THERMOREGULATORY PLASTICITY IN FREE-RANGING VERVET MONKEYS (CHLOROCEBUS PYGERYTHRUS)

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science

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DECLARATION

I declare that this dissertation is my own work, with all assistance acknowledged. This dissertation is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

(ALWYN LUBBE)

-------- day of 2013

I certify that the procedures used in this dissertation were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC clearance number 2010/41/04).

PRESENTATIONS

The conference presentation listed below is offered in support of this dissertation:

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ABSTRACT

Future climate change scenarios predict that many species will be faced with natural habitats which progressively fail to meet their niche requirements. For a species to persist in a stochastic environment, it must either adapt, move to a more suitable location, or risk becoming locally extinct. For vervet monkeys and other long-lived, slowly reproducing species, phenotypic plasticity (including phenology, morphology, behaviour, and physiology) is proposed to be the first and most robust response to rapidly changing environmental conditions. It is therefore crucial to understand the degree of behavioural and physiological plasticity employed by species in their natural environments in order to better understand the impacts of future climate change on their survival. My study is the first phase of data collection for a long-term project which aims to investigate the physiological and behavioural mechanisms employed by free-ranging vervet monkeys (*Chlorocebus pygerythrus*) in response to environmental stress. To my knowledge, my study is the first to use miniature data loggers to obtain simultaneous and continuous measurements of body temperature and activity for a free-ranging non-human primate.

Data were collected from 12 vervet monkeys over a ten month period at Samara Private Game Reserve, Eastern Cape, South Africa. Samara represents a highly seasonal environment in terms of environmental temperature and photoperiod. In winter, vervet monkeys were subjected to short day lengths (~ 10 h) and low environmental temperatures (mean ± SD 24-h minimum black-globe temperature = 2.3 ± 3.8 ^{o}C), whereas in summer day lengths were longer (~ 14 h) and the primary thermal stressor was high environmental temperatures (mean ± SD 24-h maximum black-globe temperature = 45.2 ± 6.3 ^{o}C). Uncharacteristically for the Nama Karoo region, Samara experienced high rainfall (450 mm) over the duration of my study period; approximately double the average annual rainfall for the region over the previous ten years.

In seasonal environments such as the Nama Karoo, individuals face the challenge of maintaining body temperature within a narrow range (i.e. homeothermy) whilst exposed to a thermally dynamic environment. Maintaining homeothermy is primarily expensive in terms of body water at high environmental temperatures, and metabolic energy at low

environmental temperatures. Over the duration of my study period, mean (± SD) 24-h body temperature for individual monkeys was 37.9 ± 0.1 °C. Despite being exposed to mean 24-h variations in black-globe temperature of 30.0 °C, the mean (± SD) 24-h amplitude of body temperature rhythm exhibited by individual monkeys for the duration of my study was 2.9 ± 0.1 °C, more or less within the traditionally defined limits of homeothermy (± 2 °C). However, vervet monkeys did display significant thermoregulatory plasticity (as indexed by variations in the 24-h amplitude of the body temperature rhythm) between seasons.

In summer, when the primary thermoregulatory requirement was heat dissipation at high environmental temperatures, monkeys had *ad libitum* access to free-standing water, relatively high food availability (as indexed using the sum of monthly rainfall as a proxy for food availability), and long day lengths. Vervet monkeys were limited to diurnal activity and thus long day lengths allowed for a flexible activity schedule over the diurnal period. Total time active over the diurnal period was negatively correlated with mean diurnal black-globe temperature ($r^2 = 0.26$, $n = 91$, $p < 0.0001$), indicating that high environmental temperatures inhibited activity. Periods of inactivity during the diurnal period coincided with the warmest part of the day when monkeys generally sought out cooler, shady microclimates. Reduced activity and cool micro-climate selection at high environmental temperatures are common behavioural responses amongst primates, serving to reduce thermal stress and conserve body water by reducing evaporative cooling requirements. Vervet monkeys at Samara were able to effectively maintain homeothermy, largely independent of environmental temperature in summer. This is most likely achieved through a combination of autonomic (e.g. evaporative cooling) and behavioural (e.g. shade-seeking) mechanisms.

Conversely, in winter, 24-h variations in body temperature of more than twice the traditionally defined limit of homeothermy (i.e. > 4 °C) were frequently recorded for individual monkeys and the highest recorded 24-h amplitude of the body temperature rhythm was 5.6 °C. Indeed, vervet monkeys displayed a significantly larger mean 24-h amplitude of the body temperature rhythm in winter (3.2 ± 0.4 °C) compared to summer (2.5 ± 0.1 °C; $t = 5.47$, $n = 6$, $p < 0.01$). Furthermore, the ability of vervet monkeys to maintain homeothermy was significantly compromised at low environmental temperatures; reflected by the negative correlation between 24-h amplitude of the body temperature rhythm and 24-h minimum black-globe temperature ($r^2 = 0.58$, $n = 92$, $p < 0.0001$). Due to their strictly diurnal activity scheduling, time available to complete all fundamental activities (i.e. energetic, social, and biological) was compromised by short day lengths in winter. Confounding the influence of short day lengths, the thermoregulatory challenges imposed by low environmental temperatures further reduced the time available for activity. In contrast to summer, total time active over the diurnal period was positively correlated with mean diurnal black-globe temperature ($r^2 = 0.32$, $n = 92$, $p < 0.0001$), indicating that low environmental temperatures inhibited activity. Whilst thermoregulatory challenges at low environmental temperatures promote inactive behaviours such as sunbasking and huddling to conserve energy, the coinciding mating season likely increased the requirement for monkeys to be active in order to maintain their social hierarchy and secure mating opportunities. These conflicting requirements likely resulted in a trade-off for monkeys between maintaining homeothermy and completing fundamental activities at a time when both thermoregulatory and social requirements were high. These confounding environmental (i.e. photoperiod and temperature) and social (i.e. mating season) factors are therefore likely to infer significant energetic challenges for vervet monkeys in winter, as indexed by an increase in the 24-h amplitude of the body temperature rhythm compared to summer.

After accounting for the influence of environmental temperature and photoperiod on the expression of the body temperature rhythm for vervet monkeys, I found that, over the duration of my study, periods of reduced food availability also were associated with an increase in the 24-h amplitude of the body temperature rhythm ($Z = -7.71$, $p < 0.0001$). When food availability was reduced, the energetic balance of vervet monkeys was presumably compromised by reduced food intake, so less energy was available to maintain all biological functions. The significant thermoregulatory plasticity in response to proximal environmental stressors suggests that, for vervet monkeys, maintaining strict homeothermy may be a luxury rather than a predefined norm. When environmental conditions were “good” (i.e. access to water and high food availability), monkeys were able to allocate sufficient resources towards maintaining homeothermy, which is presumably beneficial in terms of optimizing performance. In contrast, when environmental conditions were “bad” (i.e. low environmental temperatures and periods of low food availability), vervet monkeys displayed an increase in the 24-h amplitude of the body temperature rhythm. The expression of the 24-h amplitude of the body temperature rhythm may therefore provide a proxy for environmental stress experienced by an animal in its natural habitat.

My study has contributed towards a better understanding of the influence of environmental stressors on the expression of vervet monkey activity and body temperature patterns. An important future line of inquiry will be to determine the energetic implications, and ultimately the fitness consequences, of observed thermoregulatory plasticity in response to environmental stressors in order to better understand the ecological implications of climate change. Due to the marked behavioural inflexibility in activity scheduling between the diurnal and nocturnal periods, it is likely that any environmental stressors which confound the influence of short day lengths in winter may impose a significant bottleneck on survival for vervet monkeys in the Nama Karoo. Low environmental temperatures and food availability are therefore likely to be the primary environmental determinants of habitat suitability for vervet monkeys and other diurnal primates living in similar seasonal habitats. As low environmental temperatures appear to pose the primary thermoregulatory challenge for vervet monkeys under conditions during my study, the predicted increase in mean environmental temperature under future climate change scenarios may actually alleviate, to a degree, the energetic requirements of thermoregulation in winter. More pertinent to the survival of vervet monkeys occupying marginal habitat such as Samara is likely to be the frequency and duration of extreme weather events (e.g. drought, heat waves, and severe cold snaps) associated with climate change.

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TABLE OF CONTENTS **Page**

LIST OF FIGURES **Page**

LIST OF TABLES Page

CHAPTER 1

Introduction

There are 356 extant species of non-human primates (Groves 2001). Yet, given the proposed importance of thermoregulation in early hominid evolution (Wheeler 1991a,b, Chaplin *et al.* 1994, do Amaral 1996), studies addressing the ecological and behavioural implications of thermoregulation in our closest relatives are surprisingly underrepresented in the literature (Hill 2006a). As far as I am aware my study will be the first to use data loggers to obtain simultaneous, continuous measurements of body temperature and activity for a non-human primate (henceforth primate). Long-term studies are crucial if we are to determine the mechanisms available to animals which will allow them to cope with the thermal stress, aridity, and food shortage associated with climate change. My study is the first phase of data collection for such a long-term project which aims to investigate the physiological and behavioural mechanisms employed by free-ranging vervet monkeys (*Chlorocebus pygerythrus*) in response to environmental stress. Vervet monkeys are long-lived animals with slow reproductive cycles and complex social structures, making them potentially vulnerable to rapid changes in environmental conditions. My study population of vervet monkeys occurs in marginal habitat at the edge of the species' distribution range, making these monkeys the ideal subject for my study. Currently 30 % of primate species worldwide are listed as Endangered (IUCN Red List of Threatened Species), primarily due to habitat loss and degradation (Cowlishaw 1999). Measurements of body temperature and activity patterns in free-ranging vervet monkeys will provide valuable information on the thermoregulatory and behavioural plasticity employed by these animals in their natural habitat. Developing an understanding of the environmental factors which perturb body temperature or activity may help to inform managers as to the stressors which are likely to drive habitat suitability for this species under future climate change scenarios and potentially threaten the survival of populations.

1.1 Mechanisms of adapting to rapid environmental change

Climate change is not a new phenomenon. Over the past one million years the Earth has experienced cycles of glacial-interglacial periods that last approximately 100 000 years (Petit *et al.* 1999). What makes the current period of warming remarkable is the rate at which it is occurring. Global average surface temperature has increased by approximately 0.6 °C in the past 100 years, with the majority of this warming taking place over two distinguishable periods; 1910-1945 and 1976 onwards. The last decade, 2000 through 2009, was the warmest decade on record, and 2012 experienced the ninth highest mean annual temperature globally since 1880 (http://www.ncdc.noaa.gov). The current rate of anthropogenic climate warming is double that experienced in the first half of the 20th century and greater than any other time over the last 1000 years (Houghton *et al.* 2001). Climate change is a dynamic process with regionally and temporally heterogeneous impacts. Evidence suggests that globally climate change will result in higher minimum and maximum temperatures, decreased snow cover and ice extent, and increased sea level. Altered precipitation patterns will result in increased extremes of drought and wetness (Houghton *et al.* 2001, Karl and Trenberth 2003). The ecosystem impact of these trends will likely be exacerbated by anthropogenic land transformation (Mac Nally *et al.* 2009). In the face of such unprecedented environmental change, the effects of which are predicted to persist for many centuries (Houghton *et al.* 2001), many species will be subjected to natural habitats which progressively fail to meet their niche requirements. Conservative estimates predict that 18 % of plant and animal species worldwide (n = 1103) will be at acute risk of extinction under 2050 climate change scenarios (Thomas *et al.* 2004). Climate change is therefore a serious threat to global biodiversity.

Earth will lose many species as a result of climate change. Previous studies have however demonstrated the ability of organisms to rapidly adapt to recent climate change (see McCarty 2001 and Walther *et al.* 2002 for reviews). The intrinsic and often unpredictable ability of species to adapt to their environment has prompted speculation that our future is not necessarily a species depleted environment, but rather one consisting of an altered assemblage of biotic communities (Walther *et al.* 2002, Thuiller *et al.* 2006). A species' bioclimatic envelope is defined as the climatic conditions at which a given species currently persists. Using bioclimatic envelope models researchers have predicted a fundamental shift in the availability and location of suitable habitats for a wide range of species based on future climate change scenarios (Thomas *et al.* 2004, Thuiller *et al.* 2006). The concept of range shifts in response to climate change is supported by evidence that, in phase with climate warming, various species of birds, mammals, arthropods, plants, reptiles, fish, and molluscs have shifted their distributions to higher latitudes and elevations in order to remain within similar climatic niches as they currently inhabit (Chen *et al.* 2011). In this way populations may be able to "escape" the adverse effects of climate change by tracking the shift in favourable habitat distributions. The suitability of habitats based solely on climatic conditions however may be misleading, as the ability of a species to colonize a new geographical area will also be influenced by biotic interactions with resident species (Pearson and Dawson 2003). Furthermore, the feasibility of moving to a more suitable habitat may in many cases be perturbed by natural barriers such as impassable mountain ranges or, more likely, anthropogenic barriers as a result of human habitation (Thuiller *et al.* 2006). If a species cannot move in phase with shifts in its suitable habitat, that species must adapt to its current and changing environment or risk local extinction (Hughes 2000).

Micro-evolution and phenotypic plasticity are the primary mechanisms for adapting to a changing environment (Fuller *et al.* 2010). Phenotypic plasticity is defined as the capacity for a single genotype to express more than one phenotype under different environmental conditions (Garland and Kelly 2006). A phenotype will become more prevalent in a population if that phenotype infers a fitness benefit over other phenotypes, in which case directional selection will favour that trait (Garland and Kelly 2006). The relative importance of phenotypic plasticity and micro-evolution in a species' ability to adapt to climate change will be primarily dependent on that species' life history traits and the rate and extent of environmental change (Gienapp *et al.* 2007). For long-lived, slowly reproducing species such as vervet monkeys, the rate of climate change precludes the possibility of evolving adaptive heritable traits, i.e. micro-evolution (Fuller *et al.* 2010). Phenotypic plasticity however invokes more flexible mechanisms, including acclimatization, acclimation, and learning (Garland and Kelly 2006), thus facilitating a rapid adaptive response to changing environmental conditions. For long-lived species, phenotypic plasticity (including changes in phenology, morphology, physiology, and behaviour) is therefore proposed to be the primary response facilitating adaptation to the consequences of rapid climate change predicted for the future (Fuller *et al*. 2010)

Phenology refers to the timing of events, and it has been shown that across a wide range of taxa including birds, plants, insects, and amphibians, phenological shifts have occurred in phase with climate warming in the latter part of the 20th century (Walther *et al.* 2002). There is strong evidence, for example, for the earlier onset of activities such as arrival of migratory birds and egg laying being linked to warmer temperatures in the preceding winter months (Walther *et al.* 2002). For great tits (*Parus major*) there is a selective advantage for laying their eggs earlier in the year, therefore synchronizing the arrival of offspring and, consequently, a period of high food requirements for parents who must provision for younglings, with earlier peaks in insect biomass. Thus constituting a behavioural adaptation which has been attributed entirely to phenotypic plasticity associated with spring warming over the past 50 years (Charmantier *et al.* 2008). If species are unable to shift their phenology in synchrony, the timing of ecological events becomes desynchronized, which could have dire consequences for populations. For example, a population of pied flycatchers (*Ficedula hypoleuca*) have suffered a 90 % decline over the past two decades due to a mismatch between food abundance and offspring energetic requirements (Both *et al.* 2006).

With regionally changing temperature regimes, morphological variations associated with thermoregulatory benefits may also be accentuated. Coat colour, for example, can offset the energetic requirements of thermoregulation, as dark coats absorb more radiation and light coats reflect more (Maloney *et al.* 2009). For springbok (*Antidorcas marsupialis*) in South Africa, Hetem and colleagues (2009) argue that the rare black and white springbok colour morphs may be at a competitive disadvantage to the “normal” springbok because of increased energetic costs of thermoregulation in summer and winter respectively, contributing to the scarcity of these colour morphs in the natural population. A rapid change in climatic conditions may however prompt a shift in the selective advantage of a particular coat colour, depending on the direction of change in temperature. The decrease in the proportion of large, dark coloured Soay sheep (*Ovis aries*) in the population of the St. Kilda archipelago between 1985 and 2005, for example, has been attributed to selective pressures benefiting smaller, light coloured sheep in phase with the observed climate warming over that period (Maloney *et al.* 2009).

Due to the relative ease of collecting morphological data, the literature on phenotypic plasticity in response to environmental factors is characterized by a prevalence of studies which focus on morphological adaptations (Kingsolver *et al.* 2001). In contrast, physiological parameters have historically been more difficult to quantify for free-ranging animals in their natural environments, and consequently, relatively few studies have addressed the influence of environmental factors on physiological traits in mammals (Helmuth *et al.* 2005). Physiological mechanisms are however a crucial component of an organisms capacity to adapt to new or changing environments (Carey 2005), and an understanding of an organisms physiological response to its environment is therefore fundamental in order to predict the ecological consequences of future climate change (Helmuth *et al.* 2005). Whereas physiology and behaviour are often considered mutually exclusive, the inherent role of behaviour in thermoregulation has led to the conclusion that behaviour constitutes a fundamental component of an animals' physiological repertoire (Williams *et al.* 2008, Fuller *et al.* 2010), and should thus be considered intrinsically. In the next section (1.2) I will discuss the use of miniature data loggers to measure physiological and behavioural variables in free-ranging animals and the application of these data to the field of conservation physiology.

1.2 Conservation physiology

The field of research addressing the direct influence of changing environmental conditions on the capacity of organisms to maintain their physiological wellbeing, and the ecological implications of these interactions, is recognized as "conservation physiology" (Wikelski and Cooke 2006). The classical example of the application of physiological techniques to conservation efforts dates back to the 1960's, when physiologists and toxicologists combined efforts to show that the commonly used insecticide at the time, dichlorodiphenyltrichloroethane (DDT), was causing a decline in hatching success for some raptor species in the United States and resulting in drastic population declines of these species (Grier 1982). Early research on the link between DDT and hatching success in raptors was integral in the subsequent banning of DDT in the United States (Dunlap 1981, Grier 1982). More commonly now, the physiological parameters of organisms are incorporated in predictive mechanistic models, as was done for some marine (Beardall *et al.* 1998) and terrestrial (Tilman and Lehman 2001) plants to predict the effect of climate change on the distribution and diversity of these populations. The value of physiological information pertaining to species' ability to cope with environmental stress is now widely recognized and there are many examples of the practical applications of physiological techniques to informing conservation decisions and policy (see Wikelski and Cooke 2006, Cooke and O'Connor 2010).

Improvements in technology, specifically with regards to biologging, have been fundamental in the advancement of conservation physiology. Biologging is the use of implanted data loggers to remotely measure body temperature, heart rate (typically as a proxy for energy expenditure), and activity of free-ranging animals (Rutz and Hays 2009). Whereas obtaining continuous measurements of physiological variables for free-ranging animals was very difficult in the past, miniature biologgers are now suitably sophisticated to be used across the full range of animal taxa, both terrestrial and marine (Rutz and Hays 2009). The ability to obtain physiological information from free-ranging undisturbed animals has been crucial, as the body temperature rhythms and natural activity patterns of animals caged or restrained, or housed in artificial environments in the presence of observers, are unlikely to accurately reflect those of animals in their natural environment. The inability of studies based on restrained animals to accurately describe body temperature rhythms in response to environmental factors such as temperature fluctuations, for example, is due to the associated stress and restrictions of captivity which deprive the animals of their inherent social structures and behavioural traits which play an important role in thermoregulation (Mitchell *et al*. 2002).

In my study I will use biologgers to measure body temperature and activity in free-ranging vervet monkeys inhabiting the semi-arid Nama Karoo, South Africa. The Karoo is characterised by cold winters, warm summers, and low rainfall (see Chapter 2 for more details). Climate change scenarios for the Karoo predict a mean temperature increase of 1 – 3 °C, decreased rainfall, and an increase in the frequency of extreme events, particularly drought (Rutherford *et al.* 1999, van Jaarsveld and Chown 2001, Hoffman *et al.* 2009). Under future climate change scenarios, the primary environmental stressors in the Karoo and other semi-arid regions of South Africa are therefore likely to be heat stress and water scarcity. Environmental temperature and water availability are two important factors influencing the energy and osmotic balance of an organism (Boyles *et al.* 2011a). Information on the relationship between traits related to physiological maintenance (e.g. metabolic rate, thermoregulation, and thermal tolerance; Boyles *et al.* 2011a) and fluctuations in environmental conditions are therefore crucial in order to predict the ecological implications of future climate change.

1.3 Thermoregulation in terrestrial mammals

Endotherms maintain their internal body temperature by means of endogenous heat production, which requires substantial energy inputs. This significant energetic cost of endothermy has led to widespread debate amongst researchers regarding the evolution of endothermy in birds and mammals (see review by Grigg *et al.* 2004). Operating at a consistently high body temperature (relative to mean environmental temperature), with minimal variation, is proposed to enhance performance as a consequence of increased metabolic function and efficiency of chemical reactions at the cellular level (Heinrich 1977, Somero 2004, Knies *et al.* 2009). These advantages must outweigh the energetic cost of maintaining a high body temperature in evolutionary terms (Grigg *et al.* 2004, Angilletta *et al.* 2010, Boyles *et al.* 2011a). Body temperature is therefore an important determinant factor of performance for endothermic animals (Angilletta *et al.* 2010).

Heat exchange between an animal and its environment is primarily dependent on the gradient between surface body temperature and environmental temperature (Angilletta 2009). Thermal equilibrium is achieved when metabolic heat production together with heat gained from the environment equals heat lost by radiation, conduction, convection, and evaporation (Hammel 1968). Thermoregulation thus involves the active modification of the rate of endogenous and passive heat gain and heat loss between an organism and the environment, achieved by a combination of autonomic (i.e. nervous or endocrine system) and behavioural mechanisms (Hammel 1968). Autonomic thermoregulatory responses include panting and sweating (increases cutaneous evaporative water loss), regional heterothermy (alteration of local cutaneous blood flow or heat exchange), shivering, and non-shivering thermogenesis (Hammel 1968, Gordon 1985, Genin *et al.* 2003, Angilletta 2009). The thermoneutral zone (TNZ) is defined as the range of environmental temperatures within which an animal's metabolic rate and evaporative heat loss are at minimal levels (Gordon 1985). At environmental temperatures below the TNZ, an animal must increase metabolic energy expenditure to maintain body temperature, thus increasing the energetic cost of thermoregulation. At environmental temperatures above the TNZ, the primary avenue of heat dissipation is evaporative cooling, which requires use of body water, thus creating the problem of replacing lost body water and increasing the osmotic cost of thermoregulation (Gordon 1985, Hetem *et al.* 2010). Autonomic mechanisms of thermoregulation are therefore primarily costly in terms of metabolic energy at environmental temperatures below the TNZ, and body water at environmental temperatures above the TNZ. Behavioural thermoregulation on the other hand can minimize thermal stress without the costs of increased energy expenditure or water loss associated with autonomic responses (Stelzner 1988).

Behavioural thermoregulation may involve the use of suitable microclimates, in which internal body temperature can be maintained with minimal or no input from autonomic mechanisms (Hammel 1968). Animals may also manipulate the rate and degree of heat exchange between the body and the environment by means of postural adjustments or social behaviours such as huddling. These behaviours serve to mediate effective thermal conductance, by, for example, decreasing the surface area available for heat loss to the environment at low environmental temperatures (Gordon 1985, Stelzner 1988). Behaviour is therefore a highly flexible mechanism which allows animals to manipulate heat transfer and the proximal environmental conditions which they experience without necessarily increasing energy expenditure, thus likely constituting an animals most robust defense against rapid environmental change (Fuller *et al.* 2010). Illustrating the energetic cost of autonomic thermoregulation, Adair (1976) showed that squirrel monkeys (*Saimiri sciureur*) exposed to temperatures below their TNZ under laboratory conditions preferentially engaged in behavioural thermoregulation (microclimate selection) above initiating autonomic mechanisms of thermoregulation. However, in natural environments behavioural thermoregulation may have its own suite of indirect costs. For example, a habitat selected primarily for its thermal characteristics may not be optimal in terms of predation risk or access to resources; including food, water, or potential mating opportunities (Angilletta *et al.* 2010).

In humans (*Homo sapiens*), the principle autonomic mechanisms of body temperature regulation are vasodilation and sweating to dissipate heat at high environmental temperatures, and vasoconstriction to reduce heat loss followed by shivering to increase body temperature at low environmental temperatures (Gagge and Gonzalez 2011). Similarly, for non-human primates, laboratory studies have shown sweating to effectively lower body temperature in the common squirrel monkey (Stitt and Hardy 1971), Japanese macaque (*Macaca fuscata;* Nakayama *et al.* 1971), and rhesus macaque (*Macaca mulatta;* Elizondo 1977). When exposed to temperatures below their TNZ in artificial environments, shivering (e.g. rhesus macaque; Elizondo 1977) and non-shivering thermogenesis (e.g. gray mouse lemur *Microcebus murinus;* Genin *et al.* 2003) have been shown to effectively increase body temperature. Free-ranging white-faced capuchins (*Cebus capucinus*) in their natural environment have also been shown to extend their tongues more frequently during the hottest part of the day, suggesting that they do so to reduce body temperature by means of evaporative cooling; although this hypothesis was not supported by direct measures of body temperature (Campos and Fedigan 2009).

Various observational studies on free-ranging primates have demonstrated that behavioural adjustments are an important component of their thermoregulatory response to thermal stress. Chacma baboons (*Papio ursinus*) and chimpanzees (*Pan troglodytes*), for example, used caves, at least partially, for their consistently mild temperatures and shelter from wind (Barrett *et al.* 2004, Pruetz 2007). Chimpanzees also took advantage of the vertical structure of microclimates in the rainforest, where canopy cover limits the penetration of solar radiation to ground level, by being more terrestrial during warm periods than during cool periods, when they were more arboreal (Takemoto 2004, Kosheleff and Anderson 2009). Similarly chacma baboons in South Africa utilized thermal microclimates by spending the hottest part of the day resting in the shade (Hill 2006a). Certain body postures (e.g. hunching and sunbasking) and social behaviours (e.g. huddling) are also closely associated with low environmental temperatures and are proposed to have thermoregulatory implications (e.g. yellow baboons *Papio cynocephalus*; Stelzner and Hausfater 1986, Pochron 2000, colobine monkeys *Colobus polykomos*; Da Silva 1993, hamadryas baboons *Papio hamadryas*; Brent *et al.* 2003, Japanese macaques; Hanya *et al.* 2007, collared lemurs *Eulemur collaris*; Donati *et al.* 2011). These studies demonstrate that the typical behavioural response of primates to thermal stress (both low and high environmental temperatures) is to seek out appropriate microclimates and increase the frequency of resting and thermoregulatory activities such as sunbasking or huddling. Reduced activity and associated thermoregulatory behaviours in response to thermal stress are proposed to be behavioural mechanisms of energy conservation at low environmental temperatures and water conservation at high environmental temperatures (Sato 2012). The thermal environment and water availability may therefore impose a significant constraint on primate behaviour and activity patterns, as well as being an important determinant of habitat selection and day journey routes (Hill 2006a).

Studies quantifying the effect of behavioural thermoregulation on body temperature for free-ranging primates are rare and, to my knowledge, only two such examples exist. Brain and Mitchell (1999) used implanted temperature-sensitive telemeters to quantify the effect of behavioural thermoregulation on core body temperature for three male baboons, and Hanya and colleagues (2007) fitted four adult female Japanese macaques with collars which contained temperature-sensitive transmitters to measure skin temperature. During summer in the Namib Desert, the authors revealed that sandbathing, a behaviour unique to these baboons whereby they use their wrists to flick plumes of cooler subsurface sand against their ventra, significantly decreased body temperature when baboons were exposed to heat stress (Brain and Mitchell 1999). When exposed to low environmental temperatures, Japanese macaques in the coniferous forests of Yakushima employed sunbasking and huddling behaviours, which were shown to be more effective at raising skin temperature than when no behavioural thermoregulation was exercised (Hanya *et al.* 2007).

By using biologgers, body temperature and activity are easily measured variables which can provide valuable information on the influence of environmental stressors on these physiological and behavioural parameters. Sections 1.4 and 1.5 will describe the nycthemeral rhythms of body temperature and activity in primates and other terrestrial mammals, and discuss the potential ecological implications of environmental factors which perturb these variables.

1.4 The effect of environmental factors on body temperature in terrestrial mammals

Whereas body temperature was originally defined as a non-adaptive constant in mammals and birds (Scholander *et al.* 1950), subsequent research has provided resounding evidence to support the conclusion that body temperature varies within and among individuals and across populations (Angilletta *et al.* 2010). Amongst endotherms, physiologists have broadly distinguished between homeotherms and heterotherms. Homeothermic mammals maintain a consistently high body temperature within a narrow range independent of environmental temperature (Refinetti and Menaker 1992). Body temperature in homeotherms generally fluctuates by 1 – 2 °C over a 24-h period in a regulated rhythm, with the zenith and nadir of the body temperature rhythm occurring during the active and inactive phases respectively (Refinetti and Menaker 1992). Laboratory studies on rhesus macaques and squirrel monkeys report a range of 1.0 – 2.5 °C fluctuations in the 24-h amplitude of the body temperature rhythm around a mean of ~ 38 °C (Refinetti and Menaker 1992). Much larger fluctuations in the 24-h amplitude of the body temperature rhythm have however been reported for mammals which employ torpor or hibernation, referred to as heterotherms (Geiser 2004). Body size appears to be an important factor influencing the expression of heterothermy amongst mammals, with torpor and hibernation being employed almost exclusively in small (< 1 kg) mammals, with the obvious exception being black bears (*Ursus americanus;* Geiser 2004).

Body size is an important morphological characteristic which modulates the transfer of heat between an animal and its' environment. Thermal conductance decreases with increasing body mass (Aschoff 1981), and due to their relatively small surface area to volume ratio and high thermal inertia, compared to small mammals, large mammals are theoretically less susceptible to the influence of fluctuations in environmental temperature on body temperature. Conversely, small mammals have relatively high surface area to volume ratios and low thermal inertia (Aschoff 1981, McNab 1983). For small mammals, when energy (i.e.

food) availability is low and the gradient between body and environmental temperature is high, the energetic cost of maintaining a high body temperature may therefore become prohibitively high (Geiser 2004). Under such conditions of low environmental temperature or food availability many small mammals have been shown to abandon homeothermy to enter a state of torpor. Torpor is characterized by a substantial, although temporary, reduction in the set-point (defined as “the value of a regulated variable which a healthy organism tends to stabilize by the process of regulation”, IUPS Thermal Commission 2001) of body temperature, which lasts for periods of a few hours interspersed with normal foraging behaviour, thus resulting in 24-h body temperature fluctuations of between 10 – 30 °C (Geiser 2004). By down regulating the set-point at which body temperature is defended, the gradient between body and environmental temperature is reduced at low environmental temperatures, and therefore the energetic requirements of thermoregulation are reduced. Also, by reducing body temperature, the consequential reduction in metabolic rate due to the Q_{10} effect (whereby the rate of chemical reactions decrease with decreasing temperature) results in torpid metabolic rate generally being 5 – 30 % lower than resting metabolic rate, which infers significant energy savings for animals which employ torpor (Geiser 2004). Hibernation is a prolonged form of torpor which may last several days or weeks at a time (Geiser and Ruf 1995, Geiser 2004).

Torpor and hibernation are rare amongst primates, although this may be an artefact of the paucity of body temperature measurements available for free-ranging primate species. Reports of torpor in primates are largely restricted to one family of small (30 – 500 g), nocturnally active Malagasy lemurs, the Cheirogaleidae (Schülke and Ostner 2007). The only exception outside of the Cheirogaleidae family appears to be the closely related southern lesser bushbaby (~ 200 g, *Galago moholi*) inhabiting mainland Africa (Nowack *et al.* 2010). The Cheirogaleidae lemurs inhabit highly seasonal forests, with distinct warm and wet, and cool and dry periods. Torpor is common during the cool and dry period only, and has been linked with significant energy savings (Schülke and Ostner 2007). The one species of lemur known to hibernate is an anomaly in the world of hibernators. The Madagascan fat-tailed dwarf lemur (*C. Medius*) hibernates for up to seven months during which time environmental temperatures frequently exceed 30 °C. The Madagascan fat-tailed dwarf lemur is the only mammal inhabiting the tropics known to hibernate, and was also the first case of hibernation at comparatively high body temperatures, but with reduced metabolic rate characteristic of hibernation. During hibernation body temperature ranged widely between 10 and 40 °C on a daily basis, which was tightly matched to fluctuations in the temperature of the tree holes in which they sheltered. By maintaining the difference between body and tree hole temperature at a minimum, the lemurs were able to minimize metabolic energy expenditure (Dausmann *et al.* 2004). The unusually high hibernating body temperatures observed in these lemurs has prompted the re-definition of hibernation as a state of hypo-metabolism, not necessarily characterized by low body temperatures (Dausmann *et al.* 2004).

However, in recent decades research on traditionally defined homeothermic species have revealed a degree of plasticity in the body temperature rhythm of these species (see Pereira *et al.* 2002, Ostrowski *et al.* 2003, Arnold *et al.* 2004, Grigg *et al.* 2009, Maloney *et al.* 2011, Hetem *et al.* 2010) which suggests that rather than a dichotomy between homeothermy and heterothermy, thermoregulatory mechanisms in endotherms occur along a continuum (Angilletta *et al.* 2010). It would seem that “true homeothermy” is the exception rather than the norm (Arnold *et al.* 2004), and consequently variations in the body temperature rhythms of endotherms “must reflect selective pressures operating within physical, genetic, and developmental constraints” (Angilletta *et al.* 2010). Based on a model of optimal performance originally designed for ectotherms (see Huey and Slatkin 1976), Angilletta and colleagues (2010) argue that the optimal thermoregulatory strategy of endotherms is a compromise between the performance benefit of maintaining homeothermy and the energetic, and non-energetic, cost of thermoregulation. The net benefit of thermoregulation is therefore dependent on the physical properties of the organism and the environment. Any factors which increase the cost or decrease the benefit of thermoregulation would therefore favour a more labile thermoregulatory mechanism; where the maintenance of homeothermy is traded-off against energy conservation or reallocation to other biologically important activities, resulting in a more heterothermic thermoregulatory mechanism (Angilletta *et al.* 2010). The distinction between homeothermy and heterothermy is not clearly defined in the literature. Traditionally, any 24-h amplitude of the body temperature rhythm greater than 2 ^{o}C was considered to reflect heterothermy (Cabanac 1987). Typically however the term heterothermic is associated with species which enter a state of torpor or hibernation (e.g. Geiser 2004). To avoid confusion between technical terms I will use the 24-h amplitude of the body temperature rhythm as an index of “thermoregulatory precision” in my study. Any increase in the 24-h amplitude of the body temperature rhythm therefore reflects a less precise thermoregulatory mechanism (or a deviation from homeothermy). An increase in the 24-h amplitude of the body temperature rhythm (imprecise thermoregulation) could be a result of a depressed minimum body temperature (hypothermia) or an increase in maximum body temperature (hyperthermia), or both hypo- and hyperthermia.

Various studies which used biologgers to measure body temperature in free-ranging mammals have provided anecdotal support for the cost-benefit trade-off theory of thermoregulatory mechanisms. These studies have shown that traditionally defined homeothermic species may employ imprecise thermoregulatory mechanisms in response to thermal stress, water availability, or food scarcity. For example, western grey kangaroos (*Macropus fuliginosus*) were able to decrease metabolic energy requirements by nearly 7 % by lowering mean minimum body temperature by ~ 2 °C between spring and summer in an apparent response to decreased food availability in summer (Maloney *et al.* 2011). Both Alpine ibex (*Capra ibex*) and red deer (*Cervus elaphus*) employed hypothermia during cold winters when food availability was low resulting in a ~ 60 % decrease in metabolic rate compared to summer (Arnold *et al.* 2004, Signer *et al.* 2011). For Arabian oryx (*Oryx leucoryx*) the primary determinant of imprecise thermoregulation was water scarcity, which was considered to reflect a thermoregulatory mechanism to increase body heat storage (hyperthermia), thus decreasing dependence on evaporative cooling in a hyper-arid environment (Hetem *et al.* 2010). Employing a hypothermic thermoregulatory mechanism at low environmental temperatures or low food availability is proposed to infer significant energy savings by reducing metabolic heat production requirements (by reducing the gradient between body and environmental temperature) or a reduction in metabolic rate (due to the Q_{10} effect). The primary benefit of employing a hyperthermic thermoregulatory mechanism at high environmental temperatures is proposed to be the body water savings incurred by the reduction in evaporative cooling as a consequence of the reduced gradient between body and environmental temperature. Thermoregulatory plasticity (variability in the expression of an animals’ body temperature rhythm) may therefore be an important mechanism of energy or water conservation in response to environmental stressors. The influence of environmental factors on the thermoregulatory mechanism employed by mammals in their natural environments is however largely unknown. Determining these physiological-ecological interactions, and the specific environmental factors which influence these, constitutes an important line of inquiry towards understanding the energetic and ecological implications of rapid environmental change associated with future climate change.

