Under the spell of heat: use of allostatic and heterothermic pathways by an endothermic ungulate in an open landscape

Martijn J.A. Weterings1,2\*, Merlin Weiss2, Frank van Langevelde1,3, Perry Cornelissen4, Henry J. Kuipers2

1 Wildlife Ecology and Conservation Group, Wageningen University, 6708 PB Wageningen, The Netherlands.

2 Wildlife Management, Department of Animal Management, Van Hall Larenstein University of Applied Sciences, 8901 BV Leeuwarden, The Netherlands.

3 School of Life Sciences, Westville Campus, University of KwaZulu-Natal, Durban 4000, South Africa.

4 State Forestry, The Netherlands

\* corresponding author: e-mail: [Martijn2.Weterings@WUR.nl](mailto:Martijn2.Weterings@WUR.nl), [Martijn.Weterings@HVHL.nl](mailto:Martijn.Weterings@HVHL.nl); phone: +31582846333

<https://www.dlvadvies.nl/nieuws/hittestress-bij-melkkoeien-meten-is-weten/1856>

Introduction

The inquiry of how large ungulates survive the harsh winter has been a primary focus for numerous researchers in the northern hemisphere (e.g., Arnold et al., 2004; Ruf & Geiser, 2015). However, in contrast to staying warm, large-bodied ungulates have more difficulties in staying cool (Porter & Kearney, 2009; Riek & Geiser, 2013). Overheating (i.e., hyperthermia) as a result of a failure to regulate body temperature can have detrimental effects on the activity, physiology and fitness of endotherms (Gillooly et al., 2001; Lepock, 2003; Van Beest & Milner, 2013). Especially in hot conditions, large ungulates must navigate a delicate balance, as their thermal constrains interact with their need to collect resources and avoid predation (Veldhuis et al., 2019; Veldhuis et al., 2020; Peterson et al., 2022). This could be worrisome in the face of global warming (IPCC, 2023), in particular for large ungulates in open landscapes with limited possibilities to seek shade (Cain et al., 2006) or to use altitudinal shifts (e.g., Mason et al., 2017) to cool down. Consequently, there has been a shift in research focus towards examining the impacts of climate change on hyperthermia of species in temperate regions (e.g. see McCann et al., 2016; Mason et al., 2017).

While hot temperatures grow increasingly prevalent, the use and adaptation of thermoregulatory pathways of species will gain greater significance relative to other needs (Huey et al., 2012). In the face of high temperatures, large mammals usually maintain homeostasis through behavioural and metabolic adjustments. These adjustments (i.e. allostatic pathways) aid to dissipate or prevent accumulation of heat to keep the body temperature relatively stable (Ramsay & Woods, 2014) (figure 1). For example, mammals can activate evaporative cooling, seek locations that are wind-exposed or contain shade, increase heart rate and cardiovascular circulation or postpone the generation of heat (e.g. during feeding or physical exertion) to maintain homeostasis. However, activation of allostatic pathways can be costly in terms of energy and the use of water, which is why homeothermy can sometimes be discontinued (i.e., relaxed) in favor of heterothermy, especially during very hot conditions that are challenging for animals (Hetem et al., 2016). While homeothermic animals self-regulate their body temperature around a given set-point temperature, heterothermic animals allow the surrounding environment to affect their body temperature.

How to cope: allostatic pathways

Warmte vat dat energie opslaat en afgeeft.

Differences between night & day

Differences between seasons

In Singer paper -> heart rate above 70 is not reliable/accurate?

It is known that the body temperature of large ungulates varies during daily (i.e., nychthemeral) and seasonal cycles (Thompson et al., 2019), however, little is known about the underlying mechanisms (i.e. pathways) that keep the body temperature between acceptable limits during these cycles (Hetem et al., 2016). Therefore our goal was to gain insight into the differences in the use of thermoregulatory pathways by large-body sized ungulates during the day-night cycle, as well as during the seasonal cycle (figure 1, table 1).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Pathway | I | II | III | IV | V |
| Day | - | Allostatic | - | Allostatic | Allostatic |
| Night | Heterothermic | - | Heterothermic | Allostatic | Allostatic |
| Seasons | All, except winter | Summer | All, except winter | Summer | Summer |
| Type of relationship | Non-linear (+) | Linear (+/-) | Linear (+/-) | Linear (+) | Non-linear (+) |
| Hypothesis | 1 | 2 | 3 | 4 | 4 |



