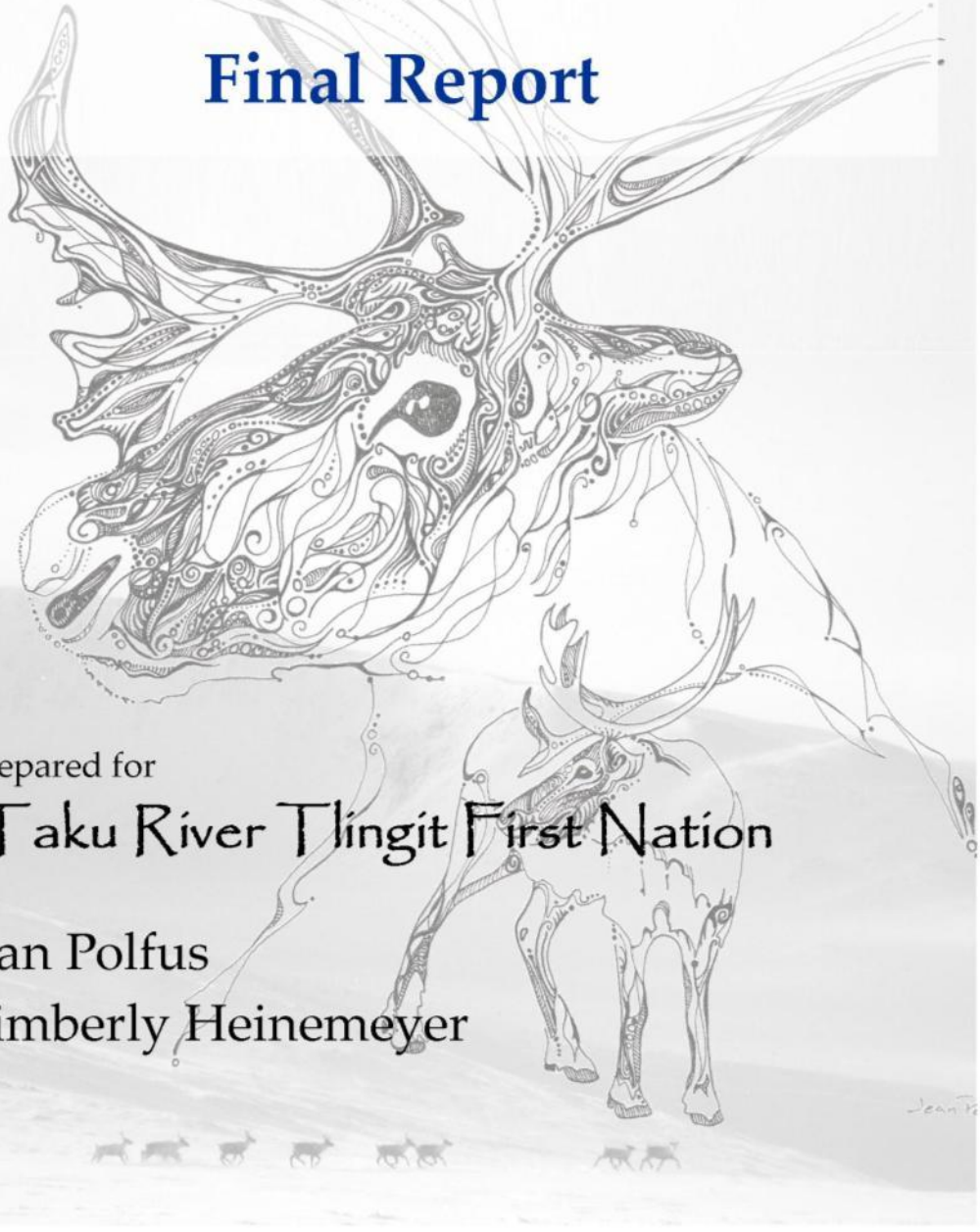
Atlin Northern Mountain Caribou Management and Monitoring Framework:

Final Report



Report prepared for

The Taku River Tlingit First Nation

by Jean Polfus
Kimberly Heinemeyer

June 2011

ATLIN NORTHERN MOUNTAIN CARIBOU MANAGEMENT AND MONITORING FRAMEWORK: FINAL REPORT 2011

Project Components

1. Habitat Modeling
2. Cumulative Effects Toolkit
3. Caribou Pregnancy
4. Predator Diet Stable Isotope Analysis
5. Lichen Sampling

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COVER PHOTO CREDITS
Jean Polfus (top left, TRTFN Territory sign, bottom left, bottom middle, middle), Wibke Peters (bottom right), Kevin Cannaday (caribou on road). Drawing of caribou by Jean Polfus.

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Taku River Tlingit
First Nation

Round River
Conservation Studies

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STUDY AREA

This study takes place within the 48,000 km^2 traditional territory of the Taku River Tlingit First Nation (TRTFN) in the Skeena region of northwest British Columbia (BC; Figure 1). Historically, tens of thousands of Tlingit maintained camps from Atlin Lake to the lower Taku River near Juneau, Alaska (McClellan 1981). During the Klondike gold rush of 1898, the Tlingit village of Atlin (59° 35' N, 133° 40' W), was populated by over 10,000 miners. Today there are approximately 450 residents in Atlin. While most of the territory remains roadless, extensive dirt roads and ATV trail systems connect local logging operations and placer and hardrock mines. The TRTFN are committed to the sustainable governance and stewardship of their land and wildlife (Taku River Tlingit First Nation 2003), and in the spring of 2008 they entered joint land use planning and wildlife management with the government of BC by establishing the *Framework Agreement for Shared Decision Making Respecting Land Use and Wildlife Management* (described in: TRTFN/BC 2008). This agreement set the stage for government-to-government discussions related to land use planning, collaborative wildlife management planning and the establishment of shared decision-making arrangements. In the fall of 2010 the TRTFN and BC developed a strategic land use plan for the Atlin Taku. The land use plan will provide a framework for culturally and ecologically sustainable management of land and resources and establish designated resource management zones.