As the cost of thermoregulation is primarily dependent on the gradient between body surface and environmental temperature (Angilletta *et al.* 2010), one might expect that the 24-h amplitude of the body temperature rhythm will decrease with increasing body size (due to decreasing thermal conductance with increasing body size, Aschoff 1981). Indeed this was the case in a meta-analysis of 57 species of mammals ranging in size from 15 g to 5 000 kg (excluding studies on free-ranging animals or species which employ torpor or hibernation; Mortola and Lanthier 2004). In their review of heterothermy in large mammals however, Hetem and colleagues (in preparation) found no relationship between the 24-h amplitude of the body temperature rhythm and body mass for 18 species of free-ranging mammals ranging in size from 21 (Springbok) to 180 kg (blue wildebeest *Connochaetes taurinus*). This dichotomy is likely explained by the interacting influence of environmental temperature, water, and food availability on the ability of animals to effectively thermoregulate. The 24-h amplitude of the body temperature rhythm of large mammals was largely independent of environmental temperature (Hetem *et al.* in preparation), and the authors argue that for large mammals, water and food availability, rather than environmental temperature may be the primary determinant factors of the expression of thermoregulatory precision. Conversely the 24-h amplitude of the body temperature rhythm has been shown to be closely related to environmental temperature in small heterothermic mammals (Geiser 2004, Dausmann *et al.* 2004, Boyles *et al.* 2012). The contrast in the relationship between environmental temperature and thermoregulatory precision between species along a size (mass) gradient suggests that, across species, the environmental factors which perturb thermoregulation, and therefore potentially influence habitat suitability, may vary greatly between species depending on their morphology, physiology, and specific suite of behavioural adaptations to coping with environmental stress.

Amongst primates, physiological research on free-ranging animals has been largely restricted to the small, nocturnal prosimians (Suborder: Strepsirrhini) of the Cheirogaleidae and *Galagidae* families, which exhibit significant heterothermy (i.e. torpor and hibernation; Schülke and Ostner 2007, Nowack *et al.* 2010). To my knowledge only two studies have collected body temperature data on any primates outside of the Strepsirrhini (see Mitchell and Brain 1999, Hanya *et al.* 2007). In both of these studies body temperature measurements were not continuous, and restricted to the diurnal period. Furthermore, Hanya and colleagues (2007) did not report any data on the amplitude of the body temperature rhythm, and acknowledged that no robust conclusions can be made based on their results, as skin temperature has been shown to deviate significantly from core body temperature. Nonetheless, Brain and Mitchell (1999) reported diurnal fluctuations in the body temperature rhythm of baboons of greater than 4 °C, more than double that previously reported from laboratory studies. For baboons in the Namib Desert water availability and environmental temperature were important determinant factors of thermoregulatory precision. Body temperature increased with increasing environmental temperature throughout the day, but body temperature was only maintained by physiological means when water was available (Brain and Mitchell 1999). The authors conclude that for these baboons, thermoregulatory precision was traded-off against water conservation on days when water was not available, resulting in increased diurnal heat storage (hyperthermia), reflected in an increase in the diurnal amplitude of the body temperature rhythm (Brain and Mitchell 1999).

Angilletta and colleagues (2010) defined adaptive thermoregulation as "any thermoregulatory pattern that is launched to counteract an environmental stressor, whether that pattern arises from genetic changes across generations (i.e. adaptation) or phenotypically plastic responses within a lifetime (i.e. acclimatization)". If labile thermoregulatory mechanisms, or adaptive thermoregulation, infer a net-benefit in terms of energy or water conservation and ultimately fitness, these physiological mechanisms may be crucial to the potential of these species to adapt to rapid environmental change associated with future climate change (Fuller *et al.* 2010). There is currently however a paucity of long-term, continuous data on body temperature rhythms for free-ranging, traditionally-defined homeothermic mammals, inhibiting the formulation of robust theories on the role that physiological plasticity may play in negating environmental stochasticity associated with future climate change for these species (Angilletta *et al.* 2010, Boyles *et al.* 2011a).

1.5 The effect of environmental factors on activity in terrestrial mammals

Activity scheduling in mammals ranges across the full spectrum from being completely nocturnal to completely diurnal, although the majority of mammals are most active in the early morning and late afternoon (i.e. crepuscular; Ashby 1972). Animals may be restricted to either diurnal or nocturnal activity by the evolutionary development of sensory organs such as eyes and ears, which may be particularly well suited to function either during the day or at night, with only limited functionality during the opposite phase of the 24-h period (Ashby 1972). Diurnal primates, including vervet monkeys, are characteristically more active in the early morning and late afternoon as they travel between their sleeping site and feeding grounds. Feeding activity peaks in the morning and mid-afternoon, and there is generally a period of increased resting and grooming around mid-day (Hall and Gartlan 1965, Clutton-Brock 1977, Baldellou and Adan 1997). Activity patterns in mammals are, however, not a clear-cut distinction between diurnal and nocturnal. Animals live in a complex environment of abiotic and biotic components which influence the decisions that they make. Biotic components such as prey items and predators may be important determinants of activity patterns, as it would, for example, clearly be beneficial for a prey-species to de-synchronize its activity with that of its primary predator. The timing of activity can therefore have important fitness consequences (Halle 2006). Abiotic factors too have been shown to be important determinant factors of shifts in activity patterns, most notably light and, as one might expect given the important function of behaviour in thermoregulation (Hill 2006a), environmental temperature (Halle 2006). Furthermore, food availability is also an important determinant of the distribution of activity over the 24-h period (Ashby 1972). Animals which rely on food items of low nutritional value or items which are widely dispersed and therefore require increased foraging time are required to distribute their activity over a larger part of each 24-h period compared to animals with concentrated, highly nutritious food sources (Ashby 1972). Behavioural flexibility in the timing of activity may therefore provide individuals with a robust solution to meeting energetic requirements and maintaining physiological wellbeing under stochastic environmental conditions associated with climate change (Halle 2006, Hetem *et al.* 2012).

Tattersall (1987), from his work primarily on the Mayotte lemur (*Eulemur fulvus*), coined the term cathemeral to describe species that devote a significant portion of their 24-h activity to both the diurnal and nocturnal phases. Amongst primates, cathemerality is widely reported for Malagasy lemurs, specifically in two genera, the *Eulemur* and *Hapalemur*, as well as neo-tropical owl monkeys *Aotus* spp (Hill 2006b). The prevalence of this behavioural strategy amongst lemurs has been attributed to optimizing resource utilization (Kappeler and Erkert 2003) as well as reducing thermal stress (Curtis *et al.* 1999) and predator avoidance (Colquhoun 2006). However, the finding that nocturnal activity is not evident amongst African primates outside of Madagascar has been attributed to the increased risk of predation by nocturnal predators such as leopard (*Panthera pardus*), which are absent from Madagascar (Hill 2006b). Species which are restricted solely to diurnal activity are limited in their behavioural options for desynchronizing activity with potentially detrimental biotic or abiotic factors. Strictly diurnal species are therefore subjected to a finite amount of time per day in which they must achieve all fundamental activities (i.e. energetic, social, and biological; Dunbar 1992). For species with inflexible activity patterns inhabiting environments with large seasonal fluctuations in day length, environmental factors which significantly inhibit activity and thus the amount of time available to achieve all fundamental activities may therefore be important ecological determinants of habitat suitability under future climate change scenarios (Korstjens *et al.* 2010). The influence of environmental factors such as temperature and food availability on activity is therefore an important ecological consideration.

For primates, the primary response to thermal stress is to reduce activity, seek out suitable microclimates, and increase the frequency of thermoregulatory behaviours. Hill (2006a) therefore proposes that the thermal environment is an ecological variable which may impose a significant constraint on primate behaviour and activity patterns. Furthermore, optimal foraging theory predicts that an animal should expend more energy on foraging when food is abundant in order to maximize the net energy gain, whereas foraging effort should be decreased in favour of energy conservation when food availability is reduced (Norberg 1977). Indeed woolly monkeys (*Lagothrix lagotricha*), colobus monkeys, and green monkeys (*Cercopithecus sabaeus*) have all been shown to decrease traveling time and increase time spent resting under conditions of low food availability or quality (Harrison 1985, Da Silva 1993, Stevenson *et al.* 1994). In a meta-analysis of 66 primate species, Korstjens and colleagues (2010) showed that environmental temperature and diet were important determinant factors of enforced resting time, defined as time required for thermoregulation and digestion and therefore not available to be converted to traveling, feeding, or social activities (Dunbar 1996). Importantly, the authors show that the increased environmental temperatures predicted under future climate change scenarios will significantly increase the requirement for enforced resting time in many habitats, thus compromising habitat suitability as these individuals will face a trade-off between maintaining physiological wellbeing and completing other biologically important activities (Korstjens *et al.* 2010).

The ability of primates to employ behavioural flexibility to cope with long-term fluctuations in resource availability and climatic conditions as a result of climate change will be key to their survival. It has already been shown for my study population of vervet monkeys that they are capable of leaving their territorial boundaries in search of water during extended periods of drought (McDougall *et al.* 2010). Such a behavioural response to the direct effect of environmental stress is likely to have knock-on socio-ecological consequences such as increased intra- and inter-troop aggression, as well as increased risk of disease transmission. Previous studies on activity patterns in primates are however limited by traditional methods of observational data collection which are largely restricted to the diurnal period. The prevalence of behavioural mechanisms such as cathemerality may therefore be underestimated. The use of activity data loggers in my study provides me with the unique opportunity to accurately describe the 24-h activity patterns of vervet monkeys and quantify the proximal influence of environmental stressors on activity.

1.6 Dissertation aims

The studies described above (sections 1.4 and 1.5) illustrate that environmental stressors (temperature, water availability, and food availability) are important determinant factors of thermoregulatory precision and activity patterns for primates. The degree of physiological plasticity and behavioural flexibility employed by these species will likely be important determinants of their ability to cope with stochastic environmental conditions under future climate change scenarios. The paucity of long-term, continuous measurements of body temperature and activity for free-ranging mammals currently inhibits the development of robust theories on the potential of physiological and behavioural mechanisms to off-set the effect of rapid environmental changes associated with climate change for primates and other species. The broad aim of my dissertation is therefore to use biologgers to investigate the influence of environmental factors on the body temperature and activity rhythms of vervet monkeys, and thereby identify the important environmental factors which may determine habitat suitability for this population under future climate change scenarios.

In chapter four I will use continuous measurements of activity to investigate whether environmental factors infer a significant constraint on vervet monkey activity and determine empirically whether monkeys are able to adjust activity patterns in order to compensate for the potential constraint of environmental factors on activity.

In chapter five I will use continuous measurements of body temperature to describe for the first time the 24-h rhythm of body temperature for any free-ranging monkey in its natural habitat and investigate the influence of proximal environmental stressors on the thermoregulatory precision of vervet monkeys.

Based on my findings I will identify the potential environmental determinants of habitat suitability for vervet monkeys and discuss the ecological implications of future climate change for the study population of vervet monkeys.

CHAPTER 2

General methodology

This chapter aims to describe the study site and provide general socio-ecological information on the study species. I provide technical details on the biologgers used to measure body temperature and activity in free-ranging vervet monkeys, as well as detail the capture and surgical procedures for the implantation of biologgers. In chapter 3 I outline the climatic data collection protocol and describe the climatic conditions for the study site for the duration of my study.

A research permit for this project was obtained from the Department of Environmental Affairs and Tourism, Graaff-Reinet (Permit number - CRO 97/10CR), and permission to work at the field-site was provided by Samara Private Game Reserve (E:32/2011/GR). All capture and surgical procedures were approved by the University of the Witwatersrand Animal Ethics Screening Committee (clearance number AESC 2010/41/04).

2.1 Study site

My study was conducted at Samara Private Game Reserve, Eastern Cape Province, South Africa (24º 50´ 19´´ E, 32º 21´ 41´´ S). Samara is situated in the semi-arid Nama Karoo (Low and Rebelo 1996), on the southern tip of the Sneeuberg mountain range 30 km east of Graaff-Reinet (Fig. 2.1). The reserve was established in 1997 and encompasses a total land area of 28 000 ha. Samara is characterized by a diverse mosaic of landscapes and vegetation types. Four biomes, namely Grassland, Nama Karoo, Savanna, and Subtropical Thicket are distinguished, as well as an azonal vegetation type which is defined by the co-existence of two or more biomes (van Cauter 2004, Fig. 2.2). The topography ranges from open Karoo plains (760 m.a.s.l.) to steep ridges and plateaus of the escarpment (1400 m.a.s.l.). The regional climate is defined by strong seasonality in temperature, rainfall (Fig. 2.3), and photoperiod. Day length (i.e. photoperiod) is shortest in June (10.1 ± 0.1 h) and peaks in December (14.2 ± 0.1 h; data from United States Naval Observatory). The region is characterized by hot summers (December, January, and February) and cold winters (June, July, and August). Maximum air temperatures exceed 40 °C in summer and minimum air temperatures frequently drop below freezing in winter. The Karoo also experiences large 24-h fluctuations in air temperature. In winter 24-h temperatures can range from minimums of ~ -5 to maximums of 30 °C, and in summer ranging from ~ 5 to 40 °C (Low and Rebelo 1996). Average annual rainfall is 307 ± 68 mm (range 243 mm in 2004 to 406 mm in 2000, for the period 2000 - 2010). Peak rainfall occurs in the summer months of January and February whereas winter is generally dry (Fig. 2.3).

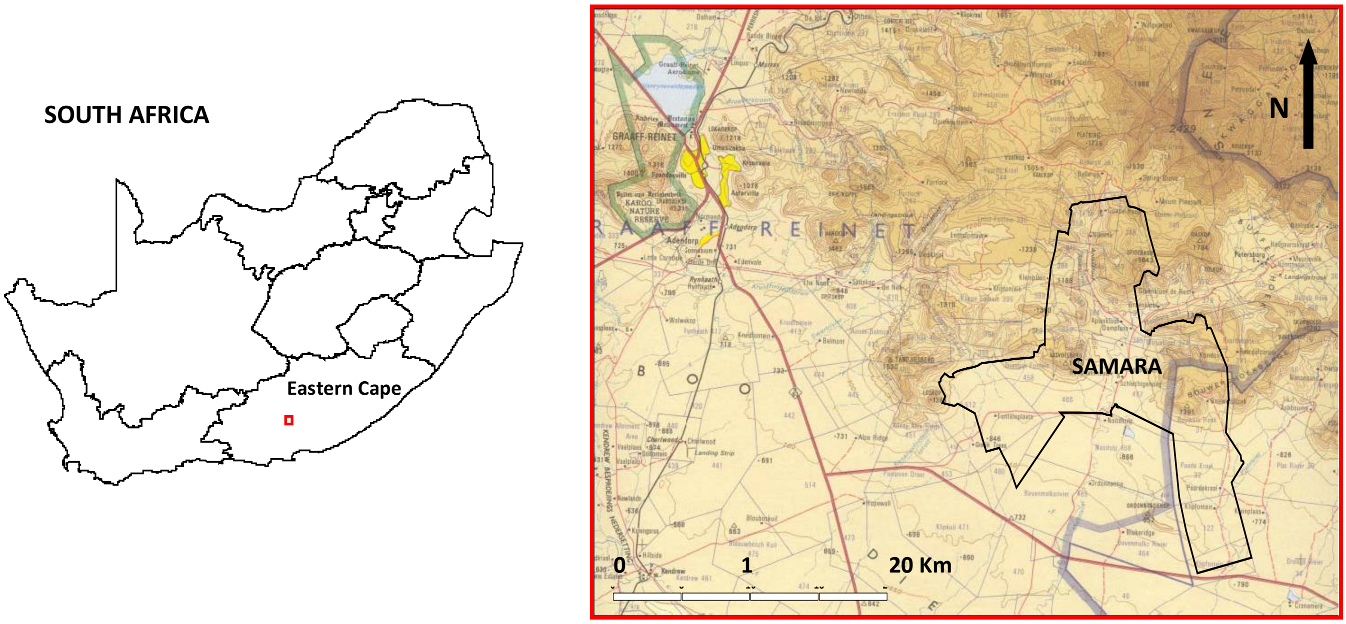

Figure 2.1: The location of Samara Private Game reserve in South Africa (*left*) and the reserve boundary illustrating the regional relief (*right*). The northern section of the reserve is mountainous, stretching out on to the Karoo plains in the southern section of the reserve. Maps courtesy South African Geographical Services and reserve boundary GPS data courtesy Ann van Cauter, Nelson Mandela Metropolitan University, South Africa.

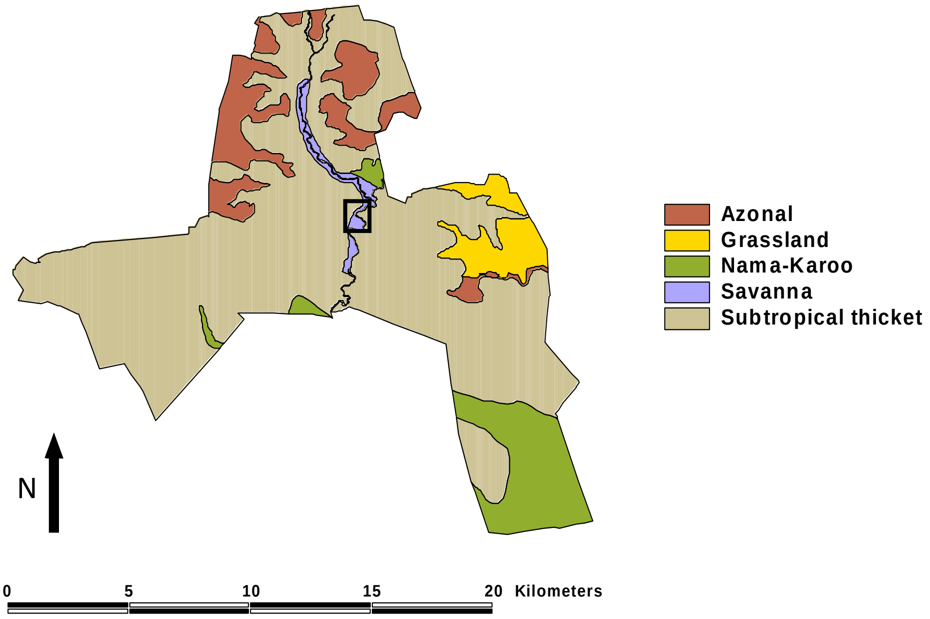

Figure 2.2: Vegetation types of Samara Private Game Reserve with location and approximate combined extent of the study troops home ranges (*clear square*) centred on the Melk river. Vegetation GPS data courtesy A. Van Cauter.

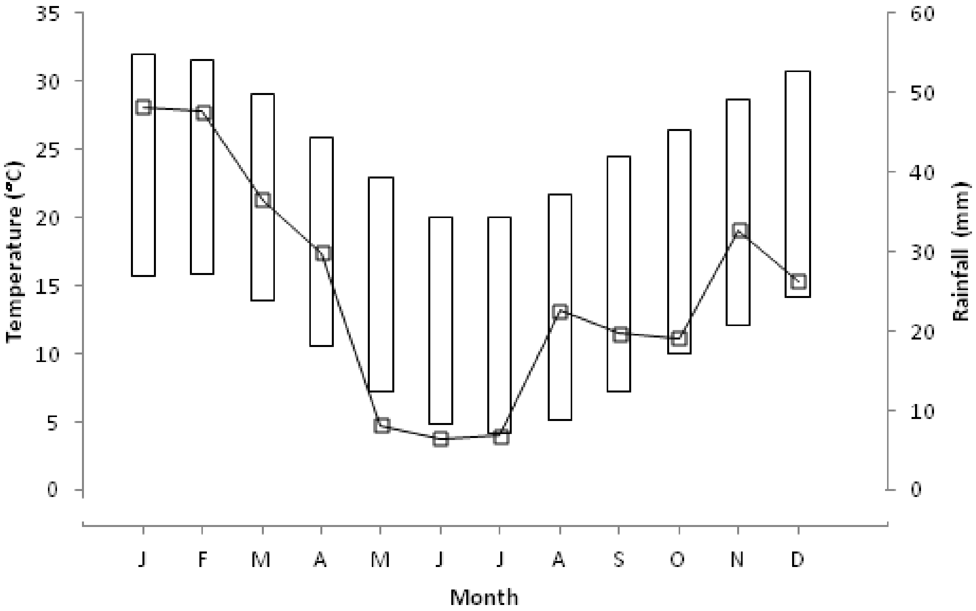

Figure 2.3: Mean monthly minimum and maximum air temperature (*bar, left axis*) and rainfall (*square, right axis*) from the Graaff-Reinet weather station, for the period 2000 – 2010. Data courtesy South African Weather Services.

The Nama Karoo falls within the southernmost distribution range of vervet monkeys (Fig. 2.4). The Karoo is marginal habitat for vervet monkeys and they are constrained to narrow corridors of suitable habitat along rivers (Pasternak *et al.* 2013). The marginal habitat and characteristically harsh environmental conditions experienced in the Karoo makes Samara the ideal site to investigate the behavioural and physiological mechanisms employed by vervet monkeys in response to environmental stress. Vervet monkeys are vulnerable to protracted periods of drought (Wrangham 1981). However, for the duration of my study the Melk river, which bisects the home range of the study population, provided monkeys with a permanent natural source of water. The riverine habitat of Samara shows few signs of transformation and is characterized by dense stands of Sweet thorn (*Acacia karroo*) woodland interspersed with predominantly Wolfthorn (*Lycium oxycarpum*), Karee (*Searsea lancea*), and Firethorn (*S. pyriodes*). The woody component of the habitat ranges in height from 1 - 5 m, with a canopy cover of 70 - 80 % in the monkeys' home range (Pasternak *et al.* 2013). The large exotic pepper tree (*Schinus molle*), mature trees ranging in height from 6 - 12 m, occurs scattered along the river bank at low densities, and is commonly utilized as sleep sites by the monkeys (personal observation). Sweet thorn trees provide the monkeys at Samara with a reliable source of food throughout the year, as they utilize the leaves, seeds, flowers, and gum of the trees, thus constituting the most important component of their diet (Pasternak *et al.* 2013). The population density of monkeys within the riverine habitat at Samara is estimated at 55 animals/km^2 (Pasternak *et al.* 2013).

Figure 2.4: The distribution range (*green shaded area*) of vervet monkeys as of 2008, from the International Union for Conservation of Nature (Kingdon *et al.* 2008).

Vervet monkeys are preyed upon by four types of predators; mammalian carnivores, avian predators, baboons, and snakes (Skinner and Chimimba 2005). On Samara chacma baboons have not been observed to prey on vervet monkeys (R. Slater, personal communication). The mammalian carnivore community consists of caracal (*Caracal caracal*) and black-backed jackal (*Canis mesomelas*), which are predominantly nocturnal hunters, and cheetah (*Acinonyx jubatus*), which are diurnal. During the summer of 2011 three vervet monkey mortalities as a result of puff adder (*Bitis arietans*) bites were confirmed by a veterinarian (G. Pasternak, personal communication). Other potentially dangerous snake species at Samara include the boomslang (*Dispholidus typus*) and Cape cobra (*Naja nivea*). Potential avian predators are the Verreaux`s (*Aquila verreauxii*) and martial (*Polemaetus bellicosus*) eagles, which occur at low densities in the region.

2.2 Study species

2.2.1 Habitat and ecology

Vervet monkeys (Fig. 2.5) are African cercopithecines which, together with baboons, are the most widely distributed and abundant primates in Africa (Struhsaker 1967a). Vervet monkeys are most abundant near riparian vegetation of the Savanna, but are highly adaptable. They are able to persist in fragmented vegetation, including cultivated lands, and are also found in both rural and urban environments (Struhsaker 1967b, Skinner and Chimimba 2005). Unlike forest dwelling primates, vervet monkeys are dependent on direct access to water, and habitat suitability is primarily dependent on access to water and adequate sleeping sites (Wrangham 1981). However, in 2010 one of the focal troops for my study was recorded going without access to freestanding water for 33 days without any observable ill-effects, possibly by obtaining the necessary quantities of water from its diet (McDougall *et al.* 2010, Pasternak *et al.* 2013). Vervet monkeys are opportunistic omnivores but feed primarily on wild fruits, flowers, leaves, seeds, and seed pods (Struhsaker 1967b, Skinner and Chimimba 2005). Insects, such as grasshoppers and termites, as well as bird eggs typically supplement the diet (Struhsaker 1967b, Skinner and Chimimba 2005).

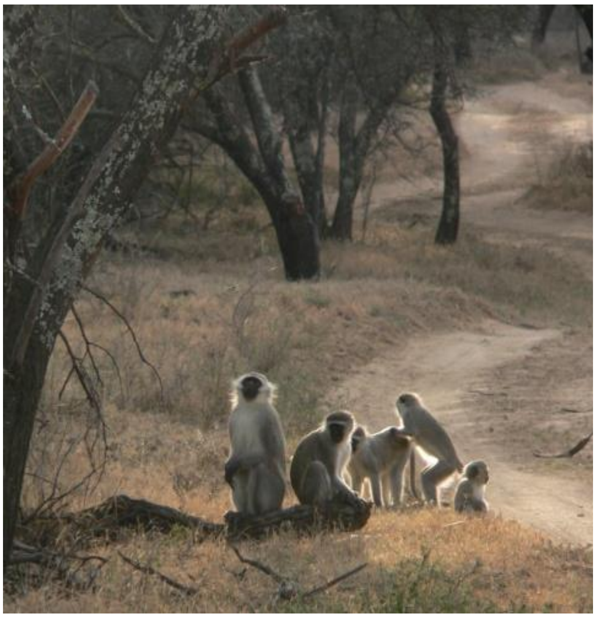

Figure 2.5: Vervet monkeys in their natural riverine habitat at Samara Private Game Reserve (Picture: Hayley Clements).

Food and water availability is also an important determinant of the home range size of vervet monkeys, with home range size typically varying between 0.06 to 1.78 km^2, but seasonal and geographical variations in home range size are common (Struhsaker 1967b, Wrangham 1981, Willems and Hill 2009). Within their home range troops defend relatively well defined territories, with both males and females, excluding infants, contributing towards the active defence of territorial boundaries against intrusion by neighbouring troops (Struhsaker 1967b). Territorial boundaries are generally all-inclusive, constituting feeding, sleeping, breeding, and rearing areas (Struhsaker 1967a).

2.2.2 Social structure

Vervet monkey social structures are typically described as multi-male, female-bonded societies (Wrangham 1980). Mean troop sizes recorded at ten sites across southern Africa varied from 12 – 33 individuals (Pasternak 2011). Vervet monkeys are sexually dimorphic, males being larger than females; the mean mass of 29 adult males recorded in northern Botswana was 5.51 kg (range: 3.86 – 8.00 kg), and the mean mass of 30 adult females was 4.09 kg (range: 3.41 – 5.22 kg; Skinner and Chimimba 2005). Females are philopatric as they do not leave their natal troop (Struhsaker 1967a). Males however commonly move between troops (Cheney and Seyfarth 1983). Typically males emigrate twice in their life time, first leaving their natal troop at puberty and once again leaving their new troop as adults (Cheney and Seyfarth 1983). Males generally emigrate to neighbouring troops, and migrations occur more frequently during the mating season (Henzi 1982, Cheney and Seyfarth 1983). The dispersal of males to neighbouring troops occurs non-randomly, and males are often accompanied by their brothers or peers (Cheney and Seyfarth 1983). Non-random dispersal reduces the risk of reproducing with closely related kin, and being accompanied by allies reduces the probability of attack by resident monkeys (Cheney and Seyfarth 1983).

Vervet monkey social organization is characterized by linear male and female dominance hierarchies which are independent of each other (Struhsaker 1967a). A dominance hierarchy is established amongst individuals of a social group when competition for priority access to resources leads to aggressive interactions (Richards 1974). Female dominance hierarchies are relatively stable, with juvenile females inheriting the rank of their mother, whereas male hierarchies are more labile (Struhsaker 1967a, Cheney and Seyfarth 1983). Male rank has been shown to change throughout the year, being primarily dependent on fighting ability (Cheney and Seyfarth 1989). In the vervet monkey dominance hierarchy, a priority relationship between dominant and subordinate animals is maintained by frequent non-aggressive behaviours such as displacement and vocal and facial threats (Struhsaker 1967a, Henzi 1982). The constant maintenance of a dominance hierarchy by non-aggressive means facilitates the reduction of physical aggression when an immediate gain is involved, such as access to food, mating, or grooming opportunities (Struhsaker 1967a). It is therefore proposed that the functional benefit of a dominance hierarchy is to reduce physical aggression and conserve energy (Struhsaker 1967a).

2.3 Study subjects

For my study data were collected from two free-ranging troops distinguished as Riverbend Mob (RBM) and Riverside Troop (RST). In 2011 RST was the larger of the two troops (see Table 2.1 for a detailed demographic description of both troops). RBM and RST occupied adjacent territories along the Melk river within the savanna biome. Although RBM was the smaller of the two troops, RBM’s home range (1.76 km^2) was larger than that of RST’s (0.64

km^2). There was significant overlap of home ranges between these and other neighbouring troops (Pasternak *et al.* 2013). The study troops relied on a completely natural diet, comprising of 26 different species of plant, with Sweet thorn products constituting 33.8 % of their total diet. Berries of the creeping saltbush (*Atriplex semibaccata*) comprised 15.3 % of their diet, and insects, primarily grasshoppers and termites, were the third largest component of their diet, accounting for 7.7 % of their diet (Pasternak *et al.* 2013).

Table 2.1: Troop composition of Riverbend Mob (RBM) and Riverside Troop (RST) in 2011. Data courtesy N. Freeman and G. Pasternak, University of Lethbridge, Canada.

Demographic parameter	RBM	RST
Adult males	9	14
Adult females	18	26
Sub-adults	22	32
Group size	49	72
Home range (km^2)	1.76	0.64
Sex ratio (M:F)	0.50	0.54

RBM and RST were first habituated to the presence of observers in 2008. Study troops were habituated by students and volunteers from the University of Lethbridge, Canada. Individual monkeys from both troops can be identified by unique morphological characteristics. Since 2008 these troops have been the subjects of a long-term study of their behavioural ecology, under the auspices of Professors Peter Henzi and Louise Barrett (see McDougall *et al.* 2010, Freeman *et al.* 2012, and Pasternak *et al.* 2013 for publications to date).

2.4 Biologgers

In my study I used temperature-sensitive and activity data loggers to obtain continuous measurements of body temperature and activity for vervet monkeys free-ranging in their natural habitat. The temperature-sensitive data loggers (model: mlog T1C, Sigma Delta Technologies, Perth, Australia) had dimensions of 30 x 25 x 10 mm and a mass of ~ 12 g when coated in wax (Fig. 2.6a). Temperature-sensitive data loggers were set to measure instantaneous body temperature at 5 min intervals at a resolution of 0.06 °C. To allow for comparison between loggers, all loggers were calibrated at the end of the study against a certified precision thermometer (Quat 100, Heraeus, Hanau, Germany) in an insulated water bath. Loggers were calibrated at 2 °C intervals between 34 and 42 °C, thus incorporating the full range of body temperatures likely for vervet monkeys. During calibration, temperature-sensitive data loggers recorded temperature at 5 min intervals and a precision thermometer reading was taken every hour, which allowed the water bath temperature time to stabilize between readings. The best fit equation for the relationship between temperature-sensitive data logger (x – axis) and precision thermometer (y – axis) readings was computed (using TableCurve 2D, Jandel Scientific, San Rafael, United States of America). A straight line equation (y = mx + c) was used for the calibration process. The accuracy of the straight line fit was assessed by examining the r-squared value and the fitted standard error (see Fig. 2.7 for example). For all loggers the r-squared values were > 0.99, and the mean standard error was 0.01 ± 0.004, below the resolution of the loggers, and therefore the application of a straight line equation provided a sufficiently good fit for the calibration. The equation derived from the fitted straight line was applied to each data point for the raw data downloaded from the temperature-sensitive data loggers at the end of my study, and these calibrated data points were used for further analyses.

The activity data loggers (model: ADXL345, Sigma Delta Technologies) had dimensions of 50 x 30 x 15 mm and a mass of ~ 21 g when coated in wax (Fig. 2.6b). Activity data loggers had an omni-directional accelerometer with sensitivity across three planes at 4 mg/LSB. The animals' movement was recorded as activity (accelerometer) counts over a 10 s period at the start of every minute. Raw activity count values ranged from 0 to 225 activity counts. For all monkeys, the activity count data was standardized by converting each count to a percentage of the maximum activity count recorded for that individual, allowing for comparison between monkeys (sensu Hetem *et al.* 2012).

Male monkeys were also implanted with tracking transmitters (African Wildlife Tracking, Pretoria, South Africa), with dimensions of 80 x 35 x 20 mm and a mass of ~ 44 g with wax, to ensure that any males that emigrated from the study troops could still be located to retrieve the temperature-sensitive and activity data loggers. The maximum combined mass of implants constituted 1.5 % of mean male body mass (5.71 ± 1.02 kg) and 1 % of mean female body mass (4.13 ± 0.49 kg) for implanted individuals in my study.

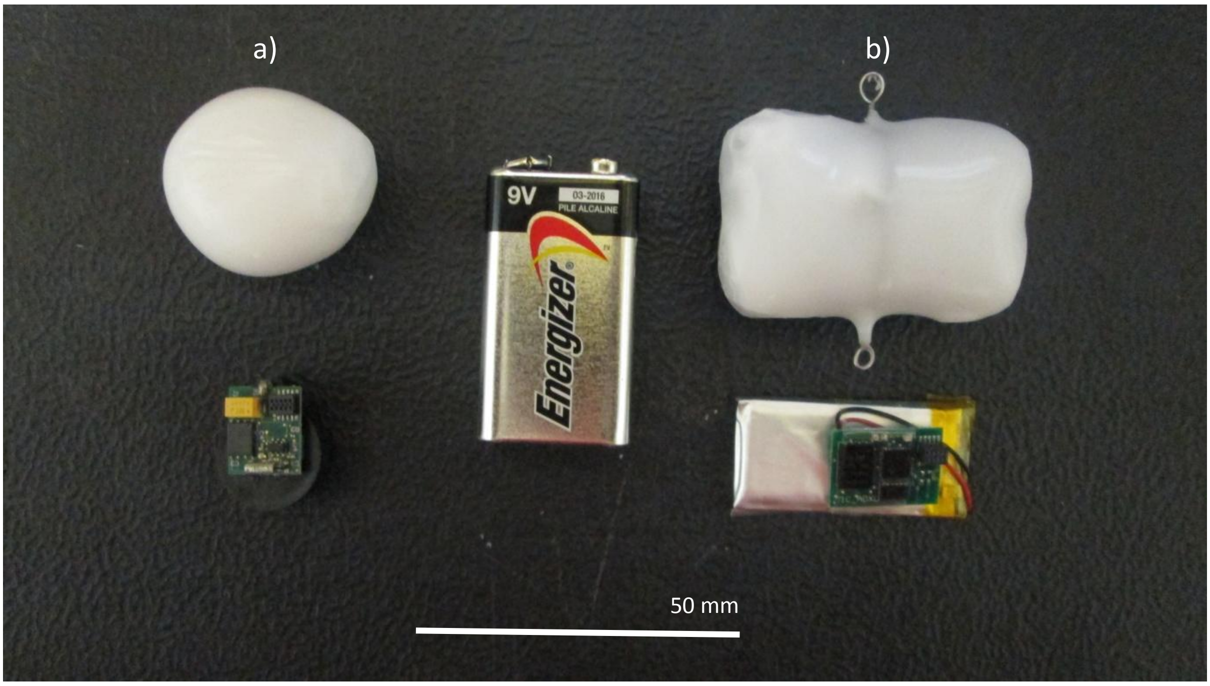

Figure 2.6: Temperature-sensitive data logger uncoated and in wax (*panel a*), and activity data logger uncoated and in wax with tethers (*panel b*). A standard 9 V battery is presented for a size reference.

