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YEAR ONE REPORT

Caribou mortality and disease prevalence in west-central Alberta

Prepared for

Petroleum Technology Alliance Canada | Alberta Upstream Research Petroleum Fund 15-ERPC-08

fRI Research Caribou Program

April 2016

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Disclaimer

This is an interim report, disease and health testing is still underway, and further analysis will be conducted for the submission of scientific journal publications, which may result in additional findings and conclusions. Any opinions expressed in this report are those of the authors and do not necessarily reflect those of project partners and funders.

Suggested citation:
Finnegan L., Macbeth B., MacNearney D., Pigeon K., Larsen T., Schwantje H., and Kutz S. 2016. Caribou mortality and disease prevalence in west-central Alberta. Year one report prepared for the Petroleum Technology Alliance Canada, Alberta Upstream Research Petroleum Fund 15-ERPC-08, April 2016. Pp vii + 39

Executive Summary

Woodland caribou are in decline across their range and although the proximate cause of decline is unsustainable rates of predation, health is increasingly recognised as a factor that may contribute to survival and reproduction in caribou. The goal of this two year project is to increase understanding of predator risk and caribou health in west-central caribou herds. In the first year of this research project our aims were 1) to use caribou mortality site visits and existing mortality databases to address the current knowledge gap surrounding predators and landscape attributes associated with caribou mortalities in west-central Alberta, and 2) to initiate the first detailed health assessment of west-central caribou herds.

Over a three year period we visited 21 caribou mortalities; 8 within 48 hours of the mortality event and 8 within 2 weeks of the mortality event. Using standardized site investigation, necropsy and sampling protocols we attributed 12 of these mortalities to probable predation, three to accidents, three to disease/health and three as unknown. Of the 12 mortalities we attributed to predation four were cougar, three were grizzly bear, two were wolf and three had multiple predator signs. Our preliminary analysis assessing the spatial distribution of mortalities revealed that mortality patterns differed across seasons, and between protected and unprotected areas. Generally mortalities occurred in wet areas and closer to seismic lines, and we found a higher probability of mortality risk on steep slopes, at lower elevations during migration and summer, and in valleys during winter. Overall these results suggest that caribou mortalities occur in habitat preferred by a range of predators.

We used biological samples collected from caribou mortalities and winter fecal pellet sampling to evaluate health and disease in west-central caribou herds. Fecal pellet surveys detected a range of gastrointestinal parasites including nematodes and tapeworm eggs, as well as dorsal spined protostrongylid larvae (muscle or lungworms). Prevalence of the parasites was similar to that reported previously for British Columbia and Alberta. Fecal surveys this winter will allow us to assess prevalence among herds, and will continue to contribute towards collation of important baseline data on pathogens in caribou. Health testing from caribou mortalities revealed the presence of ectoparasites (winter tick) and pathogenic bacteria (*Erysipelothrix rhustiopathiae*). Trace nutrients and evaluation of body fat (% bone marrow fat) suggests that some dead caribou were in poor condition. Interpretation of these results is ongoing, and will be aided by additional sample collection in the coming year.

Results from the first year of this project suggest that the predator guild associated with caribou mortalities in west-central Alberta includes not only wolves, but also cougars and bears. Continued mortality site investigations and detailed analysis of the spatial distribution of caribou mortalities relative to the distribution of a range of predators in year two will allow us to assess predation risk for west-central caribou in more detail. This information will allow land managers to direct caribou habitat restoration priorities to areas where they will have the greatest benefit for caribou. In addition, our baseline health and disease data may be used to track changes in caribou health in the future with the spread of moose, deer and elk within caribou ranges, and with climate change. This health research may contribute to caribou recovery planning by identifying priority areas for restoration based on disease transmission risk, and by identifying herds that may be at risk from disease outbreaks.

Acknowledgements

We would like to thank the numerous researchers and volunteers that assisted with caribou mortality site investigations, fecal sample collection, and fecal swabbing: Leonie Brown, Karen Graham, Kelsey Greenlay, Joe Kermish-Wells, Paul Kuchma, Cam McLelland, Brent Rutledge, Anja Sorensen, Amy Stenhouse, Gord Stenhouse, Leah Stenhouse, Becky Viejou, and members of the Aseniwuche Winewak Nation of Canada's Caribou Patrol (www.cariboupatrol.ca). Kelsey Greenlay also provided essential assistance with sample and database management, and field logistics for this project. Thanks to Steve Wotton at Peregrine Helicopters for his always exemplary flying skills and assistance in the field. Dave Mair at Sivertip aviation also carried out VHF mortality flights during early stages of this project.

Wendy Crosina and Weyerhaesuer Canada Ltd. allowed us access to up to date caribou GPS data facilitating our rapid mortality site investigations. Project collaborators Dave Hervieux, Julia Wachowski and Amy Flasko at Alberta Environment and Parks also let us know of mortalities of AEP collared caribou. We thank Wildlife Genetics International, particularly Renee Prive for genetic profiling. At the University of Calgary the members of the Kutz and Orsel laboratories carried out some of the laboratory health testing, and we extend a particular thanks to Taya Forde. The remainder of the laboratory work was completed under the direction of Manigandan Lejeune-Virapin of the Canadian Wildlife Health Cooperative, Alberta Node.

We thank Alberta Environment and Parks, the British Columbia Ministry of Environment, and Alberta Parks for logistical support, and for research permits that facilitated biological sample collection. Funding for this work was provided by the Alberta Upstream Petroleum Research Fund (15-ERPC-08), Weyerhaeuser Canada Ltd., the Safari Club International Drayton Valley Chapter, and the partners of the fRI Research Caribou Program. In-kind support was provided by the Faculty of Veterinary Medicine, University of Calgary. This project would not have been possible without the ongoing support provided by project champions Carol Barsky and Gary Sargent at CAPP, and additional support from Lorie Mayes and Tannis Such at PTAC, and Wendy Crosina at Weyerhaeuser.

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1. Project background and objectives

1.1 Introduction

Woodland caribou (*Rangifer tarandus*) are declining across their range and the ultimate cause of these declines is believed to be habitat disturbance (McLoughlin *et al.* 2003; Vors *et al.* 2007). Disturbances such as forest harvesting and oil and gas exploration and development are thought to have driven an increase in the distribution and abundance of other ungulates (moose (*Alces alces*), deer (*Odocoileus* sp.) and elk (*Cervus canadensis*)) within caribou habitat, and ultimately this has resulted in an increase in predators, notably wolves (*Canis lupus*), but also black bears (*Ursus americanus*), and potentially grizzly bears (*U.* arctos) and cougars (*Puma concolor*) (apparent competition) (James *et al.* 2004; Knopff 2010; DeCesare *et al.* 2010; Latham *et al.* 2011a). Researchers believe that the immediate threat to both boreal and mountain caribou populations is high rates of predation and unsustainable mortality (Hervieux *et al.* 2014). Because of the urgent need mandated under the federal recovery strategies for boreal and southern mountain caribou to implement habitat restoration to achieve self-sustaining caribou herds (Environment Canada 2012, 2014), it is imperative that we understand the role of predators relative to changing caribou populations.

The extent of habitat disturbance within caribou ranges means that restoration will benefit from a prioritization approach (Noss *et al.* 2009). Although fine scale restoration priorities may be based on localised factors such as the probability of restoration success (van Rensen *et al.* 2015), ultimately restoration of caribou habitat should be directed to areas that will have the greatest conservation benefit for caribou. Given that caribou declines are believed to be driven by high levels of predation, directing restoration to areas that have high caribou mortality risk may serve to expedite recovery of caribou populations. Wolves are cited as the primary predator of caribou in most boreal forest settings (Bergerud 1974; Hebblewhite *et al.* 2007; Hervieux *et al.* 2014). For this reason, research in west-central Alberta has focused on understanding factors influencing wolf predation risk (DeCesare 2012; Decesare *et al.* 2013). However, caribou ranges in west-central Alberta overlap with other primary predators such as cougar, grizzly bear, black bear and wolverine, all of which have been implicated in caribou moralities in other areas (Kinley & Apps 2001; Wittmer *et al.* 2005; Stotyn *et al.* 2007), yet it remains unknown whether these predators are a significant source of caribou mortality.

In addition to issues surrounding predation risk, caribou herds in Alberta currently exist in small isolated populations, meaning that these already fragile populations may also be at increased risk from the effects of compromised health, disease transmission or catastrophic disease outbreaks (Deem *et al.* 2001; McCallum & Dobson 2002). The relative importance of disease to wildlife is expected to increase with climate change and anthropogenic landscape change (Harvell *et al.* 2002; Hoberg *et al.* 2008), and for caribou there may already be increased risk of disease transmission associated with the incursion of moose, deer and elk into caribou ranges (Bergerud & Mercer 1989); a further layer of complexity to the apparent competition problem (Hoberg *et al.* 2008). Although health and disease have the potential to alter the timeline and overall success of current caribou recovery actions in Alberta, to date they have received little attention. Knowledge of baseline levels of disease and the overall health status of caribou herds, as well as other ungulate species (moose, deer and elk), will provide opportunities for early detection of disease and proactive management.

Through collaboration with the British Columbia Boreal Caribou Health Program (BCBCHP) and the fRI Research Grizzly Bear Program Predation Project, the broad goals of this project are: 1) determine and evaluate causes of caribou mortality, and correspondingly risk of mortality from predators, and 2) to establish comprehensive health baselines for caribou, moose, deer, and elk that occur within caribou ranges.

1.2 Study area

Our study area encompasses the range of the Narraway (NAR), Redrock-Prairie Creek (RPC), A La Peche (ALP), and Little Smoky (LSM) caribou herds in Alberta and British Columbia (Figure 1.1). NAR, RPC, and ALP caribou are central mountain woodland caribou and migrate between high elevation summer range, which encompasses both alpine and subalpine habitats, to low elevation winter range in the foothills (Edmonds & Bloomfield 1984; Brown & Hobson 1998; Natural Regions Committee 2006; COSEWIC 2014). LSM caribou belong to the boreal ecotype, occur in the foothills and boreal forest year round, and have relatively small seasonal shifts in range use (Bergerud 1992; Briand *et al.* 2009). Central mountain caribou are listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and threatened under the Species at Risk Act (SARA), while boreal caribou are listed as threatened by COSEWIC and SARA (COSEWIC 2002, 2014; Environment Canada 2012, 2014b). All caribou are listed as threatened under Alberta's *Wildlife Act* (Alberta Woodland Caribou Recovery Team 2005).

Habitat types within the range of these caribou herds are diverse. Alpine areas consist of exposed ridges and meadows with graminoid, sedge (*Carex* spp.), and herbaceous ground cover, while subalpine areas are characterized by Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*), with dwarf shrubs (*Salix* and *Betula* spp.) along riparian zones. Foothills consist of lodgepole pine (*Pinus contorta*) and white spruce (*P. glauca*) uplands and lowlands with poorly drained muskeg, black spruce (*P. mariana*), and larch (*Larix laricina*)(Natural Regions Committee 2006; Demarchi 2011). Industrial development associated with the energy (oil, gas and mining) and forest sectors are concentrated in the foothills. Oil and gas activities date to the 1950s and a coal mine has been operating in the eastern portion of the RPC range since 1969. Forestry operations date to the 1970s in Alberta and the 1980s in British Columbia (Slater 2013).

1.3 Project objectives

Our specific research objectives include: 1) determine and evaluate the causes of caribou mortality along with potential mortality risk factors associated with predation in west-central Alberta and east-central British Columbia; and 2) to establish comprehensive health baselines for caribou, and moose, deer and elk that occur within caribou ranges (Alberta only), in year one of this project we focused on the following objectives:

1. Carry out rapid mortality sites visits (within 24 hours of mortality signal) of collared caribou morality events and collect data on the cause of death (Chapter 2).
2. Complete preliminary analysis of caribou mortality distribution and mortality risk in relation to terrain and disturbance features attributed to land-use activities within herd ranges (Chapter 3).

