WATER CONSERVATION STRATEGIES OF LARGE AFRICAN UNGULATES

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, 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 ____________________, 2017

ABSTRACT

Ecosystems in the southern hemisphere are limited mainly by water availability, a resource predicted to become increasingly scarce in the region in the face of anthropogenic climate change. Understanding how the artiodactyls (e.g., sheep, goats, cattle, antelope and camels), an important grouping of animals regionally, conserve body water to facilitate their survival, is therefore of importance. The idea that selective brain cooling conserves body water has been advanced for more than 20 years, without any definitive proof that ungulates, naturally making use of selective brain cooling, actually save body water as a result of implementing selective brain cooling. Using implanted biologgers I simultaneously measure carotid arterial and hypothalamic temperature in Dorper sheep *Ovis aries.* The concomitant determination of water turnover, based on the washout rates of the stable hydrogen isotope deuterium oxide (D_2O), allowed me to measure the volume of water that a dehydrated Dorper sheep conserves during a day when exposed to heat.

Artiodactyls differ in their water requirements though, and may have selective brain cooling capabilities relative to their level of water dependency. I therefore undertook the first comparative investigation into selective brain in free-living artiodactyls by implanting, with the assistance of colleagues, biologgers to measure carotid arterial and hypothalamic temperatures in three large, sympatric artiodactyl species with varying water dependencies. Despite a clear water-dependency gradient across species, I found no difference in the magnitudes of selective brain cooling, the proportion of time that selective brain cooling was used, or the threshold temperatures for selective brain between the gemsbok *Oryx gazella*, red hartebeest *Alcelaphus buselaphus* or blue wildebeest *Connochaetes taurinus* in an environment where the animals had free access to water. I found greater variability in selective brain cooling within species, than between species and conclude that all three species had the same underlying ability to make use of selective brain cooling.

Artiodactyls, however, are likely to rely on range of water conservation mechanisms in the face of climate change. A variable body temperature and the use of appropriate microclimates are two additional strategies that could be important in the quest to conserve body water. I therefore investigated the 24h nychthemeral body temperature rhythms of the gemsbok, red hartebeest and blue wildebeest, in combination with their 24h microclimate use patterns, during the five months of a southern hemisphere calendar year most challenging physiologically: the end of the dry season through the peak of summer. I found no species differences in the 24h nychthemeral body temperature rhythms of the gemsbok, the red hartebeest or the blue wildebeest. All three species, however, were heterothermic over about the first 50 days of the study, as a result of elevated maximum and depressed minimum body temperatures. Although subtle differences were detected in microclimate use, all three species used behavioural thermoregulation and accessed microclimates cooler than that available in the full sun. Such behaviour was enhanced during the hottest days, compared to the coolest days.

In conclusion, I have investigated some of the water conservation strategies relied upon by large African ungulates in their current environments. These strategies are not mutually exclusive and will all benefit these species in the face of climate change. The long-term monitoring of these water conservation strategies will allow us to tease out the relative contributions that selective brain cooling, heterothermy and microclimate use makes to body water conservation and how that translates to individual fitness.

ACKNOWLEDGEMENTS

The research culminating in this PhD work would not have been possible without the assistance and support of a whole range of people. I am indebted to each and every one of them.

In particular I would like to thank Prof. Duncan Mitchell, who responded very enthusiastically when I emailed him about the possibility of doing a PhD in physiology, despite my limited background in animal physiology. Duncan, with Prof. Andrea Fuller and Prof. Shane Maloney (University of Western Australia) subsequently became my supervisors. Through a steep learning curve they have contributed to my development by teaching me how to think, how to question, how to research, and how to write scientifically. Thank you for your guidance and support.

I am also indebted to every one of my co-authors: Dr. Robyn Hetem, who's planning, organisational skills and attention to detail are second to none. Dr. Leith Meyer, whose excellent micro-surgery skills were put to the test – particularly during a gruelling week of field surgery on Rooipoort Nature Reserve. Dr. Haley O'Brien who, as a palaeontologist, helped to fill in many of the gaps in my understanding of artiodactyl evolutionary history and the important role that the carotid rete played in artiodactyl success.

The staff at the Central Animal Services at Wits University, particularly Sr. Mary-Ann Costello, Sr. Amelia Ramekgwa and Mr. Nico Douths without whose assistance the sheep project never would have been completed

successfully. Mary-Ann also is thanked for her unwavering support and assistance during field surgery on Rooipoort Nature Reserve.

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Prof. Andrew McKechnie from the Department of Zoology and Entomology at the University of Pretoria, and Dr. Stephan Woodborne, then at the Council for Scientific and Industrial Research (now at iThemba Laboratories), are thanked for their good-humoured guidance and support when I prepared and analysed samples for stable isotope analysis, and for access - usually after hours - to lab space and the stable isotope analyser.

I thank fellow postgraduate students and members of the Brain Function Research Group (BFRG) in the School of Physiology for their support and encouragement, especially Zipho Zwane who was there to assist with every surgery. Davison Moyo and Lisa James ably assisted with the sheep project over weekends when assistance was surprisingly hard to come by. Benjamin Rey is thanked for great sandwich lunches and general assistance during the field surgery on Rooipoort Nature Reserve.

As I have been a part-time student with a job, I’d like to acknowledge my colleagues at the University of South Africa for their support. In particular, Prof. Jimmy Hendrick who has kept on reminding me, from the first day I started at UNISA, that my research is important and that my research output will always be the final determinant of my future in academia. The Chairs of the Department of Environmental Science, first Prof. Tayo Awofolo and then Prof. Willie Nel, for allowing me time off-campus to complete my fieldwork. I certainly tested the department's "off-campus system" and like to think that I have not dropped too many balls while juggling PhD fieldwork with UNISA duties. I will always remember attending to UNISA emails and student enquiries from the top of the Rooipoort Nature Reserve water tower or, late at night after a hard day's fieldwork, from the relative comfort of my field vehicle several kilometres outside of camp. To my colleagues in the Nature Conservation Programme at UNISA: thank you for your support and understanding.

My family and friends deserve special mention. Robyn, I would not have been able to see this through without your constant support, your belief in me - especially in those times when I doubted myself - and your constant encouragement, support and unwavering love. It really helped to have you, who knew exactly what it took to get this done, by my side. My darling daughter Paige, it was an absolute pleasure to be working in my study and hearing your chatter, laughter, and occasional cries from elsewhere in and around the house. Ayden was born as I was getting close to wrapping-up this thesis and he undoubtedly provided the impetus to finally get this submitted. My parents and in-laws, brothers and sister are also thanked for their consistent support.

My friends, many of whom have also walked this path, were a constant source of encouragement. Linda Fick sacrificed her hard-earned holiday to assist with surgery at Rooipoort Nature Reserve, and has been a good friend with Lois Harden, Jerome Gaugris, Caroline Vasicek-Gaugris, Haemish Melville, Pat de Witt, Andrew and Tanya Swanepoel throughout this journey. I thank you all for your encouragement, support and continuing friendship.

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Ek maak nou klaar!

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RESEARCH OUTPUTS

Published papers

Strauss, W.M., Hetem, R.S., Mitchell, D.M., Maloney, S.K., O'Brien, H.D., Meyer, L.C.R. & Fuller, A. (2017). Body water conservation through selective brain cooling by the carotid rete: a physiological feature for surviving climate change? *Conservation* Physiology 5(1):cow078; doi:10.1093/conphys/cow078.

Strauss, W.M., Hetem, R.S., Mitchell, D.M., Maloney, S.K., Meyer, L.C.R. & Fuller, A. (2016). Three African antelope species with varying water dependencies exhibit similar selective brain cooling. *Journal of Comparative Physiology B: Biochemical, Systems, and Environmental Physiology* 186:527 – 540.

Strauss, W.M., Hetem, R.S., Mitchell, D.M., Maloney, S.K., Meyer, L.C.R. & Fuller, A. (2015). Selective brain cooling reduces water turnover in dehydrated sheep. *PLoS One* 10(2): e0115514. doi:10.1371/journal.pone.0115514.

Conference presentations

International conferences

<u>Strauss, WM,</u> Hetem RS, Mitchell D, Maloney SK, Meyer, LCR and Fuller, A. The function of the carotid rete - the unique “wonderful net” of the Cetartiodactyla. Invited speaker in the symposium: *New insights into the functional relationship between anatomy and physiology of extinct and extant vertebrates*, at the International Congress of Vertebrate Morphology (ICVM11), 29 June-3 July 2016, Washington DC, USA.

<u>Strauss, WM,</u> Hetem RS, Mitchell D, Maloney SK, Meyer, LCR and Fuller, A. Comparison of the body temperature patterns of three large, arid-zone antelope. Paper presented at the 32nd Annual Conference of the Australian & New Zealand Society for Comparative Physiology and Biochemistry (ANZSCPB), 3-6 December 2015, Fowler’s Gap Research Station (University of New South Wales), Australia

<u>Strauss, WM,</u> Hetem RS, Mitchell D, Maloney SK, Meyer, LCR and Fuller, A. Physiological flexibility of artiodactyls could buffer the effects of climate change. Paper presented at the 30th Annual Conference of the Australian & New Zealand Society for Comparative Physiology and Biochemistry (ANZSCPB), 26-29 November 2013, University of Melbourne, Australia

<u>Strauss, WM,</u> Hetem RS, Maloney SK, Mitchell D, Meyer, LCR and Fuller, A. Selective brain cooling as a water conservation mechanism in artiodactyls. Paper presented at the 4th Physiology and Pharmacology of

Temperature Regulation Symposium (PPTR 2012), 22-25 March 2012, Buzios, Rio de Janeiro, Brazil.

Strauss, WM, Hetem, RS, Maloney, SK, Mitchell, D and Fuller, A. Technological advances allow real-time quantification of selective brain cooling in artiodactyls. Poster presentation at 4th International Bio-logging Symposium, 14-18 March 2011, Hobart, Tasmania, Australia.

Local conferences

Strauss, WM, Hetem RS, Mitchell D, Maloney SK, O'Brien HD, Meyer, LCR and Fuller, A. Body water conservation through selective brain cooling: a preadaptation to climate change? 5th Oppenheimer De Beers Group Research Conference, 21-22 October 2014, De Beers Campus, Johannesburg, South Africa.

Strauss, WM, Hetem RS, Mitchell D, Maloney SK, Meyer, LCR and Fuller, A. Physiological flexibility of artiodactyls could buffer the effects of climate change. Speed presentation (5 min) at the Savanna Science Network meeting, 9-13 March 2014, Skukuza, Kruger National Park.

Strauss WM, Hetem RS, Maloney, SK, Mitchell D, Meyer, L and Fuller A. Conserving body water: the challenge faced by arid-zone ungulates. Paper presented at the 3rd Annual Diamond Route Research Conference, 30-31 October 2012, De Beers Campus, Johannesburg, South Africa.

Strauss, WM, Hetem RS, Maloney SK, Mitchell D and Fuller, A. Counter-current cooling in artiodactyls: The benefits of maintaining a cool head.

Paper presented at the Southern African Wildlife Management Association Symposium, 16-19 September 2012, Klein Kariba.

Strauss, WM, Hetem RS, Maloney SK, Mitchell D and Fuller, A. Selective brain cooling as a water conservation mechanism in artiodactyls. Paper presented at the Zoological Society of South Africa conference, 10-14 July 2011, Stellenbosch University.

AUTHOR CONTRIBUTIONS

Chapter 1

Strauss, W.M., Hetem, R.S., Mitchell, D.M., Maloney, S.K., O'Brien, H.D., Meyer, L.C.R. & Fuller, A. (2017). Body water conservation through selective brain cooling by the carotid rete: a physiological feature for surviving climate change? *Conservation Physiology* 5(1): cow078; doi:10.1093/conphys/cow078.

The idea for a review paper on selective brain cooling as a water conservation strategy originated through discussions with my South African and Australian co-authors and it is based on the literature review that I have conducted for this thesis, while also incorporating, and putting into perspective, the main findings from Chapter 2 and Chapter 3. I initiated the collaboration with H.D. O'Brien (Oklahoma State University), including discussions on how to better incorporate paleontological insights into the selective brain cooling narrative. I wrote the first draft of the manuscript. All co-authors edited the manuscript.

This paper forms part of the introduction and conclusion presented in this thesis.

Chapter 2

Strauss, W.M., Hetem, R.S., Mitchell, D.M., Maloney, S.K., Meyer, L.C.R. & Fuller, A. (2015). Selective brain cooling reduces water turnover in dehydrated sheep. *PLoS ONE 10(2)*: e0115514. doi:10.1371/journal.pone.0115514

The idea for this paper originated through discussions with my co-authors. I organised the surgery, prepared the equipment, routinely caught, monitored and weighed the animals, conducted the experiment, prepared samples for analysis, analysed the samples and all data, and wrote the manuscript. Dr. Robyn Hetem assisted with equipment preparation and organising the surgery, collected blood samples, and provided guidance on analysis of water turnover. Prof. Duncan Mitchell assisted with experimental design and surgery. Prof. Shane Maloney assisted with experimental design. Dr. Leith Meyer performed the surgical procedures. Prof. Andrea Fuller assisted with experimental design and surgery. All authors edited the manuscript.

Chapter 3

Strauss, W.M., Hetem, R.S., Mitchell, D.M., Maloney, S.K., Meyer, L.C.R. & Fuller, A. (2016). Three African antelope species with varying water dependencies exhibit similar selective brain cooling. *Journal of Comparative Physiology B: Biochemical, Systems, and Environmental Physiology* 186:527–540.

The idea for this paper originated through discussions with my co-authors. I organised the surgery, prepared the equipment, liaised with the field site, assisted with animal capture and surgery, routinely monitored the animals post release, collected and analysed all data, and wrote the manuscript. Dr. Robyn Hetem assisted with experimental design, equipment preparation, surgery organisation and logistics. Professors Duncan Mitchell, Shane Maloney and Andrea Fuller assisted with experimental design and surgery. Dr. Leith Meyer conducted the surgery. All authors edited the manuscript.

CHAPTER 1

Introduction

Some of the ideas in this chapter, related to selective brain cooling, have been published in:

Body water conservation through selective brain cooling by the carotid rete: a physiological feature for surviving climate change?

Strauss, W.M., Hetem, R.S., Mitchell, D., Maloney, S.K., O'Brien, H. D., Meyer, L.C.R. & Fuller, A.

***Conservation Physiology* 5(1): cow078; doi:10.1093/conphys/cow078.**

1.1 Large African ungulates

Ungulates are a group of hooved mammals that naturally occur on all the continents, with the exception of Antarctica and Australia. About 15 ungulate species have independently been domesticated (MacDonald, 2006) and, as a result of their economic importance have spread across the globe, including Australia. Ungulates are either even-toed (also known as cloven-hoofed) like cattle, sheep, antelope and camels, collectively referred to as artiodactyls, or they are odd-toed, i.e., horses, rhinoceroses and tapirs that collectively are referred to as the perissodactyls.

With 90 extant genera, the artiodactyls are a diverse group of mostly herbivorous mammals - the pigs often have a more general diet, including carrion, for example - with often complex social organisations. Although artiodactyls can be found in all habitats, ranging from forests to deserts, and despite their numbers having declined, artiodactyls dominate the African savanna. Southern Africa, in particular, is rich in artiodactyls, with almost a quarter (23%) of genera occurring within the region. As habitats across Southern Africa are continuously being fragmented (as elsewhere) the main stronghold of the artiodactyls is the vast network of protected areas across the region.

However, protected areas are under constant pressure as they harbour resources deemed vital for survival by a rapidly growing human population (Ripple *et al.*, 2015). Moreover the success of protected areas in protecting intact, functioning ecosystems with a complete species component has been challenged (Craigie *et al.*, 2010). In their recent assessment on the performance of 69 large mammal species across 78 African protected areas, Craigie and colleagues documented a 59% decrease in large mammals populations over the 35 year period 1970-2005 (Craigie *et al.*, 2010).

More recently, Ripple and colleagues assessed the performance of the largest 74 herbivore species in the world. The vast majority of these species (70%), all with a body mass in excess of 100 kg, are artiodactyls, and, of these, about 60% are declining in number as a result of a growing human population, unsustainable use, habitat destruction and high livestock densities (Ripple *et al.*, 2015). The artiodactyls that form the mainstay of protected areas across much of Africa – the antelope, wild pigs, buffalo and giraffe - are therefore in peril, even before one considers any potential direct impact of the greatest conservation challenge of the 21st century: anthropogenic climate change.

1.2 A changing climate

At the International Geological Congress, held in Cape Town, South Africa, during August/September 2016, the members of the Working Group on the Anthropocene voted overwhelmingly in favour of a new geological epoch: the Anthropocene (Voosen, 2016). Currently, efforts are underway to identify an acceptable global marker in stratigraphic material (e.g., sediment, rock or glacier ice) that, in addition to auxiliary markers, would pinpoint the start of human-dominated changes to the Earth system (Lewis and Maslin, 2015). While the exact start of the Anthropocene has not yet been identified, it has been suggested that the Industrial Revolution, characterised by increasing use of fossil fuels and rapid societal changes, marks the beginning of the proposed epoch (Crutzen, 2002, Steffen *et al.*, 2011, Zalasiewicz *et al.*, 2011).

Regardless of the exact starting date of the Anthropocene, anthropogenic climate change is an accepted and well-documented phenomenon (Parmesan, 2006). What still is debated is some remaining uncertainty in climate change models, particularly with regards to the downscaled modelling of rainfall patterns at local scales globally (Jakob, 2010) and also in southern Africa (Ratnam *et al.*, 2013, Sylla *et al.*, 2013, Zhang *et al.*, 2013), leading to mismatches between model projections and observations on the ground (MacKellar *et al.*, 2014, van Wilgen *et al.*, 2015). Nevertheless, continuous improvements in model development have resulted in the Intergovernmental Panel on Climate Change (IPCC) increasing their confidence in their projections in successive assessment reports.

The IPCC is, for example, highly confident that warming has increased over the African continent during the last 50-100 years, and that this warming trend is consistent with anthropogenic climate change (Niang *et al.*, 2014). Moreover, the surface temperatures over southern Africa, subtropical North Africa and parts of central tropical Africa have increased at a rate faster than the global average over the last 50 years (Engelbrecht *et al.*, 2015). Projections are that the surface temperature across subtropical Africa will increase at a rate approximately 1.5 times that of the expected global temperature increase. The annual average maximum temperatures across southern Africa and the Sahel are therefore projected to increase by 4-6°C by the year 2100 (Engelbrecht *et al.*, 2015).

Such projections into the future are based on a set of four scenarios called Representative Concentration Pathways (RCPs): RCP2.6, RCP4.5, RCP6.0 and RCP8.5 (Stocker *et al.*, 2013). These RCPs are characterised by their approximate total radiative forcing in 2100 relative to 1750 (Stocker *et al.*, 2013). RCP2.6 is the only mitigation strategy and has a very low radiative forcing (2.6 $W.m^{-2}$) that would have peaked and declined by 2100 (Fig. 1, top left). RCP4.5 and RCP6.0 are so-called stabilisation scenarios with radiative forcing of 4.5 and 6.0 $W.m^{-2}$, respectively. The RCP8.5 scenario is characterised by high greenhouse gas emissions, and while RCP4.5 stabilises by 2100, neither RCP6.0 nor RCP8.5 stabilises by that time (IPCC, 2013). The predicted mean annual temperature increase under each of these RCPs is illustrated for the periods 2016-2035 and 2081-2100, with the best (RCP2.6) and worst-case (RCP8.5) scenarios resulting in about 1.5-2°C and 5-6°C increase in surface temperature for southern Africa by 2100, respectively (Fig. 1, right hand panel).

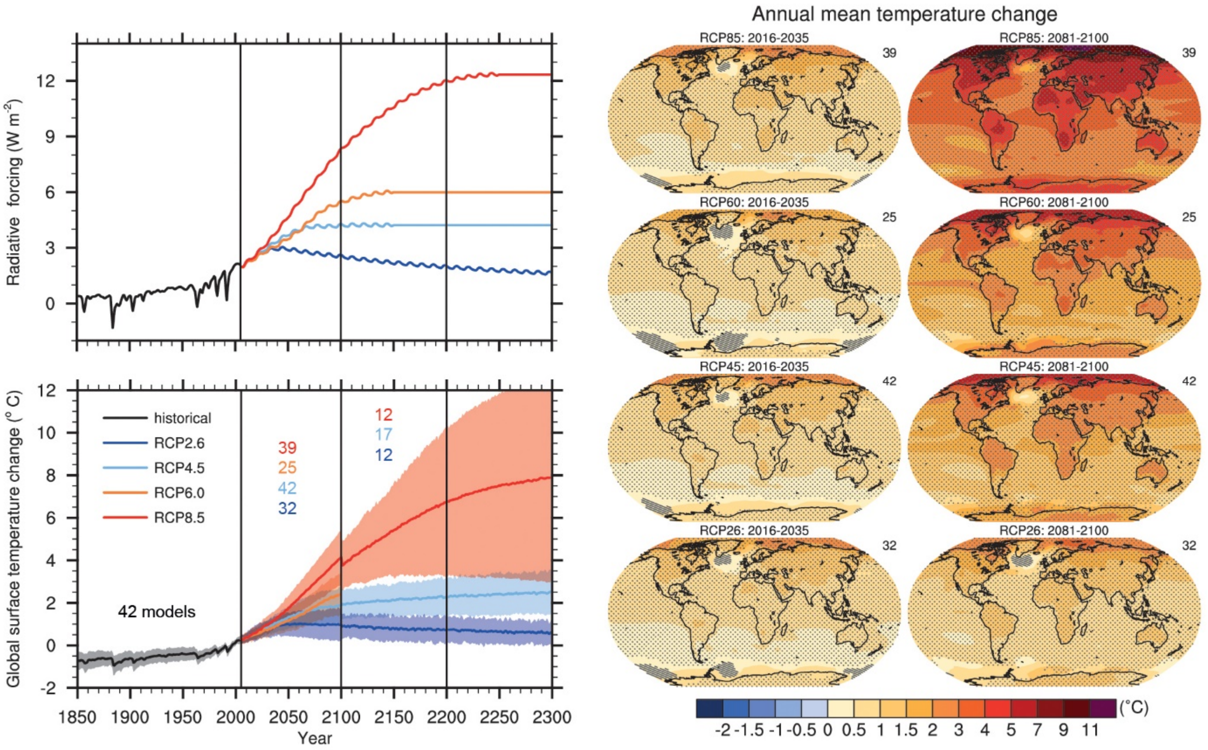

Figure 1: (Top left) Total global mean radiative forcing for the four RCP scenarios based on the Model for the Assessment of Greenhouse-gas Induced Climate Change (MAGICC) energy balance model. Note that the actual forcing simulated by the CMIP5 models differs slightly between models. (Bottom left) Time series of global annual mean surface air temperature anomalies (relative to 1986–2005) from CMIP5 concentration-driven experiments. Projections are shown for each RCP for the multi-model mean (solid lines) and ±1.64 standard deviation (5 to 95%) across the distribution of individual models (shading), based on annual means. The 1.64 standard deviation range based on the 20yr averages 2081–2100, relative to 1986–2005, are interpreted as likely changes for the end of the 21st century. Discontinuities at 2100 are due to different numbers of models performing the extension runs beyond the 21st century and have no physical meaning. Numbers in the same colours as the lines indicate the number of different models contributing to the different time periods. Maps: Multi-model ensemble average of annual mean surface air temperature change (compared to 1986–2005 base period) for 2016–2035 and 2081–2100, for RCP2.6, 4.5, 6.0 and 8.5. Hatching indicates regions where the multi-model mean signal is less than one standard deviation of internal variability. Stippling indicates regions where the multi-model mean signal is greater than two standard deviations of internal variability and where 90% of the models agree on the sign of change. The number of CMIP5 models used is indicated in the upper right corner of each panel (from Stocker *et al.* 2013).

While there is greater uncertainty in future projections of rainfall compared to the temperature projections, rainfall is generally predicted to increase over East Africa (Fig. 2, left panel), while decreases are projected across southern Africa (Engelbrecht *et al.*, 2015). However, based on the Keetch-Byram drought index (Keetch and Byram, 1968), which relates to soil moisture content, general drying is predicted across much of Africa by 2100 (Fig. 2, right panel). Crucially, the soil moisture content is predicted to decrease even in those areas where an increase in rainfall is predicted. The projected drying of soils is expected to result from enhanced evaporation because of the expected temperature increase. Water availability, therefore, will be less even if rainfall increases. The anticipated change in soil moisture regime is likely to result in an extension of the southern African dry season that typically peaks in mid-October under current climatic conditions, to last from May to December (Engelbrecht *et al.*, 2015).

Changes in ambient temperature and rainfall regimes as a consequence of anthropogenic climate change have already affected ecosystems across Africa, and there is high confidence that the predicted future effects on these ecosystems will be substantial (Niang *et al.*, 2014). The distribution and dynamics of terrestrial ecosystems, including woodlands, savanna, grasslands, shrublands and deserts have already changed, primarily as a result of anthropogenic land use change, including the expansion of agriculture and livestock grazing (Niang *et al.*, 2014).

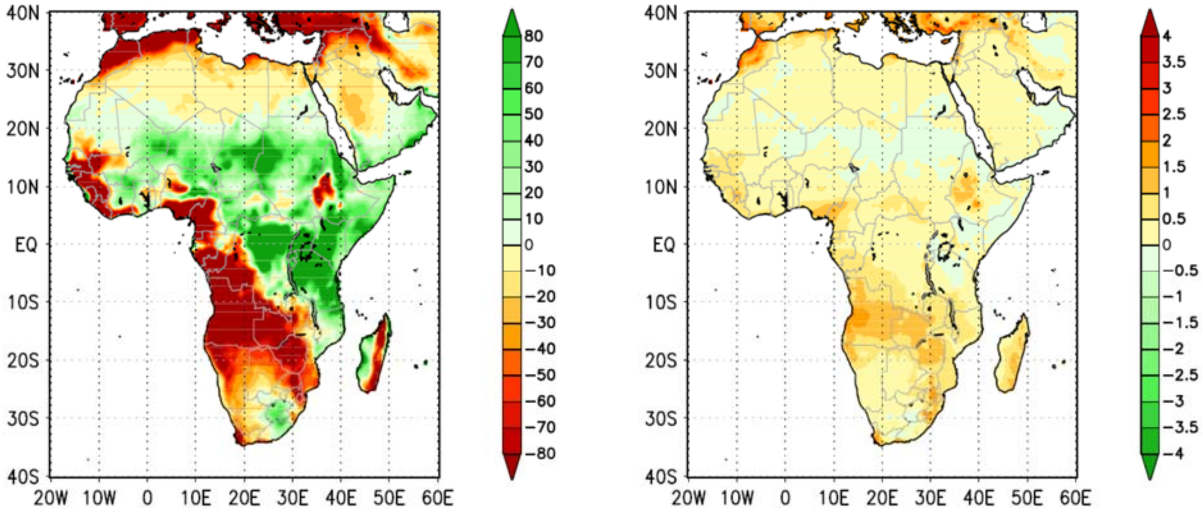

Figure 2: Predicted changes in the median annual rainfall (mm) across Africa (left) and the Keetch-Byram Index (right) by 2100 relative to 1961-1990. The Keetch-Byram Index represents the projected soil moisture levels in response to the predicted changes in rainfall. An assumption of a heterogeneous world with continuously increasing global population and economic growth that is regionally orientated underpins the projection (the so-called A2 SRES emission scenario). Adapted from Engelbrecht *et al.* (2015).

The effects of these land use changes make it difficult to discern the role that an increased concentration of atmospheric carbon dioxide – the global driver in anthropogenic climate change - plays in the structural changes that have been documented in, for example, the savanna biome (Bond and Midgley, 2012). Nevertheless, Niang and colleagues (2014) predict with high confidence that future changes in Africa's terrestrial ecosystems will very likely be driven by the increased carbon dioxide levels, and the resulting changes in ambient temperature and rainfall regimes characteristic of anthropogenic climate change.

The complexity of ecosystems makes it difficult to determine cause and effect and, consequently, it is difficult to determine specific trajectories for entire ecosystems as a result of anthropogenic climate change. However, the ecological impacts of anthropogenic climate change on fauna and flora are well-documented and coherent ecological response patterns have been identified across systems, including changes in phenology, range shifts and, as a consequence, community composition (Walther *et al.*, 2002). For example, the analysis of an extensive, long-term database (1970-2004) of documented responses in physical and biological systems has revealed temperature-related changes on all seven continents (Rosenzweig *et al.*, 2008). These authors have shown that 95% of the almost 1 000 documented changes in physical systems were in directions consistent with a warming climate, including the shrinking of glaciers (Rosenzweig *et al.*, 2008), which has been documented on all continents (Oerlemans, 2005). Equally compelling evidence, from a much larger set of about 29 000 documented changes in biological systems, showed that 90% of the documented responses of plants and animals were consistent with expectations in a warming world, for example changes in phenology, including the earlier blooming or leaf unfolding in higher plants, and changes in migratory patterns (Rosenzweig *et al.*, 2008).

The vulnerability of an organism to climate change depends on both its exposure and sensitivity to the change, as well as its resilience to perturbations, and its ability to adapt to the changing environment (Huey *et al.*, 2012). Therefore it has been suggested that the organisms that comprise biological systems have only three options when having to deal with the increasing ambient temperatures characterising anthropogenic climate change: stay and adapt, move (and possibly also adapt), or die. Those organisms, species and their populations that do not go extinct (or become extirpated) as a result of anthropogenic climate change, would have had the opportunity to either stay where they are and adapt to the changing environment, or to move to a more suitable climate (Mitchell *et al.*, 2008, Hetem *et al.*, 2014, Fuller *et al.*, 2014).

1.3 Responses to climate change

1.3.1 Extinction

Although climate change is not a new phenomenon in the Earth’s history, and the current biodiversity has evolved during successive periods of climate change (Barnosky and Kraatz, 2007), the rate at which temperatures are increasing as a result of anthropogenic climate change is unprecedented (Diffenbaugh and Field, 2013, Smith *et al.*, 2015). Moreover, the expected magnitude of the ambient temperature increase remains important. Some modelling scenarios predict an ambient temperature increase of up to about 6°C by 2100 as a result of anthropogenic climate change (Barnosky and Kraatz, 2007, Stocker *et al.*, 2013, Engelbrecht *et al.*, 2015). An ambient temperature increase of such magnitude occurred 251 Mya and resulted in the extinction of 95% of all species on earth (Benton and Twitchett, 2003). While it is not certain that all of the extinctions during this end-Permian event were a consequence of the 6°C ambient temperature increase (Hetem *et al.*, 2014), the fossil record provides an unambiguous record of species that could not cope with an increase in ambient temperature of similar magnitude to what is predicted by 2100.

Several attempts have been made to predict the persistence of species in the face of anthropogenic climate change. One study predicted that, under mid-range climatic scenarios, 25% of the more than 1 100 animal and plant species it focussed on, from different regions across the globe, would be extinct by 2050 (Thomas *et al.*, 2004). Moreover, extrapolation across the globe resulted in the prediction that an estimated 45% of terrestrial plant and animal species could be extinct by 2050 if their dispersal is curtailed (Thomas *et al.*, 2004). Predictions on the persistence of animals in the face of anthropogenic climate change have also been made on a regional scale. Focussing on 277 mammal species, representing 28 families and 12 orders across Africa, Thuiller and colleagues modelled likely species persistence in the face of anthropogenic climate change and land transformation. These authors predicted that between 25 and 40% of Africa's mammal species are likely to be critically endangered or extinct by 2080 (Thuiller *et al.*, 2006). Locally it has been predicted that South Africa's premier wildlife destination, the Kruger National Park, could lose as much as 66% of the assessed vertebrate and invertebrate animals to extinction (Erasmus *et al.*, 2002). Such predictions have contributed to the suggestion that national parks across Africa should expect changes in species composition, both through species and population losses and influxes, that are unprecedented in recent geological time (Thuiller *et al.*, 2006). Recent analyses of long-term temperature and rainfall data indicate that climatic changes have already taken place across some of southern Africa's conservation areas (van Wilgen *et al.*, 2015). Moreover, with long-term data from Kruger National Park indicating that 63% of ungulate species have declined from 1977-1996 (Ogutu and Owen-Smith, 2003), the template for species composition change is in place.

1.3.2 Range shifts

In the face of anthropogenic climate change an influx of one or more species into an area in which they have not previously occurred requires the ability to move freely in response to a changing environment. Under climate change, the direction of such movements for organisms generally is in the direction of the polar regions or to higher altitudes (Parmesan *et al.*, 1999, Parmesan and Yohe, 2003, Parmesan, 2006). A recent synthesis of movement rates in response to anthropogenic climate change across taxa and regions (with the exception of large African mammals) found that the average range shifts are 17 km.decade^{-1} poleward and 11 km.decade^{-1} towards higher altitudes (Chen *et al.*, 2011). Despite suggestions to the contrary (Warren *et al.*, 2001, Nathan *et al.*, 2011), the observed mean latitudinal shifts across taxa and regions indicate that many terrestrial species do not consistently lag behind climate shifts at present (Chen *et al.*, 2011).

The predicted movement rates of various species groups per decade and how that compares to the climate velocity expected under the IPCC's four Representative Concentration Pathways are illustrated in Figure 3 (Field *et al.*, 2014). Based on observed and modelled movement rates it is predicted that all of the species groups considered, including even the trees and herbaceous plants, would be able to disperse fast enough, to keep up with their various climatic envelopes in non-mountainous areas under RCP2.6 (the only mitigation scenario). Among terrestrial mammals, the median movement rates of rodents and primates indicate that some species would be able to track their bioclimatic envelopes, provided the mean global climate velocity, incorporating the velocity in flat areas (high) and that in mountainous areas (low), under RCP6.0 is not exceeded. However, only the artiodactyls (e.g., antelope, cattle, sheep and goats) are expected to move fast enough, on average, to keep up with their individual bioclimatic envelopes, regardless of the scenario considered (Settele *et al.*, 2014). Based on these analyses, all groups considered have to move in response to climate change and all groups but the artiodactyls, may need human assistance in order to stay within their climatic envelopes. Many of the predicted range shifts, as well as the estimates of species persistence in the face of anthropogenic climate change, are generated through niche modelling. These ecological niche models focus solely on the climatic conditions prevalent where a species currently occurs, the so-called bioclimatic envelope of a species, followed by forecasting of where that bioclimatic envelope likely will exist sometime in the future under different climate change scenarios.

Using such an approach, it has, for example, been suggested that the bioclimatic envelope of the scimitar-horned oryx *Oryx dammah*, indigenous to the Sahel of North Africa, would be located in the Kalahari Desert of southern Africa by mid-century (Thuiller *et al.*, 2006). These animals would therefore have to disperse southwards in excess of 5 000 km if they were to keep up with their bioclimatic envelope. If a terrestrial animal were to successfully track its bioclimatic envelope over thousands of kilometres it would have to be across contiguous habitat that is not fragmented or destroyed. However, the reality is that habitats are highly fragmented across much of the globe, even for organisms that have to disperse over much shorter distances, as a result of infrastructure development (e.g., road networks and fence lines), agricultural practises (e.g., land clearing for crops) and various other economic activities (e.g. mining and logging). Habitat fragmentation as well as habitat loss will therefore substantially limit the ability of species to move in response to climate change (Eycott *et al.*, 2012), regardless of their predicted movement rates (Fig. 3). While bioclimatic envelope models could project where a suitable climate might exist in the future, the limitations of these models are increasingly realised.

The use of bioclimatic envelope models to predict species persistence in the face of anthropogenic climate change has been questioned in recent years. For example, analysing the stable carbon and oxygen isotopes preserved in fossilised mammalian teeth, De Santis and colleagues (2009) showed that the assumption that niches are maintained over long time scales (“niche conservatism”), is wrong in some cases. They therefore questioned the validity of assuming a fixed relationship between an organism and its bioclimatic envelope when forecasting. Also, the existence, and indeed the maintenance of some ecosystems, like African savanna, is to a large extent driven by disturbances such as fire and grazing.

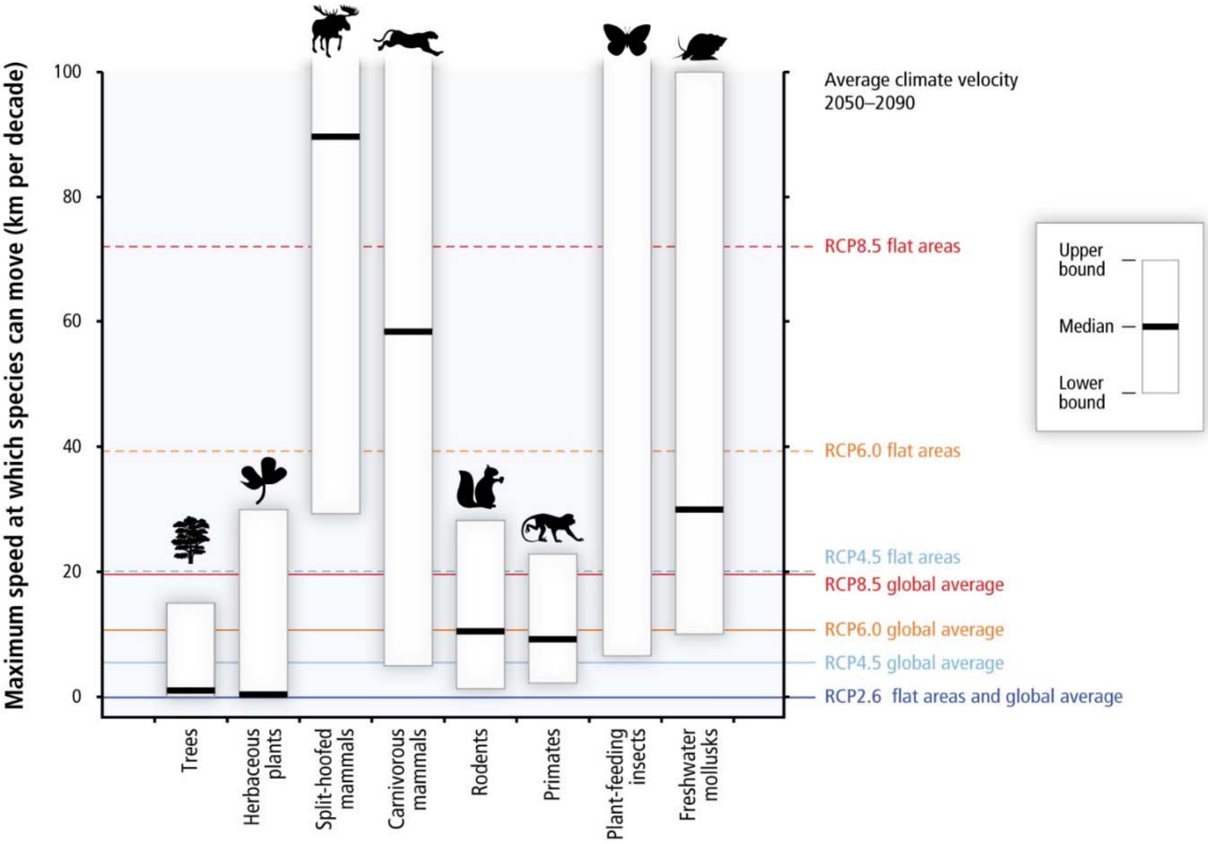

Figure 3: Maximum speeds at which species can move across landscapes (based on observations and models; vertical axis on left), compared with speeds at which temperatures are projected to move across landscapes (climate velocities for temperature; vertical axis on right). Human interventions, such as transport or habitat fragmentation, can greatly increase or decrease speeds of movement. White boxes with black bars indicate ranges and medians of maximum movement speeds for trees, plants, mammals, plant-feeding insects (median not estimated), and freshwater molluscs. For RCP2.6, 4.5, 6.0, and 8.5 for 2050–2090, horizontal lines show climate velocity for the global-land-area average and for large flat regions. Species with maximum speeds below each line are expected to be unable to track warming in the absence of human intervention (from Field *et al.*, 2014).

The usefulness and validity of selecting future conservation areas based on predicted species range shifts have therefore been questioned (Midgley and Bond, 2015). While the spatial scale at which analyses are done has also been questioned (Guisan and Thuiller, 2005), the most damning criticism of the correlative bioclimatic envelope models comes perhaps from a paper otherwise highlighting the usefulness of the models, with the results of bioclimatic envelope models predicting extinction risk, e.g., Thomas *et al.* (2004), considered debatable (Araújo and Peterson, 2012).

The ability of bioclimatic envelope models to predict extinction risk is questioned because the models generally do not make any provision for organisms adapting to their changing environment (Levinsky *et al.*, 2007, Hetem *et al.*, 2014, Muñoz *et al.*, 2015). As pointed out by Hetem *et al.* (2014), many bioclimatic envelope models mistakenly assume that a particular organism occupies all the thermally suitable habitats available, and that it cannot survive in its existing habitat should conditions depart from what it is accustomed to. Yet plasticity, which may be greatest in highly seasonal environments (Chown *et al.*, 2004) and even reversible (Garland and Kelly, 2006), may allow species to adjust to changing environmental conditions. Therefore, ignoring phenotypic plasticity in bioclimatic envelope models results in an overestimation of the number of species committed to extinction under anthropogenic climate change (Muñoz *et al.*, 2015). While the physiological responses of species to changing environmental conditions are poorly understood, a few studies have nevertheless attempted to address climatic tolerances through the incorporation of physiological parameters (Taulman and Robbins, 1996, Johnston and Schmitz, 1997). Only through the integration of physiology, ecology and evolutionary biology can a mechanistic understanding of the biological consequences of climate change be developed (Spicer and Gaston, 1999). A mechanistic understanding of the biological consequences of climate change in large mammals is unfortunately hampered by a dearth of relevant physiological knowledge.

1.3.3 Adaptation

If animals do not respond to the changing climate by shifting their distribution ranges, they will have to adapt to the changing environment. Genetic adaptation is one option, but due to the rate of climate change it probably is only viable for smaller animals with short generation times. Populations of large mammal, which typically have long generation times (about 10-15 years for large ungulates) would need to evolve faster per generation to adapt to a particular rate of environmental change (Chevin *et al.*, 2010).

The options available to mammals in response to hotter, drier environments have been the focus of a number of recent reviews (Fuller *et al.*, 2014, Fuller *et al.*, 2016, Hetem *et al.*, 2014). These reviews include detailed descriptions of how mammals could adapt to their changing climate through micro-evolution (e.g. Réale *et al.*, 2003) and phenotypic plasticity, including phenological changes (e.g. Post and Forchhammer, 2008), anatomical variation (e.g. Ozgul *et al.*, 2009), physiological acclimatisation (e.g. Hetem *et al.*, 2010), maternal effects (e.g. Stenseth *et al.*, 2002) and behavioural flexibility (e.g. Long *et al.*, 2014). Here I will briefly introduce phenotypic plasticity, with a particular focus on physiological acclimatisation and behavioural flexibility, as this mainly will be what large African mammals rely on to survive the hotter and drier conditions predicted under climate change (Fuller *et al.*, 2016).

1.3.3.1 Phenotypic plasticity

Phenotypic plasticity is defined as a single genotype giving rise to one or more phenotypes when exposed to different environmental conditions throughout a lifetime (Pigliucci, 2005) and it may involve acclimatisation in free-living organisms, acclimation in laboratory settings, and learning (Garland and Kelly, 2006, Huey *et al.*, 2012). According to Hetem and colleagues phenotypic plasticity can be expressed in a number of ways, including anatomical variation within a species, phenological changes (changes in the timing of life events), and the use of underlying physiological and behavioural capabilities to safeguard animals from the effects of climate change (Hetem *et al.*, 2014). That animals rely on phenotypic plasticity in novel environments has been documented previously. For example, experimental work has shown that the length of the hind limb of the Anolis lizard *Anolis sagrei* is a plastic trait (Losos *et al.*, 2000), thereby explaining the rapid divergence previously observed (Losos *et al.*, 1997) when Anolis lizards were introduced onto several small islands in the Bahamas. Over a 10-14 year period those lizards on islands with vegetation least similar to the area where the lizards were sourced from showed the greatest magnitude of hind leg length differentiation (Losos *et al.*, 1997). The observed plasticity in hind leg length therefore led to the establishment of phenotypes appropriate to particular environments (Losos *et al.*, 2000). Phenotypic plasticity, therefore, could be an epigenetic effect and, with the exception of maternal effects, it allows individuals to respond to a changed environment during their lifetimes. Such plasticity may allow organisms, including large mammals whose movements in particular are hampered by habitat destruction, to adapt to changing conditions without having to move.

1.3.3.1.1 Phenological changes

The most-documented examples of phenotypic plasticity, at least in part due to the relative ease of measurement, are changes in phenology. Documented changes in the timing of seasonal life events, consistent with climate change predictions (e.g., earlier spring events), include earlier arrival of migrating birds and butterflies (Ahas, 1999, Bradley *et al.*, 1999, Roy and Sparks, 2000), earlier bird nesting (Crick *et al.*, 1997, Dunn and Winkler, 1999) and earlier frog breeding (Beebee, 1995). A meta-analysis of 677 documented species responses over a median period of 45 years showed that 62% of the species displayed advanced spring events, while 27% showed no trend in phenology (Parmesan and Yohe, 2003).

