration, urine production, and defecation are down-regulated or even absent (Levin et al. 2015). Given even more advantages such as, e.g., avoidance of predation or parasite pressure, heterothermy provides mammals with a very efficient strategy to cope with demanding environmental conditions (Lyman et al. 1982). Heterothermy is best known in arctic and temperate climates, but it also occurs in the tropics (McKechnie and Mzilikazi 2011; Dausmann et al. 2012; Ruf and Geiser 2015; Nowack et al. 2020).

In the previous edition of this book, there was a contribution on what was known on the ecophysiology of Malagasy mammals from that period (Schmid and Stephenson 2003). Over the intervening years, different techniques have been developed to study how mammals change their metabolism and herein we provide a review of what has been learned on certain groups occurring on the island in the last nearly two decades. We focus on three separate orders of mammals, which include tenrecs, insectivorous bats, and lemurs.

Tenrecs – Masters of Life on a Low Flame

The masters of keeping life on a low flame are some of the Malagasy tenrecs (family Tenrecidae). They may be reminiscent of basal placental mammals (Lovegrove 2019) and have been termed protoendotherms (*sensu* Grigg et al. 2004), because of their highly labile thermoregulation. With their often reptilian-like patterns of body temperature, they might represent relicts of the transition from ectothermy to homeothermic endothermy (Levesque et al. 2014). Indeed, tenrecs have some of the lowest and most highly variable body temperatures of generally less than 35°C, as well as one of the lowest basal metabolic rates, of any eutherian mammal (Stephenson and Racey 1995).

Tenrecs have experienced a tremendous adaptive radiation that resulted in species that are terrestrial, arboreal, fossorial, and even aquatic (see Goodman et al., p. xx; Jenkins et al., p. xx), with a multitude of lifestyles and presumably many unique physiological adaptations. However, the physiology of only few species of tenrecs has been studied so far, and even less in the field, where animals show the complete spectrum of their behavioral and physiological capacity. Besides their already generally low metabolic rates, many tenrec species have been reported to be highly heterothermic and only seem to defend a stable body temperature throughout the day when they are pregnant or lactating (Thompson and Nicoll 1986). Even more pronounced, several tenrec species are known to hibernate to bridge the unfavorable dry and cold season (Gould and Eisenberg 1966; Levesque et al. 2014; Dausmann et al. 2020).

The spiny tenrec species (subfamily Tenrecinae) best studied in regard to physiological adaptations are *Echinops telfairi*, *Tenrec ecaudatus*, and *Setifer setosus*. *Echinops telfairi* (Figure 1a) has one of the lowest reported normothermic body temperatures even within the tenrecs (around 31°C) and is highly thermally labile (Lovegrove and Génin 2008; Dausmann et al. 2020). It becomes torpid every day regardless of season and ambient temperature when not reproductively active. In the warm season, *E. telfairi* uses daily fluctuations in ambient temperature to rewarm passively from daily torpor, which leads to decreased energy expenditure as well as synchrony in the activity phases and torpor bouts among individuals (Dausmann et al. 2020). This exogenous, mainly passive reheating from torpor bouts is known from several tropical heterotherms (e.g., lemurs and bats, see below) and makes rewarming comparatively energetically inexpensive with 60-100% reduction, as compared to active, endogenous warming (e.g., Ortmann et al. 1997; Geiser and Drury 2003; Bondarenco et al. 2013). This might explain why daily torpor is common in sunny regions and might occur more frequently at low latitudes than hitherto believed (Geiser and Drury 2003). Nevertheless, functional brown adipose tissue, necessary for active endogenous heat production, has been recorded in *E. telfairi* (Oelkrug et al. 2013). During the hibernation period, which is the cool dry season, this species hibernates for several months, almost regardless of the level and extent of fluctuations of (naturally occurring) ambient and respective body temperature.

Interestingly, energy expenditure during the much shorter daily torpor bouts in the warm season can be as low as during the hibernation bouts in the cold season. Both are initiated by active metabolic inhibition and reach levels of the most competent arctic hibernators such as arctic ground squirrels (*Spermophilus parryii*, Buck and Barnes 2000), despite having an approximately 10°C higher body temperature (Dausmann et al. 2020), possibly a consequence of the already low active metabolic rate. However, there seems to be a lower limit for hibernation at about 12°C, below which *E. telfairi* shows difficulties arousing from hibernation bouts (Scholl 1974; Dausmann et al. 2020). Although temperatures do drop below 10°C within the range of *E. telfairi* during the coldest nights of the cold dry season, this does not occur very frequently and is buffered even in hibernacula (space an animal seeks refuge during hibernation) with low insulation capacities (tree hollows, dead trees, and under leaf litter). More importantly, even on those coldest days ambient temperature will usually increase above 20°C in the tenrecs' habitats, ensuring passive rewarming. Thus, the 10°C-threshold is presumably rarely problematic in natural environments (Lovegrove and Génin 2008; Dausmann et al. 2020).

Tenrec ecaudatus shows the same extreme plasticity in thermoregulation and metabolism as *E. telfairi*. During the wet warm season, the animals enter daily torpor and during the cold dry season, *T. ecaudatus* enters hibernation for up to nine months (Lovegrove et al. 2014a), at a great range of ambient and body temperatures, but the depth of torpor may vary. *Tenrec ecaudatus* uses small underground burrows for hibernation (Nicoll and Goodman, p. xx) and possibly hibernates in groups. Field data on hibernating *T. ecaudatus* demonstrated that body temperature follows soil temperature for the duration of the hibernation season with no evidence of arousals, which is very exceptional among hibernators (Lovegrove et al. 2014b). Indeed, one of the most surprising discoveries from warm climate hibernators is that periodic arousals, thought to be essential to proper functioning during hibernation, are not necessarily needed. To date, the only other mammal to not exhibit these arousals outside Madagascar are black bears, *Ursus americanus*, hibernating at body temperatures above 30°C (Tøien et al., 2011). Moreover, when body temperature drops below 20°C during hibernation in *T. ecaudatus*, individuals may suspend breathing for up to 45 minutes (Treat et al. 2018). This mechanism might allow efficient gas exchange in environments that become hypoxic and hypercapnic (elevated carbon dioxide levels), such as underground burrows. Additionally, it might help to conserve water under dry conditions, which is likely critical during the pronounced dry season of southern and southwestern Madagascar. Unlike the general patterns of hibernators, the end of the hibernation season seems to be a gradual transition in *T. ecaudatus*, and occurs over a couple of weeks, at least in captivity (Treat et al. 2018). Remarkably, even when tenrecs are being active their body temperature may range from about 12°C to 34°C, fluctuating passively throughout the day. Metabolic rate can be very low in *T. ecaudatus* even while active, sometimes reaching torpid levels, despite higher body temperatures. Thus, body temperature does not necessarily dictate metabolism and tenrecs seem to be able to modify heat retention and conductance, independent of metabolism. Contrary to *E. telfairi*, *T. ecaudatus* females show variable body temperatures during gestation without apparent effects on embryonic development, and only experience more stable body temperatures above 20°C after parturition (Treat et al. 2018).

As the other two spiny tenrec species discussed so far, *Setifer setosus* shows a large degree of heterothermy and enters hibernation for five to seven months during the cold dry season. Only reproductive females have slightly higher and less variable body temperatures (Levesque and Lovegrove 2014). There is anecdotal evidence that *S. setosus* females become torpid for prolonged periods during lactation, but this needs further investigation (Levesque et al. 2013). Resting sites in this species can be below or above ground (in hollow logs or under leaf litter), thus fully or partially exposed to environmental conditions, and *S. setosus* hibernating in variable ambient temperatures show no evidence of periodic arousals (Figure 2a; Levesque et al. 2014). Since *S. setosus* can be active at body temperatures as low as 28°C (Eisenberg and Gould 1970), active rewarming is virtually dispensable, as activity itself may be used for heat production. Consequently, *S. setosus* rather relies on ambient temperature to regulate body temperature, with little energetic costs of thermoregulation, and thermolability is the norm (Levesque et al. 2014).

Tenrecs are surprisingly intolerant to ambient temperature extremes, considering their generally flexible implementation of mammalian thermoregulation. As they are capable of sustaining stable body temperature when reproductively active, this flexibility is clearly not a lack of ability for precise thermoregulation (Thompson and Nicoll 1986). General plasticity in thermoregulation reduces general energy expenditure; however, it also limits the ambient temperature range over which an organism can function (Treat et al. 2018). For many spiny tenrec species, temperatures at the high end of the spectrum may be problematic. *Echinops telfairi*, for example, lives in the hottest and driest portions of the island but can only survive temperatures above 36°C with sufficient supply of water, as the animals need to drink and wet their bodies (Scholl 1974), and *T. ecaudatus* pants and breathes irregularly above 34°C, although inhabiting the whole island (Kayser 1961; Treat et al. 2018). Torpor, on the other hand, might counterbalance this disadvantage, broadening the temperature niche, if temperatures do not drop consistently below 10-12°C. Indeed, torpor use has been found to lower the risk of extinction in highly variable and quickly changing environments (Geiser and Turbill 2009; Liow et al. 2009).

Tenrecs have clearly not read the classical physiology textbooks, when it comes to thermoregulation. They seem to be essentially ectotherms, and become endothermic only when they breed (Lovegrove 2019). With most species not even studied, there might be more surprises ahead, possible elucidating the evolution of endothermy.

Bats – Torpor Whizz Masters

The shortest, regular torpor bouts of any mammal studied so far are shown by the Malagasy bat *Macronycteris commersoni* (family Hipposideridae; Figure 1b, c). During the resting phase in warm, but not extremely hot conditions (24-34°C), they routinely shut down metabolism repeatedly for micro torpor bouts of only several minutes, still enjoying energy and water savings, while remaining alert and normothermic (S. Reher et al., unpublished data). However, individuals of this species also enter hibernation over several weeks when caves are available as roosting options. Thus, within a single population of this species, individuals span the whole spectrum of heterothermic responses endotherms are capable of and exemplify the amazing flexibility with regard to thermoregulation in Malagasy mammals.

Figure 6.1. Different species of Malagasy mammals that undergo some seasonal change in their ecophysiology. A) *Echinops telfairi* becomes torpid every day regardless of season and ambient temperature when not reproductively active. B) *Macronycteris commersoni* retreat to the warmest cave within the cave system in Tsimanapetsotse National Park as a resting site throughout the year, practically negating the need for active thermoregulation during the daily resting phase in the rainy season, and keeping body temperature high despite heterothermy in the rainy and the cool dry season. C) M*acronycteris commersoni* tolerates hyperthermic body temperatures of up to 42.9°C when resting in open vegetation on hot days during the warm rainy season. Note the temperature sensitive collar transmitter. D) *Microcebus griseorufus* use heterothermy as a very flexible response to current internal and external conditions, and daily torpor, prolonged torpor and hibernation are shown at the same time within the same population and the same habitat. Note the at the base slightly enlarged tail. E) *Cheirogaleus mediu*s are obligate hibernators and all individuals enter continuous hibernation over several months during the dry season. Body temperature patterns during hibernation can be diverse, depending on the insulation capacities of their hibernacula, from almost constant, to highly fluctuating ones. F) *Lepilemur*, in this case *L. edwardsi*, maintain one of the lowest general metabolic rates of all placental mammals and shift their thermal neutral zone to cope with the low efficiency of their food and seasonality of their habitats. Photo A, D, E by K. Dausmann, B, C by S. Reher, and F by A. Wulff.

