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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 polytetrafluoroethylene (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	$T_{Car} - T_{Hyp}$	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 ($F_{2,12} = 1.10$, $P = 0.38$).

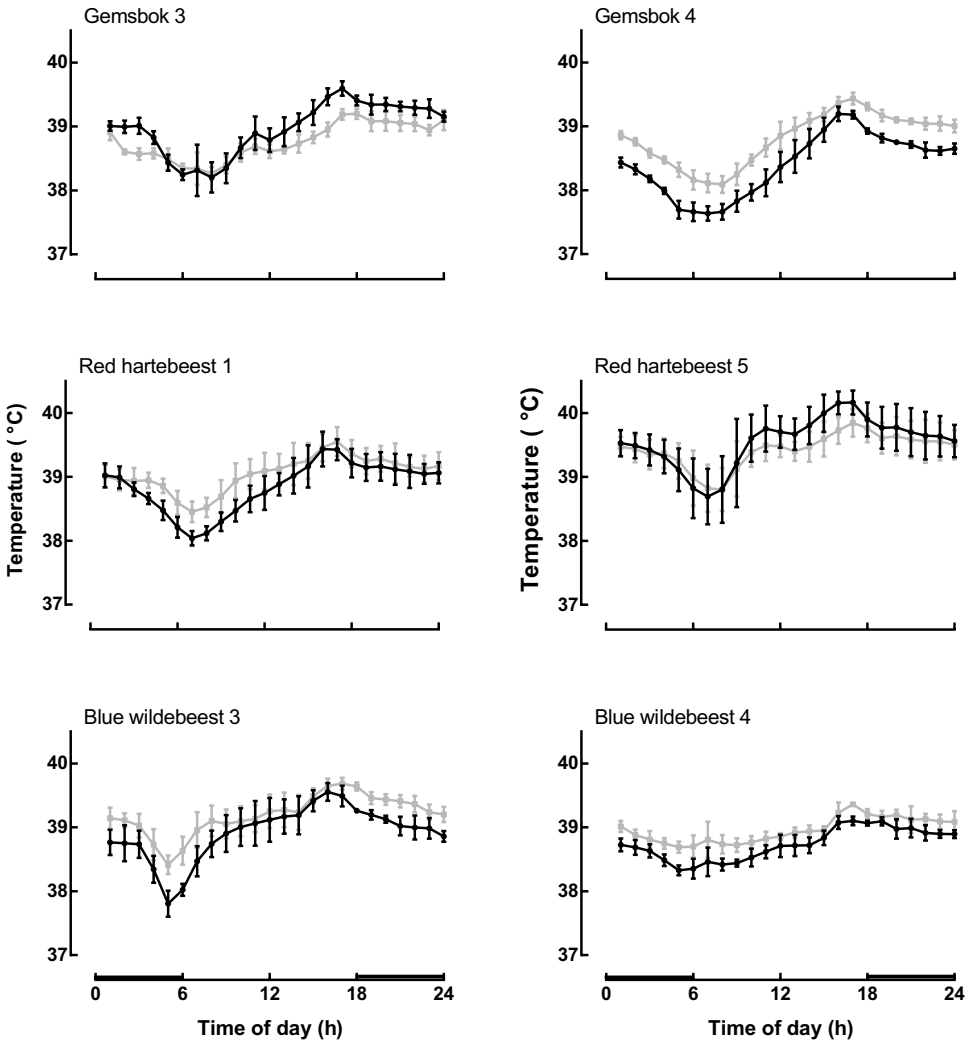

Fig. 1 The mean (± SD) hourly hypothalamic (*grey line*) and carotid blood (*black line*) temperatures of two gemsbok, two red hartebeest and two blue wildebeest females over the same 5-day period

