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Vaginal Temperature and Activity Variations in Relation to Fecal Progestagens of Captive Moose (*Alces alces*)

Variasjon i vaginaltemperatur og aktivitet sett i sammenheng med fekale progestagener hos elg i innhegning

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Class of 2016

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Contents

Summary

Title: Vaginal Temperature and Activity Variations in Relation to Fecal Progestagens of Captive Moose (*Alces alces*)

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Accurate detection of specific reproductive events presents a challenge to scientists studying wild species, including moose. There are several established methods for monitoring reproductive activity in domestic ruminants, including the use of biologging devices. The main objective of this study was to determine whether moose show a distinct thermal and activity pattern associated with luteal activity during the estrous cycle, as documented in cattle. We measured fecal progestagen, vaginal temperature, and activity of 12 captive female moose on the Kenai Peninsula, Alaska, USA, early in the breeding season to examine the relationships between these variables. Individual fecal samples were collected from August 23rd to October 15th, 2021, to classify luteal activity. Our results support that moose display an identifiable thermal pattern during the onset of luteal activity from mid-September to mid-October, which is associated with the presumed first estrous cycle of the breeding season. We developed an algorithm to detect luteal activity based on this pattern, with a sensitivity of 71%. In contrast, we did not observe a distinct pattern in activity during this period. However, subsequent, recurring patterns in both temperature and activity were identified between mid-October and mid-November, which were likely associated with following estrous cycles.

Definitions and abbreviations

CI	Confidence Interval
FSH	Follicle-Stimulating Hormone
GAMM	Generalized Additive Mixed Model
GnRH	Gonadotropin-Releasing Hormone
LH	Luteinizing Hormone
LMM	Linear Mixed Model
IFNT	Interferon-Tau
OLA	Onset of Luteal Activity
PdG	Urinary Pregnanediol
$PGF_{2\alpha}$	Prostaglandin
SD	Standard Deviation
T_a	Ambient temperature
T_v	Vaginal temperature
VIT	Vaginal Implant Transmitter

Introduction

The moose (*Alces alces*) is a large wild cervid species found in the northern forests of the United States, Canada, and Eurasia. Historically, moose are likely to have inhabited circumpolar regions ever since the retreat of Pleistocene glacial sheets, about 11,000 years ago (Hundertmark et al., 2002). They are important for the ecosystem, being an essential nutritional resource to both predators and scavengers, and by structuring the landscape as a large herbivore (Bowyer et al., 1997). Additionally, they have been hunted by humans for centuries, exploited as a food source and for crafting tools but also for spiritual, cultural and recreational purposes (Regelin & Franzmann, 1998).

Different moose populations are experiencing fluctuations. Recent studies show how populations of moose living in the southern ranges of their habitat have a reduced calf survival rate (Malmsten, 2014; Monteith et al., 2015), while other populations appear to be relatively stable (Wattles & DeStefano, 2011), expanding to new areas (Darimont et al., 2005), or increasing in size (Harris et al., 2015; Murray et al., 2012). Population declines have been attributed to influences such as parasites like brain worm (*Parelaphostrongylus tenuis*), liver fluke (*Fasciola magna*) and ticks (*Dermacentor albipictus, Ixodes ricinus*), nutritional deficiencies, predation by carnivores, anthropogenic activities and environmental conditions (Debow et al., 2021; Lenarz et al., 2009; Malmsten, 2014; Monteith et al., 2015; Murray et al., 2006).

Due to their ecological and socioeconomic importance, moose population stability is of interest to several stakeholders, such as landowners, local authorities, researchers, and hunters (Balciauskas et al., 2020; Johansson et al., 2020). Every year about 160,000 moose are

harvested during the hunting season in Norway, Finland, and Sweden (Natural Resources Institute Finland, 2022; Statistisk sentralbyrå, 2022; Svenska Jägareförbundet, 2022). Moose population and density in Fennoscandia have historically fluctuated, with harvest and management strategies among the most important drivers (Lavsund et al., 2003). Nevertheless, Fennoscandia appears to have the highest moose population density on a global scale (Jensen et al., 2020).

Determining what factors affect moose density and population dynamics is necessary from a management perspective. Detailed knowledge of a species' phenological events, such as reproduction, needs to be taken into consideration when making decisions regarding management and conservation (Wildt & Wemmer, 1999). As reproduction phenology of a species is critical for the survival and recruitment of offspring (Williams et al., 2017), it is fundamental that we have a thorough understanding of this aspect in moose.

Accurate and sufficient information of phenological events in wildlife species can be a challenge to obtain, which may be attributed to their elusive and shy behavior, and preference of remote habitats. Movement patterns of wild mammals have been remotely monitored through the use of radio-tracking technology, telemetry, and Global Positioning System (GPS) for the past 50 years (Mech & Barber, 2002). This technology is a helpful tool for researchers to register, explore, and better understand animal behavior, movement patterns and ecology in their habitat (Tomkiewicz et al., 2010). Collaring moose with GPS transmitters has enabled scientists to also determine key life events such as time of parturition and assessing calf mortality (Bergman et al., 2020; Nicholson et al., 2019; Severud et al., 2015). In addition to GPS transmitters, other biologging devices in the form of miniature sensors may be attached to or implanted in animals for remote monitoring of their physiology in their

natural habitat and can measure physiological parameters such as body temperature, activity, heart rate, blood pressure, and brain activity (Williams et al., 2021). Such high-resolution data may facilitate researchers in being able to detect and describe recurring life events, such as the estrous cycle, gestational length and parturition, and how these may respond to environmental changes (Chmura et al., 2018).

Methods for detecting and monitoring reproduction in domestic ruminants, which are based on both general and species-specific knowledge about reproduction physiology, are well-established. These include behavioral and physiological methods for determination of sexual receptivity, such as behavioral observations, measuring body temperature, or monitoring activity (Roelofs et al., 2010; Sveberg et al., 2011). As estrus expression is shown to be related to the timing of ovulation, methods which identify such behavior are helpful tools for reproduction monitoring and breeding management (Roelofs et al., 2005a; Roelofs et al., 2005b). It has been shown that dairy cattle (*Bos taurus*) which display detectable estrous signs have increased fertility and decreased pregnancy loss in comparison to those which do not display such signs (Pereira et al., 2015). More obvious or intense behavioral expressions of estrus, increases the accuracy of estrus detection (Roelofs et al., 2005b). Thus, accurate estrus detection allows enhanced reproduction management for farmers, which affects their livelihood (Britt, 1985). These methods are widely implemented in domestic ruminants and are feasible because farm animals are generally housed in controlled area and used to being handled by humans with or without the aid of physical or chemical (i.e., drugs) restraint.

In contrast, we have less experience and few established methods for monitoring the estrous cycle in wild animals, including moose. As behavioral cues in wild ungulates may be less prominent, the reliability of behavioral observations to determine or validate reproductive

events in wild species are considered to be less accurate (Sontakke, 2017). This further supports the need for more accurate reproduction monitoring techniques in wildlife. Captive animals are more habituated to human presence, which facilitate the feasibility and accuracy of reproduction monitoring and decrease the capture-related stress. Therefore, a lot of reproduction studies in wild species have been conducted in captive animals first, before being extended to free-ranging ones (Lasley & Kirkpatrick, 1991). To overcome practical and ethical challenges associated with studying non-domesticated animals, less-invasive methods are preferred, if not essential.

Regulation of the estrous cycle has been closely studied in cattle, goats (*Capra hircus*) and sheep (*Ovis aries*), which share a lot of similarities (Noakes, 2001). Based on assumptions of a chiefly shared reproductive physiology, principles of established methods for domestic ruminants may have potential to be transferrable to wild ruminant species as well.

The estrous cycle in domestic ruminants

Extensive research on reproduction physiology of domestic ruminants for the past century has expanded our knowledge and led to numerous advancements in reproduction management and technology for these species (Amiridis & Cseh, 2012; Stevenson & Britt, 2017). Cattle are non-seasonal polyestrous, meaning that they have recurring estrous cycles through the year, regardless of season. In contrast, small ruminants such as sheep and goats are seasonally polyestrous and so called “short-day breeders”, meaning that they have recurring estrous cycles only during the fall, when the duration of daylight decreases (Marshall, 1936). An estrous cycle is defined as the period between the onset of one estrus and the onset of the next, with estrus being the time a female is sexually receptive to a male. The changes which occur in ovarian activity during the estrous cycle facilitate the female to go from an inactive

to active sexual state, making her receptive to breeding, possibly establishing pregnancy, and able to produce offspring (Forde et al., 2011). The length of estrus varies between ruminant species and can last anywhere between 2 to 48 hours. Ovulation of an oocyte occurs either during or after estrus, which is also species dependent. In cattle, ovulation occurs 12 hours after the onset of estrus, while in goats and sheep ovulation occurs during or near the end of estrus, respectively (Noakes, 2001).

In transitional periods such as before and shortly after puberty (Ryan et al., 1991), postpartum (Petersson et al., 2006), or from seasonal anestrus (i.e., inactive reproductive state) to estrus (i.e., active reproductive state; Ravindra and Rawlings (1997)), the estrous cycle can be more irregular and ovulation may not be accompanied by behavioral estrus and breeding (Lauderdale, 1986). As the timing and duration of the estrous cycle is under tight hormonal control, this is taken advantage of in today's reproduction management in herds by administering hormone injections to synchronize estrous cycles, induce ovulation, or to treat ovarian cysts (Abecia et al., 2011; Smith et al., 2018).

Endocrine control of the estrous cycle

The estrous cycle is regulated by complex endocrine mechanisms, mainly through the hypothalamic-pituitary-ovarian-axis (Noakes, 2001). The hypothalamus secretes gonadotropin-releasing hormone (GnRH) which stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. FSH and LH stimulate cells in the ovaries to produce gonadal steroid hormones such as estrogens, androgens, and progestagens, affecting follicular development, ovulation, and formation of the corpus luteum. The products estradiol (an estrogen), progesterone (a progestagen) and the peptide hormone inhibin also play a role in hormonal release from the pituitary gland and the

hypothalamus (Figure 1). The positive and negative feedback effects of hormones on other endocrine glands within the hypothalamic-pituitary-ovarian-axis control and regulate follicular growth, ovulation, and corpus luteum formation. FSH stimulates the growth of follicles, while ovulation of a mature follicle is initiated by a preovulatory LH surge. Progesterone is primarily produced by the corpus luteum, and regulates the estrous cycle by inhibiting LH secretion, and ovulation of other dominant follicles. The corpus luteum is a temporary endocrine gland that develops in the ovary after ovulation of a dominant follicle. If fertilization does not occur, the corpus luteum regresses (luteolysis). Luteolysis is a process driven by prostaglandin ($PGF_{2\alpha}$), which is produced by the uterus in absence of a conceptus. If fertilization does occur, the corpus luteum persists and continues to produce progesterone, which is important to establish and maintain pregnancy (Wiltbank et al., 2014). Changes in behavior and body temperature during the estrous cycle are due to these hormonal fluctuations and their resulting effects (Allrich, 1994).

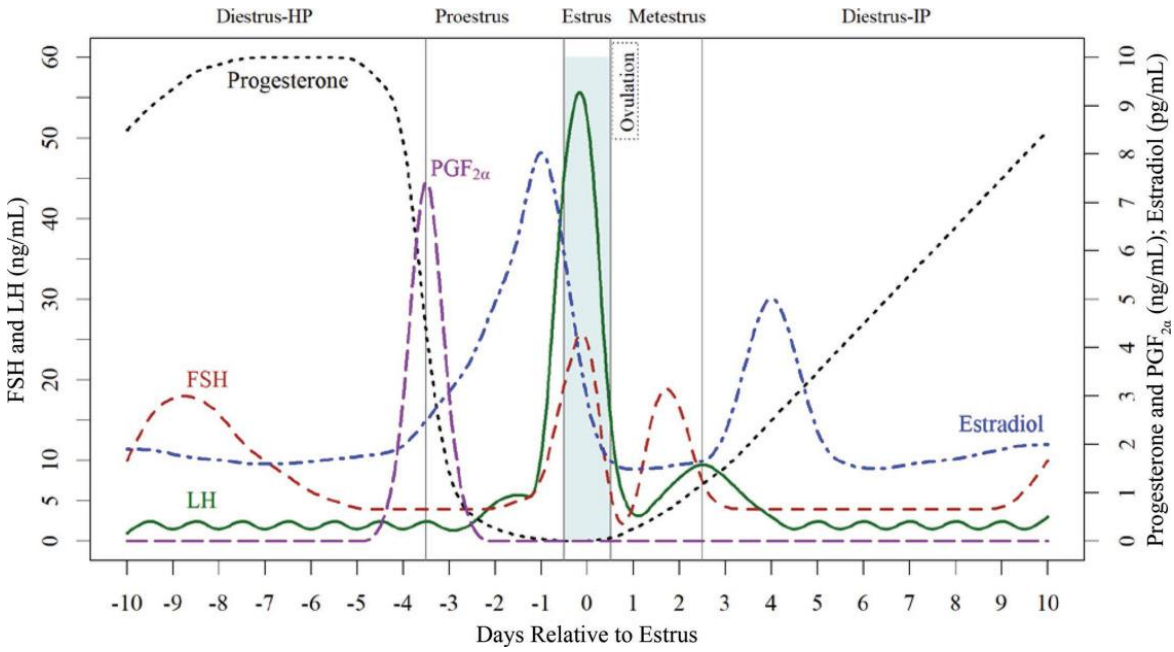

Figure 1. An overview of different hormonal concentrations in relation to the estrous cycle of the cow. Progesterone (black dashed line) is at a nadir during estrus and right before ovulation. After ovulation, the concentration increases steadily, until luteolysis and regression of the corpus luteum (initiated by $PGF_{2\alpha}$: prostaglandin, violet dashed line). The concentration decreases again before estrus. Diestrus-HP: diestrus high-progesterone. Diestrus-IP: diestrus increasing-progesterone. FSH: follicle-stimulating hormone. LH: luteinizing hormone. Reprinted from Toledo-Alvarado et al. (2018), with permission by Elsevier and Copyright Clearance Center © (2022).

Phases of the estrous cycle

The period of follicular growth, estradiol production, selection of a dominant follicle(s), and ovulation is defined as the follicular phase of the estrous cycle. Between each follicular phase is the luteal phase which begins after ovulation and formation of a corpus luteum and lasts until luteolysis. In cattle the luteal phase has a duration of 14-18 days and the follicular phase lasts for 4-6 days (Forde et al., 2011). If the ovulated oocyte is fertilized (embryo), it enters the uterus approximately 4 days later. After 12-14 days the embryo develops into a conceptus which signalizes its presence to the mother (maternal recognition of pregnancy) through secretion of interferon-tau (IFNT). IFNT prevents the production of $PGF_{2\alpha}$ in the uterus, and resultingly the corpus luteum will persist and continue to produce progesterone during pregnancy (Forde & Lonergan, 2017). Presence of a corpus luteum prevents the initiation of a new follicular phase, so luteolysis is required before a new follicular phase can occur if fertilization and pregnancy does not occur.

Progesterone metabolism

Specialized cells in the corpus luteum produce progesterone from cholesterol (Gomes & Erb, 1965). Like other steroid hormones, progesterone is released into the blood, where it circulates either freely or bound to plasma proteins. Further metabolization occurs in the liver, before excretion as conjugates in urine, bile (and subsequently feces), and saliva (Schiffer et al., 2019). Bile is released into the intestine, where the metabolites are deconjugated and partially reabsorbed and transported back to the liver again, a process called enterohepatic circulation (Russell, 2009). The excretion pathway and rate of gonadal steroid hormones differs between species and hormone type (Palme et al., 1996). Passage of steroid hormones

from blood to feces involves a delay which is approximately correlated to the intestinal passage of bile to the rectum. It has been estimated that the lag-time between blood levels of steroids and fecal steroid levels is 12-24 hours in domestic ruminants (Palme et al., 1996; Schwarzenberger, F. et al., 1996b). As previously mentioned, measurement of hormonal concentrations can be used to reflect an animal's reproductive status. Specifically measuring urinary and fecal steroid hormones offer an advantage to monitoring reproduction in wildlife species, as it can replace the more invasive method of acquiring a blood sample.

Monitoring the estrous cycle

Because of the detectable physiological and physical events which occur during the estrous cycle, it is possible to utilize measurements of these parameters (e.g., body temperature, vulva appearance, vaginal mucus, activity, mount detectors, rectal palpation, and ovarian ultrasonography) as an indicator of ovarian activity (Hanzen et al., 2000; Roelofs et al., 2010). Knowledge about what is considered a parameter's baseline value (measured outside the estrous cycle) for a species is a prerequisite to be able to identify deviations from it, which can then be monitored to detect ovarian activity and changes associated with the estrous cycle.

