ADAPTING TO CLIMATE CHANGE: THE EFFECT OF DESERTIFICATION ON THE PHYSIOLOGY OF FREE-LIVING UNGULATES

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A thesis submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy

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DECLARATION

I declare that the work contained in this thesis is my own, unless otherwise specified. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. The work herein has not been submitted before for any degree or examination in any other university.

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Signed on the _____ day of _____, 2009

ABSTRACT

For long-lived species, physiological plasticity provides the best option to counter extinction and survive climate change, yet we understand very little about long-term physiological adaptations of arid-zone artiodactyls. Variation within a morphological trait, for example, may provide pre-adaptation to changing climatic conditions. Using intra-abdominal miniature data loggers, I measured core body temperature in female springbok (*Antidorcas marsupialis*) of three colour morphs (black, normal and white) and showed that the pelt colour does indeed have thermoregulatory significance. The black springbok seem able to reduce energy expenditure in winter, but experience higher solar heat load in hot conditions. Therefore lighter coloured individuals may be selected for in the future as conditions get progressively hotter and drier with climate change.

Small individuals also may be selected for in the future. Hypothetically, small artiodactyls would have the advantage of smaller resource requirements and greater access to refuge sites than do larger artiodactyls, but they are likely to be disadvantaged by a high mass-specific metabolic rate, high water turnover and less capacity to store heat. I measured body temperature, activity and microclimate selection in free-ranging Arabian oryx (*Oryx leucoryx*, ~ 70 kg) and the smaller Arabian sand gazelle (*Gazella subgutturosa marica*, ~ 15 kg) inhabiting one of the hottest and driest regions, Arabian Desert environment, at the same time. Despite the oryx having a body mass more than four-fold that of the sand gazelle, both species responded remarkably similarly to changes in environmental conditions. Both species employed heterothermy and cathemerality, and selected the same cool microclimates during times of heat stress. In combination with high ambient temperatures, water stress appeared to be the primary driver towards heterothermy in the Arabian oryx, potentially resulting from dehydration or the combined effects of dehydration hyperthermia and starvation hypothermia.

To investigate the physiological consequences of habitat transformation, of the kind expected to occur with climate change, I monitored body temperature and activity patterns of Angora goats inhabiting both desertified and intact sites. I was able to demonstrate physiological changes in response to desertification. Following shearing, goats that inhabited the transformed site displayed an increased 24-h amplitude of body temperature rhythm and were generally less active compared to goats that inhabited the intact site, which may reflect a trend towards heterothermy and cathemerality, as was observed in the Arabian oryx and sand gazelle.

Finally, the physiology studies required to better understand the mechanisms of phenotypic plasticity underlying responses to climate change cannot be confined to the function of healthy animals as pathogens are predicted to spread with climate change. I therefore investigated the physiological consequences of infection in free-living kudu (*Tragelaphus strepsiceros*). Not only did I record quantitative evidence for autonomic and behavioural fever, but I also recorded the first evidence of sickness behaviour, in the form of decreased activity, in a free-living artiodactyl. Artiodactyl hosts are likely to have to contend with an increased costs of immunity superimposed on the chronic physiological stress of having to adapt to the climatically unsuitable areas to which they are confined.

In conclusion, I have revealed some of the physiological mechanisms that will be brought into action if long-lived artiodactyls are able to adapt phenotypically to climate change in Africa. Activity patterns and microclimate selection are flexible behavioural processes which are likely to represent an animal's primary defence to changes in climatic conditions. If behavioural processes are insufficient to maintain homeothermy, we may observe changes in an animal's body temperature, a sensitive indicator of infection, dehydration, nutrition and environmental stress. Such physiological measurements need to be incorporated into long-term physiological monitoring projects, and bioclimatic envelope models, so that we can better predict how species will respond to climate change.

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LIST OF ABBREVIATIONS

A/D	analog/digital
aut	autumn
C	pelt conductance ($W.m^{-2}.°C^{-1}$)
HFTs	Heat Flux Transducers
i.d.	inner diameter
I.M.	intramuscularly
I.V.	intravenously
o.d.	outer diameter
PE	Port Elizabeth
PTFE	polytetrafluoroethylene
$\dot{Q}$ or HF	heat flow ($W.m^{-2}$)
S.C.	subcutaneously
spr	spring
sum	summer
T_a	air temperature
T_p	plate temperature
T_s	skin surface temperatures
VPDB	Vienna Pee Dee Belemnite
V-SMOW	Vienna-Standard Mean Ocean Water
W	mass (kg)
win	winter

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RESEARCH OUTPUTS

Published papers

Hetem R.S., Mitchell D., Maloney S.K., Meyer L.C.R., Fick L.G., Kerley G.I.H. and Fuller A. (2008). Fever and sickness behavior during an opportunistic infection in a free-living antelope, the greater kudu (*Tragelaphus strepsiceros*). *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **294**: 246-254

Hetem R.S., de Witt B.A., Fick L.G., Fuller A., Kerley G.I.H., Meyer L.C.R., Mitchell D. and Maloney S.K. (2009). Body temperature, thermoregulatory behaviour and pelt characteristics of three colour morphs of springbok (*Antidorcas marsupialis*). *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology* **152**: 379-388

Hetem R.S., de Witt B.A., Fick L.G, Fuller A., Kerley G.I.H., Maloney S.K., Meyer L.C.R. and Mitchell D. Summer shearing affects body temperature of Angora goats (*Capra aegagrus*) more than does winter shearing. *Animal* **3:** 1025-1036

Submitted papers

Hetem R.S., Strauss W.M., Fick L.G., Maloney S.K., Meyer L.C.R., Shobrak M., Fuller A. and Mitchell D. Thermoregulation and cathemerality of free-living Arabian oryx (*Oryx leucoryx*) in a hyper-arid desert. *Physiological and Biochemical Zoology* (April 2009)

International conference presentations

Hetem R.S, Mitchell D., Maloney S.K., Meyer L.C.R., Fick L.G., Kerley G.I.H. and Fuller A. Fever and sickness behaviour in a free-ranging antelope, the greater kudu. Presented at the 2nd international meeting on Physiology and Pharmacology of Temperature Regulation, Phoenix, Arizona 3 - 6 March 2006 (poster presentation)

Hetem R.S., de Witt B.A., Fick L.G., Kerley G.I.H., Maloney S.K., Meyer L.C.R., Mitchell D. and Fuller A. Habitat transformation influences the physiology of Angora goats and may have conservation implications for wild ungulates. Presented at the 21st annual meeting of the Society for Conservation Biology, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa. 1 - 5 July 2007. (poster presentation)

Hetem R.S., de Witt B.A., Fick L.G., Kerley G.I.H., Maloney S.K., Meyer L.C.R., Mitchell D. and Fuller A. The effect of habitat transformation on the physiology of Angora goats. Presented at the 4th International Geosphere-Biosphere Programme Congress, Cape Town, South Africa. 5 - 9 May 2008. (poster presentation)

Hetem R.S., Strauss W.M., Fick L.G., Maloney S.K., Meyer L.C.R., Shobrak M. Fuller A. and Mitchell D. Coping with extremes: thermoregulation and cathemerality of free-living Arabian oryx (*Oryx leucoryx*). Presented at the 4th international conference of Comparative Physiology and Biochemistry in Africa, Masai Mara, Kenya. 19-25 July 2008. (invited oral presentation)

Hetem R.S., Strauss W.M., Fick L.G., Maloney S.K., Meyer L.C.R., Mitchell D., Shobrak M. and Fuller A. Coping with extremes: adaptive heterothermy and cathemerality in Arabian ungulates. Presented at the 4th international conference of Comparative Physiology and Biochemistry in Africa, Masai Mara, Kenya. 19-25 July 2008. (poster presentation)

Local conference presentations

Hetem R.S., Mitchell D., Maloney S.K., Kerley G.I.H., Meyer L.C.R., Fick L.G., de Witt B.A. and Fuller A. Feasibility of using remote measurements to assess ecological physiology in free-living ungulates. Presented at the 32nd conference of the Zoological Society of Southern Africa, Rhodes University, Grahamstown, South Africa. 13 - 15 July 2005. (oral presentation)

Hetem R.S., Strauss W.M., Fick L.G., Maloney S.K., Meyer L.C.R., Mitchell D., Shobrak M. and Fuller A. Coping with extremes: adaptive heterothermy and cathemerality in the Arabian oryx (*Oryx leucoryx*). Presented at the 33rd bi-annual conference of the Zoological Society of Southern Africa, North-West University, Potchefstroom, South Africa. 8 – 11 July 2007. (oral presentation)

Hetem R.S., de Witt B.A., Fick L.G., Fuller A., Kerley G.I.H., Maloney S.K., Meyer L.C.R. and Mitchell D. Cold stress in Angora goats after shearing. Presented at the 35th meeting of the Physiological Society of Southern Africa, Glenburn Lodge, Cradle of Humankind, South Africa. 9 – 12 September 2007. (oral presentation)

Hetem R.S., Strauss W.M., Fick L.G., Maloney S.K., Meyer L.C.R., Mitchell D., Shobrak M. and Fuller A. Coping with extremes: adaptive heterothermy and cathemerality in Arabian ungulates. Presented at the Southern African Wildlife Management Association Symposium, Didima, South Africa. 18 – 21 September 2007. (poster presentation)

AUTHOR CONTRIBUTIONS

Where chapters have been modified from submitted or published papers, the contributions of each author are listed below.

Chapter two

Hetem R.S., de Witt B.A., Fick L.G., Fuller A., Kerley G.I.H., Meyer L.C.R., Mitchell D. and Maloney S.K. (2009) Body temperature, thermoregulatory behaviour and pelt characteristics of three colour morphs of springbok (*Antidorcas marsupialis*). *Comparative Biochemistry and Physiology, A: Molecular and Integrative Physiology* **152**: 379-388.

The original idea for the project was formulated from discussions with Prof. Graham Kerley. The initial three-month winter study, with the behavioural observations, formed part of Ms. Brenda de Witt's honours (4th year) project, which I helped to supervise. We subsequently expanded the project to collect data for one year, for which I made up the equipment, organised the surgery, analysed the data. I wrote the paper encompassing both projects. Together with Ms. de Witt, I made the measurements and calculations of the heat transfers characteristics of the pelts. Dr. Shane Maloney provided guidance and invaluable expertise with analysis of the pelt heat transfer characteristics and calculations. Dr. Leith Meyer performed the surgical procedures. Dr. Linda Fick assisted with the surgery and project logistics. Prof. Andrea Fuller and Prof. Duncan Mitchell assisted with the planning and execution of the experimental work. All authors edited the manuscript.

Chapters three and four

Hetem R.S., Strauss W.M., Fick L.G., Maloney S.K., Meyer L.C.R., Shobrak M., Fuller A. and Mitchell D. Thermoregulation and cathemerality of free-living Arabian oryx (*Oryx leucoryx*) in a hyper-arid desert. Submitted to *Physiological and Biochemical Zoology* (April 2009)

Hetem R.S., Strauss W.M., Shobrak M., Fick L.G., Maloney S.K., Meyer L.C.R., Fuller A. and Mitchell D. Does size matter? Comparison of body temperature and activity of free-living Arabian oryx (*Oryx leucoryx*) and the smaller Arabian sand gazelle (*Gazella subgutturosa marica*) in the Saudi desert. Intend to submit to *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*

The idea for these projects arose from discussions with my co-authors. I set up the collaboration between the University of the Witwatersrand and the National Wildlife Research Center, Saudi Arabia, organised surgery, prepared the equipment, analysed the data and wrote the manuscripts. Mr. Maartin Strauss made the collaboration viable, assisted with animal capture and checked on the animals when I was unable to do so. Dr. Linda Fick helped to instrument the animals and assisted with surgical logistics. Dr. Shane Maloney assisted with experimental design and helped to instrument the animals. Dr. Leith Meyer performed the surgical procedures. Dr. Mohammed Shobrak took responsibility for project logistics in Saudi Arabia. Prof. Duncan Mitchell assisted with experimental design and surgery. All authors edited the manuscript.

Chapter five

Hetem R.S., de Witt B.A., Fick L.G., Fuller A., Maloney S.K., Meyer L.C.R., Mitchell D. and Kerley G.I.H. Effects of habitat transformation on the physiology of Angora goats: testing global change predictions. Intend to submit to *Journal of Arid Environments*

The idea for this project arose from discussions with my co-authors. I organised surgery, prepared the equipment, routinely caught and monitored the animals, drew blood, analysed the data and wrote the manuscript. Ms. Brenda de Witt assisted with animal capture and execution of experimental work. Dr. Linda Fick helped to instrument the animals and assisted with surgical logistics. Prof. Andrea Fuller assisted with surgery and planning of experimental work. Dr. Shane Maloney assisted

with experimental design and helped with analysis of water turnover. Dr. Leith Meyer performed the surgical procedures. Prof. Duncan Mitchell assisted with experimental design and surgery. Prof. Graham Kerley initiated the collaboration with Blaauwkrantz and provided an ecological perspective. All authors edited the manuscript.

Chapter six

Hetem R.S., Mitchell D., Maloney S.K., Meyer L.C.R., Fick L.G., Kerley G.I.H. and Fuller A. (2008) Fever and sickness behavior during an opportunistic infection in a free-living antelope, the greater kudu (*Tragelaphus strepsiceros*). *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **294**: R246–R254.

The study was designed to investigate the thermal responses of kudu to climatic stress, but after release the kudu acquired a spontaneous infection. I organised surgery, prepared the equipment, monitored the animals, analysed the data and wrote the manuscript. Prof. Duncan Mitchell assisted surgery and data interpretation. Dr. Shane Maloney helped to instrument the animals. Dr. Leith Meyer performed the surgical procedures. Dr. Linda Fick helped to instrument the animals, assisted with surgical logistics and animal monitoring. Prof. Graham Kerley initiated the collaboration with Blaauwkrantz, assisted with surgical procedures and provided logistical support. Prof. Andrea Fuller assisted with surgery. All authors edited the manuscript.

CHAPTER 1

Introduction

Some of the ideas presented in this chapter have appeared in conference proceedings: Mitchell D., Fuller A., Hetem R.S. and Maloney S.K. (2008). Climate change physiology: the challenge of the decades. In: 4th Comparative Physiologists & Biochemists meeting in Africa: Mara2008. "Molecules to Migration: The Pressures of Life". Morris S. and Vosloo A. (Eds.), Medimond Publishing Co, via Maserati 6/2, 40124 Bologna, Italy, pp. 275-281.

During his acceptance speech for the 2007 Nobel Peace Prize, Al Gore warned that climate change is "the greatest challenge we've ever faced". Many species are unlikely to survive climate change and a pivotal study by Thomas *et al.* (2004) predicted that, under mid-range climate change scenarios, a quarter of terrestrial plants and animals may be extinct by 2050. By extrapolating such predictions globally, the authors predict that well over one million species could be threatened with extinction as a result of climate change. However, these bioclimatic models may overestimate future extinction risk because the majority of these models provide no measure of species' adaptability to environmental change. For long-lived species, which are limited in their dispersal capacity as a result of the modern human-dominated landscape, phenotypic plasticity provides the only option to counter extinction and cope with climate change. But whether long-lived species display sufficient phenotypic plasticity to adapt to climate change remains obscure. This introductory chapter contextualises my research within what is currently known and relevant about climate change, identifies key gaps in the research and poses the questions that are addressed in my thesis.

The Intergovernmental Panel on Climate Change 2007 synthesis report predicts an increase in global temperatures of between 1.1ºC and 6.4ºC during the 21st century (IPCC, 2007). Such temperature projections for the end of the century may represent the warmest global climate in over 2 million years. Nevertheless, global warming has been a common occurrence throughout the Earth's 3.5 billion year history. Modelling of the current episode predicts a temperature rise of the same order of magnitude as that evident at the end of the Permian, when mass volcanism increased global temperatures by 6ºC and resulted in the extinction of nearly 95% of species (Benton and Twitchett, 2003). Since the rate of climate change may be more critical than its magnitude and duration (Davis *et al.*, 2005), the unprecedented rate of climate change predicted to occur over the next 50 years is likely to threaten the persistence of numerous species (Davis and Shaw, 2001). Although the current rate of warming has not yet exceeded the rate of past global warming episodes (Barnosky *et al.*, 2003), the

so-called “background rate”, the current episode of warming certainly is distinct from all previous episodes in one respect: because its cause is anthropogenic, it is the first of the global warming events that could be mitigated. If, as now seems inevitable, the episode will not be mitigated adequately, we must expect another major wave of extinctions of species (Myers, 1993; McLaughlin *et al.*, 2002; Thomas *et al.*, 2004; Malcolm *et al.*, 2006).

Of the nearly 30 000 documented trends in physical systems and biological characteristics of plants and animals between 1970 and 2004, 90% are in the direction consistent with environmental temperature increases (Rosenzweig *et al.*, 2008). Meta-analyses, such as those by Rosenzweig *et al.* (2008), have helped to establish a plausible link between recent changes in climate and observed changes in species and communities. Yet, climate change may exceed the natural response capacity of many species and different species are likely to show different climatic tolerance and to be affected differently by the arrival of new species. Thus, climate change is likely to have a significant impact on biodiversity (Malcolm and Markham, 2000; Sala *et al.*, 2000; Lovejoy and Hannah, 2005), and current conservation strategies, which attempt to conserve communities and ecosystems as they currently exist, will not be effective in the face of climate change (Hughes, 2000; Hannah *et al.*, 2002a; Hannah *et al.*, 2002b). We therefore need a better understanding of the potential impacts of climate changes on living organisms so that we may better anticipate the future responses of plant and animal populations (Millien *et al.*, 2006) and better conserve biodiversity.

Simplistically, species have only three prospective scenarios when faced with environmental changes. Firstly, species may migrate or shift their current distribution range to more suitable habitats where the climate is within the species’ tolerance limits. Secondly, animals may adapt to regional variation in climatic regimes either through a change in the genetic composition of the population or by a change in the expression of that genotype, so called phenotypic plasticity. Either of these adaptation strategies may result in changes in the timing of events (phenology), morphology (e.g.

colour patterns, body shape and size) or physiology of a species. Thirdly, if neither migration nor adaptation is attainable, extinction will result.

1.1 Range shifts

One of the predicted responses to climate change is that individuals will track suitable climates. In the temperate zone, for example, a 1ºC increase in mean annual temperature is predicted to correspond to a shift in isotherms of ~ 160 km in latitude or 160 m in elevation (Parmesan and Yohe, 2003; Thuiller, 2007). Thus, species are expected to follow these shifting climatic zones and move polewards in latitude and upwards in elevation (Hughes, 2000; Walther *et al.*, 2002). These predictions are based largely on palaeoecological evidence as numerous mammalian species tracked consistent climate profiles as climates warmed during the Quaternary Period (Graham and Grimm, 1990; Potts and Deino, 1995; Graham *et al.*, 1996; Lyons, 2003; Martinez-Meyer *et al.*, 2004). Such biotic responses imply that natural systems were resilient to past climate change and re-colonization following glacial retreat often exceeded expected rates of dispersal, as estimated from mean dispersal and time to reproductive maturity (Hannah *et al.*, 2002a).

Numerous recent reports have documented shifts in the geographical distribution of extant species (for review see Parmesan and Yohe, 2003; Root *et al.*, 2003) and these observations parallel expectations based on observations of warming events of the past (Barnosky *et al.*, 2003). In the Northern hemisphere, for example, recent northward movement of species' range boundaries has been observed in numerous terrestrial taxonomic groups, including plants (Grabherr *et al.*, 1994; Walther *et al.*, 2005), butterflies (Parmesan, 1996; Hill *et al.*, 1999; Parmesan *et al.*, 1999), birds (Thomas and Lennon, 1999; Brommer, 2004; La Sorte and Thompson, 2007), reptiles and amphibians (Pounds *et al.*, 1999), and mammals (Hersteinsson and MacDonald, 1992; Myers *et al.*, 2009). Global meta-analyses revealed that about 80% of these species range shifts are consistent with climate change predictions (Parmesan and

Yohe, 2003; Root *et al.*, 2003). However, of the 105 reports of range shifts in these meta-analyses, only three were included from the Southern hemisphere.

The dearth of data from long-term research in the Southern hemisphere (Midgley *et al.*, 2007; Rosenzweig *et al.*, 2008) is of concern because of hemisphere asymmetry in ecosystem processes. Whereas ecosystem processes are largely controlled by energy-related factors in the northern hemisphere, water-related factors appear to control ecosystem processes in the Southern hemisphere (Hawkins *et al.*, 2003; Chown *et al.*, 2004b). Such hemisphere asymmetries are likely to be exacerbated with climate change. While many northern species are expected to expand their ranges northwards in response to an increased primary productivity (Nemani *et al.*, 2003), longer growing season and less severe winters, the increased aridity predicted for many arid and semi-arid regions is likely to further limit the distributions of southern hemisphere species.

1.1.1 Bioclimatic envelope models

Although observed range shifts from long-term data sets in the Southern hemisphere are limited (Midgley *et al.*, 2007), various envelope models have attempted to predict species' distributions with climate change (Erasmus *et al.*, 2002; Midgley *et al.*, 2002; Meynecke, 2004; McClean *et al.*, 2005). Niche-based or bioclimatic envelope models are essentially static models that correlate current species distributions with climate variables and project future spatial shifts in species' climatic envelopes (Peterson, 2003; Huntley *et al.*, 2004; Austin, 2007). Although these models may provide a useful first estimate of the potential impact of climate change on the distribution of species, their validity has been questioned recently because of the assumptions on which they are based (Pearson and Dawson, 2003; Thuiller, 2004; Luoto *et al.*, 2005; Heikkinen *et al.*, 2006). The first assumption is that climate is the key limiting factor on species distributions (Araújo and Luoto, 2007). For simplicity, most of these models predict species range shifts based on predicted average temperature changes, but species may prove to be more sensitive to changes in precipitation than to

changes in average temperature, as has been found for birds, amphibians and reptiles (Pounds *et al.*, 1999). In addition, unlike the average temperature increases which are incorporated into the majority of these model predictions, it is likely that the predicted increase in weather extremes, such as droughts and heat waves, will impose the dominant stress on species in the future (Parmesan *et al.*, 2000; Hallett *et al.*, 2004).

Furthermore, these bioclimatic envelope models do not account for the so-called "non-climatic" influences on species' distributions, such as geology and biotic interactions. Biotic interactions between species are often overlooked in bioclimatic modelling, yet such species interactions are likely to have important consequences for future species distributions (Davis *et al.*, 1998; Mustin *et al.*, 2007). For example, the climate-driven northward range expansion of the red fox (*Vulpes vulpes*) has been associated with a decrease in the distribution range of the arctic fox (*Alopex lagopus*) as a result of an increased interspecific competition (Hersteinsson and MacDonald, 1992). Since individual species differ in their response to changing climatic conditions, species may shift their ranges independently of each other which would result in changes in community structure and ultimately lead to ecosystem disruption (Schneider and Root, 1996; McCarty, 2001; Stenseth *et al.*, 2002; Walther *et al.*, 2002). Such secondary effects of climate change may be more severe than the direct consequences of increased temperature (Humphries *et al.*, 2004) and investigation of such biotic interactions has lead to a new scope of ecological research termed "global change ecology" (Schlesinger, 2006). These responses need to be incorporated into bioclimatic models to better predict future species distributions (Guisan and Thuiller, 2005).

An additional non-climatic factor which is likely to impede the movement of many species is the modern human-dominated landscape. Human activity has resulted in habitat loss and fragmentation, and this process is likely to be exacerbated by climate change as previously fertile areas are transformed. The impassable obstacles created

by human activity lower the likelihood of species finding compatible distant habitats to colonise, ultimately increasing the risk of extinction. Under mid-range climate change scenarios, climate envelope models predict that 45% of terrestrial species are likely to be committed to extinction by 2050 if their dispersal is limited (Thomas *et al.*, 2004). Similarly, 25-40% of a representative sample of 277 African mammalian species are likely to be critically endangered or extinct by 2080 (Thuiller *et al.*, 2006). South Africa's mammalian species are predicted to be particularly sensitive to climate change because the majority of national parks are fenced, further limiting the dispersal abilities of large species. The extinction risk of South African mammals is estimated to be as high as 69% by 2050, if dispersal is limited (Thomas *et al.*, 2004). South Africa's flagship Kruger National Park, for example, may lose two thirds of its current animal species (Erasmus *et al.*, 2002) and long term population monitoring has already observed declines in seven out of 11 ungulate species studied in the Kruger National Park between 1977 and 1996 (Ogutu and Owen-Smith, 2003).

Not only do fences limit dispersal abilities of large mammals, but in some cases the future location of a suitable bioclimatic envelope may be impossible to reach. One such example where range shift is unlikely to be a feasible option is the scimitar-horned oryx (*Oryx dammah*, Fig. 1.1). In order to track its current bioclimatic envelope, this species will have to move thousands of kilometres, from the Sahara desert to Namibia, a distance unattainable without human assistance (Thuiller *et al.*, 2006). In such circumstances where range shifts are unfeasible, either as a result of unattainable travelling distances or loss of habitat connectivity, assisted colonization may provide a conservation option in the future (Hunter, 2007; McLachlan *et al.*, 2007; Hoegh-Guldberg *et al.*, 2008). Yet, moving species to areas where they do not currently occur is not without risk. The introduced organisms can carry disease, displace native species and thereby challenge ecosystem stability or alter the genetic structure of local populations. Therefore, an in-depth knowledge of species' biology and accurate climate change predictions would be required before assisted colonization can become a feasible conservation option (Davidson and Simkanin, 2008).

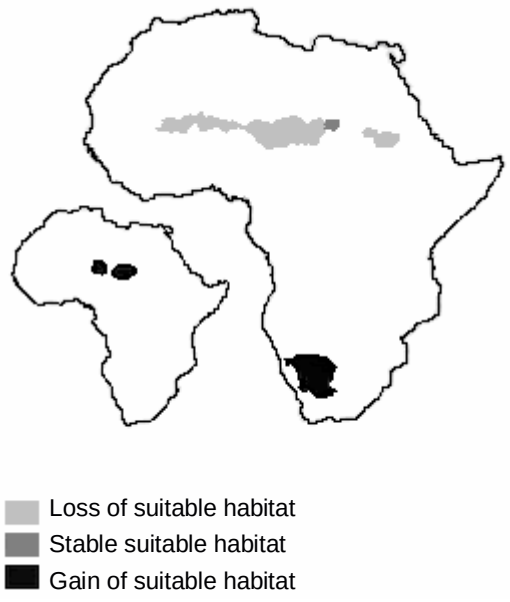

Figure 1.1. Observed current (small map) and predicted (large map) distribution of the scimitar-horned oryx (*Oryx dammah*) for 2050. Light grey areas indicate current climatically suitable habitats predicted to be unsuitable in the future. Moderate grey areas indicate the current climatically suitable habitats which are predicted to stay suitable. Dark grey areas indicate the current climatically unsuitable habitats which are predicted to be suitable by 2050 (adapted from Thuiller *et al.*, 2006).

Another limitation of bioclimatic envelope models is the assumption that species lack sufficient plasticity to adapt to climates beyond those under which a given species occurs today (Levinsky *et al.*, 2007). Yet, physiological plasticity may allow animals to adapt to changing climatic conditions. Some bioclimatic envelope models have attempted to incorporate physiological limitations to determine the climatic requirements of a diverse group of taxa including plants (Prentice *et al.*, 1992; Sykes and Prentice, 1996; Dole *et al.*, 2003), invertebrates (Crozier and Dwyer, 2006; Musolin, 2007), marine (Pörtner *et al.*, 2004; Gilman *et al.*, 2006; Osovitz and Hofmann, 2007) and terrestrial ectotherms (Pörtner, 2001; Kearney and Porter, 2004), and mammals (Taulman and Robbins, 1996; Johnston and Schmitz, 1997). Such models require an understanding of species' physiological responses to climate (Franklin, 1995; Mack, 1996; Guisan and Zimmermann, 2000), yet we lack an understanding of the ecophysiological tolerances of many species. Although these physiological models still have limitations, since they also do not take non-climatic factors into account, they are considered to be superior and more robust than those bioclimatic envelope models based on correlations between observed distributions

and current climate variables (Prentice *et al.*, 1992; Sykes and Prentice, 1996; Hodkinson, 1999).

Despite such limitations, bioclimatic envelope models reveal the potential threat that climate change poses on global biodiversity. These models, however, may overestimate future extinction risk because the majority of these models provide no measure of species' adaptability to environmental change. Natural selection, for example, may allow animals to adapt to changing climatic conditions. However, evolutionary change is often considered a slow process and the current rate of climate change may be too fast to allow evolutionary adaptation, as such, bioclimatic envelope models assume that the tolerance range of a species remains stable as it shifts its geographic range (Bennett, 1990; Jackson and Overpeck, 2000). Despite such predictions, evolutionary change is likely to have accompanied range shifts in the past, since changing climates would change the so-called “fitness optimum” for different populations throughout the species range (Davis and Shaw, 2001; Davis *et al.*, 2005), making the fundamental niche unstable over time. Indeed, numerous studies have documented morphological changes in response to past climate change events (Martin and Barnosky, 1993).

1.2 Microevolution

In combination with shifting species ranges, morphological changes are believed to constitute the first response of mammals to climate change (Barnosky *et al.*, 2003). In general, the extent of the morphological adaptation seen in the past fell within the range of morphological variation found in the species across its current geographical range (Davis and Shaw, 2001; Huntley, 2007). Thus, evolutionary responses require genetically based variation among individuals and range shifts may act to increase gene flow, thereby increasing a species chance of adapting to changing conditions. Northern range shifts, for example, may have the advantage of introducing genotypes that are better adapted to warmer conditions, thus promoting adaptation of existing

populations (Garant *et al.*, 2007; Visser, 2008). Conversely, range shifts may also decrease genetic variability as a result of outbreeding of distinct populations. For example, climate change may result in genetic mixing among subspecies of the black bear, which is likely to inhibit or even reverse sub-speciation (Kerr and Packer, 1998).

True speciation events may take millennia, leading to the perception that genetic adaptation is a slow process relative to climate change (Bradshaw and McNeilly, 1991; Etterson and Shaw, 2001; Davis *et al.*, 2005). Although the current global warming episode probably does not yet exceed the normal background rate, continued warming over the next few decades will exceed the background rate of change by one or more orders of magnitude (Barnosky *et al.*, 2003). With the unprecedented rate of climate change anticipated to occur in the future it becomes questionable whether evolutionary adaptation will be sufficient to mitigate the effects of climate change (Bradshaw and McNeilly, 1991; Davis and Shaw, 2001; Huntley, 2007). Nevertheless, a theoretical analysis recently concluded that animals must use the option of genetic adaptation to survive climate change (Rice and Emery, 2003; Visser, 2008). Such conclusions are based on evidence that genetic adaptation need not involve the slow process of speciation. Evidence has been advanced that "microevolution", that is heritable shifts in allele frequencies in a population (without speciation), already has occurred, in directions predicted by climate change (Bradshaw and Holzapfel, 2006; Bradshaw and Holzapfel, 2008). Evidence for rapid evolutionary change is particularly prevalent for short-lived species with fast generation times (for examples, see Rodríguez-Trelles and Rodríguez, 1998; Thompson, 1998; Hendry and Kinnison, 1999; Kinnison and Hendry, 2001; Reznick and Ghalambor, 2001; Yoshida *et al.*, 2003).

Such rapid evolutionary change may be an important adaptation to climate change. For example, two species of bush crickets, *Conocephalus discolor* and *Metrioptera roeselii*, are becoming more dispersive because of an increase the proportion of longer-winged (more dispersive) individuals, in Britain over the past 20 years

(Thomas *et al.*, 2001a). In addition to changes in the size of appendages, ecogeographical rules predict that animals may adapt to local conditions and changing climates by displaying variation in colour (Millien *et al.*, 2006). For example, the Western White Butterfly, *Pontia occidentalis*, in the United States and Canada, displays seasonal plasticity in wing melanin, which is believed to enhance the butterflies' thermoregulation in seasonally varying thermal environments (Kingsolver and Huey, 1998). Although an animal's colour, in itself, may have thermoregulatory advantages, often an animal's colour has fitness advantages because of an association with other morphological features. For example, the coat colour in Soay sheep is related to fitness because of an association with body size (Clutton-Brock *et al.*, 1997), and it is because of this genetic association between coat colour and body size that homozygous dark coloured sheep have decreased in frequency over a 20-year time period (Gratten *et al.*, 2008). However, analyses by Maloney *et al.* (2009) imply that increases in ambient temperatures may provide a more parsimonious explanation for the recent decline in dark-coloured Soay sheep. These authors suggest that recent warming trends would offset the potential metabolic savings associated with dark colouration, negating any selective advantage for dark-coloured sheep.

An additional ecogeographical rule which has been used to predict how animals will respond to climate change is Bergmann's rule. Bergmann's rule predicts a positive correlation between body mass of terrestrial endotherms and latitude, and, by inference, an inverse correlation between body mass and environmental temperature. With current models predicting an increase in mean surface air temperature, Bergman's rule predicts that smaller individuals would be favoured in a warmer world (Millien *et al.*, 2006). Indeed, several species of passerine birds caught in ringing operations in England over 25 recent years (Yom-Tov *et al.*, 2006) and museum specimens from Israel over 50 recent years (Yom-Tov, 2001) have exhibited a linear decrease in body mass. Similarly, the body mass of the bushy-tailed woodrat (*Neotoma cinerea*) has decreased with recent increases in temperature in New Mexico

(Smith *et al.*, 1995; Smith *et al.*, 1998; Smith and Betancourt, 2006). Although these trends have been interpreted as microevolutionary responses to climate change, the majority of these studies provided no evidence that the observed trends had a genetic basis (Teplitsky *et al.*, 2008; Wolf *et al.*, 2009). The general lack of genetic evidence to account for such morphological variations, at least partially, is because molecular genetic techniques remain inadequate to properly reveal how genetic sequences relate to ecological importance (Holt, 1990; Gienapp *et al.*, 2008).

Similar to morphological variations, variations in phenology, or the timing of life-history events, have also been attributed to a climate-driven evolutionary response. The changing migration pattern in German blackcaps (*Sylvia atricapilla)*, for example, has been attributed entirely to evolution (Berthold and Pulido, 1994; Bearhop *et al.*, 2005). Yet, to date, there are only two studies which have tested for the genetic basis of breeding time advancements in birds, and neither of these found evidence for a genetically-based response (Sheldon *et al.*, 2003; Gienapp *et al.*, 2006). The most convincing example of microevolutionary response to climate change has been provided by data from 325 marked female North American red squirrels (*Tamiasciurus hudsonicus*) in Yukon from 1989 to 2001, a period over which mean lifetime parturition date advanced by six days per generation, associated with a mean spring temperature rise of 2°C and a decreased precipitation at the location (Réale *et al.*, 2003a). Réale *et al.* (2003b) used an 'animal-model' to demonstrate that 13% (0.8 days per generation) of the observed phenological changes in parturition date could be attributed to microevolution. However, since Réale *et al.* (2003b) did not account for systematic environmental variation across years, they actually may have overestimated the amount of genetic change (Postma, 2006). Thus, potentially more than 60% of the observed changes in parturition date of the squirrel could be attributed to phenotypic plasticity.

1.3 Phenotypic plasticity

By definition, phenotypic plasticity is the process by which a single genotype gives rise to more than one phenotype when exposed to different conditions (Via *et al.*, 1995; Pigliucci, 2005; Garland and Kelly, 2006). Phenotypic plasticity to a change in environment may invoke acclimatization, acclimation and learning (Garland and Kelly, 2006). Thus, unlike genetic adaptation, phenotypic plasticity allows an organism to respond rapidly to environmental change and is thus likely to be an organism's primary response to the rapidly changing environmental conditions predicted for the future. Although the red squirrel study provides the first quantification of the role of phenotypic plasticity in climate-induced development of a functional character (Réale *et al.*, 2003b), it is not the first to document changes in phenology, or the timing of seasonal events, in response to changing climatic conditions (Harrington *et al.*, 1999a; Hughes, 2000; Walther *et al.*, 2002). In a meta-analysis of nearly 700 species, spring phenology shifted between 2.3 days (Parmesan and Yohe, 2003) and 5.1 days (Root *et al.*, 2003) earlier on average per decade over the last 50 years.

Such phenological changes are often considered as evidence that species are adapting to the changing environmental conditions. For example, over nearly 50 years, during which springs have been warming, the laying dates of great tits (*Parus major*), near Oxford in the United Kingdom, have advanced by 14 days, a change closely matched to earlier peaks in food insect biomass, and attributed entirely to phenotypic plasticity (Charmantier *et al.*, 2008). However, unlike the great tits in Britain, those in the Netherlands have not advanced their phenology to match the peak in caterpillar availability (Visser *et al.*, 1998; Visser *et al.*, 2003). A similar mismatch between food abundance and offspring needs within the Dutch population of pied flycatchers (*Ficedula hypoleuca*) led to a 90% decline in the population over the last two decades (Both *et al.*, 2006). Thus species may show different rates of phenological change than the species on which they depend, potentially resulting in asynchrony or a mistiming of key ecological events (Hughes, 2000; Thomas *et al.*, 2001b; Visser *et*

al., 2004; Visser and Both, 2005). Numerous studies have demonstrated the ecological and metabolic costs of these mistimed ecological events (Thomas *et al.*, 2001b; Stenseth and Mysterud, 2002), which may ultimately lead to loss of biodiversity. One possible cause of such a mismatch is the different environmental cues to which different species respond (Voigt *et al.*, 2003). For example, whereas most plants and insects respond to seasonal changes in temperature, most vertebrate species are more sensitive to changes in photoperiod. Thus many vertebrates electing, or forced, to stay at the historic locations may face a mismatch between photoperiod and food availability, disrupting reproduction and migration, and those dispersing latitudinally will have to adjust to an unfamiliar annual cycle of photoperiod in their new habitat (Bradshaw and Holzapfel, 2008; Visser, 2008).

Species which are unable to match the timing of key life-history events to the phenology of the species on which they depend may be forced to show plasticity in other life-history traits if they are to maintain their lifetime reproductive success. For example, flexibility in phenology of the Antarctic fur seal (*Arctocephalus gazelle*) is likely to be limited because of a long interval between conception and weaning of the pups and a highly variable environment. Instead, female Antarctic fur seals appear to be adapting their life cycles by not breeding in years of low krill supply, thus increasing adult survival and fitness (Forcada *et al.*, 2008). Another species which is changing its life-history strategy in response to stochastic environmental conditions is the pronghorn antelope (*Antilocapra americana*). Frequent severe weather events result in an increase in male mortality, which favour precocial maturation in male pronghorn and may ultimately lead to a life-history strategy of faster development (Mitchell and Maher, 2006). Not only can severe weather events directly affect the life-history strategy of male antelope, but the climatic conditions to which a female is exposed during her pregnancy can also influence life-history traits of her offspring, the so-called “maternal effects” (Bernardo, 1996).

Body mass, for example, is a major determinant of lifetime reproductive success and numerous species of antelope in the Northern hemisphere display plasticity of offspring birth mass in response to changing climatic conditions (Stenseth *et al.*, 2002). The high costs of thermoregulation during wet and windy winters is proposed to compromise the condition of pregnant ewes, to the extent that individuals born after deep-snow winters are consistently smaller and have a lower lifetime reproductive success than those born after cold dry winters (Post *et al.*, 1997; Post and Stenseth, 1998; Forchhammer *et al.*, 2001). Although these maternal effects may promote survival and enhance reproductive success of the mother, such plasticity in birth mass has long-term consequences for the offspring. Like many morphological traits, body mass at birth is a "non-labile" trait as it is expressed only once in an individual's lifetime (Nussey *et al.*, 2007). The majority of "non-labile" traits are traits which show plasticity only during development. House sparrows, for example, show morphological plasticity during development and fledglings can alter the lipid composition of the outer layer of their skin, the stratum corneum, depending on the water vapour pressure of their environment (Muñoz-Garcia and Williams, 2008). Since the lipid composition of the stratum corneum affects the cutaneous water loss (Muñoz-Garcia and Williams, 2005), these fledglings are able to reduce their cutaneous water loss, and thus conserve body water, when exposed to dry desert environments. However, such "non-labile" plasticity can only be adaptive if the trends for changes in climatic conditions at the time of development remain similar throughout the individuals' lifetime.

Although understanding the physiological mechanisms underlying changes in life-history traits is essential for predicting responses to climate change (Visser and Both, 2005; Visser, 2008), the vast majority (> 80%) of studies of phenotypic selection have focused on morphological plasticity without incorporating physiological and behavioural responses to climate change (Kingsolver *et al.*, 2001). Such a preponderance may not reflect a true biological trend but rather represent the ease of recording of morphological features, like body mass. Gathering physiological and

behavioural data, on the other hand, is labour-intensive and requires long periods of observation and monitoring. Nevertheless, given that natural selection primarily works at the level of physiology and behaviour (Berteaux *et al.*, 2004) it is concerning that we understand so little about the direct links between a species' physiology and its vulnerability to climate change. We need to improve our understanding of the physiological mechanisms which determine an animal's thermal tolerance and its acclimatory capacity in order to better predict the impact of climate change on a particular species (McCarty, 2001; Stillman, 2003; Helmuth *et al.*, 2005; Calosi *et al.*, 2008).

1.3.1 Physiological adaptation

That physiological mechanisms are responsible for the capacity of animals to adjust to new environments sounds self-evident, but according to Carey (2005) the concept is just over 50 years old; she attributes it to Adolf (1956). In order to accurately predict the direct physiological effects of climate change on a species, two elements are required. Firstly, we need an understanding of the thermal physiological sensitivity of the species and how close the species is to its thermal limit. Secondly, we need an understanding of the relationship between climate and body temperature experienced by the species and the degree to which the species can adjust, or acclimatize, its thermal sensitivity (Stillman, 2003; Gilman *et al.*, 2006). Because of the clearly defined thermal niches which occur in the marine environment, the majority of studies which have investigated the physiological principals underlying thermal limits and thermal sensitivity have focused on marine ectotherms.

Marine ectotherms have contributed substantially to our understanding of the key mechanisms of thermal adaptations and limitations, including the finding that thermal tolerance windows are limited by the capacity of circulatory and ventilatory tissues (Pörtner, 2004). Because of similarities in the molecular and cellular mechanisms of oxygen supply, numerous animal phyla are likely to show similar limitations in their capacity for adaptation (Pörtner, 2001; Pörtner, 2002). Even endotherms remain

fundamentally, and potentially detrimentally, affected by temperature (Post and Stenseth, 1999; Porter *et al.*, 2000; Humphries *et al.*, 2004). With the temperatures predicted to occur in the future, endotherms may well reach their thermal limits. In fact, endotherms may be more sensitive than ectotherms to rising ambient temperatures, since endothermy evolved during cold climatic conditions (Pörtner, 2004) and enhanced organismic complexity is often accompanied by increased thermal sensitivity (Pörtner, 2002). Theoretically, ectotherm physiology is superior to that of endotherms in warm climates as they are able to stay active for longer in the heat (Martin and Nagy, 2002), therefore ectotherms will be less compromised by global warming than global cooling. For example, a warmer climate is proposed to favour the Australian nocturnal lizard (*Heteronotia binoei*) and by 2100 its range is predicted to expand by up to 170 km as a result of the increased number of degree-days for egg development and the increased potential time available for activity (Kearney and Porter, 2004). In addition, these lizards may well be able to buffer any adverse effects associated with the increased metabolic costs of high body temperatures through appropriate thermoregulatory behaviour (Kearney *et al.*, 2009), as nocturnal ectotherms are known to select thermally suitable sites within the soil profile (Kearney and Predavec, 2000).

Nevertheless, whether endothermic or ectothermic, thermal tolerance windows differ between species and are likely to determine the range of environmental temperatures under which species can survive (Pörtner, 2002). Generalist species, with large geographic ranges, wide thermal tolerance windows and greater physiological plasticity, are less likely to be affected by climate change than are species which are physiologically specialised with respect to temperature (Potts, 1996; Bale *et al.*, 2002; Visser and Both, 2005; Codron *et al.*, 2008). Indeed, terrestrial ectotherms in the tropics appear to be operating near the upper limit of their thermal range and are believed to be particularly sensitive to climate change because they are physiologically specialised with respect to temperature and have a limited acclimation capacity (Addo-Bediako *et al.*, 2000; Deutsch *et al.*, 2008; Tewksbury *et*

al., 2008). Similarly, of the 71 European bird species monitored during the 2003 French heat wave, those species with small thermal ranges showed the sharpest decrease in population growth (Jiguet *et al.*, 2006). Mammalian species with narrow thermal tolerances, such as tropical arboreal marsupials (Isaac *et al.*, 2009), also are likely to be adversely affected by climate change. For example, the white lemuroid possum (*Hemibelideus lemuroids*), a species endemic to the mountain forests of northern Queensland, cannot maintain its body temperature when exposed to heat and risks extinction as a result of the recent 0.8ºC increase in ambient temperature. Conversely, mammalian species with wide thermal tolerances, such as the hibernating mammalian species in the Canadian Arctic region (Humphries *et al.*, 2002; Humphries *et al.*, 2004), are predicted to show an increase in abundance and distribution in response to climate change. In summary, climate change is therefore predicted to favour species with wide thermal tolerances, short generation times and genotypic variability (Pörtner and Farrell, 2008).

Although studies on short-lived species have contributed substantially to our understanding of the importance of physiological plasticity, long-lived species are likely to respond differently to changes in environmental conditions. Long-term population growth models predict that, relative to long-lived species (such as perennial plants, birds, ungulates), short-lived species (such as insects and annual plants and algae) may be particularly vulnerable to the predicted increase in climatic variability because enough members of the species must successfully survive and reproduce each year (Morris *et al.*, 2008). However, short-lived species have the advantage of fast generation times, which may improve their chance of survival as they can undergo microevolution (Hendry and Kinnison, 1999; Hendry and Kinnison, 2001; Kinnison and Hendry, 2001; Berteaux *et al.*, 2004; Visser, 2008). Large-bodied species with long generation times are predicted to have less ability to respond genetically to new selective pressures (Rosenheim and Tabashnik, 1991), possibly making them more susceptible to extinction (Gomulkiewicz and Holt, 1995), than species with short generation times. Indeed, numerous studies have demonstrated that

large mammalian species are usually more vulnerable to extinction than are smaller species because of slower reproductive rates and greater range requirements (Cardillo *et al.*, 2005). There are many species of mammals with longevity sufficient such that individuals alive now ought still to be alive in 2030, and a few species for which individuals alive now could be alive in 2100. Clearly, the survival of those individuals, and probably those species, cannot depend on genetic adaptation. Instead these species are likely to be entirely dependent on physiological plasticity to survive climate change, because, unlike developmental plasticity, physiological plasticity is "labile" and has the advantage of changing numerous times within an individuals' lifespan.

One example of a long-lived mammalian specialist which is likely to be severely affected by climate change is the polar bear (*Ursus maritimus*). Without a radical change in their behaviour, the future survival of polar bears is considered bleak (Berteaux *et al.*, 2004). They are heavily dependent on Arctic spring ice, because that is where they discover the seals (on ice to give birth) that are their primary food source at this time (Derocher *et al.*, 2004; Stirling and Parkinson, 2006). The Arctic ice is disappearing under the impact of global warming, and, if they continue with their current lifestyle, the world population of polar bears is likely to drop by two-thirds by 2050 (Amstrup *et al.*, 2007). Polar bears may well survive if they have the capacity to make a major change in lifestyle (which the fossil record shows they have done previously), namely to abandon the ice, and their current food source, and to become land-based (Derocher *et al.*, 2004). An Arctic species which is already showing such plasticity in behaviour is the humpback whale. Some humpback whales (*Megaptera novaeangliae*) are abandoning their migration habits and remaining in southeast Alaska throughout winter, seemingly in response to the availability of herring (Moore and Huntington, 2008). Although there is likely to be an energetic cost of thermoregulation in the cold waters, such costs could be offset by metabolic savings of not having to undertake one of the longest documented mammalian migrations (Rasmussen *et al.*, 2007).

1.3.1.1 Artiodactyls

An order of long-lived mammals which may be pre-adapted to the future hot and dry conditions predicted for large regions of Africa is the Artiodactyla. These even-toed ungulates have been evolutionarily successful and hugely speciose, with 90 extant genera. The artiodactyls evolved during the Eocene and speciated during a warming period in the Mid-Miocene Climatic Optimum (Barnosky *et al.*, 2003). Since these taxa evolved under hot and dry conditions of the past, and the first appearance of an artiodactyl correlates with the onset of such a warming trend, most artiodactyls are likely to be adapted to warmer and drier environments than they currently experience (Barnosky *et al.*, 2003; Mitchell and Lust, 2008). In fact, when Burns *et al.* (2003) modelled 213 mammalian species losses for national parks in the United States in response to climate change, the Artiodactyla were the only order in which no species losses were predicted. Although such a prediction may be because artiodactyls represented a small percentage (6.7%) of the total current species found within the parks, it may be that these species are better adapted to changing climatic conditions.

Traditionally, the success of artiodactyls has been attributed to improved locomotion and the evolution of a ruminant digestive tract, which enabled artiodactyls to use widely dispersed forage with a high fibre content (Janis, 1989; Codron *et al.*, 2008). More recently, however, it has been suggested that the current prevalence of artiodactyls could be attributed to the evolution of the carotid rete (Mitchell and Lust, 2008). The carotid rete is an anatomical structure consisting of an intertwining network of arteries which lies within a venous sinus at the base of the brain. Cool venous blood from the nasal mucosa drains into the sinus and cools the arterial blood destined for the brain, resulting in selective brain cooling (Baker, 1982; Mitchell *et al.*, 1987). Selective brain cooling is defined as the reduction of brain temperature below arterial blood temperature (Fig. 1.2, IUPS Thermal Commission, 2003). Although selective brain cooling originally was believed to protect the brain from a rise in body temperature (Mitchell *et al.*, 1987), recent literature proposes that

artiodactyls may employ selective brain cooling to attenuate the drive for evaporative cooling. Selective brain cooling results in a reduction in hypothalamic temperature, which reduces evaporative heat dissipation and ultimately conserves water (Jessen *et al.*, 1994; Mitchell *et al.*, 1997; Fuller *et al.*, 1999; Maloney *et al.*, 2002; Mitchell *et al.*, 2002). Such a strategy would have obvious advantages in an environment where water resources are both limited and variable (Mitchell *et al.*, 2002).

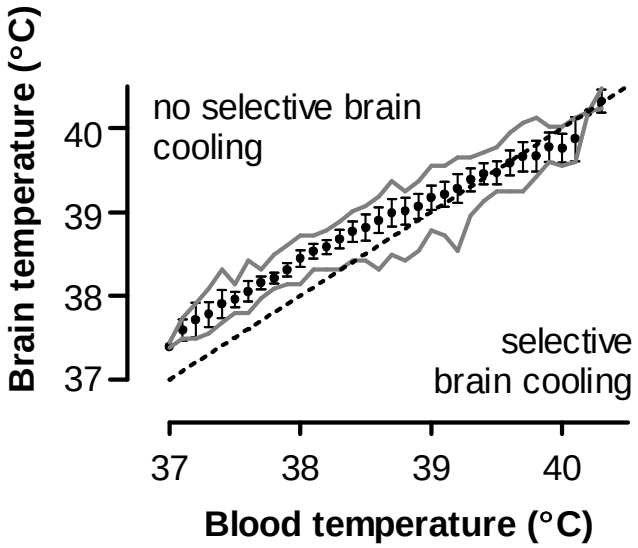

Figure 1.2. Mean brain temperature at each 0.1°C category of arterial blood temperature for a single oryx (*Oryx gazella*) over a time period of 15 days. Each point shows mean ± SD. The lines directly above and below the data points represent the maximum and minimum, respectively, recorded brain temperature for each category of blood temperature. The dashed diagonal line marks the line of identity between blood and brain temperatures; all points below the line represent periods of selective brain cooling (adapted from Maloney *et al*., 2002).

If selective brain cooling does indeed increase body temperature by attenuating thermal drive for evaporative heat loss, it may contribute to another physiological mechanism, heterothermy. Adaptive heterothermy, like selective brain cooling, is proposed to reduce evaporative cooling by desert ungulates. It is defined as the storage of body heat during the day, with a consequent rise in body temperature, to reduce both heat gain and evaporative heat loss. This stored heat then can be dissipated non-evaporatively during the colder night, when heat conservation and

generation is also suppressed, allowing body temperature to fall so that the scope for heat storage on the day is increased (Fig. 1.3). By actively widening the thermoregulatory dead band under extreme conditions, animals may be able to reduce the energetic costs of thermoregulation (Dawson, 1992) and such a strategy is likely to become increasingly important as conditions get hotter and drier with climate change.

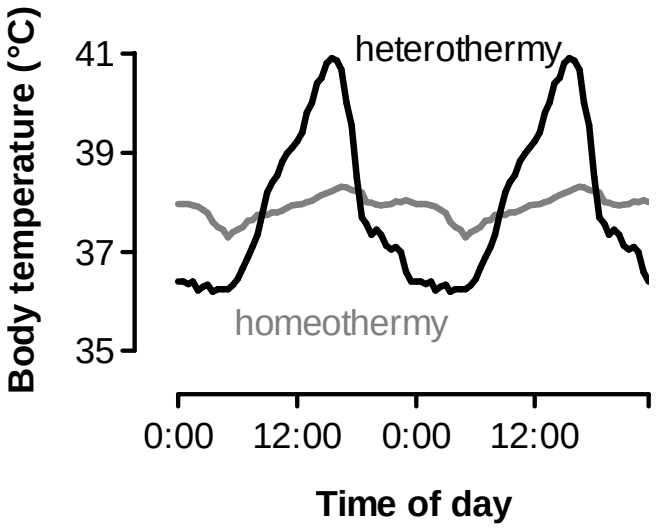

Figure 1.3. Body temperature rhythm of a well-hydrated (grey line) and dehydrated (black line) camel over a two day period. The well-hydrated camel displayed greater homeothermy than did the dehydrated camel, which displayed an increased maximum and decreased minimum body temperature with a resultant increase in daily body temperature fluctuation. Such large fluctuations of body temperature of about 6ºC, without a major change in mean 24h temperature, have been defined as adaptive heterothermy and heat stored as a result of the large increase in body temperature corresponds to a saving of nearly 5 L of water (adapted from Schmidt-Nielsen, 1957).

Yet, the presence of adaptive heterothermy rarely is evident in large free-ranging mammals exposed to hot arid environments, leading many authors to argue that it may be an artefact associated with laboratory animal studies (Finch, 1972; Hofmeyr and Louw, 1987; Mitchell *et al.*, 1997; Fuller *et al.*, 2000; Maloney *et al.*, 2002; Mitchell *et al.*, 2002). Table 1.1 summarises the findings of numerous studies of body temperatures in unrestrained artiodactyls over the last 50 years.

Table 1.1. Body temperatures of unrestrained wild artiodactyls

Species	Mass (kg)	Ambient temperature (ºC)	Body temperature (ºC)				Body site	Location	Reference
			mean	minimum	maximum	amplitude			
Arabian oryx (*Oryx leucoryx*)	88-100	29–44	38.4±1.3	36.5±1.2	40.5±0.7	4.1±1.7	abdominal	Saudi Arabia	(Ostrowski *et al.*, 2003)
		13–27	38.4±1.3	37.5±0.5	39.2±0.3	1.5±0.6	abdominal	Saudi Arabia	(Ostrowski *et al.*, 2003)
Arabian sand gazelle (*Gazella subgutturosa marica*)	12-20	25-41	39.5±0.3	38.2±0.5	40.8±0.4	2.6±0.8	abdominal	Saudi Arabia	(Ostrowski and Williams, 2006)
		11-26	39.4±0.2	38.6±0.3	40.3±0.3	1.7±0.3	abdominal	Saudi Arabia	(Ostrowski and Williams, 2006)
Black wildebeest (*Connochaetes gnou*)	130	16-31	38.6±0.0	37.0	39.7	0.8	carotid artery	South Africa	(Jessen *et al.*, 1994)
Blue wildebeest (*Connochaetes taurinus*)	120-250	-	39.3±0.2	38.4	39.8	0.9±0.1	abdominal	South Africa	(Fick *et al*., 2006)

Table 1.1. Continued

Species	Mass (kg)	Ambient temperature (ºC)	Body temperature (ºC)				Body site	Location	Reference
			mean	minimum	maximum	amplitude			
Boran cattle (*Bos indicus*)	-	-	-	36.1	39.8	-	deep muscle	Kenya	(McGinnis *et al.*, 1970)
Buffalo (*Syncerus caffer*)	350	10-25	38.7	36.9	40.1	-	lower neck tissue	Kenya	(Bligh and Harthoorn, 1965)
Camel (*Camelus dromedarius*)	450-500	21-35.5	37.1±0.4	35.1	39.1	-	hump tissue	Kenya	(Bligh and Harthoorn, 1965)
Collared Peccary (*Tayassu Tajacu*)	12-30	27-44	-	37.5	40.9	-	vaginal	AZ (USA)	(Zervanos and Hadley, 1973)
		4-18	-	37.5	40.3	-	vaginal	AZ (USA)	(Zervanos and Hadley, 1973)

Table 1.1. Continued

Species	Mass (kg)	Ambient temperature (ºC)	Body temperature (ºC)				Body site	Location	Reference
			mean	minimum	maximum	amplitude			
Eland (*Tragelaphus oryx*)	200	14-27	39.1	38.3	39.9	-	neck and back tissue	Uganda	(Bligh and Harthoorn, 1965)
	330-380	6-27	38.2±0.1	37.1	39.4	2.3±0.4	arterial blood	Namibia	(Fuller *et al*., 1999)
	-	-	-	37.0	40.8	-	deep muscle	Kenya	(McGinnis *et al.*, 1970)
Gemsbok (*Oryx gazella*)	120-180	15-30	38.8±0.1	37.0	40.5	1.8±0.3	carotid artery	South Africa	(Maloney *et al.*, 2002)
	150	12-26	38.5	36.6	40.0	-	lower neck tissue	Kenya	(Bligh and Harthoorn, 1965)
Giraffe (*Giraffa camelopardalis*)	400	10-22	38.5	37.8	39.1	-	lower neck tissue	Kenya	(Bligh and Harthoorn, 1965)
Kongoni (*Alcelaphus buselaphus cokei*)	-	-	-	37.3	39.7	-	deep muscle	Kenya	(McGinnis *et al.*, 1970)

Table 1.1. Continued

Species	Mass (kg)	Ambient temperature (ºC)	Body temperature (ºC)				Body site	Location	Reference
			mean	minimum	maximum	amplitude			
Mule deer (*Odocoileus hemionus*)	-	11-31	37.6	36.2	42.1	-	deep muscle	Wyoming (USA)	(Thorne, 1975)
Pronghorn antelope (*Antilocapra americana*)	40-50	2-38	38.6±0.3	36.4±0.8	39.5±0.7	3.1±0.4	carotid artery	Wyoming (USA)	(Lust *et al*., 2007)
	-	-7.3-4.5	38.5±0.4	37.8±0.2	39.7±0.7	1.8±0.7	carotid artery	Wyoming (USA)	(Hebert *et al.*, 2008)
	-	11.7-30.5	37.6	36.2	42.2	-	deep muscle	Wyoming (USA)	(Thorne, 1975)
	-	5-22	38.5	-	-	0.3	flank muscles	Wyoming (USA)	(Lonsdale *et al.*, 1971)
	-	-33-46	-	37.5±0.1	39.3±0.3	-	abdominal	WA (USA)	(Sargeant *et al.*, 1994)

Table 1.1. Continued

Species	Mass (kg)	Ambient temperature (ºC)	Body temperature (ºC)				Body site	Location	Reference
			mean	minimum	maximum	amplitude			
Springbok (*Antidorcas marsupialis*)	25-30	6-31	39.2±0.4	37.6	40.2	1.6	carotid artery	South Africa	(Mitchell *et al.*, 1997)
	20-35	10.0±2.1	39.2±0.1	38.6±0.1	39.9±0.2	1.2±0.2	abdominal	South Africa	(Fuller *et al.*, 2005)
	20-35	15.8±3.7	39.4±0.2	38.9±0.2	40.0±0.2	1.2±0.3	abdominal	South Africa	(Fuller *et al.*, 2005)
	20-35	17.6±2.8	39.4±0.1	38.9±0.1	40.0±0.2	1.1±0.2	abdominal	South Africa	(Fuller *et al.*, 2005)
	20-35	20.2±2.2	39.5±0.1	39.0±0.1	40.1±0.2	1.1±0.1	abdominal	South Africa	(Fuller *et al.*, 2005)

Body site = site at which body temperature was measured

Location = locality where the study was conducted

In general, those species that were exposed to high ambient temperatures displayed a larger amplitude of nychthemeral rhythm of body temperature. Using the data represented in Table 1.1, I found a weak positive correlation between body temperature amplitude and mean ambient temperature ($r^2 = 0.35$, $P = 0.02$, Fig. 1.4). Yet, despite such a relationship and a large variation in ambient conditions between the studies, only the Arabian oryx (*Oryx leucoryx*) displayed an increase in the amplitude of the nychthemeral rhythm of body temperature consistent with the definition of adaptive heterothermy (Ostrowski *et al.*, 2003). Heterothermy is proposed to be an effective water saving mechanism only for large artiodactyls (Taylor, 1970a; Mitchell *et al.*, 2002), but I found no correlation between body temperature amplitude and body mass ($r^2 = 0.04$, $P = 0.50$).

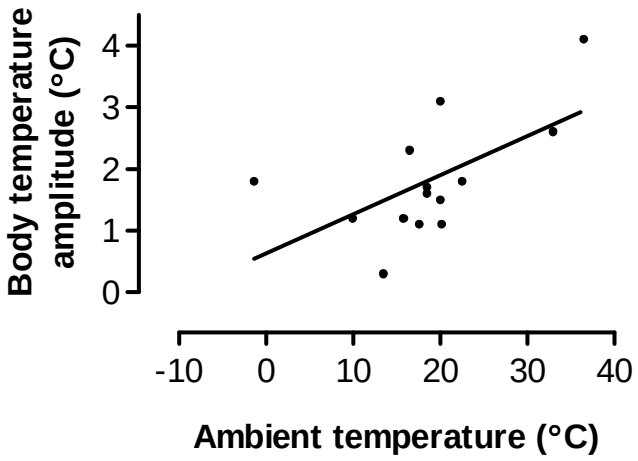

Figure 1.4. Correlation of the amplitude (the difference between maximum and minimum) of the nychthemeral pattern of body temperature versus the mean or mid-point of the range of ambient temperature over the study period (n = 14). Since the correlation was significant ($r^2 = 0.35$, $P = 0.02$), I fitted a linear regression ($y = 0.06x + 0.6$), where y = amplitude of body temperature rhythm and x = ambient temperature.

1.3.1.2 Thermoregulatory trade-offs

Since body temperature can be measured more easily than many other variables under field conditions, it may provide a convenient measure of how changes in environmental factors impact an animal's circadian rhythms (Cable *et al.*, 2007). The temperature-regulating system of artiodactyls, like that of other homeotherms, functions to buffer internal body temperature against fluctuating environmental

temperatures by controlling heat production and heat loss. When an organism is in thermal equilibrium with its surroundings, metabolic heat together with heat absorbed from the environment must equal heat loss by radiation, convection, conduction and evaporation (Finch, 1972). Thermoregulation thus involves modifying rates of heat transfer between the core and the environment through a combination of autonomic nervous system, somatic nervous system, endocrine system or behavioural responses.

Autonomic responses include polypnea (increased breathing rate, through panting), sweating (increases cutaneous evaporative water loss), vasomotor responses (alter local cutaneous blood or heat flow), ptilo- or pilomotor responses (alter the relative heat flow by controlling body hair erection, (Hammel, 1968; Bakken *et al.*, 1985). Autonomic thermoregulation also involves modifying the level of heat generation either through somatic neural responses (shivering) or endocrine (hormonal) responses. All of the mechanisms involved in the maintenance of homeothermy may disrupt the balance of other homeostatic systems. For example shivering is energetically expensive, whereas panting and sweating threaten to disrupt the osmotic and acid-base homeostasis. For species which are dependent on evaporative cooling to dissipate environmental heat, water limitations may severely compromise such a thermoregulatory strategy and animals may be forced to trade off thermoregulation, osmoregulation and acquisition of energy. Since warming trends are predicted to be accompanied by reduced precipitation and increased frequency of droughts, both in the tropics and in the Southern Hemisphere in general, including most of Africa and Australia, species inhabiting these regions are likely to have to make such trade-offs. The high cost of autonomic thermoregulation, particularly in an environment with inadequate water supplies, may result in an animal preferentially using behavioural thermoregulation (Bustamante *et al.*, 2002).

Free-living animals rely less on autonomic thermoregulation than do animals confined to a laboratory, as they have greater access to thermoregulatory behaviour. Behavioural thermoregulation, and not autonomic adaptation, is likely

to act as an animal's primary defence to adverse changes in the environmental heat load. Behavioural responses involve the co-ordinated activity of the whole animal in selecting or creating a microenvironment in which the optimal internal temperature can be achieved without, or with reduced, assistance from autonomic responses (Hammel, 1968). Terrestrial animals, because of their mobility and capacity for complex behaviours, can behaviourally select and use an array of environmental conditions, thus creating their own microenvironment (Bartholomew, 1964; Bartholomew, 1987). Desert artiodactyls, for example, are proposed to be able to maintain temperature and water balance through appropriate microclimate selection, body orientation and by restricting daily activities to time periods that reduce daily heat loads and water loss (Cain *et al.*, 2008). Since it is this microclimate selected by the individual, not the macroclimate, that has a direct influence on an animal's thermal status (Hodkinson, 1999; Porter *et al.*, 2000; Porter *et al.*, 2002), these microclimates need to be quantified and incorporated into climate change models to better predict the future survival and distribution of species.

Behaviour is thus a rapid, extremely flexible and precise mechanism, which may enhance an animal's performance, and presumably its fitness, by incorporating both morphological and physiological characteristics in a variety of ways to optimise body temperature homeostasis (Bartholomew, 1964; Bartholomew, 1987; Huey *et al.*, 2003). Since behavioural changes are generally less costly than autonomic responses, behavioural adjustments are likely to be an animal's most suitable defence against environment change (Huey and Tewksbury, 2009). However, to date, only two models have evaluated the role of behavioural thermoregulation in buffering the impact of climate change (Mitchell *et al.*, 2008; Kearney *et al.*, 2009), but whether such behavioural adjustments are actually occurring in free-living species remains to be investigated.

Nevertheless, implementing behavioural thermoregulation may involve its own costs. For example, a habitat selected purely based on its thermal properties may have an increased risk of predation, parasites, competition, as well as a decreased

resource availability, including energy, mates, food and water (Bartholomew, 1964; Huey, 1991; Bakken, 1992). Thus, in an extreme thermal environment, such as that predicted by climate change, heat loss mechanisms may be severely compromised, and obligatory thermoregulatory behaviour may compromise these inferential functions. The moose (*Alces alces*) provides an example of the potential negative costs associated with behavioural thermoregulation. In the last 20 years, the moose population in Minnesota has halved and the population in the Isle Royale National Park has declined by 75%. Moose are particularly sensitive to heat and seek shelter when ambient temperatures exceed 14ºC (Dussault *et al.*, 2004). Over the last 40 years average summer temperatures have increased by 2ºC and moose have had to forfeit valuable foraging time as they are forced to become lethargic and immerse themselves in water in an attempt to stay cool. Such behaviour leads to malnutrition and decreases their fat reserves, which are essential for winter survival. This malnutrition is likely to increase their risk of succumbing to parasites, disease and predation by wolves, all factors which are believed to have contributed to the recent decline in the moose population (Thompson *et al.*, 1998; Murray *et al.*, 2006). With further increases in summer temperatures predicted for the future, it seems likely that the moose will be extirpated from its historic southern range within the next 50 years (Murray *et al.*, 2006).