The TRTFN territory falls within the boreal mountains and plateaus ecoregion which covers northwestern BC and southern portions of the Yukon Territory (Environment Canada 2005). Mountain ranges with high peaks, broad plateaus and wide valleys characterize this ecozone. Elevations range from 660 to 2000 m. The climate is typified by long, cold winters and short, warm summers. The mean summer temperature is 10°C and the winter mean is -15°C (Environment Canada 2005). The coastal mountains remove moisture from prevailing Pacific westerly winds creating a rain-shadow effect. Annual precipitation in Atlin is approximately 33 cm (MacKinnnon et al. 1999) resulting in an average late winter snow depth of 49.5 cm, that is low compared to other regions of northern BC that can average 80 cm or more (Atlin snow station 1964-2003). Low to mid-elevation boreal forests include a mix of lodgepole pine (*Pinus contorta latifolia*), subalpine fir (*Abies lasiocarpa*) and white and black spruce (*Picea glauca* and *P. mariana*). Deciduous stands of trembling aspen (*Populus tremuloides*), black cottonwood

(*Populus balsamifera trichocarpa*), alder (*Alnus tenuifolia*) and willow (*Salix* spp.) occupy valley bottoms and riparian areas. The understory commonly consists of low shrubs and lichen species including various reindeer (*Cladina* spp.), pixie-cup (*Cladonia* spp.), foam (*Stereocaulon* spp.) and Iceland lichens (*Cetraria* spp.) and numerous forbs and mosses. White spruce and subalpine firs dominate the subalpine from 850-1500 m transitioning at mid elevations into krummholz where thick knee high spreads of willow and scrub birch (*Betula glandulosa*) dominate. Alpine habitats (above 1500 m) consist of extensive areas of rolling alpine tundra characterized by sedge and altai fescue (*Festuca altaica*) dominated meadows. Mountain heather (*Cassiope* spp.), crowberry (*Empetrum nigrum*), mountain avens (*Dryas* spp.) and lichen communities are also common.

The Atlin northern woodland caribou (*Rangifer tarandus caribou*) herd's range encompasses 11,594 km^2 east of Atlin Lake to Teslin Lake along the Yukon-BC border (Figure 1). The herd relies heavily on low-elevation mature lodgepole pine forests in the winter and high elevation alpine and subalpine habitats in the summer (Heinemeyer et al. 2003). In addition to the 555 ± 97 caribou in the Atlin herd (Marshall, BC Ministry of Environment, personal communication), other ungulates in the area include moose in valley bottoms, and mountain goats (*Oreamnos americanu*) and Stone's sheep (*Ovis dalli stonei)* in alpine habitats. The predator community consists of grizzly bears (*Ursus arctos*), black bears (*Ursus americanus*), wolverines (*Gulo gulo*), wolves, lynx (*Lynx canadensis*), martens (*Martes americana*), and fishers (*Martes pennanti*). Caribou have always been a culturally important source of meat and other animal products for the TRTFN and TEK indicates that the herd once numbered in the tens of thousands (Heinemeyer et al. 2003). As caribou numbers declined in the early 20^{th} century with the advent of firearms (Spalding 2000), many First Nation hunters switched to moose as a primary game species. In the early 1990s, concerns for population declines of the Atlin caribou herd and the Carcross/Squanga and Ibex herds (collectively known as the Southern Lakes population) led many First Nation hunters to reduce or eliminate their harvest of caribou. Monitoring efforts indicate that the two Yukon herds appear to be recovering, while aerial surveys indicate that the Atlin herd has maintained a stable or decreasing population with a low calf recruitment of 21 ± 3 calves:100 females (Bergerud and Elliott 1998, Heinemeyer 2006). The province currently allows a limited entry hunt and guide-outfitter quota of 10 males/year.