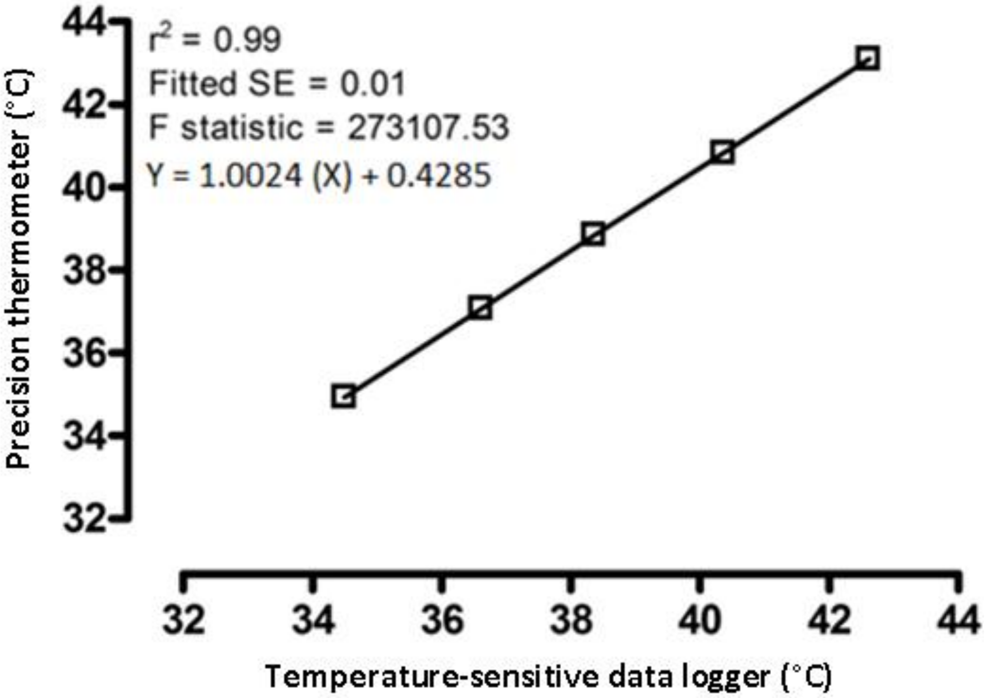

Figure 2.7: Precision thermometer temperature correlated to corresponding temperature-sensitive data logger temperature, with the linear regression line plotted. The regression statistics and calibration equation (y = m (x) + c) used to calibrate this temperature-sensitive data logger post surgery are provided.

2.5 Capture and surgery

My project included three surgical periods. The purpose of the first (14 - 20 April 2011) and second (2 - 10 July 2011) periods were to capture individuals from the two study troops to implant biologgers. The darting (capture) of monkeys and surgery was carried out by an experienced veterinarian with assistance from a vet-nurse. In total there were 25 animals implanted with biologgers, 11 from RBM (five males and six females) and 14 from RST (six males and eight females). Study animals were recaptured on the third surgery trip (15 - 20 May 2012) to retrieve biologgers.

Monkeys were darted individually with a blowpipe (Dan-Inject system, Model: Zoo, Blow 1.25, Dan-inject APS, Børhup, Denmark). Prior to surgery the troops were habituated to the blowpipe being pointed in their direction by having the observers in contact with the troop simulate use of the blowpipe with an aluminium or plastic tube of the same size, but with no projector. A drug combination was used based on the required dosage for a 5 kg monkey. The monkeys were immobilized *via* a dart containing ketamine (5 $mg.kg^{-1}$, Anaket-V, Bayer (Pty) Ltd., Johannesburg, South Africa) with midazolam (0.25 $mg.kg^{-1}$, Dormicum, Roche Products (Pty) Ltd., Johannesburg, South Africa). Midazolam has the added benefit of inducing retroactive amnesia, which was assumed *apriori* to increase the probability of successful re-darting of monkeys at the end of the study. Once recumbent (~ 5 min) the animal was transported to a temporary theatre within 5 km from the field site.

At the theatre (Fig. 2.8) anaesthesia was maintained as necessary with 0-2 % isoflurane (Isofor, Safe Line Pharmaceuticals (Pty) Ltd., Johannesburg, South Africa) administered in 100 % oxygen *via* an endotracheal tube. Respiratory rate, haemoglobin oxygen saturation, heart rate, level of carbon dioxide released at end of expiration (LifeSense® Vet LS1-10R, Medair AB, Hudiksvall, Sweden), blood pressure (Cardell® veterinary vital signs monitor, Model 9403, Sharn veterinary, Inc., Tampa, United States of America), and rectal temperature (Thermocouple thermometer, BAT-12, Physitemp Instruments Inc., New Jersey, United States of America) were monitored at 5 min intervals throughout the surgical procedure. Monkeys were kept warm during surgery with electric blankets and heating pads. Total surgery time was ~ 25 min. Monkeys were weighed, to the nearest 0.01 g, and a lubricant was applied to each eye (Terra-Cortril, hydrocortisone: Pfizer laboratories (Pty) Ltd., Johannesburg, South Africa). Prior to surgery each monkey was injected intramuscularly with a long-acting antibiotic (Penicillin: 0.001 mg.kg^{-1}, Peni LA Phenix, Virbac Animal Health, Johannesburg, South Africa), anti-inflammatory medication (Rimadyl, Carprofen: 3 mg.kg^{-1}, Pfizer Laboratories Ltd.), and analgesic (Temgesic, Buprenorphine: 0.02 mg.kg^{-1}, Kyron Laboratories Ltd., Johannesburg, South Africa).

A ~ 100 x 100 mm patch on the ventral abdominal surface of each monkey was shaved and sterilized with Hibitane (chlorhexidine gluconate, Astra Zeneca, Johannesburg, South Africa) in alcohol. A local anaesthetic was administered subcutaneously to the surgical site (Lignocaine: 40 mg/animal, Bayer (Pty) Ltd.) and within this area a 30 mm midline incision was made through the skin and *linea alba*. A temperature-sensitive data logger was placed in the abdominal cavity, where it floated freely, and an activity data logger was tethered into the suture line of the muscle and *linea alba*. Males were also implanted with a tracking transmitter. All loggers were coated with an inert wax (Sasol, Johannesburg, South Africa) and dry-sterilized in formaldehyde vapour prior to implantation. Once biologgers were in place, the muscle and skin layers were sutured closed. During surgery the animals were maintained on a drip (50 % dextrose, B. Braun Medical Ltd., Northriding, South Africa) administered at 1 drop.s^{-1}.

Post-surgery the wound was treated with a germicidal wound spray (F10, Health and hygiene, Johannesburg, South Africa). Animals were allowed to recover in a warm, dark cage, before being released out of sight of other monkeys near the rest of the troop. All study animals successfully rejoined their respective troops. The released monkeys' recovery was opportunistically monitored by the veterinarian, and in general monkeys appeared to be unhindered by the surgical procedure within two days of surgery (L. Meyer, personal communication).

Figure 2.8: A veterinarian conducting surgery to implant data loggers in an anaesthetized vervet monkey at the temporary theatre on the Samara Private Game Reserve (Picture: Hayley Clements).

In March 2012, monkeys were recaptured and a similar surgical procedure was followed to remove the biologgers. For all recaptured animals the loggers were successfully recovered, the animals' wounds had healed and there were no signs of infection from the implant surgery. Following surgery all monkeys were successfully reincorporated in to their troops.
Of the 25 implanted monkeys, two males from RST died (confirmed by observation of carcass) during the study. One female from RBM disappeared, and as females are not known to emigrate, she was presumed dead. Of the remaining animals, 12 (six males and six females) were recaptured in May 2012. Ten of these 12 recaptured monkeys had been implanted with biologgers in April 2011 and two received implants in July 2011. Five recaptured monkeys were from RST (two males and three females) and seven from RBM (four males and three females). Mean body mass of recaptured monkeys was 4.56 ± 1.42 kg (six females: 4.13 ± 0.49 kg, six males: 5.71 ± 1.02 kg).

Four of the 12 temperature-sensitive data loggers retrieved from monkeys stopped recording prematurely and three failed the day after they were launched, and thus provided no data. Nine of the activity data loggers failed prematurely. To account for the potential influence of post-surgery trauma on body temperature and activity, the first two weeks of data were discarded (sensu Maloney *et al*. 2011). Day 1 of data for analyses was therefore on the 1st May for monkeys implanted on the first surgery trip in April 2011 and the 15th July for monkeys implanted on the second surgery in July 2011. See below (Table 2.2) for a detailed description of recaptured monkeys.

Table 2.2: Study subjects, troop, sex, mass, and period of data collection (number of days, n) for temperature-sensitive and activity data loggers.

				Temperature data logger			**Activity data logger**		
ID	**Troop**	**Sex**	**Mass (kg)**	**Start date**	**End date**	**n**	**Start date**	**End date**	**n**
M1	RST	M	6.05	01/05/2011	21/12/2011	249	01/05/2011	04/07/2011	81
M2	RST	M	5.66	Failed		0	01/05/2011	09/02/2012	263
F1	RST	F	2.85	01/05/2011	29/02/2012	305	01/05/2011	29/02/2012	305
F2	RST	F	3.81	01/05/2011	29/02/2012	305	01/05/2011	09/09/2011	148
F3	RST	F	3.44	01/05/2011	29/02/2012	305	01/05/2011	27/01/2012	288
M3	RBM	M	6.36	Failed		0	01/05/2011	23/09/2011	160
M4	RBM	M	6.53	01/05/2011	29/02/2012	305	01/05/2011	07/01/2012	262
M5	RBM	M	5.95	01/05/2011	17/11/2011	211	01/05/2011	07/02/2012	288
M6	RBM	M	3.73	01/05/2011	12/02/2012	292	01/05/2011	29/02/2012	305
F4	RBM	F	3.01	01/05/2011	29/02/2012	305	01/05/2011	29/02/2012	305
F5	RBM	F	3.25	Failed		0	15/07/2011	29/02/2012	230
F6	RBM	F	4.13	15/07/2011	06/11/2011	117	15/07/2011	29/02/2012	230

RST = Riverside Troop, RBM = Riverbend Mob

CHAPTER 3

Climatic conditions at study site

3.1 Introduction

In this chapter I describe the climatic conditions (environmental temperature, photoperiod, and rainfall) for Samara Private Game Reserve for the duration of my study. In addition, I describe the development of an index for cloud cover derived from measurements of black-globe and dry-bulb temperature. Climatic data presented here will be used in subsequent chapters to investigate the influence of environmental factors on vervet monkey activity (Chapter 4) and body temperature (Chapter 5).

Environmental temperature, photoperiod, and food availability have been shown to be important determinants of activity (e.g. temperature; Hill 2006a, photoperiod; Aujard *et al.* 2007, food; Da Silva 1993) and body temperature (e.g. temperature; Boyles *et al.* 2012, photoperiod; Heldmaier *et al.* 1989, food; Humphries *et al.* 2003) patterns in mammals. As I did not quantify food availability or quality in my study, I used a measure of cumulative rainfall as a proxy for food availability. It has been shown for various sub-Saharan sites that primary production is correlated with rainfall (Barton *et al.* 1992, Le Houerou 1984). Furthermore, for baboons, the availability and quality (i.e. high energy fruits and seeds) of food has been shown to be a positive function of rainfall (Barton *et al.* 1992, Hill and Dunbar 2002). Baboons have a similarly diverse dietary composition to vervet monkeys (Skinner and Chimimba 2005), with only 19 % of the diet of baboons consisting of leaves and 24 % of the diet of vervet monkeys consisting of leaves (Korstjens *et al.* 2010). For baboons inhabiting a region of central Kenya, East Africa, which experiences similar seasonal climatic conditions to Samara, with the dominant vegetation type also being *Acacia* species (Pasternak *et al.* 2013), the sum of monthly rainfall was significantly correlated with the availability of food items (Barton *et al.* 1992). The authors note however that there was a time-lag in the effect of rainfall on biomass of food availability, with the sum of rainfall over the two previous months being the strongest predictor of food availability in the current month (Barton *et al.*

1992). Based on this finding I used the sum of rainfall over the two previous months as a proxy for food availability in the current month in my study.

I recorded black-globe and dry-bulb temperature as measures of the thermal environment which vervet monkeys experienced. There are four factors which influence the thermal environment which an animal experiences, and therefore also influence the animals' thermal balance in that environment. These factors are air temperature, solar radiation, wind speed, and water vapour pressure (Hetem *et al.* 2007). Black-globe temperature is measured in direct sunlight and incorporates the effect of air temperature, solar radiation, and wind speed, whereas dry-bulb temperature does not incorporate the influence of solar radiation or wind speed. Although black-globe temperature does not reflect water vapour pressure it is considered a physiologically more appropriate measure of the thermal environment which an animal experiences compared to dry-bulb temperature (Hetem *et al.* 2007). Hence black-globe temperature has been used as a reliable estimate of operative environmental temperature (e.g. Huey 1991, Corbet *et al.* 1993). I therefore used black-globe temperature to investigate the influence of environmental temperature on vervet monkey activity (Chapter 4) and body temperature (Chapter 5), as black-globe temperature provides the best single index of the thermal stress experienced by an animal (Hetem *et al.* 2007).

By mediating the availability of ground-level access to direct sunlight, cloud cover also influences the thermal environment which an animal experiences. Cloud cover has, for example, been shown to be an important determinant of behavioral thermoregulation in primates. In winter Japanese macaques in Yakushima, Japan, spent more time inactive engaged in sunbasking and huddling when it was clear than when it was cloudy. Both sunbasking and huddling in direct sunlight were associated with higher skin temperature compared to these behaviours when conditions were cloudy (Hanya *et al.* 2007). As black-globe temperature incorporates the influence of solar radiation and dry-bulb temperature does not, it stands to reason that the difference between black-globe and dry-bulb temperature at any given time will be higher when overhead conditions are clear compared to cloudy, as cloud cover reduces ground-level solar radiation (Liepert 2002). I therefore developed an index of cloud cover based on the difference between black-globe and dry-bulb temperature to estimate ground-level access to direct sunlight. Wind speed however also influences black-globe temperature and may therefore confound my index of cloud cover. To test the hypothesis that the difference between black-globe and dry-bulb temperature provides a reliable estimate of cloud cover, I compared the mean difference between these temperature measurements on clear days compared to overcast days (as confirmed by visual observation).

3.2 Materials and methods

3.2.1 Data collection

For the period May 2011 to February 2012, black-globe and dry-bulb temperatures (°C) were measured at 30 min intervals by a weather station (Hobo U12, Onset Computer Corporation, Pocasset, United States of America) on site, in an open clearing exposed to direct sunlight throughout the day (Fig. 3.1). Black-globe temperatures were recorded by a probe placed in a copper ball of 150 mm diameter which was painted matt black to absorb solar radiation. The dry-bulb temperature probe was housed in a ventilated air vane which was painted white to reflect solar radiation. The general weather conditions were recorded daily in four categories; namely raining, overcast, partial cloud, and clear, between 08:00 to 12:00 and 12:00 to 16:00 respectively. General weather data were only collected when I was present on site for the duration of the 08:00 to 12:00 (n = 147 days) or 12:00 to 16:00 (n = 118 days) period.

Rainfall was measured on site by means of a standard 100 mm rain gauge. Total rainfall was recorded after each rainfall event. The resources (i.e. food and water) available to monkeys were not manipulated in any way throughout my study. The availability of free-standing water within the home range of the monkeys was recorded as present or absent on a daily basis for the duration of my study.

Figure 3.1: Weather station with black-globe temperature (*black copper ball*) and dry-bulb temperature (*white vane*) probes, positioned 1 m above ground in an open clearing.

To calculate photoperiod, time of sunrise and sunset for Samara Private Game Reserve were obtained from the United States Naval Observatory (http://aa.usno.navy.mil/data) using the following co-ordinates; 24° 50´ 19´´ E, 32° 21´ 41´´ S. The distinction between the diurnal and nocturnal periods over the 24-h period was an important component of analyses throughout my dissertation. To determine the duration of the diurnal (i.e. sunrise to sunset) and nocturnal (i.e. sunset to sunrise) periods across the duration of my study period, I calculated the mean time of sunrise and sunset for each month (see Table 3.1).

Seasons were differentiated based on environmental temperature (sensu Fuller *et al.* 2005). The three coldest months (June, July, and August), determined from mean 24-h black-globe temperature, were termed “winter”, and successive three month periods named “spring” (September, October, November), and “summer” (December, January, February). For seasonal comparisons I was specifically interested in investigating the activity and body temperature patterns of vervet monkeys during the warmest (i.e. summer) and coolest (i.e. winter) periods of my study, which reflect the “extremes” in environmental temperatures which monkeys were exposed to. For comparisons between different periods of the diurnal phase, the diurnal phase was further divided in to three segments of similar duration in winter and summer respectively, referred to as “morning” (winter: 07:00 – 10:30, summer: 05:30 – 10:00), “afternoon” (winter: 10:30 – 14:00, summer: 10:00 – 15:00), and “evening” (winter: 14:00 – 17:30, summer: 15:00 – 19:30).

Table 3.1: Mean time of sunrise, sunset, and length of diurnal and nocturnal periods for respective months.

Month	Sunrise	Sunset	Diurnal (h)	Nocturnal (h)
May	07:05	17:35	10.5	13.5
June	07:20	17:25	10.1	13.9
July	07:20	17:35	10.3	13.7
August	06:57	17:55	10.9	13.1
September	06:20	18:15	11.9	12.1
October	05:40	18:35	12.7	11.3
November	05:15	19:00	13.8	10.2
December	05:10	19:25	14.2	9.8
January	05:30	19:30	14.0	10.0
February	05:58	19:10	13.2	10.8

3.2.2 Data analyses

To investigate the interrelatedness between measured environmental variables I conducted Pearsons’ correlations of the sum of rainfall over the two previous months (a proxy for food availability) against mean monthly photoperiod and mean monthly 24-h black-globe temperature, and of mean monthly 24-h black-globe temperature against mean monthly photoperiod across the duration of my study period.

To compare the thermal environment experienced by vervet monkeys between winter and summer in terms of a physiologically relevant parameter, I used the approximate TNZ of primates as a guideline to distinguish between “low” and “high” environmental temperatures. The approximate TNZ for primates is 25 – 30 °C (Elizondo 1977), which was derived from experiments on rhesus macaques, a species of similar body size to vervet monkeys. The TNZ is however defined under laboratory conditions, thus not incorporating the influence of solar radiation or wind speed on the thermal environment. As there is no data available on the TNZ of vervet monkeys, I use the identified TNZ range of primates only as a guideline for a course description of the thermal stressors which vervet monkeys were exposed to in winter compared to summer. For free-ranging animals the thermal environment is best described in terms of black-globe temperature (Hetem *et al.* 2007), and therefore I calculated the percentage of low (< 25 °C) and high (> 30 °C) 30 min black-globe temperature recordings over the diurnal, nocturnal, and 24-h periods in winter and summer respectively. Diurnal and nocturnal periods were calculated from the mean time of sunrise (winter: 07:12, summer: 05:29) and sunset (winter: 17:38, summer: 19:22), rounded-off to the nearest 30 min to coincide with the interval of black-globe temperature data collection.

To compare black-globe temperature between the morning, afternoon, and evening periods of the respective days in winter and summer, I conducted a repeated measures one-way ANOVA with Newman-Keuls post-hoc analysis on the mean morning, afternoon, and evening black-globe temperatures for all days in winter and summer.

To determine whether the difference between black-globe and corresponding dry-bulb temperature provided a reliable index of cloud cover, I conducted a one-way ANOVA with Newman-Keuls post-hoc analysis on the mean difference between black-globe and dry-bulb temperature on clear, partial sunlight, and overcast days for the 08:00 to 12:00 and 12:00 to 16:00 periods respectively.

For all analyses the Kolmogorov-Smirnov test was used to test the data for normality. All statistical analyses were conducted using IBM SPSS V.10 statistics software. If variables were significantly correlated, I conducted linear regression analysis to determine the straight line equation. Results were significant at $p < 0.05$.

3.3 Results

In winter black-globe temperatures regularly dropped below freezing (23 % of days between 1 June and 31 August 2011), whereas black-globe temperatures never were below zero in summer. During my study period the summer was very hot, with black-globe temperature exceeding 40 °C on 54 % of days between 1 December 2011 and 29 February 2012, compared to only 1 day of over 40 °C in winter. Mean 24-h black-globe temperature was lowest in July and highest in January (Fig. 3.2a). Mean photoperiod was shortest in June and longest in December (Fig. 3.2b). The sum of rainfall over the two previous months (an index of food availability) progressively decreased from the beginning of the study period to a minimum in October, before increasing again over summer (Fig. 3.2c). There was rainfall throughout the year, with only September being relatively dry (Fig. 3.2d, *left panel*). During my study period there was 450 mm of rain over 10 months (Fig. 3.2d, *right axis*).

During my study, mean monthly 24-h black-globe temperature and photoperiod varied co-linearly across months ($r^2 = 0.71$, $n = 10$, $p < 0.01$, Fig. 3.3a). There was however no significant relationship between the sum of rainfall over the two previous months and mean monthly photoperiod ($r^2 = 0.04$, $n = 10$, $p = 0.57$) or mean monthly 24-h black-globe temperature ($r^2 = 0.02$, $n = 10$, $p = 0.69$, Fig. 3.3b).

During the diurnal period in winter, the primary thermal stressor was low (< 25 °C) environmental temperatures (constituting over 50 % of black-globe temperature recordings), compared to the diurnal period in summer, when the primary thermal stressor was high (> 30 °C) environmental temperatures (constituting over 60 % of black-globe temperature recordings). During the nocturnal period, the primary thermal stressor was low environmental temperatures in both winter and summer, although mean nocturnal black-globe temperature in summer was considerably milder (~ 10 °C higher than in winter). The primary thermal stressors over the 24-h period were low environmental temperatures in winter and high environmental temperatures in summer (Table 3.2).

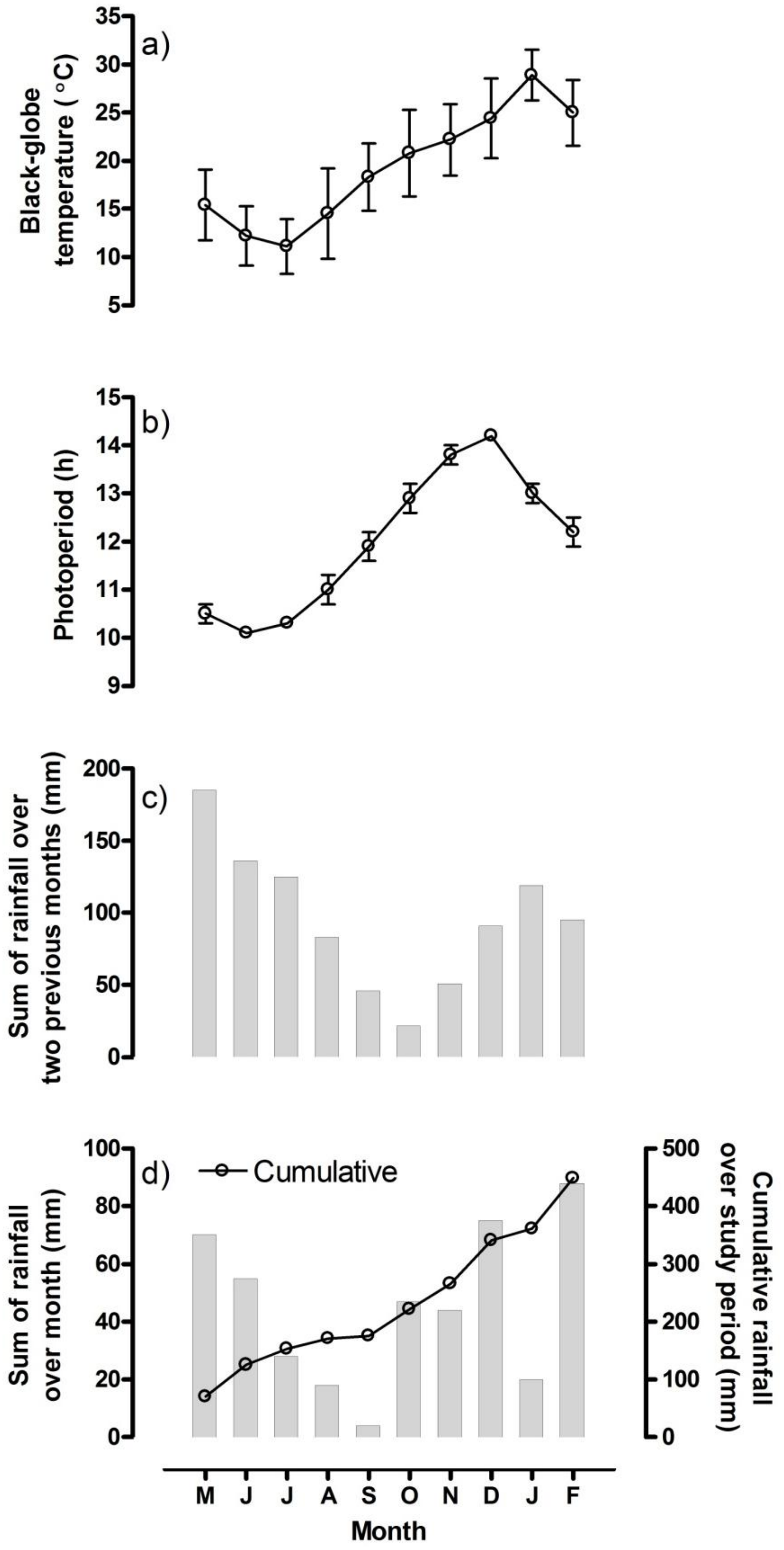

Figure 3.2: a) Mean (± SD) monthly 24-h black-globe temperature over the duration of the study period. b) Mean (± SD) monthly photoperiod over the duration of the study period. SD is the variability between days within the month. c) Sum of rainfall over the two previous months over the duration of the study period. d) Sum of monthly rainfall (*left axis*) and cumulative monthly rainfall (*right axis*) over the duration of the study period.

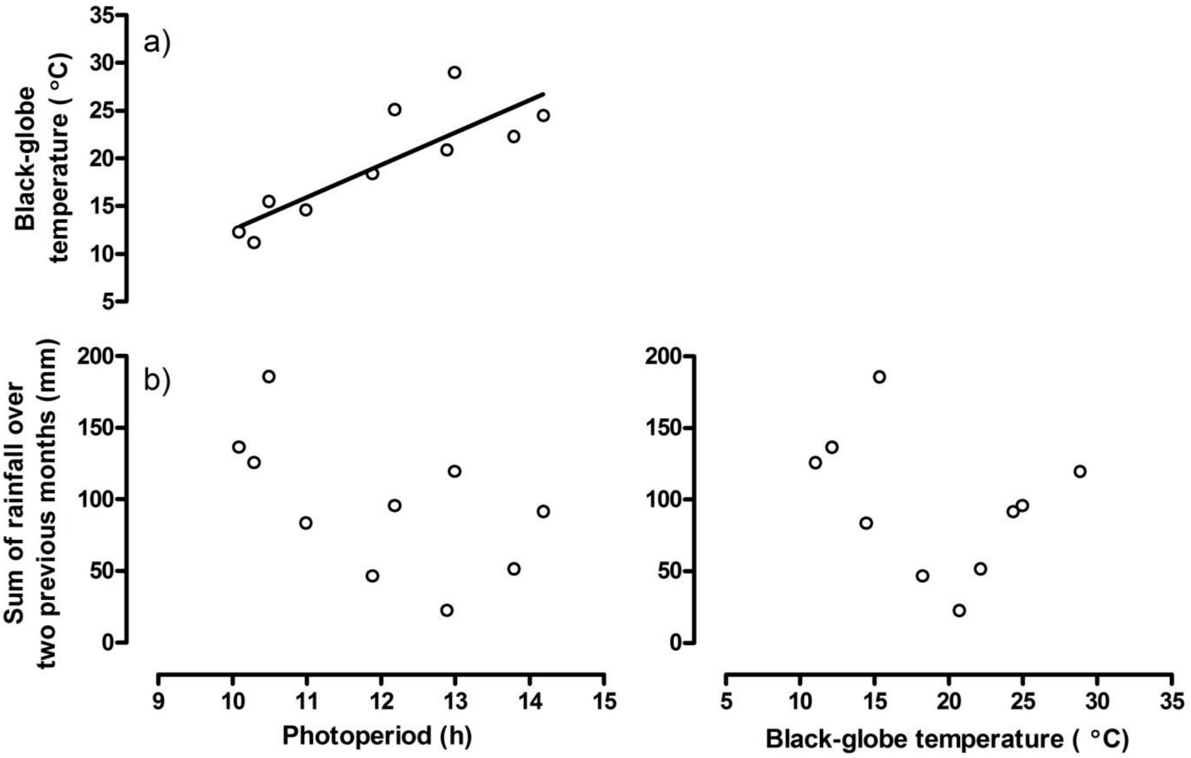

Figure 3.3: a) Mean monthly 24-h black-globe temperature in relation to mean monthly photoperiod (y = 3.39 (x) – 21.34) over the duration of the study period. b) Sum of rainfall over the two previous months correlated to mean monthly photoperiod (*left panel*) and mean monthly 24-h black-globe temperature (*right panel*).

Table 3.2: Mean (± SD) black-globe temperature and the percentage of "low" (< 25 ºC) and "high" (> 30 ºC) 30 min black-globe temperature recordings over the diurnal, nocturnal, and 24-h periods in winter and summer.

Period		**Winter**	**Summer**
Diurnal	BGT (°C)	21.5 ± 10.0	32.6 ± 11.5
	"low" (%)	51.5	28.6
	"high" (%)	22.1	61.8
Nocturnal	BGT (°C)	5.7 ± 5.6	16.2 ± 4.5
	"low" (%)	99.7	96.8
	"high" (%)	0	0.2
24-h	BGT (°C)	13.1 ± 11.1	26.1 ± 12.4
	"low" (%)	78.3	37.4
	"high" (%)	9.8	55.6

BGT = Black-globe temperature

In winter and summer, the association between mean black-globe and mean dry-bulb temperature followed similar patterns over the 24-h period. In winter, mean black-globe temperature disassociated from mean dry-bulb temperature at 08:00, shortly after sunrise (07:12 ± 0:21, Fig. 3.4a), and similarly in summer disassociation occurred ~ 1.5 h after sunrise (05:29 ± 0:22, Fig. 3.4b). Re-synchronization of mean black-globe and dry-bulb temperature occurred again in the late afternoon, ~ 30 min prior to sunset in winter (17:38 ± 0:19, Fig. 3.4a) and summer (19:22 ± 0:18, Fig. 3.4b). The maximum difference between mean black-globe and dry-bulb temperature was ~ 5 °C more in summer (12.4 °C) than in winter (7.8 °C), with the maximum difference between these variables occurring at 13:00 in both seasons, the time of day at which incident solar radiation was highest. In both winter (F (2, 273) = 269.1, $p < 0.0001$) and summer (F (2, 270) = 343.4, $p < 0.0001$), mean black-globe temperature was significantly different between the morning (winter: 07:00 – 10:30, summer: 05:30 – 10:00), afternoon (winter: 10:30 – 14:00, summer: 10:00 – 15:00), and evening (winter: 14:00 – 17:30, summer: 15:00 – 19:30) periods. In both seasons, mean black-globe temperature was lowest during the morning period (winter: 15.1 ± 5.3 °C, summer: 23.9 ± 4.7 °C), followed by the evening period (winter: 22.3 ± 6.3 °C, summer: 35.0 ± 7.3 °C), and the afternoon period was the warmest period of the day (winter: 26.9 ± 6.8 °C, summer: 39.7 ± 6.9 °C).

Between 08:00 and 12:00, the mean difference between black-globe and dry-bulb temperature was highest on clear days, followed by partially cloudy and overcast days (Fig. 3.5a), and the mean difference was significantly different between all categories of cloud cover (F (2, 144) = 42.34, $p < 0.0001$). Between the period 12:00 and 16:00, the mean difference between black-globe and dry-bulb temperature was highest on clear days, followed by partially cloudy and overcast days (Fig. 3.5b), and the mean difference was significantly different between clear and overcast days, and partial and overcast days, but not between clear and partial days (F (2, 115) = 35.32, $p < 0.0001$).

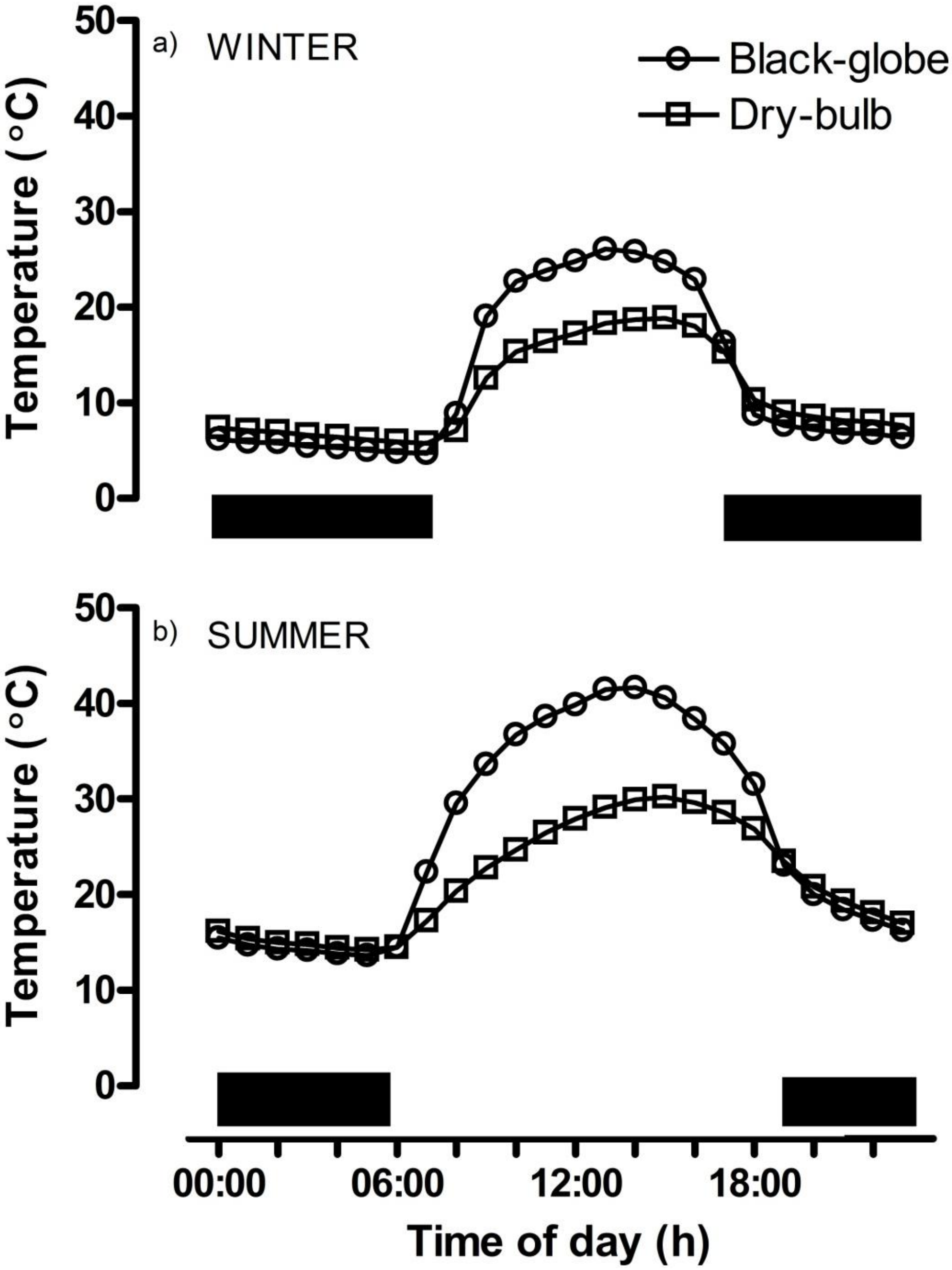

Figure 3.4: Mean hourly black-globe temperature (*circle*) and dry-bulb temperature (*square*) over the 24-h period in winter (n = 92 days, *panel a*) and summer (n = 91, *panel b*). Black bars indicate nocturnal periods.