1.3.2 Future applications

Though we know so little about it, it will be on their physiological phenotypic plasticity that the future of long-lived mammals, threatened by global warming, will depend. Many authors explicitly extend that position to behavioural plasticity, distinct from physiological plasticity, but in this context I consider behaviour to be one element, and a crucial one, of the repertoire of physiological responses available to animals to adapt to climate change (Bartholomew, 1964; Meyers and Bull, 2002; Huey *et al.*, 2003; Gilmour *et al.*, 2005). Physiological mechanisms are responsible for the capacity of animals to adjust to new environments (Feder and Block, 1991; Carey, 2005) and are fundamental to predicting the consequences of climate change (Helmuth *et al.*, 2005). Physiologists thus have

an important role to play in uncovering the mechanisms employed by animals to counter extinction and cope with climate change.

Future climate change research will require measurement of physiological characteristics of many identified individual animals for long periods, probably decades (Berteaux *et al.*, 2004; Visser, 2008). Since the responses to climate change are likely to be multifaceted responses to complex interrelated stresses, the approach will have to be that of field physiology (Costa and Sinervo, 2004), namely investigation of the mechanisms that an animal uses to maintain homeostasis in its everyday life. The more-traditional technique of characterising habitats in the field, but studying physiological responses in the laboratory (Huey, 1991), identifies what an animal can achieve physiologically, but not what it actually will do, free-living in its natural habitat, as has been demonstrated, for example, by field studies of how arid-zone antelope use selective brain cooling (Mitchell *et al.*, 2002) or how elephant shrews use torpor (Mzilikazi and Lovegrove, 2004).

The studies required fall within the sub-disciplines of conservation physiology (Carey, 2005; Wikelski and Cooke, 2006) and evolutionary physiology (Feder *et al.*, 2000). The growth of these sub-disciplines has resulted not just from the clear need for such an approach, but from the growing availability of suitable technology, such as the use of stable isotopes for field measurement of metabolic rate and water turnover, and osmotic minipumps to deliver substances to free-living animals (Goldstein and Pinshow, 2006). The primary new technology, however, has been biotelemetry or biologging (Andrews, 1998; Cooke *et al.*, 2004; Block, 2005; Wikelski and Cooke, 2006). Physiological variables such as dive behaviour and prey ingestion of marine mammals, as well as body temperature, activity and energetic expenditure of terrestrial mammals, now can be measured routinely in free-living animals. The studies will require long-term monitoring of physiological variables of animals going about their daily business in their natural habitat. Moreover, because the physiological mechanisms that animals employ in the field are confounded by the presence of human observers (Mitchell *et al.*,

2002) it is not just autonomic responses that will have to be measured by biotelemetry, but behavioural responses too. It is now possible, for example, to monitor microclimate selection by biotelemetry (Appendix 1, Hetem *et al.*, 2007). Such long-term monitoring studies also would benefit from availability of suitable equipment for sampling blood in the absence of human observers, as described 20 years ago (Hattingh *et al.*, 1988), but still seldom implemented.

1.4 Thesis aims

The general aim of the research described in my thesis was to investigate the phenotypic plasticity which may enable long-lived species to cope with climate change. I focused on the physiological plasticity of artiodactyls, which, because of their evolutionary past and the development of the carotid rete, may be particularly good at adapting to hot and arid conditions. It will become increasingly important to understand the physiological mechanisms employed by artiodactyls to adapt to arid regions so that we may better monitor and predict species responses to the increased aridity anticipated for large regions of Africa. Secondly, my thesis aims to investigate whether remote sensing technology is sensitive enough to detect physiological plasticity, such as changes in body temperature and activity. If such technology proves to be successful in detecting physiological changes in the short term it will provide confidence and motivation to extend such studies from their current durations of about a year (for example, Mzilikazi and Lovegrove, 2004; Fuller *et al.*, 2005; Gremillet *et al.*, 2005) to that of decades.

Instead of the typical modelling approach, I used an empirical or experimental approach to investigate the physiological mechanisms employed by artiodactyls under circumstances that exist today but at the extremes, and in the direction in which climate change is moving. I chose to investigate whether morphology variations, such as difference in pelt colouration and body size, have physiological consequences. Morphological changes are believed to have constituted the first response of mammals to climate change events of the past (Barnosky *et al.*, 2003)

and, because of the ease of recording of morphological features, the vast majority (>80%) of studies of phenotypic selection have focused on morphological plasticity (Kingsolver *et al.*, 2001). Yet, such morphological plasticity can only be adaptive if it results in a change in function, a condition seldom investigated.

In addition, I chose to address issues such as how artiodactyls at the current upper end of hot environments cope and the physiological consequences of desertification. Habitat transformation, in the form of desertification, will be a consequence of changing climatic conditions and there is debate about the relative detrimental effects of climate itself and its consequences for habitat. Currently, 46% of the sub-humid and semi-arid regions of southern Africa are vulnerable to desertification and, because of an increased water stress predicted for the future, the proportion of arid and semi-arid regions in Africa is predicted to increase by 5-8% by 2080 (Boko *et al.*, 2007). I therefore investigated whether similar physiological responses were exhibited by artiodactyls inhabiting one of the most stressful of current hot and hyper-arid environments, the deserts of Saudi Arabia, and those artiodactyls, which are increasingly exposed to desertified landscapes in South Africa.

Finally, the physiology studies required to better understand the mechanisms of phenotypic plasticity underlying responses to climate change cannot be confined to the function of healthy animals. Infectious pathogens have plagued animals and plants throughout their evolutionary history and have periodically caused population declines and extinctions (Wilson, 1994). According to Lochmiller and Deerenberg (2000), “immunological competence could very well be the most important determinant of life-time reproductive success and fitness for many species”. Climate change will not only bring emergent pathogens with the relocation of arthropod vectors (Rogers and Randolph, 2000; Olwoch *et al.*, 2003; Cumming and van Vuuren, 2006), but may also disrupt historically stable relationships between co-existing pathogens and their existing hosts, thus increasing mortality risks (Munson *et al.*, 2008). So conservation physiology, in the context of climate change, will need to include the study of innate immunity

and sickness behaviour, the physiological processes which animal hosts use to contend with novel pathogens (Wingfield, 2003; Carey, 2005). Yet, very little is known about the immune responses and sickness behaviours of free-living artiodactyls.

Chapter 2. Previous climate change events have resulted in changes in morphology and many studies have documented morphological changes with recent climate change. Yet, there is uncertainty about whether changes in morphology are phenotypic or genotypic. If morphological changes are genotypic, the unprecedented rate of climate change predicted to occur over the next 50 years may negate any mitigation effects of morphological adaptation, particularly for long-lived species. On the other hand, phenotypic plasticity in a morphological trait would hypothetically provide potential for long-lived species to adapt to changing climatic conditions. However, whether phenotypic or genotypic, changes in morphology are moot unless they result in a change in function. Few studies to date have investigated the physiological consequences of morphological variation. Natural variation in hair colour of springbok (*Antidorcas marsupialis*) provides an ideal opportunity to investigate the thermoregulatory advantage of a morphological trait. Anecdotal reports, mainly from game ranchers in the Eastern Cape, suggest that the black springbok are better able to survive cold winter spells than the other springbok colour morphs. If these anecdotal accounts are true, pelt colour may prove to be a functional variable capable of compensating for potential detrimental effects of climatic change. I therefore set out to investigate the effect of pelt colouration on the thermoregulation of springbok free-living in the arid Karoo. If colour variation does indeed have thermoregulatory consequences, certain colour morphs may be selected for in the future, and colour variation within a population may provide an important pre-adaptation to climate change.

Chapter 3. The deserts of Saudi Arabia represent one of the most stressful of the current hot and hyper-arid environments and, as such, provide an ideal environment in which to study the physiological mechanisms of species that have

adapted to that extreme, an extreme likely to become more prevalent under future climate change. The Arabian oryx inhabits this hot desert environment and is reputedly the most arid adapted of all antelope. It survives most of the year, including the hottest summer months, without access to drinking water. As such, the Arabian oryx provides an ideal species in which to investigate water conservation strategies. By studying the physiological mechanisms by which Arabian oryx compensate for limited water and extreme heat, I aim to gain a better understanding of the kind of physiological mechanism that other artiodactyls may employ to adapt to changing climate conditions. Since many arid regions of Africa are predicted to get both hotter and drier with climate change, understanding the physiological mechanism exhibited by this desert antelope would assist future studies in monitoring physiological adaptations to desertification. I therefore measured body temperature, activity and microclimate selection of free-living Arabian oryx occupying a hyper-arid desert environment, for a one-year period, to ascertain the importance of, and the potential interactions between, reputed water-saving mechanisms.

Chapter 4. One of the mechanisms employed by the Arabian oryx to cope with their hyper-arid environment is heterothermy. Heterothermy is proposed to be a more effective water saving mechanism for large mammals than for small mammals, since larger mammals have greater thermal inertia and also because, within a given time, large mammals would gain relatively less heat from the hot environment (McNab, 1983; Phillips and Heath, 1995) and generate less metabolic heat per gram of tissue than would small mammals (Taylor, 1970a; Mitchell *et al.*, 2002). Climate change is predicted to favour smaller individuals and numerous species of endotherms have exhibited decreased body mass in response to recent increases in ambient temperature. Yet, it is unknown whether different sized artiodactyls employ different thermoregulatory mechanisms to adapt to hot and dry environments. I therefore set out to compare thermoregulatory mechanisms of a large and small desert-adapted antelope, namely the small Arabian sand gazelle (~ 15 kg) and the larger Arabian oryx (~ 70 kg), inhabiting the same hot hyper-arid environment. Although the smaller

gazelles may have the advantage of smaller resource requirements and greater access to refuge sites than do the larger oryx (Ostrowski and Williams, 2006), they are likely to be disadvantaged by a high mass-specific metabolic rate, high water turnover and less capacity to store heat (Bartholomew, 1964; Taylor, 1970a). I therefore compared the body temperature and refuge selection of the sand gazelle and oryx living free in the same desert environment, at the same time.

Chapter 5. One of the consequences of climate change will be habitat transformation and there is debate about the relative detrimental effects of the climate itself and its consequences for habitat. I therefore set out to investigate the effects of habitat transformation for an arid-adapted artiodactyl. I chose Angora goats as my study species, because, unlike antelope, they are docile and amenable to experimental manipulation. Goats have a wide geographical distribution, surviving in a range of diverse climates and topographies. They have been able to colonise and thrive in some of the most extreme environments in the world (Shkolnik and Choshniak, 1987). Angora goats are particularly well suited to low rainfall areas (Norton and Deery, 1985) and are extensively farmed in the semi-arid regions of the Eastern Cape, helping to put South Africa on the map as being one of the biggest mohair producers worldwide. Since the mid 1800s Angora goat farming has put pressure on the natural resources, resulting in severe habitat transformation in some areas of the Eastern Cape. Such transformation of the natural flora has been classified as desertification, or dryland degradation, and is the result of overgrazing due to human mismanagement of agricultural and pastoral resources (Dean *et al.*, 1995; Kerley *et al.*, 1995). This dryland degradation as a result of overgrazing is likely to test Angora goats' ability to withstand both heat and water stress. Since the desertification within the semi-arid regions of the Eastern Cape succulent thicket habitat mimics at least some of the future consequences of climate change described earlier, it provides an appropriate location at which to analyse potential physiological consequences of habitat transformation. I therefore investigated the effect of habitat transformation, in the form of desertification, on the body temperature of Angora goats inhabiting transformed and intact sites in the Eastern Cape. The transformed and intact sites I

selected were positioned adjacent to each other, thus reducing the potential confounding variables associated with geographic location and climate.

Chapter 6. Since climate change is predicted to bring emergent pathogens, particularly with the relocation of arthropod vectors (Rogers and Randolph, 2000; Olwoch *et al.*, 2003; Cumming and van Vuuren, 2006), studies of phenotypic plasticity cannot be confined to the function of healthy animals. We need to understand the physiological mechanisms employed by free-ranging antelope to contend with novel pathogens. A herd of free-living antelope (kudu, *Tragelaphus strepsiceros*), which I had instrumented with temperature and activity data loggers for other purposes, acquired a spontaneous infection. I was able to extract quantitative data reflecting the kudu's autonomic and behavioural responses to the infection and, for the first time, show how a sick free-living artiodactyl responds to its thermal environment.

CHAPTER 2

Morphological adaptation to climate change: the effect of pelt colour on body temperature and thermoregulatory behaviour of springbok (*Antidorcas marsupialis*)

Data and ideas presented in this chapter have been published in the paper: Hetem R.S., de Witt B.A., Fick L.G., Fuller A., Kerley G.I.H., Meyer L.C.R., Mitchell D. and Maloney S.K. (2009) Body temperature, thermoregulatory behaviour and pelt characteristics of three colour morphs of springbok (*Antidorcas marsupialis*). *Comparative Biochemistry and Physiology, A: Molecular and Integrative Physiology* **152**: 379-388.

2.1 Abstract

Using intra-abdominal miniature data loggers, with the help on my colleagues, I measured core body temperature in female springbok (*Antidorcas marsupialis*) of three colour morphs (black, normal and white), free-living in the Karoo, South Africa, for one year. During winter, white springbok displayed lower daily minimum body temperatures (37.4 ± 0.5°C), than did both black (38.1 ± 0.3°C) and normal (38.0 ± 0.6°C) springbok. During spring, black springbok displayed higher daily maximum body temperatures (40.7 ± 0.1°C) than both white (40.2 ± 0.2°C) and normal (40.2 ± 0.2°C) springbok. These high maximum body temperatures were associated with larger daily amplitudes of nychthemeral rhythm of body temperature (2.0 ± 0.2°C), than that of white (1.6 ± 0.1°C) and normal (1.7 ± 0.2°C) springbok. Biophysical properties of sample springbok pelts were consistent with these patterns, as the black springbok pelt showed lower reflectance in the visible spectral range, and higher heat load from simulated solar radiation, than did the pelts of the other two springbok. Black springbok had lower diurnal activity in winter, consistent with them having to forage less because their metabolic cost of homeothermy was lower, but were disadvantaged in hot periods. White springbok, by contrast, were more protected from solar heat load, but potentially less able to meet the energy cost of homeothermy in winter. Thus energy considerations may underlie the rarity of the black and white springbok colour morphs.

2.2 Introduction

Both lay opinion and early physiological analysis associated the colour and structure of an animal's pelt with adaptation to the thermal environment (Finch, 1972). Thus, animals may adapt to local conditions and changing climates by displaying variation in colour (Millien *et al.*, 2006). Yet, the thermoregulatory consequences and adaptive significance of colour remains tentative since the thermal function for pelt colour can be confounded by other pressures, such as the requirement for concealment or communication (Cowles, 1967; Cloudsley-

Thompson, 1979; Stoner *et al.*, 2003; Caro, 2005; Caro, 2009). The natural variation in hair colour of springbok (*Antidorcas marsupialis*) provides an attractive opportunity to investigate the thermoregulatory consequences of pelt colour. There are three springbok colour morphs, commonly referred to as normal, white, and black springbok (Fig. 2.1), which show no taxonomic differences. The length, texture and thickness of hairs making up the pelt are the same (Kruger *et al.*, 1979) and so it might be expected that the morphs are similarly insulated, while varying in colour.

The familiar normal springbok of southern Africa, with a pale ventral surface, white face and rump, and a buff back, is an arid-adapted species (Estes, 1991; Skinner and Louw, 1996, see Fig. 2.1). Its colouration is proposed to have thermoregulatory importance, dependent on the assumption that the white colouration of the face and rump reflects solar radiation, thus reducing solar heat load when an animal orientates parallel to incident solar radiation, and that the darker sides absorb solar radiation, so that an animal can enhance solar heat gain by orientating perpendicular to the sun (Hofmeyr and Louw, 1987). The far less common black springbok and white springbok have the same body conformation as the normal springbok, and apparently differ only in hair colour. Black springbok are predominantly a dark dull chocolate brown, whilst white springbok are predominantly a dusty white colour (Kruger, 1976; Kruger *et al.*, 1979). Genetic studies on black and white springbok have failed to reveal the pattern of inheritance of the unusual colour morphs, though some believe that the normal colouration results from a double recessive combination (Kruger, 1976). White springbok are known to have occurred naturally, albeit at very low frequencies (Roche, 2005; Skead, 2007). The black colour morph was not recorded historically, and appears to have arisen through inbreeding of an enclosed population on a farm in the Murraysburg district of South Africa in the 1950s (Skead, 2007). Game ranchers have increased the relative abundance of black and white springbok through selective breeding (Skinner and Louw, 1996).

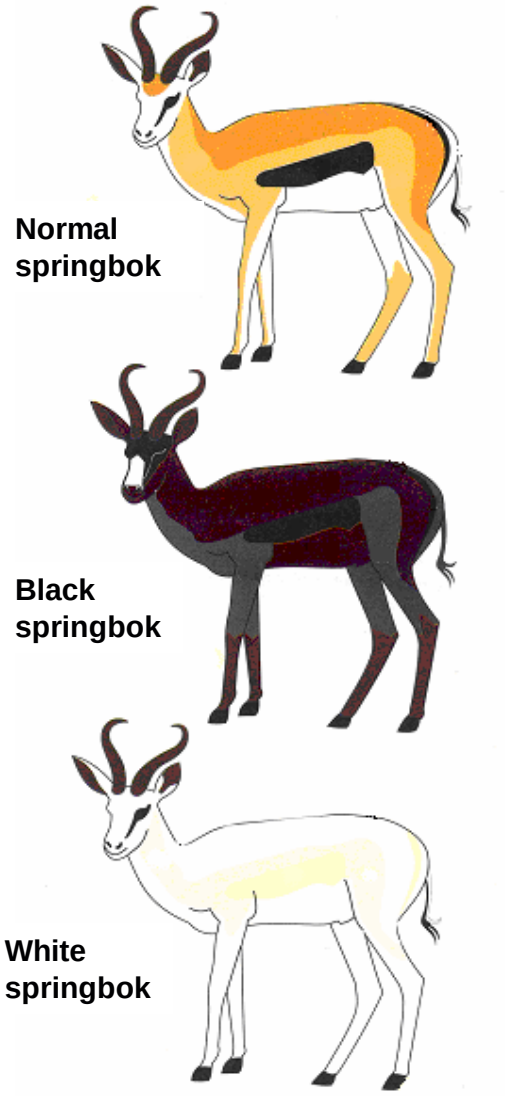

Figure 2.1. The pelt colour variations of the black, normal and white springbok (adapted from Skinner and Louw, 1996).

The adaptive significance, if any, of the different colour morphs is unknown, as the black and white animals have been bred for aesthetic purposes. However, anecdotal reports, mainly from game ranchers in the Eastern Cape, South Africa, suggest that the black springbok are better able to survive cold winter spells than are the other springbok colour morphs. If these anecdotal accounts are true, it may be that dark-coloured animals conserve metabolic energy better in cold climates by absorbing more solar radiation than their lighter-coloured counterparts, and so

offset the energetic costs of homeothermy. Although dark colouration may be an advantage during cold winters, a high absorption of radiant heat may be a disadvantage during the typically hot and cloudless Karoo summers.

The purpose of this study was to investigate whether the colour variations among the three springbok colour morphs have thermoregulatory consequences. I, together with the help of my colleagues, recorded simultaneously, for the first time, body temperature and behaviour of all three springbok colour morphs, in a small paddock near Port Elizabeth, in the Eastern Cape, for a three-month winter period. With the help of my colleagues, I subsequently expanded the study to record body temperature from animals of the three springbok colour morphs, for a year, with the animals living free in their natural environment in the Succulent Karoo, South Africa. Additionally, to determine if the pelts of the three colour morphs have different heat transfer characteristics, I measured conductance, reflectance, and radiant heat load of sample pelts of the three springbok colour morphs. As far as I am aware, this study is the first to compare body temperature, behaviour and pelt characteristics of animals with different colour morphs, but which apparently are otherwise identical.

2.3 Materials and methods

2.3.1 Body temperature and thermoregulatory behaviour

2.3.1.1 Study area

The short-term winter study, during which my colleagues and I recorded behaviour and body temperature, took place from June to August 2004 (southern hemisphere winter). Representatives of the three springbok colour morphs were housed together in a 3 ha game camp (34º00’ S, 25º39’ E, at an altitude of 60 m above sea level) at the Nelson Mandela Metropolitan University, Port Elizabeth, South Africa. The camp’s vegetation is classified as Algoa Dune Strandveld (Mucina and Rutherford, 2006). Animals had access to water *ad libitum* and

grazed on natural vegetation in the camp, which was supplemented with lucerne daily, to which all animals had access.

In the longer-term study, my colleagues and I recorded body temperatures of representatives of the three springbok colour morphs every 15 min over a one-year period (October 2004 to August 2005), at Hopedale farm, a few kilometres west of Steytlerville (33º20' S, 24º20' E, at an altitude of 480 m above sea level). The camp covered an area of 200 ha and the vegetation is classified as Steytlerville Karoo, part of the Succulent Karoo biome (Mucina and Rutherford, 2006). All animals had access to water *ad libitum* and grazed on natural vegetation.

2.3.1.2 Animals

Seventeen springbok (*Antidorcas marsupialis*), 15 of which were captured at the beginning of June 2004 at Cradock, South Africa, and supplemented by two normal springbok from the Nelson Mandela Metropolitan University's nature reserve, were housed in pens at the Nelson Mandela Metropolitan University (~ 200 km south of Cradock) before surgery and release onto the study areas. The study groups for the short-term study consisted of four female white springbok (body mass 23 ± 4 kg), five female black springbok (25 ± 3 kg) and five female normal springbok (21 ± 4 kg). For the one-year study, the study groups consisted of four female white springbok (body mass 22 ± 2 kg), four female black springbok (27 ± 3 kg) and seven female normal springbok (23 ± 3 kg). A single male white springbok (26 kg) was housed with the females during both of the studies, but no data were collected from him.

All experimental procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance number 2004/56/04).

2.3.1.3 Surgery

The springbok were anaesthetised in the holding pens by intramuscular (I.M.) injection of 3.5 $mg.kg^{-1}$ ketamine hydrochloride (Anaket-V, Bayer, Isando, South Africa) and 0.05 $mg.kg^{-1}$ medetomidine hydrochloride (Domitor, Novartis, Kempton Park, South Africa), and once recumbent were carried to a temporary operating theatre set up in an adjacent laboratory. Anaesthesia was maintained with 1-4% halothane (Fluothane, Astra Zeneca, Johannesburg, South Africa) administered in 100% oxygen via a facemask. After ~ 10 min of halothane administration, the action of medetomidine was reversed with 0.25 $mg.kg^{-1}$ (I.M.) atipamezole hydrochloride (Antisedan, Novartis, Kempton Park, South Africa). Respiratory rate, oxygen saturation, heart rate (pulse oximeters, Nonin 9847V, Nonin Medical, North Plymouth, USA) and rectal temperature (thermocouple thermometer, BAT-12, Physitemp, Clifton, USA) were monitored throughout the surgical procedure, which lasted ~ 15 min.

A 200 x 200 mm midline area on the ventral abdominal surface of each springbok was shaved and sterilized with chlorhexidine gluconate (Hibitane, Astra Zeneca, Johannesburg, South Africa) in alcohol. Within this area, a 70 mm cranial-caudal incision was made through the skin and *linea alba*, and a miniature temperature-sensitive data logger (see description below) was placed into the abdominal cavity, where it floated freely. The skin and muscle layers were sutured closed. The wound was sprayed with a topical antiseptic spray (Necrospray, Centaur Labs, Johannesburg, South Africa) and coated with a topical tick repellent (tick grease, cypermethrin 0.025% m/m, Bayer Animal Health Pty, Isando, South Africa). The springbok received a long-acting antibiotic (900 mg I.M. penicillin, Peni LA Phenix, Virbac Animal Health, Centurion, South Africa), analgesic and anti-inflammatory medication (560 mg I.M., ramiphenazone and 2 mg dexamethazone, Dexa-Tomanol, Centaur Labs, Johannesburg, South Africa), and a long-acting parasiticide (5 mg subcutaneously, doramectin, Dectomax, Pfizer Laboratories, Sandton, South Africa). Animals were marked with different coloured plastic ear tags and a small ring of coloured reflective tape (conspicuity sheeting, 3M Scotchlite Diamond Grade, Rivonia, South Africa) around their

horns. These markings enabled us to identify individual animals, even at night. After recovery from surgery, the springbok were released, as a single herd, into the game camp on the Nelson Mandela Metropolitan University campus, where they roamed freely throughout the three-month winter study period, here forth termed “Port Elizabeth winter”.

After the initial three-month study period, the springbok were recaptured, tranquillised with haloperidol (10-15 mg I.M., Kyron Laboratories, South Africa) and perphenazine (50-100 mg I.M., Kyron Laboratories, South Africa), and held in the pens at the Nelson Mandela Metropolitan University. After two weeks, animals again were anaesthetised, the data loggers removed and replaced with new data loggers under the same procedures used for the original implantation. After a two-week recovery period in the pens, the animals were transported ~ 180 km to Hopedale farm and released together. They remained as a single free-living herd.

After the one-year study period, the springbok were recaptured, tranquillised and transported back to the original holding pens at the Nelson Mandela Metropolitan University. Animals once again were anaesthetised and the data loggers were removed, under the same surgical procedures. In both studies the data loggers were all physically intact, the animals’ wounds had healed, and there were no signs of infection from the implant surgery. Most of the loggers were found intra-abdominally in the pelvic canal and were not encapsulated in adherent tissue. After surgery, the springbok were returned to the field to rejoin conspecifics.

2.3.1.4 Body temperature measurements

My colleagues and I implanted miniature temperature-sensitive data loggers (StowAway XTI, Onset Computer Corporation, Pocasset, MA, USA) with dimensions of ~ 50 x 45 x 20 mm and a mass of ~ 40 g when coated in inert wax (Sasol wax EXP986, Sasol, Johannesburg, South Africa). The data loggers had a storage capacity of 32 kb and measured temperatures within the range of + 34 to

+ 46˚C, at a resolution of 0.04˚C. All the data loggers were calibrated individually, in an insulated water bath, against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany). Calibrated accuracy was better than 0.1°C. The data loggers were set to record temperature at 5-min intervals over the short-term Port Elizabeth winter study and at 15-min intervals for the one-year study. The 15-min interval should have provided about 338 days of readings, but five of the data loggers stopped recording after about 270 days, because of premature battery failure. Such premature battery failure reduced my sample size to ten individuals during the Karoo "winter" period, comprising of two female black springbok, four female white springbok and four female normal springbok.

2.3.1.5 Behavioural observations

The springbok were observed for one week of each month of the three-month Port Elizabeth winter study period. Brenda de Witt, as part of a student project, made the actual field observations, using binoculars, from a hide on the perimeter of the game camp. Scan samples (Altmann, 1974) of behaviour were made at 5-min intervals, throughout ~ 3–6 h observation periods, which, over the week period, covered all 24 h. A one-zero sampling method (Altmann, 1974) was used to record the behaviour of each individual of the black, white and normal springbok at each scan. Ms de Witt recorded whether animals sought shelter from wind (downwind or in close proximity to vegetation) or stood out in the open, their orientation to solar radiation (long axis of body parallel, perpendicular or oblique to the sun), the proximity of the nearest conspecific (close (< 1 m) or far (> 1 m)), whether animals were in sunshine or shade, standing or lying, foraging, ruminating or neither, and the degree of their locomotor activity (inactive or active). Behaviour was not recorded during the one-year study, because it was not possible to observe the animals properly from the perimeter of the 200 ha camp, and I did not want to enter the camp as I would have disturbed the normal behavioural patterns of the free-living springbok.

2.3.1.6 Climate measurements

Black globe temperature and air temperature were measured, 1 m above ground, in an open area that was not shaded or protected by vegetation, at each of the study sites. The air temperature probe was housed in a ventilated vane which was painted white to reflect solar radiation. The measurements were made at 15-min intervals by a dual channel temperature data logger (Hobo, Onset Computer Corporation, Pocasset, MA, USA). Due to technical malfunctions, I did not obtain a complete data set, and so I supplemented my weather data with data obtained from a nearby meteorological station (South African Weather Services, Willowmore).

2.3.1.7 Body temperature analysis

For analysis of seasonal patterns of body temperature, data from the one-year study were averaged over four periods, based on prevailing climatic conditions, namely "spring" (October to November), "summer" (December to February), "autumn" (March to May) and "winter" (June to August). I also selected the 10 hottest days in summer, determined by maximum air temperatures, which averaged 37.8 ± 1.3ºC, and the 10 coldest days in winter, determined by the minimum air temperature, which averaged -1.9 ± 1.0ºC (Table 2.1). The mean ± SD of the mean, minimum, maximum and amplitude of the nychthemeral rhythm of body temperature of the groups of black, white and normal colour morphs were calculated for each "season", the 10 hottest and 10 coldest days, as well as for the short-term Port Elizabeth winter study. I calculated the rate of rise in body temperature over consecutive 4-h time intervals and analysed the maximum rate of body temperature rise. I also calculated the mean time of day at which both the minimum and maximum body temperature occurred. I performed a repeated measures two-way analysis of covariance (ANCOVA), with body mass as a covariant, to test for interaction between the body temperatures of the springbok colour morphs and "season", as well as between the body temperatures of the springbok colour morphs and hot and cold days. Newman-Keuls multiple comparisons tests were used to identify sources of significant differences in

ANCOVAs. I used a one-way ANCOVA to test for differences in the body temperature profile of the three colour morphs during the short-term Port Elizabeth winter study. I used Statistica (kernel release 5.5 for Windows, StatSoft, Inc. (1999), Tulsa Oklahoma, USA) for statistical analyses.

To further assess the influence of the ambient temperature on body temperature patterns, I performed a Pearson's linear correlation between the daily nychthemeral amplitude of body temperature, averaged for all animals in each colour morph group, and the air temperature range on the same day, over each "season". I also correlated mean daily maximum body temperature with daily maximum air temperature, and mean daily minimum body temperature with daily minimum air temperature, for each colour morph group over each "season". I fitted linear regressions, where appropriate, and compared the slopes of the regression lines among the three colour morphs and across "seasons" using a repeated measures two-way ANCOVA. A chi-squared test for independence was used to test for differences in the patterns of behaviours exhibited by the colour morphs during the short-term Port Elizabeth study. Data are expressed as mean ± SD, and $P < 0.05$ was considered to be statistically significant.

2.3.2 Pelt heat transfer characteristics

With the help of my colleagues, I measured pelt heat transfer characteristics using the technique previously described for emu pelts by Maloney and Dawson (1995), as summarized below.

2.3.2.1 Pelt samples

I purchased winter pelts of one normal, one white and one black springbok from a taxidermist in the Eastern Cape. The pelts were tanned to standards which allowed transport across international borders. A section, excised from the mid-dorsal region of each pelt and representative of each colour morphs predominant pelt colour, was used to determine spectral reflectance, thermal conductance and heat load from radiation.

2.3.2.2 Spectral reflectance

Spectral reflectance of the pelt samples was measured with an ultra-violet/visible light spectrophotometer (Varian DMS 80 UV, Belmont, Western Australia, Australia). Reflectance was measured at three sites, randomly chosen on the dorsal surface of each pelt sample, with three replicates at each site. Measurements were made between 350 nm and 950 nm, at 1 nm intervals, with reference to a barium sulphate disc. This range of wavelengths replicates the range of greatest intensity of solar energy (Gates, 1966) and the range of output of the radiation source used in the wind tunnel experiments (described below, Maloney and Dawson, 1995).

2.3.2.3 Thermal conductance

To measure thermal conductance, I mounted the pelt sample horizontally, hair upwards, on the upper surface of a metal plate (250 mm diameter), maintained at ~ 38°C via a temperature-controlled circulating water bath (Fig. 2.2). A rectangular glass wind tunnel positioned over the pelt allowed laminar-flow wind to be directed parallel to the pelt surface, at different speeds. Three heat flux transducers (20 mm x 30 mm, model HA13-18-10P, Thermonetics Corporation, U.S.A.) were embedded in the upper surface of the plate. The voltage outputs of the transducers were logged via an analog/digital (A/D) converter (Datataker, Data Electronics, Australia P/L, model 100F). The heat flux transducers were calibrated using two thicknesses of polystyrene (Boral Industries, Sydney, Australia) of known thermal conductance.

Copper/constantan (Type-T) thermocouples, 0.7 mm in diameter, were used to measure plate temperature (T_p), air temperature (T_a), and skin surface temperatures (T_s). Skin surface temperatures were measured by feeding three thermocouples to the hair/skin interface, through oblique holes from beneath the skin. Air temperature was measured near the inlet of the wind tunnel so that it was not affected by the boundary layer. Thermocouple outputs were referenced against an isothermal block (Datataker, Data Electronics, Australia P/L) and the

temperatures logged via the A/D converter, as above. Thermocouples were calibrated, against a mercury thermometer that was certified by the Australian National Association of Testing Authorities in an insulated water bath, to 0.1°C accuracy.

Thermal conductance was measured in a temperature-controlled room (air temperature 20 ± 2ºC). Once temperatures had stabilized, heat flow, skin and air temperatures were measured for at least half an hour. Mean values then were used to calculate pelt conductance (C, $W.m^{-2}.°C^{-1}$) as $C = \dot{Q}/(T_s - T_a)$, where $\dot{Q}$ is the heat flow ($W.m^{-2}$) through the pelt as measured by the transducers, T_s is skin surface temperature and T_a is air temperature. Conductance of each sample was determined at four wind speeds (~ 0, 1.5, 3 and 4.5 $m.s^{-1}$), as measured 20 mm above the pelt surface with a thermo-anemometer (Schiltknecht 39400, Technical & Scientific Equipment Co., Melbourne, Australia). Air flowed in a cranial to caudal direction across the pelt sample.

2.3.2.4 Radiant heat load

To determine the heat load on the animal resulting from radiant heat absorption, I repeated measurements of heat flow and temperature profiles across the pelt with the pelt surface subjected to a simulated solar radiant heat load (Fig. 2.2). Short-wave radiation was provided by a spotlight (ARRI daylight 575 W), containing a 575 W metal halide lamp (ILC Technology, DM1575), which radiated 590 $W.m^{-2}$ of ultra-violet/visible (300 - 1100 nm) radiation. The radiant flux was verified at the pelt surface with a radiometer (Model 8-48, Eppley Laboratory, Rhode Island, USA). The relative spectral distribution of radiation supplied by the lamp had been shown previously to be similar to that of the solar spectrum (Walsberg, 1982; Maloney and Dawson, 1995; Dawson and Maloney, 2004). Radiation was delivered through a 100 mm diameter hole in aluminium foil covering a glass pane, placed between the lamp and the wind tunnel (Fig. 2.2), so that the spotlight did not heat the wind tunnel.

The proportional heat load from radiation was calculated as $\left[HF_{without\ radiation} - HF_{with\ radiation}\right] / \ incident\ \ radiation$, where heat flow (HF) through the sample was measured by the transducers, with adjustment for the difference in air temperature and plate temperature depending on whether radiation was present or absent (Walsberg, 1982; Maloney and Dawson, 1995; Dawson and Maloney, 2004).

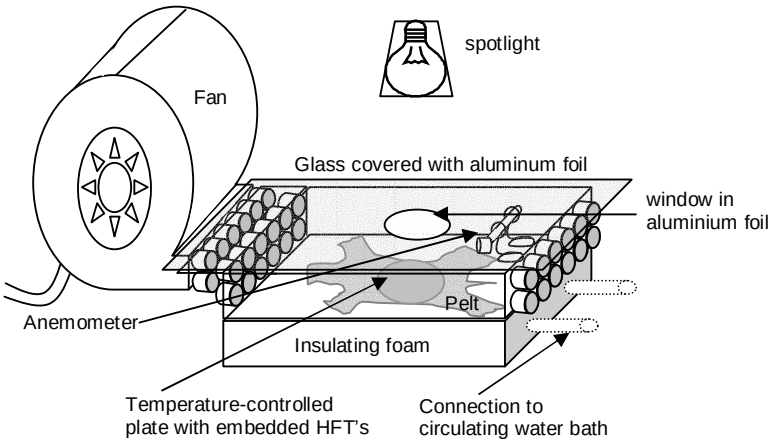

Figure 2.2. Experimental set-up for measuring thermal conductance and radiant heat load in the springbok pelts. HFT's = Heat Flux Transducers.

2.3.2.5 Data analysis of pelt characteristics

I calculated mean weighted solar reflectance by averaging measured reflectance at 50 nm intervals multiplied by the relative spectral power of sunlight at each wavelength. I could not measure reflectance above 950 nm; I assumed it to remain constant at the 950 nm value. I correlated thermal conductance with wind speed; I then fitted a linear regression and tested for differences in the slopes and intercepts using ANCOVA. I compared heat load from radiation as a function of wind speed, among the pelts, by fitting and comparing a one-phase exponential decay curve. Tukey-Kramer multiple comparisons tests were used to identify sources of significant differences in ANCOVAs. Data are expressed as mean ± SD and $P < 0.05$ was considered to be statistically significant. I used GraphPad Prism (version 4.00 for Windows, GraphPad Software, San Diego California USA) for statistical analyses.

I calculated potential energetic consequences of pelt colouration using an approach similar to that described by (Maloney *et al.*, 2005a). I used solar radiation intensity incident on a horizontal surface as measured at a nearby weather station (Blaauwkrantz Farm, 33°32'S 25°23'E, Table 2.1). I multiplied hourly solar radiation over each season by the fraction of the heat load which reaches the springbok (Fig. 2.8), at the average wind speed prevailing at the field site during that season, for each colour morph. The fraction of the total body surface area presented to incident solar radiation varies with body orientation and solar elevation. I calculated solar elevation for the latitude and longitude of my field site, using software available at http://www.susdesign.com/sunangle, for each hour of a representative day within each season. The surface area presented at different solar elevations was estimated based on data (Clapperton *et al.*, 1965) obtained for a 35 kg shorn sheep (total surface area 1 m^2) orientated either parallel or perpendicular to the solar beam. Estimating surface area (m^2) of the skin from $A = 0.09 \times W^{2/3}$, where W is mass in kg, I calculated a proportional surface area presented at different solar elevations for a 25 kg springbok model. The best fit equation was an exponential equation for parallel orientation ($\ln y = -2.02 - 0.008x$, where y is the proportional surface area of the animal exposed to solar radiation and x is the solar elevation) and a power equation for perpendicular orientation ($y = 0.27 - 1.97 \times 10^{-7} x$). For each hour of sunlight during each season, the surface area presented to incident radiation was calculated from these equations for the appropriate solar angle. The magnitude of the total absorbed solar radiation (MJ per day) was calculated by multiplying surface area presented to incident radiation by the solar radiation incident on each of the colour morphs, for each hour of sunlight within each season. Metabolic savings were calculated by expressing total absorbed solar radiation as a proportion of field metabolic rates of springbok (Nagy and Knight, 1994), within each season, for each colour morph, assuming that field metabolic rate was the same for all colour morphs.

2.4 Results

2.4.1 Body temperature and thermoregulatory behaviour

2.4.1.1 Climate

Table 2.1 shows the mean ± SD of environmental variables. Air temperature reached a maximum of 40ºC in February and dropped to as low as -4ºC in June, in the Karoo, giving a range of 44ºC. The minimum air temperatures usually occurred before dawn (03:00 - 05:00) and the maximum air temperatures usually occurred after noon (12:00 - 14:00). Notably, the Karoo "winter" was colder and drier than the Port Elizabeth winter.

2.4.1.2 Body temperature

Figure 2.3 shows mean, minimum and maximum body temperatures, and the amplitude of the nychthemeral body temperature rhythm (maximum - minimum), for the different springbok colour morphs, over the four time periods while they were living free in the Karoo. A repeated measures two-way ANCOVA of data from the one-year study revealed that mean ($F_{3,21} = 23.8$, $P < 0.0001$), daily minimum ($F_{3,21} = 14.2$, $P < 0.0001$) and daily maximum ($F_{3,21} = 42.6$, $P < 0.0001$) body temperatures decreased as ambient temperature became progressively colder from "summer" to "winter".

Table 2.1. Environmental conditions (mean ± SD) prevailing during the four seasonal periods during which the springbok were free-living in the Steytlerville Karoo region, during the three-month winter study at Port Elizabeth (PE win) and during the 10 hottest days in summer and the 10 coldest days in winter in the Steytlerville Karoo region.

	Oct-Nov "spring"	Dec-Feb "summer"	Mar-May "autumn"	Jun-Aug "winter"	Jun-Aug "PE win"	Hot days	Cold days
Mean globe temperature (°C)	no data	28.2 ± 2.8	21.2 ± 4.9	14.9 ± 2.0	17.8 ± 6.1	no data	12.5 ± 2.0
Air temperature (°C)							
daily mean	20.7 ± 6.6	23.0 ± 5.3	19.3 ± 4.1	13.6 ± 2.1	16.3 ± 2.1	28.6 ± 1.6	8.0 ± 2.4
daily minimum	12.3 ± 4.0	15.8 ± 2.2	13.6 ± 3.9	6.3 ± 6.9	9.5 ± 3.1	17.9 ± 2.5	-1.9 ± 1.0
daily maximum	27.7 ± 5.9	30.0 ± 4.4	26.5 ± 5.6	22.3 ± 3.6	22.5 ± 3.2	37.8 ± 1.2	17.1 ± 3.8
Mean monthly rainfall (mm)	15 ± 14	34 ± 12	27 ± 19	4 ± 3	35 ± 20	n/a	n/a
Mean daily wind speed ($m.s^{-1}$)*	2.7 ± 2.5	2.9 ± 2.2	1.6 ± 2.1	2.2 ± 2.7	3.6 ± 1.7	2.6 ± 1.4	1.2 ± 1.1
Mean daily radiation ($W.m^{-2}$)†	231 ± 75	244 ± 85	156 ± 62	137 ± 33	-	-	-

* Wind speed was recorded at a nearby meteorological station (South African Weather Services, Willowmore)

† Radiation was recorded at a nearby weather station located on Blaauwkrantz farm, within 100 km of the field site

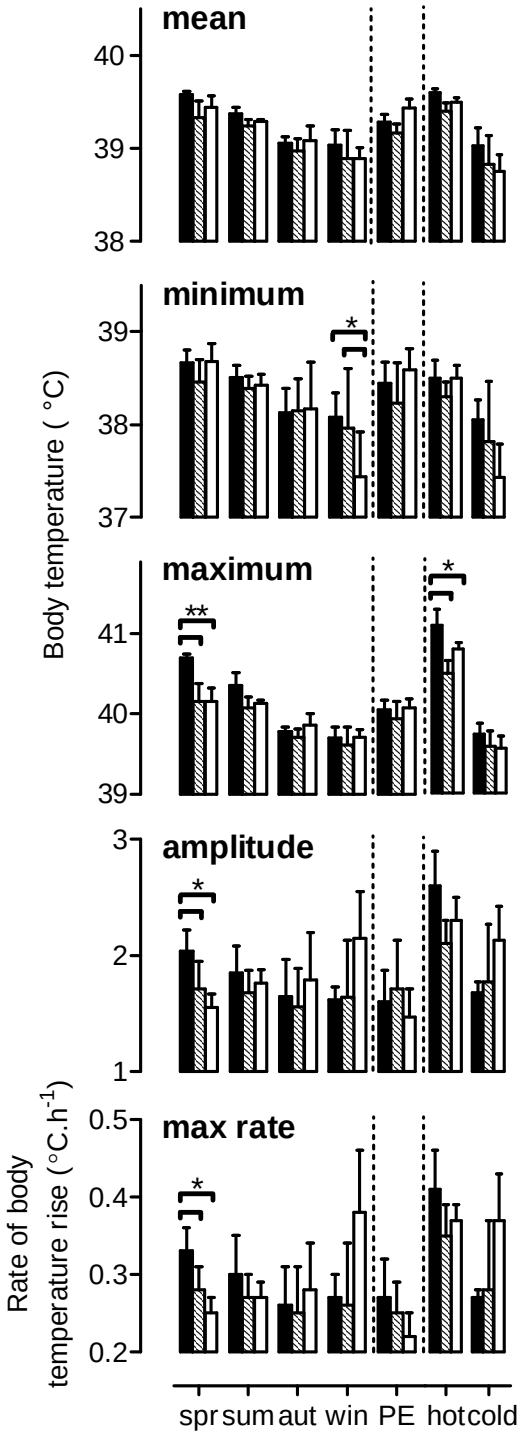

Figure 2.3. Mean ± SD of the mean, minimum, maximum, amplitude and rate of body temperature rise of the nychthemeral rhythm of body temperatures of black (closed bars), normal (hatched bars) and white (open bars) springbok over four seasonal periods (where spr = "spring", sum = "summer", aut = "autumn", win = "winter") while the springbok were free-living in the Steytlerville Karoo region, during the three-month Port Elizabeth winter study (PE) while the springbok were free-living in the Algoa Dune Strandveld and during the 10 hottest days in summer (hot) and the 10 coldest days in winter (cold) in the Karoo. Repeated measures two-way ANCOVA with body mass as the covariate, * $P < 0.05$, ** $P < 0.001$.

Post-hoc analysis of the interaction between body temperature indices and season revealed that daily minimum body temperatures of white springbok were significantly lower than those of both black ($P = 0.04$) and normal ($P = 0.04$) springbok in "winter", and that black springbok had a daily maximum body temperature significantly higher than that of both normal ($P = 0.0002$) and white ($P = 0.0002$) springbok during "spring". This higher maximum body temperature of the black springbok during "spring" resulted in an increase in the amplitude of body temperature rhythm, in black springbok compared to both white ($P = 0.005$) and normal ($P = 0.03$) springbok. I assessed whether the differences in body temperature amplitudes among the springbok colour morphs were the result of differences in the maximum diurnal rate of body temperature rise (Fig. 2.3, lowest panel). As expected, black springbok showed a faster rate of body temperature rise, compared to both white ($P = 0.01$) and normal ($P = 0.009$) springbok, during "spring". This difference in the maximum rate of body temperature rise was not the result of differences in the time at which minimum or maximum body temperatures occurred, as there were no significant differences between the time at which maximum ($F_{2,6} = 0.3$, $P = 0.75$), nor the time at which minimum ($F_{2,6} = 1.8$, $P = 0.25$), body temperature occurred among any of the springbok colour morphs, over the one-year study period.

There were no significant differences in any of the body temperature indices among the colour morphs during the short-term Port Elizabeth winter study (Fig. 2.3). The lower minimum body temperature of the white springbok compared to the other two springbok colour morphs, during the Karoo "winter" period was not evident over the short-term Port Elizabeth winter study in which the animals inhabited the Algoa Dune Strandveld, where ambient temperature was not as low as it was in the Karoo (Table 2.1), and the diet of the springbok was supplemented.

2.4.1.3 Environmental effects on the nychthemeral rhythm of body temperature

The core body temperature of the animals showed a nychthemeral rhythm with a temperature nadir shortly after sunrise and peak near sunset (Fig. 2.4). On the

coldest 10 days in winter in the Karoo, mean ($F_{1,7}$ = 48.4, P = 0.0002), daily minimum ($F_{1,7}$ = 20.9, P = 0.003) and daily maximum ($F_{1,7}$ = 194.1, P < 0.0001) body temperatures were lower than on the hottest days. The amplitude ($F_{1,7}$ = 10.1, P = 0.02) of the body temperature rhythm also was dampened on the cold days compared to the hot days (Fig. 2.4). However, the only body temperature variable which differed significantly among the colour morphs was the daily maximum body temperature ($F_{2,6}$ = 6.9, P = 0.03). Black springbok had a significantly higher daily maximum body temperature than did both white (P = 0.03) and normal (P = 0.01) springbok, and these differences were particularly apparent on the hottest days (Fig. 2.3).

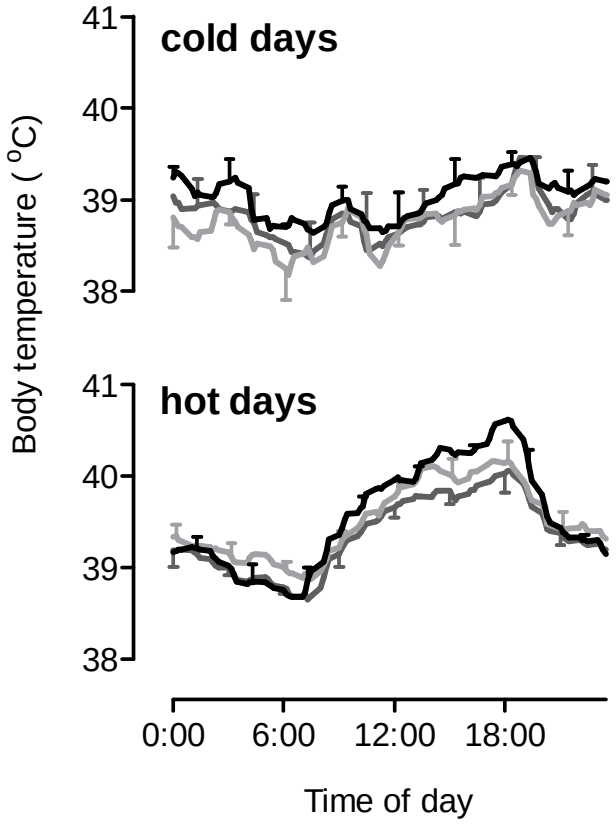

Figure 2.4. Mean ± SD of body temperature of black (black line), normal (dark grey line) and white (light grey line) springbok, as a function of time of day, over the 10 coldest days in winter (mean air temperature of 8.0 ± 2.4˚C) and the 10 hottest days in summer (mean air temperature of 28.6 ± 1.6˚C) of the one-year study period.

To further assess the influence of the ambient temperature on the nychthemeral body temperature rhythm of the different springbok colour morphs, I correlated the daily nychthemeral amplitude of body temperature, averaged for all animals in each colour morph group, with the daily air temperature range on the same day. The body temperature amplitude for each colour morph was correlated significantly with the daily range of air temperature (Fig. 2.5) during "spring" ($P < 0.001$, n = 49) and "summer" ($P < 0.0001$, n = 90), but not during "autumn" ($P > 0.15$, n = 63) or "winter" ($P > 0.05$, n = 74) in the Karoo, nor during "winter" in Port Elizabeth ($P > 0.05$, n = 42). For each of the colour morphs the slope of the regression equation did not differ between "spring" and "summer" ($F_{1,12} = 0.02$, $P = 0.90$), but these slopes were significantly different among the colour morphs ($F_{2,11} = 7.9$, $P = 0.007$). Black springbok showed the steepest slope of 0.06 ± 0.01ºC change in body temperature amplitude, per 1ºC change in the range of air temperature, versus 0.04 ± 0.01ºC for white and 0.03 ± 0.01ºC for normal springbok, in both "spring" and "summer". Similarly, for each of the colour morphs the dependence of amplitude of body temperature on range of air temperature did not differ between "spring" and "summer" ($F_{1,12} = 0.00002$, $P = 0.99$), but the dependence was significantly different among the colour morphs ($F_{2,11} = 7.1$, $P = 0.01$). Black springbok had the highest dependence of amplitude of body temperature on range of air temperature (black $r^2 = 0.34 \pm 0.09$, white $r^2 = 0.14 \pm 0.1$ and normal $r^2 = 0.13 \pm 0.09$). Daily maximum body temperature, averaged for all animals in each group of colour morphs, also was significantly correlated to daily maximum air temperature during "spring" ($P < 0.0003$) and "summer" ($P < 0.0001$). For each of the colour morphs the slopes of the regression equations were significantly steeper in "summer" than in "spring" ($F_{1,12} = 16.6$, $P = 0.002$), and the slopes of the regression equations were significantly different among the colour morphs ($F_{2,11} = 4.2$, $P = 0.04$), with black springbok showing the steepest slope. Similarly, mean daily minimum body temperature, for each group of colour morphs, also was correlated with daily minimum air temperature, but only weakly, and only during "autumn" ($P < 0.004$; black $r^2 = 0.12$, white $r^2 = 0.15$ and normal $r^2 = 0.20$).

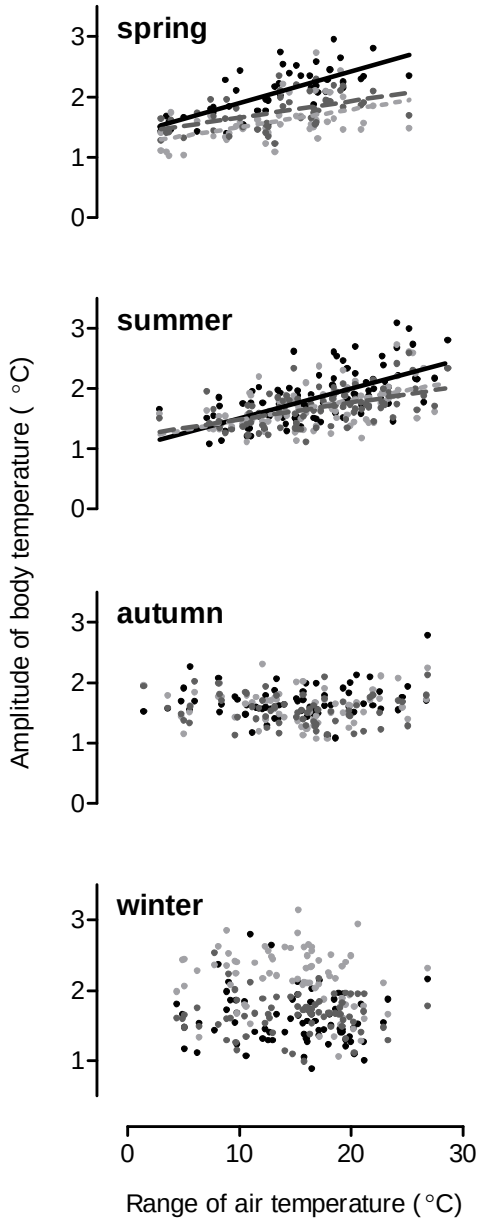

Figure 2.5. Correlation of the amplitude (the difference between maximum and minimum) of the nychthemeral pattern of body temperature, averaged for all animals in the group, for black (black circle), normal (dark grey circles) and white (light grey circles) springbok, versus the 24-h range of air temperatures on the same day, for each day in my designated "season". Regression lines were fitted for the black (solid black line), normal (dashed dark grey line) and white (dotted light grey line) springbok when the correlations were significant in "spring" (black $y = 0.06x + 1.1$; white $y = 0.04x + 1.0$; normal $y = 0.03x + 1.2$) and "summer" (black $y = 0.06x + 1.0$; white $y = 0.04x + 1.1$; normal $y = 0.03x + 1.2$), where y = amplitude of body temperature rhythm and x = 24-h range of air temperature.

2.4.1.4 Behavioural thermoregulation

During daylight hours in the short-term Port Elizabeth winter study, the white and normal springbok intermingled routinely, and appeared to behave similarly, whilst the black springbok typically stayed in a separate group. However, animals from all three colour morphs herded together at night. The white springbok seemed to initiate behavioural events, such as feeding, ruminating or lying down, and their behaviour was imitated by the normal springbok, and then by the black springbok. The three colour morphs displayed similar partitioning of behaviour, over diurnal hours, nocturnal hours and the full 24-h day (Table 2.2). The only behavioural category in which there was a significant association between springbok colour morph and behaviour was the degree of activity (Table 2.2), with the black springbok being less active during daylight hours, and consequently also over the full 24-h day, than were the other two colour morphs. Figure 2.6 illustrates the average 24-h body temperature and activity pattern for animals of each colour morph at 1-h intervals during the short-term Port Elizabeth winter study. The three colour morphs showed similar nocturnal activity profiles with distinct periods of inactivity soon after dusk and just before dawn, but the black springbok were noticeably less active between 06:00 and 09:00 (Fig. 2.6). It is clear from Figure 2.6 that the diurnal rise in body temperature was not a product of activity because the steepest rise in body temperature occurred when the animals were inactive, after the morning activity period peak, and body temperature decreased during the post-dusk activity peak.

Table 2.2. Association between partitioning of thermoregulatory behaviour and the three springbok colour morphs, over a 24-h day, as well as over nocturnal and diurnal periods, during the short term Port Elizabeth winter study.

Behaviour category	Description	*P* value		
		24-h	Diurnal	Nocturnal
Wind	Tolerance or avoidance	0.94	0.92	0.98
Sun orientation	Parallel, perpendicular or oblique	0.70	0.70	-
Location	Sun or shade	0.17	0.17	-
Position	Standing up or lying down	0.91	0.99	0.87
Proximity	>1 or <1m from each other	0.17	0.49	0.21
Feeding	Foraging, ruminating or neither	0.70	0.64	0.95
Activity	Stationary or mobile	**0.04**	**0.008**	0.80

Values are *P* values obtained from Chi-squared tests, testing association between behaviour and colour morph. Bold values represent significance.

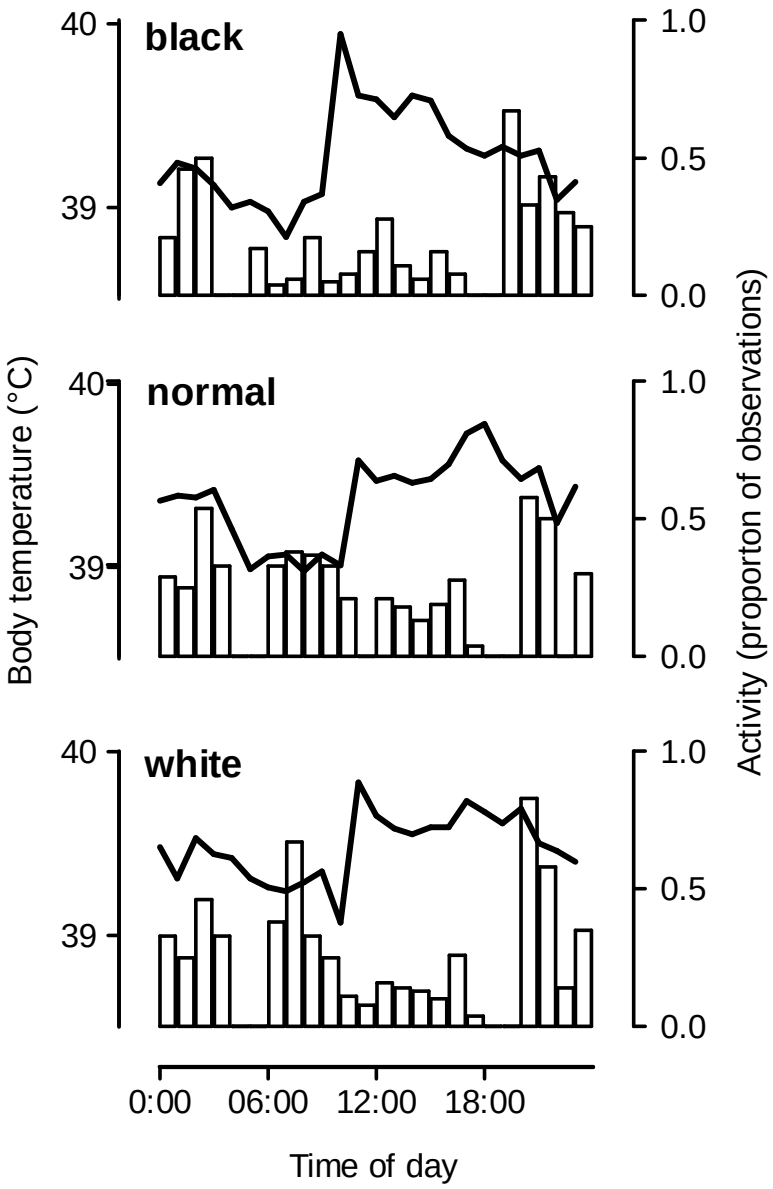

Figure 2.6. Activity, expressed as the proportion of the total number of observations for which the animals were active, during each hour of the 24-h day (open bars), and the mean body temperature (solid line) of black, normal and white springbok, as a function of time of day during the short-term Port Elizabeth winter study.

2.4.2 Pelt heat transfer characteristics

2.4.2.1 Spectral reflectance

Figure 2.7 depicts the spectral reflectance of three springbok pelts, representing each of the three colour morphs, as a function of wavelength, over a waveband simulating the solar spectrum, except for infrared radiation. Spectral reflectance increased with an increase in wavelength for all pelt samples. Mean weighted solar reflectance was different for the three pelt samples, with the black springbok pelt displaying the lowest solar reflectance (0.19 ± 0.11), the white pelt the highest reflectance (0.76 ± 0.01) and the normal springbok pelt intermediate reflectance (0.49 ± 0.06).

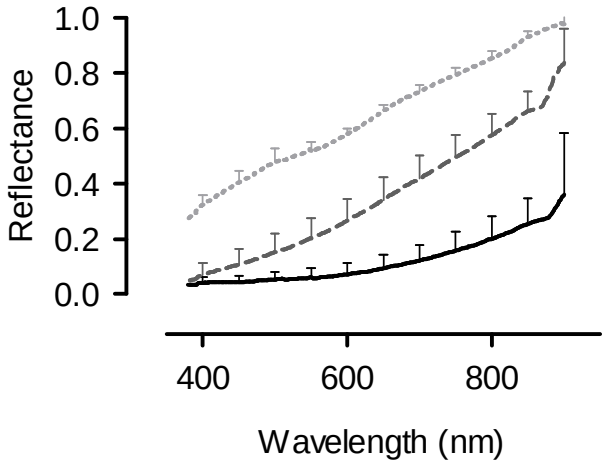

Figure 2.7. The spectral reflectance (where a reflectance of one represents total reflection and zero represents total absorption of light relative to barium sulphate) of a black (solid black line), normal (dashed dark grey line) and white (dotted light grey line) springbok pelt over the spectral range, from ultraviolet (350 nm) to infrared (950 nm).

2.4.2.2 Thermal conductance

The conductance of each pelt increased linearly as wind speed increased (Fig. 2.8A; black $r^2 = 0.96$, $P = 0.02$; normal $r^2 = 0.97$, $P = 0.01$; white $r^2 = 0.93$, $P = 0.04$). There was no significant difference between the slopes of the conductance, as a function of wind speed, among the springbok pelts (ANCOVA, $F_{2,6} = 2.6$, $P = 0.15$), but there was a significant difference in the intercepts (ANCOVA, $F_{2,8} = 23.0$, $P = 0.0005$), with the black springbok pelt having a conductance significantly higher than that of the pelts from the normal and white springbok.

2.4.2.3 Heat load from radiation

In all pelts, the fraction of the incident radiant heat load that was conveyed through the pelt to the skin decreased with increasing wind speed (Fig. 2.8B). Fitting one-phase exponential decay curves revealed significant differences among the pelts of each colour morph (ANCOVA, $F_{6,3} = 14.16$, $P = 0.03$), with the black springbok pelt having the highest, and white springbok the lowest, proportion of incident radiant heat load conveyed through the pelt.

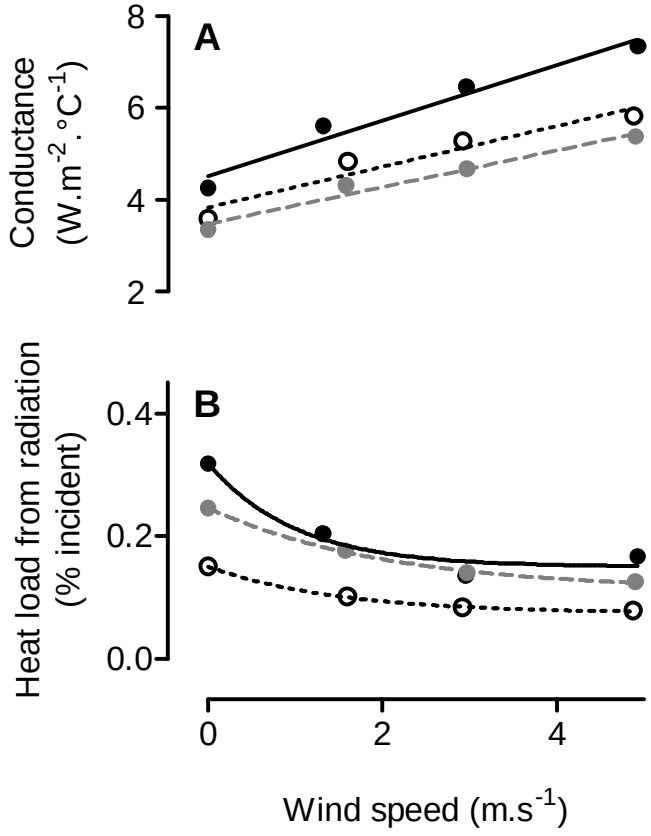

Figure 2.8. Thermal conductance of the pelt (A), and heat load from radiation conveyed through the pelt as a percentage of incident radiant load (B), for a black (black circles), normal (dark grey circles) and white (light grey circles) springbok pelt, as a function of wind speed. Linear regressions were fitted to the conductance values for black (solid black line, $y = 0.61x + 4.5$, $r^2 = 0.96$), normal (dashed dark grey line, $y = 0.40x + 3.5$, $r^2 = 0.97$) and white (dotted light grey line, $y = 0.44x + 3.8$, $r^2 = 0.93$) springbok pelts, where y = conductance ($W.m^{-2}.ºC^{-1}$) and x = wind speed ($m.s^{-1}$). One-phase exponential decay curves were fitted to the proportional heat loads for black (solid black line, $y = 16.9e^{-x} + 15.0$, $r^2 = 0.96$), normal (dashed dark grey line, $y = 13.5e^{-0.5x} + 11.1$, $r^2 = 0.99$) and white (dotted light grey line, $y = 7.6e^{-0.7x} + 7.5$, $r^2 = 0.99$) springbok pelts, where y = radiant heat load (% incident) and x = wind speed ($m.s^{-1}$).

2.4.2.4 Potential energetic consequences

If animals are employing metabolic heat to maintain homeothermy, then, theoretically, they can spare metabolic heat production by absorbing solar radiation. Based on my model, the potential energetic savings associated with pelt colour were nearly two-fold higher for the black and normal springbok compared to the white springbok (Fig. 2.9), across all seasons. The smallest potential metabolic savings were those in winter, and it was during the cool winter and autumn months when orientation to the sun had the largest effect on metabolic savings. The proportional contribution of total absorbed solar radiation to metabolic rate was low in summer, which was likely the result of the high metabolic rate for springbok during hot dry months (Nagy and Knight, 1994).

