Integrating Endocrine Biomarkers and Landscape Structure to Advance Non-Invasive Demographic and Physiological Monitoring of Woodland Caribou

By

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GENERAL ABSTRACT

Effective conservation of woodland caribou (*Rangifer tarandus caribou*) requires monitoring approaches that can provide biological information from wide-ranging, low-density populations where direct observation is difficult. Non-invasive fecal sampling is already used in caribou monitoring for genetic identification, sex determination, and population estimation, but the potential for fecal samples to provide additional information on age-class, pregnancy status, and endocrine variation remains less developed. This thesis evaluated the utility of fecal morphometric and endocrine biomarkers for deriving biological information from winter-collected fecal samples in woodland caribou from the Churchill Range of northwestern Ontario. First, we assessed whether fecal pellet morphometrics could distinguish among age-classes during winter, and whether fecal progesterone concentrations could be used to distinguish pregnant from non-pregnant females. Gaussian mixture modeling revealed no evidence of discrete morphometric size classes within sex after accounting for seasonal variation, indicating that pellet morphometrics provided limited age-class resolution. In contrast, fecal progesterone concentrations exhibited clear bimodality and supported classification of most females as pregnant or non-pregnant. Second, we evaluated whether fecal cortisol concentrations varied across gradients of anthropogenic disturbance and landscape composition. Fecal glucocorticoid concentrations were negatively associated with the proportion of the landscape burned within the previous 40 years and positively associated with the proportion of wetlands, indicating that this endocrine biomarker varied with landscape conditions. Collectively, these findings demonstrate that fecal endocrine biomarkers can strengthen non-invasive monitoring of woodland caribou by supporting pregnancy-status classification and providing insight into landscape-associated variation in fGCM concentrations.

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GENERAL INTRODUCTION

Effective wildlife management requires information on demographic structure and biologically relevant individual-level indicators, particularly for species of conservation concern (Bender, 2006; Kühl et al., 2009). For woodland caribou (*Rangifer tarandus caribou*), obtaining this information is challenging because populations occur at low density across large, often remote boreal landscapes where direct observation is difficult (Courtois et al., 2003; Flasko et al., 2017). Non-invasive fecal sampling is therefore especially valuable for caribou monitoring because winter-collected samples are already used to estimate population size, density, and sex ratios (Hettinga et al., 2012; McFarlane et al., 2020). These existing sampling programs also provide an opportunity to evaluate whether the same fecal samples can yield information relevant to demographic monitoring beyond genetic identity, including age-class and reproductive maturity (Ball, 2010; Flasko et al., 2017).

Despite these advances, the potential for fecal samples to inform age-class and reproductive maturity remains less developed. Age-class information is important because distinguishing calves, yearlings, and adults can help interpret population structure and recruitment, but age-class is not readily inferred from fecal DNA alone (Ball, 2010). As a result, fecal pellet-based indicators are needed to determine whether age-related information can be derived from non-invasively collected samples. Fecal pellet morphometrics have been explored as age-class proxies based on the expectation that pellet dimensions scale with body size (Ball, 2010; Breiter et al., 2012; Flasko et al., 2017). However, classification performance varies among populations and sampling contexts, and overlap among calves, yearlings, and adults can limit reliable assignment (Flasko et al., 2017; Morden et al., 2011).

Endocrine markers may provide complementary information when hormone concentrations can be used to identify pregnancy and, by extension, reproductive maturity among females (Morden, 2010; Sheriff et al., 2011). Fecal progesterone is particularly relevant for winter-collected samples because elevated progesterone concentrations can indicate pregnancy after the autumn breeding period (Ropstad, 2000), allowing pregnant females to be distinguished from calves and other non-pregnant individuals. Together, pellet morphometrics and fecal progesterone may improve the biological information obtained from non-invasively collected fecal samples (Flasko et al., 2017), but their utility requires evaluation under local ecological and sampling conditions before being applied for age-class or maturity-related inference in new populations.

Fecal endocrine markers may also provide information on how individuals respond physiologically to their environment (Möstl & Palme, 2002; Parker, 2003). Fecal glucocorticoid metabolites reflect circulating glucocorticoid activity over the period preceding defecation and provide a non-invasive indicator of hypothalamic-pituitary-adrenal (HPA) axis activation (Sheriff et al., 2011). Previous work has linked glucocorticoid variation in caribou to anthropogenic disturbance and habitat conditions (Ewacha et al., 2017). However, fecal glucocorticoid concentrations do not directly measure body condition or identify the specific mechanism underlying an endocrine response. Instead, they provide a non-invasive biomarker that can be evaluated in relation to landscape characteristics, helping to identify environmental contexts associated with variation in glucocorticoid activity.

For Ontario woodland caribou, the extent to which fecal samples can provide demographic and endocrine information beyond genetic identity remains unclear. Accordingly, this thesis evaluates whether fecal pellet morphometrics can provide age-related information, whether fecal progesterone concentrations can support classification of pregnancy status in winter-collected samples, and whether fecal cortisol concentrations vary in relation to landscape composition and disturbance. Together, these analyses assess the extent to which winter-collected fecal samples can be used to derive age-, pregnancy-, and endocrine-related information relevant to woodland caribou monitoring.

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CHAPTER 1

Evaluating Fecal Pellet Morphometrics and Endocrine Markers for Non-Invasive Inference of Age-Class and Pregnancy Status in Woodland Caribou

ABSTRACT

Non-invasive fecal sampling is widely used for individual identification, sex determination, and population estimation in woodland caribou (*Rangifer tarandus caribou*), but its utility for extracting recruitment-relevant information remains less clear. We evaluated whether fecal pellet morphometrics and progesterone metabolites from winter-collected fecal samples could be used to infer broad age-related structure and pregnancy status in the Churchill Range of northwestern Ontario. Pellet length, width, and weight varied strongly by sampling month, and Gaussian mixture modelling identified a single morphometric group, indicating limited support for discrete age-class classification based on pellet measurements. In contrast, fecal progesterone concentrations showed a bimodal distribution consistent with pregnant and non-pregnant females; 85% of genetically identified females were assigned to one of these two groups, while 15% remained uncertain. These results suggest that pellet morphometrics provide limited resolution for age-class inference in this population, whereas fecal progesterone offers a useful non-invasive indicator of pregnancy status when paired with genetic sex identification. More broadly, integrating endocrine assays with fecal DNA-based monitoring may improve the demographic information obtained from non-invasive sampling, particularly for low-density caribou populations where direct observation of recruitment is limited.

KEYWORDS

Woodland caribou, conservation physiology, demographic inference, endocrine assays, non-invasive monitoring, pregnancy assessment, seasonal variation.

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INTRODUCTION

The development of effective wildlife conservation and management strategies relies on understanding demographic structure, including age-class composition and the relative proportion of sexually mature individuals (Bender, 2006; Kühl et al., 2009). For slow-reproducing ungulates such as woodland caribou (*Rangifer tarandus caribou*), even subtle changes in age-class structure or recruitment can have disproportionate effects on long-term population trajectories (Ellington et al., 2020). Demographic monitoring can therefore help identify evidence of reduced recruitment or changing age-class structure, both of which are important factors for accurately interpreting population status in threatened caribou populations (Lelotte et al., 2025; Morden et al., 2011b).

Non-invasive fecal sampling has become an important tool for studying woodland caribou because it allows samples to be collected across broad spatial scales with minimal disturbance (Flasko et al., 2017; Morden et al., 2011b). Fecal DNA is now routinely used for individual identification and sex determination, enabling estimation of population size using spatial capture-recapture (SECR) methods (McFarlane et al., 2020). However, fecal samples currently provide limited information on demographic structure beyond sex. Identifying age-class structure and recruitment are important for understanding caribou population trends, yet distinguishing these demographic components from non-invasive samples remains challenging (Environment Canada, 2012; Morden et al., 2011b).

One possible way to extract age-related information from fecal samples is through pellet morphometrics (Breiter et al., 2012; Morden et al., 2011a). Pellet dimensions are expected to scale with body size, which could allow smaller calves or yearlings to be distinguished from larger adults (Breiter et al., 2012; Chame, 2003). Pellet length, width, and mass have been tested as non-invasive indicators of age-class in caribou, but results have varied among studies (Ball, 2010; Breiter et al., 2012; Flasko et al., 2017; Morden et al., 2011a). Calibration with known-age Newfoundland caribou collected from harvested and road-killed animals showed that pellet length could distinguish calves and yearlings from adults (Ball, 2010). However, subsequent studies reported more variable classification success, with separation among calves, yearlings, and adults differing among ranges, sampling periods, and analytical approaches (Breiter et al., 2012; Flasko et al., 2017). These results suggest that pellet morphometrics may help infer broad age-class differences, but their reliability likely depends on population context, sampling conditions, and local validation.

Fecal progesterone metabolites may provide complementary information by identifying pregnancy status in winter-collected samples. Since caribou breed in autumn, pregnant females are expected to maintain elevated progesterone concentrations through winter, whereas non-pregnant individuals generally exhibit lower concentrations during this period (Ropstad, 2000; Bubenik et al., 1997; Bleke et al., 2021). Fecal progesterone metabolites are therefore a reliable endocrine indicator for distinguishing pregnant from non-pregnant females under winter sampling conditions (Morden et al., 2011b). Thus, pellet morphometrics and fecal progesterone may offer complementary ways to infer broad maturity-related classes from winter fecal samples.

To our knowledge, the performance of non-invasive fecal sampling approaches for distinguishing reproductively mature from immature woodland caribou has not yet been evaluated in Ontario populations. Thus, our objective was to evaluate whether pellet morphometrics and fecal progesterone concentrations from winter fecal samples could provide information on maturity-related differences among individuals. We predicted that (i) pellet morphometric traits would exhibit detectable size-based structure consistent with smaller pellets from calves and larger pellets from mature individuals; (ii) winter progesterone concentrations would distinguish between pregnant and non-pregnant females; and (iii) pellet morphometric traits would differ between pregnant and non-pregnant females. Developing non-invasive methods to infer demographic structure would improve approaches for assessing recruitment in low-density, wide-ranging woodland caribou populations, where direct observation and repeated monitoring are often limited.

METHODS

Study Area

The fecal samples analyzed in this study were collected within the Churchill Range of northwestern Ontario, a boreal caribou range covering approximately 21 300 km² near the southern limit of continuous woodland caribou distribution (Figure 1). The south-central boundary lies approximately 10 km north of Sioux Lookout, Ontario, and represents a transition zone between more continuous boreal forest to the north and increasingly mixed land-use patterns to the south (MNRF, 2014). The Churchill Range is characterized by conifer-dominated forests, extensive wetlands, and numerous freshwater lakes. Mature forest remains widespread across much of the range, although the landscape is also influenced by forest management, mineral exploration, and associated transportation networks. Land-use intensity varies spatially, with higher levels of infrastructure and resource development in the southern and western portions of the range and comparatively lower development density further north. Historical habitat conditions across the range supported broad caribou occupancy; however, current records indicate reduced use of some southern areas and continued concentration of caribou activity in the north-central portion of the range.

Fecal Sample Collection

Fecal samples were collected across the Churchill Range of northwestern Ontario during winter 2024 (January 2024 to March 2024) as part of a government-funded monitoring program. Field sampling was conducted by contracted professionals following established safety protocols. This study did not involve animal capture or handling; therefore, animal ethics approval was not required.

Sampling occurred at locations where caribou presence was identified through aerial reconnaissance and subsequent ground verification (Orchard, 2025; Warwick, 2025). At each site, pellets were collected from discrete pellet groups; a sample was defined as a minimum of 10 pellets originating from a single defecation event. Pellets were handled using sterile, single-use tools and placed into uniquely labelled bags. Geographic coordinates were recorded using handheld GPS units. Samples were maintained frozen during field operations and stored at -20 °C until laboratory analyses. A total of 601 fecal samples were collected from 53 locations across the Churchill Range, including 132 samples in January, 107 in February, and 362 in March. DNA extraction and genotyping were conducted at Lakehead University by Warwick (2025) and Orchard (2025) following established protocols for individual identification and sex determination. These analyses resulted in the identification of 240 unique caribou, including 126 females and 114 males.

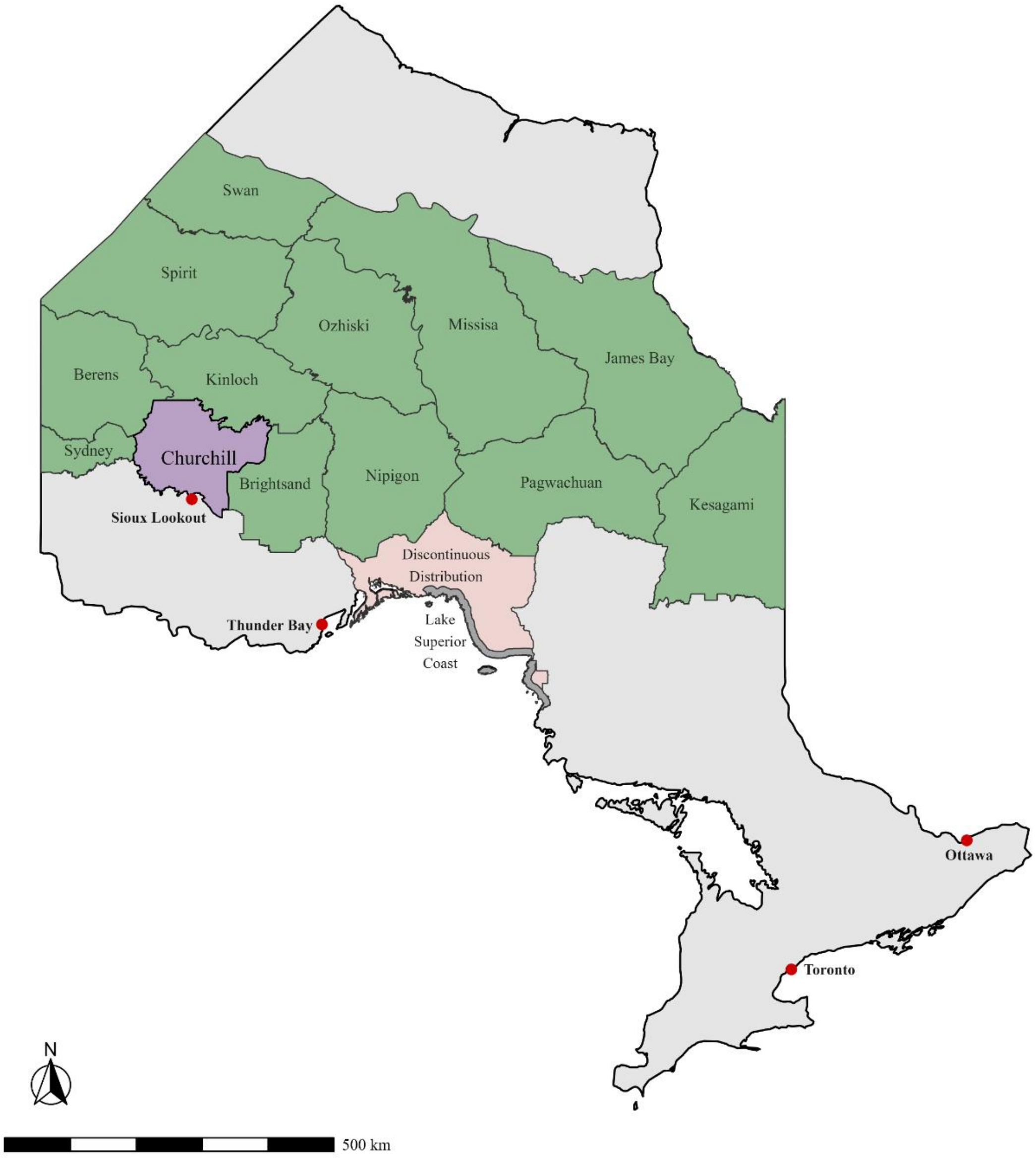

Figure 1. Spatial context of woodland caribou ranges in Ontario, Canada. The focal Churchill range is highlighted in purple, with surrounding ranges shown in green and the discontinuous distribution along the Lake Superior coast shown in light red. Major cities and towns are included for reference.

