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Faculty of Applied Ecology, Agricultural Sciences and Biotechnology

**Anne Randi Græsli**

## **Ecophysiology of moose**

**Basic physiology and responses to stressors**

PhD Applied Ecology and Biotechnology  
2022



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Basic physiology and responses to stressors



PhD Thesis

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**Photo front page:**

Moose aa\_ac\_17\_018 from the Northern coastal study area. Photo: Fredrik Stenbacka



# Sammendrag

Hvordan dyr responderer på menneskelig aktivitet og klimaforandringer, er nøkkelspørsmål i økologisk forskning. Utviklingen av biloggere og mulighetene til å kombinere forskjellige typer sensorer har gjort det mulig å undersøke slik påvirkning av viltlevende dyr, ved at de gir oss detaljert informasjon over en lengere tidsperiode. I denne avhandlingen fokuserte jeg på elg (*Alces alces*), en viktig planteeter i det nordlige økosystemet, på grunn av sin økologiske, kulturelle og økonomiske betydning. Å forstå elgens økofysiologi og påvirkningen av menneskelig aktivitet og klimaforandringer både på individuelt og populasjonsnivå er nødvendig for en bærekraftig forvaltning av elgstammen.

Jeg brukte biloggere for å samle kroppstemperatur-, hjerterefrekvens-, bevegelses- og aktivitetsdata for å studere basal fysiologi og fysiologiske og atferdsmessige reaksjoner hos elg på menneskelig aktivitet. Studiepopulasjonen bestod av 30 viltlevende voksne elgkyr ( $\geq 1,5$  år) i tre forskjellige studieområder i Sverige fra 56-67°N. Jeg undersøkte hvordan kroppstemperatur, hjerterefrekvens og aktivitet endret seg i løpet av året og døgnet, samt temperaturforandringer i forbindelse med drektighet og kalving. Denne kunnskapen brukte jeg videre til å evaluere de fysiologiske og atferdsmessige reaksjonene hos elg før, under og etter to forskjellige typer menneskelig aktivitet, løshundjakt og immobilisering fra helikopter.

Jeg dokumenterte sesongmessig hypometabolisme med lavere kroppstemperatur, hjerterefrekvens og aktivitet om vinteren sammenlignet med sommeren, noe som representerer en 60% nedgang i metabolismen. Jeg fant også høyere kroppstemperatur hos drektige kontra ikke-drektige elgkyr, samt et fall i kroppstemperatur og aktivitet omkring kalving. Menneskelig aktivitet økte elgens energiforbruk, representert med økt aktivitet, kroppstemperatur, hjerterefrekvens og lengere tilbakelagt distanse samt en nedgang i hviletid den dagen aktiviteten pågikk, sammenlignet med en referanseperiode før aktiviteten samt tiden etterpå. Responsen var avhengig av aktivitetens varighet, og jeg dokumenterte individuelle variasjoner i responsen hos elgene.

Alt i alt har denne avhandlingen, forbedret vår forståelse av elgens basalfysiologi samt hvordan elgen påvirkes av menneskelig aktivitet. Avhandlingen danner et viktig fundament videre forskning, forvaltning og bevaring av elgstammen.



# Abstract

How animals respond to human activity and a changing environment are key questions in ecological research. Recent advancements in biologging techniques and the ability to combine different types of biologgers have enabled us to investigate such impacts on free-ranging animals by providing detailed information covering longer periods of time. In this thesis, I focus on moose (*Alces alces*), a key herbivore in the northern ecosystem due to its ecological, cultural and economic importance. Understanding the ecophysiology of the moose, and the impact of human activity and climatic changes on both individual and population levels is important for sustainable management of the moose population.

I used biologgers to obtain body temperature ( $T_b$ ), heart rate (HR), movement and activity data to study the basic physiology and the physiological and behavioural responses of moose to human activity. The study population consisted of 30 free-ranging adult female moose ( $\geq 1.5$  years old) in three different study areas in Sweden ranging from 56-67°N. I investigated the annual and circadian cycle of  $T_b$ , HR and activity, and the thermal patterns during pregnancy and parturition in the basic physiology part. I further used this knowledge to evaluate the physiological and behavioural responses of moose before, during and after two different human activities; hunting with baying dogs and helicopter-based chemical immobilisations.

I documented seasonal hypometabolism with lowered  $T_b$ , HR and activity in winter compared to summer, representing a 60 % decrease in metabolic rate. I also found higher  $T_b$  in pregnant compared to non-pregnant female moose and a drop in the  $T_b$  and activity of the moose when parturition was imminent. Human activity increased the energy expenditure of the moose, represented by increased  $T_b$ , movement, activity and HR and a decreased resting time during the day of the approach compared to a reference period before the approaches and the period after the approaches. The responses were dependent on the duration of the activity and I documented individual variations in the responses among the moose.

Overall, this thesis improves our understanding of the basic physiology of moose and their responses to human activity. The thesis provides an important foundation for further research, management and conservation of moose.





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## List of papers

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**Græsli, A.R.**, Thiel, A., Fuchs, B., Singh, N.J., Stenbacka, F., Ericsson, G., Neumann, W., Arnemo, J.M., Evans, A.L. (2020) Seasonal hypometabolism in female moose. *Frontiers in Ecology and Evolution* 8:107. <https://doi.org/10.3389/fevo.2020.00107>

### *Paper II:*

**Græsli, A.R.**, Thiel, A., Fuchs, B., Stenbacka, F., Neumann, W., Malmsten, J., Singh, N.J., Ericsson, G., Arnemo, J.M., Evans, A.L. (2022) Body temperature patterns during pregnancy and parturition in moose. *Journal of Thermal Biology* 109:103334. <https://10.1016/j.jtherbio.2022.103334>

### *Paper III:*

**Græsli, A.R.**, Le Grand, L., Thiel, A., Fuchs, B., Devineau, O., Stenbacka, F., Neumann, W., Ericsson, G., Singh, N.J., Laske, T.G., Beumer, L.T., Arnemo, J.M., Evans, A.L. (2020) Physiological and behavioural responses of moose to hunting with dogs. *Conservation Physiology* 8(1):coaa122. <https://doi.org/10.1093/conphys/coaa122>

### *Paper IV:*

**Græsli, A.R.**, Thiel, A., Beumer, L.T., Fuchs, B., Stenbacka, F., Neumann, W., Singh, N.J., Ericsson, G., Arnemo, J.M., Evans, A.L. Thermal and behavioural responses of moose to helicopter-based immobilisations. *Submitted*.



# Introduction

Humans are the most dominant mammal species on the planet, and the major source of global environmental change and wildlife mortality. The coexistence of humans and wildlife is, therefore, crucial for the survival of the wildlife (Nyhus 2016). As part of wildlife conservation and management strategies, it is important to understand how human activity directly, indirectly, and through cascading effects, affects animals' ecology, physiology and behaviour (Wikelski and Cooke 2006). The recent advantages in biologging technique have enabled us to obtain detailed physiological and behavioural data from free-ranging animals, which can be used to study the impact of both human activity and global climate change on wildlife species.

This thesis aimed to use biologgers to study the basic physiology of moose (*Alces alces*), a key herbivore in northern ecosystems and a hunted species. This knowledge was further used to improve our understanding of the physiological and behavioural responses of the moose to two different types of human activity; hunting with baying dogs and helicopter-based chemical immobilisations.

## Studying physiology with biologging

The term "Bio-logging" was proposed in 2003 and defined as the "investigation of phenomena in or around free-ranging organisms that are beyond the boundary of our visibility or experience" (Boyd et al. 2004). Biologgers have traditionally been used to document athletic feats of animals, but recently there has been a rapid development of biologgers obtaining physiological data (Hawkes et al. 2021). The recent advancements in biologging techniques have enabled us to obtain unique information about the interplay between physiological and behavioural processes of free-ranging animals, and the interaction of animals with the environment, other animals, and reactions to human activities (Hawkes et al. 2021; Rutz and Hays 2009). However, to evaluate how the animals are affected, we first need to determine the normal levels of the physiological and behavioural parameters we want to study, under undisturbed conditions.

## Part I: Basic physiology of moose

Moose, the largest cervid species in the world, are ecologically, economically and culturally important. They typically inhabit boreal forests and temperate broadleaf and mixed forests in

temperate to subarctic climates of the northern hemisphere in Eurasia and North America. We find the highest moose population densities in the world in Fennoscandia, and the estimated post-harvest moose population in Fennoscandia in 2010 were 468,200 individuals (265,000 Sweden, 95,800 Finland and 107,400 Norway) (Jensen et al. 2020). The large size and high densities of moose contribute to their significant impact on the ecosystem. They may affect the ecosystem processes, especially by their feeding, but also through movement, defecation, urination and carcasses (Jensen et al. 2020). Moose are selective browsers, and their selectivity can affect the succession rate in their habitats. Habitat selection of the moose is dependent on various factors like food availability and quality, weather conditions (i.e. snow depth, precipitation, ambient temperature), predation risk, season, and human activities (Niedziałkowska et al. 2022). Scandinavian moose have a more diverse and nutritious diet in the vegetation season compared to the winter with more limited food resources available (Wam and Hjeljord 2010). The food quality in the summer range is, therefore, a major factor governing the body growth of moose (Wam and Hjeljord 2010). Morphological characteristics and body mass of the moose vary among areas, depending mainly on the environmental conditions, but also on the genetic background of the moose (Niedziałkowska et al. 2022).

Hypometabolism (a reduction in metabolic rate) has been documented as a strategy to cope with limited energy supplies and a colder climate during winter in a variety of both captive and free-ranging ungulates living in temperate climates, including moose (Arnold et al. 2006; Arnold et al. 2018; Arnold et al. 2004; Kochan 2007; Regelin et al. 1985; Riek et al. 2019; Signer et al. 2011; Turbill et al. 2011). Moose are known to tolerate cold temperatures better than warm and are easily heat stressed with rising heart rate (HR), respiration rate and energy expenditure with increasing ambient temperatures (McCann et al. 2013; Renecker and Hudson 1986). The ambient temperature alone cannot determine the threshold for heat stress, as other factors including wind speed, solar radiation, winter fur, physiological differences and immune status affect the threshold (McCann et al. 2013; Thompson et al. 2019). Increasing and fluctuating ambient temperature due to global climate change is an important concern regarding higher energetic costs, changes in habitat, food availability and quality (Beatty et al. 2008; Boyles et al. 2011; Monteith et al. 2015). This can cumulatively result in loss of body mass and following increased infection risk, lower reproduction and

survival rate (Lenarz et al. 2009; McCann et al. 2013; van Beest and Milner 2013; van Beest et al. 2012).

Moose are capital breeders, relying their reproduction on stored resources. Fecundity, recruitment and population growth rates in capital breeders are dependent on climatic factors like ambient temperature, precipitation, snow depth, and food availability and quality (Allen et al., 2017; Holmes et al., 2021). Moose are polyoestral seasonal breeders, breeding from September-November, depending on the latitude (Malmsten et al. 2014; Niedziałkowska et al. 2022). The mean reported gestation length is 231 days, resulting in a calving period in May and June (Markgren 1969; Neumann et al. 2020; Schwartz 1992; Schwartz and Hundertmark 1993). Large parts of the gestation period (up to three-fourths), therefore, overlap with the food-scarce period in winter with harsh climate and limited forage resources available, particularly at northern latitudes (Ericsson et al. 2001; Markgren 1969; Schwartz and Hundertmark 1993). The vegetation period is the time when moose build up their body reserves before the rut and the winter. As a possible strategy to utilize the short vegetation season, the timing of calving in relation to vegetation onset depends on latitude (Neumann et al., 2020). Moose in southern Sweden give birth after the onset of the vegetation period, whereas moose in the north ( $>64^{\circ}\text{N}$ ) give birth before. Reproduction, and especially parturition and lactation are energy consuming, and maintaining foetal homeothermy is a high priority for pregnant individuals (Laburn et al. 2002; Speakman 2008). Alterations in maternal  $T_b$  due to alterations in ambient temperature, exercise and stress require thermoregulatory strategies to maintain the foetal homeothermy. Changes in the maternal  $T_b$  result in disturbance of the foetal  $T_b$ , but how much the foetal  $T_b$  deviates from normal can be reduced by alterations in the thermal conductance over the placenta (i.e. changes in umbilical and/or uterine blood flow) or by foetal heat production (Laburn et al. 2002). Keeping a stable foetal  $T_b$  despite alternating maternal  $T_b$ , therefore, results in costs for both mother and foetus, as increases in blood flow require an increase in the cardiac output of the mother or foetus, while decreases in blood flow can compromise foetal oxygenation, nutrition and disposal of metabolic waste over the placenta (Laburn et al. 2002).

Warm and dry spring/summers result in reduced food availability and quality, reduced forage intake due to increased time resting in thermal shelters, in addition to the loss of energy due to increased respiratory rate, HR and metabolic rate due to heat stress (Holmes et al., 2021;



McCann et al., 2013; Monteith et al., 2015; Renecker and Hudson, 1986). This results in negative energy budgets for the moose and is important concerning reproductive rates and survival rates of calves (Holmes et al., 2021). There are also possible lagged effects of climate and/or insufficient nutritional intake affecting birth mass or fecundity in the following years (Parker et al., 2009). Variability in ambient temperatures and the weather trends in general following global climate change is therefore important in regard to overall population survival (Boyles et al., 2011).

Previous studies of moose physiology are limited with respect to time and number of moose and were usually done with anaesthetised or captive moose (Evans et al. 2012; Langvatn 1992; Renecker and Hudson 1985; Roshchevsky et al. 1999). The recent introduction of minimally invasive biologgers with high battery capacity has enabled long-term studies (>1 year) of physiological parameters like  $T_b$  and HR (Rutz and Hays 2009). The number of physiological studies evaluating simultaneous measurements of  $T_b$  and HR obtained by biologgers, in ungulates living in harsh climates is currently increasing (Arnold et al. 2006; Arnold et al. 2018; Riek et al. 2019; Signer et al. 2011; Turbill et al. 2011). Studies about the basic physiology and the physiological ability of the moose to respond to rising ambient temperatures (that are likely to come with changing climatic conditions) are important due to the ecologic, economic and cultural importance of the moose. In the first part of my thesis, I evaluated the seasonal and circadian patterns of  $T_b$ , HR and activity of moose, and the thermal patterns during pregnancy and parturition in moose.

## **Part II: Human-induced landscape of fear**

How the physiology and behaviour of free-ranging animals are affected by human activity, is a key question in wildlife research. Human disturbance may induce both immediate and long-term stress responses in an animal. Stress is a response to challenges in the body's homeostasis, and can be positive or negative – depending on the situation (McLaren et al. 2007; Moberg 2000; Reeder and Kramer 2005). A stressor is a stimulus or condition causing stress, and it could be physical, psychological or both (Reeder and Kramer 2005). The physical stressors are internal stimuli to the animal like anoxia or hypoglycaemia or external stimuli like exercise, injury or heat/cold exposure while eliciting fear, anxiety, anger or frustration are examples of psychological stressors (Reeder and Kramer 2005). The sympathetic nervous system activates immediately as a response to a stressor resulting in the secretion of

noradrenaline and adrenaline, thus increasing arousal, elevating HR, and providing energy to deal with the stressor (by promoting glycogenolysis and lipolysis) (Reeder and Kramer 2005). The hypothalamic-pituitary-adrenal (HPA) axis activates simultaneously with the sympathetic nervous system, but more slowly and with a longer lasting effect. Vasopressin and corticotrophin-releasing hormone are secreted as a response to the activation of the HPA-axis, resulting in increased secretion of adrenocorticotrophic hormone (ACTH) and thereby glucocorticoids (cortisol or corticosterone) (Reeder and Kramer 2005). Long-term exposure to stress can impair the growth, reproduction and immune function in animals since activation of the HPA-axis diverts energy away from other functions in the body (Moberg 2000). Stress results in physiological and behavioural responses of the animal depending on the situation, and objective and quantitative measurements of stress are often used to assess animal welfare (McLaren et al., 2007).

Human disturbance can affect population dynamics, causing animals to change their behaviour to a more “anti-predator behaviour” (Frid and Dill 2002). Anti-predator behaviour is an adjustment of the behaviour of the prey to predators, including humans. The indirect cost of anti-predator behaviour is often referred to as the “landscape of fear” (Brown et al. 1999). In a review, Bleicher (2017) describes the landscape of fear as “a short-term measure of the way the animal perceives its environment based on the cost-benefit analysis of the trade-off for food and safety associated with foraging in specific areas of the habitat available to it”. It is affected by different variables like predation risk, the energetic-state of the individual, inter- and intra-specific competition or the evolutionary history of the species in addition to geographical variables (Bleicher 2017). Studies regarding anti-predator behaviour have become increasingly important in both prey and predator species, and anti-predator behaviour is usually investigated from analyses of movement and activity data (Frid and Dill 2002). Stay, fight or flight when attacked, changes in home range area, and alteration of time allocated to different activities like resting, vigilance and foraging, are examples of anti-predator strategies. There are several studies documenting behavioural responses to human activity among northern ungulates species, including moose (Baskin et al. 2004; Ericsson et al. 2015b; Neumann et al. 2009, 2010, 2011a, 2011b; Sand et al. 2016; Støen et al. 2010), roe deer (*Capreolus capreolus*) (Benhaiem et al. 2008; Cederlund and Kjellander 1991), and red deer (*Cervus elaphus*) (Jarnemo and Wikenros 2014; Sunde et al. 2009).

The literature on the physiological effects of human disturbance on wild mammals is in contrast, scarce. Physiological parameters like  $T_b$ , HR, changes in body mass, cortisol levels in blood, hair and faeces, and immunological and blood parameters have traditionally been used to assess physiological stress responses in animals (McLaren et al. 2007). The main problem up to now has been to obtain reliable and sufficient information, but biologgers have been a door opener concerning this type of studies. The combined use of multiple biologgers provides a more thorough insight into the interaction between the physiological and behavioural processes of an animal. This is of high importance, as there are documented pronounced physiological responses in the absence of behavioural responses in animals in relation to human disturbance (Ditmer et al. 2015). High-resolution  $T_b$  and/or HR obtained by biologgers have been used to assess stress responses of free-ranging moose, brown bears (*Ursus arctos*), and American black bears (*Ursus americanus*) to human activities (Ditmer et al. 2015; Le Grand et al. 2019; Støen et al. 2015; Thompson et al. 2020). In part two of this thesis, I aimed to evaluate the impact of the human-induced landscape of fear in moose. I, therefore, evaluated the physiological and behavioural effects of moose on two different human activities, hunting and helicopter-based chemical immobilisations.

#### Hunting with baying dogs

Hunting is the cause of more than 80 % of moose mortality in Sweden, and the most common hunting form is hunting with baying dogs (Ball et al. 1999; Ericsson and Wallin 2001; Heberlein 2000). Around 110,000 moose are harvested in Scandinavia every year during the annual moose hunt (80,000 in Sweden and 30,000 in Norway). The hunting season in Sweden starts at the beginning of September or October and lasts for 5 to 6 months, depending on local regulations (Lavsund et al. 2003; Länsstyrelserna 2020). Concerns about animal welfare and stress in relation to hunting have been raised (Nelson et al. 2005; Paquet and Darimont 2010), but the data to address these issues are scarce.

Increased activity and movement and moose leaving the area after the disturbance, are behavioural effects documented in relation to hunting with baying dogs (Ericsson et al. 2015b; Sand et al. 2016). This is the first study evaluating the physiological responses of moose to hunting with baying dogs, and therefore also the first study evaluating the combination of physiological and behavioural responses. To measure and determine the impact of hunting

disturbance on moose, I evaluated the physiological and behavioural responses of moose to simulated hunting situations with baying dogs (hereafter approaches).

### Helicopter-based chemical immobilisations

Research on free-ranging wildlife often requires chemical capture of individuals for collaring, sample collection and health examination. For researchers and managers, it is important to use methods with the least amount of harm as possible, to minimise the stress and risk of adverse effects, and to evaluate their work afterwards. The preferred capture technique for remote populations of free-ranging moose is chemical immobilisation from a helicopter using a CO<sub>2</sub>-driven drug delivery system with a potent opioid or an opioid in combination with a sedative (Kreeger and Arnemo 2018). Etorphine has been the drug of choice in Scandinavia, either alone or in combination with xylazine and sometimes acepromazine (Evans et al. 2012; Lian et al. 2014). Etorphine alone has a high safety margin and is an effective drug for moose immobilisations (Arnemo et al. 2003), and when adding xylazine (an alpha-2 adrenoceptor agonist) to etorphine, the combination gives a synergistic effect and the dose of etorphine can be reduced. Opioids and alpha-2 adrenoceptor agonists are both respiratory depressants, and alpha-2 adrenoceptor agonists have cardiovascular side effects like hypotension and bradycardia and decreased gastrointestinal motility (Kreeger and Arnemo 2018).

Anaesthesia always comes with a risk of morbidity and mortality, even in healthy animals (Hampton and Arnemo 2022). Studies evaluating the adverse effects of immobilisations of moose have traditionally reported mortality rates, but in recent years, more studies have evaluated the non-lethal adverse effects including physiological and behavioural effects (Barros et al. 2018; Evans et al. 2012; Haga et al. 2009; Lian et al. 2014; Neumann et al. 2011b; Thompson et al. 2020). Increased activity and movement, hyperthermia, hypoxemia, hypercapnia and acidemia, and moving into more rugged terrain are examples of documented physiological and behavioural responses of moose to helicopter-based chemical immobilisations (Barros et al. 2018; Evans et al. 2012; Neumann et al. 2011b; Thompson et al. 2020). I used a combination of thermal and behavioural parameters to evaluate the short-term effect of helicopter-based chemical immobilisations on moose.

## Objectives

This PhD was divided into two parts, one concerning the basic physiology of moose and one concerning the human-induced landscape of fear of moose. The results from the basic physiology were used as a reference for the stress physiology part. The objectives of this PhD were:

1. To study the annual and circadian rhythms of heart rate and body temperature in moose, in relation to activity and ambient temperature
2. To determine if moose display a thermal pattern during pregnancy and parturition characteristic for domesticated ruminants
3. To evaluate whether calving in moose could be identified based on thermal and activity data
4. To investigate physiological and behavioural responses of moose to simulated hunting situations with baying dogs
5. To investigate the thermal and behavioural responses of moose to chemical immobilisation from a helicopter

# Material and Methods

## Study areas

The study was mainly conducted in a Northern coastal area in Sweden, hereafter the Northern coastal area, in the Nordmaling municipality and the surrounding municipalities Umeå and Vännäs in the county of Västerbotten (Paper I - IV). In addition, data from moose in two different areas in Sweden, a Northern alpine area and a Southern island area were included in Paper II. The study areas ranged from 56-67° N latitude (see Figure 1). The Northern alpine area, (Nikkaluokta) is located in a high alpine area of northern inland Sweden (67°N), the Northern coastal area (Nordmaling) is located on the northern coast, and the Southern island area is an island, Öland, located in southeast Sweden (56°N).

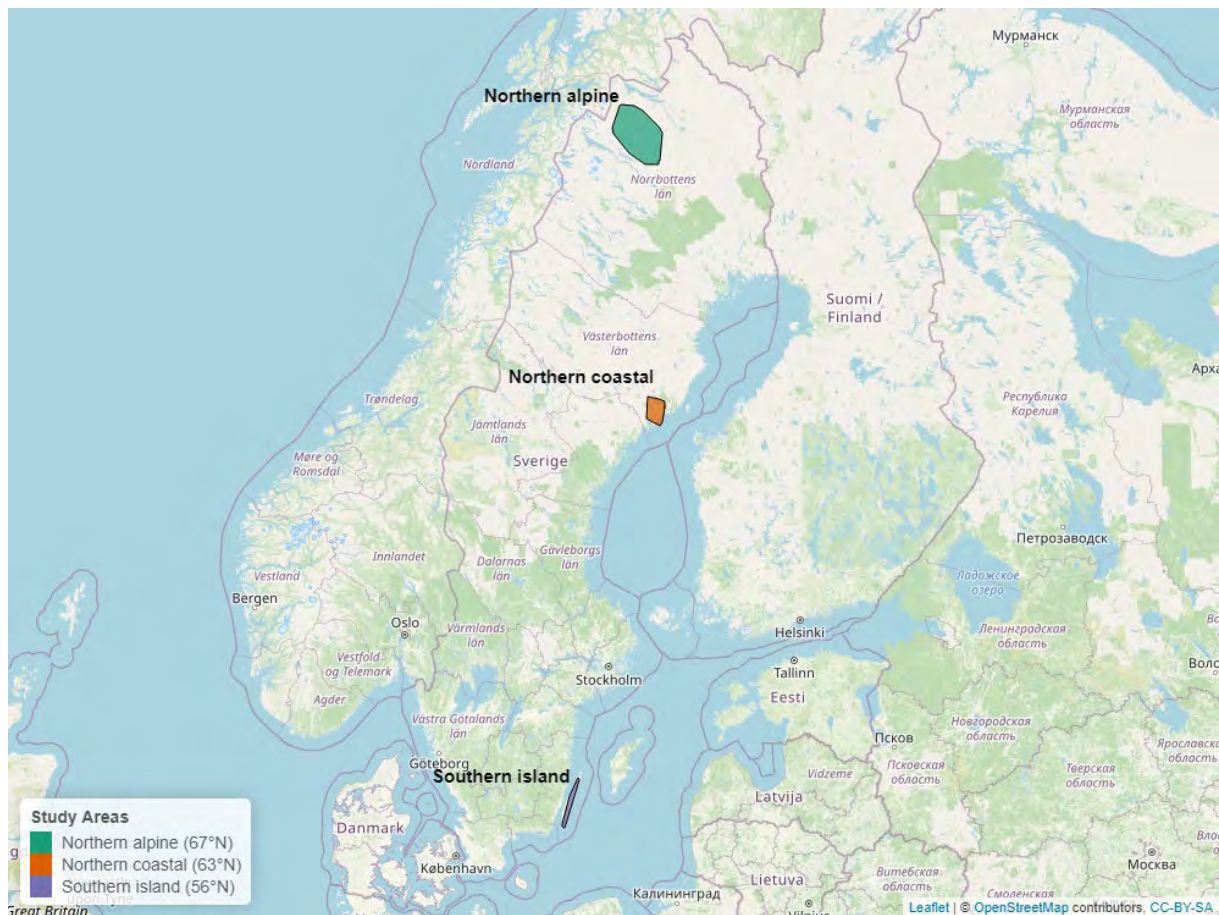


Figure 1 Overview of the study areas

The main study area, the Northern coastal, is characterised by boreal forests dominated by Scots pine (*Pinus silvestris*), Norway spruce (*Picea abies*) and birches (*Betula pendula* and *B. pubescens*). The yearly average ambient temperature is 3°C and there are >150 days with snow

cover from November to May. The mean and maximum daily snow depth from 1 November to 30 April was 0.15 and 0.5 m in 2016–2017 and 0.53 and 0.98 m in 2017–2018 (SMHI 2019). The length of the vegetation season is between 150 and 160 days from late April to mid-October (SMHI 2019).

The Northern alpine area is a mountainous area characterised by tundra vegetation and a mountain birch (*Betula pubescens*) belt. The area is covered by snow for around 200 days from the beginning of October to mid-May, and the yearly average ambient temperature is -1°C (SMHI 2019). The vegetation season starts in June and lasts 100-120 days.

The Southern island area is dominated by agricultural land, alvar grassland and forest-associated habitats. The forest stands are dominated by Scots pine and birch. The yearly average ambient temperature is 8°C, and the length of the vegetation season is between 210 and 220 days from April to November (SMHI 2019).

### Study animals

A total of 30 female moose (>1.5 years old) in three different study areas; Northern alpine (2012,  $n=10$ ), Northern coastal (2017,  $n=12$ ) and Southern island (2015,  $n=8$ ), were included in this project (Figure 2).



Figure 2 Moose aa\_ac\_17\_011 from the Northern coastal study area. Photo: Fredrik Stenbacka

Not all moose were included in all studies due to different reasons (Paper I:  $n = 12$ , Paper II:  $n = 30$ , Paper III:  $n = 8$ , Paper IV:  $n = 10$ ). The projects included in this thesis were approved by the Regional Animal Ethics Committee for Northern Sweden in Umeå (Dnr A116-09, A12-12, A50-12, A205-12, A14-15, A3-16, A28-17), and were conducted following Swedish laws concerning animal research ethics.