Changes in phenology do not take place in isolation though. Vegetation phenology, for example, is a primary environmental cue for life-history traits, including reproduction and migration, in ungulates (Ogutu *et al.*, 2014, Stoner *et al.*, 2016). Changes in the phenology of vegetation are therefore likely to impact ungulate populations. Trophic mismatches occur in ecosystems when species that depend on each other respond differently to the same environmental change. Such mismatches often result because different groups of organisms respond to different environmental cues. Vertebrates, for example, respond mostly to changes in photoperiod, while plants and insects are more responsive to changes in ambient temperature (Visser, 2008). As the annual photoperiod pattern does not change, an uncoupling between photoperiod and ambient temperature patterns arises. For example, great tits *Paus major* in The Netherlands have not advanced their egg-laying date in response to an increase in ambient temperature over a 23-year period (1973-1995), while the peak biomass of caterpillars on which they depend for feeding their young, has advanced by about 9 days (Visser *et al.*, 1998). More recently, similar trophic mismatches have been documented in ungulates (Post and Forchhammer, 2008, Kerby and Post, 2013, Plard *et al.*, 2014). A 27 year life-history data set on a population of roe deer *Capreolus capreolus* in France, for example, showed that the roe deer have not modified their phenology – in this instance the timing of births – to keep track of the earlier spring plant phenology and resultant availability of high-quality resources. Failure by the roe deer population to adapt to their changing environment already has resulted in a decrease in their fitness (Plard *et al.*, 2014).

A change in phenology is, however, not necessarily synonymous with an adaptive climate change response. Although phenological change data are scarce for mammals, a recent review of the published data indicated that almost 50% of the reported phenological changes in mammals were related to reduced fitness, and were not in response to a changing climate (Boutin and Lane, 2014). In only about a third of the studies reviewed were the reported phenological changes considered adaptive to climate change (Boutin and Lane, 2014). A phenological change in itself is therefore not adaptive, as it should be changing in the correct direction and at the correct pace in response to environmental changes.

1.3.3.1.2 Behavioural flexibility

As air temperatures continue to rise in response to anthropogenic climate change the environment will impose a heat load on animals, especially when animals are exposed to solar radiation. Once ambient temperature exceeds body temperature the only way that animals can lose heat is through evaporative water loss (Tattersall *et al.*, 2012). In a warmer and drier environment animals would therefore need to lose the water, it should be conserving, to keep cool. Because behavioural adjustments often are less costly than are physiological responses (Huey and Tewksbury, 2009), organisms are therefore likely to make use of thermoregulatory behaviour to try to buffer the effects of climate change (Kearney *et al.*, 2009).

Such thermoregulatory behaviours include the use of cooler microclimates, changes in posture or orientation, and changes in daily activity patterns, all of which would limit exposure to high heat loads and water loss (Huey, 1991). Microclimates are formed by a combination of physical factors, including solar radiation, air temperature and wind that determines thermal conditions near the ground (Porter and Gates, 1969). Although landscapes often are thermally highly heterogeneous (Carroll *et al.*, 2016), the diversity of microclimates available for use decreases with increasing body size. While small mammals have a variety of burrows, crevices and caves to select from, microclimate selection in large mammals, typically, is limited to shade-seeking. Shade use in large mammals is well-documented (e.g. Jarman, 1972, Smith *et al.*, 1985, Dean *et al.*, 1999, Seddon and Ismail, 2002, Hetem *et al.*, 2009b) and typically increases from the cool to the warm season. Similarly, large mammals are expected to increase their use of cooler microclimates in the face of climate change, and this should come at little cost, provided it does not compromise the animals' nutritional or water requirements (Fuller *et al.*, 2016).

Observations on the Arabian oryx *Oryx leucoryx*, endemic to the hyper-arid deserts of the Arabian Peninsula, showed that the animals spent more time using shade during hotter days, with the time spent foraging during the day decreasing by 60% compared to cooler days (Seddon and Ismail, 2002). More recently Hetem *et al.* (2012a) used biologging techniques to show that the Arabian oryx spent more of their time resting in the shade during the hot summer months than in warm wet months and, at times, the microclimates used were as much as 12°C cooler than the open environment. Moreover, under similar environmental heat loads the Arabian oryx made use of cooler microclimates when they had no access to surface water compared to when surface water was available (Hetem *et al.*, 2012a). Although a concomitant decrease in diurnal activity was recorded, the animals managed to maintain their overall 24h activity, as measured through biologging, by shifting from mainly crepuscular activity to nocturnal activity. Feeding for longer during the night, to compensate for decreased diurnal feeding activity as a result of high daytime temperatures, was facilitated by the absence of large predators in the Arabian Desert (Hetem *et al.*, 2012a). It is questionable whether animals that co-exist with large predators could compensate for a decrease in diurnal activity by shifting to a more nocturnal activity period in response to climate change. The long diurnal activity bouts and general lack of nocturnal foraging by the blue wildebeest in Tanzania's Manyara National Park, have, for example, been attributed to high nocturnal predation risk (Beekman and Prins, 1989). Even the obligate diurnally active animals can, however, manage their heat loads.

Changes in posture, or orientation relative to the angle of the sun, is a frequently used behavioural adjustment used by numerous types of animals to manage the solar radiation that they are exposed to (e.g. Waldschmidt, 1980, Shelly and Ludwig, 1985, Coelho, 2001, Maloney *et al.*, 2005b, Hetem *et al.*, 2009a). In a cold Northern Hemisphere environment, the Alpine ibex *Capra ibex* bask in the morning sun (Signer *et al.*, 2011). By turning the long axis of their bodies perpendicular to the sun the ibex maximise their exposure to the early morning radiant heat load, thereby increasing their body temperatures passively (Signer *et al.*, 2011). In contrast, animals in warmer environments are known to turn the long axis of their bodies parallel to the angle of the sun's rays, thereby limiting the surface area exposed to the sun's radiation and reducing their heat load (Hetem *et al.*, 2011). Among artiodactyls, clear seasonal differences in orientation have been observed, with black wildebeest *Connochaetes gnu*, as well as blue wildebeest *Connochaetes taurinus*, impala *Aepyceros melampus* and eland *Tragelaphus oryx* all spending more time oriented parallel to, and less time perpendicular to, the sun during summer than during winter (Maloney *et al.*, 2005b, Hetem *et al.*, 2011). When drinking water was available, the black wildebeest spent less time oriented parallel to the sun, indicating a change from behavioural to physiological thermoregulation such as panting and sweating (Maloney *et al.*, 2005b).

While facilitating survival in a rapidly changing environment, there are undoubtedly limits to behavioural plasticity, and it has been suggested that such plasticity ultimately will limit the physiological adaptations necessary to survive a warming climate (Buckley *et al.*, 2015). There is some evidence in lizards suggesting that the selection for heat tolerance might be compromised as a result of animals regulating their body temperatures behaviourally; the so-called Bogert effect (Huey *et al.*, 2003).

1.3.3.1.3 Physiological acclimatisation

Plasticity of physiological mechanisms determine to what extent animals can adapt to a changing environment (Carey, 2005), but the combined effects of limited environmental resources and biochemical and physical constraints limit the capacity of physiological systems to respond to change (Hetem *et al.*, 2014). Without understanding the thermal physiological sensitivity of a species, including the proximity to its thermal limits under current environmental conditions, one cannot predict the physiological effects of climate change on that species (Stillman, 2003, Kenney *et al.*, 2004). It is widely held that generalist species, which typically have large geographic ranges, greater physiological plasticity and wide thermal tolerance windows will be less affected by climate change than species physiologically specialised with respect to their thermal environment (Bale *et al.*, 2002, Visser and Both, 2005, Codron *et al.*, 2008). As thermal specialists, endotherms are therefore especially vulnerable to climate change (Huey *et al.*, 2012).

As a result of climate change, mammals across much of Africa will be exposed to not only increased air temperatures but also more frequent and extreme heat waves and droughts (Niang *et al.*, 2014). Changes in the peripheral blood flow of large mammals facilitate the establishment of a thermal gradient with the environment, which facilitates dry heat loss to, and limits heat gain from, the environment (Tattersall *et al.*, 2012). As large mammals have a smaller surface area to volume ratio than do smaller mammals, they gain heat from their environment at a slower rate than smaller mammals, while also losing heat to the environment at a slower rate. In addition, large mammals also have greater thermal inertia and lower mass-specific metabolic heat production. Consequently high air temperature and solar radiation causes less of a deviation in the core body temperature of large mammals compared with small mammals (Jessen, 2001). Nevertheless, variability in body temperature does occur, as documented in the Arabian oryx (e.g., Ostrowski et al 2003). Such variation may be a form of physiological plasticity that could allow animals to cope with climate change.

1.3.3.1.3.1 Body temperature

It has been widely held since the 1960’s, and it still is reported in some textbooks today, that large mammals can cope with heat by using heterothermy. Although the idea originated from a study on captive camels *Camelus dromedarius* (Schmidt-Nielsen *et al.*, 1956), it was popularised following subsequent work on two large African ungulates, the eland and the beisa oryx *Oryx gazella beisa* (Taylor, 1969). Based on this premise, arid-zone mammals like the camel and the beisa oryx allow their body temperatures to increase throughout the day, thereby progressively reducing the temperature gradient between the animal and the environment, and reaching a peak late in the afternoon or early evening, even when euhydrated. The enhanced temperature gradient between the animal and its environment through the evening then facilitates non-evaporative heat loss to the cooler environment throughout the night. The minimum body temperature, typically reached early morning around sunrise, then allows for substantial heat gain during the following day. Such adaptive heterothermy therefore results in wide fluctuations of 24h body temperature – up to 6.2°C in the captive camel (Schmidt-Nielsen *et al.*, 1956) and 4.0°C in the captive beisa oryx (Taylor, 1969) - as the maximum body temperature is elevated above, and the minimum reduced below, normal 24h maximum and minimum values, respectively. Adaptive heterothermy therefore facilitates the conservation of body water, as those animals employing it suppress evaporative cooling during the day, resulting in the elevation of body temperature, or the night when the temperature gradient allows for dry heat loss to the environment (Mitchell *et al.*, 2002).

More recently it was found that the gemsbok *Oryx gazella gazella*, a close relative of the beisa oryx studied by Taylor (1969), did not have widely fluctuating body temperatures. Over a measuring period of up to 15 days gemsbok body temperature had only a moderate range (1.8 ± 0.3°C) of nycthemeral rhythm. Moreover, variations in ambient heat load did not affect the range of the nycthemeral rhythm (Maloney *et al.*, 2002). Although the gemsbok study was possibly done over too-short a time frame to be conclusive, no evidence for adaptive heterothermy has been found in any of the other African ungulates studied to date (black wildebeest, Jessen *et al.* 1994; eland, Fuller *et al.* 1999; kudu *Tragelaphus strepsiceros*, Hetem *et al.* 2008; springbok *Antidorcas marsupialis*, Mitchell *et al.* 1997, Fuller *et al.* 2005; zebra *Equus quagga*, Fuller *et al.* 2000). Large fluctuations in 24h body temperature have, however, been found in the Arabian oryx (Ostrowski *et al.*, 2003, Hetem *et al.*, 2012b) as well as the diminutive Arabian sand gazelle *Gazella subgutturosa marica* with a body mass of 15 kg (Hetem *et al.*, 2012b), free-living in the deserts of Saudi Arabia.

While there is no doubt that free-living Arabian oryx and Arabian sand gazelles display heterothermy, an alternative explanation as to the cause of the variable body temperature in these animals has recently been put forward. Hetem and co-workers reported a maximum 24h range in body temperature of 7.7°C in the Arabian oryx during hot and dry summer conditions in Saudi Arabia. However, the mean 24h body temperature range of the Arabian oryx (5.5 ±0.5°C) also did not differ from that of the Arabian sand gazelle (4.1 ±0.5°C). The amplitudes of the 24h body temperatures of the Arabian oryx and sand gazelle were, however, not associated with the amplitude of the variation of air temperature (Hetem *et al.*, 2012b). The animals, therefore, did not increase their heat storage with increasing temperature. When the oryx had access to drinking water following rainfall the range in body temperature was only about 2.5°C. However, during summer, neither the Arabian oryx, nor the Arabian sand gazelle, had access to drinking water and the condition of the grazing was poor (Hetem *et al.*, 2012b). The authors argued that the absence of water, in combination with the high ambient temperatures, resulted in the elevated 24h maximum body temperatures. By inhibiting evaporative cooling when water-stressed, the Arabian oryx and sand gazelle therefore prioritised osmoregulation at the expense of thermoregulation, resulting in elevated 24h maximum body temperatures (Hetem *et al.*, 2012b, Hetem *et al.*, 2016).

The elevated body temperatures reported by Hetem and colleagues (2012b; 2016) should not be confused with dehydration-induced hyperthermia. Dehydration-induced hyperthermia is the elevation of body temperature in the absence of water, characterised by an elevated maximum body temperature due to the suppression of evaporative water loss. Unlike heterothermy, dehydration-induced hyperthermia is not characterised by an increased 24h range (maximum – minimum) in body temperature (Mitchell *et al.*, 2002). Hetem and colleagues showed decreased minimum body temperatures, in conjunction with elevated maximum body temperatures in both the Arabian oryx and the sand gazelle during the hot dry season, resulting in the increased range in 24h body temperature. The decrease in the minimum body temperature was attributed to the poor quality grazing, only sparsely available in the desert environment, potentially resulting in a nutritionally-induced drop in metabolic rate and, consequently, lower 24h minimum body temperatures (Hetem *et al.*, 2012b)

Nutritionally-induced reductions in metabolic rate and the resulting decrease in body temperature have been documented previously in domesticated animals. For example, decreasing the amount of food offered to Shetland ponies *Equus ferus caballus* by 30% was associated with a 1°C decrease in rectal temperature (Brinkmann *et al.*, 2012). Similarly Maloney and colleagues found that sheep *Ovis aries* that were fed only 70% of their maintenance diet developed nocturnal hypothermia, thereby increasing their 24h body temperature range by 70% (Maloney *et al.*, 2013). Reductions in metabolic rate, accompanied by lowered body temperature have also been recorded in wild ungulates, including the Alpine ibex (Signer *et al.*, 2011), red deer *Cervus elaphus* (Arnold *et al.*, 2004) and Przewalski’s horse *Equus ferus przewalskii* (Arnold *et al.*, 2006). A recent review article suggested that the reduction in metabolic rate and the lowering of body temperature in response to undernourishment might be an adaptation to cope with starvation (McCue, 2010).

Large mammals, regardless of their size, therefore have well-controlled, stable body temperatures when they have access to water and food of sufficient quantity and quality, with their 24h range in body temperature typically varying by less than 2.5°C (Mitchell *et al.*, 2002, Fuller *et al.*, 2014, Fuller *et al.*, 2016, Hetem *et al.*, 2016). When food becomes a limiting factor large mammals respond by reducing their metabolic rates, their energy requirements and also their minimum body temperatures. When water becomes a limiting factor mammals suppress evaporative water loss and allow their body temperatures to rise, conserving body water in the process. A reduction in both respiratory and cutaneous evaporative heat loss has previously been documented in water-stressed artiodactyls (Taylor, 1970b), while reduced respiratory evaporative heat loss has also been recorded in heat-stressed goats making use of selective brain cooling (Kuhnen, 1997). As selective brain cooling is believed to also suppress evaporative water loss (Jessen *et al.*, 1994) it is another physiological mechanism that could provide many of those animals in possession of a carotid rete the possibility for acclimatization to a changing environment.

1.3.3.1.3.2 Selective brain cooling

The carotid rete, or rostral epidural rete mirabile, is an intracranial vascular structure, near-ubiquitous and often elaborate in the Ruminantiamorpha, Whippomorpha, Camelidamorpha and Suinamorpha, collectively known as the Cetartiodactyla (Fig. 4; nomenclature *sensu* (Spaulding *et al.*, 2009). The carotid rete is present also, often in a rudimentary or primitive form, in a number of laurasiatherian mammals (Ask-Upmark, 1935, du Boulay and Verity, 1973), including cats (Kamijyo and Garcia, 1975), in which it is extracranial (Daniel *et al.*, 1953), and domestic dogs *Canis lupus familiaris* (Daniel *et al.*, 1953, Gillilan, 1976). Primates, many small-mass mammals (for example, rodents, lagomorphs, and “insectivores”), and perissodactyls (horses, tapirs and rhinoceroses), a sister group of the artiodactyls (Hassanin *et al.*, 2012), have no carotid rete (Ask-Upmark, 1935, Sisson and Grossman, 1967, Gillilan, 1974).

In artiodactyls, anatomical investigations (Ask-Upmark, 1935, Daniel *et al.*, 1953, Gillilan, 1974, Carlton and McKean, 1977, Frąckowiak *et al.*, 2015, Kiełtyka-Kurc *et al.*, 2015), including the identification of osteological correlates in extant and extinct artiodactyls (O'Brien, 2016), and physiological studies (Johnsen *et al.*, 1987, Mitchell *et al.*, 1997, Fuller *et al.*, 1999b, Maloney *et al.*, 2002, Lust *et al.*, 2007, Hetem *et al.*, 2012c) have confirmed the presence of the rete and its functionality in virtually all of the extant terrestrial artiodactyls, with informative exceptions (Fig. 4). In the vast majority of terrestrial artiodactyls, the carotid rete is found *in lieu* of the internal carotid artery and serves as the main supply of oxygenated blood to the brain (Schummer *et al.*, 1981, Wible, 1984, Frąckowiak, 2006, O'Leary, 2010, O'Brien, 2015).

An exception is the Tragulidae, which consist of three genera of small, forest-dwelling antelope that have retained an internal carotid artery instead of a carotid rete (Fukuta *et al.*, 2007, O'Brien, 2015). Whether their unique cranial vasculature is a plesiomorphic (*sensu* Janis, 1984) or apomorphic (e.g. Clauss & Rössner 2014) characteristic has not been resolved. The Hippopotamidae also are artiodactyls and a historic paper identifies hippopotamuses as having a carotid rete (Chapman, 1881), but it is indistinct (du Boulay and Verity, 1973, O'Brien, 2016). The status of the rete in Hippopotamidae requires further investigation using modern techniques, because it was identified before the anatomical techniques of resin injection and maceration for exploring vascular systems became available.

The function of the carotid rete in the Cetacea is unknown, but in the terrestrial artiodactyls, cats, and dogs, it is a heat exchanger, that can be used to cool the brain, but importantly the hypothalamic region, below the temperature of carotid arterial blood. This phenomenon, known as selective brain cooling, first was described in a domestic cat *Felis catus* 50 years ago (Baker and Hayward, 1967).

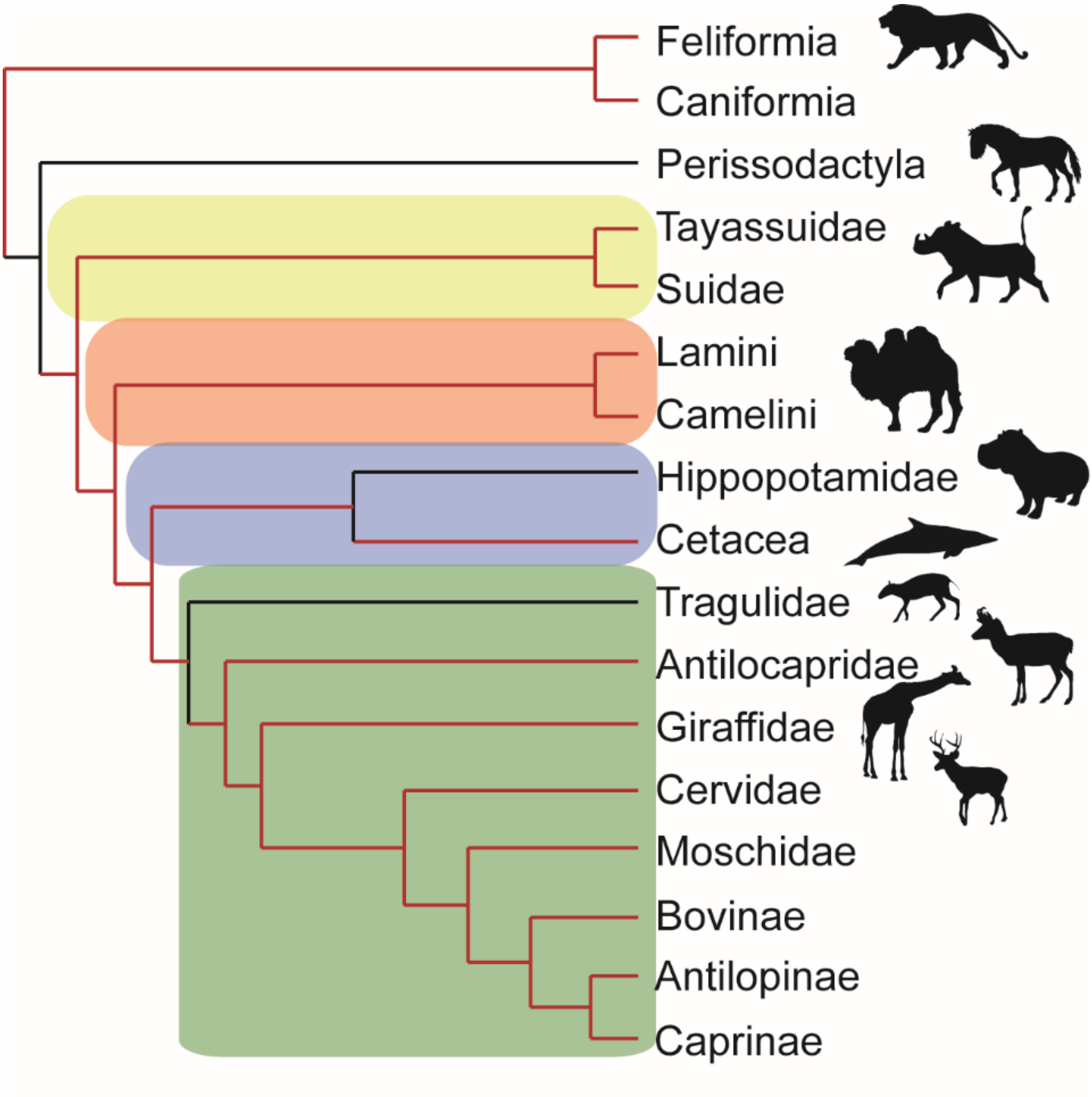

Figure 4: Phylogenetic tree indicating the relationship between the Cetartiodactyla, Perissodactyla and the Carnivora, represented by the cats and dogs (adapted from Hassanin *et al.*, 2012). Red branches indicate clades with a carotid rete, capable of selective brain cooling. Black branches designate the absence of a carotid rete (Hippopotamidae largely data deficient). Also depicted in various shades are the Ruminantiamorpha (green), Whippomorpha (blue), Camelidamorpha (orange) and Suinamorpha (yellow).

The brain, as a highly metabolically-active organ, usually has a temperature higher than that of the arterial blood perfusing it. The heat produced by the brain is removed by that blood, meaning that in the absence of a carotid rete, the brain is about 0.5°C warmer than arterial blood leaving the heart (Fuller *et al.*, 2000, Maloney *et al.*, 2009). The carotid rete, carrying arterial blood destined for the brain, is surrounded by venous blood in either a cavernous sinus (artiodactyls) or a pterygoid sinus (felids) (Daniel *et al.*, 1953). The venous blood derives from the maxilloturbinates and other mucous surfaces of the mouth and nose, where it is cooled to well below arterial blood temperature by evaporation of water into inspired air, whether or not the mammal is panting (Kuhnen and Jessen, 1991).

The thin walls and large surface area of the carotid rete vessels allow for efficient heat exchange between the arterial and venous blood, the result being that the arterial blood exiting the carotid rete into the brain, and subsequently the hypothalamic tissue in that region, can be more than 1°C cooler than arterial blood entering the rete (Maloney *et al.*, 2007).

Soon after the heat-exchange function of the carotid rete was discovered, investigations of selective brain cooling in domesticated and habituated wild mammals in captivity led to the conclusion that selective brain cooling served to protect the brain from reaching dangerously high temperatures (Baker and Hayward, 1967, Baker and Hayward, 1968, Magilton and Swift, 1968, Baker, 1972, Taylor and Lyman, 1972, Baker and Chapman, 1977, Baker, 1979, Mitchell *et al.*, 1987). That conclusion was influenced heavily by one measurement of selective brain cooling. Taylor and Lyman (1972) reported that brain temperature after induced exercise in habituated Thomson's gazelle *Gazella thomsonii* was as much as 2.7°C lower than carotid arterial blood temperature, a magnitude of selective brain cooling never seen before or since by anyone else, in any mammal.

A protectionist function fitted well with the perceived vulnerability of brain tissue to thermal damage *in vitro* (Burger and Fuhrman, 1964). Only when it was shown that goats *Capra hircus* could withstand brain temperatures of 42.5°C for an hour without any apparent ill-effect (Caputa *et al.*, 1986b) did it become apparent that the brain was not as vulnerable to thermal damage as believed previously (Mitchell *et al.*, 2002). Indeed, rather than the brain, it is the tissue of the gastrointestinal tract that is most susceptible to thermal damage (Braasch, 1964) due to reduced splanchnic blood flow and endotoxin leakage (Leon and Helwig, 2010). Subsequent studies of free-living and unrestrained mammals have revealed that selective brain cooling is not obligatory at high body temperatures (Mitchell *et al.*, 2002), and, as first noted in a laboratory study on goats, it is part of the normothermic thermoregulatory repertoire of artiodactyls (Kuhnen and Jessen, 1991). Rather than functioning primarily to protect the brain from thermal damage, selective brain cooling modulates the use of body water for thermoregulation (Jessen *et al.*, 1998). By reducing the temperature of the hypothalamus, where the temperature sensors that provide the internal drive for heat loss are located, selective brain cooling reduces evaporative water loss (Kuhnen, 1997).

Factors controlling selective brain cooling

Control mechanisms govern the onset and degree of selective brain cooling in mammals with a carotid rete. The finding that selective brain cooling typically is exhibited by tame or habituated mammals when exposed to heat or exercise indicated that a primary input to the control of selective brain cooling is the mammal's internal temperature (Jessen *et al.*, 1998). However, the absence of selective brain cooling in hyperthermic artiodactyls, particularly in free-living mammals during intense exercise, revealed that there also are non-thermal inputs in the control of selective brain cooling, and that these inputs can override thermal inputs (Mitchell *et al.*, 2002; Fuller *et al.*, 2014).

Thermal inputs

Typically at the lower carotid arterial blood temperatures, brain temperature is higher than, and runs in parallel to, carotid arterial blood temperature. In that temperature regime, in all mammals whether or not they have a carotid rete, hypothalamic temperature generally exceeds carotid arterial blood temperature by about 0.2 - 0.5ºC (Maloney *et al.*, 2007). As carotid arterial blood temperature rises, hypothalamic temperature uncouples from carotid arterial blood temperature, as selective brain cooling ensues in those species with a carotid rete. There are two variables that can be used to characterise selective brain cooling: the temperature at which hypothalamic temperature and carotid arterial blood temperature are equal, which is the threshold temperature for selective brain cooling (Kuhnen and Jessen, 1991), and the extent to which brain temperature drops below carotid arterial blood temperature, the magnitude of selective brain cooling. The threshold temperature for selective brain cooling can differ, within an individual when exposed to different environmental conditions (Hetem *et al.*, 2012).

That hyperthermia is a not a prerequisite for selective brain cooling is confirmed by measurements of the threshold temperature in many species (Table 1); it typically lies between 38°C and 39°C (Table 1), close to the modal and mean body core temperature of artiodactyls (Hetem *et al.*, 2016). In captive mammals, the magnitude of selective brain cooling typically increases with increasing carotid arterial blood temperature, but in free-living wild mammals it is more variable. Large mammals typically have a 24h rhythm of arterial blood temperature with a trough soon after dawn and a peak in the late afternoon (Mitchell *et al.*, 2002; Hetem *et al.*, 2016). Hypothalamic temperature, which is determined mainly by post-carotid rete arterial blood temperature (Hayward *et al.*, 1966), typically has a 24h pattern similar to that of carotid arterial blood temperature. As carotid arterial blood temperature approaches its 24h peak, hypothalamic temperature uncouples from carotid arterial blood temperature and selective brain cooling ensues. Although selective brain cooling could be implemented at any time of the day, its magnitude typically is greatest (0.5 – 1.5°C, Table 1) around the time of the 24h peak in carotid arterial blood temperature (Mitchell *et al.*, 2002). At that time of day, environmental heat

Table 1: The threshold temperature (mean ± SD, where originally reported) and maximum magnitude of selective brain cooling reported (or inferred) from studies of selective brain cooling on artiodactyls. Values in brackets represent sample size.

Species (sample size)	Selective brain cooling		Notes	Reference
	Threshold (⁰C)	Magnitude (⁰C)		
Domestic or habituated animals studied under controlled, laboratory conditions, unless otherwise indicated				
Goat *Capra hircus* (6)	Not reported	2.5	Heat exchanger	(Caputa *et al.*, 1986a)
Goat *Capra hircus* (3)	38.8 ± 0.1	1.2	Heat exchanger	(Kuhnen & Jessen 1991)
Goat *Capra hircus* (3)	39.05 ± 0.06	0.5	Heat exchanger & high humidity	(Kuhnen & Jessen 1992)
Goat *Capra hircus* (3)	39.2 ± 0.05	1.2	Heat exchanger & low humidity	(Kuhnen & Jessen 1992)
Goat *Capra hircus* (3)	38.9	1.5	Heat exchanger	(Kuhnen, 1997)
Goat *Capra hircus* (3)	39.0	0.3	Free-living, euhydration	(Jessen *et al.*, 1998)
Goat *Capra hircus* (3)	38.9	0.8	Free-living, dehydration	(Jessen *et al.*, 1998)
Goat *Capra hircus* (5)	39.3 ± 0.1	0.7	Hydrated and exercise	(Baker and Nijland, 1993)
Goat *Capra hircus* (5)	39.3	1.2	Dehydrated and exercise	(Baker and Nijland, 1993)
Ox *Bos taurus* (11)	39.1	0.8	Heat exposure	(Chesy *et al.*, 1983)
Ox Bos taurus (3)	40.3	1.5	Exercise	(Chesy *et al.*, 1985)
Sheep *Ovis aries* (3)	Not reported	0.6	Heat exchanger & heat exposure	(Maloney and Mitchell, 1997)
Sheep *Ovis aries* (4)	Not reported	1.0	Room temperature	(Laburn *et al.*, 1988)
Sheep *Ovis aries* (4)	Not reported	1.0	Heat exposure	(Laburn *et al.*, 1988)
Sheep *Ovis aries* (4)	Not reported	1.0	Febrile – induced	(Laburn *et al.*, 1988)
Sheep *Ovis aries* (4)	Not reported	0.5	Exercise	(Laburn *et al.*, 1988)
Sheep *Ovis aries* (4)	Not reported	0.8	Heat exposure	(Nijland *et al.*, 1990)
Sheep *Ovis aries* (4)	Not reported	0.6	Cold exposure	(Nijland *et al.*, 1990)
Sheep *Ovis aries* (4)	Not reported	0.9	Febrile – induced	(Nijland *et al.*, 1990)

Sheep *Ovis aries* (9)	39.1	0.4	Water deprivation & heat exposure	(Fuller *et al.,* 2007)
Sheep *Ovis aries* (9)	39.5 ± 0.5	1.5	Water deprivation & heat exposure	(Strauss *et al.*, 2016)
Sheep *Ovis aries* (5)	39.1 ± 0.5	0.5		(Maloney *et al.*, 2007)
Pig *Sus scrofa* (4)	38.9	0.9	Thermoneutral	(Fuller *et al.*, 1999a)
Pig *Sus scrofa* (2)	Not reported	0.8	Heat stress	(Fuller *et al.*, 1999a)
Pig *Sus scrofa* (1)	Not reported	0.3	Cold stress	(Fuller *et al.*, 1999a)
Camel *Camelus dromedarius* (2)	38.0	1.0	At rest	(Schroter *et al.*, 1989)
Camel *Camelus dromedarius* (1)	39.5	1.5	Exercise – hydrated & dehydrated	(Schroter *et al.*, 1989)
Reindeer *Rangifer tarandus* (3)	38.7 ± 0.2	1.0	Heat exchanger	(Kuhnen and Mercer, 1993)
Reindeer *Rangifer tarandus* (3)	39.5 ± 0.3	0.5	Exercise	(Kuhnen and Mercer, 1993)
Thomson's gazelle *Gazella thomsonii* (5)	39.4	2.7	Exercise	(Taylor and Lyman, 1972)
Free-living wild animals with free access to normal behaviour				
Black wildebeest *Connochaetes gnu* (4)	38.9 ± 0.2	0.4		(Jessen *et al.*, 1994)
Eland *Tragelaphus oryx* (1)	40	0.4		(Fuller *et al.*, 1999b)
Gemsbok *Oryx gazella* (4)	39.8 ± 0.4	0.4		(Maloney *et al.*, 2002)
Gemsbok *Oryx gazella* (4)	39.5 ± 0.9	0.9		(Strauss *et al.*, 2016)
Kudu *Tragelaphus strepsiceros* (4)	39.3 ± 0.7	0.5	Febrile – naturally	(Hetem *et al.*, 2008)
Kudu *Tragelaphus strepsiceros* (4)	38.8 ± 0.1	0.2	Afebrile	(Hetem *et al.*, 2008)
Arabian oryx *Oryx leucoryx* (4)	37.8 ± 0.1	1.4		(Hetem *et al.*, 2012)
Springbok *Antidorcas marsupialis* (2)	39.2 ± 0.2	0.5		(Mitchell *et al.*, 1997)
Pronghorn *Antilocapra americana* (2)	39.5	0.5		(Lust *et al.*, 2007)

Blue wildebeest *Connochaetes taurinus* (6)	39.3 ± 0.4	1.1		(Strauss *et al.*, 2016)
Red hartebeest *Alcelaphus buselaphus* (5)	39.4 ± 0.6	1.0		(Strauss *et al.*, 2016)

load is decreasing and the antelope usually are involved in low-intensity activities, such as rumination and grazing. Despite strong thermal inputs at that time of day, however, selective brain cooling in artiodactyls can be modulated by non-thermal inputs, such that its magnitude can be further increased, or it can even be completely abolished.

Non-thermal inputs

Cranial sympathetic tone

A role for sympathetic nervous system activity in the modulation of selective brain cooling was revealed through a series of elegant experiments on reindeer *Rangifer tarandus* (Johnsen and Folkow, 1988, Johnsen *et al.*, 1987, Johnsen *et al.*, 1985). Experiments on habituated artiodactyls showed that selective brain cooling could be controlled by directing the passage of venous blood draining the evaporating surfaces of the head either to the cavernous sinus, classically via the angularis oculi veins, or to the jugular vein via the facial vein, bypassing the cavernous sinus (Fig. 5). The default direction of blood flow appears to be via the cavernous sinus, so increased cranial blood flow in heat-stressed artiodactyls (Vesterdorf *et al.*, 2011, Maloney and Mitchell, 1997) led to increased flow of cooled venous blood to the cavernous sinus and, hence, increased selective brain cooling.

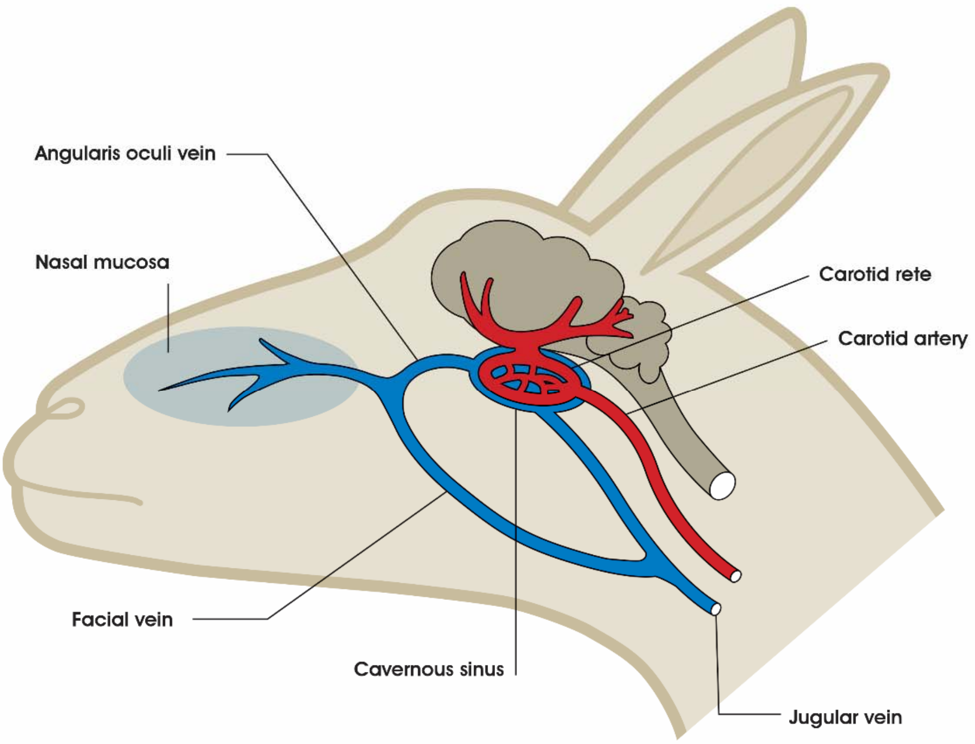

Figure 5: Diagram illustrating the position of the carotid rete in artiodactyls, located within a cavernous sinus at the base of the brain, as well as the main arterial blood supply to the brain, via the carotid rete. During selective brain cooling, cool venous blood from the nasal mucosa drains into the cavernous sinus via the angularis oculi vein (as well as some deeper veins; see Fuller *et al.* 2011). Under high sympathetic activity the cooled venous blood draining from the nasal mucosa largely bypasses the cavernous sinus as it is shunted via the facial vein, thereby attenuating selective brain cooling. Diagram adapted from Jessen (1998).

Increased cranial sympathetic nervous system activity, however, led to simultaneous contraction of a muscular sphincter in the angularis oculi vein (α-adrenergic) and dilatation of a similar sphincter in the facial vein (β-adrenergic), resulting in venous blood bypassing the cavernous sinus and returning via the jugular to the heart (Johnsen *et al.*, 1985, Johnsen *et al.*, 1987, Johnsen and Folkow, 1988). While this differential vasoconstriction can modulate the degree of selective brain cooling, it does not fully explain the control of selective brain cooling in artiodactyls. In addition to the angularis oculi veins, less superficial veins such as the sphenopalatine, external ophthalmic and ethmoidal veins also supply venous blood to the cavernous sinus (Sisson and Grossman, 1967, Carlton and McKean, 1977). As a result, severing the angularis oculi veins does not completely eliminate selective brain cooling (Fuller *et al.*, 2011). High sympathetic tone attenuates selective brain cooling not only by constricting the angularis oculi veins, but also by constricting nasal mucosal blood vessels and closing arteriovenous anastomoses.

Within the nasal mucosa, the rate of heat extraction is attenuated through a combination of reduced blood flow and the restriction of airway width (Malm, 1973). So increased cranial sympathetic nervous system activity decreases blood flow to the evaporating surfaces of the head as well as redirecting the flow of venous blood leaving those surfaces away from the cavernous sinus (Maloney and Mitchell, 1997, Fuller *et al.*, 2011). These changes result in an upward shift of the threshold temperature for selective brain cooling, as documented in springbok (Mitchell *et al.*, 1997), or complete abolishment of selective brain cooling, as documented in black wildebeest (Jessen *et al.*, 1994). Free-living mammals rarely engage in intensive exercise except for predator-prey interactions, during which there is a dramatic increase in sympathetic nervous system activity in both predator and prey. The sympathetic activity prevalent during the flight and fright response in wild artiodactyls abolishes selective brain cooling (Jessen *et al.*, 1994), overriding the drive of high body temperature. Selective brain cooling observed in tame artiodactyls exercising at low or moderate intensity likely is associated with low sympathetic activity (Mitchell *et al.*, 2002).

Though it is not as strong as in intense exercise during predator-prey interactions, there also is sympathetic activation during psychological stress. The effects of psychological stress, including rising body and brain temperatures are well-documented, even in non-rete mammals such as rats (Mohammed *et al.*, 2014). In rete-mammals selective brain cooling is absent or reduced in other circumstances likely associated with increased sympathetic tone, including nearby human presence (Maloney *et al.*, 2001), the return of drinking water to dehydrated artiodactyls (Fuller *et al.*, 2007), and vigilance in male artiodactyls (Maloney *et al.*, 2002; Hetem *et al.*, 2012). The temperament of individual mammals, a heritable trait among artiodactyls (Murphy *et al.*, 1994), plays an important role in how situations are experienced (Beausoleil *et al.*, 2008).

Hydration status

Selective brain cooling is enhanced during water deprivation (Jessen *et al.*, 1998, Fuller *et al.*, 2007). Because body temperature is elevated in dehydrated mammals exposed to heat, that enhancement could arise from a stronger thermal drive on selective brain cooling (Jessen *et al.*, 1998). However, Fuller *et al.* (2007) showed that sheep exhibited a higher magnitude of selective brain cooling during dehydration even when carotid arterial blood temperature did not increase. Water deprivation on its own therefore seems to be a sufficient stimulus to enhance selective brain cooling. That idea is supported by measurement of selective brain cooling in antelope living free in arid environments. In the hyper-arid desert of Saudi Arabia, the mean magnitude of selective brain cooling in free-living Arabian oryx peaked in the afternoon (Figure 6A), well after solar noon and maximum heat load, as measured with miniature black globe thermometers on the collar of the animals (Hetem *et al.*, 2007). Water availability, or aridity, appears to be the main factor determining the use and magnitude of selective brain cooling, with selective brain cooling being enhanced in the dry period than in the wet period (Hetem *et al.* 2012). During the hot dry period when no drinking water was available, the Arabian oryx made near continuous use of selective brain cooling during the four hours leading to sunset (Figure 6B, grey bars), compared to no more than 40% of the time during the warm wet period, when the oryx presumably had access to drinking water or food with a higher moisture content (Figure 6B, black bars).

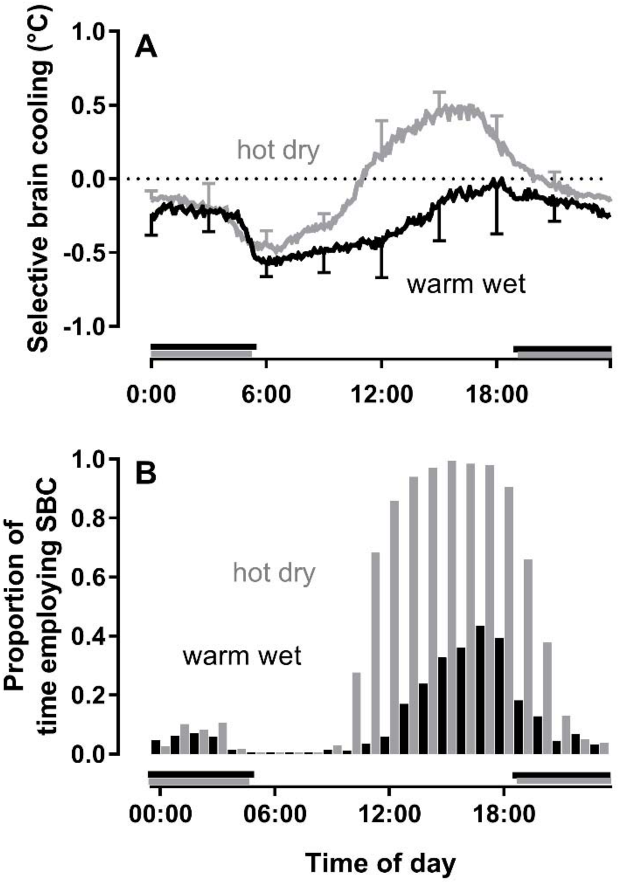

Figure 6: The effect of aridity on selective brain cooling as illustrated through differences in the mean (±SD) magnitude of selective brain cooling (carotid blood temperature minus hypothalamic temperature; A) and the proportion of time that a single Arabian oryx used selective brain cooling in the hot, hyper-arid deserts of Saudi Arabia (B). Grey depicts the hot dry and black the warm wet periods. Horizontal grey and black bars depict night-time during the two periods of interest. Data from Hetem *et al.* (2012c).

Despite being exposed to similar ambient temperatures, Arabian oryx, in the hyper-arid desert of Saudi Arabia, also showed enhanced selective brain cooling compared to the congeneric gemsbok with free access to water (Maloney *et al.*, 2002), as they initiated selective brain cooling at a lower threshold temperature, used selective brain cooling more frequently and at greater magnitude, than did the gemsbok (Hetem *et al.*, 2012c).

1.4 Thesis aims

The preceding sections have summarised some of the ways animals might respond to some aspects of climate change as well as current knowledge surrounding the physiological and behavioural responses that animals, particularly large artiodactyl mammals, could employ to adapt to the hotter and drier conditions predicted for southern Africa. In summarising the possible physiological and behavioural responses to climate change several areas worthy of further investigation have been identified and these shaped the research presented here.