Body temperature

Body temperature variation during the estrous cycle have been closely described in domestic cattle (Wrenn et al., 1958) and share similarities with women (Marshall, 1963). The general observed pattern of variation shows a decrease in temperature just prior to estrus, and then a sharp increase on the day of estrus. Following this peak there is a decrease around the expected time of ovulation before a steady increase post-ovulation, during the luteal phase

(Suthar et al. (2011); Wrenn et al. (1958); Figure 2). Theories behind the rise in body temperature around estrus include the thermogenic effect of progesterone, increased vaginal blood flow and the accompanying increase in activity (Abrams et al., 1973; Suthar et al., 2012; Walton & King, 1986; Wrenn et al., 1958). In dairy cattle, Redden et al. (1993) found that estrus could be detected with the highest accuracy when vaginal temperature increases between 0.3ºC and 1.0ºC above the previous 4-day baseline and lasting for least 3 hours (sensitivity = 81%). Similarly, Kyle et al. (1998) concluded that a vaginal temperature elevation of at least 0.4ºC which lasts for a minimum duration of 3 hours above a baseline of 3 days was particularly sensitive for detecting estrus in beef cattle (sensitivity = 89.4%, positive predictive value = 96.2%). It is not yet known whether moose show a similar relationship between body temperature and their estrous cycle.

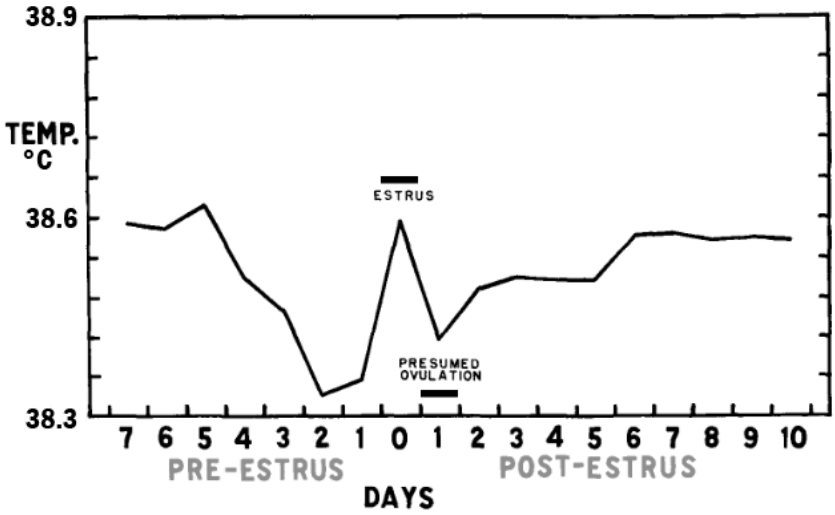

Figure 2: Changes in vaginal temperature around estrus (day 0) and presumed ovulation (day 1) in dairy cattle. Estrus was confirmed for a cow if she stood while being mounted by other cows. Reprinted from Wrenn et al. (1958), with permission by Elsevier and Copyright Clearance Center © (2022). The y-axis has been modified to show degrees of Celsius (ºC) instead of Fahrenheit (ºF).

Activity

Accelerometry registrations are used in domestic cattle to study behavior, health and reproductive events (Diosdado et al., 2015). In cattle, there has been shown an association between increased activity and the timing of estrus (Brehme et al., 2018; Løvendahl & Chagunda, 2010; Redden et al., 1993; Shahriar et al., 2016). Similar patterns have been shown in wild ungulate species, such as white tailed deer *(Odocoileus virginianus),* where peak daytime movement usually occurs on the day of mating or the day before (Ozoga & Verme, 1975). To identify increases in activity, Plenio et al. (2021) calculated a threshold value for activity in dairy cows which needed to be exceeded in order to indicate the onset of estrus (Figure 3). This threshold was defined based on the difference between the mean activity of the preceding 7 days and the cow's momentary mean activity (based on a 2-hour average), weighted by its standard deviation. In a study using a similar method, parity played a role on the amount of activity measured during estrus, where multiparous cows (i.e., cows who have calved multiple times) expressed lower activity of shorter duration than primiparous cows (i.e., cows who have calved for the first time) (Madureira et al., 2015). Knowing that several species tend to have a general increase in activity around the time of estrus, it is of interest to explore whether this could be the case for moose as well. However, it is yet to be determined whether activity levels in moose could be associated with their estrous cycle.

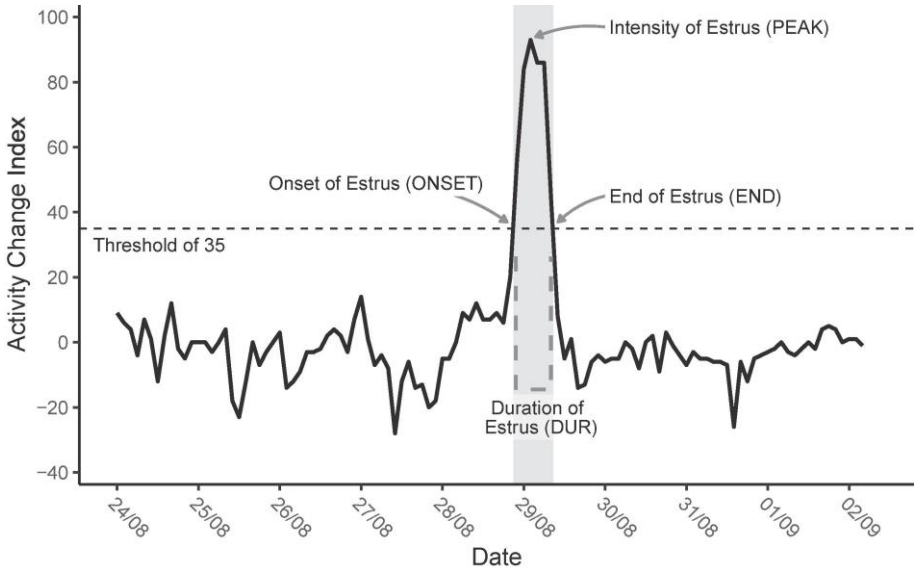

Figure 3: Changes in activity around estrus demonstrated by using a Bovine Heat Detection and Analysis Tool (BovHEAT) which illustrates a day where activity increases above a set threshold. Reprinted from Plenio et al. (2021), with permission by Elsevier and Copyright Clearance Center © (2022).

Fecal progesterone metabolites

Monitoring changes in fecal progesterone levels is a widely used non-invasive method to monitor ovarian activity in both domestic and wild mammals (Dumonceaux et al., 2006; Hirata & Mori, 1995; Kirkpatrick et al., 1993; Matsuura et al., 2004; Pereira et al., 2006; Schwarzenberger, F. et al., 1996a; Wasser et al., 1996), including moose (Monfort et al., 1993; Schwartz et al., 1995). Fecal progesterone concentration can be used as an indication of an individual's plasma progesterone, as fecal levels of the hormone demonstrate parallel changes to those observed in plasma (Hirata & Mori, 1995). As mentioned earlier, gonadal steroid hormone metabolism varies widely between species, and so the primary route of excretion is often unknown. A commonly used method to investigate excretion routes is by injecting animals with radiolabeled gonadal steroid hormones and measuring their concentration by chromatography of urine and/or feces (Graham, 2004).

Through infusion of radioactive hormones in domestic animal species, Palme et al. (1996) found a predominance of unconjugated steroids in feces, likely due to deconjugation by bacteria in the intestine. The extensive metabolism of progesterone prior to excretion in feces causes there to be very limited (if any) presence of unmetabolized progesterone in feces (Schwarzenberger, F. et al., 1996a). Therefore, assays aiming to reflect progesterone levels based on fecal samples often measure progesterone metabolites based on a certain carbon molecule position. This carbon position (C20) is common to several progesterone metabolites (20-oxo-pregnanes) present in feces, making the assay group-specific and measures the combined concentration of several metabolites instead of just one. This method has been validated for monitoring ovarian activity in cattle, and is considered to be more accurate than measuring for only one specific metabolite which could lead to considerable underestimation of progesterone levels (Schwarzenberger, F. et al., 1996b).

Nevertheless, for quantification of fecal progestagens in species with unknown steroid metabolism, it has become popular to use immune-assays where an antibody raised against progesterone cross-react with multiple common steroid hormones (Graham, 2004). This enables us to characterize steroid metabolite levels and reproductive events for a species, which can then be applied to further understand and manage the species in captivity and in the wild.

Current knowledge about the estrous cycle in moose

Sexual maturation in the female moose is speculated to occur after 1.5 years of age but varies between different cohorts and their respective environmental conditions (Garel et al., 2009; Malmsten, 2014; Sand et al., 1995; Sand, 1996; Schwartz & Hundertmark, 1993). Moose are seasonally polyestrous, meaning that they only breed during a specific time of the year.

Changes in day length (photoperiod) affect endocrine signaling pathways which are known to regulate the transition from anestrus to estrus in seasonal breeders (Shinomiya et al., 2014). The estrous cycle and breeding season in moose is reported to occur from early September and well into November (Ballenberghe & Miquelle, 1993; Malmsten et al., 2014; Schwartz & Hundertmark, 1993). Peak ovulation dates for moose in Scandinavia and North America are estimated to be synchronized and occur in late September and early October (Garel et al., 2009; Sigouin et al., 1997). Schwartz and Hundertmark (1993) estimated each estrous cycle to have a total duration of about 24 (22-28) days and a possible recurrence of 4-7 cycles during the breeding season, with primiparous females having a shorter cycle length than multiparous females, as documented for cattle (Madureira et al., 2015). Duration of sexual receptivity, or estrus, is limited to 15-26 hours at the beginning of each cycle. This has been suggested to occur on average 0.6 days prior to the nadir (95% CI = 4 days before to 3 days after the nadir) in fecal progestagen levels, based on confirmation of observed estrous behavior (copulation with a bull or ruffled rump hairs) in captive moose (Schwartz et al., 1995; Schwartz & Hundertmark, 1993). Malmsten et al. (2014) found that mating had likely occurred 2 weeks into pregnancy for a limited number of female moose, meaning that copulation may not be a reliable indicator of estrus. In addition, the time and relation between observed estrus and ovulation are not yet known for moose, so it is not possible to estimate a day of ovulation solely based on external signs of estrus. It is, however, likely that ovulation occurs during or shortly after estrus (Malmsten, 2014). A study of Norwegian moose reported that yearling moose had lower and more variable ovulation rates compared to older females (Garel et al., 2009). Prime-aged (2.5-10.5 years old) individuals tend to ovulate earlier during the breeding season than young (<2.5 years old) and old (≥11.5 years old) individuals.

Body temperature and activity in moose

Yearly body temperature in moose has been seen to range from 36.25°C to 41.25°C, with the highest frequency occurring between 37°C and 39°C (Thompson et al., 2019). Previous monitoring studies of moose have shown that moose display seasonal patterns of body temperature and activity throughout the year (Græsli et al., 2020; Thompson et al., 2019). Generally, body temperature and activity are lower during winter compared with summer and has been shown to significantly decrease between July and November. For most of the year, circadian rhythms of body temperature dominate while activity follows an ultradian rhythm with longer periods during winter compared with summer (Græsli et al., 2020). This ultradian rhythm can likely be explained by the short cycle of feeding and rumination which is characteristic for ruminant species (Scheibe et al., 1999).

Biologging devices have recently been used to investigate patterns in body temperature and activity as indicators of pregnancy and parturition in wild moose in Sweden (Græsli et al., 2022). Results from this study showed that body temperature was higher in pregnant compared to non-pregnant moose (with 0.12-0.18°C, using a datalogger with an accuracy of 0.1°C), and that body temperature and activity decreased around the time of calving. Thompson et al. (2019) also found that pregnant female moose had statistically warmer body temperatures than non-pregnant ones (0.18°C higher, using a datalogger with an accuracy of 0.5°C). These findings support that moose show identifiable patterns in body temperature and activity related to reproductive events, as has been reported for roe deer (*Capreolus capreolus*), Mediterranean mouflon (*Ovis gmelini musimon* x *Ovis* sp.), alpine ibex (*Capra ibex*), and domestic cattle (Suthar et al., 2012; Wrenn et al., 1958).

Methods for monitoring the estrous cycle in moose

A number of studies focusing on reproduction monitoring in moose have relied on strategies that do not require live animal handling, such as physical examination of reproductive organs from harvested moose (Garel et al., 2009; Malmsten, 2014; Schladweiler & Stevens, 1973; Simkin, 1965). Using this method, they determined ovarian activity based on the presence and number of corpora lutea and/or follicles in the ovaries as an indicator of whether an individual had ovulated during the current breeding season.

Monfort et al. (1993) were likely the first researchers to determine urinary and fecal gonadal hormones as reliable indicators of reproductive events in captive moose. They determined the timing of estrus through behavioral observation when a moose cow copulated with a bull. Their study suggests that urinary pregnanediol (PdG, a progestagen) is useful for tracking pregnancy and the estrous cycle in moose, while urinary estrogen could be helpful for diagnosing late pregnancies. Additionally, they reported that fecal progestagen levels above a certain threshold (1000ng/g) indicate pregnancy in moose. Two years later, Schwartz et al. (1995) further supported fecal progestagen as a method for non-invasive monitoring of pregnancy and the estrous cycle in moose. Their measurements of fecal progestagen were descriptive for both phases in the estrous cycle, showing a rapid decline during the follicular phase and a slow, gradual incline to a peak concentration during the luteal phase. Furthermore, the day of observed estrous behavior in the same study was found to occur within 1 to 2 days of when fecal progestagen was at the nadir.

Aims

Despite previous efforts to accurately monitor and describe the estrous cycle in moose, we have yet to develop more reliable methods. This likely contributes to an overall limited understanding of the species' population dynamics, which informs successful wildlife management strategies (Dolbeer, 1998; White, 2000). Through detailed description of moose physiology and phenology, we can contribute to a more solid foundation for developing sustainable management and conservation strategies.

In the present study we aimed to explore possible methods for detecting different phases of the estrous cycle in moose using body temperature and activity. Based on already established methods that are applied in domestic ruminant species, the following hypotheses were addressed in this thesis:

H1 – Fecal progestagen values can be used to classify different phases of the estrous cycle in moose.

We expected to classify different phases of the estrous cycle in moose by measuring fecal progestagen values and determining a threshold to differentiate between luteal and inter-luteal phase. This classification would then be used as a reference for luteal activity to investigate the following hypotheses.

H2 – There is an identifiable pattern in body temperature which is related to luteal activity in moose.

We expected to observe a drop in body temperature, followed by a discernible peak during the onset of luteal activity, before dropping back down to a basal temperature.

H3 – There is an identifiable pattern in collar activity which is related to luteal activity in moose.

We expected to observe an increase in collar activity at the onset of luteal activity.

H4 – Identifiable patterns in body temperature and collar activity are related to luteal activity in such a way that they can be utilized to detect phases of the estrous cycle in moose.

We expected that changes in body temperature and collar activity would be characteristic enough to utilize these to detect the different phases of the estrous cycle which were identified in H1.

Our expectations for H1 through H3 were based on characteristic patterns associated with estrus which have been previously documented in dairy cattle; a low basal level of plasma progesterone combined with an increasing trend in T_v and a peak in activity (Figure 4; Lewis and Newman (1984)).

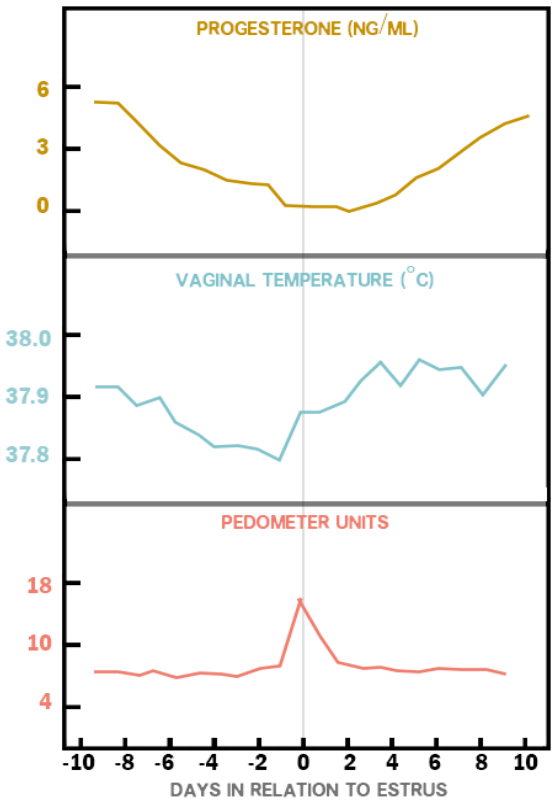

Figure 4. Plots of serum progesterone levels (nanogram per milliliter), activity (in pedometer units) and vaginal temperature (°C) plotted against days in relation to estrus (day 0) for dairy cattle. Modified from Lewis and Newman (1984), with permission by Elsevier and Copyright Clearance Center © (2022). In their study pedometer registrations were done twice a day, vaginal temperature and serum samples for progesterone were collected once a day and estrus observations were made at least twice a day for each cow.

To test our hypotheses we compared individual vaginal temperature and activity recordings to fecal progesterone levels, which is a validated method for monitoring luteal activity in captive moose (Schwartz et al., 1995). The use of captive animals allowed for regular collection of individual-specific fecal samples which would otherwise be unfeasible in wild free-ranging moose. Body temperature and activity were recorded using biologging devices, which represents a novel approach to this topic. If the above hypotheses were confirmed, this technology could offer the opportunity to do similar studies in wild free-ranging moose based on analyzing temperature and activity registrations alone. Our results would therefore have the potential to present a new approach to monitor reproduction physiology in captive moose, with the possibility of being applied to studies in wild moose.

