Fast life in the slow lane- Life history and energetics of a basal placental mammal *Setifer setosus* (Schreber, 1778)

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Abstract

There is increasing evidence that homeothermy (the maintenance of a high and stable body temperature-T_b) as observed in modern mammals was derived from an ancestral heterothermic (flexible T_b regulation) state. One of main hypotheses for why this occurred is that homeothermy benefits parental care. As such the study of the thermoregulatory physiology during reproduction in an otherwise heterothermic mammal can provide insights into the evolution of homeothermic endothermy in mammals.

This thesis presents data collected over three reproductive seasons from one such mammal, the greater hedgehog tenrec (*Setifer setosus*, Tenrecidae). Flow-through respirometry was used to measure resting metabolism over a range of ambient temperatures (T_a). At low T_a *S. setosus* demonstrated a high propensity for torpor and highly labile T_b. This high degree of heterothermy was abandoned during reproduction; pregnant and lactating females maintained higher T_b and metabolic rates. T_b obtained from free-ranging animals showed similar trends. Reproductive females had less variability in T_b, whereas non-reproductive females and males had a higher propensity for torpor as well as higher overall T_b lability. These data indicate a larger degree of homeothermy during reproduction.

Concurrent with the collection of physiological data, the use of radio-transmitters, implanted into the peritoneal cavity along with the temperature data-loggers, allowed for novel observations on the life-history of this little-studied species. The most striking finding was that *S. setosus* demonstrates an exceptional capacity to assimilate energy. In the short active season males showed high levels of activity and occupied home ranges larger than predicted for their body size. Females, in addition to maintaining a higher degree of homeothermy, can have up to three litters per year. Over this same time period individuals of both sex double their body mass in preparation for hibernation. Such high energetic outputs are thought to be incompatible with the low basal metabolic rates which this species displays. An explanation of this incongruity can be found in the high T_a at the study site, which negated most thermoregulatory costs. In reproductive females, the fitness benefits of small increments in homeothermy seem to be offset by the relatively low fitness costs involved in minimal thermoregulatory energy demands. Homeothermy during reproduction is therefore likely to have been a first step in the progressive evolution from heterothermic to homeothermic endothermy in mammals.

Preface

The data described in this thesis were collected in Madagascar from December 2009 to February 2012. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Barry G. Lovegrove. Procedures involving the use of animals were approved by Madagascar National Parks (Permit 218/09/MEF/SG/DGF/DCB.SAP/SLRSE and 158/10/MEF/SG/DGF/DCB.SAP/SCBSE) and comply with national Malagasy laws.

This thesis, submitted for the degree of Doctor of Philosophy in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any university. Where use has been made of the work of others, it is duly acknowledged in the text.

……………………………….
Danielle L. Levesque
February 2014

I certify that the above statement is correct.

……………………………….
Professor Barry G. Lovegrove (Supervisor)
February 2014

Declaration

I Danielle L. Levesque declare that:

(i) The research reported in this thesis, except where otherwise indicated, is my original research

(ii) This thesis has not been submitted for any degree or examination at any other university

(iii) This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

(iv) This thesis does not contain other peoples' writing, unless specifically acknowledged as being sourced from other researchers.

Where other written sources have been quoted, then:

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Signed:

Date: February 2014

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Acknowledgments

“...great joy arises from taking the laboratory to the study organism rather than vice versa.”
Per Scholander

First and foremost I would like to thank my supervisor Professor Barry Lovegrove for allowing me to pursue the great joy of taking the lab to the field, for all of his support and encouragement along the way, for all of the times that he and Chris welcomed me into their beautiful home and for numerous side trips, both literal and figurative over these past few years. Most of all thank you for reminding me that the reason why we study exceptions is to better understand the rule and not to point out the futility of generalisations!

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Many of the ideas found in this thesis were shaped by various advisors and lab mates over the years including, but not limited to, Glenn Tattersall, Viviana Cadena, Murray Humphries, Gordon Grigg, Peter Brice and Lyn Beard. I sincerely thank all of them for their stimulating discussions, interesting research projects, many forms of assistance and their love of science, all of which have helped to shape my views on energetics and thermoregulation. Any mistakes, missteps or misinterpretations are fully my own.

Misaotra betsika to everyone who helped me in the field especially my diligent guides Tosy, Alpha and Ndrema, the hard-working DEA students Oliva M.A. Lovasoa, Sophia N. Rakotoharimalala and Adéline Rahantamalala. I am eternally grateful to Kerileigh Lobban, Christophe Bouvier and Mark Downey all who provided considerable assistance with the day-to-day data collection, and Cliff Dearden who assisted with the construction of the radio-transmitters. *À les mesdames du* Jungle Snack; "*Misaotra betsika pour toute la sakafo matsiro*". To the staff and fellow researchers of Ankarafantiska National Park, thank you all

for making life in the field just that much more enjoyable. The staff of the Durrell Wildlife Conservation Trust and the Wildfowl and Wetlands Trust thank you for occasional logistical support as well as excellent company. The various departments and organisations who provided logistical support over the years including the Malagasy Institute for the Conservation of Tropical Environments (MICET), Madagascar National Parks, the Départment de Biologie Animale of the University of Antananarivo and Dr. Daniel Rakotondravony.

I wish to thank the funding agencies that made the research possible including the National Research Foundation (through South Africa *Ad Hoc* and incentive grants to Prof. Lovegrove) and incentive grants from the University of KwaZulu-Natal to Prof. Lovegrove, Idea Wild for the grants for the GPS equipment, and personal funding from the National Science and Engineering Research Council (Canada).

The biggest of thanks goes out to my wonderful family, who, although they do not get to see me often, have continued to support me throughout my studies. Finally to all the beasties who participated in this study for showing that statements such as the following might not be giving them enough credit.

> *"The fox knows many things," wrote Archilochus, a Greek poet of roughly 700 B.C., "but the hedgehog knows one big thing."... The hedgehog is more familiar on our side of the world as a porcupine. And the one big thing that it knows, of course, is the value of being prickly. The fox, a predator well armed with teeth and claws and speed and wit, but smallish and gracile, is obliged to be more versatile.*
>
> -David Quammen,
> *The Song of the Dodo*

Contribution of co-authors and remarks on style

This thesis is formatted as a series of manuscripts for which I am the primary author, following the style of the *Journal of Zoology*, with the exception of Chapter 4 which is formatted according to the *Journal of Experimental Biology*. Chapter 2 is published in the *Journal of Zoology*[1] and includes Daniel Rakotondravony of the Département de Biologie Animale of the University of Antananarivo, and Barry G. Lovegrove as co-authors. Chapter 3, also published in the *Journal of Zoology*[2], is co-authored by Oliva M.A. Lovasoa, Sophia N. Rakotoharimalala, two DEA students from the University of Antananarivo, and B.G. Lovegrove. Chapters 4, in press at the *Journal of Experimental Biology*[3], and 5 are authored solely by myself and B.G. Lovegrove.

The study was conceived and designed with input at all stages from B.G. Lovegrove. The data were collected with the help of a number of field assistants, including O.M.A. Lovasoa and S.N. Rakotoharimalala, and with logistical support provided by D. Rakotondravony, all who provided editorial comments on the papers for which they are listed as co-authors. All data analyses and the initial drafting of the manuscripts were performed by myself with guidance from B.G. Lovegrove.

[1] Levesque, D. L., D. Rakotondravony & B. G. Lovegrove (2012) Home range and shelter site selection in the greater hedgehog tenrec in the dry deciduous forest of Western Madagascar. J. Zool., Lond., 287, 161-168.

[2] Levesque, D. L., O. M. A. Lovasoa, S. N. Rakotoharimalala & B. G. Lovegrove (2013) High mortality and annual fecundity in a free-ranging basal placental mammal, *Setifer setosus* (Tenrecidae:Afrosoricida). J. Zool., Lond., 291, 205-212.

[3] Levesque, D.L., B.G. Lovegrove (in press) Increased homeothermy during reproduction in a basal placental mammal. J. Exp. Biol. JEXBIO-2013-098848

Original contributions to knowledge

Chapter 2 Home range and shelter site selection in the greater hedgehog tenrec in the dry deciduous forest of Western Madagascar

The Tenrecidae of Madagascar are a speciose and widely distributed group of endemic insectivorous mammals. The subfamily of larger spiny tenrecs (Tenrecinae, Order: Afrosoricida) have been relatively well studied in captivity, however studies on free-ranging populations remain rare. This chapter describes various aspects of the activity, habitat usage and distribution patterns of the greater hedgehog tenrec (*Setifer setosus*). The first measurement of home range size in a tenrec was also reported. The most novel finding presented is that home range size is much larger than predicted for their body size: 9.8 times larger for males and 4.8 larger for females. The data presented in this chapter also confirm aspects of the life history of this species that had previously been observed in a captive setting: namely that they are promiscuous and polygamous, and that the entire population in the Western deciduous forest hibernates throughout the dry austral winter. Characteristics of nest site usage of this species, most importantly that repeated use of single nest site indicated parturition, are also presented.

Chapter 3 High mortality and annual fecundity in a free-ranging basal placental mammal, *Setifer setosus* (Tenrecidae: Afrosoricida)

Mammalian life history characteristics fall along a slow-fast continuum. In general, species on the fast side mature and reproduce at an early age and produce large numbers of young, whereas those on the slow side mature later and produce only a small number of young. The life history of tenrecs, at least with regards to their reproduction, has been well studied in captive populations. The larger species of tenrec, (subfamily Tenrecinae) fall on the fast side of the life-history continuum and the largest of these, *Tenrec ecaudatus*, have one of the highest litter sizes of any eutherian mammal. Few data, however, exist on the natural history of free-ranging populations that could explain this unusual aspect of their life history. For this chapter I collated the results of three years of data collection on the phenology, reproduction and rates and causes of mortality in a population of *S. setosus*. The findings confirm that the annual activity cycle of this species includes a five to seven month hibernation period, leaving only limited time for both reproduction and pre-hibernation fattening. During the short active season females were observed giving birth to up to three litters, which places them on the faster side of the mammalian life-history continuum. Short intervals between parturition dates and simultaneous gestation and lactation provide the first evidence of post-partum oestrus in the Tenrecinae, previously observed in only one other species of tenrec (*Geogale aurita*, subfamily: Geogalinae). High levels of mortality, primarily by snakes and unidentified mammalian ground predators, were also observed and likely contribute, along with the unpredictability of Madagascar's climate, to the unusually fast life-history of these mammals. A greater understanding of the life history of this species is provided by these findings which offer some ideas as to why a fast pace of living is necessary for *S. setosus*. How this high level of energetic output is achieved, however, remains to be answered, necessitating the study of the physiology and energetics of this species presented in Chapters 4 and 5.

Chapter 4 Increased homeothermy during reproduction in a basal placental mammal

The level of homeothermy observed in most modern endotherms is likely to have been derived from an ancestral heterothermic state. One of the hypotheses for why this occurred is that homeothermy allowed for greater energetic output during reproduction (gestation and lactation) which had direct benefits to fitness. This hypothesis (the Parental Care Hypothesis) was tested by measuring resting metabolic rates over a range of ambient temperatures (T_a) in both reproductive and non-reproductive *S. setosus*. During gestation and lactation there was an increase in metabolic rate and body temperature (T_b) below the thermoneutral zone ($T_a <$ 25°C), accompanied by a decrease in T_b variability. Whereas increases in resting metabolism were substantial below 20°C, daytime rest-phase temperatures at the study site rarely decreased below the lower critical limit of the thermoneutrality. This chapter provides the first measurements of thermal profiles obtained from wild-caught reproductive basoendotherms. Contrary to previous studies on captive tenrecs, reproduction did not change the rates of metabolism within the thermoneutral zone. This observation, however, might be due to the potentially high costs of accumulating fat stores for hibernation, a process occurring in the entire population. Combining the thermal profiles with the T_a at the study site showed that the costs of thermoregulation during the active season for this population were relatively low. These data provide an example for how small increases in homeothermy, which could occur at a relatively low energetic costs, could have led to substantial increases in fitness, by allowing for the faster production of young. Mechanisms necessary for increases in thermogenesis during reproduction would have further benefited the development of homeothermy in mammals.

Chapter 5 Effects of reproductive status and high ambient temperatures on the body temperature of a free-ranging basoendotherm

Building on the thermal profiles presented in Chapter 4, this chapter presents long-term T_b recordings collected from free-ranging *S. setosus* over the course of two active seasons. In general, reproductive females had slightly higher and less variable T_b, whereas non-reproductive females and males had both a higher propensity for torpor as well as lower and more variable rest-phase T_bs. Torpor expression, defined using traditional means (a threshold or cut-off T_b) was much lower than predicted based on the high degree of heterothermy expressed by tenrecs in captivity. However, torpor defined in this manner is likely to be underestimated in habitats where T_a is close to T_b, such as in the tropics and sub-tropics. These results caution against the inference of metabolic states from T_b alone and lend support to the recent call to define torpor in free-ranging animals based on mechanistic and not descriptive variables. In addition, the decrease in T_b variability observed during gestation and lactation in this study confirms that homeothermy is essential to reproduction in this species, and likely for basoendothermic mammals in general. The relatively low costs of maintaining homeothermy in this environment might help shed light on how homeothermy could evolved incrementally from the ancestral heterothermic condition. The findings I presented in this chapter underline the importance of studying thermoregulation in a wide range of habitats, especially those where T_a is at or above T_b. Knowledge of the effects of high T_a on T_b is important because they can be considered representative of the environments in which endothermy evolved and thus help shed light on the process. Furthermore, understanding the effects of high T_a on the physiology and energetics of endotherms is increasingly relevant in the face of changing climates.

Contributions not included in this thesis

In addition to the findings presented in this thesis, data collected over the course of this study resulted in a contribution to a collaborative study on the effects of high ambient temperatures on torpor expression in the tropics and sub-tropics (Appendix A: Lovegrove et al. 2014, Physiol Biochem Zool 87, 30-45). This publication used data, also partially presented in Chapter 5, which showed that changing amplitudes in ambient temperatures during the daytime rest-phase caused body temperatures measured in resting (likely torpid) animals to rise higher than active body temperatures, resulting in a decrease in body temperatures once the animal became active. Core body temperature data measured from *Setifer setosus* helped to support similar patterns observed in the skin temperature of other species. The data on the activity and movement patterns of *S. setosus* presented in this thesis helped support the hypothesis that the animals were in torpor at this time. Most importantly, all of the data contained in this collaborative paper illustrate the importance of measuring activity in concert with body temperature and the dangers of interpreting activity patterns, as well as levels of torpor expression, from body temperature alone, especially in environments with high ambient temperatures. Similar observations, and a subset of the data obtained during the second season, were also used in a review on tropical heterothermy (Appendix B: Canale, Levesque & Lovegrove 2012. In: Living in a Seasonal World: 29-40. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin Heidelberg). These publications urge caution in the interpretation of body temperature patterns in environments with high ambient temperatures, and attempt to encourage future research to include simultaneous and accurate recordings of both activity patterns and ambient temperatures.

The methods used to create the transmitter/data-logger packages as well as an evaluation of its effectiveness are currently being drafted into a manuscript (Levesque, D.L., K.D. Lobban and B.G. Lovegrove, *in prep*. An inexpensive, implantable combination of VHF radio transmitter and Thermochron iButton(s) for simultaneous measurements of location and core body temperature in mammals). I have also made contributions towards a manuscript authored by a veterinary surgeon describing the best-practice methods for the transmitter implant surgeries (Rose, B., D.L. Levesque, C.I. Canale, and B.G. Lovegrove, *in prep.* Surgical methodology for the measurement of core body temperatures measurements in free-ranging animals). Both of these publications will provide valuable contributions to the methodology used in the study of physiology in free-ranging animals.

Chapter 1 General Introduction

The Evolution of Endothermy in Mammals

The evolution of endothermy remains one of the most enduring mysteries in mammalian biology (Bennett & Ruben 1979, Koteja 2004, Kemp 2006, Lovegrove 2012a). Endothermy is an energetically costly method of regulating body temperature (T_b) because it relies on heat produced by elevated rates of metabolism to maintain a T_b elevated above ambient temperature (T_a, Bartholomew 1972). Endothermy is believed to have developed early in the mammalian lineage, somewhere between 250 to 200 mya (Crompton, Taylor & Jagger 1978, Hillenius & Ruben 2004, Rowe, Macrini & Luo 2011). In its earliest stages, the transition from ectothermy to endothermy would have been assisted by a shift to a nocturnal niche (Crompton *et al.* 1978, McNab 1978, Malan 1996), the ancestral state in mammals (Gerkema *et al.* 2013). Indeed, marked increases in mammalian brain size (encephalization quotient) are correlated with increases in olfactory, visual and auditory enhancement and occurred in Early Jurassic mammaliaformes such as *Morganocodon* and *Hadrocodium* (~200 mya) indicating that nocturnal lifestyles and endothermy are linked in mammals (Rowe *et al.* 2011). Although their exact order of appearance is unknown, increases in baseline (basal) metabolism (primarily caused by increases in proton leak in the mitochondrial membranes), insulation and the sustained ability for increased aerobic capacity are all necessary for the maintenance of a stable T_b (Hillenius & Ruben 2004, Kemp 2006, Lovegrove 2012a).

Earlier hypotheses on the evolution of endothermy argued either that endothermy first evolved via a maintenance of high T_b (McNab 1978), or that increases in aerobic capacity came first, with the side effect of higher baseline metabolism which in turn led to higher T_b (Bennett & Ruben 1979). However, in recent years it has been proposed these changes occurred in tandem, with small increases in the one leading to proportionate increases in the other (Kemp 2006, Clarke & Pörtner 2010, Lovegrove 2012a). Excess metabolic heat produced during activity, and retained via insulation, would have attenuated nighttime decreases in T_b that occur in diurnal ectotherms, leading to an increasingly constant, albeit likely low, T_b (Crompton *et al.* 1978). A more constant internal environment would then have allowed for the optimization of biochemical processes at temperatures at or around a set-point T_b, permitting the more adaptive specializations observed in modern endotherms (Heinrich 1977, Angilletta *et al.* 2010). Further increases in the level and precision of endothermic T_b would later have occurred for a number of reasons, including providing a stable environment for foetal growth during reproduction (Farmer 2000), allowing for increases in energy assimilation and increased energy transfer from parents to young after parturition (Koteja 2000), as well as various increases in the ability for sustained aerobic capacity (Bennett & Ruben 1979, Lovegrove 2012b, Naya *et al.* 2013, Lovegrove & Mowoe 2014).

The hypothetical mammalian ancestor is postulated to have been small bodied, nocturnal and insectivorous (Crompton *et al.* 1978, Luo 2007, Gerkema *et al.* 2013, O'Leary *et al.* 2013), and likely possessed the capacity for prolonged periods of metabolic down-regulation, either in the form of daily torpor, or hibernation (Lovegrove 2012a). The plesiomorphy of torpor is supported by the prevalence of torpor expression in all major mammalian orders, including monotremes and marsupials (Grigg, Beard & Augee 1989, Geiser 1994) and eutherian lineages with small-bodied representatives (Geiser & Ruf 1995, Lovegrove 2012c). Interestingly, recent evidence suggests that only a single species of eutherian mammal survived the asteroid impact at the Cretaceous-Palaeogene boundary ~ 66 million years ago (mya) and that all modern eutherian lineages emerged within several hundred thousand years of the Early Cenozoic (O'Leary *et al*. 2013), much earlier than had been hypothesised by previous studies (eg Bininda-Emonds *et al.* 2007). The capacity for prolonged metabolic suppression would have increased the chances of this species surviving the harsh environments at the K-Pg boundary (Robertson *et al.* 2004, Lovegrove 2012c).

The classic view of endothermy is that T_b is maintained at a high and fairly constant level (homeothermy), known as the “normothermic set-point T_b”, with only small circadian variations between active and resting states (Scholander *et al.* 1950, Bartholomew 1972, Schmidt-Nielsen 1997, Clarke & Pörtner 2010). Certain endotherms also undergo periods of extended controlled decreases in T_b and metabolism (adaptive heterothermy) during hibernation or daily torpor, during which the torpor set-point T_b is regulated at a lower level (Geiser & Ruf 1995, Geiser 2004). However, from both an evolutionary and a mechanistic viewpoint, these are rather simplistic perceptions of mammalian thermoregulation. The degree of variability in T_b, as well as the level at which it is maintained, varies considerably between species (Clarke & Pörtner 2010, Lovegrove 2012a). Small mammals, in particular, show a wide range of variability in thermoregulatory patterns (Lovegrove 2005, Angilletta *et al.* 2010, Clarke & Pörtner 2010, Lovegrove 2012a, Boyles *et al.* 2013), whereas in large (> 1 kg) mammals thermal inertia reduces the opportunity for large daily changes (Refinetti & Menaker 1992, Clarke & Pörtner 2010).

Flexibility in T_b regulation, expressed either as thermolability (variations in normothermic T_b) or as torpor has many advantages, both energetic and otherwise (Canale &

Henry 2010, Geiser & Brigham 2012, Boyles *et al.* 2013). Many diurnal heterotherms take advantage of cool nighttime temperatures by entering torpor during the rest-phase and using the warming ambient temperatures in the morning to assist with returning to normothermic T_b (Lovegrove, Kortner & Geiser 1999, Geiser *et al.* 2004). In this way they benefit from the increased energy savings of torpor, while avoiding the costs associated with actively rewarming from low T_b. A number of species have also been shown to enter torpor during reproduction. Torpor use has been observed during gestation, usually to delay parturition until environmental conditions are more favorable, as well as during lactation, usually as a means to conserve energy (Geiser, McAllan & Brigham 2005, Willis, Brigham & Geiser 2006, Morrow & Nicol 2009, Canale, Perret & Henry 2012). A large degree of variability normothermic T_b similarly allows for greater opportunities for energy savings (Kuchel 2003, Canale, Levesque & Lovegrove 2012, Boyles *et al.* 2013). This high degree of thermolability is characteristic of 'protoendotherms' (*sensu* Grigg, Beard & Augee 2004) or 'basoendotherms' (Lovegrove 2012a) and was a likely mid-point in the transition from heterothermic ectothermy to homeothermic endothermy (Eisentraut 1960, Crompton *et al.* 1978, Grigg 2004, Lovegrove 2012a). Although this appears to be the current consensus (Lovegrove 2012a), an alternate hypothesis has been proposed, based on ontogenetic data, suggesting that torpor has a polyphyletic origin (Geiser 2008).

Taking the view that the ancestral condition is one of heterothermic endothermy (Eisentraut 1960, Grigg *et al.* 2004, Clarke & Pörtner 2010, Lovegrove 2012a) the high range of mean T_b, as well as differences in levels of T_b variability retained in extant mammals, can be used to test the various hypotheses proposed to explain the evolution of endothermy (Clarke & Pörtner 2010, Lovegrove 2012a, Naya *et al.* 2013). This approach, rather than pertaining directly to the evolution of endothermy *per se* serves to explain the evolution of homeothermy from the ancestral heterothermic condition, which then has the potential to shed light on the evolution of endothermy itself. Thus the study of basoendotherms, especially during periods in their life-histories where homeothermy is maintained, can provide important insights into understanding the evolution of endothermy in mammals.

The Diversity of Mammalian Life Histories: a Slow-Fast Continuum?

> "*I foresee a renaissance in life-history studies as greater accuracy is attempted in the study of energy transfer within ecosystems*."
>
> (Eisenberg 1983)

Understanding the overall importance of the evolution of endothermy in mammals is dependent on linking the effects of thermoregulatory characteristics to the transfer of energy between the organism at its environment, as well as its life history (Lovegrove 2006). Endothermy is interconnected with parental care: both major groups of endotherms, mammals and birds, show higher levels of parental care compared to the majority of ectotherms (Farmer 2000, Koteja 2004). As a result, they often produce a smaller number of young, but those they produce are more developed and have a higher probability of survival (Case 1978). The high degree of variability in the level and precision of thermoregulation found in modern mammals mirrors the wide range of variability in life-history characteristics (Harvey & Read 1988, Stearns 2000).

The term life-history refers to parameters that influence reproduction (age at first reproduction, annual fecundity, etc) and longevity (Stearns 1976, Boyce 1988). As with metabolic rate and T_b, life-history characteristics fall along a slow-fast continuum (Eisenberg 1983, Promislow & Harvey 1990). The common saying '…live fast, die young…' has its origin in the trade-off between fecundity and mortality. If short periods of time for reproduction are available, for example due to high prevailing predation pressures, then there is a fitness advantage to producing many young as early as possible (Read & Harvey 1989). On the other hand, if predation pressures are low, it is a fitness advantage to grow bigger and delay reproduction, and to produce better quality offspring over an extended lifespan.

Whereas early studies focused on the effects of body mass on life history traits (Blueweiss *et al.* 1978), later studies identified life history trade-offs using mass-independent residual-residual correlations (Read & Harvey 1989, Stearns 1989, Harvey, Pagel & Rees 1991, Symonds 1999). This approach was subsequently succeeded by a modelling approach that integrated the role of mortality regimes (Stearns, Raup & Jablonski 1986). For example, juvenile mortality has been shown to be the most important determinant of the 'speed' of life histories in mammals (Promislow & Harvey 1990).

More recently, the Metabolic Theory of Ecology (MTE) was proposed as an attempt to link the level of metabolism to both the rate of life history as well as the capacity for reproductive output (Brown *et al.* 2004, Hamilton *et al.* 2011). However, despite the intuitive appeal of linking physiology traits directly to fitness (Speakman & McQueenie 1996, Lovegrove 2003), the predictions of the MTE have failed multiple tests (Harvey *et al.* 1991, Stephenson & Racey 1995, Duncan, Forsyth & Hone 2007, Lovegrove 2009, Müller *et al.* 2012). One of the primary reasons for this is, unlike as is assumed by the MTE, many life history characteristics are not fixed within a species (Sikes & Ylönen 1998, Charnov &

Ernest 2006). The MTE relies heavily on this and other assumptions involving metabolic allometries, ignoring the impact of localised environmental factors and changing predation pressures, arguably the most important components in shaping the life history of a species (Boyce 1988, Harvey & Purvis 1999, Lahann, Schmid & Ganzhorn 2006).

It is a better understanding of the fine-grained relationships between life-history characteristics and the environment, and not multi-species comparisons relaying largely on allometries that will allow for the creation of accurate models predicting the impact of global changes in climate, an increasingly important application of physiological studies (Humphries, Umbanhowar & McCann 2004, Canale & Henry 2010, Boyles *et al.* 2011). Despite their potential failings, however, the great benefit of the MTE as well as other large-scale comparative studies lies in their ability to identify outliers (Boyce 1988, Harte 2004). The study of these unusual cases, for example those on the extreme ends of either the physiological and/or life-history slow-fast continua, can help to shed light on the general patterns in the evolution of mammalian life-history and physiology (Eisenberg 1983, Grigg *et al.* 2004, Lovegrove 2012a).

Madagascar an Island Time Machine and Muse for Evolutionary Physiologists

Islands, and their unusual flora and fauna, have long been the source of fascination for evolutionary biologists, as evidenced by the works of the fathers of evolutionary theory, Charles Darwin and Alfred Russell Wallace (Darwin 1858, Wallace 1890). Often providing a blank canvas, they are the stages for phenomenal adaptive radiations. With low concentrations of predators or competitors, they are often home to evolutionary relics, life-forms retaining ancestral characteristics unaffected by the hyper-competitiveness of mainland habitats (Quammen 1996).

Madagascar is one of the largest islands on Earth, and also has one of the highest levels of endemism (Goodman & Benstead 2003, Harper *et al.* 2007). Since its separation from mainland Africa *ca.*135 mya (Rabinowitz, Coffin & Falvey 1983) the climate on the island, at least in the lowland areas, has remained tropical (Jury 2003). However, unlike tropical rainforests at the equator, where resource levels remain relatively stable year round, Madagascar's climate, from the highlands to the seasonal dry forests, is characterised by a high degree of unpredictability (Dewar & Richard 2007). As a consequence, unusual life histories that fall on both the extreme 'slow' and extreme 'fast' sides of the life-history continuum have evolved in Malagasy mammals (Stearns 1983, Wright 1999, Richard *et al.*

2002, Dewar & Richard 2007). However, in this regard, most research to date has focused on primates (lemurs) and little on non-primates (Schmid & Stephenson 2003, Dewar & Richard 2007).

The most speciose endemic family of Malagasy mammals is the Tenrecidae (~30 species, Order: Afrosoricida). Comprised of three subfamilies; the hedgehog-like Tenrecinae and the shrew-like Geogalinae and Oryzorictinae, Malagasy tenrecs are highly variable in form and in life-histories (Olson & Goodman 2003). Since arriving in Madagascar 55 - 35 mya, tenrecs have undergone a large adaptive radiation yet have maintained a number of ancestral traits (Eisenberg & Gould 1969, Douady *et al.* 2002, Olson & Goodman 2003, Poux *et al.* 2008). All species are insectivorous, nocturnal, have limited vision, rely primarily on chemical communication for foraging, have plantigrade limbs, abdominal testes, a cloaca, and altricial young (Eisenberg & Gould 1969). Interestingly, despite similar gestation lengths, the smaller shrew-like species give birth to single young only (Eisenberg & Gould 1969, Stephenson, Racey & Rakotondraparany 1994, Symonds 1999), whereas the Tenrecinae have some of the largest litters of any eutherian mammal (Eisenberg & Kleiman 1972, Nicoll & Racey 1985, Stephenson *et al.* 1994). Differences in longevity as well as age at first reproduction place the subfamilies on opposing ends of the life-history continuum (Eisenberg & Gould 1969, Stephenson & Racey 1995, Racey & Stephenson 1996, Symonds 1999).

Tenrecs also maintain some of lowest T_bs of any extant mammal (Clarke & Pörtner 2010, Lovegrove 2012b). The Tenrecinae in particular have some of the lowest residual basal metabolic rates of any eutherian mammal (Symonds 1999, Lovegrove 2000). They therefore provide a good opportunity to test predictions regarding both the physiology of temperature regulation and the pace of life-histories in mammals and have often been used as model basoendotherms (Eisentraut 1960, Crompton *et al.* 1978, Eisenberg 1983, Stephenson & Racey 1995, Symonds 1999, Lovegrove & Génin 2008, Oelkrug *et al.* 2013).

Objectives and Study Organism

The primary objective of this thesis was to use data collected on the physiology and life-history of a free-ranging tenrec to gain insight into the evolution of endothermy in mammals. As mentioned above, tenrecs provide the ideal subjects for such a study. Despite being smaller in size, and with less extreme litter sizes than the closely related common tenrec (*Tenrec ecaudatus*), the greater hedgehog tenrec (*Setifer setosus*, Schreber, 1778) is an interesting and viable study organism. Furthermore, unlike *T. ecaudaus* it is not readily consumed by local human communities (Randrianjafy 2003), essential for studies on free-ranging populations which require high rates of recapture. Although primarily insectivorous, they are opportunistic omnivores, are known to eat fruit and carrion, and are often found foraging around refuse sites in villages (Petter & Petter-Rousseaux 1963, Eisenberg & Gould 1969, Dammhahn, Soarimalala & Goodman 2012). Populations of *S. setosus* have been studied briefly in the coastal rainforests of eastern Madagascar (Eisenberg & Gould 1969). This work showed that, unlike *T. ecaudatus*, which is an obligate hibernator (Nicoll 1985), *S. setosus* can be active year-round, albeit in smaller numbers during the colder months of the austral winter (Eisenberg & Gould 1969). Studies on captive populations indicate that *S. setosus* is likely to be a facultative hibernator (Kayser 1960, Hildwein 1964). However, no data are available on the thermoregulatory physiology of this species outside captivity. Although much is known about the distribution, habitat use and phylogeny of this species (Eisenberg & Gould 1969, Olson & Goodman 2003, Muldoon & Goodman 2010, Soarimalala 2011), long-term studies on the natural history and physiology of free-ranging populations are lacking.

By studying the interactions between the physiology and the life-history of these interesting basal mammals, I hope to shed light on aspects of both the evolution of homeothermy from heterothermy in endotherms, as well as the evolution of mammalian life-history strategies and the possible links between the two. The first two chapters (2 and 3) focus on aspects of the activity patterns and life-history of this species, highlighting a high level of energetic outputs which occur in over a short time period. The next two chapters (4 and 5) seek to quantify and characterise their thermoregulatory physiology to help explain how such high outputs are possible. Finally, in the concluding chapter (6) I combine the two to comment on thermoregulation and life history in mammals in general.

Literature Cited

Angilletta, M. J. J., B. S. Cooper, M. S. Schuler & J. G. Boyles (2010) The evolution of thermal physiology in endotherms. *Front Biosci*, **2,** 861-881.

Bartholomew, G. A. (1972) Body temperature and energy metabolism. In: *Animal Physiology: Principles and Adaptations*: 298-368. M. S. Gordon, G. A. Bartholomew, A. D. Grinnell, C. B. Jorgensen & F. N. White (Eds.). Macmillan Publishing Co., Inc., New York.

Bennett, A. F. & J. A. Ruben (1979) Endothermy and activity in vertebrates. *Science*, **206,** 649-654.

Bininda-Emonds, O., M. Cardillo, K. E. Jones, R. MacPhee, R. Beck, R. Grenyer, S. A. Price, R. A. Vos, J. L. Gittleman & A. Purvis (2007) The delayed rise of present-day mammals. *Nature,* **446,** 507-512.

Blueweiss, L., H. Fox, V. Kudzma, D. Nakashima, R. Peters & S. Sams (1978) Relationships between body size and some life history parameters. *Oecologia,* **37,** 257-272.

Boyce, M. S. (1988) Evolution of life histories: Theory and patterns from mammals. In: *Evolution of Life Histories of Mammals*: 3-30. M. S. Boyce (Ed.). Yale University Press, New Haven and London.

Boyles, J. G., F. Seebacher, B. Smit & A. E. McKechnie (2011) Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr Comp Biol,* **51,** 676-690.

Boyles, J. G., A. B. Thompson, A. E. McKechnie, E. Malan, M. M. Humphries & V. Careau (2013) A global heterothermic continuum in mammals. *Glob Ecol Biogeogr,* **22,** 1029-1039.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage & G. B. West (2004) Toward a metabolic theory of ecology. *Ecology,* **85,** 1771-1789.

Canale, C. I. & P. Y. Henry (2010) Adaptive phenotypic plasticity and resilience of vertebrates to increasing climatic unpredictability. *Clim Res,* **43,** 135-147.

Canale, C. I., D. L. Levesque & B. G. Lovegrove (2012) Tropical heterothermy: Does the exception prove the rule or force a re-definition? In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*: 29-40. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin Heidelberg.

Canale, C. I., M. Perret & P. Y. Henry (2012) Torpor use during gestation and lactation in a primate. *Naturwissenschaften,* **99,** 159-163.

Case, T. J. (1978) Endothermy and parental care in the terrestrial vertebrates. *Am Nat,* **112,** 861-874.

Charnov, E. L. & S. K. M. Ernest (2006) The offspring-size/clutch-size trade-off in mammals. *Am Nat,* **167,** 578-582.

Clarke, A. & H.-O. Pörtner (2010) Temperature, metabolic power and the evolution of endothermy. *Biol Rev,* **85,** 703-727.

Crompton, A. W., C. R. Taylor & J. A. Jagger (1978) Evolution of homeothermy in mammals. *Nature,* **272,** 333-336.

Dammhahn, M., V. Soarimalala & S. M. Goodman (2012) Trophic niche differentiation and microhabitat utilization in a species-rich montane forest small mammal community of Eastern Madagascar. *Biotropica,* **45,** 111-118.

Darwin, C. (1858) I. Extract from an unpublished Work on Species, by C. Darwin, Esq., consisting of a portion of a Chapter entitled, "On the Variation of Organic Beings in a state of Nature; on the Natural Means of Selection; on the Comparison of Domestic Races and true Species."II. Abstract of a Letter from C. Darwin, Esq., to Prof. Asa Gray, Boston, U.S., dated Down, September 5th , 1857. *Proceedings of the Linnean Society of London,* **3,** 45-62.

Dewar, R. E. & A. F. Richard (2007) Evolution in the hypervariable environment of Madagascar. *Proc Natl Acad Sci U S A,* **104,** 13723-13727.

Douady, C. J., F. Catzeflis, D. J. Kao, M. S. Springer & M. J. Stanhope (2002) Molecular evidence for the monophyly of Tenrecidae (Mammalia) and the timing of the colonization of Madagascar by Malagasy tenrecs. *Mol Phylogenet Evol,* **22,** 357-363.

Duncan, R. P., D. M. Forsyth & J. Hone (2007) Testing the Metabolic Theory of Ecology: allometric scaling exponents in mammals. *Ecology,* **88,** 324-333.

Eisenberg, J. F. (1983) *The Mammalian Radiations: An Analysis of Trends in Evolution, Adaptation, and Behavior*. Chicago, IL: University of Chicago Press.

Eisenberg, J. F. & E. Gould (1969) The tenrecs: a study in mammalian behavior and evolution. *Smithson Contrib Zool,* **27,** 1-156.

Eisenberg, J. F. & D. G. Kleiman (1972) Olfactory communication in mammals. *Annu Rev Ecol Syst,* **3,** 1-32.

Eisentraut, M. (1960) Heat regulation in primitive mammals and in tropical species. *Bull Mus Comp Zool,* **124,** 31-43.

Farmer, C. G. (2000) Parental care: the key to understanding endothermy and other convergent features in birds and mammals. *Am Nat,* **155,** 326-334.

Geiser, F. (1994) Hibernation and daily torpor in marsupials - a review. *Aust J Zool,* **42,** 1-16.

Geiser, F. (2004) Metabolic rate reduction during hibernation and daily torpor. In: *Life in the Cold. Evolution, Mechanisms, Adaptation, and Application*: 176-184. B. M. Barnes & H. V. Carey (Eds.). University of Alaska, Fairbanks.