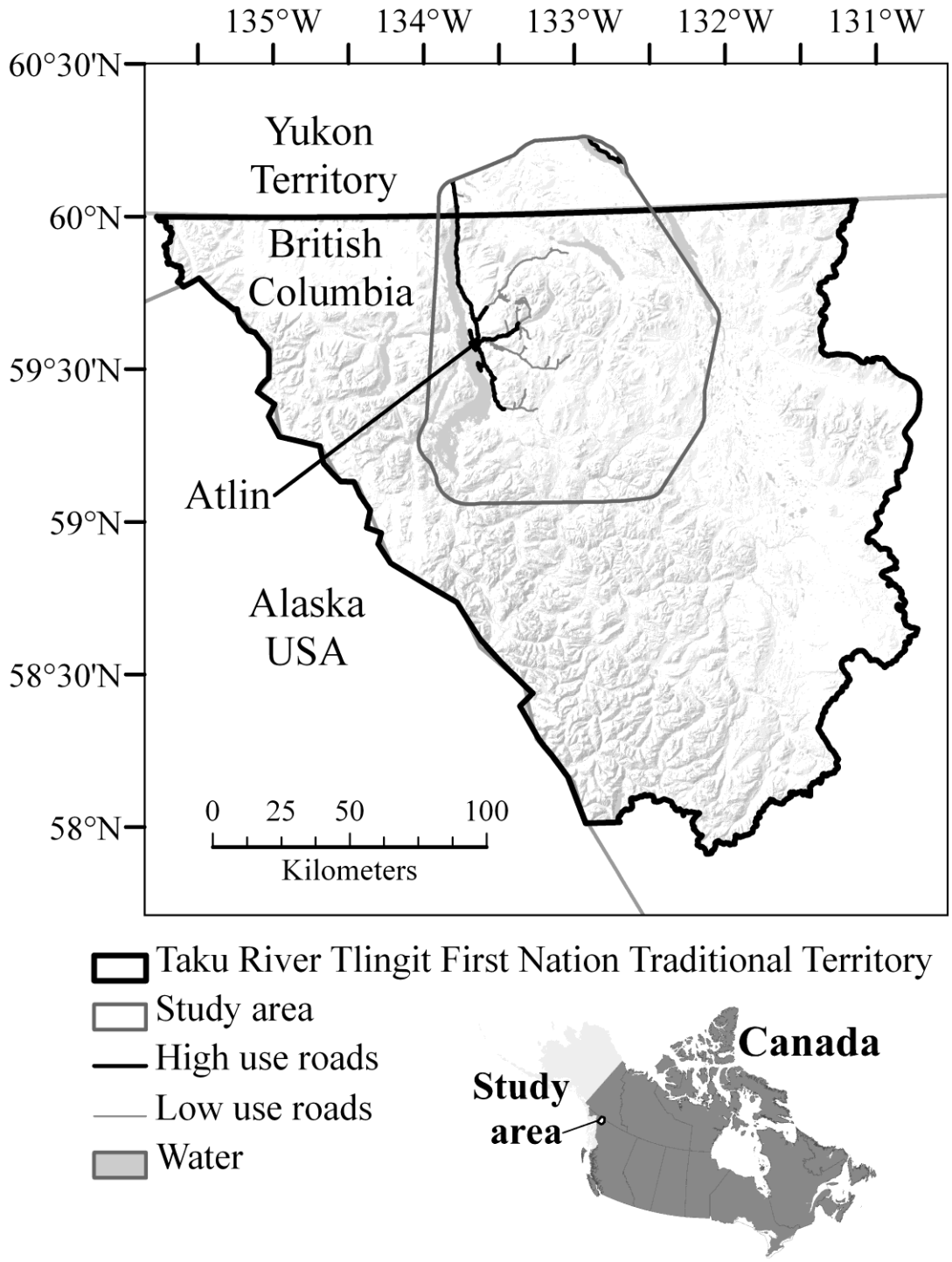
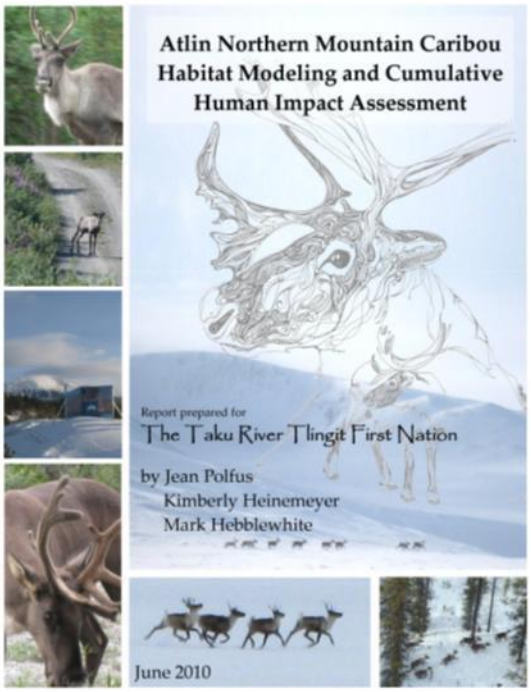


Figure 1. General location of the 11,594 km^2 home range of the Atlin northern mountain woodland caribou herd in the Taku River Tlingit First Nation territory on the boarder of the Yukon Territory and British Columbia, Canada.

1. HABITAT MODELING

Caribou habitat models were developed as part of a project in collaboration with the TRTFN, the University of Montana and Round River Conservation Studies from 2008-2010 (Polfus et al. 2010). The project objective was to use an innovative combination of habitat modeling approaches to determine the effect of cumulative human developments on the Atlin herd of northern mountain woodland caribou. To support this effort, we collaborated with the University of Calgary to develop an updated landcover classification for the range of the Atlin herd based on satellite imagery. The landcover classification improved on previous forest cover land models, and the new product was used in the development of seasonal caribou habitat models.



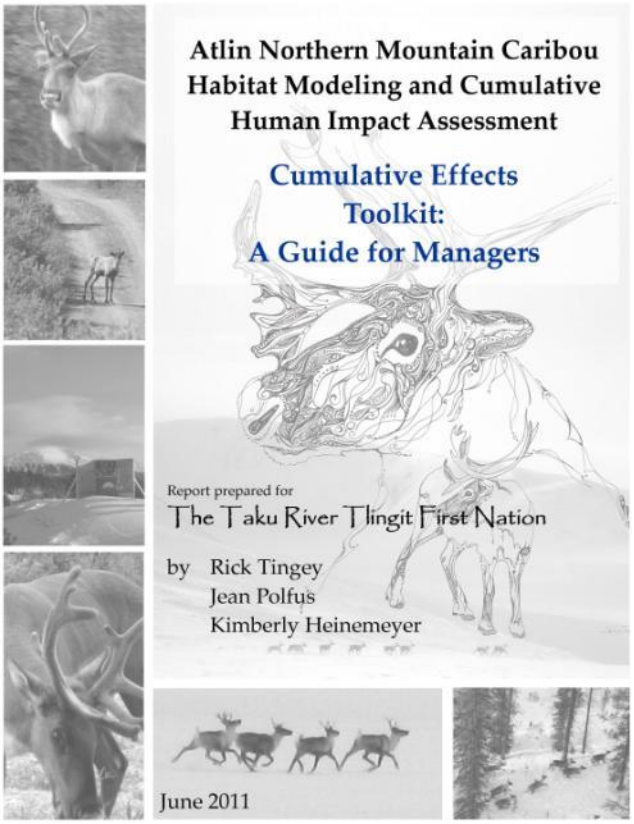
Habitat models were developed with data from GPS collared-caribou as well as with information from the Traditional Ecological Knowledge (TEK) of the TRTFN. The results of these models are available in a final report and will also be published in two academic peer-reviewed journals. Currently, results of the resource selection analysis are in review in the Journal of Biological Conservation (Polfus et al. *in review*).