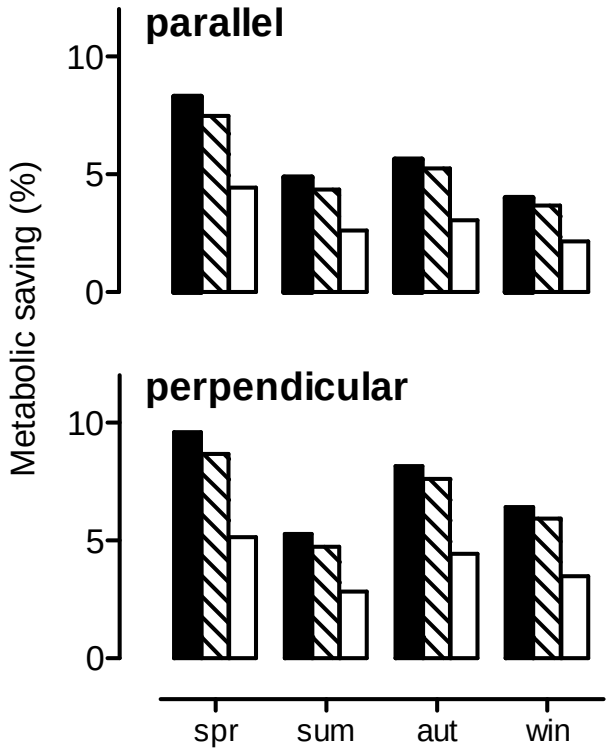

Figure 2.9. Metabolic savings of black (closed bars), normal (hatched bars) and white (open bars) springbok over four seasonal periods (where spr = “spring”, sum = “summer”, aut = “autumn”, win = “winter”), when animals were orientated parallel (upper panel) and perpendicular (lower panel) to solar radiation. Metabolic savings were calculated by expressing the total absorbed solar radiation of each colour morph per day as a proportion of the daily seasonal field metabolic rates of springbok measured by Nagy and Knight (1994).

2.5 Discussion

My study provides the first comparison of body temperature and behavioural thermoregulation of three colour morphs of a single mammalian species, while the animals were living free under the same environmental conditions. I supplemented the field studies by measuring the thermal characteristics of sample pelts from the colour morphs. The data allowed me to examine the influence of environmental thermal loads on the body temperature of representatives of the three springbok colour morphs, as well as the relationship between behaviour and thermoregulation, under natural conditions. I found that pelts from the colour morphs differed in more than just colour, and that the pelt of the black springbok conveyed more incident radiant heat to the skin than the pelts of the other springbok colour morphs. Possibly related to that finding, the black springbok displayed a higher peak body temperature, a larger 24-h amplitude of the nychthemeral body temperature rhythm, and a faster rate of body temperature rise, than did both white and normal springbok, in hot conditions. In addition, the body temperature of my black springbok was potentially influenced more by air temperature, as illustrated by a steeper slope and stronger correlation between the amplitude of the nychthemeral body temperature rhythm and the range of air temperatures, than was the body temperature of the other colour morphs.

The springbok displayed a nychthemeral rhythm of body temperature, with a minimum body temperature in the morning and a maximum in the afternoon (Fig. 2.4). These body temperature patterns were similar to those reported previously for normal springbok (Hofmeyr and Louw, 1987; Mitchell *et al.*, 1997; Fuller *et al.*, 2005). There were no differences apparent in body temperature patterns during winter between the black and normal springbok. During the Port Elizabeth winter study, my springbok, like those observed by others (Bigalke, 1972; Davies and Skinner, 1986; Skinner and Louw, 1996), foraged throughout most of the day. The black springbok displayed the lowest diurnal activity (Fig. 2.6). Since foraging was a major contribution to activity, this finding is consistent with the black springbok spending less time feeding in the winter than did the other colour morphs. Similarly, dark steers spent less time grazing than did light-coated steers

in summer (Finch *et al.*, 1984). The reduction in feeding time might reflect lower metabolic costs of homeothermy in the black colour morphs, resulting from better access to solar energy, offsetting the metabolic cost of homeothermy in cold conditions. Such a finding would be consistent with local anecdotal reports that the black springbok survive cold winters better than the other springbok colour morphs. But the higher solar heat load experienced by the black springbok, compared to the other springbok colour morphs, while of potential benefit in the cold, was associated with hyperthermia greater than that of the conspecific colour morphs in hot conditions.

In contrast to the situation with the black springbok, there was an apparent disadvantage to the white colour morph during winter, with the white springbok displaying lower minimum body temperatures than did the other springbok in the Karoo winter. This difference was unlikely to have resulted directly from the lower solar radiant input to the white morph, because the minimum body temperature occurred shortly after sunrise, when radiant heat was not a major source of warmth for any of the animals. Instead, I hypothesise that the lower morning body temperature resulted from a reduced ability of the white springbok, compared to the other springbok colour morphs, to maintain a positive energy balance in the cold Karoo. If the lower energy absorption from solar radiation during the day increased the metabolic cost of homeothermy in the white springbok, while they fed for the same time and had similar energy intake as did the normal springbok, then they were likely in deficit, relative to the other springbok colour morphs, which manifested as an exaggerated nocturnal nadir of body temperature. Evidence supporting that hypothesis is that energy restriction, or starvation, leads to a lower nadir in the nychthemeral rhythm of body temperature in many species, including sheep (Piccione *et al.*, 2002), desert goats (Choshniak *et al.*, 1995; Ahmed and El Kheir, 2004), horses (Kronfeld, 1993), many bird species (Hohtola *et al.*, 1991), rodents (Sakurada *et al.*, 2000; Bae *et al.*, 2003), and primates (Génin and Perret, 2003). My conclusions about the reduced ability of the white springbok to maintain a positive energy balance in the Karoo winter must remain tentative, however, because some of the loggers

stopped recording prematurely which resulted in a low sample size during this winter period. The minimum body temperatures of my white springbok were no different to those of the other colour morphs during the short-term Port Elizabeth winter study, where conditions were milder and animals' diets were supplemented.

To ascertain whether the differences I observed in the body temperature profile of the springbok colour morphs indeed were the result of differences in their pelt properties, I investigated pelt heat flow characteristics. The white springbok pelt had the highest reflectance in the visible spectrum, and the black springbok pelt had the highest absorbance (Fig. 2.7). These differences in absorbance contributed to the black springbok having the highest, and white springbok the lowest, heat load from radiation (Fig. 2.8B). The biophysical part of the study was limited, though, because I was able to examine only a single pelt sample of each springbok colour morph, and I do not know if differences in pelt conductance and solar heat gain can be compensated, in living animals, by autonomic responses, such as pilo-erection or vasodilation. Based on the pelt heat flow characteristics of the excised pelts, black springbok should be more prone to losing heat when ambient temperature is cooler than body temperature, because the thermal conductance of the black springbok pelt was higher than that of the other springbok pelts (Fig. 2.8A). In the presence of solar radiation, though, the characteristics of the pelt of the black springbok resulted in the transmission of more solar heat to the skin, and there was no apparent disadvantage to homeothermy of the black springbok, compared to the normal springbok, in winter.

Several earlier studies have investigated the impact of pelt colour on heat load from radiation. My data fit the traditional view that animals with darker colouration acquire a larger heat load from solar radiation than do animals with lighter colouration, because they absorb more short-wave radiation (Hamilton and Heppner, 1967; Heppner, 1970; Cloudsley-Thompson, 1979; Walsberg, 1982). Although reflectance of short-wave radiation is colour dependent, the increase in reflectance from the blue end of the spectrum to the near infrared spectrum is

independent of pelt colour, and almost all long-wave radiation is absorbed, whatever the visible colour (Dawson and Brown, 1970; Cena and Monteith, 1975; Finch *et al.*, 1984). I analysed pelt reflectance only within the visible spectrum. The reflectance of my black and white springbok pelts were similar to those reported for black goats (Finch *et al.*, 1980), white cattle (Hutchinson and Brown, 1969) and black and white finches (Heppner, 1970). The reflectance of my normal springbok pelt was similar to that reported previously for normal springbok (Hofmeyr and Louw, 1987). However, because of the way they weighted their reflectance calculation to take into account differences in reflectance between the buff and pale areas of the normal springbok pelt, Hofmeyr and Louw (1987) arrived at a slightly higher mean weighted absorbency (0.55) than I did for my normal springbok (0.51 ± 0.06). This weighted absorbency of the normal springbok pelt is lower than that reported for other African ungulates (Finch, 1972), a property proposed to offer adaptive advantages for desert life (Skinner and Louw, 1996).

The thermal consequences of pelt structure are dependent not simply on reflectance, but also on other properties that affect the depth to which radiation penetrates the fur or feathers (Kovarik, 1964; Walsberg *et al.*, 1978; Walsberg, 1983). If penetrance is low, as in dense or matted pelts, radiant heat is absorbed at the fur or feather surface and returned to the environment, reducing the potential contribution to the heat load on the animal. Such considerations become especially relevant when wind speed increases and the insulation to heat flow from the point of absorbance to the environment is reduced as the air boundary layer is reduced (see below). In such circumstances, pelt colour may have little impact on the thermal load from solar radiation that actually reaches the animal (Walsberg, 1982; Walsberg, 1990; Maloney and Dawson, 1995; Walsberg and Wolf, 1995; Dawson and Maloney, 2004). However, if all other morphological characteristics of pelts are equal, as I have been led to believe for my springbok colour morphs (Kruger *et al.*, 1979), then penetrance should be inversely related to absorbance (Hutchinson and Brown, 1969; Cena and Monteith, 1975). I discovered, though, that the pelts differed in more than just colour; the pelt of the

black colour morph also had a significantly higher conductance. Springbok pelts are thinner and have a higher conductance than predicted for an antelope of its body size (Hofmeyr, 1981). The thin pelage may well be the reason that pelt colour influenced the radiant heat load so strongly in this species. The outcome of the combination of pelt thermal properties was that the white springbok pelt prevented heat load from radiation reaching the animals more than did the normal and black pelts, an outcome that concurs with results obtained for goats (Finch *et al.*, 1980), cattle (Hutchinson and Brown, 1969; Finch *et al.*, 1984; Hansen, 1990) and pigeons (Walsberg *et al.*, 1978). The fact that the lines relating heat load from radiation to wind speed (Fig. 2.8B) converged at higher wind speeds supports the view that the effects of radiation absorbance predominated at low wind speeds, whilst small differences in penetrance exerted an effect as wind speed increased (Hutchinson and Brown, 1969; Walsberg *et al.*, 1978).

Although the radiant heat load through the colour morph pelts converged as wind speed increased, the heat load through the white pelt remained lower than that through the black pelt, even at high wind speeds. The greater access to radiant heat predicted, from the biophysical properties of the pelt, for the black animals should have thermoregulatory advantages in the cold, saving metabolic energy. My model of energetic savings (Fig. 2.9) indicates that the black springbok potentially were able to obtain almost twice the energy flux from solar radiation, in the field, as could their white counterparts. The reduced feeding activity Ms de Witt observed in the black springbok, compared to the other springbok, is consistent with such a saving. A reduced energetic cost of homeothermy in dark-coloured animals, via their better access to solar heat, has been reported often (Hamilton and Heppner, 1967; Lustick, 1969; Heppner, 1970; Dmi'el *et al.*, 1980; Finch *et al.*, 1980; Burtt, 1981; Finch *et al.*, 1984). However, as conditions get hotter with climate change dark-coloured individuals may be disadvantaged. White-coated Santa Inês ewes showed better tolerance to heat stress (McManus *et al.*, 2009) and are therefore likely to be better adapted to hot conditions in the future than their darker counterparts.

Thus I conclude that there is adaptive significance of colour morphs, and there probably are selective pressures acting against both the white and the black springbok at different times of the year. The black springbok seem able to reduce energy expenditure in winter, as predicted and in accord with anecdotal reports, but experience higher solar heat load in hot conditions. On the other hand, the white springbok apparently is living closer to the energetic edge in winter, possibly because its lower heat load from solar radiation requires a higher metabolic cost of homeothermy. From a physiological perspective, the normal springbok appears to occupy a compromise position, with better energy balance than the white springbok in winter and less overheating than the black springbok in summer, a compromise which may explain why the black and white springbok rarely occur naturally, despite the genes for those colour morphs being present. The crypsis function of the normal springbok's colouration may further explain the preponderance of its prevalence, as colour is not only important for thermoregulation but also serves an aposematic function (Caro, 2009). Nevertheless, maintaining such genetic diversity within a population, particularly diversity for a trait that adapts individual animals to different thermal environments, may provide an important pre-adaptation to climate change.

2.6 Acknowledgements

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CHAPTER 3

Adapting to arid environments: thermoregulation and cathemerality of free-living Arabian oryx (*Oryx leucoryx*) in a hyper-arid desert

Data and ideas presented in this chapter have been written up as a scientific paper and submitted to Physiological and Biochemical Zoology. Hetem R.S., Strauss W.M., Fick L.G., Maloney S.K., Meyer L.C.R., Shobrak M., Fuller A. and Mitchell D. Thermoregulation and cathemerality of free-living Arabian oryx (*Oryx leucoryx*) in a hyper-arid desert.

3.1 Abstract

In a desert environment, endotherms have to trade off thermoregulation, osmoregulation and energy acquisition. One physiological mechanism proposed to conserve body water at the expense of thermoregulation is heterothermy, a variability in body temperature beyond the limits of homeothermy. Alternatively, endotherms may reduce diurnal activity and seek shade to maintain homeothermy at the expense of energy acquisition. With the help of my colleagues, I assessed the thermoregulatory strategies of the arid-adapted Arabian oryx (*Oryx leucoryx*) by implanting data loggers to measure body temperature and activity patterns, for a one-year period, in five free-living oryx inhabiting a desert in Saudi Arabia. As predicted for adaptive heterothermy, during hot months compared to cooler months, maximum daily body temperatures were not only higher (41.1 ± 0.3°C vs. 39.7 ± 0.1°C, $P = 0.0002$) but minimum body temperatures also were lower (36.1 ± 0.3°C vs. 36.8 ± 0.2°C, $P = 0.04$), resulting in a larger 24-h amplitude of body temperature rhythm (5.0 ± 0.5°C vs. 2.9 ± 0.2°C, $P = 0.0007$). Body temperature variability was associated not only with ambient temperature, but also with water availability, with oryx displaying a greater 24-h amplitudes of nychthemeral rhythm of body temperature during warm dry months compared to warm wet months (3.6 ± 0.6°C vs. 2.3 ± 0.3°C, $P = 0.005$). Oryx displayed flexibility in their activity patterns and shifted from a continuous rhythm with crepuscular peaks during warm months to a nocturnal rhythm during hot months. The attenuation in activity over daylight hours during the hot months compared to warm months (25 ± 8% vs. 48 ± 5%, $P = 0.0004$) was accompanied by the selection of cooler microclimates, up to 12°C cooler than that experienced in the sunshine, during the heat of the day. An additional thermoregulatory strategy proposed to conserve water is selective brain cooling. Although the magnitude of selective brain cooling was small (< 1ºC), my oryx frequently employed selective brain cooling. Arabian oryx therefore employ heterothermy, cathemerality and, I believe, selective brain cooling to survive the extremely hot and arid conditions of the Arabian Desert.

3.2 Introduction

Since many arid regions of Africa are predicted to get both hotter and drier with climate change (Boko *et al.*, 2007), understanding the physiological mechanism exhibited by desert antelope would assist future studies in predicting physiological adaptations to desertification. Amongst large desert-dwelling mammals, the species which arguably faces the greatest challenge to homeostasis is the Arabian oryx (*Oryx leucoryx*). With a body mass of 80-100 kg, this artiodactyl inhabits one of the hottest deserts in the world, in Saudi Arabia, and survives most of the year, including the hottest summer months, without access to drinking water. Because of the subsequent limitations in the use of evaporative cooling to dissipate metabolic heat and heat gained from the environment, the animals have to trade off thermoregulation, osmoregulation and acquisition of energy. Desert artiodactyls display a summer nadir in metabolic rate (Nagy and Knight, 1994; Williams *et al.*, 2001; Ostrowski *et al.*, 2006a; Ostrowski *et al.*, 2006b). However, these low metabolic rates in summer are associated with low quality and quantity of food (Choshniak *et al.*, 1995; Piccione *et al.*, 2002; Ahmed and El Kheir, 2004), and it has not been determined whether the reduced metabolism occurs in response to reduced energy intake or as an adaptation to reduce the requirements for heat dissipation.

Another physiological mechanism exhibited by desert artiodactyls, with the capacity to reduce evaporative cooling, is adaptive heterothermy. It is defined as the storage of body heat during the day, with a consequent rise in body temperature, reducing both heat gain and evaporative heat loss (Schmidt-Nielsen *et al.*, 1957). This stored heat then can be dissipated non-evaporatively during the colder night, allowing body temperature to fall. Animals using heterothermy therefore would display an increase in the amplitude of nychthemeral rhythm of core body temperature under high environmental heat loads. Such heterothermy might result simply from failure of thermoregulation in the heat, but also could be an active process, termed “adaptive heterothermy”, in which the 24-h nadir of body temperature is depressed, even in well-fed animals, allowing more heat to be stored before body temperature reaches lethal levels.

Early studies reporting the existence of adaptive heterothermy were performed on captive animals, often deprived of both drinking water and behavioural thermoregulatory opportunities (Schmidt-Nielsen *et al.*, 1957; Taylor, 1969a), however, adaptive heterothermy appears to be absent in most free-living African artiodactyl species studied (Mitchell *et al.*, 2002), in environments similar to those in which captive artiodactyls do display heterothermy. Only two studies to date have reported the existence of adaptive heterothermy in free-living artiodactyls, namely the Arabian oryx (Ostrowski *et al.*, 2003) and the smaller, sympatric, Arabian sand gazelle (*Gazella subgutturosa marica*, Ostrowski and Williams, 2006). The Arabian oryx displayed an amplitude of nychthemeral rhythm of body temperature which increased from 1.5 ± 0.6°C in winter to 4.1 ± 1.7°C in summer (Ostrowski *et al.*, 2003), but the recordings of body temperature, especially at night, were sporadic, and night recordings and daytime recordings were not necessarily contiguous. Although recordings for the sand gazelle were continuous, these antelope displayed an amplitude of nychthemeral rhythm of body temperature of 1.7 ± 0.3°C in winter, which increased to only 2.6 ± 0.8°C in summer (Ostrowski and Williams, 2006). So the nychthemeral amplitudes for both of these species, in spite of the thermal stress to which they were exposed with mean daily maximum air temperature exceeding 40°C, were substantially lower than the 6ºC variation originally reported for captive camel (*Camelus dromedaries*, Schmidt-Nielsen *et al.*, 1957), eland (*Tragelaphus oryx*) and oryx (*Oryx gazella beisa*, Taylor, 1969a). Nevertheless, if adaptive heterothermy does indeed exist, it is likely to occur in a species able to survive independent of water in an arid environment, such as the Arabian oryx (Tear *et al.*, 1997; Williams *et al.*, 2001; Ostrowski *et al.*, 2002;
van Heezik *et al.*, 2003).

Another conspicuous thermoregulatory adaptation of artiodactyls, including the genus *Oryx* (Maloney *et al.*, 2002), is selective brain cooling. Selective brain cooling, the reduction of brain temperature below arterial blood temperature (IUPS Thermal Commission, 2003), originally was hypothesized to protect the

brain from a rise in body temperature, such as that associated with heterothermy (Schmidt-Nielsen *et al.*, 1957). However, recent literature proposes that arid-zone mammals possessing a carotid rete may employ selective brain cooling to attenuate thermal drive, because selective brain cooling reduces hypothalamic temperature, which reduces evaporative heat loss and ultimately conserves water (Mitchell *et al.*, 2002). Selective brain cooling therefore may be a mechanism which gives rise to heterothermy, rather than protection from it. Selective brain cooling has not been investigated in Arabian oryx.

A third thermoregulatory mechanism that might reduce the need for evaporative cooling is shade-seeking behaviour and a reduction of diurnal activity, both of which have been observed in Arabian oryx in summer (Stanley Price, 1989; Seddon and Ismael, 2002; Ostrowski *et al.*, 2003). Such behaviour, though, must reduce diurnal foraging, and, unless there is a compensatory increase in nocturnal foraging, must compromise energy acquisition, especially because resources are limited in the summer. Whether Arabian oryx increase nocturnal foraging as a result of the high heat loads during summer months is not known.

With the help of my colleagues, I therefore measured abdominal, brain and arterial blood temperature, activity and microclimate selection, of free-living Arabian oryx occupying a hyper-arid desert in Saudi Arabia to ascertain the importance of, and the potential interactions between, the reputed water-saving mechanisms. I employed data loggers to obtain regular measurements of body temperatures, activity and microclimate over one year. Such measurements have not been achieved previously for Arabian oryx, or indeed any free-living animal species.

3.3 Materials and methods

3.3.1 Animals and habitat

The study took place between March 2006 and April 2007 within the 2200 km^2 Mahazat as-Sayd Protected Area (28°15’ N, 41°40’ E) in the open steppe desert in

west-central Saudi Arabia that is both the historical and current habitat for Arabian oryx (*Oryx leucoryx*, Ostrowski *et al.*, 1998). Three male and two female, adult, wild-born oryx were captured, under veterinary supervision, in the protected area, in mid-March 2006. The oryx were habituated in outdoor pens for two weeks to reduce potential peri-operative stress. An additional male oryx was obtained from the breeding herd at the National Wildlife Research Center in Taif (21°15' N, 40°42' E). This male oryx remained in a partially open pen throughout the 11-month study period with lucerne and water available *ad libitum*. All experimental procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (protocol no. 2005/87/5).

3.3.2 Surgery

The oryx, at both locations, were darted and anaesthetised in the holding pens with etorphine hydrochloride (2.5 mg intramuscularly (I.M.), M99, C-Vet, Leyland, UK) and, once recumbent, were transported to a temporary operating theatre within 200 m of the pens. At the surgery, the animals were placed in sternal recumbency, with their heads elevated. Animals were intubated and anaesthesia was maintained with 2-6% isoflurane (Aerrane, Astra Zeneca, Johannesburg, South Africa), administered in 100% oxygen. Respiratory rate, heart rate, arterial oxygen saturation (pulse oximeters, Nonin 9847V, Nonin Medical, North Plymouth, USA) and rectal temperature (thermocouple thermometer, BAT-12, Physitemp, Clifton, USA) were monitored throughout the surgery, which lasted approximately two hours.

Under sterile surgical conditions, several miniature data loggers were implanted. All loggers were covered in an inert wax (Sasol, Johannesburg, South Africa), and dry-sterilized in formaldehyde vapour before implantation. After administration of a local anaesthetic (0.04 g lignocaine hydrochloride, subcutaneously (S.C.), Bayer Animal Health (Pty) Ltd, Isando, South Africa), incision sites were shaved and sterilized with povidone iodine antiseptic (Vetedine, Vetoquinol, Lure, France). Each oryx was fitted with data loggers connected to thermistor sensors for temperature measurement in the carotid artery, brain, and abdominal cavity. For

measurement of carotid temperature, a thermistor inserted in a blind-ended and thin-walled polytetrafluoroethylene (PTFE) catheter (o.d. 1.35 mm, i.d. 0.97 mm; Straight Aortic Flush 4F Catheter, Cordis, The Netherlands) was advanced 60 mm into the left common carotid artery towards the heart, at a position midway along the length of the neck, and secured with a purse-string suture in the artery wall. Outside the artery, the PTFE tube was sealed on a PTFE-coated co-axial cable (150 mm long, o.d. 3 mm, Belden, Richmond, USA) connecting the thermistor to the temperature-sensitive data logger (see temperature measurements). The data logger was positioned subcutaneously, dorsal to the artery. For measurement of brain temperature, a second data logger, connected to the thermistor positioned in the brain, was positioned subcutaneously, caudal to the base of the left ear. Its PTFE-coated cable was advanced subcutaneously over the skull, where it was connected to a head plate and guide tube. The guide tube, constructed from cellulose acetate butyrate tubing (40 mm long, o.d. 3.2 mm, i.d. 1.6 mm; World Precision Instruments, Sarasota, Florida, USA) sealed at the tip by a steel cap, was inserted through a 2 mm diameter burr hole, which was drilled through the cranium, at appropriate co-ordinates pre-determined from head sections of dead oryx of similar size, so that the probe tip would be positioned near the hypothalamus. The brain guide tube was connected to a polyvinyl chloride head plate ($20 \times 10 \times 5$ mm), which was secured to the skull by two bone screws and covered by skin so that it lay secured in a subcutaneous position.

For measurement of abdominal temperature, a third data logger, with an internal temperature sensor, was inserted, via an incision in the paralumber fossa, into the abdominal cavity. The muscle layer was sutured closed and an activity logger (Actical, Mini-Mitter Corporation, Bend, Oregon, USA), for measurement of activity, was tethered to the outer layer of the abdominal muscle before the skin was sutured closed over the logger.

Wounds were treated with a topical antiseptic spray (Necrospray, Centaur Labs, Johannesburg, South Africa). Each animal received a long-acting antibiotic (450 mg I.M., penicillin, Norocillin La, Norbrook Laboratories Ltd., Newry, Northern

Ireland), a non-steroidal anti-inflammatory analgesic (100 mg I.M., phenylbutazone, Dexaphenylarthrite injectable solution, Vetoquinol Veterinary Pharmaceuticals, Cedex, France), a long-acting parasiticide (2 ml S.C., Ivermectin, Noromectin, Norbrook Laboratories Ltd., Newry, Northern Ireland) and a multivitamin (9 ml I.M., Multivit injectable solution, Univet Ltd., Ireland).

Before anaesthesia was terminated, a neck collar (MOD-500 Telonics, Inc. Mesa, AZ, USA) was fitted to each oryx. In addition to a tracking radio transmitter, each collar supported a miniature black globe thermometer ("miniglobe"), to allow for the dynamic measurement of the microclimate that the oryx chose to occupy. This technique has been validated previously on other ungulate species (Appendix 1, Hetem *et al.*, 2007). Miniglobe temperature was measured by a small temperature-sensitive data logger (see temperature measurements) inserted into the centre of a matt-black hollow copper sphere (30 mm diameter, Press Spinning & Stamping Co., Cape Town, South Africa). The globe was attached to a 10-mm diameter, polyvinyl chloride rod, which in turn was attached to the outer surface of the collar. A weight on the ventral side of the collar ensured that the miniglobe remained over the dorsum of the neck and could not be shaded by the animal's body.

Following surgery, the oryx were transported back to their pens, where they became ambulatory within ~ 10 min after the effect of etorphine was reversed with diprenorphine hydrochloride (7.5 mg intravenously (I.V.), M5050, C-Vet, Leyland, UK). After a two-week recovery period, and following veterinary inspection, the five oryx in the Mahazat as-Sayd Protected Area were released into a 2 km^2 fenced enclosure with natural forage and water available *ad libitum*. Ten days later the five oryx were allowed to enter and range freely within the Mahazat as-Sayd Protected Area. The five oryx separated from each other shortly after their release and were left undisturbed, apart from the occasional serendipitous visual contact with rangers, for one year.

In April 2007, the oryx were tracked, captured and transported to the holding pens. The animals were anaesthetized once again and the data loggers were removed under a surgical procedure similar to that used for the original implantation. The animals' wounds had healed and there were no signs of infection from the initial surgery. Most of the abdominal loggers were found intra-abdominally in the pelvic canal and were not encapsulated in adhesive tissue. After surgery and a two-week recovery period in the pens, the oryx were re-released into the Mahazat as-Sayd Protected Area. A similar procedure was used to retrieve the loggers from the oryx housed at the National Wildlife Research Center in Taif.

3.3.3 Temperature and activity measurements

The miniature thermometric data loggers (StowAway XTI, Onset Computer, Pocasset, Massachusetts, USA) used to measure brain, carotid artery and abdominal temperature had outside dimensions of ~ $50 \times 45 \times 20$ mm and a mass of ~ 40 g when covered in wax. These loggers had a resolution of 0.04°C and measurement range from + 34°C to + 46°C. Temperature sensors used to measure brain and carotid blood temperatures were constructed from ruggedized glass-coated bead thermistors with insulated extension leads (bead diameter 0.3 mm; AB0E3-BR11KA103N, Thermometrics, Edison, New Jersey, USA). The scan interval of the brain and carotid blood loggers was set at 5 min and that of the abdominal logger was set at 15 min. Miniglobe temperatures were recorded every hour using a smaller thermometric data logger (iButton DS1922T, Maxim, Dallas Semiconductor, Texas, USA), which weighed ~ 10 g. These loggers had a resolution of 0.5°C and a measurement range from 0°C to 125°C.

All temperature sensors and loggers were calibrated against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany) in an insulated water bath. After calibration, the loggers and their sensors measured blood, brain and abdominal temperature to an accuracy of better than 0.05°C and miniglobe temperature to better than 0.5°C.

The activity logger recorded movement via a multidirectional, piezoelectric accelerometer which was sensitive to 0.05g. Movement was recorded over 5-min intervals at a sampling rate of 32 Hz. The logger had dimensions of 40 x 40 x 15 mm and weighed ~ 40 g when covered in wax.

3.3.4 Climatic data measurements

I collected climatic data from a portable weather station erected near the Mahazat as-Sayd Protected Area, at the Saja/Umm ar-Rimth Protected Area (23°22' N, 42°45' E), and also at the National Wildlife Research Center in Taif. I recorded wind speed ($m.s^{-1}$), solar radiation ($W.m^{-2}$), dry-bulb temperature (°C) and relative humidity (%). I also recorded miniature (30 mm diameter) black globe temperature (°C) and rainfall on site at the Mahazat as-Sayd Protected Area for the duration of the study period. Photoperiod was calculated as the daily difference between sunrise and sunset times obtained from the U.S. Naval Observatory website (http://aa.usno.navy.mil/data/).

3.3.5 Data analysis

For analysis of seasonal patterns, data from the 11-month study were averaged over four seasonal periods, based on prevailing climatic conditions (Table 3.1), namely warm wet (April to May), hot dry (June to August), warm dry (September to November) and cool dry (December to February). March was not included in my analysis to allow for post-surgical recovery. Although I called the April to May period "warm wet', only 24 mm of rain fell during this period (see Table 3.1). For successive 24-h periods, I calculated mean, minimum, maximum and amplitude of nychthemeral rhythm of body temperature as measured in the abdominal cavity. I averaged the body temperature parameters for each individual, for each period, and performed one-way repeated-measures analysis of variance (ANOVA) to test for differences in the body temperature profile across the four periods, with Newman-Keuls multiple comparison tests to identify sources of differences.

To further assess the influence of the environment on body temperature patterns, I correlated the various parameters of the body temperature profile and the corresponding prevailing environmental conditions. Since oryx seek shade during the heat of the day (Stanley Price, 1989), dry-bulb air temperature probably provides a better approximate of operative temperature than does the globe temperature in the sun. I therefore correlated the mean 24-h body temperature, averaged for all five oryx, with mean 24-h air temperature, and the amplitude of nychthemeral rhythm of body temperature against the 24-h amplitude of air temperature. I also correlated mean minimum body temperature, within each 24-h period, against the concurrent 24-h minimum and maximum air temperature, and 24-h maximum body temperature against the concurrent 24-h maximum air temperature. I fitted simple non-linear regressions where appropriate. To test the effect of season on the magnitude of the amplitude body temperature rhythm, I correlated the 24-h amplitude of nychthemeral rhythm of body temperature, averaged for all five oryx, against photoperiod.

One of my oryx (Oryx 5) died, of unknown causes in February, that is 10 months after surgery, and the data logger which had measured his carotid temperature was not recovered. The data loggers which measured carotid temperature in three oryx (Oryx 1, 3 and 4) failed within 20 days after surgery. The data logger which measured brain temperature in the captive male oryx failed within a month after surgery. I obtained a complete set of brain temperature and carotid blood temperature data for only a single free-living male oryx (Oryx 2). I analyzed the relationship between brain temperature and carotid blood temperature by sorting all 5-min measurements of carotid blood temperature into 0.1°C classes, and determining the mean, standard deviation, maximum, and minimum brain temperature at each class of carotid blood temperature.

The battery life of the activity loggers restricted measurements to the warm wet, hot dry and warm dry periods. To account for differences in the sensitivity of individual activity loggers I calculated activity as a percentage of the maximum reading each logger recorded, over the entire period. Since activity levels in light

and dark phases may be linked to the duration of the phases (Hill, 2006), I calculated “diurnal” activity between 06:00 and 18:00 and “nocturnal” activity between 18:00 and 06:00. I compared mean 24-h activity, actual diurnal activity and diurnal activity expressed as a proportion of 24-h activity, across periods, using a one-way repeated-measures ANOVA with Newman-Keuls post-hoc analysis.

The miniglobe thermometers broke off the collars of all three male oryx, so I retrieved data on the microclimate selected only for the two female oryx (Oryx 1 and 4). I correlated collar miniglobe temperatures against weather station miniglobe temperatures using linear Pearson procedures. To assess whether the animals were conforming to ambient conditions or were selecting microclimates, I tested whether the slope of the regression equation was significantly different from one (the slope of the line of identity). In addition, I tested whether the slope and elevation of the regression equations were significantly different across seasonal periods using an analysis of co-variance (ANCOVA).

I used Statistica (kernel release 5.5 for Windows, StatSoft, Inc. (1999), Tulsa Oklahoma, USA) and GraphPad Prism (version 4.00 for Windows, GraphPad Software, San Diego California USA) for statistical analyses. Values are expressed as mean ± SD and $P < 0.05$ was considered significant.

3.4 Results

3.4.1 Climate

Environmental conditions over the four periods are shown in Table 3.1. Ambient temperatures were similar during the warm wet and warm dry periods. On average, the hot dry period was 15ºC warmer than the cool dry period. Air and black globe temperature varied as a function of time of day, peaking just after solar noon (12:00) and reaching a minimum just before sunrise. Solar radiation showed the expected bell-shaped distribution and wind speed increased in the late afternoon. Rainfall totalled 31 mm over the study period, substantially lower than the ten-

year average of 100 ± 60 mm. April was the wettest month (17 mm) but some rain fell during March (7 mm) and May (7 mm). March was not included in my analysis to allow for post-surgical recovery.

Table 3.1. Environmental conditions (mean ± SD) during the four periods in which the oryx were free-living in the Mahazat as-Sayd Protected Area

	Warm wet (Apr-May)	Hot dry (Jun-Aug)	Warm dry (Sep-Nov)	Cool dry (Dec-Feb)
Globe temperature (˚C)				
24-h mean	33.8 ± 3.4	37.9 ± 1.7	32.1 ± 3.8	23.3 ± 3.6
24-h minimum	18.5 ± 3.3	22.9 ± 2.5	16.4 ± 3.8	10.3 ± 3.4
24-h maximum	53.3 ± 5.2	57.1 ± 3.9	52.3 ± 5.4	42.6 ± 6.1
Air temperature (˚C)				
24-h mean	27.7 ± 4.0	34.0 ± 1.5	26.1 ± 4.6	15.4 ± 3.7
24-h minimum	19.9 ± 4.2	25.7 ± 2.3	18.2 ± 3.8	8.9 ± 3.6
24-h maximum	34.1 ± 4.0	40.4 ± 1.3	33.2 ± 4.8	22.0 ± 4.3
Mean 24-h wind speed ($m.s^{-1}$)	4.4 ± 1.4	3.9 ± 1.0	3.9 ± 1.1	4.5 ± 1.4
Mean daytime radiation ($W.m^{-2}$)	561 ± 93	580 ± 50	456 ± 65	394 ± 79
Total rainfall (mm)	24	0	0	0
Mean time of sunrise	05:36 ± 0:15	05:29 ± 0:12	06:16 ± 0:16	06:58 ± 0:07
Mean time of sunset	18:48 ± 0:10	19:04 ± 0:11	17:48 ± 0:25	17:40 ± 0:18

3.4.2 Body temperature

The typical body temperature patterns for a single free-living male oryx and the captive male over the 11-month study period are shown in Figure 3.1. Also shown are the air temperatures prevailing near to where the animals were located. There was a clear increase in the 24-h amplitude of body temperature rhythm during the hot dry period (June to August) in the free-living male oryx, which was not evident in the captive male oryx. The nychthemeral rhythm of body temperature averaged for all five free-living oryx is shown in Figure 3.2. Although ambient temperature had an apparent effect on the nychthemeral rhythm of body temperature, as the oryx showed an increased amplitude of body temperature

rhythm during the hot dry compared to cool dry period (Fig. 3.2A), ambient temperature was not the only factor to influence body temperature rhythm. During the warm wet and warm dry periods environmental conditions were similar (Fig. 3.2D, Table 3.1), but there was a difference in the amplitude of body temperature rhythm. In particular, oryx displayed a lower minimum body temperature during the warm dry than during the warm wet period (Fig. 3.2C).

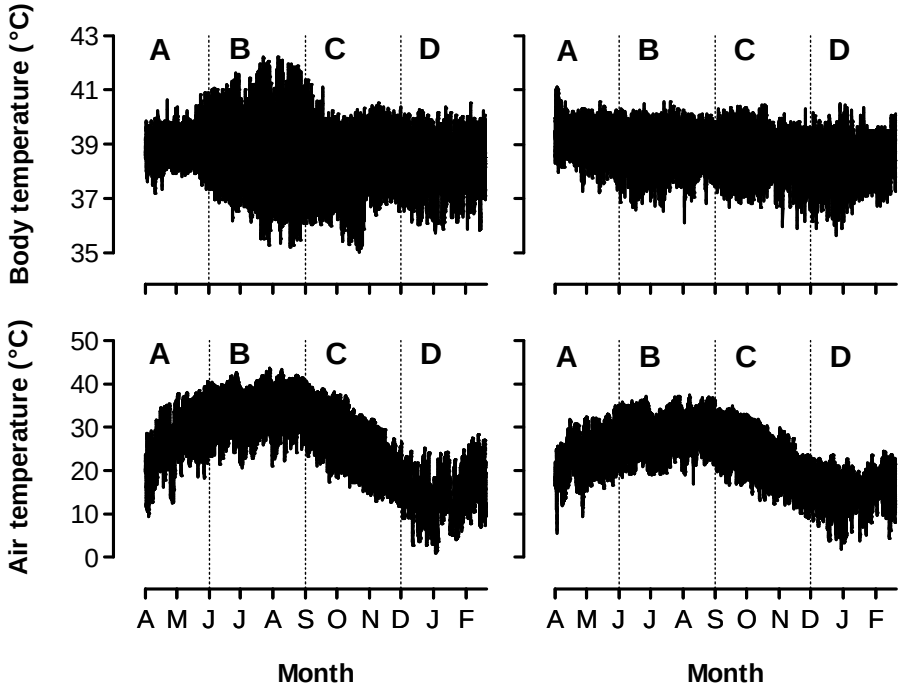

Figure 3.1. The top two panels show the original record of 15-min recordings of body temperature from a single free-living male oryx (Oryx 2, left panel) and the captive male oryx (right panel) , which had access to water *ad libitum*, over the 11-month study period (April 2006 to February 2007). The bottom two panels show the air temperature recorded at nearby weather stations, namely the Saja/Umm ar-Rimth Protected Area (left panel) and National Wildlife Research Center in Taif (right panel), over the same period. The dotted lines separate the data into the four periods analyzed, namely warm wet (panel A), hot dry (panel B), warm dry (panel C) and cool dry (panel D).

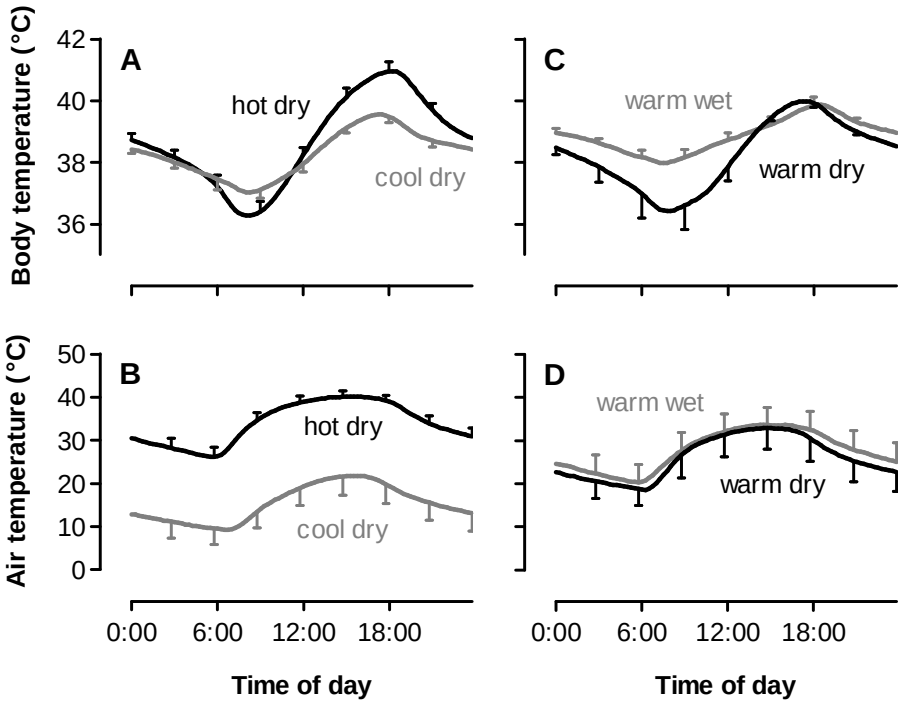

Figure 3.2. The top two panels show the nychthemeral rhythm of body temperature (mean ± SD), averaged for all five free-living oryx, over the four periods. Panel A shows data from the cool dry (light line) and hot dry (dark line) periods, and panel C from the warm wet (light line) and warm dry (dark line) periods. The bottom two panels show the nychthemeral rhythm of air temperature (mean ± SD) over the four periods. Panel B shows data from the cool dry (light line) and hot dry (dark line) periods, and panel D from the warm wet (light line) and warm dry (dark line) periods.

A one-way repeated-measures ANOVA revealed that mean (Fig. 3.3A, $F_{3,9} = 11.2$, $P = 0.002$), minimum (Fig. 3.3B, $F_{3,9} = 15.4$, $P = 0.0007$), maximum (Fig. 3.3C, $F_{3,9} = 41.4$, $P < 0.0001$) and amplitude (Fig. 3.3D, $F_{3,9} = 23.0$, $P = 0.0001$) of nychthemeral body temperature rhythm differed significantly between periods (Fig. 3.3). Maximum body temperatures (Fig. 3.3C) and amplitude of body temperature rhythm (Fig. 3.3D) appeared to be influenced by changes in air temperature, as these variables were higher during the hot dry months than during the other dry periods. However, minimum body temperatures appeared to be influenced by changes in water availability, as minimum body temperature was higher during the warm wet months than during the dry periods (Fig. 3.3B). Notably, minimum body temperature was lower during the warm dry period than during the warm wet period, despite ambient temperatures being similar. This lower minimum body temperature during the warm dry period resulted in a lower

mean body temperature (Fig. 3.3A) and a greater body temperature amplitude during the warm dry, than during the warm wet, period.

To further assess the influence of ambient temperature on body temperature, I plotted body temperature variables against air temperature (Fig. 3.4). Mean 24-h air temperature accounted for 35% of the variability in mean 24-h body temperature ($P < 0.0001$, Fig. 3.4A). There was a disjunction in the relationship, however, above a mean air temperature of about 25ºC, probably because mean 24-h body temperature was lower during warm dry than during warm wet periods (Fig. 3.3A), even though air temperatures were similar. Maximum 24-h air temperature accounted for 85% of the variability in maximum 24-h body temperature, across all 313 days of the study, when a best-fit power curve was fitted to the data (Fig. 3.4B). Above a maximum 24-h air temperature 36ºC, maximum 24-h body temperature increased by 0.25ºC per 1ºC increase in maximum 24-h air temperature. Conversely, over the full range of air temperatures, minimum 24-h body temperature was independent of both minimum 24-h air temperature (Fig. 3.4C), and maximum 24-h air temperature (Fig. 3.4D). There was a trend for minimum 24-h body temperatures to increase with increasing minimum 24-h air temperatures up to 15ºC ($r^2 = 0.57$, $P < 0.0001$), and increasing maximum 24-h air temperatures up to 30ºC ($r^2 = 0.47$, $P < 0.0001$). Again there was a disjunction in the relationship when minimum 24-h air temperature was between 15ºC and 25ºC and when maximum 24-h air temperature was between 30ºC and 38ºC, corresponding to the low minimum body temperatures displayed by the oryx during the warm dry, and not during the warm wet, period.

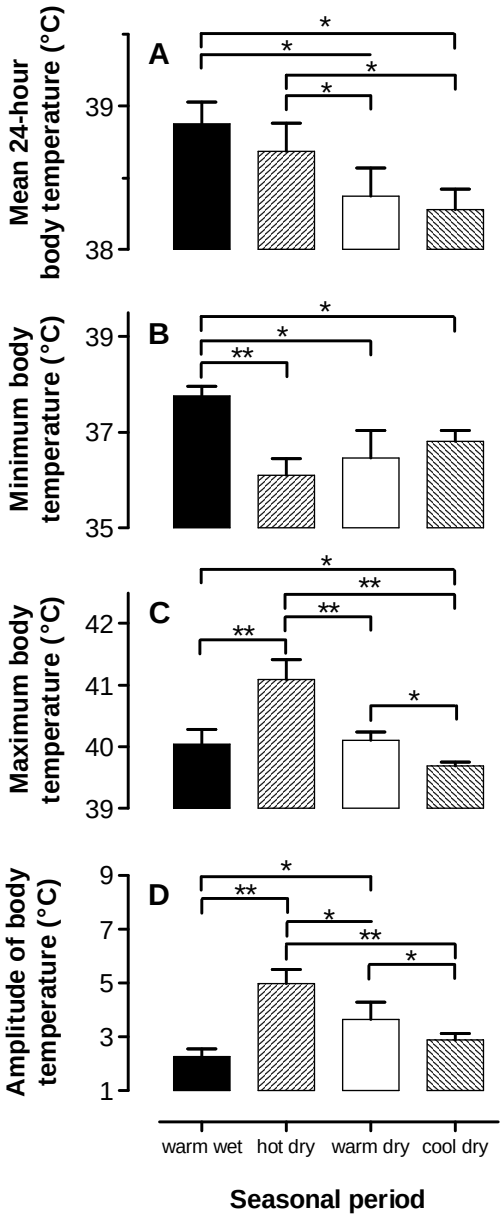

Figure 3.3. 24-h mean (panel A), minimum (panel B), maximum (panel C), and amplitude of the nychthemeral rhythm (panel D) of body temperature (mean ± SD, n = 5) over the four periods, namely warm wet (black bar), hot dry (hatched bar), warm dry (white bar) and cool dry (hatched bars). * $P < 0.05$, ** $P < 0.001$, Newman-Keuls multiple comparison tests.

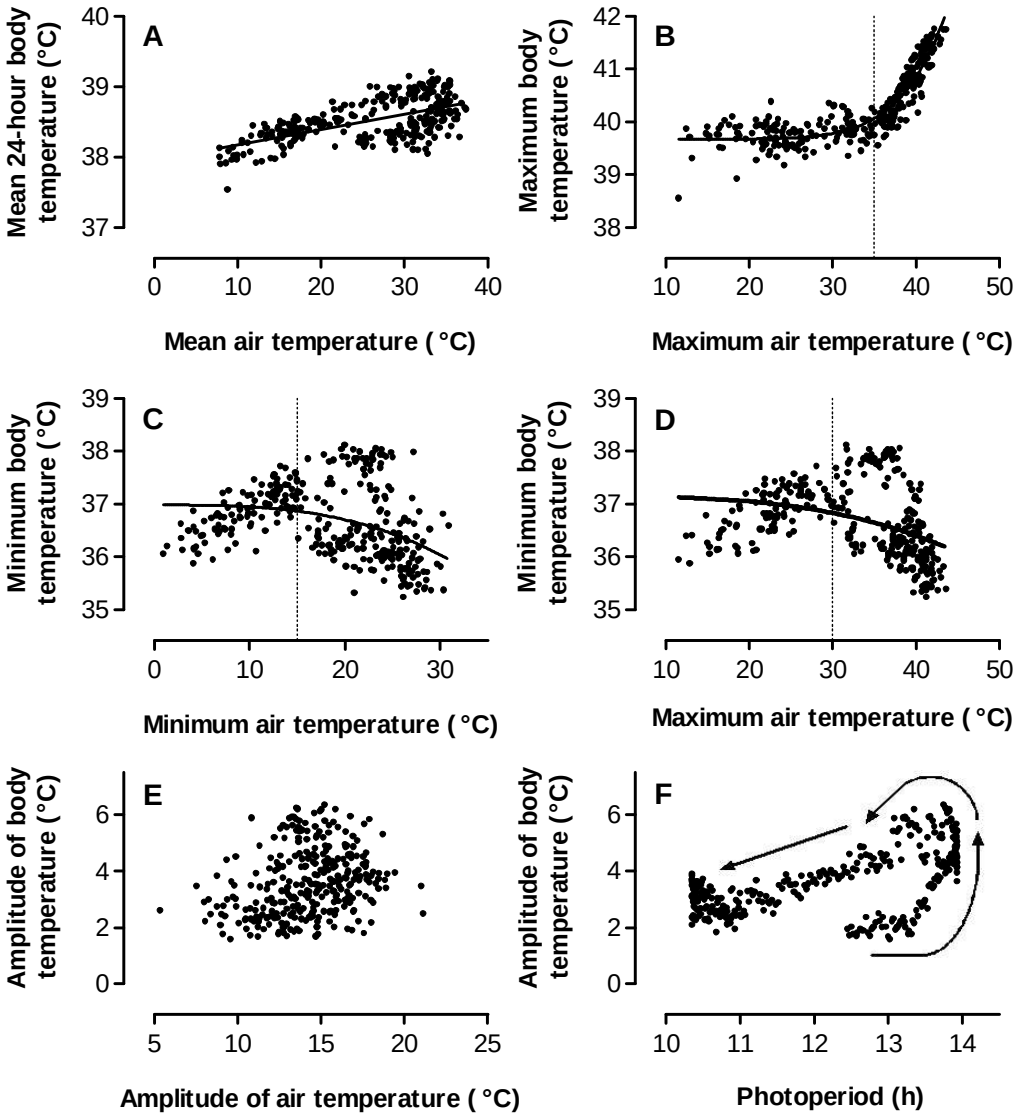

Figure 3.4. 24-h mean of body temperature (mean, n = 5) plotted against mean 24-h air temperature (panel A, $r^2 = 0.35$, $y = 0.02x + 38.0$), and the maximum daily body temperature plotted against maximum daily air temperature (panel B, $r^2 = 0.85$, $y = 39.7 + 9.2x10^{-14}x^{8.2}$) for 313 individual days, when the oryx were living free. Minimum daily body temperature (mean, n = 5) was correlated poorly with minimum daily air temperature (panel C, $r^2 = 0.13$, $y^2 = 1368 - 0.003x^3$) and with maximum daily air temperature (panel D, $r^2 = 0.15$, $y^2 = 1379 - 0.0008x^3$). So too, amplitude of nychthemeral rhythm of body temperature (mean, n = 5) was correlated poorly with the 24-h amplitude of air temperature (panel E, $r^2 = 0.07$) but better with photoperiod (panel F, $r^2 = 0.31$, $y = 0.50x - 2.5$), although there were two discrete relationships at long photoperiods. The curved arrows indicate the progression of time.

One classic measure of adaptive heterothermy is a positive correlation between the amplitude of body temperature rhythm and the amplitude of air temperature rhythm. I found no such correlation (Fig. 3.4E). On the other hand, the amplitude of body temperature rhythm was significantly correlated to photoperiod (Fig. 3.4F). Although the linear correlation between amplitude of body temperature rhythm and photoperiod was significant, the relationship was not straightforward. The amplitude of body temperature rhythm was ~ 2ºC during the warm wet period, when photoperiod was ~ 13 h, and increased to ~ 6ºC as conditions got progressively warmer and drier, which coincided with an increased photoperiod. However, the amplitude of body temperature rhythm did not return to the expected 2ºC as day length shortened and conditions got cooler. Instead, during the warm dry conditions oryx continued to have a low minimum body temperature, which resulted in the amplitude of body temperature rhythm remaining high until conditions became cooler with shorter day lengths.

3.4.3 Activity

The nychthemeral rhythm of activity also varied over the three seasonal periods analyzed (Fig. 3.5); because of a limited battery life of the activity loggers I did not obtain measurements in the cool dry period. During the warm wet period, when the amplitude of body temperature rhythm was smallest, the oryx displayed a continuous but biphasic activity pattern with crepuscular peaks (Fig. 3.5A). This pattern shifted to a predominantly nocturnal activity during the hot dry period (Fig. 3.5B), with minimal activity between 09:00 and 18:00. The pattern returned to biphasic activity as conditions got cooler in the warm dry period (Fig. 3.5C), even though the amplitude of body temperature rhythm still was greater during the warm dry period than during the warm wet period (Fig. 3.5C)

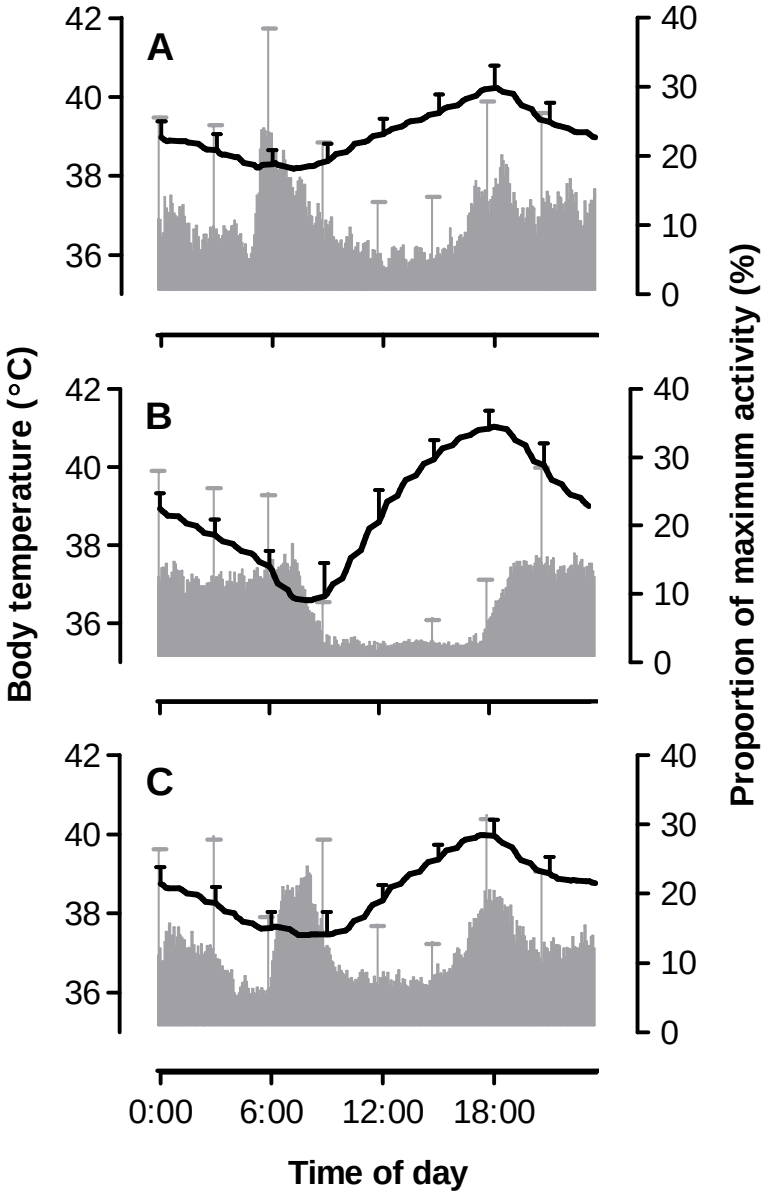

Figure 3.5. Mean ± SD of the nychthemeral rhythm of body temperature (black line) and activity (grey bars) of a representative oryx (Oryx 3) over the warm wet (panel A), hot dry (panel B) and warm dry (panel C) periods. The oryx shifted from a continuous but biphasic activity pattern with crepuscular peaks during the warm periods to nocturnal activity during hot periods. Activity was measured by a data logger, implanted subcutaneously on the left abdominal wall, as activity counts per 5 min and is shown as a percentage of the maximum count for each logger.

Mean 24-h activity did not differ across the three periods ($F_{2,8}$ = 2.2, P = 0.17). Nevertheless, mean 24-h activity of all five oryx was significantly, although weakly, correlated to both maximum daily air temperature (r^2 = 0.22, P < 0.0001, Fig. 3.6A), and photoperiod (r^2 = 0.18, P < 0.0001, Fig. 3.6B). Mean activity level between 06:00 and 18:00, however, did differ significantly over the three periods ($F_{2,8}$ = 22.4, P = 0.0005), with mean daytime activity being significantly lower during the hot dry period than during both the warm wet (P = 0.001) and the warm dry (P = 0.0007) periods. The proportion of total daily activity which occurred between 06:00 and 18:00 decreased from 48 ± 5% during warm wet periods to 25 ± 8% during the hot dry period, and returned to 48 ± 11% during the warm dry period ($F_{2,8}$ = 24.0, P = 0.0004). The proportion of total daily activity occurring between 06:00 and 18:00 was correlated strongly with maximum 24-h air temperature (Fig. 3.6C), and with photoperiod (Fig. 3.6D). Maximum 24 h air temperature accounted for 61% (P < 0.0001) of the variability in diurnal activity, and photoperiod accounted for 47% (P < 0.0001). However, a partial correlation coefficient revealed that the original correlation of activity versus photoperiod was confounded by maximum 24-h air temperature ($r_{xz.y}$ = -0.05, t_{210} = -0.75, P = 0.45) and that maximum 24-h air temperature accounted for only 27% of the proportion of diurnal activity when the effect of photoperiod was eliminated ($r_{xy.z}$ = -0.52, t_{210} = -8.89, P < 0.0001).

3.4.4 Microclimate

The periods of reduced activity during the hot dry period appeared to coincide with periods of shade-seeking behaviour. The slopes of the correlations between the miniglobe temperature on the collar of two female oryx and miniglobe temperature recorded at a nearby weather station and exposed to direct solar radiation, were significantly less than one (Fig. 3.7, ANCOVA, P < 0.0001). The regression lines intersected the line of identity, implying that oryx selected microclimates cooler than the exposed miniglobe at high environmental heat loads, across all four periods. The intersection of the regression lines of collar miniglobe temperature versus weather station miniglobe temperature with the line of identity, for the two oryx across all four periods, was 26.8 ± 3.8ºC (mean ± SD, n = 2).

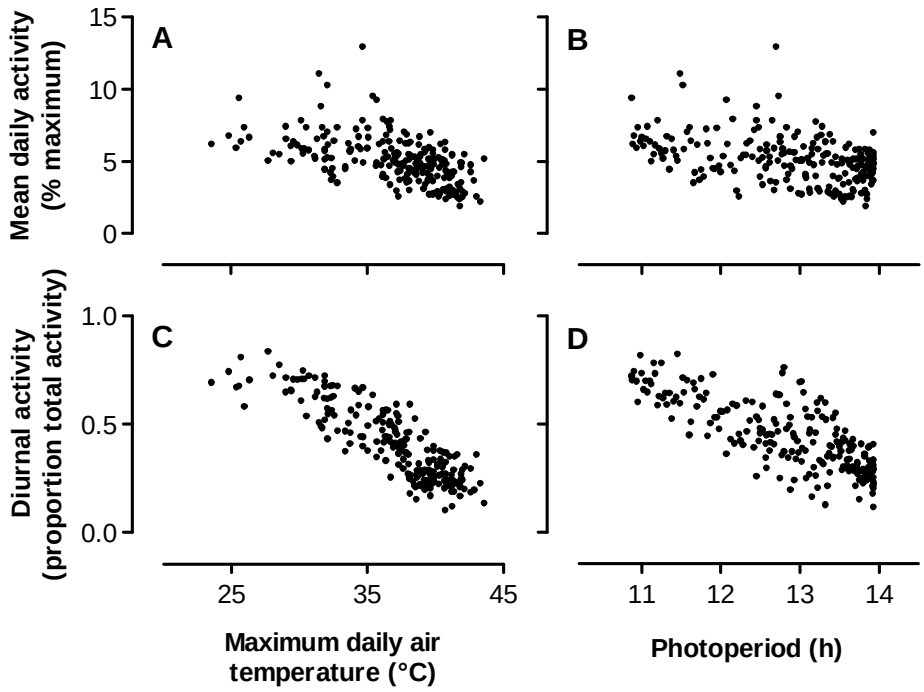

Figure 3.6. Mean 24-h activity, averaged for all five free-living oryx, plotted against maximum daily air temperature (panel A, mean, n = 5, $r^2 = 0.28$), and photoperiod (panel B, mean, n = 5, $r^2 = 0.18$), for 224 days, and mean diurnal activity, calculated as activity between 06:00 and 18:00 expressed as a proportion of total 24-h activity, plotted against maximum daily air temperature (panel C, mean, n = 5, $r^2 = 0.47$), and photoperiod (panel D, mean, n = 5, $r^2 = 0.61$).

The slopes of regression lines of collar miniglobe temperature versus that of the weather station were significantly different between the periods ($F_{3,8184} = 205.8$, $P < 0.0001$), with the regression during the hot dry period showing the flattest slope and the cool dry period showing the steepest slope. These results imply that the oryx were selecting microclimates cooler than that in direct sunlight more at high environmental heat loads, during the hot dry periods, than they were during the cool dry period. Despite similar ambient conditions, the slope of the regression line was steeper during the warm wet period compared to the warm dry period, implying that the oryx prioritized behavioural thermoregulation during the dry periods.

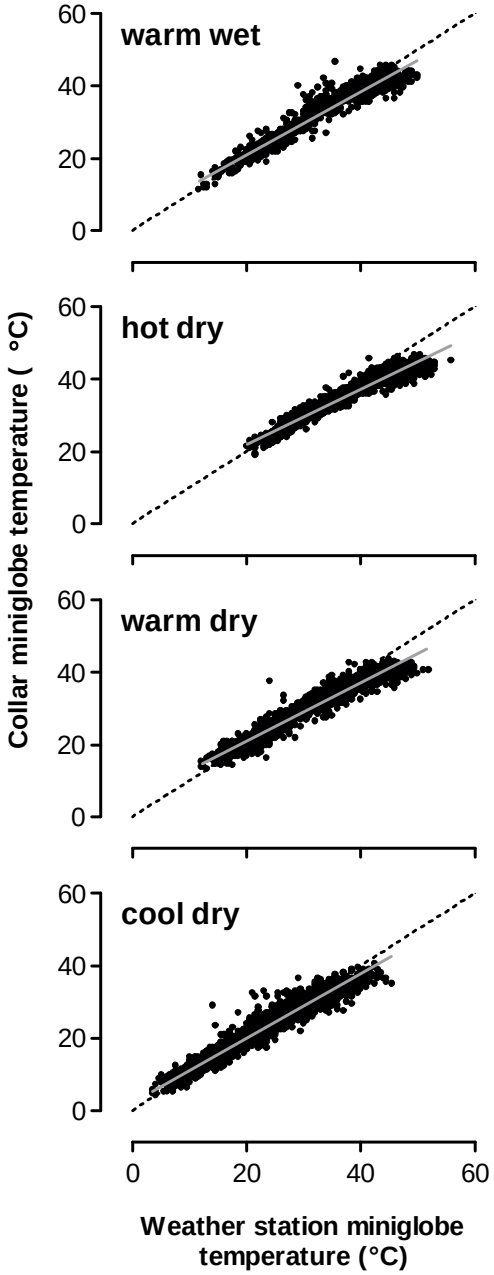

Figure 3.7. Scatter diagram showing the relationship between collar miniglobe temperatures at the site chosen by a single representative female oryx (Oryx 1) and temperatures recorded by an identical miniglobe at a nearby weather station, which was exposed to the sun, during warm wet (panel A), hot dry (panel B), warm dry (panel C) and cool dry (panel D) periods. Measurements were made hourly. The dashed line is the line of identity and the solid line is the line of linear regression. The slopes of the regression lines were significantly different between the periods ($P < 0.0001$) with the hot dry period showing the lowest slope, indicating more frequent selection of microclimates cooler than the microclimates under full solar exposure during this period. Warm wet period: $y = 0.87x + 3.5$, $r^2 = 0.97$, $P < 0.0001$, intersect 27.2ºC. Hot dry period: $y = 0.76x + 6.7$, $r^2 = 0.97$, $P < 0.0001$, intersect 28ºC. Warm dry period: $y = 0.81x + 4.7$, $r^2 = 0.97$, $P < 0.0001$, intersect 24.6ºC. Cool dry periods: $y = 0.90x + 2.0$, $r^2 = 0.97$, $P < 0.0001$, intersect 19.3ºC.

3.4.5 Selective brain cooling

Figure 3.8 shows the typical pattern of brain and carotid blood temperature recorded for a single free-living male oryx (Oryx 2) over four-day epochs during the warm wet period (Fig. 3.8A), and the hot dry period (Fig. 3.8B). The brain temperature of the oryx generally was above that of the carotid blood at low body temperatures (Fig. 3.8A), however, oryx implemented selective brain cooling near the acrophase of the endogenous nychthemeral body temperature rhythm during the hot dry period (Fig. 3.8B).

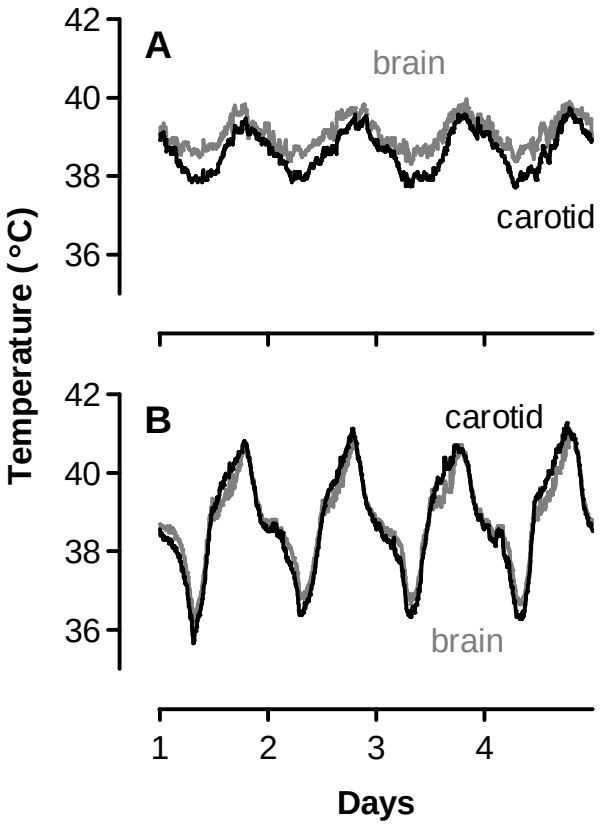

Figure 3.8. Brain (light line) and carotid blood (dark line) temperatures of a single free-living male oryx (Oryx 2) over four-day periods during the warm wet period (A) and the hot dry period (B).

Figure 3.9 shows hypothalamic temperature as a function of carotid blood temperature and the frequency distribution of blood temperature in a male oryx (Oryx 3, Fig. 3.9A) and female oryx (Oryx 1, Fig. 3.9B) under initial captive conditions when animals had free access to food and water, and in a single free-living male oryx (Oryx 2) over a month in the warm wet period (Fig. 3.9C) and in the hot dry period (Fig. 3.9D). The dashed line represents the line of identity, points below this line reflect selective brain cooling. The oryx exhibited selective brain cooling frequently under captive conditions, spending 88 ± 0.06% of their time with the hypothalamus cooler than carotid blood. On average, brain temperature was 0.25 ± 0.04ºC (mean ± SD, n = 3) cooler than carotid blood temperature throughout the study.

