ARE MINNESOTA MOOSE WARMING UP TO CLIMATE CHANGE? A
VALIDATION OF TECHNIQUES FOR REMOTELY MONITORING MOOSE
BEHAVIOR AND BODY TEMPERATURE

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Dedication

This thesis is dedicated to the memory of my father, Thomas Herberg.

Abstract

Climate change is causing rapid shifts in species distributions across the globe. Large-bodied endotherms, especially those at the edge of their bioclimatic range, are particularly vulnerable to these changes. It is critical that we understand the physiology, behavior, and energetic needs of these animals to develop effective management and conservation plans. Advancements in biotelemetry devices have greatly improved our ability to collect physiological and behavioral data from free-ranging animals; however, our understanding of how the data can be processed and used is still in its infancy. One species of conservation concern, the moose (*Alces alces*), experienced a 58% population decline in northeastern Minnesota between 2006 and 2017. To better understand behavioral and physiological responses of this species to increasing ambient temperature, the Minnesota Department of Natural Resources deployed two types of biotelemetry devices in moose throughout northeastern MN: 1) rumen boluses, known as mortality implant transmitters (MITs), capable of recording internal body temperatures, and 2) global positioning system (GPS) collars equipped with dual-axis activity sensors that detect and record changes in neck movements. The main goals of my research were to determine the accuracy of MIT-derived core body temperatures and test the efficacy of using dual-axis activity sensors for remotely predicting behavioral states of moose. Ten captive female moose (>2 years old) at the Moose Research Center in Kenai, Alaska with MITs were fit with vaginal implant transmitters (VITs) capable of recording internal body temperature, and GPS collars for 12 months starting in December 2014. A total of 384 hours of behavioral observations were collected during four, two-week windows distributed across seasons. I observed a notable effect of water intake on MIT-derived temperatures and developed an approach for censoring these observations. Using linear mixed-effects models, I predicted moose core body temperature (as measured by VITs) and found that on average, the difference between predicted and observed body temperatures was 0.05°C for winter and 0.33°C for summer, with >90% of prediction intervals containing the observed VIT-derived temperatures. Using a Dirichlet regression approach to analyze the dual-axis activity sensor data, I predicted the proportion of time

individual animals spent resting, foraging, and moving during 5-minute intervals; these results were used to understand how behavioral states change as a function of habitat, ambient temperature, and time of day. I demonstrated that combining biotelemetry devices with modern statistical approaches allows researchers to examine the physiological and behavioral responses of moose to increasing ambient temperatures and changing landscapes at finer temporal and spatial scales than previously possible.

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CHAPTER 1

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INTRODUCTION

Climate change is causing rapid shifts, poleward in latitude and upward in elevation, in organism distributions across the globe – this can result in either the extension or contraction of a species' geographic range (Parmesan 2006; Chen et al. 2011). Species whose distributions are restricted by warm temperatures are likely to experience range contractions (Parmesan and Yohe 2003; Parmesan 2006). With ambient temperatures forecasted to increase globally by as much as 4.7ºC by 2100, some species will be more frequently exposed to temperatures that may exceed their thermal thresholds (IPCC 2014). Populations at the bioclimatic edges of a given species' geographic distribution will be the first to experience climate-induced changes; large-bodied animals are thought to be particularly vulnerable (Dirzo et al. 2014; Ripple et al. 2016). Improved understanding of how individuals occupying bioclimatic edges respond to warming temperatures may provide insight into how species will respond to environmental changes and will help guide future species conservation efforts under variable climatic conditions.

Moose (*Alces alces*), a large bodied endotherm, inhabit a wide longitudinal range across their southern bioclimatic edge in North America where survival rates are lower than in their core geographic range and further north (Dodge et al. 2004; Murray et al. 2006; Maskey 2008; Lenarz et al. 2010). In Minnesota, moose in the northwestern portion of the state are all but extirpated (Murray et al. 2006), whereas the northeastern population has experienced a 55% decline between 2006 and 2016, from an estimated 8,840 moose to 4,020 (DelGuidice 2016). Past research in Minnesota found that non-hunting mortality rates of adult moose were higher (5-35%; Lenarz et al. 2009; Murray et al. 2006) than populations at the core of the species' range in North America (8–12%; Mytton and Keith 1981, Larsen et al. 1989, Ballard et al. 1991, Stenhouse et al. 1995, Modafferi and Becker 1997). While the ultimate driver of this population decline remains unknown, recent research has demonstrated that the majority of moose mortalities can be attributed to health-related causes (Murray et al. 2006; Carstensen et al. 2014). However, in addition to health-related causes such as diseases and parasites, predators, habitat

alteration, and climate change are all factors that may be contributing, alone, or in concert, to the population decline in Minnesota (Murrary et al. 2006; Lenarz et al. 2009, Lenarz et al. 2010; Carstensen et al. 2014, Mech and Fieberg 2014). Ambient air temperatures were found to significantly contribute to the northwestern population collapse (Murray et al. 2006), and survival has been shown to be negatively correlated with ambient air temperatures during winter in the northeastern population (Lenarz et al. 2009, but see Mech and Fieberg 2014).

Adapted to thrive in a cold environment, moose are known to be physiologically sensitive to heat (Renecker and Hudson 1986, Renecker and Hudson 1989; McCann et al. 2013). Renecker and Hudson (1986, 1990) found that temperatures higher than 5°C in the winter and 14-20°C during the warm season (late spring to early fall) were associated with reduced food intake and body weight, as well as increased metabolic, heart, and respiratory rates. Similar thresholds were found for late spring to early fall (17–24°C) (McCann et al. 2013). However, these studies were based on two and four captive moose respectively, and extrapolation to thermal thresholds for wild moose may not be appropriate. Ambient temperatures consistently above these thresholds may lead to elevated body temperatures of moose if they are unable to efficiently thermoregulate; this may require increased energy expenditures to mitigate excess heat. However, such conclusions are speculative, and, largely due to technological limitations, few data currently exist to support the direct or indirect effects of ambient temperature on the behavior, physiology, reproduction, and survival of wild moose living in natural settings.

Continuous monitoring of core body temperature in free-ranging ruminants poses many challenges and risks. Surgical implantation of long-term, abdominal or rectal temperature loggers is invasive and associated with increased risk of infection, adhesions, peritonitis, necrosis and death (Eagle et al. 1984; Guynn et al. 1987; Wallace et al. 1992; Moe et al. 1995). A less invasive option is the use of vaginal implant transmitters (VITs). These devices were first used as a minimally invasive means of administering drugs (Asher and Smith 1987, Rahtbone et al. 1997, Bowman and Jacobson 1998), and have since been modified with archival temperature and activity sensors. In cattle, vaginal

loggers provide a reasonable measurement of core body temperature (correlations ranging from 0.46 to 0.92 between VITs and rectal temperature measurements) but the strength of that relationship depends on the breed of cattle, pregnancy state, immune condition, rectal measurement technique, and the time difference between the two measures (Vickers et al. 2010; Burnfield et al. 2011; Burdick et al. 2012). Vaginal implant transmitters have also been used for long-term (until parturition) monitoring of physiological changes in free ranging female white-tailed deer (*Odocoileus virginianus*; Carstensen et al. 2003), moose, elk (*Cervus canadensis*; Johnson et al. 2006), black-tailed deer (*Odocoileus hemionus columbianus*; Gilbert et al. 2014), mule deer (*Odocoileus hemionus*; Bishop et al. 2011), and bighorn sheep (*Ovis Canadensis*; Patterson et al. 2013). The main limitation of these devices is that they can only monitor pregnant females (and even then, only until parturition unless the animal is recaptured).

To address some of the limitations of VITs and also examine the direct effects of ambient temperature on animals' behavior and physiology, researchers have developed a minimally invasive telemetry system capable of measuring body temperature, heart activity, and locomotive activity in ruminants (Signer et al. 2010). These devices, placed in the rumen and known as rumen boluses, have been tested in cattle (*Bos taurus*), domestic sheep (*Ovis aries*) and alpine ibex (*Capra ibex ibex*) (Signer et al. 2010); they have also been used examine the regulation of heart rate and rumen temperature in red deer (*Cervus elaphus*) (Turbill et al. 2011). Rumen temperatures in cattle and sheep were found to consistently exceed abdominal temperatures, with the magnitude of difference being related to ambient temperatures (Beatty et al. 2008a; Beatty et al. 2008b). Although the core body temperatures of the animals in these studies remained relatively stable throughout the year, rumen temperatures fluctuated (Degan and Young 1984; Nicol and Young 1990; Crater and Barboza 2007). For example, increases in rumen temperature (due to microbial fermentation) have been observed after feeding bouts (Barnes et al. 1983; Czerkawski 1980; Dehority 2003, Lawler and White 2003), and short-term declines in rumen temperature have also been observed after water consumption (Dale et al. 1954; Cunningham et al. 1964; Brod et al. 1982; Dehority 2003; Crater and Barboza

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2007). Although long-term variation in rumen-derived body temperature should be highly correlated with core body temperature (Singer et al. 2010) , short-term changes in rumen temperature need to be considered if rumen boluses are to be used to evaluate an animal's physiological response to changing ambient temperatures.

Rumen boluses allow researchers to continuously monitor the physiological state of male and female ungulates over relatively long time periods in a minimally-invasive manner. However, the only previous studies that have investigated the relationship between rumen temperature measured by these boluses and core body temperature (measured by abdominal implants) were conducted over short durations: 6 cows over 25 days (Beatty et al. 2008a), and 16 domestic sheep over 17 days (Beatty et al. 2008b). Before relying on these devices for a long-term, broad-scale study, it is important to quantify correlations between rumen temperature and core body temperature at multiple temporal scales. Our goal was to validate the use of a specific type of rumen boluses designed to record temperature, mortality implant transmitters (MIT), to determine core body temperature in moose. Specifically, our objectives were to 1) determine if MIT-recorded temperatures are affected by moose behavior, 2) establish a set of thresholds to screen out abnormal MIT readings, 3) quantify the relationship between rumen temperature measured by MITs and core body temperature measured by VITs, and 4) demonstrate the application of a MIT–VIT temperature conversion model to the MIT time series of wild moose in MN.

STUDY AREA

The study was conducted at the Kenai Moose Research Center (MRC) located on the Kenai Peninsula, Alaska. (Figure 1). The MRC, operated by the Alaska Department of Fish and Game, is a 2400-acre captive facility that was built in the 1960's to study moose's behavioral and physiological responses to their environment (Hundertmark et al. 2000). All moose in this study were maintained in outdoor enclosures, approximately 240 ha in size. Each enclosure encompassed a mix of habitat types including open meadows,

bogs, open crushed forest areas, aspen/birch (*Populus tremuloides, Betula neoalaskana*; ~25 year old), black spruce (*Picea mariana*; ~125 year old), and aspen/white spruce (*Populus tremuloides, Picea glauca*;~125 year old) stands, as well as lakes. The moose we observed cohabitated in two different enclosures and were maintained at densities of 4–8 adult females per enclosure (30–60 ha/moose); densities depended on the time of year and levels of conspecific aggression. Moose naturally forage within the enclosures and are only supplemented during times of low nutritional condition, handling (i.e., weighing), or during specific studies. Moose have access to water from lakes and wetlands throughout the enclosures. To supplement water intake during the warm season (late spring to early fall) cattle troughs are available in enclosures with fewer wetlands. While predation risks are low, encounters with brown bears (*Ursus arctos*), black bears (*Ursus americanus*), and wolves (*Canis lupus*) occur sporadically within the enclosures (Dan Thompson, pers comm.).

METHODS

Animal Handling

Five female moose (>3 years old) were chemically immobilized with 0.45 mg of carfentanil (Zoopharm, Windsor, Colorado) and 25 mg of xylazine (Henry Schein, Melville, New York) during four routine immobilization periods at the MRC (December, April, June, and September). During the same immobilizations, five additional female moose (>2 and <3 years old) were chemically immobilized with 0.30 mg of carfentanil and 15 mg of xylazine. Each moose were reversed using tolazaline (400 mg; Zoopharm, Windsor, Colorado) and naltrexone (100 mg; Zoopharm, Windsor, Colorado). Moose were fitted with a Vectronic GPS collar (GPS Plus Iridium; Vectronic Aerospace GmbH; Berlin Germany) that were uniquely marked with color tape to facilitate individual identification. Satellite communication was disabled to prolong the collar's battery life, and all data were stored on board the collar and downloaded after removal. The GPS collars collected data on activity (i.e., accelerometer counts of movements in the X and Y

plane) in 5-min intervals, geographic location in 30-min intervals, and ambient temperature in 5-min intervals. GPS collars could be removed, adjusted, and/or replaced on each captive moose throughout the study without the need for immobilization.

Mortality implant transmitters (length: 72 mm; diameter: 21 mm; weight: 120 g; Vectronic Aerospace GmbH; Berlin Germany) were deployed as a rumen bolus during the December 2014 immobilizations to measure rumen-derived body temperature in these 10 moose following a protocol outlined by Minicucci et al. 2017 (Figure 2). All adult moose were chemically immobilized by hand-injection using carfentanil and xylazine, as outlined above, and initially reversed using tolazaline. Following tolazaline application, and after consistent swallowing was observed, a V-grip applicator (Genesis Industries, Elmwood, Wisconsin) was used to place the MIT at the base of the tongue to allow for natural swallowing for inserting the MIT into the rumen. After correct placement, naltrexone was administered to reverse the carfentinil (Minicucci et al. 2017). Each MIT was programmed to transmit rumen temperature via 433 MHz ultra-high frequency (UHF) signal to the GPS collar at 296-second intervals. The collar unit was able to decode and archive the information for download upon collar removal in December 2015. Temperature transmissions were attempted for up to 12.5 seconds before being deemed unsuccessful and attempted 296 seconds later. This meant that MIT temperature intervals varied between 296 and 308.5 seconds, resulting in 11 or 12 measurements per hour, assuming 100% transmission success. To conserve battery life, the heart-rate sensor in each MIT was disabled for the duration of the study. With a small possibility of expulsion (Signer et al. 2010), MITs should remain in the moose for the duration of their life.

To validate if MIT-derived internal temperatures are representative of core body temperature, we deployed modified vaginal implant transmitters (VITs) in the 10 captive female moose during each of the routine immobilizations starting in December 2014. Eight female moose were deployed with modified vaginal implant transmitters with archival temperature and activity sensors (AVIT; diameter: 19 mm; length: 80 mm; wing span: 150 mm; Advanced Telemetry Systems (ATS); Isanti, Minnesota). Temperature and activity status for each AVIT was recorded and stored at 5-minute intervals. Two

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female moose were deployed with a modified vaginal implant transmitter capable of remotely transmitting temperature and activity data to the GPS collar (VVIT; diameter: 20 mm; length: 70 mm; wing span: 70 mm; Vectronic Aerospace GmbH; Berlin Germany). Each VVIT was programmed to transmit temperature and activity status via UHF signals to the GPS collar at 296 second intervals. Data transmissions were attempted for up to 12.5 seconds before being deemed unsuccessful and attempted 296 seconds later. This meant that VVIT temperature intervals varied between 296 and 308.5 seconds, resulting in 11 or 12 measurements per hour assuming 100% transmission success. Vaginal implant transmitter temperatures <30°C were assumed to be affected by environmental factors and data from these moose were removed from the study. Each VIT was deployed for a minimum of two weeks after each immobilization, and were allowed to remain in for a longer time period if no negative effects were observed. VITs could be manually removed at any time without sedation.

Animal Observations

To determine if the MIT-derived temperature measurements were affected by moose behavior, we conducted behavioral observations of 8 captive moose deployed with AVITs during several 6-hour long intervals. Each animal was observed twice during each of four 2-week long observation periods (January, April, July, October) to account for any seasonal effects. The observation intervals were spread randomly throughout the day from 0600 to 2200 hours, and during all weather conditions. Each of the 8 moose was observed for 48 hours, for a total of 384 observation hours for all animals. The behavioral observations were conducted from a distance ≤10 m, during which every change in behavior was recorded. The behaviors recorded included: foraging low (snout below the bottom of stomach), foraging medium (snout above the bottom of the stomach and below the top of the shoulders), foraging high (snout above the top of the shoulders), resting, ruminating, drinking/eating snow, walking, standing, running, shaking, grooming, and interacting (e.g., boxing). The exact start times of each new behavior were recorded using Recon/Juno data loggers (Trimble Navigation Limited Trimble, Sunnyvale, California) to the nearest second in a procedure similar to Moen (1996). Non-continuous 6-hour

observation intervals caused by loss of visual contact with the observed moose (e.g., spooked and ran) were possible. In this scenario, the moose being observed was relocated using VHF telemetry, and missing observations were removed from subsequent analyses. All animal handling procedures were approved by the Animal Care and Use Committee, Alaska Department of Fish and Game, and Division of Wildlife Conservation (protocol No. 09–29).

Statistical Analyses

We pre-processed the data by first identifying any missing scheduled temperature measurements using time stamps associated with each MIT and VIT temperature measurement for each moose. The missing measures were used to calculate overall and seasonal transmission success rates amongst the different devices. To avoid artificially lowering transmission rates, missing temperature measurements from collars that completely stopped transmitting before the end of the study period were removed from the analysis. Because time stamps of MIT and VIT measurements were not always exactly the same, we linearly approximated the data between consecutive time stamps. For example, if two consecutive MIT measurements were taken at 0602h and 0607h and VIT was taken at 0605h, we would linearly interpolate the MIT data to approximate its value at 0605h, the time of the VIT. We thus assumed that, within a 5-minute interval, any rise or decrease in body temperature is unlikely to be non-linear. Intervals greater than 6 minutes between consecutive temperature measures were not linearly interpolated and were removed from analyses. To determine the accuracy of both MIT and VIT temperature measurements, a water bath procedure was adapted from both Signer et al. (2010) and Vickers et al. (2010) and can be seen in Appendix 2.

To determine correlations between MIT measurements and targeted behaviors, interval lengths for each behavior were matched with the time of MIT temperatures. We targeted specific behaviors (water consumption, ruminating, running) based on knowledge from previous studies of behaviors affecting rumen temperature (Degan and Young 1984; Nicol and Young 1990; Crater and Barboza 2007). To account for differing

time stamps, MITs were linearly interpolated to match behavioral time stamps, following the procedure outlined above. For each targeted behavior, we calculated the duration of the behavior, the maximum increase or decrease in MIT-recorded temperature (Δmax) within a specified behavioral segment (i.e., start time of a specific behavior up to the end time of that behavior plus 20 minutes), the length of time from the start of a given behavior to Δmax, and length of time from the beginning of a specific behavior until temperature re-normalization (i.e., back within one standard deviation of the MIT temperature recorded when that behavior started). To determine the significance of changes in MIT-recorded temperatures, we compared Δmax in time intervals containing targeted behaviors with random intervals of the same duration from the same 6-hour observation period using a paired t-test.

To establish a set of thresholds that could be used to censor water consumption-induced changes in MIT temperatures (i.e., a drop in MIT temperature immediately following water consumption), we implemented a simplified sensitivity analysis approach as follows. Mortality implant transmitter temperatures were first separated into two groups: 1) those affected by water intake as determined by the time series calculations outlined above, and 2) those not affected by water intake according to our previous approach. We attempted to balance the need to appropriately remove behaviorally-mediated data, limiting type II error, while also avoiding bias from removing non-behaviorally mediated temperatures (type I error). We tested a given temperature threshold by calculating the percentage of MIT temperatures within each group that fell below the specified temperature threshold. We tested a range of temperatures and chose the lowest threshold that led to a type I error of ≤1%.

Once the abnormal (low or high) observations were removed from the MIT time series, we modeled the relationship between MIT and AVIT-measured temperatures using linear mixed-effect models to account for individual variability. This method accounted for the repeated measures within individuals, allowed for unequal-sized data sets, and possible among-animal differences in the relationship between AVIT and MIT measurements. To help with the interpretability of results and convergence of predictive

models, MIT temperatures were mean centered and scaled. Four seasonal models were created for MIT temperatures averaged over three different temporal resolutions: 1) 15-minutes to emulate the 15-minute resolutions of MIT recordings in a companion MN wild moose study, 2) 255-minutes to reproduce the resolution of the GPS locations in that same study, and 3) daily. Mixed models were allowed to have random slopes and intercepts and were defined as: $Y_{ij} = \beta_0 + b_{0,j} + X_{i,j}\beta_1 + X_{i,j}b_{i,j} + \varepsilon_{i,j}$. Where AVIT body temperatures (Y_{ij}) for i-th moose at the j-th time point were predicted using a fixed effect (β_1) for centered MIT temperature and random effects

$\begin{pmatrix} b_{0,j} \\ b_{i,j} \end{pmatrix} \sim MVN \begin{pmatrix} 0 \\ 0' \end{pmatrix} \begin{bmatrix} \sigma_{\beta_0}^2 & \sigma_{\beta_0,\beta_1} \\ \sigma_{\beta_0,\beta_1} & \sigma_{\beta_1}^2 \end{bmatrix} \end{pmatrix}$ that allow for the relationship between MIT

temperature and AVIT body temperature to vary by moose. Residual error was represented by $\varepsilon_{i,t} \sim N(0, \sigma_\varepsilon^2)$.

We evaluated and compared how well MIT-measured temperatures predicted core body temperatures using a k-fold cross-validation procedure for the different models fitted (i.e., each temporal resolution) (Hastie et al. 2009). Specifically, we used an 8-fold cross-validation approach in which MIT temperatures from 1 moose (test) were removed and data from the remaining 7 moose (training) were used to build mixed models. We then predicted and stored MIT temperatures for the test moose and repeated the procedure for all 8 moose. Model cross-validation results were compared using root mean

squared error (RMSE), defined as: $\sqrt{\sum_j \dfrac{\frac{\Sigma_i (y_{ij} - \hat{y}_{ij})^2}{N_j}}{k}}$. Where RMSE is the overall root mean

squared error of all cross-validations, y_{ij} is the i-th MIT temperature of the j-th cross-validation, $\hat{y}_{ij}$ is the i-th MIT predicted temperature of the j-th cross-validation, N_j is the number of observations of the j-th cross-validation, and k is the number of cross-validations. Root mean squared error was calculated for all three temporal resolutions (15-minute, 255-minute, and daily averages). To better understand how prediction confidence changed as MIT temperatures moved away from the mean (i.e., as MIT temperatures increased), we predicted VIT temperatures for a range of realistic centered

MIT temperatures (-2–6, 0.1 increments) using the fitted model for each temperature-averaging scenario and calculated 95% prediction intervals for each prediction using a bootstrap with 1000 iterations.