For the hunting approaches (Paper III) we used a total of 12 baying dogs of different breeds frequently used for moose hunting (Norwegian Elkhound:  $n = 4$ , Jämthund:  $n = 3$ , other breeds:  $n = 5$ ).

## Biologgers

### GPS-collars (Paper I-IV)

All moose were fitted with GPS Plus Collars (former version of VERTEX Plus) including a global positioning system (GPS) receiver, an ambient temperature recorder, a very high frequency (VHF) transmitter, a global system for mobile (GSM) communication modem or IRIDIUM communication, and an acceleration sensor (Vectronic Aerospace GmbH, Berlin, Germany) (VectronicAerospace 2021). We scheduled the collars to record GPS positions from 1 min to 3 h depending on the ongoing studies. The collars used the GSM cell phone network or Iridium satellite communication to send the GPS position together with the most recently recorded ambient temperature to a database server, WRAM (Wireless Remote Animal Movement), for storage (Dettki et al. 2004). The acceleration sensor integrated in the collar measured acceleration (hereafter referred to as “activity”) in three orthogonal directions six to eight times per second (6-8 Hz). For each direction, activity values were averaged for a recording interval of 5 min. The ambient temperature was recorded every 5 min, and to represent a reliable index for the actual ambient temperatures the collar-derived temperature was adjusted according to earlier described offsets (Ericsson et al. 2015a). In addition, each collar was linked to external sensors, i.e. ruminal temperature loggers and proximity sensors UHF-ID tags (the latter only in the Northern coastal study population) (VectronicAerospace 2019, 2022).

### Body temperature loggers (Paper I-IV)

To obtain  $T_b$  data, we equipped all moose with ruminal temperature loggers; mortality implant transmitters (MIT, Vectronic Aerospace GmbH, Berlin Germany, 72 x 21 mm; 100 g), hereafter



MIT (VectronicAerospace 2022) (Figure 3A). The MITs recorded the reticuloruminal  $T_b$  at 5-minute intervals and sent the information to the collar unit to be archived. The MITs have an accuracy of  $0.1^\circ\text{C}$  and a resolution of  $\pm 0.001^\circ\text{C}$  (Herberg et al. 2018; VectronicAerospace 2022). The latest  $T_b$  archived was attached when the collar sent a GPS position message, while the remaining  $T_b$  data were downloaded upon collar retrieval.

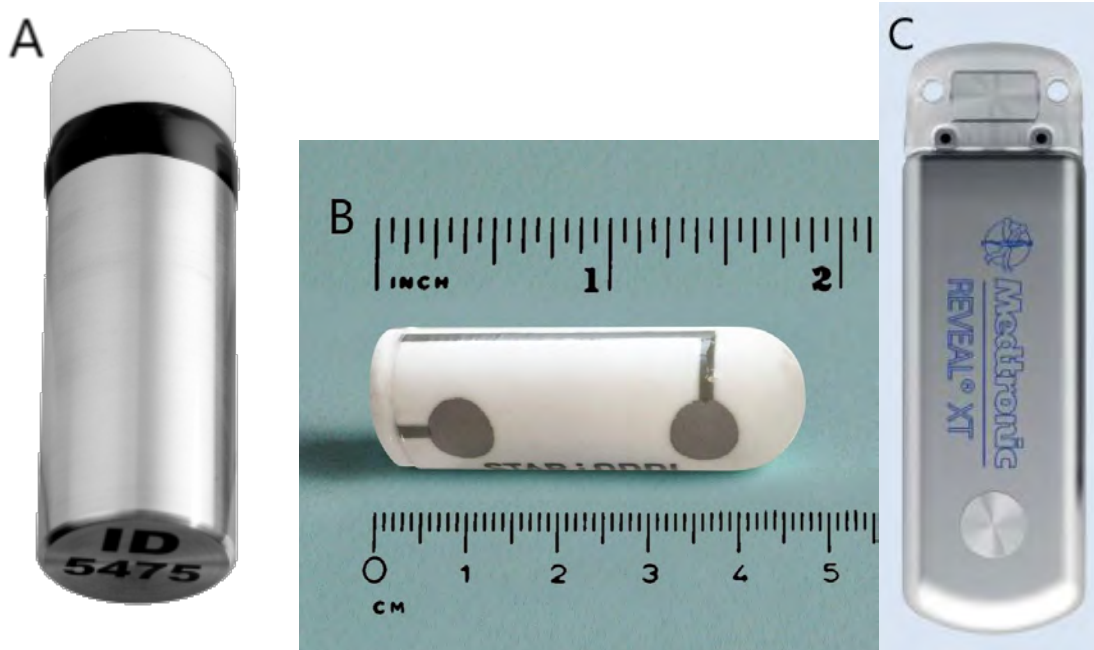


Figure 3 Internal biologgers used; Mortality implant transmitter (A) <https://www.vectronic-aerospace.com/view-all/>, DST centi HRT (B) <https://www.star-oddi.com/products/data-loggers/heart-rate-logger-animals> and Reveal XT (C) <https://news.medtronic.com/download/LINQ+Fact+Sheet+FINAL.pdf>

### Heart rate loggers (Paper I and III)

To obtain HR data, we surgically fitted the moose in the Northern coastal study area with subcutaneous loggers; DST centi HRT (Star Oddi, Gardabaer, Iceland; 46 x 15 mm; 19 g) (Figure 3B) and Reveal XT (Medtronic Inc., Minneapolis, Minnesota, USA; 62 x 19 x 8 mm; 15 g) (Figure 3C). The DST centi HRT calculated the HR automatically from a 4 s electrocardiogram (ECG) strip with a 150 Hz measurement frequency and stored along with a quality index of signal clarity and the R-R interval regularity. The logger can store up to 699,051 HR and temperature measurements or 2,785 ECG buffer measurements and, according to the manufacturer, has a battery life of up to 19 months (StarOddi 2017). Reveal XTs monitor the HR continuously using an ECG which converts the mean R-R interval (rate of a ventricular cycle) into HR, and store 2-min average values (Medtronic 2017).

### Equipment on the hunting dogs (Paper III)

All dogs included in the hunting approaches were equipped with Garmin T5 or DC50 Dog collars (Garmin Ltd, Olathe, Kansas, USA) and could be tracked directly via a hand-held GPS (Garmin Astro 320) (Garmin 2019). The dogs of the local hunting teams were in addition equipped with UHF proximity tags (Vectronic Aerospace GmbH, Berlin, Germany). The UHF proximity tags sent a signal to the UHF-ID tag on the moose collar when nearby, and switched the GPS-fix rate of the collar of the moose to 1-min positions (VectronicAerospace 2019).

### Immobilisation procedure

The moose were immobilised during wintertime (February-March) from a helicopter using a CO<sub>2</sub>-powered rifle (DANiNJECT, Kolding, Denmark) according to an earlier described procedure (Evans et al. 2012; Lian et al. 2014). We used a drug combination slightly modified from the previous work to 4.5 mg etorphine (Captivon<sup>®</sup> 98 Etorphine HCl, 9.8 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd, White River, South Africa) and 50 mg xylazine (Xylased<sup>®</sup> 500 mg, Bioveta, Ivanovice na Hané, Czech Republic). Chasing time (time from observation to successful darting), induction time (time from darting to recumbency), immobilisation time (time from recumbency to administration of reversal) and recovery time (time from administration of reversal to standing) were recorded during immobilisation. When approaching the moose we ensured that the moose was in sternal recumbency and had free airways. Afterwards, we performed a short clinical examination; measured rectal temperature, respiratory rate and HR, and classified the degree of immobilisation. The degree of immobilisation was classified as level 1 (light stage of sedation with the moose raising up and laying down again), level 2 (sedated but alert moose trying to raise up), level 3 (immobilised moose, not able to raise up, raised head, responds to stimuli and intact reflexes), level 4 (completely immobilised moose, slightly or not responding to stimuli, depressed reflexes and unable to lift the head), and level 5 (unconscious moose with absent reflexes). Pregnancy status was determined by rectal palpation (Solberg et al. 2003). Post-natal survivorship and the number of offspring (zero, single or twins) were investigated and verified the following spring by field observations a few days after the estimated calving date (calving date was based on GPS clustering) (Neumann et al. 2020). We sterilised the biologgers with ethylene oxide gas (Anprolene AN74i 60L, Andersen Europe, Kortrijk, Belgium) before surgical implantation. We gave the moose analgesics, meloxicam (Metacam<sup>®</sup>, Boehringer Ingelheim

Vetmedica GmbH, Ingelheim am Rhein, Germany) and local anaesthetics (Marcain, AstraZeneca, Cambridge, United Kingdom) prior to the surgery. Surgery was performed



*Figure 4 Implantation of heart rate loggers in a female moose*

according to best-practice guidelines (Fiorello et al. 2016). The Reveal XT and DST centi HRT loggers were surgically implanted subcutaneously at the left and right sides of the most rostral part of the sternum, respectively (Figure 4). The incisions were closed with 2-0 monofilament absorbable suture PDS II (polydioxanone) suture (Ethicon, Johnson & Johnson, New Brunswick, NJ, United States).

We fitted the moose with GPS collars before we reversed the effects of xylazine with 5 mg atipamezole (Antisedan® 5 mg/mL, Orion PharmaAnimal Health, Turku, Finland), to inhibit a swallowing reflex. We deployed MIT into the reticulorumen of the moose as previously described by Minicucci et al. (2018) (Figure 5) before we reversed the immobilisation with 50 mg naltrexone (ca 11.1 mg/1 mg etorphine given; Naltrexonhydroklorid vet. APL 50 mg/mL; Apotek Produktion och Laboratorier, Kungens Kurva, Sweden).

We recaptured the moose, according to the same procedure as the initial captures to retrieve the data obtained and to surgically remove the heart rate loggers. Moose in the Northern

coastal area were recaptured the two following years after the initial capture, while moose in the two other study areas were recaptured three years after the first capture. No capture-related morbidities or mortalities occurred during the immobilisation with the exception of one moose who died two days post-capture in the Southern island study area. The cause of death was unable to be determined.



*Figure 5 Deployment of MIT in a female moose. Photo: Jon M. Arnemo*

### Hunting approaches (Paper III)

The hunting approaches were divided into two groups; experimental and real hunts. The experimental hunts were performed after the national leash law was lifted (21 August) and until the beginning of the annual moose hunt. Real hunting situations were part of the regular moose hunt in the area. None of the real hunts included in our study resulted in a moose being killed.

For each experimental hunt, a dog handler walked with the leashed dog to a recent GPS position of a known moose. The dog was let off the leash and started tracking the moose.

Once the dog had been in close contact with the moose or was not able to find the moose, the handler leashed the dog and walked back to the car. The real hunting situations were included in the study when a UHF-ID tag of a hunting dog when near a moose included in the study, triggered the proximity sensor on the moose collar. For both types of approaches, the track log of the dogs was downloaded, and information about the hunting situation was collected.

## Data preparation

All the data for this thesis were prepared and analysed using R (RCoreTeam 2021), and  $p$ -values  $<0.05$  were considered significant.

The days of capture and recapture were excluded from the analysis in Paper I and II (and three days post-capture/recapture in Paper II) because the capture event and anaesthetics were expected to affect HR,  $T_b$  and activity (Kreeger and Arnemo 2018; Thompson et al. 2020).

In Paper III, the approaches were classified as successful, disturbed and not disturbed based on the distance between the moose and the dog, and the duration of the contact. Only approaches classified as successful and disturbed were included in the analysis.

## GPS-data

From the GPS-data, the Euclidean distance between consecutive GPS positions was calculated and I thereby calculated the total travel distance per day (Paper III and IV) and maximum travel speed per day (Paper III).

The GPS-fix rate in Paper III was 10 minutes, and the longitude and latitude values of missing positions were linearly interpolated using the “na.approx” function from the zoo package (Zeileis and Grothendieck 2005). The collars were programmed on 3 hourly positions in Paper IV.

## Body temperature data

The  $T_b$  obtained by the MITs can be influenced by water and/or snow intake, due to the reticuloruminal location of the bilogger (Herberg 2017). The ruminal temperature is shown to be a good predictor of body temperature when drinking events are filtered out (Herberg et al. 2018). I used two different methods to filter out drinking events when preparing the  $T_b$  data for analysis in this thesis. In Paper I, the R package “anomalize” was used to detect outliers

associated with drinking (Dancho and Vaughan 2018), while I used the method described by Thompson (2020) in Paper II to classify and filter out drinking events. Maximum  $T_b$  was used as the response variable in Paper III and IV, and due to that, drinking events were not filtered out. The MITs were programmed to obtain  $T_b$  at 5-minute intervals.

#### Heart rate data

The DST centi HRT (Paper I) were programmed to record raw ECG signals every second hour from the beginning of the study (February) until the 6<sup>th</sup> of August the same year. A subsample of 150 ECG strips was used to manually calculate HR from the raw ECG, that later were compared with HR measurements automatically calculated by the algorithm of the Star Oddi device. The automatic R-R interval detection was insufficient, so StarOddi adjusted the algorithm and reran the entire raw ECG data set. Due to the high noise level and insufficient automatic R-R interval detection and the decision to not record raw ECG data after the 6<sup>th</sup> of August, HR data from the Star Oddi device were not reliable after that date. I only included recalculated HR measurements with a quality index of 0 or 1 in my analysis.

The Reveal XT (Paper III) calculated the HR based on the recognition of R-peaks in the ECG. Because of the storage limit, the ECGs were deleted, while the HR data was stored in the memory of the logger. The algorithm used for the detection of the R peaks was not always correct, which resulted in the algorithm not detecting some heartbeats, while some were counted as two (double counting). There was no correlation between the activity level and the HR accuracy when comparing data from the Reveal XT, the DST centi and the accelerometer, and we concluded that the algorithm performed similarly during both active and resting phases. I, therefore, used the obtained raw HR data and removed unrealistically low HRs (based on the results from Paper I; the absolute minimum HR of a moose resting during winter was found to be 37.5 bpm) when analysing the HR data. This allowed me to calculate differences in HR and identify significant changes in HR over time, but as I was unable to account for potential double counting, I cannot report specific HR values.

#### Activity data

Overall activity was presented as the sum of the activity data on the X- and Y-axes, ranging from 0 to 510 (Paper I, II, III and IV) (Gervasi et al. 2006). The activity data (i.e. the sum of acceleration of X- and Y-axes) in Paper III and IV were used to classify the moose behaviour into two states; active and inactive (i.e. resting) by hidden Markov models (HMMs) to the

observed activity data of each individual. HMMs are time-series models that assume animals' observed movement or activity patterns to be determined by an underlying 'hidden' finite state sequence, where the states can be interpreted as proxies for the unobserved behavioural modes of an animal (Langrock et al. 2012; Patterson et al. 2009).

## Data analysis

### Paper I

#### *Seasonal patterns*

Generalised additive mixed models (GAMMs) were used to identify seasonal patterns in daily mean  $T_b$ , HR, activity and ambient temperature (Table 1). A fitted smooth term for the day since capture, a random intercept and slope for moose ID and an autoregressive model (AR1) structure to account for detected residual temporal autocorrelation were added to the models (Pinheiro and Bates 2000).

To determine the periods in which each parameter was significantly increasing or decreasing in Paper I, the first derivatives of the smooth term for the day since capture were calculated (Simpson 2018).

#### *Circadian patterns*

Lomb-Scargle periodogram analysis was used to investigate circadian rhythms in  $T_b$ , HR and activity, and I tested for significant periodicity between 0.5 and 30 h (Ruf 1999). The intensity of the  $T_b$  and activity variations over the year were visually investigated from plotted actograms including times of sunrise and sunset (Bivand and Lewin-Koh 2017; Wickham 2016)

#### *Energy expenditure*

I calculated daily energy expenditure based on the daily HR for the days with the lowest and highest daily mean HR based on a pooled equation developed from a study of captive moose (Renecker and Hudson 1985):

$$y = 4.655e^{-0.0071x}$$

Where  $y$  is the metabolic rate, and  $x$  is the HR normalised to  $BM^{-0.25}$  (body mass).

## Paper II

### *Body temperature patterns associated with pregnancy*

To investigate the  $T_b$  patterns in relation to different reproductive states throughout the year, I fitted GAMMs on daily mean  $T_b$  as described under paper I with a fitted smooth term for day of gestation (DOG), moose ID as a random intercept and slope, and an AR1 model structure. The explanatory variables used to build a set of candidate models are given in Table 1. Model selection was based on Akaike's Information Criterion (AIC), and the highest ranked model within  $\Delta AIC \leq 2$ , carried out with the lctab function from the bbmle package, was selected (Bolker and RCoreTeam 2017).

### *Body temperature and activity patterns in relation to parturition*

I used changepoint analysis among the pregnant females to detect specific time points in the calving period (calving date estimated from GPS-clustering  $\pm 15$  days), with major changes in the activity and  $T_b$  data to describe the pattern of these variables in relation to parturition (Haynes et al. 2021). The changepoint analysis identifies significant changes in time series data (Killick et al. 2012). The locations of the optimal number of detected changepoints on time series graphs were plotted, one for each parameter, to visualise changepoints in activity and  $T_b$  in the calving period. I calculated the number of individuals with the same type of pattern in relation to the parturition to identify the number of events detected as true-positive parturition events, but also the number of false-positive events and calving events not detected by this method. I repeated the procedure for non-pregnant individuals to look for the same pattern among those, and thus find the number of females identified as non-parturient, but also the number of false positive parturition events in the calving period (calving period defined as the mean calving date in the area  $\pm 15$  days for non-pregnant individuals). I thereby calculated the sensitivity (proportion of correctly identified parturient females), specificity (proportion of correctly identified non-parturient females) and accuracy (overall rate of true predictions) of the use of changepoints to detect parturition.

I also identified drops in the rolling 24 h mean  $T_b \geq 0.2^\circ\text{C}$  in relation to the calving date for pregnant females.



### Paper III

#### *Body temperature, movement and resting time in relation to hunting approaches*

Linear models were used to test for differences in the maximum daily  $T_b$  and resting time and gamma-distributed generalised linear models to test for differences in daily travel distance and maximum daily speed in relation to hunting approaches. The explanatory variables considered are given in Table 1. There were too few data points to include moose-ID as a random structure. The model selection was based on AIC corrected for small sample size (AICc) and carried out as described in paper II.

#### *Heart rate in relation to hunting approaches*

A linear mixed-effect model with the raw HR data was used to test for differences in HR in relation to hunting approaches. Due to the small sample size, we only included “period” as an explanatory variable (Table 1) and only data from successful approaches. To account for the autocorrelation within the HR data, the autocorrelation structure corCAR1 was included. Moose ID was included as a random factor to account for repeated measures of the same moose.

#### *Changepoint analysis*

Changepoint analysis was used to detect flight initiation distances (FID); how close the dog was when the moose started to react behaviourally (based on accelerometer data) and thermally (based on  $T_b$ ), and to find out how long the activity and  $T_b$  of the moose were affected by the approaches (how long it took to return to the “normal” pre-approach state). Two changepoints were identified using the Bin Seg method, which ideally should correspond to the start (moose starting to react) and end of the thermal and behavioural responses (Killick and Eckley 2014). The time the moose was affected (time affected = time between the two changepoints), the FID (how close the dog was when the moose started to react), and how long the moose was affected after the approach was finished (time difference between the approach end in the protocol and the second changepoint) was thereafter calculated.

### Paper IV

#### *Thermal and behavioural responses of helicopter-based chemical immobilisations*

To determine the potential impact of captures on moose movement, resting time and  $T_b$ , I modelled maximum  $T_b$  and total distance travelled per day using gamma-distributed

generalised linear models and the proportion of the day allocated to resting versus active behaviour using a generalised linear model with a binomial family distribution. The explanatory variables used to build a set of candidate models are given in Table 1. Model selection was carried out as described under paper II on AICc.

To determine the individual response of helicopter-based chemical immobilisations, I calculated how long it took for each response variable to return to the pre-capture level.

To determine if data from days 11-20 post-capture could be used as a proxy for pre-capture levels, I compared the levels from before and after using t-tests.

Table 1 Models used in this thesis, including the response variables, explanatory variables and random effects used in the models.

Response variable	Model <sup>1</sup>	Explanatory variable <sup>2</sup>	Random effect
<b>Paper I</b>			
Body temperature (mean)	GAMM	Day since capture	Moose-ID
Heart rate	GAMM	Day since capture	Moose-ID
Activity	GAMM	Day since capture	Moose-ID
<b>Paper II</b>			
Body temperature (mean)	GAMM	Pregnant Day of gestation Area	Moose-ID
<b>Paper III</b>			
Body temperature (maximum)	LM	Period Success	
Heart rate	LMM	Period	Moose-ID
Resting time	LM	Period Success	
Speed (maximum)	GLM	Period* Success	
Euclidean distance moved	GLM	Period* Success	
<b>Paper IV</b>			
Body temperature (maximum)	GLM	Company calf Period	
Resting time	Binomial GLM	Company calf Period	
Euclidean distance moved	GLM	Company calf Period	

<sup>1</sup>GAMM – Generalised additive mixed model, LM – Linear model, LMM – Linear mixed model, GLM – Generalised linear model

<sup>2</sup>Day since capture – continuous, number of days since the moose was captured, Pregnant – factor with two levels; pregnant and not pregnant, Day of gestation – continuous 1-231, a measure of days from the estimated conception date, Area – factor with three levels, Northern alpine, Northern coastal and Southern island study areas, Period – factor with three levels; a reference period before the hunting approach/capture, during the day of the hunting approach/capture, and the day after the hunting approach or a period of ten days after the capture, Period\* – factor with two levels; during the day of the approach and the day after the approach, Success – factor with two levels; Disturbed only: moose and dog within 240 m for < 10 min; Successful approach: moose and dog within 240 m for > 10 min. Company calf – factor with two levels; female in company with a calf, yes and no

# Results and discussion

## Basic physiology

### Seasonal variations (Paper I)

Body temperature, HR and activity of moose showed seasonal patterns, with lower levels during winter and higher levels during summer (Figure 6).

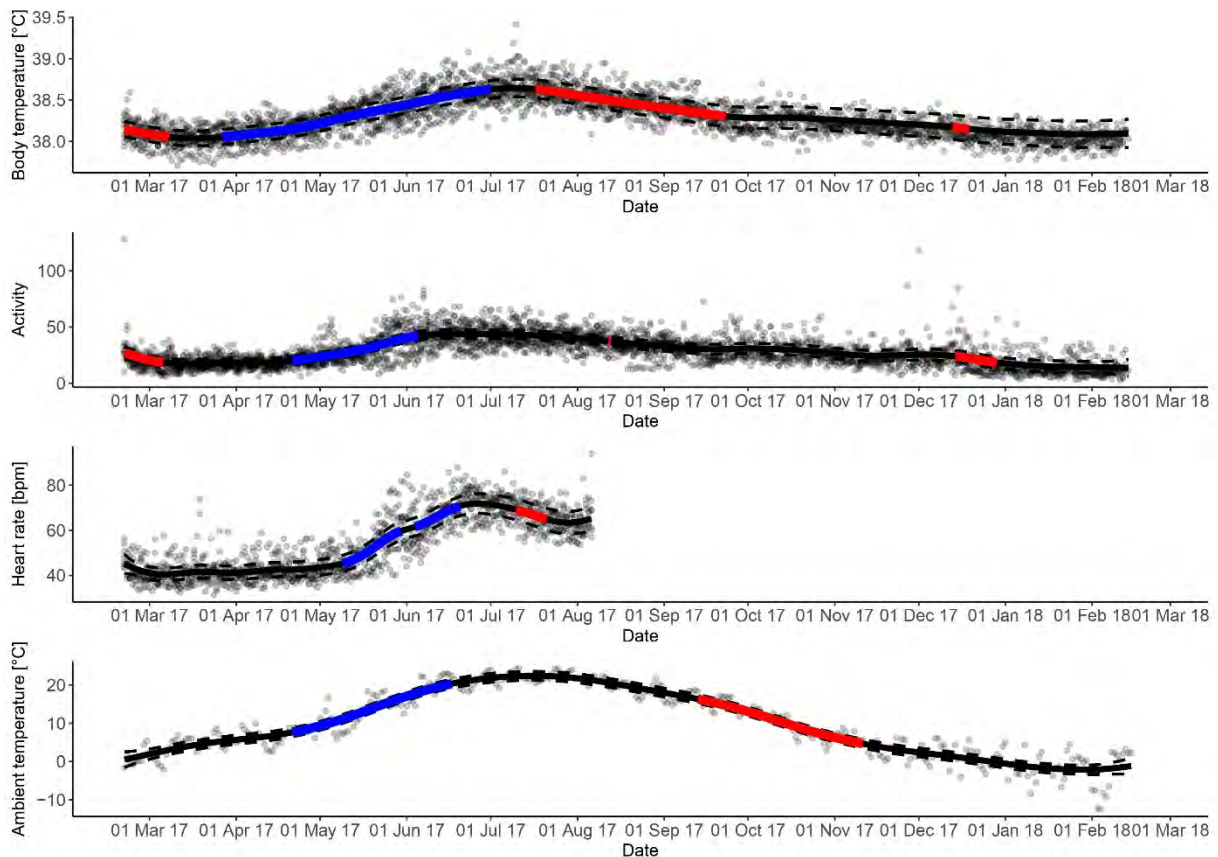


Figure 6 Daily mean body temperature, collar activity, heart rate, and ambient temperature for 12 female moose in Sweden. Solid lines represent daily mean body temperature, collar activity, heart rate, and ambient temperature, respectively, and dashed lines represent the 95% confidence interval of the daily mean. Significantly increasing and decreasing periods are presented as red and blue, respectively, on the solid line.

The highest and lowest daily mean  $T_b$  were  $38.64^{\circ}\text{C}$  (10 July, 95% confidence interval (CI);  $38.53\text{--}38.75^{\circ}\text{C}$ ) and  $38.03^{\circ}\text{C}$  (17 March, 95% CI;  $37.94\text{--}38.12^{\circ}\text{C}$ ). The difference from the highest to the lowest HR ( $71.9$  bpm (26 June, 95% CI;  $67.6\text{--}76.3$  bpm) and  $40.5$  bpm (6 March, 95% CI;  $37.7\text{--}43.2$  bpm)) represents a decrease in metabolic rate by 60%. The results demonstrate hypometabolism in moose as a strategy to cope with limited resources available and colder climates during winter. The seasonal patterns are in line with earlier reports on moose and other ungulates (Arnold et al. 2018; Signer et al. 2011; Thompson et al. 2019; Turbill et al. 2011).

Moose displayed circadian rhythmicity in activity and  $T_b$ , with  $T_b$  mainly displaying a period length ( $\tau$ ) of 24 h, while activity displayed ultradian rhythms with a period length  $\tau$  of 2-8 h throughout the year. Circadian rhythmicity in activity demonstrated is in line with previous reports in both moose (Gillingham and Klein 1992; Van Ballenberghe and Miquelle 1990) and other ungulates (Arnold et al. 2004; Ensing et al. 2014; Lowe et al. 2010; Pagon et al. 2013; Signer et al. 2011). The ultradian rhythms in activity were shorter in summer compared with winter, following the same trend as moose in Alaska and Svalbard reindeer (*Rangifer tarandus platyrhincus*) (Arnold et al. 2018; Van Ballenberghe and Miquelle 1990). Oscillation of rumination is demonstrated to be a regulatory function of activity, so changes in the rhythmicity throughout the year, could result from seasonal changes in feeding behaviour and ruminating (Scheibe et al. 1999). The circadian rhythmicity presented in  $T_b$  is similar to the patterns reported in Svalbard reindeer (Arnold et al. 2018).

#### Pregnancy and parturition (Paper II)

Body temperature varied between different reproductive states, with significantly higher  $T_b$  in pregnant compared to non-pregnant moose (Figure 7), and a decline in  $T_b$  when parturition was imminent (Figure 8).