Macronycteris commersoni is relatively widespread on the island. In Madagascar's dry spiny thicket, it gains massive fat-deposits by the end of the wet season and is not actively foraging for several months during the dry season (Goodman 2006; Rakotoarivelo et al. 2007; Reher et al. 2019). Only few juveniles, which most likely did not manage to build up a sufficiently large fat store to overcome the long dry season without foraging breaks are occasionally found outside the cave. Interestingly, in Tsimanapetsotse National Park in the extreme southwest, *M. commersoni* overwinters in large colonies in the hottest and most humid cave in the region (≥ 32°C and 95% relative humidity year-round; Figure 1b) with body temperatures closely following cave temperature (Figure 2b), although less warm caves are available. These colder caves are frequently used by other bat species and would possibly allow higher energy savings during torpor because of generally lower nightly temperatures (allowing lower metabolic rates and body temperatures), as they are less buffered resulting in greater fluctuations in temperature and relative humidity (Reher et al. 2018, 2019). Why *M. commersoni* prefers the hot cave remains unknown, however, it is feasible that stable temperatures in the thermal neutral zone (i.e., the temperature range where no additional energy expenditure to sustain high endothermic body temperature is needed) and high humidity reducing overall water loss are more advantageous compared to fluctuating conditions.

Even in the very predictable, constant conditions of the hottest cave in Tsimanapetsotse National Park, different individuals of *M. commersoni* show very different physiological responses during the cool dry season, when arthropod food resources are low. Whereas some enter hibernation with single bouts lasting up to 16 days between arousals (Figure 2b), others employ daily torpor and prolonged torpor (Figure 2c; Reher et al. 2018). Surprisingly, even the latter individuals completely cease foraging flights, although their metabolic rate is at an active level at night. This suggests that either the habitat does not offer enough food to make foraging profitable; and/or the accumulated fat combined with a favorable microclimate (high humidity and ambient temperature presumably in the thermal neutral zone) is sufficient to get through the dry season in a form of inactivity, but not necessarily continuous hibernation.

Triaenops menamena (family Rhinonycteridae) lives in the same region as *M. commersoni*, but has adapted physiologically in a very different manner to the cold dry season. Instead of long-term hibernation, it regularly enters daily torpor and forages at night. Contrary to *M. commersoni*, *T. menamena* utilizes day roost sites with very different degrees of ambient condition buffering (including the hot cave) during the cold dry season (Reher et al. 2019). The greater the roost's fluctuations in temperature and humidity over the course of the day, the higher the probability of individuals entering torpor and accordingly lower daytime energy expenditure. This makes less buffered sites the energetically more efficient choice for daytime rest (Remmers 2020). Nevertheless, individuals shift their roosting preferences towards the hot cave for the cold dry season (Reher et al. 2019), indicating that the stable, hot and humid microclimate is also beneficial for *T. menamena*, possibly because it negates the need for torpor (Reher et al. 2018; Remmers 2020).

Two more bat species rest in the hot cave in Tsimanampetsotse along with *M. commersoni* and *T. menamena*: *Paratriaenops furculus* (family Rhinonycteridae) and *Miniopterus mahafaliensis* (family Miniopteridae). No physiological data is available for the latter, but *P. furculus* repeatedly enters prolonged torpor bouts lasting up to seven days (Reher et al. 2019; H. Rabarison et al., unpublished data). Prolonged torpor is an intermediate torpor response, combining the flexibility of daily torpor with higher energy savings. Most importantly, animals are active after or between prolonged torpor bouts and carry on foraging, which eliminates the need for depositing fat as energy stores and allows for sustained social interactions during active hours. Indeed, *T. menamena* and *P. furculus* forage in-between torpor bouts and *M. mahafaliensis* is frequently trapped during the dry season. All three species primarily feed on Lepidoptera, supplemented by small Coleoptera in the dry season, whereas *M. commersoni* relies entirely on Coleoptera (Ramasindrazana et al. 2012). This might be the reason why *M. commersoni* has to resort to continuous inactivity during the dry season, a period in which beetles are scarce, to support activity, whereas the more generalist bat species can shift feeding regimes, depending on availability.

All four species are also resident in the same habitat in the warm rainy season, with greater availability of food resources (Ramasindrazana et al. 2012; Reher et al. 2019). Physiological data of the rainy season is only available for *M. commersoni* and *T. menamena.* Both species also enter torpor during this period. In addition to daily torpor, *M. commersoni* also enters prolonged torpor on occasion (Reher et al. 2018): while the exact triggers remain elusive, individuals may remain normothermic or enter torpor bouts lasting up to six days (sometimes coinciding with cyclonic rains). In both species, torpor is used more opportunistically during the warm rainy season than during the cool dry season and appears to be linked to environmental and internal factors that are assessed daily, like an insufficient foraging flight or generally low body mass (S. Reher et al., unpublished data).

As mentioned above, *M. commersoni* is widespread across the island, occupying different habitat types. In the western dry deciduous forest (Kirindy CNFEREF), it responds very differently to thermoregulatory challenges. Here, caves are not available and individuals roost solitary in understory shrubs offering no protection from daytime climatic extremes during the hot and wet season (Figure 1c). While being exposed to unbuffered high temperature and low humidity during the day, the bats respond in two different ways. In the cooler morning hours and generally on cooler rainy days (~33°C at noon), they switch between normometabolic resting and very short micro torpor bouts lasting between 3-53 min throughout the daily resting phase. If temperatures increase above a critical threshold (~ 37°C at noon), however, they extend their torpor bouts until the late afternoon and tolerate hyperthermic body temperatures near or exceeding normothermia up to 42.9°C without initiating cooling mechanisms (S. Reher et al., unpublished data). By accumulating heat instead of trying to dump it, no water is “wasted” via evaporative cooling, which is the only option for actively regulating body temperature at ambient temperatures higher than normothermia, analogous to the “camel strategy” (Schmidt-Nielsen et al. 1956). Consequently, while the key to cope with the unpredictable environment in the southwest appears to be the selection of a highly predictable cave year-round, in the seasonal west, *M. commersoni* tolerates hyperthermia in the rainy season and either hibernates or migrates to more suitable habitats during the dry season (Rakotondramanana and Goodman 2011; S. Reher et al., unpublished data).

Apart from the handful of species mentioned above, little is known on the physiological adaptations of Madagascar’s bats (Racey et al. 2010; Goodman 2011). For example, fruit bats have been reported to enter torpor, but to date no physiological data are available. Consequently, we are expecting more Malagasy bat species to be heterothermic or to make use of further physiological adaptations, such as shifts in the thermal neutral zone, intensive passive rewarming, or mechanisms that we currently do not know of.

Lemurs – Sleeping Beauties and the Dwarfs

Maybe most surprisingly was the discovery of several months-long hibernation within our own order primates, in the lemurs (Petter et al. 1977; Petter-Rousseaux et al. 1980; Dausmann et al. 2004). However, heterothermy in lemurs seems to be restricted to one family, the Cheirogaleidae (Figure. 1d, e, f). Throughout the island, Cheirogaleidae become torpid during the Malagasy cool dry season, although depth and duration of torpor bouts vary considerably, depending on species, locality, habitat, sex, and individual condition.

As far as is known, all mouse lemur species (*Microcebus* spp.) enter daily torpor, usually in the cool hours of the early mornings of the dry season within their tree hollows; however, this includes not necessarily all individuals of a population and not every day (Dausmann 2014). Occurrence of torpor episodes can be regular, irregular or daily; various patterns are often found within the same population and within one season. Thus, torpor is used as a very flexible response to current internal and external conditions. Physiological studies have verified daily torpor for *M. griseorufus* (Kobbe and Dausmann 2009; Figure 1d), *M. murinus* (Schmid 2000), *M. lehilahytsara* (Karanewsky et al. 2015; captive population), *M. rufus* (Randrianambinina et al. 2003), *M. ravelobensis* (Randrianambinina et al. 2003), and *M. berthae* (published as *M. myoxinus* in Ortmann et al. 1997). Metabolic rate can decrease to about 15% of resting metabolic rate (*M. murinus*, Schmid 2000; Schmid et al. 2000) and body temperature during daily torpor can be as low as 6.8°C, depending on ambient temperature (*M.*

berthae, Schmid et al. 2000). Maybe even more importantly in the dry deciduous forest and dry spiny thickets, water requirements are drastically reduced during torpor (Schmid and Speakman 2000).