We further evaluated how well mixed models fit to paired MIT-AVIT temperatures predicted VVIT measurements. We used a holdout cross-validation procedure in which data were divided into two sets: a training set (paired MIT-AVIT measurements) and a validation set (paired MIT-VVIT measurements). Models were fit to the training data, and were then used to predict VVIT temperatures. Before model predictions could be made, differences between VIT devices first needed to be determined. The difference (offset) between AVITs and VVITs was calculated using linear mixed effect models for each of the 4 seasons. VIT body temperatures (Y_{ij}) were predicted using a fixed effect (β_1) for device type and random effects ($\varphi_i \sim N(0, \sigma_\varphi^2)$) for the interaction between device type and moose ID. Residual error was represented by $\varepsilon_{i,t} \sim N(0, \sigma_\varphi^2)$.

Vectronic VIT temperatures were adjusted using the intercept in the model fitted above and then predicted using all 12 linear mixed-effects models (i.e., 15-minute, 255 minute, and daily models for each season). We evaluated prediction accuracy for each season using the mean difference in predicted versus observed VVIT temperatures and the percent of prediction intervals (calculated using a bootstrap with 1000 iterations) that included the observed VVIT temperatures.

Statistical significance for all analyses was determined using $\alpha<0.05$. All statistical analyses were conducted using the R statistical software (R Core Team 2017), with the lme4 (Bates et al. 2015), merTools (Knowles & Frederick 2016) and XTS libraries (Ryan and Ulrich 2013).

RESULTS

Mortality implant transmitters had an overall high rate of success transmitting temperature measurements to the GPS collars. Of 809,937 transmissions from 8 moose during the study, MITs successfully transmitted data 95.64% of the time. We observed differences amongst seasons, with the highest transmission rate observed during winter (99.56%) and the lowest during fall (87.81%; Table A.1). The overall fall transmission rate was lowered significantly by one moose who's MIT stopped transmitting on 20 October 2015, skipping 11,439 scheduled transmissions, before resuming communication on 28 November 2015. The fix rate of another moose changed from every 5 minutes to every 1 minute not long after deployment, resulting in a large number of MIT temperature measurements recorded during the winter and early spring (182,492), before the MIT stopped transmitting on 7 April 2015. The AVITs were deployed on average for 87.75 days (range 68–91), 141.63 days (range = 140–143), and 50.29 days (range = 50–51) during winter, spring/summer, and fall, respectively. One AVIT failed before the fall deployment and was removed from the data set. These AVITs recorded temperatures for 100% of the scheduled temperature measurements.

Mean MIT-recorded temperatures were 38.13°C (range = 13.65–41.14, n = 854,286) across all seasons for the moose implanted with AVITs. Mean MIT temperatures varied by season and were lowest in winter (Mean: 37.93 ± 0.21°C [SD]) and highest in summer ($\bar{x} = 38.61 \pm 0.38$°C; Table A.2; Figure A.1). We conducted 384 hours of behavioral observations across the four seasons. Three hours of observation (<1%) were censored after an observer lost visual contact with a moose during an observation period. Water intake behaviors (i.e., drinking, eating snow) had lower Δmax values than controls during all four seasons ($t = -4.21$, df = 64, P = <0.01). Water intake behaviors elicited a greater decrease in MIT temperatures during the warm seasons (spring, summer, and fall; $\bar{x} = -2.27 \pm 1.85$°C) than during winter ($\bar{x}$: -0.23 ± 0.26°C; Table 1). The mean time it took for MIT temperatures to return to within one standard deviation of pre-drinking MIT temperatures ranged from 35.95 ± 43.28 minutes during the winter season to 25.29 ± 14.33 minutes during the warm seasons (Table 1, Figure 3).

We did not detect a statistically significant effect of rumination on MIT-recorded temperatures (Δmax) in any of the four seasons ($t = 0.02$, df = 186, $P = 0.98$). However, ruminating elicited slight MIT temperature increases in all four seasons, with average increases ranging from 0.05 ± 0.06 °C during summer to 0.18 ± 0.29°C during the fall. These increases were well within normal variation observed during our observations. We could not examine the effect of running on MIT-recorded temperatures since we observed only 9 running behaviors that lasted longer than 30 seconds during our observation periods.

We determined threshold temperatures of 37.66°C and 37.54°C for winter and the warm season respectively (Figure 4). These thresholds resulted in the removal of 13% of water intake-affected temperatures during winter and 70% of water intake-affected temperatures during the warm season (Figure 4). We may have incorrectly removed up to but no more than 1% of non-water intake-affected temperatures using our threshold approach for censoring water intake-induced MIT temperatures. Removal of artificially low temperatures resulted in an overall increase of 0.04°C in mean MIT temperatures to a mean of 38.17°C ($\pm$ 1.85) across all seasons, with the smallest increase of 0.02°C during winter to a mean of 37.94°C ($\pm$ 0.18) and the largest increase of 0.09°C during spring to a mean of 38.04°C ($\pm$ 0.29) (Table A.2; Figure 5).

Abnormally low AVIT temperatures were observed in 3 moose, with frequent drops below 30°C. On average, AVIT temperatures were 38.18°C (range = 37–41.25°C, n = 403,031) across all seasons. Mean AVIT temperatures varied by season, with the lowest temperatures during fall ($\bar{x} = 38.01 \pm 0.24$°C) and highest during summer ($\bar{x} = 38.43 \pm 0.29$°C; Table A.3, Figure A.1). Across all four seasons, MIT temperatures were on average 0.03 ± 0.28°C lower than paired AVIT temperatures. Differences in paired MIT and AVIT temperatures varied by season, with MIT temperatures 0.13 ± 0.27°C lower during spring and 0.12 ± 0.28°C higher than paired AVIT temperatures during summer (Table A.3). Seasonally varying circadian patterns were observed in both MIT and AVIT temperatures (Figure 6). The temperature swings were most pronounced

during summer, with lower than average temperatures during the middle of the day and higher temperatures during the night for both MIT and AVIT temperatures.

Sensitivity analyses comparing RMSE among the different temperature averaging intervals were inconclusive and supported the temperature intervals determined *a priori* (15 minute, 255 minute, and daily) (Figure A.2). Root mean squared error decreased across all seasons as the temporal resolution over which the data were averaged went from 15 minutes to daily (Table 2). The largest model improvement occurred during the summer season, RMSE decreased by 48% (15-min RMSE = 0.25, daily RMSE = 0.13) from 15-minute averaged temperatures to daily averages and by 32% (255-min RMSE = 0.19, daily RMSE = 0.13) from 255-minute averaged temperatures to daily averages. The smallest improvements occurred during the fall season, with a decrease in RMSE of 10% (15-min RMSE = 0.30, daily RMSE = 0.27) from 15-minute averaged temperatures to daily averages and by 4% (255-min RMSE = 0.28, daily RMSE = 0.27) from 255-minute averaged temperatures to daily averages. Fall models were not as good at predicting MIT values resulting in the highest RMSE across all averaging schemes. Root mean squared error was less than or equal to 0.30 across all seasons for all temporal resolutions considered.

Mortality implant transmitters from the 2 moose deployed with VVITs had an overall transmission rate of 44.03% across all seasons. Differences were observed amongst seasons, with the highest transmission rate observed during winter (58.15%) and the lowest during fall (10.03%; Table A.5). The transmission rate for the VVITs in these 2 moose was 99.53% across all seasons, with the highest rate observed during winter (99.91%) and the lowest during summer (96.78%; table A.6); one of these animals had its VVIT fail on 6 June 2015. Mortality implant transmitter temperatures were corrected for water intake behaviors, which resulted in the removal of 365 (1.32%) and 2071 (5.25%) low temperatures during winter and the warm season respectively. After correcting for water consumption, MIT and VVIT temperatures were on average 38.15°C (range = 37.53–39.79°C; $n = 67,208$) and 37.84 °C (range = 36.90–39.50, $n = 134,867$) across all

seasons, respectively. Mean MIT temperatures in these 2 moose varied by season and followed similar patterns to the 8 moose deployed with AVITs (Table A.6).

Vectronic VIT temperatures were on average 0.31°C (SE = 0.14) lower than AVIT temperatures. Vectronic VITs were lower during every season with the difference ranging from 0.20°C (SE = 0.15) to 0.37°C (SE = 0.14) for summer and winter respectively (Table 3). After correcting for the difference in VIT temperatures, model predictions were run on 54,384 paired MIT and VVIT temperatures for these 2 moose. Winter had the smallest mean difference in predicted versus observed VVIT temperatures, ranging from 0.02°C (99.73% of PIs contained the true VVIT temperature) using 15-minute averaged temperatures to 0.07 °C (100% of PIs contained the true VVIT temperature) using daily averages. The largest differences in predicted versus observed VVIT temperatures were during summer, means ranged from 0.29°C (99.62% of PIs contained the true VVIT temperature) using 15-minute averaged temperatures to 0.35°C (100% of PIs contained the true VVIT temperature) using daily averages (Figures A.3). Predictions across a range of VIT temperatures for each temperature-averaging scenario confirmed that 95% prediction intervals decrease as temperature averaging increases, as well as showing that prediction intervals increase as MIT temperatures increase (Figure A.4).

DISCUSSION

We have established that MITs are reliable for continuously monitoring core body temperatures in moose, free-ranging or captive. While previous studies have compared the relationship between rumen-derived temperature and core body temperature over short time intervals in domestic animals (Beatty et al. 2008a; Beatty et al. 2008b), no study has evaluated and monitored this relationship over the course of several seasons in domestic or free-ranging ruminants. Our results suggest that temperature differences between the rumen and core body are minimal and constant across seasons, despite changes in ambient temperature and behavior. We have shown that behavior-induced

decreases in MIT temperatures, such as those from water intake, can be censored with minimal effort. However, short-term variation (i.e., 5-minute temperature intervals) between rumen and vaginal-derived core body temperature need to be considered when using MITs to continuously monitor core body temperature in moose.

Monitoring the physiological state of moose is difficult outside of captive settings, but rumen boluses provide a unique opportunity to monitor physiological changes over long periods of time in a minimally-invasive manner. Out of the limited body of research that has utilized rumen boluses, only a single study described the success rates of data transmission when using these devices (Ipema et al. 2008). The low transmission success (44%) with the rumen boluses utilized by these authors and non-reporting of success rates by others (Signer et al. 2010; Turbill et al. 2011) could lead to skepticism when considering the use of these devices for continuous monitoring of body temperature. The high (95.64%) transmission success rates we observed across all seasons, on the other hand, supports the use of rumen boluses to continuously monitor body temperature in free-ranging ungulates. However, we did identify some technical issues with the MITs. First, one moose had its MIT malfunction causing the fix rate to change from 296 seconds to roughly 1 minute, and it subsequently stopped transmitting half way through the study. For this particular MIT, transmissions may have ceased due to battery failure of the device (the collar continued to record GPS locations, collar temperatures, and activity measurements for the duration of the study). Overall MIT transmission success rates worsened as the study progressed, with the lowest rates observed during fall. Signer et al. (2010) reported minimal abrasions and no dissolution effects after 6 months of deployment in domestic sheep. However, we hypothesize that ruminal fluid buildup that results in a deposit layer on an MIT's outer surface could lessen successful data transmission to the collars; considerable build-up had been observed on MITs recovered after deployment in wild moose in MN. Second, we also observed lower MIT transmission success in the 2 moose deployed with VVITs. We are unaware of the specific cause; it may have resulted from a low collar battery life, MIT malfunction, MIT battery life, or behavior. Ipema et al. (2008) showed that behavior significantly affected

transmission success rate. Unfortunately, the transmission window (12.5 seconds) in our MITs limited our ability to link behavior to transmission success. However, the overall high transmission success we observed suggests behavior does not play a significant role in the transmission success rate of MITs in moose.

The use of vaginal temperature as core body temperature has been criticized in the past. Earlier studies on cattle that reported poor correlations between rectal and VIT temperature (Vickers et al. 2010; Hillman et al 2009) were conducted using hand held measurements of rectal temperature. Changes in insertion depth, thermometer movement, and air influx could have contributed to differences in temperatures between the two approaches (Vickers et al. 2010). Burnfield et al. (2011) and Burdick et al. (2012) were able to show a much greater correlation between vaginal and rectal temperature both prior to and during immune stress by using indwelling temperature loggers. Three of the moose in this study had AVIT temperatures affected by environmental factors. We observed abnormally low temperatures recorded by AVITs, presumably due to increased exposure to ambient temperature (i.e., a portion of the implant itself was extending outside of the body). This may have occurred due to a combination of factors specific to these moose (e.g., lower body condition, smaller overall size, and having never calved before). It should also be noted that the temperature sensor placement within the device differed between the two brands; the temperature sensor in AVITs is located more proximal to the antenna and thus more posterior when deployed in a moose than that of the VVITs. This likely affected the offset between the two device brands, and is an important consideration when selecting VITs for monitoring vaginal temperatures in ungulates (i.e., the closer to the cervix the better).

Studies comparing rumen and abdominally-derived core body temperatures observed that rumen temperatures were consistently higher than abdominally-derived core body temperatures by 1°C in cattle (Beatty et al. 2008a) and 0.45°C to 0.75°C in sheep (Beatty et al. 2008b). With the exception of summer, MIT-recorded rumen temperatures in our study, after correcting for behavior bias, were consistently (though minimally) lower (range = -0.01 – -0.13°C), than vaginally-derived core body

temperatures. The narrow scope of environmental conditions (i.e., climate controlled enclosures) in which this was tested in previous studies likely contributed to rumen temperatures being consistently higher than core body temperatures. Rumen temperature, moreover, has been shown to range 1.5 °C from the top of the rumen to the bottom in cattle (Dale et al. 1954). Therefore, the positon of the 120g MIT in the rumen could have influenced the temperature differences we observed. In wild moose in Minnesota, the MIT is most often recovered from the reticulum (M. Carstensen, pers comm.). The temperature gradient within the rumen could occur from both mixing and stratification of newly eaten forage, actively fermenting material, and ingesta at the bottom of the rumen (Dale et al. 1954, Beatty et al. 2008a). The seasonal effects we observed in the rumen- vs. vaginally-measured temperatures relationship were likely influenced by changes in forage intake, forage diversity, and subsequent stratification of actively fermenting material in the rumen. Increases in these factors during summer possibly induced the reversal of the rumen- vs. vaginally-measured temperatures relationship we observed during the other three seasons. Reduction in food intake has been correlated with decreases in rumen temperature (Dale et al. 1954) thus winter forage restriction is likely responsible for why we observed our lowest seasonal MIT temperatures during the cold season.

Few studies have used rumen-derived temperatures for monitoring core body temperatures in ruminants, likely due to concerns regarding the influence of animal's behavior on these temperatures. Our study indicates that rapid changes in MIT-measured rumen temperatures can be attributed to drinking events, with noticeable drops in temperatures detected immediately following water intake. The effect of water intake on MIT temperatures was significant across all seasons. Differences in Δmax amongst seasons were likely influenced by the relatively low frequency, yet copious, consumption of water during the warm season paired with the decreased effect of water intake on rumen temperature during winter. Crater and Barboza (2007) hypothesized that while cold shocks may impair substrate binding of bacteria in the rumen, muskoxen must be able to tolerate the cold shocks associated with water intake throughout the year. We

hypothesize a similar tolerance in moose, as we routinely observed temperature drops in the rumen by as much as 5°C immediately following water intake events. Moose consumed water as snow more often than they did liquid in winter. Although the availability of water during winter is limited, moose may choose snow over water to reduce the cold shock to the rumen, which has been correlated with a reduction in ruminal bacteria numbers and thus food ingestion during winter (Barboza 2007). Ruminal cooling was often rapid, and occurred at a much faster rate than ruminal warming, supporting the trend observed by Barboza (2007).

We developed thresholds (37.66°C and 37.54°C for winter and the warm season respectively) for censoring water intake-affected observations without significantly impacting sample size. After censoring these observations, MIT temperatures displayed less variation than VIT temperatures during all seasons. The approach we developed likely fails to remove a considerable amount of drinking-related temperatures that fall within the range of "normal" rumen temperature because of the slow rewarming rate after drinking as well as the minimal temperature drops observed after winter water intake events. A more conservative option would be to use our approach to identify when water intake events occurred and then remove a set number of MIT temperatures (e.g. 2 hours following water intake event). Our data suggest that, although not significant, there is a mild warming effect of prolonged rumination on MIT-measured temperatures. This was expected since microbial fermentation during rumination is known to increase rumen temperature (Barnes et al. 1983; Czerkawski 1980; Dehority 2003, Lawler and White 2003). Because of the captive nature of the moose at the MRC, paired with low predation risk, we recorded few observations where a moose walked or ran for sustained periods (>30 seconds). This limited our ability to quantify whether increased physical exertion could result in marked changes in MIT-measured temperatures compared with periods of low physical exertion. If future observations are able to capture changes in MIT-measured temperatures due to physical exertion, we could define an upper threshold that could be used to censor measurements using a similar approach to how we identified and removed MIT temperatures affected by water intake behaviors.

The linear relationship we observed between MIT and VIT temperatures corroborates Signer et al. (2010)'s hypothesis that long-term variation in rumen-derived body temperature is highly correlated with core body temperature. It also confirms that a simple model can use MIT-derived temperatures to accurately predict core body temperatures. Seasonal differences between MIT and VIT temperatures, however, highlighted the need for seasonal models. Considerable variability between paired MIT and VIT temperatures was observed with the 5-minute interval schema and suggested the need for temperature averaging. Differences in accuracy between the two devices likely contributed to this observed variability. Averaging temperatures into 15-minute intervals greatly reduced variability between paired MIT and VIT temperatures. Comparing model RMSE with varying temperature averaging schemes demonstrated continuing model improvements as temperature intervals increased, from 15-minute to daily averages. However, no clear break point existed amongst the seasons and RMSE values were low overall. This suggests that while daily temperature averaging results in seasonal models with the greatest predictive power, models utilizing temperature-averaging intervals less than 24 hours can still provide quality VIT predictions. Specific project objectives need to be considered when determining whether body temperature measurements are needed at a finer or longer time resolution.

Fall models had consistently higher RMSE than the other 3 seasons across all averaging schemes. These models were fit with fewer data points than the other seasons due to MIT failure, lower MIT transmission success, and shorter VIT deployment during this season. Further, Wrenn et al. (1958) showed that body temperatures fluctuate throughout the estrous cycle and early stages of pregnancy in cattle; the female moose in our study were bred in September 2015, thus limiting the duration the VITs were deployed as well as possibly contributing to the lower correlation between paired MIT and VIT temperatures and model RMSE during the fall season.

A cross-validation statistic, the root mean squared error (RMSE), that quantified our ability to accurately predict VVIT temperatures was our primary measure of model performance; we found that differences between predicted and observed VVIT

temperatures were on average less than 0.35°C for all seasons and all 3 temperature averaging schemes. Average temperature differences did not always decrease when temperatures were averaged to intervals greater than 15 minutes, but prediction intervals consistently decreased as these intervals increased. Prediction error was consistently higher during summer for all temperature averaging schemes and was likely the result of greater daily fluctuations in both MIT and VIT temperatures combined with increased exposure to higher ambient temperatures. Model predictions at higher VIT-derived body temperatures (>38.5°C), suggest that core body temperature is lower than MIT-derived temperatures more often than during VIT-derived temperatures less than 38.5°C. This was driven in part by the low number of MIT and VIT temperatures greater than 38.5°C that were observed. Our simulations confirmed that prediction accuracy decreases as MIT temperatures move away from the mean (i.e., increase in temperature). However, prediction error was low overall, with the biggest prediction interval (0.73°C) observed for the summer model utilizing 15 minute temperature averaging. The strong circadian rhythm observed with both MIT and VIT temperatures during summer suggests that predicted VIT temperatures during this season could be used to investigate how moose maintain a thermal balance during times of increased ruminal heat load and exposure to ambient temperatures outside of their thermoneutral zone. Beatty et al. (2008a) observed a more pronounced circadian pattern of body temperatures, both rumen and rectal-derived, as ambient temperatures increased. Using the approach we developed for correcting MIT-derived body temperatures and predicting core body temperatures, research efforts should focus on how ambient temperatures outside of moose thermal thresholds, as defined by Hudson and Renecker (1986) and McCann (2013), affect survival, habitat use, and reproductive rates in free-ranging moose.

MANAGEMENT IMPLICATIONS

We demonstrated that, with minimal censoring and data correction, minimally invasive rumen boluses such as MITs can provide unprecedented information related to a

free-ranging ruminant's physiological responses to its environment. This has direct applications for understanding, among others, moose physiological and behavioral responses to increasing ambient temperatures in Minnesota, where MITs were recently deployed in 60 free-ranging moose. On a broader scale, MITs may prove a useful tool with other large-bodied ruminants (i.e., *Ovis canadensis canadensis*) by providing the physiological evidence needed to link reduced fitness to climate change and provide guidance on how to manage imperiled populations in the future.

Table 1. Summary statistics of water intake behaviors for 8 captive adult female moose (>2 years of age) deployed with ATS vaginal implant transmitters during behavioral observations conducted in both winter and the warm season (spring, summer and fall) of 2015 at the Moose Research Center, Kenai Peninsula, Alaska. Parentheses indicate standard deviations.

Device	Season	N_d*	Mean T_d*	ΔT*	Mean Time*
MIT					
	Winter	68	44.19 ($\pm$63.85)	-0.23 ($\pm$0.26)	35.95 ($\pm$43.28)
	Warm	28	40.25 ($\pm$35.62)	-2.27 ($\pm$1.85)	25.29 ($\pm$14.33)
VIT					
	Winter	68	39.58 ($\pm$52.18)	-0.13 ($\pm$0.13)	31.60 ($\pm$16.07)
	Warm	28	13.40 ($\pm$14.64)	0	0

*N_d represents the number of water intake behaviors observed during the given season. Mean T_d represents the mean time in seconds spent consuming water during a six-hour observation time period. ΔT represents the mean temperature change associated with one water intake bout. Mean Time represents the time (minutes) it took for the device temperature to return to within one standard deviation of the device temperature at the time of the preceding water intake behavior.