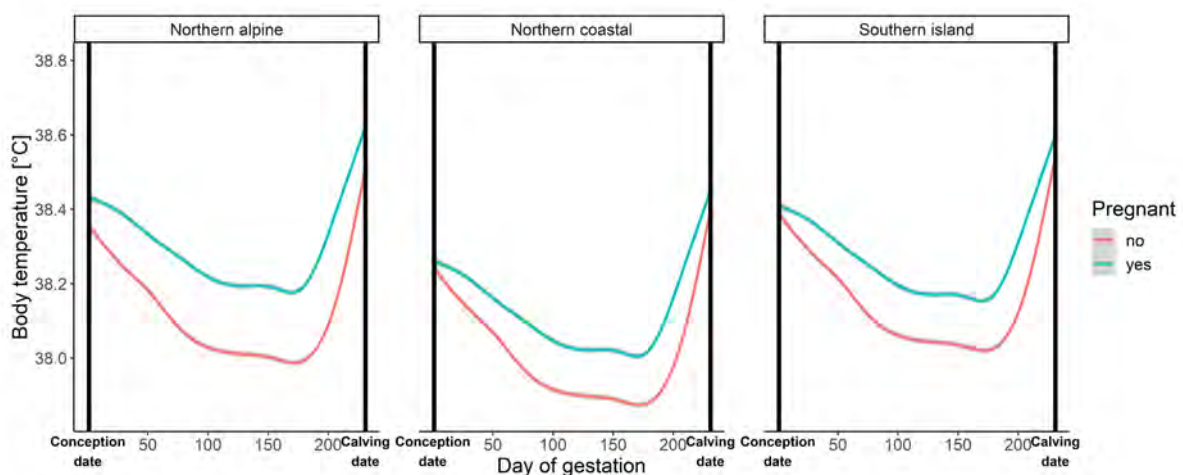


Figure 7 Daily mean body temperature patterns during pregnancy in moose between different reproductive states and areas predicted from generalised additive mixed models (GAMMs). The grey areas around the lines represent the 95% confidence intervals.

These results are similar to reports from domesticated cattle (Costa Jr et al. 2016; Suthar et al. 2012; Wrenn et al. 1958), and the differences in  $T_b$  between the reproductive states are in line with reports from moose and other capital ungulates living in cold and seasonal environments (Arnold et al. 2018; Schmidt et al. 2020; Thompson et al. 2019). Based on the

progesterone fluctuations documented during pregnancy in moose and the thermogenic effect of progesterone, I suggest that the difference in  $T_b$  between the reproductive states reflects higher progesterone levels during pregnancy. The  $T_b$  also differed between the areas with an increasing difference along a south-north gradient (i.e. from a  $0.13 \pm 0.02^\circ\text{C}$  (mean  $\pm$  SE,  $p$ -values  $<0.0001$ ) difference in the Southern island and Northern coastal areas to a  $0.19 \pm 0.02^\circ\text{C}$  (mean  $\pm$  SE,  $p$ -value  $<0.0001$ ) difference in the Northern alpine area) (Figure 7). Differences in environmental settings with respect to climatic conditions and food availability and quality could explain the differences between areas.

Changepoint detection during a decreasing phase in  $T_b$  followed by a changepoint during a decreasing phase in activity was a highly successful method of detecting parturition in moose (accuracy 91.5% (percentage of correct assessments), sensitivity 88.6% (percentage of true positive assessments) and specificity 100% (percentage of true negative assessments)) (Figure 8).

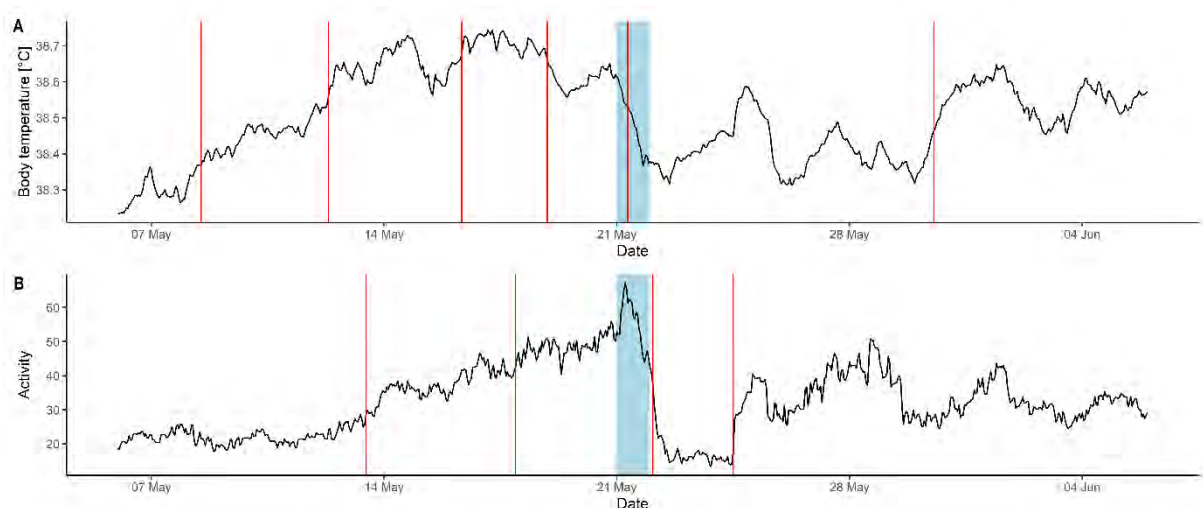


Figure 8 Changepoints (red vertical lines) in the rolling 24 h mean body temperature (A) and activity (B) in the calving period (calving date estimated from GPS-clustering  $\pm 15$  days) for one pregnant female moose. The calving date is presented as a vertical blue bar.

This type of pattern was not detected elsewhere in the calving period (estimated calving date  $\pm 15$  days) among pregnant females nor in a simulated calving period among the non-pregnant females (mean estimated calving date in the area  $\pm 15$  days). Use of activity or  $T_b$  alone would have resulted in several false positives, so I recommend a combination to get more accurate results for predicting parturition. One should though bear in mind that the parturition date is based on an estimation from GPS-clustering, and the precision of this method is not validated. The accuracy of our method is, however, in line with the accuracy of other methods using GPS

data alone or in combination with activity data to predict parturition in ungulates (DeMars et al. 2013; Marchand et al. 2021; Nicholson et al. 2019). The drop in  $T_b$  occurred on the day before the estimated calving date (mean; day -1, range day -3 – 0), while the drop in activity occurred at the estimated calving date (mean; day 0, range day -1 – 1).

### Summary basic physiology

I demonstrated seasonal hypometabolism in pregnant female moose with lower levels of  $T_b$ , HR and activity in winter compared to summer. I was also able to document the same type of pattern in  $T_b$  among female moose in different areas including both pregnant and non-pregnant ones. Lower  $T_b$  during winter is also documented in male moose, indicating that it is a strategy in moose in colder climates to save energy with limited resources available (Fohringer 2021). Even if the vegetation season, ambient temperature, wind, light-dark cycle, snow cover and snowfall are external cues known to influence HR and  $T_b$  in ungulates (Arnold et al. 2018; Signer et al. 2011), further investigation is needed to determine the role of these drivers of the fluctuations of  $T_b$ , HR and activity. Identifying the drivers of the fluctuations and their role is a key question with respect to the changing climate. Climate change, with increasing and fluctuating ambient temperatures, changes in precipitation and following days with snow cover, can result in alternated timing of plant growth, changes in habitat quality and vegetation phenology and a shortened period with forage of high nutritional quality (Monteith et al. 2015). All of these can influence the ecosystem dynamics of the moose with regards to migration, habitat selection, infection risk, the timing of reproduction, calf survival and growth and in the end the overall survival rate (Lenarz et al. 2009; McCann et al. 2013; van Beest and Milner 2013; van Beest et al. 2012).

Moose, as a capital breeder, are relying their reproductive cycle on stored energy. Little is known about the reproductive timing of the moose, and also the reported gestation lengths are highly variable (from 216-246 days) (Markgren 1969; Schwartz 1992; Schwartz and Hundertmark 1993). The suggested link between  $T_b$  and progesterone during pregnancy and the development of a method to predict parturition based on  $T_b$  and activity in this thesis could be further developed to identify other reproductive events in moose, like oestrus and mating events. This could be used to determine the length of the gestation and whether the gestation length is fixed or flexible and differs between areas. A further linking of this

knowledge and changes/differences in environmental conditions is important and will allow us to better understand how the moose will cope with a changing climate.

## Human-induced landscape of fear

### Hunting with baying dogs (Paper III)

Hunting approaches classified as successful, where the moose and dog were in contact for >10 minutes, resulted in increased HR,  $T_b$  and movement of the moose, and the moose fled away from the dogs (Figure 9). The successful approaches resulted in a  $0.88^\circ\text{C}$  (SE  $0.15^\circ\text{C}$ ,  $p$ -value  $<0.001$ ) and 24 bpm (SE 3 bpm,  $p$ -value  $<0.001$ ) higher  $T_b$  and HR, respectively, and 1.3 m/s (SE 0.2 m/s,  $p$ -value  $<0.001$ ) higher maximum speed and 4.1 km (SE 1.1 km,  $p$ -value  $<0.001$ )

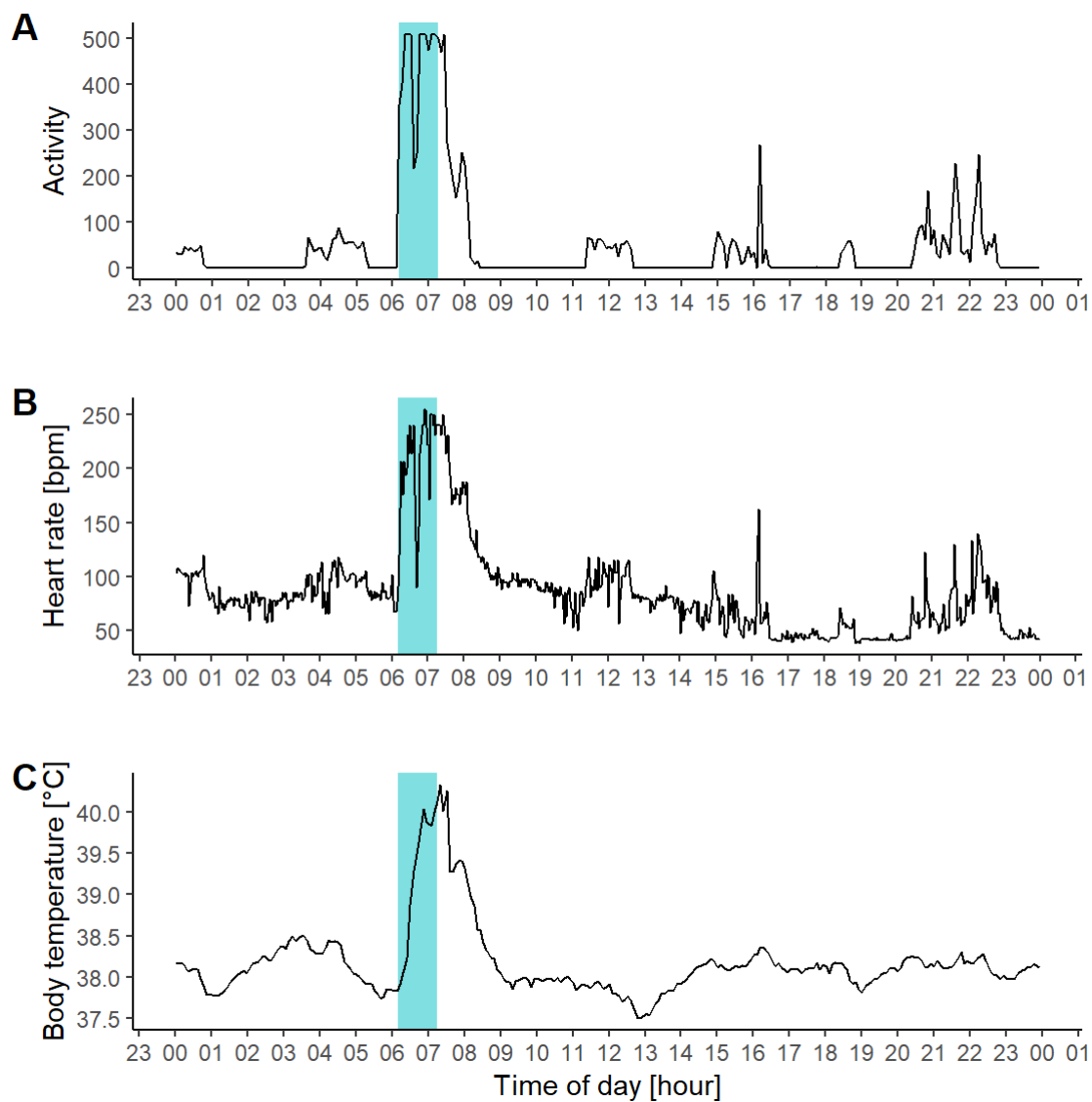


Figure 9 Graphical representation of (A) activity, (B) heart rate and (C) body temperature of a female moose when approached by a hunting dog during an experimental hunting approach. The blue ribbon represents the approach duration i.e. the time from the start to the end of the approach. For interpretation of the absolute heart rate values please see the explanation in the main text (method-section).



longer distance travelled on the day of the approach compared to the day after, and the moose rested >90 min longer ( $p$ -value <0.001) on the day after the approach compared to the day of the approach. For HR and  $T_b$  I also documented significantly higher levels on the day of the approach compared to the reference period (mean  $\pm$  SE;  $22 \pm 2$  bpm and  $0.47 \pm 0.15^\circ\text{C}$ ,  $p$ -values <0.001 and 0.031). In contrast, there were minimal physiological and behavioural changes where the moose and dog had been in contact for <10 min (disturbed only). The resting time the day after the approach was >90 minutes ( $p$ -value 0.036) longer among the successfully approached moose compared to the moose classified as disturbed only. These results indicate that the changes in moose physiology and behaviour related to hunting approaches are dependent on the time the dog and moose have been in close contact.

I demonstrated moose exceeding  $T_b$  of  $40^\circ\text{C}$  in the majority of the successful hunts, with  $40.8^\circ\text{C}$  as the highest measured  $T_b$  in relation to the approaches. This represents a  $2.3^\circ\text{C}$  increase from the baseline level at this time of the year (found in Paper I).

We were unfortunately not able to validate the exact HR values. A 22-24 bpm higher HR was found on the day of the approach compared to the reference period and the day after the approach, suggesting a considerably increased energy expenditure in relation to hunting disturbances. A method to compensate for the increase in metabolic rate could be to increase the resting time the day after the approach as documented here.

#### Helicopter-based chemical immobilisations (Paper IV)

Moose increased their  $T_b$ , activity and daily movement rate as a response to the immobilisation event (Figure 10). The maximum  $T_b$  during the day of the immobilisation was  $0.71^\circ\text{C}$  (SE  $0.11^\circ\text{C}$ ,  $p$ -value < 0.001) higher compared to the period before the immobilisation. Two moose exhibited capture-induced hyperthermia (defined as the  $T_b \geq 2^\circ\text{C}$  above the normal  $T_b$ ) with body temperatures exceeding  $2^\circ\text{C}$  above the baseline levels recorded during the reference period ( $38.02 \pm 0.02^\circ\text{C}$ ) with  $40.09^\circ\text{C}$  and  $40.23^\circ\text{C}$  as their highest measured body temperatures during the day of the immobilisation. The rest of the moose ( $n=7$ ) were normothermic (baseline  $T_b \pm 2^\circ\text{C}$ ). Capture-induced hyperthermia is documented in Scandinavian moose immobilised with a different drug-combination and capture method (Barros et al. 2018; Haga et al. 2009; Rostal et al. 2012), while other studies using the same drug-combination as my study reported normothermic moose (Evans et al. 2012; Lian et al. 2014). The reasons for moose exhibiting hyperthermia during immobilisation and also reasons

for differences between different studies could be the length of the helicopter chase, differences in environmental conditions like snow depth and ambient temperature, body condition of the moose and differences in drugs and dosages. Another important difference between the studies is the fact that the moose in our study were equipped with ruminal temperature loggers, continuously measuring the  $T_b$ , which provides more detailed information compared to traditional rectal measurements a few times during the immobilisation.

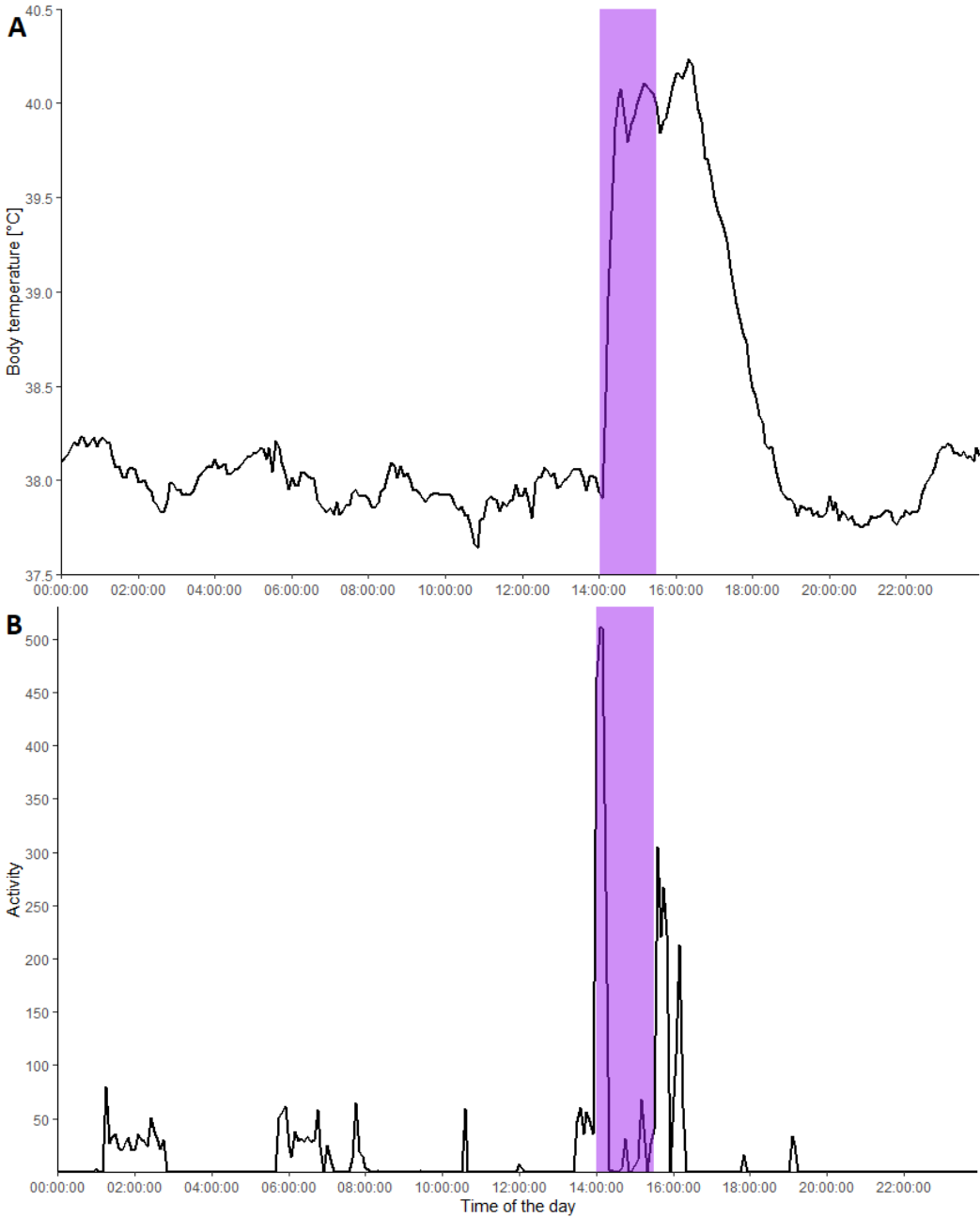


Figure 10 Graphical representation of body temperature [°C] (A) and activity (B) of a female moose during the day of the immobilisation. The purple ribbon represents the duration of the immobilisation i.e. the time from observation to the moose was standing.

The daily travel distance of the moose were 3.8 km (SE 1.4 km,  $p$ -value 0.006) and 1.8 km (SE 0.8 km,  $p$ -values 0.024 and 0.023) longer on the day of the immobilisation, and the two first days post-capture, respectively, compared to the reference period. Elevated movement following recaptures are reported earlier in female mule deer (*Odocoileus hemionus*), female bison (*Bison bison*) and female moose, which is in contrast to naïve male bison that reduced their movement and displacement rates following capture (Jung et al. 2019; Neumann et al. 2011b; Northrup et al. 2014; Thompson et al. 2020). The probability of resting was 5-6 % lower on the day of the immobilisation and the two following days ( $p$ -values <0.001) after the immobilisation compared to the reference period. The lower probability of resting and the increased movement the first days after the immobilisation could be affected by helicopter overflights in the area due to captures of other moose in the same project in the following days. The travel distance and probability of resting were also influenced by a accompanying calf, with a shorter travel distance ( $0.6 \pm 0.2$  km (mean $\pm$ SE) shorter,  $p$ -value 0.013) and a higher probability of resting (5% higher,  $p$ -value <0.001) in females with a calf on the heel.

To avoid biased results in the analysis of newly immobilised moose I recommend omitting the  $T_b$  data for at least one day and the movement and resting time data for at least three days after the immobilisation. One should though bear in mind that there were large individual variations in the time it took for each parameter to return to baseline (pre-capture) levels (maximum  $T_b$  from 0 to 6 hours (mean $\pm$  SE:  $2 \pm 1$  hours), resting time from 0 to 9 days (mean  $\pm$  SE:  $3 \pm 1$  days), movement from 1 to 6 days (mean  $\pm$  SE:  $3 \pm 1$  days)). I also documented that the data from days 11-20 post-capture were a good proxy of the reference period (before captures). The data could therefore benefit from being censored on an individual basis using days 11-20 as a reference period when including biollogger data from newly immobilised moose.

Helicopter-based chemical immobilisation with a drug combination of 4.5 mg etorphine and 50 mg xylazine resulted in a safe and effective immobilisation of the moose, both for the moose and the personnel involved in the capture. There were no capture-related morbidities nor mortalities associated with these captures, and all moose were alive one year after the immobilisation. All pregnant moose still sending data in the spring were in company with newborn calves a few days after calving. The induction times were acceptable (ca 6 min), and at the same level as for moose immobilised with a combination of etorphine, xylazine and

acepromazine, but 2 and 4 min longer, respectively than for moose immobilised with etorphine or thiafentanil as sole agents (Barros et al. 2018; Evans et al. 2012; Haga et al. 2009). It is important to have a short intensive chasing time, a quick and smooth recovery, and to decrease the duration of the anaesthesia to decrease the risk of morbidity, mortality and losing track of the moose (Kreeger and Arnemo 2018). We had a quite long immobilisation time in this study, due to surgical removal of biologgers and downloading data, which might have affected the post-capture responses of the study animals.

#### Summary human-induced landscape of fear

Moose increased their energy expenditure as a result of human disturbance deduced through increased  $T_b$ , activity, movement, HR and decreased resting time during the day of the disturbance compared to a reference period before and a period after the disturbance. I documented increased resting time as a strategy to recover and save energy after hunting disturbances, while I documented increased movement and a lower probability of resting the first days after immobilisation, compared to the reference period. I suggest that helicopter overflights when immobilising other moose in the same area could be a reason for the increased movement and activity after the captures.

This thesis shows the importance of basic physiology studies throughout the year, and the importance of comparing the results from disturbance studies with baseline values during the same period of the year. The highest measured  $T_b$  during the hunting approaches and captures were 40.8°C and 40.23°C, respectively. One could therefore think that the increase in  $T_b$  during the hunting approaches is much higher than during the captures. But the fact is that the daily mean  $T_b$  are 0.61°C higher during summer when we performed the hunting approaches, compared to during winter when we performed the captures, meaning the highest measures represent approximately the same difference from the baseline levels. In paper IV, I documented a difference between the recorded ruminal temperatures and the rectal temperatures, with all except one rectal temperature being higher than the ruminal temperature. The difference between the rectal and ruminal temperature was largest at the highest measured temperatures (up to 0.6°C difference), indicating that the highest measured ruminal temperatures are too low. The accuracy of the ruminal  $T_b$  is likely influenced by the amount and consistency of the ruminal content, the location of the transmitter, and the duration of the core  $T_b$  elevations. More research is needed to investigate the extent of this

difference, but one should bear that in mind when interpreting the highest measured ruminal temperatures.

A follow-up to this thesis would be to study the long-term effects of human disturbance. Reproductive rates, survival rates, post-natal survivorship, and slaughter weights of calves and females are examples of variables that could be evaluated in a long-term study. The problem is that I do not have data from an uncaptured control group, that other biological and environmental factors influence the results and the fact that I cannot control the environment with respect to other disturbing factors. One should also be careful when comparing results and drawing conclusions between different sources of disturbance and areas, as the environmental, biological and physiological conditions might differ. I looked at the reproductive rates and slaughter weights of the moose in the Northern coastal area in Paper III, they were quite stable over time, and the reproductive rates were fairly high in 2017 ( $>0.7$ ) (Ericsson and Wallin 1999; Svenskajägareförbundet 2019). This is in contrast to reports from the Southern island area where the moose are suffering from low calf survival rates (Allen et al. 2017). Increasing ambient temperatures and climate change are suggested as a reason for the declining moose populations in the southern edges (Allen et al. 2017; Lenarz et al. 2009; Ruprecht et al. 2016). I would therefore assume that the negative effects of human-disturbance might be more dramatic in moose in the edges of the moose range, especially on warm days as moose are easily heat stressed.

The hunting season in Sweden lasts for 5-6 months from the beginning of September or October until January or February depending on local regulations (Lavsund et al. 2003; Länsstyrelserna 2020). Most of the moose harvest in Sweden occurs at the beginning of the hunting season (September) (Singh et al. 2014). The hunting period in winter is increasingly discussed, and further studies should be performed to compare the physiological and behavioural responses between autumn and winter, where the moose are hypometabolic. The main season for immobilising free-ranging moose in Scandinavia also overlaps with the hypometabolic period. We documented successful calving and a 100 % survival rate one-year post-capture of the captured females, but further investigation is needed to determine the load for the calves, both the calves born in the spring after immobilisation and the calves following the females when captured during winter. Movement in deep snow is energy-consuming, especially for calves due to their smaller size, lower breast height and stride length

compared to adult moose (Fancy and White 1985; Lundmark and Ball 2008; Neumann et al. 2010).

### Studying ecophysiology with combined biologging techniques

Here I used biologgers to fill in some of the knowledge gaps of the basic physiology of moose and how they are behaviourally and physiologically affected by human activities. The combined use of different types of biologgers enabled a novel and deeper insight into the interaction between the physiological and behavioural processes of the moose. I experienced the importance of validating the sensor to your study species under captive conditions in a controlled environment. The Reveal XTs we used in Paper III have been successfully used in other species like brown bear and American black bear (Laske et al. 2018), but they did not work properly for moose as the algorithm detecting the heart beats sometimes did not recognise them, and sometimes it double-counted them. I also experienced that it is important to have a large enough sample size to draw statistical conclusions, especially when combining data from several loggers. I lost some data during my work due to biologgers that were lost or stopped working properly, moose that were shot during the study period and because we lost contact with a couple of moose during the study period. One should also bear in mind that there are no possibilities to obtain the same type of information from an unmarked control group when performing biologging studies. I, therefore, had to rely on that the data I collected was representative of moose in the given situations. There could however be other uncontrolled stressors affecting the results and the equipment with the sensors themselves, which might result in the animal deviating from normal conditions. It is therefore important from an animal welfare perspective that researchers and managers evaluate their work using biologgers with respect to short- and long-term effects on both individual and population levels.