Geiser, F. (2008) Ontogeny and phylogeny of endothermy and torpor in mammals and birds. *Comparative Biochemistry and Physiology A,* **150,** 176-180.

Geiser, F. & R. M. Brigham (2012) The other functions of torpor. In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations.* T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer, Heidelberg.

Geiser, F., R. L. Drury, G. Körtner, C. Turbill, C. R. Pavey & R. M. Brigham (2004) Passive rewarming from torpor in mammals and birds: energetic, ecological and evolutionary implications. In: *Life in the Cold. Evolution, Mechanisms, Adaptation, and Application*: 199-208. B. M. Barnes & H. V. Carey (Eds.). University of Alaska, Fairbanks.

Geiser, F., B. M. McAllan & R. M. Brigham (2005) Daily torpor in a pregnant dunnart (*Sminthopsis macroura* Dasyuridae: Marsupialia). *Mamm Biol,* **70,** 117-121.

Geiser, F. & T. Ruf (1995) Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiol Zool,* **68,** 935-966.

Gerkema, M. P., W. I. L. Davies, R. G. Foster, M. Menaker & R. A. Hut (2013) The nocturnal bottleneck and the evolution of activity patterns in mammals. *Proc R Soc B,* **280,** 20130508.

Goodman, S. M. & J. P. Benstead (2003) *The Natural History of Madagascar*. Chicago, IL, USA: University of Chicago Press Chicago.

Grigg, G. C. (2004) An evolutionary framework for studies of hibernation and short term torpor. In: *Life in the Cold. Evolution, Mechanisms, Adaptation, and Application*: 1-11. B. M. Barnes & H. V. Carey (Eds.). University of Alaska, Fairbanks.

Grigg, G. C., L. A. Beard & M. L. Augee (1989) Hibernation in a monotreme, the echidna (*Tachyglossus aculeatus*). *Comp Biochem Physiol A,* **92,** 609-612.

Grigg, G. C., L. A. Beard & M. L. Augee (2004) The evolution of endothermy and its diversity in mammals and birds. *Physiol Biochem Zool,* **77,** 982-997.

Hamilton, M. J., A. D. Davidson, R. M. Sibly & J. H. Brown (2011) Universal scaling of production rates across mammalian lineages. *Proc R Soc B,* **278,** 560-566.

Harper, G. J., M. K. Steininger, C. J. Tucker, D. Juhn & F. Hawkins (2007) Fifty years of deforestation and forest fragmentation in Madagascar. *Environ Conserv,* **34,** 325-333.

Harte, J. (2004) The value of null theories in ecology. *Ecology,* **85,** 1792-1794.

Harvey, P. & A. Purvis (1999) Understanding the ecological and evolutionary reasons for life history variation: mammals as a case study. In: *Advanced Ecological Theory: Principles and Applications*: 232-248. J. McGlade (Ed.). Wiley-Blackwell, Oxford UK.

Harvey, P. H., M. D. Pagel & J. A. Rees (1991) Mammalian metabolism and life histories. *Am Nat,* **137,** 556-566.

Harvey, P. H. & A. F. Read (1988) How and why do mammalian life histories vary? In: *Evolution of Life Histories of Mammals*: 213-232. M. S. Boyce (Ed.). Yale University Press, New Haven and London.

Heinrich, B. (1977) Why have some animals evolved to regulate a high body temperature? *Am Nat,* **111,** 623-640.

Hildwein, G. (1964) La dépense d'énergie minimale de deux Insectivores de Madagascar en hibernation profonde au cours de l'été boréal. *CR Hebd Seances Acad Sci Paris,* **259,** 2009-2011.

Hillenius, W. J. & J. A. Ruben (2004) The evolution of endothermy in terrestrial vertebrates: who? when? why? *Physiol Biochem Zool,* **77,** 1019-1042.

Humphries, M. M., J. Umbanhowar & K. S. McCann (2004) Bioenergetic prediction of climate change impacts on northern mammals. *Integr Comp Biol,* **44,** 152-162.

Jury, M. (2003) The climate of Madagascar. In: *The Natural History of Madagascar*: 75-87. S. M. Goodman & J. P. Benstead (Eds.). The University of Chicago Press, Chicago, IL.

Kayser, C. (1960) Cosommation d'oxygène et température centrale au cours de l'hiver austral de deux Insectivores de Madagascar, *Centetes ecaudatus* et *Setifer setosus*. *Compt Rend Soc Biol,* **154,** 1873-1876.

Kemp, T. (2006) The origin of mammalian endothermy: a paradigm for the evolution of complex biological structure. *Zool J Linn Soc,* **147,** 473-488.

Koteja, P. (2000) Energy assimilation, parental care and the evolution of endothermy. *Proc R Soc B,* **267,** 479-484.

Koteja, P. (2004) The evolution of concepts on the evolution of endothermy in birds and mammals. *Physiol Biochem Zool,* **77,** 1043-1050.

Kuchel, L. (2003) *The energetics and patterns of torpor in free-ranging Tachyglossus aculeatus from a warm-temperate climate.* PhD, University of Queensland, Brisbane.

Lahann, P., J. Schmid & J. U. Ganzhorn (2006) Geographic variation in populations of *Microcebus murinus* in Madagascar: Resource seasonality or Bergmann's rule? *Int J Primatol,* **27,** 983-999.

Lovegrove, B. G. (2000) The zoogeography of mammalian basal metabolic rate. *Am Nat,* **156,** 201-219.

Lovegrove, B. G. (2003) The influence of climate on the basal metabolic rate of small mammals: a slow-fast metabolic continuum. *J Comp Physiol B,* **173,** 87-112.

Lovegrove, B. G. (2005) Seasonal thermoregulatory responses in mammals. *J Comp Physiol B,* **175,** 231-247.

Lovegrove, B. G. (2006) The power of fitness in mammals: perceptions from the African slipstream. *Physiol Biochem Zool,* **79,** 224-236.

Lovegrove, B. G. (2009) Age at first reproduction and growth rate are independent of basal metabolic rate in mammals. *J Comp Physiol B,* **179,** 391-401.

Lovegrove, B. G. (2012a) The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol Rev,* **87,** 128–162.

Lovegrove, B. G. (2012b) The evolution of mammalian body temperature: the Cenozoic supraendothermic pulses. *J Comp Physiol B,* **182,** 579-589.

Lovegrove, B. G. (2012c) A single origin of heterothermy in mammals. In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*: 3-11. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin

Lovegrove, B. G. & F. Génin (2008) Torpor and hibernation in a basal placental mammal, the Lesser Hedgehog Tenrec, *Echinops telfairi*. *J Comp Physiol B,* **178,** 691-698.

Lovegrove, B. G., G. Kortner & F. Geiser (1999) The energetic cost of arousal from torpor in the marsupial *Sminthopsis macroura*: benefits of summer ambient temperature cycles. *J Comp Physiol B,* **169,** 11-18.

Lovegrove, B. G. & M. Mowoe (2014) The evolution of micro-cursoriality in mammals. *J Exp Biol***,** doi:10.1242/jeb.095737.

Luo, Z. X. (2007) Transformation and diversification in early mammal evolution. *Nature,* **450,** 1011-1019.

Malan, A. (1996) The origins of hibernation: a reappraisal. In: *Adaptations to the Cold*: 1-6. F. Geiser, A. Hulbert & S. Nicol (Eds.). University of New England Press, Armidale, NSW.

McNab, B. K. (1978) Evolution of endothermy in phylogeny of mammals. *Am Nat,* **112,** 1-21.

Morrow, G. & S. C. Nicol (2009) Cool sex? Hibernation and reproduction overlap in the echidna. *PLoS ONE,* **4,** e6070.

Muldoon, K. M. & S. M. Goodman (2010) Ecological biogeography of Malagasy non-volant mammals: community structure is correlated with habitat. *J Biogeogr,* **37,** 1144-1159.

Müller, D. W. H., D. Codron, J. Werner, J. Fritz, J. Hummel, E. M. Griebeler & M. Clauss (2012) Dichotomy of eutherian reproduction and metabolism. *Oikos,* **121,** 102-115.

Naya, D. E., L. Spangenberg, H. Naya & F. Bozinovic (2013) How does evolutionary variation in basal metabolic rates arise? A statistical assessment and a mechanistic model. *Evolution,* **67,** 1463–1476.

Nicoll, M. E. (1985) Responses to Seychelles tropical forest seasons by a litter-foraging mammalian insectivore, *Tenrec ecaudatus*, native to Madagascar. *J Anim Ecol,* **54,** 71-88.

Nicoll, M. E. & P. A. Racey (1985) Follicular development, ovulation, fertilization and fetal development in tenrecs (*Tenrec ecaudatus*). *J Reprod Fertil,* **74,** 47-55.

O'Leary, M. A., J. I. Bloch, J. J. Flynn, T. J. Gaudin, A. Giallombardo, N. P. Giannini, S. L. Goldberg, B. P. Kraatz, Z.-X. Luo, J. Meng, X. Ni, M. J. Novacek, F. A. Perini, Z. S. Randall, G. W. Rougier, E. J. Sargis, M. T. Silcox, N. B. Simmons, M. Spaulding, P. M. Velazco, M. Weksler, J. R. Wible & A. L. Cirranello (2013) The placental mammal ancestor and the post–K-Pg radiation of placentals. *Science,* **339,** 662-667.

Oelkrug, R., N. Goetze, C. Exner, Y. Lee, G. K. Ganjam, M. Kutschke, S. Müller, S. Stöhr, M. H. Tschöp, P. G. Crichton, G. Heldmaier, M. Jastroch & C. W. Meyer (2013) Brown fat in a protoendothermic mammal fuels eutherian evolution. *Nat Commun,* **4,** e2140.

Olson, L. E. & S. M. Goodman (2003) Phylogeny and biogeography of tenrecs. In: *The Natural History of Madagascar*: 1235-1242. S. M. Goodman & J. P. Benstead (Eds.). The University of Chicago Press, Chicago, USA.

Petter, J.-J. & A. Petter-Rousseaux (1963) Notes biologiques sur les Centetinae. *La Terre et la Vie* **17,** 66-80.

Poux, C., O. Madsen, J. Glos, W. de Jong & M. Vences (2008) Molecular phylogeny and divergence times of Malagasy tenrecs: Influence of data partitioning and taxon sampling on dating analyses. *BMC Evol Biol,* **8,** 102.

Promislow, D. & P. Harvey (1990) Living fast and dying young: A comparative analysis of life-history variation among mammals. *J Zool (Lond.),* **220,** 417-437.

Quammen, D. (1996) *The Song of the Dodo: Island Biogeography in an Age of Extinctions*. New York, NY, USA: Simon & Schuster.

Rabinowitz, P. D., M. F. Coffin & D. Falvey (1983) The separation of Madagascar and Africa. *Science,* **220,** 67-69.

Racey, P. A. & P. J. Stephenson (1996) Reproductive and energetic differentiation of the Tenrecidae of Madagasacar. In: *Biogéographie de Madagascar*: 307-319. W. R. Lourenço (Ed.). Editions de l'Orstom, Paris.

Randrianjafy, V. (2003) *Contribution à l'étude de la biologie de conservation de la communauté micromammalienne d'Ankarafantsika.* PhD, Université d'Antananarivo, Antananarivo.

Read, A. F. & P. H. Harvey (1989) Life history differences among the eutherian radiations. *J Zool (Lond.),* **219,** 329-353.

Refinetti, R. & M. Menaker (1992) The circadian-rhythm of body temperature. *Physiol Behav,* **51,** 613-637.

Richard, A. F., R. E. Dewar, M. Schwartz & J. Ratsirarson (2002) Life in the slow lane? Demography and life histories of male and female sifaka (*Propithecus verreauxi verreauxi*). *J Zool (Lond.),* **256,** 421-436.

Robertson, D. S., M. C. McKenna, O. B. Toon, S. Hope & J. A. Lillegraven (2004) Survival in the first hours of the Cenozoic. *Geol Soc Am Bull,* **116,** 760-768.

Rowe, T. B., T. E. Macrini & Z. X. Luo (2011) Fossil evidence on origin of the mammalian brain. *Science,* **332,** 955-957.

Schmid, J. & P. Stephenson (2003) Physiological adaptations of Malagasy mammals: lemurs and tenrecs compared. In: *The Natural History of Madagascar*: 1198-1203. S. M. Goodman & J. P. Benstead (Eds.). The University of Chicago Press, Chicago, USA.

Schmidt-Nielsen, K. (1997) *Animal Physiology: Adaptation and Environment*. Cambridge University Press.

Scholander, P. F., R. Hock, V. Walters & L. Irving (1950) Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation, and basal metabolic rate. *Biol Bull,* **99,** 259-271.

Sikes, R. S. & H. Ylönen (1998) Considerations of optimal litter size in mammals. *Oikos,* **83,** 452-465.

Soarimalala, V. (2011) Les afrosoricides de la fôret sèche malgache. *Afrotherian Conservation,* **8,** 4-9.

Speakman, J. R. & J. McQueenie (1996) Limits to sustained metabolic rate: The link between food intake, basal metabolic rate, and morphology in reproducing mice, *Mus musculus*. *Physiol Zool,* **69,** 746-769.

Stearns, S. C. (1976) Life-history tactics: a review of the ideas. *Q Rev Biol,* **51,** 3-47.

Stearns, S. C. (1983) The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. *Oikos,* **41,** 173-187.

Stearns, S. C. (1989) Trade-offs in life-history evolution. *Funct Ecol,* **3,** 259-268.

Stearns, S. C. (2000) Life history evolution: successes, limitations, and prospects. *Naturwissenschaften,* **87,** 476-486.

Stearns, S. C., D. M. Raup & D. Jablonski (1986) Natural selection and fitness, adaptation and constraint. In: *Patterns and processes in the history of life*: 23-46. S. Bernhard (Ed.). Springer, Berlin.

Stephenson, P. J. & P. A. Racey (1995) Resting metabolic rate and reproduction in the Insectivora. *Comp Biochem Physiol A,* **112,** 215-223.

Stephenson, P. J., P. A. Racey & F. Rakotondraparany (1994) Maintenance and reproduction of tenrecs Tenrecidae: at Parc Tsimbazaza, Madagascar. *Int Zoo Yearb,* **33,** 194-201.

Symonds, M. R. E. (1999) Life histories of the Insectivora: the role of phylogeny, metabolism and sex differences. *J Zool (Lond.),* **249,** 315-337.

Wallace, A. R. (1890) *The Malay Archipelago: the land of the orang-utan and the bird of paradise: a narrative of travel, with studies of man and nature*. Macmillan.

Willis, C. K. R., R. M. Brigham & F. Geiser (2006) Deep, prolonged torpor by pregnant, free-ranging bats. *Naturwissenschaften,* **93,** 80-83.

Wright, P. C. (1999) Lemur traits and Madagascar ecology: Coping with an island environment. In: *Yearbook of Physical Anthropology 1999, Vol 42*: 31-72.

Chapter 2 Home range and shelter site selection in the greater hedgehog tenrec in the dry deciduous forest of Western Madagascar

Abstract

Information on the spatial distribution and habitat use of the Tenrecidae (Supraorder: Afrotheria) of Madagascar are severely lacking. Here I present the first data available on home range size, as well as further data on population distribution and rest site selection of a large tenrec, the greater hedgehog tenrec (*Setifer setosus*). Data were collected over two rainy seasons in the dry deciduous woodland of Western Madagascar, in Ankarafantsika National Park. Home ranges were surprisingly large for a 200 - 300 g animal: males had an average home range (95% MCP) of 13.7 ± 4.9 ha ($n = 5$), and females of 6.7 ± 2.0 ha ($n = 5$). A high overlap between multiple individuals of the opposite sex supports a promiscuous mating system for this species. Daytime shelter sites proved to be highly variable and differed between sex and reproductive status.

Abbreviations

MCP= Minimum convex polygon
KDE= kernel density estimation
KDE_{href}= kernel density estimation using the reference bandwidth (h_{ref}) as a smoothing vector
KDE_{LSCV}= kernel density estimation using least squares cross-validation as a smoothing vector
KDE_{adj}= kernel density estimation using h_{adj} (calculated by multiplying h_{ref} by the average ratio of h_{LSCV}/h_{ref}) as a smoothing vector

Introduction

The island of Madagascar has one of the highest level of endemism in the world (Goodman & Benstead 2003, Harper *et al.* 2007). One of the most speciose endemic mammal families is the Tenrecidae (Superorder: Afrotheria). Since their colonization of Madagascar *ca.* 37 mya tenrecs have diversified, spanning wide ranges in body size, morphology and life histories (Douady *et al.* 2002, Olson & Goodman 2003). One of the largest species of tenrec, the greater hedgehog tenrec (*Setifer setosus*), is also one of most-wide spread, and yet it remains poorly-studied (Goodman, Ganzhorn & Rakotondravony 2003). They are a cryptic, strictly-nocturnal, insectivorous species with a relatively low rate of trap success (Randrianjafy 2003). Field and laboratory studies undertaken in the 1960s showed that they nest in shallow holes in the ground or in tree cavities during the day, often out of visual range (Gould & Eisenberg 1966, Eisenberg & Gould 1969). In the more seasonal parts of the country they are notably absent during the dry periods and are therefore likely to hibernate (Eisenberg & Gould 1969, Stephenson 1994, Randrianjafy 2003).

The vast majority of data available on this species is restricted to that obtained through general surveys on small mammal populations (Stephenson 1993, Randrianjafy 2003). There have been few in-depth studies and baseline data on the animal in its natural habitat are lacking (Eisenberg & Gould 1969, Randrianjafy 2003, Randrianjafy & Goodman 2008). Information on the distribution, home range and shelter sites can provide invaluable insights into the basic ecology of a species and how it will respond to various environmental stressors (Burt 1943, Brown & Orians 1970, Komers & Brotherton 1997, Wilkinson, Grigg & Beard 1998). This is especially important in Madagascar where rates of deforestation and forest fragmentation in are high (Ganzhorn *et al.* 1990, Green & Sussman 1990, Smith, Horning & Moore 1997, Agarwal *et al.* 2005, Harper *et al.* 2007).

Here I present data on the home ranges, distribution and rest site selection of a population of *S. setosus* in a segment of the western deciduous forest in Madagascar. This constitutes the first study to successfully follow multiple *S. setosus* for an extended period of time, and provides baseline data on the spatial distribution and habitat use of this species.

Materials and Methods

The study took place over two rainy seasons. The first, from January-April 2010, was a preliminary study in which eight individuals (three males, five females) were followed until hibernation commenced in March-April. The second study period was longer, from September 2010-April 2011, and resulted in the capture and radio-tracking of 14 individuals (eight males, six females).

Study site

All animals were trapped in the JBA (Jardin Botanique A) research area adjacent to the Ampijoroa Forestry Station in Ankarafantsika National Park (16º19’S, 46º48’E), one of the largest remaining intact sections of dry deciduous woodland in Madagascar (Alonso *et al.* 2002). The habitat consists of dry forest situated on sandy soils. The JBA research area used in this study includes undisturbed forest habitat as well as once-burnt forest as described in

Chouteau *et al*. (2004). Both forest types are composed primarily of trees less than 5 cm in diameter with only 8.4% of the trees in the un-burnt forest reaching a diameter of greater than 10cm (Chouteau *et al.* 2004). The site is characterized by large seasonal variation in rainfall with a distinct dry season during the Austral winter, from April-November with little or no rainfall, with the 1000-1500 mm of rain falling primarily during the remaining months (data from Durrell Wildlife Conservation Trust, Ampijoroa 1997-2010). Previous work indicated that *S. setosus* activity is limited during the dry season indicating the potential for seasonal heterothermy (hibernation) during this time (Randrianjafy 2003).

Trapping and transmitter implantation

Initial attempts were made to capture animals using Tomahawk-style and Sherman live-traps baited with sardines or dried fish, and open pitfall traps (consisting of a 10 litre plastic bucket placed in the ground with drainage holes). However neither technique had any success after a month of trapping. Trapping was abandoned in favor of catching individuals by hand. Hand-catching consisted of walking the established trails in the area at night with local guides, locating individuals by sight or by sound, chasing and catching them and then transporting them back to the research camp. Animals were housed in plastic containers lined with paper towel and provided with live insects and sardines. They were kept for a maximum of five days before surgery to allow for collection of metabolic data for a concurrent study

After several failed attempts to place external radio-transmitters both as collars (made impossible by the lack of clearly defined neck) or glued to the spines on the back (complicated by the animals' ability to pass through the smallest cracks), the radio-transmitters (2-stage collar transmitter, Merlin Systems Inc, Boise, ID, USA) were modified for implantation. This coincided with a concurrent study on long-term body temperatures in this species (Chapter 5) so the telemeters were encapsulated in surgical wax (Paramat Extra-Merck KGaA, Darmstadt, Germany) alongside two miniaturized DS1922L Thermocron iButtons (Dallas Semiconductor) as outlined in Lovegrove (2009). The telemeter package weighed around 13.0 g (mean 13.0 g, range 11.7 - 13.5 g) constituting around 5 - 8% of the animal's body mass, within a range of mass shown to have no adverse effects on locomotory behaviour in terrestrial mammals (Rojas, Koertner & Geiser 2010). Surgery was undertaken under sterile conditions in an enclosed laboratory site at the research camp. Oxygen and vaporized anesthetic (Isofluorane) were delivered to the animal through a mask at a rate of 700 ml/min. Anesthesia was induced at 1-2% isofluorane and maintained at 0.5%. The telemeter package was inserted via an incision in the peritoneal cavity which was then sutured using 3/0 catgut and sealed with Vetbond™ tissue adhesive (3m, London, ON, Canada). An intramuscular injection of antibiotics (1 µl /10 g of Duplocillin) was given to prevent post-operative infection. The animals were kept for one day of post-surgery observations and were released at the site of capture. Implanted animals were re-captured within a week of surgery to ensure proper recovery or corrective suturing if needed. Subsequent locations of the animal were made using either the R-1000 Telemetry Receiver (Communications Specialists, Orange, CA, USA) or the IC-R10 Communications Receiver (ICOM, Tokyo, Japan) connected to an RA-23K 'H' antenna (Telonics, Mesa, AZ, USA) or a standard 150 MHz Yagi antenna (manufactured by Cliff Dearden, Pietermaritzburg, KZN, South Africa) and a 150MHz power booster (Merlin Systems Inc, Boise, ID, USA). Females were captured once a week to determine reproductive status and males once every two or three weeks to assess body condition. The animals were recaptured and the transmitter package explanted after emergence from hibernation in September 2011.

Data collection

Rest site selection

Setifer setosus is strictly nocturnal (Rand 1935, Randrianjafy 2003) and can therefore be expected to stay stationary throughout the day. This allowed the easy identification of their day/rest shelter sites by locating them between dawn and dusk. Females were located every day (as repeated use of a single nest site would indicate parturition) and males every couple of days. Using only the handheld radio-receivers (without an aerial) it was possible to locate them to within a few cm of their precise location, usually in a tree. Their geographic location was recorded using a handheld GPS unit (accurate to 3 m) and included in the home range analysis. Various characteristics (height from the ground, cover and diameter at breast height [DBH] of the tree) of their shelter site were also recorded.

Collection of spatial data

Only the daytime (rest-phase) locations, as described above, were collected during the first season. The second season also included the collection of night (active-phase) locations. Night locations were made from either 18:00 - 0:00 or 0:00 - 06:00. To avoid serial autocorrelation, each individual was located once per night in a randomized order (Swihart & Slade 1985). On occasion, visual contact was made with the animal in which case its location was recorded directly into the handheld GPS. If visual contact was not possible, the animal was triangulated by recording the angle at which the signal was strongest from three to four different locations at least 10 m apart. Estimated locations from the triangulation data were obtained using Locate III (Pacer Computing, Tatamagouche NS, Canada). All points were entered into MapSource (Garmin, Olathe, KS, USA) and the latitude and longitude data were transformed into Universal Transverse Mercator (UTM) coordinates for analysis.

Data analysis

Statistical analysis

All statistics were performed using either the base program in R (R Development Core Team 2011), or using MYSTAT or Sigma Stat 3.0.1 (Systat Software, Inc, Chicago, IL, USA). Resultant probability values were compared to an α-value of 0.05, unless otherwise stated.

Rest site selection

The height of the rest sites were placed into discrete categories (< 0 m, 0 m - 0.5 m, 0.5 m -1 m, 1 m - 1.5 m, 1.5 m - 2 m and > 2 m) and then analyzed using a chi-squared test (or Fisher's Exact test for non-normal data), between the sexes and among the different reproductive statuses. Similar analyses were performed on the level of cover provided by the rest site (classed as closed, open, partially open and unclear) as well as the diameter at breast height (DBH in m binned as follows: 0 - 0.1, 0.1 - 0.2, 0.2 - 0.3, 0.3 - 0.4, 0.4 - 0.5, > 0.5) of the tree in which they were resting.

Home range analysis

Home ranges sizes were calculated in R using the *adehabitatHR* package (Calenge 2006). With the exception of lactating females, rest-sites changed daily and therefore rest-phase locations were included in all home range analyses. To account for the high variance in calculated home ranges (see Wauters *et al.* 2007 for more details) multiple estimators were used. We used the minimum convex polygon (MCP) method as it is the most robust, in order to compare data from this study to that from other home range studies. The use of 95% MCP was supported by a preliminary analysis in R (using the *mcp.area* function) indicating that excluding the most extreme 5% of the distribution points for each animal did not affect the calculated values.

However, the MCP method has been widely criticized in that it often over-estimates home range size (Worton 1989). Home ranges were therefore also calculated using fixed kernel density estimation (KDE) with three different smoothing vectors; KDE with h_{ref} (KDE_{href}), the least squares cross-validation (KDE_{LSCV}), and h_{adj} (KDE_{adj}). This last value was proposed by Wauters *et al.* (2007) as a means of decreasing the overestimation of home range size in a small mammal. The smoothing vector, h_{adj}, was calculated by multiplying h_{ref} by the average ratio of h_{LSCV}/h_{ref} (0.4 in this study). The *kerneloverlap* function in *adehabitatHR* was used to calculate the percentage of range overlap between individuals. The individuals followed exclusively between January-April 2010 were excluded from this analysis to ensure the recorded overlaps were temporal as well as spatial.

Rest-phase locations are, in general, easier to collect and more accurate, and these were the only locations that were available from animals from the first season (with the exception of a few active-phase locations). It was also of interest, and ease of future study, to determine how accurate rest-phase data were as a predictor of overall home range size. Home range size analyses were also performed on the daytime locations only and were compared to home ranges calculated including the night-locations. Differences between home range estimates and sex were calculated using a two-way repeated-measures ANOVA. After visually observing home range size based on the number of locations, home ranges of individuals with less than 30 locations were considered incomplete. Although they are reported they were excluded from the calculation of the means and the statistical analyses. The reciprocals of the data were used to satisfy the assumptions of the ANOVA. Differences among categories were determined using Holm-Sidak all pairwise multiple comparison procedures as a post-hoc test.

Results

Of the eight individuals followed (SF01-SF08) in the first year, only three animals (SF02, SF04 and SF07) remained active long enough to collect more than 30 locations (Table 2-1). The others either died (predation or unknown cause) or commenced hibernation shortly after implantation (early March 2010). An individual was considered to be hibernating when it remained in the same day site, and no nocturnal activity was observed, for more than one week. All individuals had entered hibernation by the end of April 2010. Problems with the transmitters and possible predation or dispersal meant that none of the animals from the first season, with the exception of one female (SF07), were recovered post-hibernation (September 2010). However, the transmitters of two individuals (one male and one female), who had not changed rest sites since March, continued to work until the beginning of September. Unfortunately the transmitters stopped working just as the animals started relocating, and they were both lost to the study. The one female to be recaptured (SF07) was fitted with a new transmitter and the recorded locations span the two seasons.

Of the 14 individuals implanted during the second season only 10 were followed long enough to obtain more than 10 accurate geographic locations. One male had a transmitter malfunction within a day of release and a female was killed by an unknown terrestrial predator within a week of capture. These data were excluded from all analyses. Another animal died while entering a small tree cavity (SF11) and, whereas her data are included in Table 2-4, they were excluded from statistical analyses since only 14 locations were collected before her death. A total of six individuals, three males (SF09, SF15, SF17) and three females (SF13, SF14 and SF21) were eaten by boas (either *Acrantophis madagascariensis* or *Boa manditra*). However, complete home ranges were calculated for two of these individuals (SF13 and SF14) prior to their deaths.

The activity patterns of *S. setosus* observed in this study were highly seasonal, restricted from late September to early March, for the males and to the end of April for the females. The breeding season began shortly after emergence from hibernation until just before re-entry. The first heavily pregnant females were observed in late October 2010 and at no point between that time and April 2011 were any of the females non-reproductive. Females in late lactation (30 - 40 days) were also found to be gestating. Due to the lack of external testes it was not possible to determine the reproductive condition of the males (Petter & Petter-Rousseaux 1963). Body mass was highly seasonal, fluctuating from the lowest values just after emergence from hibernation in 2010 (120 -160 g) and reaching as high as 327 g for a male one week before hibernation commenced in 2011. Females tended to be smaller than the males but reached similar masses (> 350 g) during late gestation.

Rest site selection

All individuals, with the exception of lactating females, changed rest sites daily, rarely retuning to the same area. Lactating females (four observed in this study) would remain stationary for approximately 20 - 25 days before the young were large enough to move. The mother would forage at night and return to the same day location throughout this time. On occasion individuals were found in locations where they had previously been observed and even occasionally to the exact nest site where another individual had been located. On a single occasion, in the breeding season in December, a male and a female shared a single rest location for a few days. Activity was restricted to nighttime and in no case was an individual found in a different location at dusk than at dawn.

Table 2-1 Height of day site location, in meters from the ground, percent distribution by sex, and reproductive status of individual *Setifer setosus* in Ankarafantsika National Park**.**

				Females		
Height Category (m)	**All Data %**	**Males %**	**Females %**	**Pregnant %**	**Lactating %**	**Other %**
n	**325**	**141**	**178**	**74**	**27**	**78**
<0	7.4	8.5	6.5	0	3.7	13.3
0-0.5	38.8	32.6	43.5	43.2	22.2	50.6
0.5-1	10.2	7.1	12.5	16.2	14.8	8.4
1-1.5	6.5	7.8	5.4	9.5	0	3.6
1.5-2	12.9	19.9	7.6	12.2	3.7	4.8
>2	24.3	24.1	24.5	18.9	55.6	19.3

Other refers to all females of undetermined reproductive status. Significant differences were found between sexes and between reproductive status in females.

Table 2-2 Level of cover of day site location of *Setifer setosus.*

				Females		
Level of Cover	**All Data%**	**Males %**	**Females %**	**Pregnant %**	**Lactating %**	**Other%**
n	**319**	**140**	**179**	**74**	**27**	**78**
closed	52.4	58.6	47.5	62.8	36.5	33.3
open	21	10	29.6	26.9	33.8	25.9
partially open	12.2	13.6	11.2	6.4	16.2	11.1
unclear	14.4	17.9	11.7	3.8	13.5	29.6

Level of cover refers to the portion of the animal visible to the observer. Closed: the animal could not be seen when the nest site was located. Open: the animal was fully visible. Partially open: the animal was partially visible and unclear when the nest site was too high to be evaluated by the observer. Significant differences were found between sexes and reproductive status.

Table 2-3 Frequency distribution (%) of diameter at breast height (DBH in m) of trees housing the rest sites of *Setifer setosus*

				Females	
DBH	**All Data**	**Males**	**Females**	**Reproductive**	**Non-Reproductive**
(m)	**%**	**%**	**%**	**%**	**%**
n	**208**	**113**	**95**	**73**	**22**
0 - 0.1	13.5	13.3	13.7	12.3	18.2
0.1 - 0.2	37	41.6	31.6	27.4	45.5
0.2 - 0.3	22.6	23	22.1	24.7	13.6
0.3 - 0.4	13.5	12.4	14.7	17.8	4.5
0.4 - 0.5	9.1	5.3	13.7	12.3	18.2
> 0.5	4.3	4.4	4.2	5.5	0

Differences presented according to sex, and female reproductive status. Pregnant and lactating females were combined ease in statistical analysis.

Common rest sites included hollows found in either live or dead trees and only very occasionally underground. Resting in underground or hillside holes was much more common in individuals found closer to the valley area surrounding the JBA forest site. The sandy soil of the JBA area made underground holes rare. Differences in all nest site characteristics were found between both males and females (chi-square tests resulting in $p < 0.001$, Tables 2-1, 2-2, 2-3) and between reproductive and non-reproductive females. Pregnant and lactating females were generally found higher up in the trees than non-reproductive females and pregnant females were more often found in closed nest sites.

Home range calculations

Home range size varied widely according to the estimation used. KDE_{href}, produced the largest values, and KDE_{adj} and MCP the smallest, whereas K_{LSCV} estimates had the highest inter-individual variability (Fig 2-1, Table 2-4). Males had significantly larger home ranges than females, regardless of the estimate used ($F_{1,38} = 14.82$, $p = 0.005$). Home range sizes estimated by KDE_{href}, were significantly higher than those calculated by any other estimate ($F_{3,38} = 13.98$, $p < 0.001$). Individual home ranges had a high degree of overlap with individuals of both sexes (Table 2-5, Fig 2-2). In general, the males overlapped with a higher number of individuals than females. Home range sizes were normally distributed when calculated using 95% MCP and KDE_{adj} but non-normally distributed when K_{LSCV} or KDE_{href} was used. In all cases the coefficient of variation between individuals was high.

Differences in home range estimates using day and night locations and those omitting night locations (Table 2-6), were larger than expected and much larger in males than in females ($F_{1,38} = 10.32$, $p = 0.012$), but did not differ between estimate type ($F_{3,38} = 1.52$, $p = 0.24$). Both day and night locations appear to be necessary to accurately determine home range size.

Discussion

This study is the first to establish home range size for a terrestrial Malagasy tenrec. We also provide the first conclusive evidence that *S. setosus* hibernates, at least in the highly seasonal dry deciduous woodland. The success of this study was based on the use of peritoneal implanted radio-transmitters. Although implanting reduces the transmission range of the transmitter, it is the only solution for the long-term tracking of free-ranging individuals when external mounts do not work (Pavey, Goodship & Geiser 2003, Dausmann 2005).

Table 2-4 Home ranges estimates of *Setifer setosus* in Ankarafantsika National Park using both day and night locations**.**

Animal	**Sex**	**Mass (g)**	**n**	**95% MCP (ha)**	**K_{LSCV} (ha)**	**K_{Href} (ha)**	**K_{Hadj} (ha)**
SF02†	F	198.0	33	5.29	12.85	13.92	7.09
SF04†	F	221.7	33	4.33	4.86	11.61	5.16
SF07	F	181.8	95	6.32	5.72	10.64	6.97
SF08*†	F	199.4	14	0.90	0.37	4.99	1.83
SF09*	M	148.9§	29	4.89	5.26	17.87	8.37
SF11*	F	165.6§	14	2.96	0.37	12.75	5.57
SF13	F	154.0§	107	9.17	6.93	16.59	10.49
SF14	F	158.5§	78	8.29	10.22	17.07	9.33
SF16	M	183.7§	83	14.02	18.32	28.18	16.92
SF17	M	183.9	36	14.66	42.97	37.61	21.22
SF18	M	256.5	67	21.10	11.07	53.87	26.93
SF19	M	295.7	50	9.52	20.25	24.59	12.46
SF20	M	142.0	30	9.06	7.60	24.73	12.58
SF21*	F	235.0	12	1.07	1.15	4.46	3.38
Mean		194.6 ± 44.3	58.3 ± 28.8	9.70 ± 5.1	13.28 ± 11.1	23.33 ± 12.9	12.50 ± 6.7
CV		22.8	49.4	52.5	83.7	55.3	53.6
Mean	M	201.8 ± 61.4	53.2 ± 21.9	13.67 ± 4.9	20.04 ± 13.8	33.80 ± 12.4	18.02 ± 6.2
Mean	F	189.3 ± 28.6	69.2 ± 34.62	6.68 ± 2.0	8.12 ± 3.3	13.97 ± 2.8	7.81 ± 2.1

The mean (±SD) and coefficient of variation (CV) are for all individuals excluding those marked with a * which had insufficient data to provide accurate estimates. Significant differences were found between males and females using all estimates. †Indicates individuals followed during the first season (Jan-Apr 2010 only). Reported masses are from the date the animal was implanted, §indicates that the surgery occurred within the first two months post-hibernation when the animal was at lower than average mass.

Table 2-5 Percent overlap of home range for individual *Setifer setosus* followed between September 2010-May 2011.

	Sex	SF07	SF09	SF11	SF13	SF14	SF16	SF17	SF18	SF19	SF20	SF21
SF07	F	-	0	0	0	60	0	54	94	0	0	0
SF09	M	0	-	24	0	0	0	0	0	0	17	25
SF11	F	0	33	-	0	0	2	5	10	0	55	31
SF13	F	0	0	0	-	0	22	25	47	49	0	0
SF14	F	38	0	0	0	-	0	9	68	0	0	0
SF16	M	0	0	1	13	0	-	27	62	23	25	0
SF17	M	15	0	2	11	4	20	-	78	0	24	0
SF18	M	19	0	2	15	21	33	55	-	4	24	0
SF19	M	0	0	0	33	0	26	0	9	-	0	0
SF20	M	0	12	28	0	0	28	37	52	0	-	2
SF21	F	0	48	42	0	0	0	0	0	0	6	-
Number of overlaps		3	3	6	4	3	6	7	8	3	6	3

Table 2-6 The difference in home range estimates for *Setifer setosus* based on calculations including all locations compared to estimates made using rest-phase locations only.