Materials and methods

Study area

The samples and data registrations gathered for this thesis were obtained from free-ranging captive moose at the Kenai Moose Research Center, on the Kenai peninsula, in Alaska, USA (Figure 5). The research center is operated by the Alaska Department of Fish and Game and is located on the Kenai National Wildlife Refuge (60^{o}43’ N, 150^{o} 26’ W).

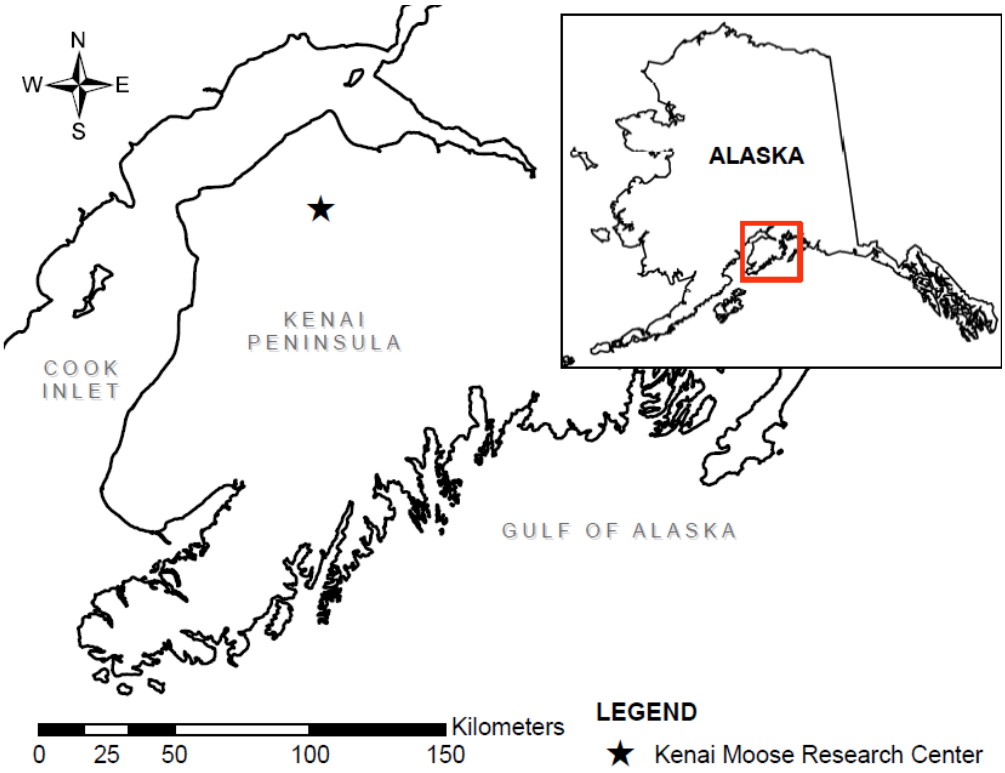

Figure 5. Map showing the location of the Kenai Moose Research Center (black star) in Alaska, USA. ©ADF&G, used with permission.

Twelve female moose were kept outdoors in two 2.6 km^2 enclosures with free access to water and natural forage (Pen 2 and 3 in Figure 6). One of these enclosures (Pen 2, Figure 6) shared a small section of fence with a third enclosure which housed three adult moose bulls (Pen 1, Figure 6). To allow for recurring estrous cycles of females, they were kept separated from the bulls. The study area is characterized as boreal forest, including dense forests, bogs, and open meadows. Present tree species were dominated by white spruce (*Picea glauca*), black spruce (*Picea mariana*), Alaska birch (*Betula neoalaskana*), willow (*Salix scouleriana*), and quaking aspen (*Populus tremuloides*). In addition to wild moose (*Alces alces gigas*), the adjacent area is also inhabited by brown bears (*Ursus arctos*), black bears (*Ursus americanus*) and grey wolves (*Canis lupus*). The design of the described study area and accessibility of animals enabled precise individual-specific collection of fecal samples.

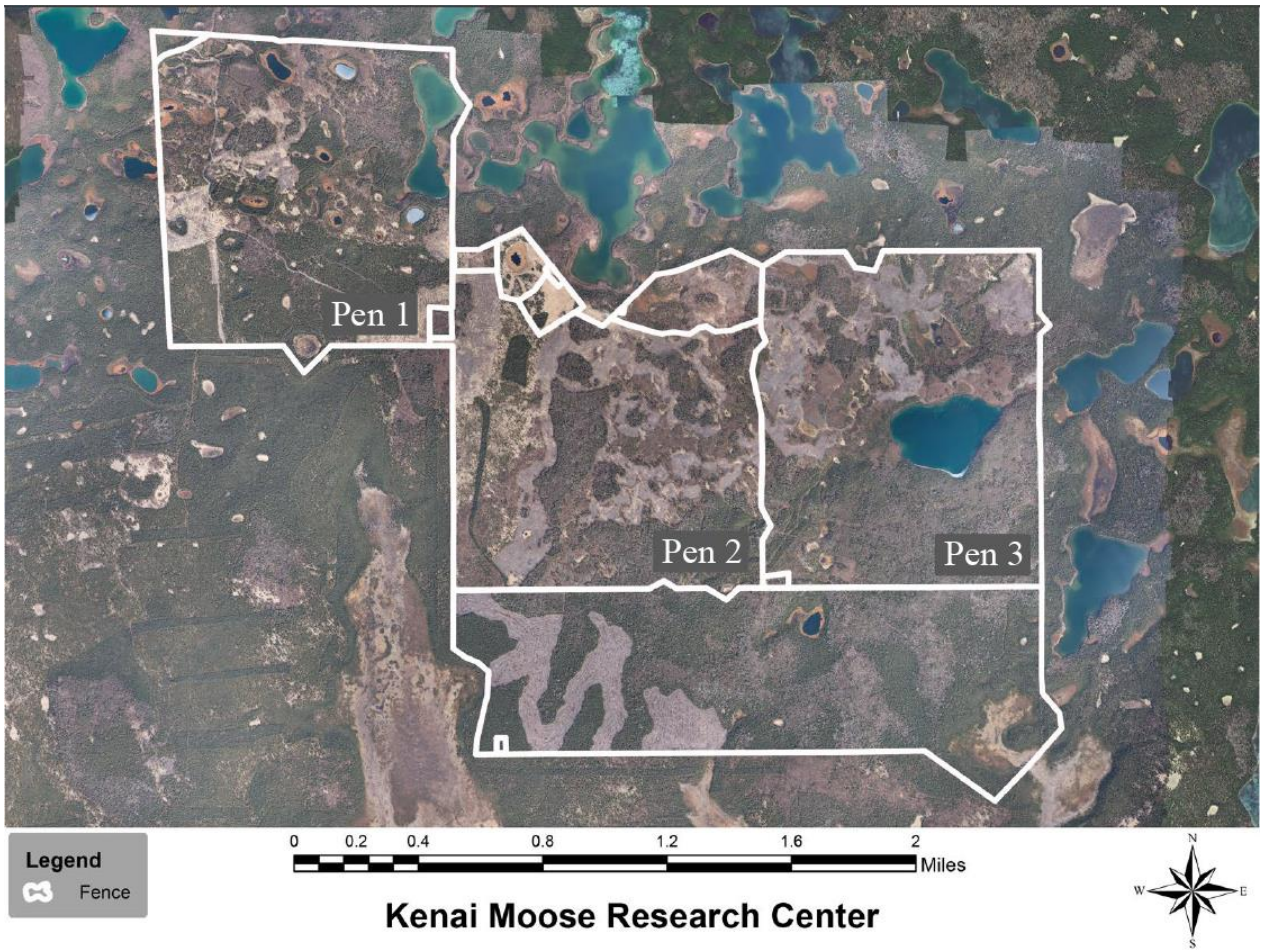

Figure 6. Detailed map of the Kenai Moose Research Center. The female moose included in this study were kept in pen 2 and 3, while the bulls were kept in pen 1. The outlined pen below pens 2 and 3 did not house any captive animals during the time of the study. ©ADF&G, used with permission.

Data collection

Study animals

In May and July 2021, 12 captive female moose, aged between 2 and 19 years old, were immobilized with a combination of Thiafentanil oxalate (0.001-0.004 mg/kg estimated body mass; 10mg/mL; Wildlife Pharmaceuticals Inc., Windsor, CO, USA) and Xylazine (0.03-0.05 mg/kg estimated body mass; 100mg/mL; Lloyd Laboratories, Shenandoah, IA, USA) by intramuscular hand-injection and equipped with Vertex Plus GPS collars (Vectronic Aerospace GmbH, Berlin, Germany) and biologging device (see below). Immobilization was

reversed by a combination of Atipamezole HCl (0.005 mg/kg estimated body mass; ¼ dose intravenous, ¾ dose intramuscular; 5g/mL; Zoetis, Parsippany, NJ, USA) and intramuscularly administered Naltrexone HCl (100mg/mg Thiafentanil oxalate intramuscular; 50mg/mL; ZooPharm LLC, Laramie, WY, USA). All animal care, handling and experimentation procedures at the Kenai Moose Research Center were approved by the Animal Care and Use Committee, Alaska Department of Fish and Game, Division of Wildlife Conservation (protocol number 0086-2020-40).

Biologging devices

Vaginal implant transmitters (VIT; size big; Vectronic Aerospace GmbH; Berlin, Germany) transmitted vaginal temperature (T_v; °C) every 5 minutes to the GPS collar, and also recorded vaginal temperature every 17.1 minutes on board the VIT. During the aforementioned immobilization procedure, a VIT was inserted into the vaginal canal of each individual by using a lubricated (OB Lube; Jorgensen Laboratories Inc., Loveland; Colorado, USA), sterilized speculum (Sterile Disposable Vaginal Speculum; Jorgensen Laboratories Inc.) following an earlier described procedures (Patterson et al., 2013). T_v was successfully downloaded from 11 individuals (downloaded from GPS-collar n=10 and from the VIT n=1). Since the VIT stopped recording for the last moose on the 12th of August 2021 (i.e., before the start fecal sample collection), we therefore excluded this individual from our T_v analysis.

GPS collars were mounted with a triaxial accelerometer which registered forward-backward, left-right, and up-down movements, stored as X, Y, and Z, respectively. Movement was recorded in 5-minute intervals as average values of each axis ranging between 0-255 at 6-8 Hz and stored in the collar. We calculated an overall activity value by summing the X, Y and Z

axes, giving values ranging from 0-765 every 5 minutes, with 0 representing no activity and 765 the highest activity level.

Both the GPS collars and the VITs had integrated very high frequency (VHF) transmitters which enabled movement tracking and identification of each individual by the use of telemetry. Individual identification was also facilitated by marking the GPS collars with specific color duct tape combination assigned to each moose.

GPS collars and VITs were manually removed from all animals without sedation on November 23rd, 2021.

Temperature validation of VITs

The VITs used in this study have an accuracy of 0.1°C according to the manufacturer, and although calibration in ice water showed no need for adjustment (Schmidt et al., 2020), these devices have not yet been validated within the body temperature of moose. A water bath was used to validate 11 of the 12 VITs that were used in this study. The remaining VIT was not included in the validation procedure because it was set to the wrong setting before being placed in the water bath. The VITs were first placed into an ice bath for an hour before transferring them to a Coliform Incubator Bath (Serial No. 10AY-5, Precision Scientific Inc., Chicago, IL, USA) with 16 liters of water preheated to 36.5°C (Thompson et al., 2018). The water bath temperature was measured with a National Institute of Standards and Technology-certified digital thermometer (Model DTR-1221A, accuracy ± 0.1°C, iProvèn USA LLC, Beaverton, OR, USA). Temperatures were recorded at 5-minute intervals for an hour, during which the water temperature was increased by 0.5°C starting from 36.5°C until 40.5°C (i.e., within the range of body temperature in moose; 36 to 41°C , Thompson et al. (2019)). To

validate the VITs temperature loggers in the water bath for body temperature in moose we used a linear mixed model (LMM) with temperature recorded by the VIT as a response variable and temperature in the water bath as the explanatory variable using the "mcgv" package in R (Wood, 2017). We included a random intercept and slope for the individual VIT over different water bath temperatures to account for the possibility that different loggers may show variability in temperature measurements over different water bath temperatures.

When the VITs were submerged in ice water, the recorded temperatures had a relatively large variation (mean = 2.16°C, 95%CI = -1.64-5.96°C), so this method was therefore not considered appropriate for validation of the loggers. However, when the VITs were submerged in the water bath which was kept within range of body temperature, their recordings were significantly lower (Wilcoxon signed-rank test, p-value = 0.25) than the water bath. The LMM predicted that temperatures recorded by the VITs were highly correlated to the water bath temperatures (deviance explained = 96.4%) with an overall accuracy within 0.3°C (95%CI = 0.1-0.3°C). The accuracy of temperature recorded by the VITs increased with rising temperature, with a difference of 0.29°C (95%CI = 0.22-0.37°C) at 36.5°C and a difference of 0.15°C (95%CI = 0.08-0.23°C) at 40.5°C.

Fecal sampling

Individual moose were tracked, localized, and identified using ground telemetry (TR-8 receiver; RA-23K antenna; Telonics Inc, Arizona, USA) and respective color combination on their GPS collar. A total number of 468 fecal samples were collected during a period of 54 days. From the 23rd of August to (and including) the 21st of September, individual samples were collected every other day. After the 21st of September, samples were collected on a daily basis for the remaining 24 days, until the 15th of October. This period was chosen based on

previous literature on the timing of the species' reproductive season, and a desire to catch the transition between reproductive inactivity (anestrus) to reproductive activity (estrus) (Garel et al., 2009; Schwartz & Hundertmark, 1993; Sigouin et al., 1997). All sampling occurred between 07:00-19:00 Alaska Daylight Time (AKDT).

Figure 7. Fecal samples from different stages of handling and processing. Top left: Fresh sample collected that day, before freezing. Top right: Freeze-dried fecal sample. Bottom left: Freeze-dried and homogenized fecal sample. Bottom right: The final subsample that was shipped to the endocrine lab.

Samples weighing between 100 to 200 grams (g) were collected in pre-labeled Whirl-Pak bags (Nasco Whirl-Pak®, Fort Atkinson, WI, USA; Figure 7) during or shortly after defecation. As steroid metabolites are shown to be unevenly distributed in fecal matter from the same feces/individual, we aimed to reduce such variability by selecting pellets from multiple fractions of feces when possible (Millspaugh & Washburn, 2003; Tanaka et al., 2019; Wasser et al., 1996). Samples were stored on ice in a cooler bag in the field, before

being transferred to a -18ºC propane freezer. The frozen samples were freeze dried (Labconco model 7752020, Kansas City, MO, USA) and homogenized to a powder in a grinder to further distribute metabolites evenly, before randomly subsampling 5g (Tanaka et al., 2019). The subsamples were shipped to the endocrine laboratory Applied Biosciences in Texas, USA, for further extraction and analysis.

In the endocrine laboratory, steroid hormones were extracted by taking 0.2g of the subsamples and adding 2ml methanol to a microcentrifuge tube. The tubes were vortexed and centrifuged at 2500*g* (*g* = gravitational force) at room temperature for 20 minutes, before the supernatant was removed and stored at -20ºC until analysis.

Progestagen concentration was determined by radioimmunoassay (RIA) (Catalog #07-270102; ImmuChem Double Antibody, 125I RIA Kit, MP Biomedicals, Costa Mesa, CA). The manufacturer's assay protocol was followed. Double antibody assays quantify a selected metabolite (in this case progesterone metabolites) in a sample by adding a known quantity of radiolabeled antigens and antibodies specific for the metabolite. The radioactive antigen will bind to the antibody, competing for binding sites with the metabolites which are present in the sample. Quantifying the bound antigen in the sample allows for metabolite quantification due to the inverse relationship between bound antigens and bound metabolites (Praither, 1985). As previously mentioned, such immunoassays will cross-react with multiple other common steroid metabolites. We were not able to acquire information regarding the percentage of cross-reactivity for our assay kit. Fecal progestagen levels are expressed as nanogram (ng) of immunoreactive fecal progestagen hormone metabolites per gram (g) dry fecal weight.

Environmental variables

Within the Kenai Moose Research Center there is a National Oceanic and Atmospheric Administration, U.S. Climate Reference Network weather station (hereafter referred to as NOAA weather station) which recorded environmental variables including ambient air temperature, relative humidity, solar radiation, windspeed and precipitation every 5 minutes (Diamond et al., 2013).

Ambient air temperature ($T_{a,}$ °C) and relative humidity (%) recorded by the NOAA weather station were used to calculate dew point temperature (°C), which was further used to calculate actual vapor pressure (expressed in hectopascal, hPa; see equation 1 and 2 in Appendix; Alduchov and Eskridge (1996)).