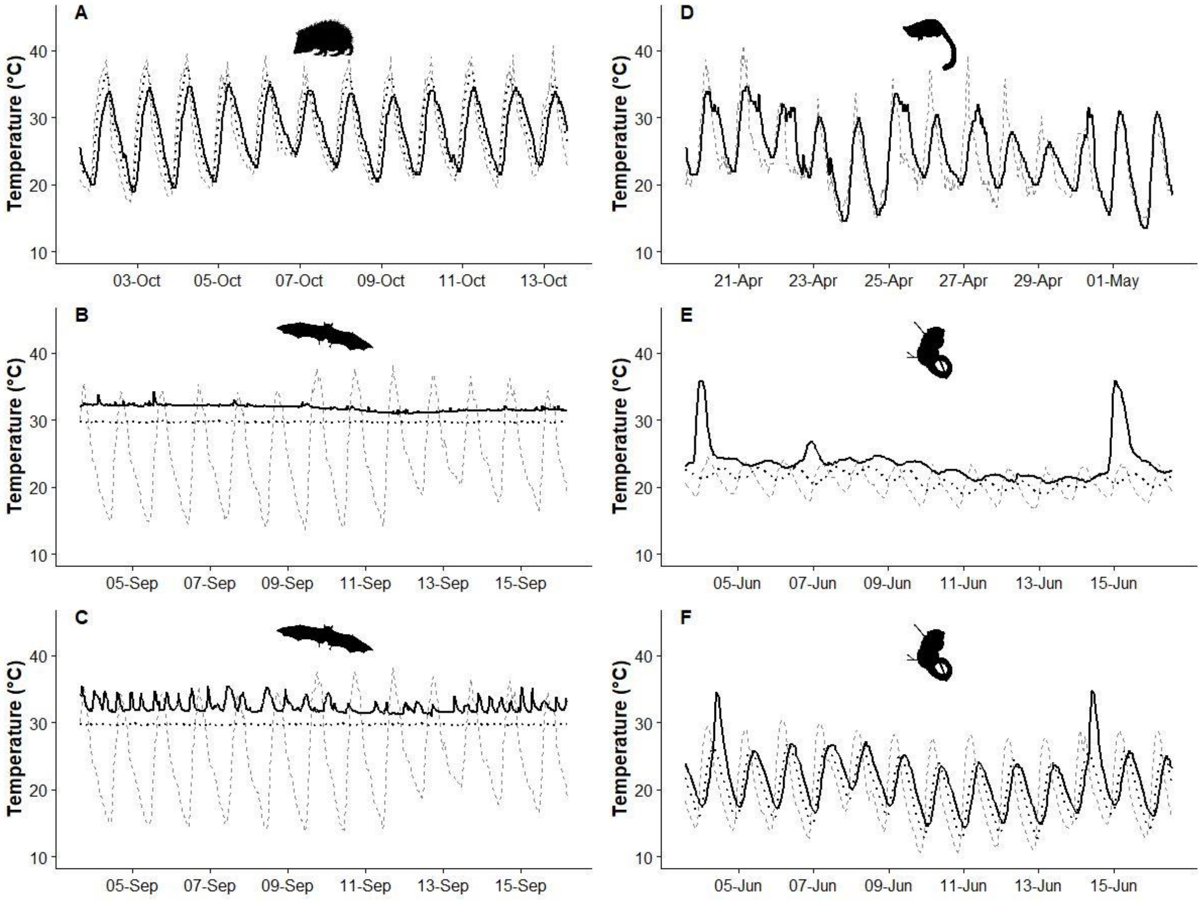

Figure 6.2. Body temperature of free-ranging individuals from three Malagasy mammalian orders during the dry season. During heterothermy, body temperature closely tracks the temperature of the resting site with its daily rise and fall, depending on insulation capacities. **A**) Continuous hibernation bout of a *Setifer setosus* in a poorly insulated hibernaculum with strongly fluctuating body temperature. **B**) Continuous hibernation bout of a *Macronycteris commersoni* in a cave with very stable resting site temperature, resulting in very stable body temperature. **C**) Body temperature of a *M. commersoni* in the same cave with very stable resting site temperature and at the same time, but employing daily torpor. **D**) Body temperature of a *Microcebus griseorufus*, showing diverse patterns of heterothermy. For the first three days, this individual entered daily torpor, still being active at night, followed by two days prolonged torpor, and followed again by a hibernation bout with fluctuating body temperature. **E**) Continuous hibernation bout of a *Cheirogaleus medius* hibernating in a well-insulated tree hollow, with almost constant body temperature; notice the two spontaneous, active arousals. **F**) Continuous hibernation bout of a *C. medius* hibernating in a more poorly insulated tree hollow, with strongly fluctuating body temperature; notice the two spontaneous, active arousals.

Black solid line: body temperature; black dotted line: temperature from the resting site; gray dashed line: ambient temperature. A, E, and F redrawn from Nowack et al. (2020); B and C from S. Reher et al. (unpublished data); and D: redrawn from Kobbe et al. (2011).

Additionally, some species utilize prolonged torpor or months-long hibernation, such as *M. griseorufus* (Figure 1d, 2d; Kobbe and Dausmann 2009; Kobbe et al. 2011) and *M. murinus* (Schmid and Kappeler 1998; Schmid 2000). The frequency and duration of torpor bouts increases with increasing body condition in *M. griseorufus* (Kobbe et al. 2011), and entry into hibernation is determined by age and body mass, with older and (the heavier) females being more likely to go into hibernation in *M. murinus* (Schmid and Kappeler 1998; Faherty et al. 2017). Whereas prolonged torpor is more an extension of daily torpor, but with increased energy savings, being employed flexibly depending on current internal and external conditions, hibernation requires preparation and can only be employed by individuals with sufficient body condition and fat reserves. *Microcebus berthae*, the smallest species of this genus (~30 g), solely uses daily torpor, as its body mass, which changes little throughout the year, does not support the accumulation of sufficient fat reserves to fuel months-long hibernation and body mass (Schmid et al. 2000). Just like the tenrecs and bats, these lemurs use the daily rise in ambient temperature to warm up passively at the end of daily torpor or hibernation bouts, before endogenous heat production kicks in, to reduce the costs of these arousals. Indeed, this two-step arousal has first been described in *M. murinus* (Ortmann et al. 1997).

The environmental cues that trigger torpor use in *Microcebus* spp. are not fully understood. Studies on a captive population of *M. murinus* identified light and food availability as potential *zeitgeber* triggering heterothermy (Perret and Aujard 2001). For example, when exposed to food restriction *M. murinus* entered torpor earlier and increased the depth and length of torpor even during gestation and lactation (Giroud et al. 2008; Canale et al. 2011, 2012). However, the decrease in body mass in food restricted and lactating females resulted in a delayed growth in the offspring (Canale et al. 2012).

Additionally, as has been shown in *M. griseorufus*, mouse lemurs may shift their thermal neutral zone to cooler temperatures during the cold dry season, to physiologically adjust to changing environmental conditions when staying normothermic (Kobbe et al. 2014). Armed with this suite of flexible responses, mouse lemurs are able to respond very individualized to changing and challenging climatic situations, availability of food and water, and body condition.

While *Microcebus* ssp. utilize all or a combination of the different heterothermic strategies, the most extreme extent of heterothermy within the lemurs are shown by the dwarf lemurs (*Cheirogaleus* ssp.). All species of this genus are obligate hibernators, and all individuals enter continuous hibernation over several months during the dry season (Petter et al. 1977, Dausmann et al. 2004; Blanco and Rahalinarivo 2010). The length of the hibernation season is variable in the different regions, longer in the dry forests (6-8 months) than in the moist evergreen forests (3-5 months), and influenced by rainfall and forest productivity (Lahann and Dausmann 2011). To fuel life during the hibernation period, *C. medius* (Figure 1e) almost doubles its body mass by storing mono-unsaturated oleic acids (synthesized from dietary carbohydrates, e.g., sugary fruits) mainly in their tails, prior to hibernation

(Fietz et al. 2003; Fietz and Dausmann 2006). Interestingly, the complex physiological underpinnings of hibernation in *C. medius* seem to be activated not by a unique set of genes, but by differential expression of genes that are present in all mammals and are involved in metabolic pathways, feeding behavior, and circadian rhythms (Faherty et al. 2016). *Cheirogaleus medius* and *C. major* hibernate in tree hollows of various insulation capacities, depending on height and thickness of the trees, and are thus exposed to differing climatic conditions (Dausmann et al. 2004). In contrast, *C. crossleyi* and C. *sibreei* in the Central Highland, as well as in lower elevation moist evergreen forest sites, hibernate buried underground in cool, but quite stable surroundings (Petter et al. 1977; Blanco et al. 2013). As body temperature usually approximates ambient temperature during hibernation, the different species' flexibilities in choice of hibernacula are also reflected in their body temperature profiles. Dwarf lemurs hibernating in well insulated tree hollows and underground show fairly constant body temperatures between about 10°C and 20°C, depending on the environmental temperature of the habitat, with regular, active arousals about once a week (Figure 2e; Dausmann and Blanco 2016). However, individuals hibernating in more poorly insulated tree hollows, as studied in *C. medius*, experience (and tolerate) daily fluctuations of body temperature during hibernation of up to more than 25°C (Figure 2f): Body temperature can drop below 10°C in the cool early mornings, but *C. medius* also continues to hibernate with body temperatures above 30°C, when being passively warmed during the day. Most notably, if these fluctuating body temperatures are passively increased above 30°C at least occasionally, expensive arousals with endogenous heating are abandoned as in *E. telfairi* and the individuals remain "ectothermic" and torpid for several months (Dausmann et al. 2004, 2005). Thus, dwarf lemurs do not hibernate continuously despite of, but precisely because of daily recurrent high ambient temperatures. These largely passive patterns also entail that huddling during hibernation does not provide energetic benefits and *C. medius* indeed mostly hibernate solitarily, although spending most of their daily resting phases in family groups during the rainy season (Dausmann and Glos 2015). Interestingly, despite these differences in physiological *modi operandi*, the different patterns yield the same level of energy savings of about 70% compared to resting metabolic rate, which probably explains the apparent lack of preference of *C. medius* for a specific choice of hibernaculum (Dausmann et al. 2009).

No physiological studies are available for the genera *Mirza* and *Allocebus*, also members of the family Cheirogaleidae, but the species of these genera are assumed to respond in a similar way as *Microcebus* spp.. For example, *Mirza coquereli* has been found curled up and cold to the touch in traps during the dry season, clearly in a torpid state (Dausmann 2014), and *Allocebus trichotis* are less active in captivity during the dry season and a body temperature of 26.2°C has been measured in *A. trichotis* at an ambient temperature of 11°C (Meier and Albignac 1991; Rakotoarison et al. 1997). The notable exception within the Cheirogaleidae is the genus *Phaner*, which seems to remain normothermic throughout the year. However, *Phaner* are most distantly related to the other Cheirogaleidae species and rely on a very specialized diet (tree exudates), which is available independent of season (Schülke 2003; see Blanco, p. xx).

Apart from heterothermy, another physiological way to save energy and water and thus to cope with hot and arid habitats, or habitats with (seasonally) sparse food availability, is maintaining a generally low metabolic rate. Many mammals living in hot and arid habitats have a 20-40% lower basal metabolic rate than would be expected from their body size (Lovegrove 2003). A low basal metabolic rate reduces energy requirements and the production of own metabolic heat, that in turn allows a higher rate of heat accumulation before expensive, water-consuming active cooling is necessary (Swanson et al. 2017). Moreover, species can adapt to seasonal changes by shifting their thermal neutral zone (e.g., *M. griseorufus*, see above).

Members of the family Lepilemuridae do both. *Lepilemur leucopus* (the study population is now considered to be *L. petteri*) living in the dry spiny thickets of southern Madagascar, not only has a very low general metabolic rate (about 50% of what would be expected from size, similar to tenrecs), but also shifts its thermal neutral zone seasonally (Bethge et al. 2017). Maybe counter-intuitively, and contrary to *M. griseorufus*, the thermal neutral zone is shifted upwards to higher temperatures in the cooler dry season. This species mainly feeds on leaves containing high concentration of potentially toxic plant secondary metabolites. One interpretation of this shifted thermal neutral zone is that plant secondary metabolites can be processed more efficiently at ambient temperatures just below the thermal neutral zone (Dearing 2013), enabling sufficient uptake of energy for the high thermoregulatory costs during the cool dry season. Alternatively, the hot daytime temperatures might be more critical for *L. petteri* during the scarce dry season, especially increased evaporative water loss, and the upward shift of the thermal neutral zone might thus counterbalance decreased energy and water content of their food (Bethge et al. 2017). *Lepilemur ruficaudatus* (Schmid and Ganzhorn 1996; K. Dausmann et al., unpublished data), *L. edwardsi* (J. Bethge et al., unpublished data; Figure 1f), and *L. leucopus* in Tsimanapetsotse (Magdziak 2017) also have exceptionally low metabolic rates, and this is likely the case for all *Lepilemur* species. Whether *Lepilemur* also becomes heterothermic remains unsolved (partly due to the difficulties of distinguishing between the already extremely low resting and torpid metabolic rates), but it does not seem to be a regular, essential response. Relatively low resting metabolic rates seem to be a general characteristic of lemur physiology, as it has been found in most lemur species studied to date, diurnal and nocturnal ones (e.g., *M. murinus*, Perret 1998, *Eulemur fulvus*, Daniels 1984, Simmen et al. 2010, *Lemur catta*, Simmen et al. 2010, and *Propithecus verreauxi*, Richard and Nicoll 1987).