The report can be found at: http://www.roundriver.org/index.php?option=com_content&view=article&id=67&Itemid=57.

2. CUMULATIVE EFFECTS TOOLKIT

There is a growing need to understand how potential future development might affect habitat selection of the Atlin herd. A GIS-based toolkit that uses information from previously developed habitat models for the Atlin herd (described in the previous section) was developed by Rick Tingey. This toolkit assesses the influence of new human infrastructure on the statistical habitat models and predicted the future reduction in habitat quality. This tool will allow managers, such as the TRTFN, to make informed decisions about the effects of proposed projects by examining the effects of future development scenarios before development occurs. The interface with ArcGIS is intended to allow easy updating of human infrastructure layers, including potential or proposed projects to determine how these projects will alter the underlying habitat quality. This provides a dynamic evaluation of proposed projects on potential caribou habitat through simple metrics that measure the loss of habitat quality.

A full report and guide for managers is available.



3. CARIBOU PREGNANCY

Understanding female ungulate pregnancy status is an important component for studying long-term population demography. Knowledge of pregnancy rates and reproductive intervals provides researchers and managers with a useful tool for detecting changes in population growth rates and calf survival (Cook et al. 2002). Analysis of metabolites progesterone (P_4), pregnanediol-3-glucuronide (PdG), and estrone conjugates (E_1C), from collected fecal samples during late pregnancy stages (March and April) has been used as a non-invasive method of detecting pregnancy status (White et al. 1995, Garrott et al. 1998, Berger et al. 1999, Stoops et al. 1999). Messier et al. (1990) found that estrogen levels of pregnant female caribou (*Rangifer tranadus*) were highly distinctive during the last trimester of pregnancy.

Methods

To collect fecal pellets for pregnancy testing we located small groups of female caribou from a helicopter. We attempted to observe caribou at a distance to roughly discern sex and age groups to avoid collection of pellet samples from males. Collection of fecal pellets focused on the freshest (wet) samples (Figure 2). We attempted to limit our sample to adult female pellets by observing herd composition and avoiding both unusually large (males) and

Figure 2. Collection of caribou scat sample in the field.

small (yearling calves) pellets. Some samples were also collect opportunistically from the road when caribou were observed close enough to obtain scat by foot. We collected 10-15 pellets per individual pile along individual caribou paths. Pellets were placed in a ziploc bag with the sample ID written on the bag. Samples were stored at -20°C as soon as possible and kept frozen at all times. To rule out collection of male samples and multiple samples from the same individuals we sent samples for genetic testing to Wildlife Genetics International. Two to three fully formed pellets were selected from each Ziploc bag and placed in a 15mL disposable sterile leak-proof centrifuge tube filled with 95% ethanol following protocol in Maudet et al. (2004). Another set of samples (4-5 pellets each) were shipped frozen (on dry ice) to the Toronto Zoo Reproductive Physiology Lab (see Appendix B). Analysis of pregnancy hormones was conducted on samples known to be individual female caribou following genetic sampling. Extra samples from each individual were kept frozen in Atlin as backup.

Results

2009

A total of 109 samples were collected from March 8–10 in 2009 (Table 1). Wildlife Genetics International determined that 20 samples were male and 88 samples were female using a pair of genes (ZFX and ZFY) that occur on both X- and Y-chromosomes. Of these 88 females, 7 microsatalite locus (*BL42, BMS745, CRH, NVHRT16, OheD, Rt1, Rt27*) were used to identify 59 individual female caribou. The 7 markers had good variability, with heterozygosity in the 80% range. The 59 individual female samples were tested for progesterone levels using enzyme immunoassay following extraction of progesterone metabolites from wet feces at the Toronto Zoo Reproductive Physiology Lab. Samples from 51 females had progesterone levels ranging from 760.9 – 2684.9 ng/g feces and were considered pregnant. Studies have shown that baseline progesterone levels can vary among the different *Rangifer* subspecies, however, levels in pregnant females are consistently 10-fold higher than non-pregnant females. Samples collected from a captive reindeer herd (*Rangifer tarandus tarandus*) at the Toronto Zoo in March 2009 were run to confirm baseline values and validate the assays for the Atlin herd. Progesterone values in the non-pregnant, non-cycling female reindeer ranged from approximately 50 – 200 ng/g feces. Non-pregnant female caribou in the Atlin herd had progesterone levels ranging from 59.4 – 125.6 ng/g feces.

2010

A total of 89 samples were collected from March 9–12 in 2010. Wildlife Genetics International determined that 10 samples were male and 79 samples were female. The multilocus microsatellite analysis used 6 of the 7 markers from 2009. The 7th marker (*BL42*) was not necessary from the perspective of match probability and was relatively error-prone due to the presence of weakly amplifying alleles. The results were strong and all 79 samples had high confidence scores for all 6 markers. Of the 79 female samples, 52 were identified as individual females, 6 of which were 'recaptures' from 2009 (Table 2). The 52 individual female samples were tested for progesterone levels at the Toronto Zoo Reproductive Physiology Lab. Samples from 51 females had progesterone levels ranging from 1265.6 – 5959.6 ng/g feces indicative of pregnancy. Only one non-pregnant female caribou was observed with a progesterone level of 119.6 ng/g feces.

Table 1. Fecal pellet collection and caribou identified during flights in the Atlin area in March 2009 and 2010.