Pellet Morphometrics

From each fecal sample, three pellets were randomly selected for morphometric analysis. Pellets were dried in an incubator at 60 °C for 24 h to remove residual moisture content prior to hormone extraction (Flasko et al., 2017; Pahuja & Narayan, 2023). Following drying, the three pellets were weighed together to the nearest 0.001 g using a precision balance, and the combined mass was used to calculate mean pellet weight per sample. Visual inspection indicated that pellet shape and structural features were retained following dehydration (Figure S1-1). The length and width of each individual pellet were measured to the nearest 0.01 mm using digital calipers (Figure S1-2). For each sample, average pellet length, width, and weight were calculated from the three measured pellets and used in subsequent analyses. Each sample was measured by the same technician to ensure consistency and reduce inter-observer measurement error.

Pellet Homogenization and Hormone Extraction

Following drying, fecal pellets were mechanically homogenized using a bead beater to ensure uniform mixing within each sample. Progesterone extraction followed Arbor Assay's DetectX® Steroid Solid Extraction Protocol (Arbor Assays, 2022). A 0.18 g subsample of homogenized fecal material was combined with 1.8 mL of anhydrous ethanol and vortexed for 1 hour to facilitate steroid extraction. Samples were then centrifuged at 5,000 rpm for 15 minutes, and 1 mL of supernatant was transferred to a clean tube and evaporated to dryness using a vacuum concentrator (approximately 2 hours). Dried extracts were reconstituted in 1 mL of anhydrous ethanol and stored at -20 °C until progesterone quantification.

Progesterone Assay Validation and Quantification

Fecal progesterone concentrations were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA; Arbor Assays DetectX® Progesterone Enzyme Immunoassay Kit, Cat. No. K025-H5) following the manufacturer's instructions. Prior to sample analysis, assay performance was evaluated through assessment of inter-assay precision, dilution linearity, parallelism, and post-extraction spike recovery. A pooled fecal extract was serially diluted (1:20-1:1280) to assess proportionality across dilutions and identify the minimum required dilution (MRD) that minimized matrix interference. Dilution parallelism was evaluated by examining back-calculated concentrations across dilutions and by estimating the slope of the log-log relationship between measured concentration and dilution factor. Post-extraction spike-recovery tests were conducted by adding known concentrations of progesterone standard to fecal extracts. Matrix blanks and assay buffer blanks were included to assess background absorbance and confirm assay specificity. Based on these assessments, a working dilution of 1:1250 was selected for all subsequent analyses.

All samples were analyzed in duplicate on 96-well microplates, with validation samples run in triplicate. Absorbance was measured at 450 nm using a BioTek Synergy H1 microplate reader. Concentrations (pg/mL) were calculated from standard curves generated for each plate using a four-parameter logistic (4PL) regression model and are reported at the final assay dilution. Analytical precision was evaluated using intra- and inter-assay coefficients of variation (CVs).

Statistical Analysis

All analyses were conducted in R version 4.5.2 (R Core Team, 2025). Pellet length (mm), width (mm), and weight (g) were treated as continuous variables. Sampling month (January, February, March) was included as a covariate to account for seasonal variation in progesterone concentrations among individuals.

To evaluate whether pellet morphometrics exhibited underlying size-based structure consistent with demographic size classes, Gaussian mixture models (GMMs) were applied using the *mclust* package (Scrucca et al., 2023). Since pellet dimensions may vary seasonally, pellet length, width, and weight were first adjusted for sampling month using linear models. Standardized residuals from these models were retained to remove seasonal effects while preserving relative size differences among individuals. Clustering analyses were conducted separately within sex to avoid confounding due to sexual size dimorphism. A composite morphometric size index was constructed by summing standardized residuals for length, width, and weight, thereby integrating multiple pellet traits into a single multivariate measure of relative size. GMMs specifying one to three components were fitted to this index. Model selection was based on Bayesian Information Criterion (BIC), with support for multiple components interpreted as evidence of underlying size-based structure. Posterior probabilities and model entropy were examined to assess clustering strength and separation among putative groups.

Progesterone concentrations were analyzed to evaluate whether winter fecal samples exhibited underlying structure consistent with pregnancy status. Since progesterone concentrations were positively skewed, values were log-transformed prior to analysis to improve distributional symmetry. GMMs were fitted to log-transformed progesterone concentrations using the *mclust* framework. Models specifying one to three components (i.e. Gaussian distributions characterized by distinct means, variances, and mixing proportions) were evaluated, and the optimal number of components was selected using BIC. Support for a two-component solution was interpreted as evidence of bimodal structure consistent with distinct high- and low-progesterone states. Posterior probabilities of component membership were calculated for every individual. The component with the higher mean concentration was designated as the high-progesterone state. Individuals were classified as High or Low progesterone based on posterior probability, and individuals with intermediate probabilities were designated as 'uncertain' and excluded from subsequent analyses evaluating morphometric differences by physiological state but were retained for visualization of progesterone distributions and morphometric patterns.

To evaluate whether pellet morphometrics differed between progesterone-defined physiological states, analyses were restricted to female samples confidently classified as High or Low progesterone. Individuals designated as Uncertain were excluded to reduce misclassification bias. Morphometric traits (length, width, and weight) were first evaluated jointly using multivariate analysis of variance (MANOVA):

$$Y_i = \beta_0 + \beta_1 \text{State}_i + \beta_2 \text{Month}_i + \varepsilon_i$$

where Y_i represents the vector of morphometric traits for individual i, state denotes progesterone-defined classification (high vs. low), and month accounts for seasonal variation.

Pillai's Trace was used as the multivariate test statistic due to its reliability to moderate deviations from multivariate normality. Trait-specific linear models were subsequently fitted:

$$Y_i = \beta_0 + \beta_1 \text{State}_i + \beta_2 \text{Month}_i + \varepsilon_i$$

Type II sums of squares were used to evaluate the effect of progesterone-defined state while accounting for sampling month.

RESULTS

Progesterone Assay Validation

Assay validation indicated acceptable analytical performance of measuring fecal progesterone metabolites. Serial dilutions 1:320 to 1:1280 met the ≤20% deviation criterion, with dilutions ranging from +8% to +12%. At the selected minimum required dilution (1:1280) was 81.9%. The estimated log-log parallelism slope was 1.19 (95% CI: 0.77-1.61), indicating proportional assay response across the dilution series. Inter-assay coefficient of variation was 10.45% (Table S1-1).

Morphometric Structure and Across Sex and Season

Across the winter sampling period, males consistently exhibited greater mean pellet length, width, and weight relative to females (Table S1-2; Figure S1-3). Seasonal variation was also evident for all traits, with morphometric values generally lowest in February and highest in March for both sexes. The greatest sex-based differentiation was observed in pellet mass, particularly during March, when mean pellet mass was highest for both males and females. Despite these trends, substantial overlap between sexes was observed within each month, suggesting that seasonal and demographic effects were modest in magnitude. Mean pellet length, width, and weight increased from February to March in both sexes, with broadly parallel seasonal trajectories evident across traits. Multivariate analyses indicated significant effects of sampling month and sex, with no significant interaction (Table S1-3).

A GMM of the composite month-adjusted morphometric size index provided no evidence of multimodal structure within either sex (Figure 2). For females, model selection based on BIC supported a single-component solution (G = 1; BIC = -585.28), with posterior probabilities uniformly equal to 1.00 and entropy approaching zero. Similarly, for males, BIC favored a single-component model (G = 1; BIC = -550.05), with posterior probabilities equal to 1.00 and negligible entropy. The morphometric size index exhibited smooth unimodal distributions in both sexes, indicating little support for morphometric-based discrimination of putative age-classes within the sampled winter population.

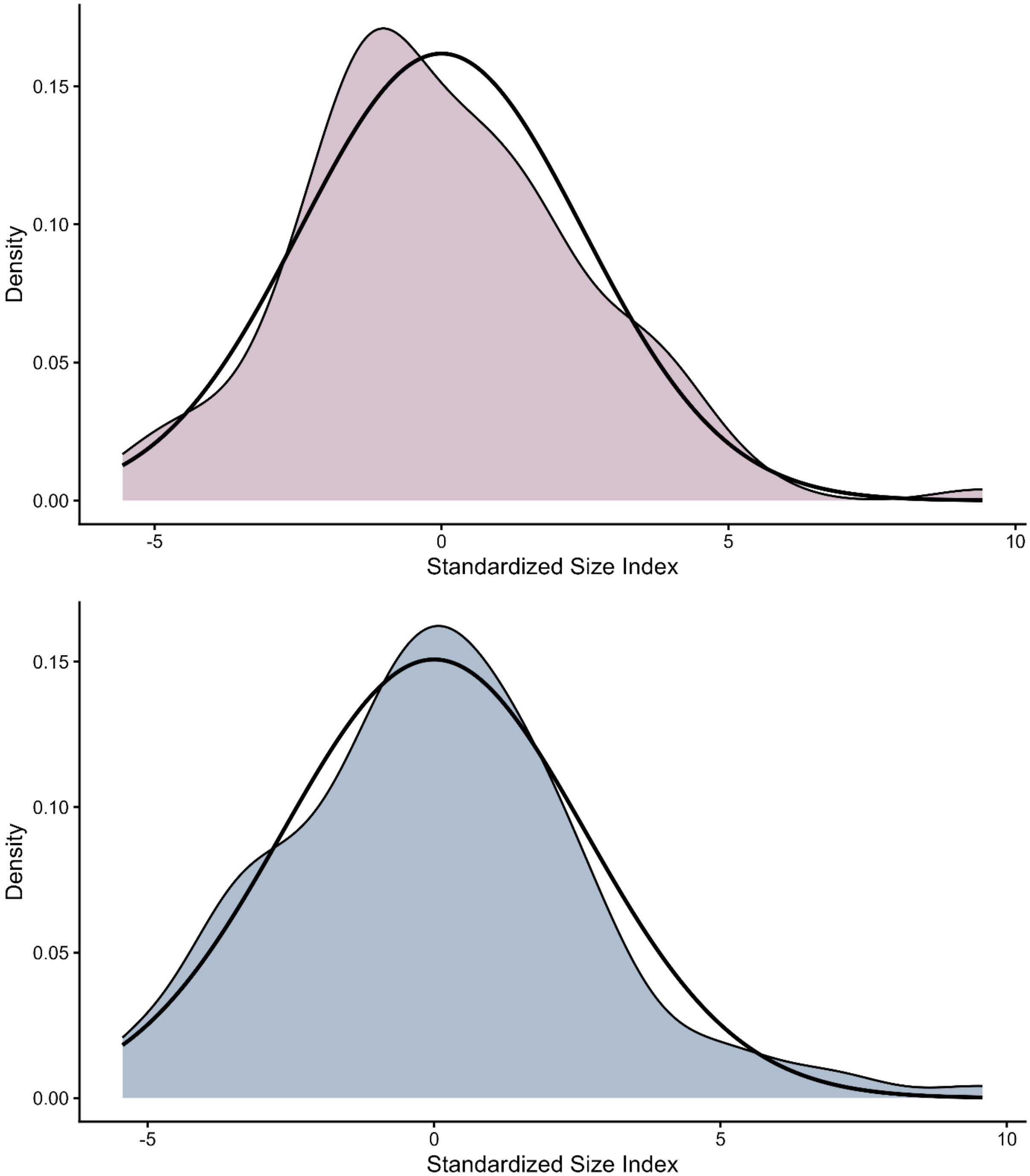

Figure 2. Density distributions of the standardized morphometric size index in female (top panel) and male (bottom panel) boreal woodland caribou. The size index was calculated as the sum of month-adjusted z-scores for pellet length, width, and weight. Shaded areas represent empirical kernel density estimates, and solid lines indicate fitted GMM densities. Distributions were evaluated to assess potential multimodality consistent with putative age-class structure.

Distributional Structure in Female Progesterone Concentrations

Log-transformed progesterone concentrations in females exhibited bimodality, consistent with two underlying physiological states (Figure 3). A GMM identified two primary progesterone distributions corresponding broadly to pregnant and non-pregnant individuals. However, overlap between distributions resulted in a subset of samples with ambiguous classification. Using posterior probability thresholds (≥0.90 for high progesterone; ≤0.10 for low progesterone), 87 of 124 females (70.2%) were classified as pregnant, 19 (15.3%) as non-pregnant, and 18 (14.5%) as uncertain. Percentile thresholds derived from confidently classified individuals further demonstrated separation between groups: the 95th percentile of the non-pregnant distribution was 231.9 pg/mL, whereas the 5th percentile of the pregnant distribution was 758.0 pg/mL. Among confidently classified samples, the maximum progesterone concentration observed among non-pregnant females (300.0 pg/mL) remained well below the minimum observed among pregnant females (711.9 pg/mL), supporting strong hormonal separation between clusters at the extremes of the distribution.