Summary

Heterothermy is not known from the other Malagasy mammalian orders. However, given the small number of species that have been physiologically studied, and even less in the field, it is conceivable that heterothermy will be detected in further species. For example, the falanouc (*Eupleres goudotii*, family Eupleridae), a highly specialized carnivoran (see Rivera et al., p. xx), feeding on soft bodied invertebrates such as earthworms, apparently can store more than 20% of its body weight as fat in its tail and is said to "go to sleep" for two to three months during the cold dry season (Lovegrove 2019). Scientific evidence is still lacking, but this might suggest that *Eupleres* hibernate to get through the period when earthworms are scarce.

The cornucopia of physiological responses found in Malagasy terrestrial mammals enables them to respond individually and flexibly to the often challenging environments of Madagascar and changes within them (Dewar and Richard 2007). Heterothermy seems to be an important component in the physiological tool kit to survive unfavorable times in numerous orders and species of mammals on the island. Moreover, the range of basic physiological approaches within the Malagasy mammals is amazing: from bats, being metabolic super performers while active, but appearing to jump at every possibility to enter, sometimes even extremely short, torpor bouts to temporarily decrease their high metabolic demands and even tolerating hyperthermia close to lethal levels; to spiny tenrecs, which take the completely opposite path and keep metabolism low most of the time, except when reproductive activities prohibit this, resembling protoendotherms in their thermoregulatory patterns. Whether the comparatively high proportion of heterothermic species within Malagasy mammals reflects concentrated scientific efforts on this topic, or whether Madagascar's unique evolutionary and geological history and challenging environment has led to selective pressures favoring this physiological response, remains a question of debate.

In contrast to arctic or temperate regions, where low temperatures and low seasonal availability of food promote heterothermy, the ultimate triggers of tropical heterothermy might be less straightforward, and more multifaceted. For example, studies in other tropical areas showed that besides the vital need of water conservation, extreme weather events, as well as environmental disasters, can elicit heterothermic responses in small mammals (cyclones, droughts, storms, floods; reviewed in Nowack et al. 2020). As climatic extremes in general and temperatures in particular are predicted to continue to increase with global climate change, with Madagascar being no exception (Hannah et al. 2008), this could push Malagasy animals near the edge. Malagasy mammals are vulnerable to extremes on either side of the climatic continuum. For example, *Echinops telfairi* might succumb when temperatures stay continuously below the temperature threshold that enables them to return to normothermia after torpor bouts during cold spells. *Macronycteris commersoni*, on the other hand, might perish during heat waves, when their body temperature is driven above the lethal level during passive hyperthermic torpor episodes and *Lepilemur petteri* might suffer from generally elevated temperature levels, because of increased toxicity of their food plants. Nevertheless, heterothermy, especially flexible shorter torpor bouts, might be a useful tool to overcome more dire and unpredictable environmental pressures for many Malagasy mammals.

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Author contribution

I hereby confirm that Stephanie Reher collected and analysed the data regarding bats, prepared figure 6.2, took lead in writing the section on bats and contributed to the introductory and summarising part of this chapter. Janina Bethge took lead in writing the section on lemurs, prepared figure 6.1 and contributed to the introductory and summarising part of this chapter. Kathrin H. Dausmann conceived and structured the chapter, took lead in writing the section on tenrecs, the introduction as well as the summary, and revised the chapter. All authors approved the final version of it.

Prof. Dr. Kathrin Dausmann

General discussion

&

Conclusions

Environmental changes can be either natural or human-induced, but they all challenge an endotherm to maintain a balanced energy budget in the long term. Especially variations in ambient temperature (T_a) and resource availability affect thermoregulation and thus physiological functioning. While endotherms usually have developed means to cope with regular fluctuations such as seasonality, unpredictable rapid changes such as extreme weather events or global warming superimposed on natural environmental variation may push them to the limits of their capacities. Species equipped with a well-assorted physiological repertoire may compensate for certain fluctuations, while others may struggle and perish. Particularly widespread species inhabiting various microclimates may possess specific traits in their repertoire that might allow them to cope with some alterations. In the scope of this thesis, I have examined how two different populations of *Macronycteris commersoni*, a widespread bat species in Madagascar, cope with changes in their environment. I specifically looked into intraspecific physiological variation over seasonal and geographic scales as well as *M. commersoni*'s potential for physiological flexibility when confronted with acute short-term fluctuations. I also studied local movements and seasonal activity in co-occurring bat species in the southern population, where several bat species share the same roosting site, to explore general patterns and possibilities for adaptation and to allow more general conclusions to be drawn.

Spatial adjustments: local shifts in microhabitat use and migration

There are principally two ways to cope with environmental change: one can either avoid new circumstances through migration or ride them out through compensatory mechanisms. In Madagascar's south-west, we found evidence that both strategies are applied by different insectivorous bat species (Chapter 1). It is one of the driest regions in Madagascar, and the harsh dry season reduces T_a, precipitation and food availability even further. Rainfall may fail for entire years (Ratovonamana et al. 2013) and low night-time temperatures occur for several months (from May to September; Kobbe et al. 2014), both of which reduce insect activity and availability sharply in the austral winter (Janzen and Schoener 1968; Pinheiro et al. 2002). In Chapter 1, we describe that three bat species appear to avoid spending this time of the year in the south and migrate seasonally (*Miniopterus griffithsi*, *Mormopterus jugularis* and *Mops leucostigma*). Migration is possible only to a limited extend for most Malagasy terrestrial mammals since almost all habitats are highly degraded and lack proper corridors, but it certainly is an option for bats. Their ability to fly enables them to cover great distances regardless of terrestrial structure. As long as suitable stopover sites are available, bats can potentially move to areas with more favourable conditions (Fleming and Eby 2003), or track their food resources (Popa-Lisseanu and Voigt 2009; McCracken et al. 2012). The three Malagasy fruit bat species *Pteropus rufus, Eidolon dupreanum* and *Rousettus madagascariensis*, for example, are reported to move among roosts regularly, presumably to track their locally changing food supply

(Jenkins et al. 2007; Racey et al. 2010; Goodman 2011). In general however, extensive migrations are less common in the tropics (Fleming and Eby 2003; Popa-Lisseanu and Voigt 2009).

Two other bats species applied a different approach to cope with the demanding southern climate. *Triaenops menamena* and *Miniopterus mahafaliensis* are resident year-round and roost in several caves during the wet season. These caves are cooler and more fluctuating in their ambient conditions than the cave to which they relocate in the lean dry season: a stable hot and humid cave (Andranolovy; Chapter 1). In addition, both supplement their diet in the dry season by eating Coleoptera and, in the case of *M. mahafaliensis*, Hymenoptera, whilst in the wet season it primary consists of Lepidoptera (Ramasindrazana et al. 2012). While sympatric species may partition available prey, for example, by hunting at different times of the night (Emrich et al. 2014) or by exploiting alternative niches and trophic levels through specific echolocation call design or wing shape (Norberg and Rayner 1987; Schnitzler and Kalko 2001), a seasonal change in diet is often resource-driven and common in insectivorous bats. Mexican *Pteronotus davyi* and *P. personatus*, for example, are more selective in their diet during the wet season too, but consume a wider variety of the remaining prey with more overlap in the dry season (Salinas-Ramos et al. 2015). This might also be the case for *T. menamena* and *M. mahafaliensis*. Through becoming more generalist in their dietary tendencies and a local shift in microclimate they adjust to the ambient conditions, allowing year-round residency and activity within the most demanding region of Madagascar. This suggests that when a range of suitable roosting sites and some prey remain available, ecological flexibility mitigates a certain degree of habitat variation and negates the need for more drastic and costly responses, such as migration.

Temporal avoidance: riding out winter

Not all species developed the prerequisites for making either migration, local habitat shifts or a more generalist diet niche beneficial. *Macronycteris commersoni*, for example, roosts within the same hot and humid cave as *T. menamena*, *P. furculus* and *M. mahafaliensis*, but is a specialised beetle predator and does not broaden its prey preference seasonally (Goodman 2011; Ramasindrazana et al. 2012). Instead, this species gains massive amounts of fat deposits by the end of the wet season, ceases foraging flights for several months during the dry season (Goodman 2006; Rakotoarivelo et al. 2007; Chapter 1) and makes extensive use of metabolic energy-saving measures (Chapter 2). Surprisingly though, individuals of this population did not necessarily rely on long-term hibernation as one would expect, but displayed various kinds of torpor responses side by side at the exact same time (Chapter 2). Metabolic measurements only reflect what an individual displayed under the given conditions within the respirometry chamber and are thus limited to a certain time period. We therefore corroborated our results with data from temperature-sensitive radio-transmitters of free-ranging individuals recorded with a remote receiver/logger system placed within the cave (figure 7.1). We acknowledge that skin temperature (T_{skin}) measurements are only a proxy for core body temperature (T_b) and potentially mask metabolic downregulation, especially at such high cave temperature (Chapter 3); yet the patterns measured imply that the bats applied different thermoregulatory responses. Some individuals entered several short torpor bouts per day (figure 7.1A), whilst others entered multi-day torpor (figure 7.1B) or hibernation with single bouts lasting up to 17 days between arousals (figure 7.1C). Thus, individuals were spanning an impressive range of heterothermic responses within a single population of this species.