The same was not true when Oryx 2 was free-living. He spent 11% of the time during the warm wet period with the hypothalamus cooler than carotid blood, and that frequency increased to 38% of his time during the hot dry period. His brain temperature was on average 0.28 ± 0.25ºC higher than carotid blood temperature during the warm wet month, and only 0.03 ± 0.32ºC higher during the hot dry month. The threshold for selective brain cooling, defined as the point at which carotid blood and mean hypothalamic temperatures are equal, decreased from 39.6°C in the warm wet month to 39.0°C in the hot dry month. Above the threshold, the average difference between carotid blood and hypothalamic temperature was greater in the hot dry period (0.28 ± 0.24ºC) than during the warm wet period (0.16 ± 0.20ºC). Yet, maximum magnitude of selective brain cooling was similar between the hot dry period (1.03ºC) and the warm wet period (0.93ºC) and seldom exceeded 1ºC at any time.

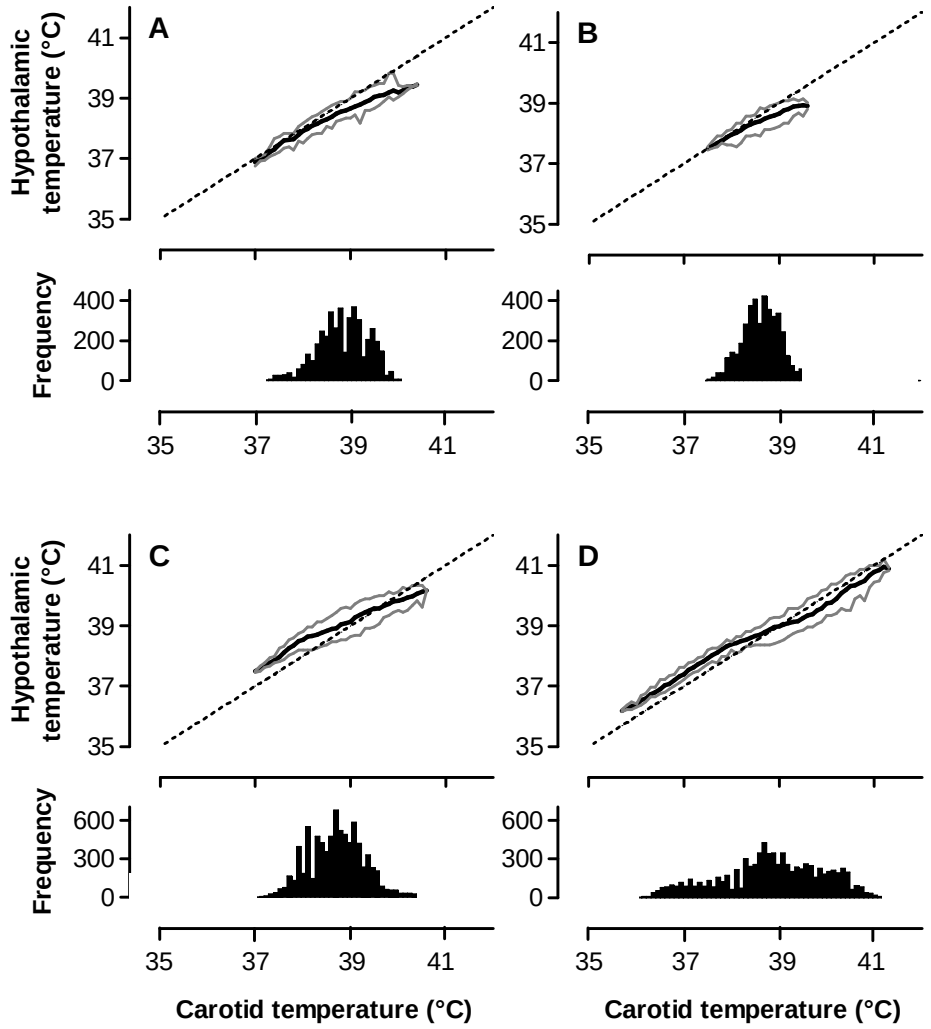

Figure 3.9. Hypothalamic temperature as a function of carotid blood temperature (top panels) and the frequency distribution of blood temperature (bottom panels), in a male oryx (Oryx 3, panel A), and a female oryx (Oryx 1, panel B), when both animals were captive and had *ad libitium* access to food and water, and a free-living male oryx (Oryx 2), in the warm wet period (May, panel C) and in the hot dry period (June, panel D). All 5-min recordings of carotid blood temperature were sorted into 0.1°C classes and the mean (solid line), minimum (lower grey line) and maximum (upper grey line) hypothalamic temperature calculated for each 0.1°C class of carotid blood temperature. The dashed line represents the line of identity; points below this line reflect selective brain cooling. Lower panels in each set show the absolute frequencies with which each 0.1°C class of blood temperature occurred; 6491 data points were obtained for the captive male oryx (panel A), 4393 data points for the captive female oryx (panel B), 8067 data points for the free-living male oryx in warm wet period (panel C) and 8355 data points in the hot dry period (panel D).

3.5 Discussion

My study provides the first remote and continuous measurement of body temperature, brain temperature, activity and microclimate selection of free-living Arabian oryx (*Oryx leucoryx*) in their natural habitat, the deserts of Saudi Arabia. The data allowed me to examine physiological and behavioural responses of this species in the hyper-arid conditions of the Arabian deserts. I could detect these responses because, together with my colleagues, I instrumented free-living oryx with data loggers capable of producing a more complete and continuous record of the thermal status and activity of undisturbed free-living oryx than had been achieved previously. The temperature data loggers, which my colleagues and I implanted in the abdomen, revealed that my oryx displayed not only higher daily maximum body temperatures (41.1 ± 0.3°C vs. 39.7 ± 0.1°C), but also lower daily minimum body temperatures (36.1 ± 0.3°C vs. 36.8 ± 0.2°C), resulting in higher daily amplitudes of nychthemeral rhythm of body temperature (5.0 ± 0.5°C vs. 2.9 ± 0.2°C), during hot dry months than during cool dry months (Fig. 3.2A), as reported previously by Ostrowski *et al*. (2003). The maximum daily amplitude of the nychthemeral rhythm of body temperature reached 7.7°C for two of the oryx during the hot dry period, the highest ever recorded for a large mammal. There was a positive power relationship between maximum daily body temperature and maximum daily air temperature. Maximum daily air temperature accounted for 85% of the variability in maximum daily body temperature (Fig. 3.4B). These results imply that the oryx indeed did implement heterothermy, but, contrary to the conventional definition of adaptive heterothermy (Mitchell *et al*., 2002), my oryx had higher mean body temperatures at night (39.0 ± 0.2°C) than in the daytime (38.4 ± 0.2°C). In addition, the classic measure of adaptive heterothermy, a correlation between the amplitude of nychthemeral rhythm of body temperature and the amplitude of ambient temperature, was not evident in my oryx (Fig. 3.4E), implying that the heterothermy was induced by something other than the prevailing air temperature.

I believe that the factor responsible for the appearance of heterothermy in my oryx was the lack of water in their environment, leading both to a reduction in water

intake and a decline in food quality and availability (Spalton, 1999). During the transitional warm wet and warm dry periods, when ambient temperatures were similar, the oryx displayed greater nychthemeral heterothermy (3.6 ± 0.6°C vs. 2.3 ± 0.3°C), mostly as a result of lower daily minimum body temperatures (37.8 ± 0.2°C vs. 36.5 ± 0.6°C) during the warm dry period than during the warm wet period (Fig. 3.2C). I believe that these low daily minimum body temperatures, which occurred in the early mornings, resulted from water and food limitations in the desert environment.

In addition to heterothermy, selective brain cooling is considered to be an important adaptation to environmental heat stress. The conventional view of selective brain cooling would predict that the magnitude of brain cooling would have increased during hot dry months, when carotid blood temperature exceeded 41ºC. Although the oryx used selective brain cooling more frequently during the hot dry period, and the threshold at which selective brain cooling was implemented was lower then, the magnitude of selective brain cooling actually was reduced in the hot dry month, compared to the warm wet month (Fig. 3.9), and brain temperature reached 41.2ºC. It is possible that 1°C is the maximal capacity for selective brain cooling in Arabian oryx, but even this small magnitude of selective brain cooling would have provided both metabolic and water conservation advantages.

My oryx appeared to have further conserved water by transferring daily activity during the hot dry period to the night, when metabolic heat could be dissipated non-evaporatively. Whereas my oryx showed continuous activity with crepuscular peaks during the warm periods, whether wet or dry, they shifted to nocturnal activity during the hot periods, without reducing total 24-h activity (Fig. 3.5). However, because the decline in forage quality during the dry season, maintaining total 24-h activity may not have been an adequate compensatory response for maintaining energy intake. Oryx have been observed to seek shade during the heat of the day and I detected the selection of cooler microclimates, without observers present, by using miniature globe thermometers attached to collars, a technique I

developed for recording microclimate selection (Appendix 1, Hetem *et al.*, 2007). As expected oryx selected cooler microclimates more frequently during the hot dry period, and at times selected microclimates where the miniglobe temperature was 12ºC lower than that in the sun (Fig. 3.7B). Selecting cooler microclimates would have further reduced evaporative heat loss, and therefore conserved body water.

Although there are obvious advantages to obtaining data from free-living, undisturbed animals, there are disadvantages in that some variables cannot yet be measured. My proposals concerning the crucial role of water in determining body temperature profiles and the magnitude of selective brain cooling in the oryx would have been strengthened if I could have measured water loss, metabolic rate, or even osmolarity by biotelemetry. Also, because it is well known that the presence of humans disrupts normal thermoregulation in wild animals (Recarte *et al.*, 1998), I stayed away from them, and so could not draw any conclusions on habitat selection, migration movements or change in body condition over the experimental period. Advancement in GPS technology combined with GIS now makes some of these measurements feasible. Hopefully it will be possible, in the future, to expand the suite of relevant thermoregulatory variables that can be measured by biotelemetry and remote observation. An additional limitation of this study was the small sample size with respect to the selective brain cooling data. Nevertheless, I reported the data because it has not previously been collected in such an arid-adapted species.

3.5.1 Heterothermy

Despite the limitations, I believe that my study expands on and refines the one previous study that has reported body temperature in free-living Arabian oryx (Ostrowski *et al.*, 2003). In that study, body temperature of six oryx was measured with temperature-sensitive radiotelemeters, which required the observer to be within 800 m of the individual animals, a difficult task in the vast terrain, especially without disturbing the animals. Although the study provided valuable insight into the adaptations of the Arabian oryx, and was the first to identify

heterothermy in a free-living antelope, the data appear to have been confined to less than one day of recordings per animal per season during the day and only about one hour of recordings per animal per season during the night, as compared to my continuous body temperature measurements, throughout the day and night, for 313 consecutive days. Not only have I recorded body temperature continuously, but I also recorded activity continuously, and, in some oryx, microclimate selection. Perhaps because conditions were drier during my study (31 mm vs. 74 ± 46 mm), water may have been less of a factor in the study of Ostrowski *et al.* (2003), and they attributed daily heat storage to the amplitude of air temperature variation. Nevertheless, my oryx displayed a larger daily amplitude of the nychthemeral rhythm of body temperature than Ostrowski's oryx during the summer (5.0 ± 0.5ºC vs. 4.1 ± 1.7°C), despite the lower maximum air temperature prevalent during my hot dry period (40.4 ± 1.3ºC vs. 43.5 ± 1.7ºC). I propose that the greater degree of heterothermy in my oryx was likely the result of the drier conditions prevalent during my study and possibly the larger data set sampling more days.

The latent heat of evaporation of water is sufficiently high, and the water vapour pressure of desert air sufficiently low, for desert mammals to be able to dissipate resting metabolic heat and environmental heat (even in direct solar radiation) by evaporation and so avoid hyperthermia. However, the evaporation that is necessary depletes body water at a rate likely to induce potentially-lethal hyperosmolarity, unless the mammal has ready access to adequate dietary water. At my experimental site, the only drinking water to which the oryx had access was in the ephemeral pools that formed when the 31 mm of rain fell between March and May; at all other times they had to rely on the pre-formed water in the sparse desert vegetation, and so likely could not afford prolific evaporative cooling. Consequently, the oryx employed autonomic and behavioural processes which reduced metabolic and environmental heat loads, and transferred responsibility for heat dissipation to non-evaporative avenues. They did so without reducing foraging time, important because reducing foraging would have

compromised energy homeostasis, especially when forage quality deteriorated during the hot dry period.

One of the processes for transferring heat loss to non-evaporative avenues is the employment of heterothermy. The capacity to store heat holds whether the heterothermy is the controlled thermoregulatory event that constitutes adaptive heterothermy, or results from failure of homeothermy (Mitchell *et al.*, 2002). But failure of homeothermy places the animal at risk of damaging hyperthermia in the absence of the suppression of the temperature nadir (a hallmark of adaptive heterothermy). One cause of failure of homeothermy is dehydration itself. Several ungulate species reduce the rate of evaporative water loss as they become dehydrated (Taylor, 1970b; Maloiy, 1973b; Finch and Robertshaw, 1979; Baker, 1989; Nijland and Baker, 1992; Silanikove, 1994; Jessen *et al.*, 1998), which results in an increased body temperature during heat exposure (Taylor, 1969a; Taylor, 1970a; Taylor, 1970b; Finch and Robertshaw, 1979; Jessen *et al.*, 1998; Alamer, 2006). Whether that elevation in body temperature constitutes dehydration-induced hyperthermia or adaptive heterothermy is not self evident. Nevertheless, whether heterothermy is dehydration induced hyperthermia or adaptive heterothermy, one expects the heterothermy to increase with increasing dehydration.

I had no way of quantifying dehydration in my free-living oryx, but previous studies have suggested that free-living Arabian oryx have a low rate of water influx of 1310 ml $H_2O.day^{-1}$ during summer (Williams *et al.*, 2001; Ostrowski *et al.*, 2002), and display higher haematocrit, plasma protein concentration and plasma osmolality during summer than during winter (Ostrowski *et al.*, 2003). My animals were exposed to harsh environments without access to free-standing water for nine months. It therefore seems plausible that the high body temperatures of my oryx, displayed during the hot dry period, were the combined result of dehydration and extreme environmental temperatures. What distinguishes adaptive heterothermy from dehydration induced suppression of evaporative heat loss, though, is that in adaptive heterothermy the amplitude of

body temperature rhythm increases without the mean 24-h body temperature necessarily increasing, because minimum body temperature is depressed (Mitchell *et al.*, 2002).

Under captive conditions of water deprivation, the dromedary camel (*Camelus dromedarius,* Schmidt-Nielsen *et al.*, 1957; Schroter *et al.*, 1987), hartebeest (*Alcelaphus buselaphus*, Harthoorn *et al.*, 1970; Maloiy and Hopcraft, 1971), Thomson's gazelle (*Gazella thomsonii*) and zebu steer (*Bos primigenius indicus*, Taylor, 1970a) have been reported to display an increased body temperature amplitude with an associated lower body temperature in the morning. Taylor found that heterothermy was not dependent on hydration state for captive eland (Taylor and Lyman, 1967; Taylor, 1969a). However, unlike the seven degree increase in body temperature, which was observed on a single occasion, and is so often referred to, the body temperature of the eland (*Tragelaphus oryx*) usually only increased by three of four degrees during the 12-h day when the animals had water available and were exposed to 40ºC (Taylor, 1969a). In fact, the single rectal temperature measurement of 33.9ºC, on which the seven degree amplitude of body temperature rhythm is based, may have been a measurement error. In addition, these rectal temperatures were based on measurements obtained from immature, captive eland and the findings may have been artefacts of studying animals in captive conditions in which they were prevented from employing behavioural thermoregulation (Finch, 1972; Fuller *et al.*, 1999; Mitchell *et al.*, 2002). Subsequent studies on free-living eland showed a much lower amplitude of nychthemeral rhythm (Bligh and Harthoorn, 1965; Harthoorn *et al.*, 1970; Finch, 1972; Finch and Robertshaw, 1979; Fuller *et al.*, 1999; Fuller *et al.*, 2004). More recently, Ostrowski and Williams (2006) argued that free-living Arabian sand gazelle (*Gazella subgutturosa marica*) displayed heterothermy at times when the animals were not dehydrated. However, this assumption was based on the finding that plasma osmolality was not different between October (319.2 ± 1.9·mOsm) and September (320.2 ± 2.3·mOsm), yet their estimates of heterothermy were made in winter (January and February) and summer (June and July). Therefore, to date, there is no substantiated evidence that heterothermy exists in free-living

animals with free access to water; several studies have shown a narrow range of body temperature in normally hydrated desert-adapted ungulates (Bligh and Harthoorn, 1965; Harthoorn *et al.,* 1970; Hofmeyr and Louw, 1987; Jessen *et al.*, 1994; Mitchell *et al.*, 1997; Fuller *et al.,* 1999; Maloney *et al.,* 2002; Mitchell *et al.*, 2002; Fuller *et al.*, 2005). When given the opportunity, oryx increase their water consumption and consume an average of 5 $l.day^{-1}$ during the summer months (Stanley Price, 1989) and preferentially select areas where new rain has fallen (Corp *et al.,* 1998). Under captive conditions, with food and water available *ad libitum*, my single male oryx maintained an amplitude of nychthemeral rhythm of body temperature of 2.6 ± 0.5ºC throughout the year (Fig. 3.1, upper right panel) despite being exposed to environmental heat loads very similar to those of the free-living animals.

Previous studies have proposed that the morning drop in body temperature is the result of vasodilation, which mixes cool peripheral blood with that of the core (Schmidt-Nielsen *et al.*, 1957; Zervanos and Hadley, 1973; Brown and Dawson, 1977; Fuller *et al.*, 1999; Maloney *et al.*, 2004). Such a drop in body temperature may allow an animal to anticipate hot ambient conditions (Maloney *et al.*, 2004) and thus pre-emptively permit additional storage of heat. In my free-ranging oryx, minimum body temperatures were low not only during the hot dry period, but also throughout the other dry periods, even though ambient temperatures were not as high during the warm dry and cool dry periods (Table 3.1). Since dehydration has been shown to decrease metabolic rates of various species including Bedouin goats (Brosh *et al.*, 1986; Dmi'el, 1986), Holstein cows (Seif *et al.,* 1973), camels (Schmidt-Nielsen *et al.*, 1967; Schroter *et al.*, 1987), eland (Taylor, 1969b) and cats (Doris and Baker, 1981), I propose that in my oryx the low body temperature in the morning may reflect these low metabolic rates associated with dehydration.

The low morning body temperatures may also reflect the so-called “nocturnal hypometabolism”, observed during winter in both red deer (*Cervus elaphus*, Arnold *et al.,* 2004) and the Przewalski horse (*Equus ferus przewalskii,* Arnold *et al.*, 2006). Such seasonal fluctuations in metabolic rates occur not only in northern

ruminants, which display a winter nadir (Nilssen *et al.*, 1984; Parker and Robbins, 1985; Mesteig *et al.*, 2000; Arnold *et al.*, 2004), but also desert ungulates, which display a summer nadir in metabolic rate (Nagy and Knight, 1994; Williams *et al.*, 2001; Ostrowski *et al.*, 2006a; Ostrowski *et al.*, 2006b). The low metabolic rates associated with low quality and quantity of food could result in a lowered body temperature, as seen in food deprived rodents (Sakurada *et al.*, 2000; Bae *et al.*, 2003), sheep (Piccione *et al.*, 2002) and desert goats (Choshniak *et al.*, 1995; Ahmed and El Kheir, 2004). During drought some grasses provide an adequate source of crude protein and water for the oryx, but these levels are predicted to be close to minimum maintenance requirements (Spalton, 1999). It therefore seems plausible that limited food and water availability during the dry seasons in a desert environment would combine to reduce the metabolic rate of desert ungulates (Brosh *et al.*, 1986; Merkt and Taylor, 1994; Williams *et al.*, 2001; Ahmed and El Kheir, 2004; Ostrowski *et al.*, 2006a; Ostrowski *et al.*, 2006b), thereby reducing endogenous heat production and energy expenditure. Similarly, Dawson *et al.* (2007) suggested that a low morning body temperature in kangaroos was probably more related to energy balance than heat storage in hot conditions. So, though as Ostrowski *et al.* (2003) first demonstrated, Arabian oryx do exhibit heterothermy in the hot dry period, and that heterothermy potentially saves water, I am not convinced that it is necessarily a consequence of ambient heat stress.

3.5.2 Cathemerality

I am more convinced that the second water-saving mechanism that my oryx employed, cathemerality, was a consequence of ambient heat stress. The term "cathemerality", coined originally for primates, described deliberate transfer of activity between the light and dark phases of the 24-h cycle in response to prevailing ecological conditions (Tattersall, 1987; Tattersall, 2006; Tattersall 2008). Chronoecological factors, such as temperature, light, competition for food resources and predation, appear to promote cathemerality by overriding endogenous clocks (Curtis and Rasmussen, 2006); the distribution of activity between day and night in my oryx was correlated with both maximum daily air temperature (Fig. 3.6C) and photoperiod (Fig. 3.6D). My oryx shifted from a

crepuscular activity pattern during warm wet periods to a nocturnal activity pattern during the hot dry period.

Previous observations on the activity patterns of Arabian oryx within the Mahazat as-Sayd Protected Area also revealed that oryx were less active diurnally on warm (maximum air temperature >35ºC) than on cool days (maximum air temperature <30ºC, Seddon and Ismael, 2002), and high ambient temperatures attenuated the diurnal activity of other tropical ungulates (Leuthold and Leuthold, 1978; Belovsky and Slade, 1986; Klein and Fairall, 1986; Owen-Smith, 1998). However, any conclusions, based on diurnal observations only, that environmental heat load reduces foraging are likely to be spurious. Identification of cathemerality requires measurement of both diurnal and nocturnal activity (Curtis and Rasmussen, 2006), which I achieved objectively by using activity loggers. Activity loggers provided a long-term, remote and unbiased assessment of activity, over successive 24-h cycles, not otherwise feasible under field conditions. Because I used activity loggers, I discovered that mean 24-h activity was not strongly correlated to either photoperiod or maximum daily air temperature, nor was there any significant difference across the three seasonal periods analyzed. Similarly, neither bushbuck (*Tragelaphus scriptus,* Wronski *et al.*, 2006) nor bighorn sheep (*Ovis canadensis mexicana,* Alderman *et al.*, 1989) showed differences in mean 24-h activity across seasons, when activity was observed during both nocturnal and diurnal hours.

My oryx, exposed to diurnal ambient temperatures often exceeding 35ºC, compensated for a reduced diurnal activity during hot periods by increasing nocturnal activity so that the magnitude of mean 24-h activity did not change. Cathemerality without loss of overall activity allowed foraging time to be maintained. Similarly, other large herbivores, including ungulates (Zervanos and Hadley, 1973; Belovsky and Jordan, 1978; Grenot, 1992; Hayes and Krausman, 1993; Berger *et al.*, 1999; Dussault *et al.*, 2004; Maloney *et al.*, 2005b), increased nocturnal activity in the face of high diurnal heat loads. Such an increase in nocturnal activity is believed to be a compensatory response, as grazing activities during the heat of the day would be limited as a result of shade-seeking behaviour,

in response to high ambient temperatures. Arabian oryx in Oman increased shade-seeking behaviour at ambient temperatures above 27ºC (Stanley Price, 1989), a threshold remarkably similar the average air temperature above which my oryx selected cooler microclimates across all periods. The selection of cooler microclimates during the heat of the day has been observed in a variety of ungulates, particularly those inhabiting desert environments, including the desert mule deer (*Odocoileus hemionus,* Sargeant *et al.*, 1994; Tull *et al.*, 2001), desert bighorn sheep (McCutchen, 1981; Hansen, 1982), eland (Taylor and Lyman, 1967), dik-dik (*Rhynchotragus kirkii*, Kamau and Maloiy, 1985), collared peccary (*Tayassu tajacu*, Zervanos and Hadley, 1973) and dorcas gazelle (*Gazella dorcas*, Wilson, 1989).

3.5.3 Selective brain cooling

The third process that I believe Arabian oryx used to facilitate homeostasis at high environmental heat loads was selective brain cooling. Arid-zone mammals possessing a carotid rete may employ selective brain cooling to attenuate thermal drive by reducing hypothalamic temperature, which reduces evaporative heat loss and ultimately conserves water by transferring cooling to non-evaporative means (Kuhnen, 1997; Jessen, 1998; Jessen, 2001; Mitchell *et al.*, 2002). Animals capable of selective brain cooling increase the magnitude and/or frequency of brain cooling during conditions of water deprivation. Both laboratory-housed sheep (Fuller *et al.*, 2007) and Bedouin goats (Jessen *et al.*, 1997; Jessen *et al.*, 1998) did so without changing the threshold body temperature at which selective brain cooling was implemented. Anomalously, captive camels showed no obvious difference in selective brain cooling between hydrated and dehydrated states at rest (Schroter *et al.*, 1989). My single free-living male oryx did not change the magnitude of selective brain cooling during the hot dry month, compared to the warm wet month, and instead appeared to decrease the threshold, therefore increasing the range of body temperatures over which selective brain cooling was implemented. The differences between my findings and those in the sheep and goats may be the result of differences in the pattern of brain cooling between laboratory and wild animals. Laboratory sheep (Fuller *et al.*, 2007) and goats

(Jessen *et al.*, 1998) display the greatest degree of selective brain cooling at night, whereas my oryx, like free-living eland (Fuller *et al.*, 1999), gemsbok (*Oryx gazella*, Maloney *et al.*, 2002), black wildebeest (*Connochaetes gnou*, Jessen *et al.*, 1994) and springbok (*Antidorcas marsupialis*, Mitchell *et al.*, 1997), displayed the greatest degree of selective brain cooling when the animal was at rest and body temperature was rising in late afternoon (Fig. 3.8).

Although my oryx employed selective brain cooling more frequently than has been reported previously for other artiodactyls, probably because its blood temperature was higher than that measured in other free-living artiodactyls, I could not conclude that selective brain cooling was enhanced in the arid-adapted oryx, since the magnitude of the difference between carotid blood and hypothalamic temperature was similar to that observed in other free-living artiodactyls (Jessen *et al.*, 1994; Jessen and Kuhnen, 1996; Mitchell *et al.*, 1997; Fuller *et al.*, 1999; Maloney *et al.*, 2002). My conclusions about selective brain cooling in the arid-adapted oryx, although noteworthy, must remain tentative, however, as I only obtained a complete data set from a single free-living male oryx. Since non-thermal factors affecting sympathetic tone may override the thermal regulation of brain temperature (Kuhnen and Jessen, 1994), particularly in free-living animals (Jessen *et al.*, 1994; Maloney *et al.*, 2002; Caputa, 2004), a high vigilance and sympathetic tone may have acted to decrease the magnitude of selective brain cooling in this individual. Although under captive conditions the magnitude and pattern of selective brain cooling was similar between male and female oryx (Fig. 3.8 upper panels), future studies aimed at assessing selective brain cooling should focus on free-living female individuals. Even though the magnitude of selective brain cooling in my single male oryx was much less than the anomalous 2.7ºC originally reported for the exercising captive Thomson's gazelle (Taylor and Lyman, 1972), it is believed that a reduction in brain temperature of even 0.5ºC is enough to substantially reduce both the respiratory water loss (Kuhnen, 1997) and the metabolic cost of thermoregulation (Kuhnen and Jessen, 1991) in goats.

3.5.4 Conclusion

The prosperity, and indeed the survival, of desert mammals depends on them managing successfully the often-competing demands of their homeostatic mechanisms. That management is particularly challenging for species too large to escape below the desert surface. I have shown how the Arabian oryx, a large artiodactyl native to one of the world's hottest deserts, is likely to manage the competing demands of thermoregulatory homeostasis, body fluid homeostasis, and metabolic energy homeostasis, and have demonstrated, I believe, that Arabian oryx give the highest priority, among those three, to body fluid homeostasis. By studying an arid-adapted artiodactyl, inhabiting one of the hottest deserts in the world, I have established the kind of physiological mechanisms required if artiodactyls are to adapt phenotypically to climate change. I have shown how the oryx uses four different physiological strategies, namely heterothermy, cathemerality, selective brain cooling and shade-seeking behaviour, to counter dehydration, hyperthermia and starvation. Yet, their current dependence on behavioural thermoregulation, combined with the loss of genetic variability as a result of a historical population bottleneck (Marshall *et al.*, 1999), may limit their capacity to adapt to future aridification and habitat transformation. The Arabian oryx already may be at the edge of its physiological limit and, with climate change predicted to increase summer temperatures in the southwest part of the Arabian Peninsula (Gitay *et al.*, 1998), its thermoregulatory competence may not be sufficient to ensure long-term survival.

3.6 Acknowledgements

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CHAPTER 4

Does size matter? Comparison of body temperature and activity of free-living Arabian oryx (*Oryx leucoryx*) and the smaller Arabian sand gazelle (*Gazella subgutturosa marica*) in the Saudi desert

Data and ideas presented in this chapter have been written up as a scientific paper and will be submitted to Journal of Comparative Physiology B. Hetem R.S., Strauss W.M., Shobrak M., Fick L.G., Maloney S.K., Meyer L.C.R., Fuller A. and Mitchell D. Does size matter? Comparison of body temperature and activity of free-living Arabian oryx (*Oryx leucoryx*) and the smaller Arabian sand gazelle (*Gazella subgutturosa marica*) in the Saudi desert.

4.1 Abstract

Heterothermy, a variability in body temperature, is widely viewed as a key adaptation of arid-adapted ungulates. However, ungulates with a small body mass, i.e. a relatively large surface area-to-volume ratio and a small thermal inertia, are proposed to be less likely to employ adaptive heterothermy than are larger ungulates. With the help of my colleagues, I measured body temperature and activity patterns, using implanted data loggers, in five free-ranging Arabian oryx (*Oryx leucoryx*, ~ 70 kg) and six smaller Arabian sand gazelle (*Gazella subgutturosa marica*, ~ 15 kg) inhabiting the same Arabian desert environment, at the same time. Compared to the oryx, the sand gazelle displayed higher (0.7ºC) mean daily body temperature ($F_{1,6} = 47.3$, $P = 0.0005$), higher minimum daily body temperature ($F_{1,6} = 42.6$, $P = 0.0006$) and higher maximum daily body temperature ($F_{1,6} = 11.0$, $P = 0.02$). Despite these differences, both species responded similarly to changes in environmental conditions. As predicted for adaptive heterothermy, maximum daily body temperatures increased ($F_{1,6} = 84.0$, $P < 0.0001$), minimum daily body temperature decreased ($F_{1,6} = 92.2$, $P < 0.0001$), and daily body temperature amplitude increased ($F_{1,6} = 97.6$, $P < 0.0001$) as conditions got progressively hotter and drier. There were no species-specific differences in activity levels. Both gazelle and oryx showed continuous activity with crepuscular peaks during the warm wet period but shifted to nocturnal activity during the hot dry period. Activity was attenuated during the heat of the day at times when both species selected cool microclimates. Therefore, the two species of Arabian artiodactyls employ heterothermy, cathemerality and shade-seeking very similarly to survive the extreme, arid conditions of Arabian deserts, despite their difference in size.

4.2 Introduction

Heterothermy reduces demands on both energy and water supply otherwise imposed by maintaining homeothermy. Theoretically, in hot arid environments, heterothermy allows animals to store heat during the day and to dissipate that heat

later non-evaporatively, so reducing evaporative cooling, and therefore waterloss. The potential water saving depends only on the degree to which the average temperature of the body tissue increases and on the animal's thermal inertia (and therefore body mass). Heterothermy is proposed to be particularly effective as a water saving mechanism for large mammals, since, in addition to their larger thermal inertia, within a given time, larger mammals would gain relatively less heat from the hot environment (McNab, 1983; Phillips and Heath, 1995) and generate less metabolic heat per gram of tissue than would smaller mammals (Taylor, 1970a; Mitchell *et al.*, 2002).

The phenomenon of adaptive heterothermy was first demonstrated by Schmidt-Nielsen and his colleagues in camels and they also confirmed the logical prediction that the degree of heterothermy would increase in animals deprived of drinking water (Schmidt-Nielsen *et al.*, 1957). Subsequently, the ability to employ adaptive heterothermy was described in several species of African antelope, but without the heterothermy always being accentuated by water deprivation (Taylor and Lyman, 1967; Taylor, 1969a). All the studies demonstrating adaptive heterothermy in antelope, however, were conducted in highly artificial circumstances, on individual or small groups of animals in climatic chambers or other confined spaces. Once biotelemetry allowed measurement of body temperature in free-living animals, it became apparent that, at the same ambient temperature at which confined mammals demonstrated adaptive heterothermy, five species of free-living African mammals did not do so (Jessen *et al.*, 1994; Mitchell *et al.*, 1997; Fuller *et al.*, 1999; Fuller *et al.*, 2000; Maloney *et al.*, 2002; Fuller *et al.*, 2005). A possible explanation for the discrepancy between confined and free-living mammals was that the low minimum body temperatures measured in confined mammals were artefacts of confinement, in animals deprived of their full suite of behavioural thermoregulation (Fuller *et al.*, 1999).

The African savannas are not the hottest and driest habitats occupied by antelope, and it remains possible that, in sufficiently stressful conditions, even free-living antelope would demonstrate adaptive heterothermy. Indeed, my colleagues and I

have demonstrated that Arabian oryx do display heterothermy (chapter 3). Yet, deserts provide a challenging habitat for not only large but also medium-sized antelope, since these antelope are too large to escape the heat by burrowing. Despite such challenges, the Arabian Peninsula supports seven species within the Bovidae family, more than half of which are gazelle (Groves, 1988). Using biotelemetry, Ostrowski and his colleagues, working in the Saudi Arabian desert, recently demonstrated heterothermy in free-living Arabian oryx (~ 70 kg, Ostrowski *et al.*, 2003) and the much smaller Arabian sand gazelle (~ 15 kg, Ostrowski and Williams, 2006). They reported a smaller amplitude of nychthemeral rhythm of body temperature in the sand gazelle, which together with a lower thermal inertia, would result in a much lower heat storage, and therefore lower water saving. One cannot draw that conclusion, however, because the sand gazelle were studied in an environment cooler and wetter than that in which the oryx were studied. Also, there clearly were problems with the body temperature measurements in the oryx, for which radiotelemetry was used. The difficulties of being close enough to the animals to pick up signal, but not so close as to disturb the animals (Ostrowski *et al.*, 2003), allowed only sporadic measurements of body temperature, especially at night, during which measurements were attained for less than 1% of the study time. The problems were evident in an unexplained change in body temperature of at least 0.5ºC between 19:00 and 19:30 and 1ºC between 05:30 and 06:00, making estimates of the amplitude of the oryx body temperature rhythm unreliable.

Although the smaller gazelles may have the advantage of smaller resource requirements and greater access to refuge sites than do the larger oryx (Ostrowski and Williams, 2006), they are likely to be disadvantaged by a high mass-specific metabolic rate, high water turnover and less capacity to store heat (Bartholomew, 1964; Taylor, 1970a). With the help of my colleagues, I therefore set out to compare the body temperature and refuge selection of the sand gazelle and oryx living free in the same desert environment, at the same time. To contribute to the open question of whether time spend foraging increases (Peters, 1983; Schmidt-Nielsen, 1984; Owen-Smith, 1988; Owen-Smith, 1992; du Toit and Yetman, 2005)

or decreases (Belovsky and Slade, 1986; Mysterud, 1998; Pérez-Barbería and Gordon, 1999) with increasing body mass, I also measure activity continuously. Ostrowski and colleagues reported activity only as observed behaviour in the oryx (Ostrowski *et al.*, 2003) and not at all in the sand gazelle. I used data loggers to make all of my measurements so that the animals were free from the disturbance imposed by human observers.

4.3 Materials and methods

4.3.1 Animals and habitat

The experiment took place between March and July 2006 within the 2200·km^2 Mahazat as-Sayd Protected Area (28°15' N, 41°40' E) in west-central Saudi Arabia. This open steppe desert is the historical and current habitat for both the Arabian sand gazelle (*Gazella subgutturosa marica*) and the Arabian oryx (*Oryx leucoryx*). Six captive-bred male Arabian sand gazelle were transported from the King Khaled Wildlife Research Center, Thumamah, Saudi Arabia (25°20' N, 45°35' E) to Mahazat as-Sayd Protected Area in mid-March 2006. During the same period, five adult wild-born Arabian oryx were captured, under veterinary supervision, from the Mahazat as-Sayd Protected Area. The oryx were habituated in pens for two weeks to reduce potential peri-operative stress. The procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (protocol no. 2005/87/5 and 2005/88/4).

4.3.2 Surgery

Sand gazelle were hand caught and placed in individual crates. Anaesthesia was induced with 8% isoflurane (Aerrane, Astra Zeneca, Johannesburg, South Africa). The oryx were darted and anaesthetized in the holding pens with etorphine hydrochloride (2.5 mg intramuscularly (I.M.), M99, C-Vet, Leyland, UK), which later was reversed with diprenorphine hydrochloride (7.5 mg intravenously (I.V.), M5050, C-Vet, Leyland, UK), once an adequate plane of inhalation anaesthesia had been established.

Once recumbent, animals were transported to a temporary sterile operating theatre within 200 m of the pens, where they were placed in sternal recumbency, supported by sandbags, with their heads elevated. Anaesthesia was maintained with 2-6% isoflurane, administered in 100% oxygen. Respiratory rate, heart rate, arterial oxygen saturation (pulse oximeters, Nonin 9847V, Nonin Medical, North Plymouth, USA) and rectal temperature (thermocouple thermometer, BAT-12, Physitemp, Clifton, USA) were monitored throughout the surgery.

For biotelemetry of body temperature and activity, miniature data loggers were implanted. The data loggers were covered in an inert wax (Sasol, Johannesburg, South Africa) and dry-sterilized in formaldehyde vapour before implantation. Incision sites were shaved and sterilized with povidone iodine antiseptic (Vetedine, Vetoquinol, Lure, France). A thermometric logger (see below) then was inserted, via an incision in the paralumber fossa, into the abdominal cavity. The muscle layer was sutured closed and an activity logger (Actical, Mini-Mitter Corporation, Bend, OR, USA) was tethered subcutaneously before the skin was sutured closed. The activity logger recorded at 5-min intervals, had dimensions of ~ 40 x 40 x 15 mm, and weighed ~ 40 g.

Wounds were treated with a topical antiseptic spray (Necrospray, Centaur Labs, Johannesburg, South Africa). The animals received a long-acting antibiotic (4.5 $mg.kg^{-1}$ I.M., penicillin, Norocillin La, Norbrook Laboratories Ltd., Newry, Northern Ireland), a non-steroidal anti-inflammatory analgesic (10 $mg.kg^{-1}$ I.M., phenylbutazone, Dexaphenylarthrite injectable solution, Vetoquinol Veterinary Pharmaceuticals, Cedex, France), a long-acting parasiticide (0.02 $ml.kg^{-1}$ subcutaneously (S.C.), Ivermectin, Noromectin, Norbrook Laboratories Ltd., Newry, Northern Ireland) and a multivitamin (0.1 $ml.kg^{-1}$ I.M., Multivit injectable solution, Univet Ltd., Ireland).

After surgery but before inhalation anaesthesia was terminated, a neck collar was fitted to each oryx (MOD-500 Telonics, Inc. Mesa, AZ, USA) and sand gazelle (African Wildlife Tracking, Pretoria, South Africa). In addition to a tracking radio

transmitter, the collar supported a miniature black globe thermometer ("miniglobe"), to allow for the dynamic ambulatory measurement of the microclimate that each animal chose to occupy. This technique has been validated previously on other ungulate species (Appendix 1, Hetem *et al.*, 2007). Miniglobe temperature was measured by a small temperature-sensitive data logger (see below) inserted into the centre of a matt-black hollow copper sphere (30 mm diameter, Press Spinning & Stamping Co., Cape Town, South Africa). The globe was attached to the collar by a 10 mm diameter polyvinyl chloride rod. A weight on the ventral side of the collar ensured that the miniglobe remained over the dorsum of the neck and could not be shaded by the animal's body.

Following surgery, the animals were transported back to their pens, where they became ambulatory within ~ 10 min of termination of inhalation anaesthesia. After two weeks of recovery, and following veterinary inspection, the oryx and sand gazelle were released into adjacent, but separate, 2 km^2 fenced enclosures with natural forage and water available *ad libitum*. Ten days later, the gate of the oryx's enclosure was opened, and the oryx were allowed to range freely within the 2200·km^2 Mahazat as-Sayd Protected Area where there was no permanent natural water and no artificial water sources. To improve the chance of recapturing the sand gazelle, they remained in their 2 km^2 enclosure for the duration of the study. The artificial water source evaporated within one month, after which it was not replaced, to replicate the dry environment to which the animals would be subjected naturally, and which was occupied at that time by the oryx. Thus, the only drinking water to which the sand gazelle and oryx had access was in the ephemeral pools that formed when 31 mm of rain fell between March and May; at all other times both species had access only to the pre-formed water in the sparse desert vegetation, and to metabolic water.

Unfortunately all six sand gazelle died in August 2006, five months after surgery, during an exceptionally hot and dry spell. I retrieved four body temperature loggers, four miniglobe temperature sensors and six activity loggers. I was unable to determine the cause of death, but saw no evidence of fever in the temperature

records. The oryx were recaptured and transported to the holding pens in April 2007. The animals were anaesthetised once again, and the data loggers were removed under a surgical procedure similar to that used for the original implantation. The animals' wounds had healed and there were no signs of infection from the initial surgery. Most of the body temperature loggers were found in the pelvic canal and were not encapsulated in adhesive tissue. Only two miniglobe sensors still were attached to the collars on the oryx. After removal of all monitoring equipment, the oryx were re-released into the Mahazat as-Sayd Protected Area.

4.3.3 Temperature measurements

The miniature thermometric data loggers (StowAway XTI, Onset Computer, Pocasset, Massachusetts, USA), used to measure body temperature, had outside dimensions of ~ 50 × 45 × 20 mm and a mass of ~ 40 g when covered with wax. These loggers had a resolution of 0.04°C and measurement range from + 34 to + 46°C. The scan interval of the body temperature loggers was set at 20-min. Miniglobe temperatures were recorded every hour, with a smaller thermometric data logger (iButton DS1922T, Maxim, Dallas Semiconductor, Texas, USA), which weighed ~ 10 g. These loggers had a resolution of 0.5°C and a measurement range from 0 to 125°C. All temperature sensors and loggers were calibrated against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany) in an insulated water bath. After calibration, the loggers and their sensors measured body temperature to an accuracy of better than 0.05°C and miniglobe temperature to better than 0.5°C.

4.3.4 Meteorological data measurements

I collected climatic data from a portable weather station erected near the Mahazat as-Sayd Protected Area, at the Saja/Umm ar-Rimth Protected Area (23°22' N, 42°45' E). I recorded wind speed ($m.s^{-1}$), solar radiation ($W.m^{-2}$), dry-bulb air temperature (°C) and relative humidity (%). I also recorded miniature (30 mm

diameter) black globe temperature (°C) and rainfall on site at the Mahazat as-Sayd Protected Area for the duration of the study period.

4.3.5 Data analysis

I analysed data within two periods, April-May and June-July, which I designated "warm wet" and "hot dry" on the basis of the prevailing ambient temperature and rainfall. I performed an unpaired Student's t-test to compare mean daily environmental conditions between the two periods. For successive 24-h periods, I calculated mean, minimum, maximum and amplitude of nychthemeral rhythm of body temperature. I averaged the body temperature parameters for each animal over each two-month period and performed a two-way repeated-measures analysis of variance (ANOVA) to test for differences in the temperature and activity profiles between the species and the two-month periods. Newman-Keuls multiple comparison tests were used to identify sources of differences.

To further assess the influence of the environment on body temperature patterns, I correlated the various parameters of the body temperature profile and the corresponding prevailing environmental parameters. Since both species sought shade during the heat of the day, dry-bulb air temperature proved to be a better index of operative temperature than did the temperature of a black globe thermometer in the sun. I therefore correlated the amplitude of the nychthemeral rhythm of body temperature, averaged for all individuals within each species, against the daily amplitude of air temperature. I fitted and compared simple non-linear regressions for minimum and maximum daily body temperature against the maximum daily air temperature. I also correlated collar miniglobe temperatures against weather station miniglobe temperatures, using linear Pearson procedures. To assess whether the animals were conforming to ambient conditions or were selecting micro-environments, I tested whether the slope of the regression equation was significantly different from one (the slope of the line of identity). In addition, I tested whether the slope and elevation of the regression equation was significantly different between the oryx and sand gazelle across the two periods, using a two-way repeated measures ANOVA.

I used Statistica (kernel release 5.5 for Windows, StatSoft, Inc. (1999), Tulsa Oklahoma, USA) and GraphPad Prism (version 4.00 for Windows, GraphPad Software, San Diego California USA) for statistical analyses. Values are expressed as mean ± SD and $P < 0.05$ was considered to be significant.

4.4 Results

4.4.1 Climate

Air and black globe temperature varied as a function of time of day, peaking just after solar noon and reaching a minimum just before sunrise (Fig. 4.1). Mean 24-h air temperature increased from 27.7 ± 4.0ºC during the warm wet period to 33.6 ± 1.5ºC during the hot dry period ($t_{120} = 10.9$, $P < 0.0001$). Similarly, mean 24-h black globe temperature increased from 33.8 ± 3.4ºC during the warm wet period to 37.8 ± 1.5ºC during the hot dry period ($t_{111} = 8.1$, $P < 0.0001$). Solar radiation showed the expected bell-shaped distribution, with a peak around solar noon, and wind speed increased in the late afternoon (Table 4.1). Rainfall totalled 31 mm during the study period, substantially lower than the preceding 10 year average of 100 ± 60 mm. April was the wettest month (17 mm) but some rain fell during May (7 mm) and March (7mm). March was not included in my analysis of temperature data. No rain fell in the hot dry period. The climatic conditions prevailing during the hot dry period of my study were similar in temperature to the summer period in which Ostrowski and Williams (2006) studied sand gazelle, but somewhat cooler than the summer conditions of their oryx study (Ostrowski *et al.*, 2003). My study period was much drier, though, than either of those that Ostrowski and colleagues encountered.

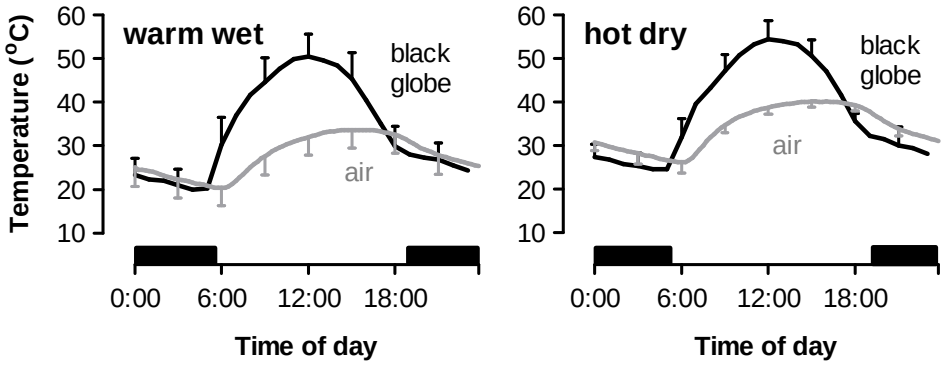

Figure 4.1. Standard black globe (mean ± SD, black line) and air dry-bulb temperature (mean ± SD, grey line) as a function of time of day, for the warm wet (left panel) and hot dry (right panel) periods, at the Saja/Umm ar-Rimth Protected Area, near the Mahazat as-Sayd Protected Area. Black bars represent night periods.

Table 4.1. Environmental conditions (mean ± SD) prevalent during the warm wet and hot dry periods.

	Warm wet	Hot dry
Air temperature (ºC)		
Mean 24 hour	27.7 ± 4.0	33.6 ± 1.5
Minimum	19.9 ± 4.2	25.3 ± 2.1
Maximum	34.1 ± 4.0	40.1 ± 1.4
Mean 24-hour wind speed ($m.s^{-1}$)	4.4 ± 1.4	4.1 ± 1.0
Mean daytime radiation ($W.m^{-2}$)	561 ± 93	604 ± 32
Total rainfall (mm)	24	0

4.4.2 Body temperature

Figure 4.2 shows the nychthemeral rhythm of body temperature for the five oryx and four sand gazelle over the warm wet and hot dry periods. Sand gazelle displayed a higher mean 24-h body temperature compared to the oryx ($F_{1,6}$ = 47.3, P = 0.0005), a higher 24-h minimum ($F_{1,6}$ = 42.6, P = 0.0006) and a higher 24-h maximum ($F_{1,6}$ = 11.0, P = 0.02) body temperature, than did the oryx (Table 4.2). However, there was no difference in the amplitude of 24-h nychthemeral rhythm of body temperature between the species ($F_{1,6}$ = 4.7, P = 0.07). Also, the environmental effects on body temperature were similar for the two species.

Compared to the warm wet period, during the hot dry period maximum 24-h body temperature increased ($F_{1,6}$ = 84.0, P < 0.0001) and minimum 24-h body temperature decreased ($F_{1,6}$ = 92.2, P < 0.0001), thus resulting in a larger amplitude of daily nychthemeral rhythm of body temperature ($F_{1,6}$ = 97.6, P < 0.0001). Mean 24-h body temperature also decreased ($F_{1,6}$ = 14.3, P = 0.009) during the hot dry period, compared to the warm wet period. However, there was no significant statistical interaction between the species and the period analysed for any body temperature parameter.

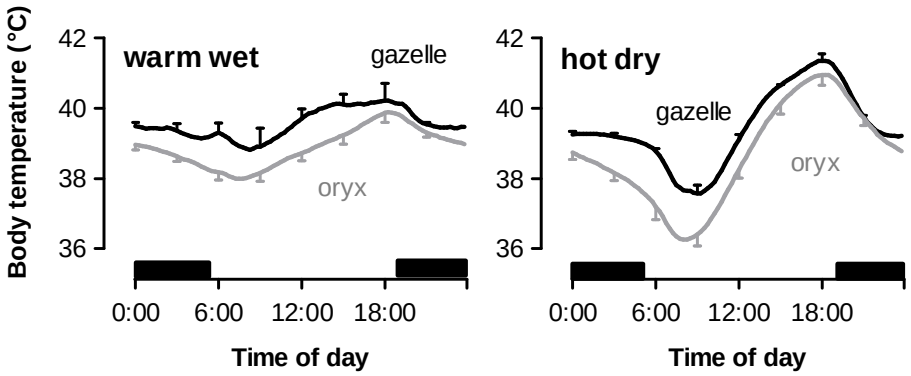

Figure 4.2. Nychthemeral rhythm of body temperature for five free-living oryx (mean ± SD, grey line) and four free-living sand gazelle (mean ± SD, black line) over both the warm wet (left panel) and hot dry (right panel) periods. Black bars represent night periods.

Table 4.2. Body temperature and daytime activity (mean ± SD) for five Arabian oryx and four Arabian sand gazelle during the warm wet and hot dry period (see text for statistical analyses).

	Warm wet		Hot dry	
	Sand gazelle	Oryx	Sand gazelle	Oryx
Body temperature (°C)				
24-h mean	39.6 ± 0.1	38.9 ± 0.2	39.4 ± 0.1	38.7 ± 0.2
24-h minimum	38.3 ± 0.1	37.8 ± 0.2	37.4 ± 0.3	36.1 ± 0.3
24-h maximum	40.8 ± 0.2	40.0 ± 0.2	41.5 ± 0.2	41.1 ± 0.3
24-h amplitude	2.5 ± 0.3	2.3 ± 0.3	4.1 ± 0.5	5.0 ± 0.5
Mean daytime activity (% maximum)	7.4 ± 1.8	5.7 ± 1.5	2.7 ± 0.8	1.9 ± 0.9

To further assess the influence of ambient temperature on body temperature, I correlated the amplitude of the nychthemeral rhythm of body temperature with the 24-h amplitude of air temperature for the sand gazelle (Fig. 4.3A) and the oryx (Fig. 4.3B). The correlation was poor and, statistically, the amplitude of nychthemeral rhythm of body temperature was independent of the 24-h amplitude of air temperature. However, the amplitude of the nychthemeral rhythm of body temperature showed a positive power relationship with the concurrent 24-h maximum air temperature for the sand gazelle ($r^2 = 0.75$, $y = 0.87 + 6.3x10^{-10} x^{6.1}$) and the oryx ($r^2 = 0.81$, $y = 0.17 + 1.8x10^{-8} x^{5.2}$) and 24-h mean air temperature for the sand gazelle ($r^2 = 0.73$, $y = 0.86 + 1.3x10^{-7} x^{4.8}$) and the oryx ($r^2 = 0.81$, $y = 0.35 + 8.7x10^{-7} x^{4.4}$). A power curve also proved to be the most appropriate simple non-linear equation for the correlation of maximum 24-h body temperature against maximum 24-h air temperature for both the sand gazelle (Fig. 4.3C) and the oryx (Fig. 4.3D). Comparison of power curves revealed significant differences between the two species ($F_{3,262} = 112.1$, $P < 0.0001$). The regression line for the sand gazelle displayed a greater elevation than did the regression line for the oryx, as a result of the higher body temperatures displayed by the sand gazelle.

Maximum 24-h air temperature not only predicted at least 75% of the variability in maximum 24-h body temperature, but also predicted minimum daily body temperature. Minimum 24-h body temperature was associated negatively with maximum 24-h air temperature for both the sand gazelle (Fig. 4.3E) and oryx (Fig. 4.3F). Comparison of the best-fit simple non-linear equation revealed significant differences between the two species ($F_{2,264} = 102.6$, $P < 0.0001$). Maximum 24-h body temperature was also correlated negatively with minimum 24-h body temperature for both the sand gazelle ($r^2 = 0.55$, $P < 0.0001$) and oryx ($r^2 = 0.69$, $P < 0.0001$). A partial correlation coefficient revealed that minimum 24-h body temperature of the oryx was more dependant on maximum 24-h body temperature than on maximum 24-h air temperature, and the latter correlation was spurious for oryx ($r_{xy.z} = -0.17$, $t_{134} = -1.93$, $P = 0.06$), but not for the sand gazelle ($r_{xy.z} = -0.39$, $t_{134} = -4.86$, $P < 0.0001$). Such findings raise the possibility that, at least for the oryx, the nadir of body temperature rhythm is set by maximum body temperature, which, in turn, is set by maximum air temperature and water stress.

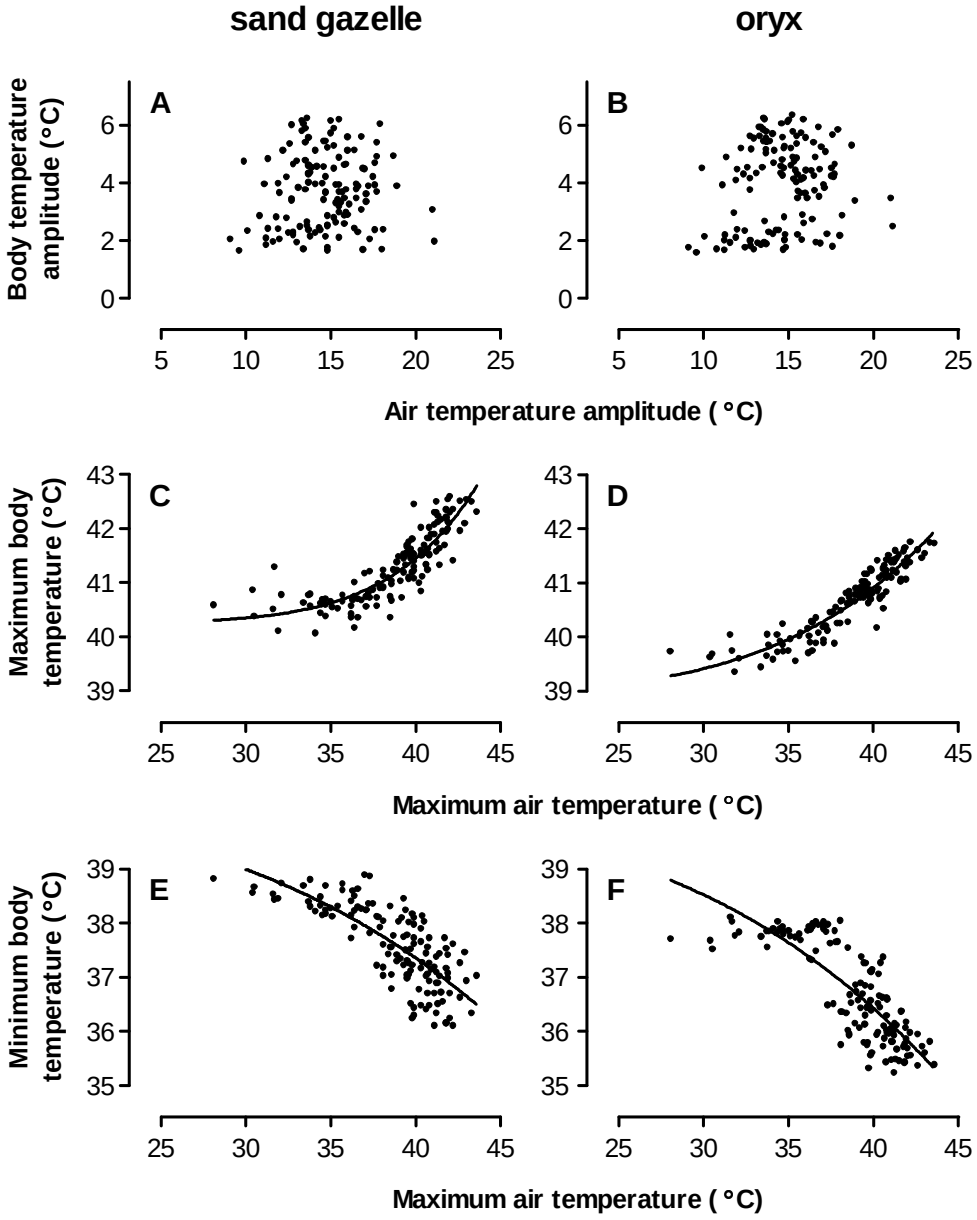

Figure 4.3. The top two panels show mean 24-h amplitude of the nychthemeral rhythm of body temperature, averaged for four sand gazelle (panel A, $r^2 = 0.008$, $P = 0.30$) and for five oryx (panel B, $r^2 = 0.03$, $P = 0.07$) plotted against the concurrent 24-h amplitude of air temperature. The bottom four panels show the mean maximum 24-h body temperature for sand gazelle (panel C, $r^2 = 0.76$, $y = 40.3 + 1.2\text{x}10^{-14} x^{8.7}$) and oryx (panel D, $r^2 = 0.86$, $y = 38.8 + 1.3\text{x}10^{-7} x^{4.5}$), and mean minimum 24-h body temperature for sand gazelle (panel E, $r^2 = 0.58$, $y^2 = 1612 - 0.003 x^3$) and oryx (panel F, $r^2 = 0.66$, $y^2 = 1599 - 0.004 x^3$), plotted against concurrent maximum 24-hour air temperature. All panels show 134 days of data.

4.4.3 Activity

Low minimum body temperatures were not related to a reduced activity. Minimum 24-h body temperature was not correlated with mean 24-h activity of the previous day, for either the sand gazelle (Fig. 4.4A, $r^2 = 0.005$, $P = 0.44$) or oryx (Fig. 4.4B, $r^2 = 0.03$, $P = 0.05$). The fraction of total activity which took place during daylight hours was correlated negatively with the concurrent maximum 24-h air temperature for both sand gazelle (Fig. 4.4C, $r^2 = 0.71$, $P < 0.0001$), and oryx (Fig. 4.4D, $r^2 = 0.65$, $P < 0.0001$). There was no difference between the slope ($F_{1,219} = 6.8$, $P = 0.14$) nor the elevation ($F_{1,220} = 0.01$, $P = 0.91$) of the regression line between species.

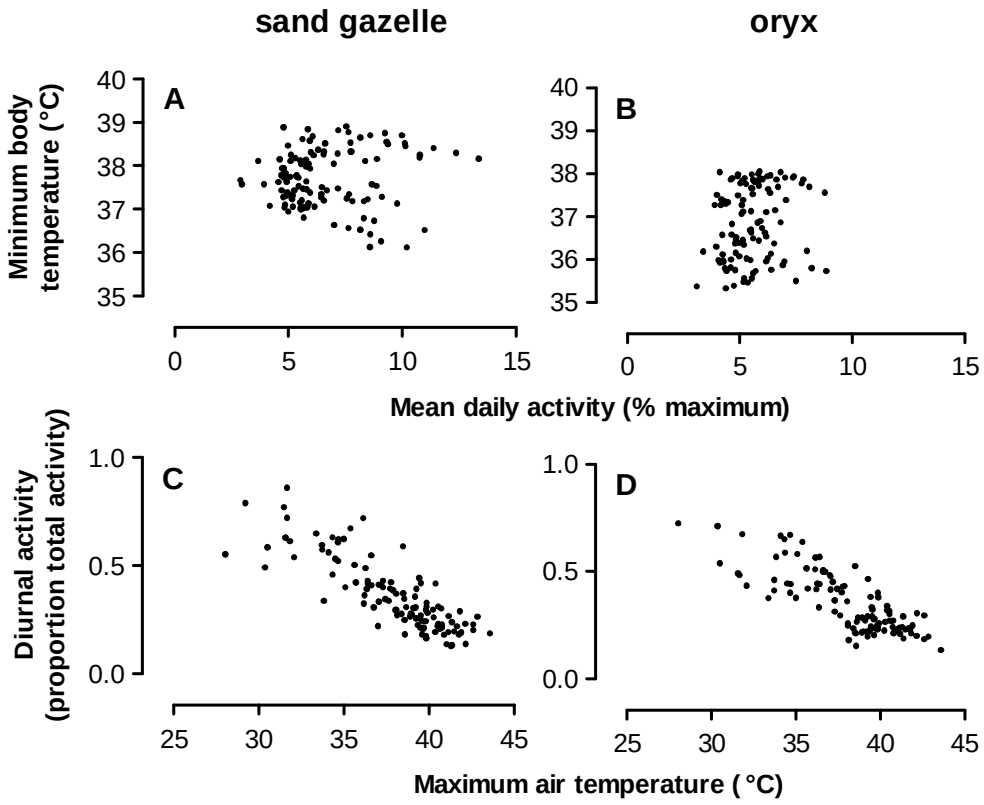

Figure 4.4. The top two panels show minimum 24-h body temperature, averaged for four sand gazelle (panel A, $r^2 = 0.005$, $P = 0.44$) and for five oryx (panel B, $r^2 = 0.03$, $P = 0.05$) plotted against the mean 24-h activity for the preceding day. The bottom two panels show the fraction of total activity which took place during daylight hours, for six sand gazelle (panel C, $r^2 = 0.71$, $P < 0.0001$) and five oryx (panel D, $r^2 = 0.65$, $P < 0.0001$), plotted against concurrent maximum 24-h air temperature.

Although the sand gazelle appeared to be more active than the oryx at all times (Fig. 4.5), the mean 24-h activity was not significantly different between the species ($F_{1,8}$ = 2.1, P = 0.19). There also were no differences between species in mean activity during daylight hours ($F_{1,8}$ = 2.3, P = 0.16), nor in the fraction of total activity which took place during daylight hours ($F_{1,8}$ = 0.3, P = 0.59). The activity patterns of both species changed between the periods. Both species shifted from a continuous activity pattern with crepuscular peaks at sunrise and sunset during the warm wet period to nocturnal activity during the hot dry period ($F_{1,8}$ = 171.8, P < 0.0001). Both species became inactive three hours after sunrise and remained inactive until an hour before sunset (09:00 and 18:00). The fraction of total activity which occurred during daylight hours decreased from 48 ± 5% to 25 ± 8% for the oryx and similarly from 47 ± 3% to 23 ± 2% for the gazelle ($F_{1,8}$ = 236.1, P < 0.0001). There was no statistical interaction between the species and the period for any of the activity parameters analysed.

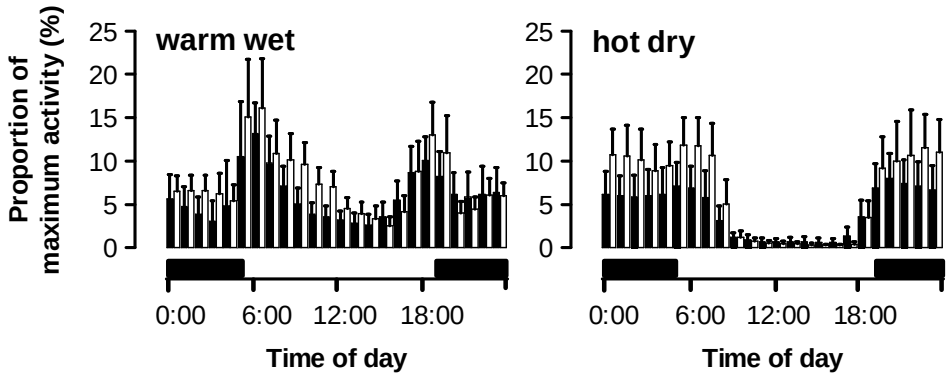

Figure 4.5. Nychthemeral rhythm of activity for six sand gazelle (white bars) and five oryx (black bars) over both the warm wet (left panel) and hot dry (right panel) periods. Both species shifted from a continuous 24-h activity with crepuscular peaks during the warm wet period to nocturnal activity during hot dry period. Activity was measured over the abdomen with a subcutaneous activity data logger, in activity counts expressed as a percentage of maximum counts for that logger. Black bars represent night periods. See text for statistical analyses.

4.4.4 Microclimate

Inactivity during the hot dry period was accompanied by increased shade-seeking behaviour (Fig. 4.6). During the heat of the day both oryx and sand gazelle selected microclimates which were cooler than the temperature recorded by an identical miniglobe exposed to the sun. There was no difference in the microclimates selected by the oryx and sand gazelle ($F_{1,3}$ = 1.9, P = 0.26), as indexed by the mean daily maximum difference between the temperature at the site chosen by the animal and the temperature of an identical miniglobe exposed to the sun at a nearby weather station. The microclimates selected by the sand gazelle and oryx were cooler during the hot dry period (6.6 ± 0.5ºC) than during the warm wet period (5.0 ± 0.3ºC, $F_{1,3}$ = 32.7, P = 0.01). There was no statistical interaction between the species and the period for microclimate selection.

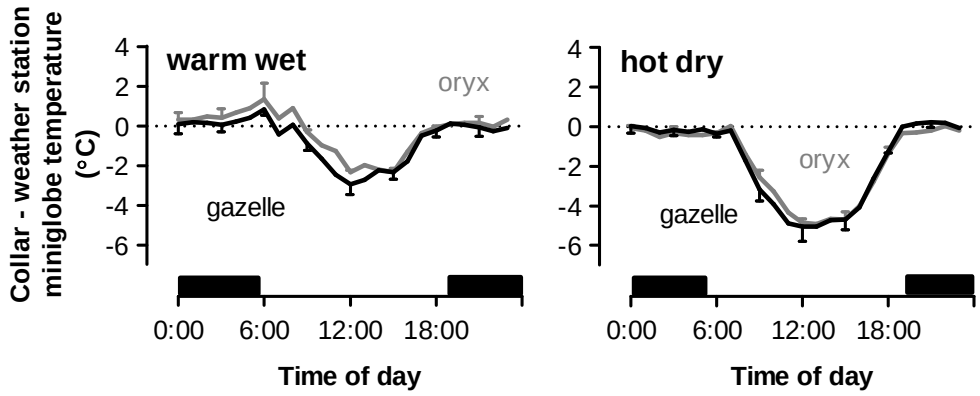

Figure 4.6. Nychthemeral rhythm of microclimate selection, expressed as the difference between miniglobe temperature at the site chosen by four free-living sand gazelle (mean ± SD, black line) and two oryx (mean ± SD, grey line) and the temperature of an identical miniglobe exposed to the sun at a nearby weather station, over both the warm wet (left panel) and hot dry (right panel) periods. Black bars represent night periods.