## Conclusion

In this thesis, I showed that the  $T_b$ , HR and activity of pregnant female moose vary seasonally with lower levels during winter and higher levels during summer. The seasonal pattern in  $T_b$  was also present among non-pregnant females. Pregnant females have higher  $T_b$  than non-pregnant ones throughout the pregnancy, and the difference between the reproductive states varied between areas with an increasing difference along a south-north gradient. The  $T_b$  and activity of the pregnant moose decreased when parturition was imminent. Detection of changepoints in the rolling daily mean  $T_b$  and the rolling daily mean activity the day before, and the day of parturition, respectively, was a highly successful method of detecting calving in moose (accuracy 91.5%: percentage of correct assessments).

Human disturbance or the human-induced landscape of fear of moose, represented by hunting approaches with baying dogs and helicopter-based chemical immobilisations resulted in increased  $T_b$ , activity, and HR (hunting approaches) during the day of the disturbance compared to a reference period before the disturbances. Hunting approaches classified as successful resulted in increased resting time the day after the approach as a possible strategy to recover and compensate for the increased energy consumption during the disturbance.

Up to now, it seems like the moose in the Northern coastal study area (the main study area) tolerate the level of human activity in the area with stable reproductive rates and slaughter weights. The survival rate of mothers and calves after immobilisation was also high. But it is important to continue to consider the physiological and behavioural consequences of the human-induced landscape of fear of moose especially due to increased human recreational activity, and climate change with increasing ambient temperatures, changes in vegetation phenology and habitat quality.

Biologgers are excellent tools for wildlife studies, providing detailed information about the animals' life under non-disturbed conditions. When performing biologging studies, I do though recommend testing and validating the biollogger to your study species, ensuring that number of animals in your study population is large enough to be able to draw statistical conclusions and to evaluate potential side effects of instrumentation (both short- and long-term) on both individual and population levels.

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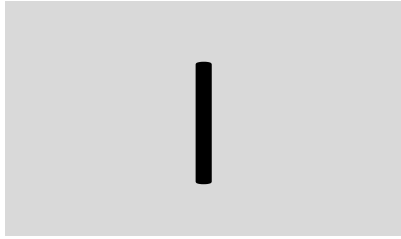
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## Dissertation articles









# Seasonal Hypometabolism in Female Moose

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How animals respond to a changing environment is a key question in ecological research. Animals living at higher latitudes are exposed to pronounced seasonal differences in both climate and in resource availability. Endotherms living in those environments have the ability to maintain a constant high body temperature ( $T_b$ ), over a wide range of ambient temperatures ( $T_a$ ). Nonetheless, many endotherms display seasonal shifts in metabolic rate (MR). Here, we studied the annual and circadian cycle of  $T_b$  and heart rate (HR) in female moose (*Alces alces*) in relation to activity and  $T_a$ . HR also can be used as a proxy of MR to calculate energy budgets. We deployed biologgers to 12 free-ranging female moose; a temperature sensor in the rumen, a HR logger subcutaneously, and a GPS collar equipped with acceleration and  $T_a$  sensors. We documented seasonal differences in  $T_b$ , HR and activity of moose, with lower levels during winter and higher values during summer. The highest daily mean  $T_b$  and HR were 38.64°C (10 July) and 71.9 beats per minute (bpm; 26 June), whereas the lowest daily mean  $T_b$  and HR were 38.03°C (17 March) and 40.5 bpm (6 March). High-resolution  $T_b$  and activity data allowed us to detect circadian and ultradian rhythmicity throughout the year. Based on previous calibration studies, MR decreased by 60% from the highest to the lowest point. Our results demonstrate hypometabolism including lower  $T_b$  and HR during winter as a strategy to reduce energy expenditure during periods with colder climate and limited availability of resources.

**Keywords:** activity, *Alces alces*, body temperature, global positioning system, heart rate, metabolic rate, moose, seasonal variation

## INTRODUCTION

Animals living in environments with ambient temperatures ( $T_a$ ) below freezing are exposed to pronounced seasonal differences in both climate and resource availability. Nevertheless, endotherms living in these environments can maintain a constant high body temperature ( $T_b$ ), over a wide range of  $T_a$ . Maintaining homeostasis in cold temperatures with limited food availability is energetically costly, and endotherms have adapted to seasonal differences through several strategies including increased insulation, hibernation, seasonal hypometabolism, and daily torpor (Geiser, 2004; Heldmaier et al., 2004; Lovegrove, 2005; Boyles et al., 2011). Hibernation in mammals usually lasts for months, during which animals rely on energy stores and reduce  $T_b$  and metabolic

rate (MR). The basal MR for hibernating mammals is on average reduced by about 95% during hibernation (Geiser, 2004; Heldmaier et al., 2004; Ruf and Geiser, 2015).

A reduction in MR hypometabolism, is not limited to organisms entering hibernation or torpor, as other endotherms also can exhibit hypometabolism. The reason for entering this stage is not only to adapt to a colder climate, but also to cope with limited energy supplies as evidenced by endotherms in tropical climates (Heldmaier et al., 2004). Seasonal variations of MR, with hypometabolism during winter, occur in a variety of both captive and free-ranging ungulates living in temperate climates, including moose (*Alces alces*; Regelin et al., 1985; Kochan, 2007), red deer (*Cervus elaphus*; Arnold et al., 2004; Turbill et al., 2011), Przewalski horse (*Equus ferus przewalskii*; Arnold et al., 2006), Svalbard reindeer (*Rangifer tarandus platyrhynchus*; Arnold et al., 2018), alpine ibex (*Capra ibex ibex*; Signer et al., 2011), and llama (*Lama glama*; Riek et al., 2019).

Circadian variations of MR also are reported in ungulates, e.g., as episodes of nocturnal hypometabolism independent of activity levels in alpine ibex and red deer (Arnold et al., 2004; Signer et al., 2011). A circadian rhythm is a biological process that displays an endogenous oscillation of about 24 h. Circadian organization in the activity pattern of different ungulate species have been reported previously (Arnold et al., 2004; Lowe et al., 2010; Signer et al., 2011; Pagon et al., 2013; Ensing et al., 2014). Studies of reindeer (*Rangifer tarandus tarandus*) living above the Arctic Circle, including animals on the Svalbard archipelago, reported only weak circadian organization of activity during constant darkness or light (Van Oort et al., 2005; van Oort et al., 2007). A more recent study of Svalbard reindeer, using biologgers, reported circadian rhythmicity in behavior, HR and  $T_b$  (Arnold et al., 2018). Circadian rhythmicity in moose could be caused by some combined adaptations to predator and/or human activity,  $T_a$ , light-dark cycles, food availability, feeding, and rumination (Van Ballenberghe and Miquelle, 1990; Gillingham and Klein, 1992; Scheibe et al., 1999; Klassen and Rea, 2008).

Several mammal species exhibit a significant correlation between heart rate (HR) and MR, which is attributed to the correlation between the rate of oxygen consumption ( $VO_2$ ) and HR, when animals are in a metabolic steady state with aerobic metabolism (Green, 2011). Because of the correlation between HR and MR, HR measurements make it possible to calculate energy expenditures of animals under free-ranging circumstances (Renecker and Hudson, 1985). Calculations of energy expenditure are important when discussing potential stressful situations, especially in capital breeders (i.e., organisms relying on stored resources for reproduction) such as moose because increased MR could result in weight loss due to increased energy consumption and decreased time spent foraging. This relationship could further result in lower survival and reproduction rates.

Biologgers have made it possible to obtain data for characterization of the physiology of an animal in their environmental setting, and their reactions to humans over long periods of time (Rutz and Hays, 2009). In this study we used ruminal implants to obtain  $T_b$ . Ruminal and vaginal transmitters have previously been used in long-term (>1 year) studies of

moose, and rumen temperature successfully predicts core  $T_b$  when censoring and filtering out drinking events (Herberg et al., 2018; Thompson et al., 2019). The few studies on moose using biologgers to monitor HR are short-term (weeks-months), usually with a limited number of animals in semi-captive conditions (Renecker and Hudson, 1985; Langvatn, 1992; Roshchevsky et al., 1999). In this study, we used the same type of HR loggers, as previously deployed in a variety of large mammals including domestic sheep (*Ovis aries*; Fuchs et al., 2019a), and Asiatic black bears (*Ursus thibetanus*) and Eurasian brown bears (*Ursus arctos*; Fuchs et al., 2019b).

There are relatively few long-term studies using biologgers that reports simultaneous measurements of  $T_b$  and HR in ungulates living in harsh climates (Arnold et al., 2006; Signer et al., 2011; Turbill et al., 2011; Arnold et al., 2018; Riek et al., 2019). Similar studies of moose are important, because similar to reindeer and caribou (*Rangifer tarandus* spp.), moose are a key herbivore in northern ecosystems that are ecologically, economically, and culturally important. Currently, we still lack knowledge on the physiological ability of moose to respond to ambient temperatures that are likely to come with changing climatic conditions. Previous studies have shown that moose are easily heat stressed with respiration rate, HR and energy expenditure rising with increasing  $T_a$  (Renecker and Hudson, 1986; Roshchevsky et al., 1999; McCann et al., 2013). In captive moose the respiration rate started to increase at 14°C and at 20°C they began open-mouthed panting (Renecker and Hudson, 1986). Thompson et al. (2019) suggested several other factors that also should be considered, including core  $T_b$  and daily variations of core  $T_b$ , in addition to the influence of body condition and season, when evaluating heat stress. Climatic changes resulting in increased  $T_a$  can be expected to result in habitat changes, decreased food availability and, therefore, poorer body condition (van Beest et al., 2012; van Beest and Milner, 2013).

In the present study, we used biologgers and global positioning system (GPS) collars to study the annual and circadian rhythms of HR and  $T_b$ , in relation to activity and  $T_a$ , in free-ranging female moose in Sweden. The obtained values and patterns are relevant background for evaluating the behavioral and physiological effects of potential stressors. Moreover, because HR can be a proxy for MR, values of HR also can be used to calculate energy budgets.

We hypothesized that moose would exhibit physiological and behavioral changes related to the dramatic seasonal differences present at northern latitudes. Firstly, we predicted lower  $T_b$  and HR in winter (Regelin et al., 1985; Langvatn, 1992; Kochan, 2007; Thompson et al., 2019). Secondly, we predicted a circadian rhythmicity in the HR,  $T_b$ , and activity of moose, as previously described in ungulates because of adaptations to feeding and rumination or environmental factors such as food availability,  $T_a$  and light-dark cycles, predation, and human activities (Scheibe et al., 1999; Arnold et al., 2004; Lowe et al., 2010; Signer et al., 2011; Pagon et al., 2013; Ensing et al., 2014). We also expected a change in these rhythms over the year (Klassen and Rea, 2008; Arnold et al., 2018). Thirdly, we predicted  $T_b$  to increase with increasing  $T_a$  because of the poor tolerance of moose to

heat-stress (Renecker and Hudson, 1986; McCann et al., 2013; Thompson et al., 2019).

## MATERIALS AND METHODS

### Study Area and Animals

The study was conducted in the Nordmaling municipality (63°34'00" N, 19°30'00" E) in the county of Västerbotten in Sweden. The study area is characterized by boreal forests dominated by Scots pine (*Pinus silvestris*), Norway spruce (*Picea abies*) and birches (*Betula pendula* and *B. pubescens*). Yearly average  $T_a$  is 3°C and there are >150 days with snow cover from November to May. Mean and maximum daily snow depth from 1 November to 30 April was 0.15 and 0.5 m in 2016–2017 and 0.53 and 0.98 m in 2017–2018 (SMHI, 2019). The length of the growing season is between 150 and 160 days from late April to mid-October (SMHI, 2019).

We immobilized 12 female moose (>1.5 years old) in February 2017. The project was approved by the Animal Care Committee for Northern Sweden in Umeå (Dnr A3/2016 2016–02–26) and was conducted in accordance with Swedish laws concerning animal research ethics. Experienced veterinarians, pilots and field personnel were responsible for captures, monitoring, handling, collaring and surgeries. All personnel were trained and certified according to the standards of the Swedish Animal Welfare Agency and the Swedish Board of Agriculture.

### Biologgers and Programming

We fitted moose with collars including a GPS receiver, a temperature recorder, an activity sensor, a mortality sensor, a very high frequency (VHF) transmitter, and a Global System for Mobile communication (GSM) modem (Vectronic-Aerospace, Berlin, Germany). Activity sensors measured acceleration in three orthogonal directions six to eight times per second. For each direction activity values were averaged for a recording interval of 5 min. To present overall activity, values of two of the three orthogonal directions ( $x$  and  $y$ ) were summed, resulting in values ranging from 0–510, with 0 representing no activity and 510 highest activity (Gervasi et al., 2006). The GPS was scheduled to record positions from 1 min to 3 h depending on the ongoing studies, and together with the recorded  $T_a$  (every 5 min), those readings were sent using the GSM network to WRAM (Wireless Remote Animal Movement) database for storage (Dettki et al., 2014). Moose also were equipped with a mortality implant transmitter (MIT, Vectronic Aerospace GmbH, Berlin, Germany) in their rumen, which recorded  $T_b$  at 5-min intervals and then transmitted information to a collar unit, where it was archived. The MIT is a cylindrical device with a diameter of 21 mm, length 72 mm, and weight ca 100 g (VectronicAerospace, 2017). The most recently stored  $T_b$  was sent together with the GPS position message and the remaining data downloaded upon collar retrieval.

Additionally, we fitted moose with subcutaneous HR loggers (DST centi HRT; Star Oddi, Gardabaer, Iceland), which can simultaneously record long-term HR and subcutaneous  $T_b$ . The DST centi HRT is a cylindrical device with dimensions

of 46 × 15 mm and weighs 19 grams. HR is automatically calculated from a 4 s electrocardiogram (ECG) strip with a 150 Hz measurement frequency and stored along with a quality index of signal clarity and the R-R interval regularity. The logger can store up to 699,051 HR and temperature measurements or 2,785 ECG buffer measurements and, according to the manufacturer, has a battery life of up to 19 month (StarOddi, 2017). We programmed the DST centi HRTs to record HR every second hour from the beginning of the study until 6 August; thereafter, they were programmed to record HR every second minute.

### Immobilization Procedure

We immobilized moose from a helicopter using a CO<sub>2</sub> powered rifle (Dan-Inject, Børkop, Denmark) with the drug combination of 50 mg xylazine (Rompun Dry Substance, Bayer AG, Leverkusen, Germany) and 4.5 mg etorphine (Etorphine® HCl 9.8 mg/mL, Vericore Veterinary Products, Novartis Animal Health UK Ltd., Litlington, United Kingdom), according to previously described procedures (Evans et al., 2012; Lian et al., 2014). Pregnancy status was determined by rectal palpation (Solberg et al., 2003). Number of offspring (zero, single or twins) was verified the following spring by field observations. Biologgers were sterilized with ethylene oxide gas (Anprolene AN74i 60 L, Andersen Europe, Kortrijk, Belgium) before surgical implantation. Prior to surgery, moose were given analgesics, meloxicam (Metacam®, Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany) subcutaneously at a dose of 0.5 mg/kg. The DST centi HRT was implanted surgically at the right side of the most rostral part of the sternum, with the moose placed in lateral recumbency with the left side up. A cell phone device (KardiaMobile EKG Monitor, AliveCor Inc., Mountain View, CA, United States) and a corresponding application Kardia App (AliveCor Inc.; AliveCor, 2018), were used to find the best ECG signal, and the most appropriate site to implant the DST centi HRT. Surgery was performed according to best-practice guidelines (Fiorello et al., 2016) and a local anesthetic, bupivacaine (Marcaïn 5 mg/mL, AstraZeneca, Cambridge, United Kingdom), was administered at a total dose of 2.0–3.0 mg/moose. A 2-cm long skin incision was made and the DST centi HRT was placed between the subcutaneous fat and the muscle with the electrodes facing away from the muscle. The incision was closed with 2-0 monofilament absorbable suture PDS® II (polydioxanone) suture (Ethicon, Johnson & Johnson, New Brunswick, NJ, United States). After surgical implantation, we reversed the effects of xylazine with 5 mg atipamezole (Antisedan® 5 mg/mL, Orion Pharma Animal Health, Turku, Finland) injected intramuscularly or intravenously, to inhibit a swallowing reflex. We deployed MIT as previously described (Minicucci et al., 2018), before we reversed the immobilization with 50 mg naltrexone (Naltrexonhydroklorid vet. APL 50 mg/mL; Apotek Produktion och Laboratorier, Kungens Kurva, Sweden) intramuscularly or intravenously. There were no mortalities or known morbidities associated with the immobilization procedure. Two moose were shot during the study period, one during the annual moose hunt (September 2017) and one as a special action by the local government because of local damage to forests (February 2018).



In both instances, the loggers were collected and downloaded. We recaptured the remaining 10 moose 1 year after instrumentation (February 2018). The DST centi HRT was surgically removed, and data were downloaded, with the Mercury software program and a Communication Box (StarOddi, 2017). We downloaded all collar data and data from MITs stored in the collar unit in the field during recaptures.

## Data Preparation

One of the instrumented moose was not pregnant the first season; the remainder gave birth in May–June 2017 (mean calving date 28 May, range 17 May to 10 June), as determined from GPS clustering and subsequent visual verification in the field. The following season two moose were not pregnant. We excluded the non-pregnant moose in the first season from the beginning of the study until 15 September 2017, and the non-pregnant moose in the second season from 15 September 2017 until the end of the study. We chose 15 September because of reports of mid-September as the beginning of oestrus in female moose in Sweden (Malmsten et al., 2014).

A strong positive correlation between temperature recorded by GPS collars on moose and  $T_a$  recorded by weather stations was previously reported, therefore collar temperatures are regarded as a reliable index for  $T_a$  (Ericsson et al., 2015). We used the offset for latitude 64°N and month reported by Ericsson et al. (2015) to correct the collar temperatures for each individual moose. Drinking and snow intake have been shown to influence ruminal temperature obtained by MITs (Herberg et al., 2018). We used the R package “anomalize” (Dancho and Vaughan, 2018) to detect outliers associated with water intake (low  $T_b$  values). To facilitate optimal outlier detection for  $T_b$  time series including visual inspection and filtering, we used the twitter method to detect outliers, set trend and frequency to 6 h and allowed for a total of 20% of outlier detection (Dancho and Vaughan, 2018).

We programmed the Star Oddi device to record raw ECG signals every second hour of which a subsample of 150 ECG strips were used to manually calculate HR. The manual ECG based HR was compared with the HR measurements automatically calculated by the algorithm of the Star Oddi device. Because of insufficient automatic R-R interval detection, the algorithm was adjusted by StarOddi and rerun on the entire raw ECG data set. Bjarnason et al. (2019) provides a more detailed description of the HR calculation. Ultimately only recalculated HR measurements with quality index 0 or 1 were included in the analysis. Limitations on data storage resulted in a decision to not record raw ECG data for the 2-min detailed HR data after 6 August. Because of the high noise level and insufficient automatic R-R interval detection, HR data were not reliable after that date.

One of the recaptured moose had lost the DST centi-HRT logger and was therefore excluded from the HR analysis. Data from two moose were excluded during the entire period because of high noise level in the ECG raw data and unsuccessful HR calculation. Days of capture and recapture were excluded from the analysis, because the capture event and the anesthetics were expected to affect HR,  $T_b$ , and activity (Kreeger and Arnemo, 2018).

## Data Analysis

To investigate the seasonal patterns of  $T_b$ , HR, and activity, we fitted separate generalized additive mixed models (GAMM) with the function “bam” on daily mean  $T_b$ , HR activity and  $T_a$  as response variables (Wood, 2017). We added a fitted smooth term for day since capture, added a random intercept and slope for moose ID and an autoregressive model (AR1) structure to account for detected residual temporal autocorrelation (Pinheiro and Bates, 2000). We applied the “gam.check” function to choose adequate basis dimensions of the parameter  $k$  (Wood, 2017). We calculated the first derivatives of the smooth term for day since capture with the “fderiv” function, to determine periods of significant increase or decrease in daily mean  $T_b$ , HR and activity (Simpson, 2018).

We used Lomb-Scargle periodogram analysis to investigate circadian rhythms in  $T_b$ , HR, and activity (Ruf, 1999). We performed analyses on the raw data on a 15-day rolling window and tested for presence of circadian rhythms between 0.5 and 30 h. Significant periodicity was detected when peaks in the Lomb-Scargle periodogram exceeded the 95% confidence limit.

We visually investigated the intensity of  $T_b$  and activity variation over the year using the R package “ggplot2” to plot actograms of raw data of  $T_b$  and activity (Wickham, 2016). We calculated times for sunrise, sunset, dusk, and dawn at the following coordinates within the study area (Bivand and Lewin-Koh, 2017). We added the times to the actograms and defined sunrise and sunset as the time when the top edge of the sun reaches the horizon. Dusk and dawn were defined as the onset and end of civil twilight.

Daily energy expenditure was calculated on the days with the lowest and highest mean daily HR based on a pooled exponential equation:

$$y = 4.655e^{0.0071x},$$

where  $y$  is the MR and  $x$  is the HR normalised to  $BM^{-0.25}$  (body mass). The equation was developed from a study of captive moose (Renecker and Hudson, 1985).

## RESULTS

From February to September 2017, we obtained  $T_b$  data from eleven moose, and from September 2017 to February 2018, we obtained  $T_b$  data from nine moose. We had valid HR data from eight moose from February until the beginning of August 2017.

Female moose exhibited seasonal variation in  $T_b$ , HR and activity with higher levels in June–July and lower levels in January–March (Table 1 and Figure 1). The seasonal trends followed the same patterns for all parameters throughout the year. Significantly increasing periods of activity, HR and  $T_b$  occurred between April and June and significantly decreasing periods occurred between July and November.

Estimated mean daily energy expenditure, for all individuals, for the days with the lowest and highest daily mean HR were 377 kJ/kg<sup>0.75</sup> and 935 kJ/kg<sup>0.75</sup>, respectively, which represented

**TABLE 1** | Overview of dates, daily mean values (Mean) and 95% CI of the daily mean (95% CI) for dates with lowest and highest values and start and stop for increasing and decreasing periods of body temperature ( $T_b$ ), heart rate (HR), collar activity (activity), and ambient temperature ( $T_a$ ) for female moose in Sweden.

	Date	Mean	95% CI	n
<b><math>T_b</math></b>				
Lowest	17 March	38.03°C	37.94–38.12	11
Increase start	27 March	38.50°C	37.96–38.14	11
Increase stop	30 June	38.62°C	38.52–38.73	11
Highest	10 July	38.64°C	38.53–38.75	11
Decrease start	17 July	38.63°C	38.52–38.74	11
Decrease stop	23 September	38.30°C	38.18–38.42	9
<b>HR</b>				
Lowest	6 March	40.5 bpm	37.7–43.2	8
Increase start	9 May	45.3 bpm	41.7–48.9	8
Break increasing period start	31 May	60.1 bpm	56.2–64.0	8
Break increasing period stop	3 June	61.2 bpm	57.2–65.2	8
Increase stop	20 June	71.2 bpm	66.9–75.5	8
Highest	26 June	71.9 bpm	67.6–76.3	8
<b>Activity</b>				
Lowest	14 February	13.7 AU	6.2–21.3	9
Increase start	21 April	19.6 AU	16.4–24.2	11
Increase stop	5 June	42.1 AU	38.1–46.1	11
Highest	17 June	44.0 AU	40.0–47.8	11
<b><math>T_a</math></b>				
Lowest	30 January	−2.1°C	−3.3 to −0.9	9
Increase start	21 April	7.6°C	6.5–8.8	11
Increase stop	17 June	20.3°C	19.2–21.5	11
Highest	16 July	22.4°C	21.2–23.5	11
Decrease start	13 September	16.3°C	15.0–17.3	11
Decrease stop	11 November	4.7°C	3.5–5.8	9

a 60% decrease in energy expenditure from summer to winter (calculations in **Supplementary Appendix A**).

Lomb-Scargle periodogram analyses detected circadian rhythmicity in  $T_b$  and activity, the schedule with HR measurements only every second hour was not adequate to detect circadian patterns in HR. Percentages of the displayed rhythms, based on the results of the periodograms for  $T_b$  and activity for one representative individual in January and September are presented in **Figure 2** (for the remainder of individuals, percentages of the displayed rhythms for each month are included in **Supplementary Appendix B**). A period length ( $\tau$ ) of 24 h of  $T_b$  was dominant during most of the year, whereas ultradian rhythms with  $\tau$  of 3–8 h were more frequent in December and January, and to a small extent in June and July. Activity displayed ultradian rhythmicity with period length  $\tau$  of 2–8 h throughout the year. Actograms of raw data of  $T_b$  and activity for the same individual in January and September are presented in **Figure 3** (for actograms for all individuals throughout the study period, see **Supplementary Appendix C**). A visual analysis of the actograms shows higher intensity of  $T_b$  and activity in September compared to January, as demonstrated in **Figure 1**.

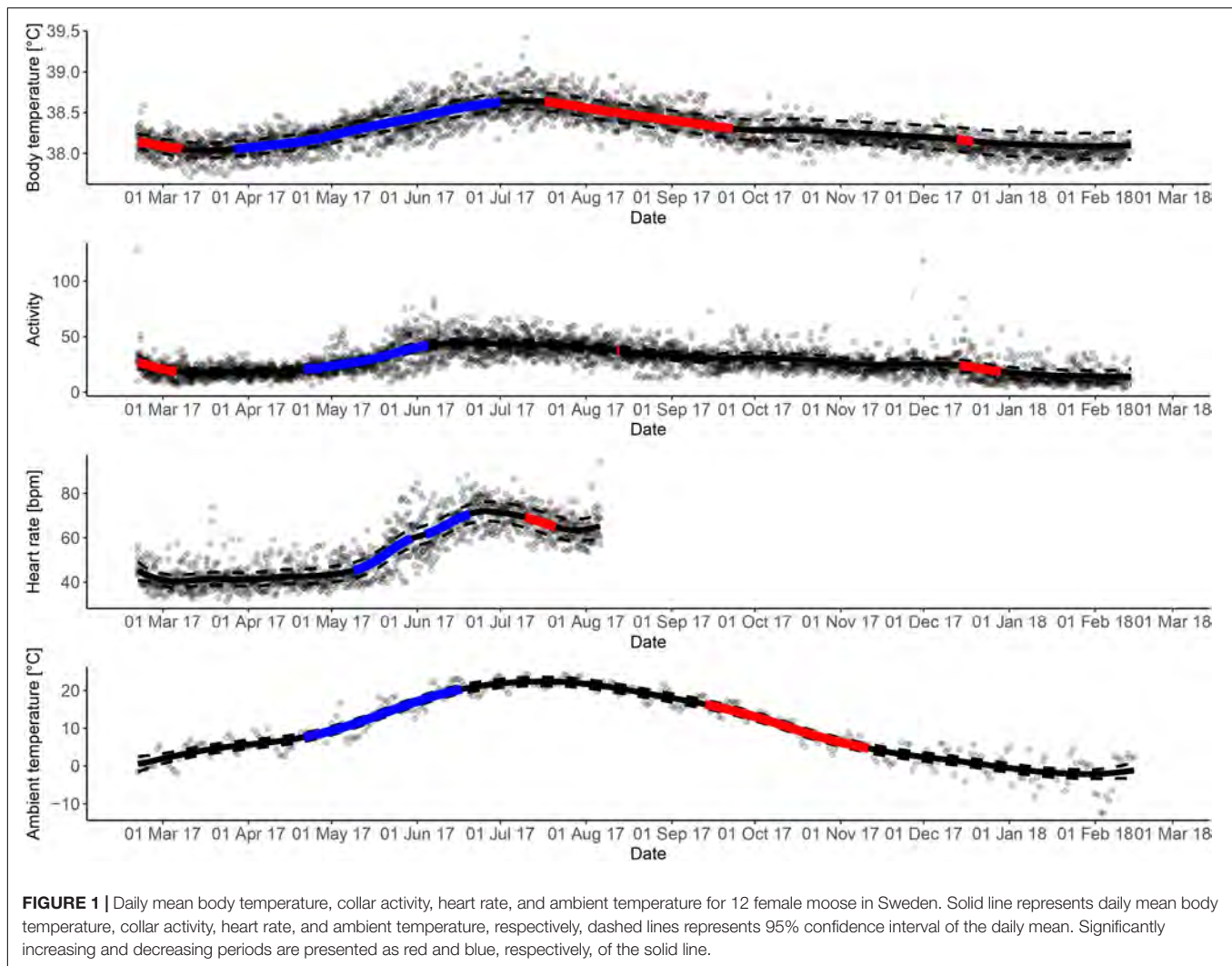
## DISCUSSION

Seasonal patterns displayed in the moose with low levels of  $T_b$ , HR and activity during winter compared with summer are similar to those reported in moose and other ungulates including alpine ibex, red deer, and Svalbard reindeer (Signer et al., 2011; Turbill et al., 2011; Arnold et al., 2018; Thompson et al., 2019). The interval from the lowest to the highest  $T_b$  recorded by vaginal implant transmitters in moose was 0.5°C (Thompson et al., 2019) compared with 0.61°C in our study using MITs, this is likely occurred because of the different types of biologgers used. The seasonal changes in  $T_b$  and HR are smaller than those reported for alpine ibex and Svalbard reindeer, which may be due to species differences, physiological state (i.e., pregnancy status, body-condition and mass, age, and sex), seasonal adaptations, the relatively mild coastal conditions and the unusually small amount of snow during the first winter of our study (mean and maximum daily snow depth was 0.15 and 0.5 m; Signer et al., 2011; Arnold et al., 2018). Maximum snow depths recorded from 2007/2008 to 2018/2019 ranged from 0.4 to 1.1 m (mean 0.66 m) and the mean snow depths from 0.13 to 0.53 m (mean of means 0.29 m; SMHI, 2019).