3. Use biological samples from caribou mortalities and non-invasive fecal surveys to complete a preliminary evaluation of caribou health and disease within the Little Smoky, A La Peche, Redrock Prairie Creek and Narraway herds (Chapter 4).

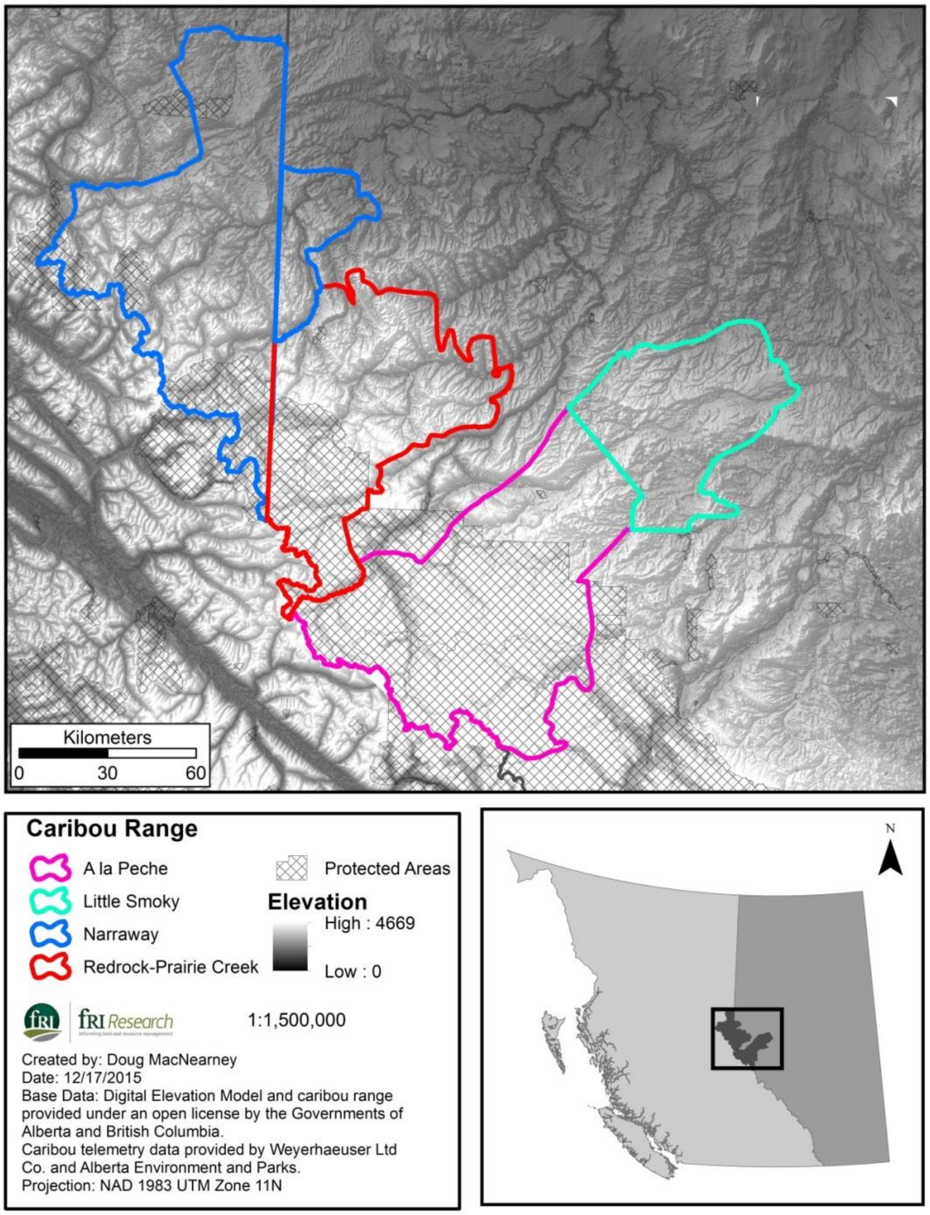

Figure 1.1. Study area map showing caribou ranges in west-central Alberta and east-central British Columbia.

2. Caribou mortality investigations

Laura Finnegan, Doug MacNearney, Karine Pigeon, Terry Larsen and Bryan Macbeth

2.1. Introduction

Previous research in west-central Alberta has focused primarily on wolves being the main predator of caribou (Kuzyk, Kneteman & Schmiegelow 2006; Whittington *et al.* 2011; Decesare *et al.* 2013a). However, west-central caribou ranges overlap with a number of other predators including cougar, grizzly bear, black bear, lynx and wolverine. Research of caribou herds in British Columbia reported cougars, bears (black and grizzly) and wolverines as predators of caribou (Kinley & Apps 2001), but it remains unknown whether these predators are a major source of central mountain caribou mortality in west-central Alberta. Knowledge of the predator guild that contributes to caribou mortality is required to determine whether that should be considered when evaluating mortality risk for caribou in west-central Alberta.

Here we describe results from caribou mortality site investigations (2013-2015) carried out within four caribou herds in west-central Alberta and east-central British Columbia. Our goal was to determine the cause of adult female caribou mortalities, and to use these data to evaluate the relative contributions of different predators as well as disease.

2.2. Methods

We identified mortality events of adult female caribou collared with Geographic Positioning System (GPS) telemetry collars (Lotek Iridium GPS 4400, Newmarket, Ontario and Televilt Global Positioning System, Lindesberg, Sweden) between 2013 and 2015. Caribou were captured and collared as part of an ongoing collaboration between Alberta Environment and Parks and Weyerhaeuser Ltd., or as part of ongoing research by Alberta Environment and Parks (Alberta Animal Care Protocol 008). The collars were programmed to send a mortality signal when no motion was registered for eight consecutive hours. No movement would indicate that the animal had stopped moving or the collar had been removed from the animal. Our goal was to visit mortality sites by helicopter < 24 hrs after receiving a mortality signal, however this was not always possible due to poor weather conditions, or avalanche risk (see Table 2.1). We also visited mortality sites opportunistically when reported by Alberta Environment and Parks (GPS and VHF collars), the fRI Research Grizzly Bear Program (collaborative predation project), or the Caribou Patrol (www.cariboupatrol.ca).

At each mortality site, at least two trained personnel used standardized field protocols to determine plausible cause(s) of death of collared caribou (e.g. predation, accident, disease, senescence). These protocols included examination of the position, condition and distribution of the carcass, habitat type, identification of predator scat and prints, and collar condition. All sites were extensively documented via photography and detailed field notes. Although signs of predator may overlap, certain characteristic wounding or feeding patterns can help narrow to the most likely species (Government of Alberta 2010).

We used necropsy and mortality sampling protocols and corresponding datasheets developed by the BCHP and CircumArctic Rangifer Monitoring and Assessment network (CARMA) to collect biological samples from carcasses. In some cases we removed partial carcasses from the site for detailed necropsy by a wildlife veterinarian. In addition to biological samples, we also collected feces (caribou and predator) and ectoparasites at mortality locations. All samples and partial carcasses were bagged and stored in a freezer at -20°C for later examination and laboratory testing (see Chapter 4).

2.3. Results

Over a three year period we investigated 21 caribou mortalities with the majority being GPS collared adult female caribou (n = 14; Table 2.1; Figure 2.1). The probable cause of death for 12 of the caribou mortalities we investigated was predation. We attributed the other 8 to accidents, disease and unknown. Of the 12 mortalities that we concluded were predation events, four were deemed to be cougar. Two of these mortalities were confirmed through visual observation of a cougar at the mortality site (F432 and F453), whereas the remaining two mortalities (F450 and 34075) were assigned by field investigation and interpretation of animal signs. Mortalities attributed to grizzly bear (F446 and F973) were based on characteristic feeding signs like peeling of the hide (Figure 2.2), burial piles, and grizzly bear scat containing caribou hair (Figure 2.2). Although both wolf and grizzly bear tracks were identified at the mortality site of F440 antemortum haemorrhage surrounding wounds more compatible with the bite pattern of a grizzly bear were identified in the hide (Figure 2.3), and offer some support for grizzly bear predation of the caribou, rather than scavenging from a dead animal.

Two of the mortalities were associated with wolf predation, which was based on clear evidence from compatible tooth marks on the hide and the pattern of carcass consumption (Figure 2.4). We visited a mortality at which signs of the presence of a wolverine were located, but the age of the animal (indicated by tooth wear), and the unknown date of death (VHF collar), suggested it was most likely a scavenging rather than predation event. Three mortalities had signs of multiple predators and we were unable to determine with confidence which predator was responsible for the mortality. One caribou died after apparently falling off a cliff (F444). Whether or not she was chased by a predator is not known. Two males from the ALP herd were killed in collisions with vehicles on highway 40N. One older caribou potentially died from heavy winter tick infestations (964), while another potentially died from disease (F786). Mortality locations were distributed in a variety of habitats throughout the herd ranges (Figure 2.1) and in Chapter 3 these mortality locations were used as part of a preliminary mortality distribution and risk modelling assessment. The appendix includes an example of one of the detailed mortality reports we produced for every mortality event. Chapter 4 details the results of our *ex situ* necropsies and detailed examination of carcass remains for health and disease assessment.

Table 2.1.Mortality dates (in chronological order) and probable cause of death for caribou in west-central Alberta and east-central British Columbia between 2013 and 2015.

ID	Mortality date	Mortality visit (days since mortality)	Remains	Herd	Collar type	Probable cause of death
F440	12 May 2013	14	Hide sections, long bones	RPC	GPS	Predation by grizzly bear, scavenging by wolf
F446	14 May 2013	12	Hide sections, rumen, skull fragment	RPC	GPS	Predation by grizzly bear
F793	7 May 2013	23	Hide sections, bones	NAR	GPS	Predation by grizzly bear
ALP_1	29 July 2013	1	Entire carcass	ALP	NA	Road traffic accident
F432	19 Aug 2013	4	Hide sections, intact bones	RPC	GPS	Predation by cougar (visual)
F786	11 Oct 2013	5	Full carcass, scavenging by birds	NAR	GPS	Potentially disease (complete test results pending)
F444	23 Jan 2014	1	Full carcass – avalanche danger prevented ground visit before August when remains were too desiccated for sampling	RPC	GPS	Accident (fall from cliff)
F439	27 Jan 2014	6	Large hide section	RPC	GPS	Predation by wolf
ALP_2	8 March 2014	0	Entire carcass	ALP	NA	Road traffic accident
1354	30 Mar to 10 Apr 2014	7-17	Hide section, front legs	LSM	VHF	Predation/Scavenging by wolverine
F453	1 May 2014	1	Limbs, intact skull, spine, pelvis	RPC	GPS	Predation by cougar (visual)
969291	unknown	unknown	Bone fragment, tooth, hair	ALP	NA	Unknown (identified during grizzly bear cluster visits)
976627	unknown	unknown	Bones	ALP	NA	Predation by grizzly bear or cougar (identified during grizzly bear cluster visits)
1068	unknown	unknown	NA	NAR	VHF	Unknown (no remains found at site, only collar)
F448	26 Oct 2014	2	Intact upper torso and head, portion of liver	RPC	GPS	Predation by wolf
F450/2199	17 Dec 2014	1	Hide section, long bones	RPC	GPS	Predation by cougar
964	5 May 2015	2	Full carcass	LSM	GPS	Potentially disease (anemia/winter tick)
F454	15 May 2015	10	Bone fragments, mandible, hair	RPC	GPS	Predation by grizzly bear or cougar
34077	28 Sept 2015	11	Skeleton, muscle, skull, hair	LSM	GPS	Potentially disease
1982	unknown	unknown	Bone fragments	NAR	VHF	Unknown
34075	20 Oct 2015	0	Spinal column, long bones, mandibles, hair	LSM	GPS	Predation by cougar