Water is the most vital limiting component in Southern Hemisphere ecosystems, as the region is relatively warm year-round, with relatively low thermal variability seasonally, as compared with the Northern Hemisphere (Hawkins *et al.*, 2003). Therefore, a particular focus of this thesis was on the conservation of body water in a region where increasing air temperatures and the associated increased evaporation rates are predicted to coincide with decreasing, less predictable, rainfall. As indicated below, much of this focus was centred on selective brain cooling in large African antelope as well as investigating variability in body temperature, subcutaneous temperature and microclimate use.

Chapter 2. For almost 20 years the evidence that selective brain cooling conserves body water hinged on a single laboratory study where the brain and core body temperatures of two goats were manipulated through the use of extracorporeal heat exchangers in 18 experiments (Kuhnen, 1997). Here it was shown that heat-stressed goats had higher respiratory evaporative water loss, when selective brain cooling was inhibited by experimentally (artificially) increasing brain temperature, than when animals were allowed to use selective brain cooling.

Although water conservation as a result of selective brain cooling has frequently been inferred in artiodactyls (Mitchell *et al.*, 1997, Jessen *et al.*, 1998, Maloney *et al.*, 2002, Fuller *et al.*, 2007, Hetem *et al.*, 2008, Hetem *et al.*, 2012c), no-one has ever measured how much water an artiodactyl, naturally making use of selective brain cooling, could save. Using a model artiodactyl, the Dorper sheep, I therefore set out to quantify the water savings that result from selective brain cooling.

Chapter 3. Although selective brain cooling has been investigated in artiodactyls inhabiting arid (e.g., Fuller *et al.* 1999; Maloney *et al.* 2002; Hetem *et al.* 2008) and even hyper-arid (e.g., Hetem *et al.* 2012c) regions, very little is known about the comparative use of selective brain cooling in artiodactyls, the relative capacities of different artiodactyl species to selectively brain cool, or whether selective brain cooling differences – if it exists - might be related to species anatomical differences in the carotid rete. Selective brain cooling has never been investigated simultaneously in more than one species inhabiting the same environment. I therefore set out to compare the use of selective brain cooling and the anatomy of the carotid rete, in three large, sympatric artiodactyl species with varying water dependencies that naturally occur in the hot and dry Northern Cape Province of South Africa.

Chapter 4. It is increasingly realised that a mechanistic understanding of how animals respond to the rapidly changing environment is needed (Seebacher and Franklin, 2012, Muñoz *et al.*, 2015, Fuller *et al.*, 2016). However, it is clear that not enough is known about the physiology of animals, particularly the physiological limits of animals (Kenney *et al.*, 2004), to understand to what extent they could adjust their physiology in a changing environment (Somero, 2010).

The identification of physiological (e.g. body temperature) and behavioural (e.g., microclimate use) signals in response to a hot and dry environment, expected to become even hotter and drier, with increased rainfall variability in the face of climate change, could serve as an important reference for future investigations into the extent and occurrence of physiological and behavioural adjustments. I therefore investigated the abdominal body temperatures, changes in peripheral blood, and microclimate use of three large artiodactyl species (gemsbok, red hartebeest and blue wildebeest) over the four warmest months of the year.

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CHAPTER 2

Selective brain cooling reduces water turnover in dehydrated sheep

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RESEARCH ARTICLE

Selective Brain Cooling Reduces Water Turnover in Dehydrated Sheep

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Abstract

In artiodactyls, arterial blood destined for the brain can be cooled through counter-current heat exchange within the cavernous sinus via a process called selective brain cooling. We test the hypothesis that selective brain cooling, which results in lowered hypothalamic temperature, contributes to water conservation in sheep. Nine Dorper sheep, instrumented to provide measurements of carotid blood and brain temperature, were dosed with deuterium oxide (D_2O), exposed to heat for 8 days (40°C for 6-h per day) and deprived of water for the last five days (days 3 to 8). Plasma osmolality increased and the body water fraction decreased over the five days of water deprivation, with the sheep losing 16.7% of their body mass. Following water deprivation, both the mean 24h carotid blood temperature and the mean 24h brain temperature increased, but carotid blood temperature increased more than did brain temperature resulting in increased selective brain cooling. There was considerable inter-individual variation in the degree to which individual sheep used selective brain cooling. In general, sheep spent more time using selective brain cooling, and it was of greater magnitude, when dehydrated compared to when they were euhydrated. We found a significant positive correlation between selective brain cooling magnitude and osmolality (an index of hydration state). Both the magnitude of selective brain cooling and the proportion of time that sheep spent selective brain cooling were negatively correlated with water turnover. Sheep that used selective brain cooling more frequently, and with greater magnitude, lost less water than did conspecifics using selective brain cooling less efficiently. Our results show that a 50kg sheep can save 2.6L of water per day (~60% of daily water intake) when it employs selective brain cooling for 50% of the day during heat exposure. We conclude that selective brain cooling has a water conservation function in artiodactyls.

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Introduction

Several mammal species, particularly artiodactyls (such as sheep, goats and antelope), use the carotid rete to lower hypothalamic temperature below arterial blood temperature, a process

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termed selective brain cooling [1]. Typically, in resting artiodactyls, when carotid blood temperature exceeds a threshold of 38.5–39.0°C [2], selective brain cooling is implemented, and brain temperature increases at a slower rate than does carotid temperature. However, selective brain cooling is not mandatory at high body temperatures and can be switched off by high sympathetic nervous system activity [3, 4]. Sympathetic nervous system activity influences blood flow to the cavernous sinus, which envelopes the carotid rete with cool blood, through the constriction of nasal mucosal blood vessels and the closure of arteriovenous anastomoses [5] located in the nasal cavity [6]. Variation in sympathetic tone between individuals of the same species appears to account for high inter-individual variability in the use of selective brain cooling [7, 8], which can be quantified by assessing the magnitude of the difference between brain and carotid blood temperature, the proportion of time spent with brain temperature lower than carotid blood temperature, and the threshold temperature for selective brain cooling [9].

Selective brain cooling initially was thought to protect a thermally vulnerable brain during hyperthermia [10, 11]. But such a protective role for selective brain cooling has been found wanting [12]. It is now thought that selective brain cooling plays a role in conserving body water by cooling the temperature sensors in the hypothalamic region of the brain, thereby reducing heat loss drive [1, 9, 12–14]. Indeed, when dehydrated, Bedouin goats [15] and Dorper sheep [8] showed enhanced selective brain cooling, both in terms of magnitude and the frequency of use. However, the higher carotid blood and brain temperatures recorded in the Bedouin goats during dehydration meant that the increased selective brain cooling also could be explained by the higher core temperature compared to the period of euhydration [8]. Conversely, the carotid blood temperature in the sheep did not increase during dehydration, yet selective brain cooling was still enhanced, showing that the increase in selective brain cooling was a function of dehydration, not simply elevated core body temperature [8]. Studies in free-ranging antelope also support the idea that selective brain cooling is enhanced in arid environments independently of heat exposure. Arabian oryx had a higher magnitude, higher frequency of use, and lower threshold of selective brain cooling in dry environments than when they had free access to water, despite ambient temperatures being similar [9].

These studies provide circumstantial evidence in support of the notion that selective brain cooling serves a water conservation function. To date, only a single laboratory study has investigated the relationship between selective brain cooling and water use. Goats, in which extracorporeal heat exchangers were used to independently manipulate carotid blood and brain temperatures, had lower respiratory evaporative heat loss when the brain was cooled artificially and the body core heated, than when the brain was not cooled [2]. Here we investigate, for the first time, whether water savings accrue in artiodactyls spontaneously using selective brain cooling. Dorper sheep were fitted with temperature probes that provided continuous, real-time, temperature measurements from the brain and carotid artery. We measured water turnover using the washout rate of deuterium oxide. We hypothesized that if selective brain cooling does play a role in water conservation it would lead to reduced water turnover in those sheep showing greater selective brain cooling.

Methods

Animals

Nine adult Dorper ewes (Dorset x Persian *Ovis aries*, initial body mass 60 ± 15 kg, mean ± SD) were used. The mean fleece length of the ewes at study termination, as measured at the shoulder and on the hip, was 18.0 ± 3.8 mm and 15.4 ± 2.2 mm, respectively. The sheep were housed indoors in a single pen at an ambient temperature of ~22°C on a 12h/12h light/dark cycle (lights on from 06:00–18:00) for two weeks before implantation surgery. Lucerne, *Eragrostis* teff and water were available *ad libitum*, while commercial sheep pellets (Epol, Johannesburg, South Africa) were provided to the sheep once per day. The water content of the food was estimated to be no more than 5%. Fresh bedding (dry straw) was provided daily after the holding pen was cleaned.

Ethics statement

The Animal Ethics Screening Committee of the University of the Witwatersrand approved all of the procedures (protocol no. 2008/55/04).

Surgery

In the holding pen the sheep were given diazepam (0.3mg kg^{-1} Valium, Roche, Nutley, NJ, USA) intra-muscularly (IM). About 15min later butorphanol (0.1mg kg^{-1}, Torbugesic, Fort Dodge, Kempton Park, South Africa) and ketamine (2mg kg^{-1}, Anaket, Bayer Animal Health, Isando, South Africa) were administered intravenously (IV) and the sheep were transported to a nearby sterile theatre. At the theatre, the sheep were maintained in sternal recumbency with the help of sandbags. Following intubation, general anesthesia was maintained with 2–3% isoflurane (Isofor, Safe Line Pharmaceuticals, Johannesburg, South Africa) in oxygen. The surgical incision sites were shaved and sterilized with chlorhexidine gluconate (Hibitane, Zeneca, Johannesburg, South Africa). Each sheep received a long-acting antibiotic (0.1ml kg^{-1}, IM, penicillin, Peni LA Phenix, Virbac Animal Health, Centurion, South Africa), an analgesic (0.01mg kg^{-1}, sub-cutaneously (SC), buprenorphine, Temgesic, Schering Plough, Isando, South Africa), a non-steroidal anti-inflammatory (2.2mg kg^{-1}, IM, flunixin meglumine, Finadyne, Centaur Labs, Johannesburg, South Africa), a broad-spectrum parasiticide (2.5ml SC, doramectin, Dectomax, Pfizer Laboratories, Sandton, South Africa), and a multivitamin (Mulitivit injectable solution, Univet Ltd, County Cavan, Ireland) injection. To anesthetize the periosteum and reduce bleeding a 1.5ml cocktail of lignocaine (0.1g, Bayer Animal Health, Johannesburg, South Africa) and adrenaline (Kyron Labs, Johannesburg, South Africa), mixed at a ratio of 10:1, was injected subcutaneously under the scalp where the brain probe would be inserted. Under sterile surgical procedures, we implanted two thermometric radio-telemeters into each animal (see below). We measured respiratory rate (visual observation), heart rate, peripheral arterial oxygen saturation (Nonin Handheld Pulse Oximeter, Plymouth, MN, USA) and rectal temperature (Cardell 9400, Midmark Corporation, Ohio, USA) throughout the ~2 hour surgical procedure. Implanted devices were covered with inert wax (Sasol, Johannesburg, South Africa) and dry-sterilized using formaldehyde vapor for at least 24 hours before implantation.

Temperature measurement

Ruggedized glass-coated bead thermistors (bead diameter 0.3mm; ABOE3-BR11KA103K-L10, Thermometrics Corporation, Northridge, CA, USA) in sealed guide tubes specific for each implantation site were used to measure temperatures in the brain and carotid artery. For brain temperature measurements, a cellulose acetate butyrate guide tube (length 44mm, OD 3.2mm, ID 1.6mm; World Precision Instruments, Sarasota, FL, USA) sealed with a stainless-steel cap at the tip was positioned near the hypothalamic region of the brain. The guide tube was inserted via a 2.0mm hole drilled through the cranium, ~3.0mm to the left of the mid-line of the skull, at previously determined anatomical markers [16]. The guide tube was connected to a polyvinylchloride head plate (20 x 10 x 5mm). The head plate was attached to the skull with two bone screws and covered with skin. A polytetrafluoroethylene (PTFE) coated coaxial cable (150mm long, OD 3mm, Belden, Richmond, VA, USA), connected to the thermistor in the head plate, was extended subcutaneously over the skull to a radio-telemeter (Africa Wildlife Tracking, Pretoria, South Africa) specifically developed for our purposes. The radio-telemeter, with dimensions ~70 x 50 x 30mm and mass of 100g when covered with wax, was placed subcutaneously, caudal to the base of the left ear.

To measure carotid arterial blood temperature, a bead thermistor, inserted into a blind-ended, thin-walled PTFE tube (OD 1.35mm, ID 0.97mm; Straight Aortic Flush 4F Catheter, Cordis, The Netherlands) was inserted 80mm into the left common carotid artery, towards the heart. It was secured in position, midway along the length of the neck, with a purse-string suture in the artery wall. Outside the artery, the PTFE tube was sealed onto a PTFE-coated coaxial cable (150mm long, OD 3mm, Belden, Richmond, VA, USA), connecting the thermistor to a second radio-telemeter, identical to that used for brain temperature measurement. Because all the transmitting units were implanted there was no external instrumentation on the animals.

The temperature sensors had a measurement range of 34°C to 50°C and a resolution of 0.03°C. They were calibrated against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany) in an insulated water bath to an accuracy of better than 0.05°C. A single receiver/transmitting unit, which logged temperature data from all animals at 5-minute intervals, was placed ~2m above ground level, and less than 8m away from any animal at any given time during the study. Logged temperature data were also transmitted to an internet server, via GPRS, at hourly intervals, with all temperature records accessible in near-real time.

Experimental procedure

The experimental procedure was repeated twice, once for four sheep and again for five sheep. The sheep were moved into a temperature-controlled climatic chamber (about 7.0m^2 floor area) ten days after implantation surgery, and allowed to acclimatize to their new environment for two days (12h/12h light/dark cycle, lights on at 06:00, temperature ~22–24°C, relative humidity ~60%). Following acclimatization to the climatic chamber, the sheep were allowed a further two days to acclimatize to the temperature and relative humidity regime that would prevail during the study: heat exposure (40°C; relative humidity ~40%) for a total of six hours per day (09:00–15:00), ~22–24°C, ~60% relative humidity for the remainder of the time (Fig. 1C). Food and water were available freely during the four-day acclimatization period. On the first two days of the study (day 1 & 2), the animals had free access to food and drinking water (as in their holding pen). Drinking water, but not food, was removed on the morning of day 3 (~09:00) and was returned on the afternoon of day 8 (~16:00) to each animal after it was individually weighed. Animals were weighed every afternoon at about 15:00.

We measured water turnover (L day^{-1}), the sum of water influx (food and metabolic water) and water loss (change in total body water), by dilution of the stable hydrogen isotope deuterium oxide, over the five days of water deprivation and heat exposure. At the time of water removal on day 3, a 4ml blood sample was collected from the right jugular vein of each sheep to determine background concentrations of deuterium oxide. Each sheep then was injected with 0.05ml kg^{-1} deuterium oxide (D_2O, 99.8 at%; Merck & Co. Ltd, Rahway, NJ, USA) IM, based on body mass measured on the previous afternoon. The blood sample collection and deuterium injection procedures were completed for all sheep within ~15 minutes of water removal. Following equilibration of the deuterium oxide in the body water pool (~ 8 hours later), another (enriched) 4ml blood sample was collected from the right jugular vein. Using the same procedures, we collected a blood sample every afternoon immediately following heat exposure from each sheep on days 4–7 of the experimental period. Shortly before heat exposure on the morning of day 8 (~08:30, 5th day of water deprivation), a background blood sample was taken, and sheep again received 0.05ml kg^{-1} deuterium oxide IM based on the body mass measured on the

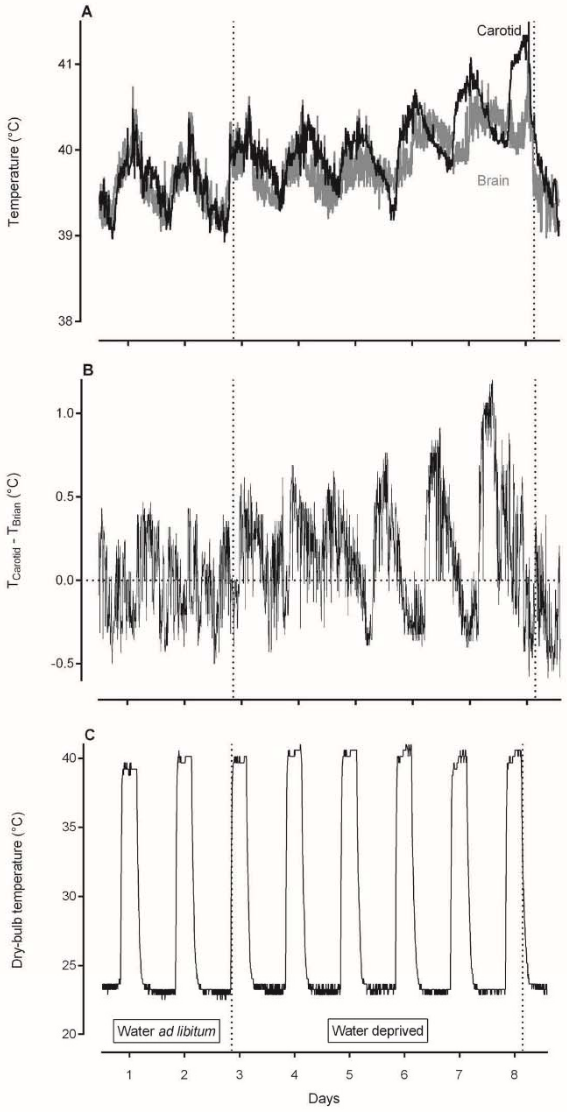

Fig 1. Carotid blood (black) and brain (grey) temperature (A) and the difference between carotid blood and brain temperatures (B; positive values indicating selective brain cooling) at 5-minute intervals for one representative sheep, and daily air dry-bulb temperature profile as recorded in the climatic chamber (C) during an 8-day experimental period. Sheep had *ad libitum* access to drinking water on days 1 and 2. Water was removed on the morning of day 3 and was returned on the afternoon of day 8 (as indicated by the dotted lines). Food was available *ad libitum* throughout the experimental period.

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previous afternoon. This second injection of deuterium oxide allowed for a measure of total body water volume at the end of the water deprivation period to allow for the calculation of change in total body water over the period of water deprivation. A final blood sample was collected ~8 hours later (i.e. ~15:00 on afternoon of day 8), following equilibration of the deuterium oxide in the body water pool and before drinking water was returned to the sheep.

The blood samples were collected in 4ml heparinized vials (Vacutainer, BD-Plymouth, UK), and placed in ice until centrifuged (Wifug Ltd, Bradford, England) at 6000g for 10 min. Plasma was separated and stored in 2ml vials (cryogenic vials 430489, Corning Inc., NY, USA) and frozen at -4°C for later analysis. A calibrated osmometer (Vapro Model 5600, Wescor Inc., Utah, USA) was used to determine the osmolality of every plasma sample. Pure water was extracted from the plasma samples through cryogenic vacuum distillation [17]. The deuterium concentration of the pure water samples was measured on a liquid water isotope analyzer (LWIA-24d, Los Gatos Research, Mountain View, CA, USA), normalized against an international reference, Vienna Standard Mean Ocean Water (V-SMOW). Individual water samples were analyzed five times and the mean concentration calculated. A comprehensive description of the underlying theory, the available methods and calculations are provided elsewhere [18]. Briefly, we used a two sample method to determine body water turnover, where the elimination rate of the deuterium oxide was calculated through the slope of a regression of the log-transformed concentration plotted against time [18]. The natural exponential function of the y-intercept of the regression line was used to estimate the isotope distribution space. We estimated total body water by dividing the known dose of injected deuterium oxide (ml) by the difference between the natural exponential function of the y-intercept (isotope distribution space) and the background enrichment (ppm) of deuterium oxide. The influx of water (through metabolism and preformed water in the diet) was determined from the deuterium oxide dilution rate over the 5-day period, when sheep did not have access to water, multiplied by the total body water.

Data analyses

The original 5-minute body temperature records from each sheep were used to find the absolute minimum and the absolute maximum carotid blood and brain temperatures each day, and to calculate the mean, standard deviation and amplitude (difference between absolute maximum and absolute minimum temperatures) for each individual. Selective brain cooling was calculated as the difference between carotid blood temperature and brain temperature, with positive values representing selective brain cooling. We categorized the 48h period before water removal and the 48h period before returning water to the sheep as periods of "euhydration" and "dehydration", respectively. We used paired Students t-tests to compare temperature data during the periods of euhydration and dehydration. Repeated measures one-way ANOVAs were used to investigate the differences in the daily magnitude of selective brain cooling (calculated as the maximum or mean positive difference between carotid and brain temperature), the proportion of time spent using selective brain cooling (calculated as the proportion of measurements in which the difference between carotid and brain temperature was ≥0.05°C), the threshold temperature (calculated as the temperature at which the regression line of carotid blood versus brain temperature crossed the line of identity) and osmolality throughout the experimental period. We used Pearson correlation analysis to investigate possible relationships between variables. To compare variability in the selective brain cooling response during water deprivation, we correlated the standard deviations of the selective brain cooling magnitude, and the standard deviations of the mean proportion of time spent using selective brain cooling, against days of water deprivation. Also, to investigate the rates at which carotid blood and brain temperatures increased with continued water deprivation we compared the slopes of the linear regression lines of the daily carotid blood and brain temperature profile against days of water deprivation. Data analysis was done using GraphPad PRISM 6. Results are reported as mean ± SD, and $P \leq 0.05$ was considered significant.

Results

Fig. 1A and B show the response of one sheep to the entire experimental protocol. During the first two days, when water was freely available, carotid blood and brain temperatures followed similar profiles and brain temperature frequently exceeded carotid blood temperature. These patterns changed following the removal of water (day 3), with brain temperature clearly decoupling from carotid blood temperature. Peak carotid blood and peak brain temperature increased with continued water deprivation (Fig. 1A), however, the increase in carotid blood temperature was larger than that of brain temperature, leading to increasing separation of the two temperatures and enhanced selective brain cooling. Indeed, the mean difference between carotid blood and brain temperatures was significantly higher during dehydration than during euhydration ($t_8 = 2.83$, $P = 0.022$). Examination of the difference between carotid blood and brain temperature revealed selective brain cooling being used for more of the day (fewer data points below the zero line), and an increased magnitude (greater positive value) of selective brain cooling, as the period of water deprivation progressed (Fig. 1B). The other eight sheep showed similar responses during the experimental protocol.

Increased carotid and brain temperature with water deprivation

The increased use of selective brain cooling with continued water deprivation (Fig. 1B) was associated with an increase in carotid blood temperature (Fig. 1A). The mean 24h carotid blood temperature was 0.6°C higher during dehydration than during euhydration ($t_8 = 3.48$, $P = 0.0082$; Table 1). Although the minimum carotid blood temperature did not change from euhydration to dehydration ($t_8 = 1.91$, $P = 0.092$), the maximum carotid blood temperature was significantly higher during dehydration than during euhydration ($t_8 = 2.85$, $P = 0.021$). The mean 24h brain temperature also was significantly higher during dehydration than during euhydration ($t_8 = 3.02$, $P = 0.017$). Unlike carotid blood temperature, the minimum brain temperature increased significantly from euhydration to dehydration ($t_8 = 2.43$, $P = 0.041$). The maximum brain temperature also increased significantly from euhydration to dehydration ($t_8 = 2.35$, $P = 0.047$).

To investigate the rate at which carotid and brain temperatures increased with continued water deprivation, we compared the slope of regression lines of the mean and maximum daily temperatures against days of water deprivation. The slope of the linear regression lines of mean daily carotid temperature ($y = 39.5 + 0.086x$, $r^2 = 0.97$) and mean daily brain temperature ($y = 39.4 + 0.037x$, $r^2 = 0.81$) against days of water deprivation were significantly different ($F_{(1,8)} = 17.37$, $P = 0.003$), with brain temperature having the lower slope. However, the slopes of the regression lines of maximum daily carotid temperature ($y = 0.093x + 40.26$, $r^2 = 0.44$) and maximum daily brain temperature ($y = 0.053x + 40.19$, $r^2 = 0.40$) against days of water deprivation did not differ ($F_{(1,8)} = 0.43$, $P = 0.53$).

Increased selective brain cooling with water deprivation

The daily magnitude (mean positive difference between carotid blood and brain temperature) of selective brain cooling increased significantly with days of water deprivation ($F_{(5,8)} = 10.26$, $P = 0.0017$) from 0.28 ± 0.06°C on day 3 (the day of water removal) to peak at 0.66 ± 0.22°C on day 8 (fifth day of water deprivation; Fig. 2A). The selective brain cooling magnitude was positively correlated with carotid blood temperatures ($r^2 = 0.61$, $P = 0.013$). The mean proportion of time that the sheep spent using selective brain cooling ranged from 52.5 ± 12.8% on day 3 to

Table 1. Mean (±SD) daily carotid blood and brain temperatures recorded every 5 minutes and the mean difference between carotid and brain temperatures in nine Dorper ewes during 48h of euhydration and 48h of dehydration.

	Carotid artery temperature		Brain temperature	
	Euhydration	**Dehydration**	**Euhydration**	**Dehydration**
Mean	39.36 ± 0.19°C	39.92 ± 0.47°C*	39.36 ± 0.18°C	39.61 ± 0.36°C*
Minimum	38.60 ± 0.21°C	38.95 ± 0.63°C	38.76 ± 020°C	38.99 ± 0.43°C*
Maximum	40.41 ± 0.39°C	40.96 ± 0.38°C*	40.37 ± 0.31°C	40.56 ± 0.29°C*
Amplitude	1.77 ± 0.40°C	1.92 ± 0.55°C	1.60 ± 0.27°C	1.69 ± 0.19°C

* indicates significant differences ($P<0.05$) between temperatures measured during euhydration and dehydration

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76 ± 15% on day 5 (the second day after water removal; Fig. 2B), but did not change significantly over the period of water deprivation, ($F_{(5,8)} = 2.23$, $P = 0.15$). Plasma osmolality increased significantly over the period of water deprivation ($F_{(5,8)} = 50.09$, $P < 0.0001$), from 282 ± 11mmol kg^{-1} on day 3 to 328 ± 10mmol kg^{-1} on day 8 (Fig. 2C). There was no significant relationship ($r^2 = 0.23$, $P = 0.34$) between the variability of osmolality, as measured by the standard deviation of mean plasma osmolality, and time since water removal. The variability in the use of selective brain cooling by different sheep (Fig. 2A & B), as measured by the standard deviation of the mean magnitude of selective brain cooling ($r^2 = 0.86$, $P = 0.008$) and the standard deviation of the mean proportion of time spent using selective brain cooling ($r^2 = 0.92$, $P = 0.003$), was correlated positively with the duration of the experimental period (days 3 to 8).

The mean magnitude of selective brain cooling increased significantly and was twice as high during dehydration (0.51 ± 0.20°C) compared to euhydration (0.26 ± 0.07°C; $t_8 = 3.78$, $P = 0.0054$; Fig. 3A). The maximum magnitude of selective brain cooling was significantly higher during dehydration (1.09 ± 0.37°C) than during euhydration (0.67 ± 0.18°C; $t_8 = 3.32$, $P = 0.0106$). The proportion of time that the sheep, on average, spent using selective brain cooling also increased significantly from their 48h state of euhydration (39 ± 12%) to that of dehydration (61 ± 24%; $t_8 = 2.57$, $P = 0.033$; Fig. 3B). The threshold temperature at which selective brain cooling was initiated did not differ between euhydration (39.31 ± 0.25°C) and dehydration (39.48 ± 0.47°C; $t_8 = 1.18$, $P = 0.27$; Fig. 3C).

Selective brain cooling and plasma osmolality

The body mass of the sheep decreased significantly, by about 16%, from 60 ± 15 kg during euhydration to 50 ± 15 kg (range of change: 7.0–11.6 kg) during dehydration ($t_8 = 17.99$, $P < 0.0001$). Concomitantly, plasma osmolality, on average, showed an increase of 15% (euhydration 287 ± 9 mmol kg^{-1} vs. dehydration 338 ± 15 mmol kg^{-1}; $t_8 = 10.96$, $P < 0.0001$). Correlating the maximum magnitude of selective brain cooling recorded per animal during the last four days of water deprivation against osmolality on the day that the maximum magnitude was recorded (i.e. one point per animal) resulted in a significant positive relationship ($r^2 = 0.70$, $P = 0.005$), indicating increased magnitude of selective brain cooling with increased osmolality (Fig. 4A). Correlating the maximum magnitude of selective brain cooling during the last four days of water deprivation against the change in osmolality (the difference between the osmolality on day of maximum selective brain cooling magnitude and the osmolality on day 3) also resulted in a significant positive relationship ($r^2 = 0.56$, $P = 0.020$; Fig. 4B). There was no relationship ($r^2 = 0.12$, $P = 0.36$) between the maximum proportion of time spent using selective brain cooling during a day on the last four days of water deprivation and osmolality on that day (Fig. 4C).

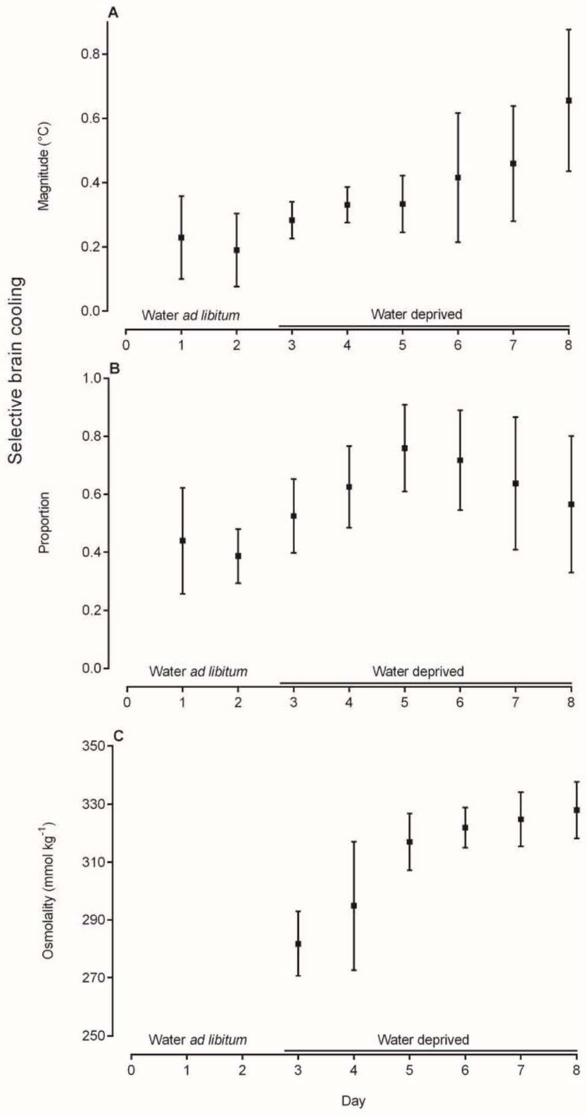

Fig 2. Mean (± SD) of the magnitude of selective brain cooling (A), the proportion of time that selective brain cooling was used (B) and the osmolality (C) as recorded daily for nine sheep over the 8-day experimental period. The five-day period in which water was removed is highlighted by the black line.

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Selective brain cooling and water loss

Total body water decreased significantly (range: -1.0–16.9L) from day 3 (41 ± 7L) to day 8 (31 ± 6L; $t_8 = 4.96$, $P = 0.0011$), that is over the five days of water deprivation. The body water fraction (% of body mass), on average, also decreased significantly over the five days of water

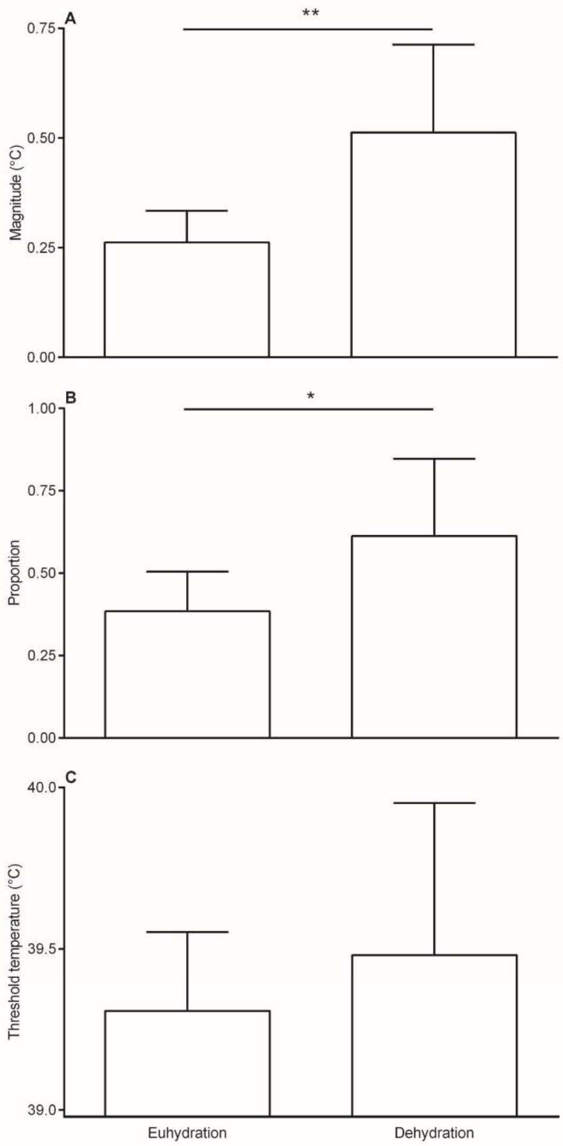

Fig 3. Mean (± SD) of the magnitude of selective brain cooling (A), proportion of time that selective brain cooling was used (B), and the threshold temperature for selective brain cooling (C) as recorded in nine Dorper sheep during euhydration (48 hours before water removal) and dehydration (last 48 hours before returning water). * P<0.05, ** P<0.01 for comparison between euhydration and dehydration.

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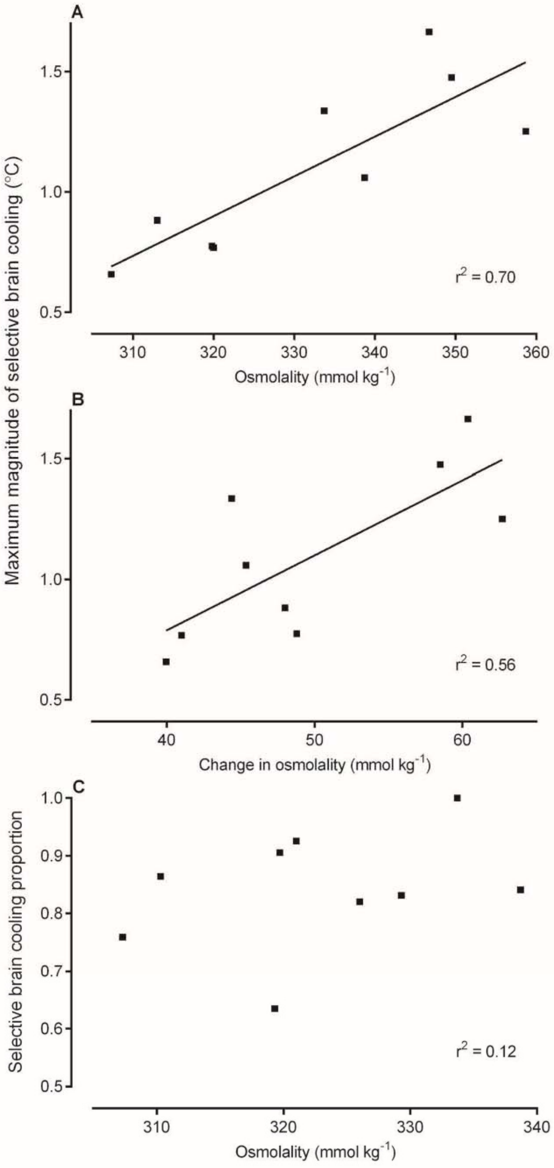

Fig 4. The relationship between the maximum magnitude of selective brain cooling (°C) during the last four days of water deprivation and plasma osmolality as recorded on the day of the maximum (A; $y = 0.02x - 4.38$ [± 1.37]), and maximum magnitude of selective brain cooling during the last four days of water deprivation and the change in osmolality (difference between osmolality on day of maximum and osmolality on day 3) (B; $y = 0.03x - 0.46$ [± 0.52]), and maximum proportion of time spent using selective brain cooling during the last four days of water deprivation and osmolality on the day of maximum proportion (C) for nine sheep. Each data point represents a single animal.

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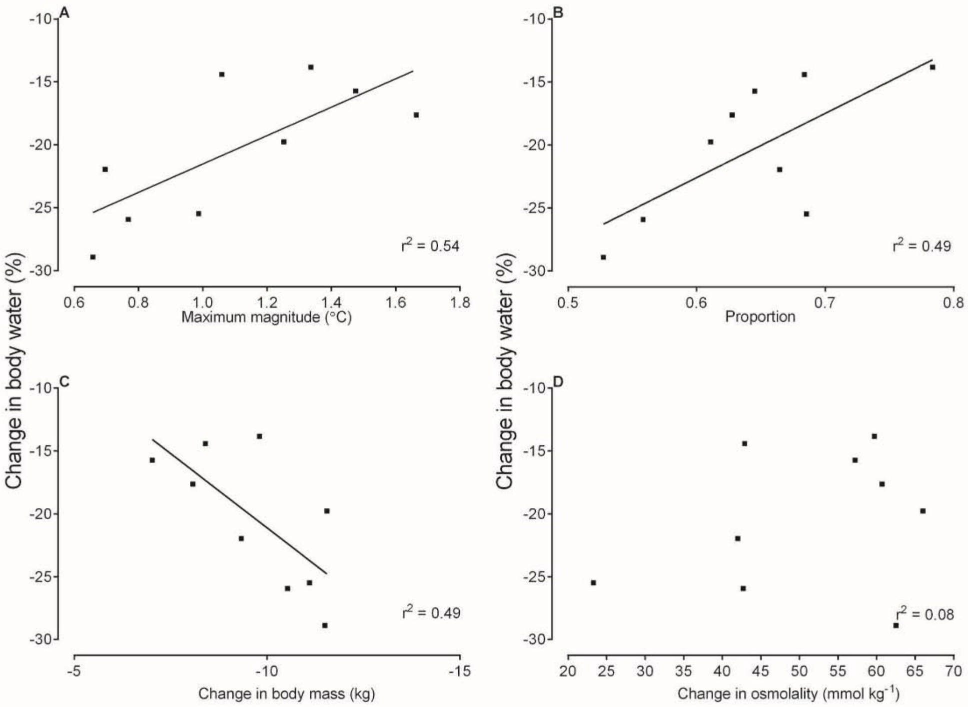

Fig 5. The relationship between change in body water (%) and the maximum magnitude of selective brain cooling (A; y = 50.96x—53.16 [± 12.67]), the proportion of time spent using selective brain cooling (B; y = 11.28x—32.80 [± 4.52]), the change in body mass (C; y = 2.36x + 2.50 [± 9.01]), and the change in plasma osmolality (D; no relationship) as recorded from day 3 to 8 (i.e., five days of water deprivation) in nine sheep. Each data point represents a single animal.

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deprivation (day 3, 70 ± 12% vs. day 8, 51 ± 7%; $t_8 = 3.88$, $P = 0.0047$). Water turnover and the magnitude of selective brain cooling correlated negatively ($r^2 = 0.54$, $P = 0.024$, Fig. 5A), as did water turnover and the proportion of time that animals spent using selective brain cooling ($r^2 = 0.49$, $P = 0.035$, Fig. 5B). A significant, negative correlation was also found between water turnover and the change in body mass of the nine sheep ($r^2 = 0.49$, $P = 0.037$) during the period of water deprivation (Fig. 5C). No relationship was found between the change in body water and change in osmolality ($r^2 = 0.08$, $P = 0.45$; Fig. 5D).

Discussion

Using stable isotope analysis to measure water turnover in sheep, we have shown, for the first time, that selective brain cooling allows an animal to conserve body water during water deprivation with heat exposure. Total body water comprised ~70% of the body mass of our sheep on the day of water removal (day 3), similar to that previously reported for large herbivores [19], and decreased to about 51% of the body mass following a 5-day period of water deprivation. Our sheep lost 16% of their initial body mass during five days of water deprivation; a decrease similar to that found in Dorper ewes previously exposed to a similar experimental protocol [8], and to Dorper rams that were water-deprived for 4 days [20]. The reduction in the total body water likely was the main cause of body mass loss in our sheep, as has been found for Marwari sheep [21], Sinai goats [22] and Dorper rams [20], with 94% of the total mass loss in the Dorper rams being attributed to water loss. Plasma osmolality of our sheep increased throughout the 5-day period of water deprivation (Fig. 2C), indicating increased water stress over time [23]. The sudden increase in osmolality from the 2nd to the 3rd day of water deprivation (day 4 to 5, Fig. 2C) is consistent with the idea that the rumen can buffer the effects of water deprivation over the short-term, but that the water reservoir of the rumen becomes depleted within a few days of water deprivation [24].

When our sheep had access to water (Fig. 1A, day 1 & 2) brain temperature and carotid blood temperature showed similar profiles, with brain temperature periodically being slightly above carotid blood temperature. Following the removal of water (day 3) both carotid blood and brain temperatures increased. Although arterial blood temperature is the main determinant of brain temperature [25], brain temperature increased by only half as much, on average, as did carotid blood temperature, resulting in an increased magnitude of selective brain cooling with progressive water deprivation (Fig. 1B & Fig. 2A).

Artiodactyls exposed to heat and deprived of drinking water appear to prioritize the conservation of body water by decreasing water loss through evaporative cooling, resulting in their body temperatures increasing, a phenomenon referred to as hypohydration or dehydration-induced hyperthermia [15, 26–28]. If selective brain cooling promotes the conservation of body water, one would expect the increased use of selective brain cooling with water deprivation to result in increased carotid blood temperature. Moreover, one would expect those animals showing the highest magnitude of selective brain cooling to also have the highest carotid blood (core) temperatures when dehydrated. In our sheep, the mean magnitude of selective brain cooling increased during the experimental period, from about 0.2°C when water was available *ad libitum*, to about 0.6°C on the 5th day of water deprivation (Fig. 2A). Concomitantly, carotid blood temperature increased by about 0.6°C during the period of water deprivation, double that previously reported for sheep exposed to a similar experimental protocol [8], but similar to the hypohydration hyperthermia previously found in dehydrated Bedouin goats [15]. We also found a strong relationship between the magnitude of selective brain cooling and carotid blood temperatures. Indeed, selective brain cooling in our sheep explained 61% of the variation in the carotid blood temperatures observed during the period of dehydration. Higher magnitudes of selective brain cooling were therefore associated with higher carotid blood temperatures.

A high carotid blood temperature is not the only factor that drives selective brain cooling [29]. Individual sheep in our study exposed to the same research and husbandry protocol showed considerable variability in the use of selective brain cooling. The variability of selective brain cooling (magnitude and frequency of use) between individuals also increased with continued water deprivation (Fig. 2A & B, day 6 to day 8). These results are likely explained by variation in sympathetic nervous system activation (for review see [1]) between animals with continued water deprivation.

Despite variability between individuals, selective brain cooling, in general, was enhanced with water deprivation. When our study animals had unlimited access to water they used selective brain cooling for 38% of the time. When dehydrated, they used selective brain cooling 61% of the time (Fig. 3B). The proportion of time that our sheep spent using selective brain cooling was not correlated to plasma osmolality, but rather to body water turnover (Fig. 5B), which explained 49% of the variability in the proportion of time spent using selective brain cooling.

The magnitude of selective brain cooling also increased in dehydrated compared to euhydrated states. We found a strong positive correlation between the maximum magnitude of selective brain cooling and both plasma osmolality (Fig. 4A) and the absolute change in osmolality (Fig. 4B), with the osmolality explaining up to 70% of the variability in the maximum magnitude of selective brain cooling. One interpretation of the relationships between selective brain cooling magnitude and osmolality could be that those animals showing the highest levels of selective brain cooling became more dehydrated. However, because the magnitude of selective brain cooling peaked on the fifth day of water deprivation (Fig. 2A) and seven of our nine study animals exhibited maximum magnitudes of selective brain cooling on the last day of water deprivation, the same day that plasma osmolality peaked, we propose that those animals that became most dehydrated showed enhanced magnitudes of selective brain cooling, and as a result had reduced evaporative heat loss drive. Indeed, we found a significant positive correlation between body water turnover and the magnitude of selective brain cooling, which explained 54% of the observed variability, confirming that larger magnitudes of selective brain cooling resulted in smaller changes in body water turnover (Fig. 5A).