We calculated a 60% decrease in MR from summer to winter by decreasing HR. The calculation is based on an equation from Renecker and Hudson (1985). Mean daily MR was 377 kJ/kg<sup>0.75</sup> in winter and 935 kJ/kg<sup>0.75</sup> in summer. Calculated MR in this study is comparable to MRs obtained in earlier studies, and the decreased MR from summer to winter in these studies varies from 33–76% (Regelin et al., 1985; Renecker and Hudson, 1986, 1989). Differences between studies are likely related to dissimilarities in methods used to measure energy expenditure, study designs, and individual and environmental variation.

Voluntarily reduced food intake in winter, was demonstrated previously in moose receiving *ad libitum* high-quality food (Schwartz et al., 1984; Regelin et al., 1985). Differences in quality and intake of digestible nutrients could result in seasonal variations of HR and  $T_b$ , but do not result from food availability alone (Mesteig et al., 2000; Theil et al., 2004; Kochan, 2007; Signer et al., 2011). Decreased food intake could result in a decrease in both endogenous heat production from fermentation and metabolism in addition to reduced blood perfusion in abdominal organs, which results in lower HR and  $T_b$  (Mesteig et al., 2000; Signer et al., 2011). Seasonal variation in organ sizes corresponding to seasonal fluctuations in food and water intake occur in Sand gazelles (*Gazella subgutturosa*; Ostrowski et al., 2006), and it is assumed that this is a common feature in other ungulates as well (Arnold et al., 2006; Signer et al., 2011). A reduction in organ size could result from lower oxygen consumption with subsequent lower HR during winter.

The period with lowest levels of HR and  $T_b$  (March–April) coincides with the time of the year we would expect least natural food availability. Shortly after reaching the lowest values in mid-March,  $T_b$  started to rise, while HR was stable at its lowest level for a longer period. A similar relationship with  $T_b$  starting to rise prior to HR also was demonstrated in hibernating brown bears prior to den exit (Evans et al., 2016). Those authors suggested that the bears experienced passive



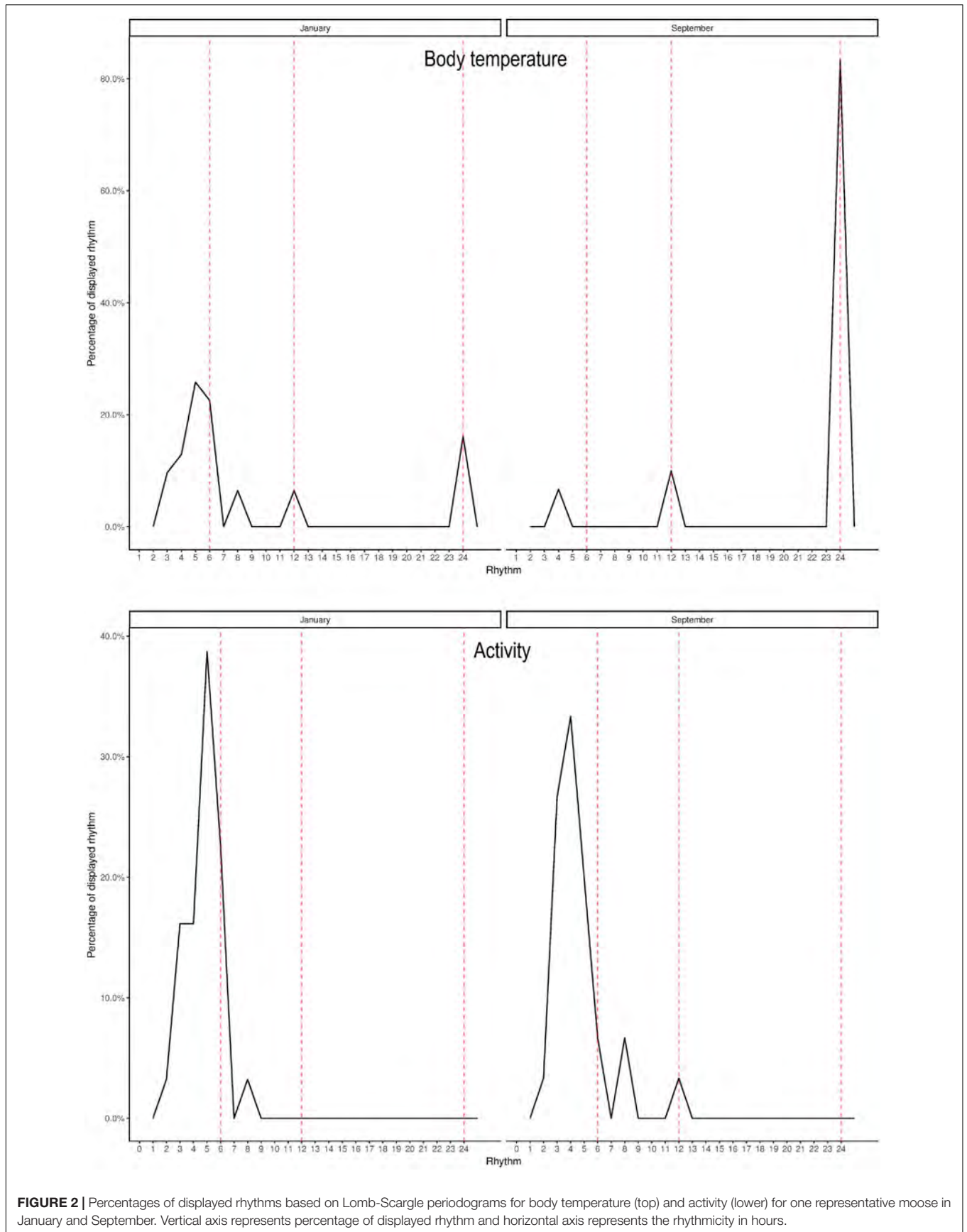
rewarming with increasing  $T_a$ , before they experienced increased activity of the sympathetic nervous system, decreased activity of the parasympathetic nervous system or a combination thereof, resulting in an increase in HR. Passive warming from basking in the sun, was also offered as a strategy in ungulates, by using exogenous heat, i.e., the sun, for thermoregulation and to reduce endogenous energy production (Signer et al., 2011). The earlier increase in  $T_b$  compared with HR and activity (and hence feeding behavior) after the lowest levels of those measurements during winter, in our study, might result from passive rewarming by the sun. Even if the  $T_a$  did not exhibit a significant increase during the period where  $T_b$  started to increase significantly, an increase in  $T_a$  was visible in the graph in March and April (Figure 1). Short days and cold nights might account for the lack of a significant increase in  $T_a$ .

Growing season, wind, light-dark cycle, snowfall, and snow cover are other external cues that influence HR and  $T_b$  in ungulates (Signer et al., 2011; Arnold et al., 2018). HR started to increase significantly at approximately the same time as the start of the growing season (mean daily  $T_a > 5^\circ\text{C}$ ; SMHI, 2019), likely

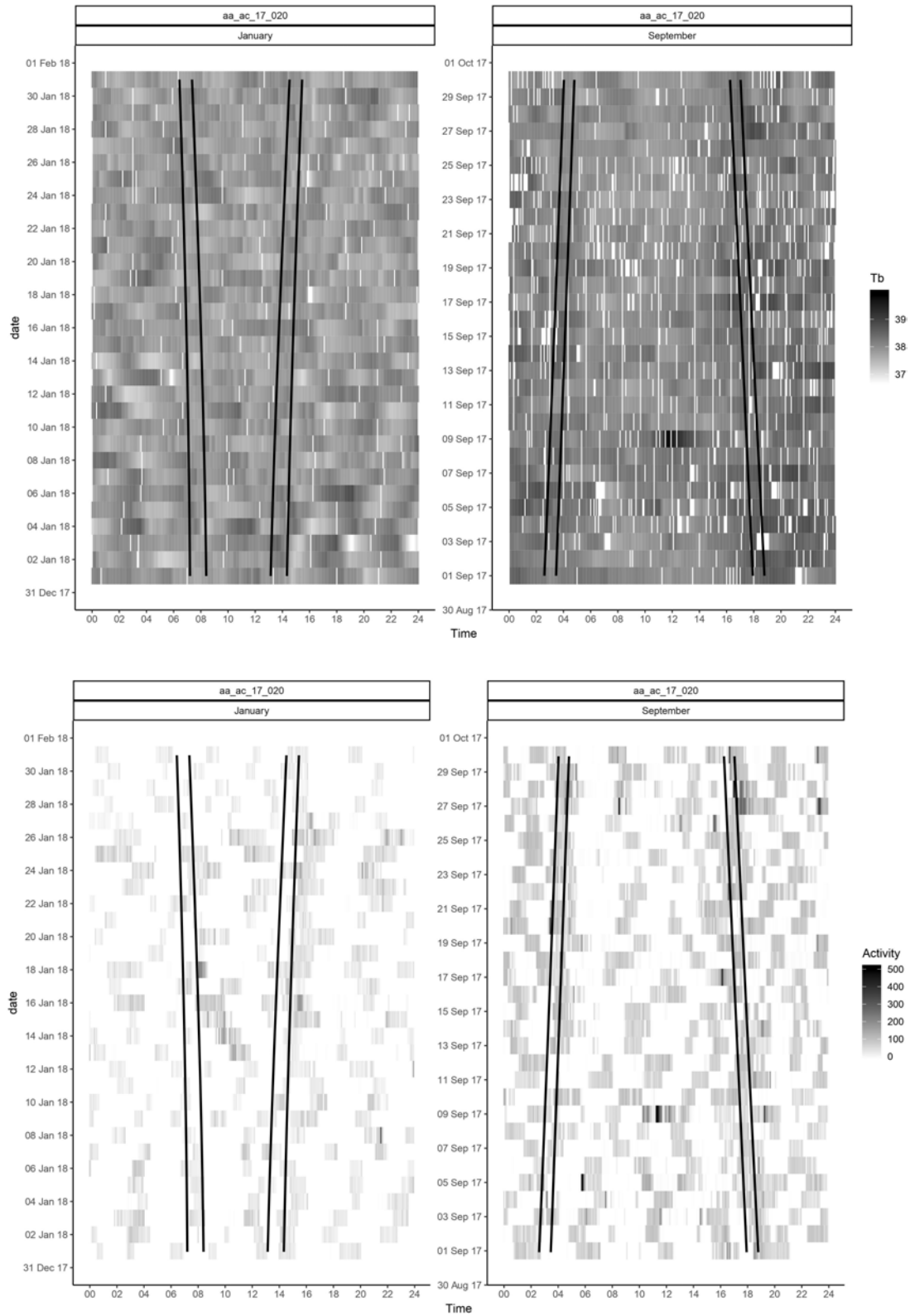
because of increased nutrition content in the food (Brosh, 2007). The last month of pregnancy could also influence the increase in HR (Mauget et al., 1997).

Activity, measured in this study with motion sensitive collars, includes head movements in addition to locomotion; consequently, feeding behavior increases activity (Gervasi et al., 2006). A rise in activity before the start of the growing season, as demonstrated in this study, could reflect increased feeding behavior because of increased food availability when snow is melting. Another moose study in the same study area reported that about 80% of the animals were migratory and that the timing of the migration differed between years, and varied with food availability and calving time (Singh et al., 2012). We did not investigate the migratory patterns in this study, but migration likely affected the seasonal patterns we observed.

We demonstrated, based on our model, a break in the increasing period of HR from 31 May until 3 June, which coincides with the calving. Nonetheless, further investigation will be necessary to determine if this break in HR resulted from calving. Reproductive status influence  $T_b$  patterns in several wild



**FIGURE 2 |** Percentages of displayed rhythms based on Lomb-Scargle periodograms for body temperature (top) and activity (lower) for one representative moose in January and September. Vertical axis represents percentage of displayed rhythm and horizontal axis represents the rhythmicity in hours.



**FIGURE 3 |** Actograms for body temperature ( $T_b$ ) and activity for one representative moose in January and September. Vertical axis represents the date and horizontal axis represents time of the day. The area between the black lines represents the twilight. Lightest color represents lowest  $T_b$  and activity, and darker color represents higher  $T_b$  and activity.

mammals. Reports exist of improved homeothermy in African lions (*Panthera leo*; Trethowan et al., 2016), wolverines (*Gulo gulo*; Thiel et al., 2019), muskox (*Ovibos moschatus*; Schmidt et al., 2020), and brown bears (Friebe et al., 2014) during gestation. Schmidt et al. (2020) also reported differences in seasonal  $T_b$  pattern between pregnant and non-pregnant muskoxen. The effect of reproductive status was not possible to detect in our study, because we only studied pregnant moose. MR in roe deer (*Capreolus capreolus*) increased during the last 2 months of gestation and during the first month of lactation, by 15 and 27%, respectively (Mauget et al., 1997). Regelin et al. (1985) documented no differences between sexes in MR in adult moose, when excluding last trimester of gestation and lactation. Thus, we would expect differences in  $T_b$ , HR and MR between pregnant and non-pregnant moose at the end of the gestation and the lactation. The skewed ratio of pregnant and non-pregnant females did not allow us to test for effects of pregnancy status on seasonal variation in HR and  $T_b$ .

Circadian rhythmicity in activity was demonstrated in this study, as previously reported in both moose (Van Ballenberghe and Miquelle, 1990; Gillingham and Klein, 1992) and other ungulates (Arnold et al., 2004; Lowe et al., 2010; Signer et al., 2011; Pagon et al., 2013; Ensing et al., 2014). In addition, we were able to demonstrate circadian organization of  $T_b$ , which has also been shown in Svalbard reindeer (Arnold et al., 2018).  $T_b$  displayed rhythms with  $\tau$  of 24 h for most of the year, except December and January, where ultradian rhythms with  $\tau$  3–8 h were more dominant. In June and July, the months with the longest daylight periods, the 24 h rhythmicity was not as dominant as in the other months. This result is similar to the pattern reported in Svalbard reindeer by Arnold et al. (2018), who documented that during the periods with midnight sun and polar night the 24-h patterns were not as dominant as during the rest of the year. Even if there are no periods with constant daylight or darkness in our study area, the differences within the year could be affected by changes in the light-dark cycle. Other reasons for deviation from the 24-h cycle could be food availability, predation, and human disturbance (Ensing et al., 2014). We observed the same trend in the ultradian rhythms in activity as in moose in Alaska and Svalbard reindeer, with shorter periods in summer compared with winter (Van Ballenberghe and Miquelle, 1990; Arnold et al., 2018). Oscillation of rumination is demonstrated to be a regulatory function of activity, so the ultradian rhythmicity of activity is likely results from feeding behavior and ruminating (Scheibe et al., 1999). Diseases and stressful situations result in impairment of the ultradian rhythmicity of activity, and periodogram analysis could be used to detect irregular patterns (Scheibe et al., 1999; Berger, 2011).

Global climate change is an important concern regarding higher energetic cost with both fluctuating and increasing  $T_a$  (Boyles et al., 2011). The threshold for heat stress cannot only be explained by the  $T_a$ , but is also affected by factors such as wind speed, solar radiation, winter fur (i.e., in spring), physiological differences (e.g., age, sex, pregnancy status, body mass, and fitness), and immune status (Renecker and Hudson, 1986; McCann et al., 2013; Thompson et al., 2019). In this

study, the highest mean daily  $T_a$  was 22.4°C, and >2 months had a mean daily  $T_a > 14^\circ\text{C}$ , one reported threshold for heat stress (Renecker and Hudson, 1986); thus, the highest mean MR calculated in our study possibly represents a MR under heat stress and hypermetabolism. As a response to the increased MR, and to reduce heat generated from feeding, ruminants reduce dry matter intake under warm conditions (Beatty et al., 2008). Increasing  $T_a$  also could result in decreased availability of high-quality forage because of changes in vegetation phenology, habitat changes, and a shortened period with forage of high nutritional quality (Monteith et al., 2015). Cumulatively, loss of body mass and failure to accumulate fat will result in lower reproduction and survival, and increased infection risk (Lenarz et al., 2009; van Beest et al., 2012; McCann et al., 2013; van Beest and Milner, 2013).

In this study,  $T_b$  started to decline significantly 1 day after  $T_a$  peaked in July. Heat stress with voluntarily reduced food intake or habitat changes to habitats with forage of poorer quality could result in decreased endogenous heat production and thereby explain the decrease in  $T_b$  (Beatty et al., 2008; Monteith et al., 2015). The lack of HR data from the beginning of August, prevent us from commenting on the HR fluctuations from summer to winter. We strongly recommend performing pilot studies when using a HR detection algorithm on new species because validation is crucial to get reliable results.

## CONCLUSION

Herein we documented the daily and annual pattern of  $T_b$  and HR of moose in northern Sweden. Annual variations of  $T_b$  and HR followed the same patterns with lowest levels in March and the highest levels from late June to early July. The decrease in HR from the highest to the lowest daily mean represents a 60% decrease in MR from summer to winter. The daily pattern of  $T_b$  varies throughout the year, with 24-h cycles as the predominant pattern in September, compared with ultradian cycles with  $\tau$  2–6 h as the predominant pattern in January. Our results indicate that hypometabolism is a vital strategy for energy saving with limited resources available in large are capital breeder in northern environment, with complexly regulated by both physiological and environmental factors. We hypothesize that hypermetabolism is also a strategy for gaining weight during summer.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The animal study was reviewed and approved by Animal Care Committee for Northern Sweden in Umeå (Dnr A3/2016 2016–02–26).

## AUTHOR CONTRIBUTIONS

AE, JA, GE, WN, and NS designed and initiated the study. AG, AE, FS, JA, BF, and AT performed the data collection; data management was done by BF and AT. AG and AT drafted the manuscript. AT and AG performed the statistical analysis with advice from BF and NS. All authors participated in writing the manuscript and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2020.00107/full#supplementary-material>

**APPENDIX A** | Energy calculations for the day with the lowest and highest daily mean heart rate.

**APPENDIX B** | Percentages of displayed rhythms based on Lomb-Scargle periodograms for  $T_b$  and activity for all individuals in all months.

**APPENDIX C** | Actograms for  $T_b$  and activity for each individual during the study.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Body temperature patterns during pregnancy and parturition in moose

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### ABSTRACT

Gestation and lactation have high energetic requirements. Up to three-fourths of the gestation period in moose (*Alces alces*) overlaps with the food-scarce period in winter. During this period, moose deal with the limited forage resources available through hypometabolism with decreased heart rate and body temperature ( $T_b$ ). Body temperature is also an indicator of oestrus, pregnancy and parturition, which is well documented in several domestic species. In this study, we sought to determine if moose displayed a similar  $T_b$  pattern during pregnancy and parturition to domesticated ruminants, and if we could detect parturition by combining  $T_b$  and activity data.

We studied the  $T_b$  pattern of 30 free-ranging adult female moose ( $\geq 1.5$  years old), equipped with ruminal temperature loggers and GPS collars. We documented a 0.13–0.19°C higher  $T_b$  in pregnant compared to non-pregnant moose, depending on the study area with the  $T_b$  difference increasing along a south-north gradient, and a drop in  $T_b$  and in activity when parturition was imminent. Detection of parturition was highly successful when combining  $T_b$  and activity data with an accuracy of 91.5%. Our findings demonstrate that  $T_b$  responses to pregnancy and parturition in a wild capital-breeding ruminant are similar to those of domesticated ruminants.

### 1. Introduction

Maintaining foetal homeothermy is a high priority in pregnant individuals. Alterations in maternal body temperature ( $T_b$ ) due to stress, exercise, and alterations in ambient temperature require thermoregulatory strategies to keep a stable foetal  $T_b$  resulting in costs for both mother and foetus (Laburn et al., 2002). Reproduction, and especially parturition and lactation are energy consuming life events (Speakman, 2008). Food scarcity combined with low ambient temperature in winter has forced many species to adapt. Strategies vary from complete inactivity and sub-freezing body temperatures to migration to more suitable habitats. Moose (*Alces alces*) are capital breeders, with reproduction relying on their stored energy reserves, and the gestation period overlaps to large degree with the periods with the lowest food availability, particularly at northern latitudes (Ericsson et al., 2001; Markgren, 1969; Schwartz and Hundertmark, 1993). Moose and other ungulates, decrease  $T_b$  during winter to drastically reduce their metabolic needs -or to hypometabolism (Arnold et al., 2018; Græsli et al., 2020; Signer et al., 2011; Turbill et al., 2011).

Body temperature, as an indicator for oestrus, pregnancy and parturition is well documented in several domesticated species like cattle, sheep, horses and dogs (Auclair-Ronzaud et al., 2020; Ewbank, 1969; Godyń et al., 2018; Verstegen-Onclin and Verstegen, 2008). Fluctuations in  $T_b$  during pregnancy in cattle have been associated with fluctuations in hormone levels, and especially progesterone levels, due to the thermogenic effect of progesterone (Kornmatitsuk et al., 2002; Nabenishi and Yamazaki, 2017; Suthar et al., 2012). Progesterone, a steroid hormone mainly secreted by the corpus luteum and/or placenta, plays an important role in the oestrus cycle, and in the establishment and maintenance of pregnancy (Wiltbank et al., 2014). During pregnancy, the corpus luteum is maintained and produces high levels of progesterone, resulting in increased  $T_b$  (Suthar et al., 2012; Wiltbank et al., 2014).

Moose are polyoestral seasonal breeders, breeding from late September to early November (Malmsten et al., 2014; Niedziakowska et al., 2022). The reported gestation lengths vary from 216 to 246 days, with a mean of 231 days resulting in a calving period in May and June (Markgren, 1969; Neumann et al., 2020; Schwartz, 1992; Schwartz and

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Hundertmark, 1993). In moose, progesterone levels increase the first 40-50 days after conception, thereafter it remains at the same level until it starts to decline 40-50 days pre-partum and in the last 4-6 days it declines sharply (Stewart et al., 1985). In cattle, corpus luteum regresses when parturition is imminent, resulting in decreased progesterone levels followed by a decrease in  $T_b$  (Cooper-Prado et al., 2011; Costa Jr et al., 2016; Kornmatitsuk et al., 2002; Suthar et al., 2012; Wrenn et al., 1958). The drop in  $T_b$  over the last days before parturition has therefore been used as a predictor of the onset of parturition in cattle (Costa Jr et al., 2016). Costa Jr et al. (2016) reported that a drop in daily mean ruminal temperature of  $\geq 0.2^\circ\text{C}$  from day -2 to -1 before parturition was a good indicator for calving in Holstein cattle. Earlier studies have used changes in movement and accelerometry data to predict parturition among ungulates, including moose (Chimienti et al., 2021; DeMars et al., 2013; Marchand et al., 2021; Nicholson et al., 2019; Severud et al., 2015). Change point analysis is a method to detect distributional changes in time series data (Killick et al., 2012). Activity derived from accelerometry data are demonstrated to decrease markedly during parturition in ungulate species like Mediterranean mouflon (*Ovis gmelini musimon*  $\times$  *Ovis* sp.) and the Alpine ibex (*Capra ibex*), where after it increase some days after the calving event (Marchand et al., 2021).

Different biologging methods have been validated for continuous measurements of  $T_b$  in moose including both ruminal and vaginal implants (Herberg et al., 2018; Thompson et al., 2018). Studies of  $T_b$  patterns during the entire pregnancy including calving in wild ruminants are, to our knowledge, limited. Thompson et al. (2019) found a statistically higher  $T_b$  in pregnant compared to non-pregnant moose ( $0.18^\circ\text{C}$  higher). Yet, the authors could not conclude if this was due to pregnancy because of the skewed ratio of pregnant and non-pregnant moose, and the  $T_b$  difference between groups was below the accuracy of the biologger. Here, we fitted female moose with ruminal temperature loggers

and GPS collars equipped with accelerometers providing fine scale data over up to two years per animal. We sought to determine if moose display a thermal pattern during pregnancy and parturition characteristic for domesticated ruminants (Costa Jr et al., 2016; Ewbank, 1969; Kornmatitsuk et al., 2002; Nabenishi and Yamazaki, 2017; Suthar et al., 2012). We also evaluated whether calving in moose could be identified based on change point analysis on a combination of thermal and activity data, as combination data has been previously documented to have higher accuracy for detecting parturition among ungulates than data from solely one source (Costa Jr et al., 2016; Ewbank, 1969; Marchand et al., 2021). We predicted higher  $T_b$  in pregnant moose compared to non-pregnant moose (Thompson et al., 2019). We also predicted a drop in ruminal temperature of  $\geq 0.2^\circ\text{C}$  when parturition is imminent (Costa Jr et al., 2016). And last, we predicted a drop in both  $T_b$  and activity in relation to calving, and thus detection of change points, due to the calving itself and the limited mobility of the calf during the first days (Costa Jr et al., 2016; Marchand et al., 2021; Testa et al., 2000).

## 2. Materials and methods

### 2.1. Study area and animals

From 2012 to 2017, we equipped 30 free-ranging female moose ( $\geq 1.5$  years old) with ruminal temperature loggers and GPS collars in three different study areas in Sweden, ranging from  $56^\circ$  to  $67^\circ$  N latitude (see Fig. 1). The Northern alpine area is located in a high alpine area of northern inland Sweden (Nikkaluokta ( $67^\circ\text{N}$ )), the Northern coastal area is located on the north-central coast (Nordmaling ( $63^\circ\text{N}$ )), and the Southern island area is located in southeast Sweden on the island of Öland ( $56^\circ\text{N}$ ). Adult female moose were visually selected and immobilised from a helicopter in the three study areas, including ten in the

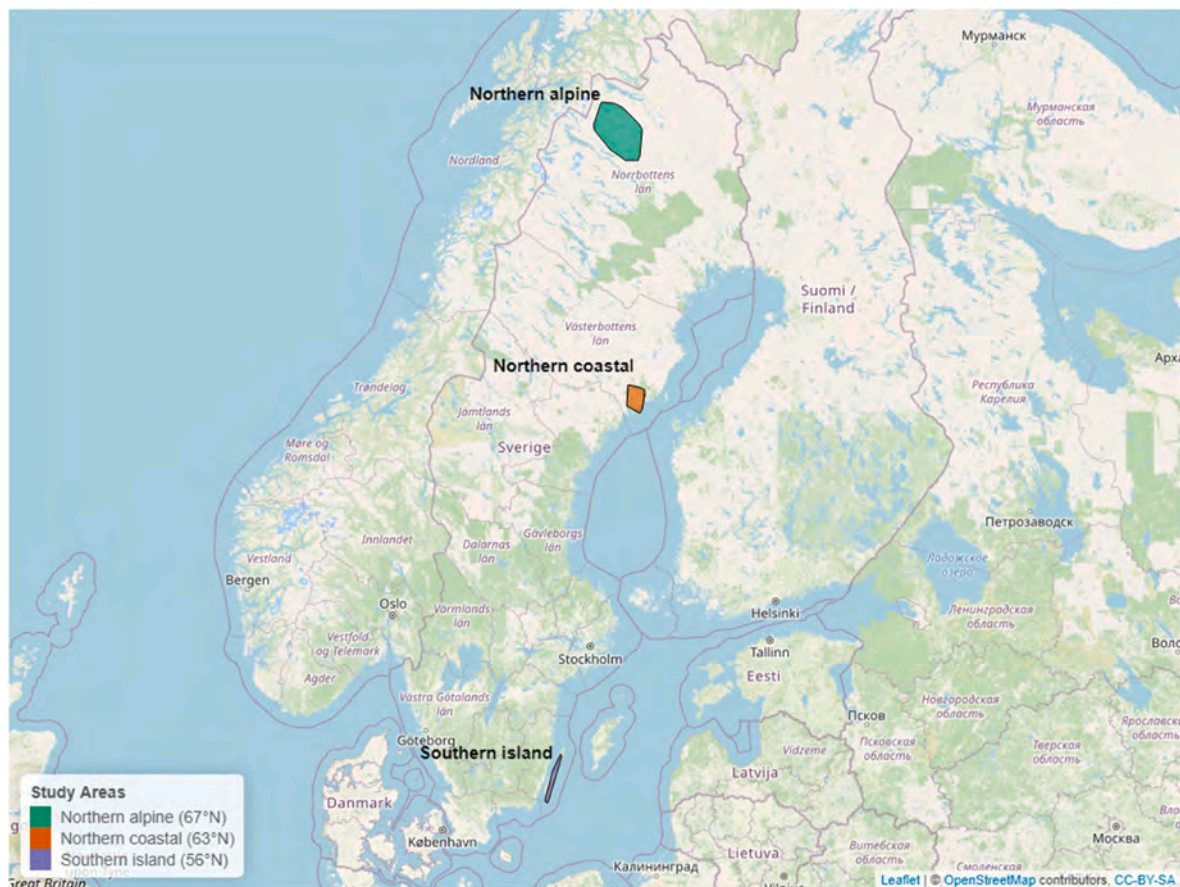


Fig. 1. Location of the study areas in Sweden.