(a)

(b)

Figure 1: Overview of (a) Conceptual model of thermoregulatory pathways of large-endothermic ungulates, and (b) characteristics of pathways during day-night and seasonal cycles. \* Ta = ambient temperature; Tb = body temperature; HR = heart rate

First, we hypothesized that large-body sized ungulates use **allostatic pathways** to maintain their thermal homeostasis (stable body temperature) during the day to avoid hyperthermia, while they **relax these allostatic pathways** during the night allowing the ambient temperature to cool down the body temperature directly (i.e., heterothermy) (pathway I in figure 1; figure 2a). We expect this to happen during all seasons, except the winter period.

(a)

(b)

(c)

(d)

Time standing

Ta

Summer day

Time standing

Tb

Night

Day

Summer day

Ta or Time standing

HR

Ta

Tb

Night

Day

Figure 2: Hypothesized interaction between (a) ambient temperature (Ta) and day- and night-time on the body temperature (Tb); (b) ambient temperature during summer day time on time standing; (c) time standing and day- and night-time on the body temperature; (d) ambient temperature and time standing on heart rate (HR) during summer day time.

Second, we hypothesized that large-body sized ungulates use **allostatic** behavioural pathways during summer day time to maximize heat loss to avoid hyperthermia from an increasing ambient temperature. For example, as a response to increasing ambient temperatures, animals can increase the time spend standing, thus maximizing heat loss by the wind to maintain thermal homeostasis (pathway II; figure 2b).

Third, in contrast to day time, we hypothesized that large-body sized ungulates **relax allostatic** pathways during the night to directly cool down their body temperature using behaviour (i.e., heterothermy) (Cain et al., 2006) and simultaneously save energy and water (Hetem et al., 2016). For example animals can increase the time spend standing to directly cool down their body temperature during night time (pathway III; figure 2c). We expect this to happen during all seasons, except the winter period.

Fourth, we hypothesized that large-body-sized ungulates can use **allostatic** metabolic pathways to avoid hyperthermia from activation of behavioural pathways (pathway IV; figure 2d). For example, animals could increase their heart rate to activate the cardiovascular system to circulate blood and radiate heat to maintain thermal homeostasis when not at rest. Fifth, similarly to our fourth hypothesis, large-body-sized ungulates can use **allostatic** metabolic pathways to avoid hyperthermia from an increase in ambient temperature (pathway V; figure 2d). We expect both hypothesis four and five to happen mainly during the summer day.

Materials & methods

Study site

We investigated Heck cattle in the open landscape of the Oostvaardersplassen (52°26’N.5°19’E), a 5600 ha Natura 2000 nature reserve in the Netherlands (figure 3). The area has a maritime climate, with precipitation in all seasons and a mild winter and summer (KNMI, 2023a). The calcareous-sea-clay area is relatively low (i.e., -4 m NAP), flat and wet, with pioneering swamp vegetation (such as elder and willow), dry and wet grasslands. Since 1983, large-bodied grazers were introduced in the area to keep the area open (i.e., Heck cattle (*Bos taurus*), Konik horses (*Equus ferus caballus*) and red deer (*Cervus elaphus*)) (Kuil et al., 2015).

Chart, diagram

Description automatically generated

Figure 3: Overview of the layout of the Oostvaardersplassen and its vegetation types (vegetation data from 2016 collected by Stateforestry, map….)

Data sampling & collection

*Capturing and collaring*

Between November 2018 and March 2022, we studied nineteen female adult heck cattle from four different herds that were equipped with various sensors. Animals were sedated and immobilized by a vet using an air-powered gun with darts. Collars (1057 g ± 25.9 g (X + SD); 0.4 ± 0.02 % of animal bodyweight) were fitted to the smallest parts of the upper neck to avoid movement of the collar (Figure 4). A ruminal bolus with a temperature and heart rate sensor (Vectronic Aerospace GmbH) was placed into the pharynx with an applicator (see Turbill et al., 2011). On the lateral side of the Vectronic collar we attached a GPS and accelerometer sensor (SODAQ-ONE). At the end of the study period, we used the same capturing procedure to remove the collars. Animal handling was approved by the animals ethical committee of Wageningen University (No. 2017.W-0052.003).