Animal	**Sex**	**n**	**95% MCP (ha)**	**K_{LSCV} (ha)**	**K_{Href} (ha)**	**K_{Hadj} (ha)**
SF02	F	24	0.96	-2.55	0.72	0.84
SF04	F	23	0.97	-9.41	0.04	-0.25
SF07	F	57	0.48	0.60	-0.65	-0.10
SF09	M	25	0.59	1.09	1.80	0.70
SF13	F	52	1.74	3.76	-0.11	2.03
SF14	F	39	0.98	2.27	-0.53	0.35
SF16	M	33	4.30	4.75	0.69	3.29
SF17	M	17	2.83	4.52	0.31	2.82
SF18	M	33	7.76	3.98	-3.71	3.42
SF19	M	30	1.94	15.61	-0.86	0.49
Mean ± SD	M		3.49 ± 2.75*	5.99 ± 5.57*	-0.36 ± 2.1*	2.14 ± 1.43*
Mean ± SD	F		1.03 ± 0.45*	-1.07 ± 5.22*	-0.11 ± 0.54*	0.58 ± 0.92*

*Significant differences were found between the sexes.

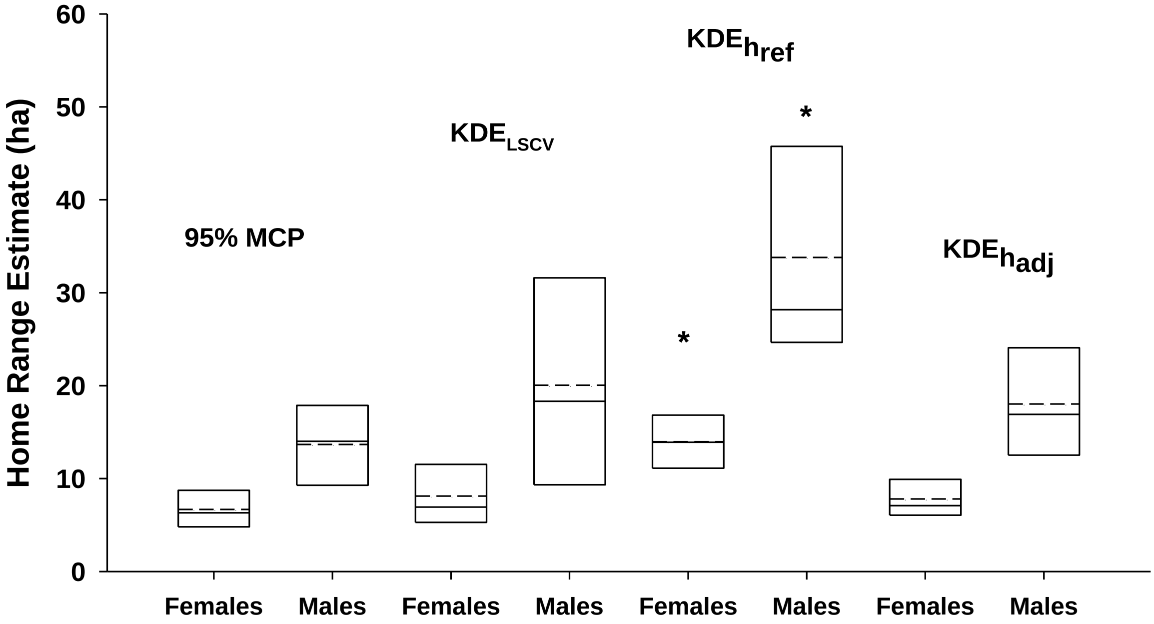


Figure 2-1 A comparison, by sex, of the various home range estimates for *Setifer setosus*. Boxplots show the median and median values (solid and dotted line, respectively), minimum and maximum values (lower and upper ends of boxes). * Indicates a significant difference in home range size between estimate type.

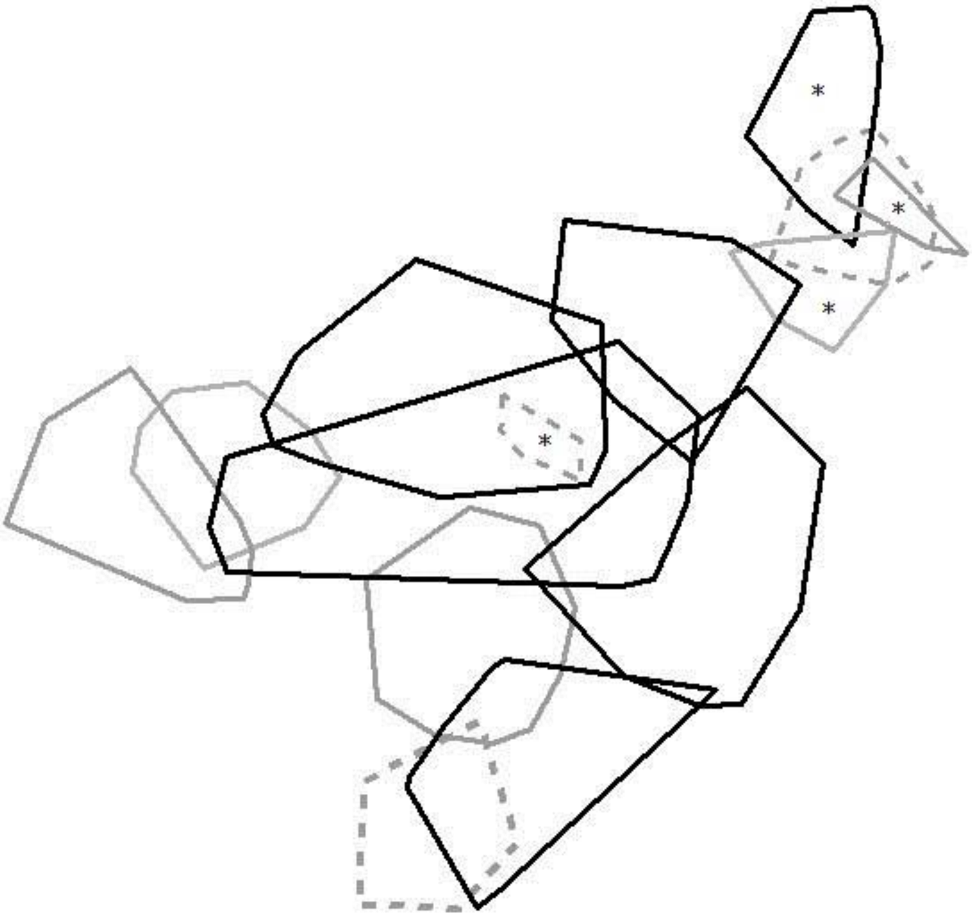


Figure 2-2 Minimum Convex Polygon (95%) home ranges of *Setifer setosus* in the JBA area of Ankarafantsika National Park, Madagascar. Male home ranges are outlined in black, females in grey. Individuals from the first season are indicated by dashed outlines and *indicates that the individual was excluded from statistical analysis.

One major disadvantage for this species, at least in the dry forest, is that there is little time during the active season when the females are not either gestating or lactating, they must therefore be caught early in their active season to ensure that their fitness is not impaired. As a result a number of females caught after October 2010 could not be tracked. Coupled with the highly unreliable trapping technique, this limitation led to part of the population being completely absent from the study. However, enough individuals with overlapping ranges were tracked to allow for some conclusions to be drawn.

Early studies in captivity noted that *S. setosus* was likely to be polygamous and promiscuous (Eisenberg & Gould 1969). This is supported by the observed distribution patterns. All individuals had range overlaps with multiple individuals of the opposite sex. In addition, one male, SF18, was observed copulating with an unknown female, and later was found in the same nest site as SF14 (300 m away) for a few days. These were the only mating events observed in this study. As only one individual, a female, was tracked over both seasons, it is unknown if the high inter-annual site-fidelity observed was representative of either her sex or the population.

The shelter site selection observed in this study, for the most part, supported early observations (Eisenberg & Gould 1969, Randrianjafy 2003). As mentioned in those studies, the primary rest sites for *S. setosus* are in tree cavities. However, the reliance of previous studies on visual observations, and locating the animal by chance, underrepresented the amount of nest sites found high in the canopy. Whereas nest site characteristics varied widely, lactating females were more commonly found in high (> 2 m) closed nest sites, likely as a means to protect the young from predators. Interestingly, Gould and Eisenberg (1966) observed a level of nest-site fidelity not observed in the present study, which may indicate differences in nest-site selection across the species geographic range. Only females with dependent young remained in the same site for multiple days, although individuals of both sexes were observed to return to previous nest sites throughout the active season. This behavior was less common in males than in females. In addition to having larger home ranges, males also travelled more, sometimes traversing the entire study grid in one evening.

The home ranges measured in the study were surprisingly large for a 200-300 g animal. Recorded home ranges were 9.8 times larger for males and 4.8 larger for females than that predicted by McNab (1963) for a 300 g animal. There are few similar sized Malagasy mammals with which to compare these data. The greater dwarf lemur, *Cheirogaleus major* (360 g) has a home range of only 4.4 ha for males and 4.0 ha for females (Lahann 2008). However, *Cheirogaleus* is strictly arboreal and therefore the comparison is likely to be misleading. Interestingly the terrestrial, 1-1.2 kg, giant jumping rat (*Hypogeomys antimena*) has only a 3.1 ha home range (Sommer 1997), almost exactly the size predicted by McNab (1963). One possible explanation for the large home range size is that the possession of body armor (in the form of dorsal spines) allows for a greater freedom of movement, without fear of predation (Lovegrove 2001). Body armor in tenrecs has proved to be an effective deterrent against the smaller Malagasy carnivores such as *Galidia elgans* (Eisenberg & Gould 1969), but clearly not against boas, as evidenced by the present study. They also constitute a small proportion of the diet for fossa (*Cryptoprocta ferox*) in the park (Dollar, Ganzhorn & Goodman 2007). In the study area, it is a local taboo to hunt *S. setosus*, but this does not hold throughout the species' range and might prove a threat (Randrianjafy 2003, Jones, Andriamarovololona & Hockley 2008).

The high variety of shelter sites and propensity for heterothermy indicate that the species is extremely adaptable and likely to perform fairly well with changing habitat and climates. Ganzhorn *et al.* (1990) came to a similar conclusion on the lesser-hedgehog tenrec (*Echinops telferi*), after observing the effects of selective logging on that species. In addition to the Western deciduous forest *S. setosus* have been observed in a wide variety of habitats including urbanized habitats, open grassland and the wetter forests of Eastern Madagascar (Petter & Petter-Rousseaux 1963, Gould & Eisenberg 1966). It would be of interest to obtain comparative data from these habitats to gain a better understanding of how various biotic and abiotic factors influence the distribution and habitat usage in this species.

Literature Cited

Agarwal, D. K., J. A. J. Silander, A. E. Gelfand, R. E. Dewar & J. G. J. Mickelson (2005) Tropical deforestation in Madagascar: analysis using hierarchical, spatially explicit, Bayesian regreesion models. *Ecol Modell,* **185,** 105-131.

Alonso, L. E., T. S. Schulenberg, S. Radilofe & O. Missa (2002) Une évaluation biologique de la Réserve Naturelle Intégrale d'Ankarafantsika, Madagascar. *RAP Bulletin of Biological Biographical sketches Assessment, Conservation International,Washington, D.C., USA.*

Brown, J. L. & G. H. Orians (1970) Spacing patterns in mobile animals. *Annu Rev Ecol Syst,* **1,** 239-262.

Burt, W. H. (1943) Territoriality and home range concepts as applied to mammals. *J Mammal,* **24,** 346-352.

Calenge, C. (2006) The package “adehabitat” for the R software: a tool for the analysis of space and habitat use by animals. *Ecol Modell,* **197,** 516-519.

Chouteau, P., R. Fenosoa & V. Rakotoarimanana (2004) Habitat selection and density of couas in Madagascar: implication for their conservation. *C R Biol,* **327,** 37-50.

Dausmann, K. H. (2005) Measuring body temperature in the field-evaluation of external vs. implanted transmitters in a small mammal. *J Therm Biol,* **30,** 195-202.

Dollar, L., J. U. Ganzhorn & S. M. Goodman (2007) Primates and other prey in the seasonally variable diet of *Cryptoprocta ferox* in the dry deciduous forest of western Madagascar. In: *Primate Anti-Predator Strategies*: 63-76. S. L. Gursky & K. A. I. Nekaris (Eds.). Springer, New York, NY.

Douady, C. J., F. Catzeflis, D. J. Kao, M. S. Springer & M. J. Stanhope (2002) Molecular evidence for the monophyly of Tenrecidae (Mammalia) and the timing of the colonization of Madagascar by Malagasy tenrecs. *Mol Phylogenet Evol,* **22,** 357-363.

Eisenberg, J. F. & E. Gould (1969) The tenrecs: a study in mammalian behavior and evolution. *Smithson Contrib Zool,* **27,** 1-156.

Ganzhorn, J. U., A. W. Ganzhorn, J. P. Abraham, L. Andriamanarivo & A. Ramananjatovo (1990) The impact of selective logging on forest structure and tenrec populations in Western Madagascar. *Oecologia,* **84,** 126-133.

Goodman, S., J. Ganzhorn & D. Rakotondravony (2003) Introduction to the mammals. In: *The Natural History of Madagascar*: 1159–1186. S. M. Goodman & J. P. Benstead (Eds.). The University of Chicago Press, Chicago, USA.

Goodman, S. M. & J. P. Benstead (2003) *The Natural History of Madagascar*. Chicago, IL, USA: University of Chicago Press Chicago.

Gould, E. & J. F. Eisenberg (1966) Notes on the biology of the Tenrecidae. *J Mammal,* **47,** 660-686.

Green, G. M. & R. W. Sussman (1990) Deforestation history of the eastern rain forests of Madagascar from satellite images. *Science,* **248,** 212-215.

Harper, G. J., M. K. Steininger, C. J. Tucker, D. Juhn & F. Hawkins (2007) Fifty years of deforestation and forest fragmentation in Madagascar. *Environ Conserv,* **34,** 325-333.

Jones, J. P. G., M. M. Andriamarovololona & N. Hockley (2008) The importance of taboos and social norms to conservation in Madagascar. *Conserv Biol,* **22,** 976-986.

Komers, P. E. & P. N. M. Brotherton (1997) Female space use is the best predictor of monogamy in mammals. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **264,** 1261-1270.

Lahann, P. (2008) Habitat utilization of three sympatric cheirogaleid lemur species in a littoral rain forest of southeastern Madagascar. *Int J Primatol,* **29,** 117-134.

Lovegrove, B. G. (2001) The evolution of body armor in mammals: plantigrade constraints of large body size. *Evolution,* **55,** 1464-1473.

Lovegrove, B. G. (2009) Modification and miniaturization of Thermochron iButtons for surgical implantation into small animals. *J Comp Physiol B,* **179,** 451-458.

McNab, B. K. (1963) Bioenergetics and the determination of home range size. *Am Nat,* **97,** 133-140.

Olson, L. E. & S. M. Goodman (2003) Phylogeny and biogeography of tenrecs. In: *The Natural History of Madagascar*: 1235-1242. S. M. Goodman & J. P. Benstead (Eds.). The University of Chicago Press, Chicago, USA.

Pavey, C. R., N. Goodship & F. Geiser (2003) Home range and spatial organisation of rock-dwelling carnivorous marsupial, *Pseudantechinus macdonnellensis*. *Wildl Res,* **30,** 135-142.

Petter, J.-J. & A. Petter-Rousseaux (1963) Notes biologiques sur les Centetinae. *La Terre et la Vie* **17,** 66-80.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rand, A. L. (1935) On the habits of some Madagascar mammals. *J Mammal,* **16,** 89-104.

Randrianjafy, V. (2003) *Contribution à l'étude de la biologie de conservation de la communauté micromammalienne d'Ankarafantsika.* PhD, Université d'Antananarivo, Antananarivo.

Randrianjafy, V. & S. M. Goodman (2008) *Setifer setosus*. *IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1. www.iucnredlist.org. Downloaded on 11 May 2010*.

Rojas, A. D., G. Koertner & F. Geiser (2010) Do implanted transmitters affect maximum running speed of two small marsupials? *J Mammal,* **91,** 1360-1364.

Smith, A. P., N. Horning & D. Moore (1997) Regional biodiversity planning and lemur conservation with GIS in western Madagascar. *Conserv Biol,* **11,** 498-512.

Sommer, S. (1997) Monogamy in *Hypogeomys antimena*, an endemic rodent of the deciduous dry forest in western Madagascar. *J Zool (Lond.),* **241,** 301-314.

Stephenson, P. J. (1993) The small mammal fauna of Réserve Spéciale d'Analamazaotra, Madagascar: the effects of human disturbance on endemic species diversity. *Biodivers Conserv,* **2,** 603-615.

Stephenson, P. J. (1994) Seasonality effects on small mammal trap success in Madagascar. *J Trop Ecol,* **10,** 439-444.

Swihart, R. K. & N. A. Slade (1985) Testing for independence of observations in animal movements. *Ecology,* **66,** 1176-1184.

Wauters, L. A., D. G. Preatoni, A. Molinari & G. Tosi (2007) Radio-tracking squirrels: performance of home range density and linkage estimators with small range and sample size. *Ecol Modell,* **202,** 333-344.

Wilkinson, D. A., G. C. Grigg & L. A. Beard (1998) Shelter selection and home range of echidnas, *Tachyglossus aculeatus*, in the highlands of south-east Queensland. *Wildl Res,* **25,** 219-232.

Worton, B. J. (1989) Kernel methods for estimating the utilization distribution in home-range studies. *Ecology,* **70,** 164-168.

Linking Statement

In the previous chapter I demonstrated that the *Setifer setosus* in the study population had a short activity period, restricted to the rainy summer season, and that they occupied relatively large territories for their size. These large home ranges are indicative of high levels of activity. In the next chapter, I seek to establish that *S. setosus* also has a fast life-history and is capable of assimilating and expending large amounts of energy in a limited time frame. To do so I analysed data, collected over three active seasons, on the natural history of this species collected via radio tracking and opportunistic recaptures. I observed a trade-off between high levels of adult mortality in this species and the need for compensatory high annual fecundity. The results of my study also allow me to comment on the evolution of fast life histories in these highly pleisiomorphic mammals.

Chapter 3 High mortality and annual fecundity in a free-ranging basal placental mammal, *Setifer setosus* (Tenrecidae: Afrosoricida)

Abstract

The spiny tenrecs, an endemic sub-family of Malagasy insectivores (Tenrecinae), are wide-ranging and fairly conspicuous, yet long term studies on free-ranging populations remain sparse. Basal to most eutherian mammals, they share many ecological and morphological traits with proposed eutherian ancestors. Understanding their unusual life histories is therefore important to the understanding of mammalian evolution. Here I present the results of a three-year study on a population of *Setifer setosus,* in the dry deciduous forest of Western Madagascar. The annual activity cycle of this species includes a five to seven month hibernation period, during the dry season, and a dramatic increase in body mass during the active season. Females, observed giving birth to up to three litters in a single season, entered hibernation later than males, after weaning their last litter. Short intervals between parturition dates and simultaneous gestation and lactation provide evidence for post-partum oestrus, previously observed in only one other species of tenrec (*Geogale aurita*, subfamily:Geogalinae). High levels of predation, primarily by snakes and unidentified mammalian ground predators, were also observed and likely contribute, along with the unpredictability of Madagascar's climate, to the unusually fast life-history of these mammals.

Introduction

Unpredictable and highly variable climatic conditions, as well as an isolated island environment, have led to high levels of endemism on the island of Madagascar (Goodman & Benstead 2003, Dewar & Richard 2007). The life-history characteristics of the island's endemic mammals are often unusual, falling on either the extreme 'slow' or 'fast' side of the life-history continuum (Stearns 1983, Wright 1999, Dewar & Richard 2007). One of most speciose groups of endemic mammals, the Tenrecidae (Superorder: Afrotheria) is a highly diverse grouping of insectivorous mammals. Although much is known about the distribution, habitat use and phylogeny of these species (Eisenberg & Gould 1969, Olson & Goodman 2003, Muldoon & Goodman 2010, Soarimalala 2011), long-term studies on the natural history of free-ranging populations are rare.

The reproductive characteristics of tenrecs are highly varied, and can be found on both the fast (early reproduction, large litters in the spiny tenrecs: Tenrecinae) and slow (late-maturing, small litters in the shrew tenrecs: Geogalinae and Oryzorictinae) side of the life-history continuum (Eisenberg & Gould 1969, Stephenson & Racey 1995, Racey & Stephenson 1996, Symonds 1999). The hedgehog tenrecs (*Echinops telfairi* and *Setifer setosus*) fall somewhere in the middle, maturing early but producing smaller litters (mean of 5.7 and 3.5, respectively) than the larger Tenrecinae (15.5 for *Tenrec ecaudatus*, Symonds 1999). The reproduction and life history of the Tenrecinae has been well studied in captivity (Eisenberg & Muckenhirn 1968, Eisenberg 1975, Stephenson, Racey & Rakotondraparany 1994) and it is known that they can be preyed on by boid snakes and a number of native and introduced Carnivora (Eisenberg & Gould 1969, Hawkins & Racey 2008). However, little information is available on either the life-history or the rates of mortality in free-ranging populations (Dewar & Richard 2007).

Baseline data on the life histories of the Tenrecinae from a wide variety of habitats are necessary to understand the evolution of these unique species. For example, because of the basal phylogenetic position of tenrecs, these data would greatly assist in reconstructing the characteristics of the ancestral placental mammal (Lovegrove 2012), which was also insectivorous and probably similar in ecology (O'Leary *et al.* 2013). It has been suggested that tenrecs may retain certain plesiomorphic characteristics of Late Cretaceous mammals (Lovegrove 2012), which allowed the ancestral mammal to survive the asteroid impact at the Cretaceous-Paleogene boundary (Robertson *et al.* 2004).

As part of a study on the physiology and temperature regulation in *Setifer setosus*, a free-ranging population inhabiting the dry deciduous woodlands of Western Madagascar was monitored and individuals were followed for three rainy seasons. I present findings on the body condition, reproduction, phenology and causes of mortality in this species. I also use these data to comment on the evolution of ‘fast’ life histories in the Tenrecidae.

Materials and Methods

Study site, capture and surgical methods

The study was conducted over three rainy seasons, from January 2010 to February 2012, in the JBA (*Jardin Botanique A*) research area in Ankarafantsika National Park (16º19’S, 46º48’E). The site is characterized by large seasonal variations in rainfall with a distinct dry season during the austral winter (April – November) with little or no rainfall, and around 1,000 -1,500 mm of rain falling primarily during the remaining months (data from the Durrell

Wildlife Conservation Trust, Ampijoroa 1997-2012, Fig 3-1). A detailed description of the study site and methods are provided in Chapter 2.

All animals were caught by hand, by walking the established trails at night with local guides, and transported them back to the research camp. Capture effort was fairly constant, averaging four to six hours, five to six nights per week between January 2010 and November 2011. The time of night varied from immediately after sunset (18:00) to midnight (0:00). Research activity during the 0:00-06:00 period by other members of the research team resulted in the occasional opportunistic capture. Animals were housed in plastic containers lined with paper towel and provided with live insects and sardines. At initial capture all animals were anesthetized using isoflurane in oxygen (induction; 1 – 2%, maintenance; 0.5%) and various morphometric measurements were taken. Each animal was marked with a small distinctive clip in the ear and injected with a transponder (Small Animal Marking System, Trovan Ltd., UK) to allow for identification at recapture.

A subset of the population (individuals larger than 180 g) was implanted with radio-transmitters for subsequent tracking and relocation. They were kept for a maximum of five days before surgery to allow for the collection of metabolic data for a concurrent study. The radio-transmitters (2-stage VHF collar-mounted transmitters, Merlin Systems Inc, Boise, ID, USA) were modified and encapsulated in surgical wax (Paramat Extra-Merck KGaA, Darmstadt, Germany) together with Thermochron iButtons (Dallas Semiconductor, Dallas, TX, USA), and implanted via a ventral midline laparotomy (Chapter 2). The telemeter package weighed around 13.0 g (mean 13.0 g, range 11.7 - 13.5 g) constituting around 5 - 8% of the animal’s body mass, within a range of mass shown to have no adverse effects on locomotory behaviour (Rojas, Koertner & Geiser 2010). The animals were kept for one day of post-operative observations, released at the site of capture, and re-captured within a week of surgery to confirm that the incision had healed.

Collection of life-history data

Females with radio-transmitters were captured once a week to monitor reproductive status (evaluated by observing changes in mass, shape of the stomach and condition of the nipples) and males once every two or three weeks to assess body condition. The rest sites of the females were located every day (as repeated use of a single nest site indicates parturition-Chapter 2) and males every couple of days. On occasion, animals without implants were opportunistically re-captured and their mass and reproductive status were recorded. If visual contact was possible the size and number of juveniles were recorded. Due to the lack of external testes it is not possible to determine the reproductive condition of the males (Petter & Petter-Rousseaux 1963).

Statistical Analysis

All statistics were performed using R version 2.15.2 (R Development Core Team 2011). Sexual dimorphism in morphometric measurements was assessed using t-tests, or Mann-Whitney U-tests, and resultant probability values were compared to an α-value of 0.05. Body mass was analysed using mixed-effects models in the R package *nlme* (Pinheiro *et al.* 2013). Pregnant females and animals from the first season were excluded from the analyses as reproductive status and date to parturition were not always known and could not be controlled for in the analyses. To standardize changes in mass between animals of different body size, body condition index (BCI- the ratio of mass to forearm length) was used. To control for time of year, as mass increased dramatically from the time of emergence in October to start of hibernation, a factor 'day' was created with 1 September as 'day' 0. Both BCI and 'day' were logarithmically transformed to control for unequal variances in the different groupings. Females were split into two groups; non-reproductive females, usually only found in the first few months of the season (day 0-100), and lactating or post-pregnant females.

Model selection was used starting with a base model of 'reproductive status',' season' and 'day', as well as interaction effects between the three factors. The model residuals were assessed graphically and no heterogeneity was observed. Model selection identified 'day' and 'animal' and their interaction (in the form ~'day|animal') as the optimal structure for random effects. The optimal fixed structure included 'reproductive status', 'day' and a 'day-season' interaction effect. Differences within the significant categorical factors were determined using a Tukey *post-hoc* test using the R package *multcomp* (Hothorn, Bretz & Westfall 2008).

Results

Population levels and rate of capture

The fates of individuals with implants from the first and second seasons are described in Chapter 2, but are included below as additional information on these individuals is presented. Despite similar capture effort, the number of new individuals captured in each season, or recaptured for the first time in subsequent seasons, varied dramatically (Fig. 3-2).

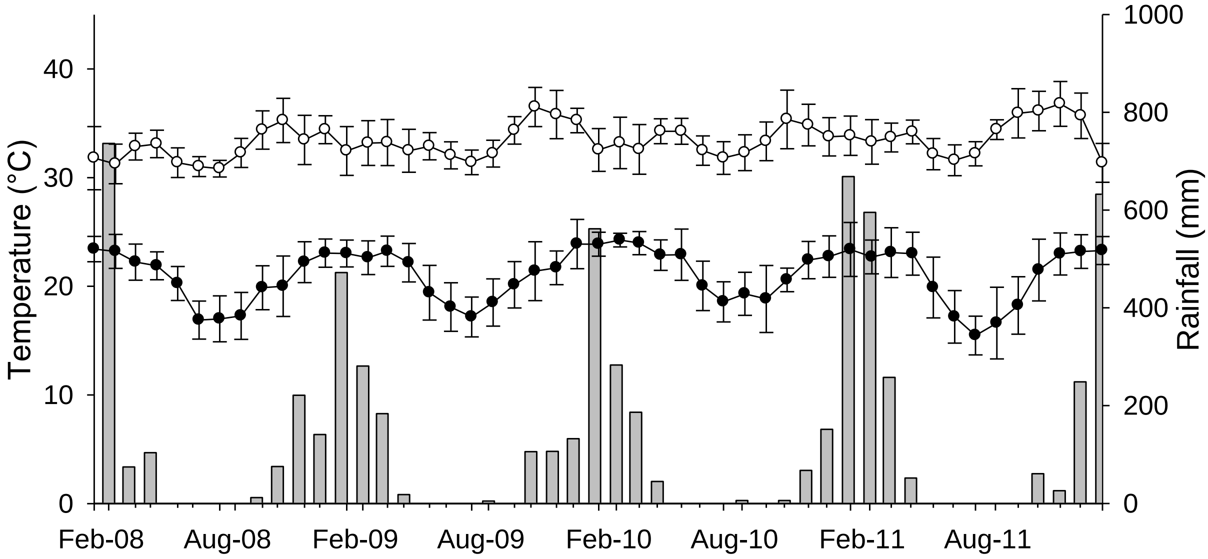


Figure 3-1 The total monthly rainfall (mm- grey bars) and the average maximum (clear circles) and minimum (black circles) ambient temperature recorded by the Durrell Wildlife Conservation Trust at the Ampijoroa Chelonian Breeding Centre, for the period of the study.

The first season (January - April 2010), was a preliminary study in which 8 individuals (3 males, 5 females) were implanted and followed until hibernation commenced in March - April. An additional 13 individuals were captured over the same time period but did not receive implants for various reasons: too small, pregnant or lactating, or caught before the surgical equipment was available. The second study period was longer (September 2010 - April 2011), and resulted in the capture and radio-tracking of 14 individuals (8 males, 6 females). An additional 30 individuals (9 females, 16 males and 5 juveniles) were captured and released without receiving implants, either because of low mass or reproductive status.

By the third study period (September 2011 - February 2012) the rate of new captures decreased and only 14 individuals (5 females, 6 males and 3 juveniles) were captured over the entire study period. All animals with the exception of two heavily pregnant females and one male caught near the end of the study, received implants. With the exception of the female from the second season which died entering a tree cavity (Chapter 2), there were no noticeable effects of the transmitter on the individuals. One good indication of success was that one single female (SF07) was implanted and followed over two seasons, and gave birth to two litters during the second season that she carried the transmitter.

Morphometrics and changes in body mass

The morphometric measurements of *S. setosus* in this study (Table 3-1) were similar those previously reported for this species in other parts of its range (Soarimalala & Goodman 2011), as well as for Ankarafantsika (Randrianjafy 2003). Repeated measures obtained from individuals captured over multiple seasons showed little differences, with the exception of a few individuals who were likely to have been yearlings at date of first capture (data not shown). There were no differences between the sexes in any of the measurements ($p < 0.05$).

Mass varied widely between individuals and over time (Fig 3-3). The animals were lightest after emergence from hibernation and heaviest immediately prior to hibernation or during pregnancy. This increase in mass was consistent between seasons and there were no overall differences found between the seasons ($F_{1,53} = 1.25$, $p = 0.27$). Model selection indicated that only reproductive status ($F_{2,53} = 28.34$, $p < 0.001$), day ($F_{1,53} = 76.30$, $p < 0.001$) and the interaction effect between day and season ($F_{1,53} = 13.43$, $p < 0.001$) were significant. Non-reproductive females had significantly smaller masses than post-reproductive females ($Z = -2.39$, $p = 0.04$) and no differences were found between males and either class of female ($p = 0.52$ and $p = 0.14$).

Seasonal activity patterns and reproduction

All members of this population restricted activity patterns to between sunset and sunrise (Chapter 2). Additional observations performed over the course of the study showed that individuals commenced nighttime activity after sunset (range 18:45 - 21:20, mean 19:31, n =13) and ceased activity before dawn (range 02:30 - 05:36, mean 04:30, n = 7).

Seasonal patterns of activity showed little change between years, although the start date for hibernation had a high level of variability both between sexes and between individuals (Table 3.2). Males entered into hibernation earlier than females: as early as 18 February by a first season male, and as late as 02 April by two males in the second season. Females were observed to enter hibernation anytime from April to May with gestating females observed as late as 17 April 2010. Similar to two individuals from the first season (reported in Chapter 2), a male from the second season remained in a single nest throughout the dry season (19 March 2011-2 September 2011). Over-wintering body temperature recordings were obtained from a single female and body temperature closely tracked ambient temperature, indicating that hibernation continued from the end of April until the end of October (data shown in Lovegrove *et al.* 2014).

Gestating females were observed shortly after emergence from hibernation (Table 3-3). Using the published gestation length of this species of ~ 60 days (Eisenberg & Muckenhirn 1968, Eisenberg 1975), the earliest estimated dates for insemination fall around mid-October. Females with implants were observed to have up to three litters per season. However, litter survival rates were low. Of the six litters followed, four were lost, and only two litters, with one pup and three pups, were observed until weaning (~ 34 days). Reduced levels of activity were occasionally observed in lactating females and one female in the second season remained at a single nest site, without evidence of nocturnal activity, for a month.

Mortality

As presented in Chapter 2, mortality among adults in this population is high (25% in the first season, 58% in the second season and 100% in the third, Table 3.4). Boid snakes (*Acranthophis madagascariensis* and *Boa manditra*) were the most common predators, although a large proportion of individuals in the third season (3/7) were killed by an unknown ground predator. In addition, one individual whose home range covered an area transected by a busy road (Route National 4) was run over by a vehicle.

Table 3-1 Summary of morphometric measurements for adult *Setifer setosus*, sample size contained within parentheses**.**

Body length (mm)	188.2 ± 12.1 (n = 39)
Head length (mm)	46.2 ± 3.4 (n = 38)
Head width (mm)	23.1 ± 2.8 (n = 38)
Tibia length (mm)	36.0 ± 2.2 (n = 39)
Forearm Length (mm)	30.1 ± 2.1 (n = 39)

Table 3-2 Confirmed hibernation start and end dates for *Setifer setosus* in the Western dry forest (Ankarafantsika National Park) over two dry seasons (Season 1: Sep 2010 - May 2011, Season 2: Sep 2011 - Feb 2012).

Animal	**Sex**	**Date of Capture**	**Season**	**Hibernation start date**	**Hibernation end date**	**Hibernation length (days)**
SF01	M	17-Feb-10	1	19-Feb-10	approx. 14-Sep-10	~ 207
SF02	F	19-Feb-10	1	after 18-May-10	lost to study	n/a
SF05	M	7-Mar-10	1	14-Mar-10	lost to study	
SF07	F	29-Mar-10	1	after 5-May-11	before 19-Nov-11	> 198
SF08	F	17-Apr-10	1	28-Apr-10	lost to study	n/a
SF18	M	23-Oct-10	2	2-Apr-11	before 19-Oct-11	< 200
SF07	F	19-Nov-10	2	26-Apr-11	22-Oct-11	179
SF16	M	22-Nov-10	2	8-Mar-11	lost to study	n/a
SF20	M	6-Dec-10	2	2-Apr-11	before 29-Nov-11	< 241
SF19	M	29-Jan-11	2	19-Mar-11	2-Sep-11	167

Table 3-3 Length of gestation and lactation of *Setifer setosus* in Ankarafantsika National Park. Start of gestation was taken either from the first day that the individuals were visibly pregnant, or taken as the parturition date from the last pregnancy*.

	Preg-nancy No	Estimated Start of Gestation*	Parturition Date	Gestation Length (Days)	Start Lactation	End Lactation	Estimated Lactation (days)	Result
SF13	1	21-Oct-10	1-Dec-10	> 41	2-Dec-10	7-Dec-10	5	lost litter
	2	13-Dec-10	23-Jan-11	41-53	23-Jan-11	8-Mar-11	44	unknown
	3	8-Mar-11	deceased 23-Mar-11	n/a	n/a	n/a	n/a	n/a
SF14	1	25-Oct-10	5-Dec-10	> 41	5-Dec-10	8-Jan-11	34	1 juvenile weaned
	2	13-Dec-10	12-Feb-11	61-69	12-Feb-11	deceased 19-Feb-11	n/a	n/a
SF07	1	6-Dec-10	16-Jan-11	> 41	16-Jan-11	11-Feb-11	26	lost litter (snake)
	2	16-Jan-11*	10-Mar-11	~ 53	10-Mar-11	13-Apr-11	34	3 juveniles weaned
SF22	1	19-Oct-11	unknown	n/a	unknown	28-Dec-11	n/a	moved out of range
	2	28-Dec-11	deceased 17-Jan-12	n/a	n/a	n/a	n/a	n/a
SF23	1	14-Oct-11	24-Nov-11	> 41	25-Nov-11	deceased 8-Dec-11	n/a	n/a
SF24	1	14-Oct-11	2-Dec-11	> 49	3-Dec-11	16-Dec-11	13	possible lost litter
	2	16-Dec-11	deceased 26-Jan-11	n/a	n/a	n/a	n/a	n/a
SB01	1	26-Oct-11	6-Dec-11	> 41	6-Dec-11	lost to study 9-Dec-11	n/a	n/a

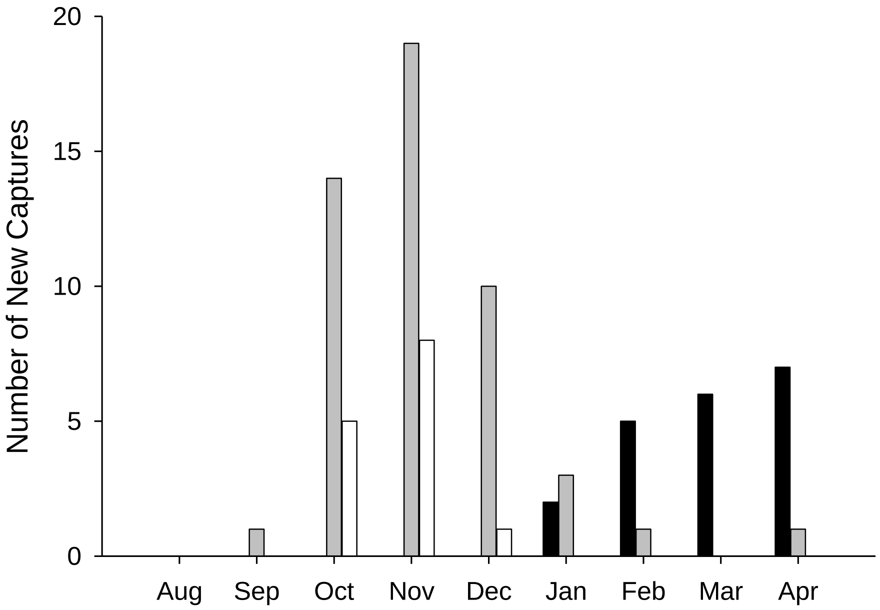


Figure 3-2 Number of new *Setifer setosus* captured each month in Ankarafantsika National Park. Black bars are from the first season (January - May 2010), light grey the second season (September 2010 - April 2011), and clear from the final season (September 2011 – January 2012. The data from the second and third season include the first date of recapture for that season. Similar sampling effort was made from January 2010 to January 2012.