Northern alpine area (2012), twelve in the Northern coastal area (2017) and eight in the Southern island area (2015). The project was approved by the Regional Animal Ethics Committee for Northern Sweden in Umeå (Dnr A116–09, A12–12, A50–12, A205–12, A14-15) and was conducted following Swedish laws concerning animal research ethics. All personnel were trained and certified according to the standards of the Swedish Board of Agriculture.

## 2.2. Biologgers; programming and data collection

We fitted all moose with GPS Plus Collars (former version of VERTEX Plus) equipped with a global positioning system (GPS) receiver, a very high frequency (VHF) transmitter, an ambient temperature recorder, a global system for mobile (GSM) communication modem or IRIDIUM communication, and an acceleration sensor (Vectronic-Aerospace, Berlin, Germany) (Vectronic Aerospace, 2021). The collars were scheduled to record GPS positions at intervals ranging from 30 min to 3 h depending on other ongoing studies and used the GSM cell phone network or Iridium satellite communication to send the GPS position together with the most recently recorded ambient temperature to a database server, WRAM (Wireless Remote Animal Movement), for storage (Dettki et al., 2004). Acceleration was measured over two axes (X and Y) by the acceleration sensor integrated in the collar (hereafter referred to as “activity”). Activity was measured at a scale from 0 to 255 at 6 to 8 Hz at both axes and average values over 5-minutes intervals are stored. The overall activity is presented as the sum of the activity on both axes, ranging from 0 to 510 with 0 representing no activity and 510 highest activity (Gervasi et al., 2006). In addition, we deployed mortality implant transmitters (MIT, Vectronic Aerospace GmbH, Berlin Germany), hereafter MIT, per os into the reticulorumen of the moose (Vectronic Aerospace, 2022). The MITs were programmed to obtain  $T_b$  at 5-min intervals, and the information was sent to the collar unit to be archived. Whenever the collar sent a GPS position message, the latest  $T_b$  archived was attached. The remaining data were downloaded upon collar retrieval. The accuracy of the MITs is 0.1°C, and they have a resolution of  $\pm 0.001^\circ\text{C}$  (Herberg et al., 2018; Vectronic Aerospace, 2022).

## 2.3. Immobilisation procedure

We immobilised moose from a helicopter during wintertime (February–March) according to earlier described procedures (Evans et al., 2012; Græsli et al., 2020; Lian et al., 2014). We deployed MITs during immobilisation as previously described (Græsli et al., 2020; Minicucci et al., 2018). During captures, pregnancy status was determined by rectal palpation (Solberg et al., 2003). To retrieve the data obtained by the MIT, we recaptured the moose, according to the same procedure as the initial captures, and downloaded the stored MIT and activity data from the collar. Moose in the Northern alpine and Southern island areas were recaptured three years after the first capture, while moose in the Northern coastal area were recaptured the two following years after the initial capture, due to other ongoing studies.

## 2.4. Data preparation and analysis

One of the moose in the Southern island area died a few days post-capture and is excluded from the analyses. A necropsy was performed and no cause of death was identified, however, the MIT was missing from the rumen and was not found elsewhere, so we cannot rule out the possibility of improper placement of the device and subsequent expulsion. Three other moose died during the study period, two were shot during the annual moose hunt, and one died due to unknown reasons over one year after device deployment. For these moose, the MIT data were downloaded from the collar, and these data were included in the analyses. In addition, we lost GPS connection with two moose during the study period, and the received MIT data of nine and twelve months

duration, respectively, were included in the analysis. In total, we used 3,935,575  $T_b$  measurements from 29 individual moose. We prepared and analysed the data using R version 4.0.0 (R Core Team, 2021), and considered  $P$  values  $< 0.05$  as significant.

We excluded the days of capture, recapture and three days post-capture/recapture from the analysis because the capture event and anaesthetics were expected to affect  $T_b$  (Kreeger and Arnemo, 2018; Thompson et al., 2020). Calving dates were determined based on GPS clustering, and subsequent visual observation of cow and offspring(s) in the field (Neumann et al., 2020). We defined the calving period as the calving date  $\pm 15$  days. We used the mean gestation length of 231 days and calculated a conception date from the calving date (Schwartz and Hundertmark, 1993). To compare the  $T_b$  patterns between different reproductive states we used the mean calving date in the given area as a simulated calving date for non-pregnant moose. An overview of the study population with pregnancy status, years and study areas are shown in Table 1. We could verify birth of twins in 32% ( $n = 12$ ) of the pregnant females (Northern alpine 9%,  $n = 1$ ; Northern coastal 39%,  $n = 7$ ; Southern island 50%,  $n = 4$ ).

We used the method described by Thompson (2020) to filter out drinking events, as the ruminal temperature is shown to be a good predictor for body temperature when drinking events are filtered out (Herberg et al., 2018). Thus, every  $T_b \leq 37.54^\circ\text{C}$  and the following 40 min, in addition to every drop in  $T_b > 0.25^\circ\text{C}$  within 5 min (from the previous regular measurement) were classified as drinking events and filtered out.

### 2.4.1. Body temperature patterns associated with pregnancy

To investigate  $T_b$  patterns, and to test for differences of  $T_b$  in relation to different reproductive states throughout the year, we fitted generalised additive mixed models (GAMMs) with daily mean  $T_b$  as the response variable (Wood, 2017). We used the following explanatory variables to build a set of candidate models: 1) Pregnant (factor with two levels; yes (pregnant) or no (non-pregnant)), describing the reproductive state of the animal; 2) “day of gestation” (DOG, continuous 1–231), a measure of days from the estimated conception date and 3) area (factor with three levels: Northern alpine, Northern coastal and Southern island), which includes our three study areas. We set up a list of candidate models with all possible additive combinations as well as the interactions between DOG and Pregnant and Area and Pregnant. We included these combinations because we expected different patterns over time between different reproductive states, due to changes in progesterone levels during the pregnancy in moose (Stewart et al., 1985), and because we expected differences in environmental conditions like climate and food availability and quality between areas, which could be reflected in differences in body condition between pregnant and non-pregnant in capital breeders like moose (Holmes et al., 2021).

We fitted a smooth term for DOG, due to the non-linear relationship with the default thin plate regression spline. Area was included due to a reported latitudinal difference in daily mean  $T_b$  (Fohringer, 2021). Due to the high number of  $T_b$  measurements, we used the function “bam” from the R package *mgcv* (Wood and Wood, 2015). We used model selection based on Akaike’s Information Criterion (AIC) to select the highest ranked model for our data within  $\Delta\text{AIC} < 2$ , carried out with the function *lctab* from the *bbmle* package (Bolker and RCoreTeam, 2017).

**Table 1**

Overview over study population, pregnancy status, year and location.

Study area	Year	$n$	Pregnant	Non-pregnant
Northern alpine	2012	10	5	5
	2013	9	6	3
Northern coastal	2017	12	11	1
	2018	8	7	1
Southern island	2015	7	6	1
	2016	4	2	2
Total		50	37	13

Moose ID was included as a random intercept and slope in all models. To account for detected temporal autocorrelation, we added an autoregressive order 1 (AR1) model structure (Pinheiro and Bates, 2000). To choose adequate basis dimensions of the parameter  $k$ , we applied the “gam.check” function (Wood, 2017). We applied a post-hoc test with estimated marginal means from the emmeans package to estimate which levels of the categorical variables were significantly different from each other (Lenth et al., 2019).

#### 2.4.2. Body temperature patterns associated with calving

We calculated hourly mean  $T_b$  during the calving period for all moose ( $n = 50$ ). From the hourly means, we calculated a rolling 24 h mean  $T_b$  for every hour during the day (from 06.00 one day to 05.59 the day after, from 07.00 to 06.59 etc.). We did that to ensure that we were able to detect drops in the variables throughout the day, as we did not have the exact time of calving. We thereafter calculated drops in the 24 h mean body temperatures for each hour during the day, and detected drops in the 24 h mean  $T_b \geq 0.2^\circ\text{C}$  in relation to the calving date estimated from GPS-clustering among the pregnant females.

#### 2.4.3. Prediction of parturition based on body temperature and activity data

We calculated rolling 24 h activity means according to the same method as used to calculate rolling 24 h  $T_b$  means for all moose. We used changepoint analysis among the pregnant females to detect specific time points in the calving period, with major changes in the activity and  $T_b$  data. We only included moose with both activity and  $T_b$  data in our changepoint analysis ( $n = 47$ ), and therefore excluded three moose lacking activity data. We used the R package “changepoint.np” due to the non-parametric distribution of the data (Haynes et al., 2021). We used the PELT (Pruned Exact Linear Time) method and the CROPS (The Changepoints for a Range of Penalties) algorithm in the “cpt.np” function of the R-package to find the optimal segmentations and locations of multiple changepoints in the time-series data (Haynes et al., 2014; Killick et al., 2012). To avoid overfitting or underfitting of the number of changepoints, we used diagnostic plots as described in the method by Lavielle (2005) to identify the appropriate penalty constant. The number of changepoints was selected as the elbow in the curve in the diagnostic plot of the unpenalised cost versus the number of changepoints. We thereby found the location of the selected number of changepoints and plotted the detected changepoints on time series graphs, one for each parameter, to visualise changepoints in activity and  $T_b$  in the calving period. We used the detected changepoints around the calving date detected by GPS-clustering to describe the pattern of  $T_b$  and activity in relation to parturition. We calculated the number of individuals with the same type of pattern in relation to the parturition to identify the number of events detected as true-positive parturition events, but also the number of false-positive events and calving events not detected by this method.

We repeated the procedure for non-pregnant individuals to look for the same pattern among those, and thus find the number of females identified as non-parturient, but also the number of false positive parturition events in the calving period. The calving period was defined as the mean estimated calving date (of the pregnant females) in the area  $\pm 15$  days for non-pregnant individuals.

We calculated the sensitivity (proportion of correctly identified parturient females), specificity (proportion of correctly identified non-parturient females) and accuracy (overall rate of true predictions) of the use of changepoints to detect parturition.

### 3. Results

#### 3.1. Body temperature patterns associated with pregnancy

Body temperature varied with reproductive states, and was higher in pregnant compared to non-pregnant moose throughout the entire

gestation period (Fig. 2). The highest ranked model to explain variation in  $T_b$  included reproductive state, area, the interaction between them, DOG and the interaction between reproductive state and the DOG (Tables 2 and 3). Post-hoc analysis revealed a  $0.19^\circ\text{C}$  (SE  $0.02^\circ\text{C}$ ,  $p$ -value  $< 0.0001$ ),  $0.13^\circ\text{C}$  (SE  $0.02^\circ\text{C}$ ,  $p$ -value  $< 0.0001$ ) and  $0.13^\circ\text{C}$  (SE  $0.02^\circ\text{C}$ ,  $p$ -value  $< 0.0001$ ) higher daily mean  $T_b$  in pregnant moose compared to non-pregnant in the Northern alpine area, Northern coastal and Southern island study areas, respectively.

#### 3.2. Body temperature patterns associated with calving

$T_b$  decreased when parturition was imminent (Fig. 3). We detected a drop in the rolling 24 h mean  $T_b \geq 0.2^\circ\text{C}$  associated with calving in 59.5% ( $n = 22$ ) of all moose (Northern alpine = 27.3%,  $n = 3$ ; Northern coastal = 77.8%,  $n = 14$ ; Southern island = 62.5%,  $n = 5$ ). Twelve of the remaining moose, which were observed with new-born calves, had a drop in the rolling 24 h mean  $T_b$  between  $0.1^\circ\text{C}$  and  $0.2^\circ\text{C}$ , while the last three moose had a  $T_b$  drop  $\leq 0.1^\circ\text{C}$  (Supplementary file 1).

#### 3.3. Prediction of parturition based on body temperature and activity data

We detected a changepoint in a decreasing trend in the body temperature followed by a changepoint in a decreasing trend in the activity in relation to the calving date (estimated from GPS clustering) in 31 of 35 pregnant individuals (Fig. 3). This gives a sensitivity of this method of 88.6%. The changepoint in  $T_b$  occurred the day before the parturition (mean: day -1, range: day -3 – 0), while the changepoint in activity occurred at the parturition date (mean: day 0, range: day -1 – 1). This pattern was only detected in relation to parturition, and not elsewhere during the calving period. All four parturient individuals not showing this pattern were from the Northern alpine area. We could not detect a pattern indicating parturition in any of the non-pregnant females ( $n = 12$ ) in the calving period (mean calving date in the area  $\pm 15$  days) (Fig. 4), resulting in a lack of parturition date for all non-pregnant females and thus a 100% specificity of the method. The overall accuracy of this method to detect parturition in moose, including pregnant and non-pregnant ones, was 91.5%.

### 4. Discussion

Our study showed that the body temperature patterns displayed in wild and free-ranging moose during pregnancy and parturition, with a significantly higher  $T_b$  in pregnant compared to non-pregnant animals, and a decline in  $T_b$  when parturition is imminent, are similar to that reported in domesticated cattle (Costa Jr et al., 2016; Suthar et al., 2012; Wrenn et al., 1958). The daily mean body temperatures were between  $0.13$  and  $0.19^\circ\text{C}$  higher in pregnant compared to non-pregnant moose, depending on the study area with body temperature difference increasing along a south-north gradient (i.e. from the Southern island and Northern coastal areas to the Northern alpine area). Our results are in line with a study of North American moose that reported a difference in core body temperature of  $0.18^\circ\text{C}$  between the pregnancy states (Thompson et al., 2019).

Our documented differences in  $T_b$  between the two reproductive states with higher  $T_b$  in pregnant than non-pregnant is in line with previous reports from muskoxen (*Ovibos moschatus*) and Svalbard reindeer (*Rangifer tarandus platyrhynchus*), two other capital breeders living in cold and seasonal environments (Arnold et al., 2018; Schmidt et al., 2020). In muskoxen, the linkage between reproductive state and  $T_b$  was suggested due to hypometabolism, as hypometabolism was only displayed in non-pregnant muskoxen (Schmidt et al., 2020). This is in contrast to moose where hypometabolism is documented in both pregnant and non-pregnant moose (Fohringer, 2021; Græsli et al., 2020). Schmidt et al. (2020) suggested that there was a trade-off in female muskoxen between hypometabolism and sustaining foetal growth. Fluctuations in maternal  $T_b$  during pregnancy disturbs the foetal  $T_b$ , but

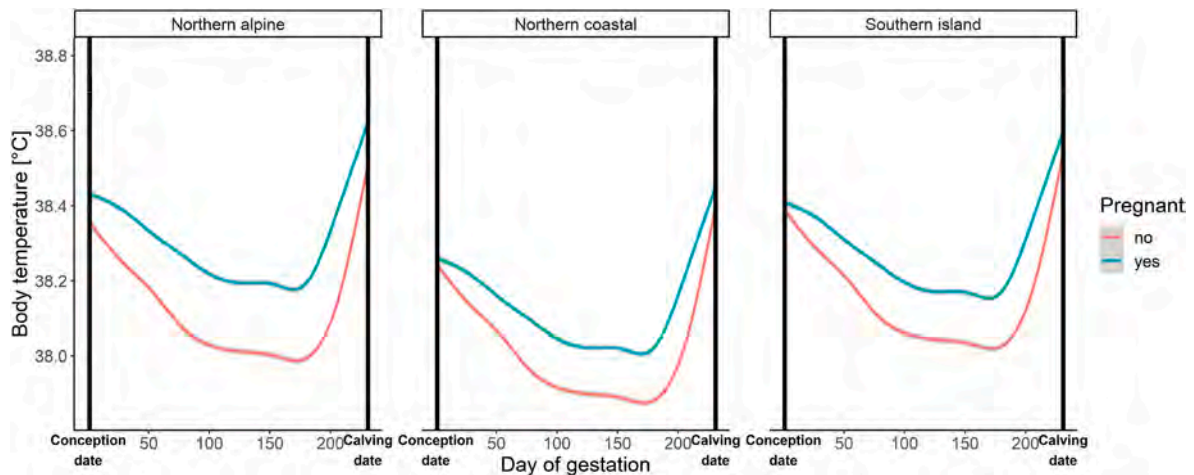


Fig. 2. Daily mean body temperature patterns during pregnancy in moose between different reproductive states and areas predicted from generalised additive mixed models (GAMMs). Grey areas around the lines represents the 95% confidence intervals.

Table 2

Log-likelihood (logLik), Akaike's information criterion ( $\Delta$ AIC), degrees of freedom (df) and model weight (weight) for the model combinations evaluating daily mean body temperature ( $T_b$ ) of female moose during pregnancy, considering the explanatory variables Pregnant (factor with two levels; yes or no), "day of the gestation" (DOG, 1–231) and Area (factor with three levels: Northern alpine, Northern coastal and Southern island). "s" is the smoother term assessing the linearity or non-linearity of the response variable. Moose ID was included as a random intercept and slope in all models. The best-fitted model were selected as the model with the lowest AIC score (most parsimonious model). To account for detected temporal autocorrelation, we added an autoregressive order 1 (AR1) in the selected model.

Model combinations	logLik	$\Delta$ AIC	df	weight
Pregnant * Area + s(DOG) + s(DOG * Pregnant)	7564.6	0.0	96.6	1
Pregnant + Area + s(DOG) + s(DOG * Pregnant)	7537.8	49.4	94.4	<0.001
Pregnant + s(DOG) + s(DOG * Pregnant)	7537.7	49.6	94.5	<0.001
Pregnant + Area + s(DOG)	7474.6	158.7	85.9	<0.001
Pregnant + s(DOG)	7474.5	158.9	86.0	<0.001
Area + s(DOG) + s(DOG * Pregnant)	7193.7	734.8	93.1	<0.001
s(DOG) + s(DOG * Pregnant)	7193.6	735.0	93.1	<0.001
Area + s(DOG)	6670.7	1760.9	83.1	<0.001
s(DOG)	6670.5	1761.2	83.2	<0.001
Pregnant * Area	3609.0	7839.9	61.0	<0.001
Pregnant + Area	3605.7	7842.6	59.0	<0.001
Pregnant	3605.6	7842.8	59.0	<0.001
Area	3277.2	8497.3	57.8	<0.001
Null model	3277.0	8497.8	57.9	<0.001

the excursions can be attenuated by changes in the heat production of the foetus or the thermal conductance of the placenta (Laburn et al., 2002). In moose, hypometabolism is a strategy to save energy during periods with colder climate and limited resources available, in both pregnant and non-pregnant moose, as well as in males (Fohringer, 2021; Græsli et al., 2020). The lowest daily mean  $T_b$  in winter was 0.6°C lower than the highest daily mean  $T_b$  in summer in pregnant females in the Northern coastal area (Græsli et al., 2020). The consequences for the foetus of maternal exposure to hot temperatures are more severe than to cold ones (Laburn et al., 2002). We therefore assume that the hypometabolism displayed during winter is well tolerated by both the foetus and the pregnant moose. The connection between fluctuations in  $T_b$  and hormone levels, and especially progesterone levels (due to the thermogenic effect of progesterone) is well documented in domestic ruminants (Suthar et al., 2012; Wrenn et al., 1958). Progesterone levels in moose increase in the beginning of the pregnancy, and then remain stable until they decline during the last part of the pregnancy (Stewart et al., 1985).

Table 3

Results of the generalised additive mixed model (GAMM) testing for effects of reproductive state, study area and day of gestation on mean daily  $T_b$ . The adjusted  $R^2$  for the model was 0.782. The intercept represents non-pregnant females in the Northern alpine area.

Term	Estimate	SE <sup>a</sup>	t-value <sup>b</sup>	p-value
Intercept	38.123	0.049	773.447	<0.0001
Pregnant (yes)	0.182	0.015	12.065	<0.0001
Area (Northern coastal)	-0.113	0.064	-1.765	0.0776
Area (Southern island)	0.032	0.073	0.440	0.6597
Pregnant (yes) Area (Northern coastal)	-0.059	0.020	-2.884	0.0039
Pregnant (yes) Area (Southern island)	-0.055	0.018	-3.009	0.0026

Term	EDF <sup>c</sup>	F-value	p-value
s(DOG)	22.75	71.75	<0.0001
s(DOG):pregnant no	7.43	6.49	<0.0001
s(DOG):pregnant yes	0.0008	0.03	0.996
s(Object_ID)	24.07	5460.39	<0.0001
s(Object_ID, DOG)	24.88	4525.36	<0.0001

<sup>a</sup> SE = standard error.

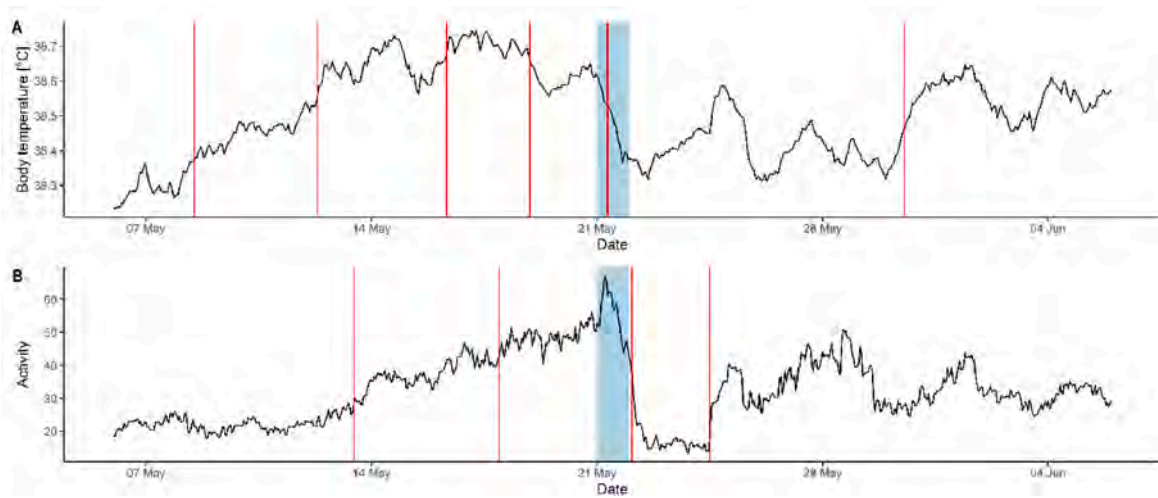
<sup>b</sup> t-value = test statistic.

<sup>c</sup> EDF = effective degrees of freedom.

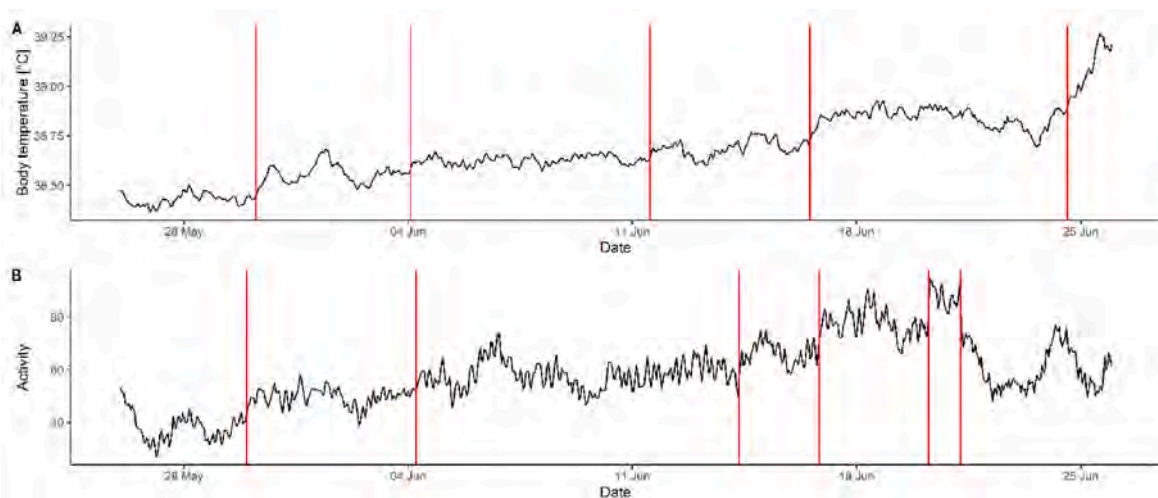
We therefore suggest that the difference in  $T_b$  between pregnant and non-pregnant moose reflects the higher progesterone levels during pregnancy, based on the progesterone fluctuations documented in moose during pregnancy and the thermogenic effect of progesterone.

About 60% of the moose in our study displayed a drop in daily mean  $T_b \geq 0.2^\circ\text{C}$  associated with calving, as earlier reported in domestic cattle (Costa Jr et al., 2016). The probability of a  $T_b$  drop  $\geq 0.2^\circ\text{C}$  associated with calving varied between areas, with highest probability in Northern coastal area (77.8%), and lowest in the Northern alpine area (7.3%). The differences between areas indicates that animals in different environmental settings encounter different limitations and margins especially with respect to food availability and quality, and climatic conditions such as ambient temperature, precipitation and snow conditions. Similarly, annual variations in environmental conditions, food availability and quality could explain differences between areas as the study years differs between the areas (Holmes et al., 2021). We recommend future research to study more deeply possible environmental factors explaining observed differences in  $T_b$  and their ecological implications. Costa Jr et al. (2016) reported an average difference of 0.32°C (primiparous) and 0.36°C (parous) in Holstein cattle. The different results between our and the study of Costa Jr et al. (2016) are likely species-specific, and/or





**Fig. 3.** Changepoints (red vertical lines) in the rolling 24 h mean body temperature (A) and activity (B) in the calving period (calving date estimated from GPS-clustering  $\pm 15$  days) for one pregnant female moose. The calving date is presented as a vertical blue bar.



**Fig. 4.** Changepoints (red vertical lines) in the rolling 24 h mean body temperature (A) and activity (B) in the calving period (mean calving date in the area estimated from GPS-clustering  $\pm 15$  days) for one non-pregnant female moose.

linked to differences in experimental set-up, methods, and ruminal sensors used.

