MOTORIZED BACKCOUNTRY RECREATION AND STRESS RESPONSE IN MOUNTAIN CARIBOU (*Rangifer tarandus caribou*)

by

NICOLA L. FREEMAN

B.Sc., University of Calgary, 1996

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Abstract

Mountain caribou (*Rangifer tarandus caribou*) are endangered in British Columbia and motorized backcountry recreation has been identified as a potential threat to their persistence. My objective was to test if fecal glucocorticoids (GCs), indicative of physiological effects of ecological stress in wildlife, could be used as a non-invasive tool to quantify stress response in free-ranging caribou exposed to motorized recreation.

I validated an enzyme-linked immunosorbent assay (ELISA) to measure concentration of fecal GCs for *R. tarandus* using an adrenocorticotropic hormone (ACTH) challenge experiment on captive reindeer exposed to extreme variation in winter weather. Female reindeer expressed elevated fecal GCs 9-11 hrs after ACTH injection. Males showed no detectable increase, perhaps due to underdosing. Fecal GCs varied markedly in both sexes in response to natural variation in weather. Overall, my results indicated fecal assays can be used to track biologically meaningful changes in adrenal activity in *R. tarandus.*

I investigated the effects of motorized recreation on stress hormone production by measuring GCs in feces of mountain caribou exposed to snowmobile and heli-ski activity. Concentrations of fecal GCs in snowmobile and heli-ski areas were higher than those measured from caribou in areas where motorized recreation was not allowed. Caribou sampled up to 4km, 8km and10 km distant from snowmobile activity showed elevated fecal GCs when compared to those sampled further from snowmobile activity areas. Other variables with a significant effect on fecal GCs included reproductive state, snow, aspect, minimum ambient temperature, and daily temperature range. My study indicates that measurement of fecal GCs provides a useful, noninvasive approach in the

evaluation of physiological effects of environment, reproductive state, and human-induced stressors on free-ranging mountain caribou. Although research on many species indicates that chronically elevated GCs carry a variety of physiological costs, more study is needed to know whether GCs can be used as an index of human impact on population health or trend.

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Co-authorship Statement

This thesis contains manuscripts that are co-written. I identified, designed, obtained funding and coordinated the research project, implemented and conducted the research, performed all data analyses and wrote the manuscripts contained in this thesis, with some assistance from Dr. Peter Arcese. For Chapters 2 and 3, coauthors Dr. Arcese and Dr. Scott Creel provided suggestions on experimental design, data analysis, reviewed drafts of the manuscripts, and assisted with editing. For Chapter 2, Dr. Sam Wasser provided laboratory services for RIA fecal analysis, reviewed the manuscript and provided editing suggestions.

1 Introduction

Expansion of recreation into natural wild areas and the potential impact on free-ranging wildlife populations is an increasing conservation concern. Wildlife managers are often faced with influencing decisions on management of recreational activities with limited information as to their effects on wildlife. Studies investigating behavioural response of wildlife to recreation are abundant, however quantifying these effects is necessary not only for establishing management guidelines, but also for acceptance and conformity to guidelines by recreation enthusiasts. Over the past twenty years, researchers have been quantifying the physiological response of wildlife to ecological and anthropogenic stressors using noninvasive endocrine field techniques. The purpose of my study was to investigate field endocrinology as a tool to assess stress response in free-ranging mountain caribou (*Rangifer tarandus caribou*) inhabiting areas influenced by motorized backcountry recreation and to provide wildlife managers with science-based information to assist in the development of recreation management guidelines.

1.1 Mountain Caribou in British Columbia

Mountain caribou, an arboreal lichen-feeding ecotype of woodland caribou, are nationally 'threatened' in Canada and listed as 'endangered' in British Columbia (British Columbia Conservation Data Centre 2006; COSEWIC 2002). An estimated 1540 mountain caribou, 98% of the global population, inhabit the mountainous terrain of the Interior Wet-Belt of southeastern British Columbia with the remaining animals occurring in northern Idaho and the northern corner of Washington (Wittmer et al. 2005a).

Population declines over the past several decades have been attributed to habitat loss and fragmentation, altered predator-prey dynamics associated with post-logging seral-stage forests, and anthropogenic disturbance associated with increased access to high elevation caribou range (Seip & Cichowski 1996; Thomas & Gray 2002; Wittmer et al. 2005a; Wittmer et al. 2005b). Recovery planning for mountain caribou, required under the Canadian Species at Risk Act (SARA), is currently underway and several gaps in knowledge have been identified, including the effects of backcountry motorized recreation on mountain caribou populations (Mountain Caribou Technical Advisory Committee (MCTAC) 2002).

Mountain caribou are an old-growth obligate species and require extensive contiguous tracts of mature forest and rounded sub-alpine mountain tops (Heard & Vagt 1998; Thomas & Gray 2002). In contrast to other ecotypes of woodland caribou, mountain caribou utilize high elevation forests and sub-alpine parkland habitat during mid- to late-winter, walking on top of the snowpack as it deepens and consolidates to feed on arboreal lichen in the forest canopy (Apps et al. 2001; Heard & Vagt 1998). This behaviour places mountain caribou in conflict with motorized recreation enthusiasts because highly suitable terrain for wintering caribou tends to also be the terrain sought by backcountry snowmobilers and heli-skiiers (Simpson & Terry 2000). The increasing popularity of backcountry snowmobile and heli-ski recreation, combined with improved access to alpine areas through extensive logging and mining road infrastructure, has resulted in increased human pressure on high elevation caribou winter range.

1.2 Caribou Response to Human Activity

Human engendered disturbance has been reported to have negative effects on behaviour and fitness of free-ranging caribou. In response to snowmobiles, aircraft overflights, and human presence caribou reaction has included increased movement and vigilance (Maier et al. 1998; Powell 2004; Simpson 1987), reduced foraging and resting (Duschesne et al. 2000), increased energy expenditure (Reimers et al. 2003), avoidance (Nellemann et al. 2000), and both short- and long-term displacement from winter range (Seip et al. 2007; Tyler 1991). Female caribou exposed to low-altitude jet flyovers have been reported to startle and run (Harrington & Veitch 1991) and have been reported to experience reduced fitness through increased energy costs and lower calf survival in calving and post-calving periods (Harrington & Veitch 1992). Given the above, it is reasonable to ask if motorized recreation acts as a behavioural or physiological stressor in mountain caribou.

1.3 Stress Response

Glucocorticoids (GCs) have become common indicators of physiological stress in wildlife due to their direct link to fitness (Creel et al. 2002; Wasser et al. 1997; Wasser et al. 2000). To clarify what is meant by stress, this research uses terminology outlined in Creel (2001), where a 'stressor' is the condition provoking a response and 'stress response' refers to changes in internal state (*i.e.*, GC concentration) as a result of external stimuli.

Basal levels of GCs, cortisol in large mammals, naturally circulate through the bloodstream and play an important role in, among others, regulation of the immune

system, gluconeogenesis, and fat storage (Wingfield 2005). However, in response to stressful stimuli the hypothalamus-pituitary-adrenal (HPA) axis rapidly increases secretion of GCs (Munck et al. 1984; Sapolsky et al. 2000). Increase in circulating GCs in response to short-term (acute) stressors may benefit individual animals for hours to days, through immediate mobilization of energy reserves (glycolysis) and reallocation of energy away from physiological processes that can be temporarily suspended without harm (Becker & Breedlove 2001; Sapolsky 1992). Once an acute stressor subsides, GCs typically return to basal levels and suspended physiological processes resume (see Wingfield 1997). However, under conditions of long-term chronic stress animals are expected to elevate their circulating GCs above normal baseline concentrations (Wingfield et al., 1998). These conditions of prolonged or repeated exposure to excess GCs may actually decrease an individual's fitness (Blas et al. 2007; Ellenberg et al. 2007; Munck et al. 1984; Pride 2005; Romero & Wikelski 2001; Sapolsky 2002; Tyler 1991). Pathologic consequences associated with chronically elevated GC levels include, among others, suppression of appetite, poor body condition, skeletal degradation, hypertension, decreased resistance to disease, and reduced reproductive output (Breazille 1987; Munck et al. 1984; Sapolsky 2002; Sapolsky et al. 2000).

1.4 Endocrinology in the Field

Glucocorticoids are metabolized in the liver and excreted as conjugates via the kidneys into the urine or via the bile into the digestive tract (Mostl & Palme 2002; Taylor 1971). Consequently, changes in circulating cortisol metabolites are paralleled in the feces following a species specific delay (Palme et al. 2005; Wasser et al. 2000). Unlike blood cortisol which provides a measure of circulating GCs at a given moment and may

be influenced by the sampling procedure itself, fecal GCs provide a integrated measure of adrenal activity over time (due to the pooling effects in the gut), such that hormone excretion reflects stress response to an event that occurred hours to days before sampling of feces (Wasser et al. 2000). Thus, while immediate GC response to acute single stress events may be most accurately detected in serum, chronic stress in wildlife may be most appropriately measured through feces.

Prior to using fecal GCs to identify stressors or interpret health status of wildlife populations, physiological and biological validation of field endocrine techniques must be conducted for the target species (Touma & Palme 2005; Wasser et al. 2000). Physiological validation involves exogenous stimulation of the HPA axis, typically through an adrenocorticotropic hormone (ACTH) challenge, to elevate circulating GCs and evaluate whether these changes are reflected in the feces (Wasser et al. 2000). Biological validation, on the other hand, involves evaluation of biologically relevant alterations in endocrine status such as animal response to challenging situations or conditions (e.g., novel disturbances, capture and handling, translocation, social interactions, environmental stressors) (for review see Touma & Palme 2005). In addition, studies investigating effects of specific stressors on animals need to account for various factors that may influence GC concentrations in blood and feces such as gender differences, seasonal variations, life history stages (e.g., reproductive state), diurnal rhythm of GC secretion, gut passage time, and environmental parameters (Touma & Palme 2005).

1.5 Thesis Objectives and Hypotheses

The use of endocrine field techniques to assess stress response to anthropogenic disturbance has not been previously explored for woodland caribou. The main goal of my research was to evaluate the feasibility of using fecal GCs to measure stress response in caribou and to determine the potential effect of motorized recreation on mountain caribou stress response. To do so, I conducted two distinct field studies between 2002 and 2005. One study involved experimental validation of fecal GC metabolites as an effective indicator of stress response in caribou, while the second study evaluated stress response in free-ranging mountain caribou exposed to motorized winter recreation. The objectives of my research are to:

- Procedurally, physiologically and biologically validate a hormone assay to quantify fecal GC metabolites in caribou.
- Estimate the lag time associated with endogenous adrenal activity and excretion of feces GC metabolites in caribou.
- Estimate baseline estimates of fecal GC metabolites in free-ranging mountain caribou over winter.
- Determine if fecal GC metabolites in mountain caribou inhabiting areas exposed to backcountry snowmobile and heli-ski recreation differed from concentrations in areas closed to motorized recreation.
- Test experimentally if mountain caribou fecal GC metabolites differ prior–to and following exposure to an acute, known helicopter disturbance.

The predictions of my research thesis are that fecal GC metabolites are an indicator of physiological stress response in caribou and that fecal GC metabolites in mountain

caribou are elevated in areas influenced by snowmobile and heli-ski recreation compared to areas with no motorized recreation. I also expect that fecal GCs in caribou will increase following exposure to an acute helicopter disturbance, then decrease to predisturbance GC levels.

1.6 Study Area

The fecal assay validation experiment was conducted on domestic reindeer during the winter of 2005 in an outdoor environment near the farming community of Groundbirch, located in the Peace Lowlands of north-eastern British Columbia (55˚46′28″ N, 120˚49′24″ W). The outdoor facility was selected to emulate winter weather conditions potentially experienced by free-ranging animals.