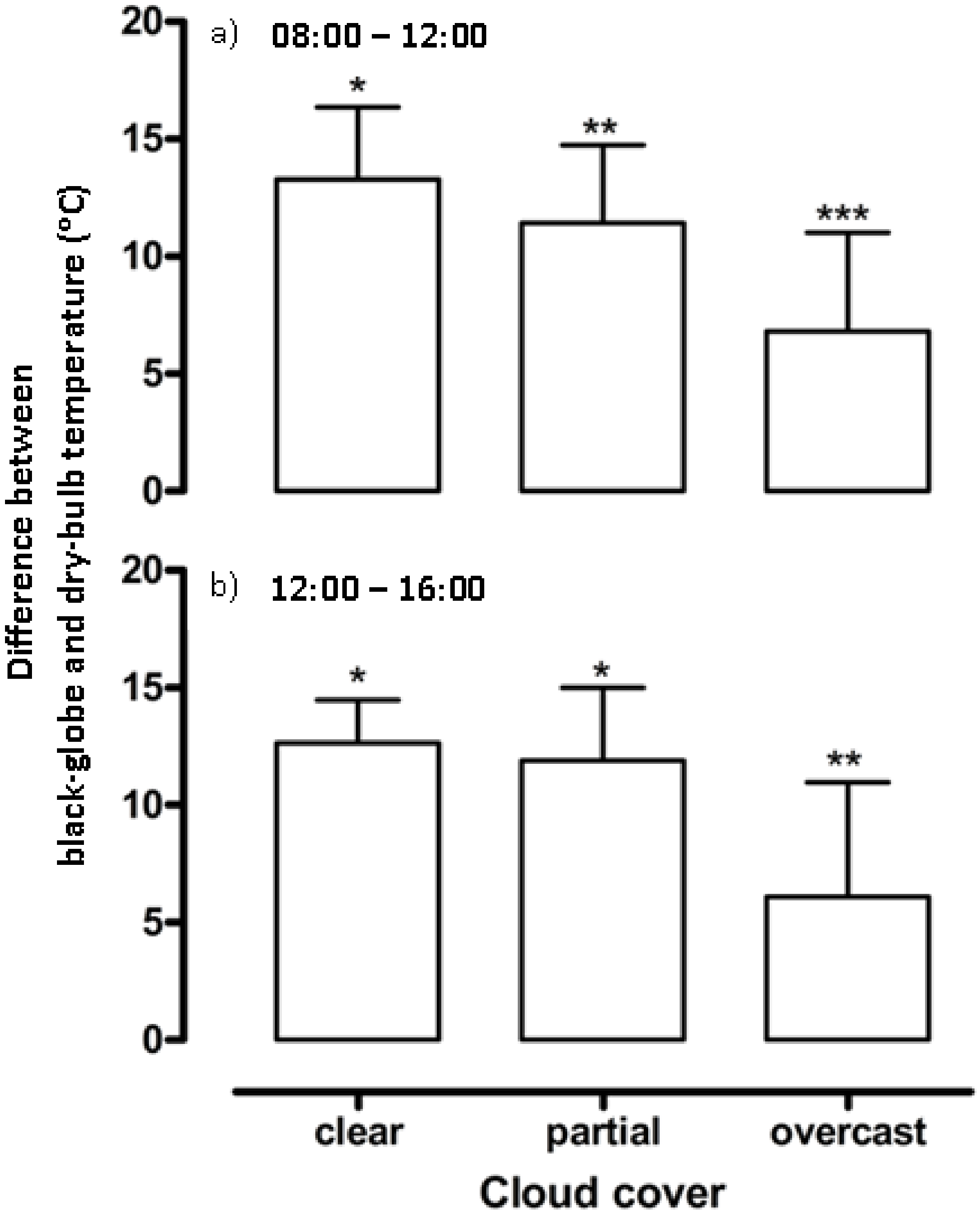

Figure 3.5: The mean (+ SD) difference between black-globe and dry-bulb temperature on clear, partial cloud, and overcast days between 08:00 and 12:00 (clear: n = 73 days, partial: n = 39 days, overcast: n = 35 days, *panel a*) and between 12:00 and 16:00 (clear: n = 50 days, partial: n = 47 days, overcast: n = 21 days, *panel b*). SD is the variability in the difference between black-globe and dry-bulb temperature in the respective categories of cloud cover. The asterisks' (*) indicate significantly different categories of cloud cover between 08:00 – 12:00 (*panel a*) and 12:00 – 16:00 (*panel b*) respectively.

3.4 Discussion

During my study at Samara, winter and summer were characterized by considerably different environmental conditions, notably in black-globe temperature and photoperiod. Over the nocturnal period the primary thermal stressor was low (< 25 °C) environmental temperatures in both winter and summer, although the mean nocturnal black-globe temperature was ~ 10 °C higher in summer than in winter. During the diurnal period, monkeys were primarily exposed to low environmental temperatures in winter and high (> 30 °C) environmental temperatures in summer. As a result of the short day lengths in winter compared to summer (photoperiod being ~ four hours shorter in winter than in summer), the 24-h temperature regime was disproportionately influenced by the cold nocturnal period in winter and the warm diurnal period in summer. Over the 24-h period in winter, low environmental temperatures constituted almost 80 % of the black-globe temperatures recorded, and in summer high environmental temperatures constituted more than half of the black-globe temperatures recorded (Table 3.1). In winter black-globe temperatures frequently dropped below freezing but on only one day exceeded 40 °C. In summer however, black-globe temperature exceeded 40 °C on more than half of the days, but never dropped below freezing. In both winter and summer, mornings were the coldest period of the diurnal phase, followed by the evening period, and the warmest period of the day was the afternoon with the zenith of black-globe temperature occurring at 13:00. The proximal thermal stressors were therefore low environmental temperatures in winter, particularly during the nocturnal period and morning, and high environmental temperatures in summer, most notably in the afternoon. The progression of black-globe temperature and photoperiod across my study period reflected a seasonal pattern, and therefore mean monthly 24-h black-globe temperature and photoperiod varied co-linearly with each other (Fig. 3.2).

Unusually for this region there was rainfall throughout the year, with only September being relatively dry (Fig. 3.1). For the last 32 years of data recorded at Graaff-Reinet, 30 km away from Samara, only 1985 (487 mm) and 1989 (590 mm) experienced more rainfall, and those measurements were both over 12 month periods compared to the ten months of my study. The high rainfall experienced during my study (450 mm) followed a severe drought during which the region experienced below average rainfall for three years in a row (2008 – 2010). Assuming that the sum of rainfall over the two previous months provided a reliable indication of food availability at Samara, my data indicates that food availability was highest at the beginning of winter, decreased to its lowest point in spring, and increased again throughout summer. There was no correlation between the sum of rainfall over the two previous months and mean monthly 24-h black-globe temperature or photoperiod.

The difference between mean black-globe and dry-bulb temperature was significantly higher on clear days compared to overcast days. This finding indicates that the difference between black-globe and dry-bulb temperature as an index of cloud cover effectively distinguishes between the extremes of cloud cover conditions observed. Between 08:00 – 12:00, the difference between mean black-globe and dry-bulb temperature also effectively distinguished partial cloud conditions from both clear and overcast conditions. However, between 12:00 and 16:00, there was no significant difference between mean black-globe and dry-bulb temperature under clear and partial cloud conditions. The difference between black-globe and dry-bulb temperature was therefore most effective as an index of cloud cover between 08:00 – 12:00.

In conclusion, Samara can be characterized as a highly seasonal environment in terms of environmental temperature and photoperiod. In winter, vervet monkeys were subjected to short day lengths (~ 10 h) and low environmental temperatures, whereas in summer day lengths were longer (~ 14 h) and the primary thermal stressor was high environmental temperatures. During my study the period of lowest food availability based on the sum of rainfall over the two previous months was during spring. Low food availability thus did not coincide with the periods of highest thermal stress, being low environmental temperatures in winter and high environmental temperatures in summer. However, uncharacteristically for this region there was high rainfall over the duration of my study period. As a result food availability was likely relatively high for the duration of my study. The difference between mean black-globe and dry-bulb temperature effectively distinguished between clear and overcast conditions, and provided the most accurate index of observed cloud cover conditions during the period 08:00 – 12:00. The difference between mean black-globe and dry-bulb temperature therefore provides a suitable measure of the availability of direct sunlight for the investigation of the influence of sunbasking behaviour in the early morning on the rate of increase in body temperature (see Chapter 5).

CHAPTER 4

The effect of environmental factors on vervet monkey activity

4.1 Introduction

For vervet monkeys, environmental (e.g. climate, day length/time of day, resource availability) and social (e.g. rank, sex, group size, breeding seasonality) factors, as well as predation, have all been shown to be important in determining activity patterns and behaviour (Struhsaker 1967b, Wrangham 1981, Isbell 1993, Baldellou and Adan 1997, Makin *et al.* 2012). In terms of environmental factors, primates typically respond to thermal stress, both at low and high environmental temperatures, by decreasing activity (i.e. spend more time resting or in sedentary activities such as grooming or huddling; Hanya 2004, Hill 2006a). Decreased activity under conditions of thermal stress is proposed to conserve energy for heat production at low environmental temperatures, and avoid hyperthermia and minimize water loss at high environmental temperatures (Sato 2012). The optimal foraging theory' (Norberg 1977) predicts that an animal should expend more energy foraging when food is abundant in order to maximize their net energy gain, whereas foraging effort should decrease in favour of energy conservation when food availability is scarce (Norberg 1977). Proximal environmental stressors (i.e. temperature and food availability) are therefore an important determinant of activity patterns in primates (Hill 2006a). If, however, environmental factors compromise activity to such an extent that it inhibits an animal's ability to acquire sufficient resources for metabolic maintenance and completing fundamental tasks (i.e. energetic, social, and biological), these environmental factors may impose a significant ecological constraint on survival (Korstjens *et al.* 2010).

One manner in which animals can avoid the detrimental energetic cost of environmental stressors, and still maintain optimal levels of activity for other energetic, social, and biological requirements, is by shifting the timing of activity over the 24-h period to desynchronize activity with periods of increased heat stress (Hetem *et al.* 2012). Among primates, cathemerality, whereby a significant portion of 24-h activity is devoted to both the diurnal and nocturnal phases, is widely reported for Malagasy lemurs and neo-tropical owl monkeys (Hill 2006b). The prevalence of this behavioural strategy amongst the lemurs of Madagascar has been attributed to optimizing resource utilization (Kappeler and Erkert 2003), as well as reducing thermal stress (Curtis *et al.* 1999) and improved predator avoidance (Colquhoun 2006). For strictly diurnal species however, there is a finite amount of time per day in which they must achieve the necessary energetic and biological requirements within their social system (Dunbar 1992). Behavioural inflexibility may therefore confound the "activity debt" imposed by environmental stressors and thus the length of photoperiod may impose a significant ecological constraint on primate activity.

4.1.2 Chapter objective, hypotheses and predictions

This chapter aims to investigate the influence of thermal stress, food availability, and photoperiod on vervet monkey activity and discuss the potential ecological implications of these environmental factors, if any, which constrain activity. Specifically, I will determine whether vervet monkeys were able to employ behavioural flexibility to maintain activity irrespective of fluctuations in day length across the study period. I will also investigate the proximal influence of thermal stress and variations in food availability (as indexed by the sum of rainfall over the two previous months) on vervet monkey activity, and determine whether these environmental factors infer a significant constraint on activity. Due to the inherent difficulties of collecting behavioural data at night, very few studies have comprehensively described primate activity patterns over the 24-h period. My study will be the first, to my knowledge, to use activity data loggers to collect continuous measurements of activity for any primate, which will give me the unique opportunity to accurately describe vervet monkey nychthemeral activity patterns.

I hypothesize that vervet monkey activity will be restricted to diurnal periods, and therefore the time available for activity (i.e. day length) will be an important determinant of activity. I predict that the total time that monkeys spend active will be inhibited by short day lengths, and will therefore be reduced in winter compared to summer. To compensate for short day lengths in winter, it is predicted that vervet monkeys will increase the percentage of the diurnal period in which they are active in order to complete all fundamental activities within limited time available. I predict that confounding the influence of day length on activity, extreme environmental temperatures (specifically low temperatures in winter and high temperatures in summer) and low food availability will compromise activity, as vervet monkeys will attempt to conserve energy or body water by reducing activity.

4.2 Materials and methods

4.2.1 Data collection

For my study 12 vervet monkeys were implanted with activity data loggers which recorded activity counts at 1 min intervals by means of an omni-directional accelerometer (see Chapter 2: section 2.4 for technical details of loggers). Ten monkeys were implanted in April 2011, with two more monkeys being implanted in July 2011. I therefore present data for 12 individual monkeys. Due to the premature failure of some activity data loggers the duration of data collection varied between individual monkeys. My sample size (i.e. monkeys) was therefore sequentially reduced from a maximum of 11 overlapping monkeys in July 2011 to five in February 2012 (see Chapter 2: Table 2.2 for details on the duration of activity data collection for all monkeys.)

4.2.2 Data analyses

Activity count data provides a longitudinal measure of the intensity of activity but no information on the specific activity in which the animal was involved. As increased sedentary activities represent the typical behavioural response of primates to environmental stressors (Hill 2006a, Korstjens *et al.* 2010), I wished to use the activity data logger readings to infer whether the animal was active or inactive. Based on observational data, low activity count values between 0 and 1 % of maximum activity counts were recorded when the animal was sedentary (e.g. as a result of grooming, scratching, postural adjustments, or breathing: McFarland *et al.* in press). The use of activity count data > 1 % of maximum activity from the activity data loggers as a proxy for an animal being "active" was validated against behavioural observations for monkeys implanted in my study (McFarland *et al.* in press), where the behavioural categories resting and grooming were considered inactive, and travelling and feeding active. In my study, an animal was considered inactive (i.e. resting or grooming) when their activity count was less than 1 % of their maximum recorded activity. An animal was considered active (i.e. travelling or feeding) when their activity count was more than 1 % of their maximum recorded activity.

In order to compare vervet monkey activity across the duration of my study period, and investigate the influence of environmental factors on activity, I calculated two activity variables:

1.) I calculated the percentage of the diurnal, nocturnal, or 24-h period active for each individual monkey for each day for the duration of my study period. To this end I calculated the proportion of time spent active during the diurnal, nocturnal, or 24-h period by dividing the sum of activity counts greater than 1 % maximum activity (i.e. active) by the sum of all activity counts (i.e. active and inactive) during the corresponding diurnal, nocturnal, or 24-h period, and converted these values to a percentage by multiplying by 100 (see equation 1).

Percentage of diurnal, nocturnal, or 24-h period active (%) = proportion of time active during corresponding period × 100 *(1)*

2.) To account for differences in photoperiod over the duration of my study period, I converted the proportion of time spent active during a given period to hours spent active during that period. Total time active over the diurnal, nocturnal, or 24-h period was calculated by multiplying the proportion of time spent active during a given period by the number of hours for that period (see equation 2). The number of diurnal and nocturnal hours was calculated for each month from the mean time of sunrise and sunset for that month (see Chapter 3: Table 3.1).

Total time active over the diurnal, nocturnal, or 24-h period (h) = (proportion of time active during given period) × (number of hours in given period) *(2)*

To illustrate how I converted the raw activity data to the above activity variables, Fig. 4.1 shows raw activity counts for a single representative male monkey over a 24-h period. All data points > 1 % of maximum activity (indicated by dotted line in Fig. 4.1) were considered active. From equation 1, the monkey was active for 67.4 % of the time over the diurnal period, 0.1 % of the time over the nocturnal period, and 32.5 % of the time over the 24-h period. From equation 2, the total time that the monkey was active over the diurnal period (10.1 h) was 6.7 h, the total time active over the nocturnal period (13.9 h) was 0.9 h, and the total time active over the 24-h period was 7.6 h. Total time active over the diurnal period therefore accounted for 88.8 % of total time active over the 24-h period in this example.

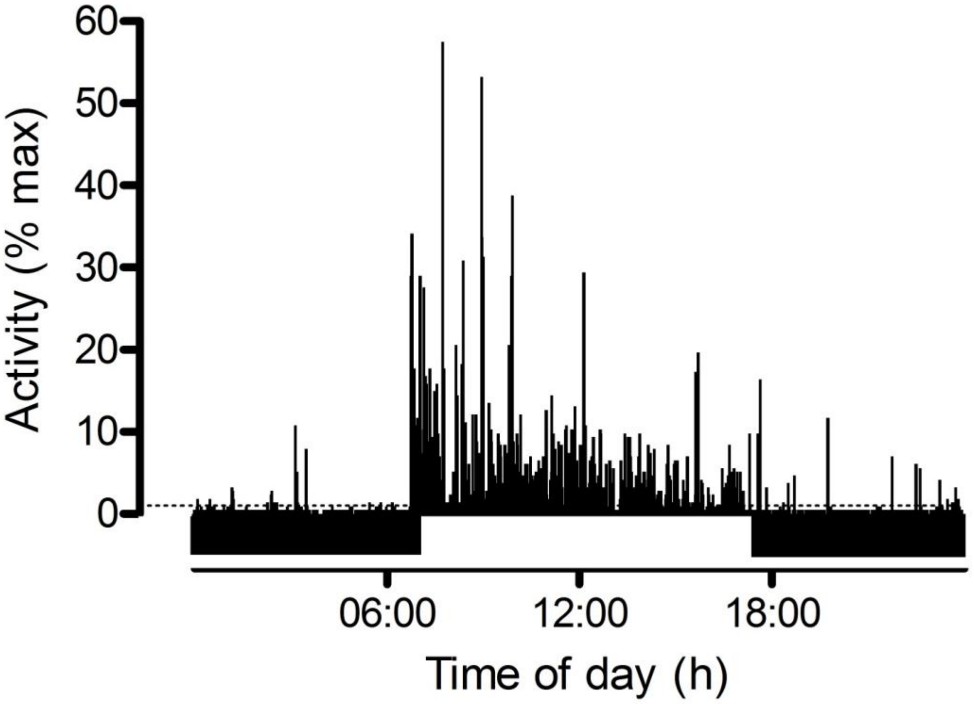

Figure 4.1: Activity logger count data expressed as the percentage of maximum activity recorded for that data logger at 1 min intervals over the 24-h period for a representative male monkey in winter. Black bars represent nocturnal periods. The 1 % of maximum activity cut-off point for active/inactive is indicated by a horizontal dotted line.

Once the activity data, expressed as a percentage of maximum activity, were converted to total time active, I checked for outliers within the total time active over the 24-h period data set. An outlier is defined as a data point which appears to deviate markedly from other data points within the sample in which it occurs. I investigated the distribution of mean total time active over the 24-h period across the study period by means of box-plots for each month. I calculated the mean total time active over the 24-h period for individual monkeys for each day, and averaged across all monkeys to obtain a single value of total time active over the 24-h period for the population for each day, which was used to construct the box-plots for each month. The box-plot analysis revealed five extreme outliers (defined as being more than three times the interquartile difference, the difference between the 75 and 25 percentiles, above or below the 75 and 25 percentiles respectively: Hodge and Austin 2004) in the data set. These outlying values corresponded to the following dates; the 5th and 25th of July, 5th of August, 2nd of October, and 24th of November. The use of data loggers for data collection in my study precludes the possibility that these outliers are an artefact of sampling protocol or human error. For the identified outliers, the data for individual monkeys were checked for incorrect entries or large discrepancies between individual monkeys possibly indicating logger failure, of which none were found. These outliers were likely a result of natural variation inherent in the population, and for this reason outliers were not excluded from any analyses. Possible causal factors of identified outliers are discussed. Note that excluding outliers from the data set did not significantly change any statistical analyses results.

4.2.2.1 Nychthemeral activity patterns

To describe seasonal 24-h activity patterns, I first calculated the percentage of time active in a given 30 min time block, for each individual monkey, by dividing the number of counts greater than 1 % maximum (i.e. active) by 30 (the number of counts in each 30 min time block), and multiplied this value by 100 to convert it to a percentage (see equation 3).

$$\text{Time active in 30 min time block (\%)} = (\text{number of counts} > 1\,\% \div 30) \times 100 \qquad (3)$$

To obtain a single mean value for each 30 min time block across the 24-h period in winter and summer for individual monkeys, I averaged the percentage of time spent active in the respective 30 min time blocks across all days in winter and summer for individual monkeys. I then averaged the single mean values of percentage time active in 30 min time blocks for individual monkeys across all monkeys to obtain a single mean value of percentage time active in respective 30 min time blocks for the population in winter and summer.

4.2.2.2 Variations in activity over the duration of the study period

To describe variations in total time active over the 24-h period across months, I calculated the mean total time active over the 24-h period for individual monkeys for each day (using equation 2), from which I calculated a mean value for each monkey for each month. I averaged across all monkeys to obtain a single mean value for the population for each month. For each individual monkey I calculated the proportion of diurnal and nocturnal activity by dividing the total time active over the diurnal period and total time active over the nocturnal period by total time active over the 24-h period for each day. The mean monthly contribution of total time active over the diurnal and nocturnal periods to total time active over the 24-h period was calculated for each monkey, and averaged across all monkeys to obtain a single mean value for the population for each month.

I compared mean total time active over the diurnal period, the nocturnal period, and the 24-h period between winter and summer for nine monkeys, which had a minimum of 30 sample days in both seasons, by means of paired t-tests. For individual monkeys, single mean values of total time active over the respective periods were calculated for winter and summer. As above, I also compared the percentage of the diurnal period active between winter and summer for nine monkeys, which had a minimum of 30 sample days in both seasons, by means of a paired t-test.

To analyze the change in total time active over the diurnal, nocturnal, or 24-h periods from mid-winter to mid-summer, I used Pearson's correlations to correlate total time active over the respective periods with days between the winter (21 June) and summer (22 December) solstices. I calculated mean total time active over the diurnal, nocturnal, or 24-h periods for each monkey for each day (equation 2) and averaged these values across all monkeys to obtain a mean value per day for the population.

4.2.2.3 The influence of environmental factors on activity

To identify the environmental factors influencing vervet monkey activity over the duration of my study period I used a linear mixed effect model. Linear mixed effect models are statistical models which contain both fixed factors and random factors. Linear mixed effect models allow analyzing the effect of a series of continuous or categorical independent variables (fixed factors) on the dependent variable. The inclusion of a random factor in linear mixed effect models also controls for the non-independence of data points, thus allowing analysis to be run at the level of the single observation (e.g. total time active over diurnal period for an individual monkey on each day over the duration of my study) while avoiding any bias from pseudoreplication. As this analysis is run at the level of the single observation, this model is also appropriate for data sets such as mine where unequal sample sizes are available for individual monkeys (Pinheiro and Bates 2000).

As monkeys were primarily diurnal (see Discussion), I considered only the influence of environmental factors (independent variables) on total time active over the diurnal period (dependent variable). The environmental factors incorporated in the model (model 1) were mean black-globe temperature, photoperiod, and sum of rainfall over the two previous months. Sum of rainfall over the two previous months was incorporated in the model as a proxy for food availability (see Chapter 3: section 3.1). Total time active over the diurnal period was calculated for each individual monkey on each day of the study period, and mean black-globe temperature was calculated for the corresponding diurnal period. Although investigating the influence of factors such as troop, mass, and sex on activity is beyond the scope of my study, due to the relatively small sample size, these factors may influence vervet monkey activity patterns (Isbell 1993, Baldellou and Adan 1997) and I therefore included them as “control” variables in the model. Control variables in the model are entered as independent variables. The model output will therefore account for the variability associated with these control variables when calculating the effect of the environmental factors of interest on total time active over the diurnal period. Individual monkeys were entered in the model as a random factor, thus controlling for bias from repeated measures.

The use of linear mixed effect models provides the most robust analysis for investigating the influence of multiple environmental factors on vervet monkey activity for my study. However, I was interested in addressing further the influence of individual environmental factors identified as important determinants of vervet monkey activity in model 1. To describe these relationships between individual environmental factors and parameters of vervet monkey activity I used Pearson’s correlations (see below).

Whilst model 1 addresses the influence of photoperiod on total time active over the diurnal period, I was also interested in whether monkeys attempted to compensate for short day lengths (i.e. reduced photoperiod) by increasing the percentage of time active during the diurnal period. To investigate the influence of photoperiod specifically on the percentage of the diurnal period for which monkeys were active, I used Pearson’s correlations to investigate the relationships between mean percentage of diurnal period active against photoperiod for all monkeys for the duration of the study period. I calculated mean percentage diurnal period active for each monkey for each day (equation 1) and averaged these values across all monkeys to obtain a single mean value per day for the population.

Whilst model 1 addresses the influence of black-globe temperature on total time active over the diurnal period for all days across the duration of the study period, I was specifically interested in the influence of extreme temperatures on vervet monkey activity. As Samara is a highly seasonal environment, vervet monkeys were subjected to opposite thermal stressors in winter compared to summer (see Chapter 3: Table 3.2). I therefore investigated whether black-globe temperature inferred a significant constraint on vervet monkey activity by means of Pearson’s correlations between total time active over the diurnal period and corresponding mean diurnal black-globe temperature, in winter and summer respectively. I calculated mean total time active over the diurnal period for each monkey for each day (equation 2) and averaged these values across all monkeys to obtain a single mean value per day for the population.

As vervet monkey activity patterns vary across the diurnal period (Baldellou and Adan 1997), I also investigated the influence of mean black-globe temperature on the corresponding total time active over the morning (winter: 07:00 – 10:30, summer: 05:30 – 10:00), afternoon (winter: 10:30 – 14:00, summer: 10:00 – 15:00), and evening (winter: 14:00 – 17:30, summer: 15:00 – 19:30) periods for all monkeys in winter and summer respectively. For the morning, afternoon, and evening respectively, total time active for a given period was calculated as per equation 2, with the number of hours in the respective periods modified to correspond with the duration of the morning, afternoon, and evening periods in winter and summer. I calculated total time active over the morning, afternoon, and evening periods respectively for each monkey for each day and averaged these values across all monkeys to obtain a single mean value for the respective periods of the diurnal period for the population. I used Pearson's correlations to correlate mean total time active for all monkeys against corresponding mean black-globe temperature for the morning, afternoon, and evening periods respectively in winter and summer.

To compare nycthemeral activity patterns of vervet monkeys at extreme temperatures in winter and summer, I described activity patterns in 30 min time blocks across the 24-h period for the ten coldest and ten warmest days in winter and summer respectively (based on mean 24-h black-globe temperature). For the ten coldest and warmest days in winter and summer I averaged the percentage of time active over 30 min time blocks across all monkeys to obtain a single mean value for each time block for the population. This analysis was conducted for ten monkeys in winter and six monkeys in summer. I used a paired t-test to compare the mean total time active over the 24-h period between cold and warm days for individual monkeys in winter and summer respectively.

For all analyses the Kolmogorov-Smirnov test was used to test the data for normality. All statistical analyses were conducted using IBM SPSS V.10 statistics software. If variables were significantly correlated, I conducted linear regression analysis to determine the straight line equation. Results were significant at $p < 0.05$.

4.3 Results

4.3.1 Nychthemeral activity patterns

Vervet monkeys shifted from a unimodal pattern of diurnal activity in winter, with time spent active peaking around mid-day (Fig. 4.2a), to a bimodal pattern in summer with activity peaks in the morning and afternoon, and a reduction in activity around mid-day (Fig. 4.2b). In both seasons vervet monkeys were primarily diurnal. Nocturnal activity was stable at ~ 10 % of time active over a 30 min time block, compared to diurnal activity levels of up to ~ 80 % of time active over a 30 min time block. In both seasons, the percentage of time active over a 30 min time block increased from the nocturnal mean during the twilight period, ~ 1 hour prior to sunrise, and decreased again to the nocturnal mean ~ 1 hour after sunset (Fig. 4.2).

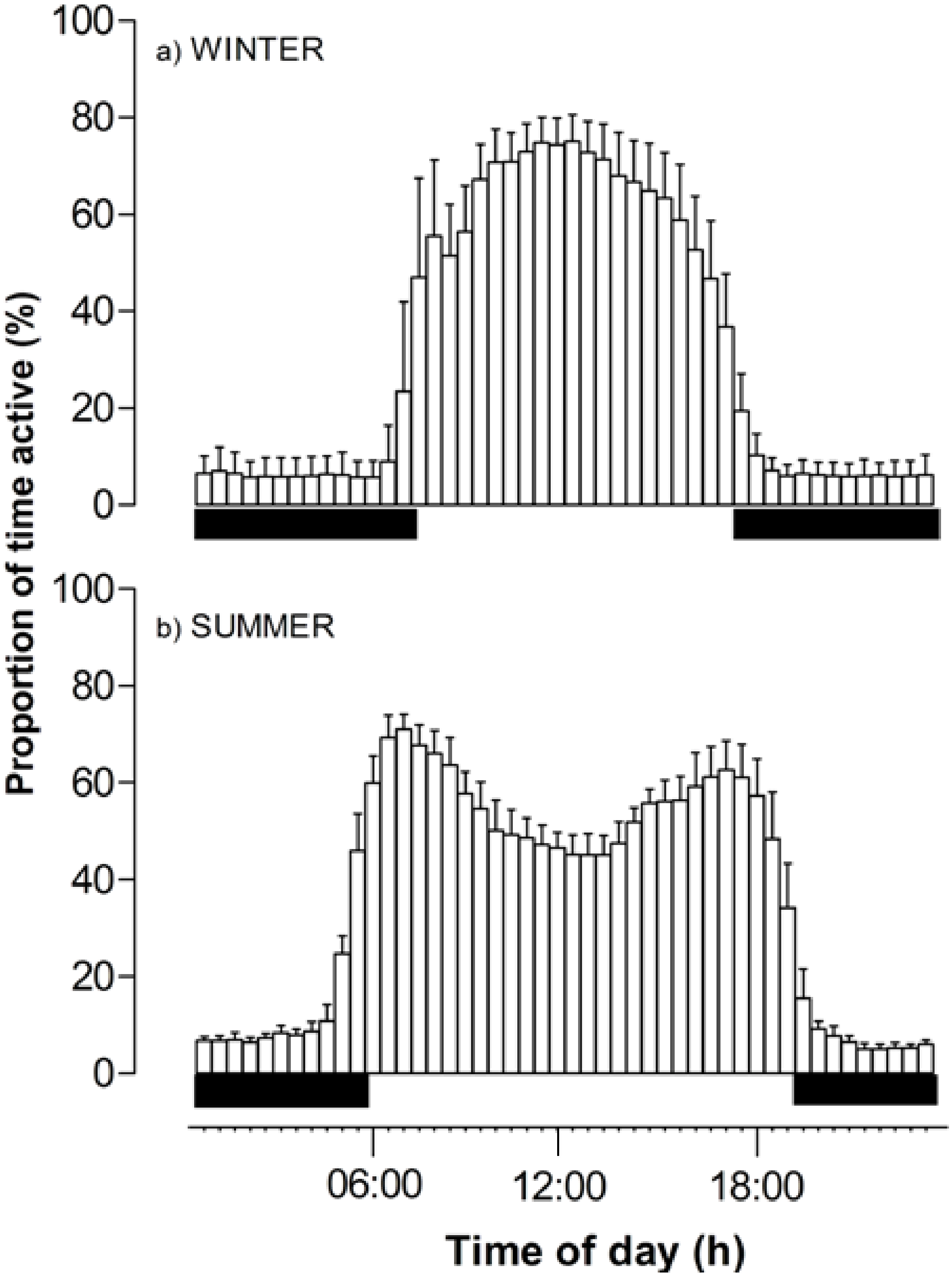

Figure 4.2: Mean (+SD) percentage of time active over 30 min time blocks for 12 monkeys in winter (*panel a,* n = 92 days) and nine monkeys in summer (*panel b,* n = 91 days). Black bars represent nocturnal periods. SD is the variability between individual monkeys.

4.3.2 Variations in activity over the duration of the study period

For individual monkeys, total time active over the 24-h period ranged from 3.1 to 14.5 h across the duration of my study period. For all monkeys, mean total time active over the 24-h period was lowest in July and peaked in December. The contribution of mean total time active over the nocturnal period to mean total time active over the 24-h period only varied from 0.9 – 1.4 h across all months, whereas mean total time active over the diurnal period varied by 2.4 h between its lowest point in July and its highest point in November. Total time active over the diurnal period constituted a minimum of 85 % of total time active over the 24-h period in June, and a maximum of 90 % of total time active over the 24-h period in November (Fig. 4.3).

Vervet monkeys spent significantly more time active over the diurnal and 24-h periods in summer compared to winter, whereas there was no significant difference in total time active over the nocturnal period between seasons (Table 4.1). Mean total time active over the 24-h period increased linearly from the winter to the summer solstice ($r^2 = 0.62$, $n = 185$, $p < 0.0001$, Fig. 4.4a). Similarly, mean total time active over the diurnal period increased linearly from the winter to the summer solstice ($r^2 = 0.70$, $n = 185$, $p < 0.0001$, Fig. 4.4b). There was however no significant relationship between mean total time active over the nocturnal period and days between the winter and summer solstices ($r^2 = 0.0001$, $n = 185$, $p = 0.88$, Fig. 4.4c). Notably, all identified outlying data points of low total time active over the 24-h period (Fig. 4.4a, *solid squares*) corresponded with cold winter days on which heavy rainfall (> 10 mm) was experienced.

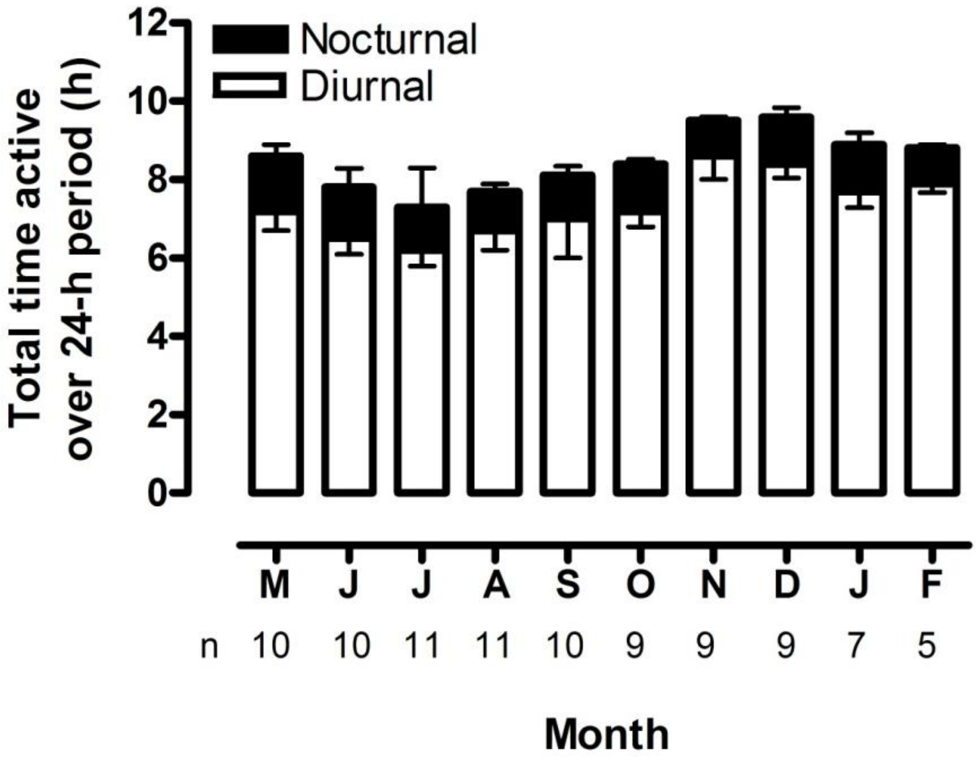

Figure 4.3: Mean total time active over the 24-h period for 12 monkeys by month over the duration of my study period. Clear (-SD) and black (+SD) bars indicate the relative proportion of total time active over the diurnal and nocturnal periods respectively. SD is the variability between individual monkeys. N indicates the sample size (monkeys) per month.

Table 4.1: Mean (± SD) total time active over the 24-h, diurnal, and nocturnal periods for nine monkeys in winter (n = 92 days) and summer (n = 91 days).