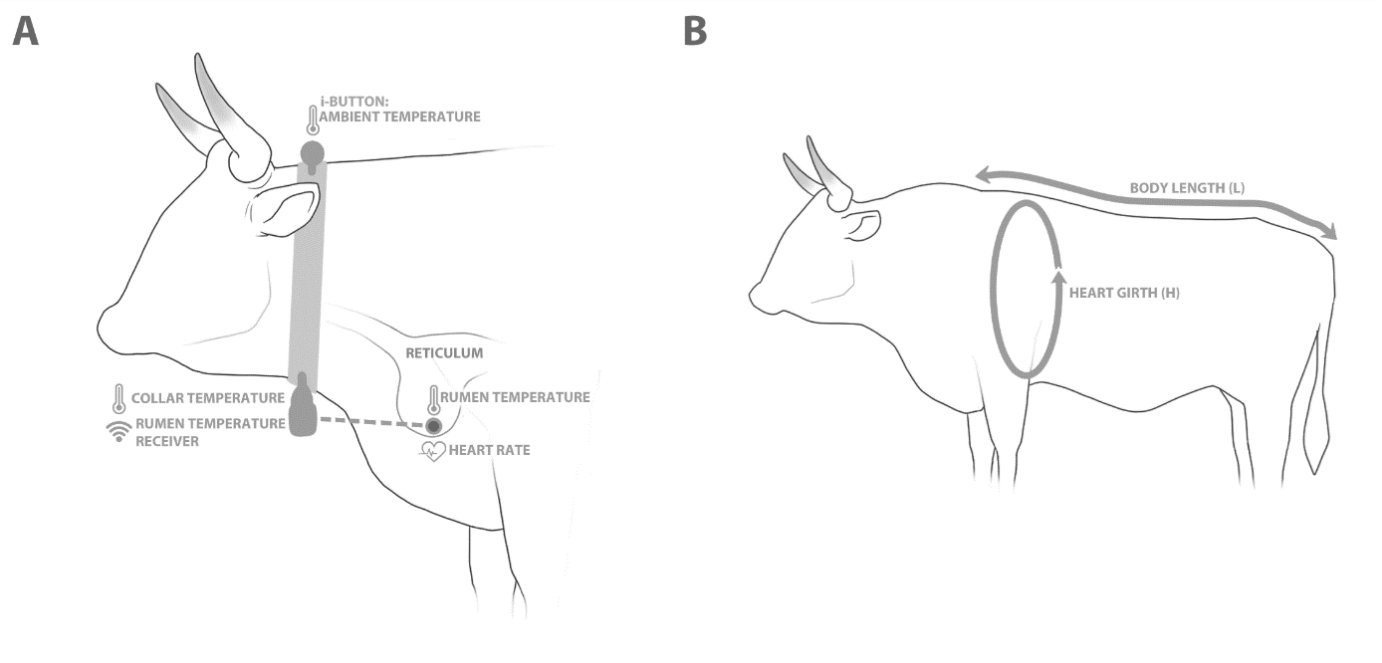


Figure 4: Collar design with (A) placements of ruminal sensor and receiver on Heck cattle (*Bos taurus*) and (B) body measurements used to estimate body mass (drawing by Bram van der Zalm, 2021)

*Ruminal sensor*

We measured rumen temperature (Tr) using a recalibrated thermistor in a ruminal sensor (22 x 80 mm cylinder, 100g) linked to a repeater unit positioned under the head of the animal (figure 4). The ruminal sensor also contained a bi-axial accelerometer, which measured acceleration caused by the beating of the heart and animal movement (Signer et al., 2010). Acceleration and temperature in the rumen were measured in three-minute intervals.

*Ambient temperature and weather characteristics*

An I-button (1-Wire, Maxim Integrated; www.maximintegrated.com) placed in a black metal globe on top of the collar (figure 4) recorded ambient temperature (Ta) in six-minute intervals. Additionally, we retrieved ambient temperature along with precipitation (mm) and wind speed (km/h) in hourly intervals from a weather station (KNMI, 2023b) approximately five kilometers away from the study site.