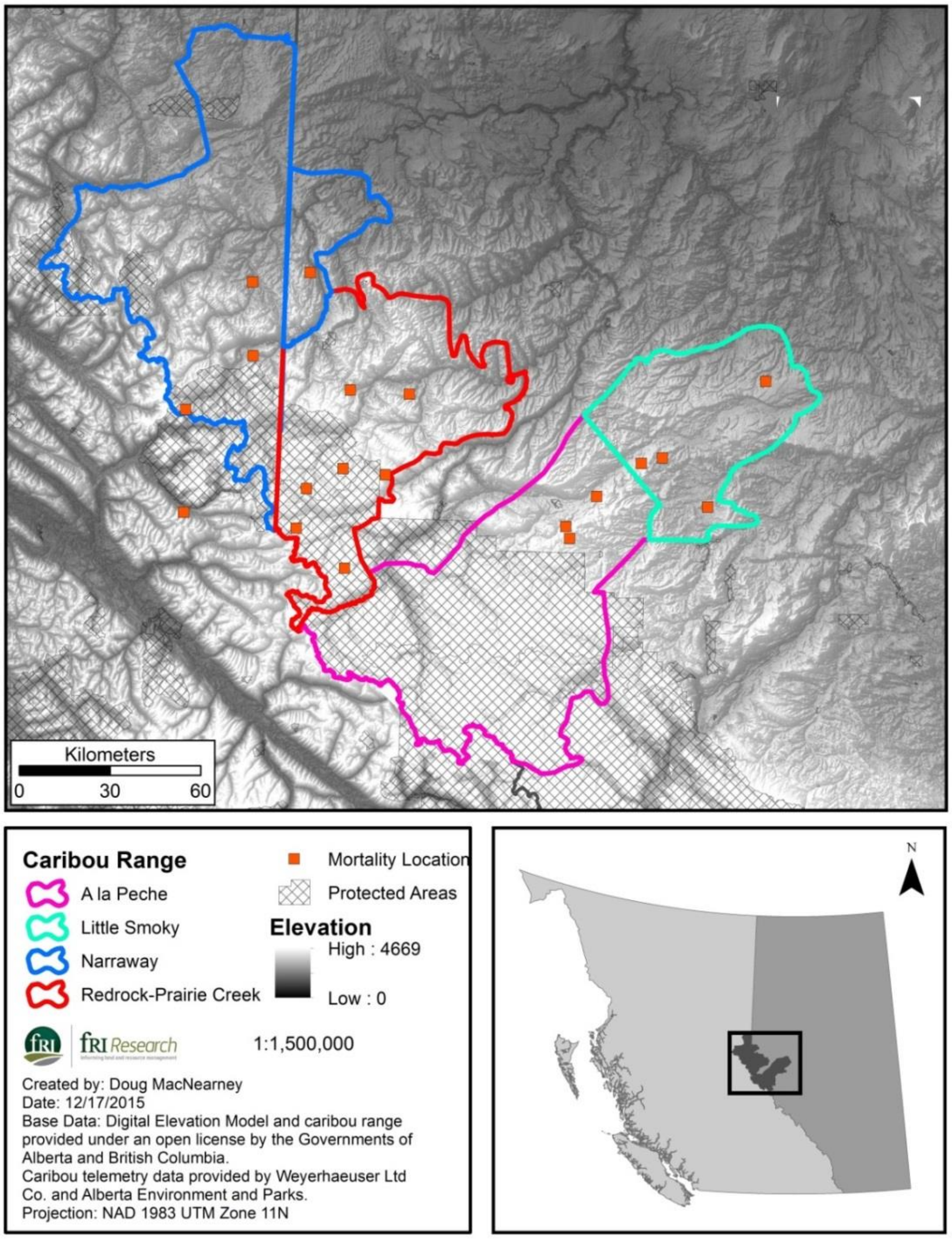

Figure 2.1. Mortality locations of collared caribou in west-central Alberta and east-central British Columbia between 2013 and 2015.

Figure 2.2. Top: Peeled caribou hide indicative of Ursid *spp. feeding; bear scat is visible in the centre of the photo. Bottom: Grizzly bear scat containing caribou hair located at caribou mortality site.*

Figure 2.3. Hide of F440 showing puncture wounds most consistent with grizzly bear.

Figure 2.4. Hide of F439 showing puncture wounds attributed to wolf. Note also probable antemortum haemorrhage around the canine puncture wounds towards the bottom right of the photograph.

2.4 Discussion

Our preliminary results suggest that the predator guild associated with caribou mortalities in west-central Alberta and east-central British Columbia includes not only wolves, but also cougars, grizzly bears and potentially black bears (DNA testing of bear scat is underway to confirm species). There were more probable predation events by cougar and grizzly bear than expected based on previous literature for west-central Alberta (McLoughlin *et al.* 2003; Kuzyk *et al.* 2006). It is possible that the relatively high grizzly bear population density in the Grande Cache population unit, when compared to other interior grizzly bear populations, results in greater degree of potential overlap between grizzly bears and caribou space use pattern than in other areas such as south Jasper. Assessing potential mortality risk based on the overlap between caribou and grizzly bears is the subject of ongoing research by the fRI Grizzly Bear and Caribou Programs.

Perhaps of more concern is the higher number of mortalities caused by cougars during the three years of data collection. Like wolves and unlike bears, cougars are active year round. Although predation rates upon female ungulates in Alberta tend to be higher during summer (Knopff *et al.* 2010), we found evidence of cougar predation year round. Increasing densities associated with range expansion (Knopff 2010), which is believed to mirror that of white-tailed deer range expansion (Dawe 2011), may be increasing the probability of an encounter between caribou and cougars in west-central Alberta. However our sample size is relatively small, thus data collection will continue. This in combination with our proposed analysis of caribou mortalities in relation to cougar probability of occurrence (see Chapter 3), may help to assess this further.

Although we could only attribute two of the probable 12 predator mediated mortalities to wolves, it is worth noting that for the first two years of this project our research was focused within the ranges of two central mountain caribou herds. Through collaboration with Alberta Environment and Parks, in the past year we expanded our study area to encompass the ranges of the Little Smoky and A La Peche caribou herds. Although previous research has found that estimated wolf numbers were similar in the Little Smoky and Redrock Prairie Creek ranges (Kuzyk *et al.* 2006), the density and distribution of habitat disturbance, and alternate prey, within central and boreal caribou seasonal ranges are likely different between the two ranges. Continued data collection into year two may help to determine whether wolf mediated predation rates within boreal herds in west-central Alberta are similar to those reported from elsewhere (Latham *et al.* 2011c).

Our field site investigations clearly show that to accurately associate mortality events to a specific predator, prompt investigation is required. For example, during our first visit to F439 four days after her death, a cougar was observed on the carcass, therefore we classified her mortality as cougar predation. However, when we revisited the site to collect biological samples one week later, there was evidence of extensive grizzly bear scavenging, which effectively masked any sign of cougar being the cause of caribou mortality. Of course, unless one is present at the time of the mortality, determining whether the predator sign is indicative of predation or scavenging is nearly impossible unless sufficient evidence remains at the mortality site. The presence of antemortum haemorrhage (F440) around the canine puncture holds indicated the animal's heart was beating when wounding occurred. Other morality events we classified as predation dependent on time that elapsed between the mortality and the site visit, and whether signs of more than one predator were present at the site. Ongoing DNA testing of predator scat may help to assign a positive predator/scavenging species identification to the remaining unknown mortality events.

Rapid mortality site investigations also allowed us to identify three mortality events that we could not attribute to predation. We carried out detailed necropsies on two of these animals and results are outlined in Chapter 4. The third animal had been scavenged by smaller predators after her death so a complete necropsy was not possible. Disease and health testing on those caribou, combined with tests from the high quality samples collected at other mortality investigations, will allow us for the first time, to assess the role of disease and health as a potential cause of death, and to collate baseline data to monitor caribou health into the future (see Chapter 4).

3. Distribution of caribou mortalities and mortality risk – preliminary analysis

Laura Finnegan, Doug MacNearney and Karine Pigeon

3.1 Introduction

The immediate threat to boreal and mountain caribou populations is elevated rates of predation linked to habitat change (Hervieux *et al.* 2014). Understanding how habitat disturbance is related to caribou mortalities, and how this in turn links to the spatial distribution of predators within caribou ranges, may be used to concurrently inform effective habitat restoration for caribou, and to inform predator management tactics that will be the most beneficial for caribou recovery.

Researchers within north-eastern and west-central Alberta have assessed caribou mortality risk and distribution relative to anthropogenic features and wolf predation risk; primarily during winter and summer (James & Stuart-Smith 2000; Whittington *et al.* 2011; DeCesare 2012). Those researchers found that caribou mortalities occur closer to linear features than random locations, and that predation risk is elevated in habitats where caribou co-occur with wolves. To date however, little research has considered the migratory behaviour of central mountain caribou when assessing mortality risk. During migration, caribou utilize valleys and areas near water to move between summer and winter ranges (Saher & Schmiegelow 2005). Increased movement rates during migration combined with a high potential overlap with caribou predators along migration paths may expose caribou to differential mortality risk during migration when compared to other seasons (Hebblewhite *et al.* 2010; DeCesare *et al.* 2013). Evaluating mortality risk during migration, and during winter and summer, will therefore improve our understanding of the distribution and probability of mortality for central mountain caribou.

In addition, because research assessing causes of mortality has to date focused upon wolves (Whittington *et al.* 2011; DeCesare 2012; Decesare *et al.* 2013), it remains unclear to what degree additional predators (e.g. bears, cougars) contribute to mortality for west-central caribou herds. For southern mountain herds in British Columbia, wolverines, cougars, and bears accounted for all the predator-caused mortalities (Kinley & Apps 2001), while later research found comparable predation rates by bears and wolves (Stotyn *et al.* 2007). Considering additional predators when evaluating mortality risk will help to inform predator management practices within west-central Alberta.

Here we used mortality location data collected between 1999 and 2015 within the Redrock Prairie Creek and Narraway herds to develop preliminary models of mortality distribution and mortality risk for west-central caribou. For this analysis, our goal was to understand the relationships between terrain, habitat and disturbances (both natural and anthropogenic), and caribou mortalities. In addition, we were also interested in how relationships varied across seasons. At the seasonal scale, we predicted that the frequency of caribou mortalities would be highest during migration and winter seasons when compared to summer (Hebblewhite *et al.* 2007, 2010; Whittington *et al.* 2011). Relative to terrain and anthropogenic features we predicted that caribou mortalities would be associated with terrain, habitat, and disturbance features preferred by caribou predators like wolves, bears and cougars. In year two of this project we will specifically evaluate this question by including probability of predator presence (RSF models) within our statistical analysis.

3.2 Methods

3.2.1. Mortality location data

Our dataset consisted of mortality events of collared female caribou in the Redrock Prairie Creek and Narraway herds that occurred between 1999 and 2015 (Lotek 2200/3300/4400, Newmarket, Ontario, Canada and Televilt Global Positioning System, Lindesberg, Sweden; n = 59; Figure 3.1). Caribou were collared as part of a long-term collaboration between Weyerhaeuser Co. Ltd and Alberta Environment and Parks. Because of advances in collar technology, the temporal precision of mortality events varied throughout the study period. In earlier years (1999 - 2010; n = 23 mortalities), mortalities were identified during the course of routine telemetry flights or other aerial activities within caribou ranges, while in later years 8 hours of animal (collar) inactivity triggered a mortality alert sent via email (n = 37 accurate mortality dates). Because accurate causes of death were only available for a small number of mortalities (see details in Chapter 2) we included all mortality events in this analysis. Later work will assess the spatial distribution of predator-caused mortalities vs. mortalities caused by other factors. We partitioned data into those that occurred inside and outside of protected areas. In addition, for the mortality risk analysis, we further partitioned data by seasons (defined using methods outlined by Rudolph & Drapeau 2012), pooling spring and fall seasons into ‘migration’, summer and calving into ‘summer’ and early and late winter into ‘winter’.