Parameter of activity	**Winter**	**Summer**	**t**	**p**
24-h (h)	7.3 ± 0.7	8.9 ± 0.7	12.25	**<0.0001**
Diurnal (h)	6.4 ± 0.5	7.9 ± 0.6	11.11	**<0.0001**
Nocturnal (h)	1.0 ± 0.2	1.0 ± 0.1	1.06	0.32

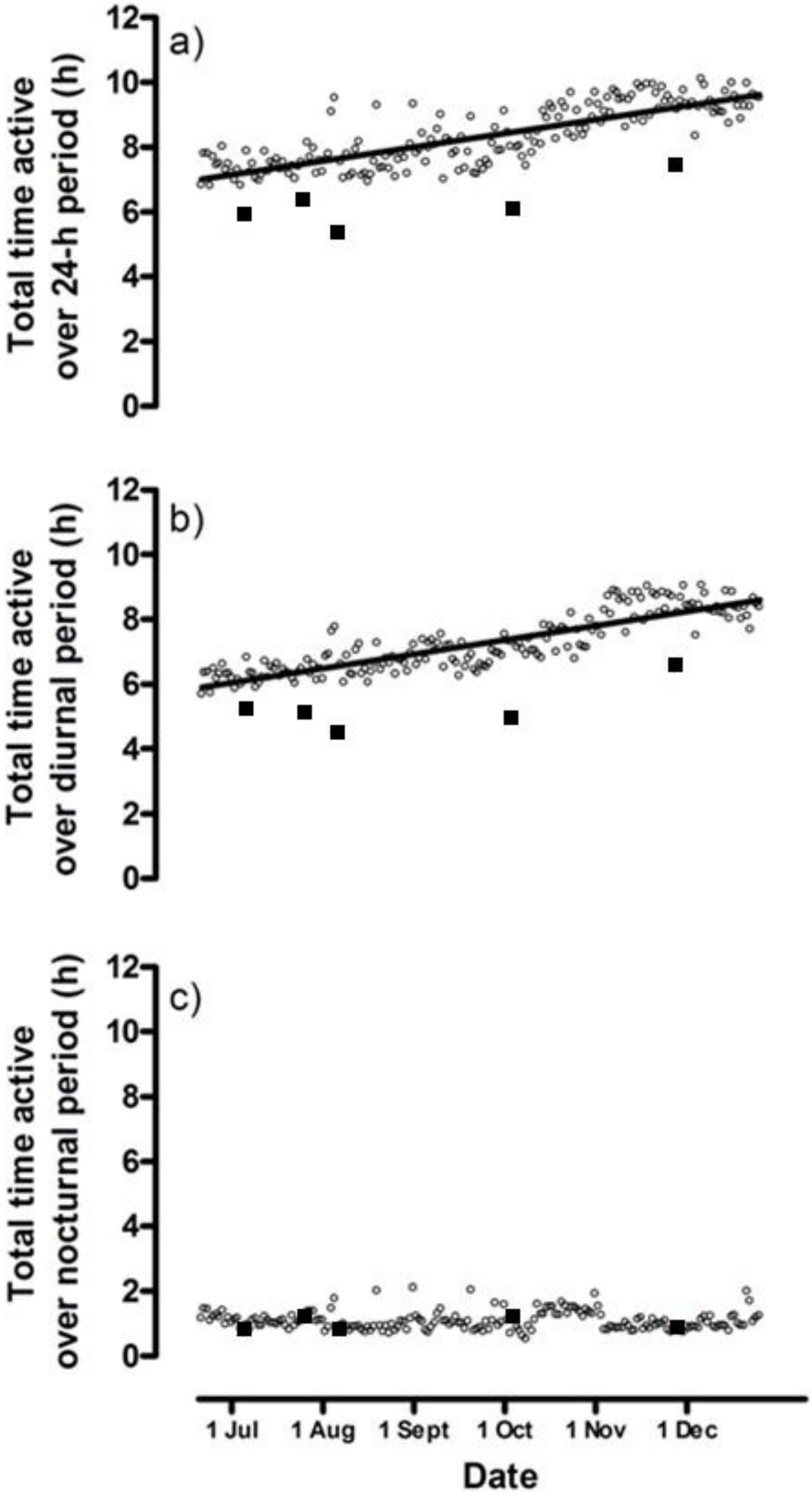

Figure 4.4: Mean total time active over the 24-h (*panel a,* y = 0.01 (x) + 5.83), diurnal (*panel b*, y = 0.01 (x) + 6.92), and nocturnal (*panel c*) periods across days between the winter and summer solstices for 12 monkeys. Identified outlying data points (*solid squares*) represent days of high rainfall.

4.3.3 The influence of environmental factors on activity

Total time active over the diurnal period increased significantly (Table 4.2; indicated by positive ß value) with increasing photoperiod, the sum of rainfall over the two previous months, and mean diurnal black-globe temperature over the duration of my study period. Photoperiod was the strongest environmental determinant of total time active over the diurnal period, followed by the sum of rainfall over the two previous months, and then mean diurnal black-globe temperature (Table 4.2; indicated by Z value).

Table 4.2: Result of linear mixed effect model investigating the influence of photoperiod, the sum of rainfall over the two previous months, and mean diurnal black-globe temperature (BGT) on total time active over the diurnal period for 12 monkeys over the duration of the study period (n = 2763). Sex, mass, and troop were included as control variables, and individual monkeys were included as a random factor.

	ß	**±SE**	**Z**	**p**	**95%CI's**		
Independent							
Photoperiod (h)	0.4536	0.0147	30.91	**<0.0001**	0.4249	to	0.4824
Rainfall (mm)	0.0018	0.0004	4.72	**<0.0001**	0.0010	to	0.0025
BGT (°C)	0.0076	0.0028	2.70	**<0.01**	0.0021	to	0.0131
Control							
Sex	-0.3845	0.9279	-0.41	0.68	-2.2031	to	1.4341
Mass (kg)	-0.2228	0.3368	-0.66	0.51	-0.8828	to	0.4373
Troop	-0.3951	0.5052	-0.78	0.43	-1.3853	to	0.5951
Random factor							
Individual	0.7798	0.1965			0.4759	to	1.2779

However, the relationship between photoperiod and percentage diurnal period active was opposite to that observed between photoperiod and total time active over the diurnal period. Over the duration of my study period there was a significant negative relationship between mean percentage diurnal period active and photoperiod (r^2 = 0.31, n = 305, p < 0.0001, Fig. 4.5). Although monkeys spent a significantly higher percentage of the diurnal period active in winter (62.5 ± 5.3 %) compared to summer (56.5 ± 4.9, t = 4.69, n = 9, p < 0.01), total time active over the diurnal period was significantly reduced at short day lengths in winter (Table 4.1).

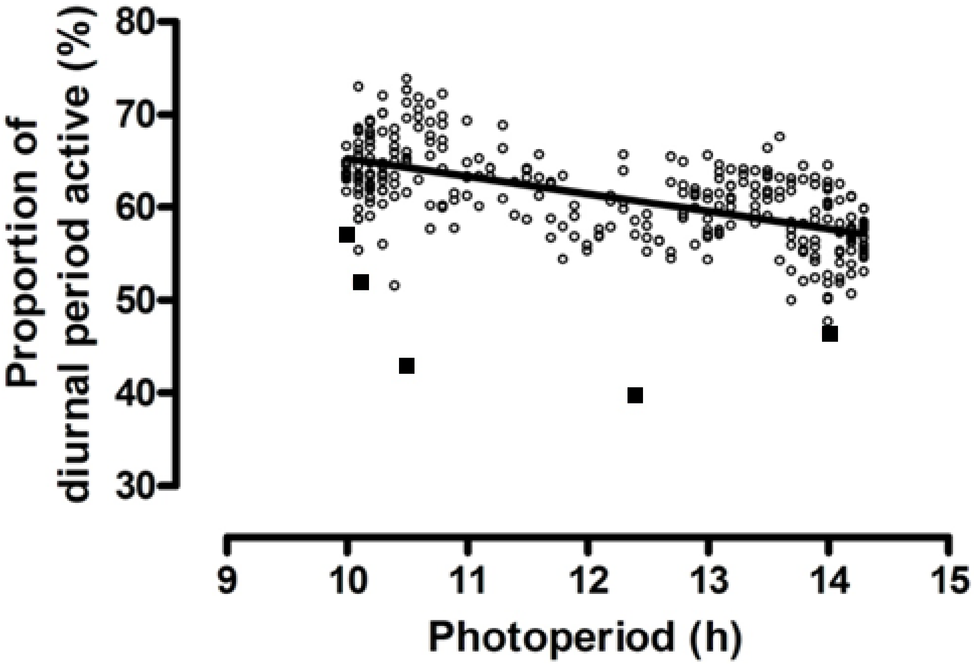

Figure 4.5: Mean percentage of time active over the diurnal period in relation to photoperiod for 12 monkeys over the duration of my study period (y = 0.37 (x) + 3.88). Identified outlying data points (*solid squares*) represent days of high rainfall.

In both winter and summer the total time active over the diurnal period was significantly correlated to corresponding mean diurnal black-globe temperature (Fig. 4.6). However, the relationships between total time active over the diurnal period and black-globe temperature were opposite in winter compared to summer. In winter, total time active over the diurnal period significantly increased with increasing black-globe temperature ($r^2 = 0.32$, n = 92, $p < 0.0001$, Fig. 4.6a), indicating that activity was constrained at low black-globe temperatures. Conversely in summer, total time active over the diurnal period significantly decreased with increasing black-globe temperature ($r^2 = 0.26$, n = 91, $p < 0.0001$, Fig. 4.6b), indicating that activity was constrained at high black-globe temperatures. Note that the three outlying data points (corresponding to days of heavy rainfall) in winter appear to have a significant influence on the correlation, but that excluding these outliers did not significantly change the slope of the relationship.

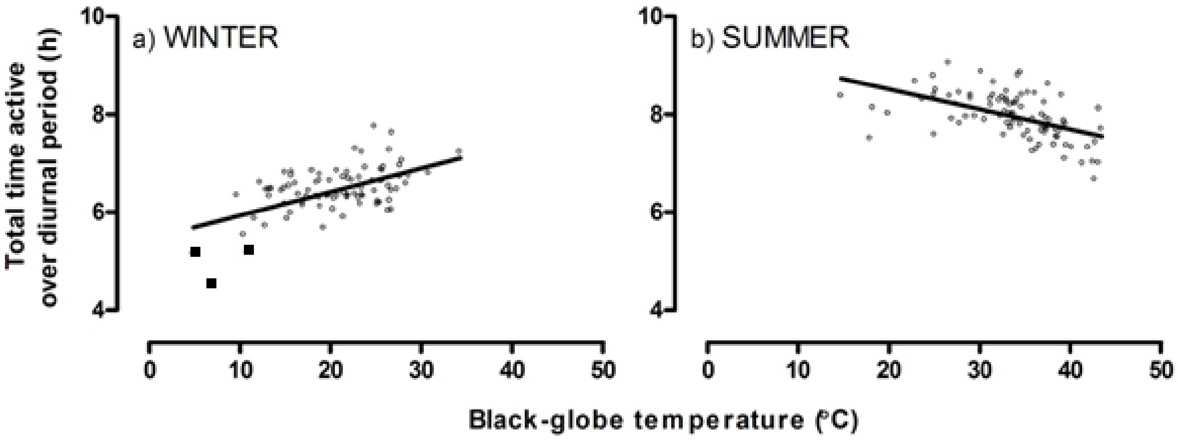

Figure 4.6: Mean total time active over the diurnal period in relation to corresponding mean black-globe temperature for 12 monkeys in winter (*panel a,* y = 0.05 (x) + 5.44) and nine monkeys in summer (*panel b,* y = -0.04 (x) + 9.34). Identified outlying data points (*solid squares*) represent days of high rainfall.

In winter (Fig. 4.7a), total time active over the morning (r^2 = 0.12, n = 92, p < 0.0001, *upper panel*) and evening (r^2 = 0.26, n = 92, p < 0.0001, *lower panel*) periods significantly increased with corresponding mean black-globe temperature, whereas total time active over the afternoon period was independent of corresponding mean black-globe temperature (r^2 = 0.01, n = 92, p = 0.43, *centre panel*). Conversely, in summer (Fig. 4.7b), total time active over the afternoon period significantly decreased with corresponding mean black-globe temperature (r^2 = 0.30, n = 91, p < 0.0001, *centre panel*), but total time active over the morning (r^2 = 0.03, n = 91, p = 0.07, *upper panel*) and evening (r^2 = 0.02, n = 91, p = 0.19, *lower panel*) periods was independent of corresponding mean black-globe temperature. Therefore, the diurnal periods during which corresponding mean black-globe temperature significantly influenced mean total time active coincided with the periods of lowest black-globe temperatures in winter (i.e. morning and evening), and highest black-globe temperatures in summer (i.e. afternoon; see Chapter 3: section 3.3).

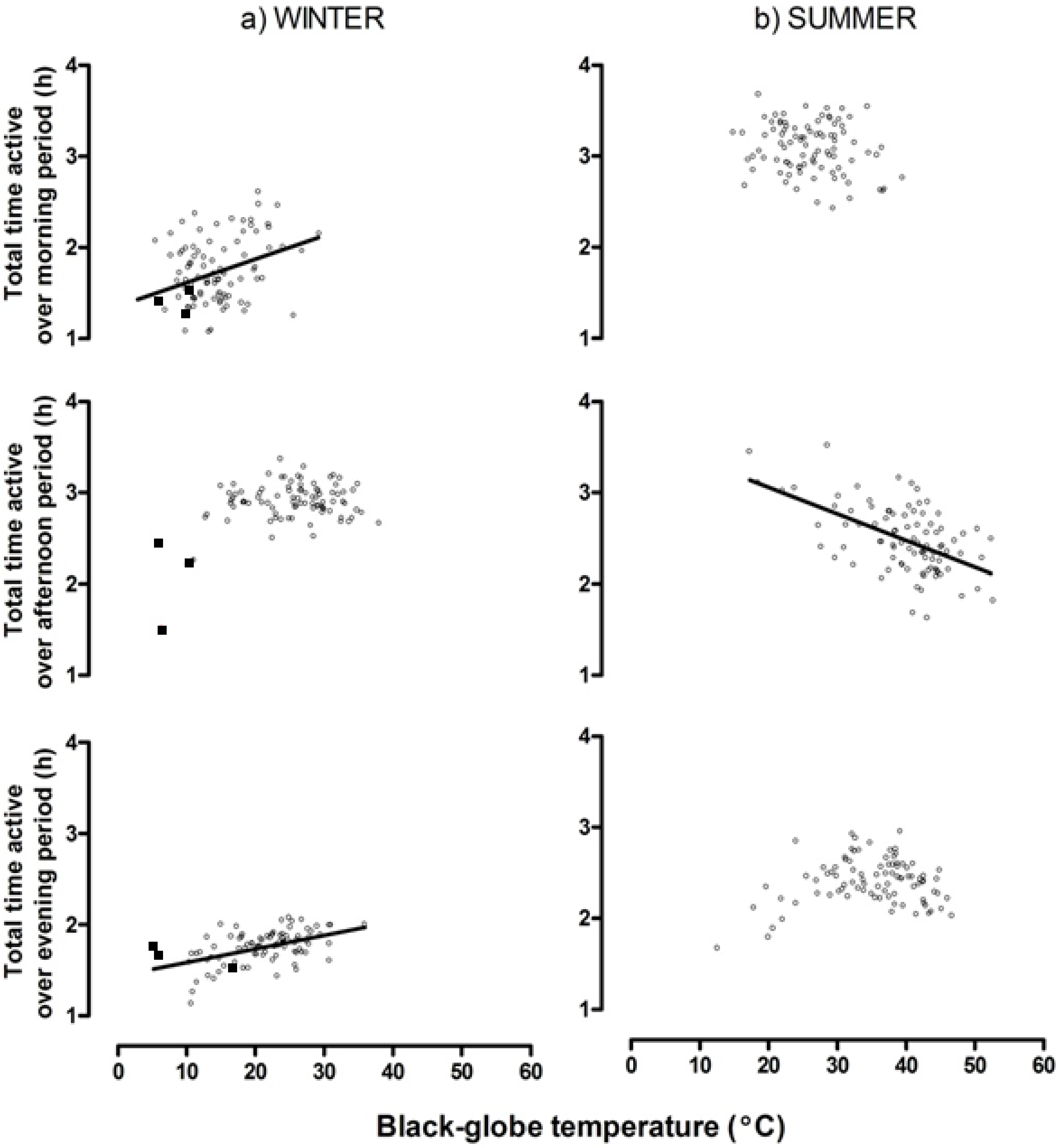

Figure 4.7: Mean total time active over the morning (*upper panel*), afternoon (*centre panel*), and evening (*lower panel*) in relation to corresponding mean black-globe temperature for 12 monkeys in winter (*panel a*) and nine monkeys in summer (*panel b*). SD is the variability between individual monkeys. Identified outlying data points (*solid squares*) represent days of high rainfall. The regression equations for winter morning and evening periods are y = 0.03 (x) + 1.36 and y = 0.01 (x) + 1.44, and the regression equation for the summer afternoon period is y = -0.03 (x) + 3.64.

In winter, on warm days monkeys became active ~ 30 min earlier and activity peaked earlier in the day than on cold days. Notably, between 07:00 and 12:00, the mean percentage of time active over 30 min time blocks was 19.8 ± 10.1 % higher on warm compared to cold days (Fig. 4.8a, *upper panel*). The ~ 10 to 15 °C higher mean black-globe temperatures on warm compared to cold mornings (Fig. 4.8a, *lower panel*) seemingly allowed monkeys to reach higher activity levels (i.e. between 60 and 80 % during a 30 min time block) more quickly and maintain these levels throughout the diurnal period on warm days. As monkeys were unable to increase activity on cold days to similar levels as on warm days at any stage over the diurnal period, or shift activity in to the nocturnal period, total time active over the 24-h period was significantly higher on warm compared to cold days in winter ($t = 6.95$, $n = 10$, $p < 0.0001$).

In summer, monkeys again became active ~ 30 min earlier on warm days than on cold days (Fig. 4.8b, *upper panel*). On both warm and cold days activity quickly increased in the early morning, but by 09:00 on warm days in summer, mean black-globe temperature had reached 42 °C, more than double the mean black-globe temperature at the same time on cold days (Fig. 4.8b, *lower panel*). Apparently as a consequence, percentage time active during a 30 min time block rapidly decreased to ~ 40 % after 09:00 on warm days, whereas activity was maintained at ~ 60 – 70 % on cold days (Fig. 4.8b, *upper panel*). On warm days, percentage time active in a 30 min time block was suppressed to ~ 40 % between 09:00 and 12:00, when black-globe temperature reached its’ zenith. After mid-day on warm days in summer, percentage time active over 30 min periods started to increase as black-globe temperatures started to decrease, more-or-less synchronizing with activity levels on cold days in the afternoon. Monkeys remained active for ~ 1 h longer in the evening on warm compared to cold days, but were unable to adequately compensate for reduced activity, notably between 10:00 and 14:00, and therefore total time active over the 24-h period was significantly higher on cold compared to warm days ($t = 5.87$, $n = 6$, $p < 0.01$).

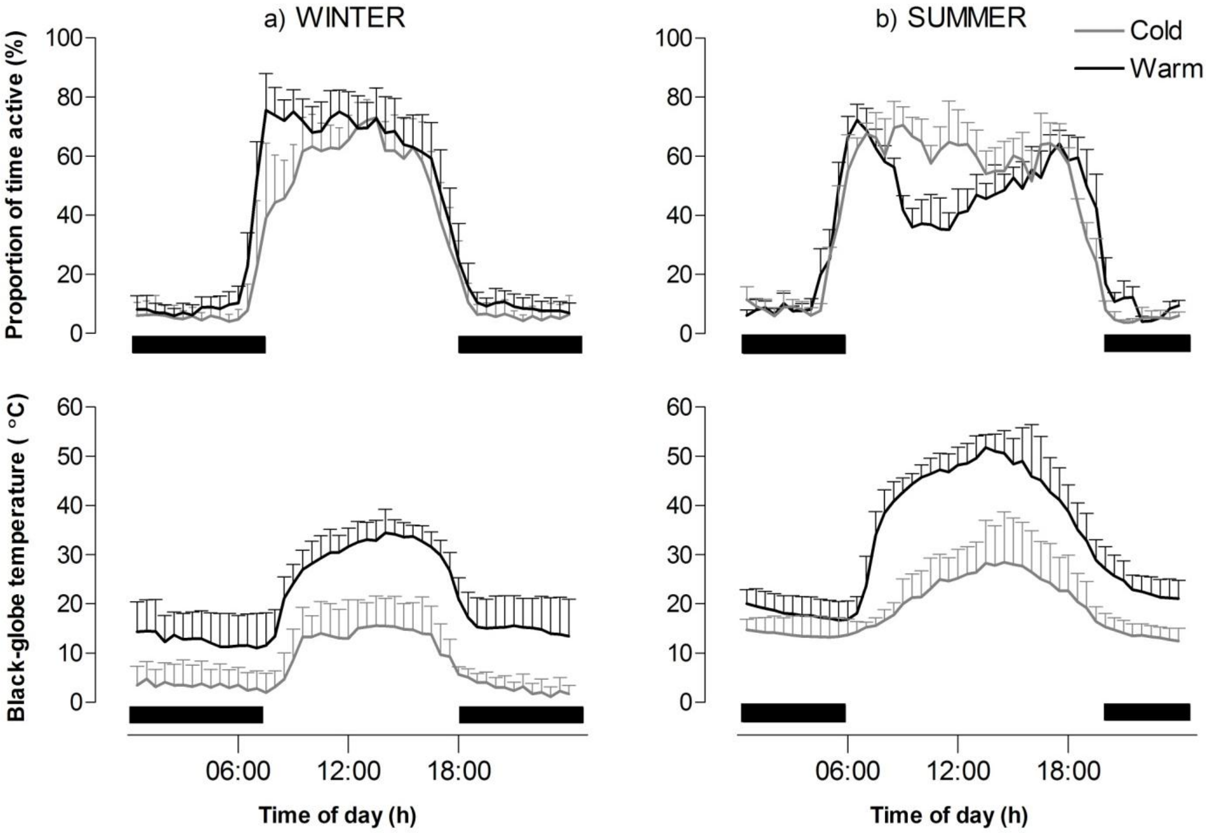

Figure 4.8: Mean (±SD) percentage of time active (*upper panel*) and black globe temperature (*lower panel*) over 30 min time blocks across the 24-h period on the ten coldest (*grey line*) and warmest (*black line*) days for ten monkeys in winter (*panel a*) and six monkeys in summer (*panel b*). SD is the variability between individual monkeys (*upper panel*) and between days (*lower panel*). Black bars represent nocturnal periods.

4.4 Discussion

My study has shown that photoperiod, rainfall, and black-globe temperature were all important determinants of vervet monkey activity. Behavioural flexibility in the distribution of activity across the 24-h period was extremely limited. Vervet monkeys were primarily diurnal, with no evidence for cathemerality. Photoperiod was the primary determinant of total time active over the diurnal period across the duration of my study period. Short day lengths in winter imposed a limitation on the time available for activity which monkeys were unable to overcome by increasing the percentage of the diurnal period for which they were active. Therefore, total time active over the diurnal period was significantly reduced in winter compared to summer. In both winter and summer, thermal stress (low environmental temperatures in winter and high environmental temperatures in summer) inferred a significant constraint on vervet monkey activity, as monkeys were unable to adequately compensate over the diurnal period for significantly reduced activity during periods of high thermal stress. Vervet monkeys did display a degree of flexibility in the distribution of activity during the diurnal period, with reduced activity during the coldest and warmest periods of the day in winter and summer respectively. Reduced activity during periods of thermal stress was likely associated with thermoregulatory behaviours such as huddling or sunbasking to conserve energy during the coldest period of the day in winter, and shade-seeking behaviour to conserve body water or avoid hyperthermia during the warmest period of the day in summer. Furthermore, over the duration of my study period, periods of estimated low food availability were associated with decreased activity, likely reflecting a behavioural mechanism of energy conservation in line with optimal foraging theory. The confounding effect of short day lengths and thermal stress in winter, coinciding with increased social demands during the mating period, may impose a significant bottleneck on the survival of vervet monkey populations at high latitudes. In the following sections I will discuss the ecological implications of each of the environmental factors which influenced vervet monkey activity as well as the potential interacting constraints of these factors.

4.4.1 Behavioural inflexibility

The nycthemeral activity cycle of vervet monkeys was tightly linked to photoperiod, with ~ 85 % of total time active over the 24-h period occurring during the diurnal period even in winter (Fig. 4.3), when day length was shortest. Total time active over the 24-h period increased significantly from mid-winter to mid-summer, driven by a significant increase in total time active over the diurnal period, whereas total time active over the nocturnal period remained unchanged for this period (Fig. 4.4). This finding indicates that nocturnal activity is an unadaptable constant, confirming that behavioural flexibility in vervet monkeys was restricted to diurnal activity. The low, but uniform levels of activity (~ 10 % over a 30 min period) recorded over the nocturnal period (Fig. 4.2) likely reflect the detection of low intensity activities such as postural adjustments which recorded activity counts of greater than 1 % of maximum activity.

An animals' activity may be restricted to a certain phase of the 24-h period by morphological limitations such as nocturnal vision (Ashby 1972), but the finding that nocturnal activity is not evident amongst African primates outside Madagascar is likely due to the increased risk of predation by nocturnal predators such as leopard (Hill 2006b). Although there were no leopard at Samara, caracal and black-backed jackal occurred at relatively high densities and these are both primarily nocturnal hunters. Predation was therefore a possible deterrent of nocturnal activity for monkeys at Samara. The finding that total time active over the nocturnal period was unchanged across the progression from the winter to summer solstice supports the theory that some uniform factor such as the threat of predation precludes the possibility of nocturnal activity. Furthermore, in winter at Samara when nocturnal activity would presumably be beneficial in terms of off-setting the limitation imposed on activity by short day lengths, nocturnal temperatures were consistently well below the TNZ recorded for primates (25 – 30 ° C, Elizondo 1977) and frequently dropped below freezing (see Chapter 3: section 3.3). The energetically optimal strategy may therefore have been to conserve energy and reduce convective heat loss by huddling during the nocturnal period (Da Silva 1993), presumably outweighing any potential benefit gained from being active during the nocturnal period in winter.

For diurnal species, day length imposes an important ecological constraint on an individual's behavioural options as it determines the amount of time available to achieve all fundamental activities (i.e. energetic, social, and biological; Dunbar 1992). The trade-off between the allocation of time to various activities is a crucial component of optimizing energetic efficiency (Norberg 1977). For a population of chacma baboons in South Africa, experiencing similar seasonal fluctuations in day length as vervet monkeys at Samara, day length was an important determinant of seasonal variations in activity patterns (Hill 2006a). In winter, short day length inferred a significant constraint on activity of baboons, with levels of foraging, travelling, social activities, and resting all decreasing during this period. In comparison, in summer, increased day length was associated with increased foraging, travelling, and social activities, as well as allowing for a more flexible behavioural strategy, and thus "excess" time was spent resting in the shade during the heat of the day (Hill *et al.* 2003, 2004). Similarly, for vervet monkeys at Samara, photoperiod was the strongest predictor of increased total time active over the diurnal period across the duration of my study period (Table 4.2), indicating that reduced day length inferred a significant limitation on vervet monkey activity. In addition, the three months with the shortest day lengths (May – July, see Chapter 3: section 3.3) coincide with the vervet monkey mating period (Baldellou and Adan 1997), when competition for mates is highest and social activities are presumably a high priority. The dynamic social interactions and increased social demands during the mating period are evidenced by the finding that the social hierarchy of male vervet monkeys in my study population is least stable and the frequency of agnostic encounters is highest during the mating period (Freeman 2011). Being active over the diurnal period in winter, which coincides with short day lengths, is therefore likely a high priority for vervet monkeys in order to complete all fundamental activities. This theory is supported by the finding that the percentage of the diurnal period for which vervet monkeys were active significantly increased with decreasing photoperiod (Fig. 4.5). Despite a significant increase in the percentage of the diurnal period active in winter compared to summer, monkeys were unable to adequately compensate for decreased day length in winter and therefore total time active over the diurnal period was significantly lower in winter than in summer (Table 4.1). These findings suggest that time available for activity (i.e. day length) may be an important commodity for vervet monkeys and other strictly diurnal species inhabiting temperate environments (Hill *et al.* 2003). It can therefore be concluded that increased time spent active over the 24-h period between winter and summer was a result of proximal restrictions on vervet monkey activity in winter, primarily from short day lengths, rather than a necessity for increased activity in summer.

4.4.2 Confounding constraints of environmental factors on vervet monkey activity

Environmental temperature and rainfall have been shown to be important determinants of primate behavioural ecology (Hill and Dunbar 2002). For baboons, which have a similar dietary composition to vervet monkeys (Skinner and Chimimba 2005), the proximate influence of rainfall seasonality on food availability is proposed to be a key factor mediating inter-population variation in foraging behaviour, as well as life history traits such as group size and interbirth intervals (Dunbar 1992). In line with optimal foraging theory it has been shown for baboons (Hill and Dunbar 2002) and green monkeys (Harrison 1985) that the percentage of time spent feeding significantly increases with increased availability of high quality food items (i.e. fruits and seeds). Similarly, in a meta-analysis of primate activity budgets, Clutton-Brock (1977) showed a negative correlation between time spent feeding on leaves (a low quality food source) and both time spent feeding and travelling.

The distinction between active (i.e. incorporating both feeding and travelling) and inactive (i.e. resting and grooming) states only in my study does not allow for a detailed investigation of the influence of food availability on vervet monkey behaviour. However, if foraging is defined as total time spent feeding and travelling (sensu Hill *et al.* 2003), an investigation of the influence of food availability on total time spent active over the diurnal period provides a useful measure of the foraging response to fluctuations in food availability for vervet monkeys. Both food availability and quality have been shown to be a positive function of rainfall (Barton *et al.* 1992, Hill and Dunbar 2002), and assuming that this relationship holds at Samara, the finding that total time active over the diurnal period was significantly reduced during periods of low food availability (as indexed by the sum of rainfall over the two previous months, Table 4.2) suggests that vervet monkeys reduced foraging effort during periods of low food availability. This finding suggests that during periods of low food availability, the total time spent feeding and travelling was reduced in favour of energy conservation, in line with optimal foraging theory, or possibly as a consequence of increased digestive requirements due to a poor quality diet (Da Silva 1993). No robust inferences on the behavioural ecology of vervet monkeys can be made based on the course behavioural categories inferred from activity count data in my study. However, it is reasonable to assume that being active is more energy consumptive than being inactive, and therefore my results suggest that decreased rainfall, and likely the consequential influence of rainfall on food availability, inferred a significant constraint on vervet monkey activity. Conversely, increased total time active over the diurnal period with increasing rainfall may reflect increased effort to optimize the utilization of high quality food availability, again in line with optimal foraging theory.

Changes in forage availability may co-vary with environmental temperature, and these may have a confounding influence on the energetic requirements of an individual. For example, for colobus monkeys the frequency of thermoregulatory body postures (e.g. hunching) during the cool wet season was dependent on both environmental temperature and food (i.e. energy) availability (Da Silva 1993). At low environmental temperatures, thermoregulation is expensive in terms of metabolic energy (Angilletta *et al.* 2010). Therefore, if an animals' net metabolic energy gain from food intake is compromised, as would be the case under conditions of low food availability or quality, this energy deficit would in turn compromise that animals' capacity to thermoregulate. However, the finding that the sum of rainfall over the two previous months, as a proxy for food availability, and mean monthly black-globe temperature were independent of each other for the duration of my study period (Chapter 3: section 3.3), suggests that the observed decrease in total time active over the diurnal period during periods of low rainfall is reflecting a direct behavioural response to food (i.e. energy) availability, independent of thermoregulatory requirements associated with environmental temperature.

Similarly, the finding that over the duration of my study period, total time active over the diurnal period significantly increased with mean diurnal black-globe temperature (Table 4.2) likely reflects a behavioural response associated primarily with thermoregulatory requirements. The majority of studies in the literature have focused on the behavioural response of primates at high environmental temperatures, which have shown that the frequency of inactive "behaviours", including resting (Stelzner and Hausfater 1986, Stelzner 1988, Hill 2006a) and other activities which can only be done while the animal is otherwise at rest (e.g. grooming and scratching, Ventura *et al.* 2005, Hill 2006a), increase with increasing environmental temperature. These inactive behaviours are typically accompanied by a preference for shady and cooler microclimates (Hill 2006a). At environmental temperatures above the TNZ, an animal must dissipate heat in order to avoid hyperthermia. The primary avenue of heat dissipation is evaporative cooling and therefore thermoregulation at high environmental temperatures is expensive in terms of body water conservation (Gordon 1985, Hetem *et al.* 2010). Decreasing activity and seeking out cool microclimates at high environmental temperatures is therefore proposed to be the optimal behavioural response to coping with heat stress and minimizing water loss (Hill 2006a, Sato 2012).

Hanya (2004) however also showed that in a seasonal environment, time spent active (i.e. feeding and travelling) decreased with decreasing environmental temperature for Japanese macaques. Travelling and feeding are energy expensive activities, and maintaining energy conserving body postures such as hunching are inhibited by being active (Bicca-Marques and Calegaro-Marques 1998, Hanya 2004). At low environmental temperatures in winter therefore, being inactive, often accompanied with postural adjustments such as hunching or huddling and preferential selection of sunny and warmer microclimates, is proposed to conserve energy for metabolic heat production (Hanya 2004, Hanya *et al.* 2007, Sato 2012).

The behavioural response of vervet monkeys along a gradient of environmental temperatures experienced in winter and summer respectively was therefore expected to be opposite. Indeed, my results support the theory that increasing time spent inactive is a direct behavioural response to thermal stress. In winter, total time active over the diurnal period was positively correlated with mean diurnal black-globe temperature (Fig. 4.6a), and conversely in summer, total time active over the diurnal period was negatively correlated with mean diurnal black-globe temperature (Fig. 4.6b). Furthermore, my results indicate that environmental temperature was a significant determinant of the distribution of activity across the diurnal period. In winter, during the morning and evening periods when environmental temperatures were lowest over the diurnal cycle, mean black-globe temperature was a significant determinant of total time active over those periods (Fig. 4.7a). Similarly in summer, mean black-globe temperature was a significant determinant of total time active over the afternoon period, the warmest part of the day (Fig. 4.7b). This temporal association between activity and environmental temperature is reflected in a comparison of the nychthemeral patterns of activity for vervet monkeys on the ten coldest and warmest days in winter and summer respectively (Fig. 4.8). The percentage of time active over a 30 min time block was most notably depressed on cold mornings in winter and warm afternoons in summer, whereas on warm days in winter and cold days in summer monkeys were able to maintain a consistent level of activity throughout the diurnal period. As observed for other vervet monkeys in a similar climatic region of South Africa to Samara (Danzy *et al.* 2012), reduced activity on cold mornings in winter were generally associated with sunbasking behaviour for vervet monkeys at Samara (personal observation). Conversely, reduced activity during the warmest part of the day in summer was typically associated with the selection of cooler, shady microclimates (personal observation). The finding that environmental temperature was an over-riding determinant of the distribution of activity over the diurnal period is reflected in observed seasonal variations in nycthemeral activity patterns (Fig. 4.2), which resemble activity patterns on the ten coldest and warmest days in winter and summer, respectively. Furthermore, the identified outliers of low total time active over the 24-h period coincided with days of heavy rainfall on cool days in winter, likely reflecting a proximal behavioural response to severe thermoregulatory requirements.

My results indicate that over the diurnal period, decreased activity was a rapid behavioural response to thermal stress, and in this way vervet monkeys did display a degree of behavioural flexibility. The likely mechanism of this behavioural flexibility is to conserve energy during the coldest period of the day in winter by selecting sunny microclimates and employing thermoregulatory behaviours such as huddling and sunbasking, and to conserve body water or avoid hyperthermia during the warmest period of the day in summer by resting in the shade (inferred from personal behavioural observations).

The positive relationship between total time active over the diurnal period and mean diurnal black-globe temperature over the duration of my study period (Table 4.2), however, suggests that the overriding thermoregulatory strategy of vervet monkeys at Samara during my study revolved primarily around energy conservation at low environmental temperatures, rather than water conservation at high environmental temperatures. In a study on captive baboons, Mitchell and colleagues (2009) demonstrated that baboons were able to effectively maintain homeothermy by autonomic means when exposed to heat stress when water was available *ad libitum*. Similarly, for free ranging baboons in the Namib Desert, water availability was the primary determinant of thermoregulatory capacity when exposed to high environmental temperatures (Brain and Mitchell 1999). Importantly vervet monkeys at Samara had access to free-standing water for the duration of my study period. Whilst behavioural thermoregulation is “cheap” in terms of energy, it is expensive in terms of time (Hill 2006a), and vervet monkeys may therefore have prioritized autonomic thermoregulation to a degree when exposed to high environmental temperatures in order to free up time for other fundamental activities.