The field study on free-ranging mountain caribou was conducted in the Quesnel Highland and Cariboo Mountains of east-central British Columbia (52°30 N, 121°00 W) during the winters of 2002 to 2004. Approximately 250 mountain caribou ranged within our study area comprising 3 of 17 sub-populations in British Columbia including the Barkerville sub-population, Wells Gray (North) sub-population, and the southern portion of the North Cariboo Mountains sub-population (Wittmer et al. 2005a; Young & Freeman 2003). The study area was characterized by mountainous terrain, cool climate, high precipitation, and high snow depths (>3 m). Four biogeoclimatic zones characterized caribou habitat including low-elevation Interior Cedar Hemlock and Sub-Boreal Spruce, mid-elevation Engelmann Spruce Sub-Alpine Fir, and high-elevation alpine tundra (Meidinger & J. Pojar 1991). Extensive high-elevation caribou winter range occurs at treeline (>1 800 m) on gently sloping plateaus and rounded sub-alpine mountain tops.

1.7 Thesis Overview

I prepared this thesis as two independent but related manuscripts to be submitted for publication. Chapter 1 introduces the study population, provides background on stress response and noninvasive endocrine techniques to measure stress, and outlines the objectives and hypotheses of this thesis. Chapter 2 describes the physiological and biological validation of the fecal glucocoticoid assay for *R. tarandus*, through an adrenocorticotropic hormone (ACTH) challenge. Chapter 3 examines the effects of snowmobile and heli-ski activity on stress response of mountain caribou through noninvasive measurement of fecal glucocorticoids. Chapters 2 and 3 follow the format of a scientific paper with abstract, introduction, methods, results, discussion, and management implications. Chapter 2 will be submitted as a research note and Chapter 3 as an article for journal publication. Chapter 4 summarizes findings from the study, lessons learned, potential for future research, conservation implications and recommendations for management of endangered mountain caribou populations in British Columbia with regards to backcountry motorized recreation.

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2 Validation of a noninvasive fecal glucocorticoid assay for *Rangifer tarandus* by ACTH Challenge[1]

2.1 Introduction

Noninvasive endocrine assays for fecal glucocorticoids (CGs) have been used to identify ecological and anthropogenic stressors in several species of free-ranging wildlife (e.g., Wasser et al. 1997, Creel et al. 2002) but have never been applied to caribou (*Rangifer tarandus* (Gmelin 1788)). We aimed to test if non-invasive hormone assays might be used to assess physiological state in free-ranging woodland caribou (*R. t. caribou*) by validating an assay for fecal GCs via an adrenocorticotrophic hormone (ACTH) challenge experiment using domestic reindeer (*R. tarandus tarandus*). Many caribou populations in Canada have experienced severe declines, some are now recognized as being at risk of extirpation, and habitat modification and human-related disturbance have been identified as a potential impediments to recovery (COSEWIC 2002). Reliable, non-invasive assays to estimate fecal CGs in caribou would be a useful tool to assess physiological stress in wild herds and test if these levels are elevated by anthropogenic stressors.

Procedural validation of a hormone immunoassay is normally achieved by measuring sensitivity (the minimum concentration that can be measured), testing repeatability (intra-assay and interassay coefficients of variation), testing specificity (by comparing serial dilutions of standards and the sample type of interest), and testing quantitative accuracy (by adding known quantities of the hormone of interest to the sample type of interest).

[1] A version of this chapter will be submitted for publication.
Freeman, N.L., P. Arcese, S. Wasser, and S. Creel. Validation of a non-invasive fecal glucocorticoid assay for *Rangifer tarandus* by ACTH Challenge.

Physiological validation of fecal GC assays is often achieved by injecting ACTH to stimulate the short-term secretion of GCs into the blood as cortisol or corticosterone, and then detecting the predicted increase and subsequent return to baseline level after a species-specific excretion delay (e.g., Wasser et al. 2000). We report the validation of an enzyme linked immunosorbent assay (ELISA) to measure endogenous GCs in woodland caribou. Substantial diurnal variation in temperature and barometric pressure also enabled us to track variation in the fecal cortisol stress response in subjects exposed to natural variation in winter weather. Our objectives were to (1) procedurally and physiologically validate an ELISA to quantify fecal cortisol metabolites in wild caribou feces, (2) estimate the lag time associated with endogenous adrenal activity and fecal cortisol excretion, and (3) determine if biologically meaningful adrenocortical function in caribou can be measured through fecal GC metabolites. We also compared the affinity of antibodies to cortisol (ELISA) and corticosterone (radio-immunosorbent assay; RIA) to track changes in fecal GCs.

2.2 Study Area

From January 2 to 9, 2005 near Groundbirch, B.C. (55˚46′28″ N, 120˚49′24″ W), 8 domestic reindeer, 4 pregnant females (2 3-yr-old and 2 4-yr-old) and 4 males (2 2-yr-old and 2 5-yr-old), were housed singly in unroofed, outdoor paddocks that allowed visual and olfactory contact. All reindeer were familiar with their facilities, used for resting, feeding and general husbandry, fed a standard grain mixture twice per day, and provided with hay and fresh snow ad libitum.

2.3 Methods

We used a crossover experimental design (Kuehl 1999) wherein half the reindeer received a control saline injection followed by an ACTH injection (S-A sequence), and the other half received ACTH followed by saline (A-S sequence). Each sequence included 2 females and 2 males; within gender, animals of the same age received the opposite treatment sequence. To minimize variation and increase precision, each animal acted as its own control for statistical analysis (a difference-to-self design). Reindeer were weighed on a platform scale prior to penning (females: 67-79 kg, males 67-127 kg) and then acclimated for 3 d. We administered first injections on Day 4, followed by a second injection 2 d later; all between 1900 and 2000 hrs to control for time of day. Each reindeer received an intramuscular ACTH injection of 1 IU/kg body mass (Acthar gel, Questcor Pharmaceuticals Inc., Union City, CA, USA) and an equivalent volume of saline, a dose comparable to other ACTH challenges in cervids (Wasser et al. 2000, Millspaugh et al. 2002). We expected peak cortisol excretion 18-24 hrs after a blood cortisol spike, with a return to pre-injection levels ~12 hrs later (e.g., Wasser et al. 2000, Millspaugh et al. 2002, Huber et al. 2003a), and considered 48 hrs sufficient to prevent carryover responses from the first injection to the second.

Fecal samples were collected from the ground within 15 min of defecation, 3-times per day (0600, 1200, and 1800 hrs) on Days 2 through 4, and at 3-hr intervals beginning 11 hrs post-injection, from 0600 to 2100 hrs. Overnight sampling was avoided to prevent inadvertent stress. We identified baseline fecal cortisol levels from the first fecal sample collected within 3 hrs of penning (Day 1), at which time new housing could not have influenced fecal GCs based on expected clearance times in ungulates. We sub-sampled

~50 mL of entire fecal masses to reduce sampling error due to uneven distribution of GC metabolites in feces (Millspaugh and Washburn, 2003). Overall, 32 fecal samples collected from each reindeer were immediately frozen and stored at -20º C. Weather data, hourly ambient temperature (C) and air pressure (kPa) was obtained via Environment Canada (2005). Our research was approved by University of British Columbia Animal Care Committee (A04-1037).

In the lab, frozen fecal samples were thawed, homogenized, sub-sampled, weighed (Mettler 5000 analytic balance), dried in a rotary evaporator without heat, and then re-weighed to estimate water content in wet samples. Ground dried feces (~0.2 g) were boiled in 10 mL 95 % ethanol for 20 min then centrifuged at 502 *g* for 15 min at 27º C. Supernatants were decanted into glass tubes, placed in a water bath (60-65º C) and dried under air; centrifuge pellets were weighed before discarding to account for variation of undigested material. Once dried, tubes were rinsed with 2 mL 95 % ethanol, vortexed for 15 sec, and placed in an ultrasonic glass cleaner for 15 sec to remove any particles adhered to the sides of the tube. Samples were re-dried, extracted with 1 mL absolute methanol, vortexed for 1 min, placed in an ultrasonic glass cleaner for 30 sec, and vortexed again for 15 sec. Extracts were then stored in airtight cryovials at -80º C until assay (Creel et al. 2002).

We measured cortisol and progesterone concentrations using ELISA (RnD Systems, Inc., Minneapolis, MN, USA). Individual samples were assayed in duplicate for cortisol, and a pooled fecal extract for each reindeer was assayed for progesterone. To compare antibody affinity for cortisol metabolites we re-assayed fecal extracts from 1 female and 1 male reindeer with ^{125}I corticosterone RIA previously used on elk (Wasser et al 2000;

Millspaugh et al. 2001); extracts were diluted 5-fold and assayed in duplicate, with an intra-assay variation of 2.90 % and manufacturer assay sensitivity of 0.2 ng (ICN Biomedical, Costa Mesa, CA, USA). Previous validation of the ^{125}I corticosterone RIA for caribou showed good parallelism and extraction efficiency of cortisol metabolites, not corticosterone per se (S.K. Wasser, unpubl. res.). For simplicity, we refer to the RIA as the corticosterone assay to distinguish it from the cortisol ELISA. All fecal hormone concentrations (hereafter fecal cortisol, corticosterone or progesterone) are expressed as ng/g dry feces.

We tested whether ACTH injection influenced fecal cortisol concentration by first comparing mean cortisol calculated from 5 treatments (baseline, pre-ACTH, post-ACTH, pre-saline, and post-saline) to test if injection sequence (A-S and S-A) influenced cortisol using a one-way ANCOVA, with the pooled progesterone included as a covariate. Second, we used a two-way ANOVA to test for gender and treatment effects (Rutherford 2001). We defined treatment as a single fecal cortisol observation with baseline representing cortisol level prior to penning (as described above), pre-ACTH and pre-saline as cortisol immediately preceding each respective injection, and post-ACTH and post-saline as transient cortisol response subsequent to injection. Both analyses employed a GLM, post-hoc comparisons were Bonferroni adjusted, and independence of observations assessed with the Durbin-Watson statistic.

Substantial diurnal changes in weather also enabled us to test if fecal cortisol varied in relation to weather. To do so, we first estimated cortisol excretion lag time by examining correlations between air temperature and fecal cortisol using Spearman's correlation coefficients, including all samples collected over the 8 d experimental period, except

samples collected within 12 hrs of injection ($N_{females}$=116, N_{males}=116). We then used backward step-wise regression to identify potential predictors on fecal cortisol, by gender, with unique values of each predictor variable corresponding to four cortisol measures (one for each reindeer). Candidate predictors were identified based on existing hypotheses and the results of published studies indicating that circulating cortisol levels vary with reproductive state (Wingfield et al. 1994, Weingrill et al. 2004), fecal water content and undigested matter (Creel 2001), and weather variables including air pressure and temperature (Romero et al. 2000, Frigerio et al. 2004; reviewed by Touma & Palme 2005). We controlled statistically for the potential influence of serial autocorrelation in samples collected from the same individuals by also including as a lagged variable cortisol level estimated in the prior sample (lag-one autocorrelation) in both models. Specific weather variables included temperature 9 hrs prior-to, and air pressure 18 hrs prior-to each sample; these lags being identified empirically as described above. Including fecal water content and indigestible matter allowed us to control statistically for the potential influence of water absorbed or evaporated after defecation and before sample collection, and to control statistically for individual or temporal variation in diet (Creel 2001). Cortisol values were transformed by natural-log prior to analysis to normalize its distribution. Model fit was assessed graphically and by examining serial autocorrelation using the Durbin-Watson statistic. An initial alpha of 0.05 was used for all tests and all statistical analyses employed SYSTAT® 11 (SYSTAT 2004).