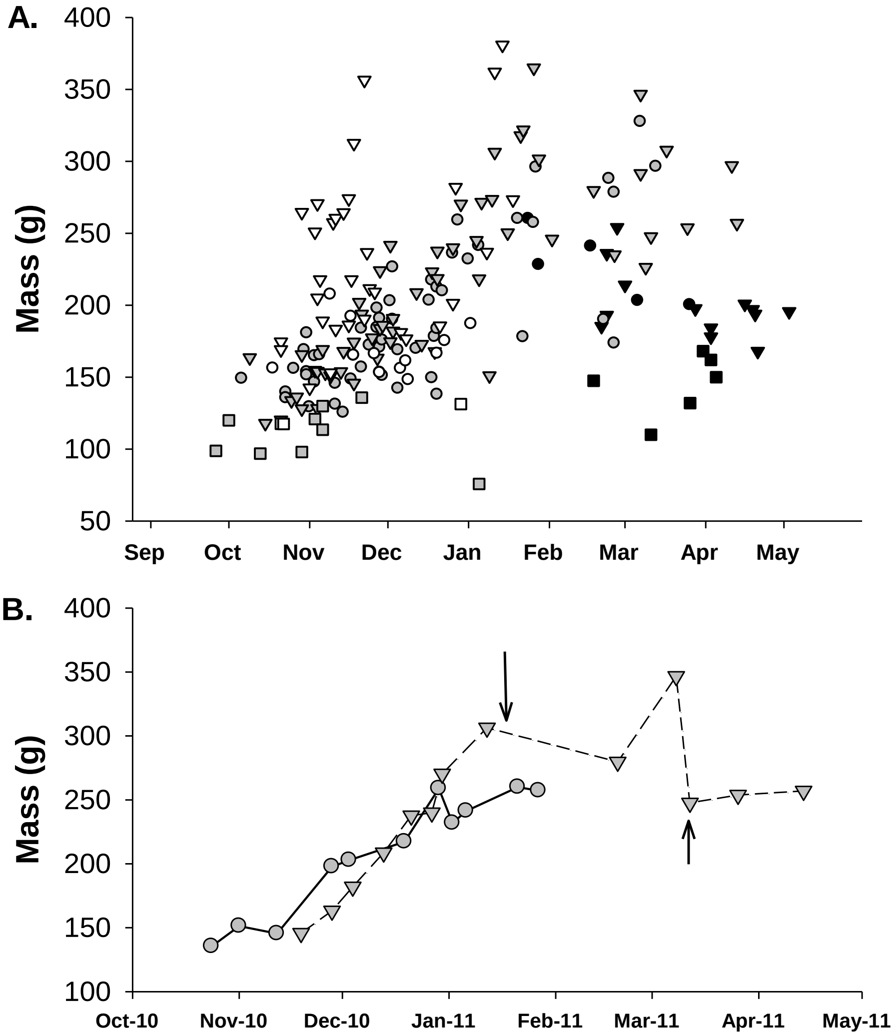


Figure 3-3 A. Annual changes in body mass of male (circles), female (triangles) and juvenile (squares) *Setifer setosus* over the three study seasons (first-black, second-light grey, third-clear). Data from females include gestating and lactating females (Nov - Mar) and multiple measures per individual are also included. **B.** Representative change in body mass of a male (circles) and female (triangles) during the second season. The female gave birth on 16 January and on 10 March (arrows). The male entered hibernation on 02 April, and the female on 26 April.

Table 3-4 Causes of mortality in *Setifer setosus* over the three study seasons. For some individuals there was clear evidence of predation by boas (transmitters found along with undigested spines) but the species was not determined. Ground predators (non-boid) could not be determined (deaths not witnessed).

Animal	**Sex**	**Date of Capture**	**Date of death**	**Cause of Death**
SF03	F	23-Feb-10	14-Mar-10	unknown ground predator
SF06	M	27-Mar-10	unknown	undetermined boa species
SF09	M	7-Oct-10	2-Feb-11	*Boa manditra*
SF10	F	10-Oct-10	20-Oct-10	unknown ground predator
SF13	F	5-Nov-10	23-Mar-11	*Boa manditra*
SF14	F	5-Nov-10	19-Feb-11	*Boa manditra*
SF15	M	19-Nov-10	16-Dec-11	*Acranthophis madagascariensis*
SF17	M	22-Dec-10	1-Feb-11	*Acranthophis madagascariensis*
SF18	M	29-Dec-10	27-Oct-11	unknown ground predator
SF20	M	24-Feb-11	12-Dec-11	undetermined boa species
SF21	F	26-Feb-11	6-Mar-11	undetermined boa species
SF22	F	19-Oct-11	17-Jan-12	*Boa manditra*
SF23	F	19-Oct-11	8-Dec-11	unknown ground predator
SF24	F	2-Nov-11	26-Jan-12	unknown ground predator
SF25	M	19-Nov-11	17-Jan-12	unknown ground predator
SF26	M	28-Nov-11	26-Dec-11	Hit by vehicle on RN 4

Discussion

The abundance of *S. setosus* in Ankarafantsika NP has always been considered low (Rakotondravony, Randrianjafy & Goodman 2002, Randrianjafy 2003) and appears to have decreased over the course of this study. Despite similar trapping efforts, capture rates decreased during the third season. Low capture rates coincided with a decrease in the number of *T. ecaudatus* (K.D. Lobban pers. comm., D.L.L. pers. obs), a species whose low abundance in the study area has been attributed to high levels of hunting for human consumption (Andriatsarafara 1981). Although *S. setosus* is generally not consumed locally (Randrianjafy 2003), reduced populations of *T. ecaudatus* could lead to higher natural predation pressure on *S. setosus*. Both species constitute a large proportion of the diet of the fossa, *Cryptoprocta ferox* (Hawkins & Racey 2008) at another site in the Western dry forest. Remains have been also found in fossa scat in Ankarafantsika (Dollar, Ganzhorn & Goodman 2007), albeit in smaller proportions as the latter study was conducted primarily during the hibernation season. Any mortalities attributed to 'unknown ground predator' in Table 3.4, could have been by fossa, however, it is equally likely to have been one of the more abundant introduced predators found at the study site, such as small Indian civets (*Viverricula indica*), African wild cats (*Felis silvestris*), and feral dogs (*Canis lupus familiaris*, Dollar *et al.* 2007). In the spiny forests of Southwest Madagascar, mortality by introduced dogs and wild cats is on the rise in diurnal lemurs (Brockman *et al.* 2008) and might equally pose a problem for the spiny tenrecs, especially since dogs are used to hunt these species (Eisenberg & Gould 1969, Andriatsarafara 1981). My research group observed a feral dog killing a female *T. ecaudatus* (D.L.L. and K.D. Lobban pers. obs); however, it is not known if *S. setosus* is common prey.

In addition to the data presented in Chapter 2, this study provides further conclusive evidence, based on long periods of inactivity, that *S. setosus* hibernates, at least in the Western dry forest. Sex differences in the timing of entrance into hibernation are similar to those found in *T. ecaudatus* (Andriatsarafara 1981), where females entered hibernation later, attributed to the need for additional time to fatten after weaning their litter. However, the lack of difference in body condition between males and females in this study suggest that it is maternal care itself, and not its effect on body condition, that delays entry into hibernation. Not enough information is available to compare emergence dates between the sexes.

Environmental conditions appeared to vary between seasons; the first season in particular was preceded by one of driest rainy seasons in recent history (Fig 3-1). No evidence of this was reflected in either hibernation start times or end times or patterns of body condition, although any existing patterns might have been obscured by small sample sizes in the first and third seasons. More data are necessary to draw any further conclusions.

The animals studied showed a remarkable ability to increase body mass throughout the active season (Fig 3-3). Although partially omnivorous, especially in urban areas (Petter & Petter-Rousseaux 1963, Eisenberg & Gould 1969), *S. setosus* are primarily insectivorous, and insect abundance increases dramatically during the rainy season coinciding with their activity periods (Nicoll 1985, Rakotoarivelo *et al.* 2007, Dammhahn & Kappeler 2008). High levels of easily assimilated energy (Bell 1990) and relatively low thermoregulatory costs (Chapter 4, 5) for this time of year, assist in rapid mass increases. Seasonal fattening is also seen in other Malagasy hibernators such as the fat-tailed dwarf lemur (*Cheirogaleus medius*) which remain more sedentary during fattening but also make use of high levels of sugar in fruit instead of protein in insects (Fietz & Ganzhorn 1999).

Gestation did not preclude mass gain in females. Despite giving birth to multiple litters, they had similar body conditions to males later in the season (Fig 3-3B). Similar gains in mass during gestation have been observed in captive *E. telfairi* (Poppitt, Speakman & Racey 1994), as well as in other non-hibernating mammals where mass stores accumulated during gestation are believed to be important for lactation (Humphries & Boutin 1999). No juveniles were caught both pre- and post-hibernation and therefore the effects of date of birth, usually a strong predictor of overwinter survival, could not be assessed.

Similar to observations on captive populations of both species of hedgehog tenrecs (Mallinson 1974, Eisenberg 1975, Künzle, Nautrup & Schwarzenberger 2007), gestation lengths were highly variable. The shortest period between subsequent parturition dates for a single individual in this study was around 53 days. This is much shorter than the 60 day average reported in comparative studies (Eisenberg & Gould 1969, Symonds 1999), but is similar to observations from a captive population held at higher-than-usual ambient temperatures (Mallinson 1974). This short inter-birth interval would indicate a period of overlap between lactation and gestation. It is therefore likely that *S. setosus* is capable of post-partum oestrus, previously thought to be unique to the large-eared tenrec *Geogale aurita* (Stephenson 1993, Racey & Stephenson 1996). The observation, on two separate occasions, one reported in Chapter 2, the other from this study, of males sharing a nest site with lactating females supports this.

In this population, in the Western deciduous forest, breeding occurred throughout the active season, and parturition dates were not synchronised. In contrast, females from a population studied in the Eastern rainforest gave birth in October, and no pregnancies were observed after that date (Eisenberg & Gould 1969). Similarly flexible breeding schedules were observed in *T. ecaudatus* introduced to the Seychelles (Nicoll & Racey 1985), where peak reproduction coincided with peak food availability. The capacity to give birth to multiple litters during a single season (max of 3 observed in this study), dramatically increases the annual fecundity of this species, especially when compared to previously reported values which only considered a single litter per year (Eisenberg & Gould 1969, Symonds 1999). In combination with the capacity for fast sexual maturation, as early as 74 days for a captive individual (Eisenberg 1975), high annual fecundity places *S. setosus* further towards the 'fast' end of the mammalian life history continuum showing more similarities with the extreme reproduction observed in *T. ecaudatus*.

Symonds (2005) posited that it is unclear if the high rate of reproduction and overall reproductive flexibility observed in the Tenrecinae relative to other insectivores is by-product of Madagascar's environment, or of their phylogeny, as little is known about the life histories of the other members of the Afrosoricida; the otter shrews (Family: Tenrecidae, Subfamily: Potamogalinae) and the golden moles (Family: Chrysochloridae). However, similar flexibility is also seen in the life history traits of other endemic Malagasy mammals, especially the cheirogaleid lemurs (Lahann, Schmid & Ganzhorn 2006, Dewar & Richard 2007, Lahann & Dausmann 2011, Canale *et al.* 2012). Therefore it is likely that the environment does play a large role in shaping the life histories of these species (Wright 1999, Dewar & Richard 2007). In addition to environmental conditions, mortality is one of the primary drivers of the 'rate' of life histories, with high rates of predation leading to high birth rates (Read & Harvey 1989). The high mortality rates observed in *S. setosus* support this hypothesis.

This study presents aspects of the natural history of a free-ranging tenrec and illustrates the importance of long-term studies across a species' range. Both the level of mortality as well as reproductive activity in this species were significantly higher than what has been reported in previous studies. The opportunistic nature of the data collection and the cryptic nocturnal character of the species precluded accurate estimations of certain population parameters (population size, juvenile growth rates, lifespan, etc.), but other aspects of the life history of *S. setosus* which were hitherto unknown have been made clear. The study was greatly limited by the inability to follow single individuals over multiple seasons. It would be of interest to quantify rates of survival of the different juvenile cohorts to determine if there are any advantages to the synchronous reproduction observed in the eastern rainforests (Eisenberg & Gould 1969) over the multiple litters observed in this western population. In addition, hibernation has been shown to increase the probability of overwinter survival, and is believed to lead to slow life histories (Turbill, Bieber & Ruf 2011). As a slow life history is not the case for this species, at least not in the habitat studied here, a comparison of the rates of mortality and the rates of reproduction with populations which are active year round could help to shed light on this exception. Greater understanding of the reproduction, phenology and rates of mortality of these basal mammals could help shed light on the evolution of mammalian life-histories.

Literature Cited

Andriatsarafara, F. R. (1981) *Quelques observation sur l'ontogenie et le comportement de Tenrec ecaudatus (Schreber 1777) en captivité: comparaisons avec les données connus chez d'autres insectivores.* Diplôme d'Études Approfondies de Sciences, Université de Madagascar, Antananarivo.

Bell, G. P. (1990) Birds and mammals on an insect diet: a primer on diet composition analysis in relation to ecological energetics. *Studies Avian Biology,* **13,** 416-422.

Brockman, D. K., L. R. Godfrey, L. J. Dollar & J. Ratsirarson (2008) Evidence of invasive *Felis silvestris* predation on *Propithecus verreauxi* at Beza Mahafaly Special Reserve, Madagascar. *Int J Primatol,* **29,** 135-152.

Canale, C. I., E. Huchard, M. Perret & P.-Y. Henry (2012) Reproductive resilience to food shortage in a small heterothermic primate. *PLoS ONE,* **7,** e41477.

Dammhahn, M. & P. M. Kappeler (2008) Comparative feeding ecology of sympatric *Microcebus berthae* and *M. murinus*. *Int J Primatol,* **29,** 1567-1589.

Dewar, R. E. & A. F. Richard (2007) Evolution in the hypervariable environment of Madagascar. *Proc Natl Acad Sci U S A,* **104,** 13723-13727.

Dollar, L., J. U. Ganzhorn & S. M. Goodman (2007) Primates and other prey in the seasonally variable diet of *Cryptoprocta ferox* in the dry deciduous forest of western Madagascar. In: *Primate Anti-Predator Strategies*: 63-76. S. L. Gursky & K. A. I. Nekaris (Eds.). Springer, New York, NY.

Eisenberg, J. F. (1975) Tenrecs and solenodons in captivity. *Int Zoo Yearb,* **15,** 6-12.

Eisenberg, J. F. & E. Gould (1969) The tenrecs: a study in mammalian behavior and evolution. *Smithson Contrib Zool,* **27,** 1-156.

Eisenberg, J. F. & N. Muckenhirn (1968) The reproduction and rearing of tenrecoid insectivores in captivity. *Int Zoo Yearb,* **8,** 106-110.

Fietz, J. & J. U. Ganzhorn (1999) Feeding ecology of the hibernating primate *Cheirogaleus medius*: how does it get so fat? *Oecologia,* **121,** 157-164.

Goodman, S. M. & J. P. Benstead (2003) *The Natural History of Madagascar*. Chicago, IL, USA: University of Chicago Press Chicago.

Hawkins, C. E. & P. A. Racey (2008) Food habits of an endangered carnivore, *Cryptoprocta ferox*, in the dry deciduous forests of western Madagascar. *J Mammal,* **89,** 64-74.

Hothorn, T., F. Bretz & P. Westfall (2008) Simultaneous inference in general parametric models. *Biom J,* **50,** 346-363.

Humphries, M. M. & S. Boutin (1999) Mass-dependent reprodution or reproduction-depedent mass? A comment on body mass and first-time reproduction in female sciurids. *Can J Zool,* **77,** 171-173.

Künzle, H., C. P. Nautrup & F. Schwarzenberger (2007) High inter-individual variation in the gestation length of the hedgehog tenrec, *Echinops telfairi* (Afrotheria). *Anim Reprod Sci,* **97,** 364-374.

Lahann, P. & K. H. Dausmann (2011) Live fast, die young: flexibility of life history traits in the fat-tailed dwarf lemur (*Cheirogaleus medius*). *Behav Ecol Sociobiol,* **65,** 381-390.

Lahann, P., J. Schmid & J. U. Ganzhorn (2006) Geographic variation in populations of *Microcebus murinus* in Madagascar: Resource seasonality or Bergmann's rule? *Int J Primatol,* **27,** 983-999.

Lovegrove, B. G. (2012) The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol Rev,* **87,** 128–162.

Lovegrove, B. G., C. I. Canale, D. L. Levesque, G. Fluch, M. Řeháková-Petrů & T. Ruf (2014) Are tropical small mammals physiologically vulnerable to Arrhenius effects and climate change? *Physiol Biochem Zool,* **87,** 30-45.

Mallinson, J. J. C. (1974) Establishing mammal gestation periods at the Jersey Zoological Park. *Int Zoo Yearb,* **14,** 184-187.

Muldoon, K. M. & S. M. Goodman (2010) Ecological biogeography of Malagasy non-volant mammals: community structure is correlated with habitat. *J Biogeogr,* **37,** 1144-1159.

Nicoll, M. E. (1985) Responses to Seychelles tropical forest seasons by a litter-foraging mammalian insectivore, *Tenrec ecaudatus,* native to Madagascar. *J Anim Ecol,* **54,** 71-88.

Nicoll, M. E. & P. A. Racey (1985) Follicular development, ovulation, fertilization and fetal development in tenrecs (*Tenrec ecaudatus*). *J Reprod Fertil,* **74,** 47-55.

O'Leary, M. A., J. I. Bloch, J. J. Flynn, T. J. Gaudin, A. Giallombardo, N. P. Giannini, S. L. Goldberg, B. P. Kraatz, Z.-X. Luo, J. Meng, X. Ni, M. J. Novacek, F. A. Perini, Z. S. Randall, G. W. Rougier, E. J. Sargis, M. T. Silcox, N. B. Simmons, M. Spaulding, P.

M. Velazco, M. Weksler, J. R. Wible & A. L. Cirranello (2013) The placental mammal ancestor and the post–K-Pg radiation of placentals. *Science,* **339,** 662-667.

Olson, L. E. & S. M. Goodman (2003) Phylogeny and biogeography of tenrecs. In: *The Natural History of Madagascar*: 1235-1242. S. M. Goodman & J. P. Benstead (Eds.). The University of Chicago Press, Chicago, USA.

Petter, J.-J. & A. Petter-Rousseaux (1963) Notes biologiques sur les Centetinae. *La Terre et la Vie* **17,** 66-80.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar & R Development Core Team (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-108.

Poppitt, S. D., J. R. Speakman & P. A. Racey (1994) Energetics of reproduction in the lesser hedgehog tenrec, *Echinops telfairi* (Martin). *Physiol Zool,* **67,** 976-994.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Racey, P. A. & P. J. Stephenson (1996) Reproductive and energetic differentiation of the Tenrecidae of Madagasacar. In: *Biogéographie de Madagascar*: 307-319. W. R. Lourenço (Ed.). Editions de l'Orstom, Paris.

Rakotoarivelo, A. A., N. Ranaivoson, O. R. Ramilijaona, A. F. Kofoky, P. A. Racey & R. K. B. Jenkins (2007) Seasonal food habits of five sympatric forest microchiropterans in Western Madagascar. *J Mammal,* **88,** 959-966.

Rakotondravony, D., V. Randrianjafy & S. M. Goodman (2002) Evaluation rapide de la diversité biologique des micromammifères de la réserve naturelle intégrale d’Ankarafantsika. *RAP Bulletin of Biological Biographical Assessment,* **23,** 83-87.

Randrianjafy, V. (2003) *Contribution à l'étude de la biologie de conservation de la communauté micromammalienne d'Ankarafantsika.* PhD, Université d'Antananarivo, Antananarivo.

Read, A. F. & P. H. Harvey (1989) Life history differences among the eutherian radiations. *J Zool (Lond.),* **219,** 329-353.

Robertson, D. S., M. C. McKenna, O. B. Toon, S. Hope & J. A. Lillegraven (2004) Survival in the first hours of the Cenozoic. *Geol Soc Am Bull,* **116,** 760-768.

Rojas, A. D., G. Koertner & F. Geiser (2010) Do implanted transmitters affect maximum running speed of two small marsupials? *J Mammal,* **91,** 1360-1364.

Soarimalala, V. (2011) Les afrosoricides de la fôret sèche malgache. *Afrotherian Conservation,* **8,** 4-9.

Soarimalala, V. & S. Goodman (2011) *Les petits mammifères de Madagascar*. Antananarivo, Madagascar: Association Vahatra.

Stearns, S. C. (1983) The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. *Oikos,* **41,** 173-187.

Stephenson, P. J. (1993) Reproductive biology of the large-eared tenrec, *Geogale aurita* (Insectivora: Tenrecidae). *Mammalia,* **57,** 553-564.

Stephenson, P. J. & P. A. Racey (1995) Resting metabolic rate and reproduction in the Insectivora. *Comp Biochem Physiol A,* **112,** 215-223.

Stephenson, P. J., P. A. Racey & F. Rakotondraparany (1994) Maintenance and reproduction of tenrecs Tenrecidae: at Parc Tsimbazaza, Madagascar. *Int Zoo Yearb,* **33,** 194-201.

Symonds, M. R. E. (1999) Life histories of the Insectivora: the role of phylogeny, metabolism and sex differences. *J Zool (Lond.),* **249,** 315-337.

Symonds, M. R. E. (2005) Phylogeny and life histories of the 'Insectivora': controversies and consequences. *Biol Rev,* **80,** 93-128.

Turbill, C., C. Bieber & T. Ruf (2011) Hibernation is associated with increased survival and the evolution of slow life histories among mammals. *Proc R Soc B,* **278,** 3355-3363.

Wright, P. C. (1999) Lemur traits and Madagascar ecology: Coping with an island environment. In: *Yearbook of Physical Anthropology 1999, Vol 42*: 31-72.

Linking Statement

In the first two chapters I established that *Setifer setosus*, at least in the dry deciduous forest in the west of Madagascar, possess large home ranges, produce a large number of young and dramatically increase body mass within a limited time frame. All of these activities require the gathering and assimilation of large amounts of energy, generally considered to be incompatible with low basal metabolic rates. As tenrecs possess some of the lowest basal metabolic rates of any eutherian mammals, how they achieve such a high energetic output is of great interest. In the next two chapters I focus on evaluating the costs of reproduction in this species with the goal of making inferences about the evolution of endothermy in mammals. In Chapter 4, I concentrate on the changes in the resting metabolic rate, of both reproductive and non-reproductive individuals, over a wide range of ambient temperatures. I examine how these costs could have resulted in the fast life history described above and comment on the link between reproduction and homeothermy in the evolution of endothermy in mammals.

Chapter 4 Increased homeothermy during reproduction in a basal placental mammal

Summary

Homeothermic endothermy, the maintenance of a high and stable body temperature (T_b) using heat produced by elevated metabolism, is energetically expensive. There is increasing evidence that the earliest endotherms were heterotherms that, rather than maintaining strict homeothermy, allowed T_b to fluctuate with large variations between active and rest-phase T_b. The high level of homeothermy observed in modern mammals is therefore likely to have evolved from an ancestral heterothermic state. One of the hypotheses for the evolution of endothermy is that homeothermy allows for greater energetic output during reproduction (Parental Care Model). I tested this hypothesis by measuring metabolic rates over a range of ambient temperatures in both reproductive and non-reproductive Greater hedgehog tenrecs (*Setifer setosus*), a physiologically primitive mammal from Madagascar. Tenrecs have some of the lowest metabolic rates and highest levels of T_b variability of any mammal and are therefore good models for the ancestral eutherian state. During pregnancy and lactation there was an increase in metabolism and T_b below the thermoneutral zone, accompanied by a decrease in T_b variability. The lower critical limit of the thermoneutral zone was estimated at ~25°C. However, whereas increases in resting metabolism were substantial below 20°C (up to 150% higher during reproduction), daytime rest-phase ambient temperatures at the study site rarely reached equivalent low levels. Thus, *S. setosus* provide an example for how relatively low-cost increases in homeothermy could have led to substantial increases in fitness by allowing for the faster production of young. The mechanisms necessary for increases in thermogenesis during reproduction would have further benefited the development of homeothermy in mammals.

Abbreviations

C_{wet} = wet thermal conductance ($mLO_2.°C^{-1}.h^{-1}$)
TNZ= thermoneutral zone
T_a = ambient temperature (respirometer temperature)
T_b = core body temperature
T_{sk} = skin temperature
T_{lc}= lower critical limit of the TNZ
ΔT = temperature differential (T_b-T_a)

TRMR= $\dot{V}O_2$ measured in the TNZ

$\dot{V}O_2$ = volumetric rate of oxygen consumed by the animal ($mLO_2.h^{-1}$)

Introduction

All extant mammals are endotherms, capable of maintaining core body temperature (T_b) above ambient temperatures (T_a) through the production of heat from metabolism (Bartholomew 1972, Schmidt-Nielsen 1997). The precision of T_b regulation varies considerably among modern mammals (Clarke & Pörtner 2010, Lovegrove 2012a), ranging from species which maintain a high degree of homeothermy (small circadian variations in T_b), to those which have highly labile T_bs (Refinetti & Menaker 1992, Angilletta *et al.* 2010, Clarke & Pörtner 2010, Lovegrove 2012a, Boyles *et al.* 2013). In addition to circadian rhythms, in which T_b typically decreases during the rest-phase (but see Lovegrove *et al.* 2014), many mammals also lower T_b and metabolic rate (MR) for extended periods of time during daily torpor and hibernation (Lyman *et al.* 1982, Geiser & Ruf 1995).

Strict homeothermy could have evolved as early as 200 mya coincident with increased encephalization and a shift to a nocturnal lifestyle, or as late as 66 mya before the crown placental groups diversified following the mass extinctions at the K-Pg boundary (Crompton, Taylor & Jagger 1978, Grigg, Beard & Augee 2004, Rowe, Macrini & Luo 2011, Lovegrove 2012b, O'Leary *et al.* 2013). Moreover, there is increasing evidence that endothermy evolved in a tropical environment from an ancestral state in which T_b was highly labile and T_a-dependent (Crompton *et al.* 1978, Grigg *et al.* 2004, Lovegrove 2012b, Lovegrove 2012a). Under this hypothesis the ancestral eutherian mammal was small, nocturnal, insectivorous, and likely to have expressed either short or long-term periods of torpor (Luo 2007, Lovegrove 2012a, O'Leary *et al.* 2013). It is this plesiomorphic heterothermic capacity which is thought to have been the most likely means by which the ancestral eutherian was able to have survived the short- and long-term devastation of the K-Pg boundary asteroid impact (Lovegrove 2012b).

A number of hypotheses have been proposed in an attempt to explain how and why endothermy, a costly method of thermoregulation and existence in general, evolved in mammals (Crompton *et al.* 1978, Bennett & Ruben 1979, Farmer 2000, Koteja 2000). In this study I argue that the study of modern mammals which putatively retained plesiomorphic heterothermic characteristics, that is, physiological characteristics that are thought to have prevailed in Cretaceous eutherian ancestors, should shed light on the transition from ectothermic-like heterothermy to homeothermy (Eisentraut 1960, Crompton *et al.* 1978, Grigg *et al.* 2004, Lovegrove 2012a). Termed ‘protoendotherms’ by Grigg, Beard and Augee (2004), and ‘basoendotherms’ by Lovegrove (2012a), extant basal eutherians, often found on low-latitude islands with little paleoclimatic history of Cenozoic cooling, are highly heterothermic, with variable T_bs and frequent use of torpor. The large amplitudes in the circadian rhythm of T_b in these animals can lead to difficulties in differentiating between torpor and normothermy using T_b alone (Poppitt, Speakman & Racey 1994, Brice *et al.* 2002, Kuchel 2003, Lovegrove & Génin 2008, Canale, Levesque & Lovegrove 2012). Also, the determination of a distinct thermoneutral zone (TNZ), a range of T_a over which MR remains minimal and constant, is highly problematic if T_b is not maintained at a constant level (Scholander *et al.* 1950, Brice 2008). The high thermolability of basoendotherms generates a relatively linear relationship between T_b and T_a, with no clear inflection points in MR at the lower and upper critical limits of thermoneutrality which typically define the TNZ in classic homeothermic endotherms (Stephenson & Racey 1993b, Nicoll pers. comms. in Stephenson & Racey 1994, Brice 2008).

Malagasy tenrecs, members of the Afrotherian order Afrosoricida, are perhaps one of the best examples of eutherian basoendotherms (Eisentraut 1960, Crompton *et al.* 1978, Lovegrove & Génin 2008). Tenrecs display some of lowest T_bs of any extant mammal and the spiny tenrecs, members of the sub-family Tenrecinae, have some of the lowest basal metabolic rates (BMR, Symonds 1999, Lovegrove 2000). They have also retained the ancestral diet (insectivory), nocturnal activity patterns, and have inhabited the relatively warm climate of Madagascar throughout their evolutionary history (Olson & Goodman 2003). Studies on free-ranging and captive Tenrecinae have indicated that these animals are highly heterothermic, with large rhythms in circadian T_b, as well as frequent (daily in the case of *Echinops telfari*) torpor bouts (Nicoll 1986, Stephenson & Racey 1994, Lovegrove & Génin 2008, Oelkrug *et al.* 2013). However, periods of homeothermy, indicated by an increase in the level and precision of T_b as well as a decrease in torpor use, have been observed in a number of tenrec species during both gestation and lactation (Thompson & Nicoll 1986, Stephenson & Racey 1993a, Stephenson & Racey 1993b, Poppitt *et al.* 1994). These observations provide strong support for the hypotheses that endothermy evolved in mammals to benefit parental care (Farmer 2000, Koteja 2000, Farmer 2003). However, to date, these studies, all on captive animals, have focused on changes in BMR or, to be more precise, the thermoneutral resting metabolic rate (TRMR) as defined by Lovegrove *et al.* (1991) to denote that resting measurements of metabolism occurred at thermoneutrality despite failing to meet all of the requirements for basal metabolism (Stephenson & Racey

1995, Symonds 1999). No study has yet to characterise changes in thermoregulatory profiles during reproduction in a basoendotherm.

Brice, Levesque and Grigg (in prep, see also Brice 2008) predicted that the higher level of homeothermy observed during reproduction in basoendotherms would result in thermal profiles that conformed more closely to classic mammalian patterns. This study provides the first test of this prediction. I sought to fully characterize the thermoregulatory patterns during reproduction in a basoendotherm by measuring RMR over a range of T_as in a free-ranging population of Greater hedgehog tenrecs (*Setifer setosus*, Schreber, 1778) in the dry deciduous forest of western Madagascar. Although *S. setosus* is known to use torpor less than its sister species *E. telfairi* (Eisenberg & Muckenhirn 1968, Eisenberg & Gould 1969), they have been shown to enter into torpor over a wide range of T_as and to express a high degree of thermolability when not torpid (Chapter 2, Lovegrove *et al.* 2014). In addition, *S. setosus* is both larger and more abundant than *E. telfairi* and, unlike the larger common tenrec (*Tenrec ecaudatus*), is not consumed by humans at the study site, making it an ideal candidate for a long-term study on the energetics in a free-ranging population (Randrianjafy 2003).

Results

Reproductive status and sample size

From October 2010 to April 2011 metabolic measurements were obtained from 22 individuals (10 females, 12 males) for a total of 92 measures. Fewer animals (4 females, and 5 males; 43 measures in total) were captured during the second season (October 2011 - February 2012). Individuals were also caught for the first time later in the year and therefore no recordings were obtained from non-reproductive females during the second season. High rates of natural mortality (Chapter 3) precluded the collection of data after January of 2012.

Model selection using Akaike weights (AICcWt, Burnham & Anderson 2002) was performed to assess the influence of time since emergence from hibernation (‘day’: calculated as number of days since 1 September, see Chapter 3), ‘reproductive status’ (male and gestating, lactating and non-reproductive females) and ‘season’ on body mass (Table 4-1). Only the values from the first day of measurements per individual were used and, as these included multiple measures per individual, a random factor (in the form ~ 1|‘animalID’, see Zuur *et al.* 2009) was included in all models. Mass was ln-transformed to ensure a normal distribution of the model residuals and heteroscedasticity within the factor ‘day’ was controlled by using varFixed (~ ‘day’) as the variance structure. The model with the highest

AICcWt (0.61) included 'day' only with a parameter estimate of 0.95 ± 0.16 g per day (Table 2). The inclusion of 'reproductive status' in the model resulted in an additional AICcWt of 0.31. The remaining AICcWt was contributed by adding 'season' to the model. A Tukey post-hoc test on the model containing 'day' and 'status', using the *glht* function in package *multcomp* (Hothorn, Bretz & Westfall 2008), provided parameter estimate sizes for differences between 'status'. There was little difference between gestating and lactating females as well as between males and non-reproductive females (parameter estimate of less than 5 g) whereas both of the latter groups were smaller than the former (differences > 25 g).

Oxygen consumption, body temperature and thermal conductance

Although torpor and normothermy are difficult to distinguish in species with a high degree of thermolability (Stephenson & Racey 1993b, Canale *et al.* 2012), it was necessary to consider 'torpid' animals separately from those that thermoregulated. Individuals were therefore classed as torpid if the ΔT ($T_b - T_a$) was less than 5°C, and thermoregulating if it was greater (Hosken & Withers 1999). Data from thermoregulating animals only were included in the analyses. Data from three data measurement sessions, two on males at 22°C and one on a male at 32°C, were excluded from the analysis because the animals remained active throughout the duration of the recording. In the first season, non-reproductive individuals entered torpor at all T_as except for a male that maintained a T_b of 29.8°C at $T_a = 22.1$°C and a female with a T_b of 30.1°C at a T_a of 25°C. In contrast, in 2011-2012 only two of the five males entered torpor during data measurements; one at all temperatures and the other at the coldest temperature (22°C) only. There was no correlation, however, between body condition (BCI, Chapter 3) and torpor expression ($F_{1,45} = 0.52$, $p = 0.48$).

Resting metabolic rate (RMR), measured as oxygen consumption ($\dot{V}O_2$), and T_b were highly variable (Table 4-1; Fig. 4-1). Piecewise linear regression indicated an inflection point in the slope of $\dot{V}O_2$ versus T_a at approximately 24.6 - 24.8°C in the model including all individuals (N = 31, n = 133), at 24.8 - 24.9°C for reproductive females only (N = 8, n = 41), and at 24.9°C for non-reproductive individuals (N = 27, n = 85). The lower critical limit (T_{lc}) of the thermoneutral zone (TNZ) was therefore estimated to be around 25°C. Initial results indicated that models containing an inflection point at 25°C had lower AICc scores, and therefore it was justified to analyse the data above (within the TNZ: $T_a \geq 25$°C) and below the T_{lc} ($T_a < 25$°C) separately.

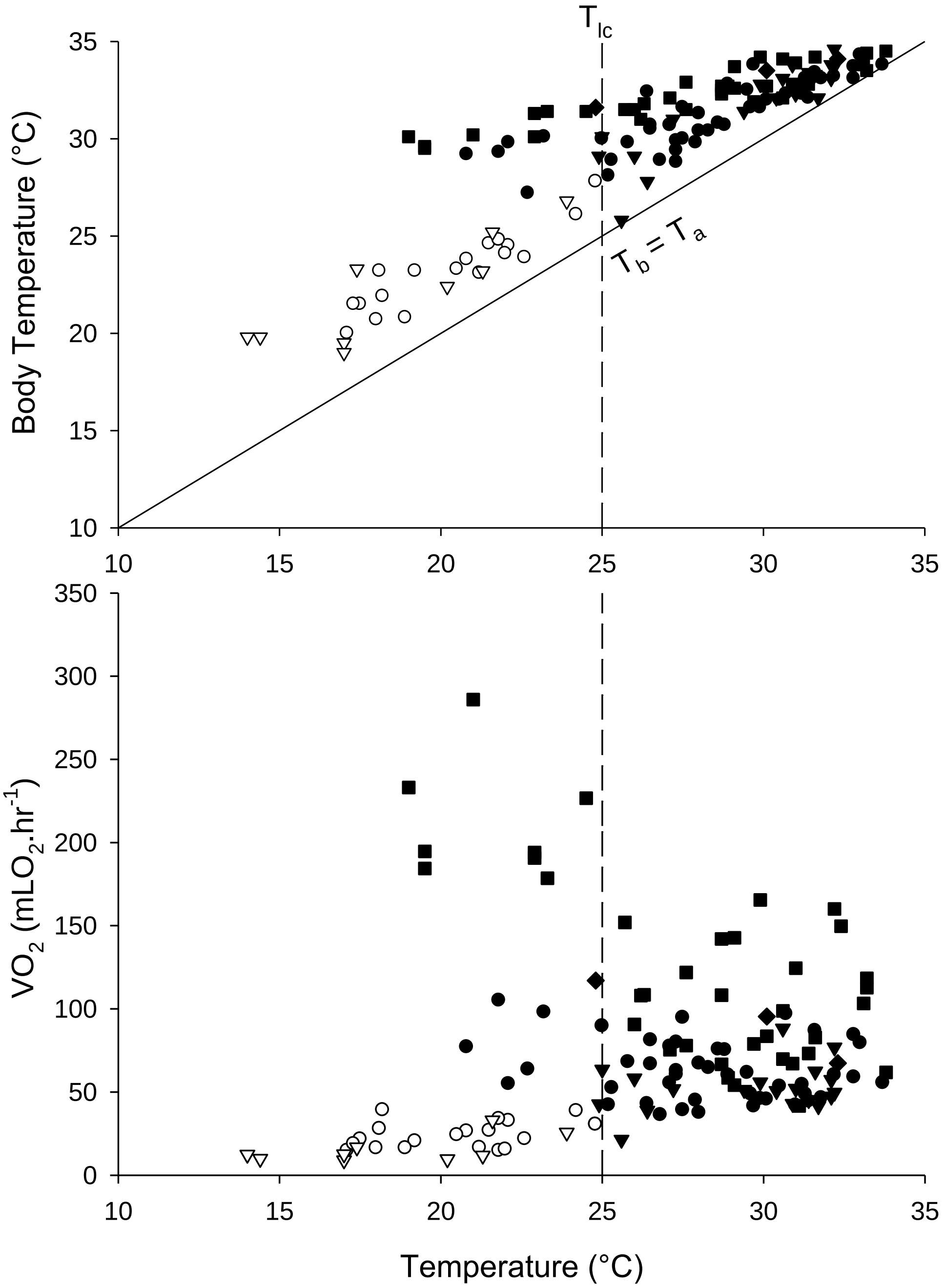


Figure 4-1 Body temperature and resting metabolic rates of *S. setosus* over two reproductive seasons in Ankarafantsika National Park. Reproductive females (gestating = squares and lactating = diamonds), males (circles) and non-reproductive females (triangles) which thermoregulated are plotted in black, torpid (T_b-T_a [ΔT] < 5°C) individuals in white. The straight line indicates $T_b = T_a$ and the dashed line the approximate lower critical limit (T_{lc}) of the thermoneutral zone. Significant differences were found between torpid and normothermic animals for both $\dot{V}O_2$ and T_b. Despite defending similar T_bs there was also a significant difference in $\dot{V}O_2$ between reproductive females and normothermic males.