Table 2. Coefficient estimates from linear mixed-effect models relating temperatures measured using ATS vaginal implant transmitters (VIT) to mortality implant transmitters (MIT). Separate models were fit to 15 minute, 255 minute, and daily averaged ATS VIT-measured and MIT-measured temperatures over four seasons. Models were built using data for 5 captive adult female moose at the Moose Research Center, Kenai Peninsula, Alaska during 2015.

Model	Season		β^*	SE^*	t^*	P^*	$RMSE^*$
15-min avg	Winter	Intercept	38.21	0.12	330.26	<0.01	0.26
		Centered-MIT	0.20	0.06	3.49	<0.01	
	Spring		38.19	0.12	308.53	<0.01	0.29
			0.18	0.06	2.78	<0.01	
	Summer		38.29	0.03	1176.06	<0.01	0.25
			0.17	0.05	3.58	<0.01	
	Fall		38.09	0.12	313.27	<0.01	0.30
			0.16	0.06	2.68	<0.01	
255-min avg	Winter	Intercept	38.24	0.11	346.38	<0.01	0.23
		Centered-MIT	0.24	0.05	4.67	<0.01	
	Spring		38.20	0.12	306.14	<0.01	0.24
			0.20	0.07	3.03	<0.01	
	Summer		38.18	0.05	784.59	<0.01	0.19
			0.24	0.06	4.303	<0.01	
	Fall		38.10	0.12	314.98	<0.01	0.28
			0.17	0.06	2.90	<0.01	
Daily avg	Winter	Intercept	38.28	0.08	462.52	<0.01	0.20
		Centered-MIT	0.25	0.02	14.43	<0.01	
	Spring		38.28	0.06	639.48	<0.01	0.15
			0.28	0.01	17.04	<0.01	
	Summer		38.17	0.04	901.79	<0.01	0.13
			0.24	0.02	13.03	<0.01	
	Fall		38.09	0.10	390.048	<0.01	0.27
			0.12	0.03	4.18	<0.01	

*β represents the coefficient estimate associated with each model. SE error represents the standard error associated with each coefficient estimate. t represents the t-statistic associated with the estimated parameter. P represents the p-value associated with the estimated parameter. RMSE represents the overall root mean squared error of all cross-validations for the corresponding season. Cross-validations were calculated using a k-fold procedure in which 1 moose was withheld from model building and the process repeated for all individuals.

Table 3. Temperature offset and standard error (SE) between ATS and Vectronic vaginal implant transmitters for 10 moose across four seasons from December 2014 to December 2015 at the Moose Research Center, Kenai Peninsula, Alaska.

	Temperature	
Season	**Difference (°C)***	**SE**
Winter	-0.37	0.14
Spring	-0.25	0.15
Summer	-0.20	0.15
Fall	-0.29	0.22
All	-0.31	0.14

*Differences were calculated from ATS VITs

Table 4. Mean difference between predicted and observed vaginal-derived body temperature in 2 moose deployed with Vectronic vaginal implant transmitters across different seasons from December 2014 to December 2015 at the Moose Research Center, Kenai Peninsula, Alaska.

Season	Averaging (min)	Mean Temperature Difference (°C)	Proportion (%)*	n
Winter	15	0.02	99.73	12159
Winter	255	0.05	99.89	945
Winter	1440	0.07	100	168
Spring	15	0.04	99.23	9649
Spring	255	0.07	99.86	702
Spring	1440	0.17	100	125
Summer	15	0.29	99.62	2123
Summer	255	0.33	99.58	474
Summer	1440	0.35	100	91
Fall	15	0.07	98.48	1719
Fall	255	0.09	98.23	452
Fall	1440	0.08	97.78	90

*Proportion (%) represents the percentage of predictions intervals that included the observed VVIT temperatures.

Figure 1. Map of the Moose Research Center in Game Management Unit 15A, Kenai Peninsula, Alaska.
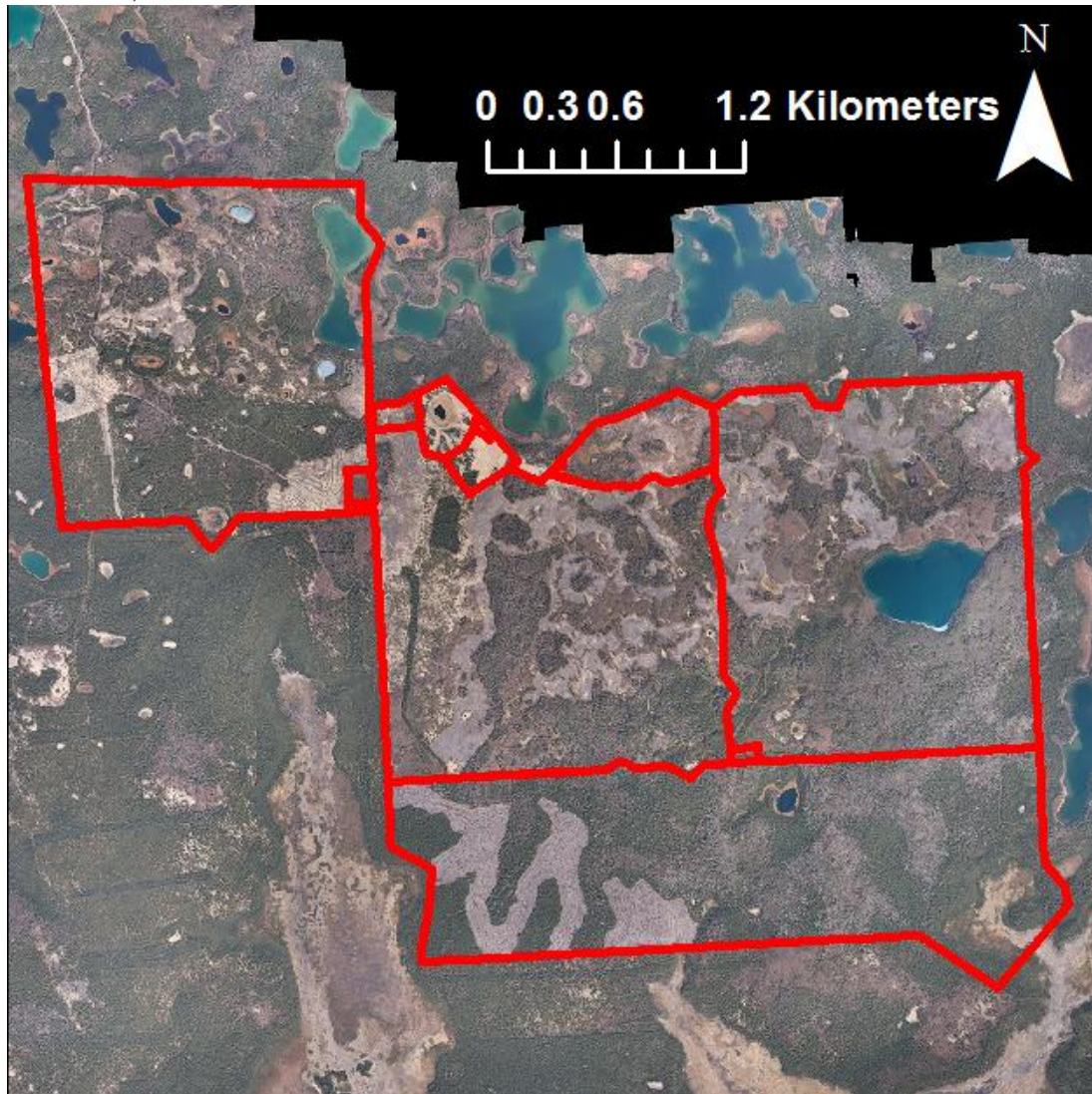
Figure 2. Oral deployment of a Mortality Implant Transmitter during the December 2014 immobilizations, Moose Research Center, Kenai Peninsula, Alaska.

Figure 3. Visualization across space (A) and time (B) of mortality implant transmitter temperatures paired with drinking behaviors during one 6-hour observation period in April 2015 at the Moose Research Center, Kenai Peninsula, Alaska.
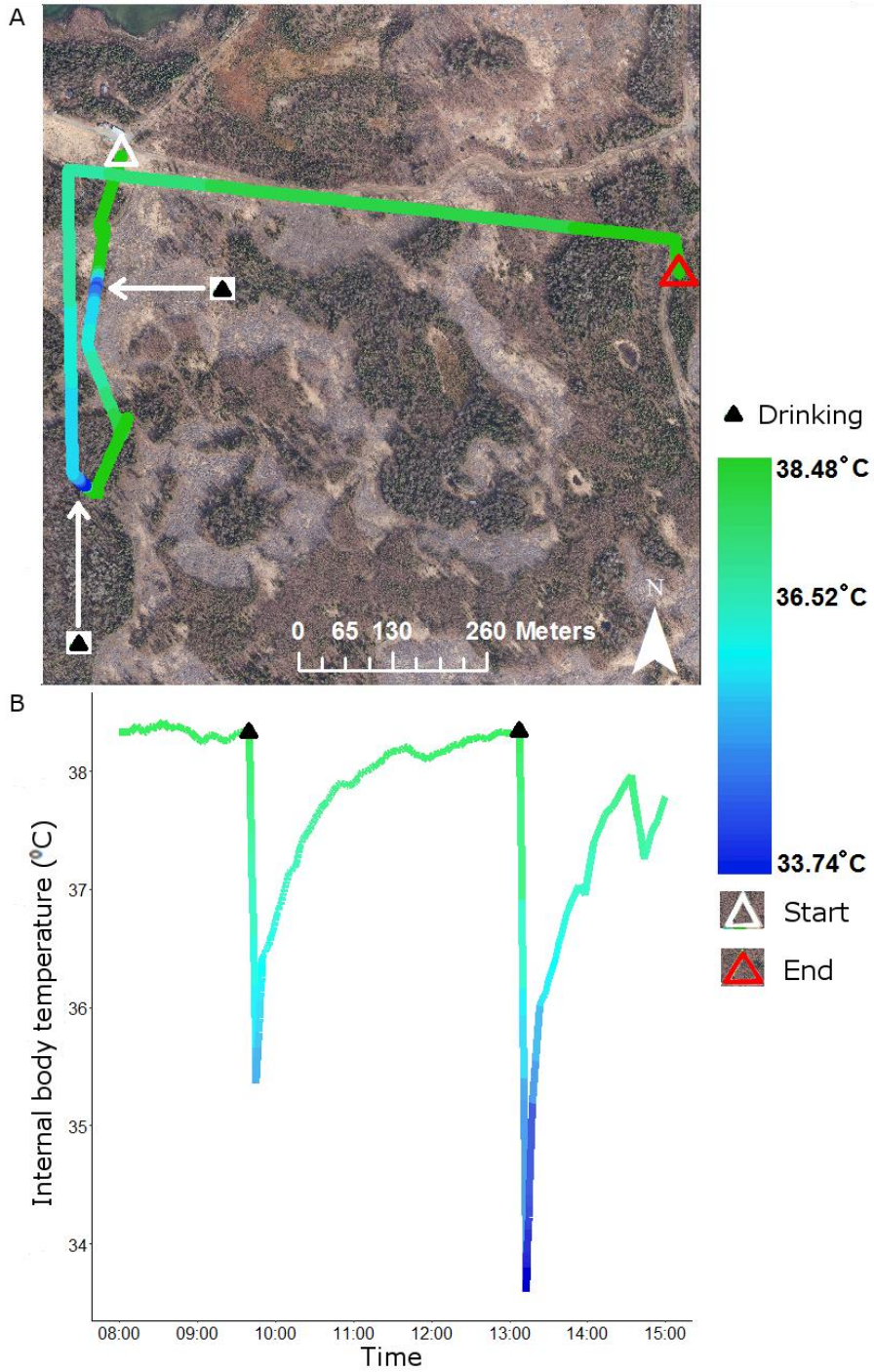
Figure 4. Visualization of temperature thresholds proposed for removing mortality implant transmitter (MIT) temperature measurements likely to be impacted by water intake during the winter and warm (spring, summer, and fall) seasons at the Moose Research Center, Kenai Peninsula, Alaska, during 2015.
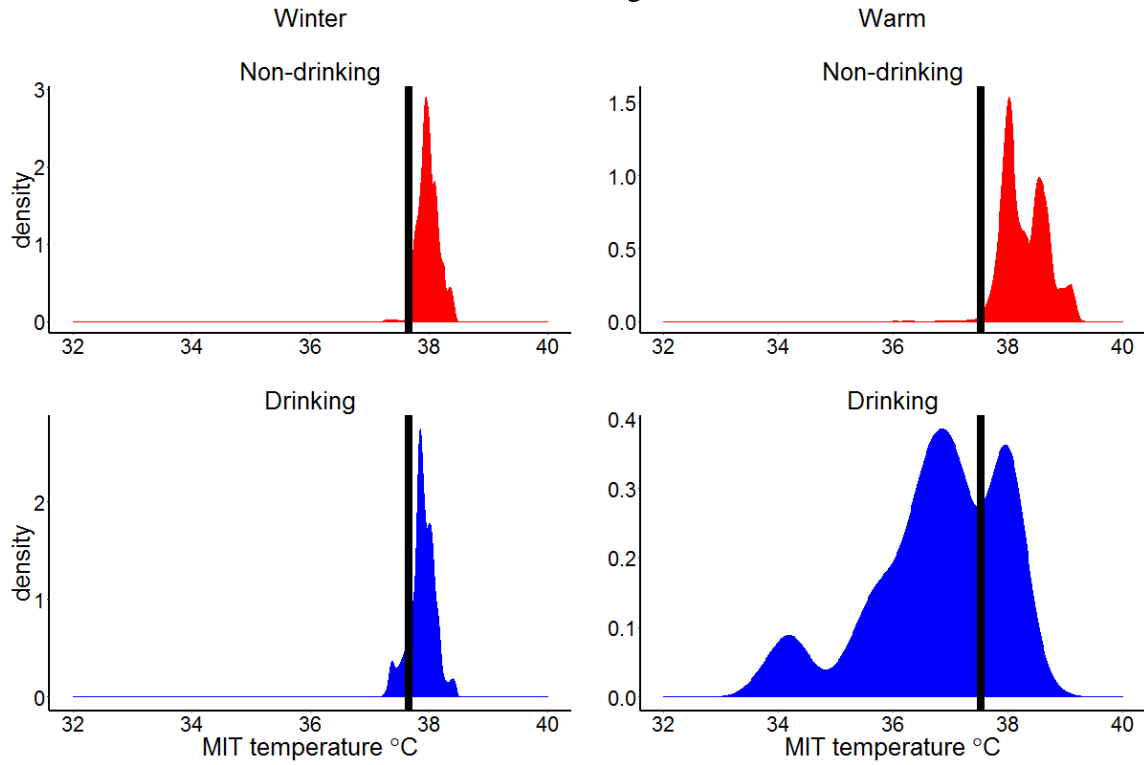
Figure 5. Variability in body temperatures measured using mortality implant transmitters before and after correcting for water intake across seasons for five moose deployed with ATS vaginal implant transmitters at the Moose Research Center, Kenai Peninsula, Alaska, during 2015.
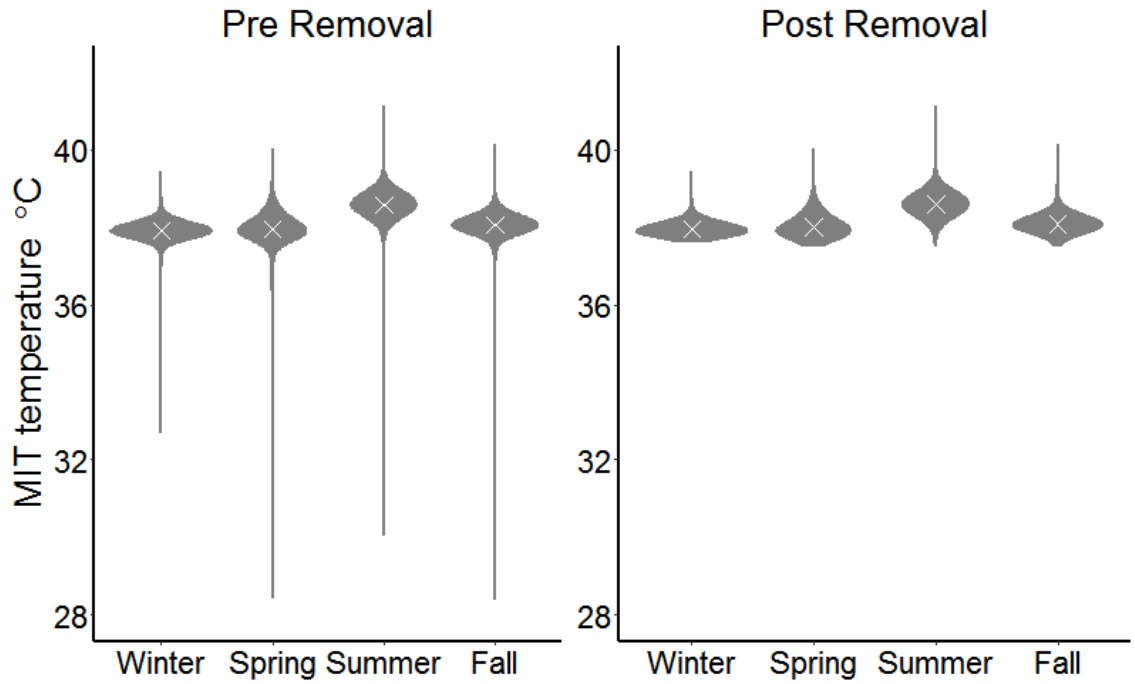
Figure 6. Visualization of the circadian rhythm of ambient temperatures, mortality implant transmitter- and ATS vaginal implant transmitter-derived temperatures of 5 moose across seasons at the Moose Research Center, Kenai Peninsula, Alaska, during 2015.
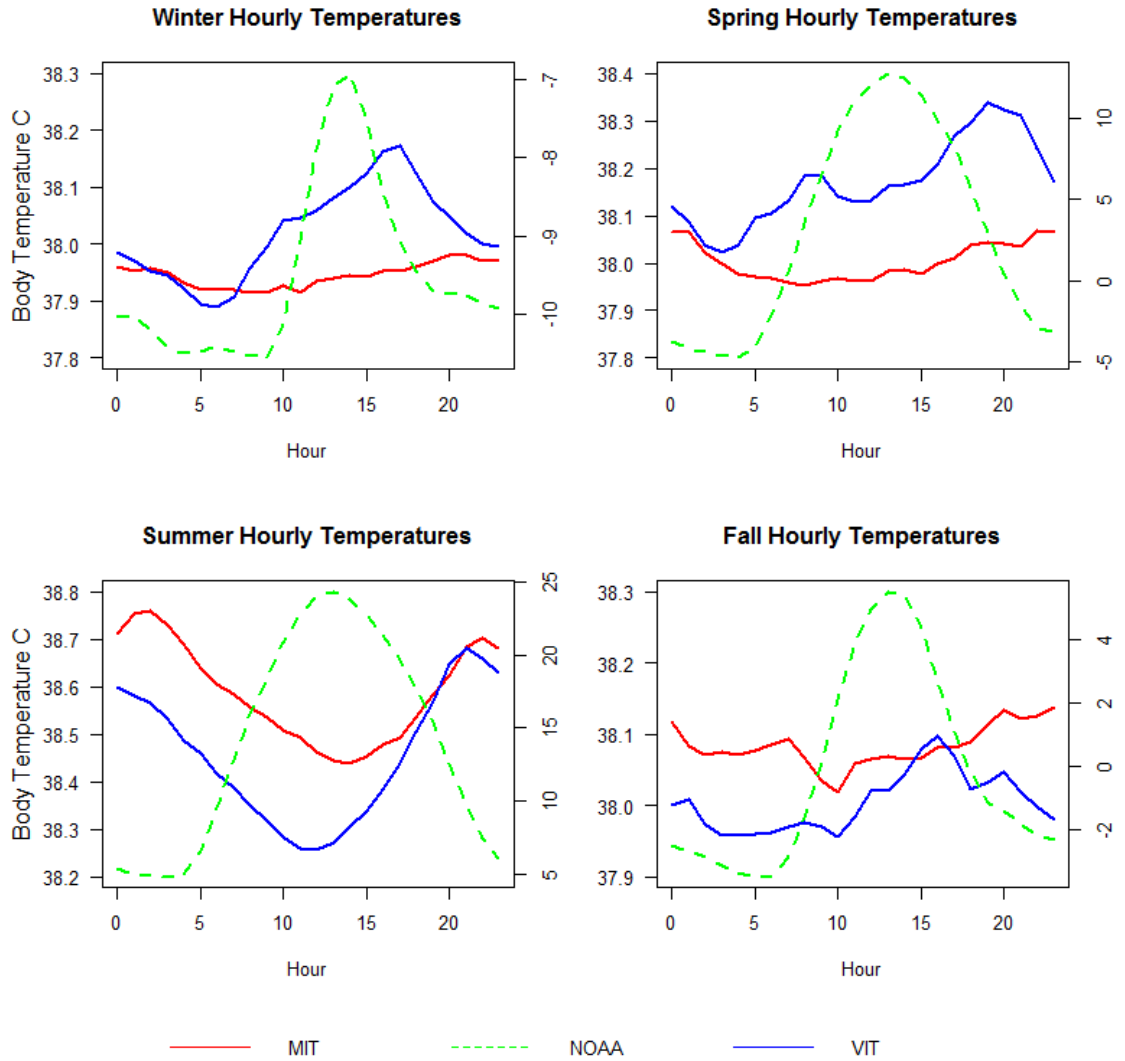
CHAPTER 2

Predicting behavioral states in moose using dual-axis

activity sensors

INTRODUCTION

Advancements in biotelemetry since the 1980's have greatly improved the ability of biologists to study the physiology, behavior, and energetic needs of free-ranging animals (Cooke et al. 2004). Researchers have gained insight into the ecology and behavior of wildlife species that are typically difficult to study, while simultaneously reducing the potential for the observation protocol to affect animal behavior (Ware et al. 2015). These methods may also be used to investigate diel and seasonal, fine-scale activity patterns in relation to broader-scale habitat use; these patterns are fundamental for understanding behavioral ecology and the complex trade-offs between balancing an organism's physiological needs in response to environmental pressure and habitat availability. Understanding fine- and broad-scale spatial and temporal patterns in activity and habitat use has direct applications for the management and conservation of imperiled species (Gervasi et al. 2006). Biometric technologies, such as Global Positioning System (GPS) collars, have reached a point where we can now study these interactions at spatial and temporal scales much finer than what was previously possible.