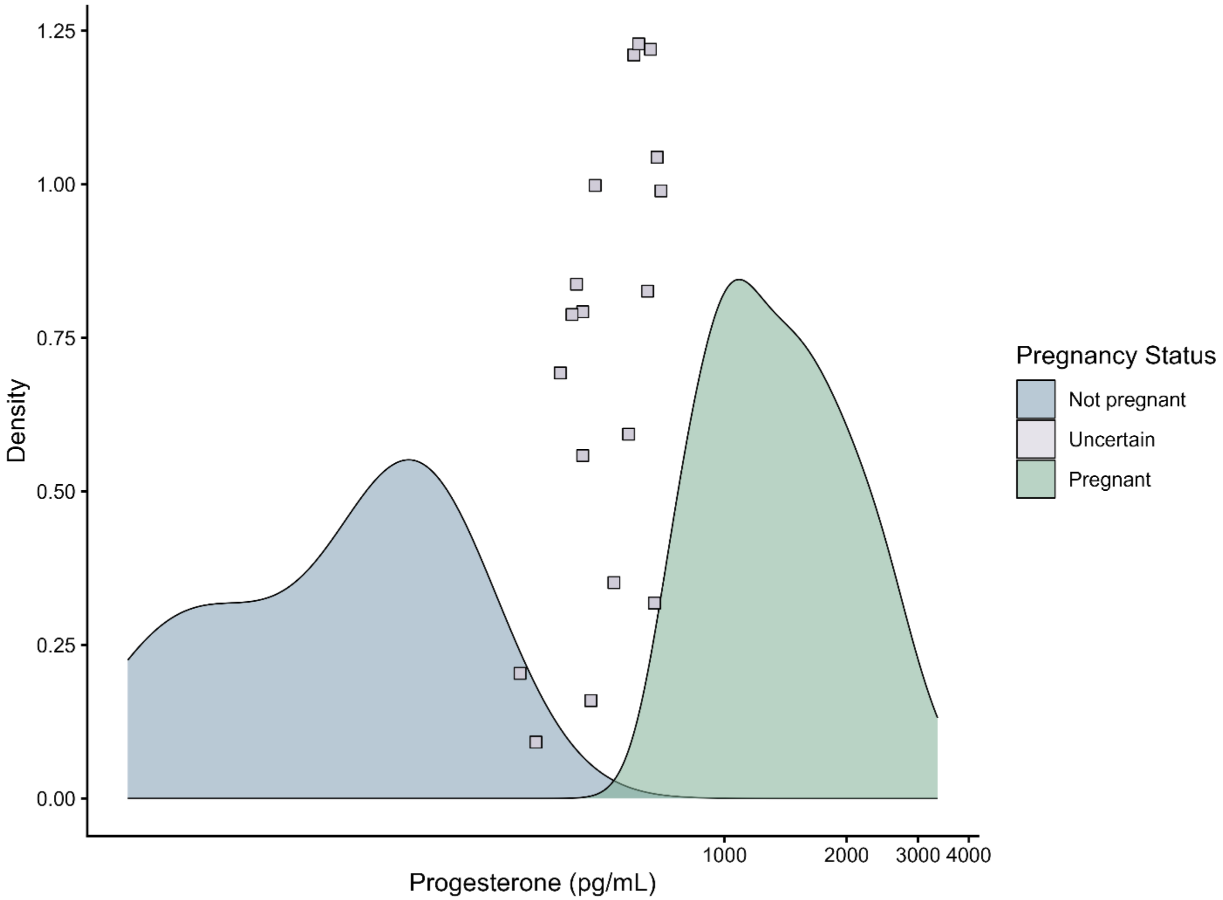

Figure 3. Density distributions of log-transformed fecal progesterone concentrations (pg/mL) in female caribou. Samples were classified as non-pregnant, uncertain, or pregnant using GMM. Points represent individuals with uncertain classification, reflecting overlap between reproductive states.

Morphometric Differences by Progesterone-Defined State

Among confidently classified females ($n = 106$), morphometric traits did not differ by progesterone-defined state. Multivariate analysis indicated no significant effect of state on the combined morphometric profile (Pillai's Trace = 0.017, $F_{3,100} = 0.56$, $p = 0.642$). In contrast, sampling month exerted a significant multivariate effect (Pillai's Trace = 0.221, $F_{6,202} = 4.18$, $p < 0.01$), indicating that seasonal variation was the primary driver of morphometric differences. Trait-specific analyses controlling for month were consistent with the multivariate results, with no significant effects of progesterone-defined state on pellet length, width, or weight (all $p > 0.31$; Table S1-4). Morphometric distributions overlapped extensively between pregnant and non-pregnant females (Figure 4), indicating that pellet morphology did not differentiate progesterone-defined physiological states during winter sampling.

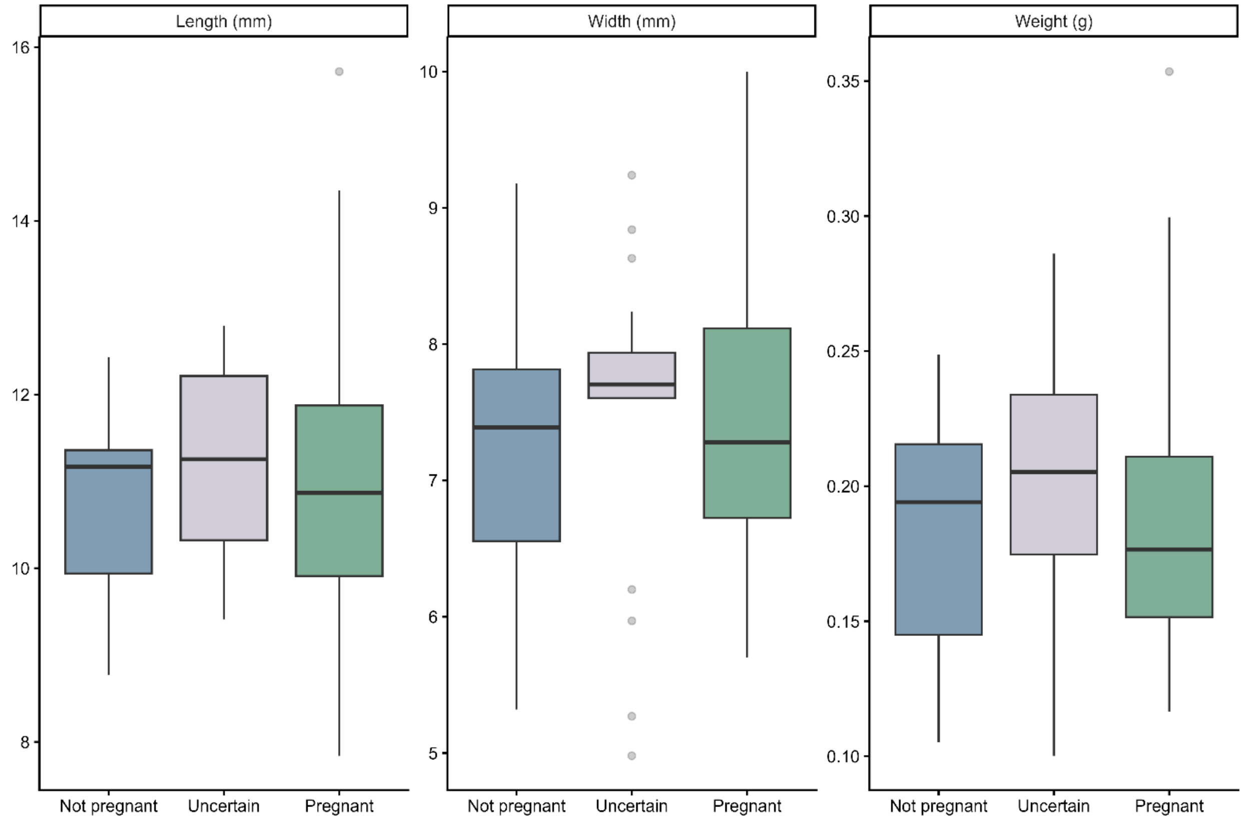

Figure 4. Pellet morphometrics of female caribou classified by pregnancy status using a GMM applied to fecal progesterone concentrations. Boxplots show pellet length (mm), width (mm), and weight (g) for individuals categorized as non-pregnant, uncertain, or pregnant. Boxes represent the interquartile range (IQR), center lines indicate medians, whiskers extend to 1.5× IQR, and points denote outliers. Pregnancy classifications were assigned based on posterior probabilities derived from the log-transformed progesterone distribution. Individuals classified as uncertain were included for visualization but excluded from statistical analyses.

DISCUSSION

This study evaluated whether winter-collected fecal samples could provide maturity- and recruitment-related information for woodland caribou in the Churchill Range. Specifically, we tested whether pellet length, width, and weight could reveal broad age-related structure consistent with immature versus mature individuals, and whether fecal progesterone concentrations could distinguish pregnant from non-pregnant females. Progesterone concentrations allowed pregnancy status to be assigned for most females, with the remaining individuals classified as uncertain. In contrast, pellet morphometrics showed no detectable structure consistent with discrete age-classes. Collectively, these findings suggest that pellet morphometrics alone provide limited resolution for demographic classification in winter, whereas fecal progesterone can provide useful non-invasive information on pregnancy status.

Age-Class Inference from Fecal Pellet Morphometrics

Our first prediction was that fecal pellet morphometrics would show size-based separation between immature individuals and larger, reproductively mature adults. This prediction was not supported. After accounting for seasonal variation, Gaussian mixture modelling of the composite morphometric size index identified a single-component solution within each sex, with no evidence of multimodality. Fecal pellet morphometrics therefore did not reveal discrete size classes consistent with immature and mature individuals during the winter sampling period.

Our findings differ from Ball (2010), who reported separation of juvenile and adult caribou based on pellet length using samples from known-age Newfoundland caribou. However, Ball (2010) relied on comparisons among pre-defined age-classes and the application of threshold values, whereas our analysis tested whether discrete morphometric groups emerged without prior age labels. Other studies have reported more limited age-class resolution (Morden, 2010; Breiter et al., 2012; Flasko et al., 2017). Although calves may show some separation from older individuals, yearlings and adults often overlap substantially in pellet measurements, and classification success varies among populations, sampling periods, and analytical approaches. This pattern is consistent with continuous variation in body size and fecal pellet dimensions rather than naturally discrete morphometric classes (Morden et al., 2011; Parker, 2003). Collectively, our findings suggest that fecal pellet size likely varies along a continuum rather than forming biologically discrete age-class groups. As a result, threshold-based age-class assignments are unlikely to be significant for unknown-age field samples, particularly when applied across populations or sampling conditions.

Structural and Seasonal Variation in Morphometrics

Although males produced slightly longer, wider, and heavier pellets than females, sex was not evaluated as a primary classification target in this study. Since sex can be reliably determined from fecal DNA in captive and wild ungulate populations (McFarlane et al., 2022; Yamauchi et al., 2000), morphometric discrimination of sex was neither necessary nor operationally relevant. Rather, sex differences were evaluated to quantify background structural variation that could confound demographic inference if not accounted for. Consistent with known sexual size dimorphism in cervids (Ball, 2010; Flasko et al., 2017; Morden et al., 2011), males exhibited modest increases in mean pellet dimensions relative to females. However, these differences were small relative to within-sex variability and did not permit reliable classification at the individual-level. More importantly, the presence of even modest sex effects underscores the necessity of controlling for sex in morphometric analyses aimed at detecting other demographic signals, such as age-class or reproductive state.

Pellet sampling month exerted a stronger influence on morphology than sex, particularly for width and weight. Morphometric values shifted across the winter sampling period, indicating sensitivity to short-term environmental conditions. Since pellet structure reflects digestive processing and forage intake (You et al., 2022), such temporal variation likely reflects ecological conditions rather than demographic structure. The absence of sex × month interactions suggests that environmental influences operated similarly across sexes. Together, these findings highlight that both sex and season represent structural sources of variation that must be accounted for prior to evaluating demographic structure in fecal morphometric studies.

Endocrine Inference of Pregnancy

Our second prediction was that winter progesterone concentrations would distinguish between pregnant and non-pregnant females; This prediction was supported. Fecal progesterone concentrations exhibited bimodality consistent with two underlying reproductive states. GMM supported a two-component solution, with posterior probability thresholds strongly differentiating individuals at the extremes of the distribution. Partial overlap between distributions resulted in an intermediate subset of females that were classified as uncertain, representing females with intermediate progesterone concentrations or posterior probabilities that did not provide strong support for assignment to either reproductive state. These individuals may reflect biological overlap between pregnant and non-pregnant females, potentially arising from variation in gestational stage or individual differences in hormone metabolism, as progesterone concentrations are known to vary within and among individuals (Ropstad, 2000). Our results are consistent with established reproductive endocrinology in caribou, where progesterone rises following conception and remains elevated throughout gestation (Čupić et al., 2021; Ropstad, 2000). Similar bimodal winter progesterone distributions have been documented in free-ranging cervids and other ungulates, supporting the reliability of fecal progesterone as a non-invasive indicator of pregnancy (Bleke et al., 2021; Flasko et al., 2017). The clear differentiation observed at the extremes of the distribution further supports the application of mixture modeling approaches for endocrine classification in wild caribou populations (Flasko et al., 2017; Morden, 2010).

Our second prediction was that winter fecal progesterone concentrations would distinguish pregnant from non-pregnant females; this prediction was supported. Gaussian mixture modelling identified two progesterone components consistent with putatively non-pregnant and putatively pregnant females, and approximately 85% of females were assigned to one of these groups with sufficient posterior support. Females with intermediate progesterone concentrations could not be reliably assigned and were therefore classified as uncertain. These uncertain classifications represent values in the region of overlap between the putative pregnant and non-pregnant distributions, where biological interpretation is ambiguous. Intermediate progesterone values could reflect pregnant females with relatively low concentrations, non-pregnant females with relatively high concentrations, recent pregnancy loss, or variation associated with gestational timing, body condition, hormone metabolism, fecal degradation, or assay response (Bleke et al., 2021; Cook et al., 2004; Messier et al., 1990; Millspaugh &

Washburn, 2004; Morden et al., 2011b; Ropstad, 2000); without independent confirmation of pregnancy status or repeated hormone measurements, these alternatives cannot be distinguished.

Similar bimodal progesterone distributions have been reported in free-ranging cervids, including caribou, where low and high progesterone groups were interpreted as non-pregnant and pregnant females, respectively (Morden, 2010; Flasko et al., 2017; Bleke et al., 2021). As in those studies, the clearest classification occurred at the extremes of the distribution, whereas females with intermediate concentrations were less reliably assigned. The separation between the lowest values assigned to the putatively pregnant group and the highest values assigned to the putatively non-pregnant group provides a study-specific indication of where classification was most reliable, but should not be treated as a universal threshold. Absolute fecal progesterone concentrations can vary among populations, sampling periods, extraction procedures, and assay kits, so the key inference is the relative separation between low and high progesterone values within this validated dataset rather than the absolute concentrations themselves (Bleke et al., 2021; Millspaugh & Washburn, 2004; Morden et al., 2011b). Thus, fecal progesterone appears useful for classifying pregnancy status when clear distributional separation exists, but intermediate values should be retained as uncertain rather than forced into discrete reproductive categories.

Morphometric Response to Reproductive State

Our third prediction was that pellet morphometrics would differ between pregnant and non-pregnant females. This prediction was not supported. Despite clear endocrine separation between pregnant and non-pregnant females, pellet length, width, and weight did not differ detectably by pregnancy status after accounting for pellet sampling month. The month of pellet sampling explained substantially more variation than progesterone-defined pregnancy status.

In large-bodied ungulates, mid-gestation pregnancy may not produce pronounced divergence in fecal morphometrics during late winter, when energy intake is constrained and body reserves are being mobilized (Parker et al., 2009; Pereira et al., 2024). Under such conditions, morphometric variation attributable solely to pregnancy would be expected to remain subtle. Since pellet dimensions primarily reflect body size and digestive processing rather than reproductive physiology directly (Ball, 2010; Maurer et al., 2021), differences among reproductive categories may be biologically small relative to environmental and seasonal influences (Cameron et al., 1993; Parker, 2003; Vors & Boyce, 2009). The absence of morphometric separation between progesterone-defined states indicates that integrating morphometrics with endocrine data did not enhance demographic discrimination under winter sampling conditions. Endocrine measures captured reproductive state directly, whereas morphometric variation appeared to reflect broader influences of body size and seasonal ecology rather than gestational status, likely reflecting seasonal shifts in forage quality and fiber content that influence intake and gut passage time (Čupić et al., 2021; Joly & Cameron, 2018).

Limitations and Future Directions

Sampling was restricted from January to March, a period characterized by constrained forage availability and relatively stable reproductive states (Parker, 2003; Ropstad, 2000). Under these conditions, variation in body size and digestive output may be biologically compressed during this period, particularly as first-year individuals approach adult size and energetic constraints limit divergence in body condition (Denryter et al., 2022; Thompson et al., 2015; Parker, 2003). As a result, the absence of detectable morphometric structure may reflect seasonal homogenization rather than a complete lack of underlying demographic differences.