Data preparation (review text)

To identify gaps in the sensor data, we merged sensor data of individual Heck cattle with a database that included a time sequence of three-minute intervals spanning the data collection in the field. The true body temperature was approximated by the temperature measured in the rumen, after filtering out ‘spikes’ (i.e., abrupt changes) in the rumen temperature. Positive spikes in rumen temperature are typically caused by microbial fermentation and other digestive processes (Barnes, 1983; Dehority, 2003; Cunningham et al., 1964; Beatty et al. 2008), whereas negative spikes may result from short-term events such as the intake of cold food and water (Brod et al., 1982; Dale et al., 1954; Cunningham et al., 1964; Crater & Barboza, 2007). We identified spikes using reference values created by the de-spike function in the OCE package (Kelly & Kelly, 2018) in R. Gaps in the time series of each animal were linearly interpolated by a running median using 61 three-minute intervals (i.e., a timespan of 3 hours). We then calculated the standard deviation between the observed and the reference values and replaced observed values with the reference values whenever they differed by at least half-a-standard deviation (Cunningham et al., 1964; Gengler et al., 1970, Lees et al., 2019).

Heart rate was recorded using a biaxial accelerometer located it the ruminal unit which detected mechanical shockwaves. The raw heart rate data was filtered by a hand-passed filter at cut-off frequencies of 5–50 Hz (Signer et al., 2010) and processed towards beats per minute (BPM) using the web application (<https://rumina.fiwi.at>) (see Signer et al., 2010 for details).

We distinguished between an animal laying on the ground (i.e., at rest) and animals standing (i.e., any other active behaviour, but standing) using the activity measurements collected from the ruminal unit. Heck cattle behaviour was classified using logistic regression (Signer et al., 2010). Standing behaviour therefore resembles a raw index of locomotion, indicating the percentage of time spent standing per observation.

To address the high degree of temporal autocorrelation between samples, we separated the data in different day phases and seasons. Day phases (dusk, dawn, day, night) were retrieved using the suncalc package in R (Thieurmel et al., 2019). However, we only included day and night phases in the analysis for reasons of simplicity and because crepuscular periods did not have sufficient data. We calculate the mean body temperature, heart rate, activity, and collar temperature for every individual per day and night phases per season.

To control for individual differences among animals, we approximated body mass based on field-measurements of the heart girth circumference behind the front leg and the body length (figure 4b) using Wangchuk et al. (2018) (Eq.1).

Eq.1: Body mass (kg) = (heart girth × body length) / Y

Where Y = 9.0 (if heart girth < 165.2 cm)

= 8.5 (if 165.2 > heart girth < 203.2)

= 8.0 (if heart girth > 203.2)

Data analysis

We used structural equation modeling (SEM) to investigate the collective indirect causal pathway connecting ambient temperature, behaviour, heart rate, and body temperature (Fan et al., 2016). The SEM model illustrated in figure 1 served as the foundation for our research. However, as our data encompassed random effects and temporal autocorrelation, which cannot be adequately addressed using conventional SEM techniques, we employed a piecewise approach (Lefcheck, 2016). Given our expectation that the pathways would vary across the four seasons and between day and night within each season (figure 1), we created eight distinct SEM models. For each of these models, we fitted separate linear mixed-effects models that accounted for random intercepts and temporal autocorrelation at the individual level. These individual models were subsequently integrated into a SEM framework.

For the heart rate and body temperature models, we examined the effects of five predictors: ambient temperature and behaviour (i.e., focus variables), as well as day of season, weight of the animal, and year (i.e., control variables). To account for potential non-linear relationships, we included quadratic effects for ambient temperature, behaviour, and day of season in these models.

In the behavioural model, we considered four predictors: the focus variable ambient temperature and the control variables day of season, weight of the animal, and year. Similarly, we incorporated quadratic effects for ambient temperature, and day of season to capture any non-linear associations.