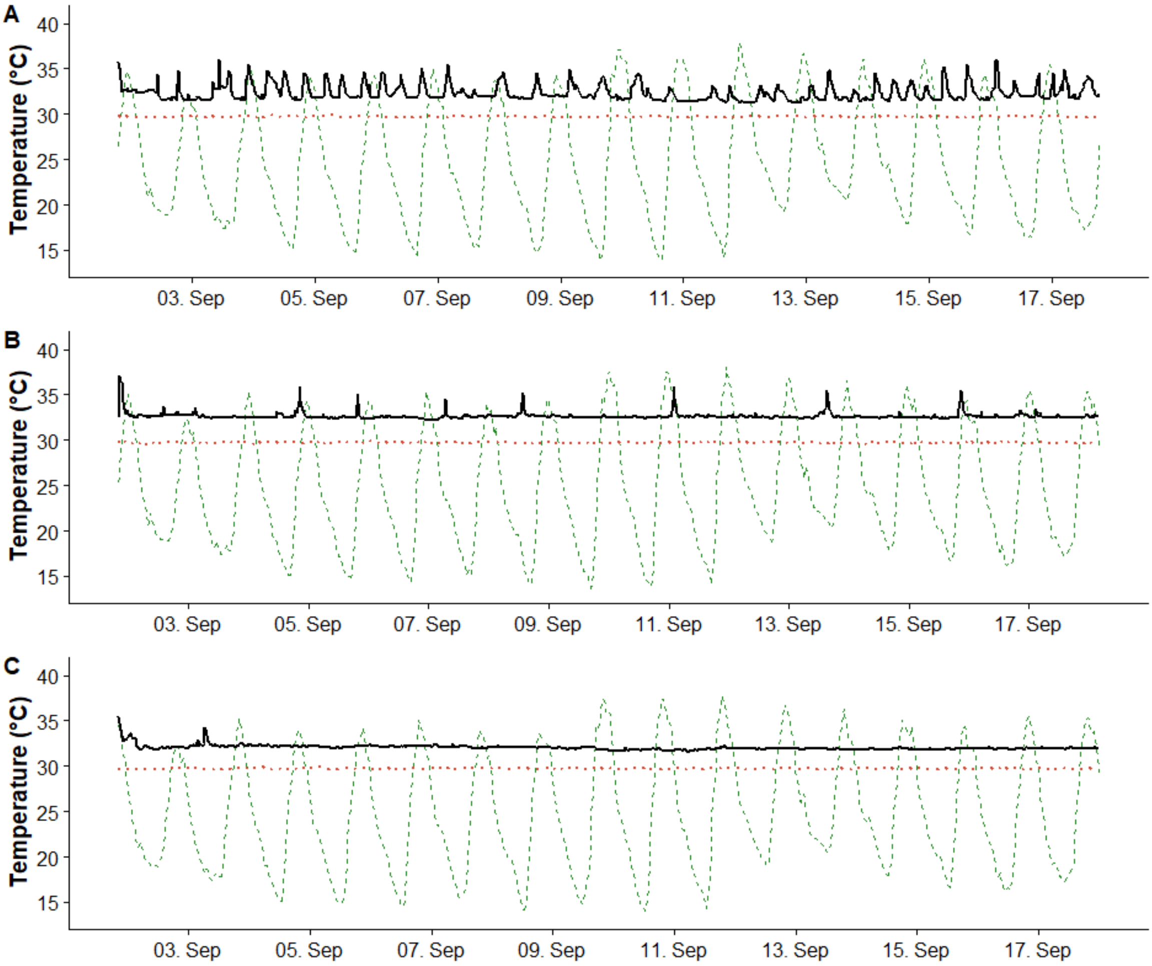

Figure 7.1. Skin temperature (black solid line) of three individuals of Commerson's roundleaf bat (*Macronycteris commersoni*) using different thermoregulatory responses in the same cave with very stable roosting conditions (orange dotted line, cave temperature; green dashed line, temperature outside the cave) and at the exact same time in the late dry season (September 2018). A, an individual entering short torpor bouts repeatedly (observed in 3 bats); B, an individual entering several multi-day torpor bouts (observed in 5 bats); C, an individual entering a continuous hibernation bout (observed in 6 bats; N_{total}=14). Please note that T_a at bat height is ~32C. Adapted and extended from Chapter 6.

Extended torpor lasting multiple hours was not always the preferred option and some individuals were even euthermic for entire days (Chapter 2). Indeed, the prospective costs of torpor bouts are the subject of lively debate and there is evidence for several negative impacts arising from torpor, such as diminished immune function and memory loss during hibernation (Millesi et al. 2001; Bouma et al.

2010; but see Clemens et al. 2009). Particularly the intensive warming during arousals is potentially harmful since it increases oxidative stress and can cause cellular damage (Carey et al. 2003; Brown and Staples 2011; Nowack et al. 2019). Reduced responsiveness to the environment, as well as missed social opportunities like reproduction or reduced territory defence (Choi et al. 1998; Mzilikazi and Lovegrove 2002), are also some of the negative aspects (reviewed in Humphries et al. 2003b; Landes et al. 2020). Consequently, not all heterotherms categorically make use of torpor (Landry-Cuerrier et al. 2008; Levy et al. 2011). The African primate *Galago moholi*, for example, enters torpor solely as last resort during acute energy emergencies (Nowack et al. 2013) and free-ranging chipmunks (*Tamias striatus*) decrease their time spent torpid in the presence of surplus food (Humphries et al. 2003a). In line with this, edible dormice with a higher body condition arouse more often during hibernation, remain euthermic for longer between hibernation bouts and enter generally shallower torpor bouts than leaner individuals (Bieber et al. 2014). Our study therefore suggests that *M. commersoni* fits into this category. As previously stated, the proportion of long-term hibernation was relatively low and more than half of the individuals studied in the dry season rather used shorter torpor bouts or remained euthermic (Chapter 2). Moreover, individuals with a higher body condition entered shorter and shallower bouts (Chapter 4). This indicates that also for *M. commersoni*, the negative impacts of long-term metabolic downregulation may outweigh the benefits of substantial energy savings in better-conditioned individuals.

Although numerous bats were regularly euthermic, they did not leave the cave for the entire duration of the dry season (Goodman 2006; Rakotoarivelo et al. 2007; Chapter 1). Given the low food availability in the austral winter in general and the even more limited options for a food specialist, staying inside a safe roost may be beneficial also in regard of limited exposure to predators (Lima 1998; Bieber et al. 2014). The particular conditions within the cave allow *M. commersoni* to roost within its thermal neutral zone (TNZ; Chapter 3; Chapter 5) and accordingly, at minimal energetic costs when the bats are active, clearly even negating the need to enter long-term hibernation bouts in better-conditioned individuals. The individuals we observed also lost their circadian rhythm in torpor timing (Chapter 4), a phenomenon typical for hibernators but also common in animals experiencing very constant environments such as seasonal continuous dark or light conditions in arctic regions (Williams et al. 2012; Appenroth et al. 2021). *M. commersoni* consequently makes use of a rather unusual overwintering strategy and relies on large fat depots as well as pronounced inactivity rather than long-term hypometabolism. Comparable observations were made in closely related species. Individuals of African *Macronycteris vittatus* (formerly *Hipposideros commersoni* s. l.; Foley et al. 2017) and Indian *H. lankadiva* did not leave their cave roost for several days during the lean season. They were warm to the touch and responsive, but without any signs of the restless behaviour typically seen in bats preparing to leave the cave (Brosset 1962; Churchill et al. 1997; Cotterill and Fergusson 1999). Most importantly, both species also selected relatively warm and humid caves, a common feature that seems to be key for this type of "active overwintering”. Certainly, this would be interesting to look into further.

The paradox of a hot cave for overwintering

Andranolovy Cave is not only the hottest, but also most humid and in both respects most stable cave in the region that we know of. We monitored seven accessible caves continuously between 2016 and 2019, and this was the largest and best-buffered one, with a year-round stable 32°C and >98% relative humidity at bat height in the chamber where the bats were roosting (Chapter 4). A small fraction of caves documented globally has the necessary geomorphology to permit such a specific microclimate and Andranolovy Cave’s distinct physical features identify it as one of them: a small entrance, little air flow, multiple chambers offering a temperature gradient to the final chamber and a certain size for housing 1,000 - 100,000 bats (De la Cruz 1992; Rodríguez-Durán 2010). Most importantly, the high bat density contributes to the characteristic microclimate through heat radiating from both the bats’ bodies and from decomposing guano (De la Cruz 1992; Peck et al.). Hot caves are therefore different from warmer caves heated by geothermal effects (Bell et al. 1986) and represent a form of ecosystem engineering, in which the exceedingly stable microclimate and abundant guano also provide a habitat for other organisms (mainly arthropods, gastropods and microbes; Hastings et al. 2007; Sakoui et al. 2020).

Overwintering in such a hot cave is intuitively a paradox and, to the best of our knowledge, it has never been described that bats would even hibernate in one. On the contrary, the benign conditions within hot caves were associated to lower basal metabolic rate reducing metabolic heat production and lower energy expenditure for thermoregulation, reducing the need for torpor (Rodríguez-Durán 1995). In our study however, about 43% of the bats from the long-term T_{skin} monitoring entered hibernation bouts. Especially in torpid animals, metabolic rate (MR) is a function of T_a owing to Q_{10}-effects and energetic savings are therefore higher in cooler environments (Heldmaier and Ruf 1992). Thus, one would expect the bats to select a relatively cool roost to decrease overall overwintering costs, and cooler caves are available in the region (Chapter 2). Most examples from classical hibernation, however, arise from temperate regions and accordingly our assumptions may be biased. The scientific effort on tropical heterothermy is increasing and the existing studies indicate that torpor at higher temperatures (1) may be more common than often assumed and (2) hold certain advantages we may not be aware of (Nowack et al. 2020). One of the most unexpected findings from tropical hibernators, for example, was that periodic arousals typical of and even thought to be essential for hibernation are suspended when T_b regularly reaches a specific threshold (Dausmann et al. 2004; Lovegrove et al. 2014b). The Malagasy lemur *Cheirogaleus medius* abandons arousing when T_b

passively rises above 30°C occasionally just like the common tenrec (*Tenrec ecaudatus*) does whilst hibernating in an underground burrow where T_b remained above 25°C (Dausmann et al. 2004; Lovegrove et al. 2014b). Whether *M. commersoni* also suspends arousals is purely speculative: the longest bout we measured in the additional radio-transmitter study (17 days) was only interrupted by us entering the cave to retrieve the equipment, so there would have been potential for longer bouts. Omitting arousals reduces oxidative stress and the associated risk of cellular damages as well as saves energy (see above). This could contribute to trade off the higher energetic costs incurred by a generally higher torpid MR (TMR) and ultimately make torpor, even hibernation, within a hot cave very beneficial.

Andranolovy Cave is also the most humid cave in the region (Chapter 4). The chamber in which most of the bats roost is completely flooded by an underground stream system (Chapter 1). The water body within the cave provides the bats with the opportunity to drink during stressful times and the high relative humidity minimises the deficit in water vapour saturation between air and individual, thereby limiting evaporative water loss (Mitchell et al. 2018). This may be vital in an arid region and particularly for bats, which have difficulties maintaining their water balance in general. Their large, hairless wing membranes expedite cutaneous evaporative water loss (Hattingh 1972), especially when they are resting (Hosken and Withers 1997, 1999; Muñoz-Garcia et al. 2012). Bats also have relatively larger lungs than terrestrial mammals or birds of comparable size (Maina 2000), increasing respiratory evaporative water loss. Even in torpor, when respiration is significantly reduced and suspended at times (e.g. Thomas et al. 1990; Hays et al. 1991), water depletion continues and metabolically produced water does not compensate for water lost by evaporation during long bouts (Thomas and Cloutier 1992). The duration of bats' torpor bouts is therefore restricted by the water budget and ambient water vapour pressure: torpor bouts shorten (Thomas and Geiser 1997) and arousal frequency increases as humidity decreases (Ben-Hamo et al. 2013), which ultimately may deplete energy stores too early and affect overwintering survival (Geiser and Broome 1993; Ehlman et al. 2013). The high relative humidity is therefore an additional aspect rendering this cave so valuable as an overwintering site out of the theoretically many caves available.

Hot caves offer exceptional conditions to the resident fauna. However, they are also rare and fragile ecosystems (Ladle et al. 2012). For example, a certain number of individual bats is essential to maintain the hot and humid microclimate. Population declines of resident bats below the critical threshold could therefore threaten the whole cave fauna, whereby the cause for the colony reduction can be manifold (e.g. hunting, loss of foraging opportunities; Ladle et al. 2012). The warming contribution of guano decay could also be reduced by intensive and unsustainable extraction by humans (De La Cruz 1992). Furthermore, even if a sufficient number of bats is present, natural or human-induced erosion may alter the geomorphologic characteristics of the cave, which in turn could rapidly and irreversibly change the cave's airflow and ultimately its microclimate (Ladle et al. 2012). Fossil records in the Caribbean, for example, indicate that in 80% of the caves once hosting hot cave communities, the ventilation today is too high to allow heat accumulation (Tejedor et al. 2004). Finally, the particular bat species roosting in hot caves may be highly specialised and rely on their roost. The availability of geothermally-heated roosts in winter, for example, allows expanding a subtropical distribution range into more temperate regions in populations of California leaf-nosed bat *Macrotus californicus* (Bell et al. 1986) and in populations of two species of mouse-tailed bats (*Rhinopoma cystops*, *Rhinopoma* microphyllum; Levin et al. 2008, 2015). Albeit heated by a different mechanism, these examples illustrate the potential dependency of hot cave inhabitants on their roosts. Consequently, although the hot cave fauna benefits from the special microclimate (e.g., thermoregulatory advantages, see above), it faces high risks of extirpation and extinction owing to its sensitivity to even small perturbations in the cave system (e.g., Morgan 2001).