2.4 Results

Serial dilutions of cortisol standards and a pooled reindeer fecal extract showed good parallelism in our ELISA across 8 points from undiluted to 256 fold. Optimal dilution

(matching antibody binding at the steepest portion of the standard curve) was 1:8. Recovery of cortisol (50 uL at 156-10 000 ng/mL) added to fecal extracts was 113 % ± 32.2 % (95% CI; $F_{1,5} = 941.5$, $p < 0.001$, $R^2_{adj} = 0.994$). For progesterone, serial dilutions of standards and pooled fecal extracts yielded parallel changes in antibody binding for 5 points from 1:128 to 1:2048 in males and for 4 points from 1:512 to 1:8192 in females. As expected, higher dilutions were required for females. Optimal dilution was 1:256 and 1:2048 for males and females, respectively. Recovery of progesterone (50 uL at 15.6-500 ng/mL) added to fecal extract was 87 % ± 9.5 % ($F_{1,4} = 1440.2$, $p < 0.001$, $R^2_{adj} = 0.997$) for females and 69 % ± 24.8 % ($F_{1,4} = 518.4$, $p < 0.001$, $R^2_{adj} = 0.990$) for males. Intra-assay variation for cortisol and progesterone was 2.8 % and 4.8 %, respectively. Inter-assay variation for cortisol was 4.9 %. Assay sensitivity was 26.4 pg/mL for cortisol and 12.0 pg/mL for progesterone, measured directly for reindeer fecal extracts.

Fecal cortisol peaked in 5 of 8 reindeer following ACTH injection (Figure 2-1), with all four females showing an elevated response to ACTH. Females also expressed higher fecal cortisol concentrations than males, which as a group showed no clear response to ACTH (Figure 2-2). Overall, 77.4% of variation in fecal cortisol was accounted for by gender ($F_{1,30} = 47.9$, $p < 0.001$), treatment ($F_{4,30} = 7.2$, $p < 0.001$), and a gender × treatment interaction ($F_{4,30} = 6.5$, $p = 0.001$). Progesterone was also closely correlated with fecal cortisol ($R^2_{adj} = 0.896$, $F_{1,5} = 40.5$, $p = 0.001$). In contrast, injection sequence did not influence cortisol ($F_{1,5} = 1.8$, $p = 0.237$).

In addition to these treatment effects, we observed a marked rise in fecal cortisol in all 8 reindeer prior to injection, followed by steep declines thereafter (Figure 2-1). This striking correlation among animals coincided with dramatic variation in ambient

temperature (max = -0.4° C, min = -36.8° C) and barometric pressure (max = 95.3 kPa, min = 92.4 kPa) over the study, as expected if these external factors affected cortisol levels similarly in all 8 reindeer (Figure 2-3). In females, cortisol varied with temperature ($\beta = -3.7$, $t = -5.3$, $p < 0.001$), air pressure^2 ($\beta = 0.004$, $t = 6.2$, $p < 0.001$), a temperature × pressure interaction ($\beta = 0.04$,$t = 5.29$, $p < 0.001$), and progesterone ($\beta = 0.0001$, $t = 2.2$, $p = 0.027$; regression including cortisol at time t-1 $\beta = 0.21$, $t = 2.4$, $p = 0.017$; $R^2 = 0.46$, $F_{5,110} = 18.8$, $SE_e = 0.24$). Cortisol in males was predicted by temperature ($\beta = -4.5$, $t = -7.2$, $p < 0.001$), air pressure^2 ($\beta = 0.004$, $t = 8.4$, $p < 0.001$), a temperature × pressure interaction ($\beta = 0.05$, $t = 7.3$, $p < 0.001$), and lagged cortisol ($\beta = 0.005$, $t = 3.9$, $p < 0.001$; $F_{4,111} = 42.2$, $R^2 = 0.60$, $SE_e = 0.21$). Fecal water content and indigestible matter were not influential predictors in male or female models.

Peak cortisol excretion occurred 11 hrs post-ACTH injection in females (Figure 2-2A), but was indistinct in males (Figure 2-2B). To estimate the lag time for variation in blood cortisol to be reflected in feces for animals of both sex we also compared change in fecal cortisol and air temperature over the over the same 6, 9, or 12-hr intervals. These changes were most strongly related to temperature 9 hrs earlier in males ($r_s = -0.54$, $n = 32$, $P < 0.002$) and females ($r_s = -0.57$, $n = 32$, $P < 0.001$), implying overall a lag of 9-11 hrs between circulating and fecal cortisol levels.

To exclude weather influences on fecal cortisol we compared post-ACTH to baseline and to post-saline samples to estimate the independent effect of exogenous ACTH. In females, post-ACTH cortisol was 160% above baseline ($p < 0.001$) and post-saline levels ($p < 0.001$; Figure 2-2A). In contrast, no statistically significant differences were observed in males (Figure 2-2B).

Comparing cortisol and corticosterone profile plots yielded similar results, with peaks in the female averaging 198 % and 214 % above baseline levels, respectively (Figure 2-4A). In the male cortisol rose 3 % and corticosterone 59 % above baseline levels (Figure 2-4B).

2.5 Discussion

Fecal cortisol rose in female reindeer within 11 hrs of ACTH injection and showed a similar lagged response to marked variation in weather, indicating that meaningful variation in cortisol can be measured in female reindeer feces using ELISA. Fecal cortisol also responded to weather in males but was unaffected by ACTH injection. Different responses of male and female reindeer to ACTH may indicate that: (a) male reindeer required a dose larger than the 1 IU/kg body mass of ACTH to elicit a spike in fecal cortisol; (b) response to ACTH injection was masked by weather more so in male than female reindeer; or (c) that a peak in fecal cortisol excretion in males occurred before sampling began. More intensive sampling designs might help resolved excretion timing, but it can also confound results of ACTH challenges (e.g., Huber et al 2003a).

Our estimated lag time for excretion of cortisol was 9-11 hrs, about half the average reported for cervids (c 22 hrs; Wasser et al. 2000, Huber et al. 2003a, Millspaugh et al. 2001; but see Denhard et al. 2001). However, excretion of corticosterone may have peaked later (see Figure 2-4A). Although immunoassays and lag times can vary by species due to differences in antibody affinities and clearance times, our results suggest that affinities for cortisol assayed by ELISA and corticosterone assayed by RIA were similar

We also observed that fecal cortisol was higher in female than male reindeer. This is expected because progesterone and cortisol both bind to carrier proteins in plasma and to cortisol binding globulin (CBG), but progesterone has a superior affinity for CBG. Pregnant animals therefore excrete unbound cortisol at higher rates than non-pregnant animals (Newcomer 1971). This affinity suggests that controlling for the influence of fecal progesterone in analyses of fecal cortisol will improve the precision of statistical models designed to test for an influence of exogenous stressors.

Like the reindeer in our study, other cervids have also been found to display variation in fecal cortisol in response to changes in weather (e.g., Creel et al. 2002, Huber et al. 2003b). In our experiment, extreme changes in air pressure and temperature coincided with marked changes fecal cortisol 9-11 hrs later. This suggests that controlling statistically for weather effects on fecal cortisol level may also improve estimates of stress in free-ranging wildlife. Overall, and in terms of caribou management, our results suggest that field sampling to allow EIA of fecal GCs could be used to identify exogenous stressors related to weather, humans or other factors, as long as gender and reproductive state, and species-appropriate lag times are known and accounted for statistically.

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Figure 2-1. Fecal cortisol (ng/g dry feces) excretion profiles of 4 female (A, B) and 4 male reindeer (*R. tarandus*) (C, D) during a winter adrenocorticotropic challenge crossed over with a saline challenge at Groundbirch, British Columbia during January 2005. Time 0 corresponds to time of baseline fecal collection at initial penning. ▲ = time of ACTH injection, □ = time of saline injection.

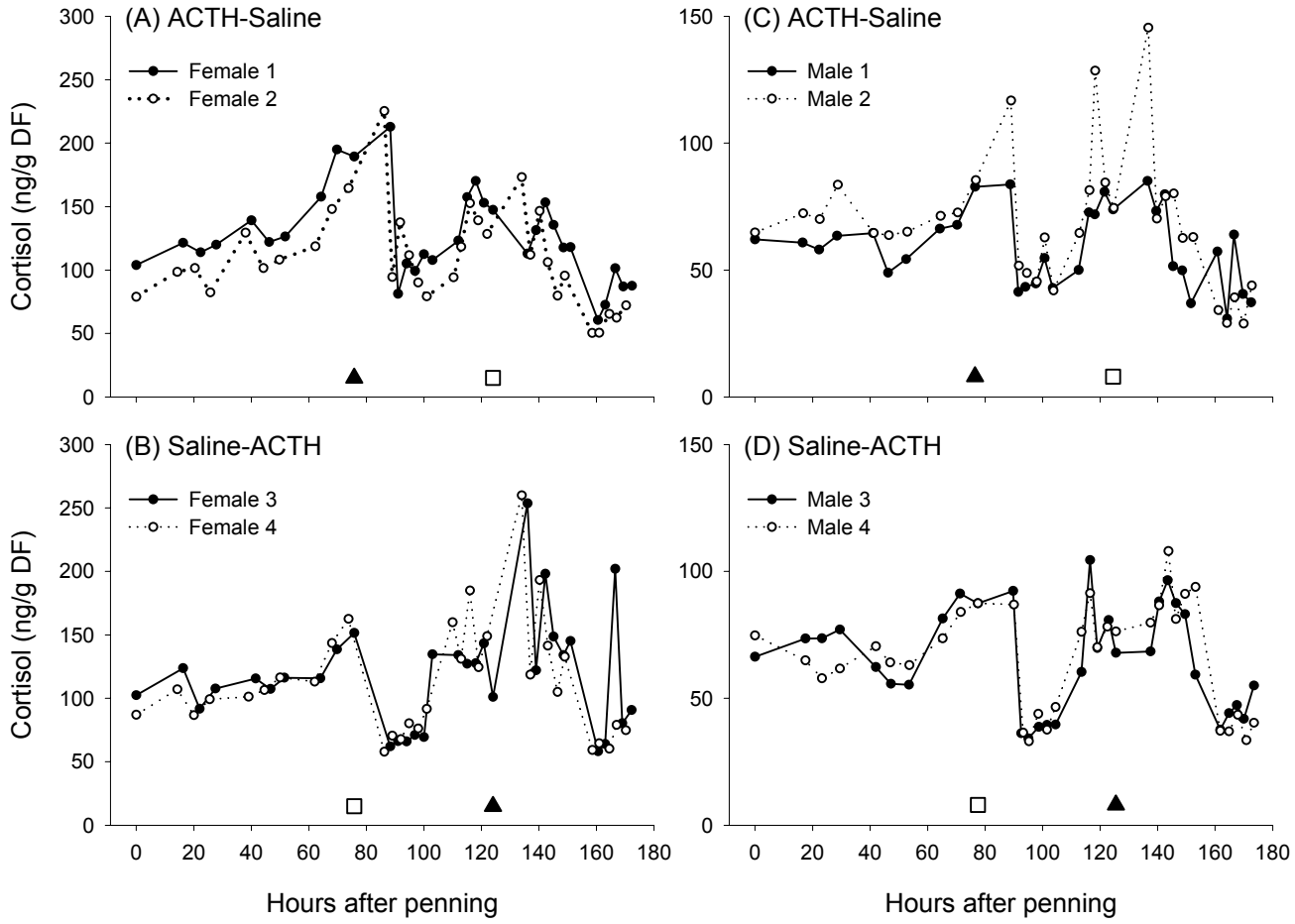


Figure 2-2. Mean fecal cortisol (ng/g dry feces) of (A) 4 female and (B) 4 male reindeer (*R. tarandus*) at baseline and 6-hr and 3-hr intervals before and after an adrenocorticotropic and saline challenge at Groundbirch, British Columbia during January 2005. Time 0 corresponds to time of ACTH or saline injection. Error bars are mean ± 95 % CI ($t_{0.05(2)3}$ =3.182).