To examine the relationships between the independent variables and the response variable, we employed Linear Mixed Models (LMMs) using the lme function of the "nlme" package in R (Pinheiro et al., 2014). To address the individual differences among study animals within each season, we incorporated random intercepts for each unique animal ID in all our models. Furthermore, we considered the temporal aspect of our data by incorporating a serial correlation structure. This structure accounted for the likelihood that measurements taken closely in time on the same animal would exhibit a higher level of correlation compared to measurements taken further apart. Given that there were gaps between the different time points, we adopted a Gaussian spatial correlation structure over time to effectively represent the serial correlation structure.

For each global model, we assessed whether including a random slope for ambient temperature, day of season and, if applicable, for behaviour would improve the model fit. This decision was based on our preliminary data exploration, which revealed that the effects of these variables could vary across individual animals. By including these random slopes, we aimed to capture the individual-specific effects of these variables on our outcome variables. We compared the Akaike Information Criterion (AIC) values between models with and without the random slopes. If the difference in AIC was greater than 2 points, it was considered indicative of a significant improvement in model fit. Conversely, if the difference in AIC was less than 2 points, the models were considered to be essentially equivalent, and we favored selecting the more parsimonious model (Burnham & Anderson, 2002; Zuur et al., 2007).

To determine the optimal model, we utilized a stepwise selection procedure with the StepAIC function from the "mass" package in R (Venables & Ripley, 2002). This function allowed us to iteratively adjust the model by adding or removing variables, while evaluating the AIC value as a measure of model fit. The aim was to obtain the model with the lowest AIC value, indicating the best balance between explanatory power and model complexity. Throughout the selection procedure, the focus variables were retained in all models. In the final model, we assessed the contribution of the quadratic terms and control variables by comparing the AIC values. If their inclusion did not improve the AIC value by at least 2 points, we removed them from the final model. This approach ensured that only the most informative and parsimonious variables were retained, enhancing the interpretability and effectiveness of our model. Parametric assumptions for the use of a linear mixed models were met.

To integrate the final models for heart rate, body temperature, and behaviour into a unified structural equation model (SEM), we employed the psem function from the "piecewiseSEM" package in R (Lefcheck, 2016). In addition to model integration, the psem function conducted Shipley's test of directed separation. This test assessed whether there were any missing relationships that could potentially exist but were not included in the initial pathway diagram. Furthermore, the psem function enabled the calculation of standardized path coefficients. These coefficients quantified the strength and direction of the relationships between variables within the SEM. By standardizing the path coefficients, we obtained a consistent measure of the magnitude of the effects, facilitating meaningful comparisons across different variables.

Discussion

Is the heck cattle homothermic or heterothermic (Hetem et al., 2016) (also present in results?).

When ambient temperature exceeds body core temperature, evaporative cooling provides the only mechanism to dissipate metabolic (internally generated) and environmental (externally intercepted) heat from an animal’s body. (Hetem et al., 2016)

When water is scarce evaporative cooling is reduced, and the body temperature rises (Hetem et al., 2016, p.198)

Heterothermic during the night, allows to save energy and does not increases the risk of pathological hyperthermia (Hetem et al., 2016, p.200)

Acknowledgements

We are grateful to Nicole Barten, Eline Dierkx, Gabi Keurntjes, Clara Köhler, Nanouk de Leng, Yorick Liefting, Eline van den Nieuwenhof, Mirjam Smits and Alana Stoker for field assistance, and Itay Dagan, Javier Ferreira Gonzalez, Peter Ebben, Jan van Loenen and Jaap de Winter for their help with soft- and hardware systems. We are indebted to Tanja de Bode, Tjibbe Hunink, Hans-Erik Kuypers, Henk Luten, Jakko Moleman, Iris Sen, Leo Smits, Bas Steltenpool, Erik de Vries and Ijsbrand Zwart that assisted us with the logistics and capturing of the Heck cattle, and Staatsbosbeheer for access to the study area. This study was funded by Nationaal Regieorgaan Praktijkgericht Onderzoek SIA (SVB/RAAK.PRO 02.048 to MaW), Van Hall Larenstein University of Applied Sciences and Wageningen University.

Author Contributions

Conception and design: MaW, MeW and HK; acquisition of data: MaW, MeW, FvL and PC; analysis and interpretation of data: MaW, MeW and HK; manuscript: MaW, MeW, FvL, and HK. All authors read, reviewed and approved the final manuscript.

Conflict of Interest

The authors declare they have no conflict of interest.

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