Inferences regarding the limited utility of pellet morphometrics for age-class discrimination should be interpreted as specific to winter conditions rather than representative of year-round patterns. Despite these limitations, winter sampling represents the most operationally feasible period for non-invasive pellet collection in boreal systems. During summer, fecal pellets are substantially more difficult to detect due to dense vegetation and rapid decomposition in warmer temperatures, and exposure to solar radiation and precipitation may accelerate hormone degradation, reducing endocrine signal reliability (Millspaugh & Washburn, 2004; Palme, 2019).

Another limitation of this study is the absence of known-age individuals, which constrained our ability to directly evaluate age-class classification accuracy. Known-age fecal samples were not available within the Churchill Range at the time of sampling, and reliance on captive individuals was avoided due to potential biases arising from differences in diet, activity, and body condition relative to free-ranging caribou. The exclusion of known-age samples reflects the realities of operational non-invasive monitoring, where individual age is rarely known a priori. As such, our approach provides a realistic assessment of whether age-class structure can be inferred directly from morphometric data in wild populations without supervised calibration.

Future research incorporating repeated sampling of individuals, multi-season sampling, or known-age individuals could help clarify the extent to which morphometric differentiation varies across ecological conditions and improve inference of demographic structure in free-ranging caribou populations.

CONCLUSION

Pellet morphometrics did not reveal discrete age-classes in winter-collected samples. In contrast, fecal progesterone concentrations exhibited a bimodal distribution consistent with two reproductive states and allowed pregnancy status to be distinguished for most females. These results indicate that pellet size provided limited resolution for age-class inference from winter fecal samples, as morphometric variation appears to reflect body size and seasonal influences rather than discrete demographic categories. More broadly, our findings suggest that the usefulness of fecal morphometrics for demographic inference is context-dependent and may be constrained by seasonal and ecological factors affecting pellet formation. In contrast, fecal progesterone provided a useful non-invasive indicator of pregnancy status in winter-collected samples. Together, these findings highlight the potential of integrating endocrine analyses with non-invasive sampling approaches to improve demographic inference in woodland caribou.

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SUPPLEMENTAL MATERIALS

Table S1-1. Serial dilution linearity, parallelism, and spike-recovery validation for progesterone ELISA.

DF	$C_{unspiked}$	% $CV_{unspiked}$	C_{spiked}	% CV_{spiked}	C_{neat}	**% Dev**	**% Rec**
1:320	1471.42	12.41	1948.59	12.20	470,854	+12%	274.50
1:640	712.39	24.70	1018.19	4.67	455,930	+8%	175.92
1:1280	355.74	14.88	498.14	3.99	455,347	+8%	**81.92**
1:2560	118.19	12.88	403.86	18.68	302,566	-28%	164.34

A pooled fecal extract was serially diluted and assayed alongside identically diluted post-extraction spikes. $C_{unspiked}$ and C_{spiked} represent measured concentrations at each dilution, reported in pg/mL. Back-calculated neat concentration (C_{neat}) was used to assess linearity across dilutions. Percent deviation (% Dev) represents deviation of C_{neat} from the mean back-calculated concentration for progesterone. Percent Recovery (% Rec) was calculated relative to the expected spike concentration ($C_{expected\ spike}$ = **173.8318 pg/mL)**. Bolded values indicate the dilution selected as the minimum required dilution (MRD) for subsequent analyses.

Table S1-2. Mean (± SD) pellet morphometric traits by sex and sampling month for woodland caribou**.**

Sex	**Month**	**n**	**Length**	**Width**	**Weight**
F	January	37	10.97 (±1.31)	7.16 (±0.90)	0.17 (±0.04)
F	February	24	10.32 (±1.20)	6.87 (±0.99)	0.17 (±0.05)
F	March	63	11.12 (±1.43)	7.70 (±0.87)	0.20 (±0.05)
M	January	31	11.59 (±1.40)	7.69 (±1.07)	0.21 (±0.05)
M	February	30	10.76 (±1.47)	7.10 (±0.94)	0.18 (±0.06)
M	March	52	11.61 (±1.56)	7.92 (±1.27)	0.24 (±0.07)

Values represent sample size (n) and mean pellet length, width, and weight (± standard deviation) for each sex-month combination.

Table S1-3. Type III ANOVA results for linear models testing effects of sex, month, and sex × month interaction on pellet length, width, and weight.

Trait	**Predictor**	***df***	**F**	***p***	**Partial η²**
Length	Sex	1,231	3.23	0.073	0.03
	Month	2,231	2.74	0.066	0.05
	Sex×Month	2,231	0.07	0.932	<0.01
Width	Sex	1,231	**4.51**	**0.035**	0.02
	Month	2,231	**7.02**	**<0.01**	0.1
	Sex×Month	2,231	0.53	0.587	<0.01
Weight	Sex	1,231	**7.59**	**<0.01**	0.06
	Month	2,231	**5.04**	**<0.01**	0.11
	Sex×Month	2,231	0.97	0.382	<0.01

Partial η² values are provided as effect size estimates. Significant values are highlighted in bold.

Table S1-4. Results of type II ANOVAs testing the effects of pregnancy status and sampling month on pellet morphometric traits (length, width, and weight) in female caribou.

Trait	**Effect**	***df***	**F**	***p***
Length	Pregnancy Status	1 102	0.19	0.664
	Month	2, 102	1.91	0.154
Width	Pregnancy Status	1 102	1.02	0.315
	Month	2, 102	**6.93**	**<0.01**
Weight	Pregnancy Status	1 102	0.11	0.739
	Month	2, 102	**5.69**	**<0.01**

Pregnancy status was derived from GMM of fecal progesterone concentrations. Degrees of freedom (df), F-statistics, and associated p-values are reported for each effect. Significant values are highlighted in bold.

Figure S1-1. Fresh (left) and dried (centre, right) woodland caribou fecal pellets. Pellets appeared to maintain their general shape and structure following dehydration, with visible retention of features such as the tapered apex and basal curvature characteristic of fresh caribou pellets. Variation in basal profile was evident among dried samples, with the three pellets in the centre panel exhibiting comparatively rounded, convex bases and those in the right panel displaying more concave basal profiles.

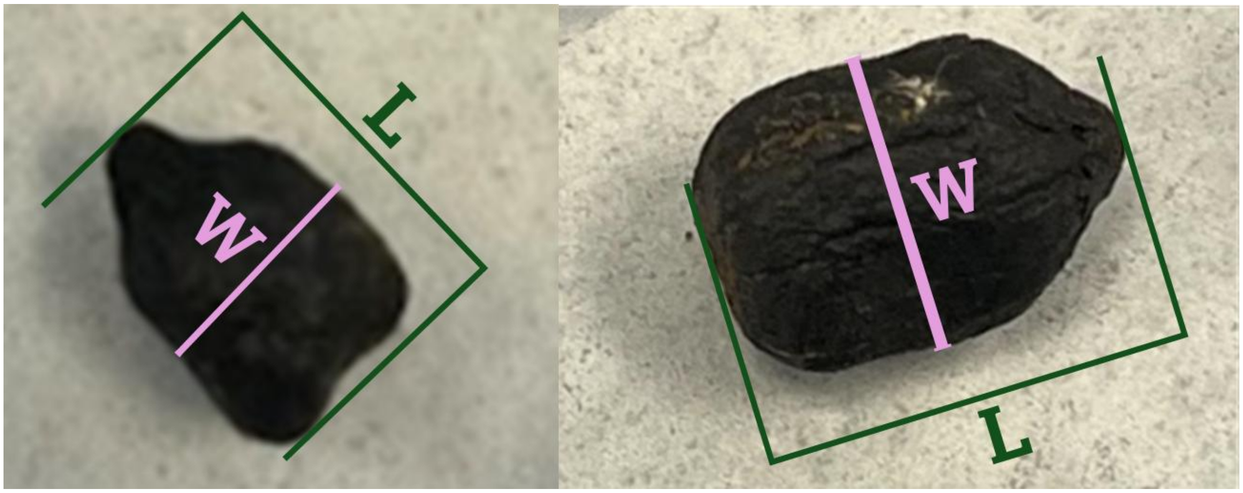

Figure S1-2. Illustration of morphometric measurements for fecal pellets exhibiting different basal profiles. A pellet with a concave base is shown on the left, and a pellet with a convex base is shown on the right. Pellet length (L; green) was measured along the longest longitudinal axis, and width (W; pink) was recorded at the widest point across the central portion of each pellet.

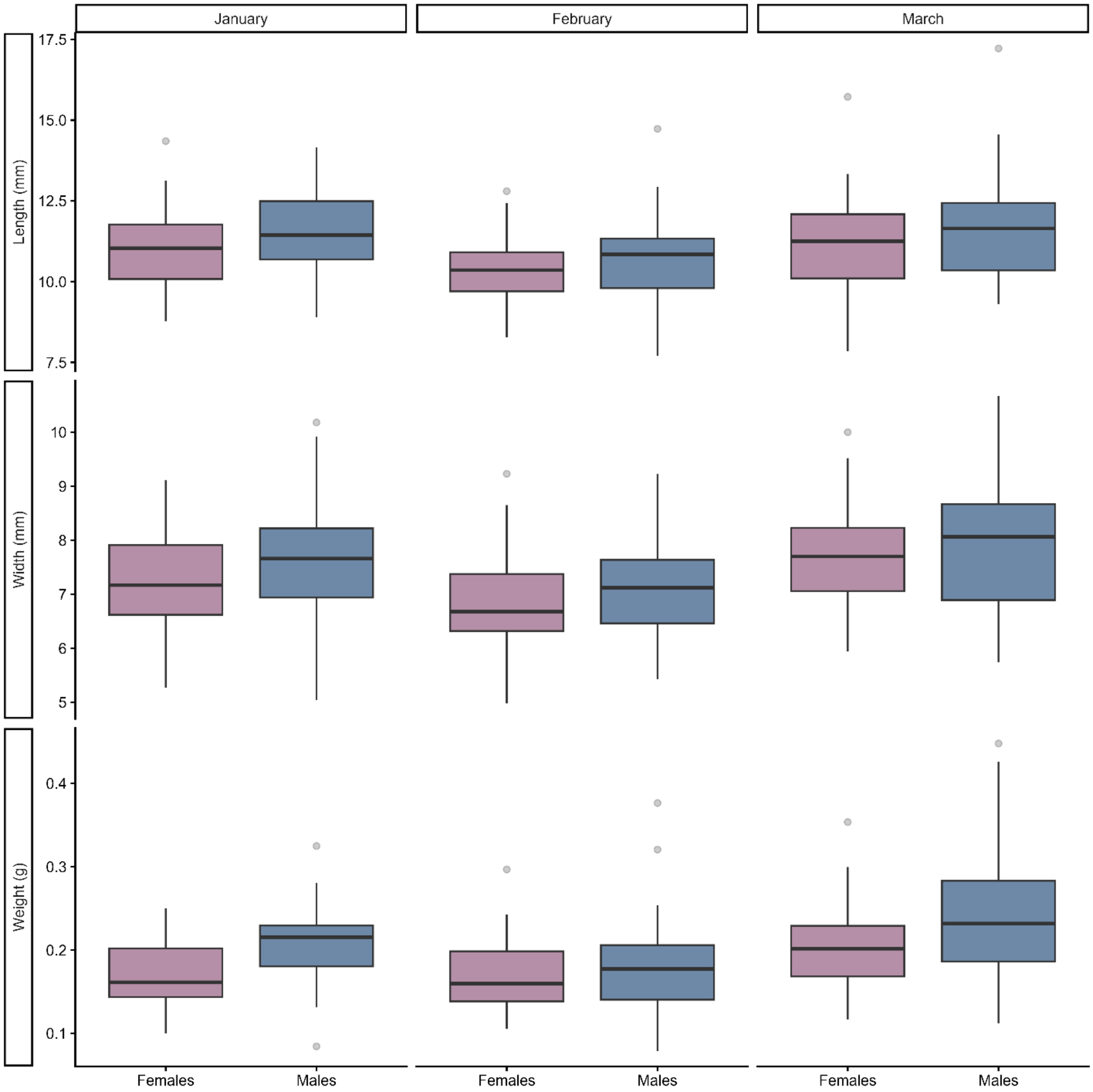

Figure S1-3. Monthly variation in pellet morphometric traits by sex. Boxplots depict pellet length (mm), width (mm), and weight (g) for female (mauve) and male (blue) caribou across January, February, and March. Boxes represent the interquartile range (IQR), horizontal lines indicate the median, whiskers extend to 1.5 × IQR, and points denote outliers.

CHAPTER 2

Successional Forest Structure and Wetlands Predict Physiological Stress in Woodland Caribou

ABSTRACT

Landscape disturbance and habitat composition can influence movement, predator-prey dynamics, forage availability, and energetic demands in woodland caribou (*Rangifer tarandus caribou*), but links between landscape context and stress-related endocrine variation remain poorly understood. We evaluated whether fecal glucocorticoid metabolite (fGCM) concentrations varied across gradients of habitat composition and disturbance in woodland caribou from northwestern Ontario, using fGCM concentrations as a non-invasive indicator of hypothalamic-pituitary-adrenal (HPA) axis activity. A total of 240 fecal samples collected during winter 2024 were analyzed, and landscape metrics describing habitat composition and disturbance were quantified at three spatial scales surrounding each sample location. In mixed-effects models controlling for sampling month, latitude, and sex, wetland cover and recent fire history emerged as significant landscape predictors of fGCM concentrations. Wetland cover was positively associated with fGCM concentrations, whereas recent fire history was negatively associated with fGCM concentrations. Elevated fGCM concentrations in wetland-dominated areas could reflect greater winter movement constraints, whereas lower fGCM concentrations in recently burned areas may reflect carry-over effects associated with summer forage availability in post-fire landscapes. Further research linking seasonal habitat use, movement, nutrition, and endocrine responses is needed to evaluate these mechanisms.

KEYWORDS

Woodland caribou, fecal glucocorticoid metabolites, anthropogenic disturbance, landscape composition, spatial scale, stress physiology, boreal forest.

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INTRODUCTION

Understanding the factors that shape variation in species fitness is essential for developing effective strategies to protect wildlife exposed to anthropogenic disturbance (Wilson et al., 2020). Across boreal ecosystems, cumulative anthropogenic pressures can influence habitat structure, landscape configuration, and ecological processes that support wildlife populations (Leblond et al., 2013). More broadly, habitat alteration and fragmentation may affect movement, connectivity, and species interactions, including predator-prey dynamics (Haddad et al., 2015; Leblond et al., 2013). These landscape-mediated effects are relevant to conservation because they can alter the environmental contexts in which animals move, forage, avoid predators, and access secure habitat (Beauchesne et al., 2014; Derguy et al., 2025; Guidobaldi Stenbacka et al., 2025).

Physiological stress responses provide one mechanism through which environmental conditions may influence wildlife (Millspaugh et al., 2001). In mammals, activation of the HPA axis leads to the release of cortisol, a glucocorticoid hormone involved in regulating energy balance, immune function, and behavioural responses to environmental challenges (Sapolsky et al., 2000; Sheriff et al., 2011). Acute increases in cortisol are adaptive and allow individuals to respond to short-term stressors, but prolonged or repeated activation of the HPA axis can have negative consequences for immune function, reproduction, growth, and survival (Wingfield et al., 1998; Sapolsky et al., 2000; Busch & Hayward, 2009). Because landscape conditions can influence movement costs, predator exposure, forage availability, and access to secure habitat, stress-related endocrine responses may provide useful information on how individuals respond to environmental pressures (Keay et al., 2006; Millspaugh et al., 2001; Sheriff et al., 2011).