Date	# of Samples	Total # animals	Bulls	Cows	Calf	Uncl	Comments
3/8/2009	9	6				6	On river bend
3/8/2009	16	16	5	9	2		Running through the trees
3/8/2009	8	7	1	6			Standing on lake
3/8/2009	2	2		2			Two cows walking along lake
3/9/2009	10	13	2	9	2		In trees standing and lying down
3/9/2009	10	4		4			Running in trees below lake
3/9/2009	7	9	1	7	1		Running in trees below lake
3/9/2009	5	4	1	2	1		Standing in opening near marsh
3/10/2009	42	52	8	30	4	10	Large group in the alpine area, some moved off before we could classify
Sub-total 2009	**109**	**113**	**18**	**69**	**10**	**16**	
3/9/2010	1	2	2				Two bulls on the road
3/9/2010	3	3		3			Three females on road
3/8/2010	2	2		2			Two females on the road
3/10/2010		5		5			Running through the trees, no place to land nearby
3/10/2010		5		5			Running through the trees, no place to land nearby
3/10/2010	7	5	2	3			Moving through marsh eating overflowed ice. Not a lot of scat in the area
3/10/2010	18	10		9		1	On lake eating ice and muskrat pushups
3/10/2010							
3/10/2010		6	6				All bulls
3/10/2010	7	5		4		1	
3/10/2010	22	14	1	13			In woods and edge of lake
3/10/2010							
3/11/2010	11	10	2	8			Two yearling bulls, with group of females, on lake digging to lick ice
3/11/2010	6	6		5	1		In marsh at end of lake. F23 could be calf
3/11/2010	10	8		8			Eight females on lake licking ice, took ice samples as well
3/12/2010	2						Scat collected, no caribou observed, but sign fresh, many animals
Sub-total 2010	**89**	**81**	**13**	**65**	**1**	**2**	
Grand Total	**198**	**194**	**31**	**134**	**11**	**18**	

Table 2. Individual female caribou pregnancy status for 2009 and 2010. Black progesterone numbers indicate pregnancy, red numbers indicate non-pregnant females. Six caribou were sampled in both 2009 and 2010 and all were pregnant both years.

	Progesterone (ng/g wet feces)	
Individual	**2009**	**2010**
A1	2684.48	
A2	2641.56	
A3	2273.55	
A4	1573.48	
A5	2481.2	
B101	1587.25	
B102	**62.12**	
B103	820.41	
B11	2441.61	
B13	1799.8	
B15	1665.5	
B18	1693.19	
C22	1566.29	
C28	920.59	
C30	1386.42	
E101	1737.77	
E33	2609.46	
E35	2083.32	
E36	**64.9**	
E37	2072.26	
E38	1703.58	
F102	760.89	
F42	1819.82	
F43	999.71	
F46	999.71	
G101	2342.7	
G102	2684.9	
G50	2002.11	
G51	1596.89	
G55	1200.33	
H101	**71.81**	
H61	**106.49**	
J102	2046.53	
J103	**59.4**	
J104	1546.21	
J105	1450.78	

	Progesterone (ng/g wet feces)	
Individual	**2009**	**2010**
J107	**59.84**	
J404	846.05	
J70	2434.75	
J71	1535.04	
J72	1417.5	
J74	2436.99	
J75	2377.34	
J76	1078.5	
J78	2322.89	
J80	**81.07**	
J85	1296.06	
J87	2095.22	
J88	**125.57**	
J91	2094.58	
J92	1949.86	
J93	1349.49	
J95	1786.15	
B17	2018.39	4708.72
C26	1631.4	2704
C29	1597.34	1810.91
G52	1528.06	2571.98
J402	1457.21	1581.36
J89	992.13	1741.15
A11		4890.11
A21		5209.83
A22		5959.62
A2b		3376.26
B1		1500.4
B10b		1957.26
B12		1752.78
B2		1450.48
B27		1862.07
B5		1265.59
C1		3583.95
C10		3371.08
C11		3637.1

	Progesterone (ng/g wet feces)	
Individual	**2009**	**2010**
C2		3451.69
D1		1728.44
D10		**119.61**
D11		3631.2
D12		1695.67
D17		2521.3
D21		2829.28
D22		1706.48
D23		3031.13
D24		3384.75
D25		3786.22
D5		2909.71
E1		1670.12
E10		3349.35
E11		3545.72
E12		3973.02
E21		2024.09
E3		2144.5
E4		2410.65
F21		4213.7
F22		4034.3
F25		1347.77
G1		1720.65
G10		2240.75
G11		3073.09
G2		1951.46
G3		3482.09
G5		3783.51
G6		3540.66
H2		1682.86
J1		2154.2
J2		1881.16
L1		3159.48
Total Females	**59**	**52**
Total Pregnant	**51**	**51**

Discussion

This study confirms findings from other studies on woodland caribou that suggest that between 88-100% of adult female caribou become pregnant each year (Seip and Cichowski 1996, Rettie and Messier 1998, Mahoney and Virgl 2003, McLoughlin et al. 2003, Wittmer et al. 2005, Gustine et al. 2006). Our results indicate that between 86 and 98% of female caribou were pregnant in the Atlin area. Though we attempted to avoid small pellets indicative of young animals, it is probable that a few of the female pellets sampled were from calves from the previous spring. Caribou calves are unlikely to become pregnant in their first year (at 4-6 months of age) and thus would not be expected to be pregnant in March. Thus, female calf samples would bias our pregnancy rates low, suggesting that almost all adult females were pregnant. Because pregnancy rates are high, the low numbers of calves observed during flights, especially in 2010 (Table 1) is concerning. More research is needed to determine why calf survival and recruitment appears to be low in the Atlin area. Stable isotope analysis (section 4) may help determine if calf survival is related to predation by wolves or bears.