4.4.3 Conclusion

My study has shown that being inactive is a direct behavioural response to thermal stress, specifically at low environmental temperatures in winter and high environmental temperatures in summer, and reduced food availability. This behavioural response is presumably enforced as a consequential trade-off of the energetic benefit and cost of being active. Over the duration of my study period, the primary thermoregulatory consideration appeared to be metabolic energy conservation at low environmental temperatures, as during my study monkeys had access to free standing water which may have facilitated autonomic thermoregulation at high environmental temperatures. My study suggests that primates living at high latitudes subjected to cold winters with short day lengths are faced with significant ecological challenges. Although thermal stress invoked a significant constraint on total time active over the diurnal period in both winter and summer, the marked behavioural inflexibility in activity scheduling displayed by vervet monkeys and the confounding effect of shortened day length and low environmental temperatures in winter imposes a potentially significant constraint on the amount of time available to complete all fundamental activities. Furthermore, this period of high environmental stress in winter also coincides with the mating season for vervet monkeys. Monkeys are therefore likely obligated to sacrifice their short-term physiological well-being, at a time when the costs of thermoregulation are highest, in favour of other biologically more important activities (i.e. reproduction).

CHAPTER 5

The effect of environmental factors on vervet monkey body temperature

5.1 Introduction

Body temperature affects nearly all physiological processes and for homeothermic endotherms, the maintenance of a high, stable body temperature increases performance at the cellular and whole organism level (Heinrich 1977, Somero 2004, Knies *et al.* 2009). Maintaining body temperature however requires considerable metabolic energy and resource (specifically water) inputs, and a growing body of evidence suggests that homeotherms exhibit more labile body temperature rhythms (see Pereira *et al.* 2002, Ostrowski *et al.* 2003, Arnold *et al.* 2004, Grigg *et al.* 2009, Maloney *et al.* 2011, Hetem *et al.* 2010) than the non-adaptive constant originally described by Scholander and colleagues (1950). These findings have led some to conclude that thermoregulatory mechanisms amongst endotherms are more accurately described as a continuum rather than a dichotomy between classical definitions of heterothermy and homeothermy (Angilletta *et al.* 2010, Boyles *et al.* 2011a).

Variations in body temperature in response to environmental stressors may off-set the energetic costs of maintaining rigorous homeothermy, and thereby serve an adaptive function (Angilletta *et al.* 2010). It is proposed that the net-benefit of maintaining a high, stable body temperature rhythm is dependent on a cost-benefit trade-off of thermoregulation; where the optimal thermoregulatory strategy of endotherms is a compromise between the performance benefit of maintaining homeothermy and the energetic, and non-energetic, cost of thermoregulation (Angilletta *et al.* 2010). Any factors which increase the cost or decrease the benefit of thermoregulation would therefore favour a more labile thermoregulatory mechanism; where the maintenance of homeothermy is traded-off against energy conservation or reallocation to other biologically important activities (Angilletta *et al.* 2010).

The primary cost of thermoregulation for small mammals is incurred by the gradient between body and environmental temperature (McNab 1983, Angilletta *et al.* 2010). In a highly seasonal environment such as Samara, individuals are faced with the challenge of maintaining homeothermy within a dynamic thermal environment. Maintaining body temperature within a narrow range (i.e. homeothermy) is primarily expensive in terms of body water at high environmental temperatures, and metabolic energy at low environmental temperatures (Gordon 1985, Hetem *et al.* 2010). Physiological adjustments such as hyper - and hypothermia may serve to off-set the cost of thermoregulation by reducing the gradient between body and environmental temperature, thus reducing evaporative cooling requirements at high environmental temperatures and metabolic energy requirements at low environmental temperatures (Angilletta *et al.* 2010, Hetem *et al.* 2010). A controlled down regulation of body temperature at low environmental temperatures may also infer significant energy savings by means of the consequential reduction in metabolic rate due to the Q_{10} effect (Geiser 2004). Thermoregulatory plasticity may therefore be an important adaptive mechanism for species living in seasonal environments, where extreme weather conditions are likely to be exacerbated under future climate change (Fuller *et al.* 2010).

An important first step in understanding how thermoregulatory plasticity may affect the adaptability of homeothermic species under future climate change scenarios is to investigate the degree to which body temperature varies within natural populations, and develop causal relationships between variations in body temperature and potential environmental stressors (Angilletta *et al.* 2010, Fuller *et al.* 2010, Boyles *et al.* 2011a). Very few studies have however recorded continuous measurements of body temperature for free-ranging animals in their natural environment and this paucity of data currently perturbs the formulation of robust theories on the role that physiological plasticity may play in negating environmental stochasticity associated with future climate change (Angilletta *et al.* 2010, Boyles *et al.* 2011a).

5.1.2 Chapter objective, hypotheses and predictions

The aim of this chapter is therefore to investigate the degree of physiological plasticity employed by vervet monkeys across seasons in their natural habitat, and to determine which, if any, environmental factors influence the thermoregulatory mechanism employed by vervet monkeys.

In line with the cost-benefit trade-off theory of thermoregulation (Angilletta *et al.* 2010), I hypothesize that environmental factors which significantly reduce the net-benefit of thermoregulation will be reflected by less precise (i.e. increased 24-h amplitude of the body temperature rhythm) thermoregulation. At high environmental temperatures the primary avenue of heat dissipation is evaporative cooling. As vervet monkeys had access to a natural source of free-standing water for the duration of my study period, I predict that monkeys will be able to effectively thermoregulate at high environmental temperatures. At low environmental temperatures however, it may be energetically optimal to minimize energetic requirements by reducing the gradient between body and environmental temperature (Angilletta *et al.* 2010), resulting in imprecise thermoregulation. Furthermore, reduced food availability may compromise the energetic balance of vervet monkeys and inhibit thermoregulatory capacity, which should again be reflected in reduced thermoregulatory precision. I therefore predict that, if vervet monkeys employ thermoregulatory plasticity in response to environmental stressors, that the 24-h amplitude of the body temperature rhythm should increase (i.e. less precise thermoregulation) with decreasing environmental temperature and food availability.

5.2 Materials and methods

5.2.1 Data collection

For my study 12 vervet monkeys were implanted with temperature-sensitive data loggers which recorded core body temperature at 5 min intervals at a resolution of 0.06 °C (see Chapter 2: section 2.5 for technical details of loggers). Three data loggers failed shortly after they were launched and therefore provided no data. Of the nine monkeys for which data are presented here, eight were implanted in April 2011 and one in July 2011. The maximum number of monkeys for which I have simultaneous data was therefore nine between July and November 2011, after which my sample size was sequentially reduced to five due to premature failure of data loggers (see Chapter 2: Table 2.2 for details on the duration of temperature data collection for all monkeys).

5.2.2 Data analyses

I conducted an initial inspection of the 24-h mean body temperature data for outliers in the data set. An outlying observation is defined as one which appears to deviate markedly from other members of the sample in which it occurs. I investigated the distribution of 24-h mean body temperature across the study period by means of box-plots for each month. I calculated the 24-h mean body temperature for individual monkeys for each day, and averaged across all monkeys to obtain a single value for the population for each day, which was used to construct the box-plots for each month. The box-plot analysis revealed no statistical outliers (defined as being more than three times the interquartile difference, the difference between the 75 and 25 percentiles, above or below the 75 and 25 percentiles respectively, Hodge and Austin 2004) in the data set, and therefore all data were included in subsequent analyses.

5.2.2.1 Nychthemeral rhythm of body temperature

To describe seasonal 24-h body temperature rhythms I calculated mean body temperature over the 24-h period for all days in winter and summer respectively, for each individual monkey. For the respective 5 min intervals across the 24-h period I averaged individual body temperature across all monkeys to obtain a mean value for each time interval for the population, in winter and summer respectively. Using mean body temperature values for the population in 5 min intervals (calculated above) I then analyzed the rate of change in body temperature over the 24-h period by means of segmented regression analyses (Davies 1987) for winter and summer months. Segmented regression analysis detects any threshold values or “break-points” at which the slope of the linear relationship between the predictor (i.e. time) and response (i.e. body temperature) variables changes significantly.

Laboratory studies have shown the circadian rhythms of body temperature and activity to be closely associated biological rhythms (Refinetti and Menaker 1992). To investigate the relationship between body temperature and activity I conducted Pearson's correlations between the change in mean body temperature from one 30 min time block to the next against the corresponding change in percentage time active from one 30 min time block to the next, in winter and summer respectively. The analyses were conducted for six monkeys with body temperature and activity data in both seasons. For each individual monkey mean body temperature and the percentage of time active were calculated in 30 min time blocks over the 24-h period. These values were averaged across all six monkeys to obtain a single mean value of mean body temperature and percentage time active for each 30 min time block. From these values for the population, the change in mean body temperature and percentage time active from one 30 min time block to the next were calculated. The correlation analyses between the change in body temperature and corresponding change in percentage time active were conducted for the period between the start of the initial early-morning increase in body temperature and the end of the late-afternoon decrease in body temperature in winter and summer, as identified by means of segmented regression analyses.

To determine the time of day associated with the minimum (nadir) and maximum (zenith) of the body temperature rhythm in winter and summer respectively, I determined the time of minimum and the time of maximum body temperature for each day for each individual monkey. A frequency distribution of the times of minimum and maximum body temperatures was constructed for each individual monkey in winter and summer. The bin size for the frequency distributions was 1 h and the bins were centered on the hour over the 24-h period. For each bin across the 24-h period I calculated the percentage of total counts in that bin ((counts in given bin ÷ total counts in all bins) x 100) for each individual monkey, and averaged the percentage of counts in each bin across all monkeys to obtain the mean percentage of counts in each bin for the population.

5.2.2.2 Variations in body temperature over the duration of the study period

The respective parameters of the body temperature rhythm used to investigate variations in body temperature over the duration of my study period and determine the relationship between body temperature and environmental stressors were; mean diurnal, mean nocturnal, 24-h minimum, 24-h maximum, 24-h mean, and 24-h amplitude of the body temperature rhythm, calculated for each individual monkey for each day over the duration of my study period. The diurnal and nocturnal periods were calculated from the time of sunrise and sunset for a given month (see Chapter 3: table 3.1), and the 24-amplitude of the body temperature rhythm was calculated as the difference between the corresponding 24-h maximum and 24-h minimum body temperatures.

I compared mean diurnal, mean nocturnal, 24-h minimum, 24-h maximum, 24-h mean, and 24-h amplitude of the body temperature rhythm between winter and summer for six monkeys which had a minimum of 30 sample days in both seasons by means of paired t-tests. For these six monkeys I also compared the nocturnal mean with the diurnal mean body temperature within both seasons by means of paired t-tests. Single mean values were calculated for each body temperature parameter per monkey in winter and summer respectively.

To describe the variation in the parameters of the body temperature rhythm across months I calculated mean 24-h minimum, 24-h maximum, 24-h mean, and 24-h amplitude of the body temperature rhythm per month for the duration of my study period. Mean monthly values for the respective parameters of the body temperature rhythm were calculated for individual monkeys and averaged across all monkeys to obtain mean monthly values for the population.

To analyze changes in 24-h minimum, 24-h maximum, 24-h mean, and 24-h amplitude of the body temperature rhythm from winter to summer, I used Pearson's correlations to correlate the respective parameters of the body temperature rhythm against days between the winter (21 June) and summer (22 December) solstices. I calculated the 24-h value for each body temperature parameter for each individual monkey, and averaged these values across all monkeys to obtain a single mean value for each parameter per day for the population.

To determine whether decreased thermoregulatory precision was a result of hypo- (i.e. depressed 24-h minimum body temperature) or hyperthermia (i.e. elevated 24-h maximum body temperature), I used Pearson's correlations to investigate the relationship between the 24-h amplitude of the body temperature rhythm, and 24-h minimum and 24-h maximum body temperature.

In addition to the above parameters of the body temperature rhythm, it has been proposed that the most commonly experienced body temperature (i.e. modal body temperature) over the period of activity approximates the preferred or optimal body temperature for performance (Boyles *et al.* 2011b). To compare modal body temperatures between seasons I constructed a frequency distribution of recorded body temperatures for individual monkeys over winter and summer respectively. The frequency distribution bin size was 0.1 °C and the bins were centered on the 0.1 °C across the range 34 – 42 °C. For each individual monkey I calculated the percentage of total counts in each bin ((counts in given bin ÷ total counts in all bins) x 100), and averaged the percentage of counts in each bin across all monkeys to obtain the mean percentage of counts in each bin for the population. Within seasons, frequency distributions of recorded body temperatures were also constructed for the nocturnal and diurnal periods respectively. To determine whether there was a significant difference between the lower (i.e. nocturnal) or upper (i.e. diurnal) modal body temperatures between winter and summer I conducted a paired t-test on the respective modes between winter and summer for six monkeys with a minimum of 30 sample days in both seasons. For these six monkeys I also compared the nocturnal modal body temperature with the diurnal modal body temperature within both seasons by means of paired t-tests.

5.2.2.3 Influence of environmental factors on parameters of the body temperature rhythm

As in Chapter 4 (see section 4.2 for description of linear mixed effect models), to identify the environmental factors influencing the respective parameters of the vervet monkey body temperature rhythm over the duration of my study period I used linear mixed effect models.

To investigate the influence of environmental temperature and food availability (as indexed by the sum of rainfall over the two previous months, see Chapter 3: section 3.1) on respective parameters of the body temperature rhythm over the duration of my study period I used linear mixed effect models and controlled for repeated measurements in each model by entering individual monkeys as a random factor. I ran separate models with 24-h minimum (Model 1), 24-h maximum (Model 2), 24-h mean (Model 3), and 24-h amplitude (Model 4) of the body temperature rhythm as the dependent variables. To determine whether the respective parameters of the body temperature rhythm "tracked" environmental temperature, as would be expected if the thermoregulatory mechanism was to minimize the difference between body and environmental temperature, corresponding measures of environmental temperature were incorporated in the respective models (e.g. when 24-h minimum body temperature was the dependent variable, 24-h minimum black-globe temperature was the independent variable, when 24-h maximum body temperature was the dependent variables, 24-h maximum black-globe temperature was the independent variable, etc.). In each model the sum of rainfall over the two previous months and photoperiod were also included as independent variables. To control for variability in the expression of the body temperature rhythm between individual monkeys, individual monkey mass, sex, and troop were included as control variables in each model.

To describe the variation in thermoregulatory precision between consecutive days I plotted 24-h amplitude of the body temperature rhythm, averaged across all monkeys per day, across days in winter and summer respectively. To compare variation in thermoregulatory precision between winter and summer I compared the mean absolute change in 24-h amplitude of the body temperature rhythm from one day to the next between seasons by means of paired t-tests for six monkeys with a minimum of 30 sample days in both seasons.

To further explore the relationship between thermoregulatory precision and environmental temperature identified in Model 4 (see Results), and determine whether variations in thermoregulatory precision were due to thermal stress, I investigated the proximal influence of the respective measures of environmental temperature on thermoregulatory precision in winter and summer respectively by means of Pearson's correlations. For winter and summer, 24-h amplitude of the body temperature rhythm was correlated against corresponding 24-h minimum, 24-h maximum, 24-h mean, and 24-h amplitude of black-globe temperature.

To determine whether vervet monkeys were able to facilitate the increase in body temperature during the initial early morning re-warming period (08:00 to 10:00 as defined by previously described segmented analysis) by means of passive re-warming in winter, I used a linear mixed effect model and controlled for repeated measurements by entering individual monkey as a random factor to investigate the influence of environmental temperature, cloud cover, and activity on the increase in body temperature over the identified two hour period. The use of a linear mixed effect model was appropriate to investigate the influence of multiple independent variables on the dependent variable. The dependent variable in the model (Model 5) was the change in body temperature (°C) between 08:00 and 10:00. The independent variables were the corresponding change in black-globe temperature (°C) (black-globe temperature at 10:00 – black-globe temperature at 08:00), the difference between mean black-globe and mean dry-bulb temperature (an index of the availability of direct sunlight, see Chapter 3: section 3.3) for the period 08:00 to 10:00, and the percentage of time spent active over the period 08:00 to 10:00 for individual monkeys ((number of counts active ÷ total number of counts active and inactive) × 100). To account for variations in photoperiod and food availability over the winter period, photoperiod and the sum of rainfall over the two previous months were included in the model as independent variables. Only six monkeys with both body temperature and activity data for winter were included in this analysis. Individual sex, mass, and troop were included in the model as control variables.

For all analyses the Kolmogorov-Smirnov test was used to test the data for normality. All statistical analyses were conducted using IBM SPSS V.10 statistics software. If variables were significantly correlated, I conducted linear regression analysis to determine the straight line equation. Results were significant at $p < 0.05$.

5.3 Results

5.3.1 Nychthemeral rhythm of body temperature

In both winter and summer the nycthemeral rhythm of body temperature was closely associated with the light:dark cycle (Fig. 5.1). Periods of uniform change in body temperature over the 24-h period in winter and summer were identified by means of segmented regression analyses. In both winter and summer the best fit segmented regression model (i.e. highest r^2 value) was achieved with six break-points (i.e. points at which the slope of the linear relationship between the predictor (time) and response (body temperature) variables changed significantly). In winter, body temperature started to increase rapidly at 08:05 (break-point identified by segmented regression). The initial rapid increase in body temperature started to taper off at 09:55 and remained stable between 11:50 and 17:00, at which time body temperature started to decrease before reaching the nocturnal mean at 19:00. Notably there was an initial, slow increase in body temperature well before sunrise, from 02:15 to 08:05. During this initial period of increasing body temperature there was substantial individual variation in mean body temperature between monkeys, and similarly during the afternoon period (Fig. 5.1a). Comparably, in summer, body temperature over the 24-h period was very uniform between individual monkeys, reflected by the relatively small and stable standard deviation over the 24-h period (Fig. 5.1b). In summer, the initial rapid increase in body temperature from the nocturnal mean started at 04:35, ~ 30 min to 1 h prior to sunrise, and continued through to 06:40, at which time the rate of increase tapered off towards mid-day (12:55). From mid-day there was a second period of increasing body temperature which resulted in the zenith of the 24-h body temperature rhythm occurring around the time of sunset (18:25), from which point body temperature rapidly decreased to reach the nocturnal mean at 21:15 (Fig. 5.1b).

In both winter ($r^2 = 0.74$, $n = 21$, $p < 0.0001$, Fig. 5.2a, *lower panel*) and summer ($r^2 = 0.51$, $n = 29$, $p < 0.0001$, Fig. 5.2b, *lower panel*), the change in mean body temperature from one 30 min time block to the next was significantly correlated with the corresponding change in the percentage of time spent active between 30 min time blocks, indicating that the change in body temperature was a function of the change in the intensity (as indexed by the percentage of time active) of activity.

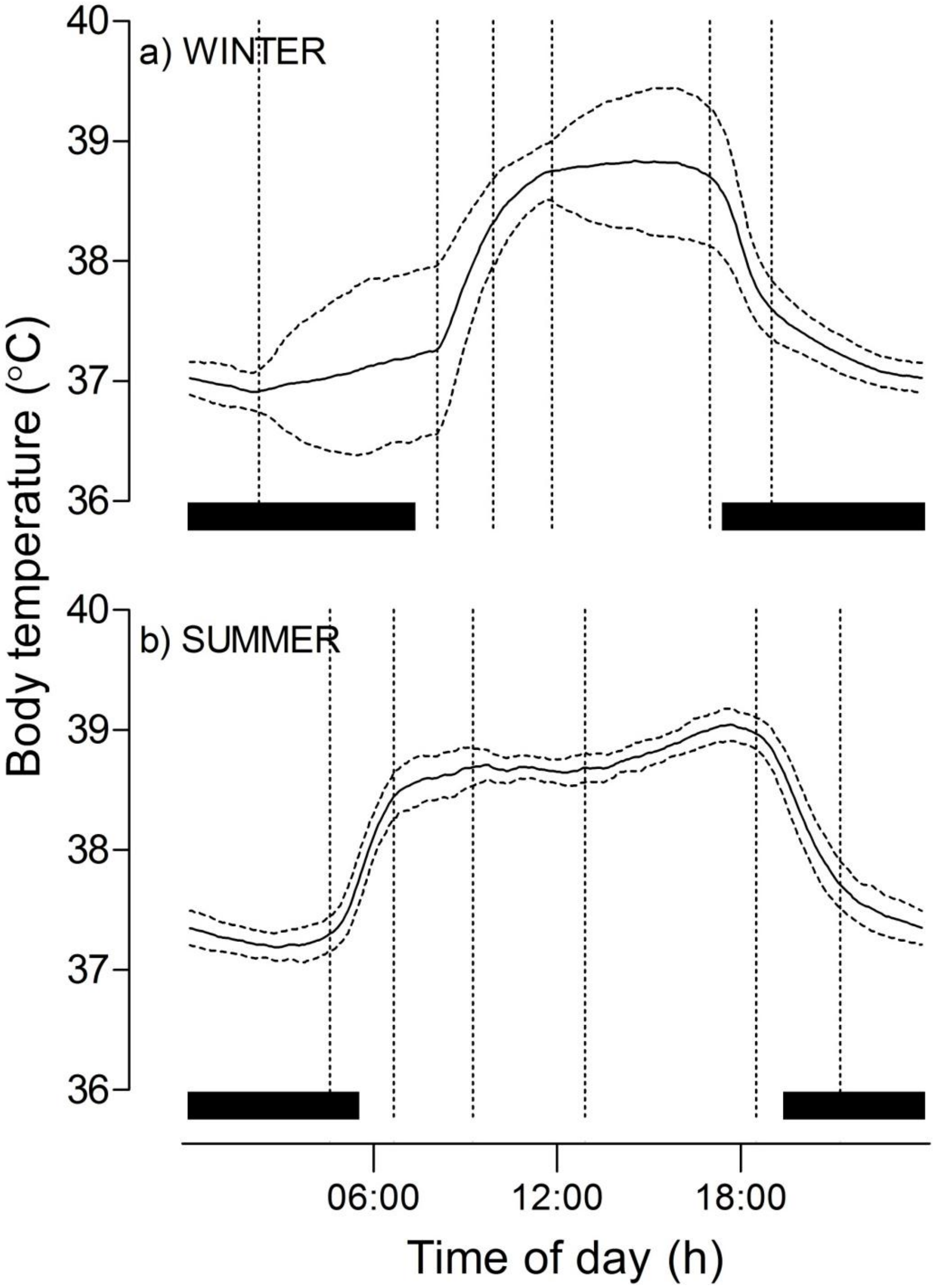

Figure 5.1: 24-h rhythm of body temperature (mean ± SD) in 5 min intervals for nine monkeys in winter (*panel a,* n = 92 days) and six monkeys in summer (*panel b,* n = 91 days). Black bars represent nocturnal periods. SD is the variability between individual monkeys for each 5 min time interval. The identified break points in the rate of change in body temperature over the 24-h period from segmented regression analyses (model fit, winter: $r^2 = 0.99$, n = 288 body temperature records over the 24-h period, summer: $r^2 = 0.99$, n = 288) are illustrated by vertical dotted lines.

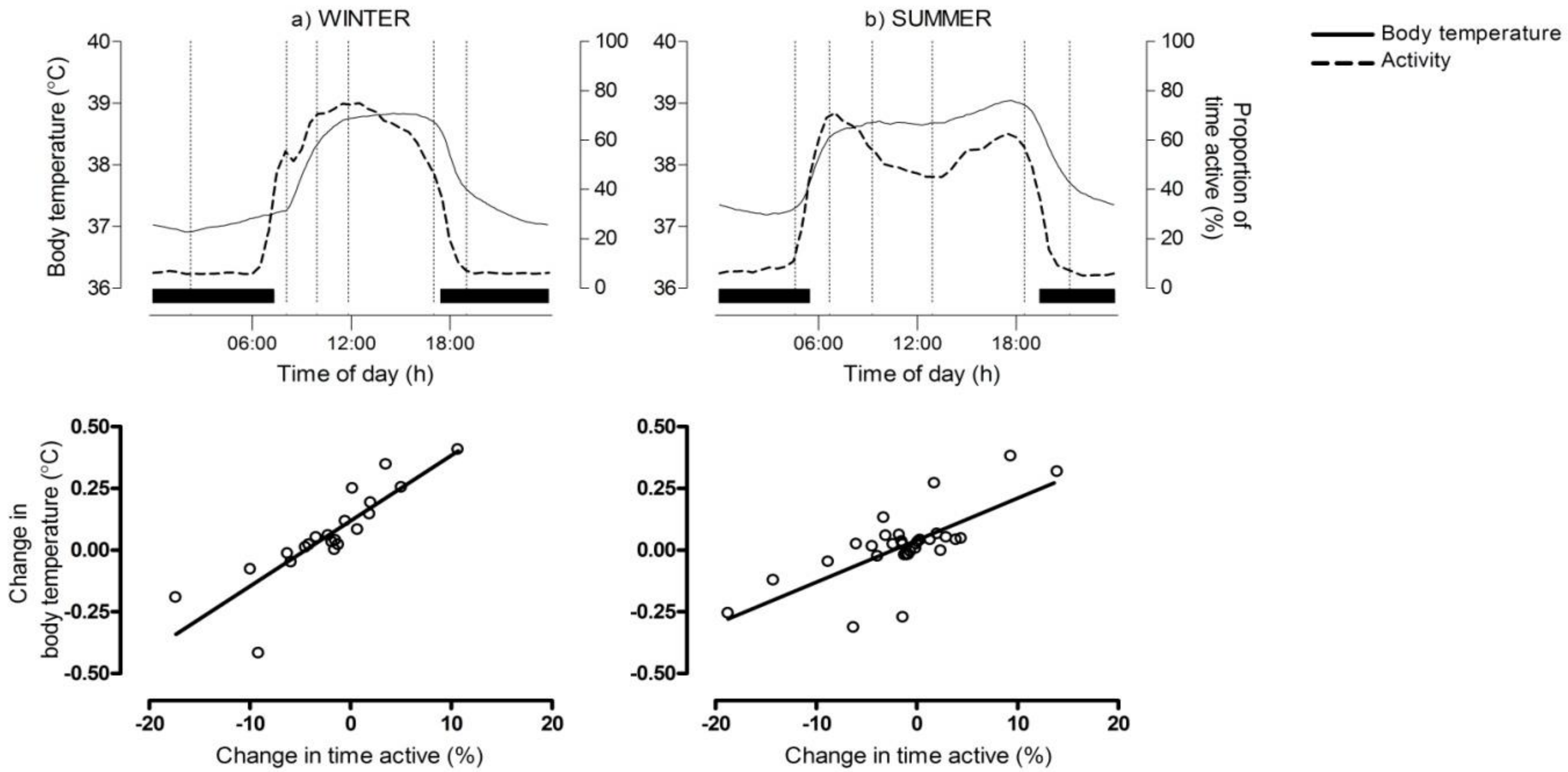

Figure 5.2: *Upper panels)* Segmented regression analyses of 24-h rhythm of body temperature for six monkeys in winter (*panel a,* r^2 = 0.99, n = 288 body temperature records over the 24-h period) and summer (*panel b,* r^2 = 0.99, n = 288). The identified break points in the rate of change in body temperature over the 24-h period are illustrated by vertical dotted lines. Black bars represent nocturnal periods. *Lower panels)* Change in mean body temperature from one 30 min time block to the next in relation to corresponding change in the percentage of time active from one 30 min time block to the next for six monkeys between 08:00 and 19:00 in winter (*panel a*, y = 0.03 (x) + 0.12) and 05:00 and 20:00 in summer (*panel b,* y = 0.02 (x) + 0.04).

Although the minimum mean body temperature occurred during the nocturnal period in winter, at ~ 02:00 (Fig. 5.1a), the modal time of minimum body temperature was at 08:00 (Fig. 5.3a), ~ 1 h after sunrise and coinciding with the time at which body temperature started to increase rapidly (Fig. 5.1a). The time of maximum mean body temperature (Fig. 5.1a) and modal maximum body temperature were more closely associated, with modal maximum body temperature occurring ~ 1 to 2 h prior to sunset at 16:00 (Fig. 5.3a). In summer the modal time of minimum body temperature occurred shortly before sunrise, at 04:00 (Fig. 5.3b), four hours earlier than in winter. Similar to winter, the modal time of minimum body temperature in summer more or less coincided with the time at which body temperature started to increase rapidly (Fig. 5.1b). As in winter, the modal time of maximum body temperature in summer also occurred in the late afternoon ~ 1 h before sunset at 17:00 (Fig 5.3b). In both winter and summer the modal times of maximum body temperature coincided more or less with the time shortly before body temperature started to decrease rapidly to the nocturnal mean.

5.3.2 Variations in body temperature over the duration of the study period

Mean 24-h minimum body temperature was significantly lower in winter compared to summer (Table 5.1). Mean 24-h minimum body temperature was lowest in July and highest in January (Fig 5.4a, *left panel*). The increase in mean 24-h minimum body temperature was significantly correlated to days between the winter and summer solstices (Fig. 5.4a, r*ight panel,* $r^2 = 0.54$, n = 185, $p < 0.0001$). Conversely, there was no significant difference in mean 24-h maximum body temperature between winter and summer (Table 5.1). Although mean 24-h maximum body temperature increased significantly across days between the winter and summer solstice (Fig. 5.4b, *right panel*, $r^2 = 0.08$, n = 185, $p < 0.0001$), the slope of the linear regression equation was close to zero and only 8 % of the variation in 24-h maximum body temperature was explained by the progression of days between the winter and summer solstice. Mean 24-h maximum monthly body temperature varied by less than 0.2 °C over the duration of my study period (Fig. 5.4b, *left panel*). Note that although not statistically identified as outliers, there were three 24-h maximum body temperature data points which were well below the mean 24-h maximum body temperature (*solid squares* in Fig. 5.4b, *right panel*). Coincidentally these relatively low 24-h maximum body temperatures occurred on the 5th and 25th of July and the 5th of August, coinciding with the identified outliers in total time active over the 24-h period on days associated with heavy rainfall (see Chapter 4: section 4.3). Since mean 24-h minimum body temperature significantly increased between winter and summer, with little change in mean 24-h maximum body temperature, the 24-h mean body temperature was significantly higher in summer compared to winter (Table 5.1) and increased significantly across days between the winter and summer solstices (Fig. 5.4c, *right panel*, $r^2 = 0.61$, $n = 185$, $p < 0.0001$). Mean 24-h body temperature was lowest in July and highest in January (Fig. 5.4c, *left panel*). Vervet monkeys also exhibited a significantly higher 24-h amplitude of the body temperature rhythm in winter than in summer (Table 5.1), and mean 24-h amplitude of the body temperature rhythm decreased linearly between the winter and summer solstices (Fig. 5.4d, *right panel*, $r^2 = 0.42$, $n = 185$, $p < 0.0001$). In summer, the highest 24-h amplitude of the body temperature rhythm recorded for an individual monkey was 4 °C, whereas in winter, the 24-h amplitude of the body temperature rhythm for individual monkeys exceeded 4 °C on 12.3 ± 10.9 % of days. The 24-h amplitude of the body temperature rhythm was lowest in January and highest in July (Fig. 5.4d, *left panel*).

Since mean 24-h minimum body temperature was associated with the inactive phase during the nocturnal period, and mean 24-h maximum body temperature was associated with the active phase during the diurnal period, mean nocturnal body temperature was significantly lower than mean diurnal body temperature in both winter ($t = 15.47$, $n = 6$, $p < 0.0001$) and summer ($t = 53.84$, $n = 6$, $p < 0.0001$). The difference between mean nocturnal body temperature and mean diurnal body temperature was 1.3 °C in winter and 1.2 °C in summer. Reflecting the seasonal trends in mean 24-h minimum and mean 24-h maximum body temperature, mean nocturnal body temperature was significantly lower in winter than in summer but there was no significant difference in mean diurnal body temperature between seasons (Table 5.1).

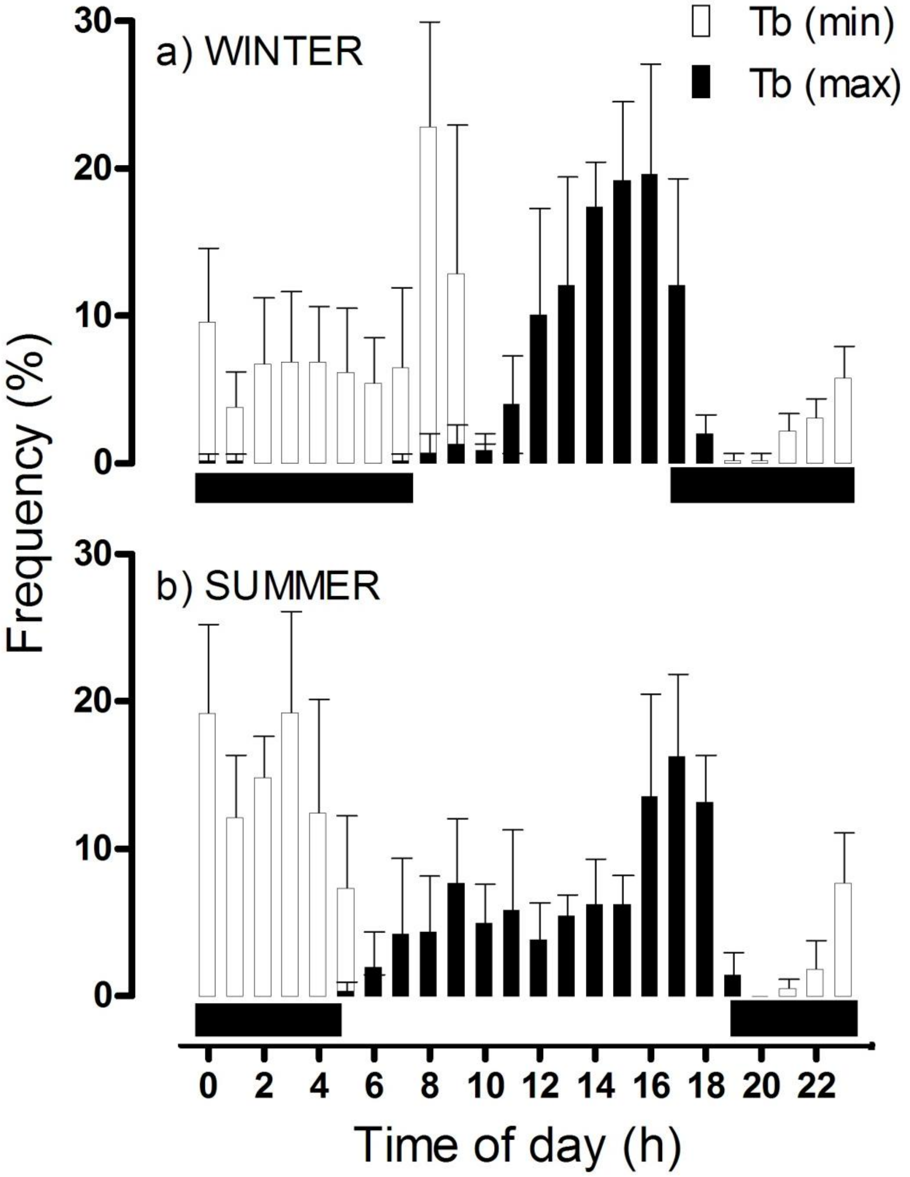

Figure 5.3: Frequency (+ SD) distribution of time of minimum (*white bars*) and maximum (*black bars*) body temperature for nine monkeys in winter (*panel a,* n = 92 days) and six monkeys in summer (*panel b,* n = 91 days). Black bars represent nocturnal periods. SD is the variability between individual monkeys for each hourly time block.

Table 5.1: Seasonal variation in 24-h minimum, 24-h maximum, 24-h mean, 24-h amplitude, diurnal, and nocturnal body temperature (mean ± SD) for six monkeys. SD is between individual monkeys.