Fecal glucocorticoid metabolites (fGCM) provide a non-invasive indicator of HPA axis activity and can be used to evaluate stress-related endocrine responses to environmental conditions without capturing or handling animals (Ashley et al., 2011; Sheriff et al., 2011). This approach is particularly useful for wide-ranging or disturbance-sensitive species because fecal samples can be collected across broad spatial extents with minimal disturbance (Flasko et al., 2017). Thus, fGCM concentrations provide a practical tool for assessing whether endocrine variation is associated with landscape contexts that may influence energetic demands, predator exposure, forage access, or habitat use (Sapolsky et al., 2000; Wingfield et al., 1998).

For woodland caribou (*Rangifer tarandus caribou*), landscape disturbance and forest composition may influence stress-related endocrine activity through effects on movement, habitat use, and predator-prey dynamics. Linear features such as roads can directly affect movement patterns, habitat use, and exposure to predators, while anthropogenic and natural disturbances such as harvest and fire can alter forest structure and create early successional conditions (Fryxell et al., 2020; Guidobaldi Stenbacka et al., 2025; Joly et al., 2003; Rettie & Messier, 1998; Whitman et al., 2017). Increased deciduous cover in these landscapes may support higher densities of alternate prey such as moose (Alces alces) and white-tailed deer (Odocoileus virginianus), potentially increasing predator densities and predation risk for caribou through apparent competition (Fortin et al., 2013; Rettie & Messier, 1998; Fryxell et al., 2020; Guidobaldi Stenbacka et al., 2025). These pathways may contribute to elevated stress-related endocrine activity in more disturbed landscapes (Ewacha et al., 2017), providing a rationale for evaluating whether fGCM concentrations vary in relation to roads, harvest, fire, and forest composition in woodland caribou.

Previous work in eastern Manitoba found that cortisol concentrations in woodland caribou were associated with disturbance history, suggesting that glucocorticoid variation may reflect landscape context in some caribou populations (Ewacha et al., 2017). However, this relationship has not been evaluated for woodland caribou in Ontario, where habitat composition, disturbance history, and predator-prey dynamics may differ from other study areas. This gap is important because endocrine responses to landscape conditions may be context dependent (Johnson, 1980; Joly et al., 2003; Merems et al., 2020), particularly near the southern portion of the species' distribution where caribou ranges are embedded in highly variable boreal landscapes (Courtois et al., 2003; Flasko et al., 2017; MNRF, 2014). Accordingly, this study evaluated whether fGCM concentrations varied across gradients of landscape disturbance and forest composition in woodland caribou from the Churchill Range of northwestern Ontario. We hypothesized that fGCM concentrations would be associated with both landscape composition and disturbance. Specifically, we predicted that fGCM concentrations would be higher in landscapes with greater road, harvest, fire, and deciduous cover, reflecting potential variation in habitat use and increased predation risk (Bowman et al., 2010; McLoughlin et al., 2005).

METHODS

Study Area

All fecal samples analyzed in this study were collected within the Churchill Range of northwestern Ontario, an area encompassing approximately 21 300 km² of boreal landscape situated near the southern limit of continuous woodland caribou distribution (Figure 1). The south-central range boundary lies 10 km north of Sioux Lookout, Ontario, and represents a transitional zone between relatively intact boreal ecosystems to the north and landscapes increasingly influenced by anthropogenic disturbance to the south (MNRF, 2014). Although historical habitat conditions across the range were sufficient to support caribou broadly, contemporary distribution has shifted northward, coinciding with expanding industrial activity and associated landscape alteration.

Fecal Sample Collection

Caribou fecal samples were collected non-invasively across the Churchill Range during winter (January to March) of 2024. Sampling was conducted without animal handling or capture. Therefore, animal ethics approval was not required. Field collection was performed by trained professionals following established safety and contamination-control protocols. At each location, fecal pellets were collected from clearly separated pellet groups using sterile, single-use tools to ensure each sample represented a single defecation event (Orchard, 2025; Warwick, 2025). Samples were placed into uniquely labelled bags, geographic coordinates were recorded using handheld GPS units (±5 m accuracy), and pellets were maintained frozen in the field before storage at -20 °C until hormone analysis. In total, 601 fecal samples were collected from 53 locations (132 in January, 107 in February, and 362 in March). Individual identity and sex were determined through DNA extraction and genotyping (Orchard, 2025; Warwick, 2025), resulting in 240 unique individuals (126 females, 114 males) across 51 locations.

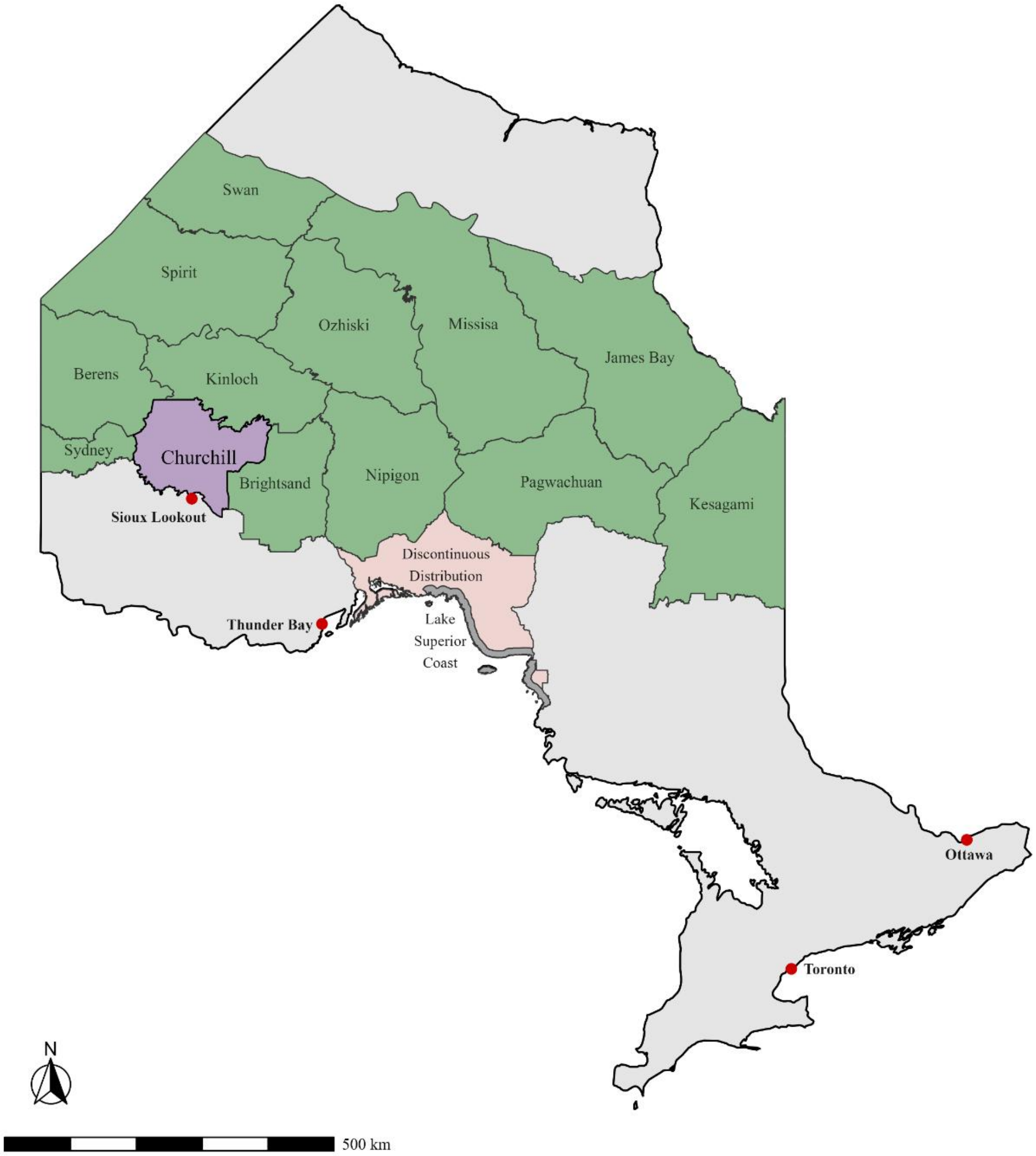

Figure 1. Spatial context of woodland caribou ranges in Ontario, Canada. The focal Churchill range is highlighted in purple, with surrounding ranges shown in green and the discontinuous distribution along the Lake Superior coast shown in light red. Major towns and cities are included for reference.

Pellet Homogenization and Hormone Extraction

From each fecal sample, three pellets were randomly selected and dried in an incubator at 60 °C for 24 h to remove residual moisture content prior to hormone extraction (Pahuja & Narayan, 2023). Following drying, the pellets were mechanically homogenized using a beadbeater to ensure uniform mixing of fecal material within each sample. Hormone extraction followed Arbor Assay's DetectX Steroid Solid Extraction Protocol (Arbor Assays, 2022). A 0.18 g subsample of homogenized fecal material was weighed and combined with 1.8 mL of anhydrous ethanol. Samples were vortexed continuously for 1 hour at room temperature to facilitate steroid extraction. The fecal samples were then centrifuged at 5,000 rpm for 15 minutes at room temperature. Following centrifugation, 1 mL of supernatant was transferred to a clean tube and evaporated to complete dryness using a vacuum concentrator at room temperature for approximately 2 hours. After complete evaporation, extracts were reconstituted in 1 mL of anhydrous ethanol and stored at -20°C prior to hormone analysis.

Corticosterone Assay Validation and Quantification

Fecal glucocorticoid metabolites (fGCM) concentrations were quantified using a corticosterone enzyme immunoassay. Although cortisol is the predominant circulating glucocorticoid in many mammals, fecal assays measure hormone metabolites rather than parent compounds, and antibody cross-reactivity may detect metabolites derived from both cortisol and corticosterone (Koren et al., 2012; Palme et al., 2019). Accordingly, measured concentrations are interpreted as an index of HPA axis activity rather than as direct measures of circulating cortisol.

Assay validation was conducted prior to sample analysis to assess dilution linearity, parallelism, spike recovery, and matrix effects. A serial dilution series (1:20-1:640) of pooled fecal extracts was prepared using assay buffer to determine the minimum required dilution (MRD) that minimized matrix interference while maintaining assay sensitivity. Spike-recovery tests were performed by adding known corticosterone standards to fecal extracts both prior to extraction (pre-spike; Table S1) and following extraction (post-spike; Table S2), with percent recovery calculated relative to expected concentrations (acceptable range: 80-120%). Based on these assessments, a working dilution of 1:80 was selected for all subsequent analyses. Matrix blanks (fecal extracts without added hormone) and true blanks (assay buffer only) were included on validation plates to assess background signal and assay specificity.

Corticosterone concentrations were quantified using a commercially available ELISA kit (Arbor Assays DetectX® Corticosterone Enzyme Immunoassay Kit, Cat. No. K014-H5) following the manufacturer's protocol. Regular samples were analyzed in duplicate, while validation samples were analyzed in triplicate on 96-well microplates. Absorbance was measured at 450 nm using a BioTek Synergy H1 microplate reader. Hormone concentrations (pg/mL) were calculated from plate-specific standard curves using a four-parameter logistic (4PL) regression model and are reported at the assay dilution used for analysis (1:80). Assay precision and reproducibility were evaluated using intra-assay and inter-assay coefficients of variation (CVs).

Spatial Data and Landscape Variables

Landscape predictors describing habitat composition and disturbance were derived for each fecal sample location to quantify environmental conditions potentially influencing physiological stress in woodland caribou. Variables included proportional cover of major landcover classes (coniferous forest, deciduous forest, mixed forest, and wetlands) as well as indicators of disturbance and linear infrastructure (wildfire, forest harvest, road density, and distance to roads). These variables represent landscape features known to influence caribou distribution, predator-prey dynamics, and predation risk through habitat composition and landscape fragmentation (Fortin et al., 2008; Bowman et al., 2010; Wittmer et al., 2007; McLoughlin et al., 2005).

Landscape variables were extracted for each fecal sample location using circular moving windows representing biologically relevant extents of caribou habitat use and disturbance exposure. Metrics were summarized at three spatial scales (5.5 km, 11 km, and 16.5 km radii) centered on each sample location. These distances correspond to one, two, and three times the spatial scale parameter (σ) estimated for woodland caribou in the Churchill range using spatial capture-recapture models (Orchard, 2025). In SECR models, σ represents the spatial scale of individual space use around an activity centre. Using multiples of σ provides biologically relevant spatial extents representing increasing areas of potential movement and environmental exposure surrounding each sampled individual.

Spatial predictors were generated from publicly available national geospatial datasets. Annual forest disturbance rasters (1985-2024; 30 m resolution) were obtained from the Canada Landsat Disturbance (CanLaD) dataset, which provides annual maps of wildfire and forest harvest derived from Landsat time-series observations (Perbert et al., 2025). Landcover information was obtained from the 2020 Land Cover of Canada dataset (Natural Resources Canada, 2022).

All spatial datasets were clipped to the Churchill caribou range with an additional 40 km buffer and projected to a common coordinate system (NAD83(CSRS) / Canada Atlas Lambert; EPSG:3979). Annual disturbance rasters were reclassified to binary presence–absence layers for wildfire and harvest. Harvest layers were buffered by 500 m to account for edge effects before temporal aggregation. Disturbance layers were then collapsed to cumulative indicators representing whether wildfire or harvest occurred within the previous 40 years. Landcover rasters were reclassified into binary grids representing wetlands, coniferous forest, deciduous forest, and mixed forest.

Road effects were represented using vector road data by generating a 500 m buffered road layer and calculating Euclidean distance to the nearest road segment. All binary rasters were resampled to a 1 km analysis grid by averaging underlying 30 m cells, producing fractional cover surfaces ranging from 0 to 1. Distance-to-road layers were resampled to the same grid resolution.

Statistical Analysis

To evaluate how landscape structure influenced fGCM concentrations, we used a two-stage mixed-effects modeling approach. First, univariate linear mixed-effects models were fit to screen individual landscape predictors while controlling for covariates. Predictors considered at each spatial scale included proportional wetland cover, proportional area burned within the previous 40 years, proportional deciduous forest cover, proportional conifer forest cover, proportional mixed forest cover, proportional area harvested within the previous 40 years, and distance to the nearest road.

All fGCM concentrations were log10-transformed prior to analysis to improve normality and homoscedasticity. Latitude (UTM northing), month of sample collection, and sex were included as control variables to account for spatial gradients, seasonal variation in glucocorticoids, and sex-specific physiological differences. Site was included as a random intercept to account for non-independence among samples collected from the same location.

$$\log_{10}(\text{fGCM}) = \beta_0 + \beta_1(\text{predictor}) + \beta_2(\text{latitude}) + \beta_3(\text{month}) + \beta_4(\text{sex}) + (1 \mid site) + \varepsilon$$

For each model, we recorded regression coefficients, standard errors, t-statistics, and p-values. Because multiple predictors were evaluated at each spatial scale, p-values were adjusted within each scale using the Benjamini-Hochberg false discovery rate procedure.