Figure 4.7 shows the correlation between the miniglobe temperatures on the collar of a single sand gazelle and a single oryx, and temperatures recorded by an identical miniglobe in the sun at a nearby weather station, during the two periods analyzed. The slopes for all the animals, over both periods, were significantly less than one (ANCOVA, P < 0.0001), and the regression lines intersected the line of identity, implying that all animals selected microclimates cooler than the exposed microclimates at higher environmental heat loads, across all four periods. There was no species difference in the slope of the regression line between sand gazelle

and oryx ($F_{1,3}$ = 0.4, P = 0.57), nor the elevation of the regression line ($F_{1,3}$ = 0.1, P = 0.75). However, the slope of the regression lines for both species decreased from 0.84 ± 0.02 during the warm wet period to 0.77 ± 0.03 during the hot dry period ($F_{1,3}$ = 34.2, P = 0.01). These results imply that both sand gazelle and oryx were selecting cooler microclimates at high environmental heat loads during the hot dry periods and were less selective of microclimates during the warm wet period. There was no significant difference between the intercept of the line of identity and regression slope between the species ($F_{1,3}$ = 0.4, P = 0.59) nor between periods ($F_{1,3}$ = 3.3, P = 0.17), implying that both sand gazelle and oryx selected cooler microclimates when the ambient air temperature exceeded 27.9 ± 1.5ºC during both periods.

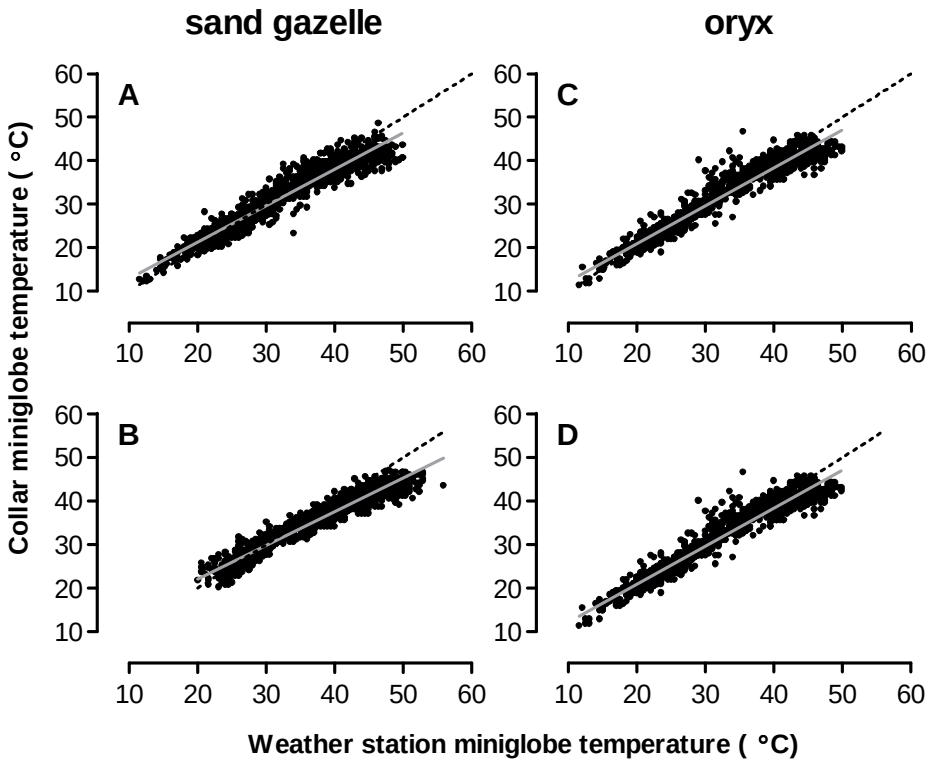

Figure 4.7. Scatter diagram showing the relationship between miniglobe temperatures at the site chosen by a male sand gazelle (left panel) and a male oryx (right panel) and the miniglobe temperatures recorded at a nearby weather station, during warm wet (upper panel) and hot dry (lower panel). Measurements were made at hourly intervals. The dashed line is the line of identity, the solid grey line is the linear regression. The slope of regression lines were not significantly different between the species, but were different between the periods. During the hot dry period both sand gazelle (panel B, $y = 0.78x + 6.3$) and oryx (panel D, $y = 0.77x + 6.2$) showed lower slopes, compared to the warm wet period for both sand gazelle (panel A, $y = 0.84x + 4.3$) and oryx (panel C, $y = 0.85x + 3.5$), indicating the selection of cooler microclimates during the hot dry period.

4.5 Discussion

My study provides the first comparison of body temperature, activity and microclimate selection of a large and a small desert-adapted antelope species, while the animals were living free at the same time and under the same hot and hyper-arid environmental conditions. Despite the oryx having a body mass more than four-fold that of the sand gazelle, the species responded remarkably similarly to changes within those environmental conditions. If anything, the heavier oryx appeared to be more influenced by environmental temperatures and maximum air temperature accounted for 86% of the variability in maximum daily body temperature of the oryx (Fig. 4.3D), compared to only 76% for the sand gazelle (Fig. 4.3C). Maximum daily body temperatures increased and minimum daily body temperature decreased as conditions got progressively hotter and drier (Fig. 4.3). The decreasing minimum body temperature with increasing environmental heat stress may seem counter-intuitive, but is exactly what is predicted by the concept of adaptive heterothermy, namely the consequence of active suppression of heat conservation and generation at night and in the early hours of the morning, in conditions in which heat storage during the rest of the day will be high, and distinguishes adaptive heterothermy from dehydration hyperthermia (Mitchell *et al.*, 2002). Theory predicts that species with more thermal inertia are more likely to employ adaptive heterothermy than those with less thermal inertia (Taylor, 1970a; Mitchell *et al.*, 2002), and indeed, the heavier oryx showed a greater decrease in minimum daily body temperature (0.9ºC vs. 1.7ºC, Table 4.2) when maximum air temperature exceeded 35ºC (Fig. 4.3F). Contrary to the conventional view of adaptive heterothermy, though, there was no association between the amplitude of the nychthemeral rhythm of body temperature, and therefore body heat storage, and the amplitude of the nychthemeral rhythm of air temperature. Thus, the nychthemeral swings in body temperature were not caused by nychthemeral swings in air temperature. Compared to that of the oryx, the thermoregulatory system of the sand gazelle appeared to operate around a higher set-point, with the sand gazelle displaying a mean 24-h body temperature an average 0.7ºC higher, a higher minimum 24-h body temperature and a higher maximum 24-h body temperature (see Fig. 4.2 and Table 4.2).

There were no significant species' differences in activity. Both gazelle and oryx were active over the full 24 h, but with crepuscular peaks, during the warm wet period, and shifted to nocturnal activity during the hot dry period (Fig. 4.5). Activity virtually ceased during the heat of the day, when both species are known to seek shade. I was able to detect the selection of cooler microclimates without observers present, by using miniature globe thermometers attached to collars, a novel technique I developed (Appendix 1, Hetem *et al.*, 2007) for recording microclimate selection by free-living ungulates. There was no difference in the pattern of microclimate selection by the two species, consistent with them being stationary, in the shade, during the hottest times of the day (Fig. 4.6). Both species selected cooler microclimates more readily during the hot dry period than during the warm wet period, and at times selected a microclimates with temperatures 12ºC below that of an identical miniglobe in the sun (Fig. 4.7).

My hot dry study period was not only significantly hotter than my warm wet period, with air temperatures being about 6ºC higher at all times of day (Table 4.1), but it also was significantly drier. Indeed no rain at all fell over the two months of the hot dry period, and rain would have been the only source of drinking water available to the animals. A lack of rain would have reduced the pre-formed water in plants, and fodder quality generally. I believe that these data support the same conclusions that I drew in chapter three, namely that the important environmental variable inducing adaptive heterothermy was the lack of water availability in conditions of heat stress, rather than the heat itself. As I have shown, the degree of adaptive heterothermy employed was not related to the daily swings in air temperature (see Fig. 4.3). Also, at the same ambient temperature, the antelope selected cooler microclimates during the hot dry period than during the warm wet period (see Fig. 4.7). I believe that, if they can access sufficient water, the antelope prefer to maintain homeothermy, which will require evaporative cooling, even in the intense desert heat, rather than implementing heterothermy, and storing heat.

The similarities in the body temperature and activity patterns of the gazelle and oryx were remarkable, given that the gazelle were captive bred and remained in a 2 km^2 enclosure throughout the study. Although the gazelle were exposed to the same environmental heat load as the oryx were, and the habitat inside their enclosure was the same as that outside it, the gazelle were not as able to seek appropriate microhabitats, including water sources, because their fenced enclosure restricted long-distance movements. Even though sand gazelle are reputed to be able to survive independent of surface water (Ostrowski *et al.*, 2006a), the scarcity of resources in the enclosure, after an exceptionally hot and dry period, may have contributed to the death of the gazelle five months after implant surgery. If so, that resource scarcity was not confined to my gazelle's enclosure, because the high mortality of sand gazelle was not specific to the gazelle in my enclosure; nearly 700 sand gazelle (50% of the estimated population) died in the Mahazat as-Sayd during 2006 (Cunningham *et al.*, 2008).

The body temperatures of sand gazelle and oryx inhabiting the Mahazat as-Sayd Protected Area have been measured previously in two studies separated by 3-6 years (Ostrowski and Williams, 2006). The body temperatures of the gazelle were measured with data loggers similar to mine, but those of the oyrx were measured by radiotelemetry, which allowed only sporadic measurements spread over three years, and in different animals, especially at night. Similar to the results of my study, sand gazelle had a higher mean daily body temperature of 39.5 ± 0.3°C (Ostrowski and Williams, 2006), compared to 38.4 ± 1.3°C for the oryx (Ostrowski *et al.*, 2003), but unlike my gazelle, they had a smaller amplitude of nychthemeral body temperature rhythm of 2.6 ± 0.8°C (Ostrowski and Williams, 2006), compared to 4.1 ± 1.7°C for the oryx (Ostrowski *et al.*, 2003), during summer. Since conditions were milder during the sand gazelle study, the differences in body temperature were attributed to differences in environmental conditions prevailing during the two studies (Ostrowski and Williams, 2006), and indeed, as I have shown (Table 4.2), sand gazelle can achieve a rhythm amplitude the same as that of the oryx. My study had the advantage of comparing these species in the same environmental conditions, so that any differences I observed

between the species were inherent, and not the result of different environmental conditions. Even given their constraints, though, what the studies of Ostrowski and colleagues did establish was that free-living antelope in the Saudi Arabian desert can employ heterothermy. On the basis of studies of African antelope my colleagues had concluded previously that adaptive heterothermy may have been an artefact of confinement (Mitchell *et al.*, 2002).

I now have confirmed the ability of free-living sand gazelle and oryx to use heterothermy, but I differ from Ostrowski *et al*. (2003), in that I found no association between the degree of heterothermy (and therefore heat storage) and the amplitude of variation in air temperature in the oryx. Ostrowski and his colleagues, like myself, found no association between the degree of adaptive heterothermy and amplitude of air temperature rhythm in sand gazelle (Ostrowski and Williams, 2006). I do not believe that the degree of heterothermy is driven by swings in ambient temperature, but rather by water availability, and consequently that the large amplitudes that I found, in both species, at ambient temperatures similar to those which Ostrowski studied sand gazelle, but cooler than those in which they studied oryx, were the result of water deprivation. Although Ostrowski and colleagues contradicted themselves later (Ostrowski and Williams, 2006), they stated that their oryx, in summer when they showed a large amplitude in rhythm of body temperature, could have been "somewhat dehydrated", showing "higher haematocrit, plasma protein concentration and plasma osmolality" (Ostrowski *et al.*, 2003). Similarly, their sand gazelle, with a plasma osmolality of about 320 mOsm in September and October (Ostrowski and Williams, 2006), that is in autumn, were likely to have been severely dehydrated in the summer (Alamer, 2006), the time at which they showed appreciable heterothermy.

The main body temperature change inducing the increase in body temperature amplitude in the hot dry period, in my oryx and sand gazelle, was the drop in minimum body temperature. While the original definition of adaptive heterothermy, and subsequent studies, imply that the drop in minimum body temperature was under active physiological control (Schmidt-Nielsen *et al.*, 1957;

Ostrowski *et al.*, 2003; Maloney *et al.*, 2004), it could result from thermoregulatory failure resulting from undernutrition. At that time, of heat without any rainfall, the forage quality available to the animals would have been poor, and, in other artiodactyl species, energy restriction leads to a lower nadir in the nychthemeral rhythm of body temperature (Choshniak *et al.*, 1995; Piccione *et al.*, 2002; Ahmed and El Kheir, 2004). However, since the maximum 24-h body temperature was a stronger determinant of the nadir of body temperature rhythm it seems more likely that, in circumstances in which animals otherwise would use evaporative cooling but don't have access to enough water, the nadir of body temperature is set by dehydration. Dehydrated animals would benefit most from the water savings associated with a lower nadir of body temperature rhythm, but such an hypothesis that the nadir of body temperature rhythm is set by ambient temperature and dehydration state, perhaps via the animal's osmolality, would need to be established through laboratory experimentation. Irrespective of the origin of the lower nadir, though, it led to increased capacity to store heat during the day, in both species. The lower minimum body temperature displayed by my oryx potentially would permit additional storage of heat during the day, compared to the sand gazelle. Such a capacity for increased heat storage combined with a greater thermal inertia may add support for Taylor's (1970a) theory that antelope with different body masses employ different strategies of heterothermy. Under simulated desert conditions in a laboratory, small gazelle allowed their rectal temperature to increase and exceed air temperature, whereas the larger antelope displayed low morning rectal temperatures, which allowed heat storage during the day negating the need for excessively high maximum body temperatures (Taylor, 1970a). However, since there was no difference in the nychthemeral rhythm of body temperature between my species, such differences in body temperature may be inherent.

Since I have established that the difference observed earlier in thermoregulatory function of sand gazelle and oryx are not only the consequence of the species previously having been exposed to different environmental conditions, I can seek an intrinsic origin for the differences, and the parsimonious explanation would be

that they relate to body size. However, the scaling of thermoregulatory function with body size is neither simple nor universally agreed. I assert caution about drawing any phylogenetic conclusions from a comparison of just two species. Given those complexities and that caution, the higher set-point for body temperature regulation displayed by the sand gazelle, both in my study and that of Ostrowski and Williams (2006), relative to the oryx, conforms to the negative scaling of body temperature with body mass reported by Clarke and Rothery (2008), particularly for the artiodactyls, and for mammals generally (Morrison and Ryser, 1952 ; McNab, 1970; Aschoff, 1982), and not with the positive scaling proposed originally by Rodbard (1950) and more recently by White and Seymour (2003). That the amplitude of the nychthemeral rhythm of body temperature of the two species, when exposed to the same environment, was not significantly different conforms to the prediction that the amplitude will be mass-independent in larger mammals (Aschoff, 1982).

That the amplitude was the same for the two species, and also that the amplitude increased by the same magnitude between the warm wet and hot dry periods, does not imply that the species were equally dependent on heterothermy. With a mass approximately four times greater, the oryx would store about four times as much heat as the gazelle as body temperature rises, and therefore save about four times as much water, that otherwise would be spent on evaporative cooling (Mitchell *et al.*, 2002). The relative benefit of that water saving would depend on factors such as metabolic rate, apportioning of cooling to evaporation, and radiant and connective heat transfer between the animal and the environment in the sun and shade, factors unknown for the gazelle and oryx.

It was not just the temperature rhythm amplitudes that did not differ between the gazelle and the oryx, despite the differences in mass, but also the nychthemeral activity pattern and its response to increasing heat and aridity (Fig. 4.5). Since time spent active was likely to correlate with time spent foraging, my data do not conform to the prediction that larger species would spend proportionally more time foraging, as a result of increased metabolic (Peters, 1983; Schmidt-Nielsen,

1984; Owen-Smith, 1988; Owen-Smith, 1992; du Toit and Yetman, 2005) and other nutritional (Demment and Van Soest, 1985) requirements. Nor do my data conform to the contrary view of the allometric scaling, namely that that time spent active, particularly time spent foraging, tends to decrease with increasing body mass (Belovsky and Slade, 1986; Mysterud, 1998; Pérez-Barbería and Gordon, 1999). Further, my data do not conform to the principle that mixed feeders which consume high quality forage, such as sand gazelle (Groves, 1988), are more active are than grazers, such as oryx (Bunnell and Gillingham, 1985; Klein and Fairall, 1986; Owen-Smith, 1992; Mysterud, 1998; Taylor *et al.*, 2006). Instead, my activity data fit best with the proposition that neither feeding style (Pérez-Barbería and Gordon, 1999) nor body mass (Jeschke and Tollrian, 2005) significantly influence activity. Body mass also has been proposed to influence the apportioning of feeding between day and night (Owen-Smith, 1988), but, once again, there is no agreement about even the slope of a relationship. Some authors have proposed that smaller species would be more vulnerable to predation and therefore forced to be more active at night (Pérez-Barbería and Gordon, 1999), but others have concluded that larger species spend less time foraging during the daylight hours than do smaller species with similar food habits (Bunnell and Gillingham, 1985).

Though the difference in activity patterns between my sand gazelle and oryx were small, and perhaps surprisingly so, there were very significant differences in the changes in pattern for both species, between the warm wet and hot dry periods. Both species shifted from continuous activity with crepuscular peaks in warm wet months to nocturnal activity during hot dry months (Fig. 4.5). The biological phenomenon of changing the timing of activity, in a way that depends on the environmental conditions, has been termed “cathemerality” (Tattersall, 2006; Tattersall, 2008). Pressure towards cathemerality is proposed to be greater for large animals, because of their greater absolute energy requirements, and all Indonesian mammals studied with a body mass above 100 kg, and most above 10 kg, were cathemeral (van Schaik and Griffiths, 1996). However, whereas in the Indonesian study the pressure towards cathemerality was likely related to foraging

requirements, my sand gazelle and oryx appeared to shift activity patterns based on prevailing ambient heat and aridity.

If thermal stress is the driving force for cathemerality, one would expect smaller species to make the greater relative adjustments to diurnal activity in hot environments, as a result of their lower thermal inertia, greater surface area to mass ratio, and smaller surface area for evaporative cooling. Several studies have reported the attenuation of diurnal activity patterns of small ungulates at high ambient temperatures (Zervanos and Hadley, 1973; Kamau and Maloiy, 1985; Wilson, 1989; Roberts and Dunbar, 1991; du Toit, 1993), commonly as a result of shade-seeking sedentary behaviour. Although high ambient temperatures also reduce the activity of large species (Jarman, 1977; Belovsky and Jordan, 1978; Leuthold and Leuthold, 1978; Bunnell and Gillingham, 1985; Belovsky and Slade, 1986; Klein and Fairall, 1986; Owen-Smith, 1998), that reduced activity was not necessarily associated with shade-seeking behaviour. Because nocturnal activity is difficult to measure without biotelemetry, which has become available only recently, it is not clear whether their adjustments of diurnal activity were accompanied by an increase in the proportion of nocturnal activity, as required if the pattern change is cathemeral, rather than just lethargy. In my oryx and gazelle, in which activity was measured by biotelemetry, the decrease in diurnal activity in the hot dry period indeed was compensated by an increase in nocturnal activity (Fig. 4.5), but also with disappearance of the crepuscular peaks.

Because my study design required observers not to be near the animals, I did not observe what they were doing while they were inactive during the day. Under observation, oryx abandon foraging and seek refuge in shade on hot days (Ostrowski *et al.*, 2003), and anecdotal evidence claims that sand gazelle do too (Ostrowski and Williams, 2006). In general, smaller diurnal animals may be forced to take refuge from temperature extremes more frequently (Peters, 1983) and are better able to seek refuge from the sun than their larger counterparts (Louw and Seely, 1982). Yet, the selection of cool microclimates during the heat of the day has been observed in a variety of desert ungulates, both large (Taylor

and Lyman, 1967; McCutchen, 1981; Hansen, 1982; Sargeant *et al.*, 1994; Tull *et al.*, 2001; Ostrowski *et al.*, 2003) and small (Zervanos and Hadley, 1973; Kamau and Maloiy, 1985; Wilson, 1989). What I have shown, again using biotelemetry, is that, in the absence of human interference, the oryx and gazelle occupied microclimates cooler than the exposed thermal environment, presumably shaded microclimates, when environmental heat load exceeded 28ºC. At any particular ambient temperature, the degree to which they avoided exposed microclimates increased when the habitat was drier. Although sand gazelle have been observed to retreat to small rock crevices during the heat of the day (Ostrowski and Williams, 2006), a microclimate proposed to provide greater thermal advantages than vegetation (Cain *et al.*, 2008), the similarity in the microclimate selection (Fig. 4.6) implies that the oryx had access to microclimates as cool as those available to the much smaller sand gazelle.

In summary, using biotelemetric techniques which free the animals from disturbance by human observers, I have confirmed that both Arabian oryx and sand gazelle employ adaptive heterothermy, and here show for the first time that, in the same environment, the amplitudes of their nychthemeral rhythm are the same, despite their different masses. I also have shown cathemerality in the activity of both species. I believe that, in very hot environments, the degree of adaptive heterothermy employed, and the degree of avoidance of exposed microclimates, are determined not by ambient temperatures alone, but by the aridity, consonant with those thermoregulatory processes saving body water. Although both sand gazelle (Ostrowski *et al.*, 2006a) and oryx (Ostrowski *et al.*, 2006b) are reported to have a low total evaporative water loss, and are believed to survive independent of surface water, the year of my study was particularly dry. Under conditions of extreme aridity in the Negev desert, the dorcas gazelle (*Gazella dorcas*), also reputed to survive independent of surface water, preferentially chose habitats with access to drinking water (Henley *et al.*, 2007). With many areas of the Africa predicted to get both hotter and drier under climate change scenarios, medium and large desert artiodactyls are likely to have to cope with increasing physiological stress. Although numerous small endotherms have

exhibited a linear decrease in body mass with recent warming trends (Smith *et al.*, 1995; Smith *et al.*, 1998; Yom-Tov, 2001; Smith and Betancourt, 2006; Yom-Tov *et al.*, 2006), such an adaptation strategy may not be appropriate for medium and large desert artiodactyls. Since water requirements scale allometrically with body mass (MacFarlane *et al.*, 1971), small ungulates may be particularly stressed in the future.

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CHAPTER 5

Effects of desertification on the physiology of Angora goats: testing global change predictions

Data and ideas presented in this chapter have been written up as a scientific paper and will be submitted to Journal of Arid Environments. Hetem R.S., de Witt B.A., Fick L.G., Fuller A., Maloney S.K., Meyer L.C.R., Mitchell D. and Kerley G.I.H. Effects of habitat transformation on the physiology of Angora goats: testing global change predictions.

5.1 Abstract

Globally, pastoralism has led to the transformation of habitat, which often leads to desertification. With climate change predicted to exacerbate desertification, phentoypic plasticity provides the best survival strategy for agriculturally important herbivores, which are long-lived and, for economic reasons, cannot be translocated. While changes in land use and regional climate have been assessed, there is little information on the plasticity of herbivores in responding to these changes. With the help of my colleagues, I investigated the physiology of Angora goats inhabiting transformed and intact sites in the Eastern Cape Province, South Africa. Although goats on both sites responded similarly under most environmental conditions, when goats were subjected to a physiological stress, imposed by shearing, goats inhabiting the transformed site had a faster rate of rise in abdominal temperature in the morning (0.38 vs. 0.31°C.h^{-1}, $P = 0.0009$), displayed an increased 24-h abdominal temperature amplitude (1.8 vs. 1.6°C, $P = 0.01$) and were generally less active (3.9 vs. 5.2 activity units) compared to goats inhabiting the intact site. Post-shearing, goats inhabiting the transformed site had higher water turnover rates ($P < 0.0001$) and selected more variable microclimates ($P < 0.0001$), than did goats inhabiting the intact site, even though they obtained less water from their diet ($P = 0.02$). Goats that inhabited the transformed site were more water dependent and appeared more susceptible to thermal stresses in their environment than were those that inhabited the intact site. Coping with such thermal challenges will be essential if livestock farming is to thrive under climate change scenarios.

5.2 Introduction

Climate change is likely to affect livestock production both because high ambient temperatures compromise the reproductive efficiency and performance of livestock (Scholes *et al.*, 1999; Nardone *et al.*, 2006), and because the predicted decrease in the quantity and quality of forage (Topp and Doyle, 1996) is likely to reduce the carrying capacity of rangelands (Hanson *et al.*, 1993; Milton and Dean, 1995; Richardson *et al.*, 2005). Since agriculture is inherently sensitive to extreme

and variable climatic conditions (Katz and Brown, 1992), it is predicted to be the economic sector most vulnerable to the risks and impact of climate change (Parry and Carter, 1989; Reilly, 1995). Agricultural production in Africa, in particular, may be compromised severely by climate change because of its heavy dependence on natural resources and a low adaptive capacity (Boko *et al.*, 2007).

In advance of the potential threats from climate change, in the subtropical thickets of the Eastern Cape of South Africa, domestic ungulates have been replacing indigenous herbivores in this area since the mid 1800s (Downing, 1978), resulting in severe habitat transformation (Aucamp *et al.*, 1980; Kerley *et al.*, 1995; Palmer *et al.*, 2004). In many places, heavy browsing by goats has transformed the indigenous thicket vegetation from a dense closed-canopy shrubland, frequently dominated by the highly palatable and nutritious forage plant *Portulacaria afra*, into an open savanna-like system (Hoffman and Cowling, 1990; Stuart-Hill, 1992; Moolman and Cowling, 1994; Kerley *et al.*, 1995; Robertson and Palmer, 2002; Sigwela *et al.*, 2003; Lechmere-Oertel *et al.*, 2005b). Such transformation of the natural flora, classified as desertification or dryland degradation, is the result of overgrazing due to human mismanagement of agricultural and pastoral resources (Dean *et al.*, 1995; Kerley *et al.*, 1995). These severely degraded areas of South Africa are likely to become even more vulnerable under predicted climate change scenarios (Meadows and Hoffman, 2003).

However, such habitat transformation is not unique to Africa and changes in the structure and dynamics of the plant community and ecosystem stability as a result of unsustainable livestock production are a world-wide phenomenon (Mattison and Norris, 2005). Climate change will exacerbate desertification (Le Houerou, 1996; Robertson and Palmer, 2002; Sivakumar, 2007), particularly because of the increased variability of climatic conditions and the frequency of extreme events, such as droughts (Mason and Joubert, 1997; Boko *et al.*, 2007). In order to meet agricultural requirements in the future it will become necessary to make use of all available pastureland (Bianca and Kunz, 1978). Domestic ungulates will have to cope with, and adapt to, climatically-unfavourable regions and an increased mean

annual temperature (Boko *et al.*, 2007), which is likely to increase energy demand (Canas *et al.*, 2003). Compared to other domestic ungulates, goats are well adapted to harsh, dry environments, as they have a relatively small body mass, low metabolic requirements, are able to use low-quality forage, are disease resistant and can survive long periods of water deprivation (Lu, 1988; Erasmus, 2000; Silanikove, 2000a; Silanikove 2000b; Lachica and Aguilera, 2003; Salem *et al.*, 2006; Tovar-Luna *et al.*, 2007). A proposed management response to climate change is the selection of heat-tolerant breeds (Nardone *et al.*, 2006), which would allow pastoralism to persist even in very hot and dry environments.

Additional management responses to climate change include changes in stocking rate, forage supplementation and alternative land uses such as game ranching (Scholes *et al.*, 1999), which already is occurring in the Eastern Cape Province, South Africa (Smith and Wilson, 2002). Nevertheless, stock farming remains the primary land use in the Eastern Cape. It is doubtful whether the transformed landscapes can be farmed profitably with goats at the low stocking rates, which are likely to result (Stuart-Hill, 1992; Stuart-Hill and Aucamp, 1993). How domestic ungulates cope with habitat transformation will become increasingly important in the face of global climate change. I therefore investigated the physiology of Angora goats inhabiting transformed and intact thicket sites in the Eastern Cape. Although Angora goats are susceptible to inanition because of their high nutritional requirement for fibre production (Hart *et al.*, 1993), they are particular well suited to low (230-560mm) rainfall areas (Norton and Deery, 1985), making them a good domestic ungulate model to investigate such responses. Most previous climate change assessments do not consider pre-adaptation to the expected conditions (Smit and Skinner, 2002), and especially not the plasticity of herbivore responses to these changes.

5.3 Materials and methods

5.3.1 Animals and habitat

Twenty-four neutered male Angora goats (*Capra aegagrus*), at Blaauwkrantz farm (33º32'S 25º23'E), near Port Elizabeth in the Eastern Cape Province, South Africa, underwent surgery (detailed below) in July 2005 before being randomly assigned to one of two habitats on the farm. Twelve goats (body mass 39.2 ± 3.5 kg, mean ± SD) were released into a 50 ha camp (intact site, comprising dense thicket vegetation) classified as Sundays Spekboomveld (Vlok *et al*., 2003) and 12 goats (body mass 39.7 ± 5.9 kg) were released into an adjacent 40 ha camp (transformed site), transformed by historical heavy browsing to form a savanna-like vegetation with a cover of ephemeral grasses and forbs and few remnant small trees (Lechmere-Oertel *et al*., 2005a). Goats had access to water *ad libitum* and foraged on the natural vegetation. Experimental procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (protocol no. 2005/45/4).

5.3.2 Surgery

For implantation of data loggers, the goats were anaesthetised by intramuscular (I.M.) injection of 2.5 $mg.kg^{-1}$ ketamine hydrochloride (Anaket-V, Bayer Animal Health Pty, Isando, South Africa) and 0.04 $mg.kg^{-1}$ medetomidine hydrochloride (Domitor, Novartis, Kempton Park, South Africa). Anaesthesia was maintained with 1-4% halothane (Fluothane, Astra Zeneca, Johannesburg, South Africa) administered in 100% oxygen via a facemask. After ~ 10 min of halothane administration, the action of medetomidine was reversed with 0.2 $mg.kg^{-1}$ (I.M.) atipamezole hydrochloride (Antisedan, Novartis, Kempton Park, South Africa). Respiratory rate, heart rate, oxygen saturation (pulse oximeters, Nonin 9847V, Nonin Medical, North Plymouth, USA) and rectal temperature (thermocouple thermometer, BAT-12, Physitemp, Clifton, USA) were monitored throughout the surgical procedure, which lasted ~ 30 min.

All implanted data loggers were covered in an inert wax (Sasol wax EXP986, Sasol, Johannesburg, South Africa) and dry-sterilized in formaldehyde vapour before implantation. Incision sites were shaved and sterilized with chlorhexidine gluconate (Hibitane, Astra Zeneca, Johannesburg, South Africa). A 70 mm cranial-caudal incision was made through the skin and *linea alba*, within the midline area on the ventral abdominal surface, and a miniature temperature-sensitive data logger (see description below) was placed into the abdominal cavity, where it floated freely. A single goat from each group also received an implantable tracking transmitter (African Wildlife Tracking, Pretoria, South Africa). The skin and muscle layers then were sutured closed. In six of the goats from each group, after administration of a local anaesthetic (0.04 g lignocaine hydrochloride, S.C., Bayer Animal Health Pty, Isando, South Africa), an additional incision was made on the upper hind leg. A smaller temperature-sensitive data logger (see below) was inserted subcutaneously. At the same subcutaneous site, a single goat from each group also had an activity logger (Actical, Mini-Mitter Corporation, Bend, OR, USA) implanted, which was tethered in a small pocket. The activity logger recorded at 10-min intervals, had dimensions of 40 x 40 x 15 mm, and weighed ~ 40 g when covered in wax. Activity counts were normalised for different logger sensitivities by expressing activity counts as a percentage of maximum counts for that logger.

Wounds were sprayed with a topical antiseptic spray (Necrospray, Centaur Labs, Johannesburg, South Africa) and coated with a topical tick repellent (Tickgrease, Cypermethrin 0.025% m/m, Bayer Animal Health Pty, Isando, South Africa). Each goat received a long-acting antibiotic (500 mg I.M. penicillin, Peni LA Phenix, Virbac Animal Health, Centurion, South Africa), an analgesic and anti-inflammatory medication (420 mg I.M. ramiphenazone, Dexa-Tomanol, Centaur Labs, Johannesburg, South Africa), and a long-acting parasiticide (5 mg S.C., doramectin, Dectomax, Pfizer Laboratories, Sandton, South Africa). Animals were marked individually with different coloured plastic ear tags.

Before halothane administration was terminated, a collar was fitted to a single goat from each group. The collar supported a miniature (diameter 30 mm) black globe thermometer ("miniglobe") to allow for the dynamic measurement of the microclimate the goat chose to occupy. This technique has proven successful on other ungulate species (Appendix 1, Hetem *et al.*, 2007). Miniglobe temperature was measured with a small temperature sensitive data logger, which was inserted into the centre of the matt-black hollow bronze sphere (Press Spinning & Stamping co., Cape Town, South Africa). The globe was attached to a 10-mm diameter, polyvinyl chloride rod, which in turn was attached to the apex of the collar.

After recovery from surgery, the goats were released into their allocated habitats where they roamed freely for a year. Within each site, goats behaved similarly and remained as a herd, so I believe that the activity and microclimate selection of the index goat reflected that of all goats in that habitat. They were caught and weighed once a month and subjected to standard husbandry practices of parasite control. In September 2005 and March 2006, the goats were shorn according to the standard management practices of the farm. The diet composition and quality, and fibre production of these animals were measured in a parallel study (Milne, 2008).

In August 2006, the goats once again were anaesthetised and the data loggers were removed under a surgical procedure similar to that used for the original implantation. The data loggers all were in perfect order, the animals' wounds had healed and there were no signs of infection from the initial surgery. Most of the abdominal loggers were found in the pelvic canal and were not encapsulated in adhesive tissue. After surgery the goats were returned to the resident herd on Blaauwkrantz farm.

5.3.3 Temperature measurements

The miniature data loggers (StowAway XTI, Onset Computer, Pocasset, USA) used to measure abdominal temperature had outside dimensions of $\sim 50 \times 45 \times 20$

mm and a mass of ~ 40 g when covered in wax. The data loggers had a storage capacity of 32 kb and measured temperatures within the range of + 34 to + 46 ˚C, at a resolution of 0.04 ˚C. The loggers measuring abdominal temperature were set to record at 20-min intervals. Subcutaneous and miniglobe temperatures were recorded every hour with a smaller thermometric data logger (iButton DS1922T, Maxim, Dallas Semiconductor, Texas USA), which had a diameter of ~ 25 mm, a height of 15 mm, and weighed about 10 g. These loggers had a resolution of 0.5°C and a measurement range from 0 to 125°C. All the loggers were calibrated, in an insulated water bath, against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany).

5.3.4 Meteorological data measurements

I collected climatic data by erecting a portable weather station at the field site (Hobo Weather Station, Onset Computer Corporation, Pocasset, Mass., USA). I recorded wind speed ($m.s^{-1}$), solar radiation ($W.m^{-2}$), dry-bulb temperature (°C), relative humidity (%), standard (150 mm diameter) and miniature (30 mm diameter) black globe temperature (°C) hourly.

5.3.5 Isotope analysis and water balance

I assessed water influx and water turnover rates, by dilution of the stable isotope, deuterium oxide, 10 days before and 10 days after the summer (March) shearing. Before the deuterium oxide was administered, animals were weighed and 10 ml jugular blood samples were taken to determine the background concentration of deuterium oxide. Animals then received a dose of 0.04 $ml.kg^{-1}$ deuterium oxide (D_2O, I.M., 99.8 atom %, Merck & Co. Ltd. Rahway, NJ, USA). A second 10 ml blood sample was taken four hours after injection, when I assumed that the administered deuterium oxide had equilibrated with body water. The goats' drinking water source was removed, and they were weighed and 10 ml blood samples were taken three, five and seven days later. Water was returned after the blood samples had been taken on the seventh day. The blood samples were collected by jugular venipuncture with Vacutainers (BD Diagnostics-preanalytical

systems, Plymouth, UK), placed in ice and centrifuged (Wifug Ltd., Bradford, England) at 6000 G for 10 min. Serum and plasma were separated and stored in 2 ml vials (cryogenic vials 430489, Corning Inc., NY, USA) and frozen at -20°C for later analysis.

Deuterium concentration of the serum was measured using a high-temperature elemental analyzer (Flinnigan elemental analyser, Thermo electron corporation, Bremen, Germany), normalized against an international references, namely Vienna-Standard Mean Ocean Water (V-SMOW). A multi-point method was used to determine water turnover in the goats (Fusch, 2000) and a log-linear regression line to establish rate constants for deuterium dilution. The measure of total body water was derived from the known dose of injected deuterium oxide divided by the difference between the y-intercept of the curve at time zero and the background concentration of deuterium oxide. Water influx was determined from the average rate of deuterium oxide dilution over the seven-day period, when goats did not have access to water, multiplied by the total body water. Water turnover was determined as the sum of water influx and the total weight loss, as all weight loss was assumed to be water loss.

To estimate the type of food consumed, the $\delta^{13}C$ values (±0.1 ‰ VPDB) of the goat serum samples were determined using a Thermo Delta V Plus mass spectrometer (Thermo Electron Corporation, Waltham, MA, USA) plumbed inline with a Flash EA 112 elemental analyser (Thermo Electron Corporation, Waltham, MA, USA), at the Council for Scientific and Industrial Research (CSIR) in Pretoria, South Africa. Laboratory standards with $\delta^{13}C$ values of -25.0 ± 0.1 ‰ VPDB and -11.4 ± 0.1 ‰ VPDB were used to correct for equipment drift.

Carbon isotope ratios can be used as an indication of diet selection, since plants which fix CO_2 by way of the Calvin C_3 cycle pathway differ in their natural abundance of ^{13}C from plants which fix CO_2 through the C_4-dicarboxylic acid pathway (Smith and Epsten, 1971). *Portulacaria afra* has a Crassulacean Acid Metabolism (CAM) photosynthetic pathway during summer and therefore has a

carbon isotope ratio of approximately -17.4 (Mills *et al.*, 2005), whereas the C_4 grasses approximate -12 and C_3 trees approximate -25.

5.3.6 Blood variables

Serum albumin, globulin and glucose concentrations were analyzed by the Department of Companion Animal Clinical Studies section of Clinical Pathology, University of Pretoria, Pretoria, South Africa. Total plasma lipids were extracted using the technique described by Bligh and Dyer (1959). Briefly, methanol and chloroform (50 ml, 2:1) were added to the sample and allowed to homogenize overnight. The sample was then filtered and rehomogenized in 15 ml of weak salt solution (0.85% NaCl). After the final biphasic system was separated into two layers, the lower (chloroform) phase was collected for evaporation and subsequent lipid estimation.

5.3.7 Data analysis

To test how the goats in the two habitats responded to climatic variation, before being shorn, I analysed data from the five hottest days and the five coldest days of my study. The five hottest days were non-continuous and determined by the highest maximum globe temperatures, which averaged 47.7 ± 3.1°C, within the two-month period before the March shearing. The five coldest days also were non-continuous and determined by the lowest night-time air temperature, which averaged 0.4 ± 0.5°C, within the month before the September shearing. The mean ± SD of the mean, minimum, maximum and amplitude of nychthemeral rhythm of abdominal temperature of the goats that inhabited the two treatments were calculated for each of these periods. I used a repeated measures two-way analysis of variance (ANOVA) to test for differences between the goats that inhabited the two habitats across hot and cold days. I also tested for differences in the time of minimum and maximum abdominal temperature, as well as differences in the maximum rate of abdominal temperature rise, between the goats that inhabited the two habitat types.

To assess how the goats that inhabited the two habitat types responded to shearing stress I analysed 10 days of data after the summer (March) shearing, hereforth termed “post-shearing”. I used an unpaired Student’s t-test to investigate differences in the temperature profiles between the goats that inhabited the two habitat types post-shearing. Over seven of those days I also performed a repeated measures two-way ANOVA on each of the blood variables to test for an interaction between the goats that inhabited the two habitat types across time. Newman-Keuls multiple comparisons tests were used to identify sources of significant differences in ANOVAs. To assess whether the animals were conforming to ambient conditions or were selecting microclimates, I tested whether the slope of the regression equation, fitted to the correlation between miniglobe temperature of the microclimate selected by the index goat and weather station miniglobe temperature, was significantly different from one (the slope of the line of identity). In addition, I tested whether the slope and elevation of the regression equation was significantly different between pre-shearing and post-shearing periods using an analysis of co-variance (ANCOVA).

I used Statistica (kernel release 5.5 for Windows, StatSoft, Inc. (1999), Tulsa Oklahoma, USA) and GraphPad Prism (version 4.00 for Windows, GraphPad Software, San Diego California USA) for statistical analyses. Data are expressed as mean ± SD and $P < 0.05$ was considered to be statistically significant.

5.4 Results

5.4.1 Climate

Black globe temperature peaked just after solar noon and reached a minimum just before sunrise. Solar radiation peaked between 12:00 and 13:00 and wind speed increased in the late afternoon. Environmental conditions over the three periods analyzed, namely cold days, hot days and post-shearing, are presented in Table 5.1. Both solar radiation and wind speed were lower on the cold than on the hot days, but both maintained a similar rhythm. Rainfall totalled 402 mm over the

one-year study period, which was 29% higher than the long term (1958 - 2005) average annual rainfall of 313 mm per annum (A. Rudman, pers. com.).

Table 5.1. Environmental conditions prevailing at the field site for the five hottest days, the five coldest days, and the 10-day period after summer shearing.

	Hot days	Cold days	Post-shearing
Black globe temperature (ºC)			
24-h mean	29.2 ± 1.1	14.6 ± 1.4	22.2 ± 1.4
24-h minimum	18.3 ± 1.7	0.4 ± 0.5	12.7 ± 3.2
24-h maximum	47.7 ± 3.1	31.1 ± 2.4	35.9 ± 3.5
Mean daytime radiation ($W.m^{-2}$)	564 ± 66	375 ± 26	379 ± 160
Maximum wind speed ($m.s^{-1}$)	4.5 ± 0.5	3.6 ± 1.1	4.0 ± 1.0

5.4.2 Hot and cold days

The abdominal temperature of the goats showed a nychthemeral (24-h) rhythm with a nadir shortly after sunrise and peak near sunset. On cold days, daily mean ($F_{1,20}$ = 129.5, P < 0.0001), minimum ($F_{1,20}$ = 91.9, P < 0.0001) and maximum ($F_{1,20}$ = 54.8, P < 0.0001) abdominal temperatures of the goats were lower, but the 24-h amplitude ($F_{1,20}$ = 6.1, P = 0.02) of abdominal temperature rhythm was higher, than on hot days (Fig. 5.1A and 5.1B). On hot days (Fig. 5.1B), goats that inhabited the intact site reached a slightly higher absolute maximum abdominal temperature (40.8 ± 0.3°C versus 40.6 ± 0.3°C, P = 0.01) than did goats that inhabited the transformed site. However, on cold days (Fig. 5.1A), goats that inhabited the transformed site displayed a higher absolute maximum abdominal temperature (40.4 ± 0.2°C versus 40.2 ± 0.2°C, P = 0.02), a greater variability of abdominal temperature, as represented by a larger standard deviation of abdominal temperature (0.47 ± 0.05°C versus 0.38 ± 0.04°C, P = 0.004), and a higher amplitude of abdominal temperature rhythm (1.98 ± 0.24°C versus 1.61 ± 0.19°C, P = 0.001) than did goats that inhabited the intact site. Goats that inhabited the transformed site also reached their minimum abdominal temperatures later in the day (09:07 ± 0:39 versus 07:39 ± 1:17, P = 0.003), which

resulted in faster maximum rate of abdominal temperature rise during the day (0.36 ± 0.07°C.h^{-1} versus 0.24 ± 0.05°C.h^{-1}, $P = 0.0002$) than goats that inhabited the intact site.

On cold days, the daily mean ($F_{1,9} = 150.9$, $P < 0.0001$), minimum ($F_{1,9} = 152.4$, $P < 0.0001$) and maximum ($F_{1,9} = 11.8$, $P = 0.007$) subcutaneous temperatures of the goats were lower, and had a greater amplitude ($F_{1,9} = 72.1$, $P < 0.0001$), than on the hot days (Fig. 5.1C and 5.1D). Daily mean subcutaneous temperatures were lower in the goats that inhabited the transformed site than in the goats that inhabited the intact site, on both hot (39.0 ± 0.5°C versus 39.4 ± 0.4°C, $P = 0.008$, Fig. 5.1D) and cold (38.0 ± 0.4°C versus 38.4 ± 0.6°C, $P = 0.007$, Fig. 5.1C) days. In addition, goats that inhabited the transformed site had a lower daily minimum (38.0 ± 0.7°C versus 38.7 ± 0.5°C, $P = 0.04$) and a lower daily maximum (40.0 ± 0.5°C versus 40.4 ± 0.4°C, $P = 0.01$) subcutaneous temperature than did goats in the intact site on hot days (Fig. 5.1D). These low subcutaneous temperatures resulted in a larger difference between abdominal and subcutaneous temperature in goats that inhabited the transformed site compared to those in the intact site, on both hot (0.79 ± 0.62°C versus 0.40 ± 0.35°C, $P = 0.03$, Fig. 5.1F) and cold (1.3 ± 0.4°C versus 1.0 ± 0.5°C, $P = 0.04$, Fig. 5.1E) days. This greater difference between abdominal and subcutaneous temperature was likely to reflect greater vasoconstriction in goats that inhabited the transformed site. On hot days the goats remain vasodilated throughout the day and night (Fig. 5.1F), but on cold days goats were vasoconstricted during the cold night (Fig. 5.1E). Shortly after sunrise on cold days there was a distinct rise in the subcutaneous temperature, likely to correspond to a period of vasodilation, which reduced the difference between abdominal and subcutaneous temperature.

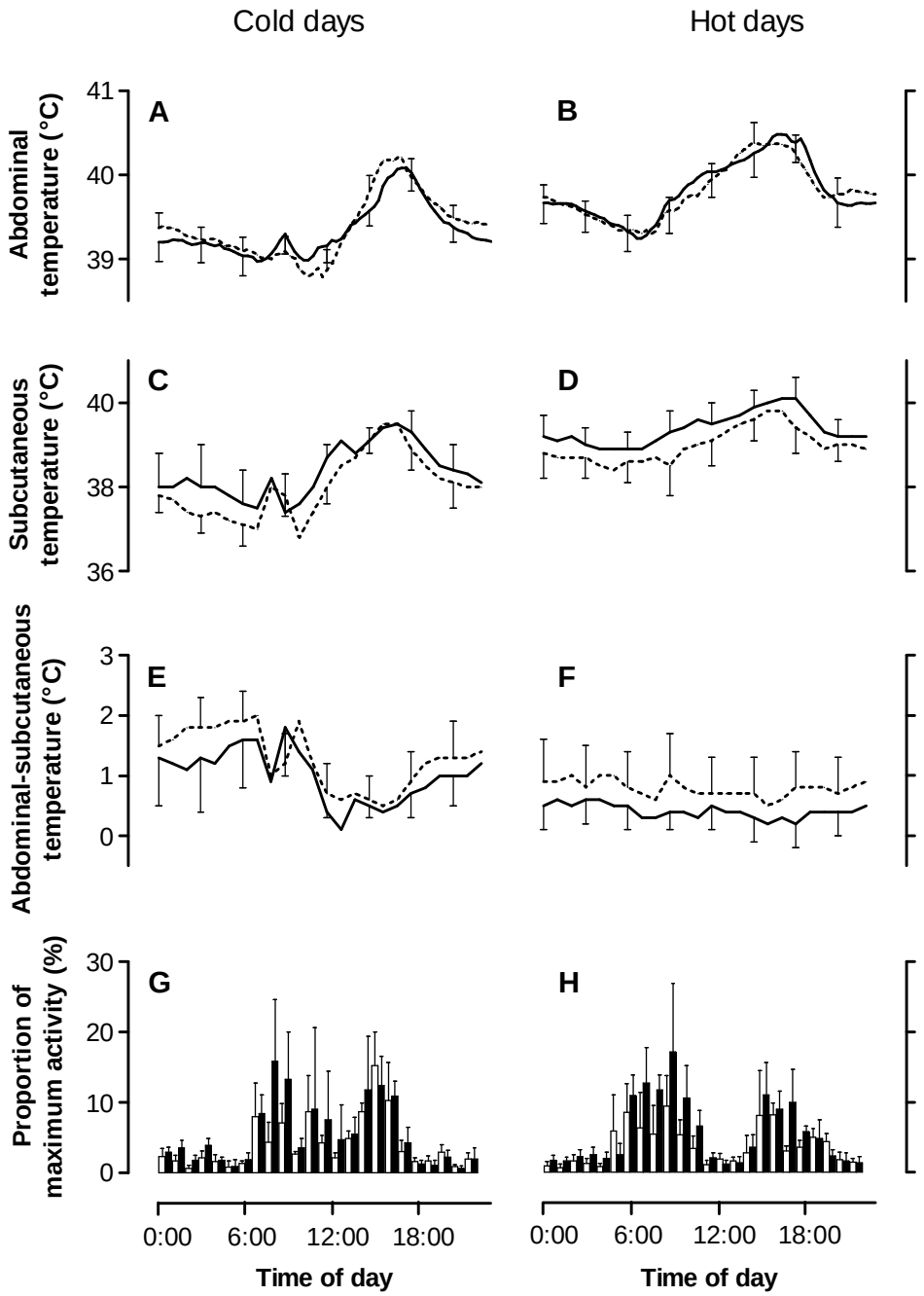

Figure 5.1. Nychthemeral rhythm (mean ± SD) of abdominal temperature, subcutaneous temperature and the difference between abdominal and subcutaneous temperature of 12 goats that inhabited the intact site (solid line) and 12 goats that inhabited the transformed site (dotted line), as a function of time of day, over the five coldest days in winter (average minimum globe temperature of 0.4 ± 0.5˚C, left panel) and the five hottest days in summer (average maximum globe temperature of 47.7 ± 3.1˚C, right panel) of the one-year study period. The lowest panel represents the nychthemeral activity rhythm (mean ± SD) of a single goat that inhabited the transformed site (white bars) and another goat that inhabited the intact site (black bars) over the same periods. The goats displayed a biphasic activity pattern with crepuscular peaks, with less activity between peaks on hot days than on cold days.

5.4.3 Shearing

Shearing imposed a thermal stress on the goats (Appendix 2, Hetem *et al.*, 2009). After summer shearing, goats that inhabited the transformed site displayed a higher variability of abdominal temperature, as represented by a greater standard deviation of abdominal temperature (0.46 ± 0.06°C versus 0.38 ± 0.04°C, t_{18} = 3.7, P = 0.002), and had a higher amplitude of abdominal temperature rhythm (1.8 ± 0.2°C versus 1.6 ± 0.1°C, t_{18} = 2.8, P = 0.01) than did goats inhabiting the intact site (Fig. 5.2A). To assess the rate of abdominal temperature rise, I calculated the rate of rise in abdominal temperature over each four hour interval and analysed the maximum rate. Shorn goats that inhabited the transformed site showed a faster maximum rate of change of abdominal temperature over the 4h interval than did the goats that inhabited the intact site (0.38 ± 0.05°C.h^{-1} versus 0.31 ± 0.03°C.h^{-1}, t_{18} = 3.9, P = 0.0009, Fig. 5.2A).

Figure 5.1G and 5.1H shows the nychthemeral rhythm of activity for an index goat that inhabited the transformed site and another that inhabited the intact site. On hot days (Fig. 5.1H), the goat, and by inference, others in the herd, displayed a biphasic activity pattern with crepuscular peaks, but there was more activity between the peaks on cold days (Fig. 5.1G), so the goats were active throughout the day. The index goat in the transformed site generally was less active than the index goat in the intact site on both hot (mean activity of 3.9 ± 3.6 versus 5.2 ± 5.6 relative activity units) and cold (4.1 ± 4.2 versus 5.3 ± 5.5 relative activity units) days.

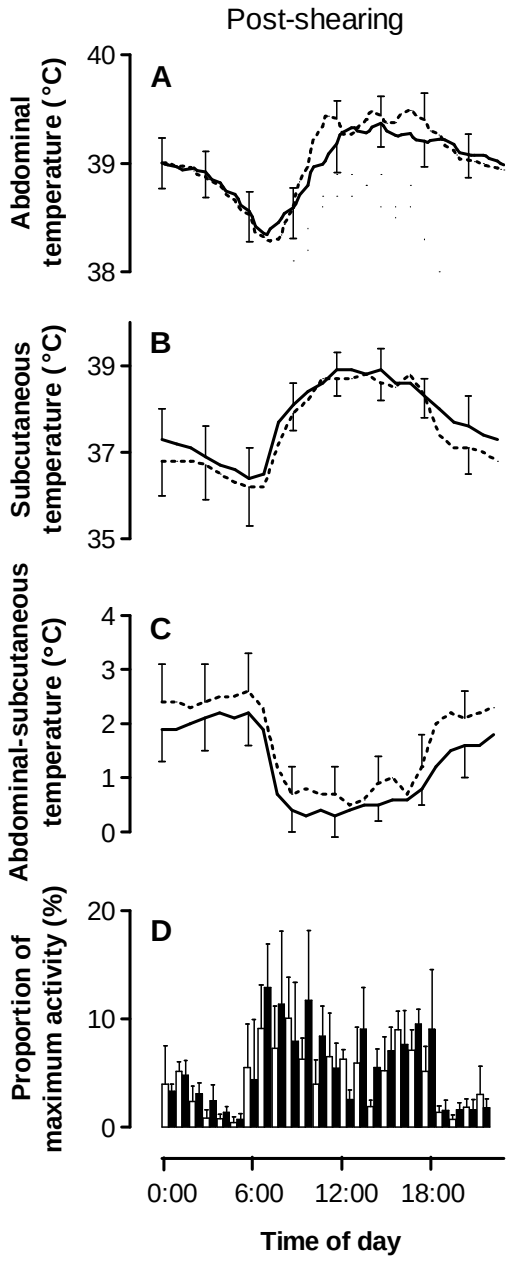

Figure 5.2. Nychthemeral rhythm (mean ± SD) of abdominal temperature, subcutaneous temperature and the difference between abdominal and subcutaneous temperature for 12 goats that inhabited the transformed site (dotted line) and 12 goats that inhabited intact site (solid line) over 10 days after summer shearing. The lowest panel shows the nychthemeral rhythm of activity for a single goat that inhabited the transformed site (white bars) and another goat that inhabited the intact site (black bars) over the same 10 day period.

There was no significant difference between subcutaneous temperatures (Fig. 5.2B), nor the difference between abdominal and subcutaneous temperature (Fig. 5.2C), for goats that inhabited the transformed site and goats that inhabited the intact site, after shearing. However, goats in both groups vasoconstricted at night (Fig. 5.2C), which they did not do on hot days when they were not shorn (Fig. 5.1F). Goats in both groups also appeared to be inactive during the night and displayed a reasonably high level of activity throughout the day (Fig. 5.2D), which was similar to that of the unshorn goats on cold days (Fig. 5.1G). In general, the shorn index goat inhabiting the transformed site was less active than was the shorn index goat inhabiting the intact site (mean activity 4.6 ± 2.9 versus 5.6 ± 3.7 relative activity units).

To assess differences in microclimate selection, after summer shearing, of goats that inhabited the two land types, I correlated miniglobe temperature at the site chosen by an index goat that inhabited the transformed site, and another index goat that inhabited the intact site with miniglobe temperature recorded at a nearby weather station, for 10 days after shearing (Fig. 5.3). The slope of the regression lines were significantly less than one, both for the goat that inhabited the intact ($F_{1,414}$ = 223.9, P < 0.0001) and the goat that inhabited the transformed ($F_{1,414}$ = 73.3, P < 0.0001) site. The regression lines both intersected the line of identity within the range of observations, implying that the goats selected microclimates cooler than the prevailing environmental conditions at high environmental heat loads on the intact site (threshold miniglobe temperature 24ºC) and on the transformed site (threshold miniglobe temperature 17ºC). The slope of the regression line was significantly lower ($F_{1,414}$ = 37.7, P < 0.0001) for the goat that inhabited the intact site (0.74 ± 0.02) than that for the goat that inhabited the transformed site (0.89 ± 0.01). The combination of lower slope and higher threshold implied that the goat that inhabited the intact site selected warmer microclimates at low environmental heat loads than did goats that inhabited the transformed site. Thus, Angora goats on the intact site appeared to select more stable microclimates than did goats that inhabited the transformed site, post-shearing.

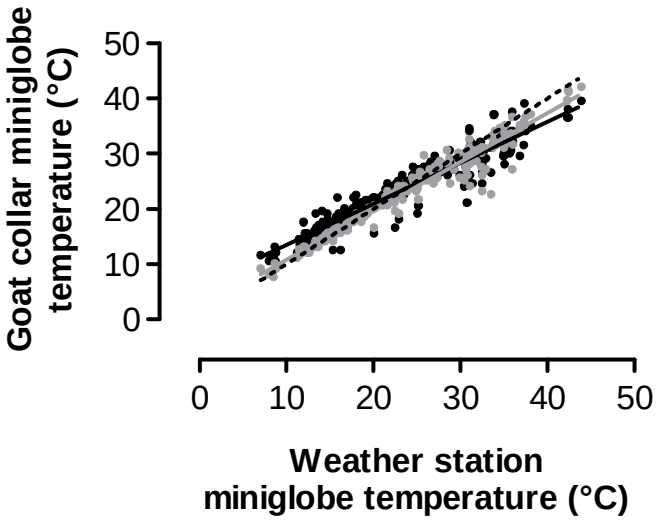

Figure 5.3. Scatter diagram showing the relationship between miniglobe temperatures recorded on a single Angora goat that inhabited the transformed site (grey dots and regression line) and another goat that inhabited the intact site (black dots and regression line) plotted against miniglobe temperatures recorded at a nearby weather station, during a 10 day period post-shearing in summer. Measurements were made hourly. The dashed line is the line of identity. Both goats exhibited microclimate selection, with the slope of both regression lines being less than one, the slope of the line of identity. The goat, and, by inference, the herd, that inhabited the intact site selected more stable microclimates as indicated by the lower slope of the regression line ($F_{1,414} = 37.7$, $P < 0.0001$), than did goats that inhabited the transformed site.

Over the post-shearing period, I assessed water influx and water turnover rates of goats that inhabited the two sites. Although there was no difference in body mass between the two groups (47.1 ± 3.6 kg versus 48.5 ± 6.5 kg, $t_{22} = 0.68$, $P = 0.50$), goats that inhabited the intact site had a significantly higher initial total body water than did goats that inhabited the transformed site (34.6 ± 4.9 L versus 29.9 ± 2.9 L, $t_{20} = 2.8$, $P = 0.01$). When total body water was expressed relative to body mass, goats that inhabited the intact site had a significantly higher relative total body water than did goats that inhabited the transformed site (77.2 ± 8.2% versus 66.8 ± 8.5%, $t_{20} = 2.9$, $P = 0.009$).On both sites, water influx ($F_{2,40} = 47.98$, $P < 0.0001$) and water turnover rates ($F_{3,51} = 203.1$, $P < 0.0001$) of goats decreased with increasing level of dehydration (Fig. 5.4). Goats in the transformed site had a higher water turnover rate (Fig. 5.4B, $F_{1,17} = 37.1$, $P < 0.0001$) despite their lower water influx (Fig. 5.4A, $F_{1,20} = 6.21$, $P = 0.02$). It therefore appears that goats in the transformed site were more dependent on

evaporative cooling to maintain their abdominal temperature in what would appear to be an environment more challenging thermally.

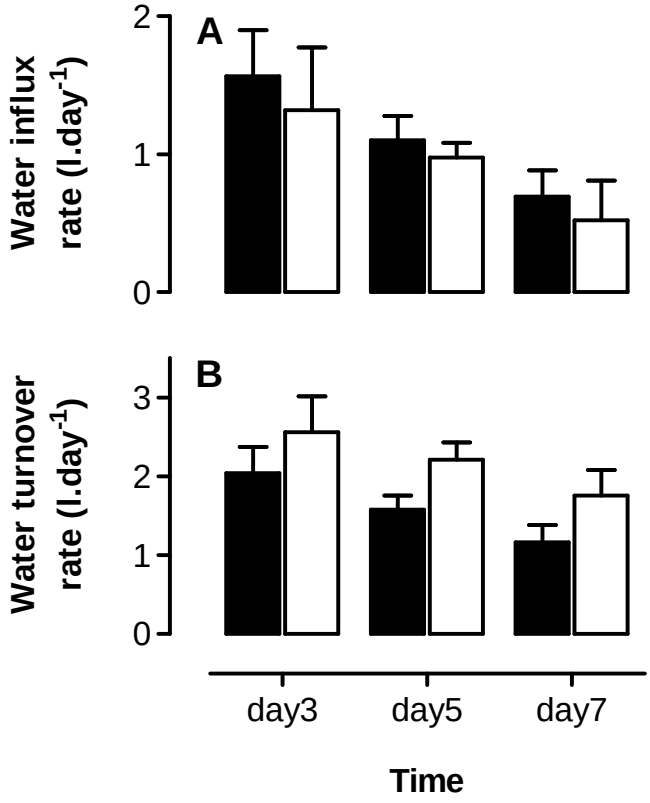

Figure 5.4. Water influx (upper panel) and water turnover rates (lower panel) of 12 goats that inhabited the transformed (white bars) and intact (black bars) site, after summer shearing, over a seven-day period during which the goats were denied access to water.

Since the goats were denied access to drinking water over the period of the water flux study, water influx could only come from pre-formed dietary water and metabolic water. I hypothesized that the difference in water influx may have arose from a difference in diet, with goats in the intact treatment obtaining a high water influx by feeding on the dominant tree-succulent in their habitat, *Portulacaria afra* (Milne, 2008). *Portulacaria afra* has a Crassulacean Acid Metabolism (CAM) photosynthetic pathway during summer and therefore has a carbon isotope ratio of approximately -17.4 (Mills *et al.*, 2005), whereas the C_4 grasses approximate -12 and C_3 trees approximate -25. Figure 5.5A shows the carbon ratio of the serum of goats that inhabited the two sites. Although the data from goats that inhabited the

intact site were consistent with them consuming a higher proportion of *P. afra* compared to goats that inhabited the transformed site before shearing, the carbon isotope ratios of goats from the two sites appeared to converge after shearing. Since dietary differences, based on carbon isotope ratios, therefore could not account for differences in water influx after shearing, I propose that goats that inhabited the intact site may have had a higher metabolic rate or metabolised a higher proportion of fats.

The total plasma lipid concentrations should provide an indication of the proportion of fats metabolised. Figure 5.5B shows the total plasma lipids at various times over the pre- and post-shearing period. Total plasma lipids changed over time ($F_{4,16} = 8.1$, $P < 0.0009$), decreasing with increasing level of dehydration. This effect was likely the result of a greater fat metabolism during dehydration, which would increase the metabolic water contribution. The effect was similar, however, between the goats from the two sites as, overall, total plasma lipids did not differ between the goats that inhabited the two land types ($F_{1,4} = 0.32$, $P = 0.60$), nor was there any statistical interaction in lipid concentrations between the goats that inhabited the two land types across time ($F_{4,16} = 2.4$, $P = 0.09$).

One of the blood variables that did turn out to be significantly different between the goats inhabiting the two sites was the albumin to globulin ratio (Fig. 5.5C), with goats inhabiting the intact site showing a lower albumin to globulin ratio than goats inhabiting the transformed site ($F_{1,11} = 6.0$, $P = 0.03$), a difference which was likely to be the result of a slightly higher globulin for goats inhabiting the intact site. Although, in general, there was no difference in serum glucose concentration between goats inhabiting the two sites, immediately after shearing the serum glucose concentrations spiked, and this spike was more pronounced for goats inhabiting the transformed site (Fig. 5.5D, $P = 0.04$).

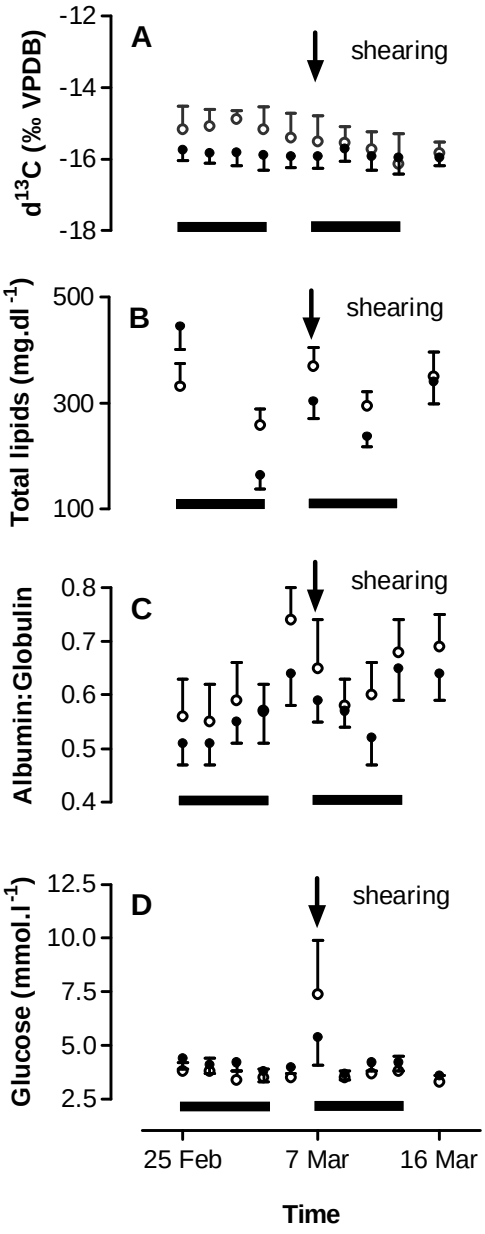

Figure 5.5. Angora goat serum carbon isotope ratios (A), total plasma lipid (B), serum albumin to globulin ratio (C) and serum glucose concentration (D) of goats that inhabited the transformed (open circles) and intact (closed circles) site. The black bars represent periods of water deprivation, and the black arrow represents time of summer shearing.

5.5 Discussion

I have shown that habitat does influence the thermoregulatory responses of artiodactyls, under conditions of thermal stress. Angora goats that inhabited my transformed site were more water dependent and appeared more susceptible to environmental thermal stresses than were those that inhabited my intact site. Post-shearing, goats that inhabited the transformed site had higher water turnover rates, even though they had a lower water influx and lower total body water, than did goats that inhabited the intact site. The goats that inhabited the transformed site had the potential advantage of greater glucose mobilization, immediately after shearing, and lower albumin to globulin ratio, than goats that inhabited the intact site. Both after summer shearing and on cold days, goats that inhabited the transformed had a higher amplitude of 24-h abdominal temperature rhythm and a faster maximum rate of abdominal temperature rise, than did the goats that inhabited the intact site. In addition, goats that inhabited the transformed site were more vasoconstricted and generally less active than were goats that inhabited the intact site, probably as a result of shade-seeking behaviour when environmental heat loads exceeded 17ºC. Although statistically significant, the differences I observed were small and, under most environmental conditions, the Angora goats responded similarly, irrespective of which site they inhabited.