Global positioning system collars offer many advantages over traditional Very High Frequency (VHF) collars including, among others: greater sampling frequency (especially where sampling is limited by personnel, diel, budget, and/or weather constraints), higher spatial accuracy, and no observation disturbance (Obbard et al. 1998). Many of today's GPS collars are also equipped with activity sensors that detect and record changes in neck movements as a measure of an animal's activity (Ungar 2005). These sensors provide a unique opportunity for researchers to understand and remotely predict behavioral states in free-ranging animals (Löttker et al. 2009, Ungar et al. 2010, Roberts et al. 2016). Though progress in biotelemetry technology has allowed activity sensors to decrease in size and weight while simultaneously recording more data (Kooyman 2004), battery life and data storage capacity are still the main constraining factors in many activity sensor applications (Hasley et al. 2009). Despite the potential to gain insight into the interactions of an animal and its environment using behavioral states classified from activity sensors, few studies have taken advantage of this technology in part due to a lack of knowledge regarding how well activity sensor measurements

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correlate with specific behaviors. Increasing our understanding of the relationships between sensor readings and animal behaviors could allow researchers to better understand these interactions while simultaneously refining recording intervals, thus maximizing battery life and storage capacity of collars and allowing for more effective study designs.

Some of the first attempts to pair animal movements with activity sensor data utilized locations obtained from VHF collars and tilt-switch activity sensors. Studies using these tilt-switch sensors have been able to distinguish active versus resting states with varying degrees of success for mule deer (*Odocoileus hemionus*; Relya et al. 1994), caribou (*Rangifer tarandus*; Maier and White 1998) and American black bears (*Ursus americanus*; Garshelis and Pelton 1980), among others. Technological advancements in the early 1990's led to the development of activity sensors capable of detecting movement on both horizontal (X-axis) and vertical (Y-axis) planes. These sensors are able to provide a measure of both intensity and duration of collar movement (Gervasi et al. 2006). Researchers have used these dual-axis activity sensors to distinguish foraging and resting bouts in white-tailed deer (*Odocoileus virginianus*; Coulombe et al. 2006), active versus inactive periods in bears (*Ursus thibetanus japonicas*, *Ursus arctos*; Gervasi et al. 2006; Yamazaki et al. 2008; Kozaki et al. 2008), elk (*Cervus canadensis*; Roberts et al. 2016), and moose (*Alces alces*; Moen et al. 1996), and resting, grazing, and traveling behaviors in cattle (*Bos taurus*; Ungar et al. 2005; Augustine and Derner 2013). More recently, activity sensors capable of continuously recording and storing raw acceleration for three axes (tri-axis) were used to accurately detect and predict fine-scale behavioral states in puma (*Puma concolor*; Wang et al. 2015), cattle (Ungar et al. 2010), Eurasian badger (*Meles meles*; McClune et al. 2014), cheetahs (*Acinonyx jubatus*; Wilson et al. 2013), and griffon vultures (*Gyps fulvus*; Nathan et al. 2012). However, tri-axis sensors are limited by battery life and have not gained popularity until recently; thus, the majority of GPS-collars continue to be equipped with less sophisticated tilt switch and dual-axis activity sensors, which are less battery intensive. Although future advancements in biotelemetry technology may eliminate or significantly reduce problems associated with limited battery life and data storage, there is still a pressing need to refine and improve

how we use data from commonly deployed sensors to increase our understanding of behavioral interactions between free-ranging animals and their environment.

Previous studies have described the numerous problems encountered when attempting to classify behaviors using activity sensor data (tilt switch, dual- and tri-axis) obtained from GPS collars (Löttker et al. 2009). The majority of studies using dual-axis activity sensors have been unsuccessful at discriminating beyond coarse (active vs rest) behaviors (Adrados et al. 2003, Coulombe et al. 2006, Gervasi et al. 2006). Löttker et al. (2009) was able to successfully classify three behavioral categories (resting, feeding/slow locomotion and fast locomotion) using dual-axis sensors but highlighted issues that arose from building models using only observation intervals in which one behavioral state was observed. For example, free-ranging ungulates rarely exhibit singular behaviors for long periods of time (Gottardi et al. 2010) and there is a greater potential for misclassification of behaviors such as vigilance (Moen et al. 1996; Ungar et al. 2005). Studies that constructed models based on observation intervals of only one behavior state or chose to convert behavioral states to the mode, observed much higher misclassification rates when applying predictive models to intervals containing more than one behavioral state (Moen et al. 1996, Löttker et al. 2009). Further, nearly all validation studies have utilized captive animals due to the difficulty of observing many free ranging species, and were often reliant upon very limited observation hours and/or just a few animals. These issues have greatly limited researchers' ability to translate behavioral classifications of captive animals' behaviors to free-ranging individuals. To address these limitations, we designed a study of moose behaviors and developed a dual-axis activity sensor-based approach that can be used to predict the species' behavioral states.

Moose (*Alces alces*), a large bodied endotherm, are experiencing lower survival rates at the southern edge of their range compared to core geographic range (Dodge et al. 2004; Murray et al. 2006; Maskey 2008; Lenarz et al. 2010). In Minnesota, moose in the northwestern portion of the state are all but extirpated (Murray et al. 2006), and the northeastern population has declined from an estimated 8,840 in 2006 to 3,710 in 2016 –

a reduction of 55% (DelGuidice 2017). Although the ultimate driver of the northeast population decline remains unknown, recent research has demonstrated that the majority of moose mortalities can be attributed to health-related causes (Murray et al. 2006; Carstensen et al. 2014). Diseases, parasites, predators, habitat alteration, and climate change are all factors that may be contributing, alone, or in concert, to the population decline in Minnesota (Murrary et al. 2006; Lenarz et al. 2009, Lenarz et al. 2010; Carstensen et al. 2014, Mech and Fieberg 2014). Moose are known to be physiologically sensitive to heat (Renecker and Hudson 1986; Renecker and Hudson 1989; McCann et al. 2013) and to alter their habitat selection when ambient temperatures increase (Schwab and Pitt 1991; van Beest et al. 2012; Street et al. 2015; Street et al. 2016) by selecting for habitats that act as thermal refuges (Dussault et al. 2004). This makes them an ideal candidate for examining how environmental changes may affect the behavior of a mammal occupying its bioclimatic edge. The ability to predict behavioral states from GPS-collars equipped with dual-axis activity sensors may offer insights into how moose behavior changes in response to its environment.

The goal of this study was to develop an approach to predict the proportion of time moose spend in different behavioral states over a given time period using activity sensor data. Specifically, our objectives were to: 1) determine if dual-axis activity sensors can accurately classify behavioral states in moose, and if so, 2) develop a predictive model that can be used to remotely infer behavioral states, and 3) examine the potential for using remotely predicted behavioral states to investigate behavioral responses of moose to changes in habitat, ambient temperatures, and time of day. We utilized multivariate multinomial regression models to examine how well dual-axis activity sensors from GPS-collared captive moose in Alaska can predict three behavioral states (resting, moving, foraging). Unlike previous studies, we did not constrain our analysis to time intervals that encompassed only one behavioral state, but focused on predicting the composition of behaviors within each observation window from the sensor. Validating the activity sensors in a captive setting has direct applications for understanding moose behavioral responses to habitat and increasing ambient temperatures in Minnesota, where

GPS collars equipped with dual-axis activity sensors were recently deployed on 173 free-ranging moose in an effort to gain a better understanding of moose behavioral and physiological responses to ambient temperatures and habitat, among others (Carstensen et al. 2014).

STUDY AREA

The study was conducted at the Kenai Moose Research Center (MRC) located on the Kenai Peninsula, Alaska (Figure 1). The MRC, a 2400-acre captive facility operated by the Alaska Department of Fish and Game, was built in the 1960's to study moose's responses to their environment (Hundertmark et al. 2000). All moose in this study were maintained in outdoor enclosures approximately 240 ha in size. Each enclosure encompassed a mix of habitat types including open meadows, bogs, open crushed forest areas, aspen/birch (*Populus tremuloides, Betula neoalaskana*; ~25 year old), black spruce (*Picea mariana*; ~125 year old), and aspen/white spruce (*Populus tremuloides, Picea glauca*;~125 year old) stands, as well as lakes. The moose we observed cohabitated in two different enclosures and were maintained at densities of 4-8 adult females per enclosure (30-60 ha/moose), depending on the time of year and conspecific aggression. Moose naturally foraged within the enclosures and were only supplemented during times of low nutritional condition, handling (e.g., weighing), or during specific studies. Moose had access to water from lakes and wetlands throughout the enclosures. To supplement water intake during the warm season (late spring to early fall) cattle troughs were available in enclosures with fewer wetlands. While predation risks were low, encounters with brown bears (*Ursus arctos*), black bears (*Ursus americanus*), and wolves (*Canis lupus*) occur sporadically within the enclosures (Dan Thompson, pers comm.).

METHODS

Captive Moose Handling

A total of 10 moose were immobilized during four routine immobilization periods at the MRC (December, April, June, and September). Five of those (>3 years old) were

chemically immobilized with 0.45 mg of carfentanil (Zoopharm, Windsor, Colorado) and 25 mg of xylazine (Henry Schein, Melville, New York), while the other five (between 2 and 3 years old) were chemically immobilized with 0.30 mg of carfentanil and 15 mg of xylazine. Tolazaline (400 mg; Zoopharm, Windsor, Colorado) and naltrexone (100 mg; Zoopharm, Windsor, Colorado) were used to reverse each moose. Each of the 10 moose was fitted with a uniquely marked (i.e., color taped) Vectronic GPS collars (GPS Plus Iridium; Vectronic Aerospace GmbH; Berlin Germany) to facilitate individual recognition by the observers. The GPS collars recorded location data on board in 30-minute intervals. We disabled satellite communication for prolonging the collars' battery life; all data were downloaded after removal. Each GPS collar was equipped with a dual axis acceleration sensor, generating acceleration values on both a horizontal (X-value) and vertical (Y-value) plane. Accelerometer counts (0–±255) for each axis are generated in quarter second intervals as an absolute value. The differences in accelerometer values between each successive quarter second interval (minus 5 to reduce accelerometer noise) are calculated and summed over the 296-second interval. The resulting cumulative value is scaled by 250, with values >255 capped at 255, so that ultimately values range from 0 to 255 for each of the two axes. Activity intervals will be referred to as 5-minute intervals going forward for simplicity. In addition to geographic location and activity, the GPS-collars also recorded ambient temperature in 5-min intervals. If needed, the GPS collars could be removed, adjusted, and/or replaced at any point in the study without the need for immobilization.

Captive Moose Observations

To determine how both horizontal and vertical activity sensors relate to moose behavior, we conducted behavioral observations on 8 captive moose during 6-hour long intervals. To account for any seasonal effect, we observed animals in 2-week long observations period in January, April, July, and October. Each animal was observed twice during each of these periods, totaling 48 hours of observations per moose over the entire study and 384 observation hours for all animals. The 6-hour observation intervals were spread randomly throughout the day from 600 to 2200 hours, and observations were

made during all weather conditions. During an observation window the observer would stand at a distance ≤10 m from a given moose, and record the exact time when a new behavior occurred using Recon/Juno data loggers (Trimble Navigation Limited Trimble, Sunnyvale, California) to the nearest second in a procedure similar to Moen (1996). The behaviors that were recorded included: foraging low (snout below the bottom of stomach), foraging medium (snout above the bottom of the stomach and below the top of the shoulders), foraging high (snout above the top of the shoulders), resting, ruminating, drinking/eating snow, walking, standing, running, shaking, grooming, and interacting (i.e., boxing).. A 6-hour observation interval might contain missing data in instances where visual contact was lost with the observed moose (i.e. spooked and ran). If this happened, the moose being observed was relocated using VHF telemetry and missing observations were removed from subsequent analyses. All animal handling procedures were approved by the Animal Care and Use Committee, Alaska Department of Fish and Game, and Division of Wildlife Conservation (protocol No. 09–29).

Wild Moose Handling

Moose were captured in northeastern Minnesota from 2013-2015 ($n = 173$) by aerial darting (Quicksilver Air Inc., Alaska) with carfentanil (4.0, 4.5mg or 6.0mg; Zoopharm, Windsor, Colorado), or thiafentanil (16mg) and xylazine (150mg or 30mg; Henry Schein, Melville, New York) from a helicopter. Moose were fitted with a Vectronic GPS collar (GPS Plus Iridium; Vectronic Aerospace GmbH; Berlin Germany). Collars collected and transmitted geographic location in 255-minute intervals to a base station (Forest Lake, MN). External temperature loggers (Length: 30 mm, diameter: 41 mm; thickness: 17 mm; Onset Computer Corporation, Bourne, Massachusetts) were affixed to the outside of each collar canister and were programmed to collect ambient temperature in 60-minute intervals. For a more detailed description of the capture methods refer to Carstensen et al. (2014). Immobilizations were reversed with naltrexone (425–575mg; Zoopharm, Windsor, Colorado) and tolazoline (400mg; Zoopharm, Windsor, Colorado).

Weather and Temporal Covariates

We obtained weather conditions from the National Oceanic Atmosphere Administration (NOAA) Climate Reference Network (CRN) weather station located at the MRC (Alaska, USA, 66.7251, -150.4493; https://www.ncdc.noaa.gov/crn/qcdatasets.html) for the captive moose. Weather data were collected in 5-minute intervals and linearly interpolated to match the exact time stamps of GPS locations and activity data, respectively. For the Minnesota wild moose analyses we used weather conditions from the NOAA weather station located at the Ely Municipal Airport (Minnesota, USA, 47.8237, -091.8293; https://www.ncdc.noaa.gov/isd/data-access). Weather data were collected approximately once per half hour and linearly interpolated to match the exact time stamps of GPS locations and activity data, respectively. The angle of the sun was calculated to further test how it might influence moose behavior; values were < 0 when the sun was below the horizon and > 0 when the sun was above the horizon. For example, crepuscular times are centered on zero with lowest values corresponding to the middle of the night and the highest values to mid-day when the sun is at its highest point. Seasons were assigned as follows for both MRC and MN moose: winter (1 November-31 March), spring (1 April-30 May), summer (1 June-31 August), and fall (1 September-31 October). Solar angles changed with each season, with larger negative values occurring during winter (i.e., less daylight) and greater positive values during summer (i.e., more daylight)

Captive Moose Analyses

Time stamps of NOAA temperature measurements, behavioral observation, GPS locations and activity sensor data were not always the same; consequently we linearly interpolated temperature measurements and GPS locations between consecutive time stamps to match activity sensor time stamps. We assumed that within a 5-minute interval, any rise or decrease in ambient temperature was most likely linear. Behaviors were classified into the following three categories due to the overlap in X- and Y-activity

values of many behaviors as well as the large number of 5-minute intervals consisting of >1 behavior: resting, foraging, and moving (Table 1).

We first calculated the proportion of time spent in each behavior category for every 5-minute activity interval by summing up the total time spent in each behavior category and dividing up by the total interval time (~5 minutes). All behavioral proportions within a 5-minute activity interval summed to 1. The incorporation of movement path characteristics (e.g., step length – the distance between two consecutive GPS relocations) into activity models was shown to allow for better distinction between resting behaviors with increased head movements and low exertion foraging/traveling behaviors (Gervasi et al. 2006). We therefore incorporated step length into some of our models. Because the temporal resolution differed between GPS locations and activity sensor data, we linearly interpolated GPS locations between consecutive 30-minute GPS locations to match the time stamps of the 5-min long behavior intervals, therefore assuming linear movements between GPS locations.

We used compositional Dirichlet regression models to quantify the relationship between the proportion of time spent resting ($R_{i,j}$), foraging ($F_{i,j}$) and moving ($M_{i,j}$) for each moose (i-th) within each 5-minute interval (j-th) as a function of X- ($X_{i,j}$) and Y-values ($Y_{i,j}$) as well as step length (Maier 2014). The Dirichlet distribution, often used as a prior in the Bayesian analysis of multinomial data, is not normally thought of as a response distribution (Gueorguieva et al. 2008). Dirichlet is well suited for both skewed and constrained data (e.g., proportions of behavioral states). The Dirichlet distribution is a generalization of the beta distribution to higher dimensions, with k-dimensional vectors x whose entries are real numbers in the bounded continuous interval (0,1) and $\sum x_i = 1$. We utilized the "common" parameterization and modeled each concentration parameter (α): where $\propto_i > 0$, $i = 1$:number of behavioral states (resting, foraging, moving), $j = 1$:number of activity intervals, and defined as:

Concentration:
$$log(\propto_{i,j}) = \beta_{0_i} + \beta_{1_i}X_j + \beta_{2_i}Y_j + \beta_{3_i}\,Step_j$$

Mean:

$$E(B_i) = \frac{\propto_i}{\sum_k \propto_k}$$

Variance:

$$Var(B_i) = \frac{\propto_i(\propto_0 - \propto_i)}{\propto_0^2(\propto_0 + 1)}$$

$$\propto_0 = \sum_k \propto_k$$

Six models within each season were built: 1) $X_{i,j}, Y_{i,j}$, 2) sum($X_{i,j}, Y_{i,j}$), and 3) $X_{i,j} * Y_{i,j}$, with or without the addition of step length for a total of 18 models across all seasons. We evaluated models using a holdout cross-validation approach in which the data were randomly divided into two sets: training (70%) and validation (30%). Models were fit to the training set and then used to predict proportions of time spent resting, foraging, and moving during each 5-minute activity interval in the validation set. The predictive accuracy of the models was assessed using root mean squared error (RMSE), defined as: $\sqrt{\sum_j \frac{\frac{\sum_i(y_{i,j} - \hat{y}_{i,j})^2}{N_j}}{k}}$. Where RMSE is the overall root mean squared error of all validations, $y_{i,j}$ is the i-th proportion of the specified behavior (i.e. resting) of the j-th validation, $\hat{y}_{i,j}$ is the i-th predicted proportion of the specified behavior (i.e. resting) of the j-th validation, N_j is the number of observations of the j-th validation, and k is the number of validations. The best model, as determined by the lowest RMSE, was then fit using all observational data and used to predict proportions of time spent resting, foraging, and moving for all 5-minute activity intervals outside of observational time periods.

We examined the effects of ambient temperature, time of day (i.e., solar angle), and habitat on the proportion of time spent resting, foraging, and moving using our best predictive model that included step length. We first predicted behavioral states for 5-minute intervals that occurred during our observation periods to better understand the accuracy of using predicted data to examine these effects. Mean and 95% confidence intervals were calculated for behavioral predictions and displayed using loess curves of

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1000 bootstrapped samples. Observed proportions were binned into 5% solar angles and compared to predictions. We then predicted behavioral states for all 5-minute intervals that occurred outside of observational time periods; predictions were binned into 5% ambient temperature quantiles to better visualize trends. Means and 95% confidence intervals were calculated for each bin using a bootstrap with 1000 iterations. The same procedure was utilized to examine trends between predicted behavioral states and solar angle. To relate habitat use to changes in behavioral states, we first determined habitat use by spatially intersecting GPS locations with habitat in ArcMAP 10.2 (ESRI 2013). Habitats were derived by the Alaska Fish and Game from a combination of satellite imagery and ground verification. Habitat types consisted of the following: Aspen, birch, water, bog, black spruce, mixed, grass, grass/black spruce, mixed closed, black spruce/birch, grass/black spruce/birch. To examine the seasonal effects of habitat in relation to changes in behavioral state and time of day, behavioral predictions were binned into 5% solar angles for each habitat within each season. Means and 95% confidence intervals were calculated for each bin using a bootstrap with 1000 iterations.

Wild Moose Analyses

We utilized the model without step length that best predicted the captive moose observations to predict behavioral states in wild moose and evaluate the feasibility of applying our models in other systems. We predicted proportions of rest, foraging, and moving during 5-minute activity intervals using data recorded for three wild moose in northeastern Minnesota during 2014. These moose, who represent only a small subset of a large number of moose GPS collared in northeastern Minnesota from 2013-2015 ($n =$ 173), were utilized as an example here because they had at least 1 full years' worth of locational and activity data. Timestamps of GPS locations and activity data were matched temporally using the aforementioned approach. The larger intervals between GPS locations (255 minutes) prevented the use of step length in model predictions.

We used the behavioral states predicted from the model to examine how changes in behavioral state relate to changes in ambient temperature, time of day, and coarse habitat class. Paired predictions of behavioral state and ambient temperature/solar angle

were binned and bootstrapped as outlined above. We spatially intersected GPS location data with land cover data derived from LANDSAT and Lidar with 1 meter resolution (J. Knight, unpublished data) using ArcMap 10.2. Habitats were classified as developed (i.e., roads and buildings), wetlands, open water, extraction (mining), forest (coniferous, deciduous, and mixed), grassland, and agriculture. Because step length could not be used in model predictions, we predicted behavioral states for the 5-minute activity interval immediately preceding each GPS location; however, this prevented the use of 98% of 5-minute activity intervals for habitat use patterns.

All statistical analyses were conducted using the R statistical software (R Core Team 2017, with the maptools (Biyand and Lewin-Koh 2015), moveHMM (Michelot et al. 2015), DirichletReg (Maier 2014) and XTS libraries (Ryan and Ulrich 2013).