Our data support the idea that osmolality enhanced the magnitude of selective brain cooling, but not the proportion of time spent using selective brain cooling. Although hormonal feedback mechanisms following the initiation of selective brain cooling have not been investigated to date, osmolality is likely located upstream of the selective brain cooling-water deprivation sequence. If another variable, such as blood volume contraction, was responsible for enhanced selective brain cooling and the resultant water saving, we would expect osmolality to decrease in those animals showing the largest magnitudes of selective brain cooling. In rats, changes in osmolality as small as 2% are much more effective in altering vasopressin secretion than are changes in blood volume [30]. Although hypovolemia does help to maintain vasopressin release over the longer term, it is not considered to be an effective stimulus for vasopressin release, unless blood volume is reduced by more than 8–10% of the normal blood volume in rats [30] and by more than 10% in sheep [31]. The lack of correlation between changes in body water and changes in osmolality (Fig. 5D) and the plateau in osmolality during the last days of water deprivation (Fig. 2C, day 6 onwards) in our sheep may be explained by the increased electrolyte excretion during water deprivation, as previously documented in ruminants [19, 32] and the decreased food intake with continued water deprivation. Even though we did not quantify daily food intake in our sheep, decreased food intake during dehydration is well documented [33].

We did not measure water turnover in our study animals before the removal of drinking water. Therefore the actual changes in water turnover from a period of euhydration to dehydration could not be quantified in our study. Although artiodactyls do use selective brain cooling when euhydrated, the use of selective brain cooling is enhanced in the absence of drinking water in goats [15], sheep [8], and in free-ranging Arabian oryx [9]. While enhanced selective brain cooling has been reported in dehydrated animals previously [8, 15], to date no one has shown that selective brain cooling results in a saving of body water in dehydrated animals. Our study is the first to show that individuals spontaneously making use of selective brain cooling have lower water turnover rates than conspecifics, living under exactly the same conditions, which use less selective brain cooling.

As shown previously in a study with similar design to ours [8] the threshold temperature for selective brain cooling (~39.4°C) did not change with hydration status. Sheep in that study [8], however, had a much smaller increase in the magnitude of selective brain cooling when dehydrated and heat exposed than sheep in our current study. The lower level of selective brain cooling in those sheep, which possibly resulted from higher sympathetic tone, implies that the animals continued to make use of evaporative water loss to maintain body temperatures [7]. Indeed, carotid blood temperature did not increase during the progressive dehydration of those sheep [8], a result which contrasts with the significant increase in mean carotid blood temperature in our sheep (~ 0.6°C, Table 1) and Bedouin goats [15]. Thus, an increase in core temperature is not a requirement for enhanced selective brain cooling during dehydration.

Rather, body water status and hyperosmolality appear to be the primary drivers of selective brain cooling [8, 9]. Hyperosmolality in cats, a species which also can employ selective brain cooling, suppressed evaporative heat loss intra-cranially under conditions of dehydration [34]. Indeed, it is the osmotic pressure of the arterial blood perfusing the brain that stimulates the inhibition of thermoregulatory responses [35]. Inhibition of evaporative heat loss mechanisms, in turn, may contribute to an increase in core temperature.

Differences in temperament and the resultant variation in sympathetic nervous system tone when exposed to the same stressors likely also accounted for the observed increase in variability in selective brain cooling use by individuals with continued water deprivation. The relationship between selective brain cooling and increased sympathetic input (i.e. psychological stress) was first suggested in a study on reindeer [36] and it is now widely accepted that increased sympathetic activity attenuates the use of selective brain cooling in domestic [4, 8, 29] and wild [1, 7, 37] artiodactyls. Merino sheep, for example, abolished selective brain cooling when an investigator entered the holding area [29], while different levels of selective brain cooling in male and female gemsbok have been attributed to the vigilance associated with territorial defense among males [7]. While sympathetic input may alter the actual time that an animal spends using selective brain cooling, the maximum magnitude of selective brain cooling may be mediated by hyperosmolality. Changes in the magnitude of selective brain cooling can, in part, be attributed to variations in the blood flow to the respiratory evaporative heat loss surfaces as well as the evaporative heat loss capacity. For example, when an animal is hyperthermic, there is increased blood flow to the nasal mucosa and other parts of the head compared to during normothermia, while the blood flow to the brain does not change [38, 39]. Increased blood flow to the nasal mucosa, as a result of an increased proportion of the cardiac output passing through arteriovenous anastomoses there [38], could explain the larger magnitude of selective brain cooling when comparing the two hydration periods (Fig. 3A). Whether there is an upper limit to the nasal mucosal blood flow that could take place, or whether no clear upper limit exists, as was found for dogs [40], is not yet known for sheep. The maximum selective brain cooling magnitude recorded in our sheep was comparable to that (1.1°C vs. 1.2°C) previously recorded in the arid-adapted Arabian oryx during summer [9]. Artiodactyls, therefore, may well have similar underlying abilities in terms of the maximum magnitude of selective brain cooling. Even small magnitudes of selective brain cooling are likely to result in the conservation of body water though.

A 50 kg sheep using selective brain cooling for 50% of the time could save an estimated 2.6 liters of water per day (Fig. 5B). Given a daily water intake of 4.2 liters for Dorper ewes in the absence of heat stress [41], such selective brain cooling would result in a ~ 60% saving of normal daily water intake. In European goats artificial cooling of the brain resulted in a water saving of 0.7L per day (or 35% of normal intake) as a result of reduced respiratory evaporative water loss [2]. It was proposed that the water savings in the European goats would be about 70% when water lost through sweating was also taken into account [2], a proportion similar to that saved by our sheep using selective brain cooling spontaneously.

In conclusion, we have measured selective brain cooling and water turnover in nine Dorper ewes deprived of drinking water for a period of five days. We have shown that selective brain cooling increases with water deprivation and that such increases are likely mediated by changes in body fluid osmolality. Increased use of selective brain cooling, either through more frequent use or an increase in the magnitude of selective brain cooling, resulted in increased core temperatures. Although a relationship between selective brain cooling and water conservation has been suggested for the past 20 years [12], ours is the first study to quantify body water savings associated with spontaneous selective brain cooling. We have shown that Dorper sheep can save a substantial amount of their daily water requirements through the use of selective brain cooling. It remains to be investigated to what extent free-ranging artiodactyls, with varying water dependencies and changing seasonal food preferences, use selective brain cooling as a water conservation mechanism in the wild.

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Author Contributions

Conceived and designed the experiments: WMS RSH DM SKM AF. Performed the experiments: WMS RSH. Analyzed the data: WMS RSH DM SKM AF. Wrote the paper: WMS RSH DM SKM LCRM AF. Surgery and veterinary expertise: LCRM.

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CHAPTER 3

Three African antelope species with varying water dependencies exhibit similar selective brain cooling

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ORIGINAL PAPER

Three African antelope species with varying water dependencies exhibit similar selective brain cooling

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Abstract The use of selective brain cooling, where warm arterial blood destined for the brain is cooled in the carotid rete via counter-current heat exchange when in close proximity to cooler venous blood, contributes to the conservation of body water. We simultaneously measured carotid blood and hypothalamic temperature in four gemsbok, five red hartebeest and six blue wildebeest to assess the extent to which these free-living animals, with varying water dependency, routinely rely on selective brain cooling. We investigated the hypothesis that innate differences in selective brain cooling exist in large, sympatric artiodactyls with varying water dependency. All three species used selective brain cooling, without any discernible differences in three selective brain cooling indices. GLMMs revealed no species differences in the threshold temperature for selective brain cooling ($z = 0.79$, $P = 0.43$), the magnitude ($z = -0.51$, $P = 0.61$), or the frequency of selective brain cooling use ($z = -0.47$, $P = 0.64$), after controlling for carotid blood temperature and black globe temperature. Comparison of anatomical attributes of the carotid retes of the three species revealed that the volume ($F_{2,9} = 5.54$, $P = 0.03$) and height ($F_{2,9} = 5.43$, $P = 0.03$) of the carotid rete, per kilogram body mass, were greater in the red hartebeest than in the blue wildebeest. Nevertheless, intraspecific variability in the magnitude, the frequency of use, and the threshold temperature for selective brain cooling exceeded any interspecific variability in the three indices of selective brain cooling. We conclude that the three species have similar underlying ability to make use of selective brain cooling in an environment with freely available water. It remains to be seen to what extent these three species would rely on selective brain cooling, as a water conservation mechanism, when challenged by aridity, a condition likely to become prevalent throughout much of southern Africa under future climate change scenarios.

Keywords Conservation physiology · Climate change adaptation · Artiodactyl · Plasticity · Water conservation · Rostral epidural rete mirabile

Introduction

The diversity of artiodactyls, the group of even-toed ungulates that includes antelope, cattle and sheep, has increased since the Eocene, particularly during the progressively warm and seasonal climates of the Miocene (Barnosky et al. 2003; Bouchenak-Khelladi et al. 2009; Cifelli 1981; Hassanin and Douzery 1999). Their rapid diversification traditionally has been attributed to the development of the ruminant digestive tract, methods of food selection, and improved locomotion, which facilitated the use of sparse fibrous vegetation in open landscapes (Janis 1976). More recently, however, Mitchell and Lust (2008) suggested that the evolution of the carotid rete, and resultant selective brain cooling (the lowering of hypothalamic temperature

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below carotid blood temperature), also may have contributed to artiodactyl diversification. By cooling the hypothalamus, selective brain cooling inhibits evaporative heat loss (Jessen et al. 1998). Selective brain cooling was increased in dehydrated goats (Jessen et al. 1998) and sheep (Fuller et al. 2007), and reduced respiratory evaporative water loss in goats (Kuhnen 1997) and total body water loss in sheep (Strauss et al. 2015).

If the adaptive significance of selective brain cooling is in the modulation of water use for thermoregulation, the capacity for and use of selective brain cooling might be expected to differ in free-living artiodactyls with different water dependencies. While measurements of selective brain cooling have been made on animals in their natural environment, to date, all studies have been on single species. To determine if selective brain cooling plays a role in water dependency, measurements on species with different water dependencies need to be made simultaneously in the same environment.

One reason to expect a physiological interspecific difference in selective brain cooling amongst artiodactyl species would be differential evolution of the rete itself, as the anatomical structure responsible for selective brain cooling in artiodactyls (Fuller et al. 2014). Under the selection pressure of aridity, those species with greater water independency may have evolved bigger and more elaborate retes. Indeed, the carotid rete is absent in the forest-dwelling mouse deer (*Tragulus javanicus* and *Tragulus napu*), which have evolved in habitats with plentiful access to water (Fukuta et al. 2007).

While the carotid rete has been described morphologically for several species (Ask-Upmark 1935), the descriptions for artiodactyls have focussed mainly on the vascularisation and arterial blood supply to the carotid rete (Daniel et al. 1953; Gillilan 1974) and the venous circulation and drainage (Carlton and McKean 1977) of the carotid rete in domesticated animals. More recently, descriptions of the arterial blood supply to the brain and the carotid rete (also known as the rostral epidural rete mirabile) have been made in giraffe *Giraffa camelopardalis* (Frąckowiak and Jakubowski 2008), selected antelope (Family: Antilopinae) (Frąckowiak et al. 2015) and deer (Family: Cervidae) (Kiełtyka-Kurc et al. 2015). However, the physical dimensions of the carotid rete in artiodactyl species with different water dependencies have not been measured.

Here we compare selective brain cooling and carotid rete size (height, length, width and volume) in three large, sympatric artiodactyl species, with varying water dependencies that occur naturally in the semi-arid Kalahari of South Africa. We hypothesized that the gemsbok *Oryx gazella* (Linnaeus, 1758), a member of the Hippotragini that is reputedly independent of surface water (Skinner and Chimimba 2005), would use selective brain cooling more and have a larger carotid rete than would two members of the Alcelaphini, the red hartebeest *Alcelaphus buselaphus* (É. Geoffroy Saint-Hilaire, 1803) and the blue wildebeest *Connochaetes taurinus* (Burchell, 1823). While both the red hartebeest and the blue wildebeest are reliant on surface water (Knight et al. 1988; Skinner and Chimimba 2005), the blue wildebeest, an obligatory grazer, is more reliant (Mills and Retief 1984) on water than is the red hartebeest, which obtains supplementary water sources in the form of melons and underground tubers (Skinner and Chimimba 2005). There may therefore also be differences in selective brain cooling within the Alcelaphini.

Materials and methods

Animals

Ten gemsbok, eight red hartebeest and ten blue wildebeest were studied; all animals were female. The animals were captured from the wild by a standard game capture technique: immobilisation via a dart fired by a veterinarian from a helicopter. The body mass of each antelope was estimated visually (Skinner and Chimimba 2005) to derive darting drug doses. The gemsbok were immobilised with darts containing etorphine hydrochloride (~0.05 mg kg^{-1}, M99, Novartis, Johannesburg, South Africa), azaperone (~0.5 mg kg^{-1}, Stresnil, Bayer Animal Health, Isando, South Africa) and ketamine (~0.5 mg kg^{-1}, Anaket, Bayer Animal Health). The red hartebeest and blue wildebeest were immobilised with etorphine hydrochloride (~0.04 mg kg^{-1}) and azaperone (0.05 mg kg^{-1}). Following immobilisation the animals all received a combination of haloperidol (~0.1 mg kg^{-1}, Kyron Laboratories, Johannesburg, South Africa) and perphenazine enanthate (~0.8 mg kg^{-1}, Kyron Laboratories, Johannesburg, South Africa).

The immobilised animals were transported to nearby animal holding pens (bomas, ~25 m^2), with each species in its own boma. They were kept in the bomas for 12 days before biologger implantation. *Eragrostis* teff and commercial game pellets (Voermol, Maidstone, South Africa) were provided once daily, and water was available ad libitum. Following biologger implantation, the animals were released into a 10,000 ha fenced portion of Rooipoort Nature Reserve, where they either joined up with conspecifics or remained solitary. After the study, the animals were culled, with a single shot, through the heart, by an experienced marksman using a high-calibre rifle as part of an annual game management quota. All the equipment was retrieved from the carcasses and the rete was perfused and isolated for measurement.

Study area

The study was conducted on the privately owned Rooipoort Nature Reserve (42,647 ha, latitude 28°30′–28°40′S, longitude 24°02′–24°25′E), located in the Kalahari, approximately 50 km west of Kimberley in the Northern Cape Province of South Africa. It receives summer rainfall, with a mean annual rainfall of approximately 400 mm. Air temperatures range from −4 °C in winter to 44 °C in the austral summer (Bezuidenhout 2009). Based on a broad-scale vegetation description, Rooipoort Nature Reserve falls within the Kimberley Thornveld and Schmidsdrift Thornveld of the Savanna Biome and the Highveld Saltpans of the Inland Azonal Vegetation (Mucina and Rutherford 2006). A comprehensive description of the vegetation of the Rooipoort Nature Reserve has been made elsewhere (Bezuidenhout 2009). Common tree and shrub species included *Vachellia erioloba*, *Vachellia tortilis*, *Ziziphus mucronata*, *Tarchonanthus camphoratus* and *Senegalia mellifera*. *Schmidtia pappophoroides*, *Themeda triandra*, *Eragrostis lehmanniana* and *Heteropogon contortus* were some of the dominant grass species. While no large predators were present, the 10,000 ha study area supported Cape buffalo *Syncerus caffer* and giraffe as well as a wide variety of African savanna antelope species.

Biologger implantation

For biologger implantation, we immobilised the animals in the bomas using a CO_2-pressurised dart gun (Dan-Inject, Børkop, Denmark, 2 ml darts). The drug combinations and dosages were as for immobilisation from the helicopter. The gemsbok, but not the other species, were given zuclophenthixol (Clopixol Acuphase, ~0.4 mg kg^{-1}, Lundbeck, Randburg, South Africa) by intramuscular injection following immobilisation. Once immobilised, the animals were blindfolded, ear-plugged, transported to a nearby temporary surgical theatre, weighed, and anaesthesia maintained with 2–3 % isoflurane (Isofor, Safe Line Pharmaceuticals, Johannesburg, South Africa) in oxygen, administered via endotracheal tube. The animals were maintained in sternal recumbency, with the use of sand bags. The red hartebeest and blue wildebeest were given butorphanol (0.12 mg kg^{-1} IV Torbugesic, Fort Dodge, Kempton Park, South Africa) following intubation.

Surgical incision sites on the left hand side of the neck, and on top of the head, between the horns, were shaved and sterilised with chlorhexidine gluconate (Hibitane, Zeneca, Johannesburg, South Africa). To anaesthetise the periosteum, a 1.5 ml bolus of lignocaine (0.1 g, Bayer Animal Health, Johannesburg, South Africa) and adrenaline (Kyron Labs, Johannesburg, South Africa), mixed at a ratio of 10:1, was injected subcutaneously under the scalp where the brain probe would be inserted. Respiratory rate, monitored visually, heart rate, arterial oxygen saturation (Nonin Handheld Pulse Oximeter, Plymouth, MN, USA), blood pressure and rectal temperature (Cardell 9400, Midmark Corporation, OH, USA) were measured throughout the ~2 h implantation procedure.

Using sterile surgical procedures, we implanted the biologgers in each animal. Temperatures in the hypothalamus and the carotid artery were measured with ruggedized glass-coated bead thermistors (bead diameter 0.3 mm; ABOE3-BR11KA103K-L10, Thermometrics Corporation, Northridge, CA, USA), in sealed guide tubes. For hypothalamic measurements, a cellulose acetate butyrate guide tube (length 44 mm, OD 3.2 mm, ID 1.6 mm; World Precision Instruments, Sarasota, FL, USA) with a stainless-steel cap at the tip was inserted via a burr hole in the cranium. Anatomical markers, previously determined for each species from skulls of adult females, were used to ensure placement of the thermistor tip in or near the hypothalamic region. The guide tube was connected to a polyvinylchloride head plate (20 × 10 × 5 mm), attached to the skull subcutaneously, with two bone screws. A polytetrafluorethylene (PTFE) coated coaxial cable (150 mm long, OD 3 mm, Belden, Richmond, VA, USA) connected to the thermistor in the head plate was extended subcutaneously over the skull to a transmitting unit (Africa Wildlife Tracking, Pretoria, South Africa) developed specifically for our purposes. The transmitting unit, with dimensions ~70 × 50 × 30 mm and weighing ~100 g when covered with inert wax (Sasol, Johannesburg, South Africa), was placed subcutaneously, caudal to the base of left ear. For carotid blood measurements, the bead thermistor, in a blind-ended, thin-walled PTFE tube (OD 1.35 mm, ID 0.97 mm; Straight Aortic Flush 4F Catheter, Cordis, the Netherlands) was inserted 80 mm into the left common carotid artery, in the direction away from the heart, and secured in position with a purse-string suture in the artery wall. Outside the artery, the PTFE tube was sealed onto a PTFE-coated coaxial cable (150 mm long, OD 3 mm, Belden, Richmond, VA, USA) connecting the thermistor to a second transmitting unit, that was positioned subcutaneously, also caudal to the base of the left ear.

The temperature sensors had a measurement range of 34–50 °C and a resolution of 0.03 °C. They were calibrated against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany) in an insulated water bath. Following calibration, the biologgers measured temperature to an accuracy of better than 0.05 °C.

Following biologger implantation each animal received a long-acting antibiotic (0.04 ml kg^{-1}, IM, benzylpenicillin, Duplocillin, Intervet, Isando, South Africa), a non-steroidal anti-inflammatory agent (0.05 mg kg^{-1}, IM, meloxicam, Mobic, Boehringer Ingelheim, Randburg, South Africa), a long-acting broad-spectrum parasiticide (0.02 mg kg^{-1}, SC, doramectin, Dectomax, Pfizer Laboratories, Sandton, South Africa), and a multivitamin injection (0.05 mg kg^{-1}, IM, Multivit injectable solution, Univet Ltd, County Cavan, Ireland).

A collar (Africa Wildlife Tracking, Pretoria, South Africa), holding a GPS/GSM tracking device, and a relay unit, was fitted around the neck of each animal at the end of surgery. Temperature data were transmitted from the implanted biologgers to the relay on the collar every 5 min, stored in a buffer, and uploaded to an internet server, via GPRS, twice daily at 06:00 and 12:00.

Following all procedures, the antelope, in sternal recumbency, were transported to the release site, located approximately 25 min from the surgical theatre and given diprenorphine hydrochloride (~0.1 mg kg^{-1}, IV, M5050, Novartis, Johannesburg, South Africa) to reverse the effects of the etorphine hydrochloride. The antelope all became ambulatory within 10 min of receiving the antagonist.

Climatic data

The study was conducted at the end of the dry season (October/November). A portable data logger (Hobo U12-013, Onset Computer, MA, USA) on site recorded black globe temperature, which provides an integrated measure of heat load imposed by radiant and convective heat exchange (Bakken et al. 1985). Other climatic data (including air temperature, wind speed and direction and relative humidity) for the town of Kimberley (28.7419°S, 24.7719°E), situated ~50 km east of the Rooipoort Nature Reserve were sourced from the South African Weather Service (Erasmusrand, Pretoria, South Africa). The water vapour pressure was calculated from the Kimberley dry-bulb temperature and humidity data. Solar radiation flux was obtained from satellite-derived data (GeoSUN Africa, Stellenbosch, South Africa).

Rete anatomy

The heads of the individual animals were removed intact immediately after death. The arterial and venous vasculature of each head was rinsed with a gravity-fed saline solution (~5 % NaCl by weight) until the solution exiting the vessels were clear of blood. A catalyst-based silicone mixture (Zhermack, Silicone Concepts, Johannesburg, South Africa) was injected into the left carotid artery and allowed to perfuse the arterial vasculature of the head. Emergence of the silicone at the contralateral carotid artery indicated complete infusion, after which both carotid arteries were ligated. The heads were stored at 4 °C for a week to allow the silicone to set, and then frozen, until they could be macerated in sulphuric acid (98 % H_2SO_4, Merck, Modderfontein, South Africa) baths over a 2 month period. The carotid arteries were used as anatomical markers to identify the retes in the silicone casts.

The left and right retes were separated; the left rete was used for microscopy and the right rete was used for morphometry. The volume of the right rete was measured by water displacement, with the mass of the displaced water measured to the nearest 0.001 g on a high-precision balance (Precisa 160A, Dietikon, Switzerland) until three values differing by <0.1 g were obtained. For consistency this process was undertaken by the same person (WMS) for all retes.

WMS used Vernier callipers (KBH, Switzerland) to measure the length (rostral/caudal), width (perpendicular to rostral/caudal axis at widest point) and height (dorsal/ventral at highest point) of the right rete to the nearest 0.01 mm, until three values differing by <0.5 mm were obtained. Means of the three values of volume and dimensions were used for comparisons.

Because of the potential influence of injection pressure and other technical aspects of cast extraction, we are reluctant to claim that our casts are faithful replicas of living retes. However, the same techniques were used for all casts, so our comparisons between casts should be valid.

Data analyses

Data from the 10 days after implantation were excluded to eliminate possible confounding effects of surgery. Complete data sets were obtained for between 3 and 35 subsequent days. There were five recording days on which we had complete data sets for two individuals from each species. We used those data to compare the degree of selective brain cooling of the different species under identical meteorological conditions; there were 1440 simultaneous measurements of carotid blood and hypothalamic temperature for each animal during these five recording days. Equipment failure curtailed data sets, but we obtained more than 40,000 simultaneous measurements of brain and carotid artery temperature. We extracted meteorological data for each day for which we obtained selective brain cooling measurements for one or more study animals. Hourly values were calculated for each meteorological variable. Over the study period, sunrise occurred at 05:39 (±00:11) and sunset at 18:32 (±00:07), so we considered 06:00–18:00 as daylight hours. We used three indices to assess the degree to which an animal used selective brain cooling (Strauss et al. 2015): the maximum magnitude of selective brain cooling (the maximum positive difference between carotid blood and hypothalamic temperature), the proportion of time spent using selective brain cooling (time that carotid blood temperature exceeded hypothalamic temperature), and the threshold temperature for selective brain cooling (the temperature at which a plot of mean hypothalamic temperature against carotid temperature crossed the line of identity). We used the original 5-min body temperature records from each animal to calculate these indices. To assess the relationship between hypothalamic and carotid blood temperatures and to calculate the threshold for selective brain cooling, the 5-min carotid blood temperature records were sorted into 0.1 °C bins, and the mean, standard deviation, minimum and maximum hypothalamic temperatures were determined for each bin of carotid blood temperature.

We used one-way ANOVAs to compare variables of the nycthemeral rhythm of hypothalamic and carotid blood temperatures across species. We used STATA (version 10) to run generalised linear mixed-effects models (GLMMs), with animal identity as a random factor, to investigate differences in selective brain cooling between species. To investigate factors associated with the three selective brain cooling indices, that is the magnitude, the frequency of use, and the threshold temperature for selective brain cooling, we included, as independent variables, the 24 h maximum or the 24 h mean carotid blood temperature, black globe temperature, and species. One data point per animal per day was therefore analysed. Because multiple measurements were made on the same animals on different days, we nested days within individual in the GLMM analyses.

Physical characteristics of the carotid rete were compared across species using one-way ANOVA. To account for the fact that the carotid rete may scale with body size, we corrected the morphological measurements by dividing the measurement (e.g., length of the rete in mm) by the mass (kg) of the relevant animal, as determined at the time of implantation surgery. In all analyses statistical significance was accepted when $P < 0.05$.

Results

Climatic conditions

Over the 35 days of selective brain cooling measurement, the dry-bulb temperature averaged 16 ± 4 °C (mean ± SD) at night and 23 ± 6 °C during the day. Solar radiation exceeded 1000 W m^{-2} on 33 days, for an average of 5 ± 2 h per day. The mean maximum solar radiation was 1051 ± 31 W m^{-2}. Black globe temperature averaged 15 ± 3 °C at night, close to dry-bulb temperature, and 38 ± 5 °C during the daytime, much higher than dry-bulb temperature, confirming radiation as a main source of heat during the day. The maximum black globe temperatures exceeded 50 °C on 16 of the days of body temperature measurement. Wind speed varied little throughout the 24 h period, with a mean wind speed of 4.1 ± 2.2 m s^{-1} at night and 5.7 ± 2.8 m s^{-1} during the day. The average hourly wind speed never exceeded 13 m s^{-1}, with a mean of 4.2 ± 0.1 and 5.7 ± 0.7 m s^{-1} during the night and daylight hours respectively. Water vapour pressure varied little throughout the 24 h period, ranging on average from 0.5 ± 0.2 kPa at night to 0.6 ± 0.2 kPa during the day. The water vapour pressure never exceeded 1.6 kPa. The water vapour pressure of the air was therefore sufficiently low to allow evaporative cooling throughout the period of data collection, as the wet evaporative surfaces of all three species would have had a water vapour pressure of ~6 kPa (i.e., 100 % relative humidity at about 37 °C) (Barenbrug 1974).

Relationship between hypothalamic and carotid blood temperature

We successfully collected simultaneous hypothalamic and carotid blood temperature data from four gemsbok, five red hartebeest and six blue wildebeest. Table 1 shows the mean 24 h carotid blood and hypothalamic temperatures, and indices of selective brain cooling, of all 15 animals, for all the days with full data sets. The carotid blood and hypothalamic temperatures of all three species exhibited a similar nycthemeral variation, with a trough around sunrise (about 05:00–08:00) and a peak just before sunset, at about 17:00 (Fig. 1). The range of the nycthemeral rhythm of the carotid blood ($F_{2,12} = 0.27$, $P = 0.77$) and hypothalamic ($F_{2,12} = 0.33$, $P = 0.73$) temperature did not differ between species. The minimum ($F_{2,12} = 1.34$, $P = 0.29$) and maximum ($F_{2,12} = 2.87$, $P = 0.10$) carotid blood temperatures also did not differ between species. Although minimum hypothalamic temperature did not differ between species ($F_{2,12} = 1.68$, $P = 0.23$), we found a species difference in the maximum hypothalamic temperature ($F_{2,12} = 4.90$, $P = 0.03$), with the red hartebeest having a higher maximum hypothalamic temperature than the gemsbok. Selective brain cooling was evident more when body temperature increased during the day than when it was decreasing at night. A rhythm in the use of selective brain cooling therefore existed, with selective brain cooling generally being initiated in the afternoon and continuing into the early evening. Over the 5 days where we had data simultaneously for all species black globe temperature consistently reached or exceeded 50 °C. In four of the antelope hypothalamic temperature seldom dropped below carotid blood temperature, that is selective brain cooling was rare during the 5 days, but one gemsbok (gemsbok 3) and one red hartebeest (red hartebeest 5) employed selective brain cooling for most of the day, every day (Fig. 2). The difference between carotid blood and hypothalamic temperatures, over the 5 days, did not differ between species ($F_{2,3} = 0.29$, $P = 0.77$). Similarly when comparing the entire data set the difference between

Table 1 Mean (± SD) carotid blood (T_{car}) and hypothalamic (T_{hyp}) temperatures recorded every 5 min and the mean difference between carotid blood and hypothalamic temperature, as well as the maximum magnitude, frequency of occurrence and threshold temperature for selective brain cooling recorded in four gemsbok, five red hartebeest and six blue wildebeest females

Species	ID	Mass (kg)	Mean temperature (°C)			Selective brain cooling indices			
			Hypothalamic	Carotid	$T_{\mathrm{Car}} - T_{\mathrm{Hyp}}$	Max. (°C)	Freq. (%)	Threshold (°C)	Days
Gemsbok	1	147	38.91 ± 0.56	38.77 ± 0.70	−0.15 ± 0.20	0.38	13	39.33	5
	2	161	38.82 ± 0.43	38.61 ± 0.57	−0.25 ± 0.16	0.86	5	39.54	4
	3	173	38.74 ± 0.33	38.91 ± 0.45	0.17 ± 0.24	0.86	63	38.42	15
	4	170	38.83 ± 0.42	38.41 ± 0.50	−0.43 ± 0.15	0.10	1	40.54[b]	20
Red hartebeest	1[a]	133	39.07 ± 0.37	38.80 ± 0.45	−0.27 ± 0.29	1.03	13	39.45	35
	2	112	39.30 ± 0.42	39.01 ± 0.46	−0.28 ± 0.20	0.35	5	40.43[b]	3
	3	110	39.22 ± 0.33	39.06 ± 0.42	−0.16 ± 0.18	0.94	12	39.53	9
	4[a]	118	39.26 ± 0.26	39.29 ± 0.29	0.03 ± 0.16	0.74	39	39.04	3
	5[a]	115	39.12 ± 0.36	39.22 ± 0.50	0.10 ± 0.21	0.99	61	38.74	23
Blue wildebeest	1[a]	178	39.09 ± 0.32	38.77 ± 0.42	−0.33 ± 0.17	0.51	1	39.75	13
	2	150	39.06 ± 0.44	39.10 ± 0.56	0.04 ± 0.19	0.57	54	38.84	6
	3[a]	157	39.15 ± 0.40	38.78 ± 0.53	−0.37 ± 0.26	0.46	6	39.83	12
	4[a]	211	39.00 ± 0.25	38.79 ± 0.30	−0.22 ± 0.14	0.41	4	39.54[b]	12
	5	170	39.23 ± 0.29	39.16 ± 0.41	−0.06 ± 0.21	1.10	25	39.24	3
	6[a]	182	38.88 ± 0.26	38.88 ± 0.32	0.01 ± 0.19	0.50	35	38.82	5

The two individuals per species that were compared for an overlapping 5-day period (see Figs. 1, 2, 3) were gemsbok 3, gemsbok 4, red hartebeest 1, red hartebeest 5, blue wildebeest 3 and blue wildebeest 4

Max. (°C)—absolute maximum difference between carotid and hypothalamic temperature, Freq. (%)—the percentage of time for which carotid temperature exceeded hypothalamic temperature, Threshold (°C)—temperature at which the plot of hypothalamic temperature versus carotid blood temperature crossed the line of identity

[a] An individual for which carotid rete morphological measures were obtained

[b] Extrapolated—plot did not cross the line of identity within the range of actual measurements

the carotid blood and hypothalamic temperatures did not differ between species ($F_{2,12} = 0.08$, $P = 0.92$). In general, the difference between carotid blood and hypothalamic temperature was small in all animals, and the average, over all days of measurement, was positive in only one gemsbok, two red hartebeest and two blue wildebeest (Table 1).

Just as for the difference between carotid blood and hypothalamic temperature, the frequency with which selective brain cooling was used was highly variable between individuals, within and between species (Table 1). One gemsbok and one red hartebeest used selective brain cooling for more than 60 % of the overlapping 5-day period, while both blue wildebeest and the remaining gemsbok and red hartebeest rarely used selective brain cooling at all (<10 % of the time). We found no difference in the proportion of time that the three species spent using selective brain cooling during the overlapping 5-day period ($F_{2,3} = 0.48$, $P = 0.66$). Similarly, when analysing all data from all animals, we found no difference between species in the proportion of time spent using selective brain cooling ($F_{2,12} = 0.08$, $P = 0.92$).

The threshold temperatures for selective brain cooling are shown for all animals in Table 1, and in Fig. 3 for the two individuals of each species over the same 5-day period. The threshold temperature for selective brain cooling also showed considerable variability between individuals within and between species. For example, gemsbok 4, which rarely exhibited selective brain cooling, had an extrapolated threshold temperature of 40.5 °C, while gemsbok 3 had the lowest threshold temperature (38.4 °C) for selective brain cooling of any of the six animals during the overlapping 5-day period. There was no difference in the threshold temperature for selective brain cooling between the three species during the overlapping 5-day period ($F_{2,3} = 0.21$, $P = 0.82$). When comparing the entire data set we also found no difference in the threshold temperature for selective brain cooling between species ($F_{2,12} = 0.06$, $P = 0.95$).

Both the largest (0.86 °C) and the smallest (0.10 °C) maximum magnitude of selective brain cooling during the overlapping 5-day period were recorded in the gemsbok, but outside that period, selective brain cooling in a blue wildebeest reached 1.10 °C. We found no species difference in the maximum magnitude of selective brain cooling during the overlapping 5-day period ($F_{2,3} = 0.45$, $P = 0.67$). When we analysed the entire data set the maximum magnitude of selective brain cooling did not differ between species ($F_{2,12} = 1.10$, $P = 0.38$).

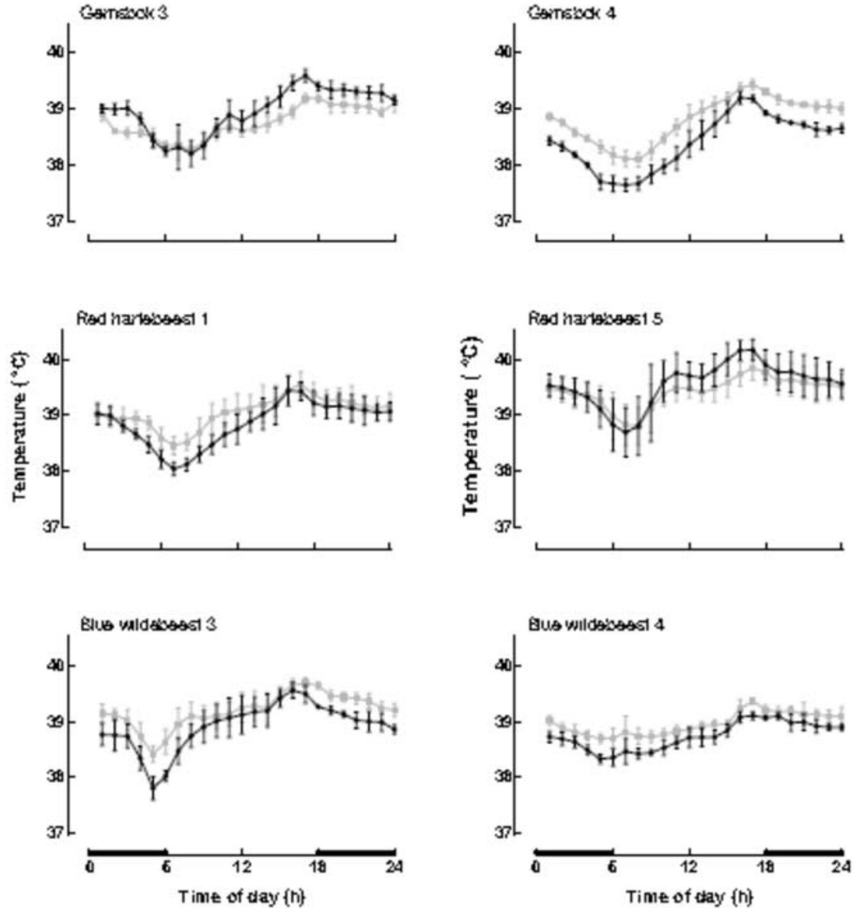


Fig. 1 The mean (± SD) hourly hypothalamic (*grey line*) and carotid blood (*black line*) temperatures of two gemsbok, two red hartebeest and two blue wildebeest females over the same 5-day period

Factors influencing selective brain cooling

We applied GLMMs to explore the factors that might influence selective brain cooling, using the entire data set. The GLMMs indicated that the maximum 24 h magnitude of selective brain cooling was associated positively with maximum 24 h carotid blood temperature ($z = 7.56$, Table 2). Also, the proportion of time spent using selective brain cooling per day was associated positively with carotid blood temperature ($z = 5.82$). The threshold temperature for selective brain cooling was associated negatively with the black globe temperature ($z = -2.55$). Species was not a significant determinant of the indices of selective brain cooling in any of our GLMMs (Table 2).

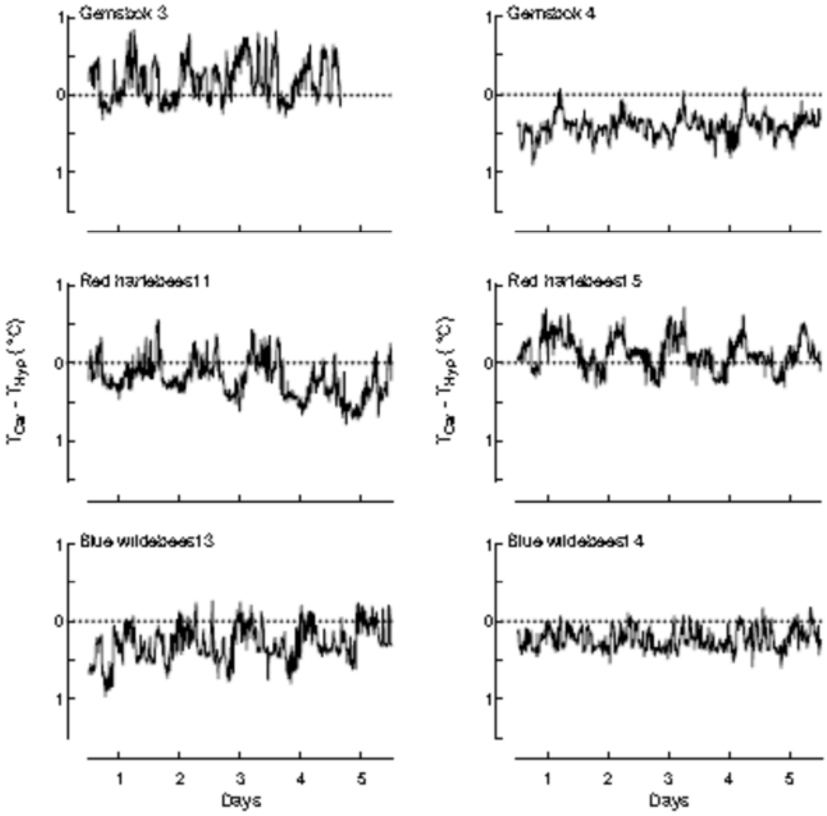
Comparison of carotid rete morphology between species

We successfully obtained carotid rete morphology measures from three gemsbok, four red hartebeest and five blue wildebeest. Both the absolute carotid rete length ($F_{2,9} = 8.01$, $P = 0.01$) and width ($F_{2,9} = 5.08$, $P = 0.03$) differed between species. Based on Tukey's post hoc test, the blue wildebeest, the largest of the three species, had a longer and wider carotid rete than the red hartebeest, the smallest of the three study species. The absolute height of the carotid rete did not differ between species ($F_{2,9} = 0.76$, $P = 0.50$).

When scaled to body mass, we found no difference in the length ($F_{2,9} = 0.42$, $P = 0.67$) or width ($F_{2,9} = 1.78$, $P = 0.22$) of the carotid rete between species. However, the height of the carotid rete differed between species ($F_{2,9} = 5.43$, $P = 0.03$) when scaled to mass (Fig. 4). Also, the volume of the carotid rete corrected for mass differed between species ($F_{2,9} = 5.54$, $P = 0.03$). Tukey's post hoc analyses indicated that the red hartebeest carotid rete had a greater volume and height, per kilogram of body mass, than did the blue wildebeest carotid rete.

For four blue wildebeest and three red hartebeest for which we determined carotid rete morphology, we also had selective brain cooling data (Table 1). None of the gemsbok

Fig. 2 The difference between carotid blood and hypothalamic temperature as recorded every 5 min for two gemsbok, red hartebeest and blue wildebeest females over the same 5-day period. *Positive values* indicate selective brain cooling



for which we obtained carotid rete measurements yielded measures of selective brain cooling. No relationships were found between selective brain cooling magnitude and carotid rete height or volume, per kilogram body mass, for either the blue wildebeest (height: $R^2 = 0.20$, $P = 0.55$; volume: $R^2 = 0.16$, $P = 0.60$) or the red hartebeest (height: $R^2 = 0.03$, $P = 0.89$; volume: $R^2 = 0.25$, $P = 0.67$) (Fig. 5).

Discussion

We document the first simultaneous measurements of selective brain cooling, determined from hypothalamic temperature and carotid blood temperature, in multiple species living free in the same environment. We also have made the first quantitative comparison of the dimensions of the carotid rete of the species, using the retes from our animals instrumented with temperature biologgers.

Hypothalamic temperature closely tracked carotid blood temperature for most of the 24 h period, with both temperatures exhibiting a nycthemeral rhythm (Fig. 1), similar to that observed previously in other artiodactyls (Mitchell et al. 2002). However, in all three species, hypothalamic temperature was lower than carotid blood temperature, on occasion, indicating selective brain cooling. Selective brain cooling was exhibited at all times of day, but was most often evident while carotid blood temperature was rising during the late afternoon (Fig. 1).

We used three indices, namely the threshold carotid blood temperature above which selective brain cooling became the norm, the maximum difference between the carotid and hypothalamic temperature (magnitude), and the frequency of use of selective brain cooling, to characterise the extent of selective brain cooling use (Table 1). The most conspicuous outcome of our analyses was the considerable variability in the use of selective brain cooling, between different individuals within the same species.