The thermoregulatory differences between the goats that inhabited the intact and the transformed sites may well have been anomalously small as a result of the benign conditions which prevailed at the study site during my study. The rainfall during the study was unusually high, 30% higher than the long-term average, and the goats that inhibited the transformed treatment accessed better quality diet items as indexed by faecal lignin content (Milne, 2008). Such an hypothesis is supported by the fact that I found no differences in mass between the goats that inhabited the two sites, and the animals did not exhibit any differences in mohair production (Milne, 2008), so that any physiological differences that I did find were not the result of nutritional stress. Since forage availability varies by orders of magnitude between wet and dry periods (Stuart-Hill and Aucamp, 1993), the

thermoregulatory differences that I observed between goats in the two habitats are likely to be exacerbated with further desertification predicted with climate change.

5.5.1 Body temperature

The high maximum abdominal temperature of the goats that inhabited the intact site on hot days may be a result of the higher specific dynamic action associated with the high proportion of browse in their diet (Milne, 2008). Similarly, desert goats maintained on a lucerne hay diet have been shown to have higher rectal temperatures than goats maintained on a grass hay diet (Ahmed and El Kheir, 2004). However, since abdominal temperatures of the goats that inhabited the intact site were not consistently higher than those of the goats that inhabited the transformed site, it seems more likely that these differences were the result of differences in the thermal environment between the two sites.

Thicket vegetation may restrict wind and create a more humid environment (Henley, 2001), resulting in a thermally more-stressful environment by reducing an animals' access to convective and evaporative cooling on hot days. The higher maximum abdominal temperature of the goats that inhabited the intact site, compared to goats that inhabited the transformed site, on hot days, may reflect those effects. The soil of the transformed habitat reached higher maximum and lower minimum temperatures than did those of the intact thicket (Lechmere-Oertel, 2003), implying more thermal variability in the transformed environment. A variable proposed to be sensitive to environmental variability is blood glucose. Immediately after shearing my goats showed a spike in blood glucose concentrations, either as a result of stress or an increased metabolism (Wentzel *et al.*, 1979), and this increase was more pronounced for the goats that inhabited the transformed site (Fig. 5.5D). Supplemental feeding and the selection for high levels of mohair production are believed to have lead to adrenal insufficiency and an inability to mobilize glucose in Angora goats (Wentzel *et al.*, 1979; Cronjé, 1992). However, Angora goats which are exposed to environments characterized by seasonal fluctuations in nutrient supply, such as my goats that inhabited the transformed site, show a superior ability to mobilize glucose precursors, at the

expense of mohair production (Cronjé, 1995). Despite the higher glucose mobilisation of goats that inhabited the transformed site, compared to goats that inhabited the intact site, after summer shearing, such a response was transient and did not result in a difference in length or diameter of hair produced by the goats in the two treatments (Milne, 2008).

Goats that inhabited the transformed site displayed higher maximum, greater variability and faster rates of rise of abdominal temperature than did goats that inhabited the intact site on cold days. In other species, the amplitude of daily body temperature rhythm depends on both habitat features and environmental temperatures, with species from more stable temperate habitats displaying smaller amplitude than species from desert and tropical habitats, which are exposed to a much greater variability in environmental temperature (Lovegrove and Heldmaier, 1994; Refinetti, 1999a; Lowe *et al.*, 2001). Although the climatic variability and habitat differences in my study were less extreme, those influences may have played out in the significant differences in the 24-h amplitude of abdominal temperature rhythm that I found between the goats that inhabited the transformed site and those that inhabited the intact site.

Despite the habitat effects, the amplitude of abdominal temperature rhythm of my goats remained within the 2°C limits of variability traditionally associated with homeothermy (Bligh and Johnson, 1973). Goats typically regulate their core body temperature within narrow limits (Appleman and Delouche, 1958; Piccione *et al.*, 2007), but Angora goats in South Africa are more labile thermally than are other livestock species in the same environment (Hofmeyr *et al.*, 1965). Such breed differences may result from differences in the insulative properties of the hair coat (Bianca and Kunz, 1978), so that the removal of the insulation by shearing makes the animal more susceptible to changes in environmental conditions, and the presence of a full coat may further stress the animal on hot days. It was around summer shearing that thermoregulatory differences between the goats that inhabited the two land types were most apparent.

Shorn Angora goats in poor body condition are more sensitive to changes in the ambient thermal conditions than are goats in good body condition (Fourie, 1984). Similarly, shorn sheep in poor condition show a lower rectal temperature (Morris *et al.*, 1962; Parer, 1963; Hopkins *et al.*, 1978), a greater rate of change and a greater daily amplitude in rectal temperature (Parer, 1963) than do shorn sheep in good condition. These differences in body temperature are similar to those reported here for shorn goats that inhabited the transformed site compared to the intact site, despite there being no evidence for a loss of body condition of goats that inhabited the transformed site. Such differences in the abdominal temperature profile are therefore likely to be the result of differences in environmental conditions between the two sites.

5.5.2 Autonomic thermoregulation

While they were fleeced, my goats remained vasodilated over both day and night, as evidenced by small differences between abdominal and subcutaneous temperature (Fig. 5.1F). After summer shearing, however, the goats vasoconstricted at night, and the degree of vasoconstriction (or at least the difference between abdominal and subcutaneous temperature) was greater for goats that inhabited the transformed site than in the goats that inhabited the intact site. Since high stocking densities decrease the availability of pasture and alter pasture composition and structure (McGregor, 1985; McGregor, 1998), and since transformed thicket has a decreased carrying capacity (Stuart-Hill and Aucamp, 1993), one would expect stocking densities to affect vasoconstriction responses in a way similar to site transformation. However, Morris *et al.* (1962) found winter skin temperature were up to 1.4°C higher in shorn sheep stocked at the high densities than in shorn sheep stocked at low densities. These low subcutaneous temperatures resulted in a difference between rectal and skin temperature of nearly 6°C for sheep stocked at low densities, compared to only 3°C for those stocked at high densities (Morris *et al.*, 1962), with the latter value similar to the difference for my shorn goats on summer nights. Similarly, Sykes and Slee (1969) found skin temperatures of sheep maintained on a low plane of nutrition to be an average 1.8°C higher than sheep maintained on a high plane of nutrition, during

cold exposure. These authors attributed the failure of heat conservation in poorly nourished sheep to a loss of insulating fat, thinning of the skin, and reduced vasomotor tone of animals on a low plane of nutrition (Morris *et al.*, 1962; Sykes and Slee, 1969). Since even my goats that inhabited the transformed site were not stressed nutritionally (Milne, 2008), and goats generally have less subcutaneous fat than do sheep (Owen *et al.*, 1977), the greater vasoconstriction of my goats that inhabited the transformed site was likely the result of heat loss to the environment potentially being greater at the transformed site, particularly at night. The reduced vegetation cover of the transformed site could have increased both the goat's radiative heat loss to the night sky and its convective cooling. Indeed, transformed sites, in general, have greater air movement than do intact thicket vegetation (Henley, 2001).

5.5.3 Activity

In addition to the environmental and autonomic influences, activity has been proposed to influence body temperature rhythm (Lowe *et al.*, 2001). However, unlike previous laboratory studies, which found synchronous rhythms in body temperature and activity (Refinetti and Menaker, 1992; Refinetti, 1999b), my index goats displayed a dissociated rhythm and were active throughout most of the day, with peaks of activity in the early morning and late afternoon. Similar patterns of activity have been shown for Angora goats on west Texas range (Askins and Turner, 1972) and may support the conclusion that climatic factors are important determinants affecting the foraging behaviour of goats (Askins and Turner, 1972), as they are with sheep (Dudzinski and Arnold, 1979; Arnold, 1982).

Since time spent foraging can be used as an index of pasture quality (Arnold and Dudzinski, 1978), I predicted that goats on the transformed site would have to walk further for an adequate supply of food and water, as found for grazing ruminants in arid areas (Young and Corbett, 1972; Allison, 1985; Manteca and Smith, 1994; Lachica and Aguilera, 2003), thus conforming to the "time minimizing" model predicted for bison (Bergman *et al.*, 2001). On the contrary, I

found that the index goat, and, by inference, the herd, that inhabited the intact site was generally more active than the counterpart that inhabited the transformed site. Stocking rates influence animal performance (Huston *et al.*, 1993a), production (Davies and Southey, 2001), and grazing time and number of steps taken have been shown to increase at high stocking densities of both sheep and goats (Animut *et al.*, 2005b). However, rather than such differences being the result of forage availability, these differences may be the effect of social feeding, with goats in larger groups displaying higher intake rates with an increased number of competitors (Shrader *et al.*, 2007; Van *et al.*, 2007). My study had the advantage of a transformed environment without altering the social structure of goats. The higher activity level of my index goat that inhabited the intact site may be explained by foraging theory, which predicts that animals should feed more selectively when high quality foods are more abundant (Stephens and Krebs, 1986). Sheep increase their forage intake at low levels of energy supplementation (Caton and Dhuyvetter, 1997) and high levels of protein supplementation (Huston *et al.*, 1993a; Huston *et al.*, 1993b). Angora goats fed more selectively on natural forage, thus increasing their foraging effort and movement rates by nearly 70%, when given a high-quality supplement (Murden and Risenhoover, 1993).

Conversely, Herselman *et al.* (1999) found that Angora goats grazed on native pasture for longer and took more steps per unit of time spent grazing than did goats grazed on improved pasture. Such differences in activity may be confounded by vegetation type. The “native pasture” provided a diet with a larger proportion of browse than the diet of animals on “improved pasture”. Since goats show a preference for browse (Huston, 1994; Rodriguez Iglesias and Kothmann, 1998; Ngwa *et al.*, 2000; Animut *et al.*, 2005a; Sanon *et al.*, 2007), the higher proportion of grass consumed by both the goats on the “improved pasture” and my goats that inhabited the transformed site and may have resulted in goats foraging less selectively. Indeed, my goats that inhabited the transformed site consumed grass in relation to its abundance (Milne, 2008), potentially resulting in a lower foraging distance.

Since energy expenditure of locomotion and grazing are a significant contributor to the energy requirements of goats in free-living conditions (Lachica and Aguilera, 2005a; Lachica and Aguilera, 2005b), one may have expected that the higher activity level of my goats that inhabited the intact site would have resulted in a higher energy expenditure and heat production (Graham, 1962; Young and Corbett, 1972; Osuji, 1974), compared to the goats that inhabited the transformed site. However, other studies have shown that heart rate, an index of energy expenditure (Puchala *et al.*, 2007), increased linearly with increased stocking rates (Animut *et al*., 2006). Although I did not measure heat production or metabolic rate directly, since my goats were denied access to water during my assessment of water influx, the higher water influx of my goats that inhabited the intact site could have been the result of a higher metabolic rate.

5.5.4 Water influx

The water influx of my goats that inhabited the transformed site was lower that that of the goats that inhabited the intact site. Morris *et al.* (1962) found that the water intake of sheep grazing at a high densities were nearly half that of sheep grazed at low densities, a difference which they attributed to the low-density sheep eating more food with a higher water content. Similarly, desert goats both acquire more water when maintained on alfalfa hay than on wheat straw (Brosh *et al.*, 1986) and gain more water from preformed and metabolic water when maintained on a lucerne hay diet than on a grass hay diet (Ahmed and El Kheir, 2004). My goats that inhabited the intact site had access to browse of high moisture content, such as *P. afra*, whereas the goats that inhabited the transformed site had a lower availability of browse and succulents (Milne, 2008), potentially making the goats that inhabited the transformed site more dependent on free water, as found for other grazing ruminants (Maloiy, 1973b; Kay, 1997). The goats that inhabited the transformed site were therefore worse-off than goats that inhabited the intact site when water was taken away. Although the ^{13}C ratio of my goats that inhabited the two sites appeared to converge over the post-shearing period (Fig. 5.5A,), such a change in diet was not the result of an increase in the proportion of succulents consumed, but rather an increase in the proportion of trees consumed

by the goats that inhabited the transformed site (Milne, 2008). Since the water content of succulents is higher than that of browse, the higher portion of succulents consumed by goats that inhabited the intact site compared to the transformed site post-shearing, 41% versus 7% (Milne, 2008), may have contributed to their higher water influx.

However, differences in diet are not the only variable which influence ^{13}C enrichment. A shift in the relative rates of oxidation of metabolic substances for thermogenesis may change post-shearing (McGraham *et al.*, 1958; Symonds *et al.*, 1986), which has been associated with a change in the background enrichment of ^{13}C (Lachica and Aguilera, 2003). Although the total plasma lipid concentration of my goats changed over time (Fig 5.5B), these changes were not consistent with changes in the background enrichment of ^{13}C. Although the total lipid concentration of the goats that inhabited the transformed site, at times, appeared slightly higher than the goats that inhabited the intact site, in general, these differences were not significant. The water contribution from fat metabolism was more than three times higher in sheep grazed at high stocking densities than at low stocking densities (Morris *et al.*, 1962), which has been attributed to the high fat metabolism associated with mild starvation. Since there is no evidence that my goats were under nutritional stress on either site (Milne, 2008), differences in total plasma lipid concentrations before shearing were likely the result of difference in the amount and type of fat in the diet (Beynen *et al.*, 2000). Since changes in fat metabolism could not account for differences in the ^{13}C enrichment, I propose that the higher water influx of goats that inhabited the intact site were the result of both the higher portion of succulents in their diet and potentially a higher metabolic rate, as indexed by the high level of activity, than goats that inhabited the transformed site.

5.5.5 Water turnover rate

Although my goats that inhabited the intact site had higher water influx, they had lower water turnover rates than did the goats that inhabited the transformed site. Morris *et al*. (1962) estimated summer water turnover rates for Merino sheep

stocked at low densities to be 26% greater than those of sheep stocked at high densities. The authors suggested that differences in the composition of the two pastures may have accounted for the difference in water turnover rates, but did not mention what these differences may have been. I believe that the higher water turnover rates of my goats that inhabited the transformed site were the result of differences in the microclimates selected by my goats. Goats which inhabited the transformed site were exposed to higher environmental heat loads than were the goats which inhabited the intact site, particularly during the heat of the day (Fig. 5.3), and would have had less access to microclimate niches. Habitat transformation of the succulent thicket is proposed to have homogenised the land surface, resulting in fewer microhabitats and greater environmental fluctuations (Lechmere-Oertel, 2003). Since maintaining homeothermy during heat stress demands water (Beede and Collier, 1986), and water turnover rates increase with increasing ambient temperature (Maloiy, 1973a), I propose that my goats that inhabited the transformed site were more reliant on evaporative cooling to dissipate their heat load, resulting in water turnover rates higher than those of the goats that inhabited the intact site.

5.5.6 Parasite load

Although the transformed site appeared to be a more stressful environment than the intact site, both in terms of thermal microclimates and nutrient availability, one potential advantage of the more open habitat is a reduced parasite load (Mooring *et al.*, 2004). My goats that inhabited the transformed site had higher albumin globulin ratio (Fig. 5.5C), resulting from lower globulin concentrations, than goats that inhabited the intact site. Although, a low albumin globulin ratio can result from a protein deficiency (Anderson *et al.*, 1962), such a deduction is unlikely since there was no difference in total serum protein concentration between the goats that inhabited the two sites (unpublished data). A low albumin to globulin ratio is also associated with inflammation and bacterial invasion (Hurwitz and Whipple, 1917), decreased immune function in heifers (Piccinini *et al.*, 2004) and low social status in pigs (Hicks *et al.*, 1998). I propose that the

goats that inhabited the transformed site were less immunologically stressed than the goats that inhabited the intact site.

5.5.7 Conclusion

Despite the potential advantage of a high albumin to globulin ratio and greater mobilisation of glucose, goats that inhabited the transformed site were more stressed thermally and more water dependent, than were goats that inhabited the intact site. I concede that the differences the differences that I observed were small. The year of my study had above-average rainfall year and even goats on the transformed site maintained good body condition. I expect that, in years of average, and particularly low, rainfall, the same trends will prevail but will have a much greater magnitude. Nevertheless, my colleagues and I are the first to demonstrate physiological changes in response to desertification, despite the relatively benign conditions of high rainfall prevalent during my study. Given that climate change is already exacerbating drought extremes (Chamaillé-Jammes *et al.*, 2007) and that current models predict a further 20-30% transformation of the succulent thicket within the next 20 years (Robertson and Palmer, 2002; Rouget *et al.*, 2003), understanding the effects of these extremes will become increasingly important. The long term viability and sustainability of agricultural herbivores will depend on maintaining appropriate stocking rates under changing climatic conditions (Richardson *et al.*, 2005). The use of sophisticated physiological measurements and biologging devices, as I have demonstrated in my goats, will allow a better understanding of phenotypic plasticity of herbivores to both the direct thermal effects of climate change and the consequences of changes in habitat.

5.6 Acknowledgements:

I gratefully acknowledge Arthur and Trinette Rudman for their hospitality and for allowing the study to take place on their farm Blaauwkrantz, and Stephan Woodborne at the CSIR for use of the high temperature elemental analyser for the determination of deuterium concentrations, Ms Elsbe Myburgh at the Department of Companion Animal Clinical Studies Section of Clinical Pathology, University of Pretoria, for serum total protein, glucose and osmolality analysis and Prof Bruce Davidson for taking time to teaching me the lipid extraction technique.

CHAPTER 6

Adapting to the spread of pathogens: fever and sickness behaviour during an opportunistic infection in a free-living antelope, the greater kudu (*Tragelaphus strepsiceros*)

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6.1 Abstract

To study their thermal responses to climatic stress, my colleagues and I implanted seven greater kudu (*Tragelaphus strepsiceros*) with intra-abdominal, brain, carotid and subcutaneous temperature data loggers as well as an activity logger. Each animal also was equipped with a collar holding a miniature black globe thermometer, which I used to assess thermoregulatory behaviour. The kudu ranged freely within succulent thicket vegetation of the Eastern Cape Province, South Africa. The kudu spontaneously developed a bacterial pneumonia, and consequent fever that lasted between six and ten days. The fever was characterized by a significant increase in mean 24-h abdominal temperature from 38.9 ± 0.2°C to 40.2 ± 0.4°C (mean ± SD, t_6 = 11.01, $P < 0.0001$), although the amplitude of body temperature rhythm remained unchanged (t_6 = 1.18, P = 0.28). Six of the kudu chose warmer microclimates during the fever than when afebrile ($P < 0.0001$). Despite the selection of a warmer environment, on the first day of fever, the abdominal-subcutaneous temperature difference was significantly higher than on afebrile days (t_5 = 3.06, P = 0.03), indicating vasoconstriction. Some kudu displayed increased frequency of selective brain cooling during the fever, which would have inhibited evaporative heat loss and increased febrile body temperatures, without increasing the metabolic maintenance costs of high body temperatures. Average daily activity during the fever decreased to 60% of afebrile activity (t_6 = 3.46, P = 0.014). I therefore have recorded quantitative evidence for autonomic and behavioural fever, as well as sickness behaviour, in the form of decreased activity, in a free-living ungulate species, and demonstrate the kinds of changes in physiological function that sick animals will display as they face the other consequences of climate change.

6.2 Introduction

Since climate change is predicted to bring emergent pathogens, particularly with the relocation of arthropod vectors (Rogers and Randolph, 2000; Olwoch *et al.*, 2003; Cumming and van Vuuren, 2006), we need to understand the mechanisms employed by free-ranging antelope in the face of infection. Animals employ a suite of autonomic and behavioural mechanisms during an infection. These mechanisms are regulated by pro-inflammatory cytokines which act centrally to inhibit the firing rate of warm-sensitive neurons in the hypothalamus, resulting in a rise in the thermoregulatory set point (Banet, 1979; Kluger, 1991; Aubert, 1999). In addition to an increased body temperature during infection, animals consistently exhibit symptoms such as lethargy, reduced engagement in social activities and reduced food intake (Aubert, 1999; Dantzer, 2004), collectively referred to as sickness behaviour. Sickness behaviour originally was thought to be the result of a weakened physiological state, but now is viewed as an adaptive reorganization of the host's priorities to facilitate recovery from an infection (Ewald, 1980; Hart, 1988; Johnson, 2002; Konsman *et al.*, 2002). Indeed, given their ubiquity among both endothermic and ectothermic vertebrates, both fever and sickness behaviour are postulated to offer a survival benefit to the host (Kluger, 1978; Kluger, 1979; Kluger, 1986; Wingfield, 2003).

Although both fever and sickness behaviour have been studied extensively in laboratory-housed animals given purified pyrogens experimentally, little is known about how free-living animals respond to opportunistic infection. Unlike naturally-occurring fevers, which last several days at least, most experimentally-induced fevers last less than 24 h and are not superimposed on the nychthemeral rhythm of body temperature (Kluger, 1991; Mphahlele *et al.*, 2004). However, in free-living impala, prolonged natural fevers did not disrupt the nychthemeral rhythm of body temperature (Kamerman *et al.*, 2001). But neither these antelope nor laboratory-housed goats with chronic fevers displayed any obvious signs of sickness behaviour

(Kamerman *et al.*, 2001; Mphahlele *et al.*, 2004). It is therefore unknown whether sickness behaviour is a component of chronic fever in free-living animals.

Laboratory-housed animals, although they may be physically active, often do not have access to the full range of natural behaviours, thermoregulatory or social. Changes in behaviour in laboratory-housed animals during fever, therefore, may not be similar to behavioural responses of a free-living animal, since wild animals would have to sacrifice parts of their usual behavioural repertoire if they employ sickness behaviour. For example, both free-living, male, north-western song sparrows and white-crowned sparrows decreased their territorial aggressive behaviour and song when exposed to an exogenous pathogen (Owen-Ashley and Wingfield, 2006; Owen-Ashley *et al.*, 2006). Such behavioural changes may be costly for wild animals, particularly if they result in an increased susceptibility to predators, decreased reproduction and parental care, loss of social position or removal from territories (Hart, 1988; Yirmiya *et al.*, 1995; Aubert *et al.*, 1997; Konsman *et al.*, 2002; Owen-Ashley and Wingfield, 2006). Sickness behaviour and immune responses to infection also are metabolically costly (Hart, 1988; Kluger *et al.*, 1998; Lochmiller and Deerenberg, 2000). Such metabolic costs may be maladaptive, particularly during times of low energy availability. Weight loss following exogenous pyrogen administration in captive male white-crowned sparrows was greater when they had high energy reserves than when they had lower reserves (Owen-Ashley *et al.*, 2006). Free-living animals therefore have to trade off any potential benefit of sickness behaviour, in supporting metabolic and physiological changes associated with infection, against associated energetic and social costs.

It is difficult to study fever and sickness behaviour in wild animals as sickness occurs opportunistically and observations of behaviour usually require human presence, which in turn may disrupt normal behaviour. Serendipitously for us, a herd of free-living antelope (kudu, *Tragelaphus strepsiceros*), which my colleagues and I had instrumented with temperature and activity data loggers for other purposes, acquired

a spontaneous infection, which lasted between six and ten days, two to five weeks after implantation of the loggers. I obtained remote and continuous measurements of temperatures at various body sites, including the brain, carotid artery, abdomen and beneath the skin, both before and during the infection. I was able later to extract quantitative data reflecting the kudu's autonomic and behavioural responses to the infection. I show that the free-living kudu exhibited sickness behaviour and implemented a suite of coordinated thermoregulatory responses that elevated body temperature during a spontaneous fever.

6.3 Materials and methods

6.3.1 Animals and habitat

The experiment took place between December 2004 and March 2005 (austral summer) at Blaauwkrantz farm (33º32'S 25º23'E), near Port Elizabeth, South Africa. The vegetation in the area has been classified as Sundays Spekboomveld (Vlok *et al.*, 2003), and the habitat is both historical and current habitat for greater kudu (*Tragelaphus strepsiceros*, Skinner and Chimimba, 2005).

Seven adult female free-living kudu (body mass 121 ± 8 kg) were captured from a large camp on Blaauwkrantz farm in November 2004. The kudu were immobilized from a helicopter, by a professional capture team under veterinary supervision, and given a long acting tranquilizer, zuclopenthixol acetate (100 mg I.M. Clopixol Acuphase, H. Lundbeck (Pty) Ltd., Randburg, South Africa), before being transported to nearby holding pens at River Bend Lodge (~ 20-min drive). The kudu were housed in these pens for a two-week habituation period to reduce potential peri-operative stress.

All experimental procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (protocol no. 2004/84/4).

6.3.2 Surgery

For surgery to implant the data loggers, each animal was immobilized with etorphine hydrochloride (6 mg intramuscularly (I.M.), M99, Novartis, Johannesburg, South Africa), together with azaperone (100 mg I.M., Stresnil, Kyron Laboratories, Johannesburg, South Africa) and transported to a veterinary surgery within 200 m of the pens. At the surgery, the animals were placed in sternal recumbency, supported by sandbags, with their heads elevated. Anaesthesia was maintained with 1 - 3% halothane (Fluothane, Zeneca, Johannesburg, South Africa), administered in oxygen via a face mask. Once an adequate plane of anaesthesia was established, the effect of the opioid, etorphine, was reversed using diprenorphine hydrochloride (15 mg intravenously (I.V.), M5050, Novartis, Johannesburg, South Africa). Respiratory rate, heart rate, arterial oxygen saturation (pulse oximeters, Nonin 9847V, Nonin Medical, North Plymouth, USA) and rectal temperature (thermocouple thermometer, BAT-12, Physitemp, Clifton, USA) were monitored throughout the surgery lasting ~ 2 h.

Under sterile surgical conditions, a suite of miniature data loggers was implanted. All data loggers were covered in an inert wax (Sasol, Johannesburg, South Africa) and dry-sterilized in formaldehyde vapour before implantation. Incision sites were shaved and sterilized with chlorhexidine gluconate (Hibitane, Zeneca, Johannesburg, South Africa). Each animal was fitted with data loggers connected to thermistor sensors for temperature measurement in the carotid artery and brain. A thermistor, inserted in a blind-ended and thin-walled polytetrafluoroethylene (PTFE) tube (o.d 1.35 mm, i.d. 0.97 mm; Straight Aortic Flush 4F Catheter, Cordis, The Netherlands), was advanced 80 mm into the left common carotid artery towards the heart, at a position midway along the length of the neck, and secured in position with a purse-string suture in the artery wall. Outside the artery, the PTFE tube was sealed on a PTFE-coated co-axial cable (150 mm long, o.d. 3 mm, Belden, Richmond, USA) connecting the thermistor to the temperature-sensitive data logger (see below). The data logger was positioned subcutaneously, dorsal to the artery. A second data logger, connected to the thermistor positioned in the brain, was positioned subcutaneously caudal to the base

of the left ear. Its PTFE-coated cable was advanced subcutaneously over the skull, where it was connected to a head plate and guide tube. The guide tube, constructed from cellulose acetate butyrate tubing (o.d. 3.2mm, i.d. 1.6 mm; World Precision Instruments, Sarasota, Fla., USA) sealed at the tip by a steel cap, was 58 mm long and was inserted through a small 2 mm diameter hole, which was drilled through the cranium, at appropriate co-ordinates, pre-determined from head sections of dead kudu of similar size, so that the probe tip would be positioned near the hypothalamus. The brain guide tube was connected to a subcutaneously implanted plastic head plate ($20 \times 10 \times 5$ mm) that was secured to the skull by two bone screws.

A third data logger, with an internal temperature sensor, was inserted, via an incision in the paralumbar fossa, into the abdominal cavity. The muscle layer was sutured closed and a smaller temperature-sensitive data logger (see below) was inserted subcutaneously before the skin was sutured closed. An additional incision was made on the upper hind limb, where an activity logger (Actical, Mini-Mitter Corporation, Bend, OR, USA) was implanted subcutaneously. The activity logger recorded at 10-min intervals, had dimensions of 40 x 40 x 15 mm, and weighed ~ 40 g.

Wounds were treated with a topical antiseptic spray (Necrospray, Centaur Labs, Johannesburg, South Africa) and coated with a tick repellent grease (cypermethrin 0.025% m/m, Bayer Animal Health Pty, Isando, South Africa). Each of the kudu received a long-acting antibiotic (22 ml I.M., penicillin, Peni La Phenix, Virbac Animal Health, South Africa), a non-steroidal anti-inflammatory analgesic (5 ml I.M., phenylbutazone, Phenylarthrite injectable solution, Bayer (Pty) Ltd. Animal Health, Isando, South Africa), a long-acting parasiticide (2.5 ml subcutaneously (S.C.), doramectin, Dectomax®, Pfizer Laboratories, Sandton, South Africa), Vitamin E and Selenium (5 ml I.M., vitESe injectable solution, Kyron Laboratories, South Africa), and perphenazine (100 mg I.M., Kyron Laboratories, South Africa).

Before halothane administration was terminated, a neck collar (African Wildlife Tracking, Pretoria, South Africa) was fitted to each kudu. In addition to a tracking radio transmitter, each collar supported a miniature black globe thermometer ("miniglobe"), to allow for the dynamic measurement of the microclimate that the kudu chose to occupy. This technique previously has proven successful on other ungulate species (Appendix 1, Hetem *et al.*, 2007). Miniglobe temperature was measured with a thermistor inserted into the centre of a matt-black hollow bronze sphere (30 mm diameter, Press Spinning & Stamping Co., Cape Town, South Africa). The thermistor leads were housed within a spring that was soldered to the miniglobe and filled with flowable silicon and covered in heat-shrink tubing to add flexibility and strength. The base of the spring was attached to the collar so that the miniglobe stood 20 mm above the collar. The miniglobe temperatures were recorded by a temperature-sensitive data logger (see below), which was attached to the collar and waterproofed with dental acrylic. A weight on the ventral side of the collar ensured that the miniglobe remained over the dorsum of the neck and could not be shaded by the animal's body.

Following surgery, the kudu were transported back to their pens, where they became ambulatory within ~ 10 min. After a three-day recovery period, and following veterinary inspection, they were transported back to Blaauwkrantz farm where they were released into a 50 ha fenced enclosure with natural forage and water available *ad libitum*. Two to five weeks post-release, the kudu spontaneously developed a lethal pneumonia, and a consequent fever that lasted between six and ten days before the kudu died. Unfortunately, my experimental design, which required that the animals be disturbed by humans as little as possible, and the data logging technology did not allow us to determine that the kudu were febrile until after they had died. Where possible I conducted gross-macroscopic and histopathological post-mortem examinations. These revealed a severe necrotizing bronchopneumonia and emphysema, with extensive neutrophil infiltrates and bacterial colonies. While it is known that wild animals do not inhabit sterile environments (Gannon *et al.*, 2007),

the deaths were unexpected; my colleagues have performed similar procedures on a variety of mammalian species without ill effects. However, since there are no reports of recovery from surgery in wild kudu, I do not know whether the species is particularly susceptible to delayed post-operative infection, or whether they were the victims of an opportunistic infection acquired at the study site.

6.3.3 Temperature measurements

The miniature thermometric data loggers (StowAway XTI, Onset Computer, Pocasset, Massachusetts, USA), used to measure brain, carotid artery and abdominal temperature, had outside dimensions of ~ 50 × 45 × 20 mm and a mass of ~ 40 g when covered in wax. These loggers had a resolution of 0.04°C and measurement range from + 34 to + 46°C. Temperature sensors used to measure brain and carotid blood temperatures were constructed from ruggedized glass-coated bead thermistors with insulated extension leads (bead diameter 0.3 mm; AB0E3-BR11KA103N, Thermometrics, Edison, New Jersey, USA). The scan interval of the brain and carotid blood loggers was set at 5 min and that of the abdominal logger was set at 20 min. Subcutaneous temperatures were recorded every hour using a smaller thermometric data logger (iButton DS1922T, Maxim, Dallas Semiconductor, Texas, USA), which had a radius of ~ 25 mm, a height of 15 mm, and weighed about 10 g when covered with wax. These loggers had a resolution of 0.5°C and a measurement range from 0 to 125°C.

The temperature of each miniglobe was measured with an uncoated bead thermistor (bead diameter 1.5 mm; 27-10K4A801 Onset Computer Corporation, Pocasset, Mass., USA). The thermistor was connected to a miniature thermometric data logger (Hobo U12-013, Onset Computer Corporation, Pocasset, Mass., USA), with a temperature range of - 20 to + 70°C and an intrinsic resolution of 0.35°C. Miniglobe temperatures were recorded instantaneously every 15 min.

All temperature sensors and loggers were calibrated against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany) in an insulated water bath. After calibration, the loggers and their sensors measured blood, brain and abdominal temperature to an accuracy of better than 0.05°C, subcutaneous temperature to better than 0.5°C and miniglobe temperature to better than 0.4°C.

6.3.4 Meteorological data measurements

I collected climatic data from a portable weather station in the enclosure into which the kudu were released (Hobo Weather Station, Onset Computer Corporation, Pocasset, Mass., USA). I monitored wind speed ($m.s^{-1}$), solar radiation ($W.m^{-2}$), dry-bulb temperature (°C), relative humidity (%) and standard (150 mm diameter) black globe temperature (°C) for the duration of the study period.

6.3.5 Data analysis

I considered the kudu to be febrile if their mean daily body temperature was at least 0.5ºC above normal. Paired Student's t-tests were used to confirm differences in body temperature between febrile and afebrile states. I obtained a complete set of brain temperature and carotid blood temperature data for four febrile kudu for analysis of selective brain cooling. The relationship between brain temperature and carotid blood temperature in each animal was analyzed by sorting all 5-min measurements of carotid blood temperature into 0.1°C classes, and determining the mean, standard deviation, maximum and minimum brain temperature at each class of carotid blood temperature.

After converting miniglobe temperature to their equivalent standard black globe temperatures (Appendix 1, Hetem *et al.*, 2007), I correlated these converted animal miniglobe temperatures against weather station standard black globe temperatures using linear Pearson procedures. To assess whether the animals were conforming to ambient conditions or were selecting micro-environments, I tested whether the slope

of the regression equation was significantly different from one (the slope of the line of identity). In addition, I tested whether the slope and elevation of the regression equation was significantly different between febrile and afebrile states using an analysis of co-variance (ANCOVA). To compare changes in activity levels during the fever, I calculated the febrile activity levels as a proportion of afebrile activity levels and compared these to a value of one using a Paired Student's t-test. I used GraphPad Prism (version 4.00 for Windows, GraphPad Software, San Diego California USA) for statistical analyses. Values are expressed as mean ± SD.

6.4 Results

6.4.1 Climate

Over the six-week study period during which the kudu were both febrile and afebrile, average dry-bulb temperature, standard black globe temperature, wind speed and solar radiation varied as a function of time of day (Fig. 6.1). Average dry-bulb temperature over 24 h was 22.5 ± 2.4ºC with a mean daily minimum of 16.8 ± 2.2ºC and mean daily maximum of 30.4 ± 5.4ºC. Standard black globe temperature was 25.0 ± 3.1ºC on average, reaching a mean daily minimum of 16.7 ± 2.3ºC and a mean daily maximum of 36.5 ± 6.0ºC (Fig. 6.1A). Solar radiation showed a bell-shaped distribution, with a mean peak of ~ 750 $W.m^{-2}$ around solar noon, with variability resulting from a combination of cloudy and clear days. Wind speed increased in the late afternoon, reaching a peak of about 4 $m.s^{-1}$ around 16:00 (Fig. 6.1B). Rainfall averaged 24 mm per month in 2004, which was slightly lower than the 50 year rainfall average of 26 mm per month. However, prevailing weather conditions were not unusual during the study period.

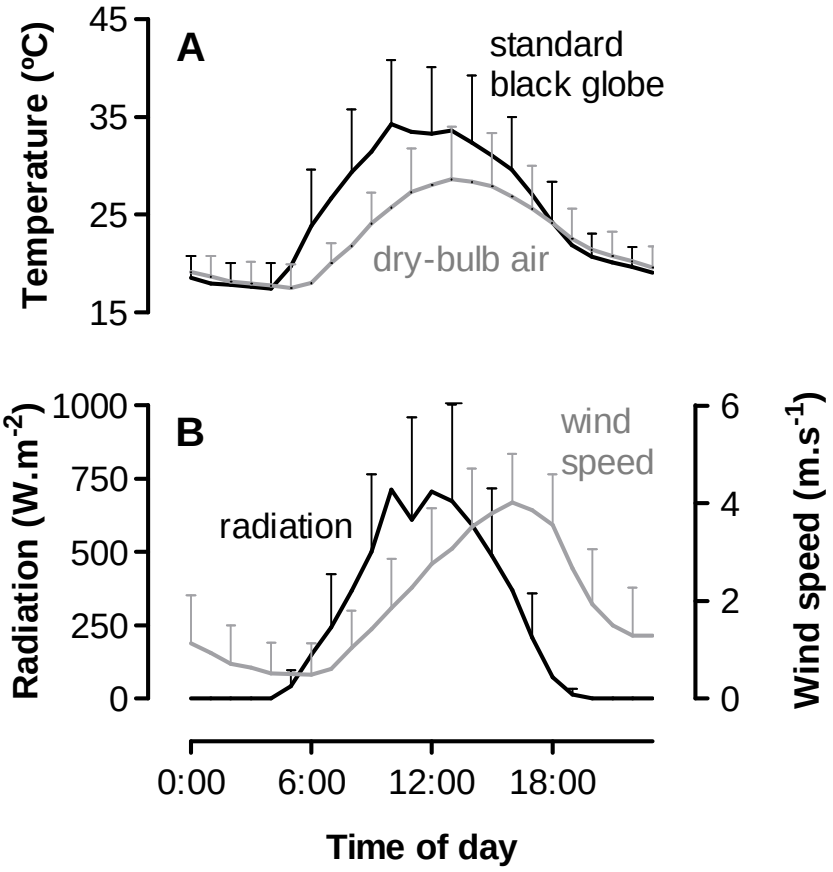

Figure 6.1. Field site weather station data, showing the standard black globe (black line) and dry-bulb air (grey line) temperatures (A), as well as radiation (black line) and wind speed (grey line) (B), as a function of time of day, as mean ± SD for the six-week study period.

6.4.2 Fever

All seven kudu developed a spontaneous fever two to five weeks after surgery. These fevers lasted between six and ten days and were characterized by an upward displacement of the nychthemeral rhythm of body temperature (Fig. 6.2). The mean 24-h abdominal temperature, for the seven animals, increased significantly from 38.9 ± 0.2°C when the kudu were afebrile to 40.2 ± 0.4°C (t_6 = 11.01, P < 0.0001) when they were febrile (Fig. 6.3). Minimum daily abdominal temperatures increased from 38.0 ± 0.4°C to 39.2 ± 0.5°C (t_6 = 5.97, P = 0.001) and maximum daily abdominal temperatures from 39.7 ± 0.3°C to 41.2 ± 0.4°C (t_6 = 19.02, P < 0.0001) during the fever. However, the amplitude of nychthemeral rhythm remained unchanged at 1.7 ± 0.5°C when the animals were afebrile and 1.9 ± 0.6°C during fever (t_6 = 1.18, P = 0.28).

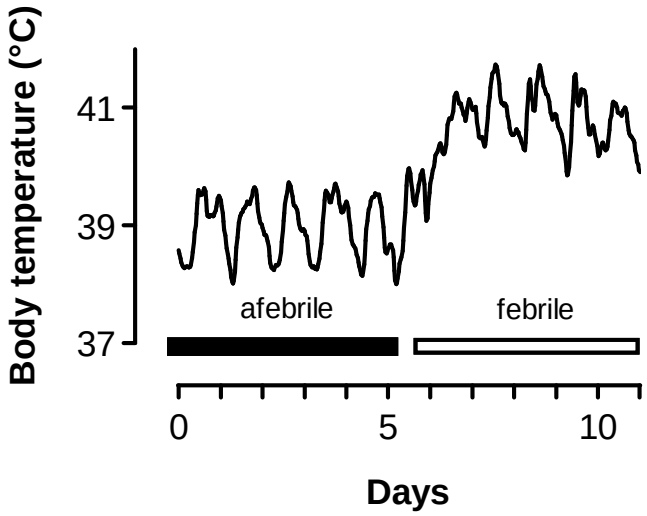

Figure 6.2. Abdominal temperature for a single free-living female kudu (kudu 6) over 11 days, including 5 days of fever. The bars at the base of the figure represent the periods I considered the kudu to be “afebrile” and “febrile”. Tick marks on the time axis represent midnight.

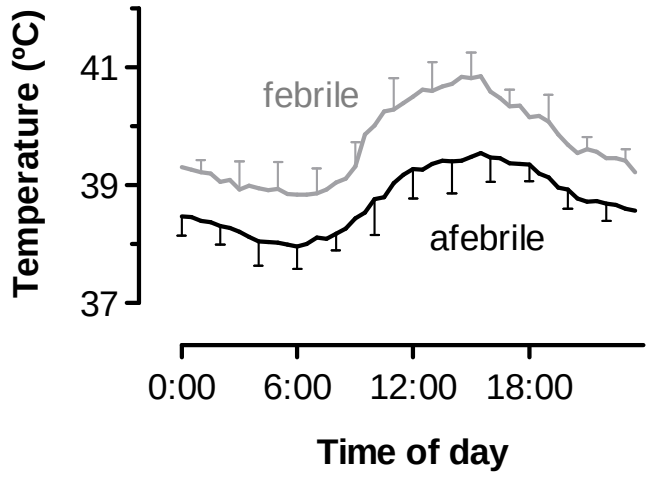

Figure 6.3. Mean ± SD of the nychthemeral rhythm of abdominal temperature for seven female free-living kudu during febrile (grey line) and afebrile (black line) states.

During febrile days, the upward shift in the nychthemeral rhythm of body temperature appeared to be associated with a change in the thermal environment that the kudu chose to occupy. Figure 6.4 shows the correlation between the standard black globe temperatures at the sites chosen by a single animal (kudu 5), converted from miniglobe temperatures recorded on the collar, and standard black globe temperatures recorded at a nearby weather station, during both febrile and afebrile states in kudu 5. The slopes for all the animals were significantly less than one (ANCOVA, $P < 0.0001$), and the regression lines intersected the line of identity, implying that kudu selected microclimates cooler than the prevailing environmental conditions at higher environmental heat loads and microclimates warmer than the prevailing environment conditions at lower environmental heat loads. Six of the seven kudu showed no difference in the slope of the regression lines between febrile and afebrile states (ANCOVA, $P > 0.17$). The remaining animal (kudu 3) showed a significantly increased slope ($F_{1,812} = 4.8$, $P = 0.03$) under the febrile state (0.41 ± 0.04) compared to the afebrile state (0.32 ± 0.01), implying that this kudu selected warmer microclimates when febrile compared to afebrile, particularly at high environmental heat loads. One animal (kudu 7) showed no change in the slope or elevation of the regression line between the febrile and afebrile state. Five of the other six kudu, which showed no difference in the slope during afebrile and febrile states, showed an average elevation in points during febrile states, with the y-intercept being significantly (ANCOVA, $P < 0.0001$) higher in the febrile state (24.0 ± 3.5ºC, n = 5) than in the afebrile state (22.8 ± 4.1ºC, n = 5). These results imply that these five kudu were selecting warmer microclimates during their fevers, irrespective of prevailing ambient temperatures.

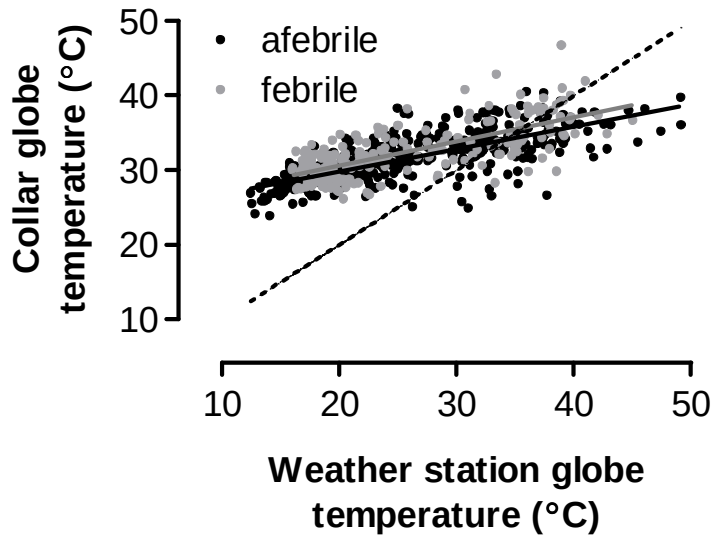

Figure 6.4. Scatter diagram showing the relationship between standard black globe temperatures at the site chosen by a single animal (kudu 5), converted from the miniglobe temperatures recorded on the collar, and standard black globe temperatures recorded at a nearby weather station, during febrile (grey) and afebrile (black) states, for a single kudu. Measurements were made at intervals of 15 min. Miniglobe temperatures were converted to equivalent standard black globe temperatures using an algorithm based on heat-transfer equations. The dashed line is the line of identity, the solid black line is the linear regression during afebrile states and the solid grey line is the linear regression during febrile states. The y-intercept of the line fitted to data obtained during fever was significantly higher ($F_{1,693}$ = 25.58, $P < 0.0001$) than that of the line fitted to data from afebrile states. Febrile data: $y = 0.31x + 24.1$. Afebrile data: $y = 0.31x + 23.8$.

Despite the selection of warmer environments in the febrile state, there was a larger temperature difference between the abdominal cavity and subcutaneous tissue, indicating a more vasoconstricted periphery, at all times of day ($t_5 = 2.74$, $P = 0.04$), compared to the afebrile state. The largest temperature differences between the abdominal cavity and subcutaneous tissue occurred on the first day of the fever. The abdominal-subcutaneous temperature differences were significantly higher ($t_5 = 3.06$, $P = 0.03$) on the first day of fever (1.8 ± 0.9ºC) than during the afebrile state (1.3 ± 0.4ºC). The average 24-h differences between abdominal and subcutaneous temperatures for the first day of fever, and for an average afebrile day, for all seven kudu, are represented in Figure 6.5.

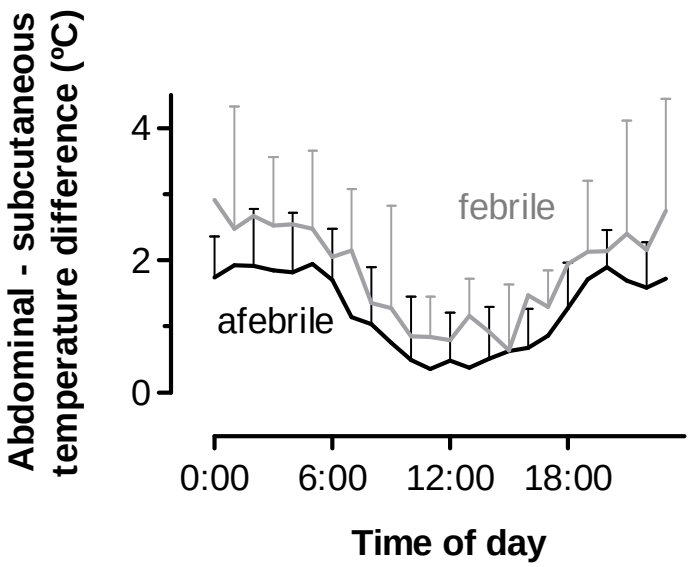

Figure 6.5. Difference between abdominal and subcutaneous temperature over the first day of fever (grey line), and for an average day of non-febrile states (black line), as a function of time of day for seven kudu (mean ± SD).

6.4.3 Sickness behaviour

In addition to peripheral vasoconstriction and selecting warmer microclimates when they were febrile, all the kudu displayed sickness behaviour in the form of decreased activity. Figure 6.6 illustrates the average 24-h activity pattern, as detected by movement of the upper hind limb, for a single kudu (kudu 2). Although all the kudu maintained a biphasic activity pattern with crepuscular peaks throughout both febrile and afebrile states, they showed a decrease in activity at all times of day when febrile. On average, daily activity of the seven kudu decreased by 40% during febrile compared to afebrile states ($t_6 = 3.46$, $P = 0.01$).

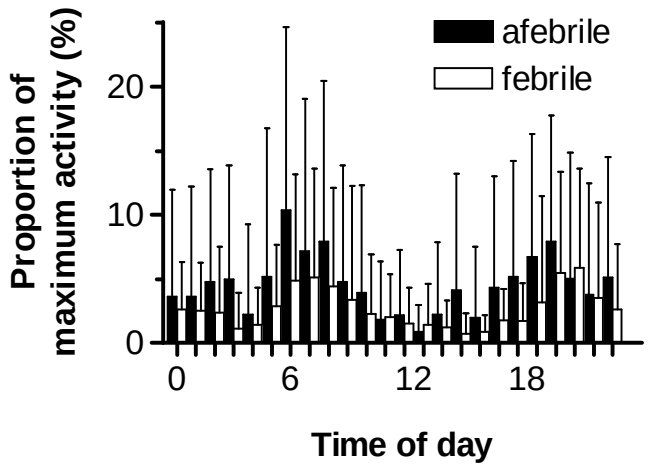

Figure 6.6. Nychthemeral activity rhythm of a single kudu (kudu 2) during febrile (white bars) and afebrile (black bars) states (mean ± SD). The kudu maintained a biphasic activity pattern with crepuscular peaks throughout their fevers, but with reduced amplitude. Activity was measured on the upper hind limb with a subcutaneous activity data logger, as activity counts as a percentage of maximum counts for that logger.

6.4.4 Selective brain cooling

Selective brain cooling is defined as a brain temperature lower than arterial blood temperature (IUPS Thermal Commission, 2003). Figure 6.7 shows the typical pattern of brain and carotid blood temperature recorded for a single kudu (kudu 3) over four days before fever was detected and for four days of fever. This kudu showed the largest magnitude of selective brain cooling at the highest carotid blood temperatures, which were recorded at the acrophase of the endogenous nychthemeral body temperature rhythm during fever (Fig. 6.7).

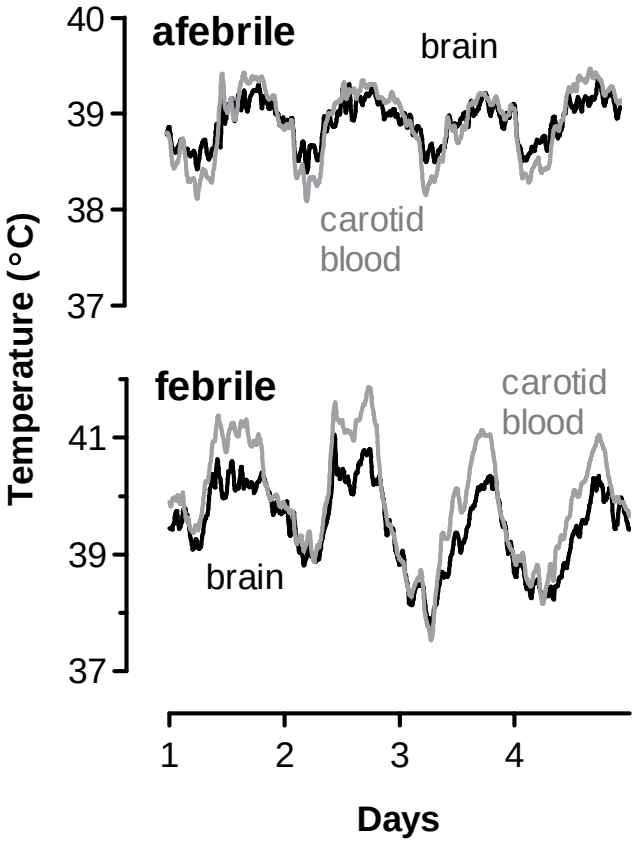

Figure 6.7. The pattern of brain (black line) and carotid blood (grey line) temperatures of a single kudu (kudu 3) over a four-day febrile period, and for the same kudu over a four-day afebrile period.

I obtained complete records of brain and carotid blood temperatures for four animals. Figure 6.8 shows mean, SD, minimum and maximum hypothalamic temperature for each 0.1°C class of carotid blood temperature during both febrile and afebrile states, for those four kudu. The dotted line represents the line of identity; points below this line reflect selective brain cooling. Three of these four kudu displayed selective brain cooling during both the afebrile and the febrile states, and one animal never displayed selective brain cooling (Fig. 6.8, kudu 1). The threshold for selective brain cooling, defined as the carotid blood temperature at which carotid blood and mean hypothalamic temperatures are equal, was not statistically different ($t_2 = 1.30$, $P = 0.32$) during afebrile (38.8 ± 0.12ºC) and febrile (39.3 ± 0.68ºC) states. However,

kudu increased the frequency with which they employed selective brain cooling during the fever ($t_2 = 4.6$, $P = 0.04$), spending 84.4 ± 7.9% of their time with the hypothalamus cooler than carotid blood during the febrile state compared to only 60.5 ± 11.3% during the afebrile state. This higher frequency of selective brain cooling resulted in significantly higher mean selective brain cooling ($t_4 = 3.2$, $P = 0.03$), as defined by the difference between carotid blood and hypothalamic temperature, during febrile (0.38 ± 0.14ºC) compared to afebrile (0.10 ± 0.07ºC) states.

Although selective brain cooling was used more often during fever, the mean brain temperature was higher for a given blood temperature when febrile than when afebrile. The slope of selective brain cooling, defined as the difference between carotid blood and hypothalamic temperature as a function of carotid blood temperature (Kuhnen and Jessen, 1991), was significantly reduced in febrile compared to afebrile states for each of the individuals ($P < 0.0001$). In addition, the mean slope of lines of regression of brain temperature on carotid blood temperature, above the threshold for selective brain cooling, was lower in afebrile (0.49 ± 0.05) than in febrile (0.63 ± 0.07, $t_4 = 2.7$, $P = 0.05$) states, indicating that the magnitude of selective brain cooling at any given carotid temperature was reduced during the febrile compared to the afebrile state.

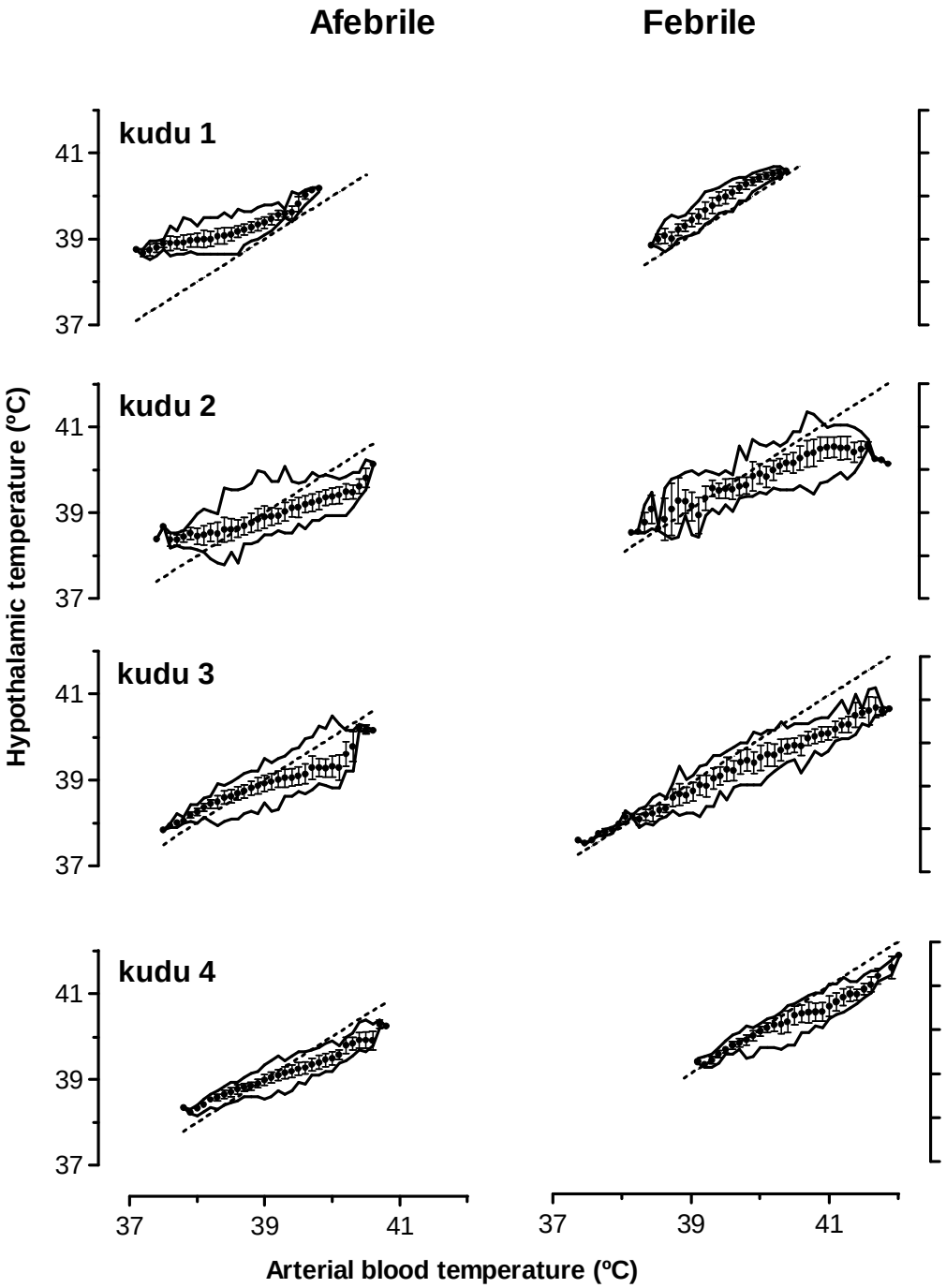

Figure 6.8. The mean, minimum, maximum and standard deviation of hypothalamic temperature for each 0.1°C class of carotid blood temperature during both febrile and afebrile states for four kudu. The dotted line represents the line of identity; points below this line reflect selective brain cooling. Kudu which display selective brain cooling when afebrile also did so when febrile, with no apparent shift of threshold temperature at which brain cooling was initiated.

6.5 Discussion

My results provide the first demonstration, in free-living animals, of certain measurable physiological processes that bring about the elevation of body temperature that is characteristic of fever. I also present the first quantitative evidence for sickness behaviour in free-living animals. I could detect these responses because my colleagues and I had instrumented free-living kudu with data loggers capable of producing a more complete continuous record of the thermal status of undisturbed free-living animals than has been achieved previously. The data loggers, which my colleagues and I implanted in the abdomen, revealed that my kudu had an average 1.3ºC increase in mean abdominal temperature and reached maximum temperatures ranging between 40.8ºC and 41.6ºC while febrile.

The elevated body temperature was achieved partially through appropriate thermoregulatory behaviour, which is less metabolically costly than is implementing autonomic mechanisms. I was able to detect such behaviour, without observers present, by using miniature globe thermometers attached to collars, a novel technique I developed (Appendix 1, Hetem *et al.*, 2007) for recording microclimate selection by animals. Irrespective of climatic conditions, or time of day, the kudu selected warmer microclimates when they were febrile (Fig. 6.4) than when they were afebrile. There was a parallel shift in the linear regression lines of the relationship between the selected microclimate and the prevailing ambient conditions, consistent with a change in set-point for behavioural thermoregulation. My technique of measuring selected microclimate also revealed that the kudu used thermoregulatory behaviour, in the form of choice of microclimate, when they were both febrile and afebrile; the slope of those regression lines were less than one, showing that the kudu selected appropriate microclimates to reduce the impact of environmental thermal stress to less than a third of what it would have been without employing thermoregulatory behaviour, over the standard black globe temperature range of 10ºC to 50ºC.

If behavioural processes alone are insufficient to increase body temperature to the elevated set-point during fever, autonomic processes are invoked. Peripheral vasoconstriction provides the least metabolically costly autonomic response in a cool environment. Using the difference between abdominal and subcutaneous temperatures as an index of peripheral blood flow, I provide evidence that the kudu implemented peripheral vasoconstriction during fever. Irrespective of the time of day, the difference between abdominal and subcutaneous temperature was higher when the kudu were febrile, particularly on the first day of the fever (Fig. 6.5), than when they were afebrile. The kudu, when febrile, therefore appeared to be more vasoconstricted at all times of day, even though standard black globe temperature reached 35ºC, on average, around solar noon (Fig. 6.1A).

An additional autonomic response which may be invoked to supplement the febrile rise in body temperature is the inhibition of evaporative heat loss (Stitt, 1973). Although I had no means of measuring evaporative heat loss continuously in the free-living animals, I do have indirect evidence that my kudu indeed did inhibit evaporation when febrile. At least three of the animals implemented selective brain cooling more frequently, and displayed a greater mean selective brain cooling, when they were febrile (Fig. 6.8). By lowering brain temperature below carotid blood temperature, selective brain cooling reduces the stimulus from hypothalamic warm receptors for evaporative heat loss. Research emanating from my research group (Mitchell *et al.*, 2002; Fuller *et al.*, 2007) and other laboratories (Kuhnen and Jessen, 1992; Kuhnen, 1997; Robertshaw, 2006) concluded that the main thermoregulatory function served by selective brain cooling is inhibition of evaporative heat loss. Even though the magnitude of selective brain cooling at any given carotid blood temperature decreased during fever, kudu spent more time using selective brain cooling. These results indicate that the control of selective brain cooling was altered during fever, in a manner which would suppress evaporative water loss and hence evaporative heat loss.

In response to the increase in set-point that occurs at the start of fever, animals increase body temperature by inhibiting evaporative heat loss, instituting peripheral vasoconstriction and selecting warmer microclimates. Once that set-point is reached, body temperature is regulated actively around the new set-point and the nychthemeral rhythm of body temperature is preserved during the "plateau" phase of fever (Fig. 6.2 and 6.3). In addition to an increase body temperature, my free-living kudu also displayed a decreased activity, characteristic of sickness behaviour. When they were febrile, my kudu reduced activity by 40% compared to that when afebrile. Because I recorded activity continuously, by implanted data loggers, I also could show that, just as they preserved the nychthemeral rhythm of body temperature when febrile, the kudu preserved the nychthemeral rhythm of activity, and specifically its crepuscular peaks and midday trough (Fig. 6.6), as if there were a downward shift in the set-point of a "regulator of activity".

Because the presence of human observers distorts the thermoregulation and behaviour of animals (Recarte *et al.*, 1998), human presence in the vicinity of the animals was kept to a minimum. Consequently, I do not know what the kudu did to reduce activity when febrile, nor how they set about selecting microclimates, however, kudu have been reported to display clear habitat preference for patches with dense cover (Fabricius and Mentis, 1992; Henley, 2001). In addition, since it was not the original intention of my study to investigate fever patterns, I cannot verify the nature of the pathogen or the cause of death. I also do not have records of other sickness behaviours, for example feeding, nor did I have any way of monitoring heat production and heat loss, in these free-living animals. I assume that the increased difference between abdominal and subcutaneous tissue temperatures, during fever, reflected peripheral vasoconstriction. But a decrease in environmental temperature would lead to the same result without a change in skin blood flow. However, given that the kudu selected warmer microenvironments during fever, the former explanation is more likely.

My data provides the first evidence for the proposition that free-living animals elevate body temperature during fever by using the same mechanisms as do human patients and laboratory animals, namely, a coordinated suite of behavioural and autonomic responses. The shape and magnitude of the body temperature increases during fever in the kudu are similar to those seen in free-living impala (Kamerman *et al.*, 2001) and springbok (Fuller *et al.*, 2005). The maintenance of the nychthemeral rhythm of body temperature also has been reported during prolonged fevers in goats (Mphahlele *et al.*, 2004) and humans (Musher *et al.*, 1979; Lell *et al.*, 2000). However, because body temperature during prolonged fever in small laboratory mammals typically is raised much more during the inactive phase than the active phase of their nychthemeral activity patterns, the nychthemeral rhythm of body temperature tends to be reduced or obliterated in small species (O'Reilly *et al.*, 1988; Gourine *et al.*, 1998; Luker *et al.*, 2000; Szelenyi *et al.*, 2004; Morrow and Opp, 2005).

Whether or not the body temperature rhythms are maintained, in environments warm enough for evaporative heat loss to be stimulated, body temperature could increase as a result of vasoconstriction or inhibition of evaporative heat loss rather than because of an increase in metabolic heat production. It is likely that evaporative heat loss would be inhibited during the genesis of fever simply because the set-point for its initiation would be raised. However, I propose that, in animals capable of selective brain cooling, the implementation of selective brain cooling during fever further inhibits evaporative heat loss. If that were the case, one would expect selective brain cooling to be implemented at the initiation of the fever, which appeared to be the case in my kudu (Fig. 6.7). However, in oxen (Chesy *et al.*, 1980) and goats (Kuhnen, 1994), selective brain cooling was most conspicuous during the defervescence phase of fever. If sheep are prevented, by upper respiratory bypass, from implementing selective brain cooling during fever, so that brain temperature rises, the elevation in rectal temperature is markedly attenuated, as would be expected if the high brain temperature stimulated evaporative heat loss (Laburn *et al.*, 1988). In species that do

not have a carotid rete, and in which there is no selective brain cooling during fever (Tegowska and Narebski, 1985), artificial cooling of the brain during fever leads to an exaggerated increase in body temperature (Hori and Harada, 1974; Banet, 1979), without increasing the metabolic cost of fever.

While I could not measure metabolic rate in my kudu, fever is metabolically costly and has been estimated to increase the metabolic heat production of humans by ~ 13% with each degree centigrade rise in body temperature (Hart, 1988; Kluger, 1991). This metabolic cost is species-specific and dependent on habitat, severity and duration of infection, nutritional and metabolic status, and the ambient temperature (Baracos *et al.*, 1987; Hart, 1988; Demas *et al.*, 1997; Elliot *et al.*, 2002). At low ambient temperatures, the increase in body temperature is achieved mainly through heat production, such as shivering, however, at higher ambient temperatures heat is stored through inhibition of heat loss (for example cutaneous vasoconstriction and the inhibition of evaporative heat loss), with little change in heat production (Hales *et al.*, 1973; Stitt, 1973; Hori and Harada, 1974; Baracos *et al.*, 1987). The selection of warm microclimates by my kudu not only facilitated an increase in body temperature directly, but may also have resulted in a preference for the employment of heat conservation mechanisms, such as increased peripheral vasoconstriction and the inhibition of evaporative heat loss.

In addition to the autonomic changes I have outlined and the selection of warmer microclimates, an additional behavioural mechanism proposed to reduce the metabolic maintenance costs of fever is a decreased activity. In theory, reduced activity acts as an energy conservation mechanism, by reducing wasteful wandering activity and convective heat loss associated with moving (Hart, 1988; Konsman *et al.*, 2002; Wingfield, 2003). Decreased activity associated with fever has been reported in various laboratory studies in both rats (Gordon, 1994; Murakami *et al.*, 1995; Luker *et al.*, 2000; Szelenyi *et al.*, 2004) and mice (Kozak *et al.*, 1994). Conversely, a decreased activity was not observed during sustained experimental fevers in penned

goats (Mphahlele *et al.*, 2004), but unlike rodents, which can be highly active in cages, penned goats have low activity, perhaps too low for a further decrease to be detected during fever. My study is the first to demonstrate reduced activity in free-living febrile animals. However, inactivity in free-living animals is not without risk as it could make them more susceptible to predation, and reduce their foraging capacity (Hart, 1988; Yirmiya *et al.*, 1995; Aubert *et al.*, 1997; Konsman *et al.*, 2002; Owen-Ashley and Wingfield, 2006).