Expanding principles of thermal physiology: torpor at high T_b

Most of the previously mentioned aspects on thermoregulatory responses derive from a single population of *M. commersoni* and a single season, the dry season. However, the hot and wet season in Madagascar's drier western formation also poses substantial challenges. While resources are plentiful and energy conservation is a lesser issue than in the lean season, daytime T_a extremes regularly exceed 40°C and are coupled with lower relative humidity (Kappeler and Fichtel 2012; Ratovonamana et al. 2013), which may overwhelm the bats' thermoregulatory system. Especially when T_a exceeds T_b, the water budget and access to water dictate the extent of evaporative cooling for heat compensation (Withers et al. 2016; Mitchell et al. 2018), a major constraint in dry areas where most organisms are living near their physiological limits (Huey et al. 2012; Lovegrove et al. 2014a) or are already suffering from acute water stress. The cave-dwelling population of *M. commersoni* avoids the pressures imposed by daytime heat by remaining in the buffered hot cave with its water reservoir year-round (Chapter 1). However, not all tropical endotherm species or populations can seek shelter, making efficient physiological mechanisms to endure thermal extremes vital (Fick et al. 2009; Fuller et al. 2021). In Chapter 3, we focused on a population of *M. commersoni* roosting solitary in open foliage to examine how it copes with being unprotected from daily fluctuating environmental conditions. Unexpectedly, the bats entered torpor despite the exposed roosting conditions and thus, limited protection from predators (Chapter 3). We generally observed two different strategies: hot torpor and micro-bouts.

Hot torpor – fate or fortune?

On hot days (mean max T_a = 37.8 ± 1.85°C), most individuals (94%) entered an extended "afternoon" torpor bout when T_a exceeded euthermic T_b (Chapter 3). Interestingly, this led to a noticeable increase in T_{skin} at the onset of the torpor bout because active regulation of T_b was suspended. Over the course of the hot afternoon, T_{skin} passively increased with T_a up to a maximum value of 42.9°C and thus, hyperthermia. The bats consequently combined both a well-known response to cold conditions, i.e. entering torpor, with a well-known response to hot conditions, i.e. tolerating hyperthermia (Chapter 3). Unlike other bat species, which take advantage of the cooler morning hours to enter torpor and probably save water in anticipation of responding to afternoon heat (Geiser et al. 2000; Bondarenco et al. 2016), in *M. commersoni* more individuals entered torpor the warmer it became and above 36°C, all bats were torpid. 36°C was about this species' upper limit of the TNZ (Chapter 3). Above it, thermoregulation required excessive water use as observed in a bat roosting in the camp, which licked its forearms extensively to enhance evaporative cooling at T_a = 36.9°C (figure 7.2). The bats consequently reduced metabolism and bodily functions to a minimum during hottest T_b, allowing for negligible internal heat production, increased external heat storage and thus substantial water savings. This makes "hot" torpor an efficient response to withstand heat.

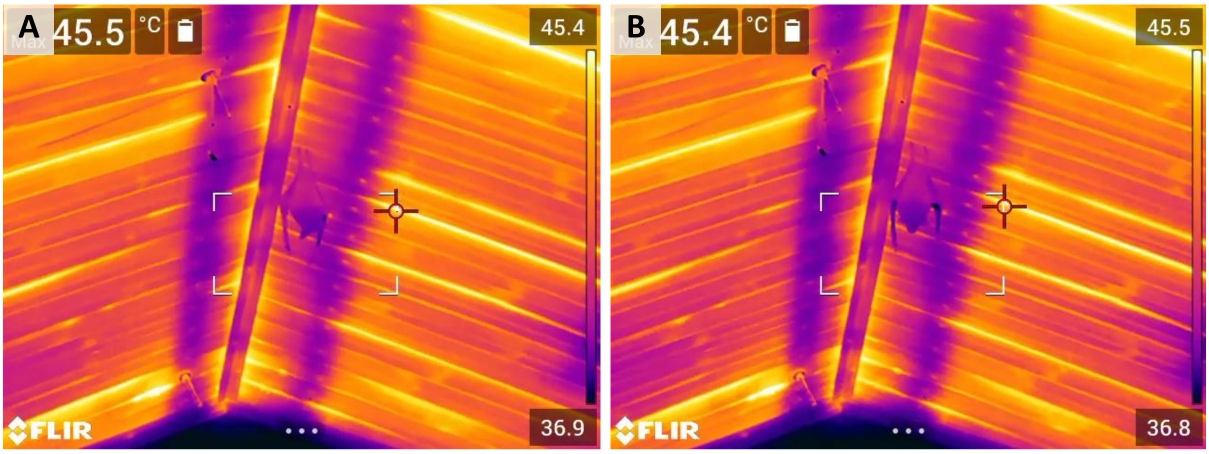

Figure 7.2. Thermal images (FLIR T540, FLIR systems, Wilsonville, USA) of a male bat resting under a hot roof next to a temperature logger (Hygrochron iButton, Maxim integrated, San Jose, USA) that recorded 36.9°C at this time. The individual extensively licked its forearms for evaporative cooling (while [A] and after [B] salivating). For scale, please note that the bat's length is ~13.6 cm from head to toe.

Our data resemble a pattern described in two species of streamertails: these hummingbirds were not reported to enter torpor, but above their TNZ, MR dropped and T_b simultaneously increased (figure 7.3; Schuchmann and Schmidt-Marloh 1979). We acknowledge that we do not know the birds' stress level during this particular study and, as T_b was obtained as T_{skin}, increasing heat dissipation enhanced by peripheral vasodilatation (Heldmaier et al. 2013; Hill et al. 2016) may have obscured the extent of T_b increase. However, the difference to euthermic T_b was substantial and even when endotherms allow hyperthermic episodes, the signal in MR is usually reversed with an increase in resting MR (RMR; e.g., Cory Toussaint and McKechnie 2012; Turner 2020; Czenze et al. 2020; Noakes et al. 2021) related to the Q_{10}-effect (Withers et al. 2016). Interestingly, in South African endotherms some species deviate from the expected impact of this effect. In two species of desert rodents and three species of passerines, there was either no increase in RMR or the increase was smaller than expected although T_b as well as evaporative water loss increased (Whitfield et al. 2015; Noakes et al. 2016; van Jaarsveld et al. 2021). The patterns suggest that some kind of active metabolic depression at high T_a was implemented (Welman 2018), especially because passerines do not use more energy saving gular fluttering as mechanism for evaporative heat dissipation (Bartholomew et al. 1968; Dawson 1982; O'Connor et al. 2017). However, since the streamertails' MR clearly decreased while T_b increased (figure 7.3), we suspect that they may have used a similar mechanism as the bats did.

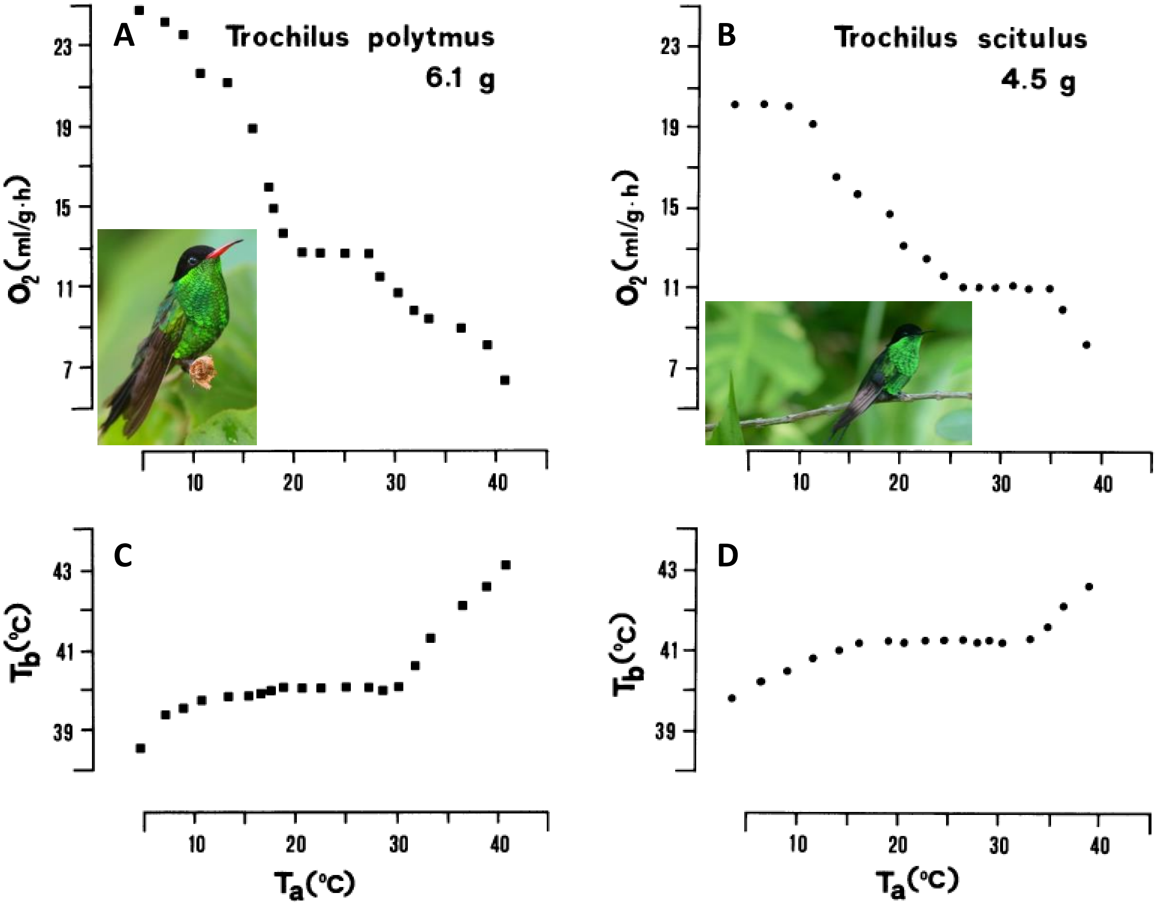

Figure 7.3. Oxygen consumption (O_2, ml g^{-1} h^{-1}; A and B) and body temperature (T_b, °C; C and D) as a function of ambient temperature (T_a, °C) in two hummingbird species, *Trochilus polytmus* and *T. scitulus*. Figure adapted from Schuchmann and Schmidt-Marloh (1979). Photo credit: *T. polytmus* - ©cotinga, *T. scitulus* - ©Paul Bowyer, observations 50787635 and 32581283, respectively from https://www.inaturalist.org/, under license (CC) BY-NC (https://creativecommons.org/licenses/by-nc/4.0/).