For example, gemsbok 3, used selective brain cooling for more than 60 % of the time, while gemsbok 4 did so for only 1 % (Table 1). Moreover, on a day when the heat load, as measured by a black globe thermometer, reached 53 °C, gemsbok 3 used selective brain cooling near-continuously for 17 h, while gemsbok 4 used it for <0.5 h (Fig. 2, day 3). Similarly, red hartebeest 1 used selective brain cooling ~5 times less frequently than did red hartebeest 5. Also, blue

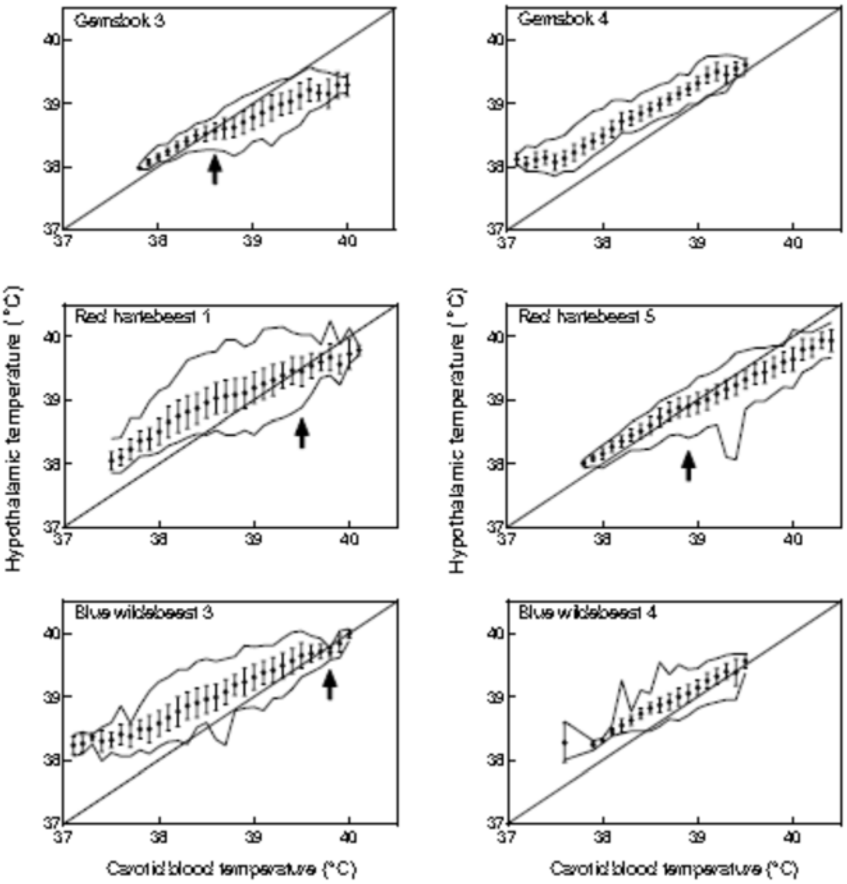


Fig. 3 Hypothalamic temperature (mean ± SD) for every 0.1 °C bin of simultaneous carotid blood temperature, in two gemsbok, two red hartebeest and two blue wildebeest females over the same 5-day period. The boundary lines demonstrate the minimum and maximum hypothalamic temperatures in that bin. The *diagonal line* is the line of identity. The *arrows* indicate the respective observed threshold temperatures for selective brain cooling; in two animals the threshold was not reached within the range of measurement

Table 2 Association between the indices of selective brain cooling and factors potentially influencing selective brain cooling, determined by generalised linear mixed models

Index of selective brain cooling	Variables modelled	$\beta \pm$ SE	z	P	95 % CI
Maximum 24 h magnitude (°C)					
	Maximum 24 h carotid blood temperature (°C)	0.253 ± 0.037	7.56	<0.0001	0.209 to 0.356
	Maximum 24 h black globe temperature (°C)	−0.0004 ± 0.002	0.21	0.835	−0.003 to 0.004
	Species	−0.035 ± 0.068	−0.51	0.611	−0.169 to 0.099
Frequency of use (%)					
	Mean 24 h carotid blood temperature (°C)	0.213 ± 0.037	5.82	<0.0001	0.141 to 0.285
	Mean 24 h black globe temperature (°C)	0.002 ± 0.002	1.04	0.298	−0.002 to 0.006
	Species	−0.031 ± 0.066	−0.47	0.641	−0.159 to 0.098
Threshold temperature (°C)					
	Mean 24 h carotid blood temperature (°C)	−0.258 ± 0.215	−1.20	0.229	−0.680 to 0.163
	Mean 24 h black globe temperature (°C)	−0.026 ± 0.010	−2.55	0.011	−0.045 to −0.006
	Species	0.198 ± 0.251	0.79	0.429	−0.293 to 0.690

Animal identity was included as a random factor and nested within days. N = 168

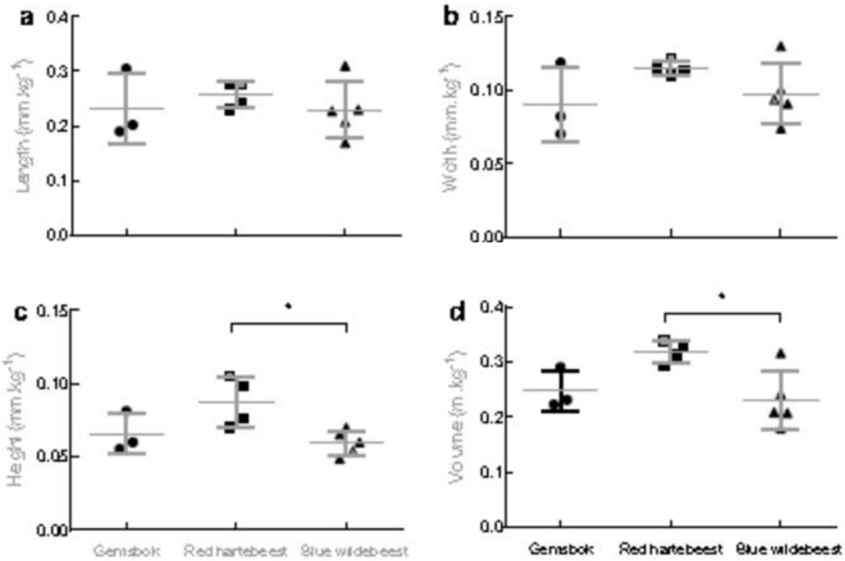


Fig. 4 Univariate scatterplots of the carotid rete length (a), width (b), height (c) and volume (d) as determined from silicone casts of the carotid retes of three gemsbok, four red hartebeest and five blue wildebeest females. The distribution of the individual values, scaled to body mass, is plotted per species. The *horizontal lines* indicate the mean with standard deviations for each species. An *asterisk* represents $P < 0.05$

wildebeest 3, and not blue wildebeest 4, showed a clear threshold temperature for selective brain cooling, during the overlapping 5-day period (Fig. 4). The high variability of use of selective brain cooling within a species masked completely any difference between species, if, indeed, any species difference exists. Some of the water-dependent blue wildebeest, for example, had a larger magnitude of selective brain cooling than did some of the water-independent gemsbok, while some of the red hartebeest initiated selective brain cooling at lower threshold temperatures, and used selective brain cooling more frequently, than did some of the blue wildebeest and gemsbok (Table 1; Fig. 2).

One explanation for the absence of physiological differences in selective brain cooling between species may be that the species had a similar anatomical substrate for selective brain cooling. Indeed, the carotid rete of the water-independent gemsbok was no bigger than that of the more water-dependent antelope. However, we did detect some differences in rete morphology, with the red hartebeest carotid rete having greater height and volume per kilogram of body mass than that of the blue wildebeest (Fig. 4). No other differences were found in rete morphology when it was scaled to body mass, and it is unlikely that the variability in rete dimensions that we detected could account for the massive variability in the indices of selective brain cooling (Fig. 5).

We used GLMMs to explore other factors that might account for the variability in the use of selective brain cooling (Table 2). Maximum heat load on the animals, as indexed by the maximum black-globe temperature on site, was not associated with the maximum magnitude of use of selective brain cooling. Neither was mean black globe temperature associated with the frequency of selective brain cooling use. The threshold for implementation of selective brain cooling was influenced significantly by black-globe temperature: the higher the heat load, the lower was the carotid blood temperature at which the animals implemented selective brain cooling. As the function of selective brain cooling is to suppress evaporative water loss (Strauss et al. 2015), that association implies that the higher the drive for evaporative water loss is likely to be, the lower the body temperature is at which evaporative water loss will be suppressed. We also explored whether the animal's own temperatures were associated with variability of selective brain cooling. Carotid blood temperature indeed was associated with two of the indices of selective brain cooling (Table 2). The higher the maximum 24 h carotid blood temperature of an individual, the greater was the 24 h magnitude of selective brain cooling, and the more frequently the individual employed selective brain cooling over the same 24 h period. So, higher carotid blood temperature was associated with enhanced use of selective brain cooling, and the variability in rete use was associated with variability in the carotid blood temperature between different animals (Fig. 3).

Despite our new biologging technology, developed specifically for studying selective brain cooling in unrestrained animals, working well in domestic sheep (Strauss et al. 2015), equipment failure in the wild herbivores reduced our sample size and duration of data collection. Nevertheless, our more than 40,000 simultaneous measurements of hypothalamic and carotid blood temperature constitute the only simultaneous data set on selective brain cooling in multiple species. Given the dependence of selective brain cooling

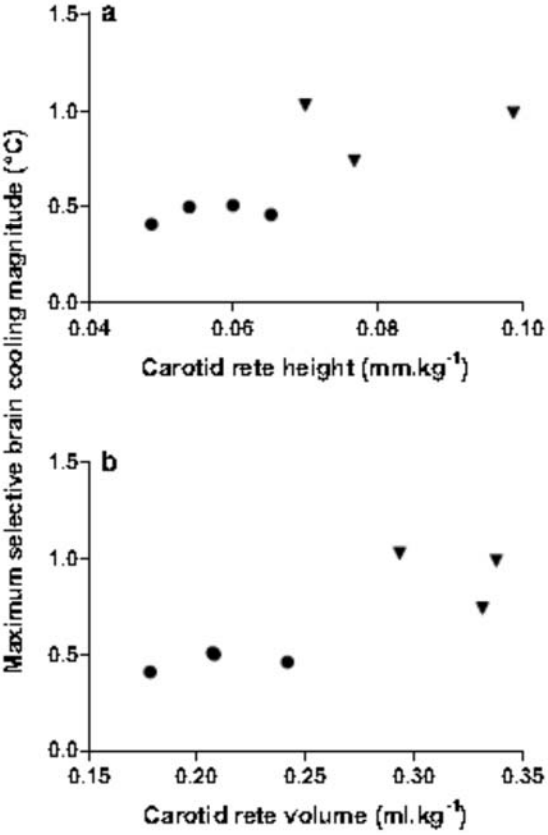


Fig. 5 The relationship between the maximum magnitude of selective brain cooling and a the carotid rete height, and b carotid rete volume when scaled to body mass of the individual blue wildebeest (*filled circle*) and red hartebeest (*filled inverted triangle*) females

on non-thermal factors, including drinking (Hetem et al. 2012) and sympathetic tone (associated with exercise and vigilance; Jessen et al. 1994; Mitchell et al. 1997; Maloney et al. 2001, 2002), behavioural differences between individual animals on the reserve may have contributed to the variability in their use of selective brain cooling. Consequently we avoided intensive behavioural monitoring, as the fear-induced increase in sympathetic drive, associated with human presence, is known to inhibit selective brain cooling (Jessen et al. 1994; Maloney et al. 2001; Mitchell et al. 2002).

We have investigated the attributes of selective brain cooling in artiodactyls from two of the bovid tribes, the Alcelaphini and the Hippotragini. Three previous studies have investigated selective brain cooling in free-living members of these tribes, namely black wildebeest (Jessen et al. 1994), a member of the Alcelaphini, and the gemsbok (Maloney et al. 2002), and the Arabian oryx (Hetem et al. 2012), members of the Hippotragini (Table 3). Climatic conditions for the previous studies (mean ambient temperature ~25 °C, mean solar radiation ~800 W m^{2}) were similar to those experienced by our study animals. Among the members of the Alcelaphini the thresholds for implementation of selective brain cooling differed by only 0.5 °C (Jessen et al. 1994) (Table 3). However, the maximum magnitude of selective brain cooling that we recorded, in both the blue wildebeest and the red hartebeest, was twice as high as that previously reported for the black wildebeest (Jessen et al. 1994). We do not know whether that difference in maximum selective brain cooling arose from intrinsic differences between species or from differences in circumstances that influenced maximum selective brain cooling.

Within the Hippotragini tribe, we can compare gemsbok and Arabian oryx; the threshold temperature for selective

Table 3 Selective brain cooling attributes measured in the Alcelaphini and the Hippotragini tribes of the Cetartiodactyla (numbers in brackets indicate sample size)

Tribe	Species	Selective brain cooling			Reference
		Magnitude (°C)	Frequency (%)	Threshold (°C)	
Alcelaphini	Black wildebeest				
	Connochaetes gnu (4)	0.5	–	38.9	Jessen et al. (1994)
	Blue wildebeest				
	C. taurinus (6)	1.1	21	39.3	This study
	Red hartebeest				
	A. buselaphus (5)	1.0	26	39.4	This study
Hippotragini	Gemsbok				
	O. gazella (4)	0.4	15	39.8	Maloney et al. (2002)
	O. gazella (4)	0.9	21	39.5	This study
	Arabian oryx				
	Oryx leucoryx (4)	1.2	87	37.8	Hetem et al. (2012)

– Variable not reported in the original publication brain cooling that we recorded for gemsbok was similar to that previously reported for that species (Table 3). Our gemsbok, all females, also used selective brain cooling for a similar proportion of time (21 vs. 23 %) as did two female gemsbok in the study of Maloney et al. (2002). Hetem et al. (2012) compared the attributes of selective brain cooling of gemsbok from southern Africa [as reported by Maloney et al. (2002)] with the Arabian oryx, from the hyper-arid deserts of Arabia. Apart from rainfall, climatic conditions were similar during the two studies. Compared to that of the gemsbok, in both the earlier study and ours (Table 3), selective brain cooling in the Arabian oryx was enhanced: the maximum magnitude of selective brain cooling exceeded 1 °C, the threshold temperature was below 38 °C, and frequency of use was above 80 % of the time (Hetem et al. 2012). The enhanced use of selective brain cooling by Arabian oryx was attributed to the greater aridity to which the Arabian oryx were exposed.

We have shown recently that depriving another artiodactyl species, the sheep (*Ovis aries*) of drinking water enhanced selective brain cooling, and the enhanced selective brain cooling was associated with reduced water loss (Strauss et al. 2015). Our study animals had access to drinking water at all times, and high maximum daily carotid blood temperatures indicative of dehydration (Hetem et al. 2012) were not evident (Fig. 1). The effects of dehydration on selective brain cooling therefore could not be determined. We expect that their selective brain cooling also will be enhanced by dehydration, perhaps more so in the water-independent species than in the water-dependent species.

In conditions in which their thermoregulation was not stressed by aridity, our animals demonstrated extraordinary variability in their use of selective brain cooling. Variability in selective brain cooling use between individuals of the same species has been documented in both domestic (Fuller et al. 2007; Maloney et al. 2007; Strauss et al. 2015) and wild artiodactyls (Fuller et al. 1999; Hetem et al. 2012; Jessen et al. 1994; Maloney et al. 2002; Mitchell et al. 1997). However, we are the first to show that this variability in use of selective brain cooling within species exceeds the variability in use between species simultaneously inhabiting the same environment. One possible cause of the variability is individual variation in activation of the sympathetic nervous system, that is, variation in the physiological response to stress (Fuller et al. 2011; Johnsen et al. 1987; Maloney et al. 2002). Individual artiodactyls have different temperaments (Beausoleil et al. 2008), and therefore different states of sympathetic activation, which may induce variability in selective brain cooling use (Strauss et al. 2015) even in the absence of any specific stressor at the time. Animal behaviour associated with increased vigilance or activity, such as that seen in a dominant male (Hetem et al. 2012), also is likely to reduce selective brain cooling. The use of selective brain cooling, therefore, is a physiological mechanism with potential for individual plasticity and, since temperament is heritable, at least in sheep (Murphy et al. 1994), it seems possible that those individuals with a greater capacity for selective brain cooling may have a competitive advantage within populations in arid environments in terms of water saving during combined thermal and osmotic stress.

In conclusion, for the first time, we have measured selective brain cooling simultaneously in more than one species living under the same environmental conditions. Our gemsbok, red hartebeest and blue wildebeest, representing a clear water dependency gradient, all used selective brain cooling, even in an environment where water readily was available. The observed intraspecific variability in the magnitude, the frequency of use, and the threshold temperature for selective brain cooling overshadowed any interspecific variability in selective brain cooling. Whether differences in selective brain cooling between the species will emerge when the artiodactyls are subjected to aridity remains to be investigated. We suggest that the observed plasticity in selective brain cooling use that exists within large artiodactyl species will allow individuals, regardless of species, to employ selective brain cooling to help cope with the increased aridity and variability in rainfall that is predicted for much of southern Africa in the face of anthropogenic climate change (Niang et al. 2014).

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Compliance with ethical standards

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. The Animal Ethics Screening Committee of the University of the Witwatersrand approved the procedures (clearance 2011/36/05). Permits to conduct the research were provided by the Department of Environment and Nature Conservation, Northern Cape Province, South Africa (permits Fauna 846/2011 and 848/2011).

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CHAPTER 4

Heterothermy and microclimate use in three large sympatric antelope with varying water dependencies

4.1 Introduction

Species across the globe are predicted to respond to climate change through, for example, shifting their distribution ranges poleward (Thomas and Lennon, 1999) or to higher altitudes (Moritz *et al.*, 2008). While many investigations focus on projecting how organisms might respond to climate change in the future, 47% of terrestrial mammal species have already been negatively impacted by climate change in at least part of their distribution range (Pacifici *et al.*, 2017). There is therefore an urgent need to understand how animals are coping in their environments that are currently undergoing change, and to identify potential behavioural patterns and physiological adaptations that could act as signals to indicate whether species have the capacity to respond to anthropogenic climate change.

Thermoregulatory signals of interest include changes in the pattern of microclimate use, as well as physiological mechanisms that animals are already using, even if only occasionally (e.g. seasonally), to facilitate their survival. Although the use of microclimates, such as shade, is well-documented in large mammals (Seddon and Ismail, 2002, Tull *et al.*, 2001), the temperature difference between various microclimates has, with a few exceptions (e.g. Hetem *et al.*, 2012, Signer *et al.*, 2011, Kinahan *et al.*, 2007) seldom been quantified for large mammals. For example, Hetem and colleagues showed that the desert-adapted Arabian oryx *Oryx leucoryx* and the Arabian sand gazelle *Gazella subgutturosa marica* made use of microclimates that were up to 12 ºC cooler than what is available in full sun during summer, in the absence of drinking water

(Hetem *et al.*, 2012b). At an environmental heat load threshold temperature of 28 ºC the oryx and gazelle therefore made use of cooler microclimates (presumably shade) more often than not (Hetem *et al.*, 2012b). By seeking shade and therefore reducing the radiant heat load, oryx and gazelles reduced the need for evaporative cooling. Although behavioural adjustment, such as increased shade use during the hot and dry season, is likely a first response to conserve body water in a warming climate (Fuller *et al.*, 2016, Cain *et al.*, 2006), it is unlikely to be sufficient on its own because of associated costs such as decreased foraging time. Moreover, once ambient temperature exceeds an animal's body temperature shade seeking won't prevent the animal from gaining heat from the environment.

A physiological water conservation mechanism that has become entrenched in the literature is adaptive heterothermy, originally defined in large mammals as the heterothermy resulting from heat storage during the day, reducing the need for evaporative water loss (Louw and Seely, 1982). First described in the dromedary camel *Camelus dromedarius* (Schmidt-Nielsen *et al.*, 1956) and later also attributed to the eland *Tragelaphus oryx* and the beisa oryx *Oryx gazella beisa* (Taylor, 1969), adaptive heterothermy allows for heat storage in the body during the day. Captive camels, water deprived under hot conditions, increased their rectal temperatures during the day to the extent that the maximum body temperatures attained were up to 6.2 ºC above the norm (Schmidt-Nielsen *et al.*, 1956). Similarly, captive, hydrated eland and gemsbok elevated their body temperatures by 6-7 °C higher than normal when exposed to temperatures of 40 °C (Taylor, 1969). The heat storage results in a greater temperature gradient between those animals with elevated body temperatures and the cool environment at night, thereby allowing for rapid non-evaporative heat loss. The minimum body temperatures attained are also lower than normal, because of a lower set point at night, thereby providing greater capacity for heat storage during the day (Schmidt-Nielsen *et al.*, 1956). The existence of adaptive heterothermy in large mammals, particularly African ungulates, has been questioned though, as the studies describing the mechanism were done in captivity where animals were deprived of their normal suite of thermoregulatory behaviours (Mitchell *et al.*, 2002).

To date, adaptive heterothermy has not been found in any of the African ungulates studied, including gemsbok (Maloney *et al.*, 2002), eland (Fuller *et al.*, 1999b), springbok (Mitchell *et al.*, 1997) and plains zebra (Fuller *et al.*, 2000). It has, however, been confirmed in free-living Arabian oryx (Ostrowski *et al.*, 2003, Hetem *et al.*, 2012b) and Arabian sand gazelle (Hetem *et al.*, 2012b) where the importance of seasonal energy deficiency (scarce, low quality food) was first recognised in the occurrence of heterothermy (Hetem *et al.*, 2012b, Hetem *et al.*, 2016). In those free-living African ungulates studied, the range in the 24h body temperature rhythm was small and not related to variations in environmental conditions (Mitchell *et al.*, 2002). However, the studies investigating the nychthemeral 24h body temperature rhythms of African ungulates, generally, has been done over the short term, limiting the inferences that can be drawn on the relationship between environmental conditions and body temperature. Moreover, a recent review of adaptive heterothermy in large mammals (Hetem *et al.* 2016) and recognition that its drivers could be reduced minimum body temperatures due to energy deficiency, elevated maximum body temperatures due to a lack of water, or a combination of both (Fuller *et al.*, 2014) necessitates a reassessment of heterothermy in African ungulates.

We therefore set out to investigate thermoregulation in three sympatric, large, African antelope species, with varying water dependencies: the water independent gemsbok (Spinage and Matlhare, 1992), the red hartebeest *Alcelaphus buselaphus* of intermediate water dependency (Crowe *et al.*, 1981) and the water-dependent blue wildebeest *Connochaetes taurinus* that drinks water daily (Berry and Jenssen, 1997). We investigated the microclimate use and the nychthemeral 24h body temperature rhythms of the three antelope species in an environment where they are currently thriving, but which is expected to become hotter and drier in the face of anthropogenic climate change (Engelbrecht *et al.*, 2015).

4.2 Methods

4.2.1 Study area

The study was conducted on the 42 000 ha privately-owned Rooipoort Nature Reserve (latitude 28°30′–28°40′S, longitude 24°02′–24°25′E). The reserve is located approximately 50 km WNW of Kimberley in the Northern Cape Province in an area where South Africa's Savanna, Grassland and Karoo biomes meet. A phytosociological study has been conducted on the reserve to describe the vegetation communities, characterised by species such as *Vachellia erioloba*, *Vachellia tortilis*, *Tarchonanthus camphoratus*, *Ziziphus mucronata*, *Schmidtia pappophoroides* and *Themeda triandra* (Bezuidenhout, 2009). The large mammal component consisted mainly of ungulates, including buffalo *Syncerus caffer*, giraffe *Giraffa camelopardalis*, eland *Tragelaphus oryx*, kudu *Tragelaphus strepsiceros*, plains zebra *Equus quagga*, blesbok *Damaliscus pygargus* and springbok *Antidorcas marsupialis*. Rooipoort Nature Reserve receives a mean annual summer rainfall of approximately 400 mm. Air temperatures range from −4 °C in the austral winter to 44 °C in the austral summer (Bezuidenhout, 2009).

4.2.2 Study animals

Gemsbok *Oryx gazella*, red hartebeest *Alcelaphus buselaphus* and blue wildebeest *Connochaetes taurinus* females were immobilised on the Rooipoort Nature Reserve. Published values of mean body mass (Skinner and Chimimba, 2005) were used to calculate drug doses for the gemsbok (210 kg), red hartebeest (120 kg) and blue wildebeest (183 kg). Etorphine hydrochloride (~0.05 mg kg^{-1}, M99, Novartis, Johannesburg, South Africa), azaperone (~0.5 mg kg^{-1}, Stresnil, Bayer Animal Health, Isando, South Africa) and ketamine (~0.5 mg kg^{-1}, Anaket, Bayer Animal Health) were used to immobilise the gemsbok. A combination of etorphine hydrochloride (~0.04 mg kg^{-1}) and azaperone (0.05 mg kg^{-1}) were used to immobilise the red hartebeest and the blue wildebeest.

Following immobilisation the animals all received a combination of haloperidol (~0.1 mg kg^{-1}, Kyron Laboratories, Johannesburg, South Africa) and perphenazine enanthate (~0.8 mg kg^{-1}, Kyron Laboratories, Johannesburg, South Africa). The immobilised animals were kept in bomas on Rooipoort Nature Reserve for 12 days before biologger implantation. *Eragrostis* teff and commercial game pellets (Voermol, Maidstone, South Africa) were provided once daily, and water was available *ad libitum*. After surgery, the animals were released into a 10,000 ha fenced-off portion of Rooipoort Nature Reserve. At the end of the study, the animals were culled as part of an annual game management quota. The implanted temperature loggers and the radio collars, each with its miniature black globe thermometer were retrieved from each carcass.

4.2.3 Biologger implantation

The animals were immobilised in their bomas using similar drug combinations and dosages as for immobilisation during the initial capture. The gemsbok were given zuclophenthixol (Clopixol Acuphase, ~0.4 mg kg^{-1}, Lundbeck, Randburg, South Africa) by intramuscular injection following immobilisation. Once immobilised the animals were blindfolded, ear-plugged and transported to a nearby temporary surgical theatre. Upon arrival at the surgery each animal was weighed. Anaesthesia was maintained with 2–3 % isoflurane (Isofor, Safe Line Pharmaceuticals, Johannesburg, South Africa) in oxygen, administered via endotracheal tube. Sand bags were used to maintain the animals in sternal recumbency. The red hartebeest and blue wildebeest were given butorphanol (0.12 mg kg^{-1} IV Torbugesic, Fort Dodge, Kempton Park, South Africa) following intubation.

The surgical incision site, on the right side of the abdomen, was shaved and sterilised with chlorhexidine gluconate (Hibitane, Zeneca, Johannesburg, South Africa). We visually measured respiratory rate, while heart rate, arterial oxygen saturation (Nonin Handheld Pulse Oximeter, Plymouth, MN, USA), blood pressure and rectal temperature (Cardell 9400, Midmark Corporation, OH, USA) were also measured throughout surgery. Using sterile surgical procedures, we implanted an abdominal temperature logger (StowAway XTI, Onset Computer Corporation, Pocasset, MA, USA) and a subcutaneous temperature logger (model: MLOG_T1C, Sigma Delta Technologies Pty. Ltd., Floreat, Australia) in each animal.

The abdominal temperature logger had a resolution of 0.03 °C across a measurement range of 34–50 °C, with dimensions of ~50×45×20 mm and mass of ~40 g when coated in inert wax (Sasol wax EXP986, Sasol, Johannesburg, South Africa). The subcutaneous data logger, with a measurement range -20 to 65 °C and a resolution of 0.06 °C, had dimensions of ~25x10x8 mm and a mass <20 g when covered in inert wax. The abdominal temperature logger was implanted into the abdominal cavity via an incision in the paralumbar fossa, where after the muscle layer was sutured closed. The subcutaneous data logger was placed underneath the skin before the skin was sutured closed. The temperature loggers were individually calibrated in an insulated water bath against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany). After calibration the biologgers measured temperature to an accuracy of better than 0.06 °C.

At the end of implantation surgery each animal received a long-acting antibiotic (0.04 ml kg^{-1}, IM, benzylpenicillin, Duplocillin, Intervet, Isando, South Africa), a non-steroidal anti-inflammatory agent (0.05 mg kg^{-1}, IM, meloxicam, Mobic, Boehringer Ingelheim, Randburg, South Africa), a long-acting broad-spectrum parasiticide (0.02 mg kg^{-1}, SC, doramectin, Dectomax, Pfizer Laboratories, Sandton, South Africa), and a multivitamin injection (0.05 mg kg^{-1}, IM, Multivit injectable solution, Univet Ltd, County Cavan, Ireland).

Each animal was fitted with a GPS/GSM tracking-collar (Africa Wildlife Tracking, Pretoria, South Africa). Dorsally, each collar also supported a miniature black globe thermometer (the “collar miniglobe”) to measure the microclimate used by the animal (Hetem *et al.*, 2007). We measured miniglobe temperature with a thermistor, individually calibrated against a high-precision thermometer in an insulated water bath, placed into a mattblack, hollow, copper sphere (i.d. 30 mm, Press Spinning & Stamping, Cape Town, South Africa). The measurement range of the miniglobe thermistors, calibrated individually as previously detailed, was 0 – 65 °C with a resolution of 0.06°C. All temperature data were recorded every 5 minutes except for the abdominal temperatures that were recorded at 15 minute intervals. All temperature data were stored on the radio-collars, with a copy of data sent via GPRS to a centralised computer server twice daily.

Following all procedures, the antelope, in sternal recumbency, blind-folded with ear plugs in place, were transported to a fenced-off 10 000 ha section of the reserve, located approximately 10 km (20 min) from the surgical theatre. Upon arrival the antelope were given diprenorphine hydrochloride (~0.1 mg kg^{-1}, IV, M5050, Novartis, Johannesburg, South Africa) to reverse the effects of the etorphine hydrochloride. The antelope became ambulatory within 10 min of receiving the antagonist. They were allowed to roam freely within the fenced-off 10 000 ha study area and, although they were translocated from elsewhere on the 42 000ha reserve, many of them joined existing herds during the study period. Our study period included the five most physiologically-challenging months for antelope in a Southern Hemisphere summer rainfall area, notably the end of the dry season (Oct/Nov) when the nutritional value of the grazing is at its lowest (Grunow *et al.*, 1980, Rutherford, 1965), followed by the hottest months of the year (Dec-Feb).

4.2.4 Climatic data

A portable weather station was deployed at the study site. Technical issues with the portable weather station resulted in inconsistent recording of data. Climatic data for the town of Kimberley (28.7419° S, 24.7719° E), situated ~50 km ESE of the Rooipoort Nature Reserve were therefore sourced from the South African Weather Service (Erasmusrand, Pretoria, South Africa). Solar radiation was obtained from satellite-derived data (GeoSUN Africa, Stellenbosch, South Africa). A miniature black globe (hereafter called the reference miniglobe), similar to that mounted on the individual radio-collars, also with a calibrated thermistor, was placed unsheltered in the veld at 1 m above the ground. The reference miniglobe, therefore, was in full sun throughout daylight hours and unobstructed at night to provide a measure of heat load in the environment.

4.2.5 Data analyses

We analysed our data for two different periods. Firstly we analysed the entire 152-day data set with the use of GLMM's, linear regression and one-way ANOVA. Secondly, we identified the 10 hottest (all during December; not necessarily consecutive) and 10 coolest days during the study period and compared animal responses under those conditions with the use of linear regression, and either one-way ANOVA or repeated measures ANOVA, where applicable.

We extracted the 24h minima and maxima for all the climatic variables. We calculated the water vapour pressure from the Kimberley dry-bulb temperature and humidity data. We extracted the 24h minima and maxima for the abdominal and subcutaneous temperature measurements obtained from each individual animal of every species. We then calculated the mean and the range (maximum – minimum) for each variable for each 24h measurement period. We also calculated a mean 24h index of peripheral blood flow (abdominal temperature – subcutaneous temperature) for each animal; it is assumed that the greater the difference between the abdominal and subcutaneous temperatures the greater the level of vasoconstriction. A small abdominal-subcutaneous difference represented vasodilation.

We used GLMM's to investigate variables associated with the following dependent variables over 152 days: abdominal temperature (minima, maxima, and range), the difference between the abdominal-subcutaneous temperature, and microclimate use. Animal identity was used as a random effect in all the GLMM analyses and, because multiple measurements were made on the same animals over 152 different days, we nested days within individual. We used the days since winter solstice (21 June) as a continuous variable to test for changes in dependent variables over time. Linear regression was used to determine the relationship between miniglobe temperature and reference miniglobe temperature. The individual regression equations were used to calculate the temperature at which each collar miniglobe (Y) equalled the reference miniglobe (X) - the threshold reference globe temperature where >50% of collar miniglobe records lie under the line of identity, i.e., that reference globe temperature at which an animal makes use of a cooler microclimate more often than not. The slopes of the regression lines and the threshold temperatures were compared across species with one-way ANOVA.

For the 10 hottest days we determined the proportion of time that each animal spent in microclimates cooler than those recorded by the reference miniglobe between 10:00 and 15:00, the hottest part of the day. The proportion of time that each species spent in cooler microclimates was compared using one-way ANOVA. As we did for the 152-day period, we regressed the miniglobe temperatures, as recorded by the individual animal collars, against the reference miniglobe temperatures for the 10 hottest days and then also for the 10 coolest days during the study period. We then calculated the shade threshold heat load for each 10-day period. The thresholds and the slopes of the individual regression lines were compared using ANOVA. We used repeated measures ANOVA to compare abdominal temperatures, and the difference between abdominal and subcutaneous temperatures, between the 10 hottest and 10 coolest days. GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA) was used for all ANOVA and linear regression analyses and STATA10 (StataCorp LP, TX, USA) was used for the GLMM analyses. The significance level was set at P<0.05 in all analyses.

4.3 Results

4.3.1 Climatic conditions

Both air temperature and black globe temperature varied over 24 hours, increasing from a minimum at about sunrise to peak about one hour after solar noon. The air and black globe temperatures therefore had a characteristic 24h bell-shape. The 24h solar radiation also showed the characteristic bell-shaped distribution, peaking around midday at 1055 ± 107 $W.m^{-2}$ (Table 1). The water vapour pressure, on average, remained lower than 1 kPa throughout the study period, a level conducive to evaporative water loss at all antelope surface body temperatures. Moderate wind speeds (mean: 4.4 ± 1.4 $m.s^{-1}$) were recorded during the study period. Rain was recorded from October through January, with 101.3 mm recorded in total.

4.3.2 Microclimate selection

As there is a linear relationship between collar miniglobe and reference miniglobe, with a slope close to one, the most important determinant of collar miniglobe temperature is environmental temperature. The relationships between the collar miniglobe temperatures for one gemsbok, one red hartebeest and one blue wildebeest and the reference miniglobe temperatures recorded during the study period are illustrated in Figure 1. The scatter of points around the line of identity in Figure 1 reflects the deviation of collar miniglobe temperatures from reference miniglobe temperatures. The mean slopes of the regression lines for all the gemsbok

(0.83), the red hartebeest (0.86), and the blue wildebeest (0.91) were less than one, indicating intervention by all three species to manage their heat loads, with a significant species difference ($F_{2,13}$ = 4.19, P = 0.04) found only between the gemsbok and blue wildebeest. As the points of intersection were high (Fig. 1), the mean difference in slope is interpreted as the gemsbok seeking warm refuges of better quality, at low reference miniglobe temperatures, than did the blue wildebeest. The threshold heatload, which is that reference globe temperature at which the majority of collar miniglobe temperatures are cooler than the reference globe temperature, differed between species ($F_{2, 13}$ = 6.90, P = 0.009). We found no difference in the threshold heat load of the gemsbok (35°C) and the red hartebeest (31°C).

However, at 48°C, the blue wildebeest threshold temperature for use of cooler microclimates, that is the reference globe temperature at which the majority of collar miniglobe temperatures would be cooler than the reference globe temperature, was significantly higher than that of either the gemsbok or the red hartebeest. Although the blue wildebeest, evidently did make use of cooler microclimates (Fig. 1 bottom panel),

Table 1: Weather conditions (mean ± SD) recorded for the Rooipoort Nature Reserve in the Northern Cape Province, South Africa.

Variable	Minimum	Maximum	Mean
Air temperature (°C)	13.2 ± 4.4	31.4 ± 3.9	22.4 ± 3.5
Black globe temperature (°C)	12.8 ± 4.0	40.0 ± 5.2	24.8 ± 3.0
Water vapour pressure (kPa)	0.6 ± 0.4	1.4 ± 0.5	1.0 ± 0.5
Wind speed (m.s^{-1})	0.9 ± 0.8	9.2 ± 2.5	4.4 ± 1.4
Solar radiation (W.m^{-2})	142	1055	752

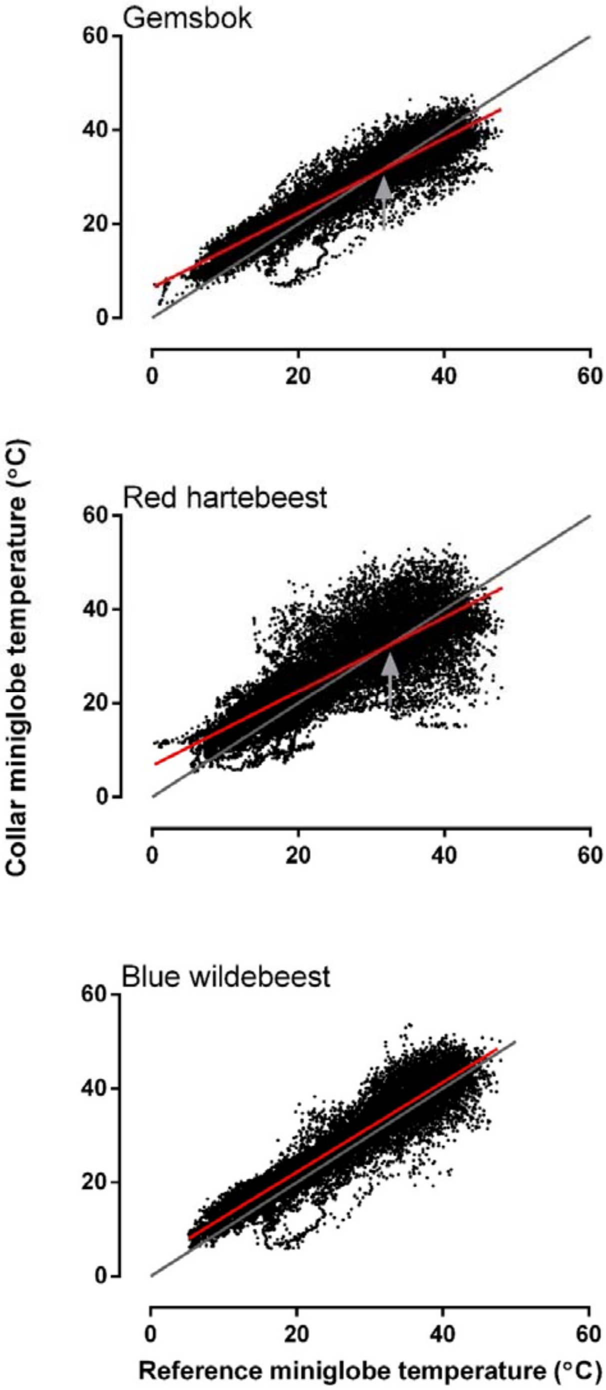

Figure 1: The relationship between the microclimates experienced by a single gemsbok, red hartebeest and blue wildebeest, as measured with the miniglobes on individual animal collars, and the heat load measured by the reference miniglobe during the study period (152 days). The grey line represents the line of identity. The arrows indicate the threshold heat load - the point where the majority of points would be below the line of identity for the individual gemsbok and red hartebeest. The threshold heat load predicted for this particular wildebeest, representing the point along the x-axis where the majority of points would be below the line of identity, i.e., in the shade, was 68°C.

it did so less predictably, on average, within the range of recorded reference globe temperatures than the gemsbok or the red hartebeest.

Although all three of our study species occupied microclimates cooler and warmer than that measured by the reference miniglobe, clear species difference in microclimate use were found. When it was cool the animals frequently made use of microclimates warmer than that measured by the exposed reference miniglobe (Fig. 1), suggesting that the animals sought shelter from wind during the day, or minimised radiative heat loss to the cold night sky. When it was hot the animals frequently made use of cooler microclimates than that measured by the reference miniglobe, but not exclusively so.

Our GLMM analyses showed that species were positively associated with the maximum 24h collar miniglobe temperatures, with the blue wildebeest exposed to higher heat loads than the gemsbok and the red hartebeest. However, species was not associated with the minimum 24h collar miniglobe temperatures (Table 2). Although the reference miniglobe temperature increased with time since winter solstice, the maximum collar miniglobe temperatures did not. Our study animals, therefore, made use of shade more with increasing time since the winter solstice as reference miniglobe temperatures increased. Minimum collar miniglobe temperatures did increase with days since winter solstice. Higher reference miniglobe temperatures were associated with higher collar miniglobe temperatures, while lower reference miniglobe temperatures were associated with lower

Table 2: Variables of interest related to microclimate use by gemsbok, red hartebeest and blue wildebeest on the Rooipoort Nature Reserve, Northern Cape Province, South Africa.

Microclimate use	Variable of interest	Z	P
Maximum collar miniglobe temperature	Species	5.09	<0.0001
	Days since winter solstice	1.21	0.228
	Maximum reference miniglobe temperature	29.52	<0.0001
	Maximum water vapour pressure (kPa)	1.59	0.112
Minimum collar miniglobe temperature	Species	-0.46	0.646
	Days since winter solstice	23.93	<0.0001
	Minimum reference miniglobe temperature	48.61	<0.0001
Cooler microclimate used (collar miniglobe – reference miniglobe = -)	Species	-0.14	0.893
	Days since winter solstice	-1.34	0.181
	Maximum reference miniglobe temperature	-28.27	<0.0001
	Maximum water vapour pressure (kPa)	6.80	<0.0001
Warmer microclimate used (collar miniglobe – reference miniglobe = +)	Species	1.75	0.080
	Days since winter solstice	5.66	<0.0001
	Minimum reference miniglobe temperature	-7.66	<0.0001

collar miniglobe temperatures. The heat load experienced by the animals was therefore influenced by the environmental heat load.

We found no species difference in the use of microclimates that were either cooler, or warmer, than that recorded by the reference miniglobe. Nevertheless, our study animals did seek cooler microclimates under higher environmental heat loads and warmer microclimates under cold conditions (Table 2). Maximum water vapour pressure also was associated positively with the use of microclimates cooler than the environmental heat load measured by the reference miniglobe. The greater use of microclimates warmer than that recorded by the reference miniglobe with increasing time since the winter solstice is attributed to the animals sheltering from wind while remaining in the sun.

When comparing microclimate use during the 10 hottest days (maximum reference globe temperatures >46.4°C) with that of the 10 coolest days (maximum reference globe temperatures <32.8°C) recorded during the study period, we found a species effect ($F_{2, 11} = 6.21$, $P = 0.02$) with significant differences in the slopes of the regression lines ($F_{1, 11} = 319$, $P<0.0001$) for all three species between the two periods. During the 10 coolest days the slopes of the regression lines were larger than one for all three species, but the slope for the gemsbok was lower than that of the red hartebeest. During the 10 hottest days (Fig. 2), the slopes of the regression lines differed significantly between species ($F_{2, 11} = 10.04$, $P = 0.003$), as did the heat load threshold temperatures for using cooler microclimates ($F_{2, 8} = 11.50$, $P = 0.004$).

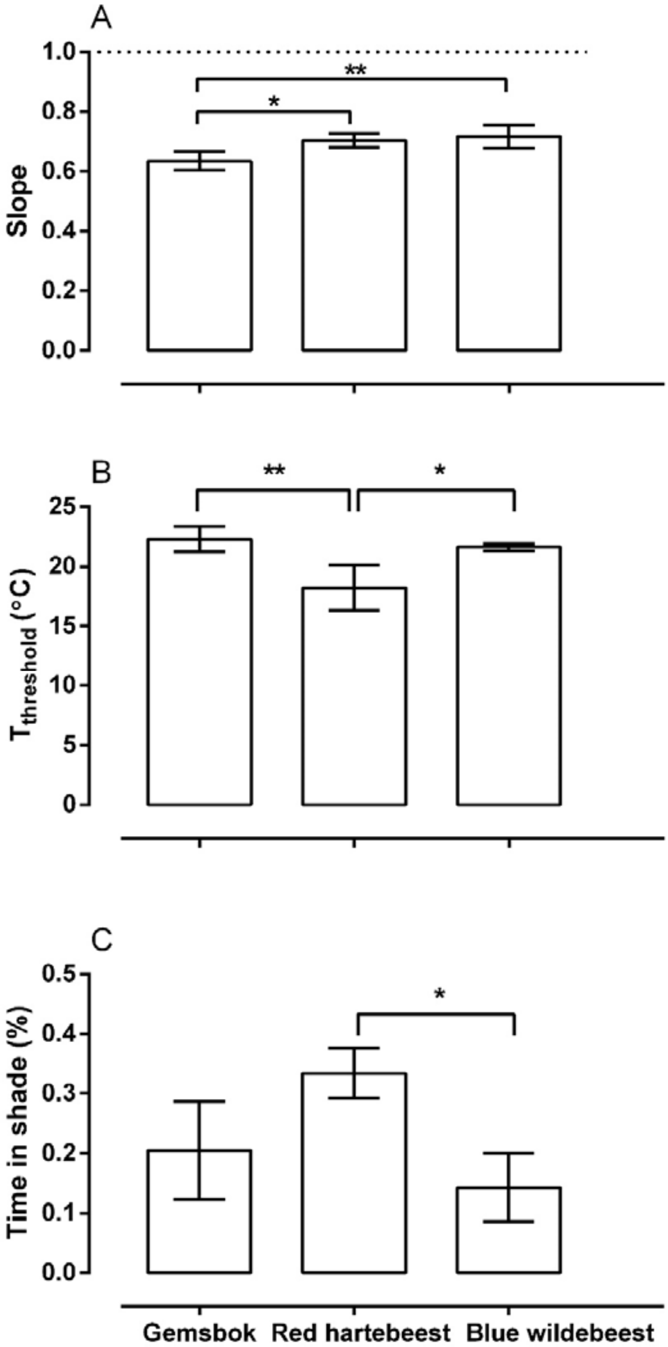

Figure 2: Comparison of microclimate use between the gemsbok, red hartebeest and blue wildebeest during the 10 hottest days ($T_{\text{reference miniglobe}}$ >46.4°C) recorded on Rooipoort Nature Reserve, Northern Cape Province, South Africa. (A) The mean slope of the individual regression lines per individual per species of collar miniglobe temperature against reference miniglobe temperature; the dotted line indicates parity between collar miniglobe and reference miniglobe temperatures (i.e., no behavioural thermoregulation to reduce heat loads). (B) The heat load threshold temperature at which the gemsbok, red hartebeest and blue wildebeest accessed cooler microclimates and, (C) the proportion of time that each species spent in microclimates cooler than that measured by the reference miniglobe during the heat of the day (10:00 - 15:00).

The red hartebeest predictably made use of microclimates cooler than that measured by the reference globe at a lower threshold temperature (18°C) than either the gemsbok (22°C) or the blue wildebeest (22°C), while the gemsbok (slope: 0.64) made use of better quality shade, than either the red hartebeest (slope: 0.70) or the blue wildebeest (slope: 0.72). The proportion of time that the gemsbok, red hartebeest and blue wildebeest spent in microclimates cooler than those measured by the reference miniglobe between 10:00 and 15:00, the hottest time of the day, differed ($F_{2,10} = 6.87$, $P = 0.01$) during the 10 hottest days of the study period. During the hottest time of the day the red hartebeest spent a larger proportion of time in cooler microclimates than did the blue wildebeest (33% vs. 14%). The gemsbok spent 21% of their time between 10:00 and 15:00 in cooler microclimates during the 10 hottest days (Fig. 2).