Predictors showing relatively strong associations with fGCM concentrations and representing distinct aspects of landscape composition or disturbance were subsequently included in multivariable mixed-effects models. Multivariable models were fit separately at each spatial scale using the same covariates and random-effects structure described above. Prior to model fitting, collinearity among retained predictors was evaluated within each spatial scale using pairwise Pearson correlation coefficients. Predictors exhibiting high correlation ($|r| \geq 0.7$) were not included together in the same model.

All analyses were conducted in R (version 4.5.2; R Core Team, 2025). Data manipulation, model tidying, and visualization were conducted using packages from the tidyverse (Wickham et al., 2019), including *dplyr*, *readr*, *tidyr*, *tibble*, *purrr*, and *ggplot2*, and *broom* for regression output. Collinearity diagnostics were performed using the *car* package (Fox & Weisberg, 2019), with GVIF-adjusted values calculated where categorical predictors were present.

RESULTS

Assay Validation

Observed corticosterone concentrations closely followed expected values across the dilution series (1:20-1:640), demonstrating acceptable parallelism with the standard curve. Back-calculated concentrations increased with dilution, consistent with matrix suppression at lower dilution factors. Dilutions of 1:80 and 1:160 met the ≤20% linearity criterion. In the pre-extraction spike test, corticosterone standards added prior to extraction were recovered at 89.1% (high spike), 101.8% (medium spike), and 111.9% (low spike), indicating minimal hormone loss during extraction (Table S2-1). Post-extraction spike recovery at the 1:80 dilution was 102.2%, confirming assay accuracy following extraction and dilution (Table S2-2). Recovery remained within acceptable thresholds (≈80-120%) from 1:80 to 1:320. The log-log parallelism slope for unspiked dilutions was 0.74 (95% CI: 0.52-0.96), indicating residual matrix effects at lower dilutions. The smallest dilution meeting both linearity and recovery criteria was 1:80 (linearity -4.6% to +11%; recovery 102-107%), which was selected as the minimum required dilution (MRD) for all subsequent analyses. All samples exhibited intra-assay coefficients of variation (CVs) below 20%. Inter-assay precision, assessed using plate mean concentrations (569.6 ± 66.1 pg/mL), yielded an inter-assay CV of 11.6%, indicating consistent performance across plates.

Sample Distribution and fGCM Concentrations

Fecal GCM concentrations varied substantially among individuals and sampling locations, ranging from 93.03 to 1291.12 pg/mL (mean ± SD = 347.13 ± 153.03 pg/mL). Following $\log_{10}$ transformation, fGCM concentrations approximated normality and were used as the response variable in all subsequent analyses. Log-transformed fGCM concentrations varied significantly with both sampling month and sex, although the effect of sex was modest and not consistent across months (Table S2-3). Pairwise comparisons indicated that concentrations declined from mid- to late winter, with March exhibiting lower values than earlier months (Table S2-4). Differences between sexes were primarily driven by higher concentrations in females during February, with no significant differences observed in January or March (Figure S2-2; Table S2-5).

Screening of Landscape Predictors Across Spatial Scales

Across spatial scales, fGCM concentrations showed consistent associations with wetland cover and recent fire disturbance at the two smaller spatial scales (Table 1). Wetland cover was the strongest predictor in the univariate models, exhibiting a positive and significant relationship with fGCM concentrations at 5.5 km and 11 km after correction for multiple testing, with effect size increasing with spatial scale. Recent fire disturbance (≤40 years) showed a negative association with fGCM concentrations and was also significant at 5.5 km and 11 km following multiple-testing correction. All other predictors (conifer forest, deciduous forest, mixed forest, harvest, and distance to roads) were non-significant across all spatial scales. In terms of control variables, sampling month occasionally influenced fGCM concentrations, with March sometimes associated with lower values relative to January, whereas February did not differ significantly (Tables S2-3 to S2-5). Latitude showed a weak positive association with fGCM concentrations in several models, while sex was not a significant predictor.

Table 1. Results of single-predictor mixed-effects models relating landscape composition and disturbance variables to $\log_{10}$-transformed fGCM concentrations of caribou (n = 240) across three spatial scales (5.5 km, 11 km, and 16.5 km).

Predictor	***β***	**SE**	***t***	***p***	***p* (adj)**
Spatial Scale	**5.5 Kilometer (1×σ)**				
Conifer Forest	0.081	0.201	0.405	0.686	0.8
Deciduous Forest	-2.976	1.914	-1.555	0.12	0.28
Distance to Roads (km)	-0.004	0.003	-1.115	0.265	0.371
Fire ≤ 40yrs	**-0.877**	0.27	-3.249	**<0.01**	**<0.01**
Harvest ≤ 40yrs	0.128	0.097	1.32	0.187	0.327
Mixed Forest	-0.157	0.625	-0.251	0.802	0.802
Wetland Cover	**1.649**	0.396	4.163	**<0.01**	**<0.01**
	11 Kilometer (2×σ)				
Conifer Forest	0.237	0.24	0.985	0.324	0.568
Deciduous Forest	-2.727	2.006	-1.36	0.174	0.406
Distance to Roads (km)	-0.003	0.005	-0.732	0.464	0.587
Fire ≤ 40yrs	**-0.984**	0.375	-2.621	**<0.01**	**0.031**
Harvest ≤ 40yrs	0.067	0.1	0.669	0.503	0.587
Mixed Forest	0	0.541	0.001	0.999	0.999
Wetland Cover	**2.655**	0.775	3.424	**<0.01**	**<0.01**
	16.5 Kilometer (3×σ)				
Conifer Forest	0.288	0.301	0.958	0.338	0.631
Deciduous Forest	-1.443	2.362	-0.611	0.541	0.631
Distance to Roads (km)	-0.004	0.006	-0.749	0.454	0.631
Fire ≤ 40yrs	-1	0.518	-1.929	0.054	0.188
Harvest ≤ 40yrs	0.083	0.114	0.729	0.466	0.631
Mixed Forest	0.258	0.557	0.463	0.643	0.643
Wetland Cover	3.678	1.439	2.556	0.011	0.074

Models include sampling month, sex, and latitude as covariates. ***β*** represents the partial regression coefficient for each predictor. ***p*** **(adj)** values were adjusted using the Benjamini-Hochberg false discovery rate (q = 0.10); significant effects are shown in bold.

Multivariable Landscape Predictors of Fecal Corticosterone

Multivariable mixed-effects models including both wetland cover and recent fire disturbance yielded results consistent with the univariate analyses. Across all spatial scales, wetland cover remained positively associated with fGCM concentrations, whereas recent fire disturbance (≤40 years) remained negatively associated with fGCM concentrations, and both predictors were significant after correction for multiple testing (Table 2, Figure 2). Coefficient estimates across spatial scales illustrate the consistent direction of these effects, with stronger positive effects of wetlands and negative effects of fire at the smaller spatial extents (Figure 3). Among control variables, sampling month was also significant across all scales, with lower fGCM concentrations observed in March relative to January. Latitude and sex were not consistently associated with fGCM concentrations.

Table 2. Multivariable mixed-effects model results relating landscape composition and disturbance to $\log_{10}$-transformed fGCM concentrations of caribou (n = 240) across three spatial scales (5.5 km, 11 km, and 16.5 km), with site included as a random intercept to account for spatial clustering.

Predictor	***β***	**SE**	***t***	***p***	***p* (adj)**
Spatial Scale	**5.5 Kilometer (1×σ)**				
Intercept	-4.884	3.293	-1.483	0.138	0.193
Fire ≤ 40yrs	**-0.764**	0.238	-3.212	**<0.01**	**<0.01**
Latitude	**0**	0	2.248	**0.025**	**0.043**
Month (February)	0.056	0.044	1.277	0.202	0.235
Month (March)	**-0.093**	0.035	-2.646	**<0.01**	**0.019**
Sex (Male)	0.009	0.026	0.351	0.726	0.726
Wetland Cover	**1.474**	0.362	4.068	**<0.01**	**<0.01**
	11 Kilometer (2×σ)				
Intercept	-4.359	3.678	-1.185	0.236	0.307
Fire ≤ 40yrs	**-0.993**	0.321	-3.097	**<0.01**	**<0.01**
Latitude	0	0	1.864	0.062	0.109
Month (February)	0.05	0.044	1.118	0.264	0.307
Month (March)	**-0.1**	0.036	-2.784	**<0.01**	**0.013**
Sex (Male)	0.01	0.027	0.377	0.706	0.706
Wetland Cover	**2.772**	0.678	4.086	**<0.01**	**<0.01**
	16.5 Kilometer (3×σ)				
Intercept	-1.982	4.768	-0.416	0.678	0.75
Fire ≤ 40yrs	**-1.08**	0.463	-2.332	**0.02**	**0.046**
Latitude	0	0	0.932	0.351	0.614
Month (February)	0.035	0.047	0.744	0.457	0.64
Month (March)	**-0.099**	0.039	-2.559	**0.011**	**0.037**
Sex (Male)	0.009	0.028	0.318	0.75	0.75
Wetland Cover	**4.073**	1.354	3.008	**<0.01**	**0.018**

β represents partial regression coefficients; ***p* (adj)** values were adjusted using the Benjamini-Hochberg false discovery rate. Significant effects are shown in bold.

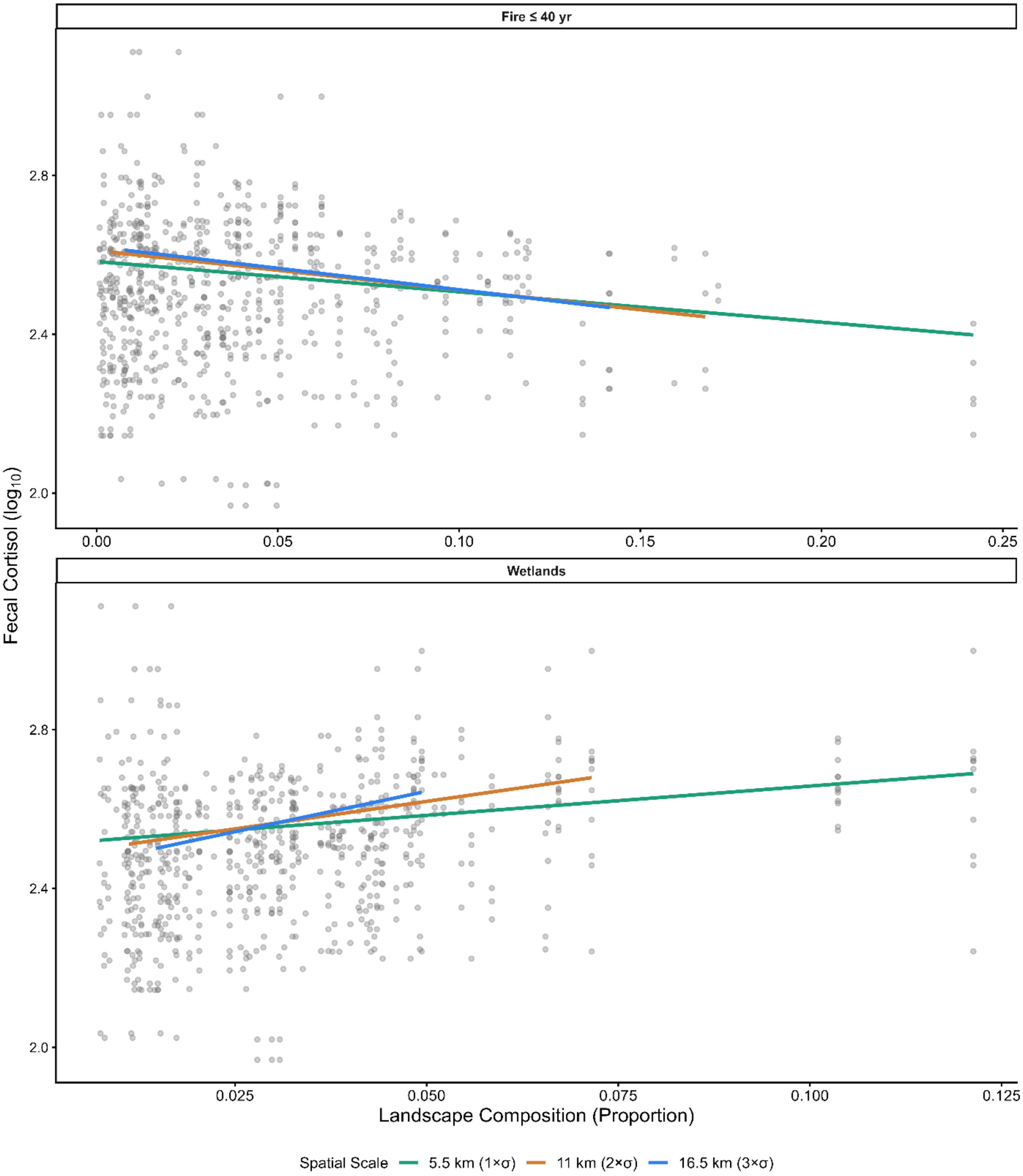

Figure 2. Partial effects of retained landscape predictors on $\log_{10}$-transformed fGCM concentrations of caribou (n = 240) across three spatial scales. Panels show relationships for proportional area burned within the last 40 years (top), and wetland cover (bottom). Coloured lines represent fitted values from multivariable linear models evaluated at three spatial extents (green for 5.5 km, orange for 11 km, blue for 16.5 km), with shaded bands indicating 95% confidence intervals. Raw data points are shown in grey. All models control for sampling month, sex, and latitude.

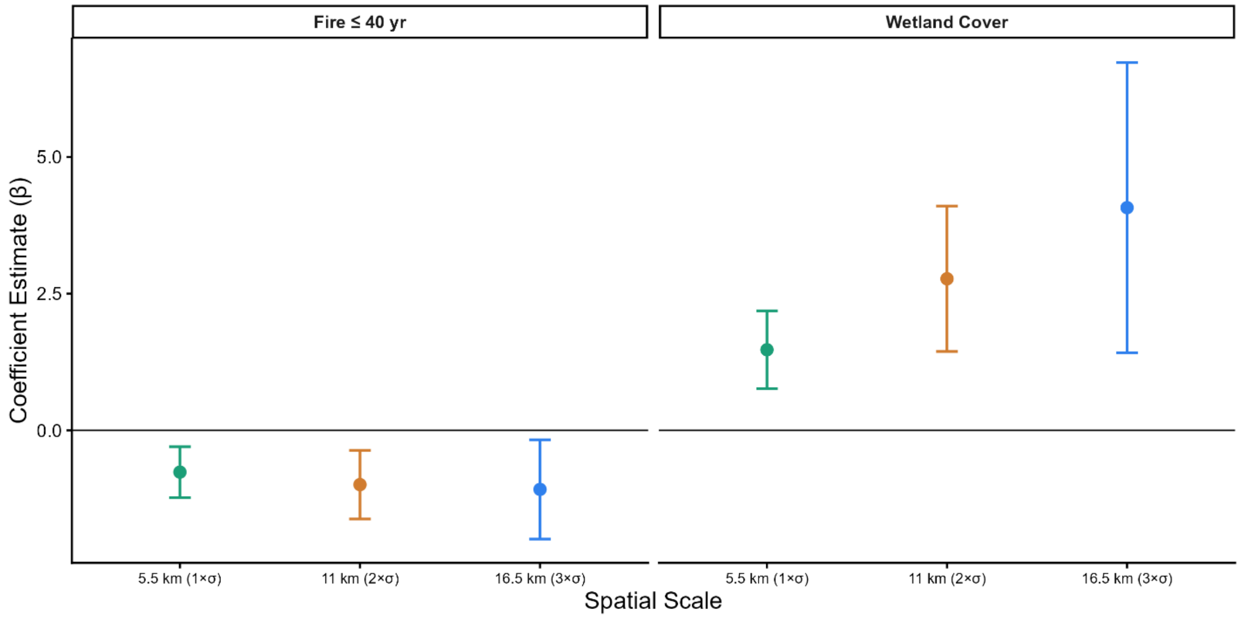

Figure 3. Standardized regression coefficients (*β*) for retained landscape predictors across three spatial scales (5.5 km, 11 km, and 16.5 km). Panels show the effects of proportional area burned within the last 40 years (left), and wetland cover (right) on $\log_{10}$-transformed fGCM concentrations in caribou (n = 240). Points represent coefficient estimates from multivariable linear models, and error bars denote 95% confidence intervals. All models control for sampling month, sex, and latitude.