Data preparation and analysis

Vaginal temperature, collar activity and fecal progestagen

All data handling and statistical analysis was performed in R version 4.1.0 (R Core Team, 2021), using RStudio version 1.4.1717 for Windows. T_v and collar activity data were filtered for the 3 following days after capture to exclude registrations where values are likely elevated due to effects of the immobilization (Thompson et al., 2019). T_v, activity, fecal progestagen and environmental datasets were checked and cleaned for duplicates and missing data, and visualized with "tidyverse" (Wickham et al., 2019), "naniar" (Tierney et al., 2019), and the "gridExtra" (Auguie & Antonov, 2016) R packages. In the raw T_v dataset, there were additional duplicate T_v recordings for specific timestamps (0.09% of registrations). This was corrected for by keeping the calculated mean of the two values. Since fecal collections occurred on alternate days for the first half of the sampling period, fecal progestagen values

were linearly interpolated between two measurements by using the "approx" function to generate daily values to include in the statistical model (Zeileis & Grothendieck, 2005). Similarly, daily values for T_v and collar activity were generated by calculating the daily mean T_v and the total daily sum of activity values (referred to as daily sum of activity hereafter).

Environmental variables

The recorded environmental variables from the NOAA weather station, ambient temperature (T_a) and relative humidity, contained outliers for one date (August 25th, 2021) during the fecal sampling period. Registrations from nearby HOBO dataloggers (HOBO U23 Pro v2 Temperature/Relative Humidity datalogger and HOBO Pendant Temperature/Light 64K datalogger, Onset Computer Corp., Pocasset, MA, USA, which recorded T_a and relative humidity, and T_a and solar radiation, respectively) were instead used to calculate daily values for this day (99.2-99.7% correlation with temperature and relative humidity recorded by the NOAA weather station during the rest of the period, respectively). As the HOBO Pendant Temperature/Light 64K datalogger recorded solar radiation using a different unit than the NOAA weather station (lux versus megajoules per square meter (MJ/m^2), respectively), we chose to only include solar radiation registrations from the HOBO Pendant Temperature/Light 64K datalogger, instead of the NOAA weather station, which contained the mentioned outliers.

Classification of luteal activity based on fecal progestagen

To determine a baseline level of fecal progestagen which was considered indicative of seasonal anestrus or an inter-luteal phase, we calculated the mean of the 2 lowest values for each individual. The term inter-luteal phase was used instead of follicular phase, as

progestagen metabolites do not directly describe the period of follicular maturation and growth (Kaneko et al., 1991). To differentiate between luteal phase, inter-luteal phase and seasonal anestrus we created a threshold defined as the baseline level of progestagen multiplied by 2. If fecal progestagen levels were below the threshold for at least one day and then increased and stayed above it for at least 14 days, we defined the day on which the threshold was exceeded as an onset of luteal activity (OLA) and the period during which fecal progestagen levels stayed above the threshold as a luteal phase (Figure 8). Anestrus and inter-luteal phases were defined as periods during which fecal progestagen levels stayed below the threshold before and after the occurrence of the first luteal phase, respectively (Figure 8). The above calculations and classifications were done by applying a rolling function using the "zoo" R package (Zeileis & Grothendieck, 2005).

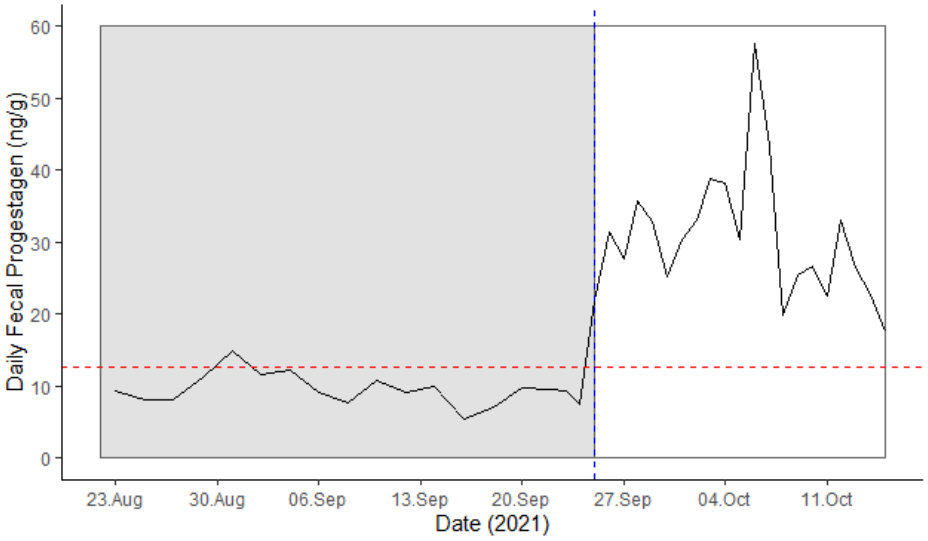

Figure 8. Example of how phases of the estrous cycle were classified based on fecal progestagen levels in one individual (30683, 12 years old). Dashed vertical blue line = day 0 of the onset of luteal activity (OLA), which was on the 25th of September 2021. White box (to the right of the dashed blue line) = period when fecal progestagen stayed above the threshold and thus fulfilled the criteria of being indicative of a luteal phase. Gray box (to the left of the blue dashed line) = period when fecal progestagen levels were below the set threshold and indicative of anestrus. The dashed horizontal red line = this individual's fecal progestagen threshold (12.62 ng/g).

For each moose, the date when the first OLA occurred was assigned as day 0. This scale was further used as a point of reference to explore variations in T_v and activity for that individual during the estrous cycle.

Characteristic thermal and activity patterns

To explore whether moose display a characteristic thermal and/or activity pattern during their estrous cycle as seen for cattle (i.e., a rise in activity and T_v around the day of estrus, prior to a rise in progesterone) we plotted graphs of daily fecal progestagen values together with daily mean values for T_v and total daily sum of activity values for each individual.

Relationship between luteal activity and vaginal temperature

To determine when changes in daily mean T_v occur in relation to the onset of luteal activity, we used Generalized Additive Mixed Models (GAMMs; using the "mcgv" R package from Wood (2017)) due to non-linear relationships between response and explanatory variables. All explanatory variables included in the model were adjusted to daily values to match up with the daily frequency of fecal progestagen values. Daily mean T_v was selected as the response variable and OLA was included as the main explanatory variable to explore the relationship between luteal activity and T_v. Additionally, we included explanatory variables that are known to influence core body temperature in moose: activity, julian day (to account for seasonal variation in T_v), T_a, vapor pressure, wind speed, precipitation and solar radiation (Thompson et al., 2019). Correlation and structure of the explanatory variables were checked using the function "ggpairs" from the "GGally" (Schloerke et al., 2018) extension to the "ggplot2" R package (Wickham, 2016). If the Pearson's correlation coefficient between OLA and another explanatory variable was above 0.50 or below -0.50, it was considered a high

correlation and this variable was excluded from further analysis (Mukaka, 2012). Additionally, if two explanatory variables other than OLA had a correlation with each other above or below this threshold, the combination of these variables were not included in the same model (see correlations in Figure 1 and 2, Appendix).

The following explanatory variables were included as a smooth term in the model selection process: OLA, daily range of T_a, daily mean relative humidity, daily mean precipitation, daily mean wind speed, daily mean solar radiation, and daily sum of activity (Table 1). Additionally, we included julian day in one of the models to test whether OLA was indeed a better predictor for T_v than the day of the year. We included the individual moose ("CollarID") as a random intercept in all models to control for inter-individual variability (Table 1, Appendix). Including this random effect improved the explained deviance greatly in comparison to when it was not included (72.3% vs. 20.6%, respectively).

Table 1. Summary of the response and explanatory variables included in the model selection process of a generalized additive mixed model to determine the factors which influence vaginal temperature in captive moose (n = 7) during the estrous cycle. M/s = meters per second. Mm = millimeters.

Variable				
Category	**Name**	**Type**	**Definition**	**Range**
Response	Daily_mean_Tv	Continuous	Daily mean vaginal temperature (°C)	37.5-38.2°C
Explanatory	OLA	Discrete	Number of days in relation to the onset of luteal activity	-21-20
Explanatory	yday	Discrete	Julian day	237-288
Explanatory	T_daily_range	Continuous	Daily range of ambient temperature (°C)	1.8-20.0
Explanatory	Daily_wind	Continuous	Daily mean windspeed (m/s)	0.39-2.80

Explanatory	RH_avg	Discrete	Relative humidity (%)	37.4-94.7
Explanatory	Prec_daily_avg	Continuous	Daily mean precipitation (mm)	0-17.8
Explanatory	Light_lux_mean	Continuous	Daily mean solar radiation (lux)	1215.5-42293.5
Explanatory	Daily_act3_sum	Continuous	Daily sum of activity	2623-17821

A gaussian distribution with an identity link function and the maximum likelihood (ML) were used. A maximum of four predictor variables were included in candidate models to avoid overfitting. We then used a model selection based on Akaike's Information Criterion (AIC, function "AICctab" from the "bbmle" R package (Bolker, 2017)), to select the highest ranked and most parsimonious model with $\Delta AIC \leq 2$. Residuals were assessed and basis dimensions for the parameter k were checked before we inspected diagnostic plots to validate model assumptions.

Relationship between luteal activity and collar activity

We also used GAMMs to determine when changes in daily sum of activity occur in relation to the onset of luteal activity. Daily sum of activity was selected as the response variable and OLA was included as the main explanatory variable to explore the relationship between luteal activity and activity values. We included the same environmental variables mentioned previously as they have been shown to affect behavior and habitat selection in moose (Thompson et al., 2021). In addition we included daily mean T_v as an explanatory variable since thermoregulation influences activity levels in moose (Thompson et al., 2021). We used a gamma distribution with a log link function and the maximum likelihood (ML) and applied the same model selection process as described above (Table 2, Appendix).

Detection of luteal activity based on vaginal temperature and collar activity

In moose for which an OLA was previously identified, we explored whether the model predictions could be applied as a tool to identify the onset of luteal activity by detecting changes in T_v and/or daily sum of activity.

Similar to what has been done in cattle, we set out to develop an algorithm that triggered an alarm when the daily mean T_v or daily sum of activity increased over a threshold (which was defined as the rolling mean of daily T_v or daily collar activity of n previous days, not including the current observation) by a set amount and specific minimum duration of days (Kyle et al., 1998; Redden et al., 1993). Based on the model predictions, a variety of criteria were tested to establish the best method to predict OLA in moose (Table 3A, Appendix).

We defined an OLA period as a period of x days around OLA and divided the rest of the fecal sampling period in non-OLA periods of x days so that each moose had the same number of periods. For each set of criteria, we tested a variety of x to get the most precise OLA prediction as possible.

Any OLA period during which one or more alarm were triggered was considered a single true-positive (TP) event and any OLA period during which no alarm occurred was considered a single false negative (FN) event. Similarly, any non-OLA period during which one or more alarms were triggered was considered a single false-positive (FP) event and any non-OLA period during which no alarm occurred was considered a single true-negative (TN) event.

We then calculated the following parameters to assess the ability of a set of criteria for a given OLA period definition to predict OLA based on T_v and activity: sensitivity (TP/TP+FN),

specificity (TN/TN+FP), predictive positive value (PPV = TP/TP+FP), predictive negative value (PNV = TN/TN+FN). Finally, we chose the combination that yielded the narrowest OLA period with the highest sensitivity and PPV, with a minimum value of 0.7 for both parameters.

We then ran the algorithm with the best combination (OLA period with the best set of criteria) over the entire period for which T_v (n = 11 moose) and activity (n = 12 moose) recordings were respectively available. If an alarm was triggered on consecutive days, these were assumed to be related to the same OLA period and counted as single event, starting on the date of the initial alarm.

Results

Classification of luteal activity based on fecal progestagen

Fecal progestagen metabolite measurements ranged from 2.2-68.3ng/g fecal matter (mean ±SD = 15.3 ng/g ±9.3 ng/g). The classification method to differ between luteal phase, inter-luteal phase and anestrus was able to identify a luteal phase and a date of OLA for 8 out of 12 individuals (Table 4, Appendix). All progestagen profiles showed some degree of fluctuation, but the remaining 4 individuals (three of these were 2 years old, and one was 19 years old) had more erratic patterns and did not successfully fulfill the set criteria to be able to identify an OLA and a coinciding luteal phase (Figure 9). The female moose for which we were not able to determine an OLA either had erratic fecal progestagen profiles (age = 2 years old; 44496, 44497 and 44498), fecal progestagen levels which did not stay above the set threshold for the minimum required days (44497), or an erratic fecal progestagen profile which stayed above the set threshold but was not preceded by a day in which the level was below the set

threshold (age = 19 years old; 30678). Thermal and activity registrations from these individuals were therefore excluded from the statistical models. The date for the OLA showed no significant interindividual variability (ANOVA, p-value = 0.585) with the mean date for OLA on the 23rd of September 2021 (SD = 4 days).

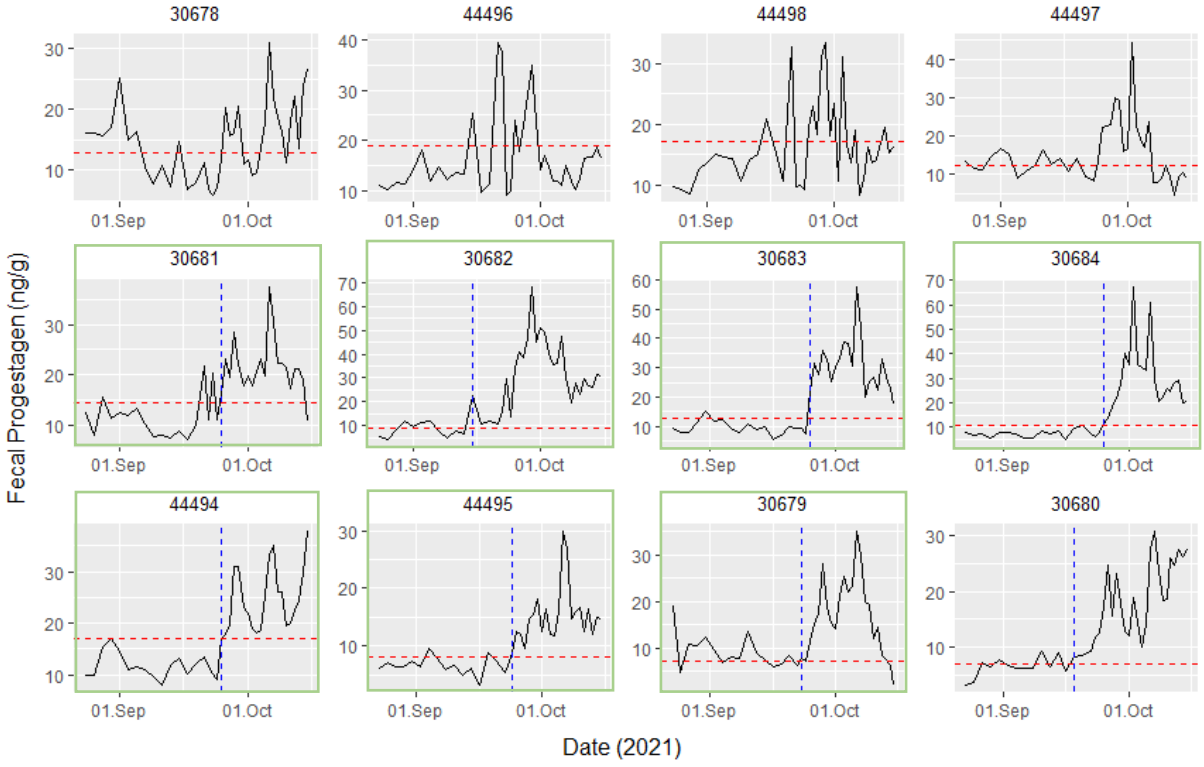

Figure 9. Fecal progestagen (ng/g) profiles from captive female moose (n = 12) from August to October 2021, at the Kenai Moose Research Center, AK, USA. The top row contains graphs from four moose (30678, 44496, 44498 and 44497) which did not fulfill the criteria for determining a date for the onset of luteal activity (OLA). The green outlined graphs indicate the 7 individuals which were included in the statistical models. Dashed red horizontal line = individual progestagen threshold. Dashed blue vertical line = date of OLA.

Vaginal temperature

Over 330,000 registrations of T_v were made between July and November 2021 for a total of 11 individuals, and over 150,000 of these occurring during the dates of fecal sampling (i.e., August 23rd to October 15th, 2021). Daily mean T_v was calculated, resulting in 594 registrations during this period, and ranged from 37.5°C to 38.6°C (mean ± SD = 37.9°C± 0.2°C). One of the 8 moose for which an OLA was detected did not have successfully stored

T_v registrations (30680) and was therefore not included in the statistical models. During the first few weeks (late August to September), daily mean T_v generally showed a decreasing trend in all individuals. From late September to early October characteristic, periodic changes in daily mean T_v were observed (i.e., a discernible increase followed by a decrease, Figure 10). Each moose had at least one such characteristic T_v pattern during the fecal sampling period. Additional patterns in T_v were observed for 8 individuals (30679, 30681, 30583, 30684, 44494, 44495, 44496 and 44498) for dates outside of the fecal sampling period. A total of 1 to 3 patterns per moose were observed during the whole period for which T_v registrations were available (July to November). The mean date for the earliest observed pattern in T_v was the 29th of September (SD = 15 days), and the mean date for the second and third observed pattern was the 18th of October (SD = 5 days) and the 13th of November (SD = 4 days), respectively. The interval between the first and second observed pattern in T_v was significantly shorter than the interval between the second and third observed pattern (paired T-test, p-value = 0.02, mean ± SD = 24 ± 2 days vs. 27 ± 2 days, respectively).