As a regulated physiological phenomenon, one would expect both fever and sickness behaviour to have survival value (Hart, 1988; Kluger *et al.*, 1996; Dantzer, 2004). Fever has evolved, however, during the so-called host-pathogen evolutionary arms race (Ewald, 1980; LeGrand and Brown, 2002), and could equally benefit the micro-organism causing the infection, as it could the host. Banet (1981) reported that the survival of rats was directly proportional to the metabolic cost during the rising phase of fever but inversely proportional to the magnitude of fever. My kudu not only appeared to reduce their metabolic costs, through vasoconstriction, warmer microclimate selection and reduced activity, but also displayed high mean maximum body temperatures of 41.2ºC. I do not know whether the fever and sickness behaviour displayed by my kudu were beneficial to them. Nevertheless, I believe that a proper assessment of the natural role of fever in thermoregulation can be made only by studying fever in free-living animals, just as the roles of other thermoregulatory strategies are revealed only by studies of free-living animals away from human observers (Mitchell *et al.*, 2002). By demonstrating, for the first time, coordinated behavioural and autonomic thermoregulatory effectors, and sickness behaviour, in free-living kudu that became infected, I believe that I have made an important first step in that direction.

With the spread of pathogens predicted to increase as the arthropod vectors increase their distribution range with climate change, it seems likely that the pathogens will have an advantage over their hosts. I have demonstrated the kinds of physiological

mechanisms that sick artiodactyls will display as they develop immunity to these novel pathogens. However, the development of such immunity is not without costs, as the elevation in body temperature during a febrile response would increase the risk of lethal hyperthermia during hot periods, which are predicted to become more frequent with climate change. The decreased activity associated with the febrile state may also compromise an animal's ability to employ cathemerality and the resultant reduction in foraging time may be particularly costly for species which are already nutritionally stressed as a consequence of the increased desertification predicted to occur in many regions throughout Africa. The loss of body condition which is likely to develop from both the reduced foraging time and anorexia that accompanies fever may further limit an artiodactyl's capacity for normal behavioural patterns. If such behavioural changes result in an increased predation risk or decrease reproductive success, they may ultimately reduce an individual's fitness. The cost of immunity and the associated sickness behaviours are therefore likely to reduce an animal's ability to employ physiological plasticity to cope with climate change.

6.6 Acknowledgements

I thank Arthur and Trinette Rudman for their hospitality and for allowing the study to take place on their farm Blaauwkrantz, Malcolm Rutherford of River Bend Lodge for use of their holding pens and surgical facilities, Dr Kennedy Erlwanger for assisting with surgical procedures, Andrè Matthee for project assistance and game management expertise, and Sophie and Martin Haupt from African Wildlife Tracking for their help in designing and making the animal collars.

CHAPTER 7

Conclusion

The general aim of my thesis was to contribute to the understanding of phenotypic plasticity of long-lived mammals, likely to be essential to cope with climate change. More specifically, I investigate the physiological strategies employed by free-ranging artiodactyls to adapt to arid regions. Since many arid regions of Africa are predicted to get both hotter and drier with climate change (Boko *et al.*, 2007), understanding the physiological mechanisms exhibited by arid-adapted artiodactyls would assist future studies in monitoring physiological adaptations to desertification. A secondary aim of my thesis was to investigate whether remote sensing technology is sensitive enough to detect physiological plasticity, such as changes in body temperature and activity. To achieve these aims, with the help of my colleagues, I implanted biologging devices and compared thermoregulatory strategies of different colour morphs of a single species (chapter two), of two different-sized artiodactyls inhabiting the same desert environment (chapter four) and of the same artiodactyl species inhabiting two different habitats (chapter five).

7.1 Morphological adaptations

Morphological changes are believed to have constituted the first evolutionary response of mammals to climate change events of the past (Barnosky *et al.*, 2003) and numerous studies have documented changes in morphology in response to recent changes in climate (Kingsolver *et al.*, 2001). One such morphological variable, which is likely to have thermoregulatory consequences, is an animal's colour. Yet, recent studies have concluded that concealment appears to be the driving force behind the evolution of colouration in ungulates, with communication and thermoregulation playing less of a role (Stoner *et al.*, 2003). Nevertheless, in chapter two, I showed the thermoregulatory consequences of different colour morphs of springbok in the arid Karoo. I concluded that there is thermoregulatory significance of pelt colour and there probably are selective pressures acting against both the white and the black springbok, that is the less successful morphs, at different times of the year. The black springbok seemed able to reduce energy expenditure in winter, but experienced

higher solar heat load in hot conditions. On the other hand, the white springbok apparently lived closer to the energetic edge in winter, possibly because its lower heat load from solar radiation required a higher metabolic cost of homeothermy. From a physiological perspective, the normal springbok appeared to occupy a compromise position, with better energy balance than the white springbok in winter and less overheating than the black springbok in summer, a compromise which may explain why the black and white springbok rarely occur naturally, despite the genes for those colour morphs being present. Although it was beyond the scope of my study to compare the relative roles of colour in thermoregulation and in crypsis, the aposematic function of the normal springbok's colouration may further explain the preponderance of its prevalence (Caro, 2009). We may find, however, that selection pressures change over time and that the white morph may be selected for in the future as conditions get progressively hotter and drier with climate change. Yet, such selection pressures would have to override any predatory pressure, which, because the conspicuousness of the white colour morph, may counter any thermoregulatory advantage.

The prediction that lighter coloured individuals would be selected for as conditions get progressively drier with climate change would be consistent with Gloger's ecogeographical rule (Millien *et al.*, 2006). Gloger's rule states that species of endotherms found in humid regions tend to be more heavily pigmented than those found in dry regions. Indeed, the Arabian sand gazelle, which inhabits one of the driest deserts in the world, is lighter in colour than the springbok and most gazelle species found throughout the tropics of Africa. Similarly, the Arabian oryx, reputed to be the most arid-adapted of all artiodactyls, is famed for its white colouration. Nevertheless, such an adaptation option would be feasible only for a species with sufficient genetic diversity for a trait that adapts individual animals to different thermal environments, and a sufficiently fast generation time to allow for that trait to be selected. Artificial selection pressures, which recently have increased the prevalence of the colour morphs of several species within South Africa, combined

with decades of predator control removing predation on conspicuous colour morphs, may be advantageous for increasing the genetic variability of populations and may ultimately provide a pre-adaptation to changing climatic conditions.

Unlike pelt colouration, body mass need not be genetically determined and can be influenced by environmental factors, making it a phenotypically plastic trait. In addition to their light colouration, endotherms of the Arabian Peninsula are generally smaller than their counterparts which inhabit more temperature regions, conforming to yet another ecogeographical rule, namely Bergmann's rule. Bergmann's rule predicts a positive correlation between body mass of terrestrial endotherms and latitude, and, by inference, an inverse correlation between body mass and environmental temperature. Thus, the higher ambient temperatures predicted to occur with climate change may provide a selective advantage for smaller individuals (Teplitsky *et al.*, 2008). However, rather than climate change favouring the selection of smaller species (genotypic adaptation) it may favour the selection of smaller individuals within a species (phenotypic plasticity), which would allow species to adapt to changing climatic conditions over a much shorter time interval. Yet temperature is not the only environmental factor which is likely to influence body mass; both reduced water availability and reduced plant productivity also would favour small individuals (Rosenzweig, 1968; Wigginton and Dobson, 1999). Thus, the primary increase in ambient temperature and the secondary reduction in water availability, and resultant lower plant productivity, predicted to occur in the future may act to reduce the body size of endotherms. Such changes in body size are observable already in small endotherms, such as birds (Yom-Tov, 2001; Yom-Tov and Yom-Tov, 2006; Yom-Tov *et al.*, 2006) and rodents (Smith *et al.*, 1995; Smith *et al.*, 1998). Similarly, smaller artiodactyls, hypothetically, would have greater access to thermal refuges, potentially giving smaller artiodactyls another favourable adaptation to hot and dry environments, over their larger counterparts. In chapter four I described the investigation of the different thermoregulatory strategies of a small and large desert-adapted artiodactyl, namely the small Arabian sand gazelle (~ 15 kg)

and the larger Arabian oryx (~ 70 kg), inhabiting the same desert environment, at the same time. Despite the oryx having a body mass more than four-fold that of the sand gazelle, the two species responded remarkably similarly to changes in environmental conditions. Both species employed heterothermy and cathemerality, and selected the same cooler microclimates during times of heat stress. Yet, because of the difference in body mass, for the same amplitude of nychthemeral rhythm of body temperature, the oryx would store about four times as much heat as the gazelle as body temperature rises, and therefore save about four times as much water, that otherwise would be spent on evaporative cooling. If small artiodactyls are unable to find appropriate refuges, they may be disadvantaged in hot and dry conditions because of their high mass-specific metabolic rates, high water turnover and less capacity to store heat. Future studies would therefore need to establish whether changes in body mass result in changes in function, such as the functional change I showed for pelt colouration in chapter two, and whether smaller individuals within a species would be advantaged in hot and dry environments.

Unfortunately all of the gazelle died within five months of my starting the project. I do not know whether the captive-bred gazelle were maladapted to the extremely hot and dry conditions of the desert environment or whether their fenced enclosure prevented them from seeking water and moving to potentially more appropriate environments. Since sand gazelle are known to travel large distances in search of suitable forage after rain (Harrison and Bates, 1991; Thouless *et al.*, 1991), restricting the dispersal ability of this species may have increased the stress associated with the exceptionally hot and dry conditions prevalent during my study and, as such, highlights the adverse effect fences may have in restricting an animal's ability to move to more appropriate environments. However, the high mortality of sand gazelle was not specific to the gazelle in my enclosure; nearly 700 sand gazelle (50% of the estimated population) died in the Mahazat as-Sayd over the same time period (Cunningham *et al.*, 2008). Whether or not the death of my sand gazelle was the result of their fenced enclosure restricting their dispersal ability, fences are likely to

be detrimental to large mammalian species in South Africa, where the majority of national parks are fenced.

7.2 Autonomic adaptations

In seeking to explore how artiodactyls will respond to climate change, I sought the most extreme of current environments and chose to investigate physiological mechanisms of an arid-adapted artiodactyl which survives there. The deserts of the Arabian Peninsula are considered to have one of the most inhospitable climates on earth. Of the seven artiodactyl species which inhabit this hot, hyper-arid environment, the species which arguably faces the greatest challenge to homeostasis is the Arabian oryx (*Oryx leucoryx*). With a body mass of 70-100 kg, this artiodactyl survives most of the year, including the hottest summer months, without access to drinking water. To investigate the thermoregulatory consequences of water limitation, in chapter three I investigated thermoregulatory strategies of the arid-adapted Arabian oryx over a one-year period during which conditions got progressively drier. I showed that heterothermy indeed was present but was dependent not only on high ambient temperatures but also on lack of water. The oryx, like other desert-adapted endotherms, would have to trade off thermoregulation, osmoregulation and energy acquisition (Cain *et al.*, 2006). I believe the data obtained from the oryx supports the view that priority is given to osmoregulation. When the oryx had access to sufficient dietary water to maintain both osmoregulation and homeothermy (by evaporative cooling), they did so. However, when they did not have access to sufficient dietary water they abandoned homeothermy.

When environmental temperature exceeds body temperature evaporative cooling provides the only mechanism to lose metabolic heat, but when water is scarce animals may attempt to conserve body water at the expense of homeothermy. Yet, adaptive heterothermy differs from dehydration-induced hyperthermia in that mean body temperature does not increase because adaptive heterothermy, by definition, also is

associated with a decreased minimum body temperature. The limited evidence for the drop in minimum body temperature associated with adaptive heterothermy in free-living animals led some researches to question whether large fluctuations in body temperature are rather the result of dehydration-induced hyperthermia (Mitchell *et al.*, 2002) and, as such reflect a compromised thermoregulatory ability during water deprivation. The capacity to store heat, and thereby conserve body water, holds whether the heterothermy is a controlled thermoregulatory event, that constitutes adaptive heterothermy, or results from failure of homeothermy, but failure of homeothermy may result in potential lethal hyperthermia. A study by Dawson *et al.* (2007) provided further evidence that, at least in kangaroos, heterothermy may represent a compromised thermoregulatory ability, rather than an adaptation. At the same level of dehydration, the desert-adapted red kangaroo (*Macropus rufus*) displayed reduced heterothermy while still reducing its evaporative water loss, compared to its mesically-adapted counterpart, the eastern grey kangaroo (*Macropus giganteus*, Dawson *et al.*, 2007). But whether heterothermy is adaptive in artiodactyls, or whether it reflects a compromised thermoregulatory ability, as in kangaroos, remains to be investigated. To do so, we would need to investigate whether desert-adapted artiodactyls are more likely to employ heterothermy than mesically-adapted species, under the same environmental conditions. In addition, we would need to investigate whether heterothermy confers any advantages in terms of water conservation and whether individuals that employ heterothermy are more or less likely to survive extremely hot and dry conditions.

I believe that, unlike adaptive heterothermy, the heterothermy observed in my oryx was the result of a failure of homeothermy as a result of a dehydration-induced hyperthermia combined with a starvation-induced hypothermia. Adaptive heterothermy predicts that the a low minimum body temperature would allow an animal to anticipate hot ambient conditions (Maloney *et al.*, 2004) and thus pre-emptively permit additional storage of heat. Yet, my oryx not only displayed a low minimum body temperature during the hot dry period, but their minimum body

temperatures remained low throughout the dry period, even though ambient temperatures were not excessively high during these periods. These low minimum body temperatures throughout the dry period may reflect a reduced metabolic heat production, as a result of the Q_{10} effect, as has been proposed for wild kangaroo (Dawson *et al.*, 2007). A low metabolic rate during the dry season could result from the combined effect of dehydration and starvation, as has been shown in other desert ungulates (Brosh *et al.*, 1986; Merkt and Taylor, 1994; Williams *et al.*, 2001; Ahmed and El Kheir, 2004; Ostrowski *et al.*, 2006a; Ostrowski *et al.*, 2006b). Similarly, low minimum body temperatures, postulated to have resulted from low metabolic rates during times of energy restrictions, were observed in both my sand gazelle during, the hot and dry period (chapter four), and the white springbok colour morph during the dry Karoo winter (chapter two). Thus, long-term monitoring of body temperature may provide a sensitive index of physiological stress as a result of changing climatic conditions, as low minimum body temperatures are likely to reflect nutritional stress and high maximum body temperatures are likely to reflect water stress.

A second autonomic mechanism that I believe my Arabian oryx used to conserve body water and facilitate homeostasis at high environmental heat loads was selective brain cooling. Arid-zone mammals possessing a carotid rete may employ selective brain cooling to attenuate thermal drive by reducing hypothalamic temperature, which reduces evaporative heat loss and ultimately conserves water by transferring cooling to non-evaporative means (Kuhnen, 1997; Jessen, 1998; Jessen, 2001; Mitchell *et al.*, 2002). If the evolution of the carotid rete indeed promoted thermoregulatory flexibility and thus facilitated the invasion of arid zones during the highly seasonal post-Eocene climate (Mitchell and Lust, 2008), such an anatomical structure may well provide a key adaptation for artiodactyls to cope with aridity and heat stress predicted to occur with climate change (Fuller *et al.*, 2008). However, not all artiodactyls exhibit the same capacity for selective brain cooling. Primitive artiodactyls, such as the mouse deer (*Tragulus javanicus*, *Tragulus napu*), do not possess a carotid rete (Fukuta *et al.*, 2007), which hypothetically restricts this species

to the climatically-stable forest environment (Whittow *et al.*, 1977). Whether all artiodactyls inhabiting arid environments have the same capacity for selective brain cooling is not known. Because the hypothalamus is exceptionally sensitive to changes in temperature, even a relatively small difference of a few tenths of a degree in selective brain cooling could result in a large difference in suppression of evaporative cooling and therefore water loss. To date, there has been no comparative investigation of selective brain cooling in sympatric species simultaneously occupying the same hot arid environment. Nevertheless, it is encouraging to note that the magnitude of selective brain cooling in the arid-adapted Arabian oryx was similar to that observed in other free-living artiodactyls (Jessen *et al.*, 1994; Jessen and Kuhnen, 1996; Mitchell *et al.*, 1997; Fuller *et al.*, 1999; Maloney *et al.*, 2002), implying that even relatively small magnitudes of selective brain cooling may be sufficient to substantially reduce evaporative water loss and thereby conserve body water. Since selective brain cooling does not disrupt other homeostatic systems, it may provide an economical form of autonomic thermoregulation. If artiodactyls are capable of phenotypic plasticity in the magnitude of selective brain cooling, the carotid rete may ultimately provide artiodactyls with greater acclimatization potential to the hot and dry conditions predicted to occur in the future.

7.3 Behavioural adaptations

An additional mechanism proposed to conserve body water and reduce metabolic costs as conditions get progressively hotter and drier would be an adjustment in the pattern of activity, termed cathemerality. The term “cathemerality” was originally coined for primates, which were observed to be active throughout the day and night, and was later defined as significant amounts of activity occurring during both light and dark phases of the 24-h daily cycle (Tattersall, 1987; Tattersall, 2006; Tattersall, 2008). It differs from both crepuscular and diel activity, which follow a 24-h periodicity (Aschoff, 1966), and, instead, describes variation in activity patterns in response to prevailing ecological conditions. Chronoecological factors, such as

temperature, light, competition for food resources and predation, appear to promote cathemerality by overriding the endogenous clock (Curtis and Rasmussen, 2006). Although seldom studied, cathemerality may be common among non-primate mammals and a study on Indonesian rain forest mammals showed all nine species of ungulates studied to be cathemeral (van Schaik and Griffiths, 1996). Identification of cathemerality requires measurement of both diurnal and nocturnal activity (Curtis and Rasmussen, 2006), which I achieved objectively by using activity loggers. Because the presence of human observers distorts the behaviour of artiodactyls (Recarte *et al.*, 1998), activity loggers provided a long-term, remote and unbiased assessment of activity, over successive 24-h cycles, not otherwise feasible under field conditions. Unlike van Schaik and Griffiths (1996), who classified animals as cathemeral based on the proportion of time spent active during diurnal and nocturnal periods, cathemerality, by definition, requires a variation in activity in response to ecological conditions. Using activity loggers, I was able to show that both the oryx and the sand gazelle shifted from a continuous activity with crepuscular peaks during warm wet periods to nocturnal activity during the hot dry period.

Such an increase in nocturnal activity is believed to be a compensatory response, as grazing activities during the heat of the day would be limited as a result of shade-seeking behaviour, in response to high ambient temperatures. Shade-seeking behaviour reduces the heat load on an animal and conserves body water by reducing the need for evaporative cooling. I was able to quantify such behaviour in my oryx and sand gazelle, without observers present, by using miniature globe thermometers attached to collars, a technique I developed (Appendix 1, Hetem *et al.*, 2007). Both cathemerality and shade-seeking behaviour represent flexible behavioural processes, which are likely to become increasingly important and may act to buffer the adverse effects of the progressively hot and dry conditions predicted to occur with climate change. However, unlike the Arabian oryx and sand gazelle, which can freely shift between diurnal and nocturnal activity since they are not exposed to natural predators,

many species throughout Africa may be exposed to a greater predation pressure should they increase their nocturnal activity.

If African species are unable to shift their activity to more appropriate times of day and are forced to be active throughout the heat of the day because of constraints by predatory pressure, they are likely to become more dependent on the already scarce and fast declining water sources, if they attempt to maintain homeothermy. When these water sources run dry, species may be forced to display heterothermy. Although, it is encouraging to note that six species of East African artiodactyls tolerated large fluctuations in rectal temperature, at least over the short term (Taylor, 1970b), it has been questioned whether these measurements were artefacts of measurement error (Mitchell *et al.*, 2002). It therefore remains to be seen whether free-living African species will be able to cope with the large fluctuations in body temperature exhibited by Arabian artiodactyls. A seemingly simple solution to the water stress associated with desertification would be to provide artificial water sources to regions where water supplies are limited (Epaphras *et al.*, 2008). However, such a management solution is not without risk and would potentially provide only a short-term solution to the problem. The recent decline in the roan antelope (*Hippotragus equinus*) population in the Kruger National Park, for example, has been attributed to an increase in the number of artificial water points (Harrington *et al.*, 1999b). It is proposed that these water points made previously dry areas, where roan antelope thrived, accessible to water-dependent species, such as wildebeest and zebra, year round. Lions followed their prey and the increased predation on adult roan antelope is believed to have caused the roan antelope to decline from about 450 to just 45 individuals between 1986 and 1993. The provision of artificial water points not only affects resident herbivores through the indirect effects of competition and predation, but the increased grazing pressure associated with the artificially increased density of herbivores can also have detrimental effects on the vegetation structure, creating so-called piospheres (Thrash, 1998; Todd, 2006). In fact, the provision of artificial water points, particularly in arid regions, may exacerbate land degradation and lead to

massive die-offs when the degraded vegetation can no longer sustain the artificially high herbivore numbers, ultimately resulting in a loss of biodiversity (Nangula and Oba, 2004; Fensham and Fairfax, 2008). Thus, the adaptive strategy with the least disruptive consequences for ecosystem function would be for species to show phenotypic plasticity and to adapt to the increasingly desertified landscapes to which they are likely to be exposed. Even those arid-adapted species which can survive independent of drinking water would need to adapt phenotypically to the reduced water availability in their forage.

7.4 Adapting to desertification

One of the consequences of climate change will be habitat transformation and there is debate about the relative detrimental effects of the climate itself and its consequences for habitat. Since I could now predict the kind of physiological mechanisms required to adapt to desert environments, in chapter five I investigated whether similar responses were exhibited by an arid-adapted artiodactyl, which is likely to have to adapt to desertified landscapes in the Eastern Cape Province, South Africa. Because Angora goats are long-lived artiodactyls, which for economic reasons cannot be translocated, they provide the ideal study species in which to investigate plasticity of herbivores in response to habitat transformation. Although, in general, goats that inhabited the transformed and intact sites responded similarly as a result of the relatively benign conditions and high rainfall prevalent during my study, I was able to demonstrate physiological changes in response to desertification when goats were subjected to a physiological stress imposed by shearing. Shearing changed the insulative property of the pelt and, as such, represents a morphological change which appeared to make the animals more susceptible to changes in environmental conditions (Appendix 2, Hetem *et al.*, 2009). Post-shearing, goats that inhabited the transformed site had a faster rate of rise in body temperature in the morning, displayed an increased 24-h amplitude of body temperature rhythm and were generally less active compared to goats that inhabited the intact site. When goats were

denied access to free-standing water, the goats that inhabited the transformed site were unable to maintain a positive water balance as a result of a suppressed dietary water intake, combined with a higher water turnover, compared to the goats that inhabited the intact site. Such an increased amplitude of body temperature rhythm and reduced activity when goats were exposed to water stress on the transformed site may reflect a trend towards heterothermy and cathemerality, as was observed in the Arabian oryx and sand gazelle. Because forage availability varies by orders of magnitude between wet and dry periods (Stuart-Hill and Aucamp, 1993), such physiological strategies are likely to become obligatory with further desertification predicted with climate change. Future studies should therefore focus on the physiological plasticity of artiodactyls exposed to habitat transformation under conditions both hotter and drier than those prevalent during my study. Given that climate change is already exacerbating drought extremes (Chamaillé-Jammes *et al.*, 2007), and that the semi-arid to arid subregions of Southern Africa are particularly vulnerable, understanding the effects of these extremes will become increasingly important in the future.

The use of sophisticated physiological measurements applied here will allow not only an understanding of the extent of adaptive responses, but also improve the predictions of species responses to climate change in the future. If we are to successfully conserve biodiversity under changing climatic conditions, we need an understanding of which species respond well to hot and dry environments so that we can prioritise those species for conservation in the future. Indeed, studies are already suggesting which breed of Borana cattle should be prioritised for cultivation in East Africa (Zander *et al.*, 2009), with the aim to prioritise species with the greatest genetic diversity to adapt to future challenges. Such recommendations are based on Weitzman's cost-effectiveness methodology (Weitzman, 1998), which ranks species based on a combination of economic factors and changes in survival probabilities. However, the predicted survival probabilities of the culturally important livestock species were fairly arbitrary and were based on the opinions of local livestock-

keepers (Zander *et al.*, 2009). Such an approach could be improved by incorporating measures of genetic diversity and a species physiological adaptive capacity.

7.5 Adapting to the spread of pathogens

Although my transformed site appeared to be a more stressful environment than the intact site, both in terms of thermal microclimates and water availability, one potential advantage of more open habitats is a reduced parasite load (Mooring *et al.*, 2004). The low albumin to globulin ratio of goats that inhabited the intact site is likely to have reflected a higher parasite load, since a low albumin to globulin ratio is associated with inflammation and bacterial invasion (Hurwitz and Whipple, 1917), decreased immune function in heifers (Piccinini *et al.*, 2004) and low social status in pigs (Hicks *et al.*, 1998). I propose that the goats that inhabited the transformed site were less immunologically stressed than the goats that inhabited the intact site. Although a reduced parasite load on the desertified site may presently be advantageous, climate change is predicted to bring emergent pathogens with the relocation of arthropod vectors (Rogers and Randolph, 2000; Olwoch *et al.*, 2003; Cumming and van Vuuren, 2006). We therefore cannot simply focus on adaptation responses of healthy individuals, but also need to include the studies of innate immunity and sickness behaviour.

In chapter six I investigated the physiological consequences of infection in free-living kudu. Although it was not my original intention to study the consequences of infection in free-living kudu, the opportunity provided the first evidence ever of the suite of responses of a sick animal in the combination of immune compromise and environmental stress likely to be the typical scenario with climate change. The fever was characterized by a significant increase in mean 24-h body temperature, but it did not alter the amplitude of nychthemeral rhythm of body temperature. Kudu reduced the metabolic cost of fever by increasing vasoconstriction and selecting warmer microclimates during the fever than when afebrile. In addition, some kudu displayed

an increased frequency of selective brain cooling during the fever, which would have inhibited evaporative heat loss and increased febrile body temperatures, without increasing the metabolic maintenance costs of high body temperatures. Not only did I record quantitative evidence for autonomic and behavioural fever, but I also recorded the first evidence of sickness behaviour, in the form of decreased activity, in a free-living ungulate species. As a regulated physiological phenomenon, one would expect both fever and sickness behaviour to have survival value (Hart, 1988; Kluger *et al.*, 1996; Dantzer, 2004), but fever evolved during the so-called host-pathogen evolutionary arms race (Ewald, 1980; LeGrand and Brown, 2002) and could equally benefit the micro-organism causing the infection, as it could the host. With the spread of pathogens predicted to increase as the arthropod vectors increase their distribution range in the future, it seems likely that the pathogens will have an increasing advantage over their hosts. In addition to the artiodactyl hosts having to develop immunity to novel pathogens, they are likely to have to contend with the increased metabolic costs of immunity superimposed on the chronic physiological stress of having to adapt to the climatically unsuitable areas to which they are confined. Although my kudu reduced the metabolic costs of immunity, even a slight increase in metabolic requirements may be too costly for artiodactyls already at their physiological limit. Thus, the predicted spread of pathogens with climate change is likely to increase extinction risk. Future studies will need to monitor physiological variables of free-living species to assess whether infections are indeed becoming more prevalent. In addition responses of sick animals need to be assessed in hot environments where the increased risk of hyperthermia may make febrile response maladaptive. Ultimately we will need to include the responses of sick animals into both models and experimental studies to better anticipate the effects of climate change.

7.6 Perspectives and significance

I have revealed the kinds of physiological plasticity which free-living artiodactyls are likely to implement as conditions in Africa get progressively hotter and drier with climate change and, in so doing, I have shown that physiological measures are an appropriate way to detect an animal's adaptation to changing environments. Activity patterns and microclimate selection are flexible behavioural processes which are likely to represent an animal's primary defence to changes in climatic conditions, particularly for large-bodied species with long generation times which cannot rely on microevolution because of the unprecedented rate of current climate change. If such behavioural processes are insufficient to maintain homeothermy, we are likely to observe changes in an animal's body temperature. Body temperature provides a sensitive indicator of physiological stress in terms of infection, dehydration, loss of body condition and nutritive stress. We need to make such measurements in individuals of several species inhabiting a variety of environments. Such studies would fall into the recently defined field of macrophysiology, defined as "the investigation of variation in physiological traits over large geographical and temporal scales and the ecological implications of this variation" (Chown *et al.*, 2004a). Incorporating such macrophysiological data into bioclimatic envelope models will allow us to better predict how species will respond to climate change (Porter *et al.*, 2002) and ultimately contribute towards effective management and conservation of Africa's biodiversity in the face of future climate change. Knowing which species demonstrate sufficient physiological plasticity to cope with the consequences of climate change will allow for more informed decisions as to which species need to be relocated (Hoegh-Guldberg *et al.*, 2008) and potentially which species should be prioritised for conservation (Zander *et al.*, 2009) in the future.

Ultimately, we require an interdisciplinary approach, combining the expertise of ecologists, botanists, zoologists, veterinarians and physiologists to provide a more complete picture of the interplay between the numerous variables affected by changing climatic conditions. As technology, such as biotelemetry, develops the

range of physiological variables which can be monitored in free-living animals is likely to increase. Within this thesis I have extended the typical biotelemetric measures of body temperature and showed the value of including remote measurements of activity and thermoregulatory behaviour. Such new technology, combined with current methodology such as Geographical Information Systems (GIS), will allow for a multidisciplinary approach to aid our understanding of animal biogeographical distributions. Since long-term monitoring programmes are already in place for ecological and environmental variables, for example the South African Environmental Observation Network (SAEON), the next logical step would be to incorporate physiological responses of individual organisms into such long-term monitoring programmes. Such long-term physiological measurements will allow us to detect when physiological thresholds are exceeded and when behavioural processes become insufficient to cope with climate change. Data from such long-term monitoring programmes will ultimately help to elucidate the causal mechanisms underlying ecosystem changes and allow us to better detect, predict and react to future climate change.

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Appendix 1

Validation of a Biotelemetric Technique, Using Ambulatory Miniature Black Globe Thermometers, to Quantify Thermoregulatory Behaviour in Ungulates

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ABSTRACT Behavioural thermoregulation is an animal's primary defence against changes in the thermal environment. We aimed to validate a remote technique to quantify the thermal environment behaviourally selected by free-ranging ungulates. First, we demonstrated that the temperature of miniature, 30 mm diameter, black globes (miniglobes) could be converted to standard, 150 mm diameter, black globe temperatures. Miniglobe temperature sensors subsequently were fitted to collars on three free-ranging ungulates, namely blue wildebeest (*Connochaetes taurinus*), impala (*Aepyceros melampus*) and horse (*Equus caballus*). Behavioural observations were reflected in animal miniglobe temperatures which differed from those recorded by an identical miniglobe on a nearby exposed weather station. The wildebeest often selected sites protected from the wind, whereas the impala and the horse sheltered from the sun. Nested analysis of variances revealed that the impala and horse selected significantly less variable environments than those recorded at the weather station ($P<0.001$) over a 20-min time interval, whereas, the microclimates selected by wildebeest tended to be more variable ($P=0.08$). Correlation of animal miniglobe against weather station miniglobe temperature resulted in regression slopes significantly less than one ($P<0.001$) for all species studied, implying that, overall, the animals selected cooler microclimate s at high environmental heat loads and/or warmer microclimates at low environmental heat loads. We, therefore, have developed an ambulatory device, which can be attached to free-ranging animals, to remotely quantify thermoregulatory behaviour and selected microclimates. *J. Exp. Zool. 307A:342–356, 2007.* © 2007 Wiley-Liss, Inc.

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Free-living terrestrial animals employ a suite of thermoregulatory behaviours in thermally heterogeneous habitats, thus selecting their microclimate (Bartholomew, '64, '87; Huey, '91). While estimating the consequences of implementing an autonomic effector, such as panting or shivering, on an animal's thermal balance is relatively straightforward, estimating how behaviour affects thermal balance is not. The heat exchange characteristics of a specific microenvironment have been assessed using hollow models (Bakken et al., 1985, Huey, '91, Hertz et al., '93), but the animal's selection of different microenvironments is more difficult to ascertain.

Often, when microclimates are assessed, the only variable measured is ambient dry-bulb

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temperature (Kinahan et al., 2007). The rate of heat exchange of an animal depends not only on dry-bulb temperature but also on wind velocity, solar radiation, long wave radiation and ambient water vapour pressure. Vernon ('30) proposed the use of the temperature of a hollow, 150 mm diameter, copper globe painted matt black, to derive the mean radiant temperature (Vernon '32; Bedford and Warner, '34; Kerslake, '72) in an attempt to assess the radiant component of the human microclimate. The temperature of the globe reaches equilibrium when radiant heat exchange is equal and opposite to convective heat exchange, which, in turn, depends on both dry-bulb temperature and wind speed. Although the temperature of the globe is not affected by ambient water vapour pressure, it is affected by three of the four factors which influence the animal's thermal balance. Hence, though it was not Vernon's original intention, users of his globe began to employ globe temperature, rather than mean radiant or dry-bulb temperatures, as a physiologically more-appropriate index of the microclimate.

While the globe thermometer was developed to assess the human microclimate, enterprising comparative physiologists saw its potential for estimating the thermal load on other species (Bond and Kelly, '55; Schmidt-Nielsen et al., '56; Kuehn et al., '70). Though it seemed implausible that a simple black globe could be useful in assessing thermal balance of animals of widely varying mass and appearance, it has been established that animal size, colour and morphology may be only minor confounders of the thermal load experienced (Walsberg and Weathers, '86; Vitt and Sartorius, '99). Thus, for many investigations, the 150 mm globe thermometer has supplanted the traditional species-specific hollow-body models as an estimate of operative environmental temperature (Huey, '91) and has been used successfully to assess the microclimates of animals, ranging from cattle, through lizards, to bees (Seely et al., '90; Thiagarajan and Thomas, '92; Corbet et al., '93).

Conventionally, when globe thermometers have been used to assess thermal load, they have been attached to fixed weather stations exposed to full radiant heat load and prevailing wind. If measurement of the microclimate selected by a free-living animal in a thermally heterogeneous habitat is needed, multiple weather stations are required in numerous habitats (Huey, '91; Kinahan et al., 2007). We suggested an alternative approach; attach the globe thermometer to the animal (Fuller et al., '99). In recent years biotelemetric collars have been successfully used to monitor a range of parameters, including GPS position, body movement and air temperature (Cooke et al., 2004; Brosh et al., 2006; Kinahan et al., 2007). Collars therefore provide the ideal location for the attachment of an ambulatory globe thermometer.

But attaching a 150 mm diameter globe to an animal's collar is impractical for all but the largest species. Smaller globes have been designed for commercially available human heat stress monitors. We know that these small globes do not reach the same equilibrium temperature as 150 mm globes in the same thermal environment, because their heat transfer coefficients for both radiant and convective heat transfer are different (Bond and Kelly, '55; Graves, '74). Many of the commercially available personal heat stress monitors appear not to address the issue of globe diameter. When globes of smaller diameters ("miniglobes") are employed, it is desirable to standardise the measurements to what the temperature attained by a standard 150 mm globe would be, otherwise comparisons between studies would be meaningless. Hey ('68) proposed a linear approximation for converting temperatures measured by miniglobes to standard globe temperatures. Later, Peters et al. ('76) proposed the manipulation of heat transfer equations as a more accurate approach to convert miniglobe to standard globe temperature, for use in personal heat stress monitors. However, neither study tested the accuracy of their conversion. We address this issue by exposing globes of different sizes to varying temperature, radiation and convection environments in a climatic chamber. Moreover, we also developed and tested a new algorithm for the conversion of miniglobe to standard globe temperature.

After successfully converting miniglobe to standard globe temperatures, we then validated an ambulatory technique to assess the microclimates selected by a free-living terrestrial animal in a thermally heterogeneous habitat. We attached 30 mm-diameter miniglobes to collars on three animals of different ungulate species (blue wildebeest (*Connochaetes taurinus*), impala (*Aepyceros melampus*) and horse (*Equus caballus*)) and measured miniglobe temperatures continually as the animals exhibited their normal behaviour undisturbed. We correlated miniglobe temperature with observed behaviour, over simultaneous time periods. To the best of our knowledge, no one has attempted to validate ambulatory globe thermometers, attached to an animal, as a means to quantify thermoregulatory behaviour.

MATERIALS AND METHODS

Agreement between miniglobe and standard globe temperatures

Estimating standard globe temperatures from miniglobe temperatures

A globe thermometer will reach thermal equilibrium when the rate at which it exchanges heat through convection with its environment is equal and opposite to the rate it exchanges heat through radiation (Kerslake, '72):

$$\sigma\epsilon(T_r^4 - T_g^4) = h_{cg}(T_g - T_a),$$

where σ is a Stefan–Boltzmann constant ($5.67 \times 10^{-8}\,W\,m^{-2}\,K^{-4}$), ϵ is the emissivity of the globe surface, T_r the mean environmental radiant temperature (K), T_g the globe temperature (K), T_a the ambient dry-bulb temperature (K) and h_{cg} the convective heat transfer coefficient of the globe ($W\,m^{-2}\,K^{-1}$). If the globe has a non-reflecting black coating, ϵ is approximately equal to 1. Setting $\epsilon = 1$, the thermal equilibrium equation can be rearranged as:

$$T_r^4 = \frac{h_{cg}(T_g - T_a)}{\sigma} + T_g^4.$$

For spheres with diameters of the order of 100 mm, the convective heat transfer coefficient is inversely proportional to the 0.4th power of diameter (Kerslake, '72), so that, comparing a miniglobe to a standard globe:

$$\frac{h_{cmg}}{h_{csg}} = \left(\frac{L_{mg}}{L_{sg}}\right)^{-0.4},$$

where h_c is the convective heat transfer coefficient ($W\,m^{-2}\,K^{-1}$), L the globe diameter (mm) and sg or mg refer to values for the standard (150 mm diameter) or miniglobe, respectively.

Convective heat transfer coefficients are dependent not only on diameter, but also on the prevailing wind speed and air pressure (Whillier and Mitchell, '68; Mitchell, '74). At sea level atmospheric pressure and air temperature of 20°C, the convective coefficient of the standard globe (Kerslake, '72) is:

$$h_{cg} = 14.1 \times V^{0.6},$$

where V is the wind speed ($m\,s^{-1}$). This convective coefficient constant remains relatively stable over a wide range of air temperatures, but not over the range of atmospheric pressures likely to be encountered by terrestrial animals at different altitudes (Mitchell, '74). Kerslake ('72) did not explicitly take account of atmospheric pressure. The convective heat transfer coefficient of a non-standard globe can be derived from the above formula by incorporating corrections for atmospheric pressure and differences in diameter, giving:

$$h_{cmg} = 14.1\left(\frac{P}{101.3}\right)^{-0.6}\left(\frac{Lmg}{150}\right)^{-0.4} V^{0.6},$$

where P is the barometric pressure (kPa).

Since the environmental mean radiant temperature is the same for globes of different diameters in the same environment, it can be calculated from their respective thermal equilibrium equations that:

$$\frac{h_{cmg}(T_{mg} - T_a)}{\sigma} + T_{mg}^4 = \frac{h_{csg}(T_{sg} - T_a)}{\sigma} + T_{sg}^4,$$

where T_{mg} is the equilibrium temperature of the miniglobe (K) and T_{sg} the equilibrium temperature of the standard globe (K).

Solving for the standard globe temperature gives:

$$T_{sg} = \left[\frac{\left(T_{mg}^3 + \frac{h_{cmg}}{\sigma}\right)T_{mg} - \left(\frac{h_{cmg} - h_{csg}}{\sigma}\right)T_a}{\left(T_{sg}^3 + \frac{h_{csg}}{\sigma}\right)}\right].$$

As a quartic equation, the equation cannot be solved easily by algebraic procedures, but by using modern computing power it can be solved by successive approximation. Thus, what the standard globe temperatures would have been, can be calculated from the temperature measured by a miniglobe of known diameter, dry-bulb temperature and wind speed, at a known barometric pressure. We wrote a software program to apply the heat transfer equations and to execute successive approximations, terminating when two successive approximations differed by less than 0.1 K, so as to calculate standard globe temperatures from that of a miniglobe of any diameter.[1]

Testing of the conversion algorithm

We conducted a laboratory study to compare the temperatures obtained from a single standard (150 mm diameter) black globe to the concurrently measured temperature of a single 30 mm diameter miniglobe, in the same environment. We assessed three methods to approximate standard globe

[1]The software was written as a macro in Microsoft Excel using the Microsoft Visual Basic Programming System and is freely available from the authors.

temperature from miniglobe temperature; (1) our algorithm as described above, (2) Hey ('68) approximation and (3) a conversion formula apparently used in commercially available personal heat stress monitors (Peters et al., '76).

Our 150 mm standard globes consisted of hollow, thin-walled copper spheres and the 30 mm miniglobes were hollow thin-walled bronze spheres (Press Spinning & Stamping Co., Cape Town, South Africa). Both globes were painted matt black and had a fine thermocouple suspended in the centre. The thermocouple voltage was referenced against an electronic ice point cell (Omega Engineering Inc., Stamford, USA) and recorded on a data logger (Hewlett-Packard data acquisition/control unit, HP 75000 series B). Each thermocouple was calibrated by water immersion against a precision thermometer (Quat 100, Heraeus, Hanau, Germany) to an accuracy of better than 0.1°C. The globes were mounted on a polyvinyl chloride base and placed along side each other in the centre of a 3 m × 3 m × 3 m climatic chamber, in which we could vary and control ambient dry-bulb temperature, wind speed and humidity. Ambient dry-bulb temperature was measured by an array of fine-tipped thermocouples placed in the free-stream environment above the globes, but not exposed to radiation. Wind speed was varied by changing the speed of a fan, and measured using a portable thermo-anemometer (Alnor GGA-65, Turku, Finland). Solar radiation was simulated using 500 W halogen lamps (Cixi Zhongfa lamps co., Ltd, Ningbo, China) placed such that both globes were irradiated equally. Net heat flow under radiation was measured at the globe surface using a calibrated Heat Flux Transducer (HA 12-18-5-P, Thermonetics corp, CA, USA) connected to a Fluke 8800A digital multimeter (Fluke Corporation, WA, USA).

A range of conditions were tested, with dry-bulb temperature (15–35°C), wind speed (0–4 m s^{-1}) and radiation (90–1,200 W m^{-2}) being varied systematically to simulate the range of conditions prevailing historically, during the summer months, at the field site where our animals were resident. The globes were allowed to stabilise to 0.1°C at a fixed combination of ambient dry-bulb temperature, wind speed and radiant temperature and then one of these variables was altered while the others kept constant. Globe temperatures were recorded for half an hour after an ambient variable was changed ("dynamic conditions"), and then for a further half an hour ("static conditions"). We also conducted a single test in an extreme condition unlikely to occur in the natural environment, but designed to challenge the validity of the algorithm, namely high wind speed (3.5 m s^{-1}), high radiation (1,148 W m^{-2}) and low dry-bulb temperature (6°C). In addition, we measured the response times of both the standard globe and miniglobe by initially allowing them to stabilise at 23°C dry-bulb and radiant temperature and then moving them simultaneously and quickly into a cold environment (dry-bulb and mean radiant temperature of 5°C) and, on a separate occasion, a hot environment (dry-bulb and mean radiant temperature of 35°C) and measuring the standard globe and miniglobe temperatures every 30 s. We set the wind speeds in the new environment at 0.3 and 35 m s^{-1}, on separate occasions. Time constants were calculated assuming an exponential approach to equilibrium.

Data analysis

The 95% limits of agreement (Bland and Altman, '86) for differences between actual miniglobe temperature and standard globe temperature were calculated, as well as between standard globe temperature and the standard globe estimates derived from Hey ('68) approximation, a conversion formula derived by the US National Institute for Occupational Safely and Health and apparently used in commercially available personal heat stress monitors (Peters et al., '76), and our algorithm and software.

Use of miniglobes to investigate thermoregulatory behaviour

Field site and animals used

The field test was performed in September 2003 (southern hemisphere spring) at the National Zoological Gardens Game Breeding Centre in Lichtenburg (26°07′S, 26°10′E, with an altitude of 1,450 m), 220 km west of Johannesburg, South Africa. We used an impala ewe (*Aepyceros melampus*, Lichenstein), a blue wildebeest bull (*Connochaetes taurinus*, Burchell) and a semi-domesticated female horse (*Equus caballus*, Linnaeus). All the animals were released into a 62 ha fenced enclosure, which consisted predominantly of flat and open grassland with a few scattered trees and shrubs.

To attach the equipment, the impala was chemically immobilised with 1 mg etorphine (M99, Logos Agvet, Johannesburg, South Africa) and 40 mg azaperone (Kyron Laboratories, Johannesburg, South Africa), and the wildebeest was

chemically immobilised with 4 mg etorphine (M99, Logos Agvet) and 100 mg azaperone (Kyron Laboratories). Collars holding a 30 mm miniglobe and tracking telemeter (Africa Wildlife Tracking, Pretoria, South Africa) were fitted to each animal (Fig. 1). Each collar was custom designed to weigh less than 1% of the animals' body mass and have a width of 30–50 mm. A weight at the base of the collar ensured that the miniglobe remained over the dorsum of the neck and could not be shaded by the animal. The effect of the etorphine then was reversed using 2 mg diprenorphine (M5050, Logos Agvet, Johannesburg, South Africa) for the impala and 8 mg diprenorphine (M5050, Logos Agvet) for the wildebeest. The animals became mobile within about 10 min. They were released into an enclosure, where they joined conspecifics to form herds of approximately ten individuals. The horse mare was restrained without sedation, collared and released into the same enclosure. All animals were recaptured after 1 month, using the same immobilisation procedure, the equipment removed and the animals released to rejoin their herds. The Animal Ethics Screening Committee of the University of the Witwatersrand approved the experimental procedures (clearance no. 2003/20/4).

The temperature of each miniglobe was measured with an uncoated bead thermistor (bead diameter 1.5 mm; 27–10K4A801, Onset Computer Corporation, Pocasset, MA, USA), positioned at the centre of the globe. The globe was attached to a 10 mm diameter, polyvinyl chloride rod, which in turn was attached to the outer surface of the collar. The rod provided thermal insulation of the miniglobe from the collar, and also housed the leads from the thermistor. Dental acrylic added strength to attachment points. The thermistor was connected to a miniature thermometric data logger (StowAway, Onset Computer Corporation), with a temperature range of −39 to +122°C, an intrinsic accuracy of 0.4°C and a mass of approximately 50 g, which was moulded into the collar (Fig. 1). All temperature sensors were calibrated against a certified precision thermometer (Quat 100, Heraeus, Hanau, Germany) in an insulated water bath. Globe temperatures were recorded every 5 min for the wildebeest and horse, and, because some of our loggers had a lower memory capacity, every 10 min for the impala, for a minimum of 2 weeks.

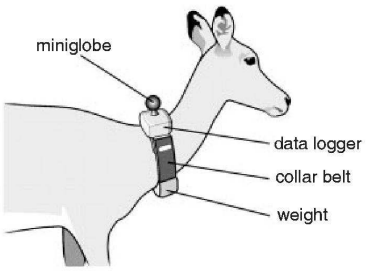


Fig. 1. Miniglobe thermometer attached to a collar and fitted on an impala.

We collected climatic data by erecting a portable weather station at the site (Hobo Weather Station, Onset Computer Corporation). We monitored wind speed, photosynthetically active radiation (PAR), dry-bulb temperature, relative humidity and standard (150 mm diameter) globe temperature, 1 m above the ground. In addition, we attached a miniglobe, identical to those on the collars, connected to a separate data logger (StowAway, Onset Computer Corporation), 1 m above the ground. Both globes on the weather station were situated on the northern side of the weather station so that they were never shaded by the remaining equipment. As wind speed can vary over short distances, an additional wind speed measurement was made in the vicinity of the animal, with a portable thermo-anemometer (Alnor GGA-65, Turku, Finland), to coincide with behavioural observations.

Behavioural observations

As the Game Breeding Centre was open to the public confined to slow-moving vehicles, the animals were accustomed to vehicle activity, so we could make behavioural observations from a vehicle, without impacting adversely on the animals' natural behavioural patterns. The herds were observed from inside a stationary vehicle parked between 10 and 250 m (typically 100 m) away, with binoculars (Nikon Travelite III 8 × 23CF). Behavioural data were collected by one observer (RSH) using instantaneous sampling methods (Altmann, '74), identifying the behaviour of the instrumented animals every 5 min, synchronously with the temperature measurement on the data loggers.

Behaviour was assigned to a sub-category within various categories, with each sub-category being mutually exclusive. The categories defined the animal's exposure to the sun (sun or shade), body posture (standing upright or lying recumbent), activity type (grazing with snout to ground;

walking, which was classified as moving with snout above ground; or little/no activity, which included time spent ruminating), and finally we defined wind exposure subjectively (exposed to or protected from the wind). Measurements for each individual were made for a minimum of 12 h, divided into 4-h sampling sessions. The timing of these behavioural sampling sessions was based on animal activity periods reported by Estes ('97) and Smithers ('83), to cover a variety of potential behaviours.

Data analysis

Miniglobe temperatures on the collars were compared to concurrent miniglobe temperatures from the nearby weather station and the animals' observed behaviour. We extracted the upper and lower quartile ranges of the miniglobe temperatures on the animal and the weather station and compared them using an Unpaired Student's *t*-tests. We also used the χ^2 test to test for associations between the likelihood of an observed behavioural category occurring when miniglobe temperature on the animal was both higher and lower than the stationary miniglobe on the nearby weather station.

We correlated weather station and animal miniglobe temperatures using linear Pearson procedures. Thereafter, we tested whether the slope of the regression equation was significantly different from one (the slope of the line of identity), to assess whether the globe temperature measured on the animals was the same as that measured at the exposed weather station, and so whether the animals were tracking ambient conditions or were selecting micro-environments. A nested analysis of variance (ANOVA) was used to test variability between the weather station and collar miniglobe temperatures on different time scales (Maloney et al., 2002). The analysis was performed over the following time scales: over all days for which data were obtained, over each day, over 4-h intervals, over each hour and at 20-min intervals. In all statistical tests values of $P < 0.05$ were considered significant.

RESULTS

Agreement between miniglobe and standard globe temperatures

Miniglobe temperatures were consistently lower than standard globe temperatures measured concurrently in the climatic chamber environment, in which dry bulb temperature, wind speed and radiation intensity were independently varied over a range of environmental conditions (Fig. 2A). Miniglobe temperatures converted to standard globe temperature by our algorithm cluster around the line of identity under both dynamic and stable conditions (Fig. 2B and C), indicating the potential for the converted miniglobe temperatures to predict actual standard globe temperatures without bias. However, the scatter around the line of identity was greater under dynamic (Fig. 2B) compared to stable conditions (Fig. 2C).

A plausible explanation for the less favourable agreement between actual standard globe temperature and those estimated from miniglobe temperature shortly after a change in conditions would be that the smaller-diameter globe responded more quickly to the changing environmental conditions. The miniglobe indeed did exhibit shorter response times (Fig. 3), with times to equilibration ($5 \times$ the time constant τ, i.e. the time taken to reach 99% of final temperature) being faster at high wind speeds. The time to equilibration of the standard globe placed at $0.3\,m\,s^{-1}$ wind speed was 25-min, compared to 20-min for the miniglobe, whereas at $3.5\,m\,s^{-1}$ wind speed, the equivalent times to equilibrium were 10 and 7.5-min, respectively. While we attribute most of the difference in time constant between the globes to the effect of size, we cannot rule out the possibility that the different thermal properties of the brass miniglobe compared to the copper standard globe had a small effect.

The accuracy of the different conversion algorithms proposed to estimate standard globe temperatures from miniglobe temperatures were assessed using the limits of agreement analysis (Bland and Altman, '86) (Table 1). The average difference between measured standard globe and the converted miniglobe temperatures was smaller under dynamic compared to static conditions, but the variability from environment to environment was much greater under dynamic conditions. All converted miniglobe temperatures reflected standard globe temperatures more accurately than did the raw miniglobe temperature. Under most environmental conditions, standard globe temperatures estimated with our algorithm were very similar to those achieved with Hey ('68) estimation, and closer to actual standard globe temperature than the algorithm of Peters et al. ('76). However, under extreme conditions of high radiation ($1{,}148\,W\,m^{-2}$) and wind speeds ($3.5\,m\,s^{-1}$)

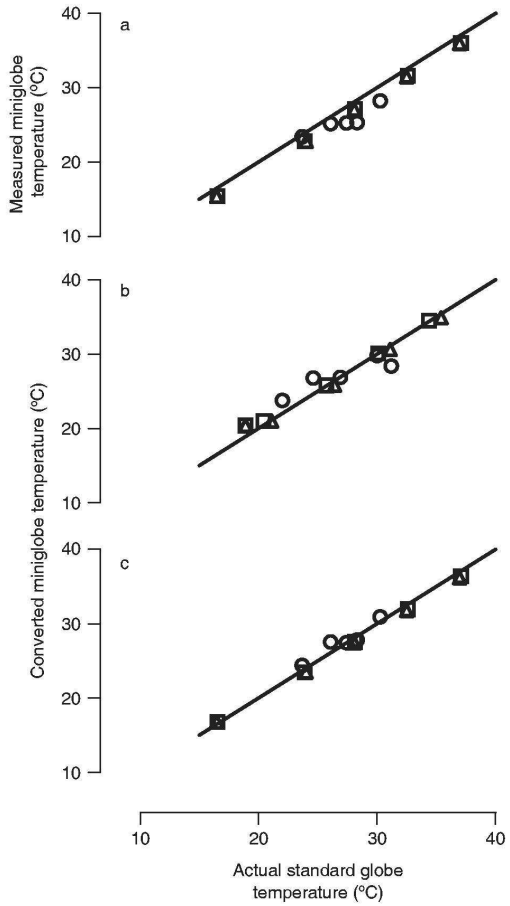


Fig. 2. Correlation between measured standard (150 mm diameter) globe temperature and measured miniglobe (30 mm diameter) temperatures (**a**), and between measured standard globe temperature and standard globe temperature estimated from miniglobe temperatures by our analysis of the heat transfer equations under dynamic conditions (**b**) (first half hour after changing ambient conditions) and under static conditions (**c**) (second half hour of measurements after a change in ambient conditions). Both globes were placed under the same environmental conditions in a climatic chamber and their temperatures measured while we independently varied dry-bulb temperature between 15 and 35°C (□), wind speed between 0 and 4 m s^{-1} (△) and radiation between 90 and 1,200 W m^{-2} (○). The solid lines are lines of identity ($y = x$).

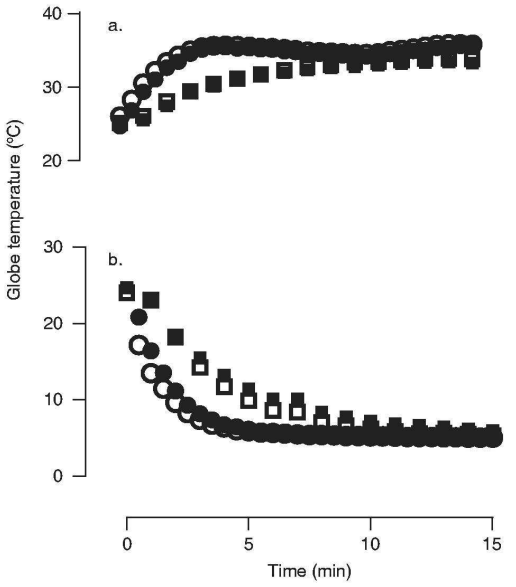


Fig. 3. Temperatures indicated by the standard globe (closed symbols) and miniglobe (open symbols) after their rapid transfer from a 23°C environment to a 35°C environment (**a**) and from a 23°C environment to a 5°C environment (b) on two occasions, with low wind speeds (0.3 m s^{-1} ■ and □) and high wind speed (3.5 m s^{-1} ● and ○). At low wind speed, calculated time constants were 6 and 7 min in the hot environment (upper panel), and 10 and 13 min in the cold environment (lower panel), for miniglobes and standard globes, respectively. At the high wind speed, time constants were reduced to 2 and 2.5 min in the hot environment (**a**), and 4 and 4.5 min in the cold environment (**b**), for miniglobes and standard globes, respectively.

accompanied by low temperatures (6°C), Hey ('68) estimation overestimated standard globe temperature by nearly 4°C, compared to only 1.8°C by our conversion algorithm. Under these extreme conditions and conditions of high radiation Peters et al. ('76) algorithm performed the best.

Use of miniglobes to investigate thermoregulatory behaviour

Field site environment

For the 1-month study period, average wind speed, dry-bulb air temperature and solar radiation varied as a function of time of day at the study site (Fig. 4). Wind speed was highest in the mid-morning, decreased progressively throughout the afternoon, but remained higher during the day than during the night. Solar radiation showed the expected bell-shaped distribution, with a mean peak of approximately 1,800 uE at 13:00, and a maximum exceeding 2,000 uE. The low standard deviations of solar radiation measurements were the consequence of predominantly cloudless skies. Hourly mean dry-bulb temperature ranged between 10 and 27°C, reaching an absolute

TABLE 1. Summary of the 95% limits of agreement (mean ± 1.96 SD) for the difference between standard globe temperatures and the raw miniglobe temperatures measured concurrently in the same environment, as well as standard globe temperatures estimated from miniglobe temperatures according to Hey ('68) approximation, Peters et al. ('76) conversion and our algorithm and software

	Raw miniglobe		Hey ('68) approximation		Peters et al. ('76) formula		Our algorithm and software	
	Static	Dynamic	Static	Dynamic	Static	Dynamic	Static	Dynamic
Temperature variation (15–35°C)	−1.04 ± 0.05	−0.82 ± 0.24	−0.42 ± 0.41	−0.12 ± 0.90	−0.59 ± 0.48	−0.39 ± 0.93	−0.42 ± 0.42	−0.11 ± 0.91
Wind variation (0–4 m s^{-1})	−1.06 ± 0.07	−0.82 ± 0.31	−0.46 ± 0.45	−0.12 ± 0.87	−0.63 ± 0.48	−0.35 ± 0.83	−0.45 ± 0.46	−0.11 ± 0.88
Radiation variation (90–1,200 W m^{-2})	−1.70 ± 1.10	−1.59 ± 1.16	0.67 ± 0.82	0.27 ± 0.91	0.48 ± 0.82	0.05 ± 0.91	0.73 ± 0.95	0.31 ± 0.92
Extreme conditions (6°C, 3.5 m s^{-1}, 1,150 W m^{-2})	−2.27 ± 0.58		3.80 ± 0.35		1.45 ± 0.42		1.77 ± 0.35	

We independently varied each of the three environmental variables under both static and dynamic conditions. Dynamic measurements were made at times 0, 10 and 20 min after the change in environmental conditions, whereas static conditions were averaged over 30–60 min following a change in environment.

minimum and maximum of 1 and 35°C, respectively. Hourly mean standard (150 mm diameter) black globe temperatures varied between 9 and 38°C, reaching an absolute minimum of 0°C and an absolute maximum of 45°C.

Ambulatory miniglobes on animals

Figure 5 shows typical examples of temperatures measured by identical miniglobes on the collar of an animal and on the fixed weather station, for the three species studied, together with records of behaviour made by direct observation. For all three species, ambulatory miniglobe temperature deviated from the miniglobe temperature on the fixed weather station for at least part of the day or night. On the days of observation, the temperature of the wildebeest's miniglobe was usually within 1°C of that recorded at the weather station (Fig. 5A). That agreement is reflected in the behavioural observations, with the wildebeest seeking shade only on two brief occasions and spending the majority of the day grazing in the sun. In the morning, however, the temperature of the miniglobe on the wildebeest exceeded that of the miniglobe on the weather station by up to 10°C just after 9:00 (see Fig. 5A). At this time the animal was protected from strong winds by assuming a lying down posture or taking refuge behind bushes. All periods when collar miniglobe temperatures exceeded the weather station miniglobe by more than 10°C were associated with high wind speeds at the weather station (3.0 ± 0.1 m s^{-1}). The mean standard globe temperatures of the microclimate selected in these circumstances was calculated, from the animal's miniglobe temperature, to be 31.7 ± 2.5°C, at a time when standard globe temperature on the exposed weather station averaged 22.3 ± 3.0°C.

The impala did not remain in any particular microclimate for long, however, even these transient changes in the behaviourally selected microclimate were reflected in corresponding changes in the animal's miniglobe temperatures (Fig. 5B). For example, impala miniglobe temperature decreased abruptly when the animal entered shade shortly after noon. The calculated standard globe temperature of the microclimate selected in the periods when collar miniglobe temperature was more than 5°C below the miniglobe at the weather station averaged 24.7 ± 0.8°C; the standard globe temperature on the weather station averaged 31.4 ± 1.3°C over the same time. The horse also entered shade on occasions. Fig. 5C shows a

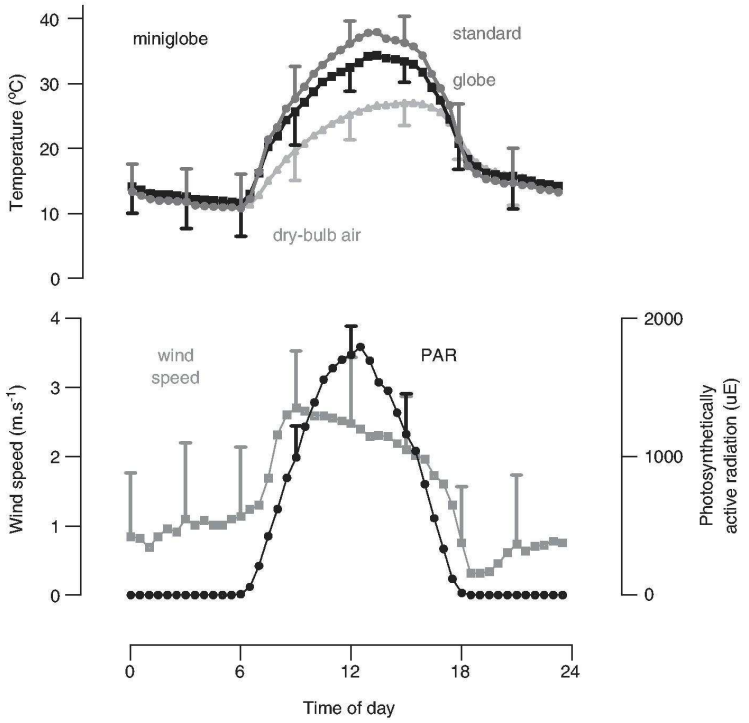


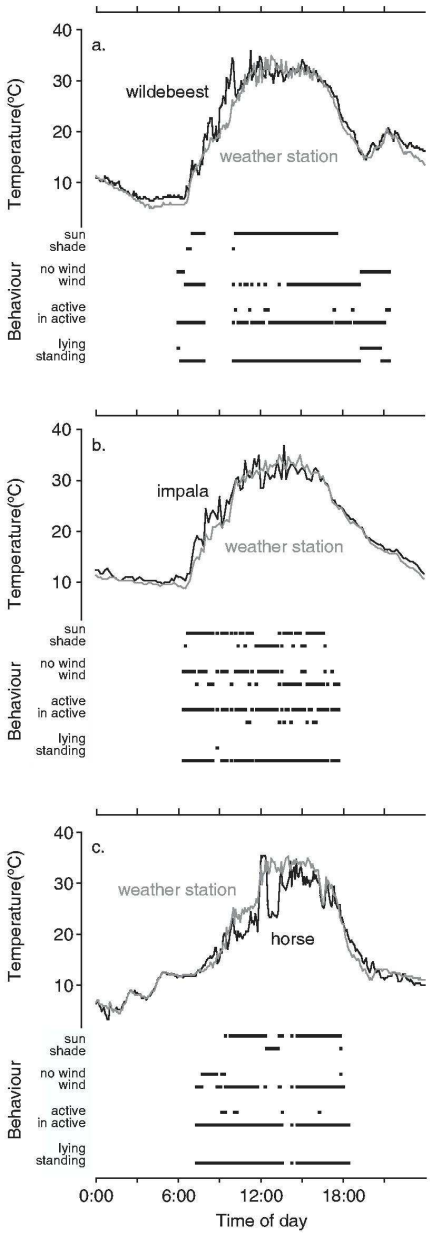
Fig. 4. Field site weather station data, showing the miniglobe, standard globe and air temperatures (upper panel), as well as photosynthetically active radiation and wind speed (lower panel), as a function of time of day, as mean and SD for the 1-month study period.

decrease of nearly 10°C in horse miniglobe temperatures, compared to the weather station miniglobe temperatures, on one such occasion when the animal entered shade at 12:25. The calculated standard globe temperature to which the horse was exposed just before shade seeking was 37°C, which decreased to 23.3°C on entering shade.

The examples shown in Fig. 5 reveal the potential for the use of the collar miniglobe as a biotelemetric measure of thermoregulatory behaviour. To confirm that potential quantitatively we plotted every measurement of collar miniglobe temperature against the weather station miniglobe temperature prevailing at the time and fitted regression lines to the data. The scatter of data around the lines of identity indicate how the animal miniglobe temperatures differed from those at the weather station. At low environmental temperatures, during the night, both impala and wildebeest scattergrams show an upward shift, consistent with the animals selecting microclimates warmer than that at the exposed weather station. During the heat of the day, the impala and horse often reduced their environmental heat load by seeking shade. This thermoregulatory behaviour was associated with a downward shift of the scattergram at the high environmental heat loads (Fig. 6B and C). Conversely, wildebeest miniglobe temperatures were higher, generally, than the weather station miniglobe temperatures throughout the day, and sometimes exceeded weather station miniglobe temperatures by more than 10°C (Fig. 6A). These shifts were consistent with our observation that the wildebeest sought protection from strong winds, and did not seek shade.

If animals use modification of their thermal environment to aid homeostasis, and if collar miniglobe temperatures reflect such a response, then the animal's miniglobe temperatures should be higher than the weather station miniglobe temperatures at low environmental heat loads, and/or lower than the weather station miniglobe temperatures at high environmental heat loads.

Such an outcome would be reflected in the slope of a regression line relating collar miniglobe to weather station miniglobe being significantly less than one. For all three animals studied, that proved to be the case ($F_{1,3240} = 10.18$, $P = 0.001$ for the wildebeest, $F_{1,1736} = 140.64$, $P < 0.0001$ for the impala, $F_{1,7616} = 588.30$, $P < 0.0001$ for the horse). However, such analysis provides no information on the elevation of the slope and further analysis may be necessary for a more complete understanding of thermoregulatory behaviour.



For the second quantitative analysis of collar miniglobe temperatures, as indices of thermoregulatory behaviour, we targeted the circumstances in which the environment of each animal was particularly hot, or particularly cold, by extracting the upper and lower quartiles of the weather station miniglobe temperatures, and then investigated whether there was a statistically significant difference between collar miniglobe temperature and weather station miniglobe temperature at that time. Upper quartile temperatures tended to occur around solar noon, and lower quartile temperatures at night. The temperature of the miniglobes on the collar of the wildebeest, impala, and horse all were significantly higher than that of the weather station miniglobe at the lower quartile range ($t_{760} = 12.26$, $P < 0.0001$, $t_{726} = 8.32$, $P < 0.0001$, $t_{1735} = 3.78$, $P = 0.0002$, respectively). These results are consistent with the animals selecting warmer environments, from amongst those accessible, at night. We were not able to observe the horse or impala at night, but observed the wildebeest lying close to the vegetation at night.