DISCUSSION

Landscape Correlates of Fecal Glucocorticoid Concentrations

This study evaluated whether fecal glucocorticoid metabolite concentrations varied systematically across gradients of landscape composition and disturbance in woodland caribou, using fGCMs as a non-invasive indicator of HPA-axis activity. We predicted that samples collected in more disturbed landscapes and landscapes with higher deciduous forest composition would have higher fGCM concentrations. Contrary to this prediction, anthropogenic disturbance and deciduous forest cover were not associated with fGCM concentrations at any spatial scale. Instead, wetland cover and recent fire history emerged as the main landscape predictors of fGCM concentrations. Fecal glucocorticoid metabolite concentrations were positively associated with wetland cover and negatively associated with recent fire history, indicating that samples collected in landscapes with greater wetland extent and lower proportional area burned had higher fGCM concentrations during winter.

The negative association between recent fire history and fGCM concentrations was contrary to our prediction that recently disturbed or early successional landscapes would be associated with higher fGCM concentrations. One possible explanation is that recent fire reflects successional or seasonal effects not captured by winter sampling alone. Recently burned areas may support early successional vegetation, including deciduous shrubs, forbs, and productive understories that provide higher-quality forage during the snow-free season (Whitman et al., 2017). Access to higher-quality summer forage can contribute to recovery of body condition after winter and accumulation of reserves before the following winter period (Cook et al., 2004; Denryter et al., 2022; Parker, 2003). Lower fGCM concentrations in landscapes with greater proportional area burned may therefore reflect carry-over effects associated with summer forage availability or broader differences in successional context. In contrast, the positive association between wetlands and fGCM concentrations may reflect movement-related constraints in wetland-dominated landscapes during winter. Wetland-rich areas may be associated with deeper snow, peatland structure, or open wetland complexes that reduce travel efficiency and increase energetic demands (Doherty et al., 2022; Whitman et al., 2017).

Anthropogenic disturbance was not a significant predictor of fGCM concentrations in this study. This result should not be interpreted as evidence that disturbance has no effect on woodland caribou, but rather indicates that road and harvest metrics were not significant correlates of fGCM concentrations in our winter-collected samples. In contrast, Ewacha et al. (2017) measured cortisol in woodland caribou hair samples from eastern Manitoba and found that concentrations were best predicted by the proportion of the home range logged in the previous 6-21 years. These contrasting results may reflect, at least in part, the different temporal windows captured by hair cortisol and fecal glucocorticoid metabolite measurements. Hair cortisol provides an integrated measure of longer-term glucocorticoid activity, whereas fGCM concentrations reflect more recent endocrine activity, typically on the order of hours to days (Ashley et al., 2011). As a result, fGCM concentrations may be more sensitive to short-term variation in winter movement conditions, local habitat context, or other environmental factors at the time of sampling. Because fGCM concentrations reflect hormone secretion over the gut passage period, they are less sensitive to stress-induced spikes than blood or urine samples, which can be influenced by rapid stress responses associated with capture or handling (Sheriff et al., 2011). Thus, while hair cortisol may better capture sustained responses to landscape disturbance, fGCM concentrations offer a complementary means of assessing more immediate physiological responses under field conditions. Differences in regional landscape composition and disturbance context may also contribute to contrasting results among studies.

Implications for Monitoring and Management

From a management perspective, our results do not support a simple relationship between anthropogenic disturbance features and elevated fGCM concentrations in the Churchill Range. Instead, the observed associations with wetland cover and recent fire history suggest that endocrine variation may be influenced by broader landscape composition and successional context, in addition to other ecological and demographic processes (Smith et al., 2023). Physiological indicators such as fGCM concentrations should therefore be interpreted alongside demographic, behavioural, and spatial information rather than as standalone indicators of population-level effects. Management frameworks that focus only on individual disturbance features may overlook broader landscape conditions that influence movement, habitat use, and energetic demands (Gengler et al., 2024; Whitman et al., 2017). These results highlight the importance of considering landscape context alongside localized disturbance when interpreting endocrine variation in woodland caribou (Hins et al., 2009; Vistnes & Nellemann, 2008).

Limitations and Future Directions

This study has several limitations that should be considered when interpreting the observed relationships between landscape context and fGCM concentrations. Fecal glucocorticoid metabolites provide a valuable non-invasive indicator of HPA-axis activity, but they reflect glucocorticoid activity over the gut passage period preceding defecation, typically on the order of hours to days (Ashley et al., 2011). Consequently, fGCM concentrations capture relatively recent endocrine responses and may not represent longer-term or cumulative glucocorticoid exposure. In addition, every individual was represented by a single fecal sample, limiting our ability to assess within-individual variability or temporal consistency in fGCM concentrations.

Sampling in this study was restricted to winter, when caribou may experience distinct movement conditions and energetic trade-offs (Parker et al., 2009). Relationships between landscape structure and fGCM concentrations may differ during other seasons, particularly during summer when forage availability, movement patterns, habitat use, and disturbance exposure may differ from winter conditions (Jachowski et al., 2018; Parker et al., 2009; Sheriff et al., 2011; Whitman et al., 2017). These results should therefore be interpreted as a season-specific assessment of landscape-associated variation in fGCM concentrations.

The absence of significant associations between anthropogenic disturbance and fGCM concentrations should also be interpreted cautiously. This result does not indicate that disturbance has no effect on woodland caribou, but rather that road and harvest metrics were not significant correlates of fGCM concentrations in these winter-collected samples. In addition, the observed relationships between landscape variables and fGCM concentrations are correlative and may reflect unmeasured underlying factors, including movement conditions, snow depth, seasonal habitat use, forage availability, predator risk, or nutritional condition (Millspaugh & Washburn, 2004; Parker et al., 2009; Sheriff et al., 2011). As a result, the mechanisms underlying landscape-associated variation in fGCM concentrations remain inferred rather than demonstrated.

Future research could address these limitations by integrating repeated non-invasive sampling with movement tracking, snow and weather data, seasonal habitat-use information, forage availability, and direct or indirect measures of nutritional condition. Repeated sampling of known individuals across seasons would provide greater insight into temporal variability in fGCM concentrations and help evaluate whether landscape-associated patterns are consistent across changing environmental conditions. Such studies would strengthen inference about the ecological pathways linking landscape context, HPA-axis activity, and caribou monitoring outcomes.

CONCLUSION

This study shows that fGCM concentrations in woodland caribou varied in relation to landscape composition and successional context. Contrary to our prediction that fGCM concentrations would increase with anthropogenic disturbance and deciduous forest cover, road, harvest, and deciduous cover were not significant correlates of fGCM concentrations in these winter-collected samples. Instead, wetland cover and recent fire history were associated with fGCM concentrations, with higher concentrations in wetland-rich landscapes and lower concentrations in landscapes with greater proportional area burned. These patterns may reflect indirect energetic and seasonal pathways, including potential winter movement constraints in wetland-dominated landscapes and possible carry-over effects of post-fire successional conditions. Overall, this study demonstrates that winter-collected fecal samples can provide useful endocrine information for caribou monitoring and highlights the importance of considering landscape composition and successional context when interpreting fGCM variation in boreal systems.

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SUPPLEMENTAL MATERIALS

Table S2-1. Results of corticosterone pre-extraction spike recovery.

Sample Type	Expected Spike (pg/mL)	Concentration (pg/mL)	% Recovery
Sample (Unspiked)	***None***	**343.69**	—
Spiked High	121.66	452.04	89.06
Spiked Medium	81.11	426.23	101.76
Spiked Low	40.55	389.08	111.93

Known quantities of hormone were introduced to fecal material before extraction to evaluate combined extraction efficiency and potential matrix effects. The expected concentration of the spike in the final assay wells was calculated by propagating the added hormone mass through all extraction, transfer, resuspension, and dilution steps. Percent recovery was determined as the difference between measured concentrations of spiked and paired unspiked samples divided by the theoretical spike value. Concentrations represent the mean of triplicate wells.

Table S2-2. Serial dilution linearity, parallelism, and spike-recovery validation for corticosterone ELISA.

DF	$C_{unspiked}$	$\% \, CV_{unspiked}$	C_{spiked}	$\% \, CV_{spiked}$	C_{neat}	% Dev	% Recovery
1:20	942.13	11.40	1440.86	9.69	18,842	-22%	98.98
1:40	398.87	10.44	1169.88	9.37	15,955	-34%	153.01
1:80	288.48	19.66	803.24	15.07	23,078	-4.6%	**102.16**
1:160	168.27	27.43	708.41	6.84	26,923	+11%	107.19
1:320	112.83	54.64	665.99	2.80	36,107	+49%	109.78

The dilution factor selected for subsequent analyses (MRD; **DF = 1:80**) is highlighted in bold. The expected increase in concentration attributable to the spike was calculated from volumetric dilution of the stock solution ($C_{expected\,spike}$) and was equal to **503.889 pg/mL** in the assay wells. C_{neat} is the back-calculated concentration at the original matrix; % Dev is deviation from the mean neat value. Percent recovery at each dilution factor (DF) was computed as the difference between the measured spiked and unspiked concentrations divided by this theoretical value. Percent coefficients of variation (%CV) are shown for both unspiked (Sample) and spiked (Spike) measurements. Concentrations represent the mean of triplicate wells. Data for the 1:640 dilution are not reported because spectrophotometric signal was insufficient for reliable quantification.

Table S2-3. Results of univariate linear mixed-effects models evaluating associations between landscape predictors and $\log_{10}$-transformed fecal glucocorticoid metabolite (fGCM) concentrations at the 5.5 km spatial scale. Each model includes latitude, sampling month, and sex as covariates. Sampling site was included as a random effect to account for non-independence among samples collected from the same location.

Predictor (5.5 km)	*β*	SE	*t*	*p*	CI low	CI high
Focal Predictor	**Conifer Forest**					
Intercept	-5.964	4.124	-1.446	0.148	-14.048	2.120
Conifer Forest	0.081	0.201	0.405	0.686	-0.312	0.475
Latitude	0.000	0.000	2.055	0.040	0.000	0.000
Month (February)	0.045	0.052	0.874	0.382	-0.056	0.146
Month (March)	-0.073	0.042	-1.731	0.084	-0.156	0.010
Sex (Male)	0.004	0.030	0.119	0.905	-0.055	0.062
	Deciduous Forest					
Intercept	-6.046	3.987	-1.516	0.129	-13.860	1.768
Deciduous Forest	-2.976	1.914	-1.555	0.120	-6.727	0.775
Latitude	0.000	0.000	2.159	0.031	0.000	0.000
Month (February)	0.053	0.051	1.045	0.296	-0.047	0.153
Month (March)	-0.078	0.041	-1.889	0.059	-0.158	0.003
Sex (Male)	0.011	0.030	0.369	0.712	-0.048	0.070
	Distance to Roads (km)					
Intercept	-4.917	4.104	-1.198	0.231	-12.961	3.126
Distance to Roads (km)	-0.004	0.003	-1.115	0.265	-0.010	0.003
Latitude	0.000	0.000	1.826	0.068	0.000	0.000
Month (February)	0.044	0.051	0.865	0.387	-0.056	0.144
Month (March)	-0.074	0.041	-1.798	0.072	-0.155	0.007
Sex (Male)	0.002	0.030	0.062	0.950	-0.056	0.060
	Fire ≤ 40yrs					
Intercept	-7.124	3.765	-1.892	0.058	-14.504	0.256
Fire ≤ 40yrs	-0.877	0.270	-3.249	0.001	-1.405	-0.348
Latitude	0.000	0.000	2.575	0.010	0.000	0.000
Month (February)	0.060	0.049	1.238	0.216	-0.035	0.156
Month (March)	-0.093	0.040	-2.337	0.019	-0.170	-0.015
Sex (Male)	0.012	0.029	0.422	0.673	-0.044	0.068
	Harvest ≤ 40yrs					
Intercept	-5.565	4.045	-1.376	0.169	-13.493	2.364
Harvest ≤ 40yrs	0.128	0.097	1.320	0.187	-0.062	0.318
Latitude	0.000	0.000	1.996	0.046	0.000	0.000
Month (February)	0.034	0.052	0.647	0.518	-0.068	0.136
Month (March)	-0.073	0.041	-1.778	0.075	-0.155	0.008
Sex (Male)	-0.002	0.030	-0.076	0.940	-0.061	0.056

Predictor (5.5 km)	***β***	**SE**	***t***	***p***	**CI low**	**CI high**
Focal Predictor				**Mixed Forest**		
Intercept	-5.174	4.709	-1.099	0.272	-14.402	4.055
Mixed Forest	-0.157	0.625	-0.251	0.802	-1.382	1.068
Latitude	0.000	0.000	1.646	0.100	0.000	0.000
Month (February)	0.048	0.052	0.916	0.360	-0.054	0.150
Month (March)	-0.071	0.042	-1.694	0.090	-0.153	0.011
Sex (Male)	0.004	0.031	0.130	0.897	-0.056	0.064
				Wetland Cover		
Intercept	-3.322	3.480	-0.954	0.340	-10.143	3.500
Wetland Cover	1.649	0.396	4.163	0.000	0.873	2.425
Latitude	0.000	0.000	1.670	0.095	0.000	0.000
Month (February)	0.036	0.046	0.782	0.434	-0.054	0.125
Month (March)	-0.079	0.037	-2.154	0.031	-0.151	-0.007
Sex (Male)	0.003	0.027	0.099	0.921	-0.051	0.056

Table S2-4. Results of univariate linear mixed-effects models evaluating associations between landscape predictors and $\log_{10}$-transformed fecal glucocorticoid metabolite (fGCM) concentrations at the 11 km spatial scale. Each model includes latitude, sampling month, and sex as covariates. Sampling site was included as a random effect to account for non-independence among samples collected from the same location.