4.3.3 Body temperature patterns

The mean abdominal temperature traces for the gemsbok, the red hartebeest and the blue wildebeest over 152 days are illustrated in Figure 3. Also shown in Figure 3 are the daily air temperature trace and the daily maximum and minimum reference miniglobe temperatures. From these abdominal temperature traces two distinct periods can be distinguished by inferring the range of body temperatures from the 15 min recording. Over the first 50 days, the maxima recorded were higher and the minima lower than over the rest of the recording period. The 24h abdominal temperature profiles of all three species were characterised by a nadir early morning just after sunrise and reaching a zenith at about sunset, well after solar

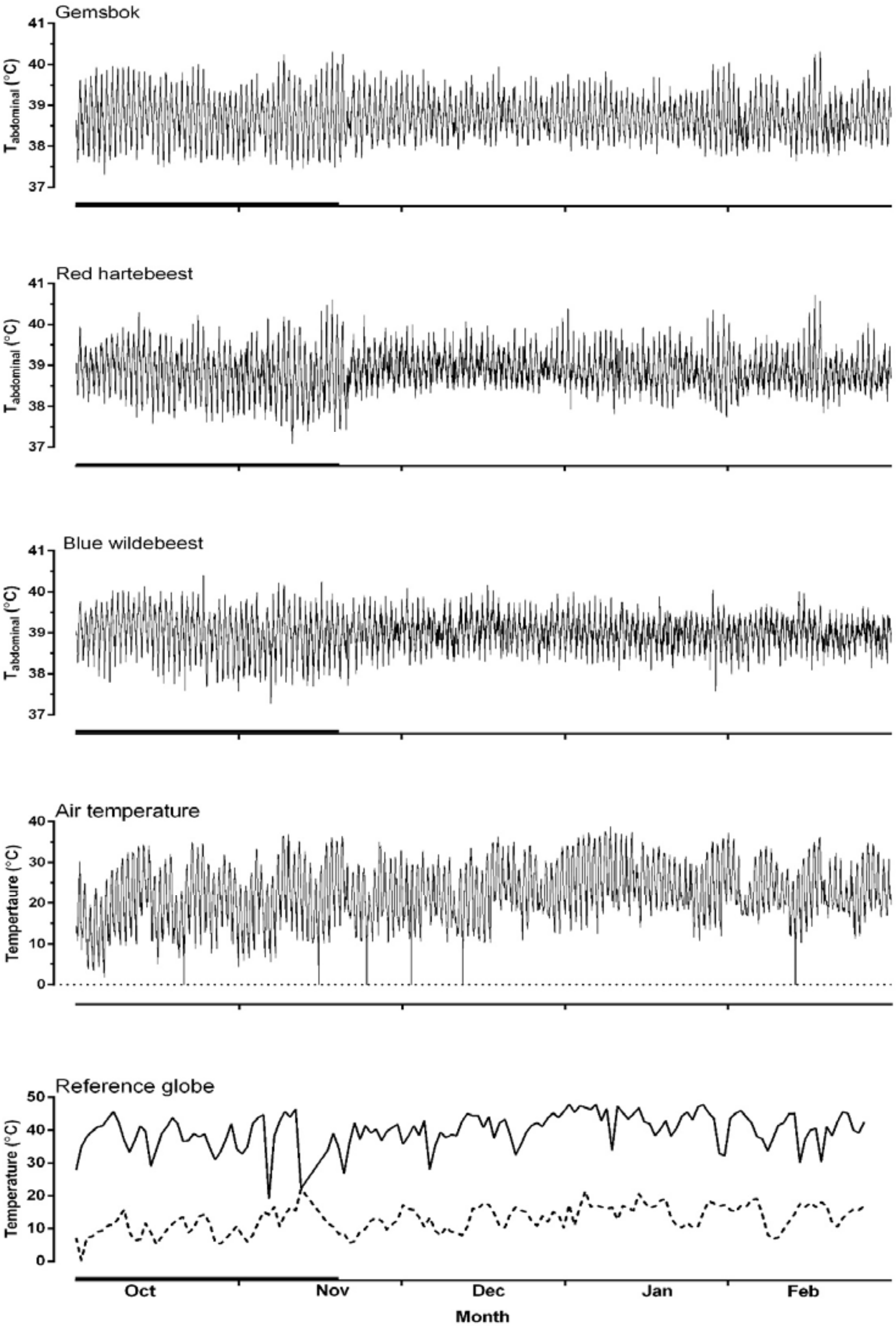

Figure 3: The mean abdominal (15 min intervals) temperature traces for all the gemsbok, red hartebeest and blue wildebeest, as well as the daily air temperature trace and the minimum and maximum daily reference miniglobe temperatures over 152 days in the Rooipoort Nature Reserve, Northern Cape Province, South Africa. The horizontal black bars highlight the period of elevated maximum and depressed minimum abdominal temperatures.

noon (Fig. 4). We found no species differences in the mean minimum or maximum 24h body temperature measures, nor in the mean range of the 24h abdominal temperature (Table 3). All three antelope species had lower mean minimum 24h abdominal temperatures, and higher mean maximum 24h abdominal temperatures closer to the winter solstice (Table 4). As a consequence, the mean 24h range of abdominal temperature (maximum – minimum abdominal temperature) also was higher closer to the winter solstice in all three species.

The mean 24h abdominal temperatures were dependent on environmental conditions. We found a positive association between the heat loads experienced by the animals, as measured with their individual collar-mounted miniglobes, and the mean 24h maximum abdominal temperatures. Collar miniglobe temperature also was associated with the mean 24h minimum abdominal temperature. Increased amplitudes in the miniglobe temperature were associated positively with increased abdominal temperature amplitudes (Table 4). All three species showed a clear 24h rhythm in abdominal-subcutaneous temperature; the profile is the inverse of that recorded for subcutaneous temperature, with the maximum difference (vasoconstriction) reached at about sunrise (Fig. 5). Shortly after sunrise, when abdominal temperature was at its lowest, peripheral blood vessels presumably dilated and the difference between the abdominal and subcutaneous temperatures decreased quickly, to reach a minimum shortly before midday. In the 24h cycle, all three species

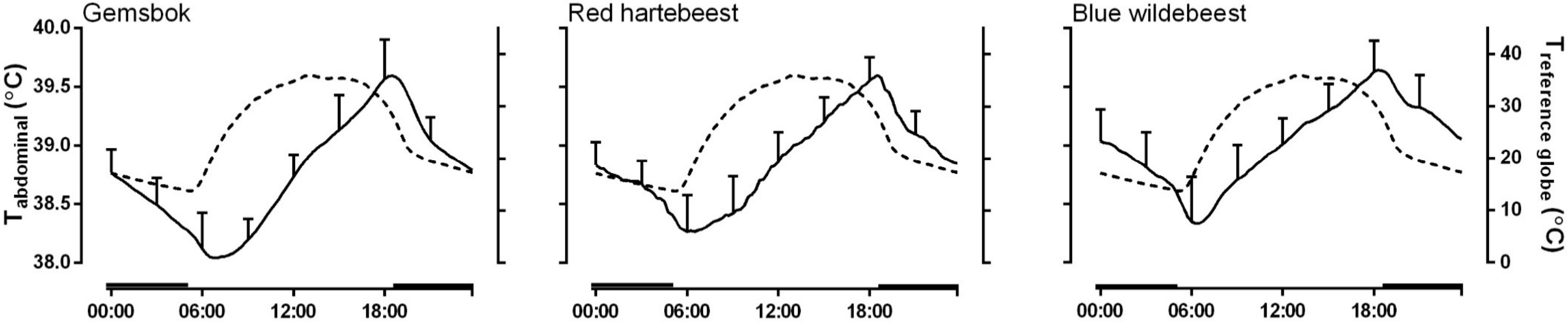

Figure 4: The mean 24h abdominal temperature profiles (±SD) for the gemsbok (left panel), the red hartebeest (middle panel) and the blue wildebeest (right panel) in relation to the mean 24h reference miniglobe temperature (dotted line; secondary y-axis) as measured over 152 days on Rooipoort Nature Reserve in the Northern Cape Province of South Africa. Horizontal black bars indicate night time.

Table 3: The mean (± SD) minimum, maximum and range of abdominal ($T_{abdominal}$) and abdominal minus subcutaneous ($T_{abdominal} – T_{subcutaneous}$) temperatures recorded in, as well as the microclimate use by, the gemsbok, red hartebeest and blue wildebeest from October to February on the Rooipoort Nature Reserve, Northern Cape Province, South Africa. $T_{threshold}$ is the heat load threshold temperature at which an animal sought a microclimate cooler than that available in full sun. The difference between the collar miniglobe and the reference miniglobe (T_{collar}-$T_{ref\ globe}$) represents the mean daily microclimate selection.

Species	$T_{abdominal}$ (ºC)			$T_{abdominal} – T_{subcut}$ (ºC)		Microclimate use		
	Min	Max	Range	Min	Max	$T_{threshold}$	Slope	T_{collar} - $T_{ref\ globe}$
Gemsbok *Oryx gazella*								
Gemsbok 1	37.4±0.4	39.5±0.3	2.1±0.5	-0.9±0.5	3.1±1.4	31.3	0.7939	1.4±1.9
Gemsbok 2	37.7±0.4	39.8±0.3	2.1±0.5	-0.9±0.6	3.5±1.1	32.1	0.8427	1.1±1.9
Gemsbok 3	37.5±0.1	39.9±0.2	2.4±0.2	-	-	37.2	0.8552	1.8±1.7
Gemsbok 4	37.7±0.4	40.0±0.4	2.3±0.7	-0.8±0.6	4.8±1.9	39.5	0.8614	2.1±1.8
Gemsbok 5	37.7±0.3	39.7±0.3	1.9±0.5	-0.7±0.6	4.2±1.7	35.4	0.8544	1.5±1.7
Gemsbok 6	38.1±0.2	39.5±0.3	1.4±0.4	-0.7±0.5	3.3±1.0	33.4	0.7998	1.6±1.9
Mean	**37.7±0.4**	**39.7±0.4**	**2.0±0.6**	**-0.8±0.6**	**3.8±1.6**	**34.8±3.1**	**0.83±0.03**	**1.6±0.3**
Red hartebeest *Alcelaphus buselaphus*								
Red hartebeest 1	38.1±0.3	39.8±0.4	1.7±0.5	-0.5±0.6	5.4±2.2	31.2	0.8972	0.6±1.9
Red hartebeest 2	38.3±0.3	40.2±0.4	1.9±0.5	-0.8±0.7	5.4±1.7	38.8	0.9144	1.2±2.0
Red hartebeest 3	-	-	-	-	-	22.9	0.8128	-0.3±0.6
Red hartebeest 4	-	-	-	-	-	32.0	0.7917	0.2±4.0
Red hartebeest 5	37.7±0.4	39.7±0.4	2.0±0.6	-0.90.8	4.61.6	28.2	0.8696	0.4±1.8
Red hartebeest 6	37.8±0.4	39.9±0.4	2.2±0.6	-1.0±0.8	5.1±2.0	33.8	0.8697	1.1±1.6
Mean	**38.0±0.4**	**39.9±0.4**	**1.9±0.6**	**-0.8±0.8**	**5.1±1.9**	**31.2±5.3**	**0.86±0.05**	**0.5±0.6**
Blue wildebeest *Connochaetes taurinus*								
Blue wildebeest 1	38.0±0.4	39.6±0.2	1.6±0.5	-1.3±0.8	6.0±3.3	42.4	0.922	1.4±2.4
Blue wildebeest 2	38.4±0.4	40.2±0.3	1.8±0.5	-0.6±0.6	3.9±1.6	37.5	0.8675	1.6±1.9

Blue wildebeest 3	37.5±0.5	39.9±0.3	2.5±0.6	-1.1±0.7	4.8±1.8	68.4	0.9548	1.9±1.7
Blue wildebeest 4	38.4±0.4	39.9±0.3	1.5±0.5	-0.7±0.7	4.1±1.9	43.3	0.8973	1.9±1.8
Blue wildebeest 5	38.1±0.4	39.7±0.2	1.6±0.4	-0.8±0.6	4.6±1.8	45.8	0.9094	1.8±1.8
Mean	**38.0±0.4**	**39.8±0.4**	**1.7±0.6**	**-0.9±0.7**	**4.7±2.3**	**47.5±12.1**	**0.91±0.03**	**1.72±0.2**

Table 4: Variables of interest related to the body temperature of three antelope species on the Rooipoort Nature Reserve, Northern Cape Province, South Africa.

Body temperature	Measurement (°C)	Variable of interest	Z	P
Abdominal	Maximum	Species	1.91	0.57
		Days since winter solstice	-5.22	<0.0001
		Maximum 24h miniglobe – maximum 24h reference miniglobe temperature (°C)	-7.38	<0.0001
		Maximum miniglobe temperature (°C)	8.06	<0.0001
		Maximum water vapour pressure (kPa)	-1.27	0.203
	Minimum	Species	1.53	0.126
		Days since winter solstice	5.67	<0.0001
		Minimum miniglobe temperature (°C)	8.72	<0.0001
	Range	Species	-0.14	0.892
		Days since winter solstice	-10.41	<0.0001
		Mean 24h miniglobe temperature range (°C)	6.93	<0.0001
		Maximum 24h miniglobe – maximum 24h reference miniglobe temperature (°C)	-10.24	<0.0001
Abdominal – subcutaneous	Vasoconstriction (large difference)	Species	2.38	0.017
		Days since winter solstice	-7.82	<0.0001
		Maximum miniglobe temperature	-8.13	<0.0001
		Maximum 24h miniglobe – maximum 24h reference miniglobe temperature (°C)	-1.90	0.058
	Vasodilation (small difference)	Species	-1.41	0.159
		Days since winter solstice	12.70	<0.0001
		Maximum miniglobe temperature	3.67	<0.0001
		Maximum 24h miniglobe – maximum 24h reference miniglobe temperature (°C)	-1.92	0.055

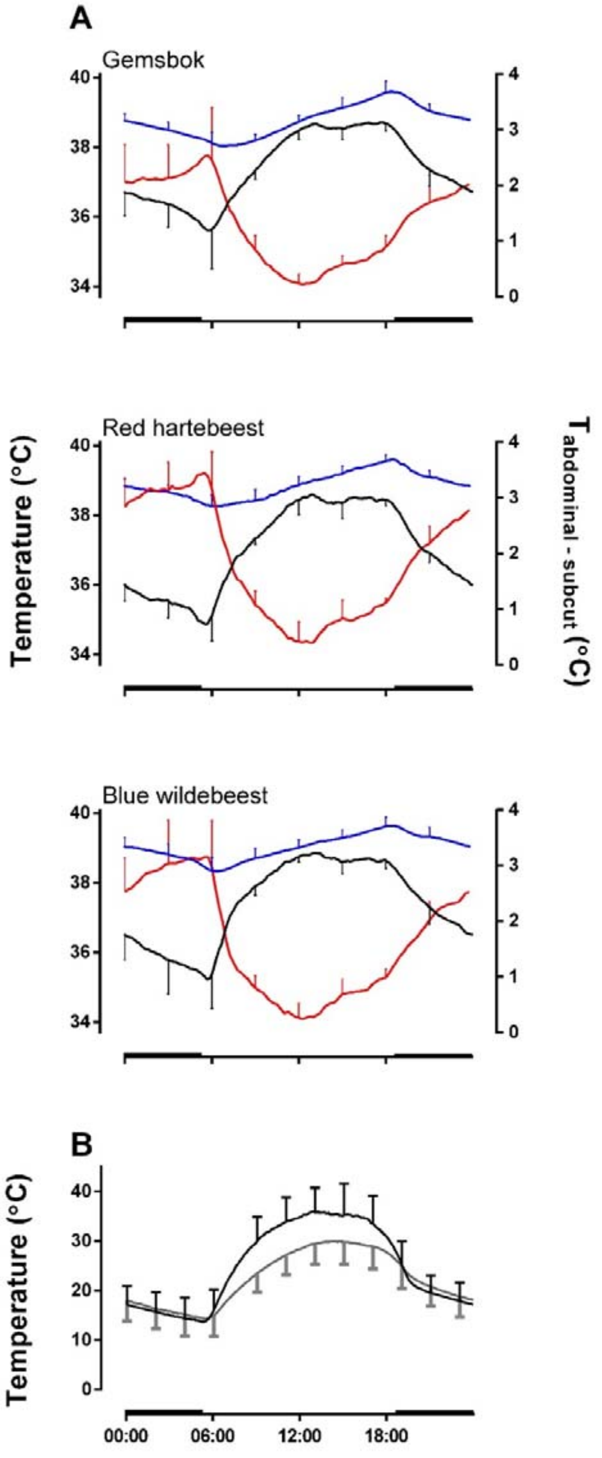

Figure 5: (A) The mean 24h abdominal (blue) and subcutaneous (black) temperatures (±SD) recorded in the gemsbok, the red hartebeest and the blue wildebeest on Rooipoort Nature Reserve, Northern Cape Province, South Africa and, on the secondary y-axis, the mean difference between the 24h abdominal and subcutaneous temperatures (red). An increasing difference between the abdominal and subcutaneous temperatures reflects increasing vasoconstriction. (B) The mean (±SD) 24h reference miniglobe (black) and air (grey) temperatures. Horizontal black bars indicate night time.

therefore reached maximum vasodilation shortly before the peak heat load (reference miniglobe temperature) was experienced (Fig. 5).

Shortly after midday, once the maximum reference miniglobe temperature was reached, the difference between abdominal and subcutaneous temperatures started increasing gradually again throughout the afternoon and evening. We found a species difference with regards to vasoconstriction, with the gemsbok showing less vasoconstriction than either the red hartebeest or the blue wildebeest (Table 4), specifically during the early morning (Fig. 5). The abdominal – subcutaneous temperature differential decreased with time since the winter solstice, was enhanced at lower miniglobe temperatures, and decreased at higher miniglobe temperatures. As for body temperature, we found no species differences with regards to vasodilation during the 152 day study period.

We found no species differences when comparing the minimum ($F_{2, 13}$ = 1.84, P = 0.2), the maximum ($F_{2, 13}$ = 1.90, P = 0.2) or the range ($F_{2, 13}$ = 0.53, P = 0.6) of body temperatures recorded during the 10 hottest days with those recorded during the 10 coolest days. The maximum abdominal temperatures differed between the two time periods ($F_{1, 13}$ = 35.2, P <0.0001) with gemsbok and red hartebeest reaching higher maximum abdominal temperatures during the 10 hottest days (gemsbok: 39.7 vs 39.5 °C; red hartebeest 40.1 vs 39.7 °C) than the 10 coolest days. No difference ($F_{1, 13}$ = 0.03, P = 0.86) was found in the range of abdominal temperature between the 10 hottest and 10 coolest days.

The minimum difference between abdominal and subcutaneous temperature was lower during the 10 coolest days compared to the 10 hottest days ($F_{1, 11} = 7.15$, $P = 0.02$), with the blue wildebeest more vasodilated during the 10 coolest days. There was no difference in the maximum abdominal - subcutaneous temperature differential (i.e., vasoconstriction) between the 10 hottest and 10 coolest days ($F_{1, 11} = 1.49$, $P = 0.25$). All three species, therefore, were maximally vasoconstricted at night, even during the 10 hottest days.

4.4 Discussion

We have used biologgers to successfully record simultaneous data on abdominal and subcutaneous temperatures in three large, sympatric antelope species with varying water dependencies. The use of a collar-mounted miniature black globe thermometer (Hetem *et al.* 2007) and a similar, unsheltered reference black globe thermometer in the open enabled us to investigate the way in which the three species made use of microclimates in the same environment. The combined data sets allowed us to investigate and compare, for the first time, abdominal temperature, abdominal - subcutaneous temperature, and microclimate use between large African antelope species inhabiting the same area. Moreover, we have collected these data sets over the physiologically most challenging time of the year, from October through February – that is the end of the dry season though the hottest period of the year.

We have shown that species differences exist in the microclimate use by the gemsbok, the red hartebeest and the blue wildebeest, both in terms of the threshold reference globe temperature at which cooler microclimates are mostly used and the quality of the shade utilised. We have also shown that the animals responded to windy conditions, by sheltering from the wind at low temperatures and also at high temperatures at the expense of shade seeking. Despite these differences in accessing microclimates cooler or warmer than that available in the full sun, we have found no species differences in the mean 24h abdominal or the difference between abdominal and subcutaneous temperatures (Table 4). While environmental conditions did affect the abdominal temperatures of all three antelope species, the effect was small. In all three antelope species, we found higher maximum and lower minimum abdominal temperatures closer to the winter solstice. For all three antelope species the range in abdominal temperature (maximum – minimum abdominal temperature) therefore was also greater closer to the winter solstice when the environmental temperature was lower.

The simultaneous recording of abdominal and subcutaneous temperatures provided us with an opportunity to compare how the animals altered peripheral blood flow to change dry heat loss during the hottest time of the year. The abdominal – subcutaneous temperature differential has previously been used as an index of peripheral blood flow in Angora goats *Capra aegagrus* (Hetem *et al.*, 2009b), and in kudu that naturally developed a fever (Hetem *et al.*, 2008). However, to the best of our knowledge we are the first to investigate the difference between abdominal and subcutaneous temperatures in more than one species simultaneously inhabiting the same habitat. Although the red hartebeest and the blue wildebeest showed lower levels of peripheral blood flow than the gemsbok, all three species decreased peripheral blood flow closer to the winter solstice, thereby limiting the transfer of heat from the core to the periphery, and, consequently, limiting heat loss to the environment. Peripheral blood flow increased in all three species with increasing days since the winter solstice, thereby facilitating dry heat loss to the environment as environmental temperatures increased.

The failure of some subcutaneous and abdominal temperature loggers limited our sample size during this study. Despite these shortcomings we collected *ca.* 15 000 abdominal, and more than 43 000 simultaneous subcutaneous and microclimate temperature records for six gemsbok, four red hartebeest and five blue wildebeest. Although our data collection spanned five months, compared to weeks or even days in earlier studies, the mean 24h minimum and maximum body temperatures, as well as the body temperature amplitudes, were similar to that previously reported for gemsbok (Maloney *et al.*, 2002), red hartebeest (Finch, 1972, Finch and Robertshaw, 1979) and blue wildebeest (Fick *et al.*, 2006, Shrestha *et al.*, 2012).

The lack of an association between days since winter solstice and maximum collar miniglobe temperature (maximum heat load experienced) reflects thermoregulatory behaviour, as an association was found between days since winter solstice and reference miniglobe temperature. Lower minimum collar globe temperatures were recorded earlier in the study when the air temperatures were lower. The minimum collar miniglobe temperatures increased with time since the winter solstice, while the maximum collar miniglobe temperatures increased with increasing reference miniglobe temperature (Table 2).

All three ungulate species made use of microclimates cooler than that measured by the reference globe, as indicated by the slopes of the regression lines being less than one (Fig. 1 and Table 3). All three ungulate species, therefore stabilised their microclimates by occupying warmer microclimates when it was cold and cooler microclimates when it was hot. The use of warmer microclimates by impala *Aepyceros melampus* and blue wildebeest during the night, when environmental temperatures were low, has been attributed to close proximity with conspecifics or recumbency (Hetem *et al.*, 2007). Lying down at night shelters animals from the wind. Moreover, lying down allows animals to make use of the boundary layer of the ground, thereby reducing convective heat loss because of the higher air temperature close to ground level (Bakken, 1992). Alternatively, the animals may have sheltered under trees or shrubs at night, thereby reducing their radiative heat loss to the night sky, which could be as cold as -30°C (Hetem *et al.* 2007).

The use of warmer microclimates during the day, even when environmental temperatures were high (Fig. 1), is possibly related to the habitats used by the animals, with dry pans, rocky and or sandy areas with relatively low vegetation cover potentially having higher levels of reradiation than the reference miniglobe habitat. The reference miniglobe habitat, characterised as *Eragrostis lehmanniana – Tarchonanthus camphoratus* shrubland was characterised by 60% herbaceous cover, <2% rocks or stones on the surface (Bezuidenhout, 2009). Alternatively the animals may have given greater priority to sheltering from the wind rather than seeking shade, thereby reducing their heat loss to the environment, as has previously been documented for blue wildebeest (Hetem *et al.* 2007).

Based on our GLMM analyses the gemsbok and red hartebeest sought microclimates cooler than that measured by the unsheltered reference miniglobe (presumably shade), at lower maximum miniglobe temperatures, than did the blue wildebeest (Table 2). Blue wildebeest are, however, known not to seek shade even when environmental temperatures are high (Hetem *et al.*, 2007). An earlier study on the behavioural thermoregulation of black wildebeest *Connochaetes gnu* suggested the existence of a threshold heat load at which orientation was initiated as a thermoregulatory behaviour (Maloney *et al.*, 2005b). While a combination of study objectives (minimum disturbance of animals), long animal flight distances and habitat structure precluded behavioural observations in the present study, we have quantified a threshold heat load for shade seeking in each of our study species (Fig. 2). The gemsbok and the red hartebeest consistently used microclimates cooler than that in full sun at threshold heat loads that were up to 25°C degrees cooler than the 48°C threshold heat load for the blue wildebeest. In the Kgalagadi Transfrontier Park gemsbok spent less time feeding and more time resting in the shade than did the blue wildebeest (Knight, 1991). Also, despite an increased proportion of blue wildebeest making use of shade from September to December, the majority of animals still rested in the sun unsheltered (Knight, 1991). The gemsbok, considered to be able to survive independent of surface water, and the red hartebeest of intermediate water dependency therefore moved into cooler microclimates before the water-dependent blue wildebeest, even when surface water was readily available.

The 10 hottest days accentuated the microclimate use signal for all three species. As for the entire study period, during the 10 hottest days the gemsbok, red hartebeest and blue wildebeest all regularly used microclimates cooler than that recorded by the reference miniglobe (slopes of the regression lines <1). However, during the 10 hottest days the threshold heatload for shade use – that reference globe temperature where more than 50% of collar miniglobe temperatures are below the reference globe temperature - was 18°C for the red hartebeest, lower than that of the gemsbok or the blue wildebeest (both 22°C). That the abdominal - subcutaneous temperature differential for the blue wildebeest was smaller during the 10 coolest days than the 10 hottest days, suggests that the animals spent more time basking in the sun during the coolest days.

The microclimate, particularly the heat loads experienced by our study animals, influenced both the minimum and the maximum abdominal temperatures. Consequently an increased range in their mean 24h abdominal temperature was associated with a greater range in the mean 24h miniglobe temperature (Table 4). The effects of heat load on the abdominal temperature were small, however, with a 1°C increase in black globe temperature, on average, resulting in a 0.01 °C increase in the abdominal temperatures of our study animals. In a year-long study Fuller and colleagues have previously reported an 0.02°C increase in abdominal temperature per 1°C increase in air temperature for the much smaller springbok *Antidorcas marsupialis* (Fuller *et al.*, 2005). By contrast, no relationship between environmental conditions and abdominal temperature was reported in earlier studies on gemsbok (Maloney *et al.*, 2002) and springbok (Mitchell *et al.*, 1997), but that could be attributed to small sample sizes (N= 1; Mitchell *et al.* 1997) and testing for relationships over short time frames (up to 15 days; Maloney *et al.* 2002), respectively (Fuller *et al.*, 2005). While Fuller *et al.* (2005) concluded that environmental conditions have only a small, weak effect on the 24h abdominal temperature rhythms of African ungulates, a more recent analysis using body temperature data from 17 ungulate species found that environmental temperature had no effect on body temperature rhythm or amplitude when animals had access to sufficient food and water (Hetem *et al.*, 2016). Moreover, the species differences that we have recorded in microclimate use did not translate into species differences in abdominal temperature (Table 4).

We did, however, find differences in the abdominal temperatures of our study animals. All three our study species had lower mean 24h minimum and higher maximum abdominal temperatures closer to the winter solstice. The range of the mean 24h abdominal temperatures were therefore also higher closer to the winter solstice (Fig. 3). We attribute the lower minimum body temperatures close to the winter solstice (Table 4) to the lower nutritive value of the grass in the Northern Cape Province at the end of the dry season. As decreasing forage quality and quantity at the end of the dry season have been documented across much of southern Africa (O'Connor *et al.*, 2001, Chamaillé-Jammes and Fritz, 2009), our study animals likely were undernourished during a part of the study period. A decrease in minimum abdominal temperature in response to nutritional stress has been documented in captive ungulates (Piccione *et al.*, 2002, Maloney *et al.*, 2013) and free-living ungulates (Hetem *et al.*, 2016, Hetem *et al.*, 2012b, Ostrowski *et al.*, 2006b, Shrestha *et al.*, 2012). All three of our study species were therefore more heterothermic, in keeping with recent evidence indicating that homeothermy is maintained only when enough water and food of sufficient quality is readily available (Hetem *et al.*, 2016).

As water was available at four artificial water points throughout the study, it is difficult to explain the higher maximum 24h abdominal temperatures observed in all three species towards the end of the dry season. It is possible that differences in activity, which we could not quantify, resulted in different levels of metabolic heat production. While the contribution of different activities to metabolic heat production in free-ranging ungulates is not known, estimates have been generated for domestic cattle. For example, in cows *Bos taurus*, feeding results in a five-fold increase and walking in a three-fold increase in metabolic heat production compared to when standing still (Malechek and Smith, 1976). Because endogenous heat production contributes to the heat load experienced by the animals (Maloney *et al.*, 2005a) increased feeding and walking activity towards the end of the dry season may therefore have contributed to the higher maximum abdominal temperatures during the period of nutritional stress. Although data are not available for all three of our study species, metabolic heat production contributed about 10% and 12% to the heat loads of captive red hartebeest and eland respectively (Finch, 1972). Alternatively, it is possible that the study animals, all of which were translocated into the 10 000 ha study area at the beginning of the study, were naïve and unfamiliar with the location of the four artificial water sources, and their naivety may have resulted in them not accessing and drinking water from these water points. Coupled with vegetation of low nutritive value, and also low water content at the end of the dry season, the animals therefore may have had a reduced water intake, thereby contributing to the increased maximum abdominal temperatures observed closer to the winter solstice (Fig. 3). With subsequent changes in ranging behaviour (A. Stainthorpe pers. comm.), greater familiarity with the distribution of water, and food of sufficient quality, ensued.

Heterothermy in large mammals is therefore not implemented in anticipation of high environmental heat loads when water is scarce (i.e., not adaptive), but rather is an inevitable response to either an energy deficiency, a scarcity of water, or as in the case of the Arabian oryx and sand gazelle, a combination of both (Hetem *et al.*, 2012b). Contrary to a suggestion by Ostrowski and Williams (2003), high air temperatures are therefore not a prerequisite for heterothermy. High environmental temperatures do, however, exacerbate the effect of a water shortage on maximum body temperature, while also influencing subcutaneous temperature directly.

A similar 24h abdominal – subcutaneous temperature rhythm was found in all three antelope species (Fig. 5, red lines). Although the antelope already were vasoconstricted, at dawn, they went through a brief period of extra vasoconstriction, with the concomitant drop in subcutaneous temperature (Fig. 5 black lines) resulting in the maximal 24h difference between abdominal and subcutaneous temperature. While air and reference miniglobe temperature gradually reached its nadir at this time (Fig. 5), none of the antelope showed the anomalous drop in abdominal temperature at dawn previously reported in eland (Fuller *et al.*, 1999b) and kangaroos (Dawson *et al.*, 2007). All three species started vasodilation, characterised by a decrease in the difference between abdominal and subcutaneous temperatures, when their abdominal temperatures were at a minimum, with vasodilation peaking at midday. Heat was therefore transferred from the body core to the periphery before abdominal temperature started increasing. At midday the difference between the mean 24h abdominal and subcutaneous temperatures were close to zero in all three species. Occasionally, as a result of solar radiation, the subcutaneous temperatures of all three species exceeded abdominal temperature. After midday, vasoconstriction was again gradually employed throughout the afternoon and evening. The greater vasoconstriction recorded in the blue wildebeest and the red hartebeest, compared to the gemsbok, is possibly related to the periodic use of more open habitats by the hartebeest (Ben-Shahar and Fairall, 1987), or earlier feeding bouts by the red hartebeest and the blue wildebeest. Exposure to cooler microclimates early morning would have resulted in the red hartebeest and blue wildebeest defending their body temperatures by reducing blood flow peripherally.

When sensible heat transfer (radiation and convection) changes from a loss to a gain, mammals have to make use of evaporative cooling to maintain their body temperatures (Tattersall *et al.*, 2012). The mean maximum 24h reference miniglobe temperature (Table 1) was higher than the mean maximum 24h abdominal temperatures of all three antelope species (Table 3). Our study species, therefore, would have gained heat from their environment during those midday periods that heat load exceeded abdominal temperature, provided they were not in shade at the time. All three species, therefore needed access to shade to reduce heat gain from their environment and to conserve body water.

All three species reduced the heat load threshold temperature for shade use during the 10 hottest days, compared to the 10 coolest days. Moreover, the slopes of the regression lines of miniglobe temperature against reference miniglobe temperature decreased from the 10 hottest to the 10 coolest days in all three species, implying the more regular use, or the use of better quality shade, during the hottest days. Both the Arabian oryx and the Arabian sand gazelle had lower threshold temperatures for shade use and used shade of better quality, during the hot dry period compared to the warm wet period in the Saudi Arabian desert (Hetem *et al.*, 2012b). Lower heat load threshold temperatures for shade use and the use of better quality shade during the hottest days compared to the coolest days likely facilitated the conservation of body water in all three species during the hottest days. In the absence of shade, the observed heterothermy – particularly the elevated maximum abdominal temperatures compared to the norm – means that all three species would have gained less heat from the environment because of a reduced temperature gradient (Hetem *et al.*, 2016), resulting in a reduced need for evaporative cooling. Under cool conditions closer to the winter solstice the animals made use of refugia warmer than the exposed reference miniglobe, thereby reducing the costs of maintaining homeothermy and conserving energy at a time that they were undernourished.

In conclusion, we have provided the first evidence of heterothermy in free-living African ungulates. Over a period of about 50 days, coinciding with the end of the dry season, and before the vegetation responded to the first summer rains, all three of our study species had reduced minimum and elevated maximum body temperatures. Although the observed heterothermy is likely not adaptive (Hetem *et al.* 2016), the elevated maximum body temperatures would have contributed to the conservation of body water under high environmental heat loads. The patterns of microclimate use, particularly during the 10 hottest days suggests behavioural flexibility. The gemsbok and the red hartebeest readily used shade, even in the presence of surface water, implying that they did not drink water daily, even though it was available. The lower threshold temperatures for shade use exhibited by the gemsbok and red hartebeest throughout the study period possibly reduced the need for evaporative cooling (e.g., panting) as it is skin temperature, not abdominal temperature that determines the onset of panting (Finch 1972). Possibly as a result of its daily water intake the blue wildebeest spent more time in the sun and had a higher heat load threshold temperature for cooler microclimate use than the gemsbok and the red hartebeest, presumably relying more on evaporative cooling for more efficient heat loss, than behavioural adjustment. Thermoregulatory signals of interest are therefore discernible in large African ungulates, even in an environment that currently is relatively benign. To what extent large African ungulates could rely on these thermoregulatory signals, and the relative contributions that each makes to water conservation, requires further investigation in a more arid region.

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CHAPTER 5

Conclusion

Some of the ideas in this chapter, related to selective brain cooling, have been published in:

Body water conservation through selective brain cooling by the carotid rete: a physiological feature for surviving climate change?

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I have investigated the water conservation strategies used by some large African antelope (i.e., artiodactyls), in an arid southern hemisphere ecosystem, a region where water is the main driver of ecosystems (Hawkins *et al.*, 2003), and a region that is expected to become hotter and drier, with increased variability in rainfall, in the face of anthropogenic climate change (Engelbrecht *et al.* 2015). More specifically, I investigated the physiological water conservation strategies that large antelope already use, and that they may rely on more in a changing environment. An improved understanding of the physiological mechanisms used intra- and inter-specifically, and insights into the capacity for, and plasticity in, its use, are urgently needed to facilitate the successful conservation management of African ungulates, which already are in decline across much of Africa (Ripple *et al.* 2015). To achieve these aims, with assistance from my colleagues, I implanted biologging devices to investigate the water conservation mechanisms in ungulates by quantifying the volume of water conserved as a result of selective brain cooling in a model ungulate (Chapter 2), by comparing selective brain cooling in sympatric, large free-living ungulates with varying water dependencies (Chapter 3), and by investigating heterothermy and microclimate use in sympatric, large free-living ungulates with varying water dependencies (Chapter 4).

5.1 Selective brain cooling

Selective brain cooling is enhanced by water deprivation (Jessen *et al.*, 1998, Fuller *et al.*, 2007, Strauss *et al.*, 2015). When Dorper sheep (Ovis aries) were deprived of drinking water and exposed to diurnal heat load, after the third day of water deprivation, when the water reservoir within the rumen would have been depleted, plasma osmolality and selective brain cooling magnitude increased in a near-linear manner and in unison (Fig. 1A; Strauss *et al.*, 2015). After 5 days of water deprivation, the 24 h mean magnitude of selective brain cooling was three times greater (0.9 vs. 0.3°C) than that during euhydration (Fig. 1B). The proportion of time during which the sheep used selective brain cooling also differed markedly depending on hydration status. Figure 1C shows that the sheep used some selective brain cooling at all times of the day regardless of hydration status. However, when dehydrated, the sheep used selective brain cooling more frequently at all times of day, but especially during the daylight hours when selective brain cooling, on average, was used 75% of the time. Water deprivation, presumably acting through hyperosmolality, therefore appears to be one driver of selective brain cooling (McKinley *et al.*, 2008).

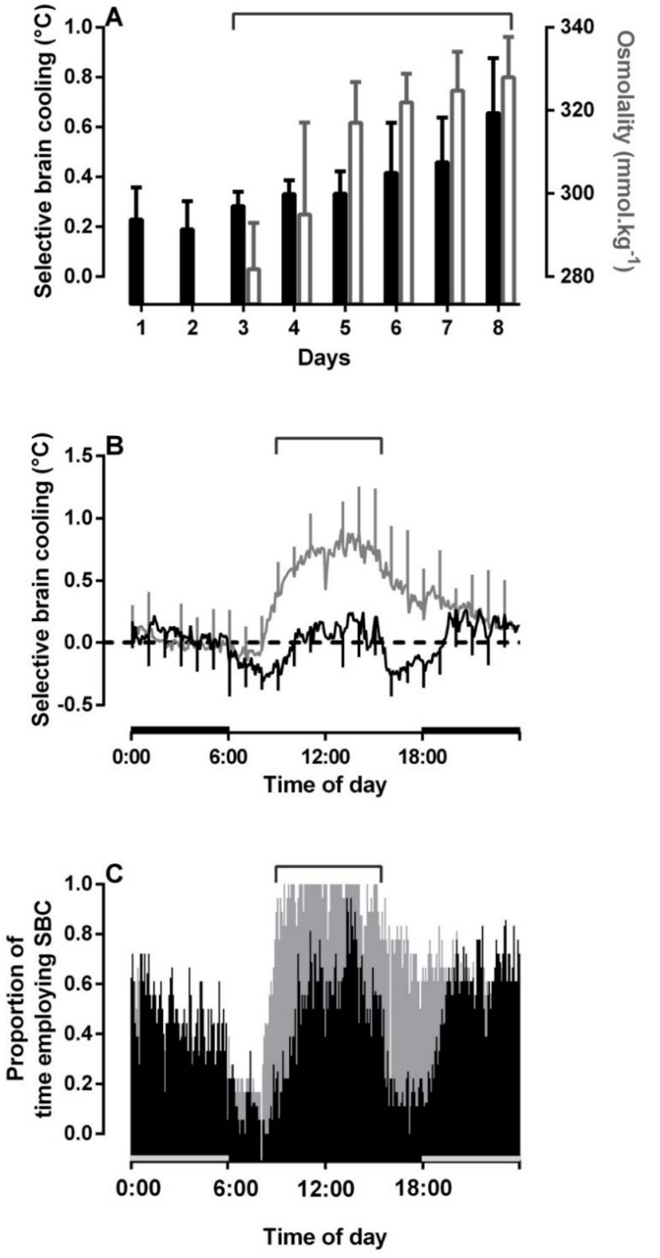

Figure 1: Summary of the effects of water deprivation on selective brain cooling in Dorper sheep. (A) The relationship between the mean (±SD) magnitude of selective brain cooling (carotid blood temperature minus hypothalamic temperature, black bars) and osmolality (grey bars) during 5 days of water deprivation; the black bracket indicates the period of water deprivation. (B) The mean (±SD) 24 h magnitude of selective brain cooling during euhydration (black line, days 1 and 2 in A) and dehydration (grey line, days 7 and 8 in A); horizontal black bars along the x-axis depict night-time. (C) The proportion of time that the sheep spent using selective brain cooling throughout the 24 h period when euhydrated (black bars; days 1 and 2 in A) and when dehydrated (grey bars; days 7 and 8 in A); the black bracket indicates the period of heat exposure (09.00–15.00 h) and the horizontal black bars along the x-axis depict night-time. Data from Strauss *et al.* (2015).

5.1.1 Selective brain cooling conserves body water

In dehydrated mammals, increased osmotic pressure of the arterial blood perfusing the brain inhibits thermoregulatory responses to heat (McKinley *et al.*, 2008), including evaporative heat loss (Doris, 1983). If hyperosmolality also enhances selective brain cooling, then what would be the benefit to artiodactyls? In goats, at least, but presumably also in other artiodactyls, the neural drive for respiratory evaporative heat loss is provided about equally by thermoreceptors in the hypothalamus and thermoreceptors within the trunk (Jessen and Feistkorn, 1984). During selective brain cooling, input from the hypothalamic temperature sensors would be attenuated. Consequently, panting and sweating, the main physiological avenues of evaporative heat loss in artiodactyls, would be attenuated. The potential water savings as a result of selective brain cooling could be substantial considering that a 1°C decrease in brain temperature resulted in a ~6-fold decrease in respiratory evaporative water loss in goats (Kuhnen and Jessen, 1994). The hypothalamic thermosensitivity for evaporative water loss in goats is therefore high.

Based on data provided by Jessen and Feistkorn (1984), the hypothalamic thermosensitivity for evaporative water loss for goats, at a trunk temperature of 40°C, was 0.35 W kg^{-1} $°C^{-1}$, or 14 g H_2O min^{-1} kg^{-1} $°C^{-1}$. In contrast, for a species lacking a rete, the rabbit, the hypothalamic thermosensitivity for evaporative water loss, calculated at an ambient temperature of 39°C, was 0.038 W kg^{-1} $°C^{-1}$, or 1.5 g H_2O min^{-1} kg^{-1} $°C^{-1}$ (Stitt, 1976). The hypothalamic thermosensitivity therefore was10 times greater in the heat-exposed goat than in the heat-exposed rabbit, a difference that I attribute to the presence of the rete in goats. A relatively small change in the hypothalamic temperature of the goat (tenths of a degree Celsius) can therefore result in significant water savings as a result of decreased evaporative water loss. In an elegant experiment on goats, Kuhnen (1997) used extracorporeal heat exchangers to manipulate selective brain cooling and measured respiratory evaporative heat loss. The experimental inhibition of selective brain cooling resulted in a reduced trunk threshold for respiratory evaporative heat loss; therefore, evaporative water loss occurred at lower body temperatures, as well as at a higher overall rate. At an aortic blood temperature of 40°C, selective brain cooling of merely 0.5°C reduced respiratory water loss by 0.72 l day^{-1}, the equivalent of 35% of the average daily water requirement of the goats (Kuhnen, 1997). Sweating is largely driven by thermal receptors in the hypothalamus (Smiles *et al.*, 1976); therefore, selective brain cooling also will reduce water loss by sweating (Strauss *et al.*, 2015), further contributing to the water savings of a mammal that uses either form of evaporative heat loss.

Recently, in the laboratory, I quantified the total water savings attributable to selective brain cooling in Dorper sheep. I used the stable hydrogen isotope deuterium oxide (D_2O) to measure water turnover in sheep that were deprived of drinking water and naturally making use of selective brain cooling. The sheep lost a quarter of their body water over 5 days of water deprivation. The threshold temperature for selective brain cooling remained unchanged, but those individuals that used selective brain cooling more frequently, or of greater magnitude, had lower water turnover rates (they therefore conserved body water better) than did conspecifics that used selective brain cooling less frequently or of smaller magnitude. I showed that a 50 kg sheep that used selective brain cooling for half of a day would save 2.4 litres of water that day, the equivalent of ~60% of the daily water requirement of a Dorper sheep not exposed to heat (Strauss *et al.*, 2015). Thus, the reduction of hypothalamic temperature by the 1°C or less that selective brain cooling can achieve reduces evaporative cooling during heat exposure sufficiently to save a substantial portion of the water that an artiodactyl would need to access each day.

How does an artiodactyl continue to maintain heat balance if its evaporative cooling is attenuated? The attenuation of evaporative cooling by selective brain cooling results in an increase in body temperature, including skin temperature (Caputa *et al.*, 1986b, Laburn *et al.*, 1988). In environments in which an artiodactyl can lose heat by radiation and convection, the higher skin temperature will enhance radiant and convective heat loss, so selective brain cooling will switch heat loss from evaporative to non-evaporative channels. If an artiodactyl is in an environment in which it is gaining heat, the rate of heat gain will be reduced by the higher skin temperature. In the process of conserving water, however, the artiodactyl may store heat during hot periods of the day. As selective brain cooling generally is used late in the afternoon (Fig.

2, left panels), the heat that is gained as a result of the suppression of evaporative cooling may be dissipated non-evaporatively during the night.