Hot torpor has been discussed as potential response to heat, but its actual existence has not been demonstrated earlier (Lovegrove et al. 2014a; Welman 2018). Welman (2018) argued that even if hyperthermic torpor existed, it would be hard to detect a visible reduction in MR owing to the

Arrhenius effect opposing the trend, i.e. forcing an increase in metabolism. We found TMR to be reduced by about 82% compared to RMR and this reduction in MR is among the highest reported for warm environments (25–84%; Song et al. 1997; Dausmann et al. 2009; Grimpo et al. 2013; Kobbe et al. 2014). Nevertheless, we acknowledge that the variation in TMR at the highest T_as was substantial, suggesting that in some individuals, and potentially in other species, the transitions between TMR and RMR may be blurred and harder to detect.

Clearly, whether hot torpor occurs more widely in the dry tropics remains to be determined. Nonetheless, our results highlight that there may exist more physiological options that we currently do not know of and the impressive capacity of small mammals to survive rising T_a. In case of *M. commersoni*, thermal tolerance is expanded during hot torpor episodes, allowing occupation of arid habitats lacking well-buffered diurnal roosts (Chapter 3). It is however, a risky response. If T_a increases too much while the bats are torpid, they could face lethal temperatures. Mammals reach their thermal limits when T_b approaches 41–44°C due to overheating or dehydration (Schmidt-Nielsen 1997; Lepock 2003), whereby the ultimate limits to life appear to be of biochemical issue, jeopardising the stability, structure and function of macromolecules (Tattersall et al. 2012; Clarke 2017). Thus, a certain T_a/T_b threshold would necessitate a premature re-arousal including evaporative cooling as a last resort. The associated and inevitable upregulation of metabolic heat production during emergency arousals could easily push individuals beyond tolerable temperature maxima and/or necessitate immediate evaporative cooling to decrease T_b to euthermia. Hot torpor consequently relies on T_a not exceeding these maxima and reliable cooling. It is a high-risk response and indeed, on “regular” warm (cloudy or rainy) but not necessarily hot days, we observed another variation of torpor that permitted bats to remain alert: micro-torpor bouts.

Micro-bouts – a hidden strategy?

Macronycteris commersoni entered hot torpor only under heat stress and maintained micro-bouts during cooler morning hours or “regular” warm days (mean max T_a = 33.9 ± 1.98°C). These are the shortest regular torpor bouts of any heterotherm studied so far, lasting between only 3 and 53 min (mean = 12-20 min depending on roost and season; Chapter 3; Chapter 4). Interestingly, micro-torpor did not lead to notable changes in T_{skin} compared to euthermia but the reduction seen in MR was considerable (~78% reduction compared to RMR, 55% savings in DREE compared to remaining euthermic; Chapter 3; Chapter 4). This very short version of torpor therefore combines energy savings (O’Mara et al. 2017a) with an increased number of euthermic phases and thus higher vigilance, which could be vital when roosting in exposed foliage to react quickly to imminent threats (Chapter 3) and beneficial in caves to maintain social and territorial activities (Kunz and Lumsden 2003). Almost all bats from both the cave and forest population used them and they appeared to be the preferred torpor mode when energy or water conservation were not vital (Chapter 3; Chapter 4).

Micro-torpor bouts usually occurred in a repetitive manner. This observed pattern also increased the number of respective arousals, which, as mentioned previously, are an expensive part of torpor (Thomas et al. 1990; Humphries et al. 2003b). Especially at low T_a, heart rate and metabolism must be driven to maximum rates to elevate T_b (Lyman 1982). This massive increase exposes the animals to oxidative stress, because the boost in reactive oxygen species can overwhelm the animal's antioxidant defences and ultimately cause severe cellular damage (Carey et al. 2003; Brown and Staples 2011; Nowack et al. 2019). An arousal at higher temperature could therefore not only reduce energy expenditure but also lower the overall stress on the cardiovascular system and cell damage (Currie et al. 2015). Interestingly, Australian desert bats can arouse from torpor without any obvious metabolic support when T_a is near euthermic T_b (Bondarenco et al. 2013) and the metabolism in *M. commersoni* rarely exceeded RMR when arousing from a micro-bout (Chapter 4). We therefore assume that the associated costs may be negligible in thermal neutral conditions, making torpor at high T_a and micro-bouts in particular an extremely efficient option.

Heterothermy at moderate T_a can be common (Song et al. 1997; Grimpo et al. 2013; O’Mara M. Teague et al. 2017b; Treat et al. 2018; Nowack et al. 2020; Chapter 6) but a drop in T_b is still a central aspect of most torpor definitions. “Traditionally”, torpor is defined by a significant reduction in both MR and T_b (Geiser 2004; Heldmaier et al. 2004). Since the work on Malagasy lemurs, we know that T_b during torpor may also vary passively by as much as 25°C depending on the insulation of the resting site (Dausmann et al. 2004, 2009; Kobbe et al. 2011). Nonetheless, T_b initially declined upon torpor entry and rarely exceeded 30°C during torpor (Dausmann et al. 2004; Dausmann et al. 2009; Kobbe et al. 2011). In our study, however, we found that the newly discovered modes of torpor deviate from previously described T_b patterns. During micro-torpor bouts, there was no clear or only little deviation in T_b, comparable to torpor within the TNZ in eastern pygmy-possums (*Cercartetus nanus*; Song et al. 1997) and golden spiny mice (*Acomys russatus*; Grimpo et al. 2013). During hot torpor by contrast, T_b even increased at the onset of the torpor bout to more than 40°C (Chapter 3), expanding the understanding of torpor that we have had so far and demanding to reconsider the original definition (Canale et al. 2012). Using a decline in T_b to define torpor is clearly not a necessity but presumably an artefact of our understanding biased by torpor patterns observed in temperate regions. A universal definition of torpor should therefore include that MR is drastically reduced while the T_b set point, defining the onset of active thermoregulation, may be shifted dramatically towards both low and high T_b, whether this ultimately entails a decrease, no change or even an increase in T_b. Different environmental conditions can result in different patterns of torpor and we need to acknowledge this with appropriate methods, particularly when studying endotherms in hot environments. For example, with the classical temperature recordings often used in ecophysiological research, micro-torpor could not have been detected and hot torpor could have been identified as adaptive/facultative hyperthermia. Recent technological advances in developing miniature biologgers, however, allow continuous recording of physiological and behavioural data from free-ranging animals (e.g., Wilson et al. 2012; Currie et al. 2014; Chmura et al. 2018; Menzies et al. 2020). Considering that almost all individuals have used micro-torpor and that torpor in general has played a central role to cope with heat in *M. commersoni*, we should include further proxies for it that are independent of potential masking by high T_b, such as heart rate or oxygen consumption, to get a complete picture of species' and/or individuals' physiological scope.

Intraspecific physiological variation in time and space

We have worked with a species that is widely distributed and masters most habitats of Madagascar, from the southern dry spiny thickets, to western dry deciduous forest and north-eastern humid rainforest (Goodman 2011). The populations seek out different microhabitats and roost in buffered caves as well as unprotected from environmental extremes in open foliage (Raharinantenaina et al. 2008; Goodman 2011; Chapter 1; Chapter 3). Prevailing conditions may also vary within and between seasons, making the complete ecological reality of this species considerably broad. Data limited to a single population and/or season consequently do not represent the species as a whole. In Chapter 4, we therefore looked in detail to which extent torpor patterns expressed by *M. commersoni* vary over seasonal and geographic scales and are thus modified to cope with respective environmental conditions to get a better understanding of its physiological scope.

Seasonal variation in physiological variables is often observed in endotherms. In *M. commersoni* though, we did not find any variation in RMR when comparing the cave population seasonally (Chapter 4). Such adjustments were also not expected given the minor variation in cave temperature and RH over the course of the year (variation: 1°C, 3%). However, the bats entered torpor more frequently and both multi-hour torpor as well as micro-torpor bouts were longer and deeper in the dry season than in the wet season (Chapter 4). This is a pattern commonly seen: for example, eastern pygmy-possum *Cercartetus nanus* and common tenrec *Tenrec ecaudatus* increase torpor bout duration during the more challenging time of the year (Turner et al. 2012; Lovegrove et al. 2014b). During the wet season, resources are plentiful and energy conservation less pressing. Cave bats entered torpor less frequently and rather flexibly to withstand the effects of a cyclone or to compensate for individual constraints (Chapter 4). Torpor as response to endure the effects of extreme events is well known (Doucette et al. 2012; Bondarenco et al. 2014; Nowack et al. 2015; Stawski et al. 2015; Barak et al. 2019). Shorter torpor bouts can also speed up fattening before hibernation (Giroud et al. 2012) as well as fattening before and during migration (Hiebert 1993; Wojciechowski and Pinshow 2009; McGuire et al. 2014) or mitigate unsuccessful foraging (Heldmaier et al. 2004) and thus benefit leaner individuals as seen in Malagasy mouse lemur *Microcebus griseorufus* (Kobbe et al. 2011). Unfortunately, we could not compare the forest-dwelling populations seasonally because we never trapped *M. commersoni* in the forest habitat in the dry season. Based on our overall results, especially the bats' reaction to lower T_a (Chapter 5), we assume that they migrate to a cave and hibernate. Flexible habitat shifts between 300-700km are regularly observed in the closely related African Hipposideridae, whereby the individuals usually have a higher body mass after migrations and thus, presumably increase either foraging success or roost quality during their trips (Vaughan 1977; McWilliam 1982 in Cotterill and Fergusson 1999).

Geographic physiological variation is less well understood (e.g., Dunbar and Brigham 2010; Stawski 2012; Noakes et al. 2016; Klüg-Baerwald and Brigham 2017; Cooper et al. 2018; Gearhart et al. 2020). Compared to the cave in the wet season (see above), the exposed foliage roosts in the forest induced extensive use of torpor related to heat stress (Chapter 3) despite the high predator abundance e.g. fossas (*Cryptoprocta ferox*) trying to attack bats during our measurements (personal observation). We even found forest bats to have the highest torpor frequency and to enter torpor bouts lasting as long as in overwintering bats. Interestingly, both RMR and TMR at the same T_a were on a similar level despite the dissimilar roosting conditions (Chapter 4). For TMR, our results are comparable to Kuhl's pipistrelle and big brown bats that display a TMR that varies very little between populations roosting under different climatic conditions (Klüg-Baerwald and Brigham 2017; Gearhart et al. 2020; but see Dunbar and Brigham 2010). For RMR, we would have expected some variation. In Namaqua rock rats (*Micaelamys namaquensis*), for example, the population inhabiting a thermally more challenging shelter has a lower RMR (van Jaarsveld et al. 2021) and many mammals inhabiting hot and arid habitats have a 20-40% lower basal MR than would be expected from their body size (Lovegrove 2003; Hill et al. 2016; Chapter 6). A low basal MR reduces energy requirements and the production of metabolic heat, which in turn allows a higher rate of heat accumulation before cooling is necessary (Swanson et al. 2017). We compared the bats' responses under their natural conditions, and thus fluctuating conditions in the forest with very stable cave conditions that did not allow us to determine a TNZ. Over the course of the study, however, we also manipulated the roosting environment commonly experienced by the bats (Chapter 5), and we found that the cave bats had a slightly narrower and lower TNZ (31-34°C) than the forest bats (32-36°C). Interestingly, we also found a tendency for the cave population to have a slightly lower RMR within the TNZ despite lower T_a and similar T_b (Chapter 5), which may be related to the difference in RH between cave and forest (see Cooper and Withers 2008; Lovegrove et al. 2014a).