Although analysis of the lower quartiles yielded similar results between the species, interesting differences in the thermoregulatory behaviour observed were picked up by analysis of the upper quartile ranges. The wildebeest miniglobe temperatures were higher than those recorded at the weather station within the upper quartile ranges ($t_{793} = 10.74$, $P < 0.0001$), a result consistent with

Fig. 5. Temperatures of miniglobe on the collar of a wildebeest (**a**), impala (**b**) and horse (**c**) and an identical miniglobe on the weather station over a 24-h period, together with a record, obtained by direct observation, of potential thermoregulatory behaviours (sheltering from the sun or wind, level of activity and posture). Gaps in behaviour bars represent periods when either the animal was not visible or the behavioural category was invalid, e.g. when the sun set or the wind was not blowing. Note that the wildebeest (**a**) did not seek shade, and instead avoided wind, in the hottest part of the day reflected in a collar miniglobe temperature higher than that of the weather station. Conversely, both the impala (**b**) and horse (**c**) sought shade for about 2 h shortly after noon but the observed decrease in collar miniglobe temperature associated with shade-seeking behaviour was far more dramatic for the horse who also remained exposed to the prevailing wind.

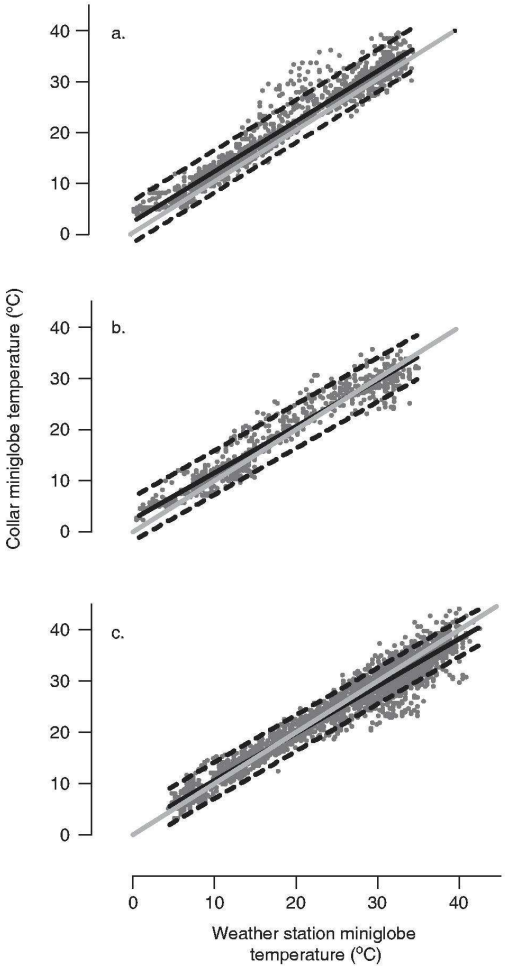


Fig. 6. Scatter diagram showing the relationship between miniglobe temperatures on the collars and that of an identical miniglobe on the weather station for wildebeest (**a**), impala (**b**) and horse (**c**). The solid grey lines are lines of identity, solid black lines are linear regressions and the dashed lines are the 95% prediction bands of the regression lines. A regression slope significantly less than one (the line of identity) was observed in all three species and implies thermoregulatory behaviour, that is the animal selecting cooler microclimates at high environmental temperatures and warmer microclimates at low environmental temperatures. For each animal, measurements were made within a 1-month period, at intervals of 5 min (wildebeest and horse) and 10 min (impala). Regression equations: wildebeest $y = 0.981x + 2.13$, $F_{1,3240} = 10.18$, $P = 0.001$, $r^2 = 0.94$; impala $y = 0.907x + 2.50$, $F_{1,1736} = 140.64$, $P < 0.0001$, $r^2 = 0.94$ horse $y = 0.922x + 1.39$, $F_{1,7616} = 588.30$, $P < 0.0001$, $r^2 = 0.96$.

the wildebeest seeking warmer microclimates from amongst those accessible. The impala miniglobe temperatures within the upper quartile range were not significantly different from weather station miniglobe temperatures ($t_{400} = 1.35$, $P = 0.17$), probably because of frequent transient changes in the impala's thermoregulatory behaviour. Nevertheless, a contingency analysis showed a significantly higher frequency of collar miniglobe temperatures below weather station miniglobe temperatures when the animal was in the shade ($\chi^2 = 13.43$, $P = 0.0002$). The horse miniglobe temperatures were significantly lower than the weather station miniglobe temperatures in the upper quartile range ($t_{1745} = 7.39$, $P < 0.0001$), a result consistent with the horse selecting cooler environments in the heat of the day. However, unlike the impala, which appeared to reduce its thermal load by seeking shade, the horse did not attain its cooler microclimate by consistently seeking shade ($\chi^2 = 2.35$, $P = 0.13$). Instead, the horse exhibited miniglobe temperatures lower than the weather station miniglobe temperatures significantly more often when it was exposed to the wind ($\chi^2 = 13.93$, $P = 0.0002$).

For the third quantitative analysis, we used nested ANOVAs to compare variability of collar miniglobe temperatures to that of weather station miniglobe temperatures. We found no difference in variability between collar miniglobe and weather station miniglobe temperatures overall for the wildebeest ($F_{1151,1151} = 1.01$, $P = 0.45$) or impala ($F_{575,575} = 0.88$, $P = 0.94$). However, the horse's collar miniglobe as a whole was significantly less variable than the weather station miniglobe temperature ($F_{2879,2879} = 1.08$, $P = 0.01$). As the horse sought protection from both wind and sun, this result supports the notion of behavioural thermoregulation through the selection of more stable microclimates. Further analysis into the variability over different time scales revealed no difference between collar and weather station miniglobe variability over the period of days ($F_{3,3} = 1.30$, $P = 0.42$; $F_{3,3} = 1.39$, $P = 0.40$ and $F_{9,9} = 1.14$, $P = 0.42$ for the wildebeest, impala and horse, respectively) or even 4 h intervals ($F_{20,20} = 1.01$, $P = 0.49$; $F_{20,20} = 1.17$, $P = 0.37$; $F_{50,50} = 1.00$, $P = 0.50$ for the wildebeest, impala and horse, respectively). All collar miniglobe temperatures, however, were significantly less variable than the weather station miniglobe over the shorter time interval of hours ($F_{72,72} = 1.57$, $P = 0.03$; $F_{72,72} = 1.65$, $P = 0.02$; $F_{180,180} = 1.34$, $P = 0.02$ for the wildebeest, impala and horse, respectively) implying that factors with a time course shorter than daily weather and circadian variation contributed to the variance. Over the

shortest epoch we analysed, 20 min, the variability manifested by the miniglobe on the collar of the horse ($F_{480,480} = 1.51$, $P < 0.0001$) and impala ($F_{192,192} = 1.56$, $P = 0.001$), was less variable than the miniglobe temperatures on the weather station, while those of the wildebeest tended to be more variable ($F_{192,192} = 1.23$, $P = 0.08$).

DISCUSSION

Our study had two objectives. The first objective was to test whether we could successfully predict standard (150 mm diameter) globe temperature from miniglobe temperature, using an algorithm derived from heat transfer equations for spheres of different diameters. The correlations in Fig. 2, together with the 95% limits of agreement in Table 1, demonstrate that the algorithm was successful, with an average accuracy better than 1°C over a wide range of environmental conditions, and often better than 0.5 °C. Although others (e.g. Peters et al., '76) had compared predicted and actual globe temperatures previously, no-one had explored a comprehensive range of ambient conditions previously, and no-one had deliberately manipulated several environmental variables systematically. The accuracy of our conversion algorithm and that of the earlier approximation of Hey ('68) should be sufficient for most research purposes. Under extreme conditions of high radiation and wind speeds accompanied by low dry-bulb temperatures, some limitations in Hey ('68) approximation were revealed (Table 1). The algorithm of Peter's et al. ('76) proved less accurate overall than either of the other conversion routines, but was better than the others under extreme conditions (Table 1). We know that Peters et al. ('76) corrected their convective heat transfer coefficient for changes in air temperature, whereas we corrected for changes in barometric pressure, because we wanted our algorithm to be applicable at different altitudes. We have not been able to determine whether commercially available human heat stress monitors, that use globes of non-standard diameter, correct for that parameter.

The reason that we, and others, derived conversion algorithms for the miniglobe was to allow comparisons between the temperatures of a miniglobe and those of the standard globe, with which many researchers are familiar. The experiments which we conducted, in a climatic chamber, to check the accuracy of the algorithm, revealed why commercial instruments may be reasonably accurate despite not correcting for differences in diameter. In most circumstances the average temperature indicated by a 30 mm diameter globe was within 2°C of the 150 mm diameter globe.

Although the miniglobe exhibited a slightly shorter response time compared to the standard globe (Fig. 3), both globes were relatively slow to reach equilibrium. These time constants, however, were similar to those found after improvements were made to globe thermometers in the middle of the last century and further reductions in the thickness of the globe is unlikely to improve the time constant (Hellon and Crockford, '59). Although a slow response time for the globe may mean that short-term changes in the thermal environment are not recorded, the thermal inertia of the animal is much larger than that of the globe, reducing the overall impact of such short-term microclimate selection on the animal's heat balance.

Our second objective was to test whether miniglobes could be attached to an animal to provide a quantitative measure of thermoregulatory behaviour, in the form of microclimate selection. For that purpose we compared the temperature of the miniglobe on the collar with that of an identical miniglobe on a nearby exposed weather station. Thus, a lower miniglobe temperature on the animal during the day would result if the animal behaviourally reduced its radiation exposure by seeking shelter from direct solar radiation. A higher miniglobe temperature on the animal would result if the animal sought shelter from strong winds, while remaining in the sun. Fig. 5 graphically represents how differences between the weather station and animals' miniglobe temperatures correspond to various behavioural observations. Further quantitative analysis revealed the capacity of the miniglobe to reflect thermoregulatory behaviour.

The elevation in the slope of the collar versus weather station miniglobe correlation in the wildebeest (Fig. 6A) and the greater variability over 20-min epochs, revealed that the animal selected microclimates warmer than the weather station in both hot and cold environments. The impala's transient shade-seeking behaviour was reflected in rapid changes in miniglobe temperatures (Fig. 5B) with a significantly higher frequency of collar miniglobe temperatures dipping below weather station miniglobe temperatures when the animal was in the shade. The downward shift in the correlation graph at high environmental heat loads (Fig. 6B), together with a slope significantly

less than one, provides further support for observations of the impala seeking cooler microclimates at high environmental heat loads. For the horse, the selection of cooler microclimates at high environmental heat loads was revealed through the horse's selection of a significantly cooler microclimate during the heat of the day, defined by the upper quartile range, and a regression slope significantly less than one (Fig. 6C). The horse, like the impala, was seen to seek shade.

Miniglobes thus appear to detect changes in the selected microclimate, consistent with the behaviour we observed during the day, for example, when the animals entered shade or were protected from wind. We were unable to observe the animals throughout the night, but comparison of lower quartile temperatures revealed that all three species selected warmer microclimates than the free-stream environment at night. This warmer microclimate may have arisen from high infrared thermal radiation from vegetation or by close association with conspecifics. Recumbency is an additional behavioural adjustment which can provide a warmer microclimate by allowing an animal to exploit the boundary layer of the ground, thus reducing convective heat loss due to the increased air temperature and decreased wind temperature closer to the ground (Bakken '92; Bustamante et al., 2002; Maloney et al., 2005a).

Although it may be beneficial for the animal to reduce convective heat loss at night by seeking protection from wind, such behaviour would not be reflected by an increase in collar miniglobe temperature if the animal were exposed to a clear night sky. The situation becomes complex because the radiation temperature of the night sky can be as low as −30°C. Exposed to such low radiation temperatures, a low wind speed would result in globe temperature close to the cold radiation temperature of the night sky, while increases in wind speed would increase globe temperature closer to the dry-bulb temperature. Thus the effect of changes in wind speed on globe temperature varies depending if radiation temperature is higher or lower than dry-bulb temperature.

While we have identified that miniglobe temperatures on an animal can indicate thermoregulatory behaviours, a major shortcoming of the technique is that a globe thermometer provides no measure of the ambient water vapour pressure, which is the main determinant of the gradient for evaporative cooling. In addition, a single miniglobe, attached to collars, provides no measure of thermoregulatory behaviours such as postural adjustments, nor the animal's orientation to wind or sun, which may profoundly affect radiant heat absorption (Mitchell et al., '97; Maloney et al., 2005b). Miniglobe temperatures also cannot reflect changes in surface areas in contact with a heat source or heat sink. For example, a standing animal exposes a greater surface area to the wind, and would lose heat more rapidly, than would a recumbent animal (Fuller et al., '99), but neither choice would be reflected in a change in miniglobe temperature.

Although miniglobes may have some shortcomings, the method provides the first, to our knowledge, data using an ambulatory technique to quantify thermoregulatory behaviour in free-living animals. A similar method was previously attempted by Fuller et al. ('99), but in that study the miniglobes were attached to the horns of eland (*Tragelaphus oryx*). The globes were dislodged from horns after only 10 days. Attaching the miniglobes to collars therefore appears to provide a more durable alternative. In both this study and that of Fuller et al. ('99), miniglobe temperatures at night regularly exceeded those recorded at a nearby weather station. However, unlike the results obtained in this study, Fuller et al. ('99) found globe temperatures on the animals to be consistently lower than that of a nearby weather station between 10:00 and 17:00. The arid-adapted eland may be more dependent on thermoregulatory behaviour as the cost of water loss through evaporative cooling mechanisms is high in their extreme thermal environments. Quantifying such differences in thermoregulatory behaviour between species and environments is a valuable future objective.

Traditionally, the study of behavioural thermoregulation in free-living animals has relied on observational studies. In many instances the presence of an observer can disrupt normal animal behaviour (Recarte et al., '98). In addition, the thermoregulatory importance of behaviour may easily be misinterpreted because temperature regulation is not the only objective of postural adjustments. A good example is that the stereotypic postures of the lizard (*Angolosaurus skoogi*), were initially attributed to thermoregulatory behaviour, but later found to serve the purpose of urination and defecation (Seely et al., '88). Huddling behaviours often have been associated with increased heat load through increased infrared thermal radiation (Finch, '72), however, this clustering may instead represent an anti-predatory defence, in which individuals use

each other as living shields for protection, termed "selfish herd" theory (Alcock, '98). It is therefore essential to develop a method that is remote and provides a quantitative measure of the heat load in the animal's microclimate, to accurately define the thermoregulatory benefit of each behaviour.

In summary, using miniature globe thermometers, attached to free-ranging ungulates, we were able to remotely quantify the thermal environment of an animal. Shade-seeking behaviour and the selection of wind-protected sites was reflected in animal miniglobe temperatures lower or higher, respectively, than those recorded at a nearby weather station. We also used a new algorithm to successfully predict standard globe temperature from miniglobe temperature. Although the difference between the measured miniature and standard globes usually was small (less than 2°C), converting miniglobe temperatures using our algorithm, to standard globe temperatures, allows for accurate comparative studies. We believe that the miniglobe provides an important advance towards developing a complete miniature weather station, which can be attached to free-ranging animals to assess their behaviourally selected microclimates. With current advances in technology, we may ultimately be able to incorporate miniature anemometers and humidity sensors to provide a more complete measure of all the physical components of the environment that influence an animals' heat balance and how thermoregulatory behaviour influences heat balance via the microhabitat an animal chooses to occupy.

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Appendix 2

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animal

Shearing at the end of summer affects body temperature of free-living Angora goats (*Capra aegagrus*) more than does shearing at the end of winter

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Angora goats are known to be vulnerable to cold stress, especially after shearing, but their thermoregulatory responses to shearing have not been measured. We recorded activity, and abdominal and subcutaneous temperatures, for 10 days pre-shearing and post-shearing, in 10 Angora goats inhabiting the succulent thicket of the Eastern Cape, South Africa, in both March (late summer) and September (late winter). Within each season, environmental conditions were similar pre-shearing and post-shearing, but September was an average 5°C colder than March. Shearing resulted in a decreased mean ($P < 0.0001$), minimum ($P < 0.0001$) and maximum daily abdominal temperature ($P < 0.0001$). Paradoxically, the decrease in daily mean ($P = 0.03$) and maximum ($P = 0.01$) abdominal temperatures, from pre-shearing to post-shearing, was greater in March than in September. Daily amplitude of body temperature rhythm ($P < 0.0001$) and the maximum rate of abdominal temperature rise ($P < 0.0001$) increased from pre-shearing to post-shearing, resulting in an earlier diurnal peak in abdominal temperature ($P = 0.001$) post-shearing. These changes in amplitude, rate of abdominal temperature rise and time of diurnal peak in abdominal temperature suggest that the goats' thermoregulatory system was more labile after shearing. Mean daily subcutaneous temperatures also decreased post-shearing ($P < 0.0001$), despite our index goat selecting more stable microclimates after shearing in March ($P = 0.03$). Following shearing, there was an increased difference between abdominal and subcutaneous temperatures ($P < 0.0001$) at night, suggesting that the goats used peripheral vasoconstriction to limit heat loss. In addition to these temperature changes, mean daily activity increased nearly two-fold after March shearing, but not September shearing. This increased activity after March shearing was likely the result of an increased foraging time, food intake and metabolic rate, as suggested by the increased water influx ($P = 0.0008$). Thus, Angora goats entered a heat conservation mode after shearing in both March and September. That the transition from the fleeced to the shorn state had greater thermoregulatory consequences in March than in September may provide a mechanistic explanation for Angora goats' vulnerability to cold in summer.

Keywords: Angora goats, body temperature, shearing, fleece

Implications

Shearing in late summer imposed a significant thermoregulatory stress on our Angora goats, which may help to explain their summer vulnerability to cold weather; in March 2007 an estimated 20 000 Angora goats died in the Eastern Cape of South Africa during a cold, wet spell. We propose that if fleeced animals are not acclimatized to cold, they may actually become more susceptible to cold stress after shearing. Such findings have animal welfare and management implications; investigating the appropriate times of year for shearing would improve husbandry and help to minimize goat mortalities from hypothermia, ultimately improving profitability of the mohair industry.

Introduction

Inclement weather results in the death of hundreds of thousands of sheep annually (Hutchinson and Bennett, 1962; Hutchinson, 1968), particularly if a cold spell occurs

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after shearing (Glass and Jacob, 1992). In an attempt to reduce such high mortalities, the annual shearing of sheep usually is timed to coincide with favorable climatic conditions (Dýrmundsson, 1991). Unlike Merino sheep, Angora goats usually are shorn twice a year, at approximately 6-month intervals. Although increasing the frequency of shearing Angora goats has advantages for fleece production and quality (McGregor and Butler, 2008a), it increases the risk of hypothermia as goats are more likely to be exposed to inclement weather at a time when their fleece insulation is reduced. Although it is recognized that high mortality among Angora goats during periodic cold spells is a serious problem in the mohair industry (Wentzel *et al.*, 1979), little is known about the thermoregulatory consequences of shearing these animals.

Angora goats may be more vulnerable than other livestock species to cold as a result of their smaller body mass, thinner subcutaneous fat deposits (Shelton, 1993; McGregor, 2001), larger surface area for cooling, and labile rectal temperatures (Hofmeyr *et al.*, 1965; Johnson, 1971; McGregor, 1985). Compared to Merino sheep, the fleece of Angora goats is more lustrous (Cena and Monteith, 1975), has a lower grease content, a lower fiber density per unit area of skin and a lower thermal insulation, thus allowing greater penetration of air and wetting of the skin (McGregor, 1985 and 2001). Such differences may explain why sheep remain susceptible to death for 2 weeks following shearing (Panaretto, 1967; Hutchinson, 1968; Donnelly *et al.*, 1974), compared to nearly 6 weeks for Angora goats. Indeed, when exposed to the same environmental conditions, the risk of hypothermia and mortality following shearing was higher for Angora goats than for Merino sheep (McGregor and Butler, 2008b). However, the known characteristics of the thermoregulatory system of Angora goats do not explain the catastrophic mortalities that occur if unseasonal cold spells occur in the summer, since those summer cold spells are milder than normal winter cold. We therefore measured the effect of September and March shearing on the body temperature and activity of free-living Angora goats in the Eastern Cape, South Africa.

Material and methods

Animals and habitat

Ten neutered male Angora goats (*Capra aegagrus*) (body mass 39.2 ± 3.5 kg) from Blaauwkrantz farm (33°32′S 25°23′E, at an altitude of 75 m above sea level), near Port Elizabeth in the Eastern Cape Province, South Africa, were used. They remained on the farm for the duration of the study. In July 2005 the goats underwent surgery (detailed below) before being released into a 50 ha rectangular paddock consisting of natural vegetation on undulating terrain. The vegetation in the area has been classified as Sundays Spekboomveld (Vlok *et al.*, 2003). This dense, semi-succulent, thorny vegetation consists primarily of perennial shrubs and trees, and is largely dominated by the tree-succulent, *Portulacaria afra*. Except for the naturally occurring trees and shrubs, no specific shelter was provided for the goats. Goats grazed on the natural vegetation and had access to fresh water *ad libitum*, supplied via a single drinking trough located in an open area near the perimeter of the camp. Experimental procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (protocol no. 2005/45/4).

Surgery

Anesthesia was induced in the goats by i.m. injection of 2.5 mg/kg ketamine hydrochloride (Anaket-V; Bayer Animal Health (Pty) Ltd, Isando, South Africa) and 0.04 mg/kg medetomidine hydrochloride (Domitor; Novartis South Africa (Pty) Ltd, Kempton Park, South Africa). Anesthesia was maintained with 1–4% halothane (Fluothane; Astra Zeneca, Johannesburg, South Africa) administered in 100% oxygen via a facemask. After ~10 min of halothane administration, the action of medetomidine was reversed with 0.2 mg/kg i.m. atipamezole hydrochloride (Antisedan; Novartis South Africa (Pty) Ltd). Respiratory rate, heart rate, percentage hemoglobin oxygen saturation (8500 Digital Handheld Pulse Oximeter; Nonin Medical Inc., Plymouth, MN, USA) and rectal temperature (laboratory digital thermometer, Physitemp, Model BAT-12; Sensortek Inc., Clifton, NJ, USA) were monitored throughout the surgical procedure, which lasted ~20 min.

Under sterile surgical conditions, a suite of miniature data loggers was implanted. All data loggers were covered in an inert wax (Sasol wax EXP986; Sasol Chemical Industries Ltd, Johannesburg, South Africa) and dry-sterilized in formaldehyde vapor before implantation. Incision sites were shaved and sterilized with chlorhexidine gluconate (Hibitane; Astra Zeneca). In all 10 goats, a 70 mm cranial-caudal incision was made through the skin and *linea alba*, within the midline area on the ventral abdominal surface, and a miniature temperature-sensitive data logger (see temperature measurements) was placed into the abdominal cavity, where it was free to migrate within the cavity. A single goat in the herd also received an intra-abdominal tracking transmitter (African Wildlife Tracking, Pretoria, South Africa). The skin and muscle layers then were sutured closed. In five of the 10 goats, an additional incision was made on the caudal aspect of the upper hind leg, after additional administration of a local anesthetic (0.04 g lignocaine hydrochloride, s.c.; Bayer Animal Health Pty), and a smaller temperature-sensitive data logger (see temperature measurements) was implanted subcutaneously. At the same subcutaneous site, these five goats also had an activity logger (Actical; Mini-Mitter Corporation, Bend, OR, USA) implanted.

All surgical wounds were treated with a topical antiseptic spray (Necrospray; Centaur Labs, Johannesburg, South Africa) and coated with a topical tick-repellent grease (cypermethrin 0.025% m/m; Bayer Animal Health Pty). Each goat received a long-acting antibiotic (500 mg i.m. penicillin; Peni LA Phenix, Virbac Animal Health, Centurion, South Africa), an analgesic and anti-inflammatory medication (420 mg i.m. ramiphenazone, Dexa-Tomanol; Centaur Labs), as well as a long-acting parasiticide (5 mg s.c., doramectin, Dectomax; Pfizer Laboratories (Pty) Ltd, Sandton, South Africa).

Before halothane administration was terminated, animals were marked with colored plastic ear tags, which enabled us to identify individual animals, and neck collars were fitted to five goats. The neck collars were 50 mm wide and were individually cut for each goat depending on its neck diameter. Each collar supported a miniature black globe thermometer ('miniglobe'), which allowed for the dynamic measurement of the microclimate that the goat chose to occupy. This technique has proven successful on other ungulate species (Hetem *et al.*, 2007). Miniglobe temperature was measured by a small temperature-sensitive data logger (see temperature measurements) inserted into the center of a matt-black hollow brass sphere (30-mm diameter; Press Spinning & Stamping Co., Cape Town, South Africa). The globe was attached to a 10-mm diameter, polyvinyl chloride rod, which in turn was attached to the outer surface of the collar.

After recovery from surgery, the goats were released into their paddock where they roamed freely for a 1-year period. Goats behaved similarly and remained as a herd. Following standard management practices of the farm, goats were shorn in mid-September 2005 and again, 6 months later, in early March 2006, the end of austral winter and summer, respectively.

In August 2006, the goats once again were anesthetized and the data loggers were removed using surgical procedures similar to that used for the original implantation. The data loggers showed no obvious defects, the animals' wounds had healed and there were no signs of infection from the initial surgery. However, four of the miniglobe thermometers had broken off the collars and three of the activity sensors were broken. After surgery the goats were returned to Blaauwkrantz farm.

Temperature and relative activity measurements

The miniature data loggers (StowAway XTI; Onset Computer Corporation, Pocasset, MA, USA), used to measure abdominal temperature, had outside dimensions of $\sim 50 \times 45 \times 20\,mm^3$ and a mass of $\sim 40\,g$ when covered in wax. The data loggers had a storage capacity of 32 kb and measured temperatures within the range of +34 to +46°C, at a resolution of 0.04°C. The loggers measuring abdominal temperature recorded at 20-min intervals. The subcutaneous and miniglobe temperatures were recorded every hour with a smaller thermometric data logger (i-Button DS1922T, Maxim; Dallas Semiconductor, Dallas, TX, USA), which had a diameter of $\sim 25\,mm$, a height of 15 mm and weighed $\sim 10\,g$ when covered in wax. These loggers had a resolution of 0.5°C and a measurement range from 0 to 125°C. All the loggers were calibrated individually, in an insulated water bath, against a high-accuracy thermometer (Quat 100; Heraeus, Hanau, Germany).

The activity loggers recorded gross body movement at 10-min intervals, had dimensions of $\sim 40 \times 40 \times 15\,mm^3$ and weighed $\sim 40\,g$ when covered in wax. Activity counts were converted to relative activity units by calculating activity counts as a percentage of maximum counts for each logger.

Meteorological data measurements

We collected climatic data by erecting a portable weather station (Hobo Weather Station; Onset Computer Corporation) at the field site, in an open area that was not shaded or protected by vegetation. We recorded wind speed (m/s), solar radiation (W/m^2), as well as standard (150-mm diameter) and miniature (30-mm diameter) black globe temperature (°C) at hourly intervals.

Water influx and turnover rate

Many thicket plant species retain their forage potential throughout the year (Stuart-Hill and Aucamp, 1993). Indeed, despite high rainfall in November, the relative forage availability was similar throughout the year of our study. The vegetation on the field site was dominated by succulents (November 46%; July 36%), woody shrubs (November 29.5%; July 31.5%) and grasses (November 23.8%; July 30.3%) and goats utilized forage in proportion to availability in both seasons (Milne, 2008). We assessed water influx and water turnover rates, by dilution of the stable isotope deuterium oxide, for 9 days before and 9 days after shearing in March. Before the isotope was administered, animals were weighed and 10 ml blood samples were taken to determine background enrichment of deuterium oxide. Animals then received an i.m. dose of 0.04 ml/kg deuterium oxide (D_2O, 99.8 at%; Merck & Co. Ltd, Rahway, NJ, USA). A second 10 ml blood sample was taken 4 h after injection, once the administered deuterium oxide had equilibrated with body water. The goats' drinking water source was removed on the day after these blood samples were taken. Goats were yarded, weighed and 10 ml blood samples were taken 3, 5, 7 and 9 days later. Water was returned after the blood samples had been taken on the 7th day. The blood samples were collected from the jugular vein with Vacutainers (BD Diagnostics-Preanalytical Systems, Plymouth, UK), then placed in ice and centrifuged (Wifug Ltd, Bradford, England) at $6000 \times \mathbf{g}$ for 10 min. Serum was separated and stored in 2 ml vials (cryogenic vials 430489; Corning Inc., New York, NY, USA) and frozen at −20°C for later analysis. Serum total protein, osmolality and glucose concentrations were analyzed by the Department of Companion Animal Clinical Studies section of Clinical Pathology, University of Pretoria, Pretoria, South Africa.

Deuterium concentration of the serum was measured on a high-temperature elemental analyzer (Flinnigan Elemental Analyser; Thermo Electron Corporation, Bremen, Germany), normalized against an international reference, namely Vienna-Standard Mean Ocean Water (V-SMOW). A multi-point method was used (Fusch, 2000) and a regression line fitted to the log-transformed data to establish rate constants for deuterium dilution. The *y*-intercept of the curve at time zero was used to estimate isotope distribution space. The total body water was derived from the known dose of injected deuterium oxide divided by the difference between isotope dilution space and the background enrichment of deuterium oxide. Water influx was determined from the average rate of deuterium oxide dilution over the 7-day period, when goats did not have access to water, multiplied by the total body water and

corrected for body mass. Water turnover was determined as the sum of water influx and total weight loss, as all weight loss was assumed to be water loss.

Data analysis

We averaged temperature data for 10 days before shearing and separately for 10 days after shearing, hereafter referred to as 'pre-shearing' and 'post-shearing', respectively, for each individual within the September and March shearing periods. We used a two-way repeated measures analysis of co-variance (ANCOVA), with live body mass of the shorn goat as a co-variant, to test for differences in the temperature profiles of the goats between the pre-shearing and post-shearing periods and across the two seasons. We also tested for differences in the time of minimum and maximum body temperature, as well as differences in the maximum rate of body temperature rise, of the pre-shearing and post-shearing periods. To test for seasonal differences in the difference between abdominal and subcutaneous temperature, we calculated the average temperature difference for all readings between 2100 and 0600 h for each goat within the September and March shearing periods and performed a two-way repeated measures ANCOVA, with live body mass of the shorn goat as a co-variant. We used a two-way repeated measures ANOVA to test for differences in blood variables between the pre- and post-shearing periods across the 9-day period of dehydration and rehydration in March. We used a two-way ANOVA to test for differences in weather conditions between pre-shearing and post-shearing periods across seasons. Newman–Keuls multiple comparisons tests were used to identify sources of significant differences in ANOVAs. We used paired Student's *t*-tests to investigate differences in water influx and water turnover rates of the goats pre- and post-shearing, during March.

To assess whether the animals were selecting microclimates different from those at the exposed weather station, we tested whether the slope of a linear regression line, fitted to the correlation of miniglobe on the index goat to weather station miniglobe temperature, was significantly different from one (the slope of the line of identity). In addition, we tested whether the slope and elevation of the regression line were significantly different between pre- and post-shearing periods using an ANCOVA. Data are expressed as mean ± s.d. and $P < 0.05$ was considered to be statistically significant.

Results

Climate

Black globe temperature peaked just after solar noon and reached a minimum just before sunrise. Solar radiation showed the expected bell-shaped distribution, and wind speed increased in the late afternoon. The September conditions were mild for the region, and March temperatures were ~5°C warmer than during September (Table 1). Environmental conditions compared between the pre- and post-shearing periods were not significantly different, in either season ($P > 0.05$, Table 1).

Abdominal temperature

Figure 1 shows, for a single goat, the decrease in abdominal temperature after shearing in both September and March. Abdominal temperatures remained lower than pre-shearing temperatures for weeks, even up to 3 months, after shearing, only gradually returning to pre-shearing values. Mean daily abdominal temperature decreased significantly from pre- to post-shearing in both seasons (Table 2 and Figure 2). The significant interaction between shearing and season revealed that 24-h mean abdominal temperature decreased significantly more after shearing in March (~0.6 ± 0.3°C) than in September (~0.4 ± 0.2°C, Table 2). Mean daily maximum abdominal temperature decreased from pre- to post-shearing in both seasons (Table 2). The interaction between shearing and season was significant, revealing that the decrease in mean daily maximum abdominal temperature post-shearing was greater in March than in September (Table 2). Mean daily minimum abdominal temperature decreased from pre- to post-shearing in both seasons (Table 2), with the decrease being similar in March and September.

A significant seasonal effect showed that the daily amplitude of the abdominal temperature rhythm was higher in September than in March. After shearing, mean daily minimum abdominal temperature decreased more than did mean daily maximum, which resulted in a significant increase in the mean daily amplitude of abdominal temperature rhythm

Table 1 *Environmental conditions (mean ± s.d.) for the 10-day periods pre- and post-shearing, in both September and March (there was no difference between pre- and post-shearing periods within each season; however, March was ~5°C warmer than September)*

	September		March		
	Pre-shearing	Post-shearing	Pre-shearing	Post-shearing	Statistics (March *v.* September)
Black globe temperature (°C)					
24-h mean	20.3 ± 2.5	18.2 ± 2.2	25.3 ± 4.0	22.2 ± 1.4	$F_{1,18} = 24.5$, $P = 0.0001$
Mean daily minimum	10.6 ± 2.9	8.7 ± 1.8	14.8 ± 4.6	12.7 ± 3.2	$F_{1,18} = 13.7$, $P = 0.002$
Absolute minimum	6.7	5.3	7.9	7.1	
Mean daily maximum	34.4 ± 6.5	32.0 ± 5.6	39.5 ± 7.9	35.9 ± 3.5	$F_{1,18} = 7.0$, $P = 0.02$
Absolute maximum	44.2	41.3	49.1	43.9	
Maximum daily solar radiation (W/m^2)	705 ± 105	816 ± 229	892 ± 222	725 ± 282	$F_{1,18} = 0.6$, $P = 0.46$
Maximum daily wind speed (m/s)	3.8 ± 0.6	3.8 ± 1.1	4.4 ± 0.7	4.0 ± 1.0	$F_{1,18} = 2.0$, $P = 0.17$

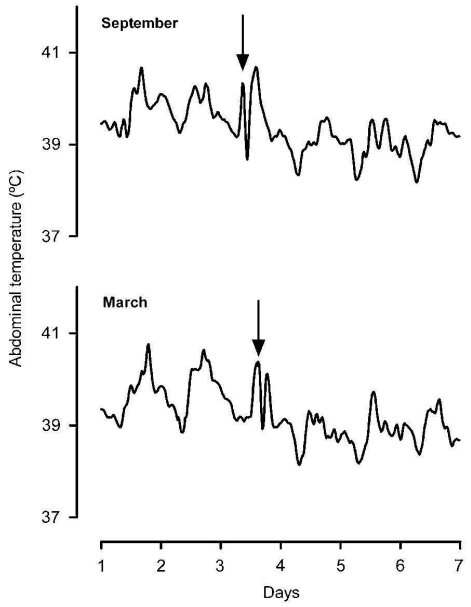

Figure 1 Nychthemeral rhythm of abdominal temperature of a representative Angora goat over a 6-day period around the time of shearing in both September (upper panel) and March (lower panel). The arrow represents time of shearing.

post-shearing, compared to pre-shearing (Table 2). A significant interaction between shearing and season showed that the increase in mean daily amplitude of abdominal temperature rhythm post-shearing was greater in September ($\sim 0.6 \pm 0.2$°C) than it was in March ($\sim 0.3 \pm 0.1$°C). This higher amplitude after the September shearing was accompanied by a faster rate of rise in abdominal temperature both during September compared to March and post-shearing compared to pre-shearing. Consistent with this faster rate of abdominal temperature rise, the peak of abdominal temperature was reached approximately 1 h earlier during the post-shearing period than during the pre-shearing period, in both seasons (Table 2 and Figure 2). However, the time of nadir of abdominal temperature was the same before and after shearing, in both seasons (Table 2).

Subcutaneous temperature

Subcutaneous temperatures decreased significantly post-shearing compared to pre-shearing in both seasons (Figure 3). There was an interaction between shearing and season with 24-h mean subcutaneous temperature decreasing more after the March shearing ($\sim 1.4 \pm 0.1$°C) than after the September shearing ($\sim 0.9 \pm 0.4$°C; Table 2). Mean daily maximum and minimum subcutaneous temperatures decreased from pre- to post-shearing in both seasons (Table 2). The significant interaction between shearing and season revealed a greater decrease in mean daily minimum subcutaneous temperature after the March shearing (Figure 3, lower panel) than after the September shearing (Figure 3, upper panel). The disproportionate decrease in mean daily minimum and mean daily maximum subcutaneous temperatures resulted in a significant increase in the mean daily amplitude of subcutaneous temperature from pre- to post-shearing in both seasons (Table 2).

The greater decrease in subcutaneous temperatures than abdominal temperature, from pre- to post-shearing, resulted in a greater 24-h mean difference between abdominal and subcutaneous temperature post-shearing ($F_{1,8} = 99.2$, $P < 0.0001$). It increased from 0.6 ± 0.4°C pre-shearing to 1.2 ± 0.4°C post-shearing, in both seasons (Figure 4). This higher 24-h mean difference was likely the result of an increase in the maximum daily difference between abdominal and subcutaneous temperature post-shearing ($F_{1,8} = 80.9$, $P < 0.0001$), namely from 1.1 ± 0.6°C pre-shearing to 2.5 ± 0.8°C post-shearing, in both seasons. Since the mean daily minimum difference between abdominal and subcutaneous temperature did not change from pre- to post-shearing ($F_{1,8} = 1.0$, $P = 0.35$), there was an increase in the mean daily amplitude of abdominal–subcutaneous temperature difference post-shearing ($F_{1,8} = 14.0$, $P = 0.006$), from 1.1 ± 0.6°C pre-shearing to 2.5 ± 0.8°C post-shearing, in both seasons. The difference between abdominal and subcutaneous temperature between 2100 and 0600 h increased from pre- to post-shearing in both seasons ($F_{1,8} = 100.2$, $P < 0.0001$). The significant interaction between shearing and season ($F_{1,8} = 5.6$, $P = 0.04$) revealed that the nighttime (2100–0600 h) difference between abdominal and subcutaneous temperature increased significantly more after shearing in March ($\sim 1.2 \pm 0.4$°C) than in September ($\sim 0.8 \pm 0.3$°C).

Relative activity

Figure 5 shows, for a single Angora goat, the average 24-h activity pattern, as detected by movement of the upper hind limb. The two goats for which we obtained activity data displayed an activity pattern with crepuscular peaks, and activity between those peaks was suppressed more in March than in September. Although there was little difference between the mean 24-h activity pre-shearing (4.7 ± 3.7 relative activity units) and post-shearing (5.4 ± 4.0 relative activity units) in September, the mean 24-h activity nearly doubled after shearing in March, from 3.2 ± 2.2 relative activity units pre-shearing to 5.6 ± 3.7 relative activity units post-shearing.

Water influx and turnover rate

In March, water influx increased significantly ($t_{10} = 4.7$, $P = 0.0008$) post-shearing, compared to pre-shearing (Figure 6, upper panel). Water turnover rate also increased significantly ($t_{10} = 5.1$, $P = 0.0005$) post-shearing compared to pre-shearing (Figure 6, lower panel).

Blood variables

Because the goats were denied access to drinking water for 7 days both pre- and post-shearing in March, their hydration state changed over time, as reflected by changes in serum protein with time (Figure 7, upper panel, $F_{4,40} = 5.2$,

Table 2 *Abdominal (n = 10) and subcutaneous (n = 5) temperature characteristics (mean ± s.d.) of Angora goats for the 10-day periods pre- and post-shearing, in both September and March (two-way repeated measures analyses of co-variance (ANCOVAs), with live body mass of shorn goats as a co-variant, were used to test for differences in the temperature profiles of the goats between the pre- and post-shearing periods (shearing) and across the 2 months (season))*

	September		March		Statistics		
	Pre-shearing	Post-shearing	Pre-shearing	Post-shearing	Shearing	Season	Interaction
Abdominal temperature (°C)							
24-h mean	39.5 ± 0.2	39.1 ± 0.2	39.6 ± 0.2	39.0 ± 0.2	**$F_{1,18} = 89.5$, $P < 0.0001$**	$F_{1,17} = 0.1$, $P = 0.80$	**$F_{1,18} = 5.2$, $P = 0.03$**
Mean daily minimum	38.9 ± 0.3	38.2 ± 0.2	38.9 ± 0.3	38.2 ± 0.3	**$F_{1,18} = 102.9$, $P < 0.0001$**	$F_{1,17} = 0.4$, $P = 0.52$	$F_{1,18} = 0.1$, $P = 0.75$
Mean daily maximum	40.3 ± 0.3	40.1 ± 0.2	40.3 ± 0.2	39.8 ± 0.2	**$F_{1,18} = 25.5$, $P < 0.0001$**	$F_{1,17} = 1.0$, $P = 0.33$	**$F_{1,18} = 7.4$, $P = 0.01$**
Amplitude (maximum−minimum)	1.4 ± 0.1	1.9 ± 0.2	1.3 ± 0.1	1.6 ± 0.1	**$F_{1,18} = 121.1$, $P < 0.0001$**	**$F_{1,17} = 10.8$, $P = 0.004$**	**$F_{1,18} = 12.9$, $P = 0.002$**
Time of nadir	7:01 ± 0:27	7:03 ± 0:23	7:54 ± 0:47	7:36 ± 0:46	$F_{1,18} = 1.1$, $P = 0.31$	**$F_{1,17} = 6.6$, $P = 0.02$**	$F_{1,18} = 2.0$, $P = 0.17$
Time of peak	15:18 ± 0:27	14:55 ± 0:46	16:17 ± 1:29	14:55 ± 1:07	**$F_{1,18} = 14.5$, $P = 0.001$**	$F_{1,17} = 1.1$, $P = 0.39$	$F_{1,18} = 4.6$, $P = 0.05$
Maximum rate of rise in abdominal temperature (°C/h)	0.25 ± 0.03	0.35 ± 0.04	0.22 ± 0.02	0.31 ± 0.03	**$F_{1,18} = 227.6$, $P < 0.0001$**	**$F_{1,17} = 10.6$, $P = 0.005$**	$F_{1,18} = 1.1$, $P = 0.30$
Subcutaneous temperature (°C)							
24-h mean	39.0 ± 0.5	38.1 ± 0.5	39.1 ± 0.4	37.8 ± 0.5	**$F_{1,8} = 183.3$, $P < 0.0001$**	$F_{1,7} = 0.04$, $P = 0.85$	**$F_{1,8} = 7.3$, $P = 0.03$**
Mean daily minimum	37.9 ± 0.8	36.2 ± 0.9	38.4 ± 0.5	36.0 ± 0.9	**$F_{1,8} = 166.6$, $P < 0.0001$**	$F_{1,7} = 0.5$, $P = 0.49$	**$F_{1,8} = 5.9$, $P = 0.04$**
Mean daily maximum	40.0 ± 0.3	39.8 ± 0.4	39.8 ± 0.5	39.4 ± 0.4	**$F_{1,8} = 16.8$, $P = 0.003$**	$F_{1,7} = 0.5$, $P = 0.50$	$F_{1,8} = 2.5$, $P = 0.16$
Amplitude (maximum−minimum)	2.1 ± 0.6	3.6 ± 0.6	1.4 ± 0.1	3.4 ± 0.5	**$F_{1,8} = 134.0$, $P < 0.0001$**	$F_{1,7} = 4.0$, $P = 0.09$	$F_{1,8} = 3.0$, $P = 0.12$

Bold values indicate significance at $P < 0.05$.

Figure 2 Nychthemeral rhythm of abdominal temperature (mean ± s.d.) for 10 free-living Angora goats over 10 days pre-shearing (dotted line) and 10 days post-shearing (solid line), during September (upper panel) and March (lower panel). Solar noon occurred between 1200 and 1300 h during both periods and black bars represent the night period.

$P = 0.002$). The goats were less dehydrated post-shearing than pre-shearing, as evident in total serum protein (Figure 7 upper panel, $F_{1,10} = 40.2$, $P < 0.0001$) and osmolality (Figure 7, middle panel, $F_{1,9} = 33.6$, $P = 0.0002$), which were higher pre-shearing than they were post-shearing. On the other hand, serum glucose concentration was significantly higher on the first day without drinking water post-shearing (Figure 7, lower panel, $F_{4,36} = 8.7$, $P < 0.0001$), than it was on the first day without drinking water, pre-shearing. There was a significant interaction in the change in glucose concentration between the number of days without water and shearing ($F_{4,36} = 3.9$, $P = 0.009$).

Microclimate selection

Figure 8 shows the correlation between miniglobe temperature at the site chosen by a single index goat and miniglobe temperature recorded at a nearby weather station exposed to solar radiation, both pre-shearing ($r^2 = 0.90$, $P < 0.0001$) and post-shearing ($r^2 = 0.90$, $P < 0.0001$) in March. The slope of the regression lines were significantly less than one, both pre-shearing ($F_{1,716} = 357.6$, $P < 0.0001$) and post-shearing ($F_{1,685} = 532.0$, $P < 0.0001$), and the regression lines intersected the line of identity, implying that the goat selected microclimates cooler than the prevailing environmental conditions at higher environmental heat loads and microclimates warmer than the prevailing environmental

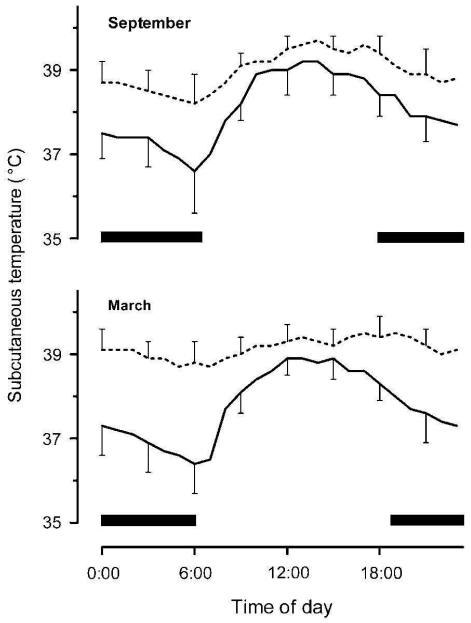

Figure 3 Nychthemeral rhythm of subcutaneous temperature (mean ± s.d.) for five free-living Angora goats over 10 days pre-shearing (dotted line) and 10 days post-shearing (solid line), during September (upper panel) and March (lower panel). Solar noon occurred between 1200 and 1300 h during both periods and black bars represent the night period.

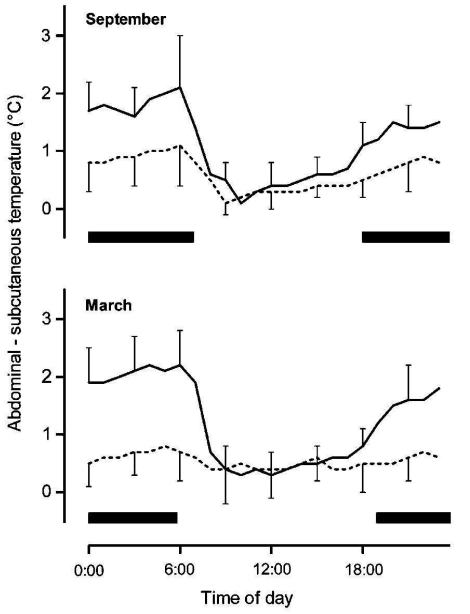

Figure 4 Nychthemeral rhythm of the difference (mean ± s.d.) between abdominal and subcutaneous temperature for five free-living Angora goats over 10 days pre-shearing (dotted line) and 10 days post-shearing (solid line), during September (upper panel) and March (lower panel). Solar noon occurred between 1200 and 1300 h during both periods and black bars represent the night period.

conditions at lower environmental heat loads. The slope of the regression line was significantly reduced ($F_{1,476} = 4.85$, $P = 0.03$) post-shearing (0.72 ± 0.02), compared with the pre-shearing period (0.77 ± 0.02).

Discussion

We have shown that the thermoregulation of free-living Angora goats is compromised after both September and March shearing. After shearing, mean daily abdominal temperature, daily maximum and daily minimum temperature decreased significantly in both seasons (Figures 1 and 2). Subcutaneous temperatures also decreased after shearing (Figure 3). Such a decrease in subcutaneous temperature is likely to be the result of both a reduced insulation of the fleece and a change in peripheral blood flow, as reflected in the difference between abdominal and subcutaneous temperature. Under the fleece the goats were consistently vasodilated (Figure 4). After shearing the goats remained vasodilated (dissipating heat) during the day, as indicated by small differences between core and subcutaneous temperatures. But the difference between abdominal and subcutaneous temperature doubled at night after shearing in September and nearly trebled in March. The difference is no doubt at least partly due to the fleece removal and increased heat loss from the skin to the environment, but also indicates that, at night, the goats were more peripherally vasoconstricted and had switched to a heat conservation strategy.

In addition to conserving heat though greater peripheral vasoconstriction, animals could prevent a further decrease in body temperature post-shearing by increasing their heat production. Although we did not measure metabolic rate directly, our two index goats, and therefore presumably the herd, increased activity levels (Figure 5, lower panel) after March shearing. The increased activity level during the early morning in March may have contributed to the increased water influx post shearing, as goats, like sheep (Birrell, 1989), are likely to consume dew on plant leaves at this time. In addition, the increased water influx (Figure 6, upper panel) and increased serum glucose (Figure 7, lower panel) after March shearing suggest that the goats increased their food intake and metabolic rate after shearing. Despite such efforts, the increased mean daily amplitude and rate of rise of abdominal temperature after shearing shows that the goats were more thermally labile after shearing, even though the index goat in the herd selected more stable microclimates after March shearing (Figure 8).

Paradoxically, the change in thermoregulatory status with shearing was greater in March than in September, despite ambient temperatures being significantly lower in September than in March. Both daily maximum and mean abdominal temperatures decreased more after the March shearing than

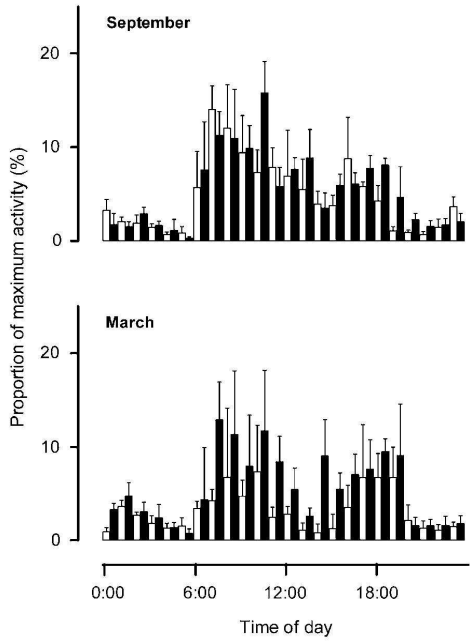

Figure 5 Nychthemeral rhythm of activity (mean ± s.d.) for an index Angora goat over 10 days pre-shearing (white bars) and 10 days post-shearing (black bars), during September (upper panel) and March (lower panel). Activity was measured on the upper hind limb with a subcutaneous data logger, and expressed as activity counts as a percentage of maximum counts for that logger.

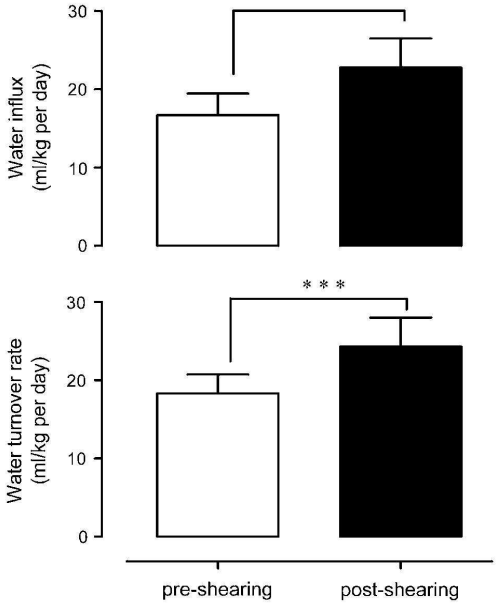

Figure 6 Mean ± s.d. of water influx (upper panel) and water turnover rate (lower panel) of 10 Angora goats over a 7-day period pre-shearing (white bars) and a 7-day period post-shearing (black bars), during March. The goats were denied access to drinking water. ***Indicates $P < 0.001$.

they did after the September shearing (Figure 2). Also, daily minimum and mean subcutaneous temperatures decreased more after the March shearing than after the September shearing (Figure 3). How might one account for the exaggerated transition in thermoregulatory status with shearing in late summer? We believe that differences resulted from seasonal acclimation; in March the predominant demands on the goats' thermoregulatory system were heat dissipation, even at night, before shearing. The goats were dehydrated before shearing in March, as evident in their relatively high serum osmolality and serum protein concentration (Figure 7). We surmise that the chronic heat stress imposed on fleeced goats in March results in a reduction in the competence of their autonomic responses necessary for heat conservation. Consequently, the goats were less well equipped in the late summer than in the late winter to cope with the loss of the insulating fleece.

If our conclusions are right, we would expect Angora goats to be compromised by shearing even more in the hotter summers to which they can be exposed. Nevertheless, we detected differences in thermoregulatory responses between seasons in which globe temperature differed by only 5°C (Table 1). Our measurements of activity were based on the behavior of two goats and microclimate selection was based on the behavior of a single index goat in the herd, and, although the animals always were observed together, that the herd behaves homogeneously would need to be confirmed. Also, we deprived the goats of drinking water in March, as could occur in summer seasons they might encounter, but not in winter. Water deprivation increases abdominal temperature in heat-stressed goats (Nijland and Baker, 1992; Jessen *et al.*, 1998), but there is no evidence that short-term water deprivation compromises thermoregulation in animals not exposed to heat stress (Baker and Doris, 1982; Fuller *et al.*, 2007).

Although the thermoregulatory responses of goats to shearing do not appear to have been investigated previously, in a thermal environment similar to those to which our goats were exposed, shearing decreased the body temperature of Merino sheep (Parer, 1963; Beatty *et al.*, 2008). However, the minimum body temperature reached by the Merino sheep was not as low (38.5 *v.* 38.1°C) and the drop in minimum body temperature post-shearing was not as great (0.4 *v.* 0.7°C) as that in our goats. If the nychthemeral pattern of body temperature in the Merino sheep was similar to that of our goats, Parer's (1963) non-continuous measurements may have overestimated minimum body temperature by measuring early-morning body temperature when it already was rising. The lower minimum body temperature of Parer's (1963) shorn Merino sheep was associated with an increased amplitude and a greater rate of rise of body temperature than those of unshorn Merino sheep, as we found in our goats. However, the Merino sheep, yarded in pens and exposed to solar radiation,

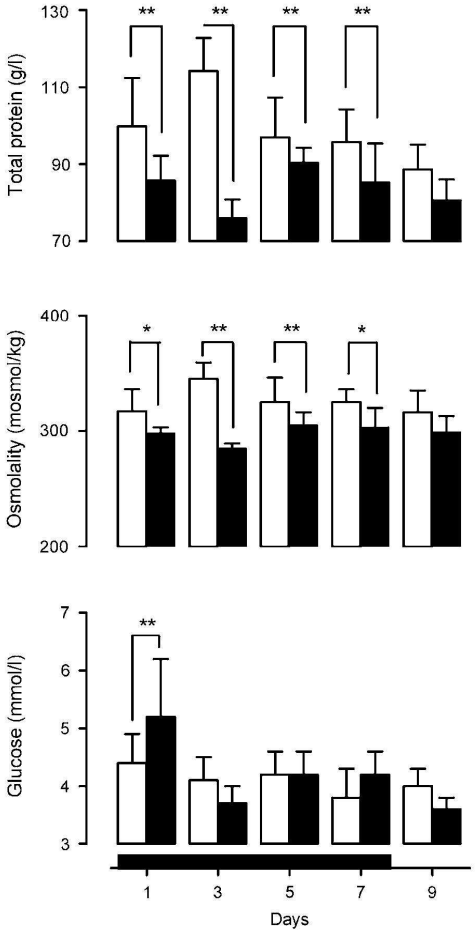

Figure 7 Mean ± s.d. of total serum protein (upper panel), osmolality (middle panel) and glucose (lower panel) for 10 Angora goats, both pre-shearing (white bars) and post-shearing (black bars) in March. Blood samples were taken every second day for 9 days. The goats were denied access to drinking water, but not food, for 7 days as represented by the black bar at the base.

showed slightly higher maximum rectal temperatures after shearing compared to before shearing (Parer, 1963; Hopkins *et al.*, 1978), while our goats had lower maximum abdominal temperature post-shearing compared to pre-shearing. In Merino sheep, the body temperatures both before and after shearing were lower than the maximum body temperature that we observed in our goats. We suspect that the Merino sheep, in spite of their full fleece, never were as heat-stressed as our goats were. Angora goats, exposed to full sun, have been shown previously to have higher, and more variable, body temperatures than Merino sheep do (McGregor, 1985 and 1998), suggesting that solar radiation provides a greater heat load on Angora goats than on Merino sheep.

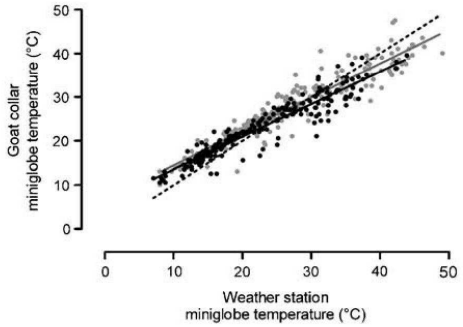

Figure 8 Scatter diagram showing the relationship between miniglobe temperatures at the site chosen by a single index Angora goat and miniglobe temperatures recorded at a nearby weather station exposed to solar radiation, during a 10-day pre-shearing (gray) and post-shearing (black) period in March. Measurements were made at hourly intervals. The dashed line is the line of identity, the solid gray line is the linear regression during the pre-shearing period and the solid black line is the linear regression during the post-shearing period.

An elevation in rectal temperature after shearing also has been reported in dairy sheep, which have an open fleece more like that of Angora goats than Merino sheep. Dairy sheep also exhibited a transient loss of body temperature rhythm after shearing, which the authors attributed to an over-reaction to mild cold stress as a result of the loss of fleece (Piccione *et al.*, 2002). However, the various studies on dairy sheep are difficult to interpret because the findings are inconsistent. Comisana ewes, housed indoors, showed hypothermia after shearing at air temperatures of 16–21°C (Piccione and Caola, 2003), hyperthermia at 16–28°C (Piccione *et al.*, 2002), but no change in morning rectal temperature when sheep were grazed in stubble pasture at an air temperature of 35°C (Pennisi *et al.*, 2004). In one instance, the post-shearing hyperthermia lasted many weeks (Piccione *et al.*, 2002). These inconsistencies may have arisen partly because the investigators measured rectal temperatures, a procedure that is likely to induce handling hyperthermia (Hargreaves and Hutson, 1990).

Although there may be a discrepancy in the way that shearing affects the body temperature of sheep and Angora goats when they are exposed to the diurnal thermal environment, there is no such discrepancy at night, when shorn animals have to implement heat conservation strategies, particularly under cool, windy conditions (Morris *et al.*, 1962; McArthur and Monteith, 1980). These night-time effects hold even, as we have shown, under relatively mild summer conditions. Like our goats, sheep have difficulty with heat conservation after shearing, as evident in the decrease in rectal temperature when shorn sheep are exposed to cold environments (Slee, 1966; Webster and Lynch, 1966; Phillips and Raghavan, 1970; Panaretto and Vickery, 1971; Bennett, 1972; Hofman and Riegle, 1977).

One of the thermoregulatory mechanisms available to animals to conserve body heat is vasoconstriction, and our

goats seemingly employed that mechanism at night, but only after shearing (Figure 4). In a previous laboratory study of a single Angora goat, the difference between skin and rectal temperature increased from 6°C in warm dry conditions to 20°C when exposed to cold, wet and wind conditions, just before the goat collapsed (Fourie, 1984). Those differences are much greater than the values we have reported, but we measured subcutaneous temperature and not skin surface temperature. Vasoconstriction leads to a reduction in heat delivery to the skin, so that, when ambient temperature is below core temperature, a low skin surface temperature would reduce the outer temperature gradient and consequently slow the sensible heat dissipation from the goat's body (Bianca and Kunz, 1978). As with our goats, skin temperature was lower in shorn than in unshorn sheep, particularly when the sheep were exposed to cold environmental conditions (Slee, 1966; Webster and Lynch, 1966; Phillips and Raghavan, 1970; Donnelly *et al.*, 1974; Glass and Jacob, 1992).

Another appropriate thermoregulatory mechanism to conserve body heat is the selection of warm microclimates. Our index goat, and therefore presumably the herd, selected warmer microclimates at low environmental heat loads after shearing (Figure 8). These results concur with results obtained for shorn sheep, which sought shelter at night (Alexander *et al.*, 1979; Mottershead *et al.*, 1982), so reducing their heat loss (Done-Currie *et al.*, 1984), more frequently than did unshorn sheep. During the heat of the day, our shorn goat, like sheep (MacFarlane *et al.*, 1966), frequently sought shaded sites and thus reduced its exposure to environmental heat load to a greater degree when shorn than when unshorn. The degree of shelter provided is of critical importance for the survival of shorn Merino sheep (Hutchinson and Bennett, 1962) and Angora goats are more dependent on shelter than are Merino sheep (McGregor, 1998).

Although shearing impairs heat conservation, it facilitates radiant heat gain during the day. When radiant heat load is high, evaporative water loss, both cutaneous and respiratory, may be required to maintain thermal balance. In goats and sheep, cutaneous evaporation (Robertshaw, 1968; Hofmeyr *et al.*, 1969) and respiration rate increased after shearing (Appleman and Delouche, 1958; MacFarlane *et al.*, 1958 and 1966; Parer, 1963). Extra evaporation demands more body water and the water turnover rates of Merino sheep nearly doubled after shearing, when maximum air temperatures averaged 36 to 38°C (MacFarlane *et al.*, 1966). Since Angora goats are more water dependent than are Merino sheep (McGregor, 1986), we would have expected a similar increase in the water turnover of our Angora goats. The water turnover rates of our Angora goats also increased significantly after shearing, though they did not double (Figure 6, lower panel). We could not measure evaporation rates in our free-living goats, but surmise that the higher water turnover after shearing was at least partly the result of higher evaporative water loss. Indeed, we suspect that the impairment of cutaneous water loss is one factor that contributed to the relatively high body temperature of our Angora goats before shearing.

In parallel with the increased water turnover post-shearing in our Angora goats, there was increased water influx (Figure 6, upper panel) after shearing. The routes for water influx available were only pre-formed water in forage or metabolic water, since we prevented access to drinking water. Shearing increases the food intake (Wheeler *et al.*, 1962; Webster and Lynch, 1966; Hutchinson and McRae, 1969; Avondo *et al.*, 2000) and metabolic rates (Hutchinson and Bennett, 1962; Panaretto and Vickery, 1971; Bennett, 1972; Hofman and Riegle, 1977; Symonds *et al.*, 1986) of sheep, and goats (Holmes and Moore, 1981; Holmes and Clark, 1989), particularly when exposed to cool environmental conditions. Similarly, cold exposure increases the metabolic rate of Angora goats (Wentzel *et al.*, 1979; Fourie, 1984), as indicated by an increased heart rate and blood glucose concentration. Our Angora goats also showed an increased blood glucose concentration immediately after shearing (Figure 7, lower panel). We caution, though, that blood glucose concentration may not be a reliable index of energy status of Angora goats, since Angora goats have an abnormal glucose homeostasis, brought about by selection for high levels of mohair production (Cronje, 1995). Nevertheless, since air temperature often fell below the lower critical temperature of 20°C reported for shorn Angora goats in dry conditions (Fourie, 1984), we believe our goats would have had increased metabolic rates, and so produced more metabolic water. This increased metabolic rate is likely to be an important factor contributing to the increased energy requirements of Angora goats (Lachica and Aguilera, 2003) after shearing.

Though shearing itself would have caused an increased metabolic rate, a further increase in metabolic rate would have resulted from the increased activity post-shearing. Our two index goats, and so, presumably, the herd, showed an increase in daily activity from pre- to post-shearing during March (Figure 5). Similarly, the foraging time for shorn Merino sheep is higher than for unshorn Merino sheep (Hutchinson and McRae, 1969; Birrell, 1989). The activity pattern of our goats was biphasic, with most activity, which presumably was foraging activity, taking place after sunrise and in the late afternoon. Biphasic patterns of foraging activity have been reported previously for both Merino sheep (MacFarlane *et al.*, 1966) and Angora goats (Askins and Turner, 1972). Goats also show seasonal variation in daily activity rhythm, with the highest activity levels reported during vernal equinox (Piccione *et al.*, 2008). Yet, the increased activity observed after the March shearing was not observed after the September shearing. Similar results were found for sheep in cold weather, in that daily movement patterns remained unaltered or even decreased after shearing (Webster and Lynch, 1966). These results are likely to correspond to the depression in food consumption reported for newly shorn sheep exposed to extreme cold (Webster and Lynch, 1966; Panaretto and Vickery, 1971; Donnelly *et al.*, 1974), which is proposed to be a consequence of acute cold stress (Sykes and Slee, 1969b). The reduction in food intake coupled with the increased metabolic

costs of thermoregulation may place the animals into negative energy balance, putting Angora goats at a greater risk of lethal hypothermia (McGregor and Butler, 2008b).

In summary, therefore, shearing induced autonomic and behavioral thermoregulatory responses in our Angora goats, as has been reported previously for both sheep and goats, and our goats' thermoregulatory responses were congruent with post-shearing responses observed previously. What we have added is the paradoxical observation that the transition from the fleeced to the shorn state had greater thermoregulatory consequences in late summer than in late winter, despite colder conditions in late winter. We believe that, in summer, the goats lost some capacity for thermoregulation in the cold, because they had been chronically heat-stressed before shearing, and had adapted to that stress. Similarly, sheep are unable to adapt to sudden weather changes after shearing and exposure to cold stress after summer shearing results in higher mortalities than after winter shearing (Glass and Jacob, 1992). The resilience of sheep after shearing in winter may be the result of cold adaptation in winter (Sykes and Slee, 1969a; Slee, 1972; Young, 1981). One of the few beneficial potential outcomes of global warming, associated with climate change, is a decrease in the statistical probability of stock losses due to cold, wet weather after shearing (Scholes *et al.*, 1999; Burton and Lim, 2005). However, if fleeced animals are not acclimated or acclimatized to cold, they may actually become more susceptible to cold stress after shearing in a warmer future. That summer shearing imposes significant thermoregulatory stress on Angora goats may help explain their summer vulnerability to cold weather; in March 2007 an estimated 20 000 Angora goats died in the Eastern Cape of South Africa during a cold, wet spell. If, as we propose, adaptation to heat stress imposed on fleeced goats in summer compromises their heat conservation strategies, they will be more vulnerable to cold in summer. With interannual variability in rainfall and heavy rainfall events predicted to increase with future climate change (Boko *et al.*, 2007), we can anticipate increased Angora goat mortalities in summer if the practice of biannual shearing continues.

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