4. PREDATOR DIET STABLE ISOTOPE ANALYSIS

Jean Polfus, Ethan Rubenstein and Leif Olson

Woodland caribou (*Rangifer tarandus caribou*) are declining across Canada. The northern mountain ecotype occurs in local populations throughout the Yukon, Northwest Territories and northwestern British Columbia. The northern mountain population was assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2002 and listed as a species of *special concern* in 2004 by the Species at Risk Act (SARA). There is considerable evidence that woodland caribou populations are limited by predation (Stuart-Smith et al. 1997, Bergerud and Elliott 1998, McLoughlin et al. 2003). Almost all northern mountain caribou populations exist in multi-predator, multi-prey systems. Caribou are known to use an "isolation" strategy to avoid predators by spatially segregating themselves from other prey species and predators. By maintaining low population densities caribou may reduce their risk of incidental detection by predators (Stuart-Smith et al. 1997). There is concern that the spatial segregation tactic used by caribou to decrease predation risk is not sufficient in human altered systems. Human development can alter predator-prey relationships by providing young seral forests that are preferred by moose *(Alces alces)* and wolves *(Canis lupus)* which increases caribou vulnerability to predation (James and Stuart-Smith 2000, COSEWIC 2002, James et al. 2004, Environment Canada 2007). Linear developments such as roads and seismic lines may also increase the mobility of wolves. In northeastern Alberta, James and Stuart-Smith (2000) found that caribou have higher risk of predation from wolves near linear corridors. Seismic lines, which have low human use, may be preferentially used by wolves, increasing their travel efficiency and the ease of caribou detection. Even a small increase in predation through altered spatial relationships between caribou, predators and alternate prey could lead to population level effects in herds with low growth rates.

The draft recovery plan for northern mountain caribou calls for an increased understanding of the dynamics of multi-species predator-prey systems and competition with other herbivores. For example, in east-central Yukon, Hayes et al. (2000) found that moose composed 94% of the biomass of ungulates killed by wolves. Wolves did not prey heavily on caribou even when caribou outnumbered moose. Similarly, in the North Columbia Mountains in southeastern BC, Stotyn (2008) found that the relative proportion of caribou within wolf diet was

not related to caribou density. Rather, caribou may use spatial or temporal refuges to avoid wolves, or wolves may preferentially prefer moose and other prey items. However, more information is needed to understand which predators may be limiting caribou populations and how the levels of human development and alternative prey species affect these predator-prey interactions in the Atlin area.

Stable isotope analysis can be used to understand predator-prey dynamics. Stable isotope ratios (δ^{15}N and δ^{13}C) have recently been used to describe relative fitness of ungulate prey species (Darimont et al. 2007), diet composition of predators (Mowat and Heard 2006), trophic relationships (Urton and Hobson 2005) and interspecific interactions (Caut et al. 2006) in mammalian predator-prey systems. Stable isotope assays can provide a continuous measure of feeding ecology. Different ^{13}C and ^{15}N isotope signatures can be used to determine the relative contribution of different foods to an animal's diet (DeNiro and Epstein 1978,1981). Metabolically inactive tissue, such as hair, reflects diet during its growth phase and so can represent diet up to many months. Previous studies have determined wolf diets using hairs (Darimont and Reimchen 2002, Urton and Hobson 2005). Noninvasively sampled guard hairs can be collected during the annual molt between May and July, and contain a record of a wolf's diet for the period of hair growth of the previous year. Grizzly bear (*Ursus arctos*) and black bear (*Ursus americanus*) hair contains similar information since bears also molt once a year (Jacoby et al. 1999). To examine predator diets, baseline prey signatures are first established for each potential prey species through analysis of prey hair samples. Prey values are then compared to the isotope values of the predators to determine what proportions of prey species contributed to the total dietary composition (Urton and Hobson 2005, Stotyn et al. 2007). To determine the trophic relationships for northern mountain caribou herds we will perform stable isotope analysis on hair samples from wolves, bears (grizzly and black) and prey species.

Methods

Predator hair samples were collected using non-invasive hair snares set up in various locations throughout the study area (Figure 3). Hair was also opportunistically collected in the field on rub trees, at kill sites or from local hunters and trappers when available. Wolf hair was collected with non-invasive rub pads (Figure 4). We based our wolf hair snare design on a study conducted by Fannin and Ausband (2009) of the Montana Cooperative Wildlife Research Unit in

Missoula, Montana. The wolf snares were constructed out of a 33 x 14 cm piece of plywood with two metal-bristled brushes (barbeque grill cleaning brushes) attached to each end with wire wrapping around the plywood. We buried the plywood part of the hair snare to help avoid detection and camouflaged the bristles with brush and debris. As a further precaution we boiled the snares as well as clothes and tools that were used when setting the snares to avoid leaving human scent. The snares were lured using lures purchased from Halfordsmailorder.com. We used the following lures: Forsyth wolf call, Forsyth wolf gland, Forget's cachotier call (canine), freshwater fish oil and commercial wolf urine. We set up snares in areas that appeared to be movement corridors, based on field observations (tracks and scat) and information from local hunters and trappers who had local knowledge of animal locations.

We collected bear hair from rub trees found in the field and also from barbed wire corral stations with lure in the center (Boulanger and McLellan 2001). The corrals were constructed by placing a strand of barbed wire approximately 50 cm from the ground around several trees to create an enclosed area (Figure 4). In the center, a log or clump of large branches was lured with a non-reward bait of homemade mixture of salmon oil, beaver castor and Forget's cachotier call (canine). The height of the barbed wire was intended to force the bears to crawl under it, leaving hair on the barbs. Rub trees were wrapped with a few strands of barbed wire to collect hair as bears naturally used the trees. Bear hair was also collected opportunistically from rub trees that were encountered in the field that were not wrapped with barbed wire. Wolf and bear snares were checked and re-lured routinely, ideally at an interval of ten days.