The benefits of torpor appeared to outweigh its costs and our results suggest that the intraspecific variation in overall torpor patterns may be more plastic than the level of metabolic reduction at a given T_a (but see Dunbar and Brigham 2010 and Chapter 5). Interestingly, despite marked environmental differences between seasons and sites, flexible torpor use, and not roosting conditions *per se*, dictated energy expenditure (Chapter 4). Individual bats could balance DREE and body condition by adjusting bout duration and frequency, allowing flexible energetic savings between 45 and 84% compared to remaining euthermic (Chapter 4). The broad physiological repertoire observed in *M. commersoni* allows the regulation of energy consumption and water depletion in direct response to prevailing conditions at a fine scale and enables the species to effectively compensate for contrasting environmental pressures. Physiological traits are apparently not fixed within a species over seasonal and geographic scales and conclusions drawn from limited datasets may under- or overestimate a species' full natural capabilities. However, accurate insight is critical for predicting the effects of ongoing disturbance events or rapid climatic changes on wildlife, and when defining suitable habitats that might serve as refugia and ensure the long-term viability of both populations and species (Irwin et al. 2010; Cooke et al. 2013; Rezende and Bacigalupe 2015; Cooper et al. 2018).

Limits of species' flexibility

Differences on a population level as discussed in Chapter 4 reflect a species' capacity for phenotypic variation and/or local adaptation, and may even hint at incipient speciation (Violle et al. 2012; Richardson et al. 2014). However, we do not know to what extent single individuals can flexibly take advantage of this intraspecific variation to mitigate rapid environmental disturbances and buffer local extirpation, or whether these are local adaptations of the respective populations. *M. commersoni* is disturbed in caves due to extensive hunting (Goodman 2006; Jenkins et al. 2011) and guano extraction (Reher, unpublished data). Simultaneously, the forest population is increasingly confronted with habitat destruction (Zinner et al. 2014), associated warming of the remaining fragments (Tuff et al. 2016) as well as generally rising daytime and night-time temperature maxima through global warming and its concomitants, i.e. more frequent heat-waves and droughts (Nematchoua et al. 2018). A forced shift in microhabitat is consequently a likely future scenario for either population. Understanding their capacities for fast and flexible responses is therefore vital to estimate species' and particularly populations' persistence in the long-term. To study the effects of rapid environmental changes on the metabolic responses in *M. commersoni*, we pushed the populations out of their commonly experienced environment by exposing them to the respective contrasting roosting conditions (Chapter 5). Although these manipulated environmental conditions were within the ecological range faced by the species as a whole throughout its distribution, and despite considerable intraspecific variation, most bats appeared to struggle with the unfamiliar conditions.

The forest bats usually experience daily fluctuations in both T_a and RH_a, and the combination of high T_a with high RH_a may have hampered their thermoregulatory capacities (Gerson et al. 2014; Lovegrove et al. 2014; Chapter 5). Many endotherms inhabiting drier environments have adaptations to conserve water (Heldmaier et al. 2013; Clarke 2017). Birds (Muñoz-Garcia and Williams 2005; Champagne et al. 2012) and bats (Muñoz-Garcia et al. 2012; Ben-Hamo et al. 2016) from arid habitat, for example, have a modified lipid composition in their skin, which reduces the permeability of the *stratum corneum* and thus the rate of cutaneous evaporative water loss (Haugen et al. 2003). This could be true for the forest population of *M. commersoni*. Interestingly, we know that some individuals salivate their forearms when T_a approaches the upper critical limit of the TNZ (Chapter 3). Combined with the hyperthermic T_{skin}, which we have observed during the short euthermic periods when exposed to the cave conditions (Chapter 5), our data indicate that the bats tried to keep up cooling mechanisms. Even at high RH_a, cooling via evaporation is possible: it requires an efficient system to keep the water vapour pressure on the skin surface higher than that in the air, which can be accomplished by a combination of higher T_{skin} through vasodilatation and higher skin humidity (Schmidt-Nielsen 1997; Withers et al. 2016). While a high T_{skin} also allows for passive regulation though convection and radiation, hyperthermia may have serious physiological consequences in the long-term. Unexpectedly, the high T_{skin}-T_a differential persisted during torpor, when vasomotor regulation as active mechanism is considered to be less functional (Muñoz-Garcia et al. 2016), and despite lower metabolic heat production than in cave bats (Chapter 5). The hot cave conditions likely inhibited heat loss in forest bats and, just like in their natural surroundings, the bats entered torpor episodes to cope with heat stress (Chapter 3). We therefore propose that the forest individuals are acclimatised or adapted to conditions that are more arid.

Cave *M. commersoni* had difficulties maintaining euthermia when confronted with T_a below or above their commonly experienced 32°C (Chapter 5). This population overwinters in the hot cave for the entire dry season (Rakotoarivelo et al. 2007; Raharinantenaina et al. 2008; Goodman 2011; Chapter 1,2) and while T_a may decrease to ~20°C in the wet season, they only experience this low T_a during foraging. Costly flapping flight generates substantial amounts of heat (Carpenter 1986; Speakman and Thomas 2003; Voigt and Lewanzik 2011) and T_b regulation during the active phase is further substituted by digestion (MacArthur and Campbell 1994; Beale et al. 2018). It is therefore likely that these individuals rarely need to actively upregulate T_b and maintain it at a constant level at low T_a. Indeed, with decreasing T_a, T_b decreased steadily although maximum MR increased (Chapter 5), suggesting that inside the respirometry chamber without physical activity, the bats had only limited capacity to generate heat sufficiently such as through non-shivering thermogenesis and thus, to cope with low T_a. The cave population appeared to be narrowly acclimatised to its buffered cave microclimate (Chapter 5). A similar observation has been made in different populations of hairy-footed gerbils (*Gerbillurus paeba*) and Namaqua rock rats (*Micaelamys namaquensis*), in which the direction of intraspecific variation is consistent with their commonly experienced microclimatic conditions and not the climate on larger scale (van Jaarsveld et al. 2021). While the hot cave certainly holds many advantages, it also imposes risks (see above; Chapter 2): the stable environmental conditions are rarely found outside the cave (Ladle et al. 2012), rendering its specialised fauna highly susceptible to minor deviations in roosting conditions.

When confronted with higher T_a than commonly experienced, however, the observed responses were surprisingly homogeneous; all cave bats entered hot torpor just like the forest bats when heat stressed, although the torpor patterns varied with respect to entry timing and T_a-threshold. Tropical bats experiencing high T_a in their diurnal roosts appear to have a greater capacity for coping with heat (Cory Toussaint and McKechnie 2012; Bondarenco et al. 2013, 2014; Czenze et al. 2020). Furthermore, Cooper et al. (2020) found that prior experience with high T_a reduced metabolic heat production in zebra finches (*Taeniopygia guttata*) during heat exposure, suggesting that even previous short-term exposure to certain environmental conditions may confer a more favourable physiological response subsequently. However, the southern population roosts in a highly insulated roost year-round and we never could have recorded hot torpor in their natural environment (Chapter 2, 4). We therefore assume that despite relatively homogeneous environmental conditions, the cave population has a broader physiological repertoire than it typically uses. Exposure of individuals to conditions outside their natural prevailing environment may therefore provide insight into their potential for flexible physiological responses. For golden spiny mice (*Acomys russatus*), for example, it was discovered somewhat coincidentally that they can enter multi-day torpor more than seven times longer than previously reported for this species during a flooding event (Barak et al. 2019).

We found evidence that local adaptation or acclimatisation may hamper flexible responses when the environment changes rapidly. Even though the bats found means to cope with the new situations, they all showed patterns that are not necessarily sustainable in the long-term (Chapter 5). While some endotherm species acclimatise to novel microclimate within weeks (Heldmaier and Hoffmann 1974; Noakes and McKechnie 2020), necessary time spans may not always be given. The forest population already tolerates hyperthermic T_{skin} close to mammalian lethal limits (Schmidt-Nielsen 1997; Lepock 2003; Chapter 3) and the cave population already declined substantially due to anthropogenic disturbance at their roost (Goodman 2006) and has no alternative hot cave nearby. Since physiological responses may be manifold and hard to predict (e.g. uniform hot torpor), we need to work on broad environmental scales and ideally combine field research with more experimentally-driven approaches to understand what species and populations currently do and potentially could do when they are pushed out of their commonly experienced environment.

Conclusions

Understanding how endotherms function in their current environment and to what extent they can cope with changes in it, is a pressing concern in contemporary biological research. Ongoing disruptive human interference in natural habitats is superimposed by global warming, which in turn is accompanied by an increase in the frequency of irregular weather events (IPCC 2014). Physiological flexibility can help species tolerate certain environmental fluctuations, but physiological traits are often considered to be fixed. Consequently, the results of my thesis highlight the need for examining species in a broader ecological sphere to obtain a clearer picture of their tolerance range, potential limits and ultimately a more accurate insight into their chances of enduring.

We described two new torpor patterns, both related to coping with warm or even hot ambient conditions: micro-torpor and hot torpor. To cope with acute heat stress through torpor and thereby conserving energy and water usually needed to downregulate T_b may be a huge advantage for heterotherms over homeotherms that do not have the option to reduce internal heat production as effectively. Both newly discovered patterns broaden our general idea of hypometabolism, demand for a redefinition of torpor that does not fundamentally include a decrease in T_b and indicate that we are far from grasping the actual breadth of heterotherms' possibilities. Some species may have developed proper compensatory mechanisms that we are simply not aware of. Since these characteristics cannot necessarily be provoked or even predicted, physiological data in the context of environmental questions should be collected in a framework that is as natural as possible and thus, ecologically relevant.

We quantified an impressive intraspecific physiological variation in a single species, probably allowing *M. commersoni* to inhabit a range of different habitat and climate zones. However, when pushing the single populations out of their native environmental conditions, we observed limited capacity to cope with rapidly changing microclimate in both populations, suggesting local adaptation and/or acclimatisation. This is alarming considering that disturbance in natural habitats is high in Madagascar and that forced habitat shifts are not far-fetched for either population. Based on our results, we argue that a wide distribution over a broad range of microhabitats and -climates may not represent the adaptive scopes of species. Discrete populations may be locally adapted or acclimatised, ultimately hampering flexibility if rapid responses to environmental change are required.

When we are analysing how organisms function within their respective environments, we need to consider that we are often only looking at snapshots taken from their entire ecological reality and thus only small samples of their (physiological) capacity. Comparative studies across seasonal and geographic scales, including extreme and less demanding habitats, can therefore provide indications on species' intraspecific variation and their potential tolerance range to environmental challenges. Understanding to what extent this variation is driven by local adaptation, developmental plasticity or phenotypic flexibility is then essential for accurately determining extirpation risk of populations, for managing conservation actions such as species translocation or reintroduction, and for predicting wildlife's chances for persisting in future environments and climates.

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Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Stephanie Reher

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