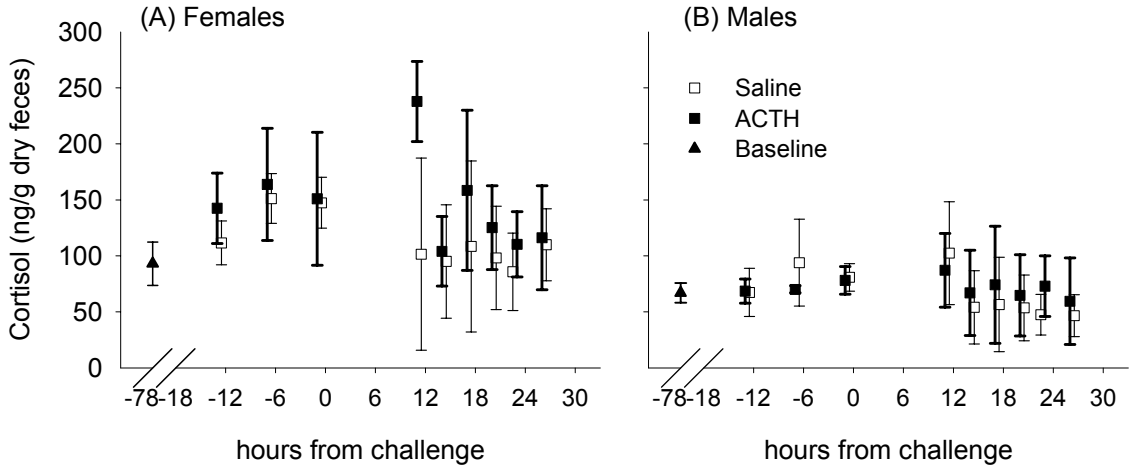


Figure 2-3. Covariance of fecal cortisol (ng/g dry feces) profiles in 4 female and 4 male reindeer (*R. tarandus*) and (A) ambient temperature (°C) 9 hrs prior-to the cortisol observation and (B) air pressure (kPA) 18 hrs prior-to the cortisol observation during an adrenocorticotropic and saline challenge at Groundbirch, British Columbia in January 2005. Fecal cortisol observations corresponding to the 11-hr post-ACTH and post-saline injection response were excluded. Time 0 corresponds to time of baseline fecal collection at initial penning. ● = females, ○ = males, air temperature (solid line), air pressure (dashed line).

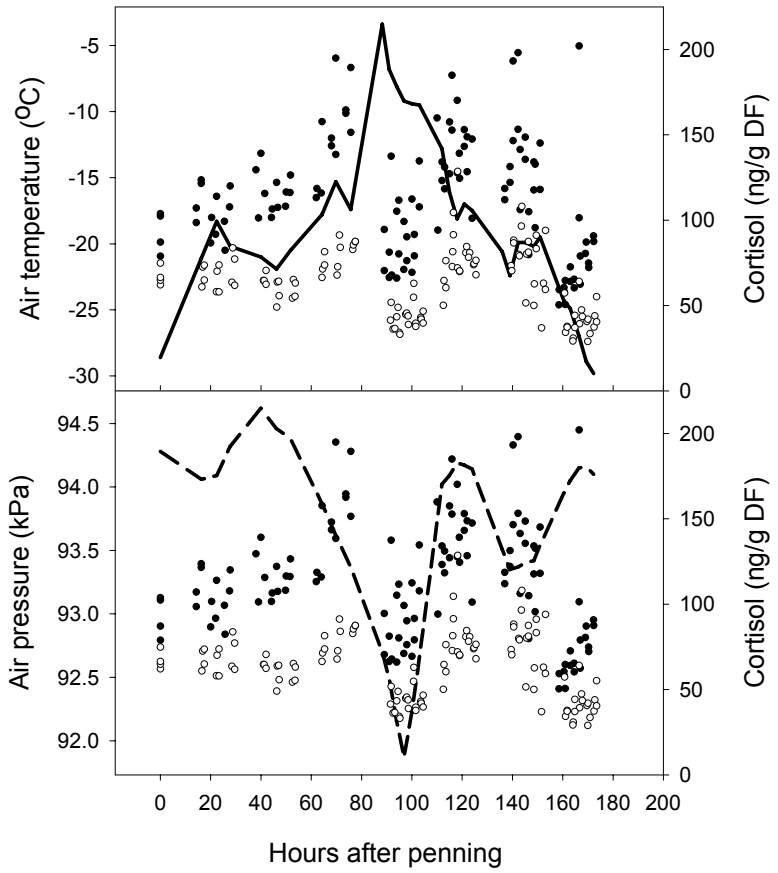
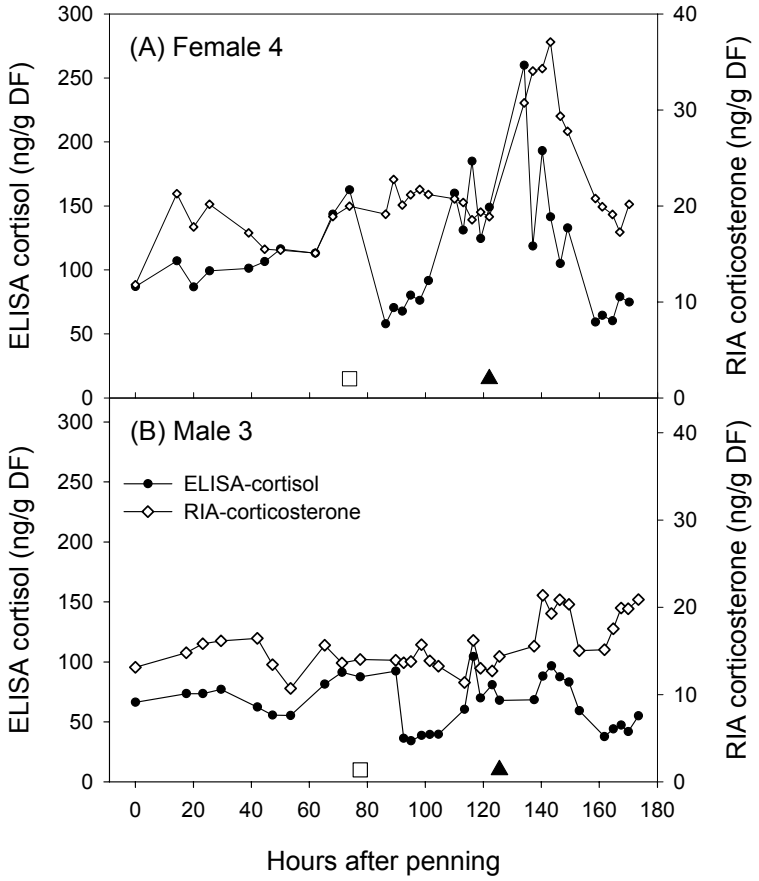


Figure 2-4. Comparison of reindeer (*R. tarandus*) fecal glucocorticoids (ng/g dry feces) measured by ELISA (cortisol) and RIA (corticosterone) immunoassay techniques for excretion profiles of (A) Female 4 and (B) Male 3 during an adrenocorticotropic and saline challenge at Groundbirch, British Columbia in January 2005. Time 0 corresponds to time of baseline fecal collection at initial penning. □ = time of saline injection, ▲ = time of ACTH injection.



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3 Motorized Backcountry Recreation and Stress Response in Mountain Caribou[2]

3.1 Introduction

Increasing tourism and recreation in wilderness areas are conservation concerns because of their potential impact on wildlife populations. In British Columbia, Canada, mountain caribou (*Rangifer tarandus caribou*) have declined dramatically in number and are considered threatened (COSEWIC 2002; Heard & Vagt 1998; Thomas & Gray 2002; Wittmer et al. 2005a). Motorized backcountry winter recreation has been identified as a potential threat to this ecotype of woodland caribou (MCTAC 2002) because both caribou and backcountry snowmobilers and heli-skiers rely on high elevation forest and parkland habitat (Simpson & Terry 2000).

Human-related disturbances are also known to affect behaviour and reproduction in free-ranging caribou. In response to snowmobiles, aircraft and humans caribou may startle and run (Harrington & Veitch 1991), increase avoidance, movement and vigilance behaviors (Maier et al. 1998; Powell 2004; Simpson 1987; Tyler 1991), reduce foraging and resting (Duschesne et al. 2000), increase energy expenditure (Reimers et al. 2003) and become displaced from a former range (Seip et al. 2007). Reproductive success may also be impacted by human disturbance in caribou. Female caribou exposed to low-altitude jet flyovers showed increased energy expenditure and reduced calf survival (Harrington & Veitch 1992). Despite these findings, it is not yet known whether snowmobile and helicopter activity also influence caribou stress physiology. Our goal here was to test if glucocorticoids (GCs), which are known to indicate physiological

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effects of stress in other wildlife species (Creel 2001; Palme et al. 2005; Romero et al. 2000; Walker et al. 2006), also become elevated in free-ranging caribou exposed to backcountry motorized recreation.

Secretion of GCs by the adrenal gland is a stress response with physiological consequences that may differ depending on the duration of the response (Sapolsky 2002; Wingfield 1997). Increase in circulating GCs in response to short-term (acute) stressors (hours to days) allow immediate mobilization of energy reserves and reallocation of energy away from physiological processes that can be temporarily suppressed without harm (Becker & Breedlove 2001; Sapolsky 1992). Once the stressor is resolved, GCs typically return to basal levels and suspended physiological processes resume (Wingfield 1997). Repeated or prolonged exposure to elevated GCs (due to long-term chronic stressors) however, can shift short-term benefits to long-term detriments and reduce individual fitness (Blas et al. 2007; Ellenberg et al. 2007; Munck et al. 1984; Pride 2005; Romero & Wikelski 2001; Sapolsky 2002; Tyler 1991). Chronically elevated GCs can negatively affect feeding behavior, growth and body condition, resistance to disease, reproductive output, and survival (Breazille 1987; Sapolsky et al. 2000). Thus, chronic elevation of GCs is one mechanism that may link behavioral responses to environmental disruption, to changes in population demography (e.g., Clinchy et al. 2004; Cockrem & Silverin 2002; Ellenberg et al. 2007; Scheuerlein et al. 2001).

If mountain caribou do experience chronic stress when exposed to motorized recreational vehicles in late winter, when energy reserves are low and females are late in pregnancy, it may also influence individual fitness and population trend. We therefore tested if caribou showed a stress response to motorized recreation by measuring GC

concentration in mountain caribou feces collected on winter ranges that were well-separated in space, and either open or closed to snowmobile and heli-ski activity. We aimed to 1) estimate baseline levels of fecal GCs in mountain caribou fecal GCs over winter, 2) determine if fecal GCs in caribou inhabiting areas subject to regular snowmobile and heli-ski activities differed from levels estimated in areas closed to motorized recreation, and 3) to test experimentally if caribou fecal GCs rose in response to an acute helicopter disturbance.