Body temperature (°C)	**Winter (n = 92 days)**	**Summer (n = 91 days)**	**t**	**p**
Diurnal	38.5 ± 0.2	38.7 ± 0.1	2.04	0.10
Nocturnal	37.2 ± 0.2	37.5 ± 0.1	4.06	**< 0.01**
24-h minimum	34.6 ± 0.5	36.2 ± 0.1	7.45	**< 0.0001**
24-h maximum	40.3 ± 0.6	40.6 ± 0.4	0.87	0.42
24-h mean	37.7 ± 0.2	38.2 ± 0.1	5.37	**< 0.01**
24-h amplitude	3.2 ± 0.4	2.5 ± 0.1	5.47	**< 0.01**

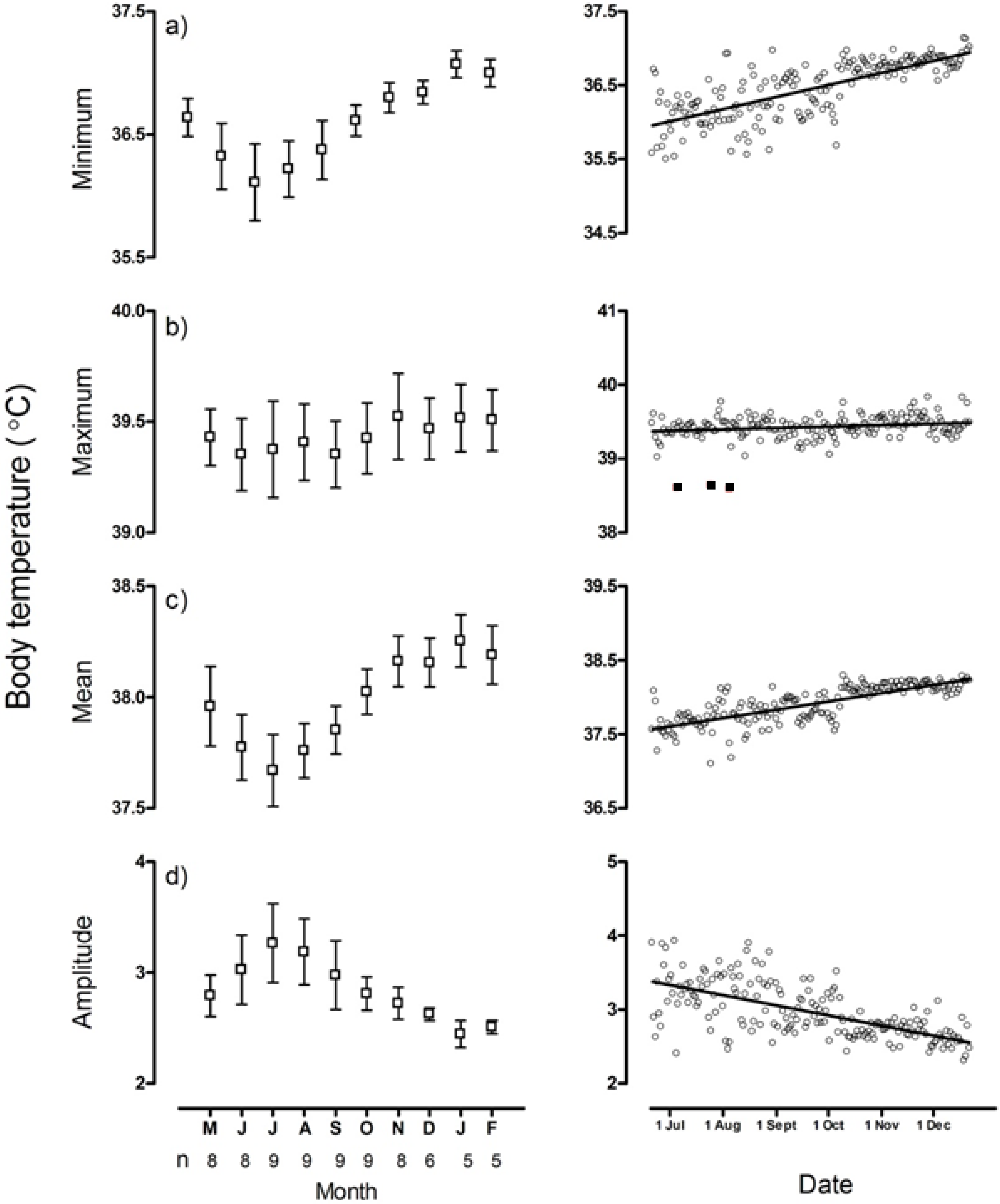

Figure 5.4: *left panels*) Mean (± SD) 24-h minimum (*panel a*), 24-h maximum (*panel b*), 24-h mean (*panel c*), and 24-h amplitude (*panel d*) of the body temperature rhythm across months for the duration of the study period. SD is the variability between individual monkeys. N is the sample size (monkeys) per month. *Right panels)* Mean 24-h minimum (*panel a*: y = 0.005 (x) + 35.95), 24-h maximum (*panel b*: y = 0.001 (x) + 39.33), 24-h mean (*panel c*: y = 0.004 (x) + 37.57), and 24-h amplitude (*panel d*: y = -0.004 (x) + 3.38) of the body temperature rhythm across days between the winter and summer solstices.

In winter (Fig.5.5a) and summer (Fig. 5.5b), thermoregulatory precision was a function of both 24-h minimum and 24-h maximum body temperature. The 24-h amplitude of the body temperature rhythm decreased with increasing 24-h minimum body temperature (Fig. 5.5, *left panels,* winter: $r^2 = 0.76$, n = 92, $p < 0.0001$, summer: $r^2 = 0.42$, n = 91, $p < 0.0001$) and increased with increasing 24-h maximum body temperature (Fig. 5.5, *right panels,* winter: $r^2 = 0.18$, n = 92, $p < 0.0001$, summer: $r^2 = 0.36$, n = 91, $p < 0.0001$). In both seasons 24-h minimum body temperature was the stronger predictor of variation in mean 24-h amplitude of the body temperature rhythm compared to 24-h maximum body temperature, as illustrated by the higher r^2 values.

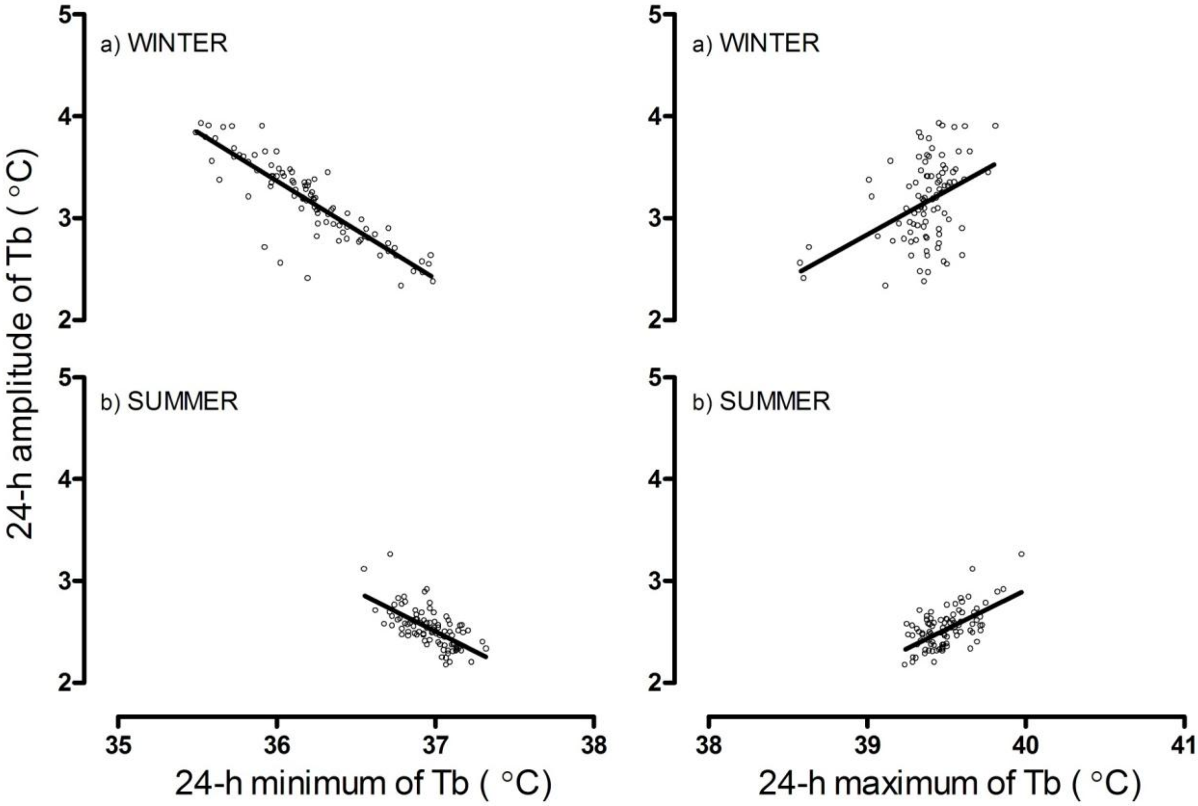

Figure 5.5: Mean 24-h amplitude of the body temperature (Tb) rhythm in relation to mean 24-h minimum body temperature (*left panel*) and mean 24-h maximum body temperature (*right panels*) in winter (*panel a,* n = 92 days, minimum: $y = -0.96 (x) + 37.86$, maximum: $y = 0.86 (x) – 30.59$) and summer (*panel b,* n = 91 days, minimum: $y = -0.79 (x) + 31.71$, maximum: $y = 0.77 (x) – 27.89$).

Vervet monkeys exhibited a bimodal distribution of body temperatures across the duration of the study period. The lower modal body temperature for all monkeys was 37.1 °C in winter (Fig. 5.6a) and 37.3 °C in summer (Fig. 5.6b). For six monkeys with data in both seasons there was no significant difference in the lower modal body temperature between winter and summer ($t = 2.10$, $n = 6$, $p = 0.09$). The upper modal body temperature for all monkeys was 39.0 °C in winter (Fig. 5.6a) and 38.6 °C in summer (Fig. 5.6b). For six monkeys with data in both seasons the upper modal body temperature was significantly higher in winter than in summer ($t = 5.94$, $n = 6$, $p < 0.01$). Note that this comparison of modal body temperature between seasons yields the exact opposite result compared to the comparison of mean 24-h minimum and mean 24-h maximum body temperature between seasons (Table 5.1).

The lower and upper modal body temperatures effectively reflected nocturnal (or inactive phase, Fig. 5.7a) and diurnal (or active phase, Fig. 5.7b) modes respectively. In line with the result derived from the comparison of mean nocturnal and diurnal body temperatures within seasons (Table 5.1), the nocturnal modal body temperature was significantly lower than the diurnal modal body temperature in winter ($t = 20.82$, $n = 6$, $p < 0.0001$) and summer ($t = 16.74$, $n = 6$, $p < 0.0001$). The difference between diurnal and nocturnal modal body temperatures was 1.7 °C in winter and 1.3 °C in summer. Note that in both winter and summer, the difference between the nocturnal and diurnal modal body temperatures was within the traditionally defined limits of homeothermy (i.e. < 2 °C, Cabanac and Simon 1987). The range of body temperatures recorded in winter was however ~ 1.5 °C greater than in summer, due to the pronounced tail in lower body temperatures experienced during winter compared to summer (Fig. 5.6).

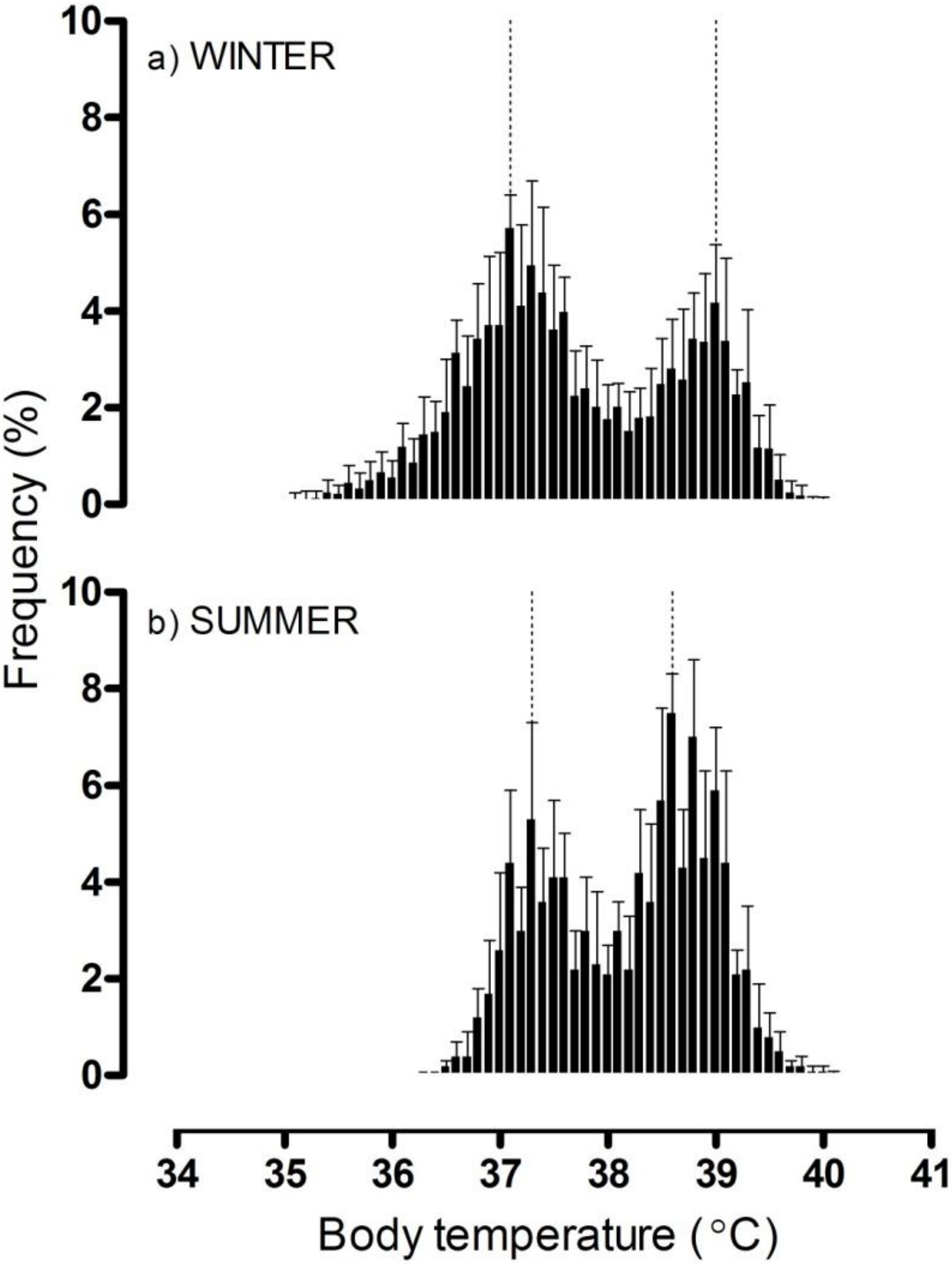

Figure 5.6: Mean (+ SD) frequency distribution of observed body temperatures, divided in to 0.1 °C categories, over the 24-h period for nine monkeys in winter (*panel a,* n = 92 days) and six monkeys in summer (*panel b,* n = 91 days). Vertical dotted lines indicate lower and upper modal body temperatures. SD is the variability between individual monkeys.

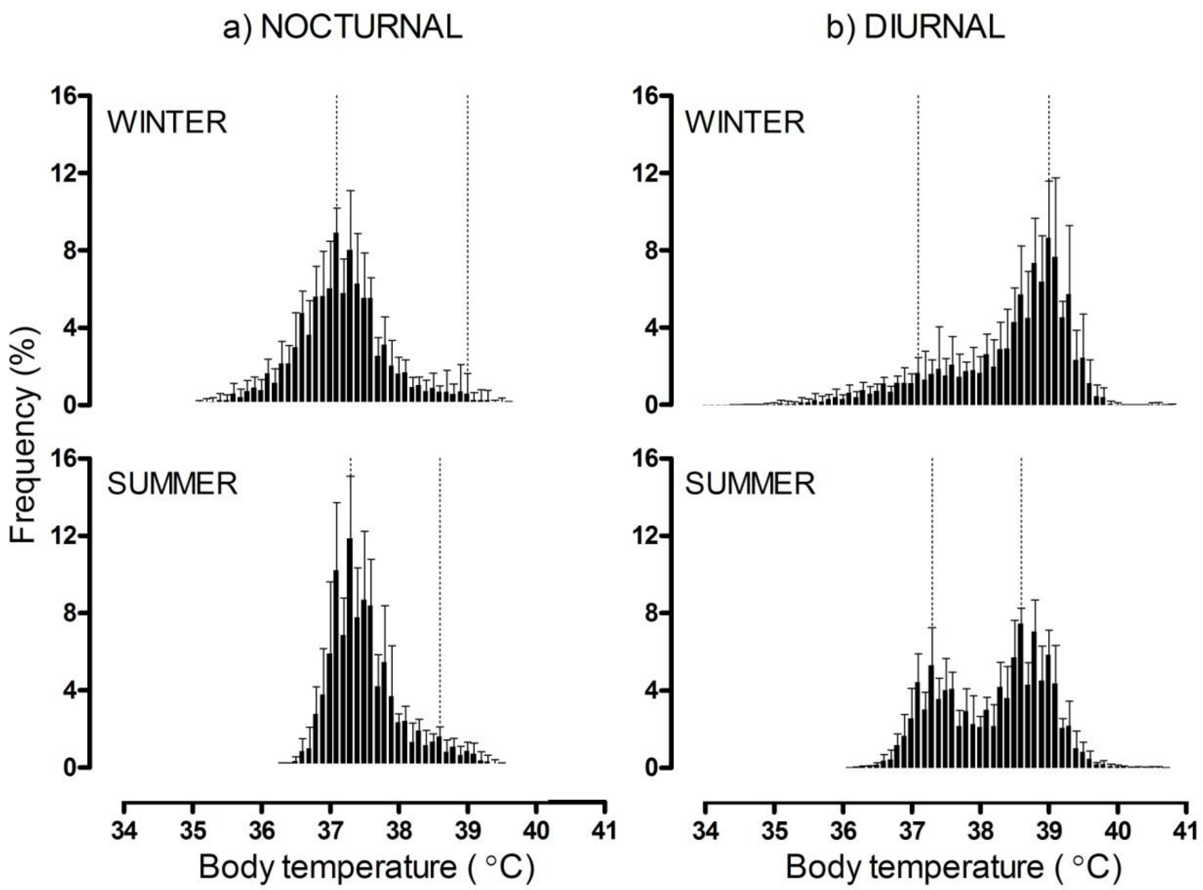

Fig. 5.7: Mean (+ SD) frequency distribution of observed body temperatures, divided in to 0.1 °C categories, over the nocturnal (*panel a*) and diurnal (*panel b*) periods for nine monkeys in winter (*upper panels,* n = 92 days) and six monkeys in summer (*lower panels,* n = 91 days). Vertical dotted lines indicate lower and upper modal body temperatures. SD is the variability between individual monkeys.

5.3.3 Influence of environmental factors on parameters of the body temperature rhythm

Across the duration of my study period, 24-h minimum body temperature increased with increasing 24-h minimum black-globe temperature, photoperiod, and the sum of rainfall over the two previous months as a proxy for food availability (positive ß, Table 5.2). 24-h Maximum body temperature increased with 24-h maximum black-globe temperature and the sum of rainfall over the two previous months, but was unrelated to photoperiod (Table 5.2). The finding that there was no significant relationship between 24-h maximum body temperature and photoperiod supports the finding that there was no significant difference in mean 24-h maximum body temperature between seasons (Table 5.1), as photoperiod is a highly seasonal parameter. 24-h Mean body temperature increased with 24-h mean black-globe temperature, photoperiod, and the sum of rainfall over the two previous months. In contrast to the above measures of body temperature, the 24-h amplitude of the body temperature rhythm significantly decreased with increasing photoperiod and the sum of rainfall over the two previous months, photoperiod being the strongest predictor of variation in the 24-h amplitude of the body temperature rhythm (Table 5.2; highest Z value). Similar to other parameters of body temperature, the 24-h amplitude of the body temperature rhythm also “tracked” changes in environmental temperature, significantly increasing with increasing 24-h amplitude of black-globe temperature across the duration of my study period. These analyses have revealed that environmental temperature, rainfall (a proxy for food availability), and photoperiod were all important determinants of the body temperature rhythm for vervet monkeys.

In winter the mean absolute change in mean 24-h amplitude of the body temperature rhythm between consecutive days was 0.6 ± 0.1 °C, compared to the mean absolute change of 0.4 ± 0.1 °C between consecutive days in summer. For six monkeys with data in both seasons the mean change in 24-h amplitude of the body temperature rhythm between consecutive days was significantly greater in winter than in summer ($t = 6.28$, $n = 6$, $p < 0.01$). These findings indicate that in winter (Fig. 5.8a), the 24-h amplitude of the body temperature rhythm was highly labile between consecutive days compared to summer (Fig. 5.8b), when variation in the 24-h amplitude of the body temperature rhythm was relatively stable over the duration of the season.

Table 5.2: Result of linear mixed effect model investigating the influence of black-globe temperature (BGT), photoperiod, and the sum of rainfall over the two previous months on 24-h minimum, 24-h maximum, 24-h mean, and 24-h amplitude of the body temperature (Tb) rhythm over the duration of the study period for nine monkeys (n = 2358). Sex, mass, and troop were included as control variables, and individual monkeys were included as a random factor.

Dependent variable		**ß**	**±SE**	**Z**	**p**	**95% CI's**		
Minimum Tb	***Independent***							
	BGT$_{(min)}$ (°C)	0.0457	0.0022	20.37	**<0.0001**	0.0413	to	0.0500
	Photoperiod (h)	0.0979	0.0091	10.77	**<0.0001**	0.001	to	0.1158
	Rainfall (mm)	0.0009	0.0002	3.95	**<0.0001**	0.0005	to	0.0014
	Control							
	Sex	-0.2134	0.1030	-2.07	**0.04**	-0.4152	to	-0.0116
	Mass (kg)	0.1290	0.0368	3.51	**<0.0001**	0.0570	to	0.2011
	Troop	0.1308	0.0660	1.98	0.05	0.0015	to	0.2601
	Random factor							
	Individual	0.0667	0.0241			0.0328	to	0.1356
Maximum Tb	***Independent***							
	BGT$_{(max)}$ (°C)	0.0104	0.0010	10.01	**<0.0001**	0.0084	to	0.0125
	Rainfall (mm)	0.0004	0.0001	2.76	**<0.01**	0.0001	to	0.0007
	Photoperiod (h)	-0.0121	0.0067	-1.80	0.07	-0.0253	to	0.0010
	Control							
	Sex	-0.1584	0.1932	-0.82	0.41	-0.5371	to	0.2203
	Mass (kg)	0.0550	0.0690	0.80	0.43	-0.0803	to	0.1902
	Troop	0.1601	0.1241	1.29	0.20	-0.0831	to	0.4034
	Random factor							
	Individual	0.1340	0.0432			0.0712	to	0.2522
Mean Tb	***Independent***							
	BGT$_{(avg)}$ (°C)	0.0226	0.0012	18.94	**<0.0001**	0.0203	to	0.0249
	Photoperiod (h)	0.0569	0.0058	9.83	**<0.0001**	0.0456	to	0.0683
	Rainfall (mm)	0.0006	0.0001	4.61	**<0.0001**	0.0003	to	0.0009
	Control							
	Sex	-0.1580	0.0776	-2.04	**0.04**	-0.3100	to	-0.0060
	Mass (kg)	0.0814	0.0277	2.94	**<0.01**	0.0271	to	0.1357
	Troop	0.0826	0.0498	1.66	0.10	-0.0149	to	0.1801
	Random factor							
	Individual	0.0521	0.0180			0.0265	to	0.1023
Amplitude Tb	***Independent***							
	Photoperiod (h)	-0.2191	0.0085	-25.83	**<0.0001**	-0.2358	to	-0.2025
	BGT$_{(amp)}$ (°C)	0.0174	0.0016	10.80	**<0.0001**	0.0142	to	0.0205
	Rainfall (mm)	-0.0020	0.0003	-7.71	**<0.0001**	-0.0026	to	-0.0015
	Control							
	Sex	0.0662	0.2616	0.25	0.80	-0.4465	to	0.5790
	Mass (kg)	-0.0756	0.0934	-0.81	0.42	-0.2587	to	0.1076
	Troop	0.0268	0.1680	0.16	0.87	-0.3025	to	0.3561
	Random factor							
	Individual	0.1804	0.0588			0.0953	to	0.3416

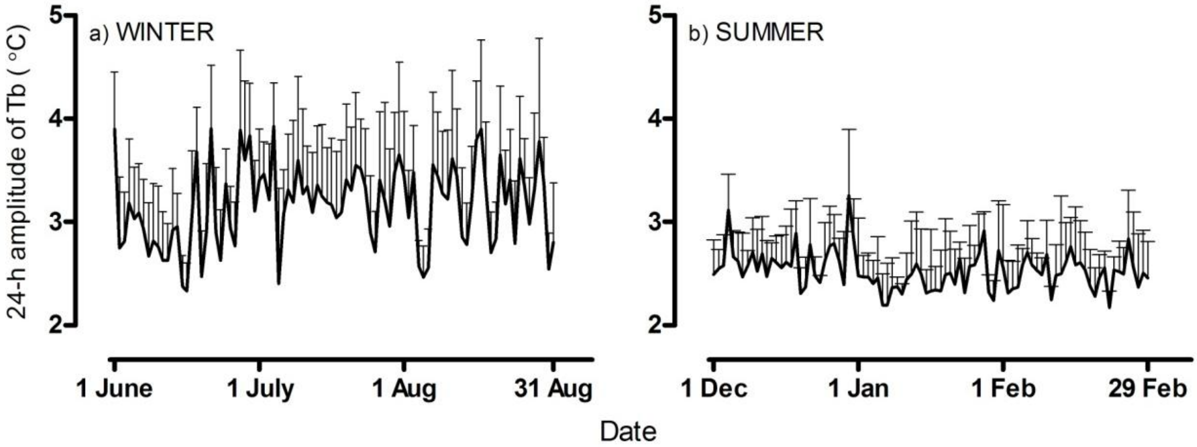

Figure 5.8: Absolute change in 24-h amplitude (+SD) of the body temperature rhythm across consecutive days for nine monkeys in winter (*panel a*) and six monkeys in summer (*panel b*). SD is the variability between individual monkeys.

In winter (Fig. 5.9, *upper panels*), the 24-h amplitude of the body temperature rhythm was directly related to corresponding measures of 24-h minimum (Fig. 5.9a), 24-h maximum (Fig. 5.9b), 24-h mean (Fig. 5.9c), and 24-h amplitude (Fig. 5.9d) of black-globe temperature. In winter, the 24-h amplitude of the body temperature rhythm decreased with increasing 24-h minimum (r^2 = 0.58, n = 92, P < 0.0001) and 24-h mean (r^2 = 0.11, n = 92, P < 0.01) black-globe temperature and increased with increasing 24-h maximum (r^2 = 0.05, n = 92, P = 0.03) and 24-h amplitude (r^2 = 0.35, n = 92, P < 0.0001) of black-globe temperature. 24-h Minimum black-globe temperature was the strongest predictor of variation in the 24-h amplitude of the body temperature rhythm in winter, explaining 58 % of the variation observed in 24-h amplitude of the body temperature rhythm. In summer (Fig. 5.9, *lower panels*), 24-h minimum black-globe temperature (Fig. 5.9a) explained only 11 % of the variation in 24-h amplitude of the body temperature rhythm (r^2 = 0.11, n = 91, P < 0.01), and was the only measure of environmental temperature to significantly influence the 24-h amplitude of the body temperature rhythm. These findings indicate that in winter, the 24-h amplitude of the body temperature rhythm (a measure of thermoregulatory precision) of vervet monkeys was highly labile between days, and that thermoregulatory precision was significantly influenced by environmental temperature. Whereas in summer thermoregulatory precision of vervet monkeys remained relatively stable and were largely independent of environmental temeprature.

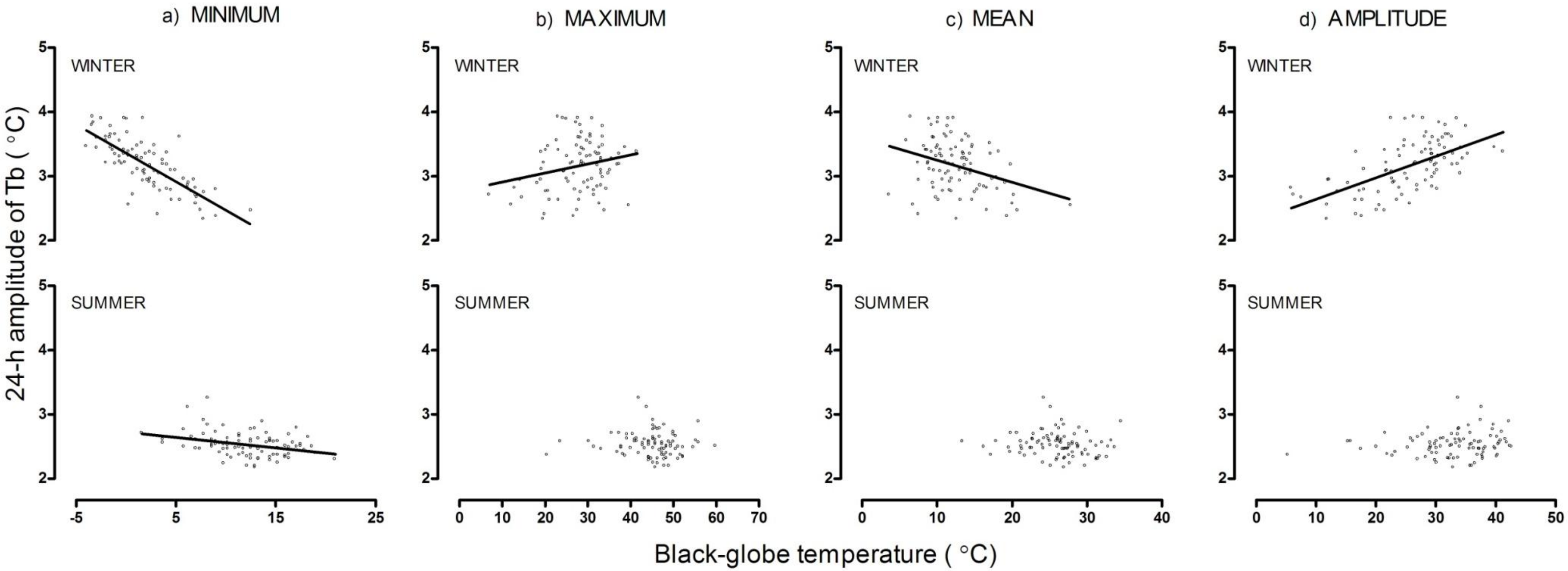

Figure 5.9: Mean 24-h Amplitude of the body temperature (Tb) rhythm in relation to the corresponding 24-h minimum (*panel a,* winter: y = -0.09 (x) + 3.36, summer: y = 0.02 (x) + 2.73), 24-h maximum (*panel b,* winter: y = 0.01 (x) + 2.77), 24-h mean (*panel c,* y = -0.03 (x) + 3.59), and 24-h amplitude (*panel d,* winter: y = 0.03 (x) + 2.31) of black-globe temperature for nine monkeys in winter (*upper panels*) and six monkeys in summer (*lower panels*).

In winter, the change in body temperature over the early-morning re-warming period between 08:00 and 10:00, as identified by segmented regression analysis (Fig. 5.1), increased significantly with increasing availability of direct sunlight and corresponding change in black-globe temperature (Table 5.3; positive ß value). The availability of direct sunlight, as indexed by the difference between mean black-globe and mean black-bulb temperature between 08:00 and 10:00, was the strongest predictor of the change in body temperature during the re-warming phase in winter (Table 5.3; highest Z value). There was no relationship between the change in body temperature and the percentage of time spent active over the re-warming period, indicating that passive heat gain from the environment rather than increased physical effort was employed to facilitate early-morning re-warming in winter.

Table 5.3: Result of linear mixed effect model investigating the influence of the availability of direct sunlight (mean black-globe temperature, BGT – mean dry-bulb temperature, DBT), the change in black-globe temperature, and the percentage of time spent active on the change in body temperature during the early-morning re-warming phase (08:00 to 10:00) for six monkeys in winter (n = 546). The sum of rainfall over the two previous months and photoperiod were included as predictor variables. Sex, mass, and troop were included as control variables, and individual monkeys were included as random factors.

	ß	**±SE**	**Z**	**p**	**95 % CI's**		
Independent							
BGT-DBT (°C)	0.103	0.016	6.59	**<0.0001**	0.073	to	0.134
Change in BGT (°C)	0.038	0.007	5.73	**<0.0001**	0.025	to	0.051
Rainfall (mm)	0.006	0.003	1.91	0.06	-0.001	to	0.012
Activity (%)	-0.003	0.002	-1.66	0.10	-0.007	to	0.001
Photoperiod (h)	-0.297	0.184	-1.61	0.11	-0.659	to	0.064
Control							
Sex	-0.089	0.515	-0.17	0.86	-1.098	to	0.920
Mass (kg)	-0.046	0.200	-0.23	0.82	-0.438	to	0.346
Troop	0.144	0.379	0.38	0.71	-0.599	to	0.887
Random Factor							
Individual	0.671	0.021			0.632	to	0.712

5.4 Discussion

My study has provided the first continuous measurements of 24-h body temperature for any Cercopithecine primate. Despite black-globe temperatures ranging from below freezing to > 50 °C over the duration of my study period, with mean 24-h variation in black-globe temperature of 30.0 °C, the mean 24-h amplitude of body temperature rhythm exhibited by individual vervet monkeys for the duration of my study was 2.9 ± 0.1 °C, more or less within the traditionally defined limits of homeothermy (± 2 °C, Cabanac and Simon 1987). However, vervet monkeys did display significant seasonal variations in body temperature rhythm. The 24-h amplitude of the body temperature rhythm decreased significantly from mid-winter to mid-summer, by ~ 0.8 °C. 24-h Variations in body temperature of more than twice the traditionally defined limit of homeothermy were frequently recorded for individual monkeys in winter and the highest recorded 24-h amplitude of the body temperature rhythm was 5.6 °C. Effective thermoregulation in summer, even at environmental temperatures well above the approximate TNZ of primates, was likely maintained by behavioural thermoregulation (shade-seeking) and evaporative cooling, facilitated by *ad libitum* access to a natural source of water. In winter, however, the ability of vervet monkeys to maintain homeothermy was compromised, when low environmental temperatures were associated with a hypothermia induced increase in the 24-h amplitude of the body temperature rhythm. Furthermore, evidence suggests that the energy deficit imposed by low food availability compromised the thermoregulatory capacity of vervet monkeys resulting in less precise (i.e. increased 24-h amplitude of the body temperature rhythm) thermoregulation. Evidence suggests that reduced thermoregulatory precision reflects a physiological response to environmental stress, which corroborates my findings of Chapter 4, that low environmental temperature in winter, and periods of low food availability over the duration of my study period, imposed potentially severe energetic challenges for vervet monkeys at Samara. The next sections discuss the proximal influence of environmental stressors on the expression of the body temperature rhythm of vervet monkeys, and the potential energetic implications of thermoregulatory plasticity in response to environmental stochasticity.

5.4.1 Nychthemeral rhythm of body temperature

In both winter and summer, the nycthemeral rhythm of body temperature was closely associated with the light:dark cycle. In both seasons the periods of rapid increase and decrease between the nocturnal and diurnal means of body temperature more or less coincided with the time of sunrise and sunset (Fig. 5.1). The finding that the change in body temperature was significantly correlated with the corresponding change in activity between the start of the initial early-morning increase in body temperature and the end of the late-afternoon decrease in body temperature (Fig. 5.2) is in line with previous research which has shown body temperature to correlate positively with activity levels (Pereira *et al.* 2002). The association between body temperature and activity may be explained by the energy released during ATP hydroxylation to power muscle contractions during exercise, which is lost as heat (Glanville and Seebacher 2010). My data therefore suggest that increasing activity facilitates an increase in body temperature. As the nycthemeral rhythms of body temperature and activity were closely associated with each other, and both tightly coupled to the light:dark cycle, photoperiod appears to be an important zeitgeber of the nychthemeral rhythm of body temperature and activity for vervet monkeys.

In winter, however, the start of the early-morning increase in body temperature was more closely associated with the time at which access to direct sunlight became available in the valley (reflected by the disassociation between black-globe and dry-bulb temperature, see Chapter 3: Fig. 3.4), rather than the time of sunrise. Whereas in summer, when early-morning environmental temperatures were ~ 10 °C milder than in winter, the initial increase in body temperature started one to two hours prior to sunrise. These findings suggest that photoperiod alone is not the only determinant of the nycthemeral rhythm of body temperature for vervet monkeys. The delayed increase in body temperature relative to the time of sunrise in winter is likely due to the importance of passive radiant-heat assisted re-warming for increasing body temperature during the early-morning re-warming period (Table 5.3). These results suggest that milder early-morning environmental temperatures in summer facilitated an increase in activity and body temperature prior to sunrise, and conversely the relatively low early-morning environmental temperatures, and subsequent dependence on the availability of direct sunlight to increase body temperature, inhibited an increase in activity and body temperature prior to sunrise in winter. In this manner both photoperiod and environmental temperature were important zeitgebers of the nycthemeral rhythm of body temperature and activity for vervet monkeys.