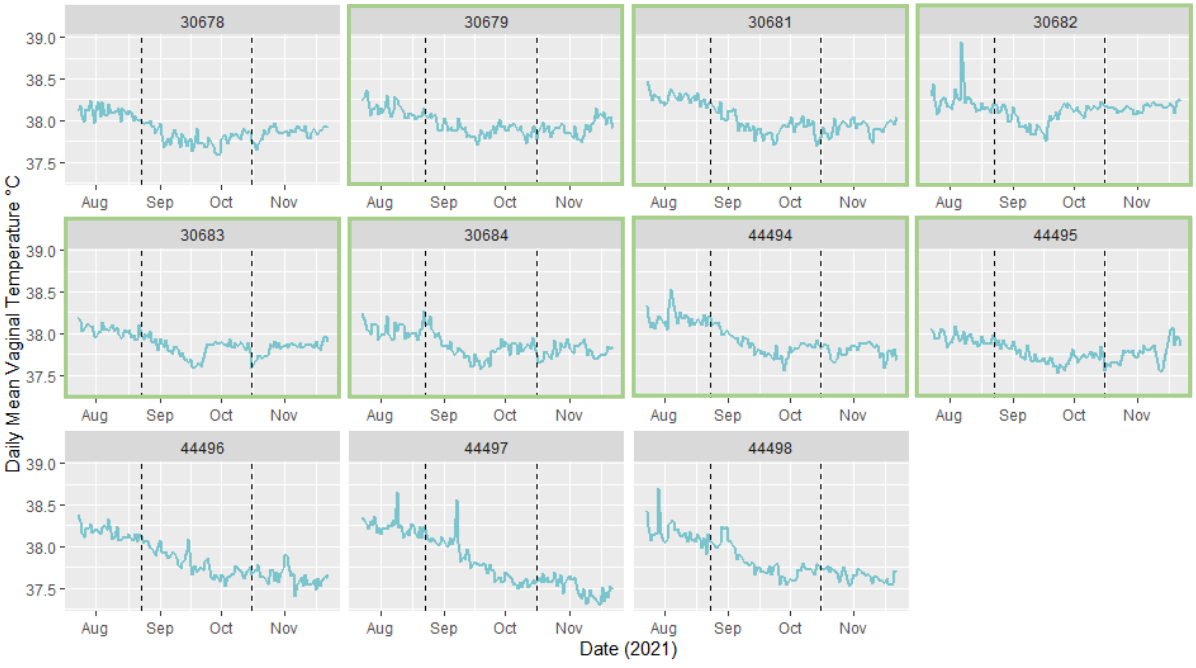

Figure 10. Daily mean vaginal temperature (°C) registrations from captive female moose (n = 11) between July and November 2021, at the Kenai Moose Research Center, AK, USA. The area between the two vertical dashed black lines in each graph indicates the dates for the fecal sampling period which took place from August 23rd to October 15th, 2021. The green outlined graphs indicate the 7 individuals which were included in the statistical models.

Activity

Activity registrations were successfully stored for all 12 individuals resulting in over 645,800 registrations for each axis (X, Y, and Z) between May and November 2021, with 185,000 registered during the period of fecal sampling (i.e., August 23rd to October 15th, 2021). Daily sum of activity during the fecal sampling period ranged between 1427 to 21,042 (mean ± SD = 7901 ± 3489) and consisted of 234 to 288 registrations a day. There was a general decline in activity from May towards November (shown from late July in Figure 11). This pattern was more obvious for the three youngest individuals (2 years old; 44496, 44497 and 44498) and they generally had slightly higher activity levels between May and September. There were only two moose with discernible but relatively small peaks of activity during the fecal sampling period (30679 and 30680, on the 13th and the 1st of October, respectively). However, a total of nine moose (30679, 30680, 30681, 30683, 30684, 44494, 44495, 44496, and 44498) had one or two more obvious activity peaks from mid-October until the end of activity registrations in November, which were found 27 ± 1 day (mean ± SD) apart from each other. The mean date for when the first and second most obvious peaks in activity occurred was the 18th of October (SD = 8 days) and the 12th of November (SD = 9 days), respectively.

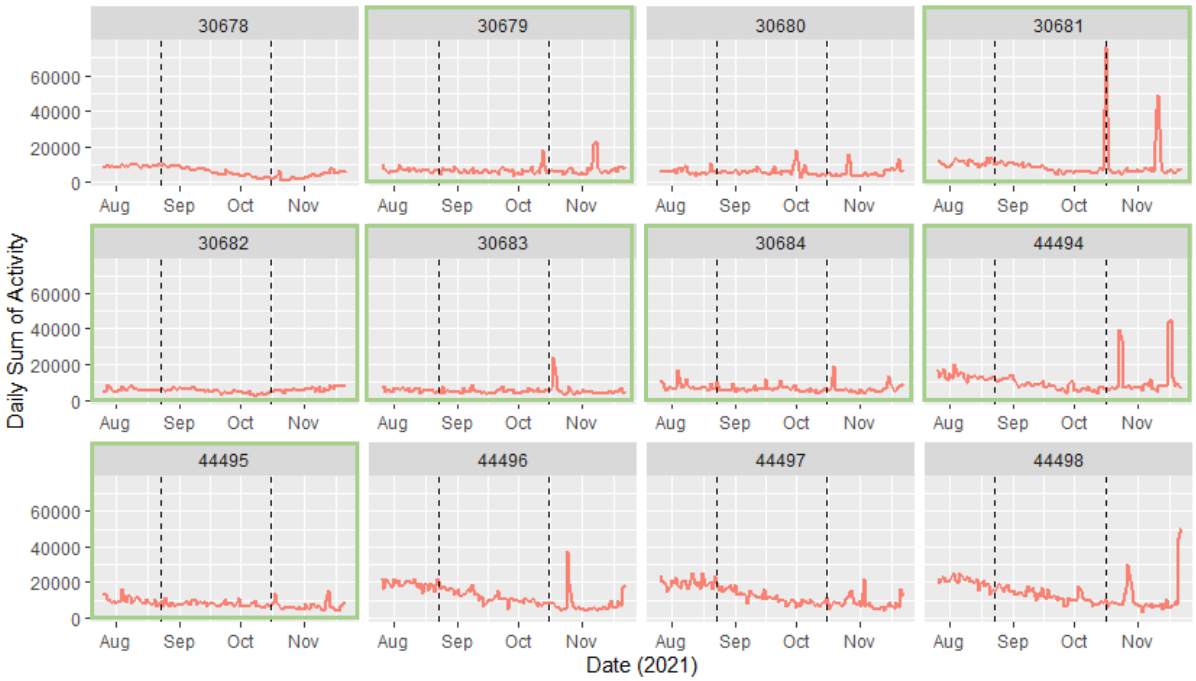

Figure 11. Daily activity registrations from captive female moose (n = 12) between July and November 2021, at the Kenai Moose Research Center, AK, USA. The area between the two vertical dashed black lines in each graph indicates the dates for the fecal sampling period which took place from August 23rd to October 15th, 2021. The green outlined graphs indicate the 7 individuals which were included in the statistical models. Daily sum of activity = daily sum of X-, Y- and Z-axis values registered on the GPS-collar.

Characteristic thermal and activity patterns

For the 7 moose which had T_v, activity and an OLA available during the fecal sampling period, the general observation was that T_v started to increase a few days before or after day 0 of OLA (ranging from 7 days before OLA and 3 days after OLA), whereas activity values did not appear to show as much of a distinct pattern around this time (Figure 12). Activity peaks were 1.4 to 2.7 times greater (mean ± SD = 1.8 ± 0.4) than the individual mean of activity values during the fecal sampling period.

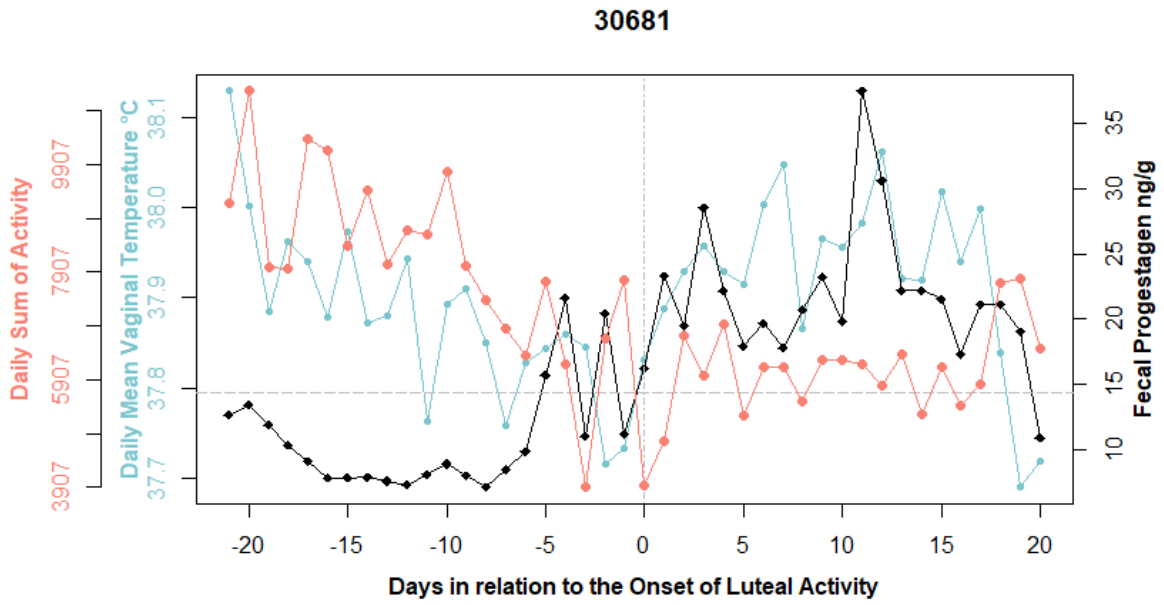

Figure 12. Graph from a 9-year-old captive female moose (individual 30681) containing daily values of mean vaginal temperature (blue line), sum of activity (red line) and fecal progestagen levels (black line) between August and October 2021. Temperature and activity values can be read on the left y-axes, and fecal progestagen levels can be read on the right y-axis. The x-axis shows days in relation to the onset of luteal activity (OLA). Dashed vertical gray line = day 0 for OLA. Dashed horizontal gray line = fecal progestagen threshold associated with luteal activity for this individual (14.3 ng/g).

When daily mean T_v and daily sum of activity were plotted against dates exceeding the period of fecal sample collection (i.e., after October 15th), we observed that a drop in T_v would periodically align with a peak in activity. The peak in activity was generally seen on the same day as the drop in T_v, or one to two days after, with an interval of 27 ± 2 days (mean ± SD, Figure 13). Activity peaks were 2.3 to 6.8 times greater (mean ± SD = 4.0 ± 1.4) than the individual mean of activity values for this time period, which was significantly higher than for the previous period with fecal sample collection (paired T-test, p-value =

0.002).

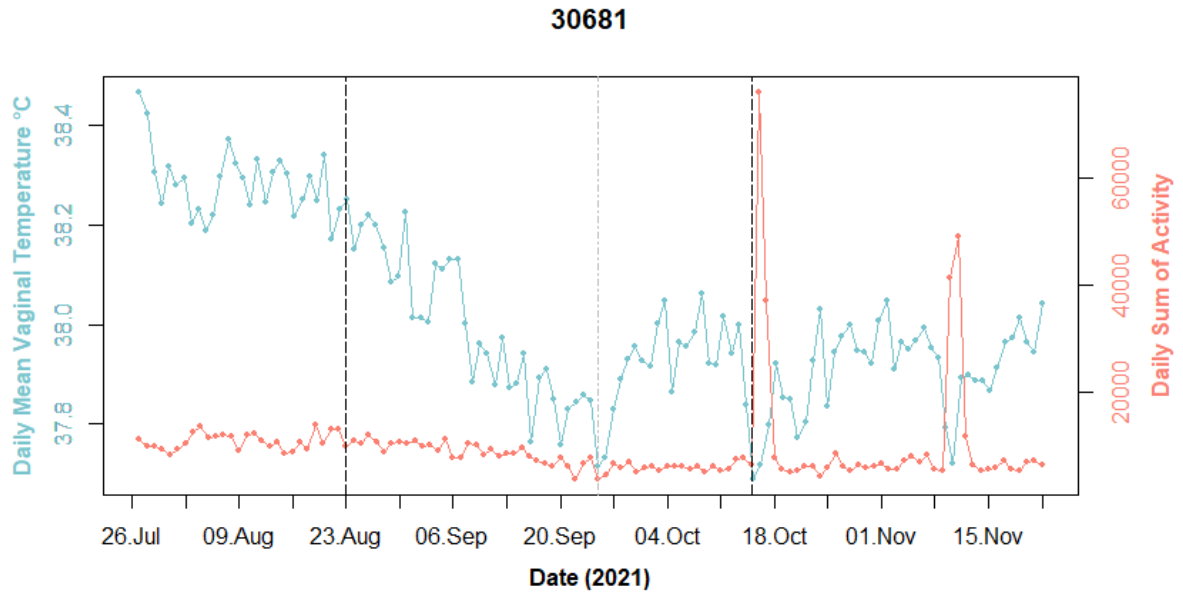

Figure 13. Graph from a 9-year-old captive female moose (individual 30681) containing daily values for mean vaginal temperature (blue line) and daily sum of activity (red line) recorded between July and November 2021. The area between the two vertical dashed black lines in each graph indicates the dates of the fecal sampling period which took place from August 23rd to October 15th, 2021. Dashed vertical gray line = day 0 for OLA, which is on the 25th of September for this individual.

Relationship between luteal activity and vaginal temperature

In the end we included registrations from 7 moose which all had T_v, activity and an OLA available to be included in the statistical models. The top model for predicting T_v only included the variable OLA and explained 72.3% of the deviance (Table 5 and 6, Appendix). It predicted that T_v dropped by 0.2°C over a 17-day period, before stabilizing at 37.8°C (with a 95% confidence interval (CI) of 37.7-37.8°C) 5 days before OLA (Figure 14). Subsequently, T_v increased by 0.2°C over a second 17 day-period before entering a new decreasing phase after reaching 37.9°C (95% CI = 37.9-38.0°C) 12 days after OLA (Figure 14).

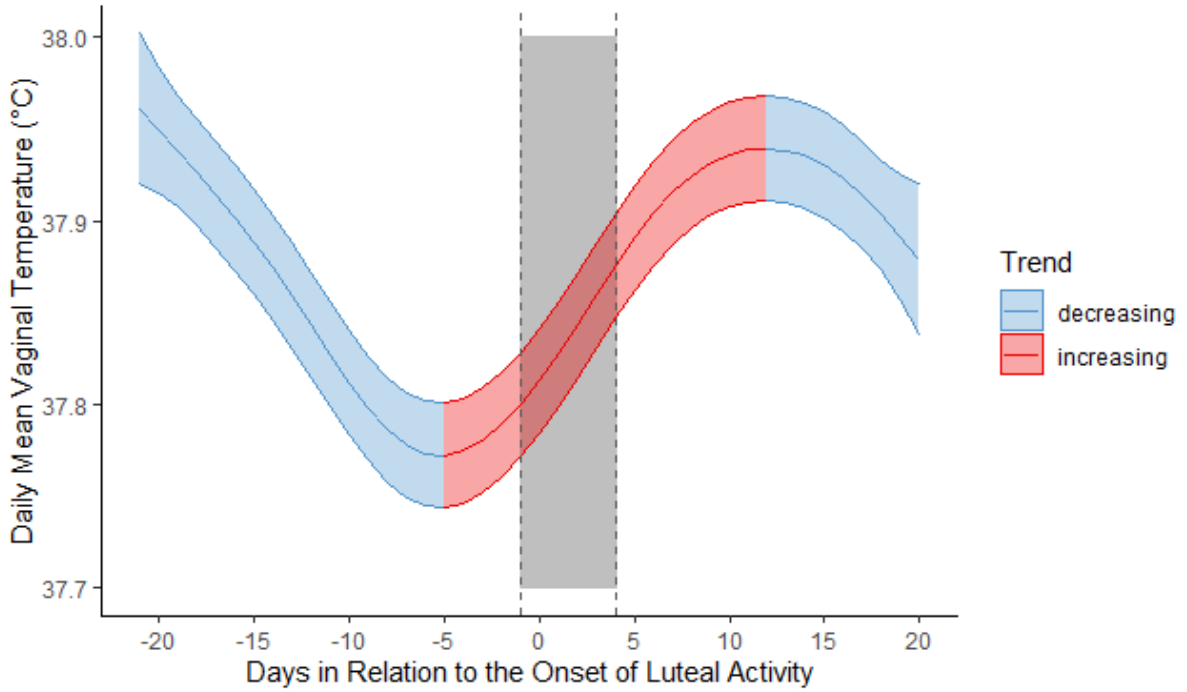

Figure 14. Predicted values of a generalized additive mixed model predicting daily mean vaginal temperature (T_v; °C) from captive female moose plotted against days in relation to the onset of luteal activity (OLA). Solid central line = the predicted daily mean vaginal temperature over time. Shaded colored area = 95% CI. Blue = decreasing trend. Red = increasing trend. Gray shaded area = the OLA period that was used in the final algorithm to identify OLA based on daily mean T_v (between -1 to +4 days in relation to OLA).