We demonstrated a changepoint in a decreasing trend in  $T_b$  the day before the calving, followed by a changepoint in a decreasing trend in activity on the day of the calving. This was a highly successful method of detecting parturition in moose (accuracy 91.5% (percentage of correct assessments), sensitivity 88.6% (percentage of true positive assessments) and specificity 100% (percentage of true negative assessments). When interpreting this result one should bear in mind that the parturition date is based on an estimation from GPS-clustering, and subsequent visual observation of mother and offspring(s) in the field (Neumann et al., 2020), and the precision of this method is not validated. The accuracy of our method are, however, similar to the accuracy of other methods using GPS data alone or in combination with activity data to predict parturition in ungulates (DeMars et al., 2013; Marchand et al., 2021; Nicholson et al., 2019). By combining  $T_b$  and activity data as a combination of the individuals' physiological and behavioural response, we were able to get more accurate results than the use of activity or  $T_b$  alone, as the use of them alone would have resulted in several false positives. This is likely due to other sources of fluctuations in activity and thus mainly movement, and ruminal  $T_b$  such as heat increment of feeding, drinking events, the location of the ruminal sensor, increased

activity, stress and infections (Beatty et al., 2008). We cannot rule out that there might be more efficient and less invasive methods to infer the parturition in moose, more research is needed to investigate this.

Age, body mass, population density and climatic conditions are known to affect fecundity in female moose, and moose in northern-Sweden living under harsh climatic conditions have a lower number of offspring per female moose compared to moose further south (Sand, 1996; Sæther and Haagenrud, 1983). Progesterone concentrations during pregnancy are higher in female moose with twins compared to singletons (Stewart et al., 1985). Due to the thermogenic effect of progesterone, one would expect that this was reflected in the  $T_b$ , as shown in cattle where cows with twins had higher  $T_b$  compared to those with singletons (Scanavez et al., 2018). Due to the skewed ratio between females with singletons and twins within the different areas, we were not able to investigate the correlation between the number of offspring per female and the  $T_b$  pattern. Considering the potential connection between  $T_b$  and progesterone levels, we also expect that the differences in progesterone levels during oestrus will influence the  $T_b$  levels, as reported in cattle (Cooper-Prado et al., 2011; Wrenn et al., 1958). A variety of gestation lengths of moose have been reported, varying from 216 to 246 days (Markgren, 1969; Schwartz, 1992; Schwartz and Hundertmark, 1993). We would suggest further development of this study,

by investigating the  $T_b$  and activity patterns during oestrus in captive moose, to see if the conception date could be identified by  $T_b$  and activity and possibly determine a more accurate gestation length based on the conception and parturition dates. This is relevant information for monitoring reproductive patterns in moose in a changing climate and vegetation phenology.

## 5. Conclusion

Here we documented  $T_b$  patterns of female moose in three different environmental settings in Sweden during the entire pregnancy, including parturition. We found a significant higher  $T_b$  in pregnant compared to non-pregnant moose, and  $T_b$  decreased when parturition was imminent. We also found site-specific change in temperature with larger change at more northern latitudes. About 60% of the pregnant moose displayed a drop in daily mean  $T_b \geq 0.2^\circ\text{C}$  associated with calving. A combination of  $T_b$  and activity data allowed successful detection of parturition with a high accuracy (91.5%).

## Author statement

**Anne Randi Græsli:** Conceptualisation, Investigation, Formal analysis, Writing – Original Draft, Writing – Review & Editing **Alexandra Thiel:** Investigation, Formal analysis, Writing – Review & Editing **Boris Fuchs:** Investigation, Writing – Review & Editing **Fredrik Stenbacka:** Investigation, Writing – Review & Editing **Wiebke Neumann:** Conceptualisation, Writing – Review & Editing **Jonas Malmsten:** Conceptualisation, Writing – Review & Editing **Navinder Singh:** Conceptualisation, Writing – Review & Editing **Göran Ericsson:** Conceptualisation, Investigation Writing – Review & Editing, Project administration, Funding acquisition **Jon M. Arnemo:** Conceptualisation, Investigation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition **Alina Lynn Evans:** Conceptualisation, Investigation, Writing – Review & Editing, Supervision.

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## Declaration of competing interest

There are no conflicts of interest.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2022.103334>.

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# Physiological and behavioural responses of moose to hunting with dogs

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Optimal management of hunted species requires an understanding of the impacts of hunting on both individual animal and population levels. Recent technological advancements in biologging enable us to obtain increasingly detailed information from free-ranging animals, covering longer periods of time, and providing the data needed to assess such impacts. In Sweden, more than 80 000 moose are harvested annually, mostly hunted with the use of baying dogs. The effects of this hunting method on animal welfare and stress are understudied. Here, we evaluated 6 real and 17 experimental hunting approaches with baying dogs [wearing global positioning system (GPS) collars] on 8 adult female moose equipped with ruminal temperature loggers, subcutaneous heart rate (HR) loggers and GPS collars with accelerometers. The obtained data were used to analyse the behavioural and physiological responses of moose to hunting with dogs. Successful experimental approaches (moose and dog were within 240 m for > 10 min) resulted in higher maximum body temperature ( $T_b$ , 0.88°C higher) and a mean increase in HR of 24 bpm in moose at the day of the approach compared to the day after. The moose rested on average > 90 min longer the day after the approach compared to the day of the approach. The moose travelled on average 4.2 km longer and had a 1.3 m/s higher maximum speed the day of the approach compared to the day after. Our results demonstrate that hunting with dogs increase moose energy expenditure and resting time (and consequently decrease time available for foraging) on an individual level. This could possibly affect body condition and reproduction rates if the hunting disturbances occur frequently.

**Key words:** Activity, *Alces alces*, body temperature, GPS (global positioning system), heart rate, human disturbance

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## Introduction

Understanding how human disturbance affects behaviour and physiology of animals is important for wildlife conservation

and management (Wikelski and Cooke, 2006). Human disturbance may induce both acute and chronic stress. Stress is a nonspecific response to challenges in the body's homeostasis, which can be either positive or negative depending on the type

and length of the exposure (McLaren *et al.*, 2007, Reeder and Kramer, 2005, Reh binder, 1990). A stressor is a stimulus causing stress, and the stressor could be physical, psychological or both (Reeder and Kramer, 2005). As a response to a stressor, the sympathetic nervous system is activated immediately, also known as the fight-or-flight response, while the hypothalamic–pituitary–adrenal axis is activated more slowly and lasts longer (Reeder and Kramer, 2005, Reh binder, 1990). Stress results in physiological (i.e. metabolic, neuroendocrine and immunological) and behavioural responses of the animal, and objective and quantitative measurements of stress are often used to assess animal welfare (McLaren *et al.*, 2007). Both immediate and long-term responses to stressful situations could result in life-threatening consequences, including hyperthermia in acute stress situations and immune suppression, loss of body weight and decreased reproductive rates in long-term situations (Moberg, 2000).

In Sweden, more than 80 000 moose (*Alces alces*) are harvested annually. The hunting season lasts for 5 to 6 months from the beginning of September or October until January or February, depending on local regulations (Lavsund *et al.*, 2003; Länsstyrelserna, 2020). Hunting with dogs, especially baying dogs, is a common practice (Ball *et al.*, 1999; Heberlein, 2000). Concerns about animal welfare and stress related to hunting have been raised (Nelson *et al.*, 2005, Paquet and Darimont, 2010), but so far, data are lacking to assess these issues. Different physiological and behavioural variables help to measure and to determine if hunting disturbances result in increased energy expenditure.

Stress can be measured through physiological variables like body temperature ( $T_b$ ) and heart rate (HR) (McLaren *et al.*, 2007). However, the identification of changes during potentially stressful situations requires a baseline for these variables. Continuous measurements of HR and  $T_b$  could indirectly assess the sympathetic nervous system and the acute stress response as the activation results in the release of catecholamines (adrenaline and noradrenaline), which increases HR and  $T_b$ . Increased HR (>200 bpm) and  $T_b$  (>42.0°C) have been recorded in brown bears (*Ursus arctos*) chased by baying dogs and black bears (*Ursus americanus*) hunted using bait (Laske *et al.*, 2011; Støen *et al.*, 2018). Extremely high  $T_b$  is cytotoxic, and even short periods of exposure to high  $T_b$  can be lethal (Lepock, 2003, Tansey and Johnson, 2015). Increased HR during hunting situations could be due to the combination of both fleeing and stress, and it is difficult to differentiate the role of each as both are normal physiological reactions. Persistently elevated HR can be dangerous when lasting for a long time, and even fatal, due to arrhythmias, infarction or myopathy of the heart (McLaren *et al.*, 2007; Stephenson, 2007).

Behavioural responses to stress caused by hunting, also known as anti-predator behaviour, have been studied in different game species including moose (Baskin *et al.*, 2004; Neumann *et al.*, 2009; Ericsson *et al.*, 2015b; Sand *et al.*, 2016), red deer (Jarnemo and Wikenros,

2014, Sunde *et al.*, 2009), roe deer (*Capreolus capreolus*) (Benhaïem *et al.*, 2008, Cederlund and Kjellander, 1991) and brown bears (Ordiz *et al.*, 2013; Hertel *et al.*, 2016). In ungulates, displacement from typical home range, increased home range area and nocturnal behaviour and stay, fight or flee when attacked are examples of anti-predator strategies (Benhaïem *et al.*, 2008, Cederlund and Kjellander, 1991, Jarnemo and Wikenros, 2014, Lingle and Pellis, 2002, Stankowich, 2008, Sunde *et al.*, 2009). Predation risk can drive prey to be more vigilant and to shift to safer habitats, which can result in lower fitness due to poorer forage quality and less time spent on feeding (Brown *et al.*, 1999). How individuals respond varies among habitat characteristics, hunting pressure and hunting method (Jarnemo and Wikenros, 2014). Hunting disturbance can result in increased resting time to compensate for increased energy consumption or to recover from the exhaustion (Le Grand *et al.*, 2019).

While several studies have evaluated behavioural responses (Ericsson *et al.*, 2015b; Sand *et al.*, 2016), none have studied the physiological responses of moose to baying dogs. Behavioural studies found that exposure to baying dogs resulted in increased activity, i.e. increased maximum speed and flight distance, and in moose leaving the area after disturbance (Ericsson *et al.*, 2015b, Sand *et al.*, 2016). Sand *et al.* (2016) found differences in the flight patterns between females with and without calves, as well as differences in flight patterns and reactions of moose in response to different dogs. Behavioural impacts, together with physiological responses, have been assessed in simulated hunts with baying dogs in brown bears (Le Grand *et al.*, 2019). During experimental days, compared to control periods, the authors noted higher HRs and  $T_b$ , with the bears also travelling longer distances and at higher speeds.

Here, we investigated the behavioural and physiological responses of moose to simulated hunting situations with baying dogs (hereafter approaches). We tested for changes in five variables; maximum  $T_b$ , HR, Euclidean distance travelled, maximum speed of movement and time allocated to resting versus active behaviour. We measured moose response during three periods (reference period (14–20 August), day of the approach and day after the approach). Based on previous studies of the effect of hunting on moose and other game species, we tested the following predictions:

Effects on physiology; maximum  $T_b$  and mean HR will be higher during the day of the approach compared to the reference period and the day after the approach.

Effects of behaviour:

1. Total distance travelled (per day) will be longer and the total time spent resting (minutes inactive/day) will be lower on the day of the approach compared to the day after.
2. Maximum speed will be higher during the day of the approach compared to the day after.

## Material and methods

### Study area and animals

The study was conducted in north-eastern Sweden, in the county of Västerbotten in Nordmaling, Vännäs and Umeå municipalities. During the study years of 2017 and 2018, a total of 675 and 782 moose were shot in the three municipalities, respectively (SvenskaJägareförbundet, 2019). The study area is characterized by boreal forest, dominated by Scots pine (*Pinus silvestris*), Norway spruce (*Picea abies*) and birch (*Betula spp.*) in addition to agricultural land and marshland. The elevation level in the area ranges from 0 to 300 m.a.s.l., and the total land area of the three municipalities are 4076.2 km<sup>2</sup> (SCB, 2019). During the management period 2016–2018, the estimated moose density was on average 8.2 moose per 1000 ha in the winter (i.e. after hunting) (CAB, 2019). We studied the responses of eight adult female moose (>1.5 years old) (not each of the eight females was studied every year) and 12 baying dogs of different breeds frequently used for moose hunting (Norwegian Elkhound: n = 4, Jämthund: n = 3, other breeds: n = 5). In 2017, all five females had calves in spring, and in 2018, six had calves and one was without calf in spring.

Moose were equipped with global positioning system (GPS) Plus collars (Vectronic Aerospace GmbH, Berlin, Germany), ruminal temperature and mortality transmitters (MIT; Vectronic Aerospace GmbH, Berlin, Germany) and subcutaneous HR loggers (Reveal XT; Medtronic Inc., Minneapolis, Minnesota, USA and DST centi HRT; Star Oddi, Gardabaer, Iceland) during immobilization in February 2017. Moose were immobilized with the drug combination of 50 mg xylazine (Xylased<sup>®</sup> 500 mg, Bioveta, Ivanovice na Hané, Czech Republic) and 4.5 mg etorphine (Captivon<sup>®</sup> 98 Etorphine HCl, 9.8 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd, White River, South Africa), from a helicopter using a CO<sub>2</sub> powered rifle (Dan-Inject, Børkop, Denmark). The immobilization procedure is described in detail elsewhere (Evans *et al.*, 2012; Lian *et al.*, 2014; Græsli *et al.*, 2020).

### Biologgers, programming and data collection

Collars included a GPS receiver, an ambient temperature recorder, a triaxial acceleration sensor, a mortality sensor, a very high frequency (VHF) transmitter and a global system for mobile communication modem (VectronicAerospace, 2019a). In addition, each collar was linked to external sensors, i.e. the MIT and proximity sensors UHF-ID tags (VectronicAerospace, 2019b; VectronicAerospace, 2019c). We adjusted collar-derived ambient temperatures according to the offsets described by Ericsson *et al.* (2015a) to represent a reliable index for the actual ambient temperatures. The acceleration sensor integrated in the collar measures activity as back–forward and right–left movement over two axes (X and Y) on a scale from 0–255 at 6 to 8 Hz, and stores average

values over 5-min recording intervals. Overall activity was presented as the sum of the activity data on the X- and Y-axes, ranging from 0 to 510 (Gervasi *et al.*, 2006). GPS positions were recorded every 3 h during the study period, and we changed it manually for the day of the approach to 10-min positions. This schedule was kept until the end of the study period for that year. Additionally, in 2017, the schedule switched to 1-min positions (n = 2) when in contact with a proximity sensor (attached to a dog). All GPS positions with associated information (ambient temperature, the most recently stored ruminal temperature and proximity contact) were sent to the wireless remote animal movement (WRAM) database for storage (Dettki *et al.*, 2014). At recapture of the moose, the remaining data stored on board the collar were manually downloaded and sent to the WRAM database.

To record moose physiological responses, we deployed a MIT in the rumen as described earlier (Minicucci *et al.*, 2018; Græsli *et al.*, 2020). MITs record the ruminal temperature at 5-min intervals and transmit the information for storage to the collar unit (VectronicAerospace, 2019b). In addition, moose were fitted with surgically implanted subcutaneous HR loggers (Reveal XT and DST centi HRT). Reveal XTs continuously monitor the HR using an electrocardiogram (ECG), which converts the mean R-R interval (rate of a ventricular cycle) into HR, and store 2-min average values (Medtronic, 2017). DST centi HRT calculates a mean HR from a 4-s ECG strip with a 150 Hz measurement frequency and stores the HR with a quality index of the signal clarity and the R-R interval regularity (StarOddi, 2017). Prior to the surgery, an analgesic, meloxicam (Metacam<sup>®</sup>, Boehringer Ingelheim Vetmedica GmbH, Germany), was given subcutaneously at a dose of 0.5 mg/kg. The DST centi HRT was surgically implanted as earlier described (Græsli *et al.*, 2020), and the Reveal XT was surgically implanted subcutaneously at the left side of the most rostral part of the sternum, according to the same procedure. We programmed and activated the Reveal XT after implantation, prior to the anaesthetic reversal.

Data recorded by the collars, MITs and Reveal XTs were downloaded in the field during recaptures in February 2018 and in February 2019. The recaptures were carried out in the same way as the initial captures. During the recaptures in 2019, we surgically removed the Reveal XTs according to the same procedure as used for implantation.

The dogs included in the real hunts belonged to local hunting teams, while the experimental hunts were conducted by trained field personnel (experienced hunters) with trained hunting dogs. All dogs were equipped with Garmin T5 or DC50 Dog collars (Garmin Ltd, Olathe, Kansas, USA) and could be tracked directly via a hand-held GPS (Garmin Astro 320) (Garmin, 2019). In addition, the dogs of the local hunting teams were equipped with UHF proximity tags (Vectronic Aerospace GmbH, Berlin, Germany), which send a signal to the moose collar when in close proximity (VectronicAerospace, 2019c).



## Experimental protocol

The experimental hunts were performed after the national leash law (i.e. dogs should be kept on leash/under supervision from 1 March to 20 August) was lifted (21 August) and until the beginning of the annual moose hunt (first Monday in September, i.e. 4 September in 2017 and 3 September in 2018, [Länsstyrelserna, 2020](#)). Real hunting situations were part of the regular moose hunt in the area from 4–24 September 2017. None of the real hunts included in our study resulted in a moose being killed. The experiments were approved by the Animal Care Committee for Northern Sweden in Umeå (application numbers: Dnr A 3-16, Dnr A 28-17). Experienced hunters, hunting dogs and field personnel carried out the real and experimental hunts, while experienced field personnel, pilots and veterinarians carried out captures, handling and surgeries.

## Experimental hunts

For each experimental hunt, a dog handler walked with the leashed dog in an upwind direction to a known moose position given by its latest GPS position. The current position of the moose was determined using a VHF receiver, aiming to release the dog close enough to the moose to avoid that the dog would pick up the scent of other, unmarked animals, while at the same time avoiding that the target moose would become aware of the human presence. Within a distance of  $200 \pm 50$  m the dog was let off the leash and started tracking the moose while the handler monitored the dog using a hand-held GPS, thus simulating a real hunting situation (see [Fig. 1](#)). Once the dog had been in close contact with moose or was not able to find the moose, the handler leashed the dog and walked back to the car. Dog handlers participating in experimental hunts filled out a field protocol noting date and time, coordinates, moose ID, dog ID, hunting situation and weather. The dog's GPS track was later downloaded from the hand-held GPS.

## Real hunts

When near, the UHF-ID tag of a hunting dog triggered the proximity sensor on the moose collar, and a message with the ID tag number of the dog was sent together with the GPS data from the moose. The signal strength depends on distance and topography between the UHF-ID tags and the moose collar and is expected to be between 300 and 400 meters ([VectronicAerospace, 2019c](#)). We then contacted the corresponding dog handler and met to download the tracklog of the dog, using a laptop computer and the basecamp software (Garmin Ltd, Olathe, Kansas, USA), and to collect information about the hunting situation. The field protocol was the same for both experimental and real hunts. The dog collars were programmed to collect a GPS fix every 1 s.

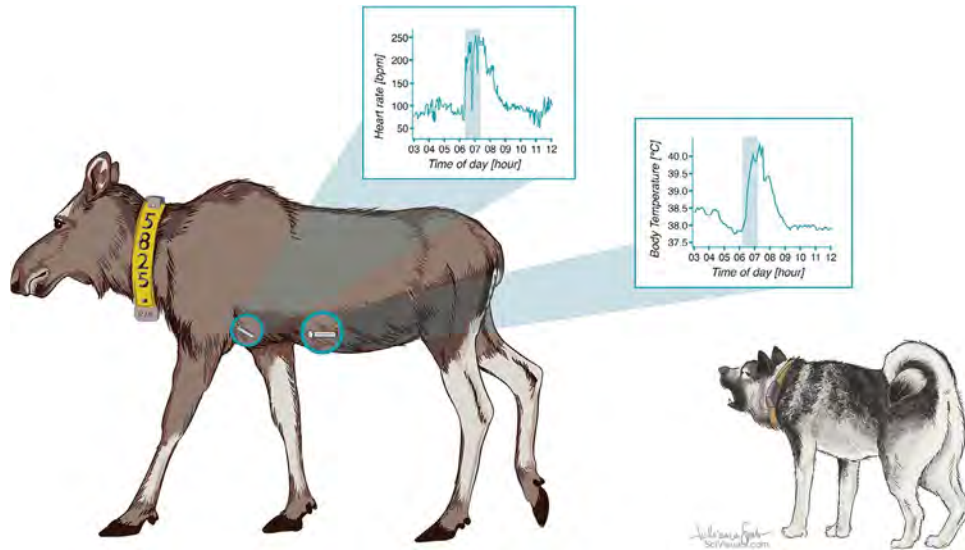
## Data preparation and analyses

Twenty-three dog–moose encounters (hereafter, called ‘approaches’) occurred during real ( $n=6$ ) and experimental ( $n=17$ ) hunting situations on eight individual female moose (overview in the Supplementary material ([Appendix 1](#))). Based on the distance between the moose and the dog as well as the duration of the contact, we classified approaches as successful (moose and dog within 240 meters for at least 10 min), disturbed (moose and dog were within 240 meters for less than 10 min) and not disturbed (moose and dog were never within 240 meters). The 240-meter distance threshold was based on maximum flight initiation distance reported from a study of the same moose approached by a skier in wintertime ([Viljanen, 2019](#)), and the 10-min threshold was based on the 10-min resolution of GPS positions. We tested for differences in moose behaviour (i.e. activity) and physiology (i.e.  $T_b$ , HR) between the day of the approach (24 h) and a 7-day reference period before the approach and to 1 day after the approach using thereby each moose as its own control. We used 1 week prior to the beginning of the dog-training period (14–20 August) as the before-approach reference period, assuming moose under non-disturbed conditions, but within similar seasonal conditions. To test for difference in moose movement, we excluded the reference period, because only GPS data at 3-h intervals were available for this period. The distance travelled per day and the speed would, therefore, have been underestimated and would have led to a bias in our model. HR,  $T_b$  and activity values from the reference period were generally higher than values from later in the hunting season, due to seasonal variations ([Græsli et al., 2020](#)).

We only included approaches classified as successful or disturbed in the analyses and excluded approaches that had missing data ( $n=4$ ), were classified as not disturbed ( $n=2$ ), proximity sensor triggered by a dog in a car ( $n=1$ ) and approaches carried out the day before another approach on the same moose ( $n=2$ ). This resulted in data from 14 approaches (successful  $n=10$ , disturbed  $n=4$ ) on eight individual moose. In three of these approaches, the same moose was approached twice the same day because the field personnel considered the first approach of that day as unsuccessful. Consequently, this day was considered as one approach event.

## Body temperature ( $T_b$ )

We used a linear model from the lme4 package ([Bates et al., 2015](#)) to test for difference in the maximum daily  $T_b$  (response variable), considering the explanatory variables Period (factor with three levels (reference period, day of approach and day after approach)) and Success (factor with two levels (disturbed and successful)). Maximum  $T_b$  instead of mean  $T_b$  was selected as the response variable to minimize the influence of drinking periods on our results, as drinking cold water drastically decreases rumen-measured  $T_b$  ([Herberg et al., 2018](#)). Not every moose was experimentally approached repeatedly, which resulted in too few data points to include the



**Figure 1:** When approached by a baying hunting dog, the moose can flee in order to escape the hunting dog (as illustrated here), stand its ground or confront the dog. When the moose stands its ground the dog is typically barking and running around the moose (baying), and the hunter sneaks in and shoots the moose. Scientific Illustration by Juliana D. Spahr, [SciVisuals.com](https://www.scivisuals.com) (included with permission).

individual moose ID as a random structure. We based model selection on Akaike's information criterion corrected for small sample size (AICc) and carried it out with the function `lctab` from the `bbmle` package (Bolker and R Core Team, 2017). We picked the highest ranked model with the highest AICc weight within  $\Delta\text{AICc} \leq 2$  and applied a *post-hoc* test with estimated marginal means from the `emmeans` package (Lenth *et al.*, 2019) to estimate which levels of the categorical variables were significantly different.

### Movement of the moose

We used GPS locations collected every 10 min when analysing the movement data. In the case of missing positions or delays in switching to 10-min positions (0.08% of the positions), we used the `'na.approx'` function from the `zoo` package in R to linearly interpolate the longitude and latitude values (Zeileis and Grothendieck, 2005). We excluded one of the approaches because the GPS position frequency was not appropriate as the GPS failed to switch to 10-min intervals. In total, we analysed movement data from 13 approaches on eight moose.

We used the `AdehabitatLT` R package to calculate the Euclidean distance between consecutive GPS positions and calculated the total distance travelled per day (m) and the maximum speed (m/min) per day (Calenge, 2006). We modelled the maximum speed and the total distance travelled per day (response variables), using a gamma-distributed generalized linear model with the identity link function. We applied Period (factor with two levels; day of approach and day after approach) and Success (factor with two levels; disturbed and successful) as explanatory variables. A random structure for individual moose ID was not included (see the explanation

for the body temperature model), and the model selection followed the same approach as for body temperature.

### Heart rate

The Reveal XT calculated the HR based on recognition of R peaks in the ECG, and the ECGs were deleted (because of the storage limit) while the HR data were stored in the memory of the logger (Medtronic, 2017). The algorithm used for detection for R peaks was not always correct, likely because the Reveal XT is designed for use in human medicine, and heart anatomy and physiology of humans and moose differ. Because of that, some of the heartbeats were not detected and sometimes one heartbeat was detected as two (double counting). The absolute minimum HR of a moose resting during winter was found to be 37.5 bpm (Græsli *et al.*, 2020), so all values lower than this were removed. Based on comparisons of the HR data from the Reveal XT with manually calculated HRs from ECGs obtained by another HR logger (DST centi HRT, which was deployed additionally as part of a separate study) and accelerometer data from the same moose, we found no correlation between activity level and HR accuracy and concluded that the algorithm performed similarly during both active and passive phases. Consequently, we used the obtained raw HR data and removed unrealistically low HRs. By doing this, we were able to calculate differences in HR and identify significant changes in HR over time. However, we were unable to account for potential double counting and can therefore not report specific mean or maximum HR values.

All experiments were carried out between 08.00 and 14.00 local time, and we therefore only assessed the HR measurements during this period of the day. To compare the HR data

between the different periods, we built a linear mixed-effects model (nlme package) with the raw HR data and included the variable period (reference time, day of approach and day after approach) as a fixed factor (Pinheiro *et al.*, 2019). To account for the autocorrelation within the HR data, we included the autocorrelation structure corCAR1. Moose ID was included as a random factor to account for repeated measures of the same moose. Due to the small sample size, the model could not include the explanatory variable Success and was therefore only built using the data of successful approaches.

### Resting time (active versus inactive behaviour)

To classify moose behaviour into (1) inactive (i.e. resting) and (2) active, we fitted separate hidden Markov models (HMMs) to each individuals' observed activity data (i.e. summed acceleration of X- and Y-axes). HMMs are time-series models that assume animals' observed movement or activity patterns to be determined by an underlying 'hidden' finite state sequence, where the states can be interpreted as proxies for the unobserved behavioural modes of an animal (Patterson *et al.*, 2009; Langrock *et al.*, 2012). We modelled activity using a state-dependent gamma distribution. To account for potential effects of temporal patterns and temperature on moose behaviour, the state transition probabilities were expressed as functions of the time of day, light conditions (dark, light or twilight at time of observation), day of the year and collar-derived ambient temperature using a logit link function (i.e. with the categories representing the two different states the process might switch to). We used forward selection based on Akaike's Information Criterion (AIC) to determine the influence of these covariates considered in each of the individual HMMs. An overview of the individuals and models chosen are presented in the Supplementary material (Appendix 2). For cyclic covariates (day of year and time of day), we included sine and cosine terms. All HMMs were fitted via numerical likelihood maximization using the momentuHMM R package (McClintock and Michelot, 2018). Each model was run with 30 sets of random starting values to avoid local maxima (always choosing the model with the highest log-likelihood value). We then determined the most likely state sequence from each HMM using the Viterbi algorithm and calculated the total time spent resting per day (min) based on these results. Subsequently, we ran a linear model with resting time as the response variable and performed the model selection, following the same approach as for body temperature.