3.2.2. Landscape variables

Terrain, habitat, and natural disturbance variables: We used vegetation cover derived from a combination of Moderate Resolution Imaging Spectroradiometer (MODIS) and Landsat imagery mapped at a 30m x 30m resolution (McDermid *et al.* 2009) to determine the landcover that intersected each location (Table 3.1). Because of small sample sizes, we grouped landcover into a binary variable (forest = 1; non-forest = 0) for logistic regression. We used a 30m x 30m resolution digital elevation model to extract values of elevation (DEM), slope, terrain wetness (compound topographic index, CTI; Gessler *et al.* 2000), and terrain ruggedness (topographic position index, TPI; Jenness 2006). In addition, we calculated the distance to water, distance to wildfires less than 25 years old, 25 to 39 years old, and greater than 40 years old. Details of GIS data layers are in Table 3.1. To represent the diminishing effect of these features at larger distances, we applied an exponential decay: (1-exp $^{(-0.002 \times \text{distance (m)})}$). This decay causes the effect of distance to decrease rapidly beyond 500m, and to become constant at distances greater than 2km.

Anthropogenic disturbance variables: We calculated the distance to anthropogenic disturbances (seismic lines, cut blocks (0-25 years, >25 years), roads and well sites) using the exponential decay function. For roads, cut blocks, and well sites, we used annual maps of disturbance derived from SPOT imagery to match mortality events to year-specific disturbances. Built-before-year was not available for pipelines in BC and we therefore excluded pipelines from our analysis.

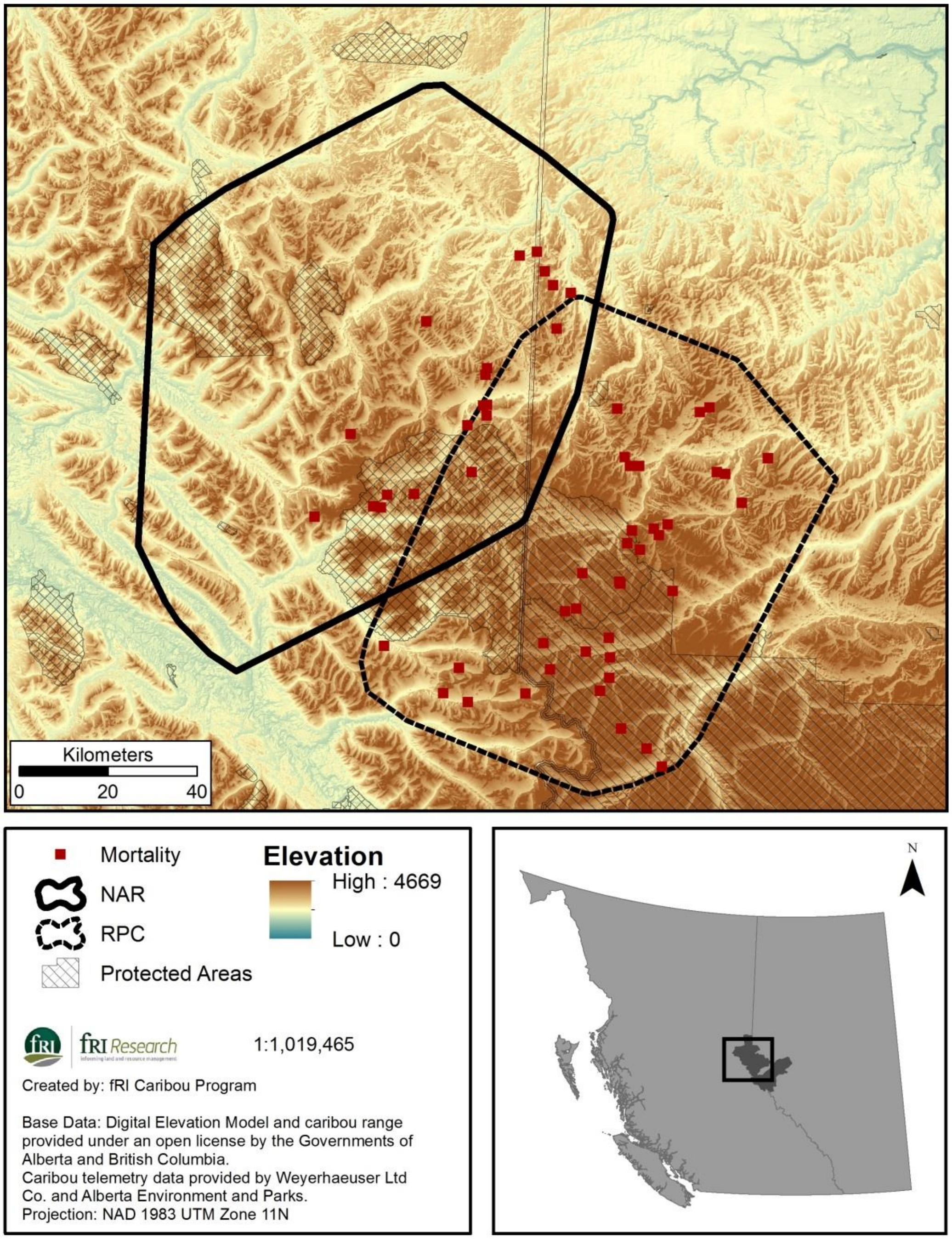

Figure 3.1. Mortality locations of collared caribou in the Redrock Prairie Creek (RPC) and Narraway (NAR) ranges between 1999 and 2015.

Table 3.1: Terrain, habitat and disturbance variables used to explain caribou mortality distribution and mortality risk in west-central Alberta between 1999 and 2015.

Variable	Description
Elevation	Elevation of a 30m x 30m pixel, m
Slope	Slope of a 30m x 30m pixel, degrees
CTI	Index of soil wetness of a 30m x 30m pixel; low values are dry, high values are wet, unitless.
TPI	Terrain ruggedness of a 30m x 30m pixel; high values are ridges, values near 0 are flat areas, low values are valleys and drainages, unitless
EStream	Distance to water (streams, rivers), exponential decay
EFire40	Distance to wildfires > 40 years old, exponential decay
EFire25	Distance to wildfires 25 to 39 years old, exponential decay
EFire0	Distance to wildfires < 25 years old, exponential decay
ESeismic	Distance to seismic lines, exponential decay
ECutO	Distance to cutblocks ≥ 25 years old, exponential decay
ECutY	Distance to cutblocks < 25 years old, exponential decay
ERoad	Distance to roads, exponential decay
EWell	Distance to well sites, exponential decay

3.3.3. Data analysis

We carried out data exploration following Zuur *et al.* (2010); excluding variables from the same models if variables had a correlation coefficient ≥ 0.6 or a variance inflation factor > 3, and testing for non-linear relationships amongst variables using generalized additive models. We carried out data exploration and statistical analyses within R and RStudio (2015) and we visualised results using the R package 'ggplot2' (Wickham 2009).

Mortality distribution models: We used generalized linear models to compare the spatial distribution of mortality events (n = 60) to random locations within each herd range. We drew random locations at a density of 1 per km^2 within a minimum convex polygon (MCP) bounding all GPS locations for each of the herds (Geospatial Modelling Environment; Beyer 2012). Because accurate month of death was not available for 23 caribou, we could not draw availability within seasonal MCPs. We pooled data across herds and built two separate models; one included all natural variables, and one included all anthropogenic disturbance variables (see Table 3.1). We used the 'drop1' function within R to identify the most parsimonious combination of variables from each model set and combined the remaining natural and anthropogenic variables into a global model. We assessed global model fit using conditional R^2 values (Nakagawa & Schielzeth 2013) estimated with the R package 'MuMin' (Bartón 2015), and evaluated the ability of global models to predict mortality events using k-fold cross validation (Boyce *et al.* 2002).

Mortality risk models: We used generalized linear mixed models (Gillies *et al.* 2006) to evaluate mortality risk (R package 'lme4'; Bates *et al.* 2014). We standardized DEM, CTI, and TPI to improve model convergence. We compared mortality events (n = 37) to temporally matched caribou GPS locations collected from collared caribou within the same herd and on the same date (same 24 hour period; 2 to 24 GPS 'alive' locations per mortality location), and included mortality date as a random effect. Following methods outlined above, we built two separate models using either natural or anthropogenic variables, and used the 'drop1' function to build a global model. We assessed global model fit and the ability of global models to predict mortality events using the methods described above. All results are presented as β (beta) coefficients ± standard errors.

3.3 Results

Between 1999 and 2015, 22 of 59 collared caribou mortalities occurred within protected areas and 37 occurred outside of protected areas (Figure 3.2a). Seven mortalities occurred during early winter, eight during late winter, nine during spring, two during calving, six during summer, and seven during fall. The frequency of mortalities pooled across seasons used for statistical analysis is shown in Figure 3.2b.

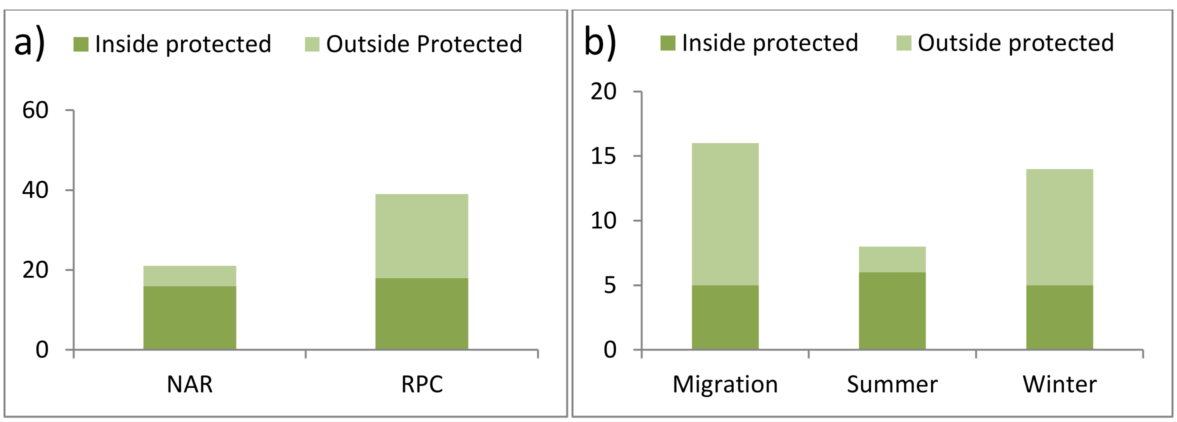

Figure 3.2. Frequency of collared adult female caribou mortalities within the Narraway and Redrock Prairie Creek herds a) inside and outside of protected areas (n = 59) and b) inside and outside of protected areas by season (n = 37 mortalities with accurate date of mortality).

Mortality distribution:

Within protected areas, mortalities were positively associated with wet areas (cti; β = 0.92 ± 0.27; Figure 3.3), and were closer to young fires than random locations (β = -1.65 ± 0.57). Outside protected areas, mortalities were also positively associated with wet areas (cti; β = 0.94 ± 0.27; Figure 3.3), and were closer to seismic lines (β = -1.48 ± 0.49; Figure 3.4), and further from well sites (β = 4.59 ± 2.36; Figure 3.4) when compared to random locations. K-fold cross validation revealed poor model performance ($r_s < 0.5$).