Relationship between luteal activity and collar activity

The selected top model for predicting activity included the explanatory variables OLA and daily mean precipitation (Table 7 and 8, Appendix) and explained 38.9% of the deviance. It predicted that activity decreased by 1373.5 from 15 days before OLA (95% CI = 6655.8-7868.3) to 11 days after OLA (95% CI = 5392.0-6375.5, Figure 15), and then increased by 755.7 from 11 days after OLA to 20 days after OLA (95% CI = 5891.0-7436.7). Additionally, when daily mean precipitation increased from 0 mm to 17.8 mm, activity increased from 6418.3 (95% CI = 5902.4-6979.4), to 7554.3 (95% CI = 6579.3-8673.8).

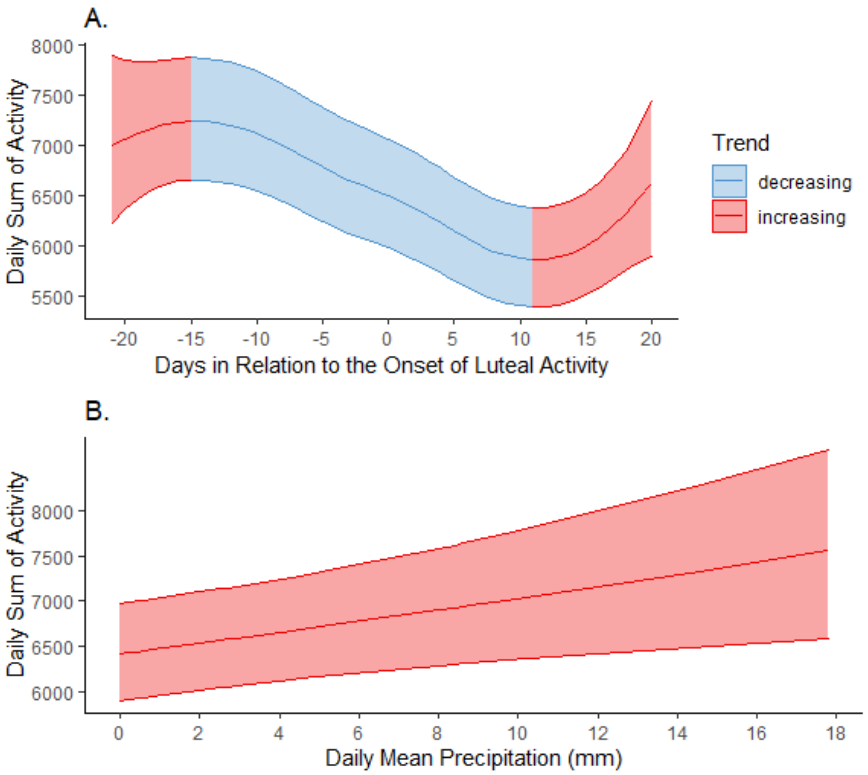

Figure 15. Predicted values for the daily sum of activity from captive female moose plotted against days in relation to the onset of luteal activity (A) and daily mean precipitation (B). Solid central line = predicted daily sum of activity over time. Shaded area = 95% CI. Blue = decreasing trend. Red = increasing trend.

Detection of luteal activity based on vaginal temperature and collar activity

According to the GAMM with daily sum of activity as a response variable, activity did not appear to be a particularly precise parameter for predicting OLA. Therefore, we only developed an algorithm for identifying OLA based on daily mean T_v.

The period in relation to OLA which was available for all moose was [-21; 20] days around OLA. Based on our model predictions with T_v as a response variable (i.e., 0.2°C increase in T_v over 17 days, from 5 days before to 12 days after OLA), we tested different sets of parameters that are shown in Table 3B (Appendix).

The optimal combination of set criteria for triggering an alarm for OLA based on T_v changes was an increase in daily mean T_v which was above the rolling mean calculated over the 6 previous days (not including the current one) with a duration of at least 7 days and an OLA period comprised from 1 day before to less than 4 days after the OLA. Consequently, our final algorithm predicts an OLA to occur with a precision of 5 days (i.e., an OLA is predicted to occur from 1 day after and less than 4 days before the day of an alarm based on an increase in T_v, Figure 16). This combination yielded a sensitivity of 0.71, a specificity of 0.93, a PPV of 0.63 and a PNV of 0.96.

When running the final algorithm over the entire period during which T_v registrations were available for all 11 moose (from the 26th of July to the 22nd of November 2021), the alarm was triggered once or twice per individual with a distance between each trigger ranging from 19 to 53 days (38 ± 13 days = mean ± SD, figure 16). The algorithm triggered a total of four T_v alarms indicating an OLA period for the moose which we did not identify an OLA for based on fecal progestagen profiles (two alarms for 30678, and one alarm each for 44496, 44497 and 44498). Three of the triggered T_v alarms occurred during the fecal sampling period but less than 14 days before the end of the fecal collections (one on the 1st of October for 30678 and two on the 4th of October: one for 44496 and one 44497). Thus, we could not determine whether these alarms were connected to an OLA period since the duration of fecal progestagen data was too short to apply our classification method.

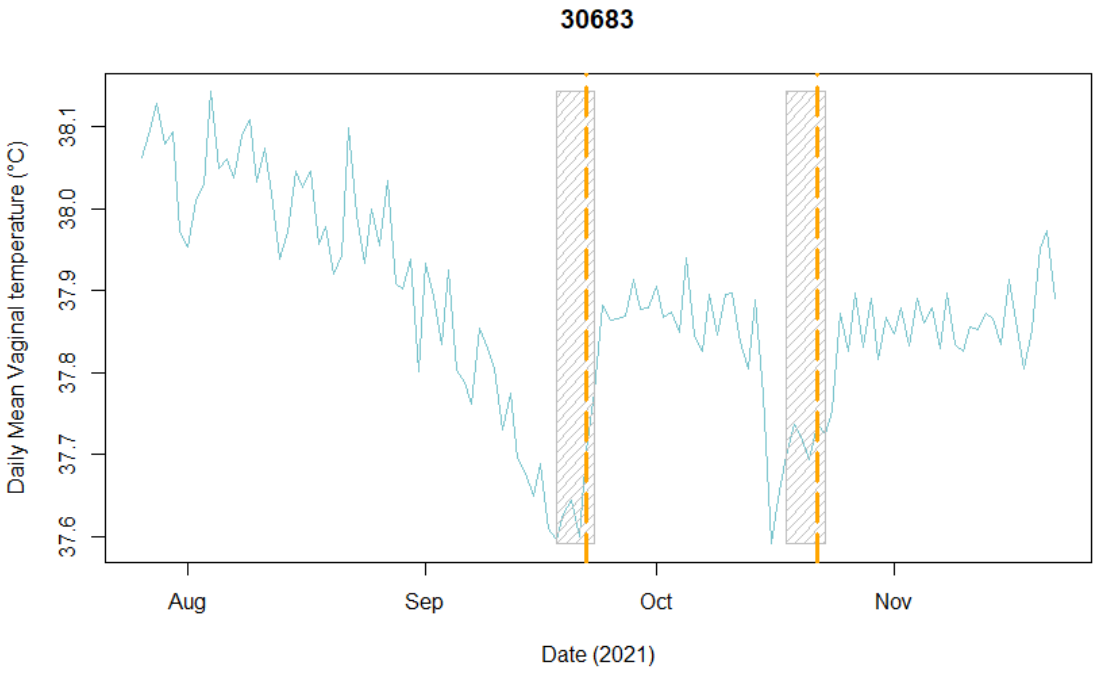

Figure 16. Example of the detected OLA periods for a captive female moose (30683, 12 years old) from July to November 2021. The days on which the vaginal temperature (T_v; °C) alarms were triggered are indicated as yellow dashed vertical lines. Gray, dashed rectangles = the corresponding 5-day periods during which OLA is predicted to occur (from 1 day after and less than 4 days before the day of a T_v alarm).

Discussion

Currently, there are still several aspects regarding the estrous cycle of moose which are yet to be described. The goal of this study was to gain better insights about the moose estrous cycle and investigate a novel approach to monitor luteal activity based on biologging technology. We observed clear, periodic patterns in T_v from mid-September and into November in prime-aged moose, which align well with the previously reported duration of the estrous cycle in moose (Schwartz & Hundertmark, 1993). Clear patterns in collar activity were visible in October and November but were less clear or absent in August and September, when fecal progestagen data was available. Based on data from the presumed first estrous cycle of the breeding season, we found that daily mean T_v could be used to predict the onset of luteal

activity in moose, similar to what has been shown in domestic cattle (Kyle et al., 1998; Redden et al., 1993). This was not the case for detecting the presumed first estrous cycle with the daily sum of activity. Our findings support previous literature on the reproductive characteristics of moose and provide new perspectives on physiological parameters during the breeding season and their relation to the estrous cycle.

Classification of luteal activity based on fecal progestagen

All progestagen profiles showed some degree of fluctuation, which is likely due to both steroid metabolite instability due to the chosen sample medium and actual fluctuations in corpus luteal activity (Palme et al., 1996; Schwarzenberger, F. et al., 1996a). Similar patterns have been seen in progestagen profiles during the estrous cycle and pregnancy for several wild ruminant species including sable antelope (*Hippotragus niger*), brown brocket deer (*Mazama gouazoubira*), reticulated giraffe (*Giraffa camelopardalis reticulata*), and scimitar-horned oryx (*Oryx dammah*) (Dumonceaux et al., 2006; Morrow et al., 1999; Pereira et al., 2006; Thompson et al., 1998). Fluctuating patterns could also be a demonstration of luteal activity in a transitional period from anestrus to estrus, which has been described for seasonal breeders such as sheep, where progesterone levels increase prior to the first ovulation of the breeding season and before the onset of full-length estrous cycles (Bartlewski et al., 1999; Ravindra & Rawlings, 1997).

Since our study period lasted for 54 days, combined with the fact that cycle length in moose is estimated to last between 22-28 days (Schwartz & Hundertmark, 1993), we anticipated that we would catch at least one estrous cycle per moose. Due to the early timing of our sampling period (i.e., starting date on the 23rd of August) we likely captured the first estrous cycles of the season, as cycling in moose has been reported to occur between September and November

(Ballenberghe & Miquelle, 1993; Garel et al., 2009; Malmsten et al., 2014; Schwartz & Hundertmark, 1993; Sigouin et al., 1997). This assumption is strengthened by investigating the measured fecal progestagen levels during the first half of the sampling period (i.e., from the 23rd of August until mid-September). During this time frame, progestagen levels stayed below or near the individually calculated threshold and thus deviated from the expected periodic pattern that is associated with multiple estrous cycles in captive moose (Schwartz et al., 1995).

Monitoring progesterone concentrations alone is not considered sufficient to predict the specific timing of ovulation in cattle (Roelofs et al., 2006). Therefore, without a method to specifically determine whether the study animals demonstrated signs of estrus, like observing overt behavioral cues such as copulation with a bull (Schwartz et al., 1995), measuring additional gonadal steroid hormones, or examining the ovaries through transrectal palpation and/or ultrasonography (Bartlewski et al., 1999; Hanzen et al., 2000), we have taken care not to draw too many assumptions regarding estrus or ovulation, but rather identify when individual fecal progestagen profiles were indicative of luteal activity.

We were able to classify the luteal phase of the estrous cycle in moose based on fecal progestagen values. Because the duration of luteal phase is currently unknown for moose, we based our OLA classification method on existing approaches for classifying normal luteal activity in cattle, where progesterone levels are required to be below a set threshold for less than 7 days, followed by staying above the set threshold for more than 14 days (Martin et al., 2010). The duration for which progesterone levels are required to stay above the set threshold also corresponds to the average luteal phase length in other seasonal breeding ruminants such

as marsh deer (*Blastocerus dichotomus*), sheep, and goats (15, 14 and 16 days, respectively, Polegato et al. (2018), Brown et al. (2014), Fatet et al. (2011)).

We found that the timing of OLA was not significantly different between individuals, which supports the theory that female moose are to a certain degree synchronized, in respect to estrous cyclicity (Garel et al., 2009; Schwartz et al., 1995). In Scandinavia, 95% of ovulations in wild moose have been reported to occur within an interval of less than 10 days from late September to early October (Garel et al., 2009). On a global scale, most of mating events in moose have been reported to occur over a 15-day period, from the 23rd of September to the 8th of October (Sigouin et al., 1997). The mean date of OLA in our study animals was the 23rd of September (range = 15th to the 25th of September, i.e., 10 days), which corresponds well with previously reported dates for the estrous cycle and breeding season in moose (from 24th of September to the 8th of October: Ballenberghe and Miquelle (1993), from the 23rd of September to the 8th of October: Sigouin et al. (1997), from the 28th of September to the 27th of October: Schwartz and Hundertmark (1993), from end of September to early October: Garel et al. (2009), and from mid-September to early November: Malmsten et al. (2014)).

Thompson et al. (2019) estimated the mean conception date in wild Alaskan moose to be on the 2nd of October, based on a 231-day gestation length and subtracting this duration from the dates of when VITs were expelled from reproductive tracts on the day of parturition. Similarly, Ballenberghe and Miquelle (1993) reported that the earliest observed mating of wild Alaskan moose occurred on the 24th of September, with the highest count of mating observations occurring during the first week of October before markedly declining. The 24th of September coincides well with the dates for OLA in our study animals (i.e., 23rd of

September ± 3 days = mean ± SD), which is likely also the date of their initial estrous cycle that season.

Furthermore, we observed that females in Pen 2 (30679, 30683, 30684 and 44495) showed interest in and allowed bulls in Pen 1 to approach them by the fence between the 22nd and the 28th of September, which is within 2 to 3 days of the calculated dates for OLA (the 24th of September for 30683, and the 25th of September for 30679, 30684 and 44495). This adds confidence to our assumption that the dates we calculated for OLA are related to the estrous cycle and ovulation which is required to occur before corpus luteum formation and following luteal activity (Noakes, 2001).

Malmsten et al. (2014) found that estrous cycles of nulliparous moose occurred later in the breeding season than estrous cycles of parous moose. Of the individuals we were able to determine OLA for, only one was a nulliparous 9-year-old (30684), with an OLA that occurred within two standard deviations of the mean OLA date (23rd of September ± 3 days). However, our study population was too small and unequally distributed to compare the onset and length of luteal phase between nulliparous and parous females. Besides, it is rather unlikely that a 9-year-old female would be nulliparous in the wild, as Sand and Cederlund (1996) reported that 99.9% of wild Swedish moose over 6 years old showed signs of earlier pregnancy (i.e., size and structure of the uterus, only one 12-year-old of 718 females in this age-group showed no signs of earlier pregnancies).

Similarly, Garel et al. (2009) reported that young (<2.5 years old) and old (≥11.5 years old) individuals tend to ovulate later during the breeding season than prime-aged (2.5-10.5 years old) individuals. Although timing of OLA was not significantly different in our dataset, we

observed that the four individuals which were older than 11.5 years old had an OLA which occurred earlier and had a higher variability than what was observed for prime-aged females (mean ± SD = 20th of September ± 5 days vs. 25th of September ± 1 day, respectively). We were not able to define an OLA for the individuals that were less than 2.5 years old in our study and we could therefore not test this group.

The abnormal and/or short fecal progestagen patterns observed in female moose for which we were not able to determine an OLA (4446, 44497, 44498 and 30678) may be due to or influenced by the transitional period from anestrus to estrus. Following seasonal anestrus in sheep, short luteal phases have been hypothesized to be caused by short-lifespan corpora lutea which are unable to produce sufficient amounts of progesterone (Philippe et al., 2006). This hypothesis may also explain the patterns observed in our study.

Age could also be a contributing factor to the observed abnormal progestagen patterns, as these were identified in data from moose that were either 2 (44496, 44497 and 44498) or 19 years old (30678), while the moose we were able to calculate an OLA for were between 9-18 years old. It has been shown that middle aged cattle (in their second to fifth lactation, i.e., from about 3 to 6 years old) have a higher incidence of abnormal ovarian function when compared to young (first lactation, i.e., between 2 to 3 years old) and old (sixth lactation onwards, i.e., above 7 years old) (Claire Bulman & Wood, 1980). In contrast, our results showed that the youngest and oldest individuals had irregular fecal progestagen profiles. Ericsson et al. (2001) found that senescence (i.e., when litter size decreases) in wild Swedish moose occurs after 11 years of age, with 15 years of age considered to be the age of last reproduction. This could explain why the 19-year-old individual had an irregular fecal progestagen profile for which we did not identify any OLA. In addition, it has been reported

that ovulation rate is usually lower, more variable, and occurs later for moose younger than 2.5 years old (Garel et al., 2009), which could explain why our fecal collection period did not identify an OLA for the three 2-year-old moose in our study.