Table 4-1 Mean body mass, resting metabolic rates at thermoneutrality (TRMR, T_a = 25 - 33.5°C) and minimum thermal conductance (C_{wet}, T_a < 30.5 °C) of reproductive, and non-reproductive *Setifer setosus*.

	Male	**Female**		
		Non-reproductive	**Gestating**	**Lactating**
Mass (g)	172.7 ± 46.2 (21)	147.1 ± 17.7 (9)	272.3 ± 7.3 (11)	222.1 ± 74.8 (2)
TRMR (mLO2.hr^{-1})	60.7 ± 17 (41)	53 ± 14.6 (19)	99.9 ± 34.4 (29)	81.4 ± 19.8 (3)
TRMR (mLO2.g^{-1}.hr^{-1})	0.36 ± 0.11 (41)	0.36 ± 0.11 (19)	0.40 ± 0.07 (29)	0.30 ± 0.08 (3)
Min C_{wet} ($mLO_2.hr^{-1}.°C^{-1}.g^{-1}$)	0.10 ± 0.05 (14)	0.12 ± 0.11 (7)	0.10 ± 0.02 (7)	0.08 (1)
	N = 17	N = 8	N = 7	N = 2

The number in parentheses indicates the total number of measures obtained.

Preliminary analysis of $\dot{V}O_2$ in the TNZ (TRMR, $T_a > 24.5°C$) indicated a single outlier, a reproductive female 33 days from parturition, which expended twice as much energy per gram while defending a similar T_b, as any other individual at 25°C. This datum was excluded from all subsequent analyses. Mean values for TRMR appeared to differ between reproductive and non-reproductive individuals (Table 4-1; Figs 4-1 and 4-2) at the whole-animal level. However, the best model predicting TRMR (AICcWt = 0.96) contained 'mass' (parameter estimate = 0.37 ± 0.04 $mLO_2.hr^{-1}$) only as a fixed factor, and 'day' and 'animal ID', as well as their interaction (in the form ~'day|animal ID'), as random factors (Table 4-2). A model containing 'reproductive status' only, as well as one with 'reproductive status' and 'T_a', explained the remaining AICcWt (0.02, and 0.01 respectively).

The highest ranking model describing the $\dot{V}O_2$ of non-torpid animals (5 males, 9 gestating females) below the T_{lc} contained 'T_a', 'reproductive status' and 'mass' as fixed factors, and 'animal ID' as the sole random factor (Table 4-2). Of the fixed effects 'reproductive status' had the largest impact on $\dot{V}O_2$ with gestation resulting in an increase of 186.53 ± 35.4 $mLO_2.hr^{-1}$ higher than that of males. The RMR of reproductive females also increased as T_a decreased below T_{lc} at a rate of 14 $mLO_2.hr^{-1}°C^{-1}$ (Fig. 4-1). The relationship between T_a and RMR in thermoregulating males was not significant ($t_3 = -0.39$, $p = 0.72$), although sample size was very small.

Inflection points in the regression of T_b against T_a were located at similar temperatures to those for $\dot{V}O_2$ (~ 25°C), but their inclusion in the model produced no improvement in AICc scores; one set of models only, containing all data from non-torpid individuals, was used in the T_b analysis. Fixed factors showing the most influence on T_b were 'reproductive status', 'T_a' and 'day' (Table 4-2; Fig. 4-1), although the effect of the latter was slight (< $0.01°C.day^{-1}$). 'Animal ID' was also included as a random effect. Contrary to the patterns seen in $\dot{V}O_2$, T_b was dependent on T_a : T_b increased by 0.4 ± 0.02°C per °C change in T_a. Males and non-reproductive females had lower T_bs than reproductive females (by 13.2 ± 2.9 and 4.8 ± 1.5°C, respectively), although this difference decreased with increasing T_a. The second best fitting model replaced 'day' with 'mass' although the effect of this was once again small (< $0.001°C.g^{-1}$).

An initial inflection point in measures of wet thermal conductance (C_{wet}; Fig. 4-3) was found at 32.5 - 32.8°C, after which C_{wet} increased dramatically. All analyses of C_{wet}, ln-transformed to conform to model assumptions, included data below this inflection point only.

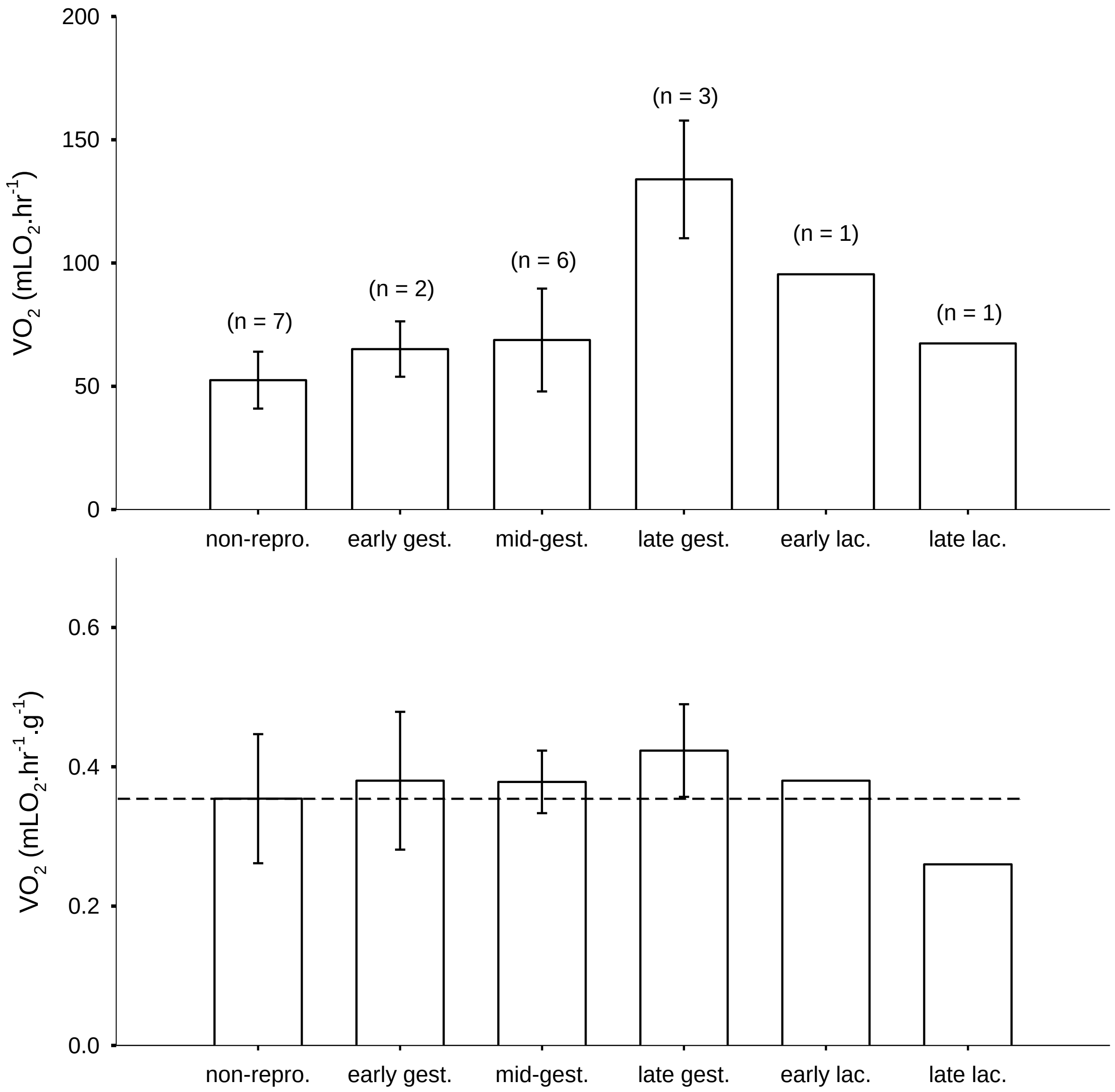


Figure 4-2 Resting metabolic rate in the thermoneutral zone (T_a > 25°C) for female *Setifer setosus* according to reproductive status. Categories describe the number of days prior to parturition (early = 60 – 35 days, mid = 35 – 10 days, late = 10 – 0 days), the dashed line indicates the mean value from the non-reproductive females. The female measured during late lactation was also in mid-gestation. Repeated measures were available for some, but not all individuals. There were no differences in $\dot{V}O_2$ between groups when mass was used as a covariate.

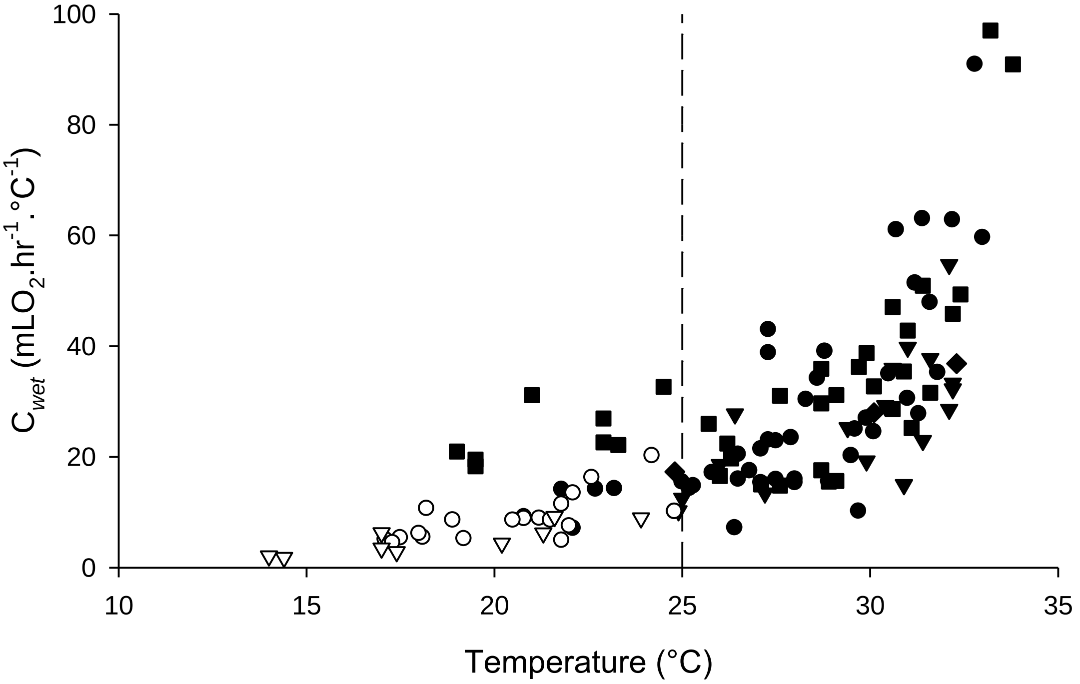


Figure 4-3 Wet thermal conductance of *Setifer setosus* over a range of ambient temperatures. Reproductive females (gestating = squares and lactating = diamonds), males (circles) and non-reproductive females (triangles) which thermoregulated are plotted in black, torpid (T_b-T_a [ΔT] < 5°C) individuals in white. The straight line indicates the lower critical limit of the thermoneutral zone.

Table 4-2 Best fitting linear mixed models evaluating the effect of various factors on the physiological parameters of reproductive and non-reproductive *Setifer setosus*. Ranking was performed using corrected Akaike Information Criterion (AICc) scores and Akaike weights (AICcWt). All models with an AICcWt > 0 are presented and the number of parameters contained in the model are included (*k*). ‘Status’ refers to the reproductive status of the individual (Male and gestating, lactating and non-reproductive female). ‘Day’ is the number of days since September 1, a proxy for the start of the active season.

Fixed Factors	Random Factors	Variance Structure	*k*	AICc	AICcWt
Body mass					
‘day’	~ 1|‘animalID’	varFixed (~ ‘day’)	4	431.12	0.57
‘day’ +’status’	~ 1|‘animalID’	varFixed (~ ‘day’)	7	432.10	0.35
‘day’ + ‘status’ + ‘season’	~ 1|‘animalID’	varFixed (~ ‘day’)	8	435.07	0.08
$\dot{V}O_2$ in TNZ (Ta ≥ 25°C)					
‘mass’	~ 'day'|‘animalID’	varFixed (~ ‘day’)	6	745.47	0.96
‘status’+’mass’	~ 'day'|‘animalID’	varFixed (~ ‘day’)	9	752.84	0.02
‘T_a’+’status’+’mass’	~ 'day'|‘animalID’	varFixed (~ ‘day’)	10	754.43	0.01
$\dot{V}O_2$ below TNZ (Ta < 25°C)					
‘T_a’+’status’+’mass’	n/a	varFixed (~ ‘day’)	5	139.75	0.96
‘T_a’+’status’+’mass’+’day’	n/a	varFixed (~ ‘day’)	6	147.17	0.02
‘mass’	n/a	varFixed (~ ‘day’)	3	148.52	0.01
Body temperature					
‘T_a’ x ‘status’ +’day’	~ 1|‘animalID’	varIdent (~ 1|‘status’)	11	263.97	0.51
‘T_a’ x ‘status’ + ‘mass’+’day’	~ 1|‘animalID’	varIdent (~ 1|‘status’)	12	265.11	0.29
‘T_a’ x ‘status’ +’mass’	~ 1|‘animalID’	varIdent (~ 1|‘status’)	11	266.11	0.18
‘T_a’ x ‘status’	~ 1|‘animalID’	varIdent (~ 1|‘status’)	10	270.99	0.02
Wet thermal conductance (Ta < 32.5 °C)					
‘T_a’ x ‘status’ +’mass’	~ 1|‘animalID’	varFixed (~ ‘T_a’)	9	66.97	0.72
‘T_a’ x ‘status’ +‘mass’ +’day’	~ 1|‘animalID’	varFixed (~ ‘T_a’)	10	68.89	0.28

An additional inflection point in the data at $T_a < 32.5$°C was located at $T_a = 30.5$°C under which point C_{wet} was at a minimum (Table 4-1). Values for C_{wet} were at a minimum at the lowest temperatures, increased slightly with increases in T_a below the TNZ, and dramatically above 32.5°C (Fig. 4-3). Similar to the T_b dataset, 'reproductive status', 'T_a' and their interaction as well as 'mass' were the fixed factors in the best model describing C_{wet} below 32.5°C, along with 'animal ID' as a random factor (Table 4-2). Gestating females had the highest C_{wet}: 7.3 ± 1.5 $mLO_2.hr^{-1}.°C^{-1}$ greater than males and 12.5 ± 1.5 $mLO_2.hr^{-1}.°C^{-1}$ greater than non-reproductive females. Although, similar to T_b, the difference was less at higher T_a.

Discussion

Previous studies on the thermoregulation of basoendotherms found thermal profiles that significantly deviated from the Scholander-Irving model (Stephenson & Racey 1993b, Nicoll in Stephenson & Racey 1994, Brice 2008). The results from this study, however, are mixed. Although TRMR was constant over a wide range of T_as in both reproductive and non-reproductive individuals, indicative of a TNZ between ~25°C and 32.5°C, T_b was highly correlated with T_a at all temperatures. This observation deviates from the classical Scholander-Irving model which assumes that normothermic T_b is maintained within a narrow range. Similarly, high levels of variability in T_b seen in both reproductive females and the small number of thermoregulating males, led to unusual relationships between $\dot{V}O_2$ and T_a below the TNZ. The classical model predicts that the slope of $\dot{V}O_2$ below the TNZ should intersect with the y-axis ($\dot{V}O_2$) at $T_a = T_b$ (Scholander *et al.* 1950). This was not the case in either group of non-torpid animals. For the thermoregulating non-reproductive individuals, a lack of correlation between T_a and $\dot{V}O_2$ below the T_{lc} meant that the $\dot{V}O_2$ would never intersect with T_a within biologically viable T_bs. Similarly, reproductive females would have to have a T_b of > 36°C for the classical model to be an accurate representation of their thermoregulation.

The pattern of C_{wet} also indicates some level of deviation from the classic mammalian model. In most homeothermic mammals C_{wet} reaches a minimum at the T_{lc}, and increases with increasing T_a within and above the TNZ (Scholander *et al.* 1950, Brice 2008). In this study C_{wet} never reached a minimum and started to increase only well into the TNZ, above around 32.5 - 32.8°C. Such a delayed deployment of heat loss mechanisms would indicate that T_b is flexible until a certain point, after which it is defended against potential hyperthermia, and efforts are made by the animal to offload stored heat. The T_b

measurements from *S. setosus* presented in Lovegrove *et al.* (2014) would indicate that high T_bs can be reached during torpor, although this is not always the case. The high levels of C_{wet} observed in a few individuals (Fig. 4-3) indicates a capacity to withstand high temperatures, however, the tolerance of high temperatures by tenrecs is not currently known. Furthermore, evaporative water loss was not measured in this study. It would be of interest for future studies to measure this species' capacity for evaporative cooling, as well as its reliance upon these mechanisms for thermoregulation. Water loss considerations may be especially important in tropical species whose entire period of activity coincides with periods of high T_a and simultaneous high levels of ambient humidity (Chapter 3, Krockenberger, Edwards & Kanowski 2012, Lovegrove *et al.* 2014).

Previous studies on the thermal profiles of basoendotherms found the delineation between torpid and non-torpid animals problematic (Stephenson & Racey 1994, Brice 2008). Although the distinction between the two states were clear at $T_a < T_{lc}$, three males, removed from the full data analysis, defended a lower body temperature (~ 26°C). These males were in a steady state since this T_b was maintained for the length of the measurement period. The maintenance of such intermediate rest-phase T_bs appears to be characteristic of tenrecs. Similar observations have been made on a shrew tenrec, *Microgale dobsoni* (Stephenson & Racey 1993b) and on *S. setosus* McNab (1980a), observed defending T_bs ranging between 23 - 29°C. However, the large and very likely obese (530 g) animals used in the latter study render those results unreliable. Nevertheless, as shown in Chapter 3, there were no differences in body condition index (BCI) between seasons, or between torpid versus thermoregulating males in this current study. It is therefore unlikely that differences in body mass alone can account for the different thermoregulatory states of non-reproductive individuals.

In accordance with previous studies on *T. ecaudatus* (Eisentraut 1960), lethargy and unresponsiveness, usually requirements for the diagnosis of torpor (IUPS Thermal Commission 2003), were not a characteristic of torpor in tenrecs. Similarly, a number of species of tenrec have been found to be active at T_bs as low as 25°C (Eisenberg & Gould 1969, Crompton *et al.* 1978, Poppitt *et al.* 1994). Activity at low T_b has similarly been observed in monotremes (Kuchel 2003), marsupials (Rojas, Körtner & Geiser 2012, Turner *et al.* 2012), placental mammals (Wooden & Walsberg 2004) and birds (Merola-Zwartjes & Ligon 2000). As a consequence, differentiating torpor from normothermy in these species using T_b alone is complicated (Brice *et al.* 2002, Canale *et al.* 2012). Without a discernible pattern in the thermal profiles of the reproductive females, I would be reluctant to accept the inflection point found at 24.9°C in the non-reproductive individuals as a valid T_{lc} as prescribed by the Scholander-Irving model. The consistent relationship observed between T_b and T_a, and a lack of corresponding data on activity or responsiveness, make any distinction between torpid and thermoregulating individuals in this context arbitrary. In addition, the Scholander-Irving model was formulated to describe a mammal defending a narrow T_b setpoint, which is evidently not the case for non-reproductive *S. setosus*. As has been reported in previous studies on tenrecs (Poppitt *et al.* 1994, Lovegrove & Génin 2008), there is no threshold normothermic T_b under which all animals can be considered to be torpid. However, a coarse definition of torpor using the difference between T_b and T_a (ΔT, Arlettaz *et al.* 2000, Canale *et al.* 2012) was supported somewhat by the $\dot{V}O_2$ data. As an approximation, an animal with a ΔT of less than 5°C could be considered torpid, but only reliably at T_as less than 25°C, and only if other methods for diagnosis (such as activity, metabolic rate or heart rate) are unavailable.

Previous studies on captive tenrecs (Nicoll & Thompson 1987, Poppitt *et al.* 1994, Racey & Stephenson 1996) have shown increases in TRMR during reproduction. This was not the case in the current study. One explanation for the observed differences is that none of the recordings in this study can be considered to be truly basal because the population was both reproductively active and in the process of accumulating fat stores for hibernation (Chapter 3). During early pregnancy, as well as during lactation, the captive tenrecs of previous studies did not show significant gains in mass (Stephenson & Racey 1993a, Poppitt *et al.* 1994, Stephenson & Racey 1994), whereas individuals at all stages of reproduction in this study showed steady increases in body mass (Chapter 3). Potential increases in $\dot{V}O_2$ during reproduction were possibly masked by simultaneous increases in mass. Although there is a possibility that handling stress would cause wild-caught individuals to have higher RMRs than captive, the high incidence of torpor observed in this study would suggest that the animals were comfortable with the experimental set-up. Furthermore, the average TRMR (0.36 ± 0.09 $mLO_2.hr^{-1}.g^{-1}$ for non-reproductive individuals) falls within the reported values for this species (0.34-0.46 $mLO2.hr^{-1}.g^{-1}$, Kayser 1960, Eisenberg & Gould 1969, Stephenson & Racey 1995).

Interestingly, one of the lowest TRMR values measured in a reproductive female was from an individual who was both in late lactation and mid-gestation (Fig 4-2). This low value is similar to those found during a study on reproduction in a species of shrew tenrec, *Geogale aurita* (Stephenson & Racey 1993a), where simultaneous gestation and lactation did not result in combined increases in TRMR. However, sample sizes of the different reproductive stages in the current study were too small to determine the fine-grain relationship between reproduction and TRMR. The earlier studies on captive individuals also indicated that tenrecs can enter torpor during pregnancy (Nicoll & Thompson 1987, Stephenson & Racey 1993a). This was not observed during either the constant environment trials (this study) or in free-ranging animals (Chapter 5), with the exception of a single female 46 - 49 days prior to parturition. This individual was in very poor body condition, having recently emerged from hibernation, and entered torpor at the lowest temperatures (14 - 21 °C). However, despite poor body condition early on in the season, a single young was raised successfully (SF14, Chapter 3) and a body condition comparable to that of the population was attained within a few weeks. Laboratory-based studies on the shrew *Crocidura russula monacha* (Mover, Hellwing & Ar 1988, Mover, Ar & Hellwing 1989) also found that TRMR during gestation and lactation, as well as during simultaneous gestation and lactation, showed little change. There was, however, a significant increase in food intake, and therefore in daily energy expenditure. This was also the case in reproductive *E. telfairi*, where increases in TRMR during gestation and lactation were less than in those in which daily energy expenditure was measured over 24 hrs (Poppitt *et al.* 1994). It is therefore possible that the costs of maintaining a higher degree of homeothermy during reproduction in *S. setosus* were underestimated by the methods used in this study.

Conclusions; reproduction and the evolution of homeothermy via heterothermy

This study provides further evidence that homeothermy is sustained in otherwise heterothermic mammals during reproduction. Interestingly, unlike in laboratory populations fed *ad libitum*, the only increases in TRMR observed during reproduction in *S. setosus* were due to increases in mass (therefore in total, whole-animal metabolic rate), and not due the reproductive condition *per se*. Increases in homeothermy are therefore likely to have higher effects on foetal development than increases in TRMR. Presumably, the reliance upon homeothermy can at least partly be attributed to a relationship between T_b and foetal growth rate (Farmer 2000). As an illustration, a captive population of *S. setosus* held at higher-than-average ambient temperatures (> 25°C) had shorter gestation lengths than those housed in colder conditions (Mallinson 1974). This shortened gestation period matched those observed in the current study population (Chapter 3), where environmental temperatures were similarly warm. The mean minimum daily T_a during the active season (October-May) since 1997 was 21.9 ± 2.3°C (n = 3572) and daytime (rest-phase) T_a decreased below 20°C only on 0.01% of study days during the second season. The costs of maintaining homeothermy in this environment are therefore relatively low which is, presumably, how females in this population can give birth to up to three litters per year while also accumulating fat stores for hibernation.

Assuming that the climate of the tropical forests of Madagascar have changed very little since the time when the placental mammal radiations occurred (~ 65 mya, Jury 2003, Lovegrove 2012a, O'Leary *et al.* 2013), *S. setosus* provide a useful model to test a crucial trade-off associated with the evolution of endothermy in general. The trade-off balances the fitness benefits of small increments in homeothermy during reproduction with the relatively low fitness costs involved in minimal thermoregulatory energy demands. Such a trade-off can occur in tropical environments only, which is the climate which prevailed broadly across the globe from the Jurassic ~ 200 mya at the putative establishment of endothermy in small, nocturnal mammaliaformes (Rowe *et al.* 2011), until at least the Eocene Thermal Maximum ~50 mya (Zachos 2001). Continental global cooling occurred thereafter in the late Cenozoic, but the climates of the current tropics remained much the same as they were in the Late Eocene. Thus in both ancestral and contemporary small, tropical mammals, low-cost increments in homeothermy provide(d) a stable environment for foetal development which, by increasing the rate of development, would have increased fitness (Farmer 2003) and allowed for greater energy reserves to be used in provisioning the young, either via milk production, or fat storage (Koteja 2004). Thus apart from the support for the Parental Care hypothesis, my data also support the predictions of the Plesiomorphic-Apomorphic Endothermy hypothesis, which argues that basoendotherms such as *S. setosus*, display plesiomorphic endothermic traits that implicate stabilizing selection (Lovegrove 2012a).

The presence of fully functional uncoupling proteins (especially UCP1), in the brown adipose tissue of tenrecs (Oelkrug *et al.* 2013), indicates that the physiological capacity to maintain homeothermy has existed in the eutherian mammal lineage for at least 66 million years. UCP1, found in mammalian mitochondrial membranes, facilitates non-shivering thermogenesis and constitutes an important part of heat produced by non-shivering thermogenesis necessary for rewarming from hibernation in eutherian mammals (Carey, Andrews & Martin 2003, Cannon & Nedergaard 2004, Jastroch *et al.* 2005). When housed at cold T_as, tenrecs periodically maintain high T_b similar to periods of normothermy observed in all mammalian hibernators (Willis 1982, Oelkrug *et al.* 2013). They therefore have the capacity to maintain high and relatively stable T_bs when necessary. However, the fact that homeothermy is observed solely during reproduction indicates that, at least in warm climates, it is not necessary for a day-to-day existence, especially in tropical Madagascar. Increased homeothermy during reproduction has also been observed in a monotreme (Beard & Grigg 2000, Nicol & Andersen 2006, Morrow & Nicol 2009) as well as in a number of eutherian and marsupial mammals (Morrison 1945, Audet & Fenton 1988, Geiser, Körtner & Schmidt 1998) indicating that it is likely to have been one of the first steps in the evolution of homeothermy in ancestral heterothermic mammals (Farmer 2000).

Methods and Techniques

Study site, capture and surgical methods

The study was conducted over two rainy seasons, from September 2010 to April 2011 and from September 2011 to February 2012, in Ankarafantsika National Park (16º19'S, 46º48'E), Madagascar. A detailed description of the study site and general methods is provided in Chapters 2 and 3. Briefly, all animals were caught by hand, in the Jardin Botanique A research area adjacent to the Ampijoroa Forestry Station, by walking the established trails in the area at night with local guides. Upon capture individuals were transported to the research camp where they were housed in plastic containers lined with paper towel and provided with live insects and tinned sardines. At initial capture all animals were anesthetized using isoflurane in oxygen (induction; 1 – 2%, maintenance; 0.5%) and morphometric measurements were taken. Each animal was marked with a small distinctive clip in the ear and injected with a transponder (Small Animal Marking System, Trovan Ltd., UK) to allow for identification at recapture. They were kept for a maximum of five days for the collection of metabolic data after which selected animals had a combination of radio-transmitter and body temperature data logger (DS1922L Thermochron iButtons, Dallas Semiconductor, Dallas, TX, USA) implanted into the peritoneal cavity as described in Chapter 2. Females with radio-transmitters were captured once a week to determine reproductive status, and males once every two or three weeks to assess body condition. In addition, the rest sites of the females were located every day as repeated use of a single nest site would indicate parturition (Chapter 2), and males every couple of days. Pregnant females (within 10 days to parturition) were captured in the field and brought back to the laboratory for a repeat of the metabolic measurements. If both the lactating female and her pups were accessible they were caught on the morning of the experiment and released back into the nest by sunset of the same day. Ambient temperatures for the study site were obtained from the Durrell Wildlife Conservation Trust.

Respirometry data

Flow-through respirometry was used to obtain values for $\dot{V}O_2$ over a range of ambient temperatures (15 - 34°C). To avoid injury through pathological hypothermia, measurements at T_as < 10°C were not attempted (Lachiver pers. comms. in Kayser 1961). Measurements were conducted between 04:30 and 18:00, to coincide with the natural rest phase, and were made over a period of 4-6 hours. The animal was placed in a 700 mL respirometer consisting of an air-tight plastic container (Komax Industry Co, Seoul, Korea) with three small air holes at the base and an outlet near the top. The respirometer was placed inside a modified refrigerator in which the temperature was controlled using heat lamps activated via a programmable temperature controller (TC410, Rhomberg Instruments, Johannesburg, RSA). Ambient air, partially dried using silica gel, was pulled through the chamber at a rate of 500 mL.min^{-1}. The air was pulled through the chamber, into a mass flow meter and pump (MFS Mass Flow System, Sable Systems, Las Vegas, NV), dried using Drierite™, and pushed into a manifold. A subsample of the air from the respirometer was pulled at 150 mL.min^{-1} through scrubbers containing soda lime to remove CO_2 and Drierite™ to remove water released by the soda lime, and a mass flow meter before entering the pump and being pushed through an O_2 analyser (FoxBox-C Field Gas Analysis System, Sable Systems, Las Vegas, NV). Channels were configured in Sable System's data acquisition software, Expedata (v 1.1.15), to record the fractional concentrations of O_2, the flow rate, and the barometric pressure every two seconds. The precise temperatures experienced by the animals were monitored using pre-calibrated iButtons taped to the insides of the respirometer and programed to record T_a once every minute with a resolution of 0.0625°C. To control for O_2 analyser drift, a baseline measurement from an empty reference respirometer, was used at regular intervals (5 min every 20 - 40 min) throughout the experiment. Before each measurement the animal was weighed and T_b was measured by inserting a calibrated Cu-Cn thermocouple 2 cm into the cloaca. At the end of the $\dot{V}O_2$ measurement period the position of the animal within the chamber was recorded and the T_b measurement was repeated. During the second season skin temperature (T_{sk}) was measured by securing an iButton to the stomach of the animal using surgical tape. Core T_b data, obtained from implanted iButtons, was only available for a small number of individuals (N = 4, n = 16). A mixed model, using measurement type as a fixed factor and 'animal ID' as a random effect, indicated that T_{sk} was significantly different from both core T_b ($t_{30} = 3.02$, $p = 0.005$) and T_b at the end of the experiment ($t_{30} = 2.30$, $p = 0.028$), whereas the two methods of T_b did not differ from each other ($t_{30} = 0.72$, $p = 0.47$). T_b at the end of data measurement was therefore used in all subsequent analyses.

Most experiments were conducted during the initial capture period of each year (October-November) and for each individual usually consisted of a single measurement at a low temperature (18 - 25°C) and one in the TNZ estimated at around 30 - 33.5 °C based on values used by Nicoll and Thompson (1987) for *E. telfairi*. If possible, individuals with radio-transmitters were recaptured later in the season (December - April) for additional measurements. Upon recapture the animals were kept in captivity for a total of two days, allowing for measurements to take place at 4 - 5 different T_as. Lactating females were measured only if it was possible to capture both the mother and the pups. Thus measurements were obtained from two females only, both in the first season; one with three pups 13 - 14 days old (two weeks from weaning) the other with a single pup aged 30 - 33 days old (within days of weaning). The latter female was also gestating (38 days from parturition: SF14 in Chapter 3). Measurements on these females were conducted at two temperatures and the mother was returned to the pups for at least an hour between measurements.

Data analysis

To prepare the raw data files for analysis, O_2 concentrations were corrected for analyser drift throughout the experimental period using the recorded baselines and the drift correction function in Expedata. To obtain steady-state values, a pre-recorded macro was used to locate multiple 10 minute sections (300 samples) of data with the most stable trace. The lowest of these values was used as the RMR for that temperature. T_{sk} values were used to confirm that the animal was in a steady state at this time. Only values after the first two hours of measurements were used to ensure that the animals were fully acclimated to the temperature. $\dot{V}O_2$ was calculated using the proportion of O_2 entering and leaving the respirometer, flow rate, chamber temperature and T_b and equations modified from Withers (2001). $\dot{V}O_2$ and the T_b measured at the end of the experiment were used to calculate C_{wet} using Equation 3 from McNab (1980b).

Statistical analysis

All statistics were performed using R version 3.0.1 (R Development Core Team 2011) and linear mixed modelling was implemented using the R package *nlme* (Pinheiro *et al.* 2013). Piecewise linear regression (p. 425 Crawley 2007), with mass as a covariate, was used to determine inflection points in the slope of $\dot{V}O_2$ versus T_a, which would typically identify the T_{lc}. This analysis was repeated for the T_b and C_{wet} data.

To quantify the importance of various factors on $\dot{V}O_2$, T_b, C_{wet} and body mass, model selection was performed using Akaike Information Criterion scores corrected for small sample size and Akaike weights (AICc and AICcWt, Burnham & Anderson 2002) implemented in the R package *AICcmodavg* (Mazerolle 2013) with respirometer temperature (‘T_a’), ‘season’, ‘day’, and ‘reproductive status’, as fixed factors, and ‘animal ID’ as a random factor. The assumptions of the models, i.e. normally distributed residuals with a mean of zero, were verified by observing qq-plots and histograms of the residuals.

Literature Cited

Angilletta, M. J. J., B. S. Cooper, M. S. Schuler & J. G. Boyles (2010) The evolution of thermal physiology in endotherms. *Front Biosci,* **2,** 861-881.

Arlettaz, R., C. Ruchet, J. Aeschimann, E. Brun, M. Genoud & P. Vogel (2000) Physiological traits affecting the distribution and wintering strategy of the bat *Tadarida teniotis*. *Ecology,* **81,** 1004-1014.

Audet, D. & M. B. Fenton (1988) Heterothermy and the use of torpor by the bat *Eptesicus fuscus* (Chiroptera: Vespertilionidae): a field study. *Physiol Zool,* **61,** 197-204.

Bartholomew, G. A. (1972) Body temperature and energy metabolism. In: *Animal Physiology: Principles and Adaptations*: 298-368. M. S. Gordon, G. A. Bartholomew, A. D. Grinnell, C. B. Jorgensen & F. N. White (Eds.). Macmillan Publishing Co., Inc., New York.

Beard, L. A. & G. C. Grigg (2000) Reproduction in the short-beaked echidna, *Tachyglossus aculeatus*: Field observations at an elevated site in South-east Queensland. *Proc Linn Soc N S W,* **122,** 89-99.

Bennett, A. F. & J. A. Ruben (1979) Endothermy and activity in vertebrates. *Science,* **206,** 649-654.

Boyles, J. G., A. B. Thompson, A. E. McKechnie, E. Malan, M. M. Humphries & V. Careau (2013) A global heterothermic continuum in mammals. *Glob Ecol Biogeogr,* **22,** 1029-1039.

Brice, M. P. (2008) *Heat tolerance and cold indifference in the short-beaked echidna, Tachyglossus aculeatus: Body temperature management in a mammalian constitutional eurytherm.* PhD, University of Queensland, Brisbane, Qld.

Brice, P. H., G. C. Grigg, L. A. Beard & J. A. Donovan (2002) Patterns of activity and inactivity in echidnas (*Tachyglossus aculeatus*) free-ranging in a hot dry climate: correlates with ambient temperature, time of day and season. *Aust J Zool,* **50,** 461-475.

Burnham, K. & D. Anderson (2002) *Model Selection and Multimodal Inference: A Practical Information-Theoretic Approach* 2nd edn. New York, NY: Springer-Verlag.

Canale, C. I., D. L. Levesque & B. G. Lovegrove (2012) Tropical heterothermy: Does the exception prove the rule or force a re-definition? In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*: 29-40. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin Heidelberg.