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

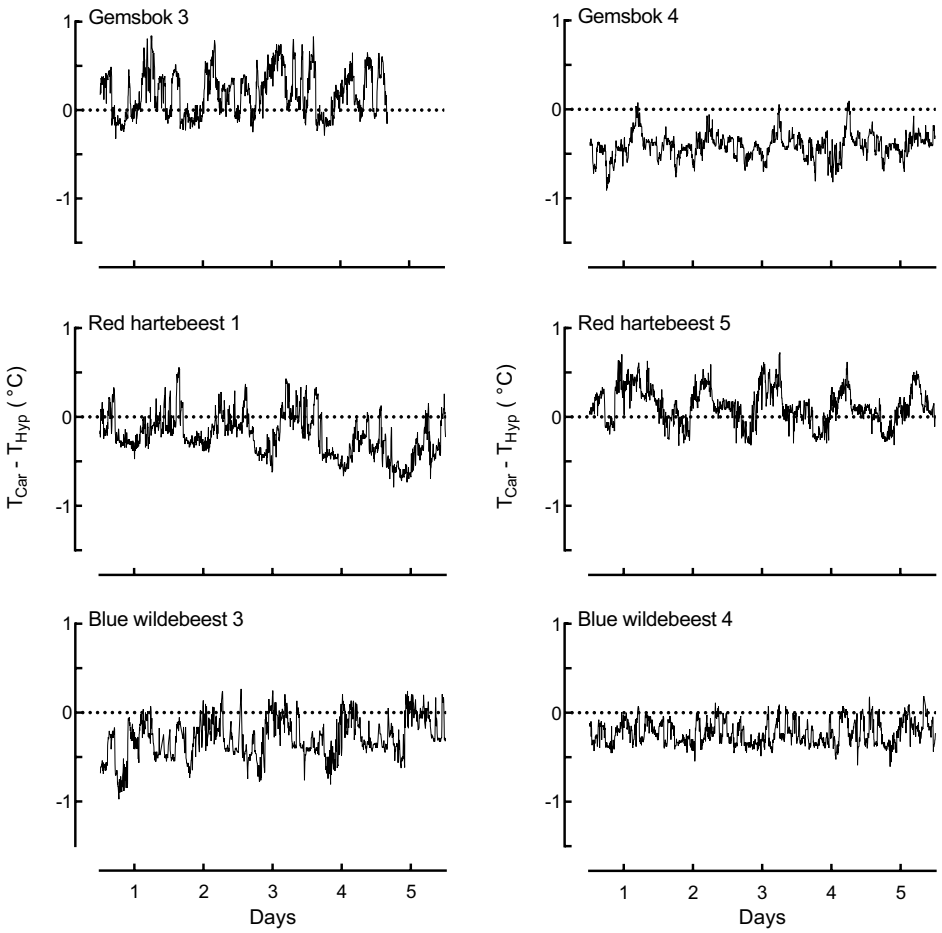

Fig. 2 The difference between carotid blood and hypothalamic temperature as recorded every 5 min for two gemsbok, red hartebeest and blue wildebeest females over the same 5-day period. *Positive values* indicate selective brain cooling

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

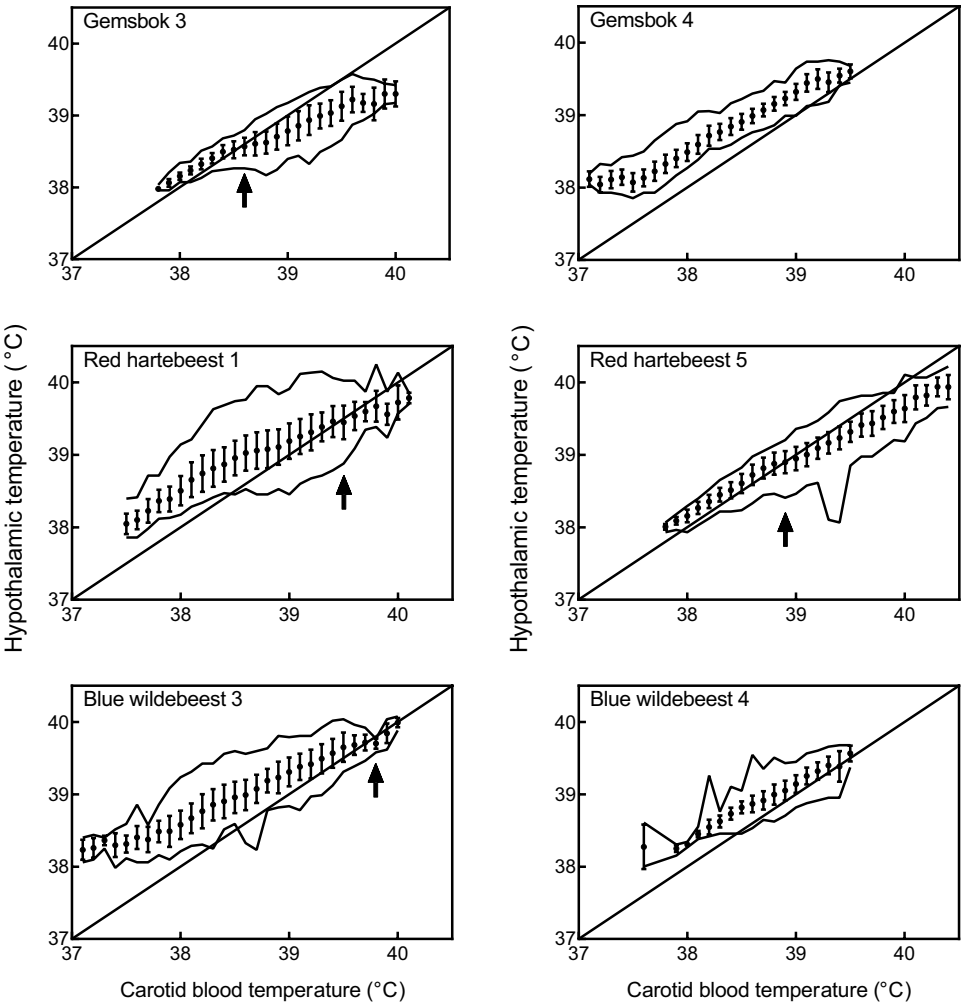

Fig. 3 Hypothalamic temperature (mean ± SD) for every 0.1 °C bin of simultaneous carotid blood temperature, in two gemsbok, two red hartebeest and two blue wildebeest females over the same 5-day period. The boundary lines demonstrate the minimum and maximum hypothalamic temperatures in that bin. The *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 0.005
	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