Characteristic thermal and activity patterns

Expectations for identifiable patterns in daily mean T_v and daily sum of activity in relation to fecal progestagen and luteal activity were met to a certain degree during the fecal sampling period. An identifiable pattern was observed for daily mean T_v, which generally increased around the same time as fecal progestagen did. There were fewer and less clear identifiable patterns (i.e., smaller peaks) observed for the daily sum of activity during the fecal sampling period. However, for dates exceeding the fecal sampling period we observed clear, coinciding patterns of daily sum of activity and daily mean T_v, which resembles the typical pattern that has been described for cattle (i.e., a peak in activity when T_v and progesterone are low and about to increase, Lewis and Newman (1984)). As our statistical models were based on data collected during the fecal sampling period, we were able to predict the first luteal activity of the breeding season by changes in T_v, but not activity. However, the observed subsequent, coinciding patterns in both parameters indicate that an algorithm for detecting subsequent luteal activity in the breeding season would likely benefit from including both T_v and activity.

As fecal progestagen values were not available for the dates when both activity and T_v patterns were more obvious and coinciding, we suspect that the values registered during the fecal sampling period might be less optimal to include in a statistical model to explore their relationship to subsequent luteal activity and base predictions on.

As our fecal collection period likely caught the first luteal activity of the breeding season, a possible explanation for less clear patterns could be that the initial luteal activity after a period of anestrus might not be preceded by the expected behavioral estrus and associated changes in vaginal temperature and activity. After a period of anestrus, normal estrous behavior has been reported to occur only at the end of the first estrous cycle in ewes (Ravindra & Rawlings, 1997). This can be termed a "silent" cycle, which refers to how the first postpartum cycle is often not accompanied by overt estrous behavior (Gonzalez et al., 1987). Since an appropriate balance of ovarian hormones are required for the expression of estrus (Karsch et al., 1980), perhaps ovarian hormone levels during the transitional period are too variable to stimulate a more intense expression of thermal and activity patterns associated with estrus until the subsequent cycle.

In years where breeding is desired, female moose at the Kenai Moose Research Center share a pen with a male from mid-September to mid-October to allow breeding, with most parturitions occurring between the 3rd of May and the 4th of June the following year (personal observations, Dan P. Thompson, and John A. Crouse). Based on a gestational length of 231 ± 5 days (mean ± SD, range = 216-240) as suggested by Schwartz and Hundertmark (1993), these parturition dates coincide well with the same study's range of dates for when the first overt behavioral estrus has been documented to occur in captive moose (28th of September to the 12th of October). However, we cannot confirm whether the first overt behavioral estrus in captive moose coincides with the first ovulation of the breeding season, or if signs of estrus are only observed or more obvious in the subsequent cycles, when behavioral estrus may be more prominent.

It has been hypothesized that sex pheromones produced by bull moose likely play an important role in stimulating the onset of estrus and ovulation in moose cows (Miquelle, 1991; Schwartz et al., 1990). The study animals were kept in enclosures that were near captive bulls and exposed to wild bulls in the area, but physical contact was limited by fences. This could therefore potentially have affected timing of sexual receptivity and ovarian activity of the moose in our study. It could be that the physical separation from bulls leads to a weaker expression of estrous behavior, meaning that potential indicative parameters (e.g., body temperature and activity) may not be as clear.

When exploring temperature and activity registrations which occurred after the fecal sampling period, we found a periodic pattern of a peak in activity around the same time that T_v dropped and was about to rise. The interval of this pattern (about 27 days) aligns well with the expected duration of the estrous cycle in moose (22-28 days, Schwartz and Hundertmark (1993)). Moreover, we found that the interval length between the first two T_v patterns (between September and October) was significantly shorter than the following two (between October and November). If the interval between observed T_v patterns reflects the length of the estrous cycle in moose as is reported for cattle (Piccione et al., 2003), it is possible that the initial estrous cycle of a breeding season may be shorter than the following ones, similar to other seasonal breeders (Camp et al., 1983; Gonzalez et al., 1987; I'Anson & Legan, 1988). It has been suggested for ewes that this is because the first LH-surge after a period of acyclicity may not be sufficient to initiate a full-length estrous cycle (I'Anson & Legan, 1988), but that the following increase in progesterone ensures that the next LH-surge can (Legan et al., 1985).

Relationship between luteal activity and vaginal temperature

The high explained deviance (72.3%) of the model with daily mean T_v as a response variable supports that there is a relationship between luteal activity and the identifiable pattern which was observed for T_v. According to our model predictions, the rise in collar activity occurs 17 days after the predicted rise in T_v. Despite that the model with activity as a response variable had a lower explained deviance, this indicates that the elevation in T_v is likely not associated with an increase in activity but rather an increase in progesterone hormone, which is known for its thermogenic effects (de Mouzon et al., 1984).

Despite a high explained deviance, it appeared difficult to generalize the predictions of our model as some moose exhibited a rise in T_v either before or after OLA (range = 7 days before to 3 days after OLA), whereas our final model predicted that T_v would rise 5 days before OLA. As T_v patterns in relation to the rise in fecal progestagen and OLA were inconsistent within our study population, we were unable to identify a potential pattern of delay between a rise in T_v and fecal progestagen values. This is in reference to a 12-24-hour lag-time between plasma progesterone and fecal progesterone metabolites which has been documented in domestic ruminants and likely caused by factors such as the thermogenic effect of progesterone and an increased vaginal blood flow (Abrams et al., 1973; Palme et al., 1996; Schwarzenberger, F. et al., 1996b; Suthar et al., 2012).

Relationship between luteal activity and collar activity

The low explained deviance of our final model (38.9%) including OLA and daily mean precipitation indicates that activity is not a great predictor for the onset of luteal activity in captive moose during the first estrous cycle of the season. Redden et al. (1993) found that T_v and activity monitoring yielded similar estrus detection rates in cattle (81% and 80%,

respectively) and some authors have even found that monitoring activity can identify low-intensity estrous behavior which would otherwise be labeled as "silent ovulation" (Shipka, 2000). However, other authors have found that monitoring temperature can yield better detection rates than monitoring activity (Sakatani et al., 2016; Walton & King, 1986), which also seems to be the case in our study. Perhaps activity would have been a good predictor for the subsequent estrous cycles if we had been able to include the activity data beyond the fecal collection period, when significantly higher activity peaks were observed.

Another aspect to consider is the difficulty of setting a threshold or definition for when activity values should be regarded as high, low, or associated with certain behaviors, as specific behaviors and their relationship to activity values have not yet been validated for moose. Also, in order to match the fecal sampling frequency, the activity values included in the model were summed to daily values, meaning that higher resolution variations within a day were probably lost. These uncertainties made it challenging to include collar activity data in a statistical model and interpret the results. To increase the interpretability and application of collar activity, future studies should investigate the relationship between accelerometry registrations and behavioral observations in moose, similar to what has been done for both domestic (Alvarenga et al., 2016; Vázquez Diosdado et al., 2015) and wild ruminants (Heurich et al., 2012; Löttker et al., 2009).

Detection of luteal activity based on vaginal temperature

Our algorithm for detecting luteal activity based on T_v was able to identify 71% of OLAs during the first estrous cycle, which is lower than what has been previously reported for detection of estrus in cows (81% for Redden et al., 1993 and 89.4% for Kyle et al., 1998). A relevant difference between such algorithms for detecting estrus in cows and our algorithm is

that they are based on hourly values for T_v and activity, instead of lower resolution daily values. In addition, our algorithm returned a low number of false positives (3 out of 49 non-OLA periods) but a relatively high number of false negatives (2 out of 7 OLA periods). The combination of these factors explains the relatively long interval which was observed between two detected OLAs for some moose (over 28 days for 5 out of 7 moose) by the algorithm over the entire period for which T_v was recorded (i.e., between July and November). Another explanation could be that thermal patterns were more moderate during the first cycle compared to the following cycles. Because of this, an algorithm based on registrations from the first estrous cycle might not contain the most relevant range in parameters for detecting subsequent OLAs.

Because our variables were of a daily frequency (instead of hourly) and since the timing of the rise in T_v in relation to OLA was not consistent among moose, the detected OLA period based on T_v in our final algorithm was relatively broad (5 days) when compared to similar alarms in cattle which can predict estrus to occur on the same day (Kyle et al., 1998; Redden et al., 1993). Additionally, studies in cattle have been based on a higher sample size and multiple individual estrous cycles (n = 21 with a total of 47 cycles, Kyle et al. (1998) and n = 10 with a total of 26 cycles, Redden et al. (1993)), which ultimately yields a narrower confidence interval. However, our final algorithm is the first reported attempt at offering a specific method of detecting ovarian activity using biologging devices in captive moose. It can therefore be argued that a highly precise method for predicting ovarian activity is not as crucial in captive or wild moose as it is for cattle, in which artificial insemination plays a major role (Smith et al., 2018). A possible use of our algorithm could be an estimation of estrus and conception dates in free-ranging populations as part of conservation or management studies.

When we ran the algorithm on T_v registrations from moose which we were not able to classify an OLA for based on fecal progestagen profiles (n = 4), it triggered a total of four alarms based on an increase in T_v. Three of these were triggered during the fecal sampling period (one on the 1st of October for 30678 and two on the 4th of October for 44496 and 44497) and one afterwards (20th of October for 30678). Since these alarms occurred at the end of the fecal sampling period, and our classification method for OLA required that fecal progestagen level stayed above the individual threshold for a minimum of 14 days, we could not confirm whether these alarms were associated with an OLA or not. An additional month of fecal collection would have enabled us to do so. If we consider that these T_v alarms were associated with OLAs, the 19-year-old individual (30678) had an estrous cycle of 19 days (interval between the first and second trigger of the alarm, which was triggered on the 1st, and the 20th of October, respectively) and the two 2-year-old individuals (44496 and 44497) started an estrous cycle on the 4th of October. This could further support that young individuals tend to ovulate later during the breeding season (Garel et al., 2009).

Limitations

We were able to validate the accuracy of temperature registrations for all but one VIT which we included data from in our study. Prior to publishing our results in a scientific journal, a second validation procedure is planned to be performed on all VITs that were used in this study to confirm that all our temperature registrations were within the same accuracy.

Despite our efforts to collect fecal pellets from multiple fractions of excreted feces from an individual in addition to thorough homogenization, our subsamples may not be entirely representative of serum progesterone levels since steroid metabolites are known to be

unevenly distributed in fecal pellets (Millspaugh & Washburn, 2003; Tanaka et al., 2019; Wasser et al., 1996).

Additionally, the concentration of fecal progestagen in our study was considerably lower than what has been reported for moose during their estrous cycle previously (i.e., 0.3-7 μg/g (Schwartz et al., 1995) compared to 0.002-0.07μg/g (2-70ng/g) in our study). However, as absolute values of progesterone metabolites may vary considerably between assays, it makes it less feasible to compare these values across studies (Schwarzenberger, Franz et al., 1996).

Nevertheless, validation of all protocols involved in the fecal sampling and analysis is crucial and would have improved our ability to select the most accurate methods and interpret their results (Touma & Palme, 2005). Because we lacked information about the cross-reactivity of our immunoassay, it is difficult to determine the validity of this method to measure progesterone metabolites in feces as it could include the concentration of other steroid metabolites that are not directly associated with the luteal phase of the estrous cycle. A more specific characterization of the radiolabeled antibody and antigen used in our assay along with validation of this assay method in moose would give a better indication of whether the selected approach is suitable for this species. (Möstl et al., 2005; Palme et al., 1996; Touma & Palme, 2005).

As our study was based on fecal progestagen levels, we could only identify luteal activity and were therefore unable to pinpoint a more specific timing of estrus or ovulation. However, the characteristic thermal patterns that were visible in our dataset are likely associated with ovulation as seen in domestic ruminants, where a decrease in T_v and an increase in activity occurs prior to ovulation and is followed by a postovulatory increase in T_v (Suthar et al.,

2011; Wrenn et al., 1958). Future studies should include more specific methods to be able to estimate the timing of estrus and ovulation in captive moose, such as registration of behavioral cues, either directly through observation or indirectly by using devices that detect when a female is mounted (Alhamada et al., 2016). Another strategy could be the application of remote blood collection technology (Fønss & Munksgaard, 2008; Ingram et al., 1994; Säkkinen et al., 2004; Voigt et al., 2004) in captive moose to directly measure levels of hormones associated with the estrous cycle (i.e., progesterone, estradiol, LH and FSH, Ravindra and Rawlings (1997)). Measuring LH in saliva was recently validated for detecting estrus in Murrah buffalo (*Bubalus bubalis*; Srinivasan et al. (2020)), which may be a relevant method to apply in other ruminants such as captive moose.

Furthermore, we observed that the mean date for clear patterns in both T_v and activity was not observed until the 18th of October. Based on this, along with fecal progestagen values indicating that the first estrous cycle occurred around the 23rd of September, an additional month of fecal sampling would have enabled us to look at how patterns in T_v and collar activity may be associated with luteal activity in the following estrous cycles. Also, it would have been beneficial to be able to register multiple estrous cycles to be able to classify the inter-luteal phase and better describe and compare estrous cycle phases within and between individuals.

Conclusion

Based on fecal progestagen levels, we were able to classify the luteal phase of the estrous cycle and estimate that the mean date for the onset of luteal activity in captive moose was the 23rd of September. An associated T_v increase was identified around the same time and can be used to detect the initial onset of luteal activity in a breeding season. We did not observe a

similar, identifiable pattern in collar activity related to luteal activity. However, subsequent, recurring patterns in both T_v and collar activity were identified between mid-October and mid-November and are likely associated with following estrous cycles. The first interval length between T_v patterns was significantly shorter than the following one, which may reflect that the initial estrous cycle is of a shorter duration. Our algorithm for detecting OLA periods had a sensitivity of 71% and offers a novel approach to estimate the timing of luteal activity during the initial estrous cycle of a breeding season in moose. We advise that our results should be interpreted with care regarding assumptions about the timing of estrus and ovulation, as these events were not directly assessed or confirmed in our study. Additional trials which include a method for confirmation of estrus, ovulation and/or copulation should be conducted to investigate how these events align with the findings of this study. Including this information would enhance our current knowledge about reproductive characteristics of moose and serve a pivotal role in making well-informed management and conservation decisions for the species.

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Sammendrag

Tittel: Variasjon i vaginaltemperatur og aktivitet sett i sammenheng med fekale progestagener hos elg i innhegning

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Det kan være en utfordring å kartlegge spesifikke aspekter ved reproduksjonsfysiologien hos ville arter. For våre husdyr finnes det flere etablerte metoder for å overvåke reproduksjonsaktivitet, inkludert bruken av biologgere til å registrere fysiologiske parametre som kroppstemperatur og aktivitet. Hovedformålet med denne studien var å avgjøre om elg viser tydelige mønster i temperatur og aktivitet som er tilknyttet lutealaktivitet i løpet av brunstsyklusen, slik som er påvist hos kyr. Tidlig i brunstsesongen målte vi fekalt progestagen, vaginaltemperatur og aktivitet hos 12 elgkuer som gikk i innhegning på Kenai halvøya, Alaska, USA, for å studere sammenhengen mellom disse variablene. For å klassifisere lutealaktivitet, ble individuelle avføringsprøver samlet inn fra 23. august til 15. oktober 2021. Resultatene våre støtter at elg viser et tydelig temperaturmønster ved begynnende lutealaktivitet fra midten av september til midten av oktober, som er assosiert med den antatte første brunstsyklus i brunstsesongen. Vi utviklet en algoritme med 71% sensitivitet for å detektere lutealaktivitet basert på en stigning i vaginaltemperatur. I motsetning til dette, fant vi ikke et tilsvarende mønster for aktivitet i perioden som samtidig

hadde korresponderende fekale progestagenmålinger. Vi så derimot etterfølgende og gjentagende mønstre i både temperatur og aktivitet sent i oktober og november som sannsynligvis var tilknyttet etterfølgende brunstsykluser.

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Appendices

Equation 1. Equation for dew point temperature:

$$T_d = \frac{b\left[ln\left(\frac{RH}{100}\right) + \frac{aT}{b+T}\right]}{a - ln\left(\frac{RH}{100}\right) - \frac{aT}{b+T}}$$

Where: T_d = dew point temperature (°C), a = 17.625, b = 243.04, T= ambient temperature (°C) RH = relative humidity.

Equation 2. Equation for actual vapor pressure:

$$e = 6.11 \times 10^{\left(\frac{7.5 \times T_d}{237.3 + T_d}\right)}$$

Where: e = actual vapor pressure (in units of millibars (mb) or hectoPascals (hPa), T_d = dew point temperature (°C).