Prey hair was opportunistically collected from mammalian prey species when we came upon hair in the field at kill sites, hunting camps or from local hunters and trappers. We collected samples from moose, caribou, mountain goat (*Oreamnos americanu*), Stone's sheep (*Ovis dalli stonei)* and beaver (*Castor canadensis*). When hair samples were detected we collected as many strands as possible with tweezers and placed them in small manila envelopes. The envelopes were then placed in plastic bags with desiccant beads to prevent moisture build-up. We collaborated with the Kluane Ecological Monitoring Project in the Yukon who provided snowshoe hare (*Lepus americanus*) hair samples collected during annual monitoring efforts and the Museum of Southwestern Biology at the University of New Mexico who provided small mammal samples (least chipmunk; *Tamias minimus* and northern red backed voles; *Myodes rutilus*) collected in the new Agay Mene Territorial Park near Tarfu Lake just off the Atlin road.

For the bear diet analysis we also collected samples from 15 species of plants important to bears from across the study area (Fuhr and Demarchii 1990, Wellwood 2003, Nielsen et al. 2004). These included above-ground foliage from *Equisetum spp.*, *Taraxacum spp.*, *Trifolium spp.*, *Carex spp.*, *Festuca spp.*, *Heracleum lanatum*, *Lupinus spp.*, *Rosa spp.*, *Senecio triangularis*, and berries from *Amelanchier alnifolia*, *Actaea rubra*, *Arctostaphylos uva-ursi*, *Shepherdia canadensis*, *Vaccinium caespitosum*, *Empetrum nigrum*. Samples were desiccated in a drying oven and ground into a fine powder for stable isotope analysis.

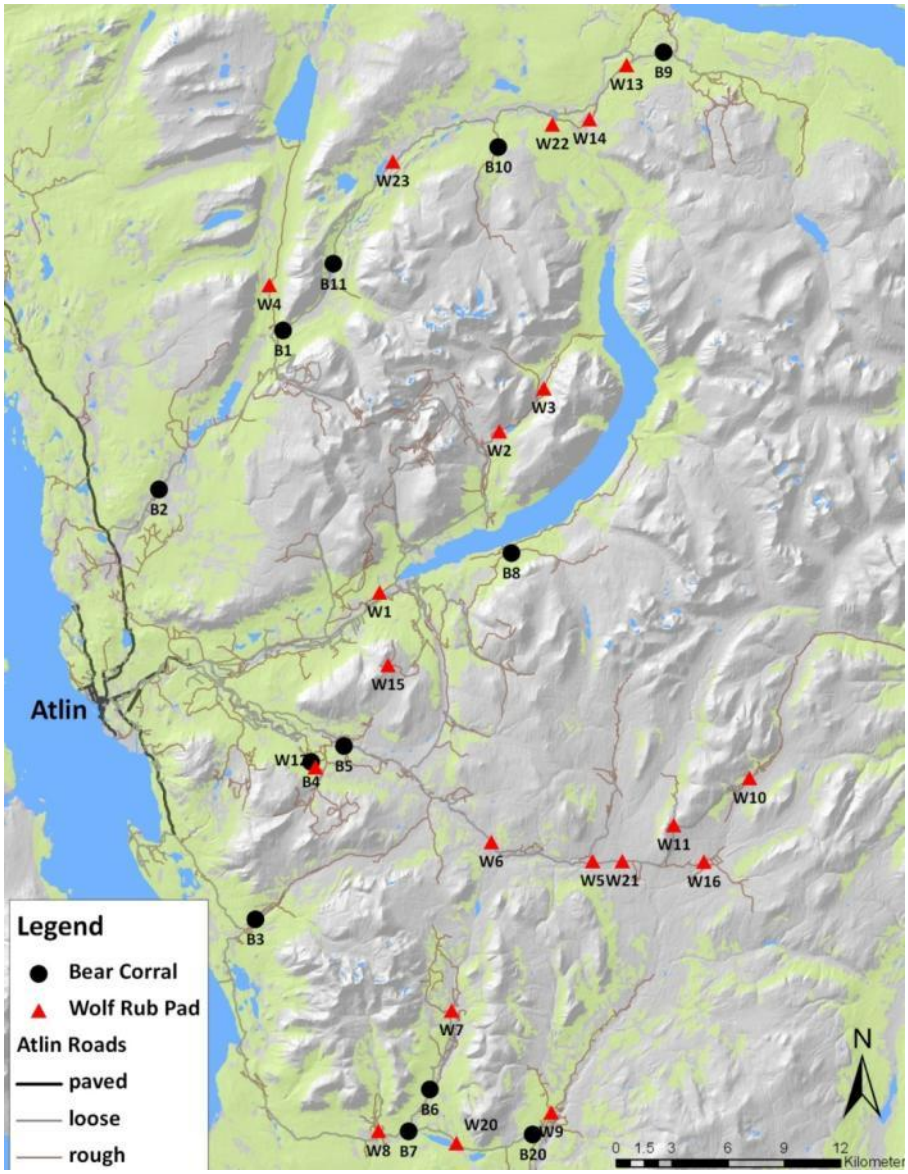


Figure 3. Locations wolf and bear hair snares near Atlin, British Columbia in 2009 and 2010. Bear and wolf snares are denoted by symbol and the letters “B” and “W”, respectively.