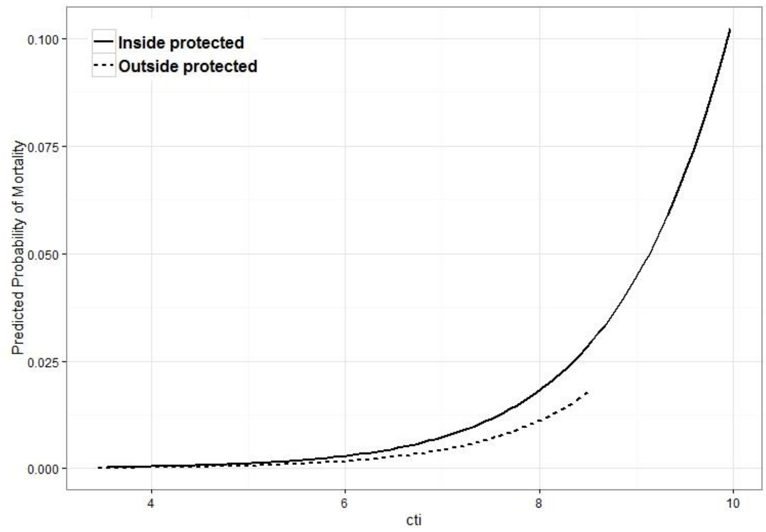

Figure 3.3. Predicted probabilities of adult female caribou mortalities inside and outside of protected areas within the Narraway and Redrock Prairie Creek herds relative to cti values. All other values within the model were held at their mean for graphical predictions.

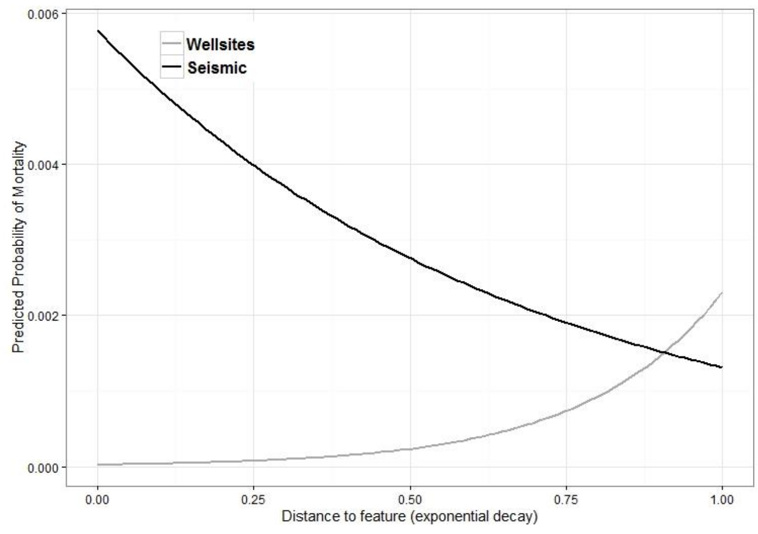

Figure 3.4. Predicted probabilities of adult female caribou mortalities outside of protected areas within the Narraway and Redrock Prairie Creek herds relative to proximity to well sites and seismic lines. All other values within the model were held at their mean for predictions.

Mortality risk:

Because of seasonal shifts in herd ranges throughout the year, there were insufficient data to assess mortality events outside of protected areas during summer, or mortality events inside of protected areas during winter. We assessed mortality risk during migration inside of and outside of protected areas. We did not consider anthropogenic disturbances for models built within protected areas.

During migration, caribou that died within protected areas were at lower elevations ($\beta = -5.39 \pm 0.001$, conditional $R^2 = 0.75$; Figure 3.5), while caribou that died outside of protected areas were on steeper slopes ($\beta = 2.49 \pm 0.013$; conditional $R^2 = 0.39$) and further from streams ($\beta = 4.48 \pm 0.015$) when compared to alive caribou. During summer, caribou that died were also at lower elevations ($\beta = -2.55 \pm 0.006$, conditional $R^2 < 0.01$; Figure 3.5), and during winter, caribou that died were in areas with lower TPI values ($\beta = -1.95 \pm 0.027$; conditional $R^2 < 0.01$; Figure 3.6) when compared to alive caribou. K-fold cross validation indicated revealed poor model performance (r_s <0.5 in all cases).

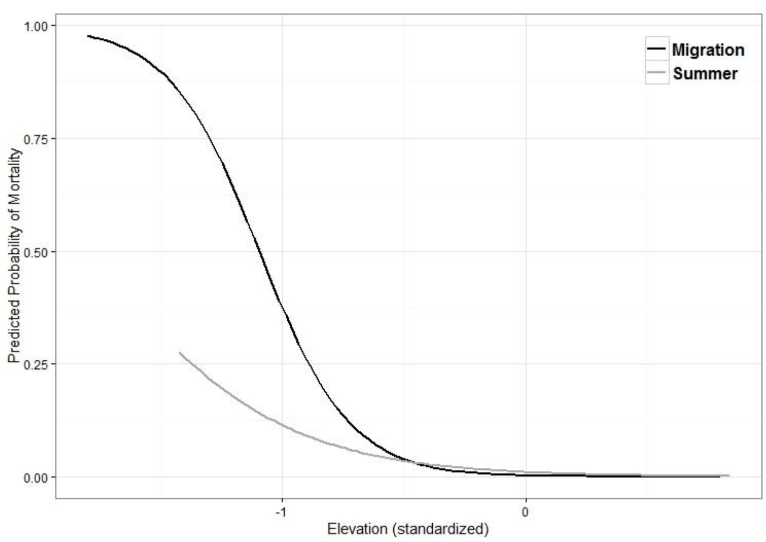

Figure 3.5. Predicted probabilities of adult female caribou mortalities inside of protected areas within the Narraway and Redrock Prairie Creek herds during the migration and summer seasons relative to standardized elevation.

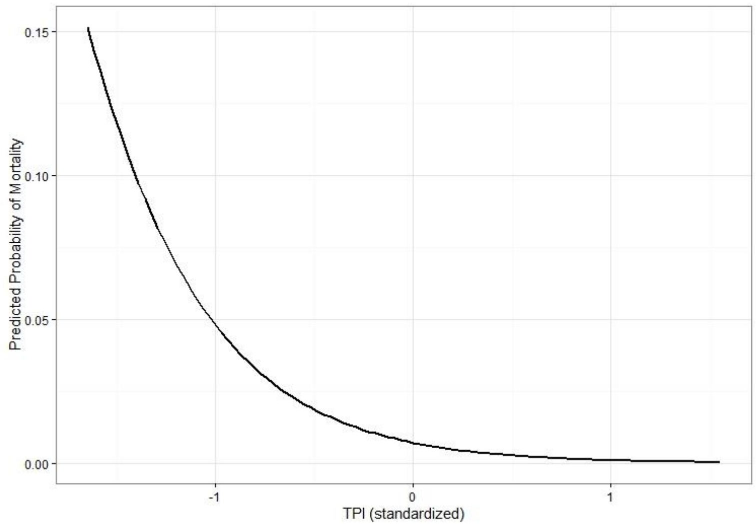

Figure 3.6. Predicted probability of adult female caribou mortalities outside of protected areas within the Narraway and Redrock Prairie Creek herds during winter relative to standardized TPI. All other values within the model were held at their mean for predictions.

3.4 Discussion

Our preliminary analysis suggests that localised topography and anthropogenic disturbance may influence caribou exposure to mortality risk although results were variable across seasons, and inside vs. outside of protected areas. In addition models performed poorly under cross validation so our results should be interpreted with caution. We found no strong seasonal pattern in mortalities although there were slightly higher frequencies of mortalities during migration, as described previously by Hebblewhite *et al.* (2010). In addition, we found similar numbers of mortalities inside and outside of protected areas. The mortality patterns observed may be driven by the complex predator guild within the range of west-central Alberta caribou (Redrock Prairie Creek and Narraway) herds, and the migratory patterns of our study animals. For example, during winter, when central mountain caribou are at low elevations and in drainages, these caribou may be at a higher mortality risk from wolves (Hebblewhite *et al.* 2010; DeCesare 2012). However, during summer, when central mountain caribou are at higher elevations, these caribou may be at higher mortality risk from wolverines, bears, and cougars (Bergerud, Butler & Miller 1984; Kinley & Apps 2001; Wittmer *et al.* 2005; Stotyn *et al.* 2007). As planned in the coming year, including the probability of predator presence within our models, may help further explain the spatial and seasonal distribution of mortalities as was observed by Hebblewhite *et al.* (2010) and DeCesare (2012) when including wolf predation risk.

We found contrasting patterns when we assessed the relationship between anthropogenic features, terrain and mortality risk, and the spatial distribution of mortalities. Outside of protected areas, when we compared mortality locations to available locations within each herd range, we found that mortalities occurred in wetter areas and closer to seismic lines during winter. However, when we compared mortality locations to alive caribou locations, we found that caribou died on steeper slopes and further from streams during migration, while during winter, caribou died in areas with lower TPI values. These results are largely similar to previous research. For example James & Stuart-Smith (2000) found that caribou mortalities also occurred closer to seismic lines although this pattern was only evident when mortality locations and alive locations were compared. In addition, Hebblewhite *et al.* (2010) also found a significant negative relationship between caribou survival and TPI. Because wolves use areas closer to seismic lines than expected by random (Latham *et al.* 2011b), and because they also select for drainages and areas with lower TPI (Hebblewhite *et al.* 2010), is it possible that these caribou mortality patterns are driven by wolf predation risk, particularly during winter. Mortality patterns during migration outside of protected areas were less clear: caribou mortalities occurred on steeper slopes and further from streams. During spring migration, caribou select for less rugged terrain (lower TPI) and areas close to water (Saher & Schmiegelow 2005), it is therefore possible that caribou mortalities during spring are a consequence of caribou selecting habitat with higher mortality risk. Comparison of habitat selection coefficients between caribou that died during migration and those that survived (year two of this project) will help to assess this further.

Within protected areas, caribou mortalities were distributed in wetter areas and closer to burned areas while mortality risk was highest during all seasons at lower elevations. The distribution of mortalities in wet areas and closer to regenerating areas may reflect increased predation risk from cougars, grizzly bears, and potentially wolves in those areas. Regenerating areas like burns are attractive to moose, deer, and elk, which in turn makes them attractive to predators (Nielsen *et al.* 2004a, b; Lesmeries *et al.* 2012; Peters *et al.* 2013). In addition, wetter areas may also contain more vegetative food which may attract grizzly bears (Nielsen *et al.* 2004b). Selection of caribou for these same habitats may increase the probability of encounters between caribou and predators. Therefore, the observed associations between elevation and mortality may be driven by caribou behaviour rather than elevation itself. For instance, during summer, caribou prefer high elevation habitat (Bergerud *et al.* 1984) but movements are restricted because of calves at heel (DeMars *et al.* 2013). Therefore, despite the presence of wolverine, bear, and cougar within high elevation caribou habitat during summer (Bergerud *et al.* 1984; Kinley & Apps 2001), restricted movements may decrease the probability of encounter between caribou and predators, resulting in relatively high survival in alpine habitats (Hebblewhite *et al.* 2010).

Understanding mortality risk for caribou in relation to terrain, anthropogenic features, and specific predators, will help inform habitat restoration and approaches to predator management that will be most beneficial for caribou. Our preliminary analysis suggests that seismic lines are associated with the distribution of caribou mortalities but at a larger scale, seasonal and spatial patterns of mortality appear to be associated with areas preferred by wolves and potentially grizzly bears and cougars. Our ongoing work integrating mortality risk with grizzly bear probability of overlap, and our proposed work to assess potential overlap with other significant predators in west-central Alberta will help to not only identify areas that are potential predator refugia for caribou, but also areas with the highest predation risk for caribou that could be used to prioritize areas for restoration within herd ranges. In addition, ongoing work comparing fine scale seasonal habitat selection patterns of caribou that lived versus those that died will increase our understanding of trade-offs between caribou habitat selection and predation risk (Decesare *et al.* 2013).