3.2 Study Area

We conducted our study in the Quesnel Highland and Cariboo Mountains of east-central British Columbia, northeast of Williams Lake (121°00W, 52°30 N) between January and March, 2002 to 2004 (Figure 1). Our 7 540 km^2 study area was characterized by mountainous terrain, cool climate, high precipitation, and high snow depths (>3 m). Caribou habitat occurs in four biogeoclimatic zones in our study area including low-elevation Interior Cedar Hemlock and Sub-Boreal Spruce, mid-elevation Engelmann Spruce Sub-Alpine Fir, and high-elevation alpine tundra (Meidinger & J. Pojar 1991). Extensive high-elevation caribou winter range occurs at treeline (>1 800 m) on gently sloping plateaus and rounded sub-alpine mountain tops. Approximately 250 mountain caribou range within our study area, comprising 3 of 17 identified sub-populations: the Barkerville sub-population, Wells Gray (North) sub-population, and the southern portion of the North Cariboo Mountains sub-population (Wittmer et al. 2005a; Young & Freeman 2003).

3.3 Methods

3.3.1 Fecal Collection

We collected 360 fecal samples from mountain caribou within our snowmobile, heli-ski, and control areas during January through March, 2002-2004. Weather permitting, we recorded the locations of up to 16 radio-collared caribou biweekly, scouted for uncollared caribou groups, and recorded observations of concentrated snowmobile and heli-ski activity to identify core areas of recreation use using a fixed-wing Cessna 182. Caribou group locations were re-visited the same day using a Bell 206 helicopter. Because telemetry and helicopter flights occurred within 2 hrs of each other, they cannot have influenced fecal GCs collected that day because fecal GCs reflect circulating levels roughly 9-11 hrs earlier in caribou (Freeman 2008, Chapter 2), and roughly 24 hrs earlier in other cervids (e.g., Millspaugh et al. 2001; e.g., Wasser et al. 2000). To minimize the potential influence of our sampling technique on GC levels and the possibility of resampling individual caribou, we sampled different parts of the study area during biweekly sampling trips. The only exception to this rule occurred during annual, late winter aerial censuses of the entire study area. During this period, we sampled resident herds systematically in each area to avoid collecting duplicate samples from individual animals. Autocorrelation plots of time series data for fecal GCs in individual caribou, after controlling for progesterone, indicated functional independence of samples (i.e., no serial autocorrelation) within 24 hrs (Freeman 2008, Chapter 2). As a consequence, we considered each fecal sample we collected to be an independent observation of GC level in each area of interest.

Fecal pellets were collected from the snow surface after animals had moved away from the area. To minimize the chance that two samples were taken from one animal, we preferentially collected pellets from individual caribou 'beds' or, when bedding areas were not found, from on top of the snow on the clearly visible trails of individual caribou. We estimated time of defecation for all samples to control for diurnal variation in GC secretion (Millspaugh & Washburn 2004; Touma & Palme 2005) based on direct observation, sample state, or freshness of caribou tracks and time since last snowfall; the majority (75%) of samples were fresh and all frozen samples were < 3 d old. Sub-zero temperatures prevailed through the study periods and froze pellets quickly, thus minimizing potential steroid degradation, if any, of frozen samples (Washburn & Millspaugh 2002). Sampling error was reduced further by homogenizing ≥ 50 pellets from each sample before analysis (e.g., Millspaugh & Washburn 2003). All samples were stored at -20º C until processing.

Snow water equivalent (SWE) and ambient temperature data were obtained from 4 high-elevation Automated Snow Pillow stations (ASP, elevation range 1 460 m - 1 670 m) within and bordering our study area (River Forecast Centre 2005). We used inverse distance weighting to interpolate SWE and temperature at each fecal collection site, correcting horizontally (straight line distances) and vertically (elevation differences) between sample sites and ASP stations. We accounted for the lag in fecal GC excretion by using ASP data recorded 1 d prior to fecal excretion (Freeman 2008, Chapter 2). We also recorded aspect at each fecal collection site as supplementary information potentially influencing snow state and distribution.

3.3.2 Motorized Recreation

Snowmobile recreation occurred in the western portion of our study area and commercial heli-skiing in the more rugged terrain to the east; neither recreation activity was permitted in the northern-most portion of the study area (Figure 3-1). Heli-skiing occurred January through March in 2003 and 2004, but not in 2002. No commercial heli-ski operation was active prior to 2003 within our study area, nor was there spatial overlap of snowmobile and heli-ski recreation for the duration of our study. Snowmobilers accessed trailheads via logging/mining roads while the heli-ski area was accessible only by helicopter during the winter. Movement of radio-collared caribou between snowmobile (3 660 km^2), heli-ski (2 230 km^2) and control (1 650 km^2) areas was not observed during winter 2002 through 2004, however populations are not discrete and shifts between the snowmobile and heli-ski areas by caribou have been recorded (Young & Freeman 2003).

We tested if caribou GC levels were greater in areas of motorized recreation by comparing GCs from heli-ski, snowmobile, and control areas. We collected caribou fecal samples in winter 2002 through 2004 in the snowmobile areas and in winter 2003 and 2004 in the heli-ski areas. Our control areas comprised mountain complexes undisturbed by motorized recreation where potential for recreation exposure was a minimum of 10 km away (i.e., straight-line distance) and were separated by lakes, rivers, and low elevation forested terrain not suitable for wintering caribou. In 2002 we collected control samples from the area designated for a commercial heliski operation, but without previous snowmobile or heliski recreation. In 2003, collared animals were absent from control areas and below-normal snow depths caused caribou to remain in dense mid-

elevation forests (e.g., Kinley et al. 2007), which we could not access for sampling. In 2004, we collected control samples in the northern portion of the study area (Figure 3-1).

We also used a finer-scale approach to test if caribou GCs were elevated in proximity to recreation activity. For snowmobile activity, we identified core areas of concentrated use within our study area using snowmobile track locations recorded during fixed-wing and helicopter flights. Snowmobile activity was widespread throughout sub-alpine meadows consequently we identified centers of intensive snowmobile activity, such as play areas or hill climbs (Figure 3-1). We then determined straight-line distance (m) from caribou fecal collection sites to nearest snowmobile activity center and grouped fecal samples into distance categories of <4 km, 4-8 km, and 8-10 km. Categories were selected based on repeatedly documented caribou avoidance zones, ranging from 1 to 4 km in the vicinity of anthropogenic activity (e.g., Cameron et al. 1992; Cameron et al. 2005; Mahoney & Schaefer 2002; Nellemann & Cameron 1996, 1998; Nellemann et al. 2001; Smith et al. 2000; Vistnes & Nellemann 2001; Weir et al. 2007). Fecal samples 8-10 km from snowmobile activity were from areas where access was restricted either by topographical barriers or long-standing recreation closures; these samples represent an 'intermediate' between caribou exposed to snowmobile activity and controls. Our initial plan to relate GC levels to the season-long distribution of heli-skiing activities was compromised when an agreement to share data with a commercial skiing operation fell apart, thus these analyses were limited to the coarse scale approach outlined above.[3]

[3] We were unable to test if caribou GCs varied with proximity to heli-ski routes or the frequency of heli-ski activity because a potential provider of these data required that we agree to financial compensation in the event that any conclusion of our study had negative economic consequences. This requirement prevented

3.3.3 Helicopter Disturbance Trial

To assess caribou GC response to acute helicopter disturbance, three caribou groups of 7, 9, and 11 adults were exposed to 3-5 min of direct and 6-11 min indirect Bell 206 helicopter disturbance during the aerial caribou census of March 2003. Mountain caribou population counts are conducted in late March when caribou are utilizing open, high elevation sub-alpine habitat and sightability is high (Wittmer et al. 2005a). Given the endangered status of mountain caribou, we restricted the amount of helicopter disturbance to that normally experienced by caribou during a late winter aerial census. Direct helicopter disturbance represented survey time required by experienced observers to visual detect, count, age-classify and determine radio-collar presence within caribou groups while indirect exposure was helicopter search activity within 500 meters of a caribou group. Immediately following classification, we collected fresh fecal samples from the ground (*see* fecal collection methodology). Due to delay in expression of circulating cortisol in the feces, these samples represented pre-stress GC concentrations. We hypothesized that GCs would increase following exposure to helicopter disturbance, then decline (*i.e.*, recover) to levels observed prior to disturbance. We relocated caribou groups by radio-telemetry approximately 24 hrs later to collect post-stress fecal samples, and again 72 hrs later for recovery samples. We thought 72 hrs between post-stress and recovery samples was sufficient to minimize potential of a) carryover effects from helicopter disturbance on day 2 and b) unknown stressors influencing GCs prior to recovery sampling (*e.g.*, weather; Freeman 2008, Chapter 2). After pre-stress sampling

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us from asking if variation in heli-ski activity influenced caribou GCs at spatial scales finer than 'presence' or 'absence' of heli-skiing.

and exposure to the helicopter disturbance caribou in 2 of the 3 groups formed a single herd (n = 20), thus were combined for analysis. All field and experimental methods were approved by the B.C. Ministry of Environment's Animal Care Committee (C06-011).

3.3.4 Extraction and Determination of Fecal Cortisol Concentration

We measured fecal cortisol using a commercial enzyme-linked immunosorbent assay (ELISA, RnD Systems, Inc., Minneapolis, MN, USA). A physiological validation of this assay technique has demonstrated, through an adrenocorticotropic (ACTH) challenge, that changes in steroid concentration can be detected in feces of *Rangifer tarandus* (Freeman 2008, Chapter 2). Because concentrations of cortisol and the sex hormone progesterone are correlated in most mammals (Wasser et al. 1996), including caribou (Freeman 2008, Chapter 2), we accounted for effects of reproductive state by measuring progesterone concentration in the feces, also by ELISA.

Methodology for fecal hormone extraction followed those outlined in Freeman (2008, 2). We calculated and included water content of feces in statistical analyses to account for water absorbed or desiccated after defecation (Creel 2001). All fecal extracts were assayed in duplicate and if >15 % CV, extracts were rediluted and re-assayed. Serial dilutions of cortisol standards and a pooled fecal extract showed good parallelism across 8 points from undiluted to 256 fold. Optimal dilution (at the steepest portion of the standard curve) was 1:16. Recovery of cortisol (50 uL at 156-10 000 ng/mL) added to fecal extracts was 156 % ± 61.5 % ($F_{1, 4} = 661.8$, $p < 0.001$, $R^2_{adj} = 0.992$). For progesterone, serial dilutions of standards and a pooled fecal extract yielded parallel changes in antibody binding for 5 points from 1:128 to 1:2048 with optimal dilution of 1:512. Extraction efficiency of progesterone (50 uL at 31-2 000 ng/mL) was 106 % ±

46.8 % ($F_{1, 2} = 45\ 395.2$, $p < 0.001$, $R^2_{adj} = 0.999$). Intra-assay variation for cortisol and progesterone was 4.80 % and 3.73 %, respectively. Inter-assay variation for cortisol and progesterone was 15.8 % and 10.5 %, respectively. Assay sensitivity was 50.5 pg/mL for cortisol and 11.2 pg/mL for progesterone, measured directly for caribou fecal extracts. Hormone concentrations (hereafter fecal cortisol and progesterone) were corrected for dilution ratios and expressed as ng/g of dry feces.