Cannon, B. & J. Nedergaard (2004) Brown adipose tissue: function and physiological significance. *Physiol Rev,* **84,** 277-359.

Carey, H. V., M. T. Andrews & S. L. Martin (2003) Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol Rev,* **83,** 1153-1181.

Clarke, A. & H.-O. Pörtner (2010) Temperature, metabolic power and the evolution of endothermy. *Biol Rev,* **85,** 703-727.

Crawley, M. J. (2007) *The R Book.* Chichester, West Sussex, UK: John Wiley & Sons Ltd.

Crompton, A. W., C. R. Taylor & J. A. Jagger (1978) Evolution of homeothermy in mammals. *Nature,* **272,** 333-336.

Eisenberg, J. F. & E. Gould (1969) The tenrecs: a study in mammalian behavior and evolution. *Smithson Contrib Zool,* **27,** 1-156.

Eisenberg, J. F. & N. Muckenhirn (1968) The reproduction and rearing of tenrecoid insectivores in captivity. *Int Zoo Yearb,* **8,** 106-110.

Eisentraut, M. (1960) Heat regulation in primitive mammals and in tropical species. *Bull Mus Comp Zool,* **124,** 31-43.

Farmer, C. G. (2000) Parental care: the key to understanding endothermy and other convergent features in birds and mammals. *Am Nat,* **155,** 326-334.

Farmer, C. G. (2003) Reproduction: the adaptive significance of endothermy. *Am Nat,* **162,** 826-840.

Geiser, F., G. Körtner & I. Schmidt (1998) Leptin increases energy expenditure of a marsupial by inhibition of daily torpor. *Am J Physiol,* **275,** R1627-R1632.

Geiser, F. & T. Ruf (1995) Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiol Zool,* **68,** 935-966.

Grigg, G. C., L. A. Beard & M. L. Augee (2004) The evolution of endothermy and its diversity in mammals and birds. *Physiol Biochem Zool,* **77,** 982-997.

Hosken, D. J. & P. C. Withers (1999) Metabolic physiology of euthermic and torpid lesser long-eared bats, *Nyctophilus geoffroyi* (Chiroptera: Vespertilionidae). *J Mammal,* **80,** 42-52.

IUPS Thermal Commission (2003) Glossary of terms for thermal physiology - Third edition (Reprinted from the Japanese Journal of Physiology). *J Therm Biol,* **28,** 75-106.

Jastroch, M., S. Wuertz, W. Kloas & M. Klingenspor (2005) Uncoupling protein 1 in fish uncovers an ancient evolutionary history of mammalian nonshivering thermogenesis. *Physiol Genomics,* **22,** 150-156.

Jury, M. (2003) The climate of Madagascar. In: *The Natural History of Madagascar*: 75-87. S. M. Goodman & J. P. Benstead (Eds.). The University of Chicago Press, Chicago, IL.

Kayser, C. (1960) Cosommation d'oxygène et température centrale au cours de l'hiver austral de deux Insectivores de Madagascar, *Centetes ecaudatus* et *Setifer setosus*. *Compt Rend Soc Biol,* **154,** 1873-1876.

Kayser, C. (1961) *The Physiology of Natural Hibernation*. New York, NY: Pergamon Press Oxford.

Koteja, P. (2000) Energy assimilation, parental care and the evolution of endothermy. *Proc R Soc B,* **267,** 479-484.

Koteja, P. (2004) The evolution of concepts on the evolution of endothermy in birds and mammals. *Physiol Biochem Zool,* **77,** 1043-1050.

Krockenberger, A. K., W. Edwards & J. Kanowski (2012) The limit to the distribution of a rainforest marsupial folivore is consistent with the thermal intolerance hypothesis. *Oecologia,* **168,** 889-899.

Kuchel, L. (2003) *The energetics and patterns of torpor in free-ranging Tachyglossus aculeatus from a warm-temperate climate.* PhD, University of Queensland, Brisbane.

Lovegrove, B. G. (2000) The zoogeography of mammalian basal metabolic rate. *Am Nat,* **156,** 201-219.

Lovegrove, B. G. (2012a) The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol Rev,* **87,** 128–162.

Lovegrove, B. G. (2012b) A single origin of heterothermy in mammals. In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*: 3-11. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin

Lovegrove, B. G., C. I. Canale, D. L. Levesque, G. Fluch, M. Řeháková-Petrů & T. Ruf (2014) Are tropical small mammals physiologically vulnerable to Arrhenius effects and climate change? *Physiol Biochem Zool,* **87,** 30-45.

Lovegrove, B. G. & F. Génin (2008) Torpor and hibernation in a basal placental mammal, the Lesser Hedgehog Tenrec, *Echinops telfairi*. *J Comp Physiol B,* **178,** 691-698.

Lovegrove, B. G., G. Heldmaier & T. Ruf (1991) Perspectives of endothermy revisited - The endothermic temperature-range. *J Therm Biol,* **16,** 185-197.

Luo, Z. X. (2007) Transformation and diversification in early mammal evolution. *Nature,* **450,** 1011-1019.

Lyman, C. P., J. S. Willis, A. Malan & L. C. H. Wang (1982) *Hibernation and Torpor in Mammals and Birds*. New York: Academic Press.

Mallinson, J. J. C. (1974) Establishing mammal gestation periods at the Jersey Zoological Park. *Int Zoo Yearb,* **14,** 184-187.

Mazerolle, M. J. (2013) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.28.

McNab, B. K. (1980a) Energetics and the limits to a temperate distribution in armadillos. *J Mammal,* **61,** 606-627.

McNab, B. K. (1980b) On estimating thermal conductance in endotherms. *Physiol Zool,* **53,** 145-156.

Merola-Zwartjes, M. & J. D. Ligon (2000) Ecological energetics of the Puerto Rican tody: heterothermy, torpor, and intra-island variation. *Ecology,* **81,** 990-1003.

Morrison, P. R. (1945) Acquired homiothermism in the pregnant sloth. *J Mammal,* **26,** 272-275.

Morrow, G. & S. C. Nicol (2009) Cool sex? Hibernation and reproduction overlap in the echidna. *PLoS ONE,* **4,** e6070.

Mover, H., A. Ar & S. Hellwing (1989) Energetic costs of lactation with and without simultaneous pregnancy in the white-toothed shrew *Crocidura russula monacha*. *Physiol Zool,* **62,** 919-936.

Mover, H., S. Hellwing & A. Ar (1988) Energetic cost of gestation in the white-toothed shrew *Crocidura russula monacha* (Soricidae, Insectivora). *Physiol Zool,* **61,** 17-25.

Nicol, S. & N. A. Andersen (2006) Body temperature as an indicator of egg-laying in the echidna, *Tachyglossus aculeatus*. *J Therm Biol,* **31,** 483-490.

Nicoll, M. E. (1986) Diel variation in body temperature in *Tenrec ecaudatus* during seasonal hypothermia. *J Mammal,* **67,** 759-762.

Nicoll, M. E. & S. D. Thompson (1987) Basal metabolic rates and energetics of reproduction in therian mammals: marsupials and placentals compared. In: *Reproductive Energetics in Mammals*: 7-27. A. S. I. Loudon & P. A. Racey (Eds.). Clarendon Press, Oxford, UK.

O'Leary, M. A., J. I. Bloch, J. J. Flynn, T. J. Gaudin, A. Giallombardo, N. P. Giannini, S. L. Goldberg, B. P. Kraatz, Z.-X. Luo, J. Meng, X. Ni, M. J. Novacek, F. A. Perini, Z. S. Randall, G. W. Rougier, E. J. Sargis, M. T. Silcox, N. B. Simmons, M. Spaulding, P. M. Velazco, M. Weksler, J. R. Wible & A. L. Cirranello (2013) The placental mammal ancestor and the post–K-Pg radiation of placentals. *Science,* **339,** 662-667.

Oelkrug, R., N. Goetze, C. Exner, Y. Lee, G. K. Ganjam, M. Kutschke, S. Müller, S. Stöhr, M. H. Tschöp, P. G. Crichton, G. Heldmaier, M. Jastroch & C. W. Meyer (2013) Brown fat in a protoendothermic mammal fuels eutherian evolution. *Nat Commun,* **4,** e2140.

Olson, L. E. & S. M. Goodman (2003) Phylogeny and biogeography of tenrecs. In: *The Natural History of Madagascar*: 1235-1242. S. M. Goodman & J. P. Benstead (Eds.). The University of Chicago Press, Chicago, USA.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar & R Development Core Team (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-108.

Poppitt, S. D., J. R. Speakman & P. A. Racey (1994) Energetics of reproduction in the lesser hedgehog tenrec, *Echinops telfairi* (Martin). *Physiol Zool,* **67,** 976-994.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Racey, P. A. & P. J. Stephenson (1996) Reproductive and energetic differentiation of the Tenrecidae of Madagasacar. In: *Biogéographie de Madagascar*: 307-319. W. R. Lourenço (Ed.). Editions de l'Orstom, Paris.

Randrianjafy, V. (2003) *Contribution à l'étude de la biologie de conservation de la communauté micromammalienne d'Ankarafantsika.* PhD, Université d'Antananarivo, Antananarivo.

Refinetti, R. & M. Menaker (1992) The circadian-rhythm of body temperature. *Physiol Behav,* **51,** 613-637.

Rojas, A. D., G. Körtner & F. Geiser (2012) Cool running: locomotor performance at low body temperature in mammals. *Biol Lett,* **8,** 868-870.

Rowe, T. B., T. E. Macrini & Z. X. Luo (2011) Fossil evidence on origin of the mammalian brain. *Science,* **332,** 955-957.

Schmidt-Nielsen, K. (1997) *Animal Physiology: Adaptation and Environment*. Cambridge University Press.

Scholander, P. F., R. Hock, V. Walters, F. Johnson & L. Irving (1950) Heat regulation in some arctic and tropical mammals and birds. *Biol Bull,* **99,** 237-258.

Stephenson, P. J. & P. A. Racey (1993a) Reproductive energetics of the Tenrecidae (Mammalia: Insectivora). I. The large-eared tenrec, *Geogale aurita*. *Physiol Zool,* **66,** 643-663.

Stephenson, P. J. & P. A. Racey (1993b) Reproductive energetics of the Tenrecidae (Mammalia: Insectivora). II. The shrew-tenrecs, *Microgale* spp. *Physiol Zool,* **66,** 664-685.

Stephenson, P. J. & P. A. Racey (1994) Seasonal variation in resting metabolic rate and body temperature of streaked tenrecs, *Hemicentetes nigriceps* and *H. semispinosus* (Insectivora: Tenrecidae). *J Zool (Lond.),* **232,** 285-294.

Stephenson, P. J. & P. A. Racey (1995) Resting metabolic rate and reproduction in the Insectivora. *Comp Biochem Physiol A,* **112,** 215-223.

Symonds, M. R. E. (1999) Life histories of the Insectivora: the role of phylogeny, metabolism and sex differences. *J Zool (Lond.),* **249,** 315-337.

Thompson, S. D. & M. E. Nicoll (1986) Basal metabolic rate and energetics of reproduction in therian mammals. *Nature,* **321,** 690-693.

Turner, J. M., G. Körtner, L. Warnecke & F. Geiser (2012) Summer and winter torpor use by a free-ranging marsupial. *Comp Biochem Physiol A,* **162,** 274-280.

Willis, J. S. (1982) The mystery of the periodic arousal. In: *Hibernation and Torpor in Mammals and Birds*: 92-103. C. P. Lyman, J. S. Willis, A. Malan & L. C. H. Wang (Eds.). Academic Press, New York.

Withers, P. C. (2001) Design, calibration and calculation for flow-through respirometry systems. *Aust J Zool,* **49,** 445-461.

Wooden, K. M. & G. E. Walsberg (2004) Body temperature and locomotor capacity in a heterothermic rodent. *J Exp Biol,* **207,** 41-46.

Zachos, J., Pagani, M., Sloan, L., Thomas, E. & Billups, K. (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science,* **292,** 686–693.

Zuur, A. F., E. N. Ieno, N. Walker, A. A. Saveliev & G. M. Smith (2009) *Mixed Effects Models and Extensions in Ecology with R*. New York, NY, USA: Springer.

Linking statement

The previous chapter presented data which determined the cost of gestation and lactation in *S. setosus* held at constant ambient temperatures within a respirometer. The next chapter utilizes T_b as a proxy for energy expenditure to determine the energetics of reproduction in free-ranging individuals. With this information I can provide a more thorough characterisation of thermoregulation in *S. setosus*, as well as estimate the costs of reproduction in this species. By observing the effects of reproduction on thermoregulation in a free-ranging basoendotherm, I will also be able to comment on the evolution of homeothermy in mammals. In addition, the data collected on the interactions between high T_a and T_b will help further the understanding of the effects of high T_a on the physiology and energetics of endotherms, which is increasingly relevant in the face of changing global climates.

Chapter 5 Effects of reproductive status and high ambient temperatures on the body temperature of a free-ranging basoendotherm

Abstract

Tenrecs (Order Afroscoricida) have some of the lowest body temperatures (T_b) of any eutherian mammal. They also have a high level of variability in both active and resting T_bs and, at least in cool temperatures in captivity, frequently employ both short- and long-term torpor. However, this high degree of heterothermy is generally reduced during gestation and lactation. Previous studies have measured the T_b of free-ranging tenrecs during the dry, austral winter, when many of the larger species hibernate. To date, however, no recordings have been obtained from individuals during the reproductive season. This study presents data collected over two rainy seasons in Ankarafantiska National Park, a dry deciduous forest in western Madagascar.

Reproductive females had slightly higher daily minima (T_b ~ 32°C) and less overall variability in T_b, whereas non-reproductive females and males had both a higher propensity for torpor as well as a lower (T_b ~ 30.5°C) and more variable rest-phase T_b. Torpor expression, either defined as T_b decreasing below a certain threshold (T_b ~ 28°C) or by a set T_b-ambient temperature differential ($T_b – T_a < 5$°C), was much lower than predicted compared with the heterothermy shown by tenrecs in captivity. However, torpor defined via T_b alone is likely to underestimate the overall use of torpor, which been shown to occur at ambient temperatures close to normothermic T_b in species from tropical and sub-tropical habitats.

The results of my study caution against inferring metabolic states from T_b measures alone and lend support to the recent call to define torpor in free-ranging animals based on mechanistic and not descriptive processes. In addition, the decrease in T_b variability observed during gestation and lactation in this study is in agreement with the parental care models for the evolution of endothermy, and confirms that homeothermy is essential to reproduction in this species and likely in mammals in general. The relatively low costs of maintaining homeothermy in the tropical climates of present day Madagascar help to shed light on how homeothermy could have evolved from the ancestral heterothermic condition in similar climates.

Abbreviations

T_a = ambient temperature measured via a black body apparatus
T_b = core body temperature
T_{soil} = ambient temperature measured at a depth of 250 mm below the surface
T_{tree}= ambient temperature measured in a tree cavity
ΔT = temperature differential (T_b - T_a)
T_{bmax} = average of the 5 highest T_b data for each day
T_{bmin} = average of the 5 lowest T_b data for each day
ΔT_b = difference between the daily maximum and minimum T_b

Introduction

The hypothetical mammalian ancestor is postulated to have been small bodied, nocturnal and insectivorous (Crompton, Taylor & Jagger 1978, Luo 2007, Gerkema *et al.* 2013, O'Leary *et al.* 2013), and likely possessed the capacity for prolonged periods of metabolic down-regulation (Lovegrove 2012a). The plesiomorphy of torpor is supported by the prevalence of torpor expression in all major mammalian orders, including monotremes and marsupials (Grigg, Beard & Augee 1989, Geiser 1994) and eutherian lineages with small-bodied representatives (Geiser & Ruf 1995, Lovegrove 2012b). The capacity for prolonged heterothermy, either in the form of daily torpor or hibernation (*sensu* Geiser & Ruf 1995), would have increased the chances of mammals surviving the harsh environments that followed the asteroid impact at the Cretaceous-Palaeogene boundary (Robertson *et al.* 2004, Lovegrove 2012b). Furthermore, a propensity for large circadian variations in T_b as well as the capacity for torpor, are a likely mid-point on the continuum between ectothermy and the highly regulated and rigid homeothermy seen in many modern mammal lineages (Crompton *et al.* 1978, Grigg, Beard & Augee 2004, Lovegrove 2012b, Lovegrove 2012a).

The study of extant mammals retaining ancestral characteristics, so-called “basoendotherms” (*sensu* Lovegrove 2012a), can help in understanding how and why homeothermy evolved, given that it is an energetically costly means of thermoregulation. The spiny tenrecs of Madagascar (Order Afrosoricida, Subfamily Tenrecinae) are some of the best examples of extant eutherian basoendotherms (Eisentraut 1960, Crompton *et al.* 1978, Lovegrove & Génin 2008, Oelkrug *et al.* 2013). Members of this subfamily are insectivorous, primarily nocturnal, and have inhabited the sub-tropical island of Madagascar, where climates have remained fairly stable, since 55-35 mya (Eisenberg & Gould 1969, Douady *et al.* 2002, Olson & Goodman 2003). Well studied in captivity, the Tenrecinae have some of the lowest and most highly variable body temperatures (T_b), as well as the lowest basal metabolic rates, of any eutherian mammal (Stephenson & Racey 1995, Lovegrove 2000, Clarke & Pörtner 2010). It has recently been demonstrated that the lesser hedgehog tenrec (*Echinops telfairi*) possesses functional brown adipose tissue and has the capacity to actively rewarm from low T_bs during torpor (Oelkrug *et al.* 2013). However, the maintenance of a stable T_b in captivity is rare outside of gestation and lactation (Stephenson & Racey 1993a, Stephenson & Racey 1993b, Poppitt, Speakman & Racey 1994).

The restriction of homeothermy to periods of reproductive activity, more specifically to gestation and lactation, lends support to previous hypotheses which postulated that homeothermy evolved in mammals partially to benefit reproduction. Homeothermy could have evolved either via higher T_bs that promoted the maintenance of high and stable foetal growth rates (Farmer 2000), or by increasing the capacity for high levels of energy assimilation needed to provision the young (Koteja 2000). To date, the only data available on the T_b patterns of free-ranging tenrecs are from the austral winter (Lovegrove & Génin 2008, Lovegrove *et al.* 2014), with no data available from reproductively active individuals. Thus one of the primary aims of this study was to obtain T_b data from free-ranging reproductive basoendotherms, to test hypotheses for the link between parental care and homeothermy. Here I present the results of a two-year study on the thermoregulation of a free-ranging population of greater hedgehog tenrecs (*Setifer setosus*, Schreber, 1778) inhabiting the dry deciduous forest of western Madagascar.

Materials and Methods

Study site, capture and surgical methods

The study was conducted over two rainy seasons, from September 2010 to February 2012 (coinciding with the second and third seasons presented in Chapter 3), in the ‘Jardin Botanique A’ research area adjacent to the Ampijoroa Forestry Station in Ankarafantiska National Park (16º19’S, 46º48’E). A detailed description of the study site, population and general methods are provided in Chapters 2 and 3. Detailed T_a data were recorded at various locations throughout the study site using DS1922L Thermocron iButtons programmed to record once every 30 minutes at a resolution of 0.0625°C (Dallas Semiconductor, Dallas, TX, USA). A variety of configurations were used: black body and Stevenson Screens were placed 1 m from the ground in shaded areas and soil temperature was recorded at 0 mm, 250 mm and 500 mm. In 2011 - 2012 iButtons were also placed, on opposing ends of the study area, in two tree cavities which had previously been used as nest sites by *S. setosus*.

All animals were caught by hand, by walking the established trails in the area at night with local guides. Individuals were located, captured and transported back to the research camp where they were housed in plastic containers lined with paper towel and provided with live insects and tinned sardines. They were kept for a maximum of five days before surgery to allow for the collection of metabolic data (Chapter 4). A subset of the population of sufficient body mass (> 150 g) were subsequently implanted with a combination of radio-transmitter and body temperature data logger (DS1922L Thermocron iButtons; Chapter 2). During the first season, two miniaturized iButtons (Lovegrove 2009) were encapsulated in surgical wax (Paramat Extra-Merck KGaA, Darmstadt, Germany) alongside a modified 2-stage collar transmitter (Merlin Systems Inc., Boise, ID, USA). The resulting packages had a total mass of around 13.0 g (mean 13.0 g, range 11.7 - 13.5 g). During the second season (September 2011-February 2012) a single unmodified iButton was used which increased the mass of the implant to a maximum of 14 g while not affecting the size. The iButtons were programmed to record body temperature at 30 or 36 min intervals with an accuracy of 0.5°C. All iButtons were calibrated against a mercury thermometer prior to implantation as well as post-recovery to the nearest 0.1°C, no drift was observed. The package was implanted via ventral midline laparotomy undertaken under sterile conditions in an enclosed laboratory site at the research camp. Animals were observed for one day post-surgery and released at the site of capture. Implanted animals were re-captured within a week of surgery to ensure proper recovery or corrective suturing if needed.

The transmitters used in a preliminary season as well as the first season of this study had a high level of malfunction, leading to the majority of the animals being lost to the study over the hibernation period. If, however, the animal was successfully recaptured the data loggers were recovered using similar surgical procedures that were used to implant the loggers. A high level of natural mortality during the second season (Chapter 3) meant that recovery surgery was unnecessary and all data loggers were collected in the field after the animal’s death.

Data analysis

All statistics were performed using R version 3.0.2 (R Development Core Team 2011) and linear mixed modeling was implemented using the *lme* function in the R package *nlme* (Pinheiro *et al.* 2013). The assumptions of the models, i.e. normally distributed residuals with a mean of zero, were verified by observing qq-plots and histograms of the residuals.

Ambient temperature

The T_a, at 30 - 45 min intervals, was obtained from iButtons: in the black body configuration, from those buried at a depth of 250 mm in the soil (T_{soil}) and, from the second season only, in tree-cavities (T_{tree}). All data covering the period of activity for each season (September-May) were compiled. Exact sunrise and sunset times were obtained for each day from The United States Naval Observatory website (http://www.usno.navy.mil/USNO/). To account for the nocturnal activity patterns of this species each day was shifted to comprise a period starting from sunrise to sunrise. T_a recorded from 1 October to the 10 February for each season were analysed for a) trends in the mean daytime (rest phase) and nighttime (active phase) temperatures, b) the maximum and minimum T_a (T_{amax}, T_{amin}, taken as an average of the 5 highest and 5 lowest, respectively), c) the amplitude of daily temperature variability ($T_{amax} - T_{amin} = \Delta T_a$), and d) the time of day of T_{amin} and T_{amax}. Frequency distribution tables were created with a bin size of 0.5°C. The amount time at which T_a was greater than the lower critical limit of the thermoneutral zone (25°C, Chapter 4) was calculated, first for the entire day, and then for the daytime and nighttime periods separately. Differences between seasons as well as the effect of the 'day' (with 1 September as day 0, see Chapter 3) were tested via linear mixed models. T_a and T_{soil}, available from both seasons, constituted paired data which were controlled for using (~ 1|'day/season') as the random structure. Autocorrelation between the data points was accounted for using the correlation structure 'corCAR1' with day as the time covariate (Pinheiro & Bates 2000).

Body temperature analysis

The data from three males, one from the first season and two from the second season, have been previously analysed with regards to torpor at high T_as, as presented in Lovegrove *et al.* (2014), but are included in the current analyses as different aspects are covered. All data were placed into spreadsheets similar to those used in Lovegrove *et al.* (2014): each T_b datum was assigned the following markings, 'light' (nighttime or daytime based on sunrise and sunset times), 'day', 'sex', and, if female, 'reproductive status' (gestating, lactating, unknown). Data from the first week following the surgeries, the day of parturition (n= 2), as well as from the three to four day period in which the animals were housed in the laboratory for metabolic rate measurements (Chapter 4), were excluded from the analyses. In addition, a single high data point recorded in a gestating female (36.2°C) was found to coincide with the exact moment of recapture in the field. This outlier was deemed to be the result of a stress-induced rise in T_b

(Careau *et al.* 2011) and not representative of overall thermoregulatory patterns, and was therefore also excluded from the analyses.

Using similar protocols to that of the T_a data analysis, the mean, max, min and ΔT_b as well as the time of day in which the maximum and minimum T_b occurred were calculated for each day for all individuals. Differences between seasons (using males only) and reproductive status (using data collected from the second season only) as well as the effects of T_a and T_{tree} on T_b were performed via model selection using Akaike Information Criterion scores corrected for small sample size and Akaike weights (AICc and AICcWt, Burnham & Anderson 2002) implemented in the R package *AICcmodavg* (Mazerolle 2013). Repeated measures were controlled for, as per Chapter 4, using 'animal ID' as a random factor, and autocorrelation was corrected for using the correlation structure 'corAR1' in the form of ~1|'animal ID'. All females of unknown reproductive status were excluded from this portion of the analysis. Torpor expression was quantified using the criteria determined in Chapter 4: a difference in T_b and T_a of less than 5°C at T_as below the TNZ (< 25°C). A second analysis was performed using the method outlined in McKechnie *et al.* (2007), where a normal distribution with a standard deviation of 1 was created around the mode T_b for each animal and the lower 99% confidence limit of this distribution was used as the threshold T_b between torpor and normothermy. These analyses were performed three times, the first on full-day datasets, followed by a separate analysis of nighttime and daytime values.

Results

Ambient temperature

T_a at the study site varies little, despite dramatic changes in precipitation between the dry austral winter and the wet summer (Chapter 3: Fig 3-1) Maximum daily T_as remained greater than 30°C throughout the year, although T_{amin} was much less during the dry season. Analyses performed on T_a collected during the active (summer) season showed that mean T_a did not differ between the two study seasons ($F_{1,129} = 0.05$, $p = 0.183$) but significantly decreased over time ($F_{1,131} = 169.81$, $p < 0.001$). Similar decreases were seen in T_{amax} ($F_{1,131} = 550.43$, $p < 0.001$) which had had a small (0.6°C) but significantly higher mean in the first season ($F_{1,131} = 6.00$, $p = 0.016$). The opposite pattern was found in T_{amin} with the second season having slightly higher daily minima ($F_{1,131} = 27.5$, $p < 0.001$) and T_{amin} increasing significantly as the season progressed ($F_{1,131} = 62.86$, $p < 0.001$). T_{tree} was only available from the second season but showed similar decreases in both mean and maximum levels over time (mean: $F_{1,131} = 181.83$, $p < 0.001$, max: $F_{1,131} = 409.2$, $p < 0.001$) and an increase in minimum T_{tree} until day

100 ($F_{1, 131}$ = 13.57, $p < 0.001$), when it began to decrease. Patterns in T_{soil} were a much dampened version of T_{tree} patterns with a mean daily ΔT_{soil} of 0.65 ± 0.41°C. All values reached a peak around day 60 - 80 and decreased as the season progressed. Only 40% of all recorded T_as were less than the lower critical limit of the thermoneutral zone of *S. setosus* (T_a ~ 25°C) and of those only 1.2 % were below T_a = 20°C. A large percentage of the recorded T_a was above 30°C (28.1 %). Similarly, 54.9 % of all T_{tree} recordings fell into the TNZ, with only 15.8 % above 30°C and 29.1 % below the TNZ. The variability in T_{soil} was much less and 93.1 % of all recordings were in the TNZ with only 4.5 % falling above and 2.4 % below.

The mean time of day at which T_a reached its maximum was 13:32 (± 1.2 h, range: 08:43 - 17:02) and minimum T_a occurred at a mean time of 04:45 (± 1.6 h, range: 20:13 - 09:35). Visual observations of the data indicated a large degree of overlap between seasons and an increase in variability as the season progressed, coinciding with an increases in foliage and rainfall at the study site (Sato 2012). T_{tree} showed similar patterns to T_a, although with a slight delay, peaking at a mean time of 16:33 (±1.4 h, range: 12:28 - 23:28) and reaching a minimum at 05:55 (±2.1 h, range: 21:43 - 07:51). However, the time at min T_{tree} did not change throughout the season. T_s reached a maximum on average around 0:02 (± 3.2 h, range: 15:27 - 06:35), and a minimum at around 11:05 (± 3.6 h, range: 22:06 - 18:11). As with T_a, there did not appear to be differences between seasons but both max and min times became earlier as the season progressed.

Reproductive status and sample size

Detailed life histories of members of the study population are presented in Chapter 3. The use of modified iButtons for the first season (September 2010 – September 2011) resulted in a high rate of data-loss. Of the three animals (two males, one female) recovered post-hibernation, T_b data from the hibernation period were recovered from the female only (Fig. 5-1, Chapter 3, Lovegrove *et al.* 2014). Only two functional data-loggers were recovered from the active period of this first season. One, covering a period of 38 days, was recovered from a male after the transmitter package was regurgitated by a boa (*Acranthophis madagascariensis*). The second was obtained from a female who died while entering a narrow tree cavity (Chapter 2) after 28 days. The reproductive status of this female throughout the study is unknown.

Switching to a single unmodified iButton for the second season (September 2011-February 2012) resulted in an improved recovery rate of T_b data. However, a smaller number of animals were captured throughout the season and high natural mortality (Chapter 3) limited the capacity for data collection. Recordings were successfully obtained from two males and three females resulting in 92 animal-days for males and 180 for females, of which 121 were during gestation and 28 during lactation. The post-hibernation data from the female from the first season (18 days) are the only data available from the period immediately preceding hibernation in either season. As this individual was not followed for the remainder of the season her reproductive status at the time of the recordings is unclear, although she was likely in the early stages of gestation at recapture (11 November).

The female with the longest period of data available (72 days) moved out of range of the study site for an extended period of time (~ 40 days) during which she was presumed to have given birth. She was heavily pregnant before disappearing and, upon recapture appeared in the early- to mid-stages of pregnancy. It is not known if she was successful in suckling her first litter until weaning. She fell prey to a snake (*Boa manditra*) in the later stages of the second pregnancy. Neither of the females who remained in the study area throughout the recording period successfully reared a litter. One was killed by an unknown ground predator within 11 days of giving birth whereas the other is believed to have lost a litter to unknown causes after 12 days, and was later killed by a ground predator during the early stages of second pregnancy (Chapter 3).

Daily variations in T_b

T_b was highly variable and the difference between the daily maxima and minima (ΔT_b) ranged from 0.6 to 8.1°C (mean: 2.6 ± 0.9°C). Circadian rhythms were highly variable and while the majority of values of maximum daily T_b were recorded during the active phase and minimums during the rest phase, no overall pattern is visible either between or among individuals (Fig. 5-2). Active phase T_b showed slightly less variability than resting phase T_b (Fig. 5-3), ranging between 30 - 35°C, compared with 26 - 36°C during the rest phase. Daily modal T_b measured during the active phase ranged from 31.6°C (the male from the first season) to 34°C in a gestating female from the second season (Table 5-1) and were generally lower in the rest phase.

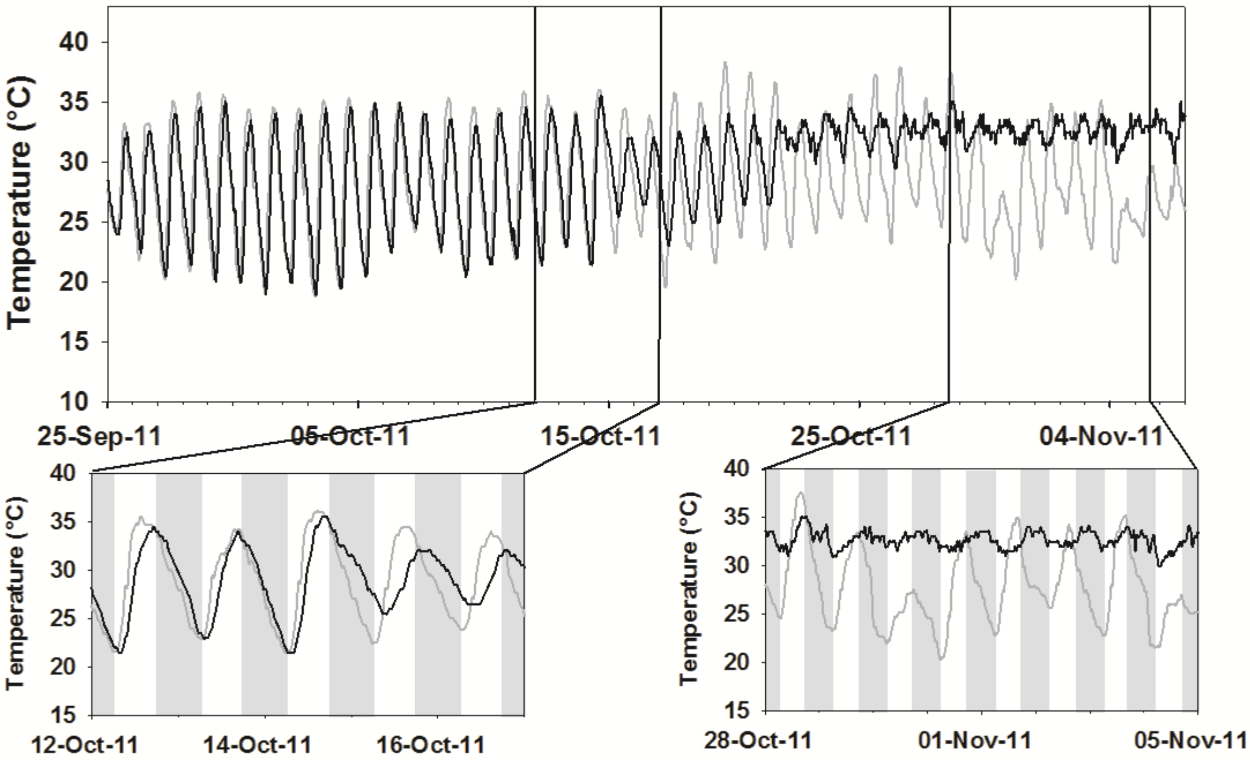
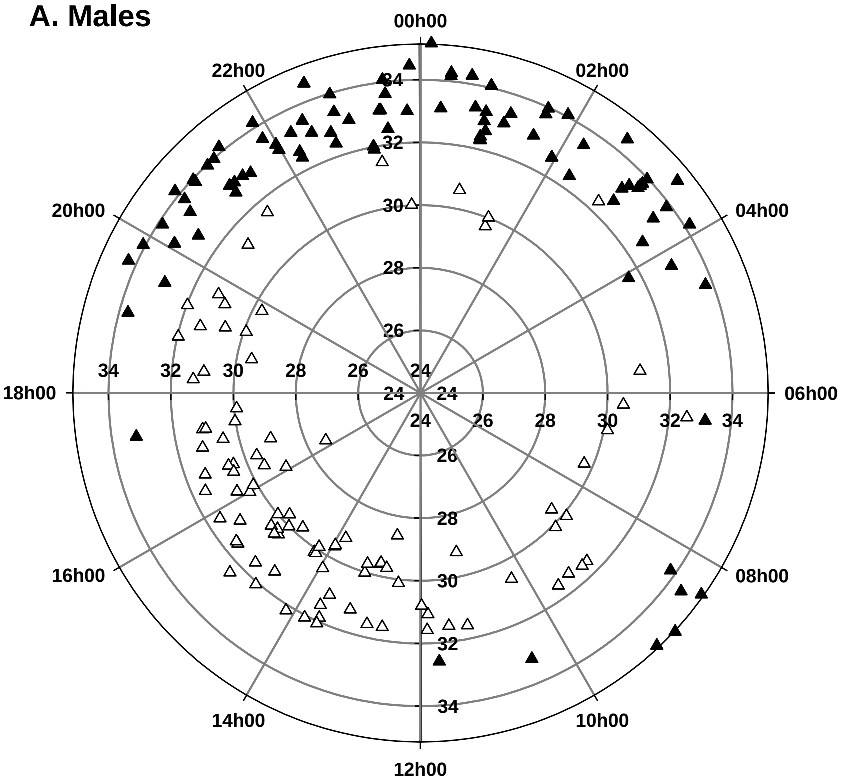


Figure 5-1 Reproduced from Lovegrove *et al*. (2014). T_b (black line) recordings from a hibernating *Setifer setosus* during the dry austral winter in Ankarafantsika National Park along with the ambient temperature (grey line) measured at a neighboring nest site in a tree cavity with similar thermal properties to the hibernacula. A. Recordings from a few days during hibernation showing a change in T_b amplitude, likely caused by a change in nest site. B. T_b after immergence from hibernation. The animal (an adult female) was recaptured on 7 November 2011 and was likely in the early stages of gestation.

A. Males



B. Females

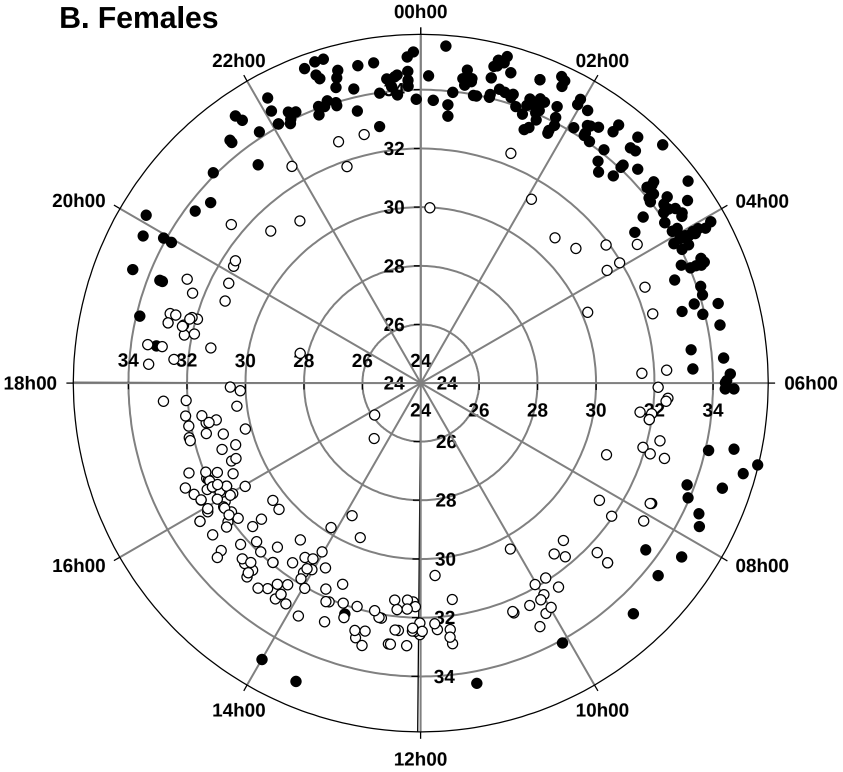


Figure 5-2 Time of day and T_b (in °C) at which maximum (black) and minimum (hollow) T_b (°C) were measured in both A. male (triangles) and, B. female (circles) *Setifer setosus*.