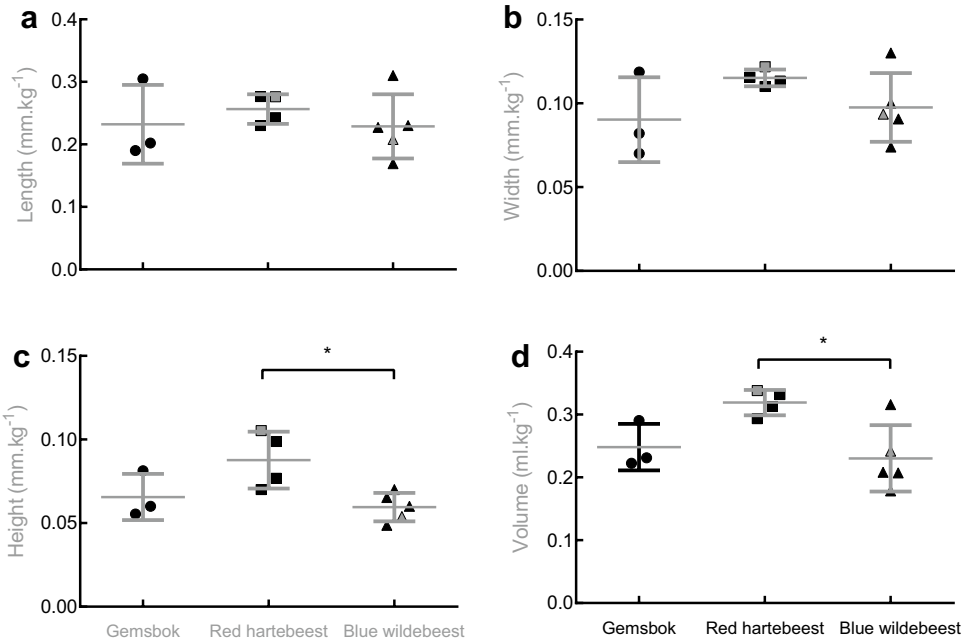

Fig. 4 Univariate scatterplots of the carotid rete length (**a**), width (**b**), height (**c**) and volume (**d**) as determined from silicone casts of the carotid retes of three gemsbok, four red hartebeest and five blue wildebeest females. The distribution of the individual values, scaled to body 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

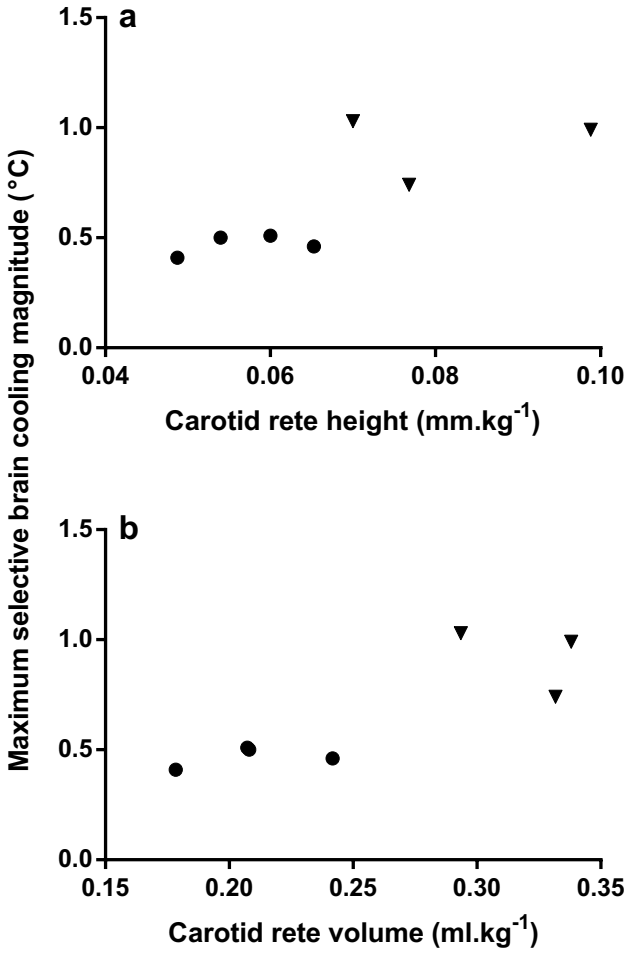

Fig. 5 The relationship between the maximum magnitude of selective brain cooling and **a** the carotid rete height, and **b** carotid rete volume when scaled to body mass of the individual blue wildebeest (*filled circle*) and red hartebeest (*filled inverted triangle*) females

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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