Predictor (11 km)	***β***	**SE**	***t***	***p***	**CI low**	**CI high**
Focal Predictor	**Conifer Forest**					
Intercept	-6.511	4.100	-1.588	0.112	-14.546	1.525
Conifer Forest	0.237	0.240	0.985	0.324	-0.234	0.708
Latitude	0.000	0.000	2.183	0.029	0.000	0.000
Month (February)	0.041	0.051	0.801	0.423	-0.060	0.142
Month (March)	-0.080	0.042	-1.889	0.059	-0.163	0.003
Sex (Male)	0.008	0.030	0.252	0.801	-0.051	0.067
	Deciduous Forest					
Intercept	-4.845	4.067	-1.191	0.234	-12.817	3.127
Deciduous Forest	-2.727	2.006	-1.360	0.174	-6.658	1.204
Latitude	0.000	0.000	1.826	0.068	0.000	0.000
Month (February)	0.049	0.051	0.959	0.338	-0.051	0.149
Month (March)	-0.077	0.041	-1.869	0.062	-0.158	0.004
Sex (Male)	0.011	0.030	0.352	0.725	-0.049	0.070
	Distance to Roads (km)					
Intercept	-5.391	4.101	-1.315	0.189	-13.430	2.647
Distance to Roads (km)	-0.003	0.005	-0.732	0.464	-0.012	0.006
Latitude	0.000	0.000	1.941	0.052	0.000	0.000
Month (February)	0.043	0.052	0.826	0.409	-0.059	0.144
Month (March)	-0.074	0.042	-1.769	0.077	-0.156	0.008
Sex (Male)	0.002	0.030	0.052	0.958	-0.057	0.060
	Fire ≤ 40yrs					
Intercept	-9.217	4.088	-2.254	0.024	-17.229	-1.204
Fire ≤ 40yrs	-0.984	0.375	-2.621	0.009	-1.719	-0.248
Latitude	0.000	0.000	2.884	0.004	0.000	0.000
Month (February)	0.053	0.050	1.059	0.289	-0.045	0.150
Month (March)	-0.093	0.041	-2.279	0.023	-0.173	-0.013
Sex (Male)	0.010	0.029	0.340	0.734	-0.047	0.067
	Harvest ≤ 40yrs					
Intercept	-5.829	4.094	-1.424	0.155	-13.853	2.195
Harvest ≤ 40yrs	0.067	0.100	0.669	0.503	-0.129	0.263
Latitude	0.000	0.000	2.040	0.041	0.000	0.000
Month (February)	0.041	0.052	0.790	0.430	-0.061	0.143
Month (March)	-0.072	0.042	-1.718	0.086	-0.153	0.010
Sex (Male)	0.000	0.030	-0.015	0.988	-0.060	0.059

Predictor (11 km)	***β***	**SE**	***t***	***p***	**CI low**	**CI high**
Focal Predictor				**Mixed Forest**		
Intercept	-5.753	4.834	-1.190	0.234	-15.228	3.723
Mixed Forest	0.000	0.541	0.001	0.999	-1.060	1.061
Latitude	0.000	0.000	1.722	0.085	0.000	0.000
Month (February)	0.046	0.052	0.885	0.376	-0.056	0.148
Month (March)	-0.070	0.042	-1.675	0.094	-0.152	0.012
Sex (Male)	0.002	0.031	0.078	0.937	-0.057	0.062
				Wetland Cover		
Intercept	-0.961	3.846	-0.250	0.803	-8.499	6.576
Wetland Cover	2.655	0.775	3.424	0.001	1.135	4.174
Latitude	0.000	0.000	0.891	0.373	0.000	0.000
Month (February)	0.036	0.047	0.765	0.444	-0.056	0.129
Month (March)	-0.080	0.038	-2.119	0.034	-0.155	-0.006
Sex (Male)	0.004	0.028	0.134	0.893	-0.051	0.058

Table S2-5. Results of univariate linear mixed-effects models evaluating associations between landscape predictors and $\log_{10}$-transformed fecal glucocorticoid metabolite (fGCM) concentrations at the 16.5 km spatial scale. Each model includes latitude, sampling month, and sex as covariates. Sampling site was included as a random effect to account for non-independence among samples collected from the same location.

Predictor (16.5 km)	***β***	**SE**	***t***	***p***	**CI low**	**CI high**
Focal Predictor				**Conifer Forest**		
Intercept	-5.834	4.043	-1.443	-13.759	2.090	0.149
Conifer Forest	0.288	0.301	0.958	-0.301	0.877	0.338
Latitude	0.000	0.000	2.025	0.000	0.000	0.043
Month (February)	0.043	0.051	0.829	-0.058	0.143	0.407
Month (March)	-0.077	0.042	-1.843	-0.159	0.005	0.065
Sex (Male)	0.007	0.030	0.247	-0.052	0.067	0.805
				Deciduous Forest		
Intercept	-4.510	4.569	-0.987	-13.465	4.444	-4.510
Deciduous Forest	-1.443	2.362	-0.611	-6.073	3.186	-1.443
Latitude	0.000	0.000	1.553	0.000	0.000	0.000
Month (February)	0.048	0.052	0.928	-0.053	0.149	0.048
Month (March)	-0.071	0.042	-1.715	-0.153	0.010	-0.071
Sex (Male)	0.006	0.030	0.189	-0.054	0.065	0.006
				Distance to Roads (km)		
Intercept	-5.719	4.068	-1.406	-13.693	2.255	-5.719
Distance to Roads (km)	-0.004	0.006	-0.749	-0.016	0.007	-0.004
Latitude	0.000	0.000	2.037	0.000	0.000	0.000
Month (February)	0.040	0.052	0.779	-0.061	0.142	0.040
Month (March)	-0.075	0.042	-1.790	-0.157	0.007	-0.075
Sex (Male)	0.001	0.030	0.043	-0.057	0.060	0.001
				Fire ≤ 40yrs		
Intercept	-9.789	4.509	-2.171	-18.627	-0.951	-9.789
Fire ≤ 40yrs	-1.000	0.518	-1.929	-2.016	0.016	-1.000
Latitude	0.000	0.000	2.738	0.000	0.000	0.000
Month (February)	0.045	0.051	0.894	-0.054	0.145	0.045
Month (March)	-0.085	0.042	-2.043	-0.167	-0.003	-0.085
Sex (Male)	0.006	0.030	0.213	-0.052	0.064	0.006
				Harvest ≤ 40yrs		
Intercept	-6.048	4.107	-1.473	-14.096	2.001	-6.048
Harvest ≤ 40yrs	0.083	0.114	0.729	-0.140	0.306	0.083
Latitude	0.000	0.000	2.087	0.000	0.000	0.000
Month (February)	0.041	0.052	0.778	-0.062	0.143	0.041
Month (March)	-0.073	0.042	-1.740	-0.154	0.009	-0.073
Sex (Male)	0.000	0.030	-0.013	-0.060	0.059	0.000

Predictor (16.5 km)	***β***	**SE**	***t***	***p***	**CI low**	**CI high**
Focal Predictor			**Mixed Forest**			
Intercept	-7.322	5.320	-1.376	-17.750	3.105	0.169
Mixed Forest	0.258	0.557	0.463	-0.834	1.350	0.643
Latitude	0.000	0.000	1.859	0.000	0.000	0.063
Month (February)	0.043	0.052	0.835	-0.058	0.145	0.404
Month (March)	-0.071	0.042	-1.699	-0.153	0.011	0.089
Sex (Male)	0.000	0.030	0.007	-0.059	0.060	0.995
			Wetland Cover			
Intercept	1.581	4.732	0.334	-7.694	10.856	0.738
Wetland Cover	3.678	1.439	2.556	0.858	6.497	0.011
Latitude	0.000	0.000	0.181	0.000	0.000	0.857
Month (February)	0.035	0.049	0.719	-0.061	0.131	0.472
Month (March)	-0.081	0.039	-2.067	-0.159	-0.004	0.039
Sex (Male)	0.004	0.029	0.153	-0.052	0.061	0.878

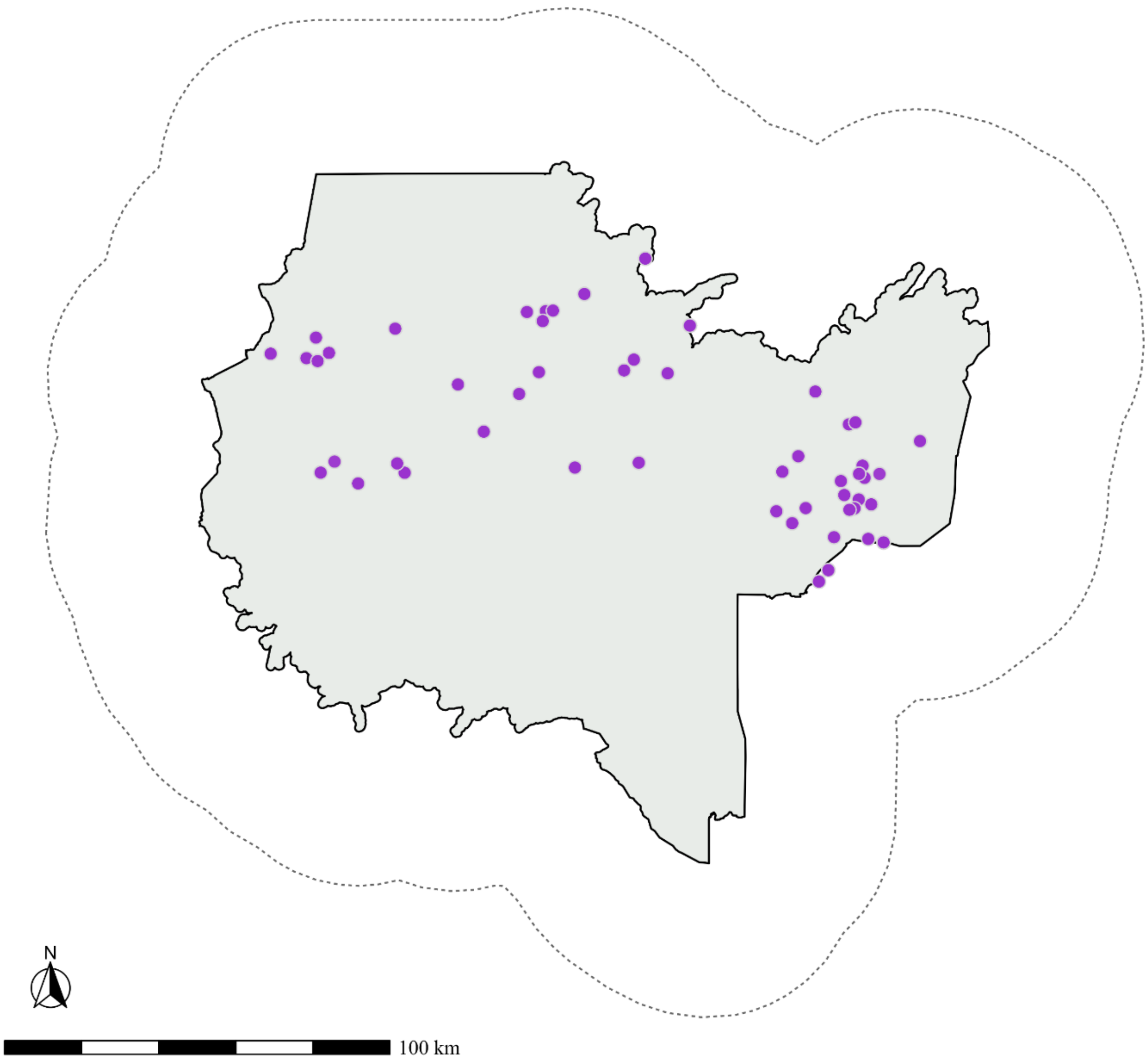

Figure S2-1. Spatial distribution of winter 2024 fecal sample collection locations relative to the Churchill woodland caribou range in Ontario, Canada. The solid outline delineates the Churchill range boundary, and purple circles indicate fecal sampling locations (n = 240). The dashed outline represents the 40-km sampling extent used to define the study area surrounding the range.

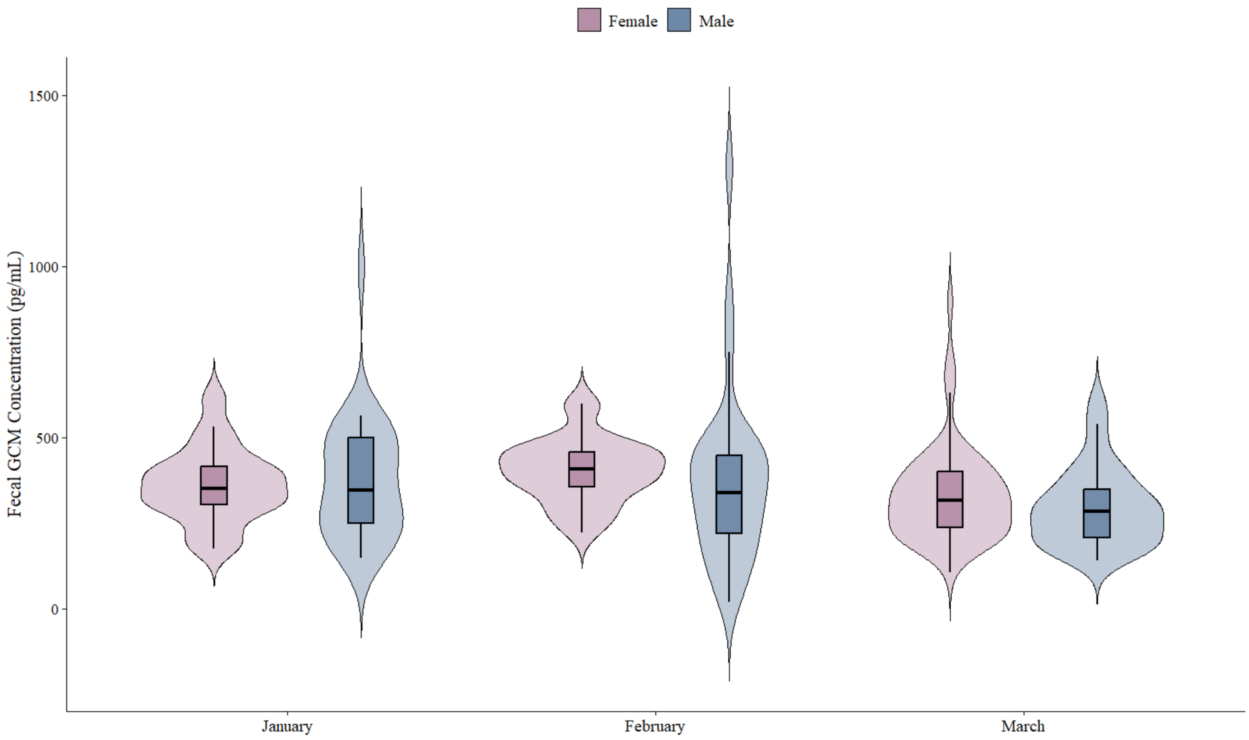

Figure S2-2. Distribution of fGCM concentrations (pg/mL) across sampling months (January-March), separated by sex. Violin plots illustrate the density distribution of values, with overlaid boxplots indicating the median, interquartile range, and spread of the data.

GENERAL CONCLUSION

The collective findings of this thesis demonstrate both the potential and limitations of fecal samples for deriving biological information relevant to woodland caribou monitoring. Fecal pellet morphometrics provided limited age-class resolution, indicating that pellet size alone is unlikely to reliably distinguish immature from reproductively mature individuals under local sampling conditions. In contrast, endocrine biomarkers provided more informative insights, with fecal progesterone concentrations supporting classification of pregnancy status for most females, and fecal glucocorticoid metabolite concentrations revealing associations with landscape composition and successional context. These findings demonstrate that fecal endocrine biomarkers can strengthen non-invasive monitoring of woodland caribou by supporting pregnancy-status classification and providing insight into variation in fGCM concentrations as an indicator of hypothalamic-pituitary-adrenal axis activity.