4. Evaluation of caribou health in west-central Alberta

Laura Finnegan, Bryan Macbeth, Helen Schwantje and Susan Kutz

4.1 Introduction

Wildlife health reflects the cumulative effects of interactions between a species' evolutionary history and recent or past biotic and abiotic factors acting on both individuals and populations (Stephen 2013, 2014). As such health may be considered as an indicator of an individual's or a population's capacity to cope with the combined effects of natural and anthropogenic challenges (Busch & Hayward 2009; Linklater 2010). Understanding and tracking the health status of free-ranging wildlife may, therefore, provide valuable information for the management and conservation of species-at-risk (Pedersen & Babayan 2011; Ellis *et al.* 2012; Kutz *et al.* 2013).

Health is increasingly recognized as a factor that may contribute to diminished survival and reproduction in free ranging caribou. Some determinants of caribou health such as certain pathogens and severe nutritional defects may kill caribou or affect their reproductive output directly, while others may act through more subtle, chronic or cumulative effects (compromised immunity, reduced body condition) (Crête & Huot 1993; Hughes *et al.* 2009; Gustine *et al.* 2012). Accordingly, the consequences of compromised health may lead to population level impacts associated with direct mortality or reproductive failure, and/or indirect effects related to increased predation risk, reduced pregnancy rates, low calf survival and juvenile recruitment, or a compromised ability to cope with natural or anthropogenic stressors (e.g. severe weather, industrial development) (Bradshaw *et al.* 1998; Millspaugh *et al.* 2001; Ashley *et al.* 2011). The relative importance of health as a factor affecting caribou population dynamics is expected to increase as the degradation and fragmentation of caribou ranges continues and with climate change. A related increase in range overlap with other ungulate species (that may carry pathogens also affecting caribou), and an interaction between declining caribou health and increasing predation risk may be particularly important (Hoberg *et al.* 2008). In Alberta, all caribou herds are in decline and many exist as small isolated populations (Hervieux *et al.* 2013). Within these already fragile populations there is an increased risk of compromised health, disease transmission or catastrophic disease outbreaks (McCallum & Dobson 2002). This risk has the potential to alter the timeline and overall success of current caribou recovery actions in Alberta.

To date there has been little research on the disease and health status of woodland caribou in western Canada. Understanding of the diversity, distribution, and prevalence of pathogens, the overall health status of caribou herds in Alberta, and the relationship between caribou herd health and landscape features (including sympatric ungulates and predators), will provide an important contribution to recovery planning.

Here we present preliminary results from the first intensive health survey of boreal and central mountain caribou herds in west-central Alberta and east-central British Columbia. Ultimately we aim to establish comprehensive herd health baselines that will provide insight into the overall health status of caribou in our study areas, and that may be used to help guide provincial recovery initiatives.

4.2 Methods

4.3.1. Sample collection

4.3.1.1. Collection of biological samples from caribou mortalities

We collected biological samples from radio-collared caribou mortalities either in the field (Figure 2.1), or *ex situ*, using standardized protocols developed by the British Columbia Boreal Caribou Health Research Program (BCHRP, Schwantje *et al.* 2014) and the CircumArctic Rangifer Monitoring and Assessment (CARMA) Network (Kutz *et al.* 2013). We also opportunistically collected intact carcasses of two male A La Peche caribou killed on highway 40 (one in 2013, one in 2014). Samples collected from caribou mortalities were dependant on the quality and quantity of carcass remains available, and included intact long bones, mandibles, plucked hair, hide and muscle, sections of major organs (e.g. lung, liver, heart, spleen, kidney, lymph nodes and reproductive organs), blood (as clots collected from the heart), rumen contents, and fecal samples. Where possible, we carried out a complete necropsy including a comprehensive external and internal examination. Of the five mortalities where complete necropsies were possible, three were performed by a wildlife veterinarian, the others were carried out by experienced biologists from the fRI Research Caribou and Grizzly Bear Programs. All tissue and fecal samples were stored in sterile Whirlpaks® at -20ºC until laboratory analysis, hair was stored with desiccant at room temperature in paper envelopes in the dark, and any parasites recovered were stored in 70% ethanol at room temperature.

4.3.1.2. Non-invasive fecal sampling

We collected caribou fecal samples in February 2014 and between January 1st and March 31st (helicopter based) or May 31st (ground based) 2015 (n = 217 fecal piles; Figure 4.1). We located the majority of our sampling sites with helicopter flights to GPS locations of collared female caribou approximately one week after the collared animal had left the area, and then searching for evidence of track networks and evidence of cratering. We also opportunistically sampled sites by searching for tracks and cratering *en route* to the collared animal's location. If caribou were still present at the site upon arrival the helicopter did not land and if possible the site was revisited at a later date. At each site we estimated the number of animals present by counting unique sets of tracks approaching or leaving the area. Following existing Alberta Environment and Parks protocols we sampled 1.5 times the number of fecal piles as caribou estimated to have visited the site. The Caribou Patrol (www.cariboupatrol.ca) also collected opportunistic samples from A La Peche and Little Smoky caribou encountered during their winter and spring patrols (2015 only). Patrollers noted the location and number of caribou and revisited the site (within 24 hours) to collect samples after caribou had left the area. At both helicopter and ground based survey locations three sets of fecal pellets were collected from each fecal pile and samples were stored in sterile Whirl-Paks® for genetic (~10 pellets per Whirl-Pak ®) and pathogen testing (>20 pellets per Whirl-Pak®). Samples were kept in a cooler with ice/snow during field collection and then kept at -20ºC in a freezer to maintain quality until submitted for diagnostic testing.

We identified fecal samples from unique individuals for pathogen testing using genetic profiling (Wildlife Genetics International, Nelson; www.wildlifegenetics.ca).

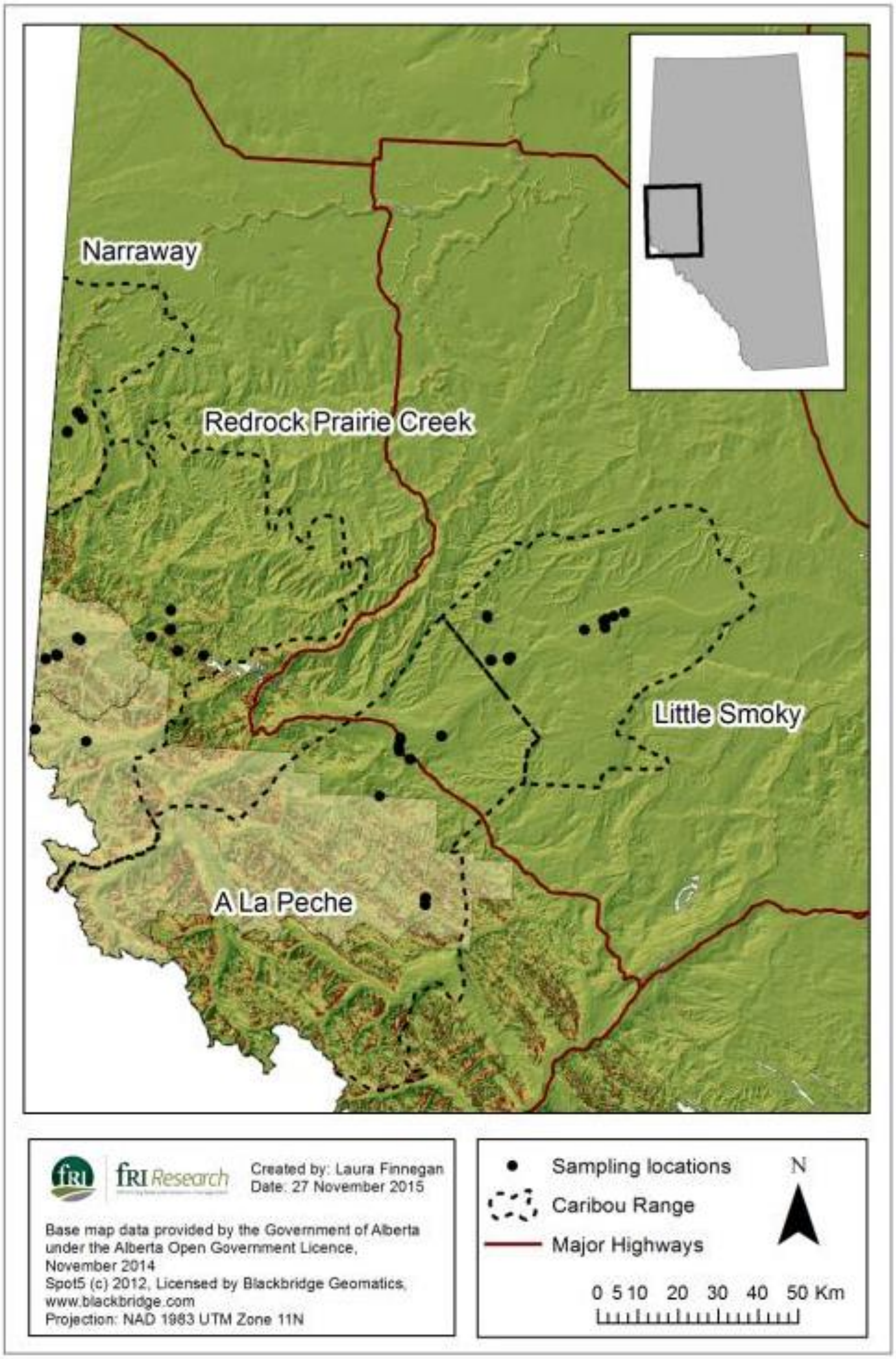

Figure 4.1. West-central Alberta caribou ranges showing provincial range boundaries and fecal pellet sampling locations in 2014 and 2015.

4.3.2. Health testing

4.3.2.1. Baseline health and disease parameters to test

Through research collaboration with the BCHRP and *Rangifer* health specialists at the Canadian Wildlife Health Cooperative (CWHC), the University of Calgary Faculty of Veterinary Medicine (UCVM) and elsewhere, we developed a diagnostic testing strategy to determine the baseline diversity, distribution, and prevalence of selected pathogens and parasites in caribou from west-central Alberta (Table 4.1). Other health indicators including marrow fat, trace nutrient status, and chronic stress levels were also assessed. Tests selected focused on those pathogens, parasites, and health indicators that could be evaluated using samples gathered from mortality site investigations, non-invasively, or opportunistically (from road kills).

Table 4.1.Selected health indicators, type, potential significance, and sample(s) required to detect occurrence or evaluate status in caribou from west-central Alberta. Table adapted from Table 1 in British Columbia Boreal Caribou Health Program Year 1 Synthesis Report (Schwantje et al. *2014) and further details regarding the potential significance of each selected health indicator can be found therein.*

Health indicator	Type	Potential significance	Applicable samples
Erysipelothrix rhusiopathiae	Bacterium	Recently implicated in widespread mortalities of muskoxen in Canadian Arctic archipelago (See Kutz *et al.* 2015) First detected in caribou in BC in 2013 (see Schwantje *et al.* 2014). Also detected in moose in BC in 2013 Growing body of evidence this bacterium may be associated with mortalities in woodland caribou and other free-ranging ungulates. However disease ecology and overall importance in caribou and other wildlife is poorly understood In domestic ruminants case of chronic disease, fatal septicemia, also implicated in abortions	Serum; blood; tissue; marrow; feces
Protostrongylid nematodes	Nematode parasites	Lung, muscle, and neurotrophic nematodes of cervids Significance depends on parasite species and intensity of infection Effects may range from subclinical/mild to fatal	Feces, various tissues
Gastrointestinal nematodes	Nematode parasites	Associated with weight loss, reduced pregnancy rates, altered grazing behavior in caribou Cumulative effects with other parasites and health determinants probable	Feces; gastrointestinal tracts
Fascioloides magna (Giant liver fluke)	Trematode parasite	Causes severe liver pathology in caribou No reports of mortalities in caribou to date Potential subclinical effects Caribou may serve as definitive host Causes severe disease in moose	Feces; liver

Table 4.1. continued

Health indicator	Type	Potential significance	Applicable samples
Dermacentor albipictus (Winter tick)	Ectoparasite	Severe pathology and heavy infestations recently identified in woodland caribou from BC (Schwantje *et al.* 2014). May lead to hair loss, poor body condition (Figure 4.2) Anemia, death	Hide section
Cortisol	Hormone	Indicator of physiological stress May be associated with parasite burden, body condition, other natural or anthropogenic stressors (e.g. habitat fragmentation, predation risk) Known linkages between chronic stress, poor health, and diminished fitness in other species Hair cortisol may serve as a practical integrative health biomarkers in caribou	Hair; feces
Marrow fat content	General	Generally accepted as an indicator of body condition and nutritional stress in free-ranging cervids Established linkages between body fat levels and survival and reproductive success (pregnancy) in caribou	Intact long bone; mandibles
Trace nutrients	General	Indictor of total nutritional status Trace nutrients status affects growth, immunity, reproductive performance of ungulates	Liver; blood

*Figure 4.2. Severe hair loss in an adult female boreal caribou from NE British Columbia heavily infested with winter tick (*Dermacentor albipictus*). Picture credit: B and D Culling, Diversified Environmental Service Inc., Fort St. John, BC.*

4.3.2.2. Laboratory work

All laboratory work was carried out at the UCVM and the CWHC in Calgary Alberta by trained laboratory technicians or wildlife veterinarians. Diagnostic tests included enzyme-linked immunosorbent assays (ELISAs), molecular techniques (PCR, full genome sequencing) and general and selective bacterial cultures. All diagnostic tests employed had previously been evaluated in *Rangifer* spp., at the CWHC or as part of the BCHRP (Table 4.2).