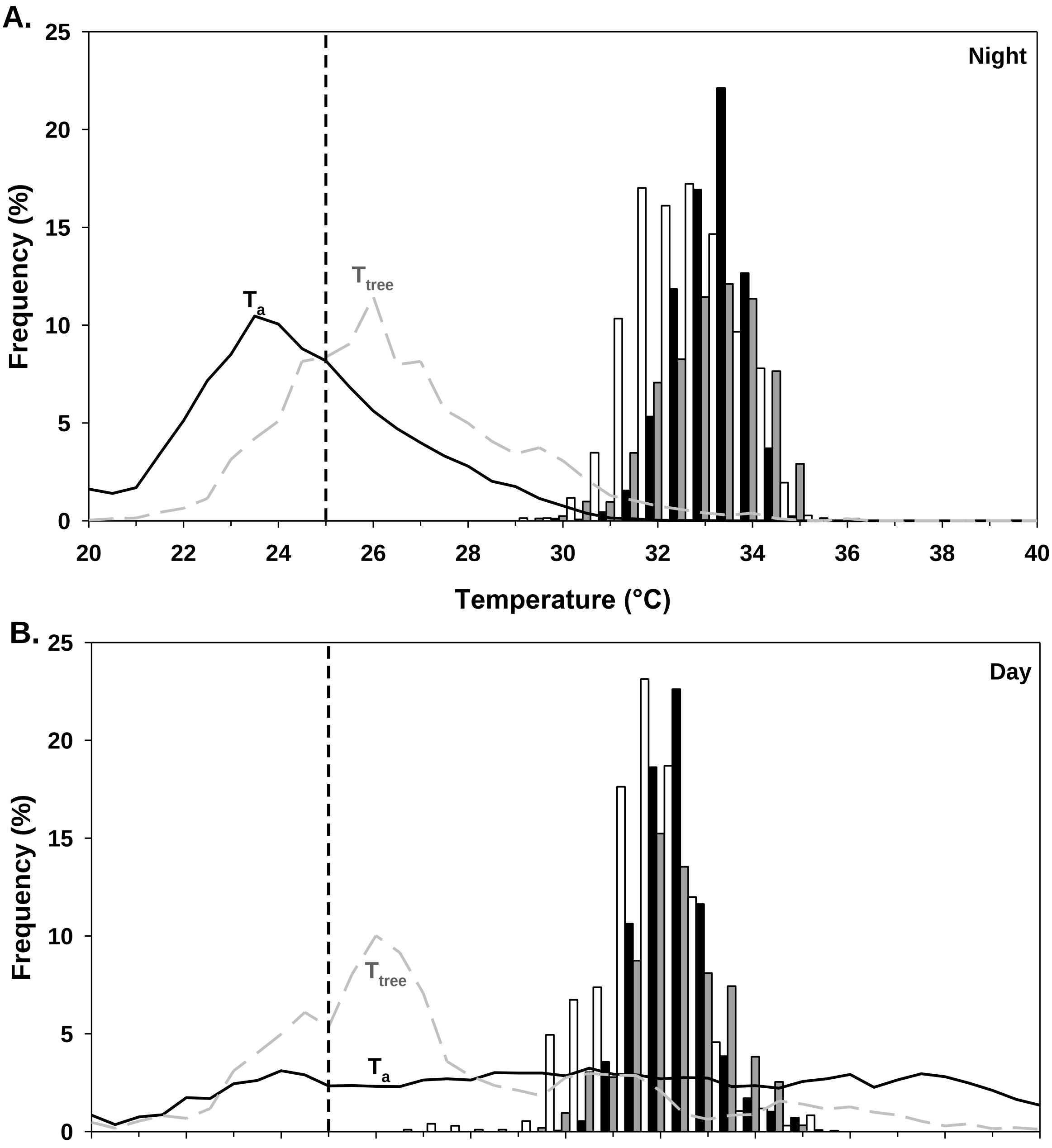


Figure 5-3 Distribution of nighttime (active phase, A.) and daytime (rest-phase, B) body temperature of male (hollow bars) and gestating (black bars) and lactating (grey bars) female *Setifer setosus*. Environmental temperature distributions are indicated by the black lines (T_a) and the dashed grey lines (T_{tree}) and the lower critical limit of the thermal neutral zone in marked via a dashed vertical line.

Table 5-1 Modal T_b for each individual *Setifer setosus* during the active season. Values are provided for nighttime (active phase) and daytime (rest phase) and full day. The threshold T_b for torpor expression was calculated using the 99% confidence limit of a normal distribution around the modal T_b with a standard deviation of 1.

	First Season		**Second Season**					
	SF11	SF17	SF07	SF22	SF23	SF24	SF25	SF26
	n = 27	n = 36	n = 17	n = 68	n = 31	n = 73	n = 42	n = 18
Sex	female	male	female	female	female	female	male	male
Modal T_b (°C)								
Full 24h	32.6	30.6	33	33	33.8	33.4	32.6	32
Night	33.6	31.6	33.5	34	33.8	33.4	33.1	32
Day	32.6	30.6	32.5	33	32.8	32.4	32.1	31.5
Threshold T_b (°C)								
Full 24h	30.0	28.0	30.4	30.4	31.2	30.8	30.0	29.4
Night	31.0	29.0	30.9	31.4	31.2	30.8	30.5	29.4
Day	30.0	28.0	29.9	30.4	30.2	29.8	29.5	28.9
Time T_b < Threshold T_b (%)								
Full 24h	4.0	0.0	4.9	0.0	0.7	0.8	0.5	1.1
Night	3.3	0.0	1.5	0.1	0.3	0.8	0.0	0.0
Day	12.2	0.9	11.8	0.1	1.0	0.8	1.6	2.0

‘Animal ID’ was contained in all of the best ranking linear models analysing the mean, max and min T_b of males (Table 5-2). ID was the only factor contained in the best fitting model for T_{bmax} and T_{bmin} whereas mean T_b was also influenced by Ta and ‘day’. Only ‘day’ was contained in the best fitting model for ΔT_b of the males (AICcWt = 0.56), although the second model containing only ‘animal ID’ had a large AICcWT (0.17). Insufficient data were available to make a firm comparison between seasons, however, and all three males differed from each other.

Comparing the data obtained from males to that of females during the second season provided more conclusive results (Table 5-3). In all cases except for the standard deviation, T_{tree} provided a better fit to the data than T_a. However, while contained in the top ranking models, neither T_{tree} nor T_a influenced T_b by more than ± 0.08°C. Gestating females had higher mean and modal T_b than males and the non-reproductive female (Tables 5-3 and 5-4, Fig. 5-2) and mean T_b was influenced by T_{tree} (Table 5-3). ‘Reproductive status’ was not contained in the top ranking model describing T_{bmax} but was similarly influenced by T_{tree}. In contrast, neither of the environmental temperatures were included in the best model for T_{bmin}, and both gestating and lactating females had higher minimum T_bs (by 0.8 - 1.1°C). Interestingly, only gestating, and not lactating females, had less variable T_bs than the non-reproductive individuals, supported by the analysis of both of the standard deviations and the ΔT_b (Tables 5-3 and 5-4).

Torpor during the reproductive season

Using the criteria determined in Chapter 4, torpor, outside of hibernation, was observed only on 11 out of 272 animal days. Torpor bouts defined in this manner were observed in males, 6 by the first season male lasting 0.5 - 3 h with an average torpid T_b of 29.4°C. A single bout was also observed in a male in the second season (visible in Fig 5-4 A.) which lasted 3 h before increasing T_a decreased the T_b - T_a differential to above the 25°C T_a threshold limit for torpor. Additionally, torpor was expressed by the first season female on a single day with T_b decreasing as low as 25.6°C and by the non-reproductive female from the second season (Fig 5-1) on the first two days following emergence from hibernation. A single bout lasting for a single recording point (30 min or less) was observed in one of the second season females on the day of parturition.

Table 5-2 Rank of linear models evaluating the effects of various factors on the daily mean T_b of free-ranging male *Setifer setosus*. Ranking was performed using corrected Akaike Information Criterion (AICc) scores and Akaike weights (AICcWt). All models with an AICcWt > 0 are presented and the number of parameters contained in the model are included (*k*). 'Day' is the number of days since September 1.

Model Parameters[a]	k	AICc	AICcWt
Mean T_b			
'meanT_a' + 'day' + 'animal ID'	7	40.23	0.56
'meanT_a'+ 'animal ID'	6	42.11	0.22
'meanT_a' x 'day' + 'animal ID'	8	42.36	0.19
'animal ID'	5	46.12	0.03
T_{bmin}			
'animal ID'	5	194.89	0.58
'day' + 'animal ID'	6	197.01	0.2
'minT_a' + 'day' + 'animal ID'	7	197.42	0.16
'minT_a' x 'day' + 'animal ID'	8	199.63	0.05
T_{bmax}			
'animal ID'	5	150.63	0.34
'day' + 'animal ID'	6	150.96	0.29
'maxT_a' + 'day' + 'animal ID'	7	151.43	0.23
'maxT_a' x 'day' + 'animal ID'	8	152.40	0.14
ΔT_b ($T_{bmax} – T_{bmin}$)			
'day'	4	260.60	0.56
'animal ID'	5	262.91	0.17
'day' + 'animal ID' + 'ΔT_a'	7	263.75	0.12
'day' + 'animal ID'	6	264.07	0.1
'day' x 'animal ID' + 'ΔT_a'	8	265.19	0.06

[a] All models were corrected for autocorrelation using the correlation structure corCAR1 (form = ~1|animal ID).

Table 5-3 Rank of models evaluating the effects of various factors on predicting the daily mean T_b of male and female *S. setosus* during the second season of the study. Ranking was performed using corrected Akaike Information Criterion (AICc) scores and Akaike weights (AICcWt). All models with an AICcWt > 0 are presented and the number of parameters contained in the model are included (*k*). ‘Day’ is the number of days since September 1.

Model Parameters[a]	k	AICc	AICcWt
Mean T_b			
‘meanT_{tree}’ + ‘reproductive status’	8	100.56	0.46
‘meanT_{tree}’	5	101.73	0.26
‘meanT_{tree}’ + ‘reproductive status’ + ‘day’	9	102.44	0.18
‘meanT_a’ + ‘reproductive status’	8	105.05	0.05
‘meanT_a’	5	106.25	0.03
‘meanT_a’ + ‘day’ + ‘reproductive status’	9	107.17	0.02
‘meanT_a’ + ‘day’	6	108.27	0.01
T_{bmin}			
‘reproductive status’	7	475.44	0.41
‘minT_{tree}’ + ‘reproductive status’	8	476.88	0.20
‘minT_a’ + ‘reproductive status’	8	477.19	0.17
‘minT_{tree}’ + ‘reproductive status’ + ‘day’	9	478.35	0.10
‘minT_a’ + ‘reproductive status’ + ‘day’	9	478.97	0.07
‘minT_{tree}’	5	481.57	0.02
‘day’	5	481.78	0.02
‘minT_a’	5	482.34	0.01
T_{bmax}			
‘maxT_{tree}’	5	295.07	0.39
‘maxT_{tree}’ + ‘reproductive status’	8	296.56	0.19
‘maxT_a’	5	296.69	0.18
‘maxT_{tree}’+ ‘reproductive status’ + ‘day’	9	297.84	0.1
‘maxT_{tree}’+ ‘day’	6	298.18	0.08
‘maxT_{tree}’	7	300.51	0.03
‘maxT_{tree}’+ ‘day’ + ‘reproductive status’	9	300.57	0.03
‘day’	5	303.73	0.01
ΔT_b ($T_{bmax} - T_{bmin}$)			
‘ΔT_a’ + ‘reproductive status’	8	475.48	0.63
‘ΔT_a’ + ‘day’ + ‘reproductive status’	9	477.46	0.23
‘ΔT_{tree}’+ ‘reproductive status’	8	479.58	0.08
‘ΔT_{tree}’+ ‘reproductive status’ + ‘day’	9	481.68	0.03
‘reproductive status’	7	483.99	0.01
‘ΔT_a’	5	484.21	0.01

[a] All models were corrected for autocorrelation using the correlation structure corCAR1 (form = ~1|animal ID).

Table 5-4 Means ± standard deviations of various parameters of the T_b of *Setifer setosus* measured over two rainy seasons.

Status	**Male** **male**	**Female** **non-repro**	**gestating**	**parturition***	**lactating**	**unknown***
	N = 3, n = 96	N = 1, n = 17	N = 3, n = 112	N = 2, n = 2	N = 2, n = 27	N=2, n=58
Mean T_b (°C)						
Mean	31.9 ± 0.7^{a}	32.5 ± 0.6^{a}	33.1 ± 0.3^{b}	32.8 ± 0.8	33.1 ± 0.7^{a}	33.2 ± 0.8
Range	30.2 - 33.1	30.5 - 33.2	32.5 - 34.1	32.2 - 33.3	31.8 - 33.8	30.6 - 34.1
S.D. T_b (°C)						
Mean	0.9 ± 0.3^{a}	1.0 ± 0.5^{a}	0.7 ± 0.2^{b}	1.4 ± 0.2	0.8 ± 0.2^{a}	0.9 ± 0.4
Range	0.4 - 2.3	0.5 - 2.5	0.3 - 1.3	1.3 - 1.5	0.4 - 1.0	0.3 - 3.1
Modal T_b (°C)						
Mean	31.6 ± 1.0^{a}	32.7 ± 0.6^{a}	32.9 ± 0.6^{b}	33.1 ± 1.6	33.0 ± 0.9^{b}	33.0 ± 0.9
Range	29.6 - 35.1	31.5 - 34.0	31.5 - 34.5	31.9 - 34.2	31.0 - 34.7	29.1 - 35.0
Min T_b (°C)						
Mean	30.6 ± 1.0^{a}	30.9 ± 1.3^{a}	32.1 ± 0.5^{b}	30.4 ± 0.6	31.8 ± 0.7^{b}	32.0 ± 1.3
Range	27.4 - 32.6	26.5 - 32.5	30.7 - 33.3	30.0 - 30.8	30.2 - 32.8	25.9 - 33.4
Max T_b (°C)						
Mean	31.9 ± 0.7^{a}	32.5 ± 0.6^{a}	33.1 ± 0.3^{a}	32.8 ± 0.8	33.1 ± 0.7^{a}	33.2 ± 0.8
Range	30.2 - 35.1	30.5 - 33.2	32.5 - 34.1	32.2 - 33.3	31.8 - 3.8	30.6 - 34.1
ΔT_b (°C)						
Mean	2.8 ± 0.9^{a}	3.0 ± 1.3^{a}	2.2 ± 0.6^{b}	4.4 ± 0.3	2.5 ± 0.5^{a}	2.5 ± 1.1
Range	1.1 7.1	1.5 - 6.8	1.0 - 3.9	4.2 - 4.6	1.3 - 3.4	0.6 - 8.1

Different letters indicate significant differences according to reproductive status. *Day of parturition was excluded from the statistical analysis due to a small sample size. Unknown refers to two females from different seasons, one which left the study site for an extended period of time during mid-late gestation, returning midway through a second pregnancy.

Torpor expression, evaluated using the threshold T_b obtained from the normal distributions around the modal T_b, provided slightly different results (Table 5-1). Using this definition, all but one of the bouts by the first season male (with a threshold T_b of 28°C) mentioned above were considered to be rest phase decreases in T_b and not torpor. Higher incidences of torpor were reported in all other animals, all of which, with higher modal T_bs, had higher torpor threshold T_bs (30.0 - 31.2°C). Torpor was more common in the daytime than at night. Both the lone female from the first season, and the non-reproductive female from the second season, expressed torpor on over 10% of all recording days. The percent time spent in torpor was low (less than 1%) for most of the remaining individuals. However, the total percentage of daytime T_as that fell below 28°C (the lowest threshold T_b) over the entirety of two study seasons was only 34% and the mean T_a recorded at the time of each daily T_b minima was 28.1 ± 5.0 °C (range: 19.2 - 39.1 °C) and T_{tree} was 25.8 ± 2.1 (range: 20.9 - 32.1 °C).

A re-analysis of respirometry measurements on the thermoconforming individuals presented in Chapter 4 indicated that it took anywhere from 1.75 - 7.74 h for animals to reach a steady-state T_b once exposed to an experimental temperature. The time to a steady state was dependent on T_a ($F_{1, 32} = 6.20$, $p = 0.018$) and the interaction between T_a and T_b ($F_{1, 32} = 6.23$, $p = 0.018$), but not body mass, which was not included in the best-fitting model. A subset of these data from 21 - 24°C, matching the average minimum full-day T_a for the study site (~ 22°C), showed that, regardless of T_b or mass, it took a mean time of approximately 3.77 ± 1.46 h (n= 14, range: 1.75 - 6.99 h) for the animals to reach a steady state. Assuming that nighttime activity ceased between 04:30 and 05:30 (Chapter 3), T_b would reach equilibrium with T_a anywhere between 08:00 and 09:40, by which time the mean T_a at the site was already 27.3 ± 2.3 °C (range 22.4 - 32.5 °C) and T_{tree} was 26.6 ± 2.4 °C (range: 22.5 - 29.7); both temperatures which fall within the measured TNZ for this species. Similarly, the temperature 250 mm below the ground surface (at a similar depth to 7.4% of all nest sites occupied during the second season, Chapter 2) rarely decreased below 25°C, equal to the lower limit of the thermoneutral zone, at any time. T_a increased throughout the day, leading to a corresponding increase in T_b (Fig 5-1, and 5-4).

In terms of potential torpor, increases in T_a prevented T_b decreasing far below 27 °C, making it almost impossible to detect torpor accurately unless, as was likely the case with the male Fig 5-4A, activity ceased early at night (before 03:00). In addition, on a number of occasions, mainly in the early-to-middle parts of the season (before the decrease in T_a seen around day 100), multiple instances of putative “hyperthermic daily torpor” were observed.

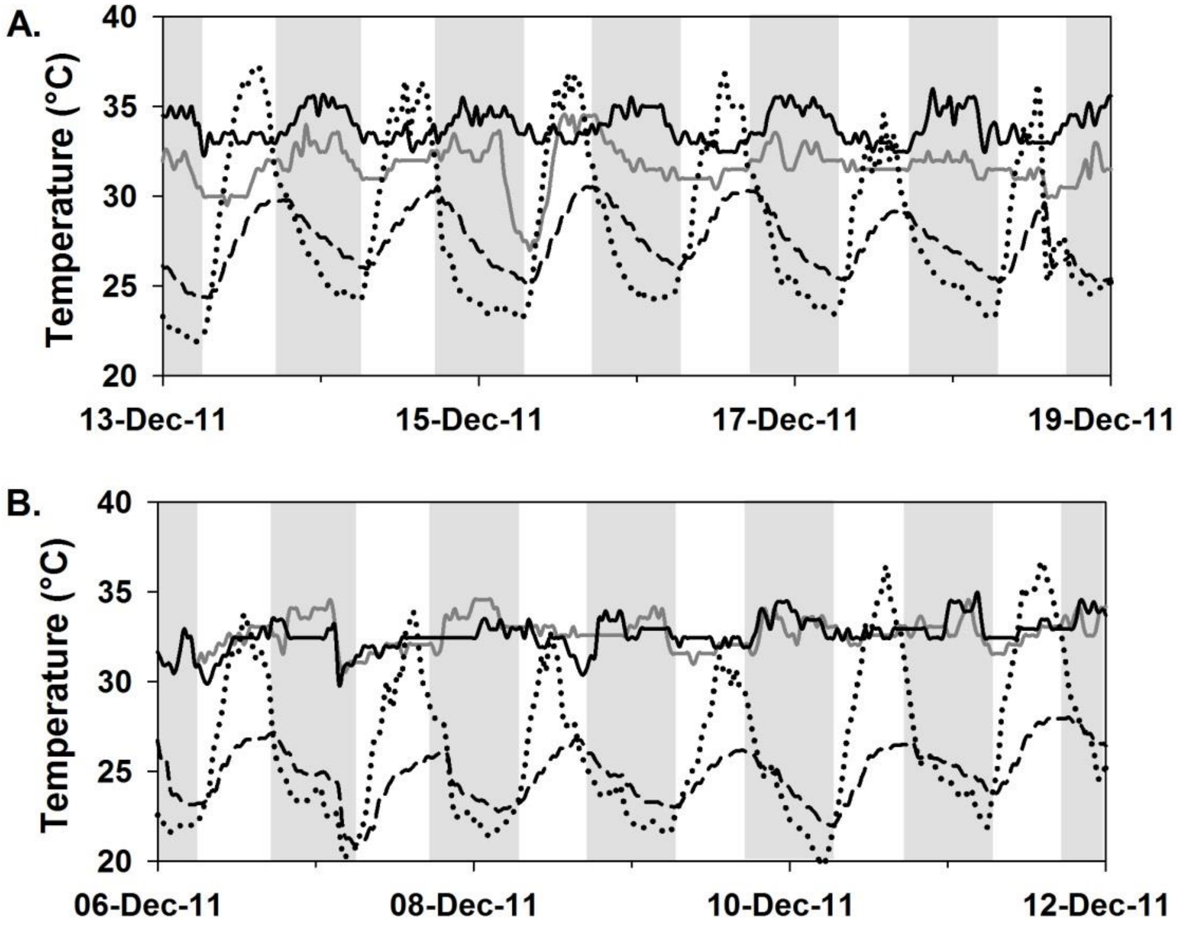


Figure 5-4 Representative traces of T_b of free ranging *Setifer setosus*. Females (black lines) had generally higher, less variable, T_b than males (grey lines), more so during gestation (A.) than during lactation (B.). Torpor expression was highly limited by high environmental temperatures, both air (dotted line) and from a former nest site in a tree cavity (dashed line).

This hypothetical form of torpor has been described in more detail elsewhere (Lovegrove *et al.* 2014) but, briefly, torpor bouts initiated at the end of the nocturnal activity period continue beyond the point where T_a increases above normothermic T_b leading to greater T_bs during the rest phase. In fact, on 13.3% of all recording days, the max rest-phase T_b was higher than the highest individual modal T_b for the active phase (34°C). These are clearly visible in Fig 5-3 and fall mostly between noon and 18:00. On many of these days, as described in Lovegrove *et al.* (2014), T_b decreased with the commencement of activity, as can be seen around 15 December in the trace from the male in Fig 5-5A.

Discussion

T_b datasets from free-ranging animals in warm climates are valuable but rare. This study is one of the first to present a long-term dataset of core T_b collected from a free-ranging basoendotherm during reproduction, as well as from a mammal inhabiting an environment where T_a routinely increases above T_b. I report a number of novel findings. First, torpor use by free-ranging *S. setosus* during the active (reproductive season) was much lower than was expected based on previous studies in the laboratory. Second, data obtained from reproductive females confirmed the results of laboratory studies on similar species and demonstrated an increase in homeothermy during both gestation and lactation (Stephenson & Racey 1993b, Poppitt *et al.* 1994).

Circadian rhythms in T_b are a characteristic of all endotherms, with T_b decreasing during the rest phase, and usually increasing at the onset of activity. The level of T_b variability during the rest phase is often dependent on T_a (Aschoff 1981, Refinetti & Menaker 1992), although this is amplified in some species compared to others (Refinetti 1998). One of the characteristics of basoendotherms (Lovegrove 2012a) is that T_b is highly variable and that the distinction between normothermy and rest phase decreases in T_b is often indistinct (Kuchel 2003, Grigg *et al.* 2004, Canale, Levesque & Lovegrove 2012). A study on *E. telfairi* held in captivity over a range of T_as showed rest-phase decreases in T_b that ranged from slight transient decreases (to ~ 28°C from 31°C) at a T_a of 27°C, to larger more profound decreases (clear incidences of torpor) at lower T_as. The patterns observed in this study of *S. setosus* more closely mirrored that of *E. telfairi* housed at 27°C, despite a wider range of T_a present at the study site. Interestingly, the time of day at which T_b reached either a maximum or a minimum was unrelated to the peaks in the T_a data, and all the parameters of environmental temperature showed very little influence on the extreme T_bs. However, thermal inertia and the large daily changes in amplitude in both T_a and T_{tree} can explain these patterns. As has been described in Lovegrove *et al.* (2014), these large increases in environmental temperatures occasionally led to rest-phase T_b increasing above the active phase T_b. Although this pattern is fairly novel, it has now been observed in a number of species (Lovegrove *et al.* 2014) and is likely to become more common as T_b data is collected from more species of nocturnal, tree-nesting mammals inhabiting warm climates.

In addition to smaller daily variations in T_b, torpor expression in this species was much lower than was expected based upon previous studies on captive animals. Despite the fact that torpor expression is often underestimated in the laboratory (see Geiser *et al.* 2000 for a review), this study presents one of the few cases where the opposite pattern was observed. This is primarily due to the interactions with environmental temperatures, however, and not the physiological characteristics of this species *per se*. The results clearly demonstrate that the T_a of the study site was not conducive to low T_b during torpor. Even during hibernation at this study site, T_b closely tracked T_a throughout hibernation and increased to above 30°C every day (Fig 5-1). Interestingly, the highest T_bs measured in *S. setosus* (> 36°C) occurred on two occasions during hibernation, both immediately preceding a change in T_b amplitude, which, in *C. medius*, was indicative of a change in nest site (Dausmann *et al.* 2005). However, high T_b measured during torpor does not necessarily negate metabolic down-regulation, and significant energy savings from torpor at high T_as (32 - 35°C) have been demonstrated in a species of spiny mouse (*Acomys russatus*, Grimpo *et al.* 2013).

The use of heterothermy in warm climates comes at a relatively low cost as high temperatures facilitate passive rewarming compared with the more energetically costly active rewarming necessary in colder climates (Lovegrove, Kortner & Geiser 1999, Canale *et al.* 2012). Nevertheless, despite a reliance upon exogenous passive heating, functional brown adipose tissue has been observed in *E. telfairi* (Oelkrug *et al.* 2013) and earlier studies indicate that *S. setosus* also possess the ability to rewarm from torpor (Kayser 1960, Hildwein 1964). However, the need for active rewarming from torpor seemed completely negated in this study, and in all cases, even during hibernation, passive rewarming was employed. *S. setosus* can be active at T_bs as low as 28°C (Eisenberg & Gould 1969, Crompton *et al.* 1978), after which point activity itself may be used as a means of heat production (see Humphries & Careau 2011). Thus arousal from torpor in this species occurs at a comparatively lower cost than it does in in typical daily heterotherms. It should be noted, however, that the body mass of the animals used in the Crompton study were suspiciously low (~ 120 g), indicating the use of either juveniles or *E. telfairi* which can easily be mistaken for *S. setosus* where their ranges overlap (Eisenberg & Gould 1969).

Similar difficulties in distinguishing resting T_bs from active T_bs and torpor from normothermy occurred during a study of another basoendothermic mammal, the echidna (*Tachyglossus aceulatus*, Order: Monotremata) in semi-tropical Queensland (Kuchel 2003). Echidnas are characterised by their low and highly variable T_b (Grigg & Beard 2000, Nicol & Andersen 2006, Brice 2009) and during the warmer summer months the distinction between normal rest-phase decreases in T_b and short torpor bouts is difficult to discern. The T_as measured in those studies were lower than those measured in the current study, and echidnas shelter underground, where T_as remain below normothermic T_bs year-round allowing for larger decreases in T_b than was observed in *S. setosus*. The high level of variability in echidna T_b led Kuchel (2003) to caution defining torpor based on T_b patterns (i.e. T_b above or below a certain threshold) and suggested the use of mechanistic properties, such as metabolic rate or heart rate, instead of T_b. A greater push for mechanism-based definitions of torpor has received more attention as T_b patterns of free-ranging individuals from a wider range environments become available (Boyles, Smit & McKechnie 2011, Canale *et al.* 2012). As a single value for T_b can occur during many different metabolic states (Brice *et al.* 2002, Canale *et al.* 2012) and activity can occur at surprisingly low T_b (Kuchel 2003, Rojas, Körtner & Geiser 2012, Turner *et al.* 2012), it has been suggested that more studies should attempt to measure field metabolic rate or employ other more accurate proxies for metabolism such as heart rate (Anderson & Jetz 2005, Speakman & Krol 2010, Canale *et al.* 2012, Boyles *et al.* 2013).

Even in a torpid state, it does seem possible that individual *S. setosus* have some control over their T_b during the rest phase, as the choice of nest site has a large impact on their thermal environment (Dausmann *et al.* 2005, Dausmann, Glos & Heldmaier 2009). Behavioural means of avoiding torpor have been observed in a primate from mainland Africa, *Galago moholi* (Nowack *et al.* 2013), as well as from a number of temperate heterotherms whose choice of hibernacula can vary according to sex or reproductive conditions (Buck & Barnes 1999, Willis & Brigham 2005). Interestingly, only a very small percentage (7.4%) of nest sites observed during the first season were below ground (Chapter 2). A large number of nest sites were fully or partially exposed such that T_bs were equal to the T_{tree} measurements or somewhere between T_{tree} and T_a. Thus, on the majority of days, the animals chose a thermal environment that would actively prevent the opportunity for low T_b during torpor. The increased costs of low T_b – high costs of rewarming and potentially lower vigilance due to increased lethargy – presumably outweigh the minimal benefits of a slightly lower T_b during torpor (Bieber *et al.* 2014).

Respirometry measurements obtained from this population demonstrated that gestation and lactation resulted in a decrease in heterothermy, both in terms of an overall decrease in T_b variability (during gestation), as well as a decrease in torpor use (during both stages of reproduction), in female *S. setosus* (Chapter 4). Torpor has been observed in pregnant tenrecs in captivity, but it is rare compared with heterotherms from more temperate climates (Nicoll & Thompson 1987, Stephenson & Racey 1995, Willis, Brigham & Geiser 2006). Temperate heterotherms have been shown to use torpor to extend gestation so that lactation, the most energetically costly stage in the life history of female mammals (Clutton-Brock, Albon & Guinness 1989), coincides with peak food availability (Richard *et al.* 2002, Willis *et al.* 2006) or with more favourable environmental conditions (Morrow & Nicol 2009). In the current study torpor was only observed on two occasions during reproduction, both in the same female in the early stages of gestation during the second season 46 - 49 days prior to parturition (Chapter 4) and at T_as much lower than those commonly observed at the study side (14 and 21 °C).

Torpor in lactating females, however, cannot conclusively be ruled out. Two of the females studied during the second season showed a pronounced decrease of around 1 - 2°C below normal rest phase T_bs on the estimated date of parturition. T_b recordings from these individuals during lactation were only available for short periods of the time as both mothers were killed by an unknown ground predator before their litter was weaned. Interestingly, two females during the first season spent very little time active during lactation. One female did not leave the nest site at night for an entire month (Chapter 3). Although no T_b data are available to confirm the use of torpor over this time period, given the environmental conditions and the fact that both females maintained a relatively steady body mass throughout lactation, it seems likely. More data are necessary to conclude whether or not torpor is common during lactation in tenrecs.

The effects of reproduction on the thermoregulation of males was more difficult to determine, as it is not possible to measure the reproductive status of males due to the lack of external testes (Petter & Petter-Rousseaux 1963, Kleisner, Ivell & Flegr 2010). Elevated levels of testosterone in reproductively active males have been shown to reduce torpor expression (Mzilikazi & Lovegrove 2002, Fietz, Klose & Kalko 2010). Likewise, warm temperatures are necessary for spermatogenesis (Barnes *et al.* 1986, Kleisner *et al.* 2010). However, only short periods at normothermic temperatures are necessary for adequate sperm production (Fowler & Racey 1987) and the high rest-phase T_as experienced by this population would negate any potential negative effects of torpor on spermatogenesis.

Therefore, torpor avoidance for these reasons was not likely in this population. Brown adipose tissue deposits have been found surrounding the reproductive organs of *E. telfairi*, which indicates the potential utility of warming this area in habitats with colder T_as (Oelkrug *et al.* 2013) but the high T_as at the current study offset the necessity of physiological means of maintaining high T_bs.

Conclusion

This study confirmed that the thermoregulatory patterns of *S. setosus* show a large degree of heterothermy, in terms of both torpor and in daily T_b variations. The thermoregulatory patterns of this species, a tropical dwelling basoendotherm, stand in sharp contrast with those of most high latitude species. Whereas the latter group (meso- and supraendotherms sensu Lovegrove 2012a) maintain elevated T_bs over a wide range of T_a, primarily through physiological mechanisms, and homeothermy is the norm, *S. setosus* rather rely on T_a to regulate T_b, and thermolability is the norm. As seen in this study and in Chapter 4 reproduction and high T_a were cause for an increase in the level of homeothermy. Increases in homeothermy during reproduction has been observed in other species displaying basoendothermic characteristics, such as echidnas (Beard & Grigg 2000, Nicol & Andersen 2006) and sloths (*Bradypus griseus*, Morrison 1945). This study, along with a small sample of skin temperatures from tarsiers (*Tarsius syrichta*) presented in Lovegrove *et al.* (2014) are the only studies, to my knowledge, observe how T_as at or near T_b affect thermoregulation in basoendotherms. Both of these datasets show that high T_a can reduce the opportunities for low T_b during torpor and potentially mask the physiological state of the animal (Canale *et al.* 2012, Lovegrove *et al.* 2014).

Interestingly, females in this population hibernate for at least five months and have been shown to give birth to up to three litters in a single season, while simultaneously accumulating sufficient fat reserves (> 100 g) for the subsequent hibernation period (Chapters 2 and 3). Low thermoregulatory costs, combined with high levels of food availability, could have contributed to the high energetic outputs observed in this species. The findings show that the increase in homeothermy observed in captive tenrecs during reproduction does indeed occur in the wild, where, although food availability is not likely to be equal to *ad libitum* food, T_a can be much more favourable. Along with data collected from incubating echidnas (Beard & Grigg 2000, Nicol & Andersen 2006) as well as gestating sloths (Morrison 1945), these data provide further support for the parental care hypotheses for the evolution of endothermy in mammals. The thermal environment of the study site was conducive to the maintenance of a high and stable T_b, with very little added energetic costs to thermoregulation. Slight increases in homeothermy during reproduction in similarly warm habitats were therefore a probable first step along the progressive evolution from heterothermic to homeothermic endothermy (Kemp 2006, Lovegrove 2012a).

Literature Cited

Anderson, K. J. & W. Jetz (2005) The broad-scale ecology of energy expenditure of endotherms. *Ecol Lett,* **8,** 310-318.

Aschoff, J. (1981) Thermal conductance in mammals and birds: Its dependence on body size and circadian phase. *Comp Biochem Physiol A,* **69,** 611-619.

Barnes, B. M., M. Kretzmann, P. Licht & I. Zucker (1986) The influence of hibernation on testis growth and spermatogenesis in the Golden-mantled ground-squirrel, *Spermophilus lateralis*. *Biol Reprod,* **35,** 1289-1297.

Beard, L. A. & G. C. Grigg (2000) Reproduction in the short-beaked echidna, *Tachyglossus aculeatus*: Field observations at an elevated site in South-east Queensland. *Proc Linn Soc N S W,* **122,** 89-99.

Bieber, C., K. Lebl, G. Stalder, F. Geiser & T. Ruf (2014) Body mass dependent use of hibernation: Why not prolong the active season, if they can? *Funct Ecol***,** online first. DOI: 10.1111/1365-2435.12173.

Boyles, J. G., B. Smit & A. E. McKechnie (2011) Does use of the torpor cut-off method to analyze variation in body temperature cause more problems than it solves? *J Therm Biol,* **36,** 373-375.

Boyles, J. G., A. B. Thompson, A. E. McKechnie, E. Malan, M. M. Humphries & V. Careau (2013) A global heterothermic continuum in mammals. *Glob Ecol Biogeogr,* **22,** 1029-1039.

Brice, P. H. (2009) Thermoregulation in monotremes: riddles in a mosaic. *Aust J Zool,* **57,** 255–263.

Brice, P. H., G. C. Grigg, L. A. Beard & J. A. Donovan (2002) Patterns of activity and inactivity in echidnas (*Tachyglossus aculeatus*) free-ranging in a hot dry climate: correlates with ambient temperature, time of day and season. *Aust J Zool,* **50,** 461-475.

Buck, C. L. & B. M. Barnes (1999) Temperatures of hibernacula and changes in body composition of arctic ground squirrels over winter. *J Mammal,* **80,** 1264-1276.

Burnham, K. & D. Anderson (2002) *Model Selection and Multimodal Inference: A Practical Information-Theoretic Approach* 2nd edn. New York, NY: Springer-Verlag.

Canale, C. I., D. L. Levesque & B. G. Lovegrove (2012) Tropical heterothermy: Does the exception prove the rule or force a re-definition? In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*: 29-40. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin Heidelberg.

Careau, V., D. Réale, D. Garant, J. R. Speakman & M. M. Humphries (2011) Stress-induced rise in body temperature is repeatable in free-ranging Eastern chipmunks (*Tamias striatus*). *J Comp Physiol B,* **182,** 403-414.

Clarke, A. & H.-O. Pörtner (2010) Temperature, metabolic power and the evolution of endothermy. *Biol Rev,* **85,** 703-727.