RESULTS

Captive observations

We classified behaviors during direct observations for 4608 5-minute intervals from 8 moose spread across 4 seasons. Two 6-hour observation windows had to be removed from the analysis due to a misalignment of the Trimble clock and the GPS collar time. Two GPS collars failed over the course of the study, 1 during April 2015 and the other during September 2015. After accounting for these issues as well as removing any intervals in which visual contact was lost between the observer and the moose, 3501 5-minute intervals (291.75 hours) remained. Moose rested more during summer observation periods (Table C.1), with 67% of the time moose were observed at rest, 25% as foraging, and 8% as moving. During spring (Table C.1), the observed moose spent more time foraging relative to the other seasons; we classified 40% of the time moose were observed foraging, 54% as resting, and 6% as moving. The proportion of time spent moving was similar for all seasons and ranged from 6% to 8% (Table C.1). Of these 3501 5-minute intervals, 1559 consisted entirely of resting behaviors, 106 foraging behaviors, while none consisted of only moving behaviors. The majority of 5-minute intervals (*n* = 1836) consisted of more than one target behavior category (resting, foraging, and/or moving; hereafter referred to as mixed intervals) (Table C.2). Mean X and Y values were

lowest during pure resting intervals ($\bar{x}_X = 1.71 \pm 6.21$ [SD]; $\bar{x}_Y = 0.84 \pm 5.09$) and highest for mixed intervals (X-value $\bar{x}$ 39.13 ± 23.75; Y-value $\bar{x}$ 28.90 ± 24.18; Figure 2; Table C.2). Average X- and Y-activity values for all behavioral categorizations (resting, foraging, and mixed) varied significantly across seasons (ANOVA$_X$: $F_3 = 22.13$, p < 0.001; ANOVA$_Y$: $F_3 = 35.53$, p < 0.001). X and Y values were consistently higher for all behavioral categories during spring and summer compared to fall and winter, with the highest values observed during summer (Table C.3). A post-hoc Tukey test showed that accelerometer data were significantly different among all seasons (adjusted p-value < 0.001) with the exception of winter and fall season X-values (adjusted p = 0.78) (Figure C.1). These results justified the need to build different models for spring, summer, and combined fall/winter seasons. We tested for and found no statistically significant variability in X and Y values among individuals within each season (results not shown).

Captive models

Dirichlet models were built utilizing a total of 2449 5-minute intervals from 8 moose spread across the combined fall/winter (n = 1199), spring (n = 578) and summer (n = 672) seasons. Models were evaluated using 1052 5-minute intervals withheld from model building from the same 8 moose spread across fall/winter (n = 515), spring (n = 249), and summer (n = 288). The best model for all 3 seasons predicted the proportion of time spent resting, foraging, and moving as a function of X and Y values as well as step length (Table 2). The lowest RMSE was observed for the winter/fall model (RMSE: 0.1640), followed by summer (RMSE: 0.1871) and spring (RMSE: 0.2045). Small differences in RMSE between seasonal models using activity values and step length as predictors and those using only activity values as predictors suggests that X and Y values alone can provide good predictions of proportions of behaviors for studies utilizing larger time gaps between GPS locations. However, models without step length were found to consistently over predict proportion of time spent resting during observed foraging bouts, especially during summer.

MRC moose predictions

Comparisons were made between predicted and observed behavioral states in relation to time of day for 3,501 5-minute activity intervals. We found that prediction

intervals for the proportion of time spent resting and foraging within a 5-minute interval encompassed the majority of observed behavioral proportions across a range of observed solar angles (Figure 3). The proportion of time spent moving within a 5-minute interval was consistently over predicted, although minimally, for a range of observed solar angles (Figure 3). Using the best predictive models based on RMSE, we predicted the proportion of time spent resting, foraging, and moving for 789,957 5-minute intervals during 2015 for the same 8 moose used in modeling building. Predicted intervals covered all seasons and did not overlap with behavioral observations. The 8 MRC moose altered their behavior across seasons based upon time of day (i.e., solar angle) (Figure C.2). During spring, summer, and fall, MRC moose were more likely to increase the proportion of time they spent resting during the middle of the day (greatest angle of the sun) and the middle of the night (lowest angle of the sun) and were more likely to be foraging and moving during crepuscular periods (Figure C.2). This pattern differed for winter; moose activity (foraging and moving) peaked during crepuscular times as well as the middle of the night (Figure C.2). Along with the sun's position, we observed changes in behavior in association with variation in ambient temperature. During all seasons, with the exception of winter, we observed a positive association between the mean proportions of time spent resting and higher ambient temperature. This association varied by season, with increases in rest occurring at higher temperatures during spring (>18°C) than summer (>16°C) and fall (10°C) (Figure C.3). We observed a slight increase in moving behavior at temperatures >25°C during summer. Moose were more active in aspen and birch stands during the summer season; as solar angle increased moose utilizing both black spruce stands and bogs displayed the highest proportions of resting behaviors (Figure C.4). Moose utilizing black spruce stands were less active during all solar angles during spring compared to those utilizing bogs, aspen and birch stands. Activity patterns did not vary much between habitat type and time of day during the winter and fall seasons (Figure C.4).

Wild Moose Predictions

We used seasonal models developed with the captive moose's X and Y activity values to predict the proportion of time spent resting, foraging, and moving for 285,201

5-minute intervals during 2014 for 3 free-ranging moose in northeastern MN. These 3 MN moose altered their behaviors across all four seasons based on time of day, ambient temperature, and habitat. During all four seasons, MN moose were more active (i.e., an increase in foraging and moving behavioral states) during the crepuscular periods of the day and the least active during the middle of day (Figure 4). Resting activity increased during the night and peaked during the darkest periods (i.e., middle of the night) for spring and summer, while peaking closer to sunrise/sunset during fall and winter (Figure 4). In response to increasing ambient temperatures, moose reduced their activity during spring, summer and fall. This change from a positive association of activity with increasing ambient temperature to a strongly negative one occurred at a higher ambient threshold during spring (16°C) and summer (14°C) compared to fall (5°C) (Figure 5). We were unable to demonstrate fine-scale seasonal and time of day patterns related to habitat due to an overall lack of paired activity-habitat data (n = 5592), with >75% of those data points lying in three habitat types (coniferous forest, mixed forest, and forest/shrub wetland) . Moose utilizing emergent wetlands and regenerating forest stands displayed higher proportions of active behavioral states (i.e., moving) than those in coniferous and deciduous stands when all four seasons were combined (results not shown).

DISCUSSION

We established that dual-axis activity sensors programed to record activity values in 5-minute intervals can be used to predict the proportion of time spent resting, foraging and moving in either captive or free-ranging moose. We observed slight improvements in behavioral state predictions with the addition of step-length derived from GPS fixes less than or equal to 30 minutes. While previous studies have utilized behavioral observations of captive animals to validate collar activity sensors, most have chosen to use time intervals consisting of only purely active or inactive behaviors to build predictive models (Ungar et al. 2005; Löttker et al. 2009). Studies that did utilize time intervals encompassing more than one behavioral state typically converted intervals to the mode behavior observed within that time period (e.g., Moen et al. 1996), which often resulted in substantial increases in error when predicting intervals of mixed behaviors (Moen et al.

1996, Löttker et al. 2009). Nearly all of our observed active 5-minute time intervals contained a mix of active behaviors (foraging, walking, running, interacting, drinking) and inactive behaviors (standing, vigilance). Only 3% of our observed 5-minute intervals consisted of purely active behaviors, with all 106 of these consisting of purely foraging behaviors. These observations were consistent with findings in captive roe deer (*Capreolus capreolus*), where nearly all observed active intervals contained inactive behaviors (Gottardi et al. 2010) – this confirms the need for a modeling approach that incorporates a natural mix of behaviors in ruminants. The Dirichlet modeling technique we used allowed us to incorporate mixed intervals (i.e., intervals that contained two or three of the target behaviors), which increased our prediction accuracy of intervals containing a variety of behavioral states. The absence of observed intervals consisting of purely moving behaviors highlights why models that assume a single behavioral state within each interval may be misleading when classifying mixed intervals. This also highlights the limitations of captive observational studies; few predator encounters and enclosure size likely contributed to the lack of observed moving behaviors over 1 minute in duration.

Few studies have collected activity sensor data in conjunction with behavioral observations across multiple seasons, and we found no other multi-season study conducted on moose. The significant differences we observed in activity values between all four seasons suggest that a need to develop season-specific (i.e., spring, summer, fall/winter combined) predictive models in this system. Several factors may affect, alone or in concert, the seasonal differences we observed. First, differences in a moose's body condition throughout the course of a year have been observed to affect GPS collar fit, with the loosest fit occurring during spring and transitioning to the tightest fit during late fall/early winter (Dan Thompson, pers comm.). We therefore expected higher activity values in the spring with a looser collar fit. Physiological differences between individuals could also explain some of seasonal variation in activity sensor values. Previous studies have found that collar fit can vary depending upon sex and/or age class (Gremse 2004, Coulombe et al. 2006, Gervasi et al. 2006, Löttker et al. 2009). Löttker et al. (2009) found that activity values from male red deer were considerably lower than females,

especially during high locomotion behaviors, but pooled data from both genders produced models that fit well for all of them. The inability to maintain close proximity to male moose for extended periods of time as well as the low number of males housed at the MRC limited our study to female moose. Because of this and previous findings we recommend that activity values from wild male moose should be compared to females over the course of all seasons to adjust the predictive models if necessary. We did not observe significant differences in X and Y values resulting from variation in individual moose collar fit and behavior. However, this could be due to the captive nature of the moose at the MRC. Ideally the potential for variation in collar fit and behavior would be accounted for in the modeling process, this is generally not feasible outside of captive conditions. Looser collar fit combined with increased foraging activity during spring and summer could explain the higher activity values observed during both resting and active 5-minute behavioral states during those seasons compared to winter and fall. Moen et al. (1996) found increased activity counts during summer due to a combination of increases in browsing, head movement needed to strip leaves, and head movement from insect harassment.

To gain a better understanding of how collar fit affects activity values, we performed two different collar fit experiments. At the MRC we manually altered collar fit on two adult female moose to be either "tight" (1-2" gap between the collar and moose's neck) or "loose" (>4" gap) and performed 2 hour behavioral observations in the same manner as outlined in the methods for a total of 10 observation hours. Comparing activity values from the two fit types was inconclusive, but can likely be contributed to the low number of observation hours performed (results not shown). For example, two observation periods consisted almost entirely of resting behaviors, for which we would expect similarly low activity values between the two fit types. The second experiment was two part with phase 1 testing how collar activity values change as a function of the degree of collar rotation and fit. The collar fit, number of rotations and degree of rotation were controlled for and tested using a machine built specifically for collar testing. Phase 2 utilized trained therapy horses to test whether differences in activity values could be observed when behaviors were controlled and consistent for the entirety of the activity

interval. For more detailed methods see McGraw et al. (In prep). Significant differences were found in both phases of this experiment; increased rotation ("movement") and looser collar fit on the collar machine resulted in increased activity values. Significant differences were found between behavioral type using the trained horses, with standing behaviors exhibiting the lowest activity values and trotting exhibiting the highest values (McGraw et al. In prep). The results of this experiment corroborate with the finding of Moen et al. (1996).

We observed considerable overlap in X- and Y-axis activity values when comparing purely resting and foraging intervals. We found that during times of low exertion foraging (primarily foraging low) when browse was plentiful, neck movements were minimal and corresponding X- and Y-values were often smaller than 10. Sustained periods of walking with little to no neck movement could also produce intervals with similarly low activity values. We observed periods of sustained walking along enclosure fence lines interspersed with standing behaviors that resulted in activity values lower than expected (<100). It should be noted that resting was not always associated with zero or very low activity sensor counts, we observed activity values as high as 116 and 81 for X- and Y-values respectively for 5-minute intervals consisting of only resting behaviors. We observed considerable head movement during periods of vigilance both in standing and bedded positions, corroborating the findings of Ungar et al. (2005) and Moen et al. (1996) that rest behaviors are not always associated with low activity counts. Resting intervals with the highest recorded activity values occurred during late spring and summer corresponding with periods of high insect harassment (Moen et al. 1996). Moose which were observed bedding during these times displayed increased head movement, differentiated from shaking behaviors which were also often displayed during these times. Much of the overlap we encountered between behavioral categories can be attributed to the fact that activity counts are "averaged" over a 5-minute interval. Therefore, a number of the issues we encountered could be alleviated by simply shortening the interval or getting rid of the averaging process all together and instead storing all raw acceleration values (Löttker et al. 2009). We recommend using activity intervals shorter than 5 minutes, ideally a short time interval that also sufficiently

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balances study data storage and battery longevity needs. Vectronic and other collar companies now offer GPS collars equipped with activity sensors capable of storing raw acceleration values in a continuous manner, but this comes at the cost of a shorter battery life. Future efforts focused on studying very fine-scale behavioral responses to environmental changes should, whenever possible, utilize these newly developed activity sensors.

Using the Dirichlet regression modeling approach we were able to predict proportions of resting, foraging and moving across the different seasons with relatively good accuracy (RMSE <0.21 for all seasons, respectively). We observed prediction error from two main sources. Proportions of rest were over predicted and foraging under predicted during intervals in which low exertion foraging behaviors were the dominant behavior observed (i.e., foraging low during winter and early spring). The overlap in activity values between foraging and behaviors categorized as moving but with minimal neck movements (e.g., head down walking) also introduced error, resulting in over prediction of the proportion of time spent foraging during these intervals. The addition of GPS movement path data to our models allowed for better distinction between periods of high-exertion foraging (e.g., stripping browse during summer) and moving behaviors with minimal neck movements. We expected step length to partially mitigate prediction error observed between resting and low exertion foraging behaviors, but we obtained only minimal improvements. We suspect that this was due to the length of time between fixes (i.e., 30 min), and hypothesize that fix rates ≤10 minutes would help reduce this source of error. The addition of movement paths should only be used when GPS fixes are ≤30 minutes apart. Attempts to utilize 60 and 120 minute fixes (via coarsening our locational data) resulted in minimal prediction improvements and induced new sources of prediction error. We were unable to use movement paths when examining behavioral states in the three free-ranging MN moose due to the much longer time intervals between GPS locations (255-minutes). We encourage researchers to pay close attention to these potential sources of error when interpreting results.

Both captive MRC and wild MN moose appear to modify their behavior in response to changes in ambient temperature, solar angle, and habitat type. Moose are

known to be physiologically sensitive to heat (Renecker and Hudson 1986, Renecker and Hudson 1989; McCann et al. 2013). Renecker and Hudson (1986, 1990) found that temperatures greater than -5°C in the winter and 14-20°C during the warm season (late spring to early fall) were associated with increased metabolic, heart, and respiratory rates, reduced food intake, and reduced body weight. McCann et al. (2013) found similar thresholds for late spring to early fall (17-24°C). These were based on two and four captive moose respectively, and highlight the difficulty of assessing thermal thresholds outside of a captive setting. Temperature-dependent changes in behavior were the least pronounced during winter for both MRC and MN moose. Street et al. (2015) found slight increases in activity values at moderate temperatures during winter. These findings corroborate with the slight increase in activity we observed as ambient temperatures increased toward 0°C. Well adapted to tolerate cold temperatures, moose are limited by both forage quantity and quality during the winter; this could explain why we observed relatively constant activity levels across much of the ambient temperature gradient during this season (Schwartz et al. 2007). During both the summer and fall we saw marked decreases in the proportion of active behaviors as temperatures increased. The mean proportion of active behaviors decreased considerably at temperatures exceeding 15°C and 5°C during summer and fall respectively, suggesting that moose during these seasons are faced with the tradeoff between resting more frequently to reduce thermal stress and seeking quality food sources and foraging. Forced to rest during times of increased ambient temperatures, moose forfeit feeding opportunities and this deficit has been shown to reduce weight and overall body condition (Renecker and Hudson 1992).

We found that wild MN moose increased activity as temperatures warmed in the spring until temperatures exceeded 15°C after which marginal decreases in activity were observed. Thermal tolerances are known to shift seasonally for many species (Pörtner 2002); we therefore expected a lower thermal threshold followed by marked decreases in activity during spring as a result of natural acclimation from the winter season hindered by remaining winter coats. Our results suggest, however, that when experiencing warm temperatures during late spring (i.e., May), moose may choose to take advantage of increased forage quality and abundance at the cost of potential thermal stress. As spring

advances, rapid plant growth occurs and nutritional quality peaks. This time period also corresponds with peak energetic demands on gestating and lactating female moose (Schwartz et al. 2007); Gasaway and Coady (1974) indeed found that the metabolizable energy requirement by the end of the gestation period is six-fold compared to March. Parturition initiates an even more energy-demanding phase, two- to three-fold that of gestation. Energy needs therefore peak during the early summer and gradually decline as the young are weaned (Schwartz et al. 2007).

Behavioral responses of moose to thermal conditions are consistent throughout much of North American moose range. Moose occupying the boreal forest in Québec utilized conifer forest as a thermal refuge more frequently when ambient temperatures were high (Dussault et al. 2004). Likewise, moose in British Columbia were found to select for mature forest when temperatures exceeded critical limits (Schwab and Pitt 1991). We observed similar patterns at the MRC, where moose utilizing conifer stands during both spring and summer rested more than those utilizing aspen and birch stands, especially during the middle of the day when the sun and ambient temperatures were peaking. Additionally, moose in Alberta were found to bed in wet meadows during summer to reduce both respiration rates and energy expenditure (Renecker and Hudson 1990). At the MRC, we found that the captive moose that were using bogs during summer displayed high proportions of resting behaviors, which indicates that they may be using bogs as thermal refuges. We did not observe strong patterns with the Minnesota moose; only small increases in the proportions of time spent foraging and moving were associated with emergent wetlands and regenerating forests. These results are likely an artifact of the low number of activity values observed in some habitats (e.g., regenerating forests). It is important to recognize that our results are just a small example of the behavioral changes Minnesota moose appear to exhibit in response to changes in ambient temperatures, habitat, and time of day. Future efforts should focus on incorporating more data from the Carstensen et al. (2014) study to investigate fine-scale behavioral patterns of moose in northeastern Minnesota in response to habitat and ambient temperature.

MANAGEMENT IMPLICATIONS

With ambient temperatures forecasted to increase globally by as much as 4.7℃ by 2100 (IPCC 2014), it is crucial that we improve our understanding of how moose occupying bioclimatic edges respond to warming temperatures. While we were able to capture strong behavioral changes in relation to habitat, ambient temperature, and time of day, we are unable to state how any of these changes relate to heat stress and not just changes in activity without physiological data. The ability to now pair behavioral changes with physiological measurements taken by mortality implant transmitters (MIT) will hopefully give researchers new insights into how ambient temperatures outside of moose thermal thresholds, as defined by Hudson and Renecker (1986) and McCann (2013), affect survival, habitat use, and reproductive rates in free-ranging moose. Our approach highlights how researchers can more fully utilize the data collected from GPS collars equipped with dual-axis activity sensors, providing new insights into how animals respond behaviorally to changes in their environment at very fine spatial and temporal scales. Future research should utilize new MIT technology in conjunction with GPS and activity data to better understand the behavioral and physiological responses of moose to changes in their environment. Our approach highlights how researchers can more fully utilize the data collected from GPS collars equipped with dual-axis activity sensors, providing new insights into how animals respond behaviorally to changes in their environment at very fine spatial and temporal scales. Future research should utilize new MIT technology in conjunction with GPS and activity data to better understand the behavioral and physiological responses of moose to changes in their environment.

Table 1. Description of the three behavior categories that we used to evaluate the performance of dual-axis accelerometer values for predicting moose behavior. Observations were made on 8 GPS-collared captive female moose at the Moose Research Center, Kenai Peninsula, Alaska during 2015.

Behavior	Classification	Description
Laying	Resting	Bedded position with legs generally tucked, head could be up or down.
Ruminating	Resting	Predominantly bedded position, with or without head movement. Infrequently while standing.
Standing	Resting	Upright quadruped position, with or without head movement (i.e. vigilance).
Drinking	Foraging	Consumption of water during the warm season (spring, summer, fall). Could be standing or lying.
Snow Intake	Foraging	Consumption of snow during the winter season. Could be standing or lying.
Foraging Low	Foraging	Consumption of foraged plants, with mouth below the bottom of the stomach while standing or while lying.
Foraging Medium	Foraging	Consumption of foraged plants with the mouth above the bottom of the stomach but below the shoulder hump.
Foraging High	Foraging	Consumption of foraged plants with the mouth above the top of the shoulder hump.
Walking	Moving	Slow methodical movement, forward or backward.
Running	Moving	Accelerated movement, forward.
Shaking	Moving	Accelerated up-down/side-to-side head and body movement while walking, foraging, standing, or lying.
Grooming	Moving	Self-grooming with hind hooves and/or rubbing against trees.
Interaction	Moving	Social interaction with other moose, with forelegs leaving the ground (i.e. boxing).

Table 2. Coefficient estimates from seasonal Dirichlet models predicting proportions of resting, foraging, and moving behaviors as a function of the interaction between X- and Y-activity values as well as step length. Models were built using data for 8 captive adult female moose at the Moose Research Center, Kenai Peninsula, Alaska during 2015.

Season	Model[a]	Rest			Forage			Moving			RMSE
		β	SE	p-Value	β	SE	p-Value	β	SE	p-Value	
Winter/Fall											0.1574
	Intercept	1.155	0.087	<0.001	-1.723	0.045	<0.001	-1.838	0.046	<0.001	
	X[b]	-0.078	0.004	<0.001	0.085	0.003	<0.001	0.004	0.003	0.161	
	Y[c]	0.032	0.006	<0.001	0.010	0.007	0.170	0.045	0.007	<0.001	
	Step[d]	-0.021	0.004	0.57	-0.006	0.002	0.019	0.004	0.003	0.119	
	X:Y[e]	<0.001	<0.001	<0.001	-0.001	<0.001	<0.001	<0.001	<0.001	0.001	
Spring											0.2040
	Intercept	2.103	0.148	<0.001	-1.463	0.069	<0.001	-1.605	0.069	<0.001	
	X	-0.077	0.006	<0.001	0.042	0.005	<0.001	-0.012	0.005	0.011	
	Y	-0.032	0.009	<0.001	0.045	0.008	<0.001	0.049	0.008	<0.001	
	Step	0.004	0.004	0.283	-0.013	0.003	<0.001	0.003	0.003	0.312	
	X:Y	<0.001	<0.001	<0.001	-0.001	<0.001	<0.001	<0.001	<0.001	0.001	
Summer											0.1869
	Intercept	4.499	0.124	<0.001	-1.065	0.068	<0.001	-1.040	0.066	<0.001	
	X	-0.082	0.005	<0.001	0.017	0.005	<0.001	-0.094	0.005	<0.001	
	Y	-0.082	0.008	<0.001	0.004	0.006	0.457	0.011	0.006	0.098	
	Step	-0.035	0.004	<0.001	-0.009	0.003	0.005	-0.002	0.003	0.51	
	X:Y	0.001	<0.001	<0.001	<0.001	<0.001	0.659	<0.001	<0.001	0.001	

[a]Predictors associated with the model. [b]X-activity value associated with each 5-minute activity interval. [c]Y-activity value associated with each 5-minute activity interval. [d]Step length (m) associated with each 5-minute activity interval. [e]Interaction between the X- and Y-activity value associated with each 5-minute activity interval.