Table 4.2.Health indicators and diagnostic tests used to evaluate disease prevalence and the overall health status of caribou in west-central Alberta. Adapted from Table 5 in Schwantje et al. *2014.*

Health indicator	**Diagnostic test(s)**
Erysipelothrix rhusiopathiae	Indirect Protein A/G-HRP ELISA to detect antibodies (in house assays: UCVM and CWHC, Calgary, AB) Direct PCR and selective tissue culture followed by full genome sequencing
Protostrongylid nematodes	Fecal Baermann with *a posteriori* PCR identification of dorsal-spine larvae (DSL) (in house assays: UCVM and CWHC, Calgary, AB)
Gastrointestinal nematodes	Fecal floatation with morphological identification of parasite eggs (in house assays: UCVM and CWHC, Calgary, AB)
Fascioloides magna (Giant liver fluke)	Fecal sedimentations (Flukefinder®, Soda Springs, ID, USA) with morphological identification of fluke eggs (in house assays: UCVM and CWHC, Calgary, AB)
Dermacentor albipictus (Winter tick)	Morphological identification, , collection of voucher specimens, KOH digestion
Hair cortisol	Oxford EA-65 Cortisol Competitive EIA kit (Oxford Biomedical, Lansing, MI, USA) (Western College of Veterinary Medicine (WCVM), University of Saskatchewan, Saskatoon, SK)
Marrow fat content	CWHC ungulate marrow fat assessment protocol (WCWM, University of Saskatchewan, Saskatoon, SK)
Trace nutrients and toxicology*	High-Performance-Liquid-Chromatography (HPLC) (in house assays: Prairie Diagnostic Services Inc, Saskatoon, SK)

*Trace nutrients and toxicology panels included Vitamin A and E, Beryllium (Be), Magnesium (Mg), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se), Strontium (Sr), Molybdenum (Mo), Cadmium (Cd), Tin (Sn), Antimony (Sb), Barium (Ba), Thallium (Tl), Bismuth (Bi) and mercury (Hg).

4.3 Results

4.3.1. Summary of main findings and ectoparasites identified in caribou

Necropsy of the male A La Peche animal killed as a result of a motor vehicle collision in 2013 detected no notable abnormalities, although severe decomposition of the internal organs prevented a detailed examination. The animal was approximately 16 months old and died from a broken neck. The A La Peche male that died as a result of a motor vehicle collision in 2014 was approximately 3 to 4 years old. He was in relatively poor condition with diminished internal fat deposits and had a mild to moderate winter tick (*Dermacentor albipictus*) infestation with no associated hair loss. Traumatic diaphragmatic hernia, with rupture of the rumen into the thoracic and abdominal cavities, together with compound fracture of the left tibia and fibula, and comminuted fracture of the right mandible, were consistent with per acute trauma (vehicle strike) as the proximate cause of death. Necropsy of caribou F786 revealed she was not emaciated (Figure 4.3). Two lungworms (*Dicyocaulus* spp.) were recovered from one lung, however advanced decomposition precluded definitive identification of these parasites. We detected two winter ticks on the hide of caribou 1354, but there were insufficient remains to quantify the true extent of tick infestation in this animal. Caribou 964 was heavily infested with winter tick, notably around the hind quarters (Figure 4.4 top). A 10cm x 10cm section of hide was collected from this caribou and subjected to KOH digestion. A total of 95 mature winter ticks were collected from this hide section. Subsequent necropsy revealed diffuse muscle pallor that was most likely indicative of severe anemia (Figure 4.4 bottom) and possibly related to the heavy tick infestation. Caribou 964 was also emaciated and had evidence of extensive tooth wear associated with advanced age (Figure 4.4 top). No winter ticks were identified on the hide of caribou F448.

Figure 4.3. Carcass of F786 in situ at mortality site

Figure 4.4. Top: View of the inside hind haunch of 964 showing winter ticks. Bottom: Left mandible of 964 (top of photograph) with right mandible of F450/2199 (bottom of photograph) for comparison. Note the severe tooth wear, and the diffuse pallor of muscles attached to the mandibles of 964.

4.3.2. Bacteria

Using a combination of PCR and bacterial culture we identified the bacterium *Erysipelothrix rhusiopathiae* in 6 of 11 caribou mortalities examined between 2013 and 2015 (Table 4.3).

*Table 4.3.*Erysipelothrix rhusiopathiae *status of caribou sampled as part of mortality site investigations within west-central Alberta and east-central British Columbia between 2013 and 2015.*

ID	Sex	Herd	Probable cause of death	*Erysipelothrix rhusiopathiae* tissue culture status
ALP_1	Male	ALP	Vehicle	Negative
Un-collared grizzly kill	Male	ALP	Predation	Positive
ALP_2	Male	ALP	Vehicle	Testing ongoing
1354	Female	LSM	Predation/Disease?	Negative
964	Female	LSM	Ticks/starvation	Negative
34075	Female	LSM	Disease?	Testing ongoing
F440	Female	RPC	Predation	Positive
F444	Female	RPC	Accident	Negative*
F446	Female	RPC	Predation	Positive
F453	Female	RPC	Predation	Positive
F454	Female	RPC	Predation	Testing ongoing
F448	Female	RPC	Predation	Testing ongoing
F450/2199	Female	RPC	Predation	Testing ongoing
F786	Female	NAR	Disease	Positive
F793	Female	NAR	Predation	Positive
1068	Female	NAR	Predation	Negative*

*marginal sample quality – significant autolysis or dried out

4.3.3. Parasites

Parasite results reported here are from fecal samples only. A total of 74 fecal samples from individual caribou were available for testing (Table 4.4).

4.3.3.1. Abomasal parasites

5% of caribou tested (n = 4/74) had Nematodirinae eggs in their feces, while 23% (n = 17/74) had eggs with the typical 'strongyle' morphology (most likely representing *Ostertagia gruehneri* or *Teladorsagia boreoarcticus,* although other species are possible)(see Kutz *et al.* 2012). The intensity of infection was low in both cases (1 - 9.64 eggs/gram of feces). We detected tapeworm eggs (*Moniezia* spp.) in 12% (n = 9/74) of caribou tested, and the intensity of infections was relatively high (15 – 125 eggs/gram of feces). Neither *Marshallagia* spp. eggs nor *Eimeria* spp. oocysts were detected within our samples. Table 4.4 summarizes results by herd.

Table 4.4.Number of caribou samples collected across four west-central Alberta herds with positive gastrointestinal parasite results detected using fecal floatation (Nematodirinae, Strongyle and Moniezia *spp) and Baermann larval counts (Protostrongylid dorsal spined larvae, DSL). The intensity of infection, shown as the range of eggs or larvae per gram of feces, is given in parenthesis.*

Herd	Number of samples	Nematodirinae (eggs/g)	Strongyle (eggs/g)	*Moniezia spp* (eggs/g)	DSL (larvae/gr)
ALP	15	1 (<1)	6 (<1 – 1.43)	0	1 (10.43)
LSM	21	0	7 (<1 – 9.94)	1 (112.68)	7 (1.48 – 37.69)
RPC	35	3 (2.5 – 6.92)	2 (<1)	8 (15.37 – 125.38)	5 (<1 – 24.51)
NAR	3	0	2 (<1 – 1.71)	0	0

4.3.3.2. Protostrongylid nematodes

We recorded dorsal spined Protostrongylid larvae (DSLs) in 16% (n = 12/74) of fecal samples. Both prevalence and intensity of infection of DSLs varied across herd ranges (Table 4.4). Molecular identification of DSLs is ongoing.

4.3.3.3. Giant liver fluke (*Fascioloides magna*)

We detected no fluke eggs in 74 of 74 fecal samples tested.

4.3.4. Hair cortisol

Hair cortisol concentration is currently being evaluated as an integrated biomarker of chronic physiological stress levels in samples collected from caribou mortalities. Results will be provided in year 2.

4.3.5. Nutrition and toxicology

We used criteria developed by BCHRP collaborators Drs. J. and R. Cook (NCASI, LA Grande OR, USA) to interpret marrow fat % recorded in AB caribou where femur marrow fat <85% is indicative of evidence of nutritional stress, and femur marrow fat <12% is indicative of starvation. Marrow fat % derived from long bones collected from dead caribou indicated that most caribou examined were nutritionally stressed to some degree (Table 4.5). Caribou F793 and ALP_1 had particularly low % marrow fat (21.6% and 44.4%) and caribou 964 was approaching emaciation/starving (marrow fat 17.9%).

Table 4.5 Bone marrow fat % determined in Alberta caribou.

ID	Sex	Herd	Probable cause of death	Date of Death	Bone Tested*	% Marrow fat
ALP_1	Male	ALP	Vehicle	29 July 2013	Femur	44.4
Un-collared grizzly kill	Male	ALP	Predation	Unknown	Radius/Ulna	82.1
964	Female	LSM	Ticks/Starvation	5 May 2015	Femur	17.9
1354	Female	LSM	Predation/Disease	30 March- 10 April, 2014	Metatarsus	74.4
F793	Female	NAR	Predation	7 May, 2013	Femur	31.6
F786	Female	NAR	Disease	11 October, 2013	Femur	80.6
F450/2199	Female	RPC	Predation	17 Dec 2014	Femur	64.9

F440	Female	RPC	Predation	12 May, 2013	Femur	58.8
F446	Female	RPC	Predation	14 May, 2013	Femur	62.5
F453	Female	RPC	Predation	1 May, 2014	Femur	70.6
F448	Female	RPC	Predation	26 October, 2014	Radius	77.1
F454	Female	RPC	Predation	15 May 2015	Radius/Ulna	82.6

* Marrow fat % measured in femurs is generally considered to be the most reliable index of condition that can be estimated using bones obtained from ungulate mortality sites. Values presented for bones other than femurs may represent a less reliable estimate of marrow fat %. To better establish the utility of using alternate bones when femurs are not available or are in poor condition (e.g. cracked and/or dried), we are working to establish a comparative database of marrow fat % measured in multiple bones collected from the same caribou.

Trace nutrient levels were also evaluated in liver samples recovered from four caribou mortalities (F786, F448, 964 and ALP_2; Table 4.6). Marginal or deficient Selenium levels were identified in all caribou with the exception of ALP_2. Caribou F448 was also deficient in Magnesium, Manganese, Copper, and Zinc. With the exception of caribou 964 mercury levels were high-normal in all animals tested.