5.1.2 Selective brain cooling as a physiological feature for surviving hotter and drier environments

Water economy strategies in artiodactyls provide compelling evidence that selective brain cooling mitigate negative population responses to warming and aridification. The use of selective brain cooling offers significant water savings to artiodactyls, but it is not the only mechanism by which they can save water. Dehydration itself reduces evaporative water loss via osmosensitive neurons in the hypothalamus, even in the absence of selective brain cooling (Baker and Doris, 1982).

However, unlike dehydration or blood osmolality, selective brain cooling can be switched off rapidly (probably within seconds) by high cranial sympathetic activity. That property of selective brain cooling potentially conveys a survival benefit (Mitchell *et al.*, 2002). For example, should an artiodactyl in a hot environment and implementing selective brain cooling be confronted by a flight-or-fight situation, its selective brain cooling would be abolished immediately by increased sympathetic tone. Consequently, hypothalamic temperature would increase, and the hypothalamic drive on evaporative cooling would be restored immediately, with the full power of evaporative cooling invoked to dissipate the extra metabolic heat. Immediate survival outweighs the longer-term benefits of body water conservation, and the artiodactyl makes temporary use of full evaporative cooling to avoid a potentially lethal hyperthermia. When the threat has receded, selective brain cooling can again be initiated and evaporative water loss suppressed. Thus, selective brain cooling may bestow benefits for survival in arid environments in two ways: (i) switching selective brain cooling on conserves body water, improving long-term survival; whereas (ii) switching selective brain cooling off rapidly accelerates evaporative cooling to avoid lethal hyperthermia, thereby supporting immediate survival.

The ability to modulate evaporative water loss through the use of selective brain cooling may have contributed to the evolutionary success of artiodactyls (Mitchell and Lust, 2008). It can be assumed that the carotid rete, and therefore selective brain cooling, evolved concomitantly with the emergence of the modern artiodactyl orders, at least 45 million years ago (Janis, 2009). When the diversity trends of Artiodactyla (presumably all rete bearing) and their sister clade Perissodactyla (presumably not rete bearing) are compared across the Cenozoic, discrepancies in generic richness are established in three pulses, each of which corresponds to a trend of aridification, whether warming or cooling (Fig. 2). In the earlier half of the Cenozoic, global climate is best described as a ‘tropical hothouse’ (Buchardt, 1978, Wolfe, 1978, Wing, 1987, Huber and Sloan, 2001, Bowen and Zachos, 2010, Galeotti *et al.*, 2010). During this period of abundant moisture, artiodactyls and perissodactyls enjoyed similar generic richness (Fig. 2). However, a pronounced period of cooling and drying across the Eocene to Oligocene transition began to shift this relationship (Diester-Haass and Zahn, 1996, Lear *et al.*, 2008). Although many large-bodied mammals faced extinction throughout this period (Prothero, 1985, Hooker, 1992, Legendre and Hartenberger, 1992), artiodactyls survived better both during and after this event. Throughout the drier, temperate Oligocene (Kennett, 1985, Ehrmann and Mackensen, 1992), artiodactyl generic richness remained elevated relative to perissodactyls (Fig. 2). Across the Late Oligocene to Early Miocene transition, another period of aridification swept the globe, this time accompanied by warming (Kennett, 1985, Retallack, 2013). This period of warming and aridification saw a pulse in generic richness of artiodactyls capable of selective brain cooling, immediately before the Mid-Miocene expansive radiation of C_4 grasslands. It is often the expansion of grasslands and the artiodactyl rumen that have been associated and promoted as the driver of artiodactyl success (Retallack, 1991, Jacobs *et al.*, 1999, Retallack, 2001, Retallack, 2007a, Retallack, 2007b, Retallack, 2013), but, as Figure 2 shows, the artiodactyls proliferated before the grasslands expanded.

The C_4 grasslands, which are now dominant in the tropics, are characterised by a photosynthetic pathway different to that of the more common, ancestral C_3 photosynthetic pathway (Edwards *et al.*, 2010). In many terrestrial plants, the C_4 photosynthetic pathway, which evolved under conditions of depleted CO_2, has resulted in reduced efficiency and rate of carbon uptake, especially under hot and arid conditions (Sage 2004). The C_4 grasslands therefore represent diet of a lower quality than the C_3 grasses, as they have a higher fibre content and lower concentrations of nitrogen than C_3 grasses, and because leaf anatomy makes nutrients less accessible in the C_4 grasses than in the C_3 grasses (Caswell *et al.*, 1973, Ehleringer and Monson 1993, Heckathorn *et al.* 1999).

Although the late Cenozoic (Mid-Miocene to present) diversification patterns of artiodactyls generally have been attributed to the ruminant digestive physiology of bovids (Janis *et al.*, 1998, Clauss *et al.*, 2003, Janis, 2007, Janis, 2009, Clauss and Rössner, 2014, O'Brien, 2016), as well as their ability to survive on smaller amounts of grass selectively chosen (Janis, 2008), this highly specialized mode of digestion is variably present and cannot solely be responsible for earlier Cenozoic survivorship and diversification patterns. Indeed, it is likely that the influence of selective brain cooling insulated artiodactyls from extensive periods of aridification, whether warming or cooling, that saw declines among ungulates that do not use selective brain cooling (O'Brien, 2016). This pattern of selective-brain-cooling-related survivorship is corroborated by evolutionary patterns of the Tragulidae.

Although they are small-bodied artiodactyls with a rudimentary capacity for rumination (Clauss and Rössner, 2014), tragulids do not possess a carotid rete (Fukuta *et al.*, 2007). Therefore, they can be considered to ruminate but not to use selective brain cooling. The earliest record of tragulids and other closely related small-bodied primitive ruminants is the Late Eocene to Early Oligocene (Webb and Taylor, 1980), when swamps and temperate forests dominated much of Europe and North America (Mai, 1989, Pross *et al.*, 2001, Retallack, 2007a, Retallack, 2007b, Kunzmann, 2012, Retallack, 2013). Since that period, these taxa without selective brain-cooling have experienced declines in generic richness even as more advanced ruminants have increased in diversity (Clauss and Rössner, 2014). Thus, although diet undoubtedly plays a role in the extinction and survivorship capacity of any taxon, failure to incorporate selective brain cooling not only leaves early artiodactyl evolution unsatisfactorily explained, but it renders an incomplete evaluation of these species' abilities to persist into the Anthropocene.

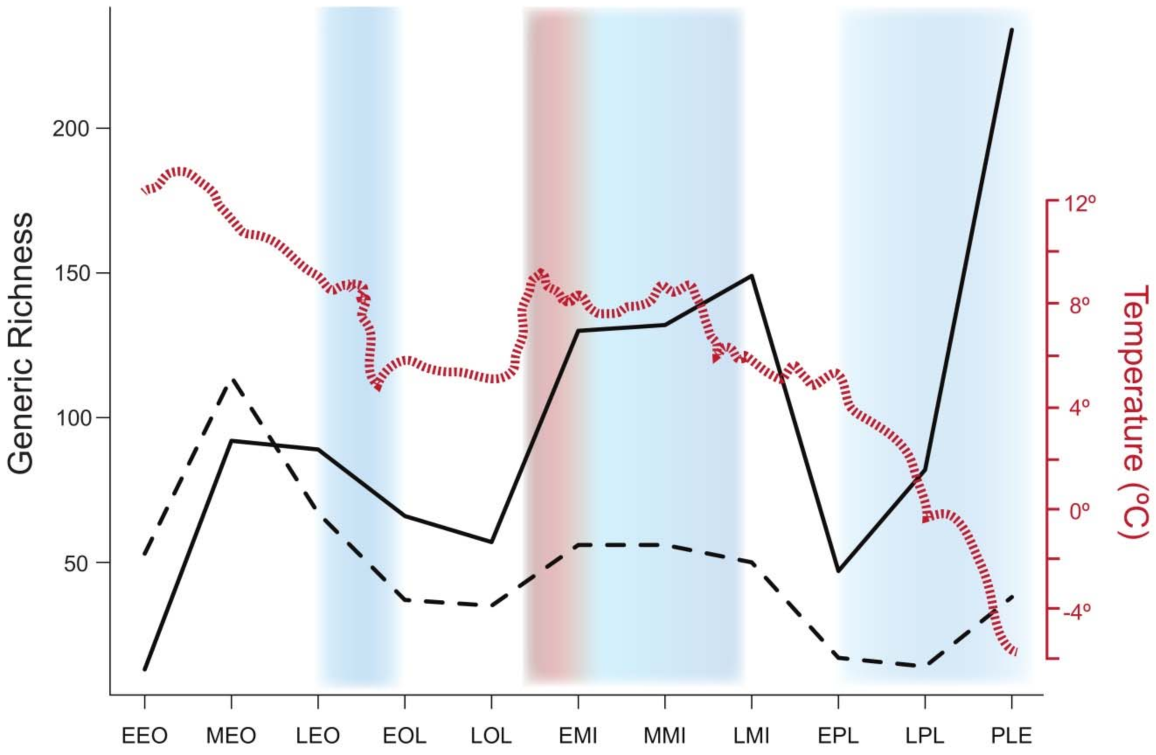

Figure 2: Diversity curve for the artiodactyls (continuous line) and perissodactyls (dashed line) from the early Eocene (EEO) to the Palaeocene (PLE) relative to global temperature (red line; Zachos *et al.*, 2001) and an indication of the relative hydrological regimes (Janis, 2008); blue and red shading represent aridification combined with cooling and warming temperatures, respectively. Occurrence data were downloaded from the Fossilworks/Paleobiology Database in August 2016. The epochs along the x-axis are as follows: EEO, Early Eocene; MEO, Mid-Eocene; LEO, Late Eocene; EOL, Early Oligocene; LOL, Late Oligocene; EMI, Early Miocene; MMI, Mid-Miocene; LMI, Late Miocene; EPL, Early Pleistocene; LPL, Late Pleistocene; and PLE, Paleocene.

Among artiodactyl species, there may be innate differences between species in their capacity for selective brain cooling, which may facilitate the persistence of those species with enhanced selective brain cooling in a hotter and drier Anthropocene. To date it has not been feasible to measure the water savings that result from selective brain cooling in free-living artiodactyls, but I recently investigated selective brain cooling in three sympatric antelope species with different water dependencies and determined the dimensions of their carotid retes (Strauss *et al.*, 2016). Individuals of all three species, living free in their natural habitats in the same environmental conditions, used selective brain cooling. However, I found no differences in selective brain cooling use between the gemsbok, independent of surface water, the red hartebeest of intermediate water dependency or the blue wildebeest that is dependent on surface water and has to drink water daily. In fact, I found more variability in selective brain cooling use within those species than between those species in a habitat in which surface water was available ad libitum. Should this pattern of variability hold true in a more arid region, the result implies that with progressive aridification (as predicted with climate change), individuals in each species will be favoured rather than one species being favoured over another.

Although earlier descriptions of carotid rete morphology documented variability in the vascularization of the rete between species (Daniel *et al.*, 1953, Carlton and McKean, 1977), I found little quantifiable difference in the rete volume of those three artiodactyls with varying ecological water requirements (Strauss *et al.*, 2016). The observed variability in use of selective brain cooling within these three species living in exactly the same environmental conditions reaffirms the concept that selective brain cooling is not simply under thermal control.

5.2 Body temperature

Because selective brain cooling reduces heat loss drive by inhibiting evaporative water loss through panting and sweating (Jessen *et al.*, 1994, Kuhnen, 1997, Strauss *et al.*, 2015) it should, in theory, contribute to heterothermy as it would result in the elevation of body temperatures. In chapter 2 I showed that heat-exposed sheep conserved body water, at the expense of hyperthermia, when they switched from evaporative cooling to non-evaporative cooling (i.e., selective brain cooling). The elevation of maximum body temperatures, first described in captive dromedary camels (Schmidt-Nielsen *et al.*, 1956), eland and beisa oryx (Taylor, 1969), was originally termed adaptive heterothermy and identified as a water conservation strategy vital to large, arid-adapted mammals. More recently, however, it has been questioned whether heterothermy in large mammals is adaptive or whether it is an indication that animals are unable to cope with their circumstances. When water deprived, mammals tend to reduce evaporative water loss (Taylor, 1970b, Finch and Robertshaw, 1979, Silanikove, 1994), thereby conserving body water. However, when water deprivation coincides with heat exposure, the reduced evaporative water loss often leads to an elevation in the maximum body temperatures (Finch and Robertshaw, 1979, Jessen *et al.*, 1998), leading to the risk of hyperthermia.

A recent analysis, by Hetem and colleagues, of body temperature data from 17 ungulate species showed that these large mammals were able to maintain their body temperatures independent of ambient temperature, provided they had access to sufficient food and water (Hetem *et al.* 2016). In their comprehensive review on heterothermy and its drivers, the authors discounted the older arbitrary limits previously used to define heterothermy – e.g., an increase in amplitude of 2°C or more despite larger fluctuations in ambient temperature (Bligh and Johnson, 1973), or, in the case of small torpid mammals, a decrease in body temperature of 5°C or more below that of normal resting temperature (Cooper and Geiser, 2008). Instead they considered heterothermy simply as perturbations from the underlying 24h body temperature rhythm when animals that are not heat stressed have access to sufficient food and water (Hetem *et al.* 2016).

Many mammals and birds show a 24h body temperature rhythm; a so-called nychthemeral rhythm, when influenced by environmental signals (Hetem *et al.* 2016). My sheep, housed in a climatic chamber, displayed a 24h rhythm in body core temperature (Chapter 2), as did the free-living gemsbok, red hartebeest and blue wildebeest (Chapter 3).The free-living ungulates also showed a nychthemeral rhythm in subcutaneous temperature (chapter 4), despite subcutaneous temperatures being more influenced by environmental conditions than was core body temperature. As shown in Chapter 4, the 24h nycthemeral rhythm of subcutaneous temperature therefore differs substantially from that of core body temperature. Moreover, the simultaneous measurement of subcutaneous and core body temperature in captive goats *Capra hircus* revealed a discrepancy of up to 3.5°C between measurement sites (Torrao *et al.*, 2011), similar to that reported in three free-living ungulate species (chapter 3).

In chapter 4 I used the difference between the abdominal and the subcutaneous temperatures as an index of peripheral blood flow throughout the day. A similar rhythm in vasoconstriction/dilation was found in all three species. The observed early morning vasoconstriction, indicates that the animals were defending their body temperatures, more so closer to the winter solstice when they were nutritionally stressed, by decreasing peripheral blood flow and therefore the flow of heat from the core to the periphery. At about sunrise there was a pre-emptive diurnal increase in peripheral blood flow, transporting heat from the core to the periphery before body core temperature started to rise, thereby attenuating the rise in core temperature.

In chapter 4 I also showed that the body temperatures of the gemsbok, red hartebeest and blue wildebeest were dependent on ambient conditions, suggesting, according to Hetem *et al.* (2016), an inadequate supply of food or water, or a combination of both. Seasonal food shortages have previously been implicated in reduced metabolic rates in desert ungulates (Williams *et al.*, 2001, Ostrowski *et al.*, 2006a, Ostrowski *et al.*, 2006b) and, while animals could try and compensate for a reduced metabolic rate, through for example behavioural changes, body temperature will decrease if the attempted compensation is insufficient (Hetem *et al.* 2016). I attribute the heterothermy observed in the gemsbok, red hartebeest and blue wildebeest to a combination of starvation-induced hypothermia and dehydration-induced hyperthermia at the end of the dry season.

Although they were heterothermic seasonally (chapter 4), there is no indication that the gemsbok, red hartebeest or blue wildebeest were compromised. The body temperature that constitutes a pathological hyperthermia is not known for the ungulates, but, at least in humans, that would be around 45°C (Kenney *et al.*, 2004), substantially higher than the maximum body temperatures recorded in the three ungulate species on Rooipoort Nature Reserve. Nevertheless, the predicted concomitant increase in ambient temperature and reduced, more variable, rainfall are likely to result in fewer food resources, which, in turn, are expected to make the animals more susceptible to the increased ambient temperatures. All three species are therefore expected to become more heterothermic under more extreme conditions. The degree of heterothermy that the animals would be able to withstand is not known. The animals may, however, use behaviour to buffer the expected changes.

5.3 Microclimate use

Climates are changing globally and considerable effort has gone into predicting how the climates of various world-regions will change and also how ecosystems are likely to be affected. Large parts of southern Africa, for example, are predicted to become hotter and drier, with increased variability in rainfall and, only recently, has it been realised that the region is warming at a rate faster than the global average (Engelbrecht *et al.* 2015). Bioclimatic envelope model predictions are commonly used to model the future suitability of habitat in the face of climate change. However, the usefulness of such correlative models has been questioned as they do not incorporate factors such as dispersal, adaptation or behavioural plasticity (Pearson and Dawson, 2003). Moreover, a recent meta-analysis highlighted the degree of spatial mismatch between the sizes of organisms and the scale at which climate is modelled, with the grid-length in species distribution models 10 000 fold larger on average than the animals they study (Potter *et al.*, 2013). The biological relevance of bioclimatic envelope model predictions also has been questioned, as animals make use of microclimates and not broad-scale climates (Varner and Dearing, 2014).

At the smaller terrestrial landscape scale there is considerable variation in temperature, and thermal environments at a fine scale influence the daily activity patterns and the thermoregulatory behaviour of organisms (Angilletta, 2009). Recently, Carroll and colleagues, using black globes similar to those used in chapter 4, concluded that thermal landscapes, and the occurrence of thermal extremes in the landscape, are subject to high temporal variability, with thermal conditions spatially homogenous at night and increasingly variable during the day, until about 14:00 when thermal heterogeneity peaks (Carroll *et al.*, 2016). The observed thermal homogeneity at night implies that an animal is likely to experience the same thermal conditions regardless of the habitat patch it chooses at night. However, Carroll *et al.* (2016) did not measure microclimate use by animals in conjunction with their characterisation of microclimates. In chapter 4 I showed that the gemsbok, red hartebeest and blue wildebeest occupied warmer microclimates when the reference miniglobe temperature was low. By laying in the warmer air boundary layer close to the ground, sheltering from radiant heat loss at night (Mount, 1979), and by avoiding wind, my study animals therefore occupied warmer microclimates than those that they would have experienced in the open.

Sheltering is a common thermoregulatory strategy, also among small mammals. Most small mammals make use of burrows, which buffer them against temperature extremes, with daytime burrow temperatures commonly about 10°C cooler than air temperature (Stark, 1963, Johnson, 1970, Taylor and Skinner, 2004, Wilson *et al.*, 2010). While few large mammals can access underground thermal refuges, they do adjust the timing of their activity and seek appropriate microclimates, such as shade during the heat of the day (Kinahan *et al.*, 2007, Hetem *et al.*, 2012b).

In chapter 4 I showed that three large ungulates with varying water dependencies differed in their use of microclimates, with the gemsbok and red hartebeest consistently using microclimates cooler than in full sun at lower threshold temperatures, than did the water dependent blue wildebeest. I also showed that all three species had lower heatload threshold temperatures during the 10 hottest days of the study period, and made use of better quality shade, compared to the 10 coolest days. Despite having access to water to facilitate evaporative cooling (panting and sweating) my study animals, therefore, also used thermoregulatory behaviour. The management of environmental heat loads, for example, through shade seeking, likely to be the first response for ungulates in environments predicted to become both hotter and drier, will increase in importance but would impede feeding activity. In the hyper-arid Arabian desert, the Arabian oryx compensated for increased shade use during the hot dry season by becoming largely nocturnal in an area devoid of natural predators (Hetem *et al.*, 2012b). In African ecosystems animals may not be able to shift their activity and become more nocturnal because of the prevalence of predators in these systems. A recent camera trap study in South Africa’s Addo Elephant National Park revealed that buffalo *Syncerus caffer*, kudu and warthog *Phacochoerus aethiopicus* are more active diurnally in the presence of nocturnal predators, than in the absence of such predators (Tambling *et al.*, 2015). The ungulates in the Addo National Park, therefore reduced their activity overlap with the nocturnal predators, suggesting a higher susceptibility to predation at night. Biotic and abiotic factors influence habitat selection and new relationships, including biological costs, are likely to emerge as species respond to climate change (Martin, 2001).

Simultaneous, long-term conservation physiology studies are therefore needed in the same thermal landscape on both ungulates and their predators, to ascertain the extent of diurnal and nocturnal overlap in microclimate use currently, and in the face of climate change. Such studies should not only focus on the characterisation of microclimates at scales suitable to both the temporal and spatial heterogeneity of the habitat and the range use patterns of the species of interest, but should also include the simultaneous quantification of the microclimates being used by the species of interest. The shade provided by trees is especially important, for large mammals, in maximising thermal buffering across landscapes (Carroll *et al.* 2016) yet, in African disturbance-dependent ecosystems, tree cover is reduced through disturbance by both fire and herbivores (Midgley and Bond, 2015). Predictions related to the future of African ecosystems vary greatly (Midgley and Bond, 2015) reinforcing the need for long-term monitoring of species and their microclimates. The effects of climate change on local communities are, however, likely to exacerbate landscape degradation and habitat transformation, including the loss of trees (IPCC, 2013). Homogenisation of the landscape following the loss of trees will result in a shift in landscape thermal patterns to be more homogenous (Carroll *et al.* 2016). The expected loss of thermal heterogeneity is likely to result in trade-offs between the thermal environment, foraging and predation risk.

5.4 Perspectives and significance

In my thesis I have investigated water conservation strategies of large African ungulates, with a particular focus on selective brain cooling, as well as heterothermy and microclimate use. I have shown that selective brain cooling does indeed conserve body water in a model ungulate naturally making use of selective brain cooling and that selective brain cooling did not differ between three antelope species with varying water dependency when water was freely available in their habitat. As I could not challenge the animals in terms of their selective brain cooling use it is not clear whether the similar magnitudes of selective brain cooling, regardless of species or water dependency, reflects a similar underlying ability for use of this water conservation mechanism among artiodactyls. Nevertheless, the plasticity in the use of selective brain cooling may provide a physiological feature for selection in the face of anthropogenic climate change, as numerous regions are expected to become hotter and drier, with increased variability in rainfall (Niang *et al.*, 2014). Large mammals will be unable to adapt genetically given the rapid rate of climate change and will be unlikely to be able to move to new, suitable habitats, leaving them dependent on phenotypic plasticity if they are to counter such climate change (Hetem *et al.*, 2014). Having survived and diversified dramatically during periods of pronounced aridification, the artiodactyls may be insulated, to a degree, from global warming and drying (Mitchell and Lust, 2008). Moreover, the observed inter-individual variability in selective brain cooling use within species implies that individual artiodactyls within populations, regardless of species or ecological water dependency, might have a relative evolutionary advantage in hotter, drier, less predictable environments. Representatives of the characteristic large herds of artiodactyls across various landscapes, which often also form the mainstay of large tracts of land under conservation management, therefore could persist through the Anthropocene.

However, considering the continuing decrease in antelope populations across the globe (Ripple *et al.*, 2015) and the poor performance of artiodactyls during mid-latitude glaciation of the Miocene to Pliocene transition, persistence through the Anthropocene may partly depend on the degree of intra-specific selective brain cooling plasticity and resilience generally. Indeed, artiodactyls such as the Arabian oryx may already be living close to their physiological tolerance levels. With a 24h body temperature range (maximum—minimum 24 h body temperature) of up to 7.7°C during the summer, these animals are seasonally losing control of homeothermy as a result of water and nutritional stress (Hetem *et al.*, 2012b, Hetem *et al.*, 2016). As a consequence of a paucity of similar data from other artiodactyls, and also from perissodactyls, we know little about the physiological performance of these mammals in current environmental conditions and, ultimately, their ability to cope with a changing climate. While I expect the first response to a warming and drying environment to be one of behavioural adaptation, particularly increased shade use, we do not know to what extent ungulates would be able to compensate for reduced feeding time by changing to nocturnal activity patterns. Moreover, we do not know the magnitude of heterothermy that free-living African ungulates would be able to withstand, or indeed, whether that differs between species.

It seems likely that African ungulates would need to rely on an array of water conservation strategies if they are to survive climate change. As perissodactyls, many of which are evolutionarily distinct (Zoological Society of London, 2014), cannot use selective brain cooling to conserve body water, concerted conservation efforts may be required as conditions become drier under anthropogenic climate change. Yet such conservation efforts should not be to the detriment of other species sensitive to disturbance (see, for example, Harrington *et al.*, 1999). Survival of those predators depredating artiodactyls and perissodactyls may also depend on the predators' capacity for selective brain cooling, as selective brain cooling has been observed in heat-stressed domestic cats (Baker and Doris, 1982) and dogs (Baker, 1984). The degree to which free-living felids and canids use selective brain cooling is currently not known. We also do not know, therefore, whether the evolutionary success of carnivores can be attributed to the development of their carotid rete, selective brain cooling and the conservation of body water. A better understanding of selective brain cooling, which provides some mammals with flexibility in terms of water conservation, is integral to efforts to predict how mammals will respond to changing environments and how best to conserve them.

The studies that are required are long-term investigations undertaken on a range of artiodactyl and carnivore species (Fuller *et al.*, 2014, Hetem *et al.*, 2014). Such studies should focus on identifiable individuals and their progeny, free-living in their natural environment, with the aim of relating varying levels of evolutionary success to the observed flexibility in microclimate selection, body temperature regulation and selective brain cooling and resulting water savings. Measuring water turnover in large, free-living mammals remains a challenge though. In chapter 2 I was able to quantify the amount of water that a heat-exposed, water-deprived Dorper sheep conserves as a result of selective brain cooling. The use of the stable hydrogen isotope, deuterium oxide (D_2O), and the associated sampling intervals were feasible in a laboratory setting where relatively small, habituated animals could readily be captured and handled for the collection of blood samples. Considering the cost (of doubly labelled water or indeed deuterium oxide) and the sampling requirements, which are particularly impractical when working with large, free-ranging mammals, it becomes clear why water-turnover has been measured only in one free-living African antelope, the springbok (Nagy and Knight, 1994). If we are to tease out the relative importance of the various water conservation strategies used by African ungulates we need to develop an affordable, logistically feasible technique to measure water turnover in large ungulates. A next step therefore would be the development of a non-invasive technique to gain insights into the water turnover of large ungulates.

A possible non-invasive technique to measure water turnover would require weighing individually identifiable animals (equipped with bio-loggers to measure body, brain, subcutaneous and microclimate temperatures) individually on their way to and from water. By controlling access to water through a series of access-controlled points, each equipped with a scale that individual animals have to walk over, and a passive integrated transponder (PIT tag) reader to identify the individual animals (fitted with a PIT tag), one could gain insight into not only the drinking frequency of specific individuals, but also the volume of water consumed by each individual. Once water turnover can feasibly be measured, and with biologgers in place, we would be much closer to attributing actual water savings to specific water conservation strategies such as shade seeking, heterothermy, or selective brain cooling in free-living ungulates.

In chapter 3 I investigated selective brain cooling in three sympatric artiodactyl species with varying water dependency in an environment where water was available. It would be most insightful to repeat that study, also including a measure of metabolic rate (e.g. heart rate monitors), in a more arid area where the artiodactyls would not have access to surface water. Only by challenging the animals physiologically will we be able to discern whether species with varying water dependency have different selective brain cooling capabilities and to what extent they may rely on those abilities.

The African antelope can also be challenged physiologically by studying them in one of the harshest environments available – the Arabian Desert. As there are large numbers of African antelope, semi free-living in extensive animal collections in the Middle East, the comparative water conservation strategies – selective brain cooling, heterothermy and microclimate use - of African and Arabian antelope could be investigated in the same hyper-arid environment. Such a comparative study where animals simultaneously inhabit the same environment would provide important insights into the extent that African antelopes could adapt to a warming and drying environment.

There are a number of other questions related to selective brain cooling that remains unanswered and could be investigated further. These include selective brain cooling in cats, such as the lion *Panthera leo* free-living in their natural environment. The circumstances under which these animals use selective brain cooling is not known at present, while the function remains unclear as prey items are a main source of water to these carnivores. Moreover there also is a pressing need to further investigate the relationship between rete dimensions, incorporating modern anatomical procedures e.g., three- dimensional scanning and printing, and selective brain cooling use. For example, do similar sized species showing variable development in the carotid rete (e.g. warthog vs. blesbok) have similar selective brain cooling abilities?

In chapter 4 I investigated the microclimate and body temperature profiles of free-living African ungulates. To get a better idea of the spatial and temporal availability of microclimates and how animals are making use of these, future studies should deploy multiple arrays of reference globes throughout the landscape. These reference globe arrays need to sample, at a scale suitable to the animals being studied, microhabitats across the entire range used by the animals of interest. In conjunction with the collar mounted miniglobes on the study animals, more detailed insight would thus be gained into microclimate use relative to availability, compared to the sun/shade (or open/shelter) comparison in chapter 4. Also in chapter 4, I attributed the elevation in maximum body temperature, and, to some extent, also the suppression of minimum body temperature to the translocated animals being naïve about resource distribution (water and food, respectively). Translocation of animals for reintroduction purposes is an important conservation management tool throughout the world. In this way, organisms, either captive-bred or sourced from other free-living populations, are reintroduced back into areas that they previously occupied, but from which they have become extirpated (Seddon *et al.*, 2012). Monitoring of reintroduced animals typically revolves around ecological parameters such as range use and breeding success (e.g. Stanley-Price, 1989), with time to first offspring or time to first offspring reproducing used as measures of short-term success. There is, however, a need for a mechanistic approach to understanding why reintroductions fail (Tarszisz *et al.*, 2014). Incorporating the measurement of body temperature into reintroductions, could provide valuable insights with regards to animal performance and stress (see Meyer *et al.*, 2008) following release into an area, especially if those measures are available in real time (chapter 2). Such data also would help to quantify how reintroduced animals respond to specific reintroduction management approaches (e.g., hard release vs. sift release), thereby contributing to improved conservation management.

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Appendix 1

Body water conservation through selective brain cooling by the carotid rete: a physiological feature for surviving climate change?

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Perspective

Body water conservation through selective brain cooling by the carotid rete: a physiological feature for surviving climate change?

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Some mammals have the ability to lower their hypothalamic temperature below that of carotid arterial blood temperature, a process termed selective brain cooling. Although the requisite anatomical structure that facilitates this physiological process, the carotid rete, is present in members of the Cetartiodactyla, Felidae and Canidae, the carotid rete is particularly well developed in the artiodactyls, e.g. antelopes, cattle, sheep and goats. First described in the domestic cat, the seemingly obvious function initially attributed to selective brain cooling was that of protecting the brain from thermal damage. However, hyperthermia is not a prerequisite for selective brain cooling, and selective brain cooling can be exhibited at all times of the day, even when carotid arterial blood temperature is relatively low. More recently, it has been shown that selective brain cooling functions primarily as a water-conservation mechanism, allowing artiodactyls to save more than half of their daily water requirements. Here, we argue that the evolutionary success of the artiodactyls may, in part, be attributed to the evolution of the carotid rete and the resulting ability to conserve body water during past environmental conditions, and we suggest that this group of mammals may therefore have a selective advantage in the hotter and drier conditions associated with current anthropogenic climate change. A better understanding of how selective brain cooling provides physiological plasticity to mammals in changing environments will improve our ability to predict their responses and to implement appropriate conservation measures.

Key words: Artiodactyl success, brain temperature, carotid arterial blood temperature, osmoregulation, physiological plasticity, rostral epidural rete mirabile

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Introduction

The carotid rete, or rostral epidural rete mirabile, is an intracranial vascular structure, near-ubiquitous and often elaborate in the Ruminantiamorpha, Whippomorpha, Camelidamorpha and Suinamorpha, collectively known as the Cetartiodactyla (Fig. 1; nomenclature *sensu* Spaulding *et al.* (2009)). The carotid rete is present also, often in a rudimentary or primitive form, in a number of laurasiatherian mammals (Ask-Upmark, 1935; du Boulay and Verity, 1973), including cats (Kamijyo and Garcia, 1975), in which it is extracranial (Daniel *et al.*, 1953), and domestic dogs (*Canis lupus familiaris*; Daniel *et al.*, 1953; Gillilan, 1976). Primates, many small-mass mammals (for example, rodents, lagomorphs and 'insectivores') and perissodactyls (horses, tapirs and rhinoceroses), a sister group of the artiodactyls (Hassanin *et al.*, 2012), have no carotid rete (Ask-Upmark, 1935; Sisson and Grossman, 1967; Gillilan, 1974). In artiodactyls (e.g. antelopes, cattle, sheep and goats), anatomical investigations (Ask-Upmark, 1935; Daniel *et al.*, 1953; Gillilan, 1974; Carlton and McKean, 1977; Frąckowiak *et al.*, 2015; Kiełtyka-Kurc *et al.*, 2015), including the identification of osteological correlates in extant and extinct artiodactyls (O'Brien, 2016), and physiological studies (Johnsen *et al.*, 1987; Mitchell *et al.*, 1997; Fuller *et al.*, 1999b; Maloney *et al.*, 2002; Lust *et al.*, 2007; Hebert *et al.*, 2008; Hetem *et al.*, 2012; Strauss *et al.*, 2016) have confirmed the presence of the rete and its functionality in virtually all of the extant terrestrial artiodactyls, with informative exceptions (Fig. 1). In the vast majority of terrestrial artiodactyls, the carotid rete is found in lieu of the internal carotid artery and serves as the main supply of oxygenated blood to the brain (Schummer *et al.*, 1981; Wible, 1984; Frąckowiak, 2006; O'Leary, 2010; O'Brien, 2015). An exception is the Tragulidae, which consist of three genera of small, forest-dwelling antelope that have retained an internal carotid artery instead of a carotid rete (Fukuta *et al.*, 2007; O'Brien, 2015). Whether their unique cranial vasculature is a plesiomorphic (*sensu* Janis, 1984) or apomorphic characteristic (e.g. Clauss and Rössner, 2014) has not been resolved. The Hippopotamidae also are artiodactyls, and a historic paper identifies hippopotamuses as having a carotid rete (Chapman, 1881), but it is indistinct (du Boulay and Verity, 1973; O'Brien, 2016). The status of the rete in Hippopotamidae requires further investigation using modern techniques, because it was identified before the anatomical techniques of resin injection and maceration, for exploring vascular systems, became available.

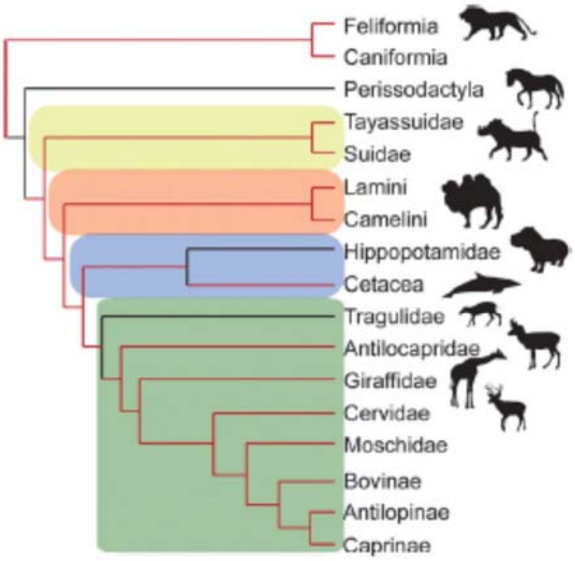

Figure 1: Phylogenetic tree indicating the relationship between the Cetartiodactyla, Perissodactyla and the Carnivora, represented by the cats and dogs (adapted from Hassanin *et al.*, 2012). Red branches indicate clades with a carotid rete, capable of selective brain cooling. Black branches designate the absence of a carotid rete (Hippopotamidae largely data deficient). Also depicted in various shades are the Ruminantiamorpha (green), Whippomorpha (blue), Camelidamorpha (orange) and Suinamorpha (yellow).

The function of the carotid rete in the Cetacea is unknown, but in the terrestrial artiodactyls, cats and dogs, it is a heat exchanger that can be used to cool the brain, but importantly, the hypothalamic region, below the temperature of carotid arterial blood. This phenomenon, known as 'selective brain cooling', first was described in a domestic cat (*Felis catus*) almost 50 years ago (Baker and Hayward, 1967). The brain, as a metabolically active organ, usually has a temperature higher than that of the arterial blood perfusing it. The heat produced by the brain is removed by that blood, meaning that in the absence of a carotid rete, the brain is –0.5°C warmer than arterial blood leaving the heart (Fuller *et al.*, 2000; Maloney *et al.*, 2009). The carotid rete, carrying arterial blood destined for the brain, is surrounded by venous blood in either a cavernous sinus (artiodactyls) or a pterygoid sinus (felids; Daniel *et al.*, 1953). The venous blood is derived from the maxilloturbinates and other mucous surfaces of the mouth and nose, where it is cooled to well below arterial blood temperature by evaporation of water into inspired air, whether or not the mammal is panting (Kuhnen and Jessen, 1991). The thin walls and large surface area of the carotid rete vessels allow for efficient heat exchange between the arterial and venous blood, the result being that the arterial blood exiting the carotid rete into the brain and, subsequently, the hypothalamic tissue in that region, can be more than 1°C cooler than arterial blood entering the rete (Maloney *et al.*, 2007).

Soon after the heat-exchange function of the carotid rete was discovered, investigations of selective brain cooling in domesticated and habituated wild mammals in captivity led to the conclusion that selective brain cooling served to protect the brain from reaching dangerously high temperatures (Baker and Hayward, 1967, 1968; Magilton and Swift, 1968; Baker, 1972, 1979; Taylor and Lyman, 1972; Baker and Chapman, 1977; Mitchell *et al.*, 1987). That conclusion was influenced heavily by one measurement of selective brain cooling. Taylor and Lyman (1972) reported that brain temperature after induced exercise in habituated Thomson's gazelle (*Gazella thomsonii*) was as much as 2.7°C lower than carotid arterial blood temperature, a magnitude of selective brain cooling never seen before or since by anyone else, in any mammal. A protectionist function fitted well with the perceived vulnerability of brain tissue to thermal damage *in vitro* (Burger and Fuhrman, 1964). Only when it was shown that goats (*Capra hircus*) could withstand brain temperatures of 42.5°C for an hour without any apparent ill effect (Caputa *et al.*, 1986b) did it become apparent that the brain was not as vulnerable to thermal damage as believed previously (Mitchell *et al.*, 2002). Indeed, rather than the brain, it is the tissue of the gastrointestinal tract that is most susceptible to thermal damage (Braasch, 1964), attributable to reduced splanchnic blood flow and endotoxin leakage (Leon and Helwig, 2010). Subsequent studies of free-living and unrestrained mammals have revealed that selective brain cooling is not obligatory at high body temperatures (Mitchell *et al.*, 2002) and, as first noted in a laboratory study on goats, it is part of the normothermic thermoregulatory repertoire of artiodactyls (Kuhnen and Jessen, 1991). Rather than functioning primarily to protect the brain from thermal damage, selective brain cooling modulates the use of body water for thermoregulation (Jessen *et al.*, 1998). By reducing the temperature of the hypothalamus, where the temperature sensors that provide the internal drive for heat loss are located, selective brain cooling reduces evaporative water loss (Kuhnen, 1997; Strauss *et al.*, 2015). In the context of this alternative role for selective brain cooling, now described in textbooks of animal physiology (for example, Willmer *et al.*, 2009; Withers *et al.*, 2016), we provide here a perspective on the evolutionary and functional significance of selective brain cooling and its potential to provide physiological plasticity for artiodactyls facing hotter and drier environments associated with current climate change.

Factors controlling selective brain cooling

Control mechanisms govern the onset and degree of selective brain cooling in mammals with a carotid rete. The finding that selective brain cooling typically is exhibited by tame or habituated mammals when exposed to heat or exercise indicated that a primary input to the control of selective brain cooling is the mammal's internal temperature (Jessen *et al.*, 1998). However, the absence of selective brain cooling in hyperthermic artiodactyls, particularly in free-living mammals during intense exercise, revealed that there also are non-thermal inputs in the control of selective brain cooling, and that these inputs can override thermal inputs (Mitchell *et al.*, 2002; Fuller *et al.*, 2014).

Thermal inputs

The typical relationship between hypothalamic temperature and pre-rete carotid arterial blood temperature in artiodactyls with carotid retes is shown in Fig. 2 (right panels). At the lower carotid arterial blood temperatures, brain temperature is higher than, and runs in parallel to, carotid arterial blood temperature, as illustrated for one gemsbok (*Oryx gazella*, upper panel), one red hartebeest (*Alcelaphus buselaphus*, middle panel) and one blue wildebeest (*Connochaetes taurinus*, lower panel) over the same 5 day period. In that temperature regime, in all mammals, whether or not they have a carotid rete, hypothalamic temperature generally exceeds carotid arterial blood temperature by about 0.2–0.5°C (Maloney *et al.*, 2007). As carotid arterial blood temperature increases, hypothalamic temperature uncouples from carotid arterial blood temperature, as selective brain cooling ensues in those species with a carotid rete. Figure 2 also illustrates two variables that can be used to characterize selective brain cooling (red arrows): the temperature at which hypothalamic temperature and carotid arterial blood temperature are equal, which is the threshold temperature for selective brain cooling (Kuhnen and Jessen, 1991), and the extent to which brain temperature drops below carotid arterial blood temperature, the magnitude of selective brain cooling. The threshold temperature for selective brain cooling can differ, not only between species simultaneously exposed to the same environmental conditions (Fig. 2), but also both between individuals within a species (Strauss *et al.*, 2016) and within an individual when exposed to different environmental conditions (Hetem *et al.*, 2012), with the threshold temperature for selective brain cooling being reduced under high heat loads (Strauss *et al.*, 2016).

The finding that hyperthermia is a not a prerequisite for selective brain cooling is confirmed by measurements of the threshold temperature in many species (Table 1); it typically lies between 38 and 39°C (Table 1), close to the modal and mean body core temperature of artiodactyls (Hetem *et al.*, 2016). In captive mammals, the magnitude of selective brain cooling typically increases with increasing carotid arterial blood temperature, but in free-living wild mammals it is more variable. Figure 2 (left panels) illustrates the carotid arterial blood and hypothalamic temperatures of three individual artiodactyls, a gemsbok (upper panel), a red hartebeest (middle panel) and a blue wildebeest (lower panel), living free in the same habitat and measured over the same 24 h period (Strauss *et al.*, 2016). Like other large mammals (Mitchell *et al.*, 2002; Hetem *et al.*, 2016), these antelope had a 24 h rhythm of arterial blood temperature, with a trough soon after dawn and a peak in the late afternoon (Fig. 2, grey line). Hypothalamic temperature (Fig. 2, black line), which is determined mainly by post-carotid rete arterial blood temperature (Hayward *et al.*, 1966), had a 24 h pattern similar to that of carotid arterial blood temperature, and the individuals from the three species exhibited a pattern of selective brain cooling similar to that typically exhibited by other free-living artiodactyls (Jessen *et al.*, 1994; Mitchell *et al.*, 1997; Hetem *et al.*, 2012; Strauss *et al.*, 2016). As carotid arterial blood temperature approached its 24 h peak, hypothalamic temperature

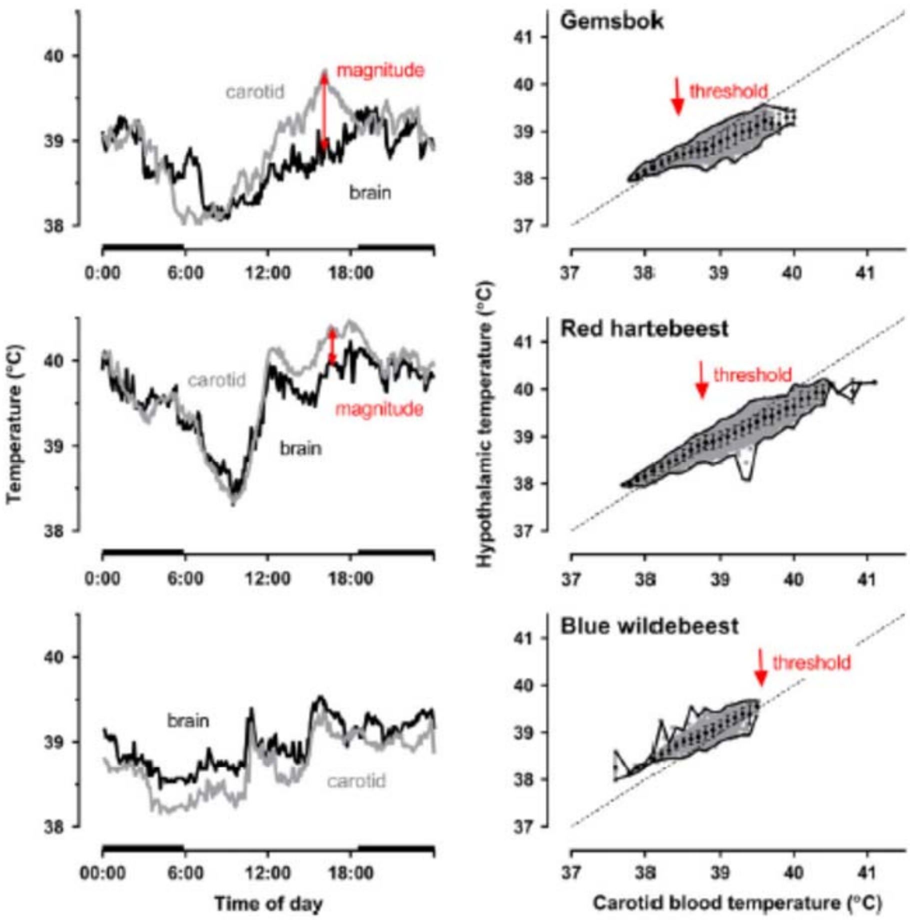

Figure 2: Left panels show the 24 h carotid blood and hypothalamic temperature profiles of a single gemsbok *Oryx gazella* (upper), red hartebeest *Alcelaphus buselaphus* (middle) and blue wildebeest *Connochaetes taurinus* (lower), for a single day, when the animals were free living in the same conditions in the Northern Cape Province, South Africa. Red arrows represent the magnitude of selective brain cooling within the 24 h period. Horizontal black bars indicate night time. Right panels show the correlation of hypothalamic temperature against carotid arterial blood temperature (grey circles) as well as hypothalamic temperature (mean ± SD) for every 0.1°C bin of simultaneous carotid arterial blood temperature, in the same gemsbok (upper), red hartebeest (middle) and blue wildebeest (lower) over a 5 day period during which they were exposed to the same environmental conditions. The boundary lines demonstrate the minimum and maximum hypothalamic temperatures in each bin. The diagonal line is the line of identity. Red arrows indicate the respective observed threshold temperatures for selective brain cooling; in the blue wildebeest the threshold was not reached within the range of measurement. Data from Strauss *et al.* (2016).

uncoupled from carotid arterial blood temperature, and selective brain cooling ensued. Although selective brain cooling could be implemented at any time of the day (e.g. Fig. 2, middle left panel), its magnitude typically was greatest (0.5–1.5°C; Table 1) around the time of the 24 h peak in carotid arterial blood temperature. At that time of day, environmental heat load was decreasing, and the antelope usually were involved in low-intensity activities, such as rumination and grazing. Despite strong thermal inputs at that time of day, however, selective brain cooling in artiodactyls can be modulated by non-thermal inputs, such that its magnitude can be further increased or it can even be completely abolished.