Clutton-Brock, T. H., S. D. Albon & F. E. Guinness (1989) Fitness costs of gestation and lactation in wild mammals. *Nature,* **337,** 260-262.

Crompton, A. W., C. R. Taylor & J. A. Jagger (1978) Evolution of homeothermy in mammals. *Nature,* **272,** 333-336.

Dausmann, K. H., J. Glos, J. U. Ganzhorn & G. Heldmaier (2005) Hibernation in the tropics: lessons from a primate. *J Comp Physiol B,* **175,** 147-155.

Dausmann, K. H., J. Glos & G. Heldmaier (2009) Energetics of tropical hibernation. *J Comp Physiol B,* **179,** 345-357.

Douady, C. J., F. Catzeflis, D. J. Kao, M. S. Springer & M. J. Stanhope (2002) Molecular evidence for the monophyly of Tenrecidae (Mammalia) and the timing of the colonization of Madagascar by Malagasy tenrecs. *Mol Phylogenet Evol,* **22,** 357-363.

Eisenberg, J. F. & E. Gould (1969) The tenrecs: a study in mammalian behavior and evolution. *Smithson Contrib Zool,* **27,** 1-156.

Eisentraut, M. (1960) Heat regulation in primitive mammals and in tropical species. *Bull Mus Comp Zool,* **124,** 31-43.

Farmer, C. G. (2000) Parental care: the key to understanding endothermy and other convergent features in birds and mammals. *Am Nat,* **155,** 326-334.

Fietz, J., S. M. Klose & E. K. V. Kalko (2010) Behavioural and physiological consequences of male reproductive trade-offs in edible dormice (*Glis glis*). *Naturwissenschaften,* **97,** 883-890.

Fowler, P. & P. Racey (1987) Relationship between body and testis temperatures in the European hedgehog, *Erinaceus europaeus,* during hibernation and sexual reactivation. *J Reprod Fertil,* **81,** 567-573.

Geiser, F. (1994) Hibernation and daily torpor in marsupials - a review. *Aust J Zool,* **42,** 1-16.

Geiser, F., J. C. Holloway, G. Körtner, T. A. Maddocks, C. Turbill & R. M. Brigham (2000) Do patterns of torpor differ between free-ranging and captive mammals and birds. In: *Life In The Cold: 11th International Hibernation Symposium*: 95-102. G. Heldmaier & M. Klingenspor (Eds.). Springer-Verlag, Berlin.

Geiser, F. & T. Ruf (1995) Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiol Zool,* **68,** 935-966.

Gerkema, M. P., W. I. L. Davies, R. G. Foster, M. Menaker & R. A. Hut (2013) The nocturnal bottleneck and the evolution of activity patterns in mammals. *Proc R Soc B,* **280,** 20130508.

Grigg, G. C. & L. A. Beard (2000) Hibernation by echidnas in mild climates: hints about the evolution of endothermy? In: *Life in the Cold: Eleventh Internation Hibernation Symposium*: 5-20. G. Heldmaier & M. Klingenspor (Eds.). Springer, New York.

Grigg, G. C., L. A. Beard & M. L. Augee (1989) Hibernation in a monotreme, the echidna (*Tachyglossus aculeatus*). *Comp Biochem Physiol A,* **92,** 609-612.

Grigg, G. C., L. A. Beard & M. L. Augee (2004) The evolution of endothermy and its diversity in mammals and birds. *Physiol Biochem Zool,* **77,** 982-997.

Grimpo, K., K. Legler, G. Heldmaier & C. Exner (2013) That’s hot: golden spiny mice display torpor even at high ambient temperatures. *J Comp Physiol B,* **183,** 567-581.

Hildwein, G. (1964) La dépense d'énergie minimale de deux Insectivores de Madagascar en hibernation profonde au cours de l'été boréal. *CR Hebd Seances Acad Sci Paris,* **259,** 2009-2011.

Humphries, M. M. & V. Careau (2011) Heat for nothing or activity for free? Evidence and implications of activity-thermoregulatory heat substitution. *Integr Comp Biol,* **51,** 419-431.

Kayser, C. (1960) Cosommation d'oxygène et température centrale au cours de l'hiver austral de deux Insectivores de Madagascar, *Centetes ecaudatus* et *Setifer setosus*. *Compt Rend Soc Biol,* **154,** 1873-1876.

Kemp, T. (2006) The origin of mammalian endothermy: a paradigm for the evolution of complex biological structure. *Zool J Linn Soc,* **147,** 473-488.

Kleisner, K., R. Ivell & J. Flegr (2010) The evolutionary history of testicular externalization and the origin of the scrotum. *J Biosci,* **35,** 27-37.

Koteja, P. (2000) Energy assimilation, parental care and the evolution of endothermy. *Proc R Soc B,* **267,** 479-484.

Kuchel, L. (2003) *The energetics and patterns of torpor in free-ranging Tachyglossus aculeatus from a warm-temperate climate.* PhD, University of Queensland, Brisbane.

Lovegrove, B. G. (2000) The zoogeography of mammalian basal metabolic rate. *Am Nat,* **156,** 201-219.

Lovegrove, B. G. (2009) Modification and miniaturization of Thermochron iButtons for surgical implantation into small animals. *J Comp Physiol B,* **179,** 451-458.

Lovegrove, B. G. (2012a) The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol Rev,* **87,** 128–162.

Lovegrove, B. G. (2012b) A single origin of heterothermy in mammals. In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*: 3-11. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin

Lovegrove, B. G., C. I. Canale, D. L. Levesque, G. Fluch, M. Řeháková-Petrů & T. Ruf (2014) Are tropical small mammals physiologically vulnerable to Arrhenius effects and climate change? *Physiol Biochem Zool,* **87,** 30-45.

Lovegrove, B. G. & F. Génin (2008) Torpor and hibernation in a basal placental mammal, the Lesser Hedgehog Tenrec, *Echinops telfairi*. *J Comp Physiol B,* **178,** 691-698.

Lovegrove, B. G., G. Kortner & F. Geiser (1999) The energetic cost of arousal from torpor in the marsupial *Sminthopsis macroura*: benefits of summer ambient temperature cycles. *J Comp Physiol B,* **169,** 11-18.

Luo, Z. X. (2007) Transformation and diversification in early mammal evolution. *Nature,* **450,** 1011-1019.

Mazerolle, M. J. (2013) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.28.

McKechnie, A. E., R. A. M. Ashdown, M. B. Christian & R. M. Brigham (2007) Torpor in an African caprimulgid, the freckled nightjar *Caprimulgus tristigma*. *J Avian Biol,* **38,** 261-266.

Morrison, P. R. (1945) Acquired homiothermism in the pregnant sloth. *J Mammal,* **26,** 272-275.

Morrow, G. & S. C. Nicol (2009) Cool sex? Hibernation and reproduction overlap in the echidna. *PLoS ONE,* **4,** e6070.

Mzilikazi, N. & B. G. Lovegrove (2002) Reproductive activity influences thermoregulation and torpor in the pouched mouse, *Saccostomus campestris J Comp Physiol B,* **172,** 7-16.

Nicol, S. & N. A. Andersen (2006) Body temperature as an indicator of egg-laying in the echidna, *Tachyglossus aculeatus*. *J Therm Biol,* **31,** 483-490.

Nicoll, M. E. & S. D. Thompson (1987) Basal metabolic rates and energetics of reproduction in therian mammals: marsupials and placentals compared. In: *Reproductive Energetics in Mammals*: 7-27. A. S. I. Loudon & P. A. Racey (Eds.). Clarendon Press, Oxford, UK.

Nowack, J., M. Wippich, N. Mzilikazi & K. H. Dausmann (2013) Surviving the cold, dry period in africa: Behavioral adjustments as an alternative to heterothermy in the african lesser bushbaby (*Galago moholi*). *Int J Primatol,* **34,** 49-64.

O'Leary, M. A., J. I. Bloch, J. J. Flynn, T. J. Gaudin, A. Giallombardo, N. P. Giannini, S. L. Goldberg, B. P. Kraatz, Z.-X. Luo, J. Meng, X. Ni, M. J. Novacek, F. A. Perini, Z. S. Randall, G. W. Rougier, E. J. Sargis, M. T. Silcox, N. B. Simmons, M. Spaulding, P. M. Velazco, M. Weksler, J. R. Wible & A. L. Cirranello (2013) The placental mammal ancestor and the post–K-Pg radiation of placentals. *Science,* **339,** 662-667.

Oelkrug, R., N. Goetze, C. Exner, Y. Lee, G. K. Ganjam, M. Kutschke, S. Müller, S. Stöhr, M. H. Tschöp, P. G. Crichton, G. Heldmaier, M. Jastroch & C. W. Meyer (2013) Brown fat in a protoendothermic mammal fuels eutherian evolution. *Nat Commun,* **4,** e2140.

Olson, L. E. & S. M. Goodman (2003) Phylogeny and biogeography of tenrecs. In: *The Natural History of Madagascar*: 1235-1242. S. M. Goodman & J. P. Benstead (Eds.). The University of Chicago Press, Chicago, USA.

Petter, J.-J. & A. Petter-Rousseaux (1963) Notes biologiques sur les Centetinae. *La Terre et la Vie* **17,** 66-80.

Pinheiro, J. & D. Bates (2000) *Mixed-effects models in S and S-PLUS Springer*. New York, NY: Springer Verlag.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar & R Development Core Team (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-108.

Poppitt, S. D., J. R. Speakman & P. A. Racey (1994) Energetics of reproduction in the lesser hedgehog tenrec, *Echinops telfairi* (Martin). *Physiol Zool,* **67,** 976-994.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Refinetti, R. (1998) Body temperature and behavior of tree shrews and flying squirrels in a thermal gradient. *Physiol Behav,* **63,** 517-520.

Refinetti, R. & M. Menaker (1992) The circadian-rhythm of body temperature. *Physiol Behav,* **51,** 613-637.

Richard, A. F., R. E. Dewar, M. Schwartz & J. Ratsirarson (2002) Life in the slow lane? Demography and life histories of male and female sifaka (*Propithecus verreauxi verreauxi*). *J Zool (Lond.),* **256,** 421-436.

Robertson, D. S., M. C. McKenna, O. B. Toon, S. Hope & J. A. Lillegraven (2004) Survival in the first hours of the Cenozoic. *Geol Soc Am Bull,* **116,** 760-768.

Rojas, A. D., G. Körtner & F. Geiser (2012) Cool running: locomotor performance at low body temperature in mammals. *Biol Lett,* **8,** 868-870.

Sato, H. (2012) Diurnal resting in brown lemurs in a dry deciduous forest, northwestern Madagascar: implications for seasonal thermoregulation. *Primates,* **53,** 255-263.

Speakman, J. R. & E. Krol (2010) Maximal heat dissipation capacity and hyperthermia risk: neglected key factors in the ecology of endotherms. *J Anim Ecol,* **79,** 726-746.

Stephenson, P. J. & P. A. Racey (1993a) Reproductive energetics of the Tenrecidae (Mammalia: Insectivora). I. The large-eared tenrec, *Geogale aurita*. *Physiol Zool,* **66,** 643-663.

Stephenson, P. J. & P. A. Racey (1993b) Reproductive energetics of the Tenrecidae (Mammalia: Insectivora). II. The shrew-tenrecs, *Microgale* spp. *Physiol Zool,* **66,** 664-685.

Stephenson, P. J. & P. A. Racey (1995) Resting metabolic rate and reproduction in the Insectivora. *Comp Biochem Physiol A,* **112,** 215-223.

Turner, J. M., G. Körtner, L. Warnecke & F. Geiser (2012) Summer and winter torpor use by a free-ranging marsupial. *Comp Biochem Physiol A,* **162,** 274-280.

Willis, C. K. R. & R. M. Brigham (2005) Physiological and ecological aspects of roost selection by reproductive female hoary bats (*Lasiurus cinereus*). *J Mammal,* **86,** 85-94.

Willis, C. K. R., R. M. Brigham & F. Geiser (2006) Deep, prolonged torpor by pregnant, free-ranging bats. *Naturwissenschaften,* **93,** 80-83.

Chapter 6 Summary and General Conclusions

Life History and Energetics of a Free-Ranging Basoendotherm: *Setifer setosus*

Setifer setosus hibernate for up to seven consecutive months, and their short active season is used to accumulate large fat stores while simultaneously achieving high reproductive outputs. Free-ranging *S. setosus* make full use of a limited period of activity by way of surprisingly large energetic outputs. Females produce up to three litters in a single season (Chapter 3). Males cover large home ranges in order to overlap with those of multiple females (Chapter 2), potentially leading to significant reproductive outputs for both sexes. Previous studies have also shown that *S. setosus* is capable of maturing early (Mallinson 1974) and produces litters with larger masses than other species of similar body size (Symonds 1999). Although the former is a characteristic of 'fast' species, the latter is usually associated with 'slow' life-histories (Charnov & Ernest 2006). Early maturation, when added to the high number of litters produced per year observed in the current study, places *S. setosus* further towards the 'fast' side of the mammalian life-history continuum. The high level of adult and juvenile mortality observed in this study (Chapter 3) is certainly the primary driver for this shift into the fast lane for *S. setosus,* as well as for the other spiny tenrecs species that make up the Tenrecinae (Read & Harvey 1989, Promislow & Harvey 1990, Symonds 2005). In contrast, members of the other two tenrec subfamilies endemic to Madagascar, whose small size and cryptic nature reduce the risk of predation, fall into the 'slow' category for their size, maturing late, and producing only a single litter of 1-3 young per year (Eisenberg & Gould 1969, Stephenson & Racey 1995, Symonds 1999). Such extremes in life-history characteristics are believed to be a result of the high level of unpredictability and variability of resource availability on the island of Madagascar (Wright 1999, Symonds 2005, Dewar & Richard 2007).

The high energetic outputs of *S. setosus* can be attributed to measures used to counteract high mortality and unpredictable energy availability. These reasons do not however, explain how such high outputs are possible, especially given that high energy assimilation is usually considered to be incompatible with low metabolic rates (Koteja 2000, Brown *et al.* 2004, Koteja 2004). The characterisation of the thermoregulatory physiology of *S. setosus* presented in this thesis can help shed light on this apparent disconnect. Although both gestating and lactating females maintained a higher degree of homeothermy, the costs of this added level of thermoregulation were much lower than expected. The thermal profiles of both reproductive and non-reproductive *S. setosus* described in Chapter 4 illustrate that energetic cost to increases in homeothermy within the thermoneutral zone are negligible. The thermoneutral zone itself was also rather large, spanning from 25°C to at least 33.5°C, which is much larger than would be predicted for a 250-300 g animal (Riek & Geiser 2013). Although the relative costs of maintaining T_b at temperatures below the lower limit of the thermoneutral zone were high, these temperatures were only rarely reached during the daytime rest phase (Chapter 5). Presumably, with so little energy allocated to temperature regulation, most available energy is allocated towards increases in body mass and reproductive output, in the form of multiple litters and large neonates for females, and a high level of activity in males, resulting in large home ranges and the potential for multiple partners. The ability to amass large energy stores would be necessary both in order to survive the dry austral winter, as well as to cope with potential lulls in resource availability (Jönsson 1997, Dewar & Richard 2007).

The Energetics of Basoendothermy

The low thermoregulatory costs of living in the warm environment of Madagascar and the capacity for the accumulation of sufficient energy to survive colder periods, explain, at least partially, why *S. setosus*, as well as other tenrecs, have maintained pleisiomorphic thermoregulatory characteristics. Freed from the need to maintain a high and constant T_b, energy reserves can be directed to non-thermogulatory demands, in the case of *S. setosus*, towards increasing fecundity to counter high predation pressure. The data presented in Chapter 4 are unique in that they present the first full characterisation of the thermal profile from a ‘basoendothermic’ (*sensu* Lovegrove 2012a) mammal during reproduction. Previous studies on the thermal profiles of basoendotherms failed to locate a thermoneutral zone in the classic sense of the Scholander-Irving Model (Nicoll & Thompson 1987, Stephenson & Racey 1994, Brice 2008, Oelkrug *et al.* 2013). In those studies T_b closely tracked T_a leading to the difficulties both in determining basal metabolic rates as well as delineating torpor from normothermy. Oelkrug *et al.* (2013) attempted to circumvent this problem by agitating the animals and providing them with excess to food at all temperatures below 30°C. Although the procedure allowed for the measurement of ‘resting’ rates, and the animals did indeed defend an elevated T_b at low T_a, the results cannot be considered truly representative of the resting state of these animals.

Forced arousal from a torpid state in order to attain a thermal profile resembling the classical Scholander-Irving model merely masks a thermoregulatory reality, namely that there is a lack of a T_b set-point that differentiates thermoregulation in basoendotherms from species which show a higher degree of homeothermy. My data show that by allowing T_b to decrease along with T_a basoendotherms are capable of reducing the T_b - T_a gradient which allows for lower rates of thermal conductance as well as a widening of the range of T_a over which minimum rates of metabolism are measured. Such flexible thermoregulation usually corresponds with a parallel ability to be active over a larger range of T_bs (Brice *et al.* 2002, Kuchel 2003, Wooden & Walsberg 2004, this study). Interestingly, the idea that endotherms are capable of operating over a wide range of T_bs and may display similar specialist/generalist thermal adaptations to ectotherms has only recently been proposed (Angilletta *et al.* 2010, Boyles *et al.* 2011). Under this classification system basoendotherms, such as tenrecs, are the best examples of thermal generalists because optimal performance is not confined to a narrow range of T_bs. The various advantages of these modes of thermoregulation, however, have yet to be fully evaluated (Boyles *et al.* 2013), and the diversity displayed by modern mammals may provide important clues on the evolution of endothermy (Lovegrove 2012a).

Grigg *et al.* (2004) and Brice *et al*. (in prep, see also Brice 2008) have predicted that the higher degree of homeothermy during gestation and lactation such as observed in *S. setosus* would result in a thermal profile with a closer fit to the Scholander-Irving model. Presumably, thermal profiles measured in other species of basoendotherms during reproduction should show similar patterns. Conversely, torpor during reproduction has also been shown in a number of species (Geiser, McAllan & Brigham 2005, Willis, Brigham & Geiser 2006, Canale, Perret & Henry 2012) including the basoendothermic echidna (Morrow & Nicol 2009) indicating a trade-off between energy use and length of time devoted to reproduction. The shift to homeothermy during reproduction, and the ability to actively rewarm from torpor, with or without functional brown adipose tissue, has now been observed in all major mammal groupings, and indicates that they all possess the functional ability to maintain a high T_b when necessary (Geiser & Baudinette 1990, Poppitt, Speakman & Racey 1994, Beard & Grigg 2000, Nicol & Andersen 2008). Additional studies on other instances of homeothermy in these species, as well as intra-specific variability in the level of precision of T_b regulation, will help further the understanding of the evolution of endothermy and its diversity in extant mammals (Lovegrove 2012a, Boyles *et al.* 2013).

Linking Metabolism to the Rate and Scope of Life-History Characteristics

S. setosus, as well as other spiny tenrecs, provide interesting challenges to the Metabolic Theory of Ecology which claims that basal metabolism determines the pace of life in mammals. Despite the intuitive appeal of this premise, a species' position on the slow-fast metabolic continuum does not dictate its position on the slow fast life-history continuum (Harvey, Pagel & Rees 1991, Duncan, Forsyth & Hone 2007, Lovegrove 2009, Müller *et al.* 2012). This does not necessarily mean that the underlying concept, that energetic outputs are determined by basal metabolism, is faulty. It merely illustrates that the original MTE equations present an oversimplification, and fail to account for the complexities of the interactions between metabolism, the environment and energy use (Müller *et al.* 2012, Humphries & McCann 2013, Naya *et al.* 2013). Interestingly, whereas, inter-specific studies have led to mixed, though generally negative results (Harvey *et al.* 1991, Duncan *et al.* 2007, Lovegrove 2009, Hamilton *et al.* 2011), there exists some conclusive evidence for a link between BMR and reproductive output at an intra-specific level (see Sadowska, Gębczyński & Konarzewski 2013 for a review).

One of the biggest strengths of comparative studies has been the description of the high level of variability in various mammalian traits and the identification of outliers. As outliers on the slow side of the metabolic rate continuum (Lovegrove 2003), and the fast side of the life-history continuum (Eisenberg 1983), tenrecs provide an interesting test case. My results highlight important factors that are often omitted from macroecological studies, namely, the immediate effects of environmental conditions, and morphological and physiological mammalian diversity. Numerous studies have found that both metabolic and life-history traits are plastic and vary between individuals as well as between populations (Sikes & Ylönen 1998, Lahann, Schmid & Ganzhorn 2006, Nilsen *et al.* 2009, Borries, Gordon & Koenig 2013, Konarzewski & Książek 2013). Mammals also differ vastly in locomotory capacity and their attendant form and function (Lovegrove 2012a, Lovegrove & Mowoe 2013). This variability is often ignored in larger inter-specific studies. It is also questionable whether species with year-long activity periods can be realistically compared with those placed under extreme seasonal constraints (see McNamara & Houston 2008). There is therefore a greater need for both more holistic models that can account for phenotypic plasticity and overall variability, especially as we seek to predict species responses to changing global climates (Canale & Henry 2010, Boyles *et al.* 2011).

Another difficulty with large comparative studies is they assume that thermoregulation in all endotherms is equal, and that strict homeothermy is the norm. Some studies have attempted to control differences in the level of T_b between species by standardising metabolism to a high T_b (Savage *et al.* 2004, White *et al.* 2008) but, as demonstrated in this thesis, it is often the degree of variability in T_b that will determine the costs of thermoregulation, not the level itself. This is especially true in animals living in environments where T_as are close to T_b (Lovegrove *et al.* 2014). Many studies, primarily those undertaken on species from the Northern Hemisphere, assume that a high degree of homeothermy is the defining characteristic of mammalian thermoregulation. This mode of thinking has been perpetuated by a lack of data from tropical and sub-tropical environments (Lovegrove 2003, Lovegrove 2006, Canale, Levesque & Lovegrove 2012, Lovegrove 2012a). Homeothermy is much less common than expected, and is likely to be a derived form of thermoregulation, having evolved via an ancestral heterothermic condition (Crompton, Taylor & Jagger 1978, Grigg *et al.* 2004, Lovegrove 2012a, Lovegrove 2012b, Naya *et al.* 2012, Naya *et al.* 2013). Future hypotheses on the interactions between metabolism and life-history should take the evolutionary history of endothermy into account.

Implications for Future Studies on the Evolution of Endothermy in Mammals

The existence of multiple, and potentially conflicting, hypotheses has made it clear that there is no single cause that led to the evolution of the many forms of endothermy found in extant mammals. Clarke and Rothery (2008, p. 66) state that incongruities between the level of scaling of metabolism and of T_b at the ordinal level points towards "… a complex relationship between mass, T_b and resting metabolic rate and leaves open the intriguing question of whether evolution has adjusted resting metabolic rate through changes in T_b or whether T_b is simply a consequence of resting metabolic rate that has evolved for a particular environment and ecology." Although, there has been more success linking BMR to environmental factors than T_b, indicating that is it is the more likely to be under direct selection (Lovegrove 2000, Clavijo-Baque & Bozinovic 2012, Naya *et al.* 2013), the interactions between the two remain unclear.

As the first study to observe both the thermal physiology and life history characteristics of a free-ranging basoendotherm inhabiting a warm environment, the data presented in this thesis provide some interesting insights. Resting metabolic rates measured over a range of T_as showed that the increases in homeothermy in gestating and lactating females were accompanied by only minor changes in RMR within the thermoneutral zone. The environmental conditions at my study site provided conditions where T_a during the rest phase rarely decreased below thermoneutrality. Thus the fitness benefits of small increments in homeothermy could be offset by the relatively low fitness costs involved in minimal thermoregulatory energy demands. During the fixed temperature trials presented in Chapter 4 homeothermy in *S. setosus* was only observed during reproduction. It is therefore not essential for the everyday functioning of these mammals, at least in warm climates. Increases in homeothermy, in otherwise heterothermic mammals, during reproduction has now been observed in a number of mammalian species (Morrison 1945, Audet & Fenton 1988, Geiser, Körtner & Schmidt 1998, Beard & Grigg 2000, Koteja 2000, Farmer 2003, Nicol & Andersen 2006). It represents a likely first step in the progressive evolution from heterothermic to homeothermic endothermy (Kemp 2006, Lovegrove 2012a). Further study of the relationships between thermoregulation and life-history in free-ranging mammals, especially from basoendotherms inhabiting warm, non-seasonal environments, is needed to further elucidate the complicated relationship between T_b, metabolism and life-history.

Literature Cited

Angilletta, M. J. J., B. S. Cooper, M. S. Schuler & J. G. Boyles (2010) The evolution of thermal physiology in endotherms. *Front Biosci,* **2,** 861-881.

Audet, D. & M. B. Fenton (1988) Heterothermy and the use of torpor by the bat *Eptesicus fuscus* (Chiroptera: Vespertilionidae): a field study. *Physiol Zool,* **61,** 197-204.

Beard, L. A. & G. C. Grigg (2000) Reproduction in the short-beaked echidna, *Tachyglossus aculeatus*: Field observations at an elevated site in South-east Queensland. *Proc Linn Soc N S W,* **122,** 89-99.

Borries, C., A. D. Gordon & A. Koenig (2013) Beware of primate life history data: A plea for data standards and a repository. *PLoS ONE,* **8,** e67200.

Boyles, J. G., F. Seebacher, B. Smit & A. E. McKechnie (2011) Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr Comp Biol,* **51,** 676-690.

Boyles, J. G., A. B. Thompson, A. E. McKechnie, E. Malan, M. M. Humphries & V. Careau (2013) A global heterothermic continuum in mammals. *Glob Ecol Biogeogr,* **22,** 1029-1039.

Brice, M. P. (2008) *Heat tolerance and cold indifference in the short-beaked echidna, Tachyglossus aculeatus: Body temperature management in a mammalian constitutional eurytherm.* PhD, University of Queensland, Brisbane, Qld.

Brice, P. H., G. C. Grigg, L. A. Beard & J. A. Donovan (2002) Patterns of activity and inactivity in echidnas (*Tachyglossus aculeatus*) free-ranging in a hot dry climate: correlates with ambient temperature, time of day and season. *Aust J Zool,* **50,** 461-475.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage & G. B. West (2004) Toward a metabolic theory of ecology. *Ecology,* **85,** 1771-1789.

Canale, C. I. & P. Y. Henry (2010) Adaptive phenotypic plasticity and resilience of vertebrates to increasing climatic unpredictability. *Clim Res,* **43,** 135-147.

Canale, C. I., D. L. Levesque & B. G. Lovegrove (2012) Tropical heterothermy: Does the exception prove the rule or force a re-definition? In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*: 29-40. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin Heidelberg.

Canale, C. I., M. Perret & P. Y. Henry (2012) Torpor use during gestation and lactation in a primate. *Naturwissenschaften,* **99,** 159-163.

Charnov, E. L. & S. K. M. Ernest (2006) The offspring-size/clutch-size trade-off in mammals. *Am Nat,* **167,** 578-582.

Clarke, A. & P. Rothery (2008) Scaling of body temperature in mammals and birds. *Funct Ecol,* **22,** 58-67.

Clavijo-Baque, S. & F. Bozinovic (2012) Testing the fitness consequences of the thermoregulatory and parental care models for the origin of endothermy. *PLoS ONE,* **7,** e37069.

Crompton, A. W., C. R. Taylor & J. A. Jagger (1978) Evolution of homeothermy in mammals. *Nature,* **272,** 333-336.

Dewar, R. E. & A. F. Richard (2007) Evolution in the hypervariable environment of Madagascar. *Proc Natl Acad Sci U S A,* **104,** 13723-13727.

Duncan, R. P., D. M. Forsyth & J. Hone (2007) Testing the Metabolic Theory of Ecology: allometric scaling exponents in mammals. *Ecology,* **88,** 324-333.

Eisenberg, J. F. (1983) *The Mammalian Radiations: An Analysis of Trends in Evolution, Adaptation, and Behavior*. Chicago, IL: University of Chicago Press.

Eisenberg, J. F. & E. Gould (1969) The tenrecs: a study in mammalian behavior and evolution. *Smithson Contrib Zool,* **27,** 1-156.

Farmer, C. G. (2003) Reproduction: the adaptive significance of endothermy. *Am Nat,* **162,** 826-840.

Geiser, F. & R. V. Baudinette (1990) The relationship between body-mass and rate of rewarming from hibernation and daily torpor in mammals. *J Exp Biol,* **151,** 349-359.

Geiser, F., G. Körtner & I. Schmidt (1998) Leptin increases energy expenditure of a marsupial by inhibition of daily torpor. *Am J Physiol,* **275,** R1627-R1632.

Geiser, F., B. M. McAllan & R. M. Brigham (2005) Daily torpor in a pregnant dunnart (*Sminthopsis macroura* Dasyuridae: Marsupialia). *Mamm Biol,* **70,** 117-121.

Grigg, G. C., L. A. Beard & M. L. Augee (2004) The evolution of endothermy and its diversity in mammals and birds. *Physiol Biochem Zool,* **77,** 982-997.

Hamilton, M. J., A. D. Davidson, R. M. Sibly & J. H. Brown (2011) Universal scaling of production rates across mammalian lineages. *Proc R Soc B,* **278,** 560-566.

Harvey, P. H., M. D. Pagel & J. A. Rees (1991) Mammalian metabolism and life histories. *Am Nat,* **137,** 556-566.

Humphries, M. M. & K. S. McCann (2013) Metabolic Ecology. *J Anim Ecol***,** in press.

Jönsson, K. I. (1997) Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos,* **78,** 57-66.

Kemp, T. (2006) The origin of mammalian endothermy: a paradigm for the evolution of complex biological structure. *Zool J Linn Soc,* **147,** 473-488.

Konarzewski, M. & A. Książek (2013) Determinants of intra-specific variation in basal metabolic rate. *J Comp Physiol B,* **183,** 27-41.

Koteja, P. (2000) Energy assimilation, parental care and the evolution of endothermy. *Proc R Soc B,* **267,** 479-484.

Koteja, P. (2004) The evolution of concepts on the evolution of endothermy in birds and mammals. *Physiol Biochem Zool,* **77,** 1043-1050.

Kuchel, L. (2003) *The energetics and patterns of torpor in free-ranging Tachyglossus aculeatus from a warm-temperate climate.* PhD, University of Queensland, Brisbane.

Lahann, P., J. Schmid & J. U. Ganzhorn (2006) Geographic variation in populations of *Microcebus murinus* in Madagascar: Resource seasonality or Bergmann's rule? *Int J Primatol,* **27,** 983-999.

Lovegrove, B. G. (2000) The zoogeography of mammalian basal metabolic rate. *Am Nat,* **156,** 201-219.

Lovegrove, B. G. (2003) The influence of climate on the basal metabolic rate of small mammals: a slow-fast metabolic continuum. *J Comp Physiol B,* **173,** 87-112.

Lovegrove, B. G. (2006) The power of fitness in mammals: perceptions from the African slipstream. *Physiol Biochem Zool,* **79,** 224-236.

Lovegrove, B. G. (2009) Age at first reproduction and growth rate are independent of basal metabolic rate in mammals. *J Comp Physiol B,* **179,** 391-401.

Lovegrove, B. G. (2012a) The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol Rev,* **87,** 128–162.

Lovegrove, B. G. (2012b) A single origin of heterothermy in mammals. In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*: 3-11. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin

Lovegrove, B. G., C. I. Canale, D. L. Levesque, G. Fluch, M. Řeháková-Petrů & T. Ruf (2014) Are tropical small mammals physiologically vulnerable to Arrhenius effects and climate change? *Physiol Biochem Zool,* **87,** 30-45.

Lovegrove, B. G. & M. O. Mowoe (2013) The evolution of mammal body sizes: responses to Cenozoic climate change in North American mammals. *J Evol Biol,* **26,** 1317-1329.

Mallinson, J. J. C. (1974) Establishing mammal gestation periods at the Jersey Zoological Park. *Int Zoo Yearb,* **14,** 184-187.

McNamara, J. M. & A. I. Houston (2008) Optimal annual routines: behaviour in the context of physiology and ecology. *Phil Trans R Soc B,* **363,** 301-319.

Morrison, P. R. (1945) Acquired homiothermism in the pregnant sloth. *J Mammal,* **26,** 272-275.

Morrow, G. & S. C. Nicol (2009) Cool sex? Hibernation and reproduction overlap in the echidna. *PLoS ONE,* **4,** e6070.

Müller, D. W. H., D. Codron, J. Werner, J. Fritz, J. Hummel, E. M. Griebeler & M. Clauss (2012) Dichotomy of eutherian reproduction and metabolism. *Oikos,* **121,** 102-115.

Naya, D. E., L. Spangenberg, H. Naya & F. Bozinovic (2013) How does evolutionary variation in basal metabolic rates arise? A statistical assessment and a mechanistic model. *Evolution,* **67,** 1463–1476.

Naya, D. E., L. Spangenberg, H. Naya, F. Bozinovic, B. O. Brilot, M. Bateson, D. Nettle, M. J. Whittingham, J. C. A. Read & J. Bueno (2012) Latitudinal Patterns in Rodent Metabolic Flexibility. Latitudinal Patterns in Rodent Metabolic Flexibility. *Am Nat,* **179,** E172-E179.

Nicol, S. & N. A. Andersen (2006) Body temperature as an indicator of egg-laying in the echidna, *Tachyglossus aculeatus*. *J Therm Biol,* **31,** 483-490.

Nicol, S. C. & N. A. Andersen (2008) Rewarming rates and thermogenesis in hibernating echidnas. *Comparative Biochemistry and Physiology, Part A,* **150,** 189-195.

Nicoll, M. E. & S. D. Thompson (1987) Basal metabolic rates and energetics of reproduction in therian mammals: marsupials and placentals compared. In: *Reproductive Energetics in Mammals*: 7-27. A. S. I. Loudon & P. A. Racey (Eds.). Clarendon Press, Oxford, UK.

Nilsen, E. B., J. M. Gaillard, R. Andersen, J. Odden, D. Delorme, G. Van Laere & J. D. Linnell (2009) A slow life in hell or a fast life in heaven: demographic analyses of contrasting roe deer populations. *J Anim Ecol,* **78,** 585-594.

Oelkrug, R., N. Goetze, C. Exner, Y. Lee, G. K. Ganjam, M. Kutschke, S. Müller, S. Stöhr, M. H. Tschöp, P. G. Crichton, G. Heldmaier, M. Jastroch & C. W. Meyer (2013) Brown fat in a protoendothermic mammal fuels eutherian evolution. *Nat Commun,* **4,** e2140.

Poppitt, S. D., J. R. Speakman & P. A. Racey (1994) Energetics of reproduction in the lesser hedgehog tenrec, *Echinops telfairi* (Martin). *Physiol Zool,* **67,** 976-994.

Promislow, D. & P. Harvey (1990) Living fast and dying young: A comparative analysis of life-history variation among mammals. *J Zool (Lond.),* **220,** 417-437.

Read, A. F. & P. H. Harvey (1989) Life history differences among the eutherian radiations. *J Zool (Lond.),* **219,** 329-353.

Riek, A. & F. Geiser (2013) Allometry of thermal variables in mammals: consequences of body size and phylogeny. *Biol Rev,* **88,** 564-572.

Sadowska, J., A. K. Gębczyński & M. Konarzewski (2013) Basal metabolic rate is positively correlated with parental investment in laboratory mice. *Proc R Soc B,* **280**.

Savage, V. M., J. F. Gillooly, W. H. Woodruff, G. B. West, A. P. Allen, B. J. Enquist & J. H. Brown (2004) The predominance of quarter-power scaling in biology. *Funct Ecol,* **18,** 257-282.

Sikes, R. S. & H. Ylönen (1998) Considerations of optimal litter size in mammals. *Oikos,* **83,** 452-465.

Stephenson, P. J. & P. A. Racey (1994) Seasonal variation in resting metabolic rate and body temperature of streaked tenrecs, *Hemicentetes nigriceps* and *H. semispinosus* (Insectivora: Tenrecidae). *J Zool (Lond.),* **232,** 285-294.

Stephenson, P. J. & P. A. Racey (1995) Resting metabolic rate and reproduction in the Insectivora. *Comp Biochem Physiol A,* **112,** 215-223.

Symonds, M. R. E. (1999) Life histories of the Insectivora: the role of phylogeny, metabolism and sex differences. *J Zool (Lond.),* **249,** 315-337.

Symonds, M. R. E. (2005) Phylogeny and life histories of the 'Insectivora': controversies and consequences. *Biol Rev,* **80,** 93-128.

White, C. R., J. S. Terblanche, A. P. Kabat, T. M. Blackburn, S. L. Chown & P. J. Butler (2008) Allometric scaling of maximum metabolic rate: the influence of temperature. *Funct Ecol,* **22,** 616-623.

Willis, C. K. R., R. M. Brigham & F. Geiser (2006) Deep, prolonged torpor by pregnant, free-ranging bats. *Naturwissenschaften,* **93,** 80-83.

Wooden, K. M. & G. E. Walsberg (2004) Body temperature and locomotor capacity in a heterothermic rodent. *J Exp Biol,* **207,** 41-46.

Wright, P. C. (1999) Lemur traits and Madagascar ecology: Coping with an island environment. In: *Yearbook of Physical Anthropology 1999, Vol 42*: 31-72.

Appendix A

Lovegrove, B. G., C. I. Canale, D. L. Levesque, G. Fluch, M. Řeháková-Petrů & T. Ruf (2014) Are tropical small mammals physiologically vulnerable to Arrhenius effects and climate change? *Physiol Biochem Zool,* 87, 30-45.

Appendix B

Canale, C. I., D. L. Levesque & B. G. Lovegrove (2012) Tropical heterothermy: Does the exception prove the rule or force a re-definition? In: *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*: 29-40. T. Ruf, C. Bieber, W. Arnold & E. Millesi (Eds.). Springer Berlin Heidelberg.