Figure 1. Map of the Moose Research Center in Game Management Unit 15A, Kenai Peninsula, Alaska.

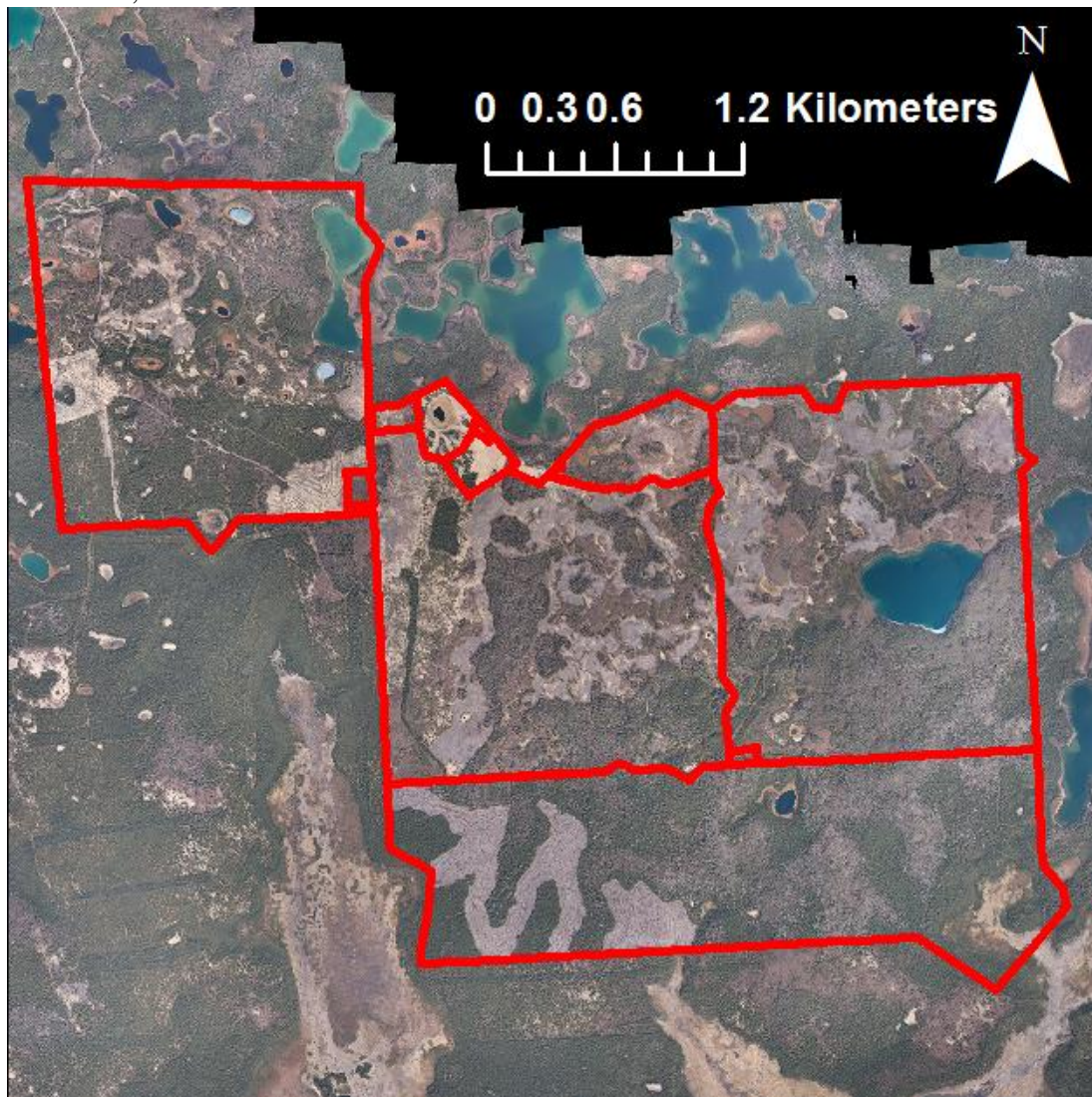**Figure 2.** Visualization of X- and Y- accelerometer values from a total of 3,501 5-minute intervals across all seasons from 8 adult captive moose at the Moose Research Center, Kenai Peninsula, Alaska. 1560 intervals contained only resting behaviors, 98 only foraging behaviors, but none contained only walking/resting behaviors. The majority (1843) were mixed and contained more than 1 behavior.
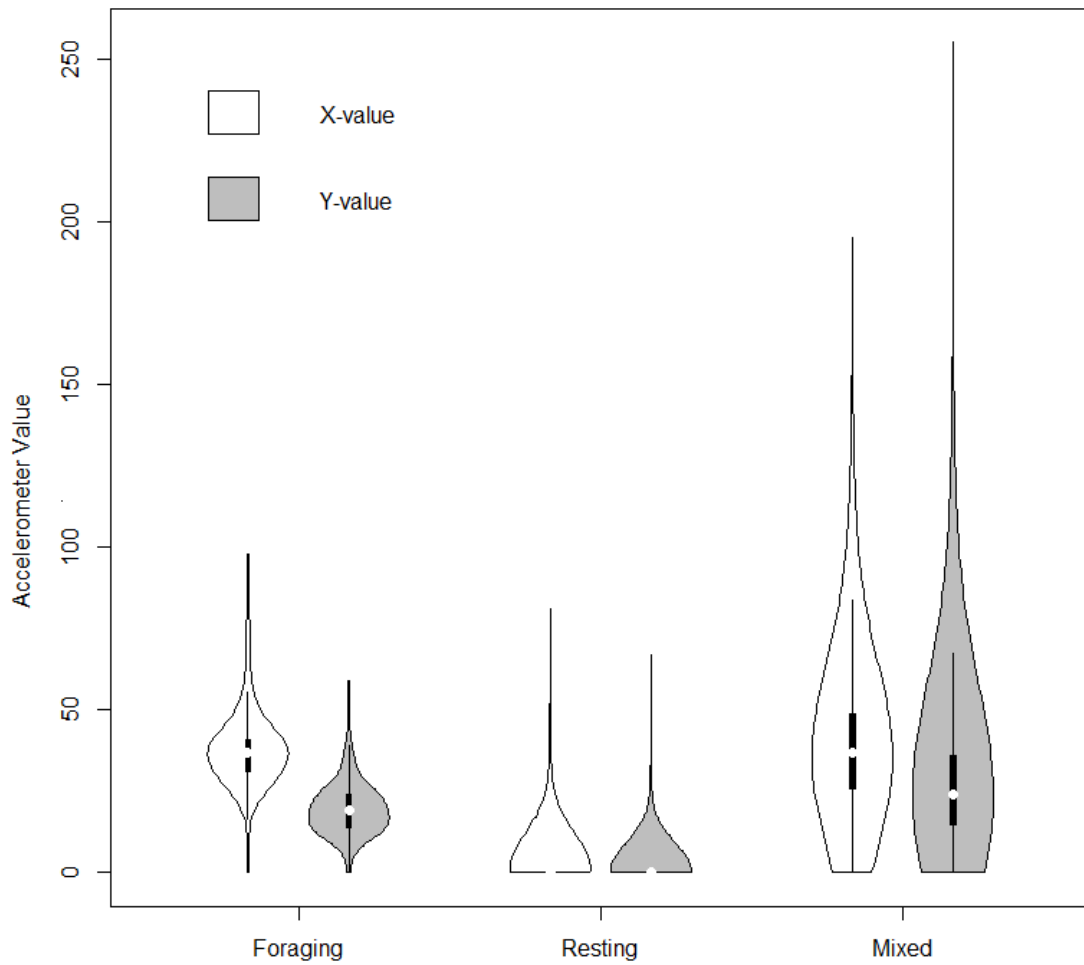
Figure 3. Visualization of predicted versus observed proportions of time spent foraging, moving and resting within a 5-minute interval in response to changing solar angle for 8 captive moose at the Moose Research Center, Kenai Peninsula, Alaska. Observed proportions were binned into 5% quantiles and are depicted by black points. Prediction means and 95% confidence intervals were calculated using bootstrapping with 1000 iterations and are depicted using locally weighted smoothing curves.
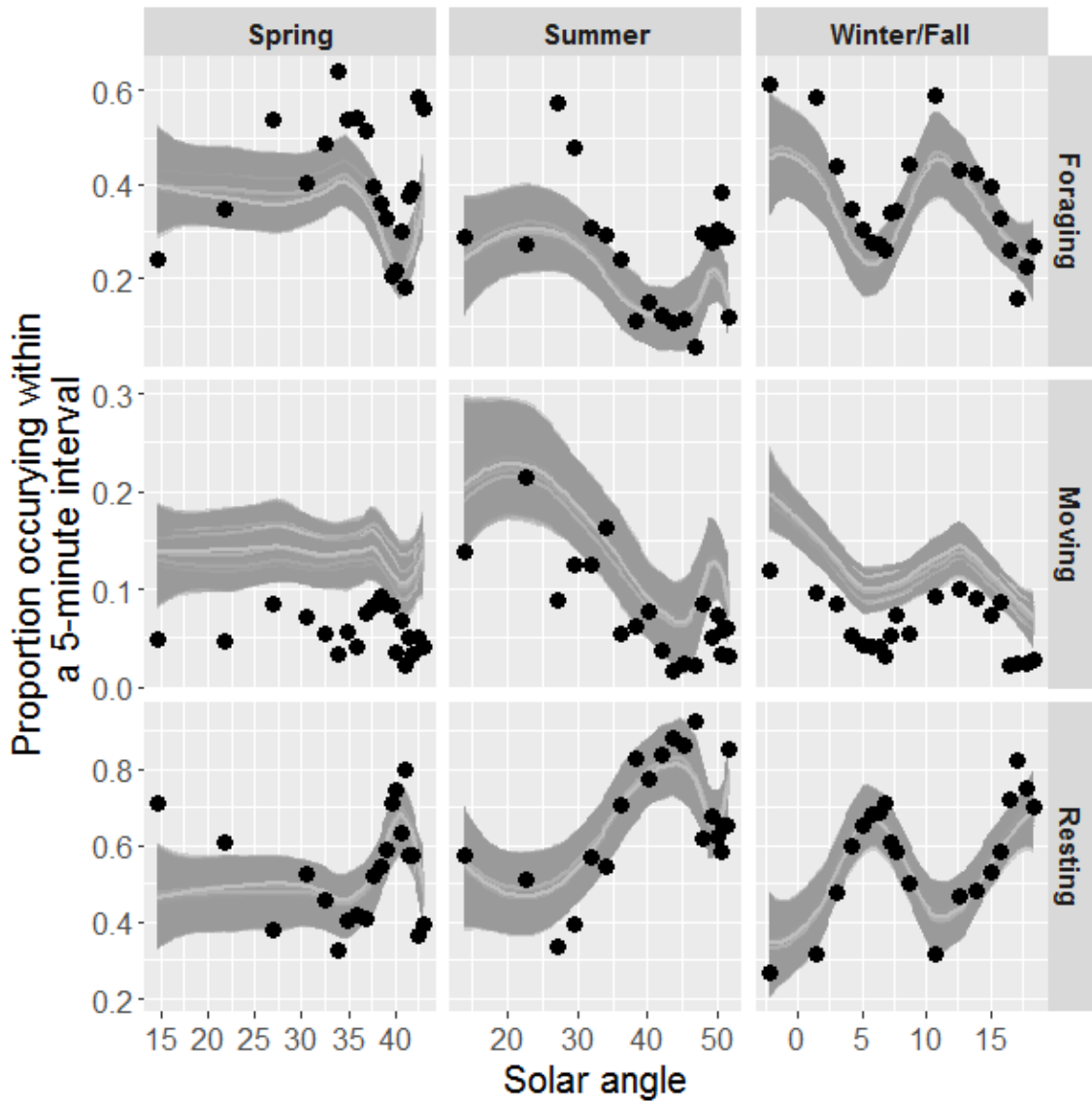
Figure 4. Mean predicted proportions of time spent foraging, moving, and resting within a given 5-minute interval in response to changing solar angles for 3 free-ranging moose in northeastern Minnesota using data collected in 2014. Crepuscular times are centered on zero with lowest values corresponding to the middle of the night and the highest values to the middle of the day when the sun is at its highest point. Predictions were binned into 5% quantiles. Means and 95% confidence intervals were calculated using bootstrapping with 1000 iterations. Trends are depicted using locally weighted smoothing curves.

Figure 5. Mean predicted proportions of time spent foraging, moving, and resting within a given 5-minute interval in response to changing ambient temperature for 3 free-ranging moose in northeastern Minnesota during 2014. Predictions were binned into 5% quantiles. Means and 95% confidence intervals were calculated using bootstrapping with 1000 iterations. Trends are depicted using locally weighted smoothing curves.
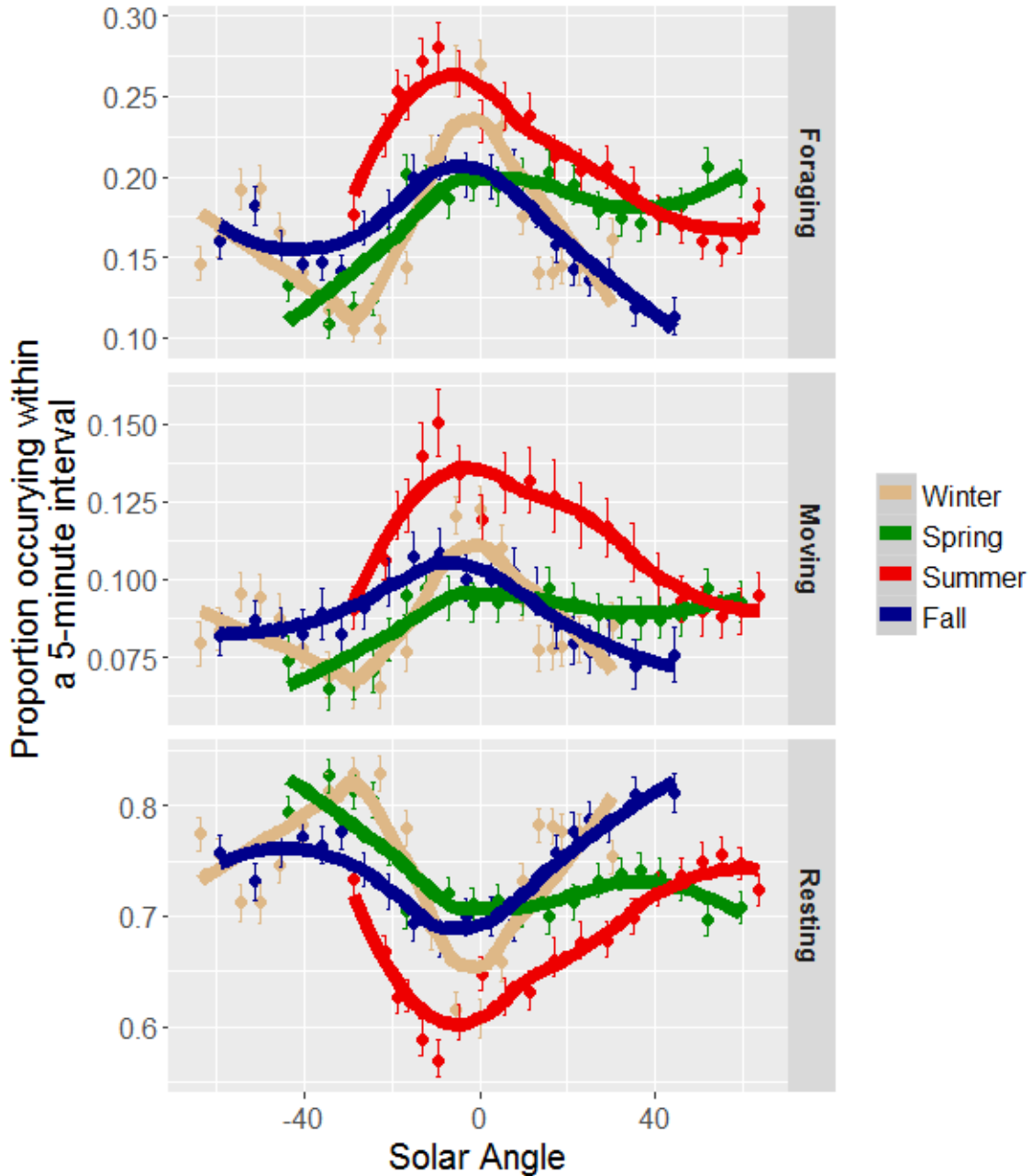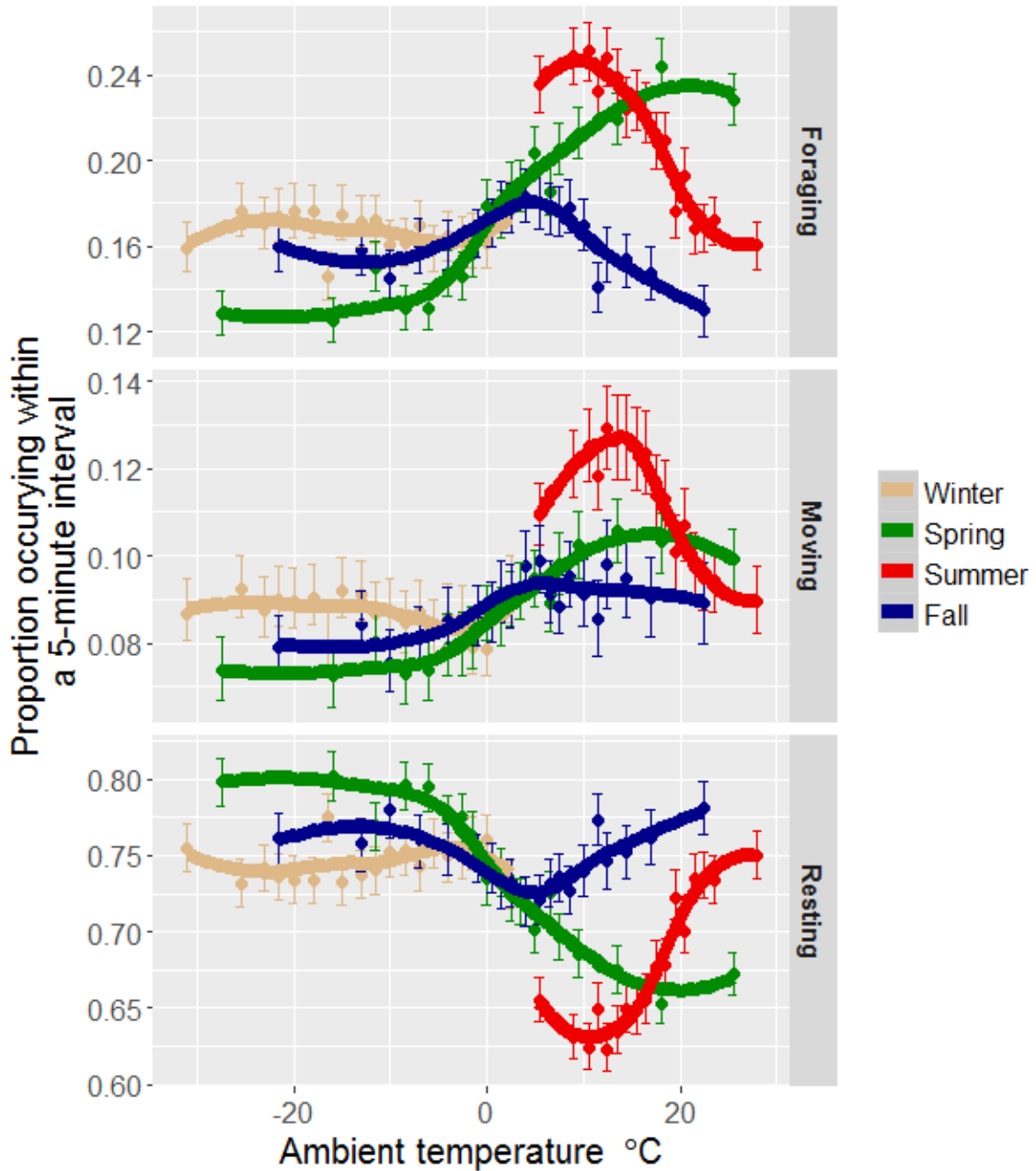
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APPENDIX A

Chapter 1 supplemental tables and figures.

Table A.1. Scheduled and recorded mortality implant transmitter measurements and successful transmission rates for 8 moose deployed with ATS vaginal implant transmitters across different seasons from December 2014 to December 2015 at the Moose Research Center, Kenai Peninsula, Alaska.

Season	Recorded Measurements	Scheduled Measurements	Transmission Rate (%)	Max Gap*
Winter	271,894	273,089	99.56	57,193.07
Spring	177,321	182,726	97.04	35.47
Summer	175,457	183,316	95.71	4,100.56
Fall	149,989	170,806	87.81	1,023.08
All	774,661	809,937	95.64	57,193.07

*Max Gap represents the maximum gap in minutes between mortality implant transmitter measurements.

Table A.2. Average rumen-derived body temperature pre- and post-correction for water intake in 8 moose deployed with ATS vaginal implant transmitters across all seasons from December 2014 to December 2015 at the Moose Research Center, Kenai Peninsula, Alaska. Parentheses indicate standard deviations.

Season	Pre-Correction Mean MIT Temperature (°C)	Post-Correction Mean MIT Temperature (°C)
Winter	37.92 ($\pm$0.21)	37.94 ($\pm$0.18)
Spring	37.95 ($\pm$0.42)	38.04 ($\pm$0.29)
Summer	38.61 ($\pm$0.38)	38.63 ($\pm$0.28)
Fall	38.10 ($\pm$0.38)	38.11 ($\pm$0.23)
All	38.13 ($\pm$0.43)	38.17 ($\pm$0.37)

Table A.3. Average rumen-derived and vaginal-derived (VIT) body temperatures pre- and post-correction for water intake in 5 moose deployed with ATS VITs across different seasons from December 2014 to December 2015 at the Moose Research Center, Kenai Peninsula, Alaska. Parentheses indicate standard deviations.