3.3.5 Statistical Analysis

Statistical analyses were performed using SAS® software (SAS Institute Inc. 1995) and SYSTAT® software (Systat Software Inc. 2002). We used general linear models (GLM) to determine the effects on caribou GCs of motorized recreation and included all potentially influential variables (reproductive state, year, SWE, daily minimum temperature and temperature range, aspect, and percent water content of feces) in initial models, unless indicated otherwise. We selected candidate variables using *a priori* hypotheses and the results of previous studies indicating that reproductive state (Wingfield et al. 1994, Weingrill et al. 2004), fecal water content (Creel 2001), and climate variables, such as snow water equivalent and temperature (Creel et al. 2002, Romero et al. 2000, Frigerio et al. 2004) influence cortisol. Final models were estimated using GLM and backward stepwise variable selection, retaining only those variables identified statistically significant as the $p = 0.05$ level. We used the arcsine-square-root transformation to normalize proportion data. Aspect was binned into north, east, south, and west at 90° intervals (e.g. north representing 315°–45°). To meet statistical assumptions, we tested for homogeneity of slopes for all factor × covariate interactions

(Engqvist 2005). Strong bimodality in the distribution of progesterone was used to categorize samples into high (pregnant) and low progesterone (non-pregnant) groups to meet the assumption of parallel slopes. Before testing for effects of recreation, we first tested for diurnal variation in circulating fecal CGs using a sub-sample of fresh fecal samples collected in control areas. Using these control samples, we then tested if time of defecation influenced CG concentration after controlling statistically for the predicted effects of reproductive state, SWE, aspect and temperature. Last, we used factorial ANOVA to estimate the effects of our acute helicopter disturbance on fecal CGs, while controlling statistically for reproductive state, in two caribou herds sampled 0 (pre-stress), 1 (post-stress) and 4 days (recovery) after to exposure to our helicopter stress. We did not use repeated measures ANOVA because fecal samples were collected anonymously within groups. We used critical $\alpha = 0.05$ for all statistical analyses, estimated Bonferroni adjusted p-level for post-hoc comparisons, and assessed normality and model fit graphically. Fecal GC values are reported as means ± SE.

3.4 Results

3.4.1 Motorized Recreation

Diurnal variation of circulating GCs was not detectable in feces ($F_{[1, 83]} = 1.82$, $P = 0.18$), which is not surprising given the long gut-passage time of ungulates. Consequently, we used both fresh and frozen samples in all model analyses. Fecal GC levels were higher in mountain caribou sampled in areas exposed to snowmobile and heli-ski activity than in areas without motorized backcountry recreation (2002-2004 dataset, $F_{[12, 359]} = 58.9$, $P < 0.0001$, $R^2 = 0.67$). After controlling for the effects of

reproductive state, aspect, SWE, and an aspect by area interaction fecal GCs were an average of 18% greater in areas of snowmobile activity (weighted mean for aspect by area158.2) and 20% greater in areas of heli-ski activity (wmean 155.3) than in controls areas (wmean 132.0, Figure 3-2). Fecal GCs in control areas were greater on eastern aspects; these samples were collected from two caribou groups (n=17) on the same mountain ridge on the same day ($wmean_{excluding\ east\ aspect}$ 124.5). Differences were similar but much more pronounced in 2004, the only year in which samples were simultaneously collected from all snowmobile (172.0 ± 6.3), heli-ski (195.0 ± 22.4) and control areas (110.8 ± 9.4; $F_{[7, 179]} = 47.9$, $P < 0.0001$, $R^2 = 0.66$) (Figure 3-3). In 2004, fecal GCs were 77% higher in snowmobile areas ($P < 0.0001$) and 55% higher in heliski areas ($P = 0.002$) than controls. Fecal GCs were 14% higher in snowmobile than heli-ski areas ($P = 0.89$). As expected, reproductive state explained a significant portion of variation in fecal GCs in all models and ~40% of caribou fecal samples were from pregnant individuals (Figure 3-4). We verified that GC trends for recreation and control areas were consistent for pregnant and non-pregnant caribou by re-running the analysis of the 2002-2004 dataset as two models and finding trends comparable for both reproductive states.

Fecal GCs in caribou were significantly greater within 4 km (167.8 ± 5.4), 4–8 km (161.2 ± 6.4) and 8–10 km (152.3 ± 10.0) of a snowmobile activity center compared to controls (138.3 ± 5.46; $P = 0.001$; $F_{[17, 293]} = 36.7$, $P \leq 0.0001$, $R^2 = 0.69$). After we controlled for effects of reproductive state, snow, aspect, and an aspect by area interaction fecal GC levels of caribou within 4 km and 4–8 km of snowmobile activity were 17–21% higher than controls and 10% higher within 8–10 km. Potential covariates

not included in any of our final models investigating effects of recreation were year, minimum ambient temperature and water content of feces.

3.4.2 Helicopter Disturbance Trial

Caribou stress response to an experimental, acute helicopter disturbance yielded inconsistent GC responses across groups and a statistically significant group by time interaction ($F_{[2, 69]} = 4.34$, $P = 0.017$). Fecal GCs increased 11% following helicopter disturbance in Group 1 (n = 7), but did not decline to pre-stress levels during the sampling period (Figure 3-5). In contrast, Group 2 (n=20) had higher fecal GC levels than Group 1 in pre-stress samples, but showed declining GCs thereafter. The predisturbance GC levels of Group 2 were substantially higher than mean GC levels for any of the study areas in any year, strongly suggesting that this herd had been subjected to an undocumented stressor prior to inclusion in our experiment.

3.5 Discussion

Glucocorticoids have been used to infer physiological effects of ecological stressors on wildlife with the supposition that elevated GC levels are indicative of long-term chronic stress (Creel 2001; Wingfield 1997). Our results show that mountain caribou GC levels were higher in winter in both snowmobile and heli-ski areas compared to those without motorized recreation. Caribou GCs were also elevated in areas up to 10 km distant from snowmobile activity suggesting the potential effects of snowmobile disturbance may extend a substantial distance.

While chronically elevated stress levels in snowmobile and heli-ski areas were evident, caribou stress response to an acute stressor was less clear, with inconsistent GC

responses to direct helicopter disturbance. We propose that fecal GCs may be better suited for measurement of chronic rather than acute stressors in free-ranging mountain caribou given the difficulty associated with controlling for lag times and external confounding factors. Inconsistent caribou response to a known helicopter disturbance suggests that a) disturbance duration may not have been enough to elicit a detectable GC response in feces, b) the ~24 hr lag time selected for fecal collection missed the expected spike in fecal GC concentration and/or c) unknown external stressors influenced caribou fecal GCs prior-to and during our experimental challenge confounding interpretation of results. This last interpretation is supported by the unusually high GC levels of experimental group 2 in the predisturbance sample.

Our data provides quantitative assessment of caribou physiological response to prolonged exposure to human disturbance. Prior research on disturbance caused by skiers, snowmobiles and helicopters focused on behavioral changes, flight distances or displacement of caribou from winter range. For instance in British Columbia, long-term displacement of mountain caribou from high quality winter range has been attributed to intensive snowmobile recreation (Seip et al. 2007). The presence of skiers and snowmobiles has been determined to have negative energetic effects on caribou (e.g., Duschesne et al. 2000; Powell 2004; Reimers et al. 2003; Reimers et al. 2006) and result in avoidance of these areas (Nellemann et al. 2000; Vistnes & Nellemann 2001). Wilson and Hamilton (2003) reported that mountain caribou use of heli-ski areas was lower during months and years when heli-skiing activity was highest. Although caribou may show no overt behaviour and appear to habituate to human presence in certain situations (e.g., Johnson & Todd 1977; Mahoney et al. 2001; Tyler 1991), ungulates in remote

mountainous environments may not readily adapt to unpredictable human disturbance (e.g., Bleich et al. 1994; e.g., Cote 1996; Frid 2003; Goldstein et al. 2005). MacArthur et al. (1982) used behavioural response to human approach and heart rate to monitor bighorn sheep response to human-related harassment (road traffic, aircraft, humans) and noted cardiac responses (i.e., energy expenditure) persisted even when sheep appeared to exhibit behaviour consistent with habituation. These authors reported that overt behaviour alone may be a poor indicator of stress response to human disturbance. Given the above, it is not unexpected that we detected evidence of chronic stress in mountain caribou exposed to motorized winter recreation.

The physiological response detected in mountain caribou may be one mechanism linking behavioral responses to demographic responses, though further work is needed on the relationship between chronic elevation of GCs and fitness in the wild. In Yellowstone National Park, fecal GC levels in elk were positively correlated with increases in snowmobile traffic for both daily and annual time scales (Creel et al. 2002). Although reproductive success of elk sampled for GCs was not considered by Creel et al, avoidance of snowmobiles by elk has been reported (Borkowski et al. 2006), as have declines in calf production in response to human disturbance (Phillips & Alldredge 2000; Shively et al. 2005). Together, these studies suggest that adverse effects of human-related recreation on behaviour, physiology and reproductive success occur in some cervid populations.

Glucorticosteroid values recorded here should be interpreted as conservative estimates of the effect of motorized recreation on mountain caribou GCs and we expect the magnitude of physiological stress response to be underestimated. Observed GC levels

represent caribou response to a range of snowmobile and heli-ski recreation intensity and frequency *(i.e.*, minimal to intensive, daily to weekly). Our results are further conservative in that: a) caribou usually avoid areas of intensive snowmobile use (e.g., Kinley 2003; Seip et al. 2007) making collection of fecal samples difficult in these areas, b) proximity of caribou to snowmobile activity did not account for topographical barriers creating visual barriers, reducing noise disturbance, and limiting potential for interaction between caribou and humans and c) radio-collared caribou locations were known to the heli-ski operation active within our study area, thereby enabling avoidance of the caribou and heli-ski interaction we were attempting to test. In addition, core snowmobile areas likely differed in frequency of use, but we were unable to estimate use precisely due to the existence of multiple access routes into sub-alpine terrain and survey cost. Although attempts to choose caribou groups well-removed from human disturbance were made, it remains possible that some caribou in control areas, such as the two caribou groups sampled on eastern aspects, may inadvertently represent GC response to acute stress, such that caribou may have: a) had interactions with humans in areas where humans are typically absent, b) recently traveled from areas where they were in contact with humans, or c) had interactions with predators or other environmental stressors. Despite these qualifications, differences in caribou GCs between recreation and control areas were still detected.

Although elevated GCs may not always indicate harmful stress (Romero 2004), the effects of chronic stress may not manifest in fitness measures during the time frame of a disturbance, but rather become apparent over the long-term (Walker et al. 2005). Fecal GC levels have been used to predict survival at the individual level while differences in

GC levels among populations have been used to predict population persistence and declines (e.g., Arlettaz et al. 2007; Blas et al. 2007; Cabezas et al. 2007; Ellenberg et al. 2007; e.g., Pride 2005; Romero & Wikelski 2001). Given the potential predictive power of GCs to anticipate health status of free-ranging wildlife populations, elevated GCs in mountain caribou may forewarn reduced reproduction, survival and population decline.

Our observations of elevated GCs in mountain caribou exposed to snowmobile and heli-ski recreation are consistent with the hypothesis that these activities induce a chronic stress response in mountain caribou. By comparison, an extreme weather event preceded a rise in fecal GCs comparable to those recorded in recreation areas, but declined thereafter (Chapter 2; Fig. 2-1), indicating that severe weather led to an acute stress. High average GC levels, however, are more consistent with an hypothesis of chronic stress. It remains uncertain at what point chronic elevation of GCs begins to affect the health of caribou populations. Until such work is completed, however, our results indicate that the health of mountain caribou may be linked to the presence of snowmobile and heli-skiing activity.