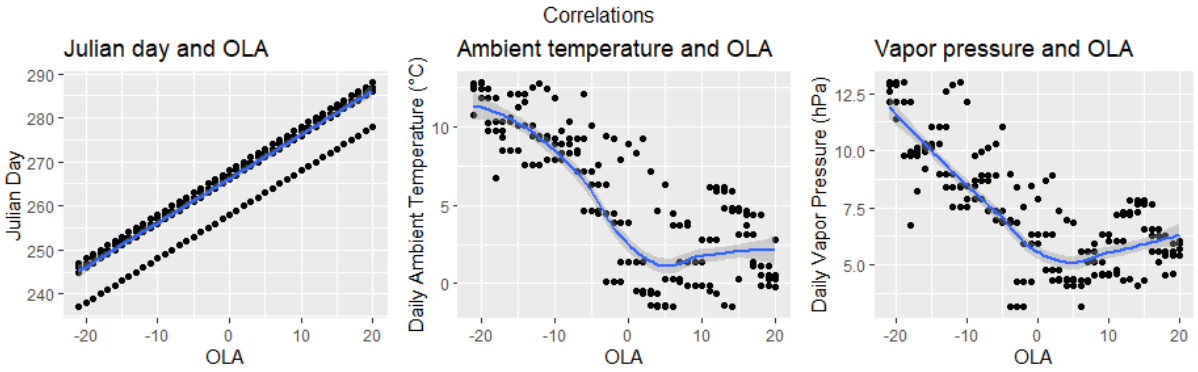

Figure 1. Correlations between the explanatory variable onset of luteal activity (OLA) and julian day, daily ambient temperature (°C) and daily vapor pressure (hPa) (0.96, -0.77 and -0.71, respectively).

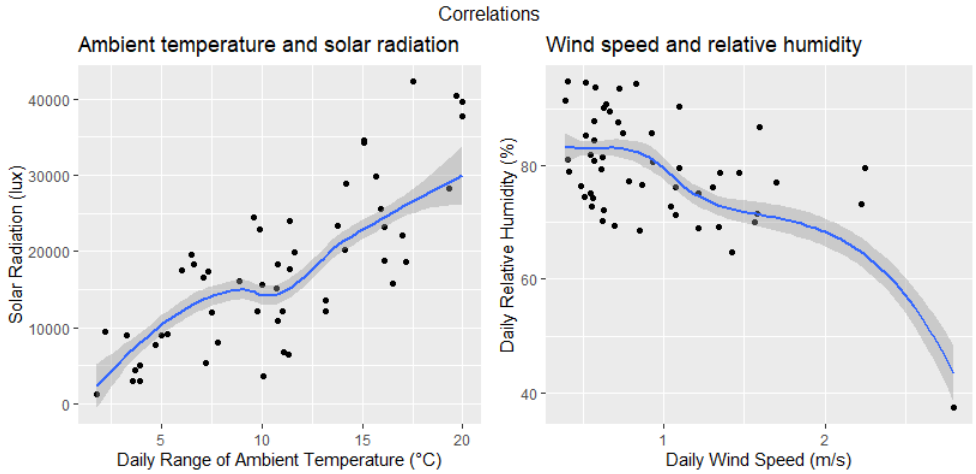

Figure 2. Correlations between the explanatory variables daily range of T_a and solar radiation, and between windspeed and relative humidity (0.72 and -0.63, respectively).

Table 1: Summary of candidate generalized additive mixed models fitted to determine which variables are better at explaining vaginal temperature in captive moose during their estrous cycle. The top section contains parameters that were included across all models, while the bottom section contains the explanatory variables included in each corresponding model. Daily_mean_Tv = daily mean vaginal temperature (°C). OLA = onset of luteal activity. Yday = julian day. T_daily_range = daily range of ambient temperature (°C). Daily_wind = daily mean windspeed (m/s). RH_avg = relative humidity (%). Prec_daily_avg = daily mean precipitation (mm). Light_lux_mean = daily mean solar radiation (lux). Daily_act3_sum = daily sum of activity. CollarID = animal identification number.

Response variable:	Daily_mean_Tv
Random effect:	CollarID
Family:	Gaussian
Link:	Identity
Smoothing parameter:	Maximum likelihood

Model	
m0	1
m1	s(OLA)
m2	s(yday)
m3	s(OLA) + s(T_daily_range)
m4	s(OLA) + s(RH_avg)
m5	s(OLA) + s(Prec_daily_avg)
m6	s(OLA) + s(Daily_wind)
m7	s(OLA) + s(Light_lux_mean)
m8	s(OLA) + s(Daily_act3_sum)
m9	s(OLA) + s(T_daily_range)+ s(RH_avg)
m10	s(OLA) + s(T_daily_range)+ s(Prec_daily_avg)
m11	s(OLA) + s(T_daily_range)+ s(Daily_wind)
m12	s(OLA) + s(T_daily_range)+ s(Daily_act3_sum)
m13	s(OLA) + s(RH_avg)+ s(Prec_daily_avg)
m14	s(OLA) + s(RH_avg)+ s(Light_lux_mean)
m15	s(OLA) + s(RH_avg)+ s(Daily_act3_sum)
m16	s(OLA) + s(Prec_daily_avg)+ s(Daily_wind)
m17	s(OLA) + s(Prec_daily_avg)+ s(Light_lux_mean)
m18	s(OLA) + s(Prec_daily_avg)+ s(Daily_act3_sum)
m19	s(OLA) + s(Daily_wind)+ s(Light_lux_mean)
m20	s(OLA) + s(Daily_wind)+ s(Daily_act3_sum)
m21	s(OLA) + s(Light_lux_mean)+ s(Daily_act3_sum)

m22	s(OLA) + s(T_daily_range)+ s(RH_avg)+ s(Light_lux_mean)
m23	s(OLA) + s(T_daily_range)+ s(RH_avg)+ s(Daily_act3_sum)
m24	s(OLA) + s(RH_avg)+ s(Prec_daily_avg)+ s(Light_lux_mean)
m25	s(OLA) + s(RH_avg)+ s(Prec_daily_avg)+ s(Daily_act3_sum)
m26	s(OLA) + s(Prec_daily_avg)+ s(Daily_wind)+ s(T_daily_range)
m27	s(OLA) + s(Prec_daily_avg)+ s(Daily_wind)+ s(Light_lux_mean)
m28	s(OLA) + s(Prec_daily_avg)+ s(Daily_wind)+ s(Daily_act3_sum)
m29	s(OLA) + s(Daily_wind)+ s(Light_lux_mean)+ s(Daily_act3_sum)
m30	s(OLA) + s(Light_lux_mean)+ s(Daily_act3_sum)+ s(RH_avg)
m31	s(OLA) + s(Light_lux_mean)+ s(Daily_act3_sum)+ s(Prec_daily_avg)
m32	s(OLA) + s(T_daily_range)+ s(Prec_daily_avg)+ s(Daily_wind)
m33	s(OLA) + s(T_daily_range)+ s(Prec_daily_avg)+ s(Daily_act3_sum)
m34	s(OLA) + s(Daily_wind)+ s(Daily_act3_sum)+ s(T_daily_range)

Table 2: Summary of candidate generalized additive mixed models fitted to determine which variables are better at explaining activity in captive moose during their estrous cycle. The top section contains parameters that were included across all models, while the bottom section contains the explanatory variables included in each corresponding model. Daily_act3_sum = daily sum of activity. OLA = onset of luteal activity. Yday = julian day. T_daily_range = daily range of ambient temperature (°C). Daily_wind = daily mean windspeed (m/s). RH_avg = relative humidity (%). Prec_daily_avg = daily mean precipitation (mm). Light_lux_mean = daily mean solar radiation (lux). Daily_mean_Tv = daily mean vaginal temperature (°C). CollarID = animal identification number.

Response variable:	Daily_act3_sum
Random effect:	CollarID
Family:	Gamma
Link:	Log
Smoothing parameter:	Maximum likelihood

Model	
m0	1
m1	s(OLA)
m2	s(yday)
m3	s(OLA) + s(T_daily_range)
m4	s(OLA) + s(RH_avg)
m5	s(OLA) + s(Prec_daily_avg)
m6	s(OLA) + s(Daily_wind)
m7	s(OLA) + s(Light_lux_mean)
m8	s(OLA) + s(Daily_mean_Tv)
m9	s(OLA) + s(T_daily_range)+ s(RH_avg)
m10	s(OLA) + s(T_daily_range)+ s(Prec_daily_avg)
m11	s(OLA) + s(T_daily_range)+ s(Daily_wind)
m12	s(OLA) + s(T_daily_range)+ s(Daily_mean_Tv)
m13	s(OLA) + s(RH_avg)+ s(Prec_daily_avg)
m14	s(OLA) + s(RH_avg)+ s(Light_lux_mean)
m15	s(OLA) + s(RH_avg)+ s(Daily_mean_Tv)
m16	s(OLA) + s(Prec_daily_avg)+ s(Daily_wind)
m17	s(OLA) + s(Prec_daily_avg)+ s(Light_lux_mean)
m18	s(OLA) + s(Prec_daily_avg)+ s(Daily_mean_Tv)
m19	s(OLA) + s(Daily_wind)+ s(Light_lux_mean)
m20	s(OLA) + s(Daily_wind)+ s(Daily_mean_Tv)
m21	s(OLA) + s(Light_lux_mean)+ s(Daily_mean_Tv)
m22	s(OLA) + s(T_daily_range)+ s(RH_avg)+ s(Light_lux_mean)

m23	s(OLA) + s(T_daily_range)+ s(RH_avg)+ s(Daily_mean_Tv)
m24	s(OLA) + s(RH_avg)+ s(Prec_daily_avg)+ s(Light_lux_mean)
m25	s(OLA) + s(RH_avg)+ s(Prec_daily_avg)+ s(Daily_mean_Tv)
m26	s(OLA) + s(Prec_daily_avg)+ s(Daily_wind)+ s(T_daily_range)
m27	s(OLA) + s(Prec_daily_avg)+ s(Daily_wind)+ s(Light_lux_mean)
m28	s(OLA) + s(Prec_daily_avg)+ s(Daily_wind)+ s(Daily_mean_Tv)
m29	s(OLA) + s(Daily_wind)+ s(Light_lux_mean)+ s(Daily_mean_Tv)
m30	s(OLA) + s(Light_lux_mean)+ s(Daily_mean_Tv)+ s(RH_avg)
m31	s(OLA) + s(Light_lux_mean)+ s(Daily_mean_Tv)+ s(Prec_daily_avg)
m32	s(OLA) + s(T_daily_range)+ s(Prec_daily_avg)+ s(Daily_wind)
m33	s(OLA) + s(T_daily_range)+ s(Prec_daily_avg)+ s(Daily_mean_Tv)
m34	s(OLA) + s(Daily_wind)+ s(Daily_mean_Tv)+ s(T_daily_range)

Table 3. Set of criteria tested in the algorithm (A) and (B) OLA period definitions tested in the algorithm. OLA = Onset of luteal activity.

A.

Criterion	Tested values
Baseline (days)	2, 3, 4, 5, 6
Amount (°C)	0, 0.05, 0.1, 0.15, 0.2
Duration (days)	1 to 17

B.

OLA period	Number of days in an OLA period (x)	Number of OLA periods in total (and per moose)	Number of non-OLA periods in total (and per moose)
[-2;2[	4	7 (1)	56 (8)
[-1,4[	5	7 (1)	49 (7)
[-2,3[	5	7 (1)	42 (6)
[-2,4[	6	7 (1)	35 (5)

Table 4. ID, age, parity, P4 range, individual threshold, and OLA for female moose (n=12) from August to October 2021. ID = animal identification number. Parity = number of pregnancies, including total number of offspring in parenthesis. P4 range = range of fecal progestagen, from minimum to maximum values, including the mean in parenthesis. Threshold = specific threshold calculated based on our criteria. OLA = Onset of luteal phase, indicating the first day fecal progestagen fulfilled the set criteria for luteal phase.

* = individual missing temperature data.

ID	Age (years)	Parity	P4 range	Threshold (ng/g)	OLA
44496	2	1(1)	4.6-44.5 (14.5)	18.9	-
44497	2	0	9.2-39.6 (16.1)	12.4	-
44498	2	1(1)	8.4-33.5 (15.7)	17.1	-
30681	9	3(6)	7.1-37.5 (15.3)	14.3	25/09
30684	9	0	4.9-67.3 (16.0)	10.5	25/09
44494	9	2(4)	8.1-37.7 (16.8)	17.1	25/09
44495	9	2(3)	3.3-29.8 (10.2)	8.2	24/09
30680*	12	3(6)	3.3-30.7 (12.4)	6.9	18/09
30683	12	4(5)	5.5-57.5 (17.7)	12.6	25/09
30679	13	3(6)	2.2-34.8 (12.4)	7.1	23/09
30682	18	2(3)	4.0-68.3 (21.1)	9.0	15/09
30678	19	2(3)	6.0-31.1 (14.4)	12.8	-

Table 5. Model selection with daily mean T_v as a response variable based on Akaike's information criterion (AIC). ΔAIC = delta AIC, the difference in AIC score between the highest ranked model and the model being compared. df = degrees of freedom. ω = model weight.

Model	ΔAIC	Df	ω
m1	0.0	14.2	0.1026
m7	0.5	15.2	0.0816
m19	0.6	16.1	0.0772
m4	1.4	15.2	0.0513
m8	1.4	15.2	0.0499
m6	1.5	15.1	0.0485
m17	1.7	16.1	0.0444
m27	1.9	17.1	0.0404
m5	1.9	17.2	0.0398
m14	2.1	16.1	0.0358
m3	2.1	15.1	0.0358
m21	2.1	16.2	0.0356
m29	2.2	17.2	0.0347
m20	2.8	16.2	0.0248
m15	2.9	16.2	0.0239
m24	2.9	17.1	0.0237
m18	3.1	18.3	0.0220
m31	3.1	17.1	0.0218
m13	3.1	16.1	0.0215
m11	3.2	16.1	0.0209
m9	3.6	16.1	0.0172
m12	3.6	16.2	0.0171
m16	3.7	18.1	0.0162
m30	3.8	17.1	0.0152
m10	3.9	16.1	0.0143
m25	3.9	20.2	0.0143
m23	4.1	20.4	0.0132
m22	4.3	20.3	0.0117
m34	4.6	17.2	0.0104
m28	4.8	19.2	0.0091
m32	5.0	17.1	0.0085
m26	5.0	17.1	0.0085

m33	5.1	19.3	0.0079
m2	46.7	14.3	<0.001
m0	151.3	7.9	<0.001

Table 6. Estimated parameters for the explanatory variables that were included in the final general additive mixed model (m1) evaluating when changes in daily mean vaginal temperature occur in relation to the onset of luteal activity in moose. SE = standard error. Edf = degrees of freedom. Ref.df = reference degrees of freedom. k' = number of basis functions. R-sq.(adj) = adjusted R-squared. OLA = onset of luteal activity. CollarID = animal identification number.

Parametric coefficients	Estimate	SE	t-value	p-value
Intercept	37.84	0.04	1041.00	<0.01

Approximate smooth terms	edf	Ref.df	k'	F-value	p-value
s(OLA)	6.00	7.16	9.00	28.71	<0.01
S(CollarID)	5.92	6.00	7.00	85.95	<0.01

R-sq.(adj) = 0.71, deviance explained = 72.30%, Maximum likelihood = -331.51.

Table 7. Model selection with daily sum of activity as a response variable based on Akaike's information criterion (AIC). ΔAIC = delta AIC, the difference in AIC score between the highest ranked model and the model being compared. df = degrees of freedom. ω = model weight.

Model	ΔAIC	Df	ω
m5	0.0	13.9	0.1914
m16	0.8	14.2	0.1301
m18	1.8	14.1	0.0796
m13	1.8	14.2	0.0761
m17	1.9	14	0.0731
m10	2.1	14.9	0.0663
m32	2.5	18.2	0.0543
m26	2.5	18.2	0.0543
m28	2.9	17.9	0.0448
m25	3.8	15.7	0.0285
m31	3.8	15.8	0.0282
m24	3.8	15.6	0.0281
m33	3.9	15.9	0.0275
m22	3.9	15.7	0.0268
m27	4.2	15.7	0.0234
m6	5.3	16.2	0.0132
m7	6.1	13.4	0.0089
m1	6.5	12.5	0.0074
m4	6.8	13.3	0.0062
m14	7.1	14.1	0.0055
m3	7.6	13.4	0.0043
m20	7.8	17	0.0038
m11	7.9	16.5	0.0037
m19	8.4	14.3	0.0029
m9	8.4	14.2	0.0028
m8	8.8	13.4	0.0023
m21	9.4	13.6	0.0017
m15	9.7	13.9	0.0015
m12	10.1	14.1	0.0012
m34	10.9	17	<0.001
m30	13.6	11.7	<0.001
m2	11.6	8.8	<0.001

m23	13.6	11.7	<0.001
m29	14.2	11.7	<0.001
m0	35.4	7.8	<0.001

Table 8. Estimated parameters for the explanatory variables that were included in the final general additive mixed model (m5) evaluating when changes in daily sum of activity occur in relation to the onset of luteal activity in moose. SE = standard error. edf = degrees of freedom. Ref.df = reference degrees of freedom. k' = number of basis functions. R-sq.(adj) = adjusted R-squared. OLA = onset of luteal activity. Prec_daily_avg = daily mean precipitation (mm). CollarID = animal identification number.

Parametric coefficients	Estimate	SE	t-value	p-value
Intercept	8.77	0.059	148.90	<0.01

Approximate smooth terms	edf	Ref.df	k'	F-value	p-value
s(OLA)	3.97	4.91	9.00	6.52	<0.01
S(Prec_daily_avg)	1.00	1.00	9.00	6.71	0.01
S(CollarID)	5.68	6.00	7.00	18.17	<0.01

R-sq.(adj) = 0.34, deviance explained = 38.90%, Maximum likelihood = -2559.50.

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