Season	Pre-correction MIT Temperature (°C)	Post-correction MIT Temperature (°C)	VIT Temperature (°C)	Mean Temperature Difference (°C)
Winter	37.92 (±0.20)	37.94 (±0.16)	38.08 (±0.28)	- 0.08 (±0.26)
Spring	37.93 (±0.40)	37.98 (±0.26)	38.18 (±0.32)	- 0.13 (±0.27)
Summer	38.58 (±0.35)	38.59 (±0.28)	38.43 (±0.29)	0.12 (±0.28)
Fall	38.08 (±0.35)	38.11 (±0.22)	38.01 (±0.24)	- 0.01 (±0.26)
All	38.06 (±0.41)	38.10 (±0.33)	38.18 (±0.64)	- 0.03 (±0.28)

Table A.4. Summary statistics of ruminating behaviors for 8 captive adult (> 2 years of age) female moose deployed with ATS vaginal implant transmitters (VIT) during behavioral observations conducted during all four seasons at the Moose Research Center, Kenai Peninsula, Alaska from December 2014 to December 2015. Parentheses indicate standard deviations.

Device	Season	N_r*	Mean T_r*	ΔT*	Mean Time*
MIT					
	Winter	41	30.79 ($\pm$22.95)	0.09 ($\pm$0.11)	0
	Spring	37	22.04 ($\pm$18.13)	0.06 ($\pm$0.14)	0
	Summer	70	18.50 ($\pm$14.85)	0.05 ($\pm$0.06)	0
	Fall	22	37.09 ($\pm$29.82)	0.18 ($\pm$0.29)	0
VIT					
	Winter	30	34.15 ($\pm$22.66)	0.04 ($\pm$0.09)	0
	Spring	25	20.75 ($\pm$17.44)	0.10 ($\pm$0.16)	0
	Summer	50	18.46 ($\pm$13.39)	0.05 ($\pm$0.13)	0
	Fall	15	36.52 ($\pm$31.67)	0.05 ($\pm$0.09)	0

*N_r represents the number of ruminating behaviors observed during the given season. Mean T_r represents the mean time in minutes spent ruminating during a six hour observation time period. ΔT represents the mean temperature change associated with one ruminating bout. Mean Time represents the time (seconds) it took for the device temperature to return to within one standard deviation of the device temperature at the time of the preceding ruminating behavior.

Table A.5. Scheduled and recorded mortality implant transmitter (MIT) measurements for 2 moose deployed with Vectronic vaginal implant transmitters (VIT) across different seasons from December 2014 to December 2015 at the Moose Research Center, Kenai Peninsula, Alaska.

Device	Season	Recorded Measurements	Scheduled Measurements	Transmission Rate (%)	Max Gap (Minutes)
MIT	Winter	27,756	47,730	58.15	369.92
	Spring	33,434	72,212	46.30	2179.98
	Summer	3,398	26,575	12.79	806.60
	Fall	2,620	26,120	10.03	1075.03
	All	67,208	152,637	44.03	2179.98
VIT	Winter	47,599	47,640	99.91	15.22
	Spring	35,185	35,694	98.57	2169.85
	Summer	50,274	51,948	96.78	55.75
	Fall	25,778	25,830	99.80	17.07
	All	134,867	135,501	99.53	2169.85

Table A.6. Average rumen-derived and vaginal-derived body temperatures pre- and post-correction for water intake in 2 moose deployed with Vectronic vaginal implant transmitters across different seasons from December 2014 to December 2015 at the Moose Research Center, Kenai Peninsula, Alaska. Parentheses indicate standard deviations.

Season	Pre-correction MIT Temperature (°C)	Post-correction MIT Temperature (°C)	VIT Temperature (°C)	Mean Temperature Difference (°C)
Winter	38.00 (±0.19)	38.02 (±0.17)	37.73 (±0.16)	0.28 (±0.13)
Spring	38.16 (±0.45)	38.24 (±0.27)	37.94 (±0.24)	0.30 (±0.20)
Summer	38.63 (±0.51)	38.69 (±0.29)	38.05 (±0.26)	0.64 (±0.23)
Fall	38.02 (±0.52)	38.13 (±0.22)	37.71 (±0.18)	0.42 (±0.17)
All	38.10 (±0.39)	38.15 (±0.28)	37.84 (±0.23)	0.32 (±0.19)

Figure A.1. Variability in mortality implant transmitter-derived body temperature and ATS vaginal implant transmitter-derived body temperature of 5 moose across seasons at the Moose Research Center, Kenai Peninsula, Alaska, during 2015.
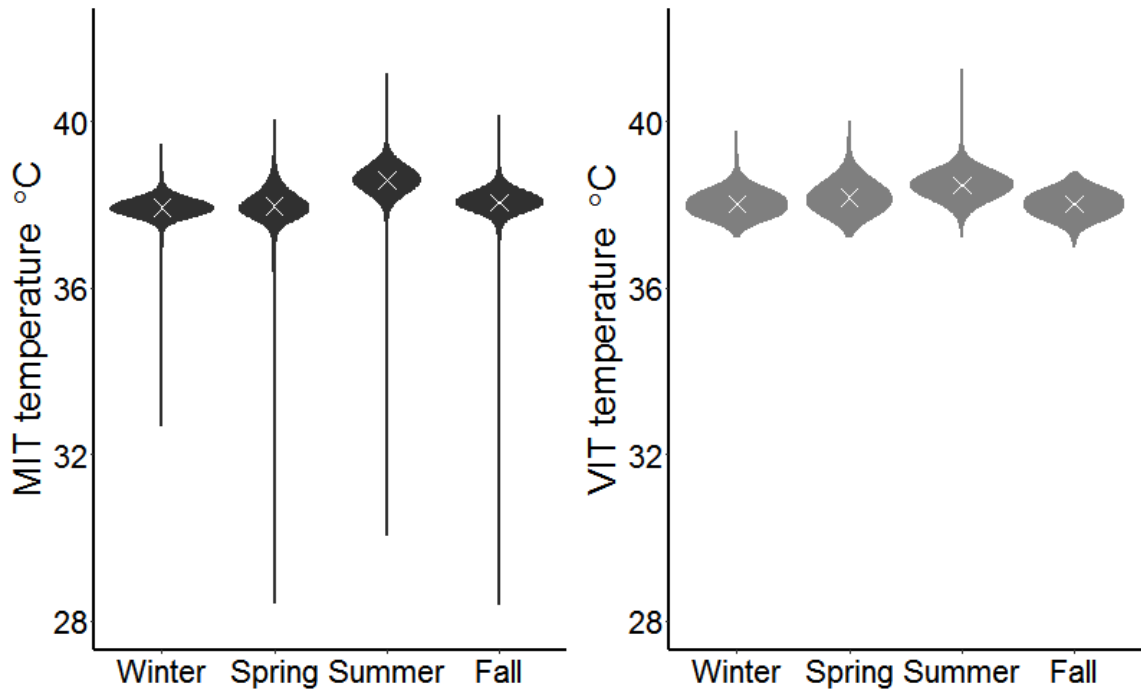
Figure A.2. Overall linear mixed-effects model root mean squared error (RMSE) as a function of body temperature averaging across seasons.
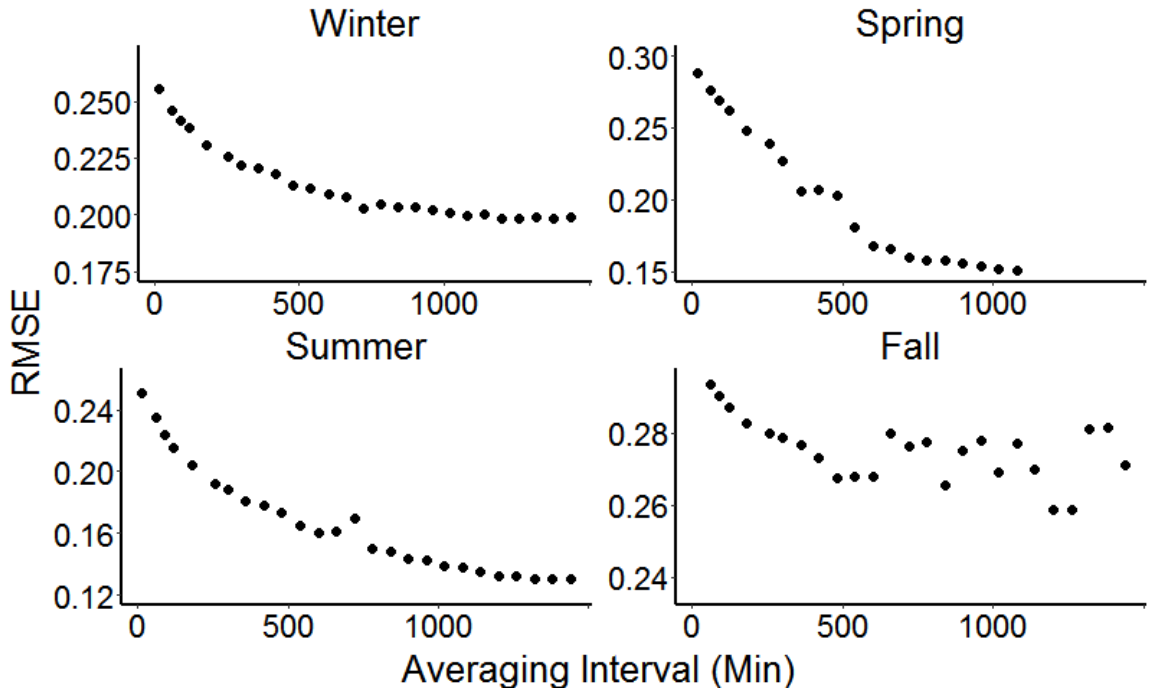
Figure A.3. Difference in predicted and observed averaged 15-min body temperatures (measured by Vectronic vaginal implant transmitters) and bootstrapped 95% prediction intervals during winter and summer at the Moose Research Center, Kenai Peninsula, Alaska.
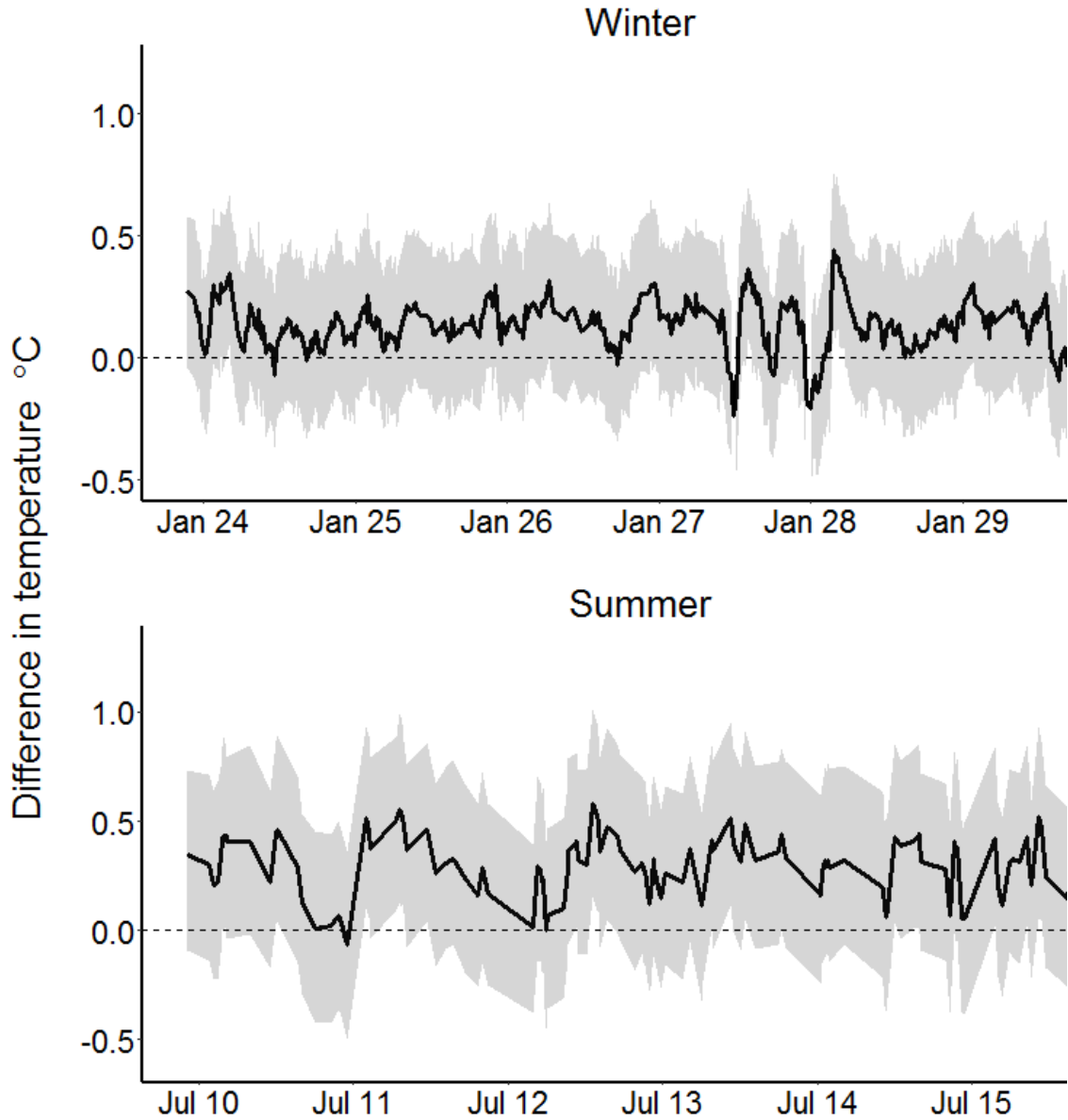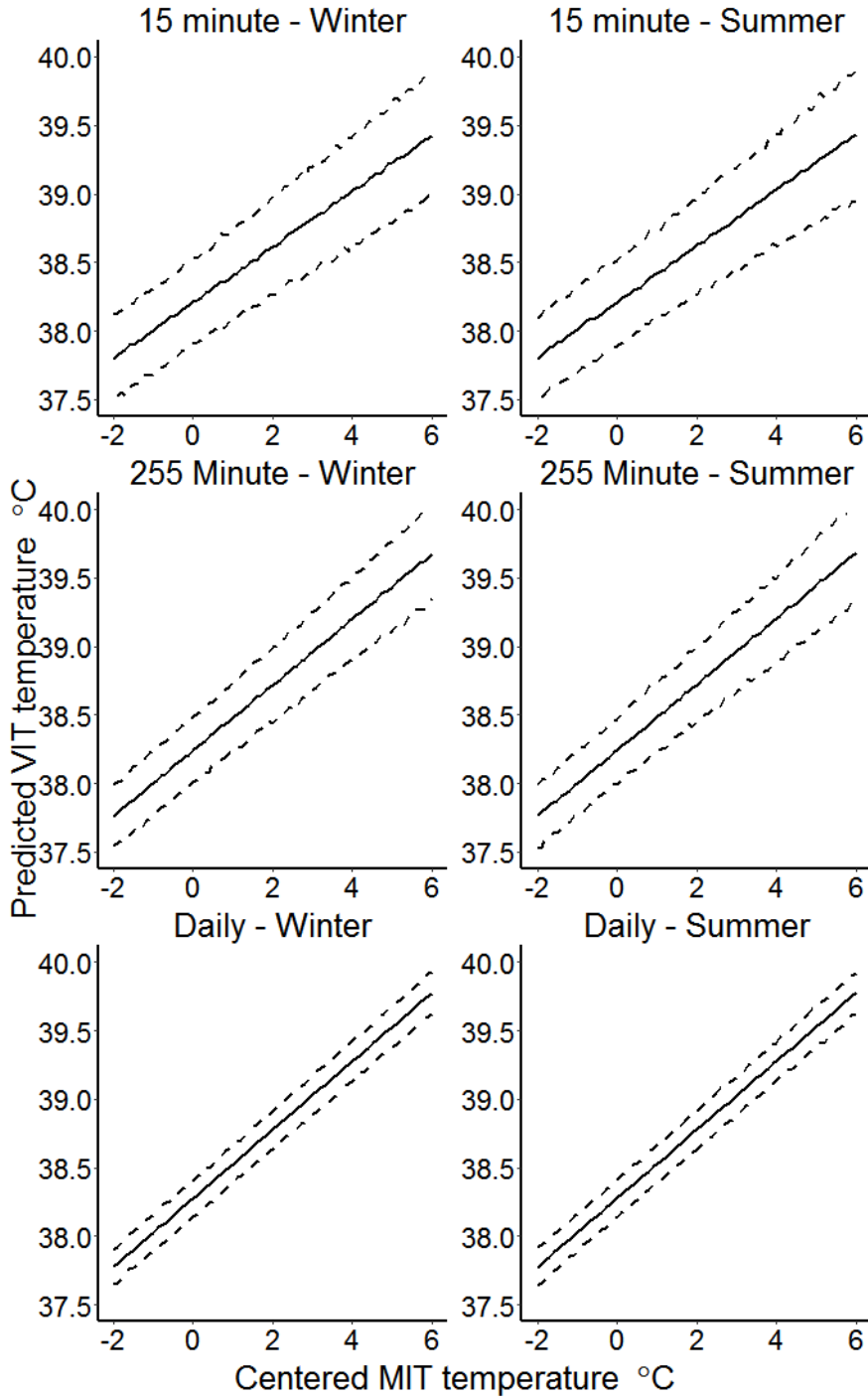
Figure A.4. Predicted vaginal implant transmitter (VIT) temperatures as a function of centered Mortality Implant Transmitter (MIT) temperatures for a range of realistic centered MIT temperatures (-2°C –6°C, 0.1°C increments). Dashed lines represent the lower and upper 95% prediction intervals.

APPENDIX B

To validate the accuracy of both mortality implant transmitter (MIT) and vaginal implant transmitter (VIT) temperature measurements a water bath validation procedure was adapted from Signer et al. (2010) and Vickers et al. (2010). The objective was to test the sensor accuracy of each device at 5°C increments between 30-45°C. Six MITs and fourteen VITs were tested using a VWR 28L Digital Water Bath (WB28; uniformity: ±0.2°C; stability: ±0.1°C) and a Fisher Scientific Traceable Platinum Ultra-Accurate Digital Thermometer (accuracy: ±0.05°C; resolution: ±0.001°C). We tested the accuracy using the following procedure:

1. Warm the water bath to 30°C and take temperature measurement using the thermometer
2. Take thermometer temperature reading at 5 minute intervals starting on the hour (e.g., 12:00,12:05)
3. Place sensor devices in the water bath once the water has balanced at 30°C, allow sensors to sit for 30 minutes
4. Increase water bath temperature to 35°C for 30 minutes
5. Increase water bath temperature to 37°C for 30 minutes
6. Increase water bath temperature to 37.5°C for 30 minutes
7. Increase water bath temperature to 38°C for 30 minutes
8. Increase water bath temperature to 38.5°C for 30 minutes
9. Increase water bath temperature to 39°C for 30 minutes
10. Increase water bath temperature to 39.5°C for 30 minutes
11. Increase water bath temperature to 40°C for 30 minutes
12. Increase water bath temperature to 45°C for 30 minutes

Mortality implant transmitter and VIT results were analyzed separately. For each transmitter type (MIT or VIT) the difference (accuracy) between the transmitter-measured temperature and the water bath temperature as determined by the Fisher thermometer was calculated using linear mixed effect models. Temperatures (Y_{ij}) were predicted using a fixed effect (β_1) for measurement device (e.g., MIT or Fisher thermometer) and random effects ($\varphi_i \sim N(0, \sigma_\varphi^2)$) for the interaction between device type and transmitter ID. Residual error was represented by $\varepsilon_{i,t} \sim N(0, \sigma_\varphi^2)$. On average, MIT and VIT measured temperatures were found to be 0.11°C (SE=0.31) and 0.02°C (SE=0.01) lower than the water bath temperature as determined by the Fisher thermometer, respectively. Both of these temperature differences fall within the accuracy of the devices themselves and do no warrant any temperature correction.

APPENDIX C

Supplemental tables and figures.

Table C.1. Proportion of time 8 captive adult (>2 years of age) were observed in each behavioral state during 3,501 5-minute intervals at the Moose Research Center, Kenai Peninsula, Alaska distributed across four, user-defined seasons.

Season	Number of 5-minute Intervals	Rest	Forage	Moving
Winter (1 Nov-31 Mar)	872	0.56	0.38	0.07
Spring (1 Apr-30 May)	827	0.54	0.40	0.06
Summer (1 June-31 Aug)	960	0.67	0.25	0.08
Fall (1 Sep-31 Oct)	842	0.60	0.34	0.06

Table C.2. Mean (±SD) X- and Y-activity values of 5-minute intervals of pure behaviors such as resting or foraging, mixed behaviors, or for all 3,501 intervals obtained from observing 8 adult captive female moose at the Moose Research Center, Kenai Peninsula, Alaska in . None of the intervals we observed contained strictly moving behaviors.

Behavior	Number of 5-minute Intervals	Mean Activity X	Mean Activity Y
Resting	1559	1.71 (±6.21)	0.84 (±5.09)
Foraging	106	38.94 (±13.31)	20.25 (±8.60)
Mixed	1836	39.13 (±23.75)	28.90 (±24.18)
All	3501	22.46 (±25.77)	16.15 (±22.59)

Table C.3. Mean ($\pm$SD) X- and Y-activity values across four seasons of 5-minute intervals of pure behaviors such as resting or foraging, mixed behaviors, or for all 3,501 intervals obtained from observing 8 adult captive female moose at the Moose Research Center, Kenai Peninsula, Alaska. None of the intervals we observed contained strictly moving behaviors.