At least two factors also limit the interpretation of our study. First, we typically sampled caribou in herds of 5 to 6 animals in size. If all caribou within herds respond similarly to heli-skiing and snowmobiling, our sampling design may have lead to some non-independence of data, inflated our estimates of the degrees of freedom in statistical tests, and overestimated the statistical significance of some of our results. Second, because we do not know the individual histories of the animals we sampled, we cannot relate fecal GCs to individual fitness traits or the health of individual animals. Unfortunately, the current low density of mountain caribou on the landscape probably

makes the cost of larger, fully randomized trials prohibitive. Managers must therefore decide if our evidence of elevated fecal GCs in mountain caribou exposed to snowmobile and heli-skiing activity as compared to those inhabiting areas closed to motorized recreation, constitutes a biologically significant result. If we assume, however, that caribou, like several other species do incur fitness costs when experiencing chronic stress, then it is reasonable to assume that chronic elevation of GCs in caribou may also reduce individual fitness.

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Figure 3-1. Map of study area with snowmobile, heli-ski, and control (no motorized recreation) areas, core areas of snowmobile activity, and locations of mountain caribou where fecal samples were collected over 3 winters (2002-2004) in the Quesnel Highland and Cariboo Mountains of east-central British Columbia, Canada. ● = caribou fecal collection site ▲ =snowmobile activity.

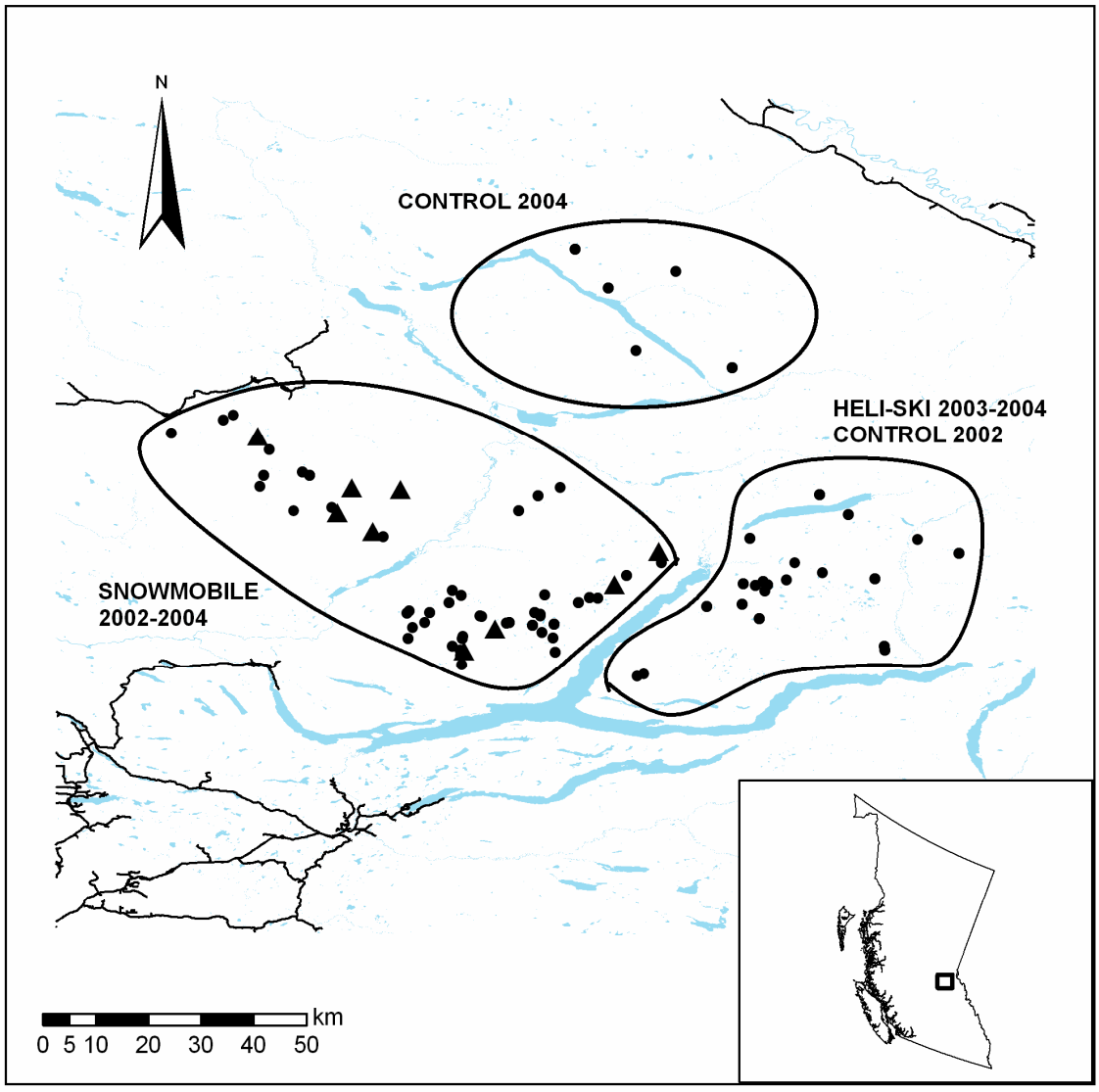


Figure 3-2. Fecal GC concentration (ng/g dry feces) of mountain caribou exposed to snowmobile and heli-ski activity in the Quesnel Highland and Cariboo Mountains of east-central British Columbia. Fecal GCs over 3 winters (2002-2004, n=360) is shown for recreation activity by aspect after controlling for effects of snow, temperature and reproductive state using GLM analysis of variance. Error bars are mean ± SE; labels above error bars indicate sample size.

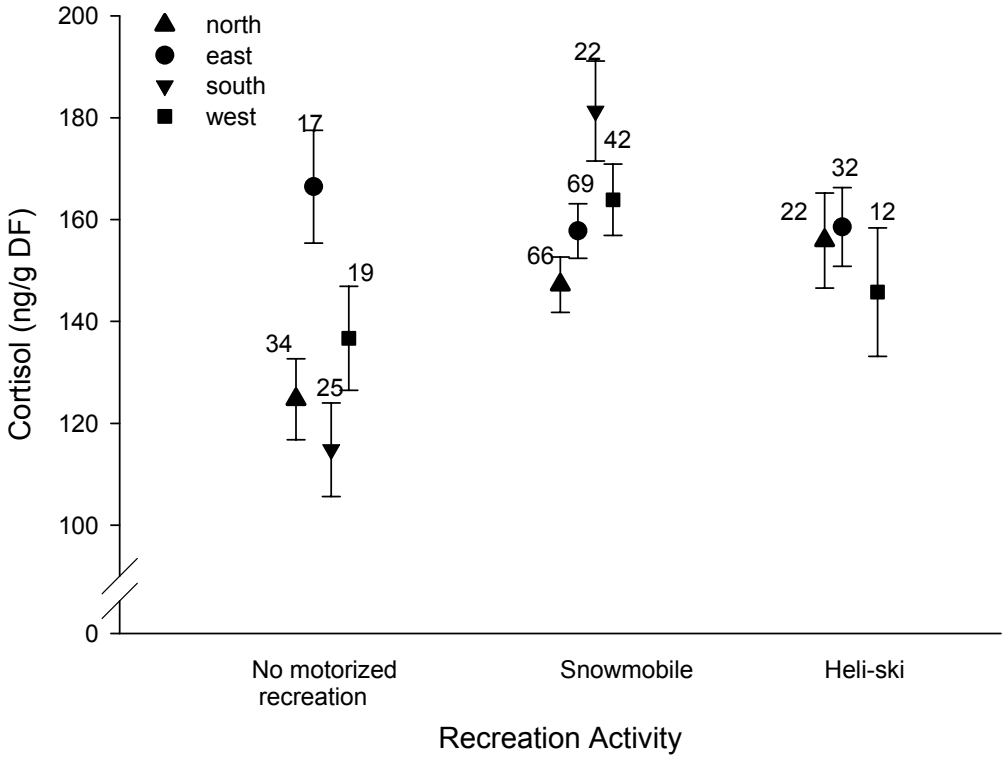


Figure 3-3. Fecal GC concentration (ng/g dry feces) of mountain caribou exposed to snowmobile and heli-ski activity in the Quesnel Highland and Cariboo Mountains of east-central British Columbia in winter 2004 (n=180) after controlling for effects of snow, temperature and reproductive state using GLM analysis of variance. Error bars are mean ± SE; labels above error bars indicate sample size.

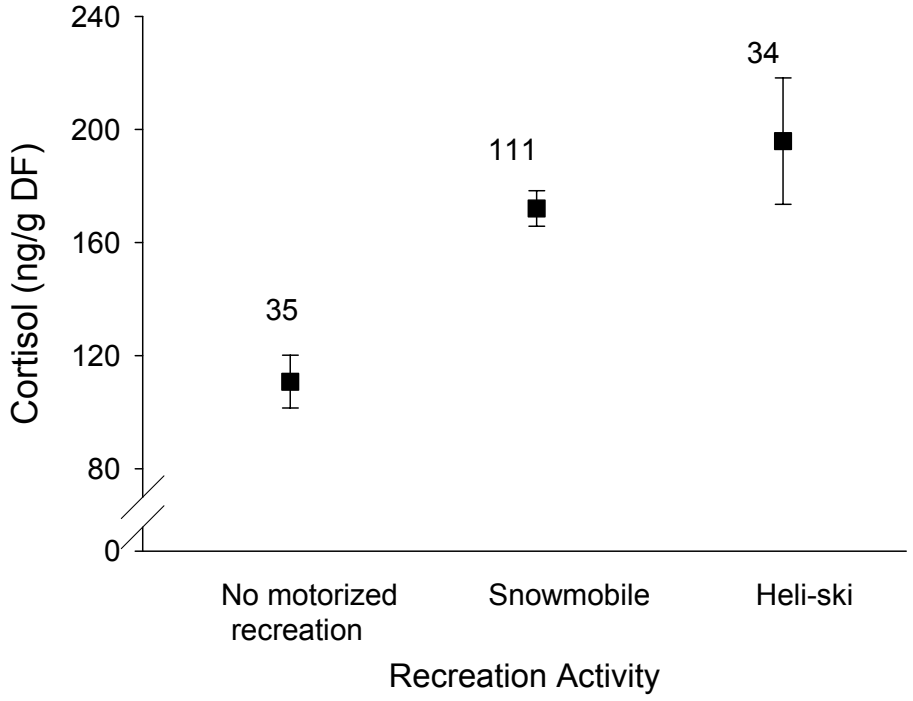


Figure 3-4. Fecal progesterone concentration (ng/g dry feces) of mountain caribou between January and March, 2002-2004, in the Quesnel Highland and Cariboo Mountains of east-central British Columbia. Progesterone concentrations (n=360) are plotted on a natural logarithmic scale, with the bimodal distribution corresponding to non-pregnant (ln progesterone <6 ng/g DF) and pregnant (ln progesterone ≥6 ng/g DF) caribou.