### Behavioural responses

In order to detect flight initiation distances (FID—i.e. how close the dog was when the moose started to react behaviourally (based on collar activity (accelerometer) data) or physiologically (based on body temperature) and to estimate for how long collar activity levels and  $T_b$  were affected by the approaches (i.e. how long it takes for both metrics to return back to the 'normal' pre-approach state),

we applied change point analysis (R package changepoint), (Killick and Eckley, 2014). We applied the analysis over the course of the day of the approach and used collar activity data because it was recorded at a higher resolution than the GPS data (5- vs 10-min resolution). We identified two changepoints based on the variance in the data, which should ideally correspond to the start (time of FID) and end (settling down of the moose) of the approach.

We then calculated the time the moose was affected (hereafter: time affected = time between the two changepoints), compared the times of the changepoints with the GPS data of the dogs and calculated FID (Euclidean distance). In addition, we calculated the time affected after an approach ended (the difference between the time of the second changepoint and the time the approach ended (found in the protocol)). We included approaches carried out the day before another approach on the same moose ( $n=2$ ) in the changepoint analysis. We excluded those approaches where the moose was moving before the dog found the moose ( $n=2$ ) or where data were missing ( $n=1$ ). In total, we performed changepoint analysis on 16 approaches (disturbed  $n=6$ , successful  $n=10$ ).

All the data were prepared and analysed using R version 3.6.1 (R Core Team, 2019). We considered  $P$  values  $<0.05$  as significant.

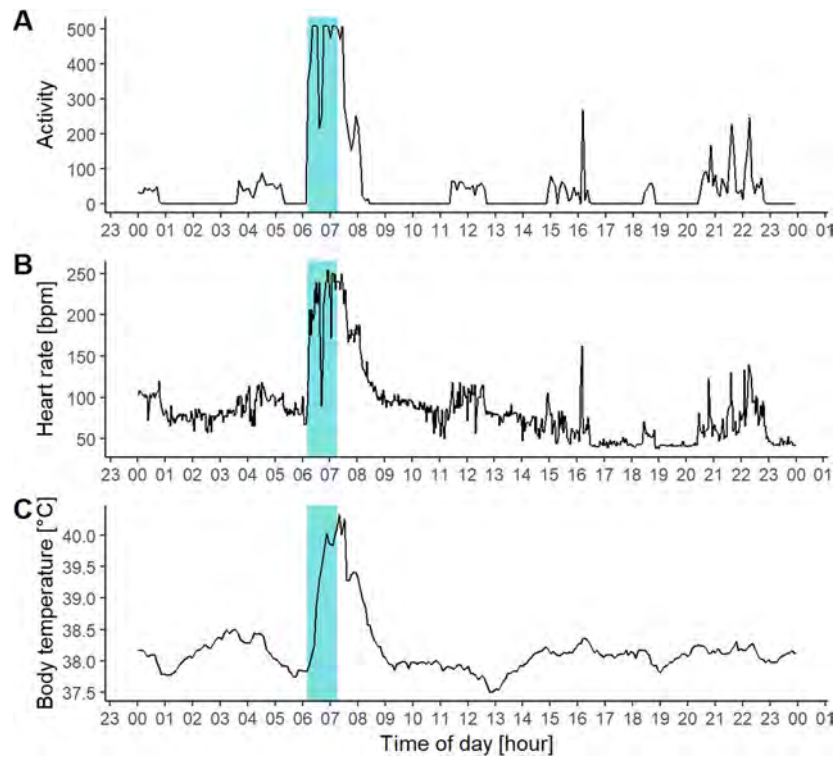
## Results

### Body temperature ( $T_b$ ), movement of the moose, heart rate and resting time

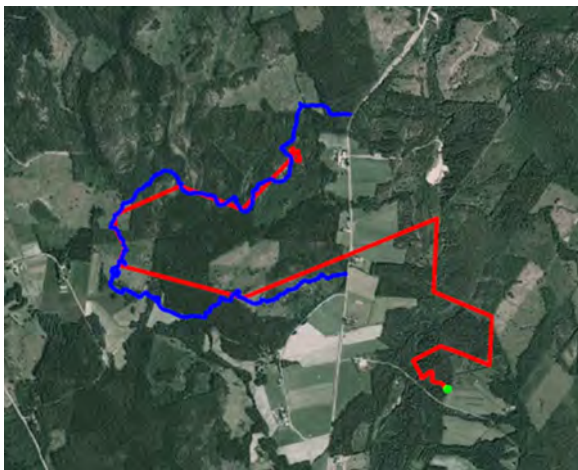
All successfully approached moose fled away from the dogs and increased their HR,  $T_b$  and movement when approached (see Fig. 2). None of the moose stood its ground or confronted the hunting dogs. Hunting dogs followed moose tracks closely (e.g. Fig. 3). The highest ranked model for each response variable included period for the movement models and the interaction between period and success for  $T_b$  and resting (see Table 1).

We observed a significantly higher maximum  $T_b$  ( $0.88^\circ\text{C}$  higher—SE  $0.15^\circ\text{C}$ ,  $P$  value  $<0.001$ ), at the day of the approach compared to the day after for the successful approaches (see Table 2 and Fig. 4). In addition, the maximum  $T_b$  on the day of the approach was  $0.47^\circ\text{C}$  higher (SE  $0.15^\circ\text{C}$ ,  $P$  value  $0.031$ ) compared to the reference time. Highest  $T_b$  measured in the study was  $40.8^\circ\text{C}$ , which was recorded during an approach. We did not detect any differences in  $T_b$  between the reference period, the day of the approach and the day after the approach among the moose that had been in contact with the dog  $<10$  min (approaches classified as disturbed).

We found a higher maximum speed (mean  $\pm$  SE:  $76.5 \pm 14.5$  m/min faster;  $P$  value  $<0.001$ ) and a longer distance travelled (mean  $\pm$  SE:  $4.1 \pm 1.1$  km longer;  $P$  value  $<0.001$ )



**Figure 2:** Graphical representation of (A) activity, (B) heart rate and (C) body temperature of a female moose when approached by a hunting dog during an experimental hunting approach. The blue ribbon represents the approach duration i.e. the time from the start to the end of the approach. For interpretation of the absolute heart rate values please see explanation in the main text.



**Figure 3:** Map presenting the movement of a female moose (red line—green dot represents the last position of the moose) when approached by a hunting dog (blue line) during an experimental hunting approach.

at the day of the approach compared to the day after the approach for all moose (see Table 2 and Fig. 5).

HR values for the day of the approach were significantly higher compared to the reference period and the day after the

approach (22 bpm (SE 2 bpm,  $P$  value  $<0.001$ ) and 24 bpm (SE 3 bpm,  $P$  value  $<0.001$ ), respectively) (see Table 3 and Fig. 6). The highest HR measured, which could be confirmed from stored ECG episodes, was 195 bpm.

Moose rested significantly longer (94.5 min (SE 20.6 min,  $P$  value  $<0.001$ )) the day after a successful approach compared to the day of the approach. In addition, we demonstrated that the successfully approached moose rested 96 min longer (SE 30.3 min,  $P$  value 0.036) the day after the approach than the moose in approaches characterized as disturbed (moose in contact with the dog  $<10$  min) (see Table 2 and Fig. 7). The mean resting time per day in the reference period was 13.5 h.

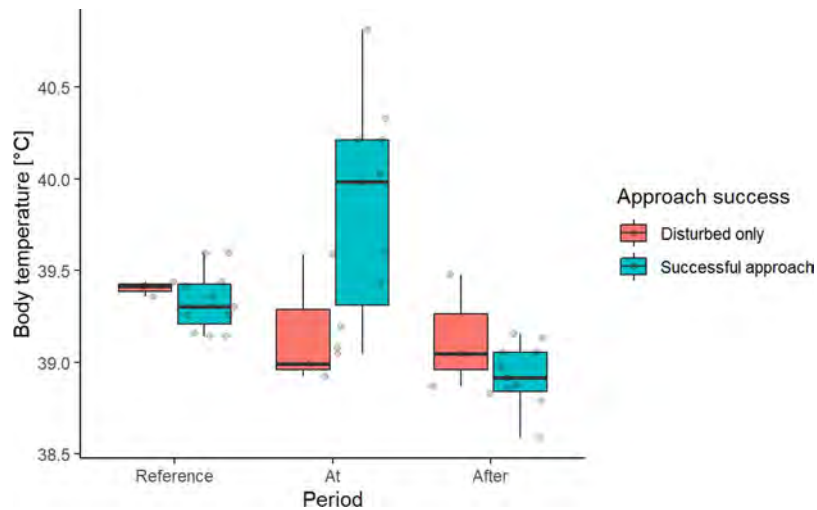
### Behavioural responses

Based on collar activity (accelerometer) data, we detected changepoints in 10 of the 16 approaches (disturbed  $n=1$ , successful  $n=9$ ). Changepoints on  $T_b$  data were detected in three successful approaches.

For the successful approaches, the average flight initiation distance was 142 m (median) (range 86–248 m). The collar activity reached a similar level as before the approach 54 min (median) after an approach ended (range 27–114 min).

**Table 1:** Log-likelihood (logLik and  $\Delta\text{logLik}$ ), Akaike’s information criterion corrected for small sample size (AICc and  $\Delta\text{AICc}$ ), number of parameters (n) and model weight (weight) for the top-ranked, linear model combinations evaluating body temperature, movement (distance travelled and maximum speed) and resting behaviour (active vs inactive) of moose approached by hunting dogs

Linear model combinations	logLik	AICc	$\Delta\text{logLik}$	$\Delta\text{AICc}$	n	Weight
Body temperature						
Period * success	−12.1	41.5	14.9	0.0	8	0.55
Period	−16.8	42.6	10.3	1.1	5	0.32
Period + success	−16.3	44.4	10.7	2.8	6	0.13
Distance						
Period	−235.0	477.0	7.9	0.0	4	0.49
Period * success	−232.2	477.5	10.6	0.5	6	0.38
Period + success	−234.9	479.7	7.9	2.7	5	0.13
Speed						
Period	−119.8	246.7	12.7	0.0	4	0.62
Period * success	−118.0	248.9	14.5	2.2	6	0.20
Period + success	−119.7	249.2	12.8	2.5	5	0.18
Resting						
Period * success	−201.4	420.5	11.4	0.0	8	0.56
Period + success	−205.2	422.2	7.7	1.7	6	0.24
Period	−206.7	422.6	6.1	2.2	5	0.19



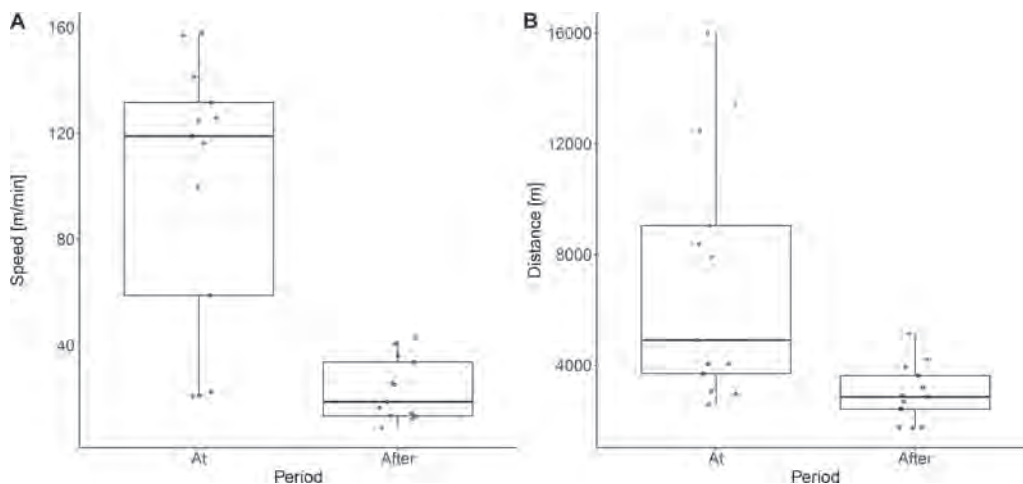
**Figure 4:** Maximum body temperature  $T_b$  (°C) during different periods (Reference; reference period (14–20 August), At; the day of the approach (24 h), After; the day after the approach (24 h)) between different success states (Disturbed only: moose and dog within 240 m for <10 min; Successful approach: moose and dog within 240 m for >10 min), for moose approached by hunting dogs.

Moose  $T_b$  was affected 14.5 min longer than the moose activity (median total time  $T_b$  132 min; range 124–160 min, versus collar activity 117.5 min; range 25–180 min). Similarly, we found that moose  $T_b$  was on average affected 8 min longer than activity, after an approach ended (median time

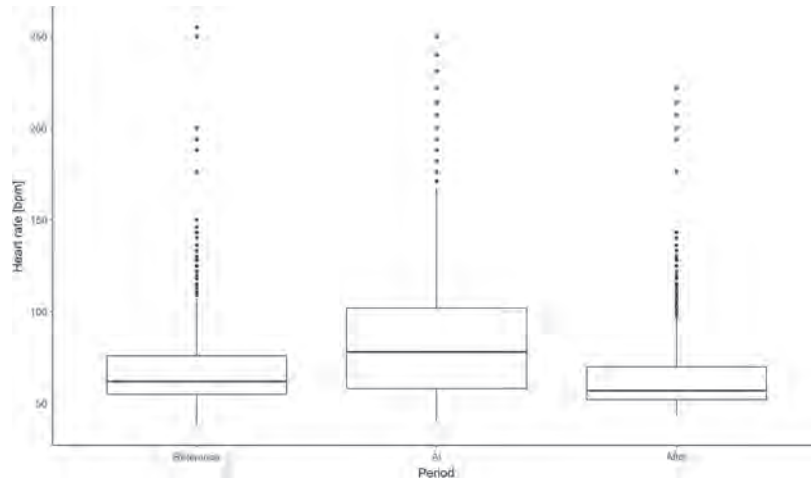
$T_b$  62 min (range 36–81 min) vs activity 54 min (range 27–114 min)). Drinking episodes had an impact on the changepoint detection in  $T_b$  (based on graphical visualization of the  $T_b$  dropping around 2°C in a short period of time) after the end of an approach.

**Table 2:** Model parameter estimates, standard errors (SE), *t* values and *P* values for variables in the linear models evaluating body temperature, movement (distance travelled and maximum speed) and resting behaviour (active versus inactive) of moose approached by hunting dogs

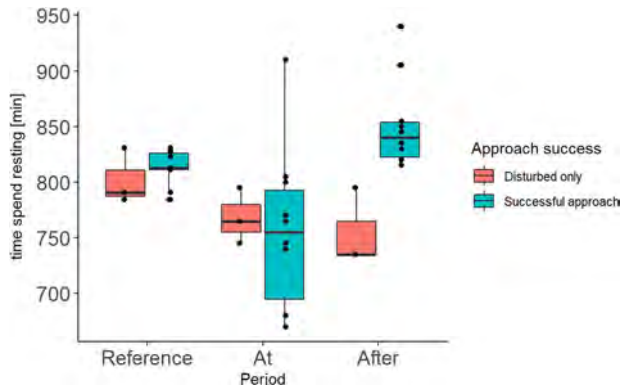
	Estimate ( $\beta$ )	SE	<i>t</i> value	Pr(>  <i>t</i>  )
<b>Body temperature</b>				
(Intercept)	39.40	0.20	195.68	<0.001
Period at	-0.24	0.28	-0.83	0.41
Period after	-0.27	0.28	-0.96	0.34
Success successful	-0.07	0.23	-0.31	0.76
Period at: success successful	0.71	0.32	2.22	0.03
Period after: success successful	-0.13	0.32	-0.41	0.69
<b>Distance</b>				
(Intercept)	7123	1008	7.06	<0.001
Period after	-4145	1093	-3.79	<0.001
<b>Speed</b>				
(Intercept)	99.6	14.1	7.07	<0.001
Period after	-76.5	14.5	-5.28	<0.001
<b>Resting</b>				
(Intercept)	801.9	26.6	30.18	<0.001
Period at	-33.6	37.6	-0.89	0.378
Period after	-46.9	37.6	-1.25	0.221
Success successful	11.4	30.3	0.38	0.709
Period at: success successful	-23.2	42.8	-0.54	0.591
Period after: success successful	84.6	42.8	1.97	0.057



**Figure 5:** Maximum speed ( $m \cdot min^{-1}$ ) (A) and overall distance travelled ( $m/day$ ) (B) during different periods (At; the day of the approach (24 h), After; the day after the approach (24 h)) for moose approached by hunting dogs.



**Figure 6:** Heart rate (bpm) during different periods (Reference; reference period (14–20 August), At; the day of the approach (24 h), After; the day after the approach (24 h)), for successful approaches (moose and dog within 240 m for >10 min) of moose approached by hunting dogs. For interpretation of the absolute heart rate values please see explanation in the main text.



**Figure 7:** Time spent resting (minutes/day) during different periods (Reference; reference period (14–20 August), At; the day of the approach (24 h), After; the day after the approach (24 h)) and between different success states (Disturbed only: moose and dog within 240 m for <10 min; Successful approach: moose and dog within 240 m for >10 min), for moose approached by hunting dogs.

**Table 3:** Model parameter estimates, standard errors (SE), degrees of freedom (DF), *t* values and *P* values for variables in the linear mixed model evaluating heart rate of moose approached by hunting dogs

	Value	SE	DF	<i>t</i> value	<i>P</i> value
(Intercept)	65.25	4.32	9712	15.10	<0.001
Period at	24.46	3.29	9712	7.43	<0.001
Period reference	2.85	2.49	9712	1.15	0.25

where the moose and dog had been in contact for <10 min (approaches classified as disturbed). Our results thus indicate that significant changes in moose behaviour and physiology dependent on the time the dog have been in close contact with the moose. However, we are aware of the small sample size of our study and that this limits the interpretation of our results.

We demonstrated  $T_b$  in moose exceeding 40°C in the majority of the successful hunts, and the highest  $T_b$  measured was 40.8°C. Brown bears hunted with baying dogs are documented to have an increased  $T_b$  of 4.7°C from their baseline (mean) levels to the maximum  $T_b$  during hunting (37.5°C versus 42.2°C) (Evans *et al.*, 2016; Støen *et al.*, 2018). In moose, we found an increase of 2.3°C from the mean  $T_b$  baseline levels (Græsli *et al.*, 2020) to the maximum  $T_b$  (38.5°C versus 40.8°C). Long-time exposure to high  $T_b$  could be life-threatening because of cytotoxicity. Damage of mammalian cells due to hyperthermia starts after a relatively short period with temperatures >40–41°C, and the degree of damage depends on the exposure time and other stress factors (Lepock, 2003). It results in protein denaturation and impairment of the DNA synthesis in the cell, and long-term exposure leads to organ failure and death (Tansey and Johnson, 2015). Occasionally, we found a rapid decrease in

## Discussion

### Physiological and behavioural responses

This study, combining different types of biologgers, provides novel insight in the correlation between physiological and behavioural responses of moose to hunting with dogs. Combining  $T_b$ , HR and movement data from biologgers uncovers the underlying mechanisms behind stress responses in moose and adds to the field of conservation medicine. We documented significant physiological and behavioural responses in approaches where the moose and dog had been in contact for >10 min. In contrast, we found minimal changes in behavioural and physiological parameters in approaches

rumen temperature shortly after the end of the approaches (similar to the pattern visualized by Herberg *et al.* (2018)), suggesting that some of the moose drank water when reaching the highest  $T_b$  levels. Drinking can, therefore, act as a behavioural thermoregulation strategy to avoid overheating (Tansey and Johnson, 2015).

We found a significant increase in HR, indicating increased energy consumption during the day of the approach. A significant correlation between HR and metabolic rate is demonstrated in a variety of animals, including moose (Green, 2011, Renecker and Hudson, 1985). The lack of validated HR measurements (not possible to validate the exact HR values) and the non-linear relationship between HR and metabolic rate in moose (Renecker and Hudson, 1985) prevented us from making a valid estimate on the increased energy consumption in this study. Even though it was not quantified, our findings (22–24 bpm difference) suggest a considerably increased energy expenditure due to hunting related disturbances when comparing our findings with seasonal differences found in moose (Græsli *et al.*, 2020). More specifically, the difference of 31.4 bpm in daily mean HR from the highest levels in summer (71.9 bpm) to the lowest daily mean in winter (40.5 bpm) represents a 60% decrease in metabolic rate from summer to winter (Græsli *et al.*, 2020). Moose might compensate for the increased energy consumption from hunting by increasing the resting time the day after, as we demonstrated. Another reason for the increased resting time is likely to be recovery from exhaustion as suggested in bears (Le Grand *et al.*, 2019). We recommend further studies to determine the effect of these events on the moose's time spent foraging the day after a hunting situation.

Moose populations have been declining along the edges of the moose range, and increasing ambient temperatures and climate change are suggested as a reason for that (Lenarz *et al.*, 2009; Ruprecht *et al.*, 2016; Allen *et al.*, 2017). Moose are easily heat stressed with increasing ambient temperatures, resulting in increased respiratory rate, HR and body temperature (McCann *et al.*, 2013, Renecker and Hudson, 1986, Thompson *et al.*, 2019). When evaluating heat stress in moose one should consider core body temperature and the daily variations, body condition, solar radiation, vapour pressure and season in addition to ambient temperature (Thompson *et al.*, 2019; Græsli *et al.*, 2020). High ambient temperatures during the hunting season might, therefore, result in extra energy consumption in hunted moose. Increasing ambient temperatures from climate change can further result in changes in food resources and increased infection risk in addition to increased energy consumption, and all of these can result in lower survival and reproductive rates (McCann *et al.*, 2013, van Beest and Milner, 2013, van Beest *et al.*, 2012). We therefore suggest that the negative effects of hunting disturbance might be more dramatic in moose in the edges of the moose range, because of the negative effects of climate change, especially on warm hunting days.

None of the moose stayed and confronted the attacker (dog or human)—which is in line with earlier findings (Ericsson *et al.*, 2015b). In North American moose, confronting the attacker is a common anti-predator strategy towards fight off attacks from wolves (Ballard and van Ballenberghe, 2007). In Sweden, the main source of mortality for adult moose is hunting (Ericsson and Wallin, 2001). Ericsson *et al.* (2015b) suggested that female moose may have altered their anti-predator behaviour towards hunting. According to Swedish hunting laws any accompanying calf has to be shot before the female (SOU, 2009), providing a learning experience for long-living female moose. As a result, Swedish female moose might be more prone to flee compared to their North American conspecifics. Our results support previous research that hunting disturbances increase travel distance and higher maximum speed on the day of the approach compared to the day after disturbance in moose (Ericsson *et al.*, 2015b; Sand *et al.*, 2016), red deer (Jarnemo and Wikenros, 2014, Sunde *et al.*, 2009) and brown bear (Le Grand *et al.*, 2019). In red deer, it is documented that escape strategies are linked to habitat type: red deer in fragmented and more open landscapes fled more often for longer distances, and at a higher speed, than red deer in homogenous forest landscape (Jarnemo and Wikenros, 2014, Sunde *et al.*, 2009). It is likely that moose have the same type of response, yet, this aspect was beyond the scope of our study, and we suggest further research on the interplay between habitat type and physiological responses.

Moving in a sinuous pattern is possibly an antipredator strategy by moose, increasing the chance that the dog loses the track or switches to the track of another moose (Baskin *et al.*, 2004, Cederlund and Kjellander, 1991, Ericsson *et al.*, 2015b). Even if not quantified by our GPS analysis, the tracks of the moose and the hunting dog indicated that moose often moved in a sinuous pattern when approached. Our results might, therefore, have been more precise if we used one instead of 10-min GPS intervals when calculating the FID. Including wind as a parameter is valuable in explaining the antipredator strategy of moose and other prey species, as a variable to sense the location of the predator. Sometimes the hunting dogs picked up non-target moose instead; in these cases, the target moose was within 100 meters but did not move away even though a hunting dog was in close vicinity. These results are in contrast to the results of a study on the effects of hunting with dogs on roe deer as a non-target species, demonstrating that roe deer behaviour was significantly affected (Grignolio *et al.*, 2011). The differences between the studies might be due to different hunting strategies such as the use of different types and numbers of dogs, former experiences with hunting, species-specific behaviour and anti-predator strategies, and that some of the roe deer actually were pursued by the dogs (even if non-targeted) (Grignolio *et al.*, 2011).

About 45% of the moose observed by hunters in our study area were shot, meaning that >50% survived a hunting approach (Länsstyrelserna, 2019). There is not any data available on the number of surviving moose that have been



chased within a given area. Yes, we assume that the amount of stress from the hunting approaches, for a given surviving moose, will vary and depends on the length of the chase, if moose were aware of hunters/dogs, their previous hunting experience, and if any in their company were shot. In spite of the documented overlap in oestrus and moose hunt in southern Sweden (Malmsten *et al.*, 2014), moose in our study area had a fairly high and stable (same level the past 20 years) reproductive rate in 2017 ( $>0.7$ ) (Ericsson and Wallin, 1999, SvenskaJägareförbundet, 2019). The slaughter weights of the moose in our study area are also stable over time (SvenskaJägareförbundet, 2019), suggesting that the hunting disturbances must be stable. Most harvest occurs in the beginning of the hunting season (Singh *et al.*, 2014). Yet, to improve moose management reducing moose density in areas with high browsing damages in winter, hunting at the end of the hunting period (in winter) is increasingly discussed. Further studies should include evaluation of the effects of hunting during wintertime when the moose display hypometabolism and compare the physiological and behavioural responses during autumn and winter.

## Conclusion

Based on the physiological and behavioural results, we can conclude that hunting with baying dogs represents a notable stress event for the individual moose. Moose might compensate for the increased energy consumption by increasing the resting time on the day after being chased by a hunting dog. Yet, increased frequency of hunting disturbances and higher ambient temperature due to climate change may likely increase the energy consumption and thereby enhance the risk of negative effects on reproduction and body condition. As hunting methods and breeds of hunting dogs used are continuously evolving, managers need to continue to consider the physiological consequences for moose.

## Author contributions

J.M.A., A.L.E., G.E., W.N., N.J.S., B.F., F.S. and A.R.G. designed and initiated the study. Data collection was performed by A.R.G., A.L.E., F.S., J.M.A. and B.F. T.G.L. contributed with input for data collection and provided equipment. Data management was done by A.T., B.F., L.G. and A.R.G. A.T., L.G. and A.R.G. did the statistical analysis with advice from B.F., O.D. and L.T.B. A.R.G. led the writing of the manuscript. All authors reviewed the manuscript and approved the submitted version.

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## Conflict of interest

T.G.L. is an employee of Medtronic Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

Supplementary material is available at *Conservation Physiology* online.

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# IV











































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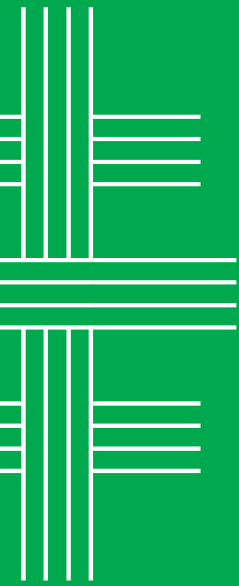












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How animals respond to human activity and a changing environment are key questions in ecological research. Recent advancements in biologging techniques and the ability to combine different types of biologgers have enabled us to investigate such impacts on free-ranging animals by providing detailed information covering longer periods of time. This thesis focus on moose (*Alces alces*), a key herbivore in the northern ecosystem. Understanding the ecophysiology of the moose, and the impact of human activity and climatic changes on both individual and population levels is important for sustainable management of the moose population. This thesis includes a collection of papers using biologgers to study the basic physiology of moose and the physiological and behavioural responses of moose to human activity. Moose displayed seasonal hypometabolism with lowered body temperature, heart rate and activity in winter compared to summer, representing a 60% decrease in metabolic rate. Pregnant females had a higher body temperature compared to non-pregnant females, and a drop in the body temperature and activity when parturition was imminent. Human activity increased the energy expenditure of the moose, represented by increased body temperature, movement, activity and heart rate and a decreased resting time during the day of the activity compared to the periods before and after. The response of the moose was dependent on the duration of the activity. Overall, this thesis improves our understanding of the ecophysiology of moose and provides an important foundation for further research, management and conservation of moose.