In both seasons, mean nocturnal (i.e. inactive phase) body temperature was significantly lower than mean diurnal (i.e. active phase) body temperature (Table 5.1). The difference between nocturnal and diurnal body temperatures was also reflected in the bimodal distribution of body temperature for vervet monkeys, with the lower and upper modal body temperatures being significantly different and occurring during the nocturnal and diurnal periods respectively (Fig. 5.6, 5.7). Amongst small mammals bimodal distributions of body temperature are common (Boyles *et al.* 2011b), whereas in contrast, some large mammals have been shown to display unimodal distributions of body temperature (Hetem *et al.* in preparation). Based on the assumption that body temperature is adaptive, the most commonly experienced body temperature (i.e. modal body temperature) is proposed to reflect the “preferred” body temperature (i.e. the body temperature at which the system tends to stabilize, Boyles *et al.* 2011b). Preferred body temperature may therefore also be referred to as the set-point of body temperature (defined as “the value of a regulated variable which a healthy organism tends to stabilize by the process of regulation”, IUPS Thermal Commission 2001). The set-point of body temperature is not a static parameter, but rather an adjustable reference value (Refinetti and Menaker 1992). Whether the circadian rhythm of body temperature is an artefact of the adjustment of the set-point of body temperature over the 24-h cycle (i.e. different nocturnal and diurnal set-points), or a result of heat gain and loss from a single set-point value is unclear (Refinetti and Menaker 1992). For small mammals, which have relatively high thermal conductance compared to large mammals (Aschoff 1981), defending body temperature at two distinct set-points during the cold (i.e. nocturnal) and warm (i.e. diurnal) phases of the diel cycle respectively may have evolved to minimize the energetic requirements of maintaining body temperature by reducing the gradient between body and environmental temperature.

The most notable difference between the nychthemeral rhythms of body temperature for vervet monkeys in winter compared to summer was the extent of individual variability over the period ~ four hours prior to sunrise and over the afternoon in winter compared to the relatively small and stable variation in mean body temperature over the 24-h period between individual monkeys in summer (Fig. 5.1). This finding suggests two causal relationships; external environmental factors which varied between winter and summer, and the physical properties of individual monkeys, were important determinants of the expression of the 24-h body temperature rhythm.

5.4.2 Influence of environmental factors on parameters of the body temperature rhythm

For vervet monkeys at Samara, the 24-h amplitude of the body temperature rhythm significantly decreased between mid-winter and mid-summer (Fig. 5.4), driven primarily by depressed 24-h minimum body temperatures (i.e. hypothermia) in winter (Fig. 5.5, and Table 5.1). Maloney and colleagues (2011) also observed hypothermia induced imprecise thermoregulation for western grey kangaroos in Australia, with 24-h minimum body temperature also exhibiting a seasonal progression. The authors concluded that the reduction in 24-h minimum body temperature in the dry season was best explained as a seasonally entrained thermoregulatory adjustment, potentially in response to seasonal fluctuations in food availability, rather than a proximal response to fluctuations in environmental temperature (Maloney *et al.* 2011). For springbok in South Africa inhabiting a similar climatic environment to vervet monkeys at Samara, Fuller and colleagues (2005) reported a similar increase in mean 24-h body temperature with increasing photoperiod as observed for vervet monkeys (Table 5.2). The authors proposed that in a highly seasonal environment, where the generally dry winter months are associated with poor food availability or quality, a lower body temperature in winter compared to summer may be related to energy availability, whereby body temperature is correlated with season *via* the influence of season on food availability (Fuller *et al.* 2005). For both kangaroos and springbok, body temperature was unrelated to environmental temperature (Fuller *et al.* 2005, Maloney *et al.* 2011). Maloney and colleagues (2011) propose the theory that seasonal adjustments of the body temperature rhythm may be an endogenous circannual pattern of body temperature variability which has evolved to optimize energy expenditure in response to the “normal” seasonal fluctuations in environmental conditions to which the kangaroos were exposed. My data provides strong evidence for the seasonal entrainment of thermoregulatory mechanisms, as the 24-h amplitude of the body temperature rhythm was linearly correlated with days between mid-winter and mid-summer (Fig. 5.4), and photoperiod was the strongest predictor of the 24-h amplitude of the body temperature rhythm over the duration of my study period (Table 5.2). This finding suggests that photoperiod may itself be an entrained zeitgeber for the adjustment of the thermoregulatory system. By experimentally manipulating photoperiod length, Haim and Levi (1990), for example, were able to induce changes in the body temperature rhythms of the fat jird (*Meriones crassus*) in an apparent adjustment to the differing metabolic demands of winter and summer in the jird's natural habitat. I however propose that for vervet monkeys at Samara, an entrained seasonal adjustment in thermoregulatory strategies alone is insufficient to explain the variation observed in the body temperature rhythm of vervet monkeys (see below).

5.4.2.1 The proximal influence of environmental stressors on vervet monkey thermoregulation

For small mammals the primary cost of thermoregulation is imposed by the gradient between body and environmental temperature (McNab 1983, Angilletta *et al.* 2010). If vervet monkeys were obligatory homeotherms, any increased cost of thermoregulation should have been off-set by increased energy expenditure to maintain body temperature, and therefore body temperature would be maintained independently of fluctuations in environmental temperature. However, the cost-benefit theory of thermoregulation (Angilletta *et al.* 2010) predicts that the energetically optimal thermoregulatory mechanism will be to minimize the gradient between body and environmental temperature, thereby employing a more heterothermic (i.e. less precise) thermoregulatory mechanism as thermal stress increases. Indeed, across the duration of my study period, 24-h minimum, 24-h maximum, 24-h mean, and 24-h amplitude of the body temperature rhythm increased with corresponding measures of black-globe temperature (Table 5.2), indicating that vervet monkeys employed a labile thermoregulatory mechanism in response to fluctuations in proximal environmental temperature.

Vervet monkeys were subjected to vastly different thermal stressors in winter compared to summer (see Chapter 3: Table 3.2). The 24-h amplitude of the body temperature rhythm of vervet monkeys was significantly higher (Table 5.1) and more variable between consecutive days in winter compared to summer (Fig. 5.8), indicating that in winter monkeys employed a more labile and less precise thermoregulatory mechanism than in summer. The highest 24-h amplitude of the body temperature rhythm recorded for any monkey in summer was 4.0 °C, compared to in winter when the 24-h amplitude of the body temperature rhythm for individual monkeys exceeded 4 °C on 12.3 ± 10.9 % of days. Vervet monkeys therefore displayed distinct thermoregulatory patterns in winter compared to summer, being able to effectively maintain homeothermy in summer, whereas they employed less precise thermoregulation in winter. To determine whether proximal thermal stress influenced the thermoregulatory capacity of vervet monkeys, I investigated the relationship between the expression of the body temperature rhythm and black-globe temperature in winter and summer respectively.

At high environmental temperatures in summer, vervet monkeys would have to overcome the challenge of dissipating body heat at environmental temperatures above their TNZ in order to maintain homeothermy. The primary avenue of heat dissipation at high environmental temperatures is evaporative cooling. For rock hyrax' (*Procavia capensis*), a species of similar body mass to vervet monkeys (~ 3 kg), inhabiting a region of South Africa with similar seasonal fluctuations in environmental temperature to that of Samara, the 24-h amplitude of the body temperature rhythm was significantly higher in summer (~ 6.0 °C) than in winter (~ 4.5 °C; Brown and Downs 2006). The authors hypothesize that the thermoregulatory mechanism of rock hyrax's was beneficial in terms of body water conservation in summer, when body temperatures reached hyperthermic levels, presumably delaying the activation of body water expensive autonomic cooling mechanisms (Brown and Downs 2006). In contrast, vervet monkeys at Samara were able to thermoregulate effectively in summer, the mean 24-h amplitude of the body temperature rhythm for all monkeys being 2.5 ± 0.1 °C. In both artificial (Mitchell *et al.* 2009) and natural (Brain and Mitchell 1999) conditions, baboons have been shown to effectively maintain homeothermy at high environmental temperatures when access to water was available, in contrast to when access to water was denied. Similarly, the 24-h amplitude of the body temperature rhythm for vervet monkeys was largely independent of environmental temperature in summer (Fig. 5.9), when monkeys had access to a natural source of water. It is therefore likely that for vervet monkeys at Samara effective thermoregulation in summer was facilitated by a combination of behavioral thermoregulation, whereby monkeys spent the hottest part of the day resting in the shade (see Chapter 4: section 4.4), and unperturbed access to free-standing water. Summer was also characterized by high food availability, as indexed by the sum of rainfall over the two previous months, and these environmental conditions (*ad libitum* access to water and high food availability) may therefore be representative of "good" environmental conditions, under which vervet monkeys were able to allocate sufficient resources towards thermoregulation and maintain the 24-h amplitude of body temperature within a narrow and stable range.

However, in winter, variation in thermoregulatory precision was significantly related to environmental temperature, with 24-h minimum black-globe temperature accounting for 58 % of the variation in the 24-h amplitude of the body temperature rhythm. At low environmental temperatures, due to their relatively high thermal conductance and low thermal inertia, small mammals are subjected to severe energetic challenges. The energetic requirements of maintaining homeothermy at low environmental temperatures may become prohibitively high, and under these conditions (often in conjunction with low food availability) many small mammals enter a state of torpor to conserve energy (Geiser 2004). Torpor is characterized by a temporary, but substantial reduction in body temperature resulting in an increase in the 24-h amplitude of the body temperature rhythm (i.e. heterothermy). The "cut-off" body temperature considered to represent a state of torpor is a reduction of body temperature below 35 (Willis 2007) to 30 °C (Barclay *et al.* 2001). By reducing body temperature, and consequently reducing the gradient between body and environmental temperature, the metabolic energy requirements for endogenous heat production are significantly reduced (Geiser 2004). My data indicate that increased 24-h amplitude of the body temperature rhythm in vervet monkeys was driven primarily by nocturnal (i.e. inactive phase) hypothermia (i.e. reduced 24-h minimum body temperature, Fig. 5.5). Although the degree of the reduction in body temperature is much reduced, the thermoregulatory plasticity observed for vervet monkeys in winter may be considered an attenuated version of the physiological mechanism employed during torpor. Mean nocturnal body temperature for vervet monkeys in winter was 37.2 ± 0.2 °C, which is above the proposed upper cut-off body temperature for torpor of 35 °C (Willis *et al.* 2007). Body size plays an important role in thermoregulation due the relationship between thermal conductance and thermal inertia and body size (Aschoff 1981). The majority of studies on body temperature in free-ranging mammals have been done for small (< 1 kg) heterothermic (i.e. torpid) species or large (> 10 kg) antelopes. Vervet monkeys therefore represent a size class (mean mass in my study = 4.6 ± 1.4 kg) for which few studies have recorded 24-h body temperature rhythms. Compared with other "small" (< 10 kg) free-ranging mammals which did not employ torpor for which 24-h body temperature rhythms were recorded in winter, also in South Africa, the mean inactive phase body temperature of vervet monkeys was similar to those reported for southern lesser galagos (36.8 ± 0.7 °C, ~ 0.2 kg; Mzilikasi *et al.* 2006), Cape ground squirrels (*Xerus inauris,* 36.3 ± 0.3, ~ 0.8 kg; Wilson *et al.* 2010), and rock hyrax' (36.8 ± 0.1, ~ 3.0 kg; Brown and Downs 2006). Although vervet monkey body temperatures did frequently drop below 35 °C in winter (mean 24-h minimum body temperature = 34.6 ± 0.5 °C), the nocturnal modal body temperature of 37.1 °C suggests that these lower (< 35 °C) body temperatures were not defended and may therefore reflect periods when monkeys "lost" thermoregulatory capacity (i.e. were unable to maintain body temperature at the preferred level). Moreover, the modal time of 24-h minimum body temperature in winter was 08:00 (Fig. 5.3), which coincides with the time at which access to direct sunlight became available. On cold winter mornings, monkeys typically remained inactive in their sleeping huddles until access to direct sunlight became available, at which time they moved to the top of trees to sunbask (personal observation). When monkeys are clustered in sleeping huddles they are effectively reducing their thermal conductance by limiting the body surface area available for heat transfer with the environment. The association between the time of modal 24-h minimum body temperature and the time at which monkeys move from their sleeping huddles may therefore be due to the sudden increase in thermal conductance caused by moving away from their sleeping huddles.

Various authors propose the theory that thermoregulatory imprecision, whereby the 24-h amplitude of the body temperature rhythm is increased to reduce the gradient between body and environmental temperature, can be explained as an adaptive mechanism to reduce the energetic requirements of thermoregulation (Angilletta *et al.* 2010, Glanville and Seebacher 2010, Boyles *et al.* 2011a). If reducing body temperature to decrease the gradient between body and environmental temperature does infer a significant energetic benefit for vervet monkeys, a pertinent question is why not reduce body temperature further (i.e. employ torpor)? Maintaining the defence of body temperature, although at a lower level, may be explained by the trade-off between energy conservation (achieved by reducing the gradient between body and environmental temperature) and performance. In endothermic species, high body temperatures are associated with increased performance at the cellular and whole organism level (Heinrich 1977, Somero 2004, Knies *et al.* 2009). For the southern lesser galago, for example, the absence of torpor was proposed to be as a consequence of the mating season coinciding with the period of low environmental temperatures and food availability (i.e. winter), under which conditions the galagos were expected to employ torpor to conserve energy. In line with the cost-benefit theory of thermoregulation, the authors conclude that the ecological costs of torpor outweighed the potential energetic savings, and therefore torpor was inhibited to increase fecundity (Mzilikasi *et al.* 2006). At Samara, short day lengths, low environmental temperatures, and the coinciding mating season in winter imposes a significant constraint on the activity budget of vervet monkeys (see Chapter 4: section 4.4). The imprecise thermoregulatory mechanism employed by vervet monkeys in winter therefore supports my theory that vervet monkeys may be forced to trade-off homeothermy in order to complete other fundamental (i.e. energetic, social, and biological) activities.

In addition to thermal stress, food availability may also effect the energetic balance of vervet monkeys and therefore influence thermoregulatory capacity. The cost-benefit theory of thermoregulation proposed by Angilletta *et al.* (2010) predicts that employing a less precise thermoregulatory strategy during periods of low food availability may significantly reduce the energetic costs of thermoregulation thus conserving energy during a time of low energy intake. In line with this theory, the 24-h amplitude of the body temperature rhythm decreased with increasing food availability (as indexed by the sum of rainfall over the two previous months), indicating that vervet monkeys thermoregulated more precisely when food availability was high. The finding that the sum of rainfall over the two previous months, and therefore food availability, was not correlated with other climatic factors which vary seasonally (i.e. environmental temperature and photoperiod, see Chapter 3: Fig. 3.3), suggests that the observed thermoregulatory response to proximal food availability was independent of any endogenous seasonal adjustment. This finding, in conjunction with the finding that thermoregulatory precision varied significantly in winter in response to environmental temperature, but was relatively stable and independent of environmental temperature in summer, indicates that the thermoregulatory mechanism employed by vervet monkeys is a labile parameter in response to proximal environmental stressors, rather than an entrained seasonal adjustment. The observed thermoregulatory plasticity in response to proximal environmental stressors suggest that maintaining strict homeothermy may be a luxury rather than predefined norm. When environmental conditions were “good”, monkeys were able to allocate sufficient resources towards maintaining homeothermy, which is presumably beneficial in terms of optimizing performance. In contrast, when environmental conditions were “bad”, vervet monkeys employed less precise thermoregulation, possibly in a trade-off to conserve energy or prioritize energy allocation for other biologically important activities such as reproduction.

5.4.3 Thermoregulatory plasticity

My data suggests that vervet monkeys may be able to reduce the energy requirements of thermoregulation at low environmental temperatures by reducing body temperature (i.e. hypothermia), thus reducing the gradient between body and environmental temperature. Reducing body temperature however also creates the problem of having to subsequently increase body temperature from these depressed levels to achieve “optimal” active phase body temperature. One of the primary energetic costs associated with torpor and hibernation has been shown to be the metabolic requirements of arousal, whereby hypothermic torpid body temperature must be increased to normothermic levels (Geiser and Drury 2003). The lowest 24-h minimum body temperature recorded for an individual monkey was 34.2 °C, which is 2.9 °C below the lower modal body temperature for vervet monkeys, and similarly the energetic requirements to increase body temperature from such depressed levels may infer a significant energetic constraint on vervet monkeys. One way in which vervet monkeys may overcome the energy deficit imposed by depressed 24-h minimum body temperatures is by the active utilization of exogenous heat for thermoregulation by means of sunbasking. Sunbasking has been shown to significantly reduce endogenous metabolic heat production requirements in small heterothermic mammals when arousing from torpor (Lovegrove *et al.* 1999, Geiser and Drury 2003), and

Singer and colleagues (2011) demonstrated for the first time the central role of radiant-heat assisted re-warming from depressed overnight minimum body temperatures in a large ungulate, the Alpine ibex. For vervet monkeys, the strongest predictor of the increase in body temperature during the initial early-morning re-warming phase in winter was the availability of direct sunlight, followed by the corresponding change in environmental temperature, indicating that radiant-heat gain from the environment may be an important behavioural mechanism for overcoming the potential energetic deficit imposed by nocturnal hypothermia. By the combination of nocturnal hypothermia at low environmental temperatures, and subsequent utilization of radiant-heat assisted re-warming, vervet monkeys may be able to significantly reduce the energetic requirements of thermoregulation at low environmental temperatures.

The theory that reducing body temperature in response to low environmental temperature serves to conserve energy by reducing the gradient between body and environmental temperature is, however, dependent on the reduction in body temperature being a regulated adjustment in the set-point at which body temperature is defended. If inactive phase hypothermia is a regulated adjustment, as is postulated for torpor, then thermoregulatory energy requirements would be reduced due to the reduced gradient between body and environmental temperature, and metabolic rate would be reduced due to the Q_{10} effect associated with the reduced body temperature. If however, inactive phase hypothermia at low environmental temperatures was a consequence of an inability to thermoregulate (i.e. an animal is attempting to defend body temperature, therefore presumably operating at maximal cold induced metabolic rate, but the level of heat production is insufficient to balance heat loss), the energetic implications would be very different (Maloney *et al.* in preparation). The mechanism of hypothermia is therefore an important consideration for the interpretation of the ecological significance of the altered physiology (Maloney *et al.* in preparation).

My finding that there was no significant difference in the modal nocturnal body temperature between winter and summer for vervet monkeys suggests that there was no regulated downward adjustment in the preferred, or set-point, at which body temperature was defended in winter. The significantly lower nocturnal mean body temperature observed in winter compared to summer may be an artifact of the depressed minimum body temperatures experienced during the nocturnal period (illustrated by the wider range of body temperatures recorded during the nocturnal period in winter compared to summer, Fig. 5.7), and therefore not a true reflection of an adjustment in the thermoregulatory system. Supporting the theory that the observed low body temperatures (i.e. below lower modal body temperature) experienced by monkeys in my study was a consequence of a loss of thermoregulatory capacity, is the finding that 24-h minimum body temperature significantly increased with mass of individual monkeys over the duration of my study period (Table 5.1), indicating that larger monkeys were able to maintain 24-h minimum body temperature at higher levels than were smaller monkeys. This finding may be explained by the relationship between thermal conductance and body size, whereby smaller monkeys would require increased energetic inputs to maintain body temperature compared to larger monkeys, and may suggest that the observed hypothermia in winter was a consequence of loss of thermoregulatory capacity at low environmental temperatures. Body mass/size may therefore be the physical property explaining the significant variation in the expression of the 24-h body temperature rhythm between individual monkeys in winter (Fig. 5.1).

Determining the energetic implications of imprecise thermoregulation is beyond the scope of my study. Whether nocturnal hypothermia is a regulated adjustment of the thermoregulatory system to conserve energy at low environmental temperatures, or an indication of physiological stress (whereby monkeys are “unable” to maintain homeothermy), will however have significant implications for the interpretation of the energetic and ecological consequences of thermoregulatory plasticity in response to environmental stressors.

5.4.4 Conclusion

My data have shown that the thermoregulatory mechanism employed by vervet monkeys is a highly labile parameter, which fluctuates in response to proximal environmental stressors.

The seasonal comparison of thermoregulatory mechanisms suggests that under “good” environmental conditions (i.e. summer) vervet monkeys were able to effectively maintain homeothermy, whereas at low environmental temperatures in winter, and low food availability over the duration of my study period, vervet monkeys employed less precise thermoregulation. It can therefore be concluded that imprecise thermoregulation in vervet monkeys is a direct physiological response to proximal environmental stressors. Whether imprecise thermoregulation reflects an adaptive physiological mechanism, to conserve energy or reallocate energy for maintaining homeothermy to other biologically important activities such as reproduction, or alternatively reflects a physiological stress due to the inability to maintain homeothermy remains to be elucidated. Regardless of the thermoregulatory mechanism, my data supports my findings of Chapter 4 that the climatic conditions experienced in winter, in combination with the coinciding mating season, may impose severe energetic challenges for vervet monkeys.

CHAPTER 6

Conclusion

My study has shown that for vervet monkeys at Samara, proximal environmental stressors inferred a significant constraint on activity and thermoregulatory precision. Vervet monkeys were subjected to low environmental temperatures and short day lengths in winter, whereas in summer the primary thermoregulatory requirement was heat dissipation at high environmental temperatures. Due to the unusually high rainfall for the Nama Karoo experienced during my study period, monkeys had *ad libitum* access to free-standing water. In summer, unperturbed access to a natural source of water likely facilitated evaporative cooling when necessary, and long day lengths allowed for a flexible activity schedule whereby the warmest period of the day was spent resting in the shade. Shade-seeking behaviour at high environmental temperatures is common amongst primates (see Takemoto 2004, Hill 2006a, Kosheleff and Anderson 2009), serving to reduce thermal stress and conserve body water by reducing evaporative cooling requirements. Likely through a combination of autonomic (e.g. evaporative cooling) and behavioural (e.g. shade-seeking) mechanisms, vervet monkeys at Samara were able to effectively maintain homeothermy, largely independent of environmental temperature in summer.

Due to their strictly diurnal activity scheduling, time available to complete all fundamental activities (i.e. energetic, social, and biological) was severely compromised by short day lengths in winter. Confounding the influence of short day lengths, the thermoregulatory challenges imposed by low environmental temperatures further reduced the time available for activity. Activity was most notably inhibited on cold winter mornings, when, as observed for other vervet monkeys in a similar climatic region of South Africa to Samara (Danzy *et al.* 2012), vervet monkeys at Samara commonly employed sunbasking behaviour to passively increase body temperature. Whilst thermoregulatory challenges at low environmental temperatures promote inactive behaviours such as sunbasking and huddling, the coinciding mating season likely increased the requirement for monkeys to be active in order to maintain their social hierarchy and secure mating opportunities. These conflicting requirements likely resulted in a trade-off for monkeys between maintaining homeothermy and completing fundamental activities at a time when both thermoregulatory and social requirements were high. Indeed, the thermoregulatory precision of vervet monkeys was significantly compromised at low environmental temperatures in winter.

Furthermore, over the duration of my study, periods of reduced food availability were associated with reduced activity and decreased thermoregulatory precision. When food availability was low, the energetic balance of vervet monkeys was presumably compromised by reduced food intake, which meant that less energy was available to maintain all biological functions. Periods of low food availability were desynchronized with periods of high thermal stress, as food availability was lowest in spring. These findings indicate that reduced thermoregulatory precision may reflect the energetic state of an individual monkey.

My study period was however an anomaly, in that there was unusually high rainfall at Samara; approximately double the average annual rainfall for the region. In the future, the stress experienced as a result of the cold winters may be augmented by low food availability, which historically for this region also coincides with the dry season in winter. The general climatic trend predicted for the Karoo under future climate change scenarios is an increase in mean environmental temperatures and a decrease in rainfall (Rutherford *et al.* 1999, van Jaarsveld and Chown 2001, Hoffman *et al.* 2009). Ironically, the predicted increase in environmental temperature associated with climate change may actually be beneficial for vervet monkeys in off-setting the energetic cost of thermoregulation at low environmental temperatures in winter. An increase in early-morning and late-afternoon environmental temperatures in winter may also allow vervet monkeys to increase activity in this twilight period, thus effectively increasing the amount of time available in a day to achieve all fundamental activities. However, what is likely to be more pertinent for the survival of vervet monkeys in the Karoo is the frequency and duration of extreme weather events such as drought, heat waves, or severe cold snaps associated with climate change. Particularly coinciding extreme weather events may have severe implications for vervet monkeys inhabiting marginal habitats such as Samara. My study, for example, showed that the activity of vervet monkeys was significantly compromised on cold days which experienced heavy rainfall in winter. It has also been shown for baboons in the Namib desert that thermoregulatory precision was significantly reduced during periods of high environmental temperature coinciding with water scarcity (Brain and Mitchell 1999). Due to the high rainfall during my study I was unable to investigate the influence of high environmental temperatures in conjunction with water scarcity on the thermoregulatory precision and activity scheduling of vervet monkeys. However, it is likely that the primary determinants of the persistence of vervet monkey populations in the Karoo will be the confounding influence of environmental stressors at short day lengths, coinciding with the mating season in winter. The behavioural flexibility afforded by long day lengths in summer may allow for a greater resilience to the energetic challenges imposed by high environmental temperatures, low food availability, or water scarcity, although this is speculative without further data under these conditions. The general trends in environmental conditions predicted for this region under future climate change scenarios may therefore not necessarily be detrimental to the survival of vervet monkeys in the Karoo.

Although my study indicates that current environmental stressors in winter may impose a significant bottleneck on survival for vervet monkeys, this population shows few signs of ecological stress (e.g. reduced body weight or increased foraging effort, Pasternak *et al*. 2013). These findings suggest that vervet monkeys are relatively well adapted to coping with the “normal” seasonal variations in environmental conditions which they currently experience. The fact that vervet monkeys are one of the most widely distributed primate species in the world (Struhsaker 1967a) is testament to their adaptability. For vervet monkeys at Samara, the abundance of sweethorn trees, which provide a highly nutritious source of food throughout the year, possibly accounts for the lack of ecological stress observed for this population (Pasternak *et al*. 2013). Another, not necessarily mutually exclusive explanation for the lack of evidence for ecological stress at the population level may be that vervet monkeys are able to off-set the energetic requirements imposed by proximal environmental stressors by employing a labile thermoregulatory mechanism, or “adaptive thermoregulation”, to conserve energy.

6.1 Is thermoregulatory plasticity an indicator of stress or adaptability?

My data indicate that imprecise thermoregulation is a direct response to proximal environmental stressors. When environmental conditions were "good" (e.g. access to water and high food availability), monkeys were able to allocate sufficient resources towards maintaining homeothermy. However, in line with the cost-benefit theory of thermoregulation, environmental stressors (in this case low environmental temperatures and low food availability) which increased the cost, or decreased the net-benefit of thermoregulation, resulted in less precise thermoregulation. These findings suggest that the maintenance of strict homeothermy is a luxury afforded by favourable environmental conditions, supporting the theory that thermoregulatory mechanisms in endotherms occur along a continuum, rather than a dichotomy between "heterothermy" and "homeothermy" (Angilletta *et al.* 2010). One theory proposes that a labile thermoregulatory mechanism may serve to decrease the energetic costs of thermoregulation and allow energy to be reallocated for other biologically important activities (Angilletta *et al.* 2010). Such a labile thermoregulatory mechanism may be considered "adaptive" if it infers a net-fitness benefit, for example, if a short-term reduction in homeothermy (i.e. imprecise thermoregulation) increased growth, fecundity, or survival. Indeed, it has been shown that small mammal species which employ torpor or hibernation live longer, and are less vulnerable to extinction than larger or strictly homeothermic species (Geiser and Turbill 2009, Turbill *et al.* 2011).

The fitness benefit of torpor or hibernation is primarily incurred by the energetic savings of dormancy during periods of low resource availability, and decreased exposure to predators when inactive (Geiser and Turbill 2009, Turbill *et al.* 2011). If the observed nocturnal hypothermia for vervet monkeys is an attenuated form of torpor, this physiological plasticity, in combination with subsequent passive radiant-heat assisted re-warming, may infer significant energetic savings at low environmental temperatures in winter, with potential fitness benefits. The assumption of "adaptive" thermoregulation is that the maintenance of homeothermy is traded-off for energy conservation or reallocation to some other activity which ultimately increases net-fitness. However, it is likely that homeotherms have evolved to perform optimally within a narrow range of body temperatures (Boyles *et al.* 2011a), and therefore any deviation (i.e. imprecise thermoregulation) from homeothermy may reflect negatively on performance, which may impact fitness. Therefore, the question is whether the reduction in body temperature observed for vervet monkeys in winter is a controlled down regulation, as in torpor (i.e. implying adaptation), or an inability to maintain a high body temperature because of low energy reserves (i.e. implying stress).

An alternate interpretation of the observed nocturnal hypothermia for vervet monkeys at Samara is that nocturnal hypothermia reflects a loss of thermoregulatory capacity rather than a regulated adjustment in the thermoregulatory system, in which case the observed imprecise thermoregulation may be associated with a significant energetic cost (i.e. monkeys are attempting to maintain body temperature, thus operating at maximal cold-induced metabolic rate, but are unable to do so; Maloney *et al.* in preparation). For example, an increase in the 24-h amplitude of the body temperature rhythm is associated with decreased mass gain for Alpaca's (*Vicugna pacos*), and a reduction in the number of pregnancies in female rabbits (*Oryctolagus cuniculus*) compared to individuals which maintained homeothermy (S Maloney, unpublished data). These findings infer that homeothermic individuals in a population grow faster and are more successful breeders than less homeothermic individuals. Imprecise thermoregulation may therefore be reflecting an "energy deficit". Based on modal body temperature as a proxy for set-point body temperature, my data indicates that for vervet monkeys at Samara there was no controlled down regulation of body temperature in winter. Rather, imprecise thermoregulation in winter appears to reflect an inability to maintain homeothermy at low environmental temperatures (i.e. a physiological indicator of stress).

Furthermore, there was significant individual variability in thermoregulatory precision in winter. Specifically during the coldest periods of the 24-h cycle, when some individuals displayed increased hypothermia compared to others. Body mass appeared to be an important physical aspect influencing the degree of hypothermia experienced for individual monkeys. Smaller (i.e. lighter) monkeys would have required increased energetic inputs to defend body temperature compared to larger (i.e. heavier) monkeys, due to their relatively higher thermal conductance and lower thermal inertia (Aschoff 1981). The finding that smaller monkeys had lower 24-h minimum body temperatures than larger monkeys therefore supports the theory that imprecise thermoregulation reflected a physiological stress response to low environmental temperatures; related to the relationship between energetic requirements for thermoregulation and body mass. Besides physical factors such as body mass, social factors such as rank may be important factors influencing the thermoregulatory precision of individual monkeys. High ranking individuals may, for example, be privy to optimal overnight huddling positions on cold winter nights which may help to conserve body heat and significantly reduce energy requirements of thermoregulation. The observed individual variability in thermoregulatory precision highlights the importance of incorporating detailed behavioural observations and collecting individual morphological data in addition to biologger data, as is done for the long-term study which my project is contributing towards.

Determining the energetic and ecological implications of thermoregulatory plasticity for vervet monkeys was however beyond the scope of my study, and indeed with the exception of unpublished data from S. Maloney, the relationship between physiology, environmental stressors, and some measure of fitness (e.g. energy, reproduction, or growth) has not been empirically tested for any free-ranging “homeothermic” species. Anecdotal evidence suggests that there is an increase in mortalities for vervet monkeys at Samara towards the end of winter (P. Henzi, personal communication), which supports the conclusion based on my data that winter infers significant energetic challenges for monkeys and that imprecise thermoregulation may reflect a physiological stress response to unfavourable environmental conditions. To date however very little is known about how body temperature influences parameters of performance or fitness amongst mammals. This line of enquiry will be crucial in developing robust theories on the energetic and ecological implications of physiological plasticity in response to environmental stress.

6.2 Future research

Further studies need to ascertain whether the hypothermia observed for vervet monkeys in winter is adaptive, thus inferring a net-fitness gain, or alternatively reflects a physiological stress response (i.e. net-fitness is compromised). To determine the energetic and ecological implications of physiological plasticity, long-term studies monitoring physiological parameters (e.g. body temperature) of individual animals in conjunction with direct measures of fitness (e.g. reproduction, growth, survival) and environmental parameters are necessary. A valuable addition to the current long-term project on vervet monkeys at Samara would be to incorporate a direct measure of energy expenditure. A measure of heart rate (achievable by using existing biologging technology), for example, could provide a valuable proxy for metabolic rate (sensu Arnold *et al.* 2004. Signer *et al.* 2011) which would contribute towards understanding the energetic implications of nocturnal hypothermia observed in winter.

Given the apparent influence of food availability on activity and thermoregulation of vervet monkeys in my study, future data collection protocols should also endeavour to quantify variations in food quantity and quality. Due to the relative ease with which vervet monkeys and other primate species are habituated (allowing for detailed observation without influencing their natural behaviour), these species provide the ideal model for investigating the relationship between physiological stress, the ecological factors which perturb physiological wellbeing, and the ultimate fitness consequences of these interactions. The collection of detailed social, demographic, and behavioural data, facilitated by habituation, also provides scope for incorporating the influence of factors such as rank, sex, reproductive state, population density, or disease on physiological parameters and fitness.

The large 24-h and seasonal fluctuations in environmental conditions at Samara also make this the ideal study site. It is hypothesized that species occupying narrow environmental niches such as those in the tropics, where environmental conditions are relatively stable across seasons, will be more vulnerable to changes in environmental conditions than species occupying more seasonal environments, as these species are inherently adapted to larger fluctuations in environmental conditions (Janzen 1967, McCain 2009). A valuable line of inquiry would therefore be to compare physiological/thermoregulatory mechanisms within and amongst populations ranging from tropical to temperate habitats. Under optimal environmental conditions, it is proposed that homeotherms will preferentially maintain body temperature within a narrow range, characteristic of strict homeothermy. The maintenance of homeothermy likely is associated with increased performance which ultimately results in a net-fitness gain (Boyles *et al.* 2011a). Imprecise thermoregulation may therefore provide a valuable indicator of environmental, or other (e.g. social or disease), stress which may provide an index of how “comfortable” an animal is in its current environment.

Such an indicator of environmental stress may provide a valuable “tool” for implementing conservation strategies. The designation and maintenance of priority conservation areas is perceived as fundamental in perturbing habitat transformation and biodiversity loss under future climate change scenarios. The identification of these priority areas is typically based on modeled information on the spatial distribution and abundance of key species (Chown and Gaston 2008). The majority of models use a “top-down” approach to predict species distributions and range shifts, whereby the current climatic conditions under which the species persists is assumed to reflect the fundamental niche of that species (e.g. bioclimatic envelope models; Chown and Gaston 2008). These models are however limited in that they do not account for the effects of biotic interactions or dispersal limitations, or the potential for organisms to adapt to changing environmental conditions (Chown and Gaston 2008). Already the incorporation of a biophysical “bottom-up” approach, which can provide information on the mechanistic response of species to the environment (e.g. thermal tolerance, see Beardall *et al.* 1998), is considered a valuable contribution to refining “top-down” predictive models. Measuring parameters such as body temperature and activity in free-ranging animals (i.e. conservation physiology) can provide information on the adaptive potential (i.e. plasticity) at the level of the individual, within the context of the complex natural systems which these individuals inhabit; information not obtainable under laboratory conditions. Understanding the potential for behavioral (e.g. microclimate selection or sunbasking) or physiological (e.g. hypothermia) plasticity to off-set the energetic costs inferred by environmental stressors may provide valuable insights in to the important ecological determinants of habitat suitability, and how these may vary under future climate change scenarios. The level of physiological data required to develop mechanistic models should not be a deterrent. Advances in biologging technology now allow for physiological data (e.g. body temperature) to be obtained from free-ranging individuals with relative ease. It is likely that physiological mechanisms will be fairly uniform within groups (i.e. several broad “rules” may apply, see Chown *et al.* 2002, Blomberg *et al.* 2003), and therefore the physiological-environmental-fitness interaction need only be estimated for representatives of many taxa, not all species (Chown and Gaston 2008).

I hypothesise that the overriding limitation on the ability of vervet monkeys to adapt to environmental stressors at Samara is their marked behavioural inflexibility in activity scheduling between the diurnal and nocturnal periods. It is therefore likely that any environmental stressors which confound the influence of short day lengths in winter may impose a significant bottleneck on survival. Low environmental temperatures and food availability are therefore likely to be the primary environmental determinants of habitat suitability for vervet monkeys and other diurnal primates living in similar seasonal habitats. Understanding physiological plasticity of representative taxa across a range of environmental conditions may help to prioritize appropriate conservation areas and ultimately conserve biodiversity under changing environmental conditions.

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