Non-thermal inputs

Cranial sympathetic tone

A role for sympathetic nervous system activity in the modulation of selective brain cooling was revealed through a series of elegant experiments on reindeer (*Rangifer tarandus*; Johnsen *et al.*, 1985, 1987; Johnsen and Folkow, 1988). Experiments on habituated artiodactyls showed that selective brain cooling could be controlled by directing the passage of venous blood draining the evaporating surfaces of the head either to the cavernous sinus, classically via the angularis oculi veins, or to the jugular vein via the facial vein, bypassing the cavernous sinus

Table 1: The threshold temperature (mean ± SD, where originally reported) and maximum magnitude of selective brain cooling reported (or inferred) from studies of selective brain cooling in artiodactyls

Species (sample size, *n*)	Selective brain cooling		Notes	Reference
	Threshold (°C)	Magnitude (°C)		
Domestic or habituated animals studied in controlled laboratory conditions, unless otherwise indicated				
Goat *Capra hircus* (6)	Not reported	2.5	Heat exchanger	(Caputa *et al.*, 1986a)
Goat *Capra hircus* (3)	38.8 ± 0.1	1.2	Heat exchanger	(Kuhnen and Jessen, 1991)
Goat *Capra hircus* (3)	39.1 ± 0.1	0.5	Heat exchanger and high humidity	(Kuhnen and Jessen, 1992)
Goat *Capra hircus* (3)	39.2 ± 0.1	1.2	Heat exchanger and low humidity	(Kuhnen and Jessen, 1992)
Goat *Capra hircus* (3)	38.9	1.5	Heat exchanger	(Kuhnen, 1997)
Goat *Capra hircus* (3)	39.0	0.3	Free-living, euhydration	(Jessen *et al.*, 1998)
Goat *Capra hircus* (3)	38.9	0.8	Free-living, dehydration	(Jessen *et al.*, 1998)
Goat *Capra hircus* (5)	39.3 ± 0.1	0.7	Hydrated and exercise	(Baker and Nijland, 1993)
Goat *Capra hircus* (5)	39.3	1.2	Dehydrated and exercise	(Baker and Nijland, 1993)
Ox *Bos taurus* (11)	39.1	0.8	Heat exposure	(Chesy *et al.*, 1983)
Ox *Bos taurus* (3)	40.3	1.5	Exercise	(Chesy *et al.*, 1985)
Sheep *Ovis aries* (3)	Not reported	0.6	Heat exchanger and heat exposure	(Maloney and Mitchell, 1997)
Sheep *Ovis aries* (4)	Not reported	1.0	Room temperature	(Laburn *et al.*, 1988)
Sheep *Ovis aries* (4)	Not reported	1.0	Heat exposure	(Laburn *et al.*, 1988)
Sheep *Ovis aries* (4)	Not reported	1.0	Febrile, induced	(Laburn *et al.*, 1988)
Sheep *Ovis aries* (4)	Not reported	0.5	Exercise	(Laburn *et al.*, 1988)
Sheep *Ovis aries* (4)	Not reported	0.8	Heat exposure	(Nijland *et al.*, 1990)
Sheep *Ovis aries* (4)	Not reported	0.6	Cold exposure	(Nijland *et al.*, 1990)
Sheep *Ovis aries* (4)	Not reported	0.9	Febrile, induced	(Nijland *et al.*, 1990)
Sheep *Ovis aries* (9)	39.1	0.4	Water deprivation and heat exposure	(Fuller *et al.*, 2007)
Sheep *Ovis aries* (9)	39.5 ± 0.5	1.5	Water deprivation and heat exposure	(Strauss *et al.*, 2016)
Sheep *Ovis aries* (5)	39.1 ± 0.5	0.5		(Maloney *et al.*, 2007)
Pig *Sus scrofa* (4)	38.9	0.9	Thermoneutral	(Fuller *et al.*, 1999a)
Pig *Sus scrofa* (2)	Not reported	0.8	Heat stress	(Fuller *et al.*, 1999a)
Pig *Sus scrofa* (1)	Not reported	0.3	Cold stress	(Fuller *et al.*, 1999a)
Camel *Camelus dromedarius* (2)	38.0	1.0	At rest	(Schroter *et al.*, 1989)
Camel *Camelus dromedarius* (1)	39.5	1.5	Exercise, hydrated and dehydrated	(Schroter *et al.*, 1989)
Reindeer *Rangifer tarandus* (3)	38.7 ± 0.2	1.0	Heat exchanger	(Kuhnen and Mercer, 1993)
Reindeer *Rangifer tarandus* (3)	39.5 ± 0.3	0.5	Exercise	(Kuhnen and Mercer, 1993)
Thomson's gazelle *Gazella thomsonii* (5)	39.4	2.7	Exercise	(Taylor and Lyman, 1972)

(Continued)

Table 1: continued

Species (sample size, *n*)	Selective brain cooling		Notes	Reference
	Threshold (°C)	Magnitude (°C)		
Free-living wild animals with free access to normal behaviour				
Black wildebeest *Connochaetes gnu* (4)	38.9 ± 0.2	0.4		(Jessen *et al.*, 1994)
Eland *Tragelaphus oryx* (1)	40.0	0.4		(Fuller *et al.*, 1999b)
Gemsbok *Oryx gazella* (4)	39.8 ± 0.4	0.4		(Maloney *et al.*, 2002)
Gemsbok *Oryx gazella* (4)	39.5 ± 0.9	0.9		(Strauss *et al.*, 2016)
Kudu *Tragelaphus strepsiceros* (4)	39.3 ± 0.7	0.5	Febrile, naturally	(Hetem *et al.*, 2008)
Kudu *Tragelaphus strepsiceros* (4)	38.8 ± 0.1	0.2	Afebrile	(Hetem *et al.*, 2008)
Arabian oryx *Oryx leucoryx* (4)	37.8 ± 0.1	1.4		(Hetem *et al.*, 2012)
Springbok *Antidorcas marsupialis* (2)	39.2 ± 0.2	0.5		(Mitchell *et al.*, 1997)
Pronghorn *Antilocapra americana* (2)	39.5	0.5		(Lust *et al.*, 2007)
Blue wildebeest *Connochaetes taurinus* (6)	39.3 ± 0.4	1.1		(Strauss *et al.*, 2016)
Red hartebeest *Alcelaphus buselaphus* (5)	39.4 ± 0.6	1.0		(Strauss *et al.*, 2016)

(Fig. 3). The default direction of blood flow appears to be via the cavernous sinus, so increased cranial blood flow in heat-stressed artiodactyls (Maloney and Mitchell, 1997; Vesterdorf *et al.*, 2011) led to increased flow of cooled venous blood to the cavernous sinus; hence, increased selective brain cooling. Increased cranial sympathetic nervous system activity, however, led to simultaneous contraction of a muscular sphincter in the angularis oculi vein (α-adrenergic) and dilatation of a similar sphincter in the facial vein (β-adrenergic), resulting in venous blood bypassing the cavernous sinus and returning via the jugular vein to the heart (Johnsen *et al.*, 1985, 1987; Johnsen and Folkow, 1988). Although this differential vasoconstriction can modulate the degree of selective brain cooling, it does not fully explain the control of selective brain cooling in artiodactyls. In addition to the angularis oculi veins, less superficial veins, such as the sphenopalatine, external ophthalmic and ethmoidal veins, also supply venous blood to the cavernous sinus (Sisson and Grossman, 1967; Carlton and McKean, 1977). As a result, severing the angularis oculi veins does not completely eliminate selective brain cooling (Fuller *et al.*, 2011). High sympathetic tone attenuates selective brain cooling not only by constricting the angularis oculi veins, but also by constricting nasal mucosal blood vessels and closing arteriovenous anastomoses. Within the nasal mucosa, the rate of heat extraction is attenuated through a combination of reduced blood flow and the restriction of airway width (Malm, 1973). Thus, increased cranial sympathetic nervous system activity decreases blood flow to the evaporating surfaces of the head as well as redirecting the flow of venous blood leaving those surfaces away from the cavernous sinus (Maloney and Mitchell, 1997; Fuller *et al.*, 2011). These changes result in an upward shift of the threshold temperature for selective brain cooling, as documented in springbok (*Antidorcas marsupialis*; Mitchell *et al.*, 1997), or complete abolishment of selective brain cooling, as documented in black wildebeest (*Connochaetes gnu*; Jessen *et al.*, 1994). Free-living mammals rarely engage in intensive exercise except for predator–prey interactions, during which there is a dramatic increase in sympathetic nervous system activity in both predator and prey. The sympathetic activity prevalent during flight and fright in wild artiodactyls abolishes selective brain cooling (Jessen *et al.*, 1994), overriding the drive of high body temperature. Selective brain cooling observed in tame artiodactyls exercising at low or moderate intensity is likely to be associated with low sympathetic activity (Mitchell *et al.*, 2002).

Although it is not as strong as in intense exercise during predator–prey interactions, there is also sympathetic activation during psychological stress. The effects of psychological stress, including an increase in body and brain temperatures, are well documented, even in mammals lacking a carotid rete, such as rats (Mohammed *et al.*, 2014). In mammals with a carotid rete, selective brain cooling is absent or reduced in other circumstances likely to be associated with increased sympathetic tone, including nearby human presence (Maloney *et al.*, 2001), the return of drinking water to dehydrated artiodactyls (Fuller *et al.*, 2007) and vigilance in male artiodactyls (Maloney *et al.*, 2002; Hetem *et al.*, 2012). The temperament of individual mammals, a heritable trait among artiodactyls (Murphy *et al.*, 1994), plays an important role in how situations are experienced (Beausoleil *et al.*, 2008). For example, individual roe deer (*Capreolus capreolus*) manifest different responses to stress (Monestier *et al.*, 2016) and therefore, presumably, different levels of sympathetic activation. Individual variability in sympathetic responses to the same stressor may contribute to an underlying plasticity in the control of selective brain cooling (Strauss *et al.*, 2016).

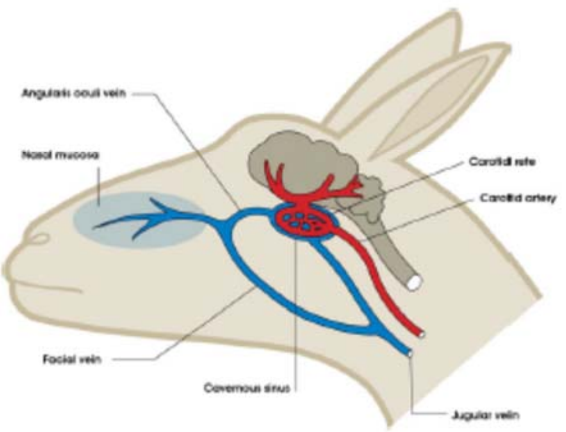

Figure 3: Diagram illustrating the position of the carotid rete in artiodactyls, located within a cavernous sinus at the base of the brain, as well as the main arterial blood supply to the brain, via the carotid rete. During selective brain cooling, cool venous blood from the nasal mucosa drains into the cavernous sinus via the angularis oculi vein (as well as some deeper veins; see Fuller *et al*, 2011). During high sympathetic activity, the cooled venous blood draining from the nasal mucosa largely bypasses the cavernous sinus as it is shunted via the facial vein, thereby attenuating selective brain cooling. Diagram adapted from Jessen (1998).

Hydration status

Selective brain cooling is enhanced during water deprivation (Jessen *et al.*, 1998; Fuller *et al.*, 2007; Strauss *et al.*, 2015). Given that body temperature is elevated in dehydrated mammals exposed to heat, that enhancement could arise from a stronger thermal drive on selective brain cooling (Jessen *et al.*, 1998). However, Fuller *et al.* (2007) showed that sheep exhibited a higher magnitude of selective brain cooling during dehydration even when carotid arterial blood temperature did not increase. Water deprivation on its own therefore seems to be a sufficient stimulus to enhance selective brain cooling. That idea is supported by measurement of selective brain cooling in antelope living free in arid environments. In the hyper-arid desert of Saudi Arabia, the mean magnitude of selective brain cooling in free-living Arabian oryx (*Oryx leucoryx*) peaked in the afternoon (Fig. 4A), well after solar noon and maximal heat load, as measured with miniature black globe thermometers on the collar of the animals (Hetem *et al.*, 2007). Water availability, or aridity, appeared to have been the main factor determining the use and magnitude of selective brain cooling, with selective brain cooling being enhanced in the dry period compared with the wet period (Hetem *et al.*, 2012). During the hot dry period when no drinking water was available, the Arabian oryx made near-continuous use of selective brain cooling during the 4 h leading to sunset (Fig. 5B, grey bars), compared with no more than 40% of the time during the warm wet period, when the oryx presumably had access to drinking water or food with a higher moisture content (Fig. 4B, black bars).

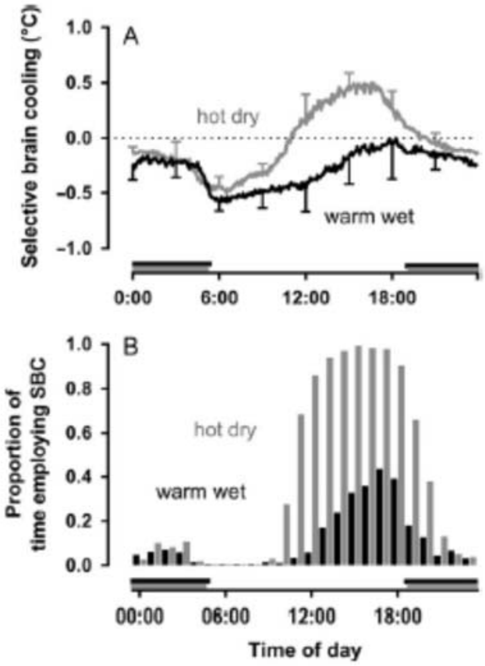

Figure 4: The effect of aridity on selective brain cooling as illustrated through differences in the mean (±SD) magnitude of selective brain cooling (carotid blood temperature minus hypothalamic temperature; A) and the proportion of time that a single Arabian oryx used selective brain cooling in the hot, hyper-arid deserts of Saudi Arabia (B). Grey depicts the hot dry and black the warm wet periods. Horizontal grey and black bars depict night-time during the two periods of interest. Data from Hetem *et al.* (2012).

Despite being exposed to similar ambient temperatures, Arabian oryx, in the hyper-arid desert of Saudi Arabia, also showed enhanced selective brain cooling compared with the congeneric gemsbok with free access to water (Maloney *et al.*, 2002), as they initiated selective brain cooling at a lower threshold temperature and used selective brain cooling more frequently and at greater magnitude than did the gemsbok (Hetem *et al.*, 2012).

The enhancement of selective brain cooling by water deprivation has been confirmed in more controlled conditions, in which domestic artiodactyls can be handled for sample collection (Jessen *et al.*, 1998; Fuller *et al.*, 2007; Strauss *et al.*, 2015). When Dorper sheep (*Ovis aries*) were deprived of drinking water and exposed to diurnal heat load, after the third day of water deprivation, when the water reservoir within the rumen would have been depleted, plasma osmolality and selective brain cooling magnitude increased in a near-linear manner and in unison (Fig. 5A; Strauss *et al.*, 2015). After 5 days of water deprivation, the 24 h mean magnitude of selective brain cooling was three times greater (0.9 vs. 0.3°C) than that during euhydration (Fig. 5B). The proportion of time during which the sheep used selective brain cooling also differed markedly

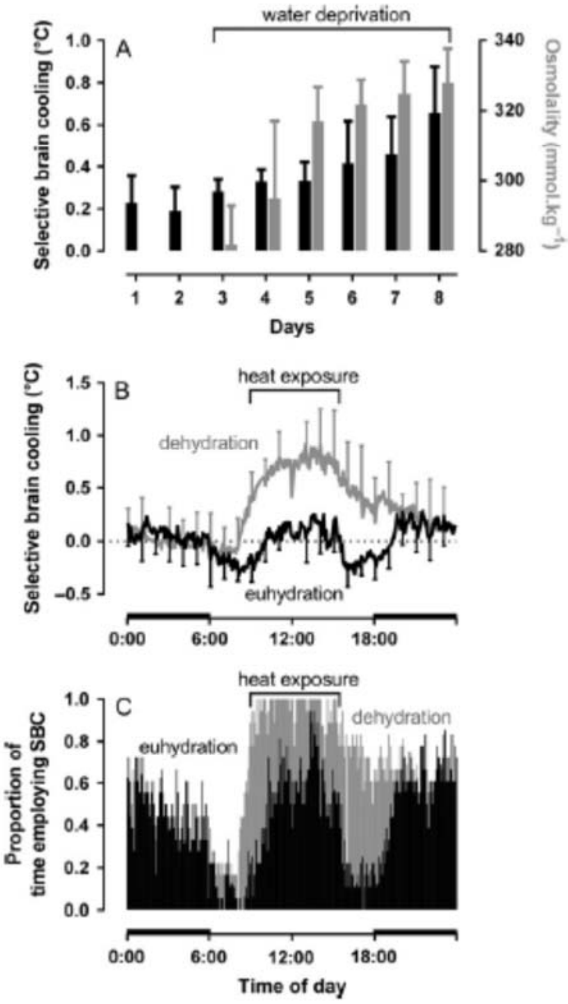

Figure 5: Summary of the effects of water deprivation on selective brain cooling in Dorper sheep. (**A**) The relationship between the mean (±SD) magnitude of selective brain cooling (carotid blood temperature minus hypothalamic temperature, black bars) and osmolality (grey bars) during 5 days of water deprivation; the black bracket indicates the period of water deprivation. (**B**) The mean (±SD) 24 h magnitude of selective brain cooling during euhydration (black line, days 1 and 2 in A) and dehydration (grey line, days 7 and 8 in A); horizontal black bars along the *x*-axis depict night-time. (**C**) The proportion of time that the sheep spent using selective brain cooling throughout the 24 h period when euhydrated (black bars; days 1 and 2 in A) and when dehydrated (grey bars; days 7 and 8 in A); the black bracket indicates the period of heat exposure (09:00–15:00) and the horizontal black bars along the *x*-axis depict night-time. Data from Strauss *et al.* (2015).

depending on hydration status. Figure 5C shows that the sheep used some selective brain cooling at all times of the day regardless of hydration status. However, when dehydrated, the sheep used selective brain cooling more frequently at all times of day, but especially during the daylight hours when selective brain cooling, on average, was used 75% of the time. Water deprivation, presumably acting through hyperosmolality, therefore appears to be a driver of selective brain cooling.

Selective brain cooling conserves body water

In dehydrated mammals, increased osmotic pressure of the arterial blood perfusing the brain inhibits thermoregulatory responses to heat (McKinley *et al.*, 2008), including evaporative heat loss (Doris, 1983). If hyperosmolality also enhances selective brain cooling, then what would be the benefit to artiodactyls? In goats, at least, but presumably also in other artiodactyls, the neural drive for respiratory evaporative heat loss is provided about equally by thermoreceptors in the hypothalamus and thermoreceptors within the trunk (Jessen and Feistkorn, 1984). During selective brain cooling, input from the hypothalamic temperature sensors would be attenuated. Consequently, panting and sweating, the main physiological avenues of evaporative heat loss in artiodactyls, would be attenuated. The potential water savings as a result of selective brain cooling could be substantial considering that a 1°C decrease in brain temperature resulted in a ~6-fold decrease in respiratory evaporative water loss in goats (Kuhnen and Jessen, 1994). The hypothalamic thermosensitivity for evaporative water loss in goats is therefore high. We calculated the hypothalamic thermosensitivity for evaporative water loss for goats, at a trunk temperature of 40°C, to be 0.35 W kg^{1} °C^{1}, or 14 g H_2O min^{1} kg^{1} °C^{1} using data provided in Jessen and Feistkorn (1984). In contrast, for a species lacking a rete, the rabbit, the hypothalamic thermosensitivity for evaporative water loss, calculated at an ambient temperature of 39°C, was 0.038 W kg^{1} °C^{1}, or 1.5 g H_2O min^{1} kg^{1} °C^{1} (Stitt, 1976). The hypothalamic thermosensitivity is therefore 10 times greater in the heat-exposed goat than in the heat-exposed rabbit. A relatively small change in the hypothalamic temperature of the goat (tenths of a degree Celsius) therefore can result in significant water savings as a result of decreased evaporative water loss.

In an elegant experiment on goats, Kuhnen (1997) used extracorporeal heat exchangers to manipulate selective brain cooling and measured respiratory evaporative heat loss. The experimental inhibition of selective brain cooling resulted in a reduced trunk threshold for respiratory evaporative heat loss; therefore, evaporative water loss occurred at lower body temperatures, as well as at a higher overall rate. At an aortic blood temperature of 40°C, selective brain cooling of only 0.5°C reduced respiratory water loss by 0.72 l day^{1}, the equivalent of 35% of the average daily water requirement of the goats (Kuhnen, 1997). Sweating also is driven largely by thermal receptors in the hypothalamus (Smiles *et al.*, 1976); therefore, selective brain cooling also will reduce water loss by sweating (Strauss *et al.*, 2015), further contributing to the water savings of a mammal that uses both forms of evaporative heat loss.

Recently, in the laboratory, we quantified the total water savings attributable to selective brain cooling in Dorper sheep. We used the stable hydrogen isotope deuterium oxide (D_2O) to measure water turnover in sheep that were deprived of drinking water and naturally making use of selective brain cooling. The sheep lost a quarter of their body water over 5 days of water deprivation. The threshold temperature for selective brain cooling remained unchanged, but those individuals that used selective brain cooling more frequently, or of greater magnitude, had lower water turnover rates (they therefore conserved body water better) than did conspecifics that used selective brain cooling less frequently or of smaller magnitude. We showed that a 50 kg sheep that used selective brain cooling for half of a day would save 2.4 litres of water that day, the equivalent of ~60% of the daily water requirement of a Dorper sheep not exposed to heat (Strauss *et al.*, 2015).

Thus, the reduction of hypothalamic temperature by the 1°C or less that selective brain cooling can achieve reduces evaporative cooling during heat exposure sufficiently to save a substantial portion of the water that an artiodactyl would need to access each day. How does an artiodactyl continue to maintain heat balance if its evaporative cooling is attenuated? The attenuation of evaporative cooling by selective brain cooling results in an increase in body temperature, including skin temperature (Caputa *et al.*, 1986b; Laburn *et al.*, 1988). In environments in which an artiodactyl can lose heat by radiation and convection, the higher skin temperature will enhance radiant and convective heat loss, so selective brain cooling will switch heat loss from evaporative to non-evaporative channels. If an artiodactyl is in an environment in which it is gaining heat, the rate of heat gain will be reduced by the higher skin temperature. In the process of conserving water, however, the artiodactyl may store heat during hot periods of the day. As selective brain cooling generally is used late in the afternoon (Fig. 2, left panels), the heat that is gained as a result of the suppression of evaporative cooling may be dissipated non-evaporatively during the night.

Selective brain cooling as a physiological feature for surviving hotter and drier environments

Water economy strategies in artiodactyls provide compelling evidence that selective brain cooling can mitigate negative population responses to warming and aridification. The use of selective brain cooling offers significant water savings to artiodactyls, but it is not the only mechanism by which they can save water. Dehydration itself reduces evaporative water loss via osmosensitive neurons in the hypothalamus, even in the absence of selective brain cooling (Baker and Doris, 1982). However, unlike dehydration or blood osmolality, selective brain cooling can be switched off rapidly (probably within seconds) by high cranial sympathetic activity. That property of selective brain cooling potentially conveys a survival benefit (Mitchell *et al.*, 2002). For example, should an artiodactyl in a hot environment and implementing selective brain cooling be confronted by a flight-or-fight situation, its selective brain cooling would be abolished immediately by increased sympathetic tone. Consequently, hypothalamic temperature would increase, and the hypothalamic drive on evaporative cooling would be restored immediately, with the full power of evaporative cooling invoked to dissipate the extra metabolic heat. Immediate survival outweighs the longer-term benefits of body water conservation, and the artiodactyl makes temporary use of full evaporative cooling to avoid a potentially lethal hyperthermia. When the threat has receded, selective brain cooling can again be initiated and evaporative water loss suppressed. Thus, selective brain cooling may bestow benefits for survival in arid environments in two ways: (i) switching selective brain cooling on conserves body water, improving long-term survival; whereas (ii) switching selective brain cooling off rapidly accelerates evaporative cooling to avoid lethal hyperthermia, thereby supporting immediate survival.

The ability to modulate evaporative water loss through the use of selective brain cooling may have contributed to the evolutionary success of artiodactyls (Mitchell and Lust, 2008). It can be assumed that the carotid rete, and therefore selective brain cooling, evolved concomitantly with the emergence of the modern artiodactyl orders, at least 45 million years ago (Janis, 2009). When the diversity trends of Artiodactyla (presumably all rete bearing) and their sister clade Perissodactyla (presumably not rete bearing) are compared across the Cenozoic, discrepancies in generic richness are established in three pulses, each of which corresponds to a trend of aridification, whether warming or cooling (Fig. 6). In the earlier half of the Cenozoic, global climate is best described as a 'tropical hothouse' (Buchardt, 1978; Wolfe, 1978; Wing, 1987; Huber and Sloan, 2001; Bowen and Zachos, 2010; Galeotti *et al.*, 2010). During this period of abundant moisture, artiodactyls and perissodactyls enjoyed similar generic richness (Fig. 6). However, a pronounced period of cooling and drying across the Eocene to Oligocene transition began to shift this relationship (Diester-Haass and Zahn, 1996; Lear *et al.*, 2008). Although many large-bodied mammals faced extinction throughout this period (Prothero, 1985; Hooker, 1992; Legendre and Hartenberger, 1992), artiodactyls enjoyed a comparatively higher degree of survivorship both during and after this event. Throughout the drier, temperate Oligocene (Kennett, 1985; Ehrmann and Mackensen, 1992), artiodactyl generic richness remained elevated relative to perissodactyls (Fig. 6). Across the Late Oligocene to Early Miocene transition, another period of aridification swept the globe, this time accompanied by warming (Kennett, 1985; Retallack, 2013). This period of warming and aridification saw a pulse in generic richness of artiodactyls capable of selective brain cooling, immediately before the Mid-Miocene expansive radiation of C_4 grasslands. It is often the expansion of grasslands and the artiodactyl rumen that have been associated and promoted as the driver of artiodactyl success (Retallack, 1991, 2001, 2007a, b, 2013; Jacobs *et al.*, 1999). Although the late Cenozoic (Mid-Miocene to present) diversification patterns are largely attributable to the ruminant

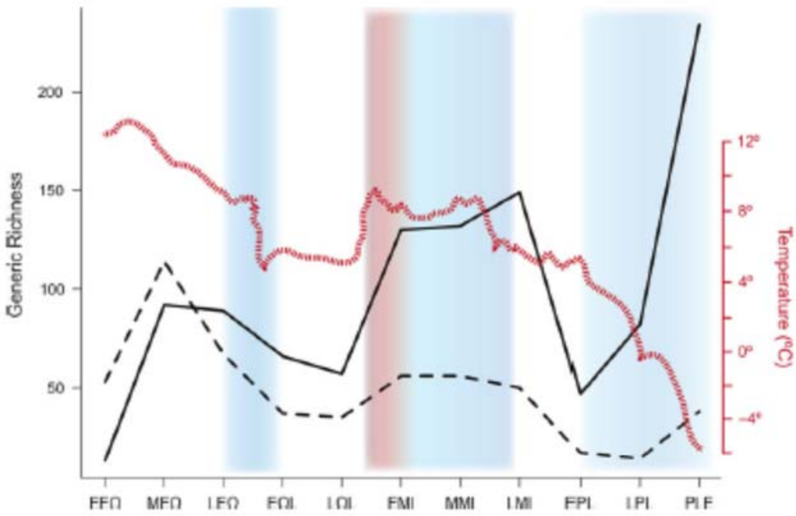

Figure 6: Diversity curve for the artiodactyls (solid line) and perissodactyls (dashed line) from the early Eocene (EEO) to the Palaeocene (PLE) relative to global temperature (red line; Zachos *et al.*, 2001) and an indication of the relative hydrological regimes (Janis, 2008); blue and red shading represent aridification combined with cooling and warming temperatures, respectively. Occurrence data were downloaded from the Fossilworks/Paleobiology Database in August 2016. The epochs along the *x*-axis are as follows: EEO, Early Eocene; MEO, Mid-Eocene; LEO, Late Eocene; EOL, Early Oligocene; LOL, Late Oligocene; EMI, Early Miocene; MMI, Mid-Miocene; LMI, Late Miocene; EPL, Early Pleistocene; LPL, Late Pleistocene; and PLE, Paleocene.

digestive physiology of bovids (Janis *et al.*, 1998; Clauss *et al.*, 2003; Janis, 2007, 2009; Clauss and Rössner, 2014; O'Brien, 2016), this highly specialized mode of digestion is variably present and cannot solely be responsible for earlier Cenozoic survivorship and diversification patterns. Indeed, it is likely that the influence of selective brain cooling insulated artiodactyls from extensive periods of aridification, whether warming or cooling, that saw declines among non-selective-brain-cooling ungulates (O'Brien, 2016).

This pattern of selective-brain-cooling-related survivorship is corroborated by evolutionary patterns of the Tragulidae. Although they are small-bodied artiodactyls with a rudimentary capacity for rumination, tragulids do not possess a carotid rete. Therefore, they can be considered to ruminate but not to selective brain cool. The earliest record of tragulids and other closely related small-bodied primitive ruminants is the Late Eocene to Early Oligocene (Webb and Taylor, 1980), when swamps and temperate forests dominated much of Europe and North America (Mai, 1989; Pross *et al.*, 2001; Retallack, 2007a, b, 2013; Kunzmann, 2012). Since that period, these non-selective-brain-cooling taxa have experienced declines in generic richness even as more advanced ruminants have increased in diversity (Clauss and Rössner, 2014). Thus, although diet undoubtedly plays a role in the extinction and survivorship capacity of any taxon, failure to incorporate selective brain cooling not only leaves early artiodactyl evolution unsatisfactorily explained, but it renders an incomplete evaluation of these species' abilities to persist into the Anthropocene.

Among artiodactyl species, there may be innate differences between species in their capacity for selective brain cooling, which may facilitate the persistence of those species with enhanced selective brain cooling in a hotter and drier Anthropocene. Although it has not been possible logistically, to date, to measure the water savings that result from selective brain cooling in free-living artiodactyls, we recently investigated selective brain cooling in three sympatric antelope species with different water dependencies and determined the dimensions of their carotid retes (Strauss *et al.*, 2016). Individuals of all three species, living free in their natural habitats under the same environmental conditions, used selective brain cooling, but we found no differences in selective brain cooling use between the gemsbok that is independent of surface water, the red hartebeest of intermediate water dependency and the blue wildebeest that is dependent on surface water and has to drink water daily. In fact, we found more variability in selective brain cooling use within those species than between those species in a habitat in which surface water was available *ad libitum*. Although earlier descriptions of carotid rete morphology documented variability in the vascularization of the rete between species (Daniel *et al.*, 1953; Carlton and McKean, 1977), we found little quantifiable difference in the rete anatomy of those three artiodactyls with varying ecological water requirements (Strauss *et al.*, 2016). The observed variability in use of selective brain cooling within these three species living in exactly the same environmental conditions reaffirms the concept that selective brain cooling is not simply under thermal control (in the sense that there is a threshold temperature at which it is initiated). Moreover, the plasticity in the use of selective brain cooling may provide a physiological feature for selection in the face of anthropogenic climate change, as numerous regions are expected to become hotter and drier, with increased variability in rainfall (Niang *et al.*, 2014). Large mammals will be unable to adapt genetically given the rapid rate of climate change and will be unlikely to be able to move to new, suitable habitats, leaving them dependent on phenotypic plasticity if they are to counter such climate change (Hetem *et al.*, 2014).

Having survived and diversified dramatically during periods of pronounced aridification, the artiodactyls may be insulated, to a degree, from global warming and drying (Mitchell and Lust, 2008). Moreover, the observed inter-individual variability in selective brain cooling use within species implies that individual artiodactyls within populations, regardless of species or ecological water dependency, might have a relative evolutionary advantage in hotter, drier, less predictable environments. Representatives of the characteristic large herds of artiodactyls across various landscapes, which often also form the mainstay of large tracts of land under conservation management, therefore could persist through the Anthropocene. However, considering the continuing decrease in antelope populations across the globe (Ripple *et al.*, 2015) and the poor performance of artiodactyls during mid-latitude glaciation of the Miocene to Pliocene transition, persistence through the Anthropocene may depend on the degree of intra-specific selective brain cooling plasticity. Indeed, artiodactyls such as the Arabian oryx may already be living close to their physiological tolerance levels. With a 24 h body temperature amplitude (maximum minus minimum 24 h body temperature) of up to 7.7°C during the summer, these animals are seasonally losing control of homeothermy as a result of water and nutritional stress (Hetem *et al.*, 2012, 2016). As a consequence of a paucity of similar data from other artiodactyls, and also from perissodactyls, we know little about the physiological performance of these mammals under current environmental conditions and, ultimately, their ability to cope with a changing climate. Because perissodactyls, many of which are evolutionarily distinct (Zoological Society of London, 2014), cannot use selective brain cooling to conserve body water, concerted conservation efforts may be required as conditions become drier under anthropogenic climate change. Yet such conservation efforts should not be to the detriment of other species sensitive to disturbance (see, for example, Harrington *et al.*, 1999).

Survival of those predators depredating artiodactyls and perissodactyls may also depend on the predators' capacity for selective brain cooling, as selective brain cooling has been observed in heat-stressed domestic cats (Baker and Doris, 1982) and dogs (Baker, 1984). The degree to which free-living felids and canids use selective brain cooling is currently not known. We also do not know, therefore, whether the evolutionary success of carnivores can be attributed to the development of their carotid rete, selective brain cooling and the conservation of body water. A better understanding of selective brain cooling, as a form of physiological plasticity that is available to some mammals, is integral to efforts to predict how mammals will respond to changing environments and how best to conserve them. The studies that are required are long-term investigations undertaken on a range of artiodactyl and carnivore species (Fuller *et al.*, 2014; Hetem *et al.*, 2014). Such studies should focus on identifiable individuals and their progeny, free-living in their natural environment, with the aim of relating varying levels of evolutionary success to the observed flexibility in selective brain cooling and resulting water savings.

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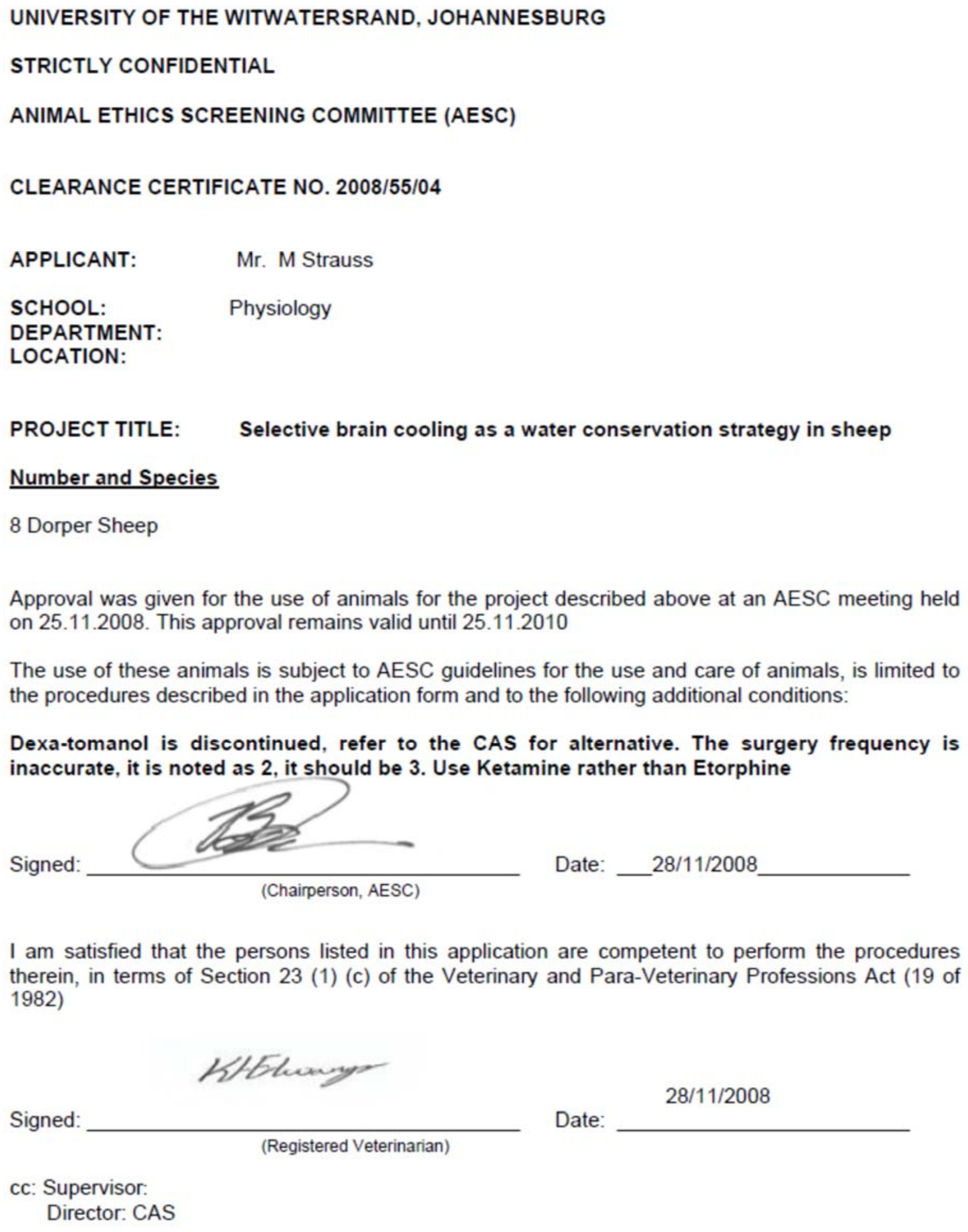
Appendix 2

Ethical clearance certificates



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2008/55/04

APPLICANT: Mr. M Strauss

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: **Selective brain cooling as a water conservation strategy in sheep**

Number and Species

8 Dorper Sheep

Approval was given for the use of animals for the project described above at an AESC meeting held on 25.11.2008. This approval remains valid until 25.11.2010

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

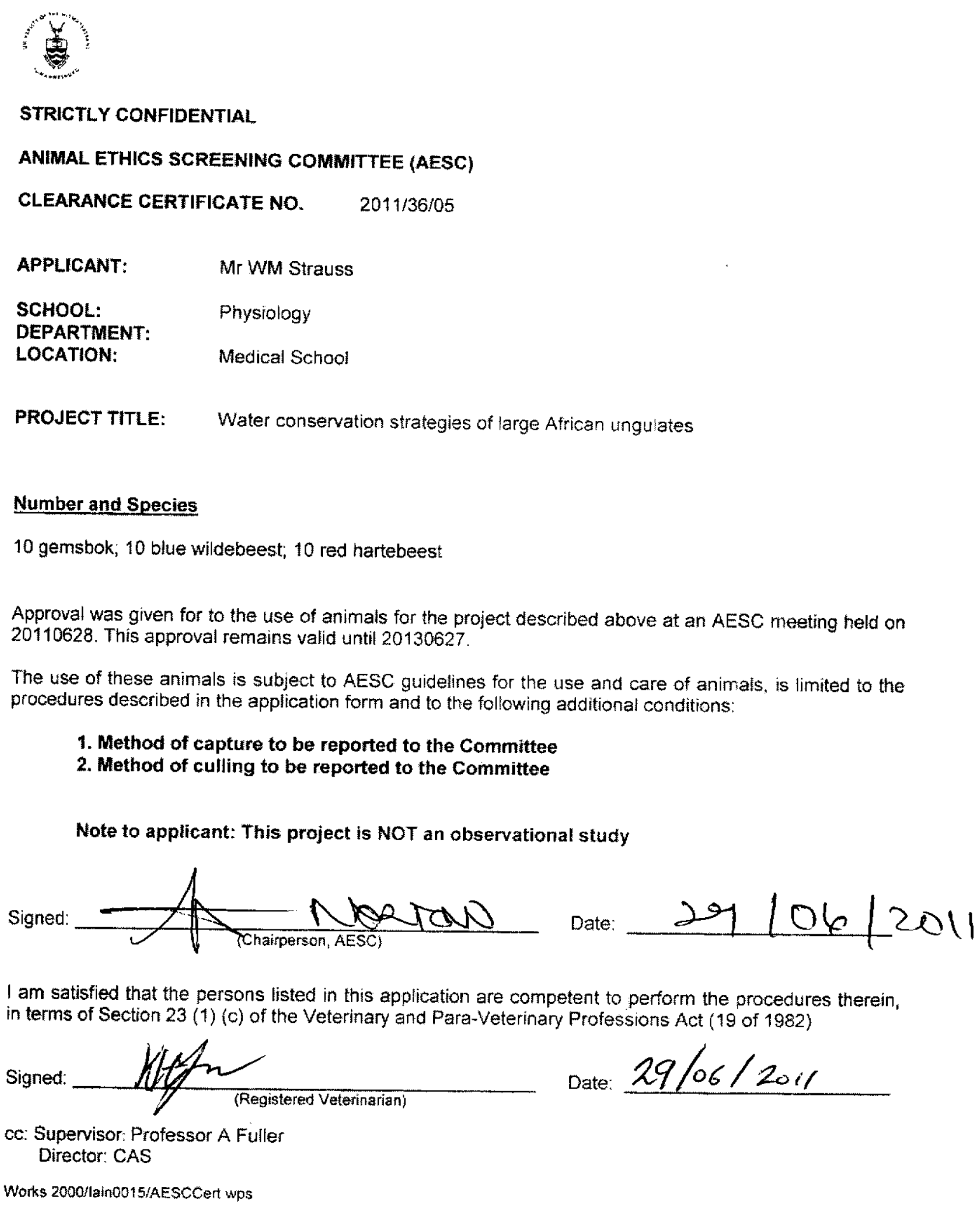
Dexa-tomanol is discontinued, refer to the CAS for alternative. The surgery frequency is inaccurate, it is noted as 2, it should be 3. Use Ketamine rather than Etorphine

Signed: ______________________ Date: 28/11/2008
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: ______________________ Date: 28/11/2008
(Registered Veterinarian)

cc: Supervisor:
Director: CAS



STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2011/36/05

APPLICANT: Mr WM Strauss

SCHOOL: Physiology
DEPARTMENT:
LOCATION: Medical School

PROJECT TITLE: Water conservation strategies of large African ungulates

Number and Species

10 gemsbok; 10 blue wildebeest; 10 red hartebeest

Approval was given for to the use of animals for the project described above at an AESC meeting held on 20110628. This approval remains valid until 20130627.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

1. **Method of capture to be reported to the Committee**
2. **Method of culling to be reported to the Committee**

Note to applicant: This project is NOT an observational study

Signed: ______________________ (Chairperson, AESC) Date: 29/06/2011

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: ______________________ (Registered Veterinarian) Date: 29/06/2011

cc: Supervisor: Professor A Fuller
Director: CAS

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