Behavior	Season	Number of 5-minute Intervals	Mean Activity X	Mean Activity Y
Resting	Winter	334	0.19 ($\pm$2.02)	0.13 ($\pm$1.44)
	Spring	316	1.23 ($\pm$5.93)	1.02 ($\pm$7.51)
	Summer	521	3.94 ($\pm$8.91)	1.64 ($\pm$6.20)
	Fall	389	0.42 ($\pm$22.44)	0.24 ($\pm$1.81)
Foraging	Winter	20	33.10 ($\pm$9.71)	15.60 ($\pm$5.92)
	Spring	44	38.84 ($\pm$7.62)	22.80 ($\pm$5.82)
	Summer	5	59.17 ($\pm$18.58)	41.00 ($\pm$10.56)
	Fall	29	39.17 ($\pm$17.90)	14.97 ($\pm$4.89)
Mixed	Winter	518	31.85 ($\pm$18.13)	22.95 ($\pm$18.18)
	Spring	467	37.27 ($\pm$21.30)	27.57 ($\pm$23.19)
	Summer	434	55.17 ($\pm$28.97)	45.18 ($\pm$27.54)
	Fall	424	33.77 ($\pm$18.26)	21.01 ($\pm$19.82)

Figure C.1. Distribution of X- and Y-activity values across all four seasons for all 8 captive female moose that were observed at the Moose Research Center, Kenai Peninsula, Alaska during 2015. Among-season differences in X- and Y- activity values were tested using Tukey's test. Seasons sharing the same letter are not significantly different at the α=0.05 level. The first and third quartiles are depicted by the boxes, with the whiskers representing the maximum non-outlier values. Possible outliers are depicted by the dots.
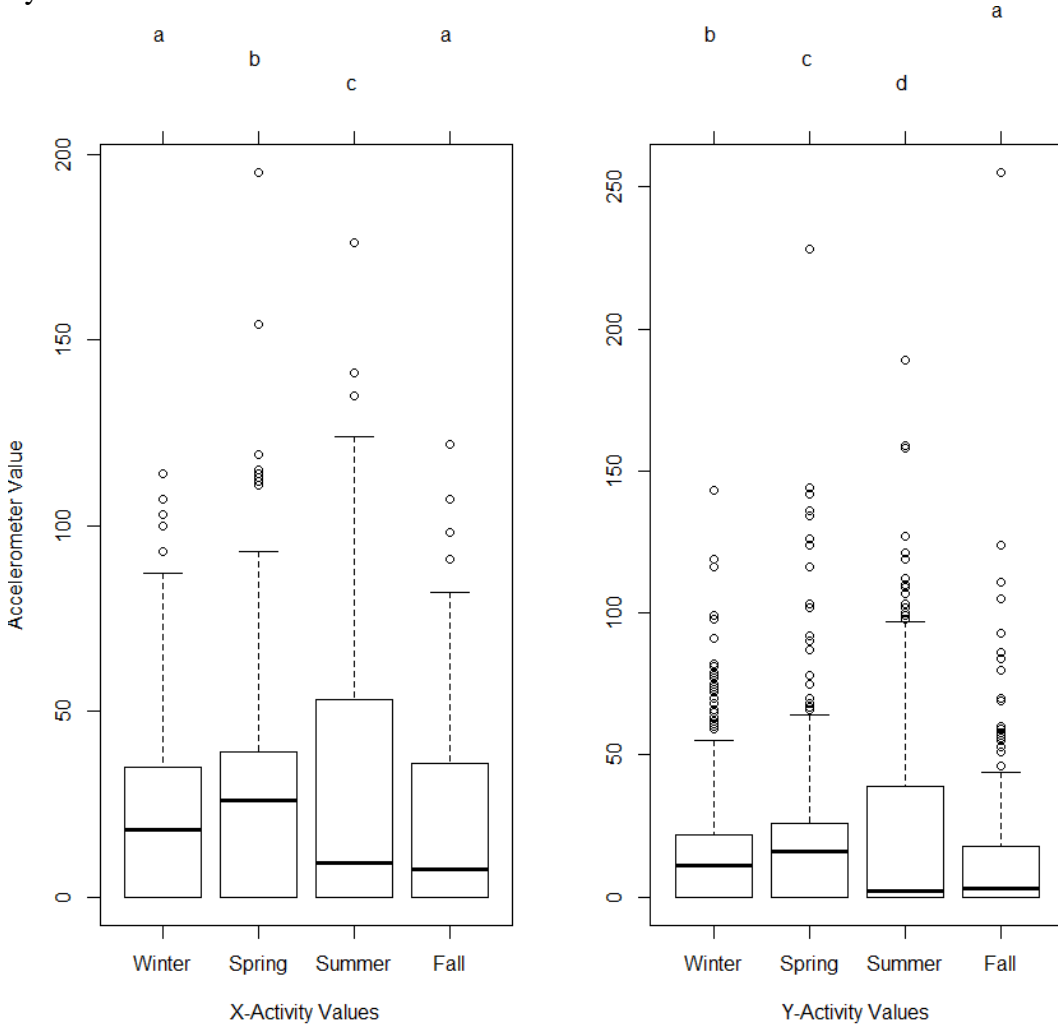
Figure C.2. Mean predicted proportions of time spent foraging, moving and resting within a 5-minute interval in response to changing solar angles for 8 captive moose at the Moose Research Center, Kenai Peninsula, Alaska. Crepuscular times are centered on zero with lowest values corresponding to the middle of the night and the highest values to the middle of the day. Predictions were binned into 5% quantiles. Means and 95% confidence intervals were calculated using bootstrapping with 1000 iterations. Trends are depicted using locally weighted smoothing curves.
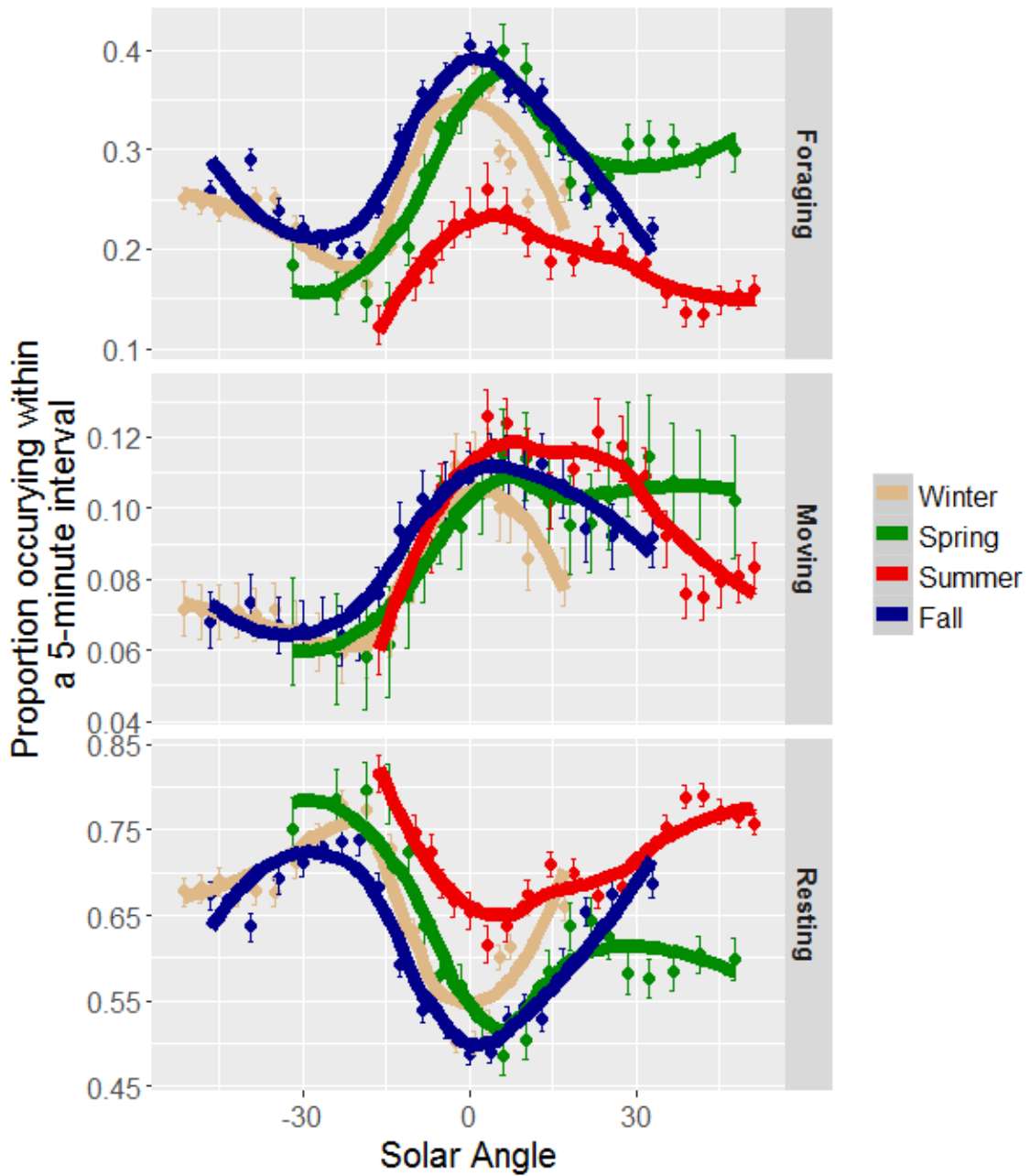
Figure C.3. Mean predicted proportions of time spent foraging, moving and resting within a 5-minute interval in response to changing ambient temperature for 8 captive moose at the Moose Research Center, Kenai Peninsula, Alaska. Predictions were binned into 5% quantiles. Means and 95% confidence intervals were calculated using bootstrapping with 1000 iterations. Trends are depicted using locally weighted smoothing curves.
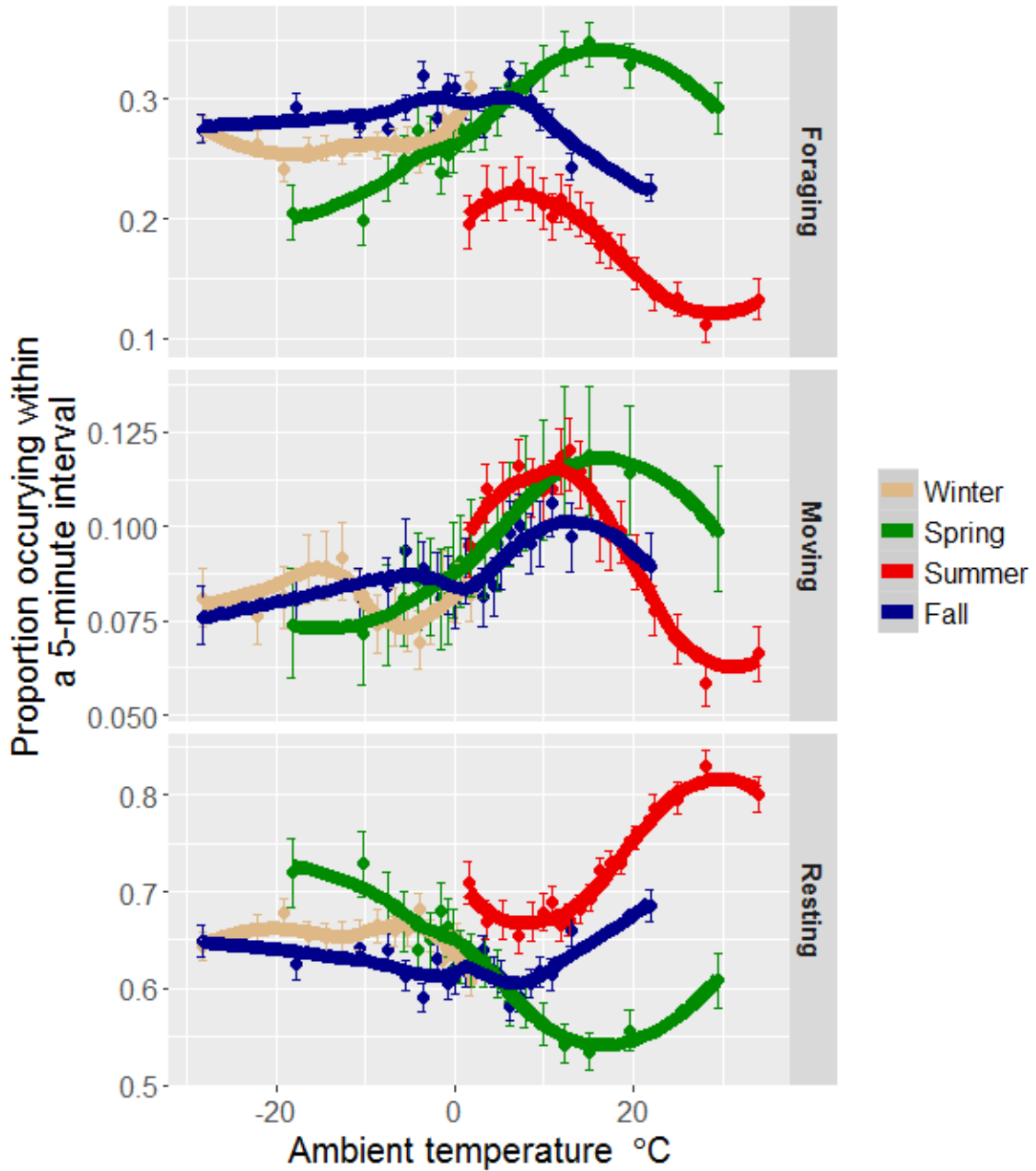
Figure C.4. Mean combined predicted proportions of foraging and moving behavioral states (active) within a 5-minute intervals collected within each habitat in response to changing solar angles for 8 captive moose at the Moose Research Center, Kenai Peninsula, Alaska. Crepuscular times are centered on zero with lowest values corresponding to the middle of the night and the highest values to the middle of the day when the sun is at its highest point. Predictions were binned into 5% quantiles. Means and 95% confidence intervals were calculated using bootstrapping with 1000 iterations. Trends are depicted using locally weighted smoothing curves.
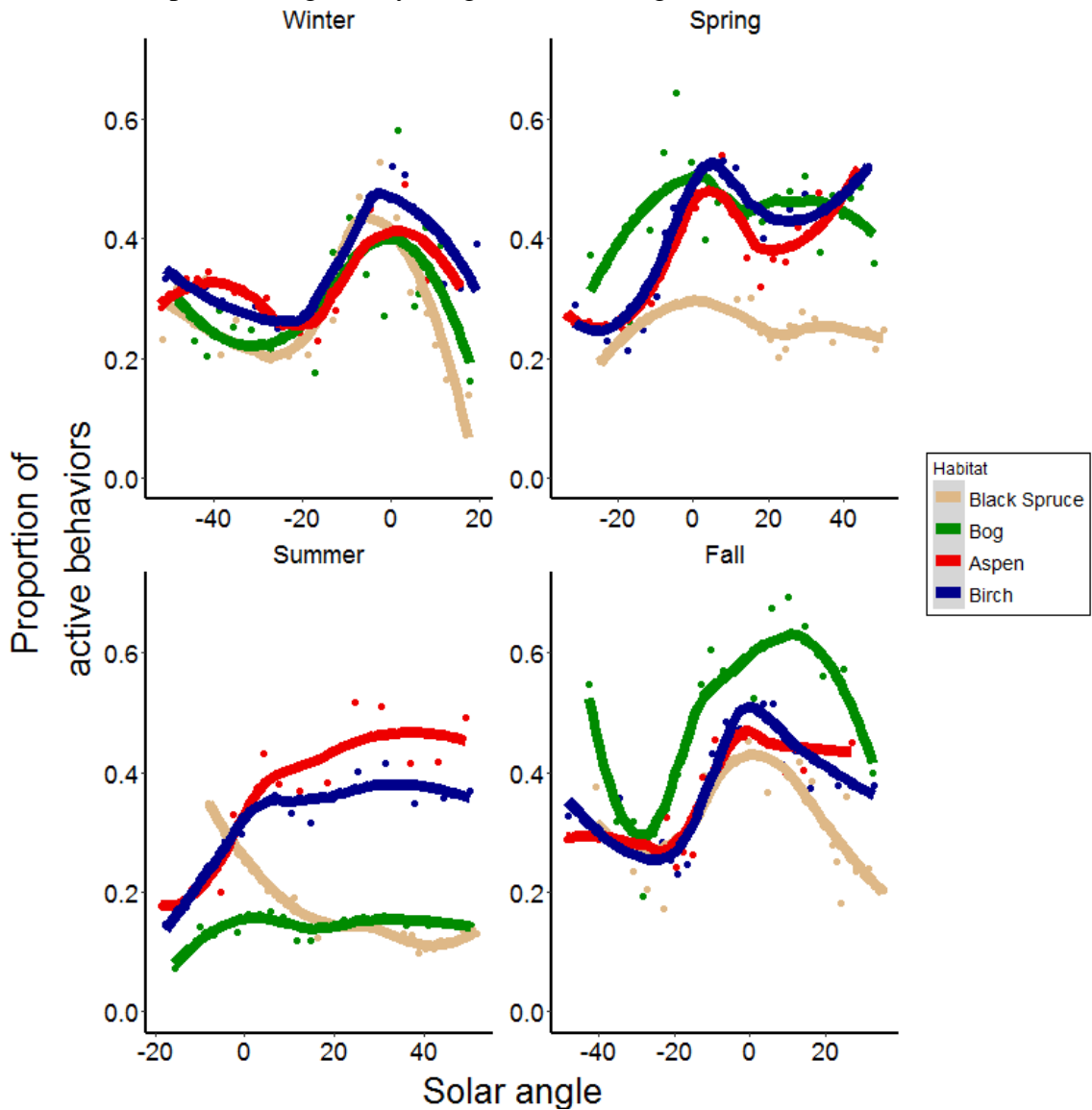
APPENDIX D

Noting the potential bias associated with using our best Dirichlet model without step length, specifically the over prediction of the proportion of time spent resting during times of sustained foraging low behaviors and over prediction of foraging behavior during times of walking with little to no neck movement, a conservative approach would be to predict wild moose behavioral states only as active or inactive during 5-minute intervals. To test this approach, we followed methods similar to Gottardi et al. (2010) and categorized 5-minute intervals as active if at least 20% (60 seconds) of the time the moose was observed foraging or moving in a specific interval. Predictions from our Dirichlet models were used to examine changes in behavioral state (active vs. inactive) in relation to changes in ambient temperature and time of day. This method could be simplified by using logistic regression models to predict active vs. inactive behavioral states instead of post-processing Dirichlet prediction results as active vs. inactive. Paired predictions of behavioral state and ambient temperature/solar angle were binned and bootstrapped as outlined in the paper. Using our same cross-validation procedure, we were able to correctly classify 95% of active intervals (n = 421) and 90% (n = 631) of inactive intervals out of a total of 1,052 5-minute intervals evaluated. We predicted both behavioral proportions and active versus resting states for 285,201 5-minute intervals during 2014 for 3 free-ranging moose in northeastern MN. Comparisons between the two methods can be seen in Figures B.1 and B.2. While the interpretation of the results between the two methods is different, emergent seasonal patterns were similar between proportional (Dirichlet) and discriminant methods in relation to ambient temperature and solar angle.

Figure D.1. A comparison of proportional (Dirichlet) versus discriminant methods. Mean combined predicted proportions of foraging and moving behavioral states (active) within a 5-minute interval in response to changing ambient temperature (Dirichlet) were compared to predictions of the mean proportion of active 5-minute intervals (discriminant), for 8 captive moose at the Moose Research Center, Kenai Peninsula, Alaska. Predictions were binned into 5% quantiles. Means and 95% confidence intervals were calculated using bootstrapping with 1000 iterations. Trends are depicted using locally weighted smoothing curves.
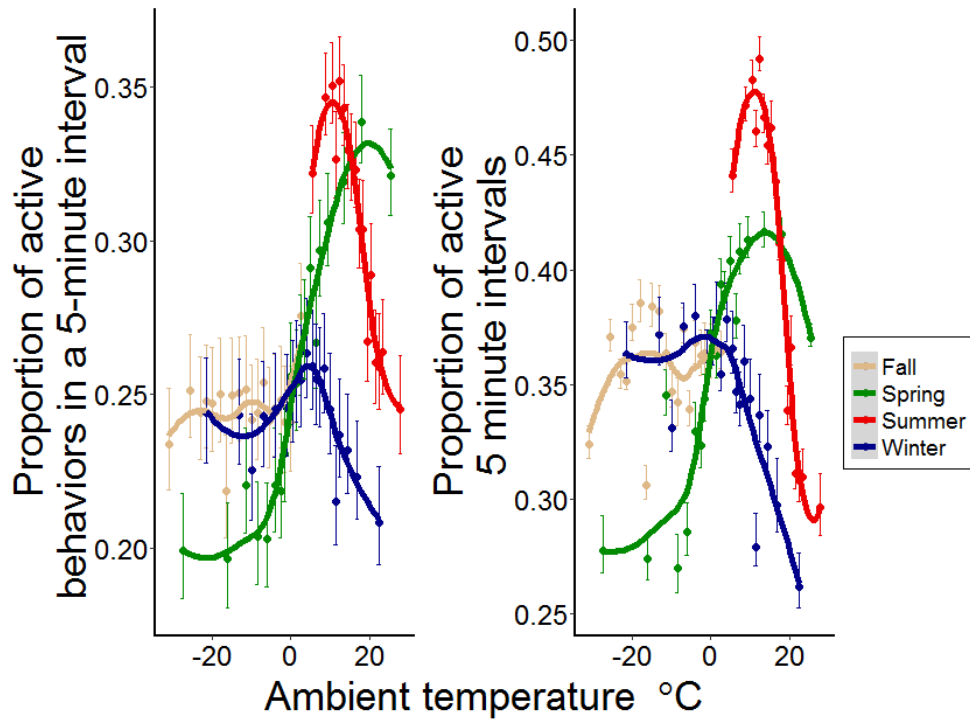
Figure D.2. A comparison of proportional (Dirichlet) versus discriminant methods. Mean combined predicted proportions of foraging and moving behavioral states (active) within a 5-minute interval in response to changing solar angles (Dirichlet) were compared to predictions of the mean proportion of active 5-minute intervals (discriminant), for 8 captive moose at the Moose Research Center, Kenai Peninsula, Alaska. Crepuscular times are centered on zero with lowest values corresponding to the middle of the night and the highest values to the middle of the day when the sun is at its highest point. Predictions were binned into 5% quantiles. Means and 95% confidence intervals were calculated using bootstrapping with 1000 iterations. Trends are depicted using locally weighted smoothing curves.