Table 4.6 Trace Nutrient status determined in liver samples collected from caribou F786 (NAR), F448 (RPC), 964 (LSM), and ALP_2 (ALP).

Target (Units)	F786 (NAR)		F448 (RPC)		964 (LSM)		ALP_2 (ALP)	
	Ppm/t/b	Result	Ppm/t/b	Result	Ppm/t/b	Result	Ppm/t/b	Result
Vitamin E (ppm)	30.1	Normal	12.6	Normal	196.4	High Normal	9.80	Normal
Beryllium (ppt)	<4.00	Normal	<4.00	Normal	<4.00	Normal	0.235 ppb	Normal
Magnesium (ppm)	186.0	Normal	41.6	**Deficient**	246.0	High Normal	136.9	Normal
Vanadium (ppb)	1.98	Normal	11.9	Normal	14.8	Normal	3.67	Normal
Chromium (ppb)	44.5	Normal	116.4	Normal	78.3	Normal	162.3	Normal
Manganese (ppm)	2.66	Normal	0.108	**Deficient**	6.80	High Normal	4.41	Normal
Iron (ppm)	321.3	Normal	499.0	Normal	570.0	Normal	558.8	Normal
Cobalt (ppb)	49.7	Normal	4.89	Normal	55.3	Normal	61.7	Normal
Nickel (ppb)	35.9	Normal	34.1	Normal	23.0	Normal	43.6	Normal
Copper (ppm)	102.3	High Normal	1.20	**Deficient**	39.6	Normal	143.3	High Normal
Zinc (ppm)	29.7	Normal	2.15	**Deficient**	110.5	Normal	22.9	Normal
Arsenic (ppb)	1.28	Normal	1.97	Normal	7.56	Normal	8.54	Normal
Selenium (ppm)	0.363	**Marginal**	0.142	**Deficient**	0.411	**Marginal**	0.599	Normal
Strontium (ppb)	262.8	Normal	306.1	Normal	233.1	Normal	494.0	Normal
Molybdenum (ppm)	0.379	Normal	0.018	Normal	1.22	Normal	0.846	Normal
Cadmium (ppb)	221.3	Normal	33.3	Normal	1332	Normal	445.0	Normal
Tin (ppb)	4.95	Normal	3.09	Normal	2.92	Normal	109.1	Normal
Antimony (ppb)	0.452	Normal	<7.00 ppt	Normal	0.480	Normal	0.380	Normal
Barium (ppb)	112.6	Normal	137.8	Normal	829.0	Normal	553.0	Normal
Thallium (ppb)	1.20	Normal	0.350	Normal	0.800	Normal	1.43	Normal
Lead (ppm)	0.031	Normal	0.016	Normal	0.121	Normal	0.059	Normal
Bismuth (ppt)	<1.00	Normal	<1.00	Normal	<1.00	Normal	<1.00	Normal
Mercury (ppb)	175.2	High Normal	3.52	Normal	340.1	High Normal	604.5	High Normal

4.4 Discussion

Using a combination of mortality site visits and non-invasive fecal sampling this ongoing project is providing new knowledge and understanding of factors which may adversely affect declining woodland caribou populations in west-central Alberta. Through collaborations with ongoing caribou health research programs in BC, the NWT and elsewhere we are also contributing towards a broader understanding of the role that health may play in woodland caribou population dynamics across their distributional range.

The bacterial pathogen *Erysipelothrix rhusiopathiae* was first identified in tissues collected from a number of radio-collared boreal caribou from NE British Columbia which died during a period of unusually high mortality in the spring through fall of 2013 (reviewed in Schwantje *et al.* 2014). Although this pathogen had been previously identified as the cause of severe disease and mortality in moose, deer, muskoxen, and European reindeer this was the first record of *E. rhusiopathiae* in North American caribou. Since this initial finding the BCHRP has been critically evaluating *E. rhusiopathiae* as a potential cause of morbidity and mortality in woodland caribou (and moose) in BC. To date evidence of exposure to this pathogen has been recorded in both live-captured and dead caribou and probable evidence of infection causing disease has been detected in dead caribou (including ante mortem seroconversion, recovery of the bacterium, and associated histopathological lesions) (reviewed in Schwantje *et al.* 2014, B. Macbeth pers. comm). We partnered with the BCHRP to evaluate the occurrence of this potentially important novel pathogen in caribou from AB. Our rapid response to the mortalities of radio-collared caribou provided an extremely unique opportunity to obtain high quality tissues for bacteriological and other health related analyses.

We cultured *E. rhusiopathiae* from the tissues of 6 of 11 AB caribou examined to date, including one caribou (F786) which was found intact beside a lake and most likely died as the result of disease (Figure 4.3). The ecology, pathogenesis, and epidemiology of *E. rhusiopathiae* in caribou and other free-ranging ungulates are currently poorly understood. However, based on knowledge from domestic species, infection is most likely to occur following ingestion of the bacteria, and this pathogen may cause an array of adverse effects ranging from chronic arthritis and abortions to per acute septicemia and death. In some species, some animals may carry *E. rhusiopathiae* without developing clinical disease (Wang *et al.* 2010). Although the pathogenesis of *E. rhusiopathiae* is unclear, multiple serotypes (strains) of the bacteria are also known to exist in a variety of species and different virulence factors occurring in different strains are associated with the type and severity of disease seen in infected animals (e.g. chronic vs. per acute fatal) e.g. (Bender *et al.* 2011; Ho *et al.* 2012). Moreover, multiple genotypes of *E. rhusiopathiae* may be found in the same animal which may suggest that some are host adapted while others may be associated with a range of disease states. Importantly, clinical disease caused by *E. rhusiopathiae* is often associated with "stress" (e.g. environmental stressors, co-infections with other pathogens) or compromised immunity and may occur in both asymptomatic carriers and newly infected hosts (Wang *et al.* 2010). *Erysipelothrix rhusiopathiae* infections are also known to be transmitted between different species (Wang *et al.* 2010). The BCHRP has also identified this bacterium in moose (B. Macbeth pers comm) and we are pursuing similar analyses in moose, deer, and elk that inhabit AB caribou ranges. Complete genome sequencing has been performed on all *E. rhusiopathiae* isolates collected from AB caribou and will be used to advance our understanding of the overall importance of this bacterium in free-ranging caribou in Year 2.

Our assessment of parasites within caribou feces detected a low prevalence of nematodes (strongyles or Nematodirinae) and was similar to results reported for boreal caribou in NE British Columbia (14% infected, Schwantje *et al.* 2014). Freezing of feces destroys strongyle eggs, thus although presence can be determined, absence and quantification is not reliable in previously frozen feces. Prevalence of *Moniezia* spp. (tapeworm) infection in west-central Alberta (12%) was similar to that reported for boreal caribou in British Columbia (8% infected, Schwantje et al. 2014). Using fecal samples collected during the winter of 2015/16 we will augment this dataset in the coming year. In addition, because prevalence and intensity of gastrointestinal nematodes may vary with respect to life history stage and season, additional data collected in year two will allow us to assess patterns of parasite infections between sexes, reproductive status, and throughout the winter.

It should also be noted that although the eggs of some gastrointestinal parasites (e.g. cestodes, *Nematodirus* spp., *Nematodirella* spp., and *Marshallagia*) are relatively resistant, the eggs of other important gastrointestinal parasites (e.g. the strongyles *Ostertagia* and *Teladorsagia*) are adversely affected by freezing and freeze thaw cycles. All fecal samples evaluated in this pilot study had been stored frozen for at least one month prior to analysis and it is likely that these results underestimate the true prevalence and intensity of strongyle infections in caribou from west-central AB. Similar considerations are likely for other parasites (e.g. *Dicytocaulus*, a lung worm) larvae of which may also be found in caribou feces, but are killed by freezing. The collection and evaluation of fresh, or formalin fixed, fecal samples will be pursued opportunistically to more accurately establish gastrointestinal parasites baselines in our study herds.

Prevalence of DSLs in caribou from west-central Alberta in 2014-15 (16%) were lower than previous records from west-central Alberta in 1976-1982 (28.4%, Gray & Samuel 1986) and British Columbia (35%; Schwantje *et al.* 2014), but higher than previous records from north-east Alberta (8.3%; Gray and Samuel 1986). Like previous research we found that the intensity of infection varied across herds (Gray & Samuel 1986; Schwantje *et al.* 2014). *Paraelaphostrongylus odocoilei*, a muscle worm commonly found in mule deer, was previously reported in caribou feces collected in west-central Alberta (Gray & Samuel 1986). Ongoing molecular identification of DSL will confirm the identity of species recovered in this study. The occurrence, prevalence, intensity and identify of DSLs will continue to be evaluated in year two of this research.

In 1990 Welch *et al.* identified 4, 8, and 132 ticks on hides collected from three free-ranging woodland caribou in west-central AB (caribou from the A La Peche and Little Smoky herds). In this study we recovered 95 mature winter ticks from a single 10 x 10 cm section of hide obtained from caribou 964 (LSM) representing a ten-fold greater infestation. Although not explicitly quantified, 100's of mature winter ticks were also observed on the carcass of the male ALP caribou killed on HWY 40 in 2014 (B. Macbeth pers comm.). This level of infestation has recently been noted in both live-captured and dead boreal caribou from NE BC, where some heavily infested animals have also been recorded with significant hair loss and in poor body condition (Figure 4.2; B. and D. Culling Diversified Environmental Services Inc. pers comm). Although little work has been done on the impact of winter tick in caribou, the effects on moose health and survival is well established. *Dermacentor albipictus* infestations are irritating and excessive grooming may lead to significant hair loss in heavily infected animals (Samuel 1991; Mooring & Samuel 1998). Grooming behaviour may also interrupt foraging and together these responses may lead to a decrease in body condition and a diminished probability of overwinter survival in affected individuals (Glines & Samuel 1989; Mooring & Samuel 1998). Heavy infestations may also cause anemia (due to blood loss) and winter ticks have recently been found to carry and transmit microorganisms which have the potential to cause severe/fatal disease in cervids (Baldridge *et al.* 2009). Climate change leading to longer, drier, and warmer periods in autumn and earlier snowmelt in spring is predicted to improve conditions for winter ticks (Drew & Samuel 1986), and may increase the risk of infestation and related disease in woodland caribou in the near future. Likewise, recent landscape change may also enhance the risk of winter tick transmission to caribou due to an increase in the number of moose inhabiting caribou range.

We found that most caribou examined had experienced at least some level of nutritional stress prior to death (femur marrow fat <85%). Most bone samples were collected in the spring and it is probable that these findings reflect nutritional stress experienced during the preceding winter in the majority of cases. Caribou 964 (marrow fat 17.9%) was approaching levels considered to reflect starvation (marrow fat <12%) which may have been related to the heavy winter tick infestation or extensive tooth wear in this aged animal. Caribou F793 and ALP_1 were also in very poor condition (marrow fat % 31.6 and 44.4%). We will continue to evaluate marrow fat and features of caribou with varying levels of marrow fat in year two of this study.

To our knowledge this is the first study which has evaluated trace nutrient levels and toxicology in caribou from west-central AB. In domestic and free-ranging ungulates, trace nutrients are critically important (but often overlooked) determinants of immunity, health, growth, reproductive output, and survival (e.g. Hyvarinen *et al.* 1977; O'Hara *et al.* 2001; Murray *et al.* 2006). Although the number of liver samples we were able to analyze was small, our preliminary findings suggest that trace nutrient deficiencies may occur in some caribou from west-central AB. The trace nutrient status of caribou may also vary across herd ranges. The multiple deficiencies recorded in caribou F448 are notable. The high-normal mercury values recorded in caribou from the RPC, LSM and NAR herd ranges are unlikely to have been clinically significant. Further research into the trace nutrient status and toxicology of both live and dead caribou from west-central AB is warranted to clarify the overall importance of these findings.

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