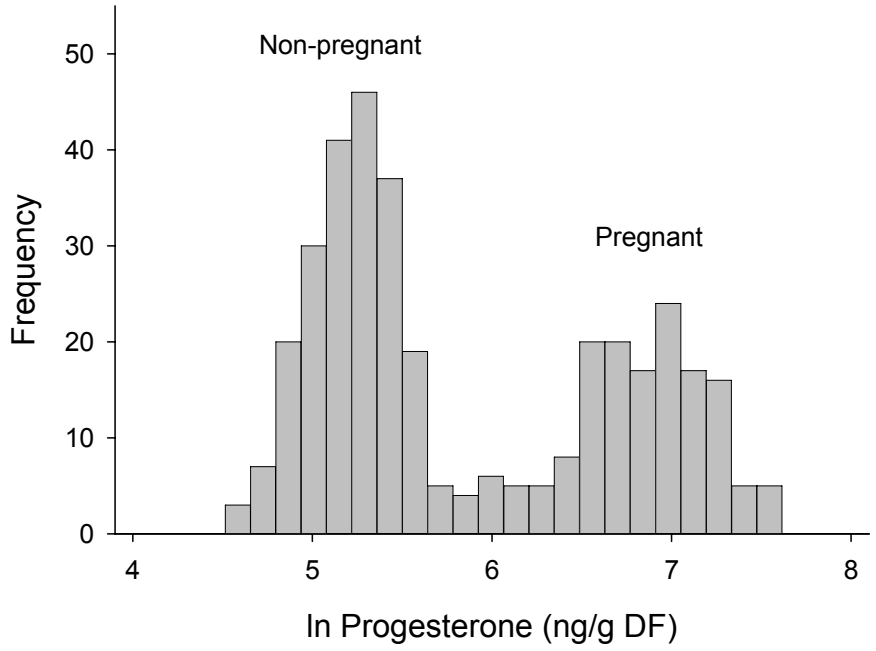
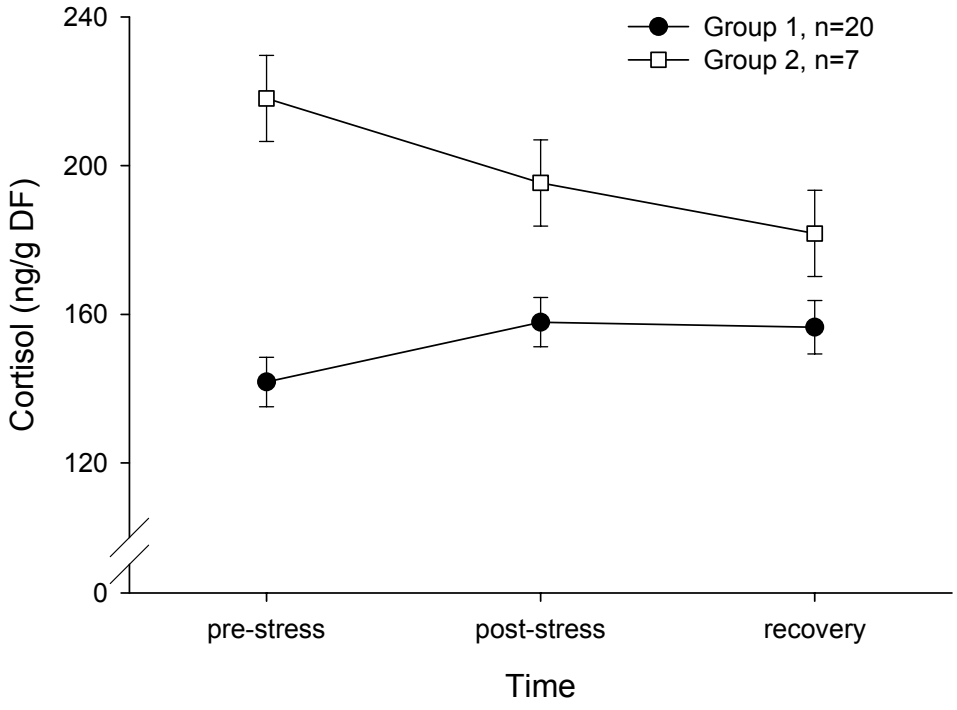


Figure 3-5. Fecal GC concentration (ng/g dry feces) of mountain caribou during pre-stress, post-stress and recovery periods of a helicopter disturbance trial conducted in March 2003 in the Quesnel Highland and Cariboo Mountains of east-central British Columbia. After controlling for effects of reproductive state using GLM analysis of variance, fecal GCs are mean ± SE.



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4 Discussions and Conclusions

4.1 Summary of Research Findings

Concerns about declining North American caribou (*R. tarandus*) populations have created a need to quantify the response of caribou to anthropogenic stressors now thought to be involved in these declines. Studies of elk (*Cervus elaphus*) have demonstrated that noninvasive assays for the metabolites of circulating corticosteroids can be used to assess physiological stress levels in free-ranging animals (Creel et al. 2002; Millspaugh et al. 2001; Wasser et al. 2000). Prior to application of noninvasive endocrine techniques in free-ranging wildlife stress studies, validation of suitable, species-specific assay techniques is required (Millspaugh & Washburn 2004; Touma & Palme 2005). My research investigated the use of noninvasive endocrine techniques as a tool to assess stress response in endangered mountain caribou populations exposed to motorized backcountry recreation.

My study reported the first validation of a noninvasive, fecal endocrine assay designed to quantify stress hormones in *R. tarandus*. Using an ACTH Challenge I was able to estimate the effects of natural variation in winter weather and gender on fecal hormone profiles; each of these are potentially influential factors affecting hormone excretion profiles in free-living caribou. The results showed that extreme variation in weather can influence fecal stress hormone profiles in response to ACTH stimulation, and that this response may vary in males and females. Female reindeer expressed elevated fecal GCs 9-11 hrs after ACTH injection while males showed no detectable increase, perhaps due to underdosing. Fecal GCs varied markedly in both sexes in response to natural variation in

weather; this variation was consistent for all the reindeer hormone profiles. The ability to track extreme variation of winter weather in reindeer feces suggests field studies need to account for potential variation in weather parameters, such as ambient temperature, temperature changes, barometric pressure and snow. I also showed that fecal progesterone was highly correlated with fecal cortisol in *R. tarandus*. As a consequence, my study provided an essential validation of a fecal hormone assay for glucocorticoids in female caribou, but also raised important issues related to potential gender and weather-related influences on hormone profiles of free-living caribou.

I applied this noninvasive field endocrine technique to a free-ranging mountain caribou population to investigate the potential effects of snowmobile and heli-ski recreation on physiological stress response in caribou. The results indicated that concentrations of fecal GCs in snowmobile and heli-ski areas were higher than those measured in caribou in areas closed to motorized recreation. I investigated effects of snowmobiles at a finer scale and found caribou sampled up to 4km, 8km and10 km distant from snowmobile activity showed elevated fecal GCs compared to those sampled further from snowmobile activity areas. Further investigation of heli-ski recreation beyond this coarse scale approach was not possible due to unavailability of heli-skiing activity data. I included several potential predictor variables in our analyses to account for variation in fecal GCs and found that reproductive state, snow, aspect, minimum ambient temperature, and daily temperature range exerted a significant effect on fecal GCs. A component of the field study included investigation of effects of an ecologically relevant disturbance on mountain caribou GCs. I observed inconsistent caribou response to a known helicopter disturbance possibly due to inadequate sampling of feces following

disturbance, missing the window to detect a stress response (i.e., uncertainty in excretion lag time), or the presence of external stressors during implementation of the disturbance trial. I suggest that fecal GCs may be better suited for measurement of chronic rather than acute stressors in free-ranging mountain caribou given the difficulty associated with controlling for lag times and external confounding factors.

Overall, my research indicates that measurement of fecal GCs provides a useful, noninvasive approach in the evaluation of physiological effects of environment, reproductive state, and human-induced stressors on free-ranging mountain caribou. I expect my research to be of broad interest to wildlife ecologists interested in applying noninvasive monitoring of fecal stress hormones to assess effects of anthropogenic disturbance to free-ranging wildlife.

4.2 Caribou Conservation and Management Implications

Elevated GCs in mountain caribou exposed to snowmobile and heli-ski recreation suggest existing levels of recreation activity within the Quesnel Highland and Cariboo Mountains of British Columbia may induce chronic stress response in mountain caribou. My research also suggests that potential effects of snowmobile recreation on caribou may be detected through elevated GCs in caribou up to 10 km distant from snowmobile recreation areas. This distance is a straight line distance and does not take into consideration topographical barriers (e.g., mountain ridges, valleys) that may reduce anthropogenic noise and/or visual disturbance. Defining a threshold distance for proximity of recreation activity to caribou or high quality caribou habitat may be a difficult task given that snowmobile and heli-ski disturbance effects may extend beyond

mountain ranges where recreation activity occurs. Thus, interpretation of the effect of snowmobile proximity on caribou GCs ought to be conservative.

These results lead to two important questions: a) whether chronically elevated GCs are having a negative effect on caribou fitness at the individual or population level and b) at what point do chronically elevated GCs become detrimental to mountain caribou populations? Researchers have recently begun to use the predictive power of GCs to anticipate health status of a population and several studies have correlated chronically elevated GCs with reduced individual survival, population persistence and population declines (e.g., Arlettaz et al. 2007; Blas et al. 2007; Cabezas et al. 2007; Ellenberg et al. 2007; Pride 2005; Romero & Wikelski 2001). While understanding the range of GCs that is deleterious to wildlife is necessary to assess population health (Millspaugh & Washburn 2004); it is unreasonable to consider identifying a stress threshold given the endangered status of mountain caribou and the ongoing efforts to recover populations. Rather, my results reveal the vulnerability of mountain caribou to disturbance, such that elevated GCs may forewarn detrimental physiological consequences associated with chronic stress response, including reduced reproduction, survival and population decline.

Because mountain caribou are currently listed are endangered, and our results suggest that motorized winter recreation elevates fecal GCs in caribou, it may be prudent for managers to take measures to reduce human-related stress. For example, managers might consider restricting access to areas used most intensively by caribou seasonally, by designating flight paths that minimize disturbance, and by providing some areas of winter range where caribou populations are undisturbed.

Future research on the potential effects of chronic GC elevation on fitness or population trend in mountain caribou might ask if mean GC levels estimated in herds or longitudinally in individual animals, is linked to population performance, reproduction or survival. Because of the difficulty and likely cost of such studies, however, an alternative approach may be to link fecal CGs to other short-term physiological indices of individual heath.

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5 Appendices

5.1 Appendix 1. Animal Care Certificate for Reindeer ACTH Challenge

The University of British Columbia

Animal Care Certificate

Application Number: A04-1037

Investigator or Course Director: Peter Arcese

Department: Forest Sciences

Animals Approved: Caribou Rangifer tarandus 8

Start Date: **April 1, 2002** Approval Date: **March 11, 2005**

Funding Sources:

Funding Agency: Habitat Conservation Trust Fund
Funding Title: Quesnel Highland Mountain Caribou Project: Impact of Backcountry Recreation

Unfunded title: N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility

Office of Research Services and Administration
102, 6190 Agronomy Road, Vancouver, V6T 1Z3
Phone: 604-827-5111 Fax: 604-822-5093

5.2 Appendix 2. Animal Care Certificate for Mountain Caribou Research

ACC# C06-011

Received

FEB 0 6 2007

BRITISH COLUMBIA
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102 Industrial Place
Penticton, BC V2A 7C8

February 2, 2007

Nicola Freeman
A/Ecosystem Biologist
Environmental Stewardship Division
102 Industrial Place
Penticton BC V2A 7C8

Re: Ministry of Environment/Ecosystem Branch Animal Care Application

Dear Nicola:

Your application "*Quesnel Highland Mountain Caribou Project: Impact of Backcountry Recreation*", describing field studies on woodland caribou, was received and reviewed by the Ministry of Environment Ecosystem Branch Animal Care Committee.

I am please to inform you that the application is approved.

Sincerely,

[redacted]

Helen Schwantje, DVM, M.Sc.
Wildlife Veterinarian
Chair, Ministry of Environment Animal Care Committee

Ministry of Environment Ecosystems Branch

Mailing Address:
PO Box 9338 Stn Prov Govt
Victoria BC V8W 9M1
Locaton Address:
4 – 2975 Jutland Rd
Victoria BC V8T 5J9

Telephone: 250-387-9731
Facsimile: 250-356-9145
Website: www.gov.bc.ca/env