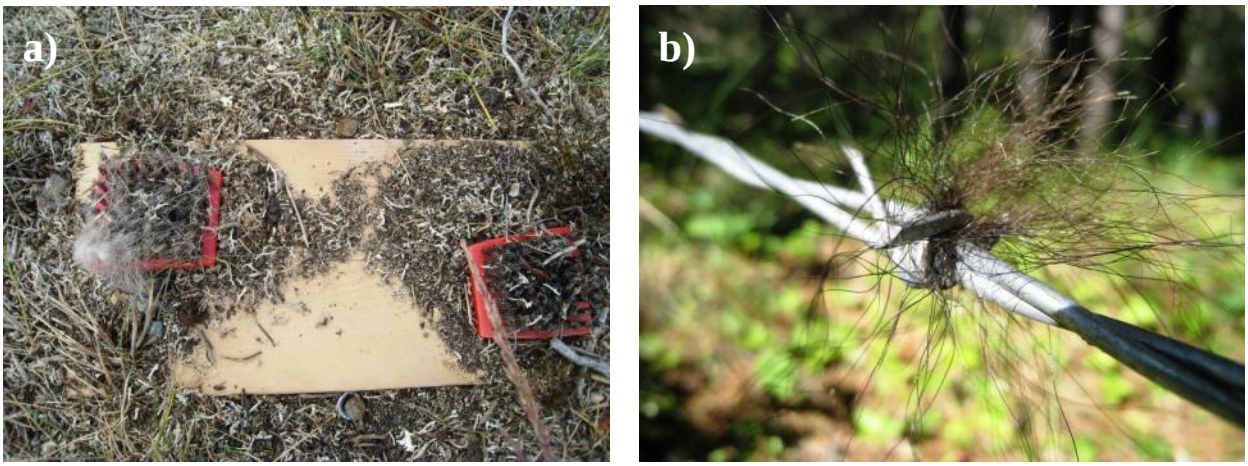


Figure 4. Wolf snare (a) and bear hair on barbed wire corral (b). The wolf snare is disguised under dirt and dead foliage, but is partially visible because it was rolled on (notice hair sample on left bristle).

To confirm the detection of grizzly bear, black bear and wolf hair we performed DNA analysis of hair samples collected at rub pads, bear hair snares and rub trees. Hair samples were sent to the USFS Rocky Mountain Research Station, located in Missoula, Montana, to identify species and individuals. This facilitated the analysis of unique individual bear and wolf samples in the subsequent stable isotope analysis. After genetic analysis, all hair samples were cleaned of surface oils in a 2:1 chloroform:methanol solution for 24 hours and dried at low heat in a drying oven for 24 hours (see Appendix A for full hair preparation methods). Predator hairs were cut into three equal sections representative of different seasons during hair growth (Milakovic and Parker 2011). Assuming carnivore coats grow at a relatively constant rate (Jacoby et al. 1999), the base portion will reflect most recent growth (late summer) and the middle will reflect earlier growth (summer) and the tip will reflect new hair growth (spring/summer). However, wolves and bears have different molt patterns, which are currently debated in the literature. Generally, it is assumed that wolves have one annual molt that begins in late spring when the old coat is shed and new hair grows until late fall (Darimont and Reimchen 2002). Bears likely begin molt in late spring after emerging from the den and continue into the fall (Stotyn et al. 2007). The rate and timing of hair growth is expected to differ between individual bears (B. Milakovic pers. comm.), so by splitting hairs into three equal sections based on each guard hair length each section is representative of a specific time period relative to each bear. This makes comparisons between bears possible assuming that body condition is similar within the population.

Samples were sent to the University of California Davis analytical lab to be cut into small pieces and loaded into miniature tin-cups (5x8 mm; Costech Analytical Technologies Inc., Valencia, CA, USA) for combustion. Replicates were included approximately every 8-12 samples to check the instrument precision when enough hair was available. Stable-isotope ratios of carbon and nitrogen were measured on a continuous flow isotope-ratio mass spectrometer in the Stable Isotope Facility at the University of California Davis. Stable isotopes are expressed in delta notation (δ) in parts per thousand (‰) as $\delta^{13}C$ and $\delta^{15}N$ using laboratory standards (Milakovic and Parker 2011). Bayesian mixing models will be used to determine the proportions of prey in predator diets (Milakovic and Parker 2011).

Results

Hair Collection

During the summers of 2009 and 2010 we collected 127 bears hair samples, 41 wolf hair samples, 21 wolf pelt samples, 4 beaver, 24 caribou, 21 moose, 16 mountain goat, 16 Stone's sheep, 27 snowshoe hare and 37 small mammal samples. After trying multiple wolf lures on our wolf rub pads, we had the greatest success with Forsyth wolf gland. Due to sample quantity and quality only the largest samples of complete wolf and bear hairs were sent to the lab for genetic analysis. Similarly, approximately 10-20 of the highest quality sub-samples of each prey species were selected for stable isotope analysis.

Genetic Analysis

2009

Species Identification: In 2009, DNA extractions were preformed on 42 bear samples and 18 wolf samples. Care was taken to retain at least 10 hair shafts from each sample for subsequent stable isotope analysis. In most cases DNA extraction used only the hair follicle and a minimal amount of the hair shaft. Species identification was performed on all samples using mitochondrial DNA. We obtained DNA for species identification from 27 of the 42 suspected bear samples (64%); 22 samples were from grizzly bear and 5 samples were from black bear. We obtained DNA for species identification from 17 of the 18 wolf samples (94%); and all were

identified as either wolf/domestic dog (*Canis lupus sp*). Since sample collection was remote, we assume that all samples were from wolves.

Individual Identification: All samples identified to species were further evaluated for individual using DNA microsatellite analysis. We used a panel of nine variable loci on bears and eight variable loci on wolves (both the bear and wolf markers had been used previously at the USFS Rocky Mountain Research Station). We obtained high quality DNA from 18 of the 22 grizzly bear hair samples, allowing us to conduct individual identification using the microsatellite panel. Thirteen unique individuals were identified. Four bears were detected from multiple sites and/or dates, while nine individual bears were each detected once. We obtained high quality DNA from all five of the hairs identified as black bear allowing us to conduct individual identification. All five black bear samples were from unique individuals. We obtained high quality DNA for individual identification from 14 of the wolf hair samples. Three wolves were detected from multiple sites and/or dates. Seven other individuals were each detected once from a single sample.

2010

Species Identification: In 2010, DNA extractions were preformed on 22 bear samples and 13 wolf samples. We obtained DNA for species identification from 20 of the 22 suspected bear samples (94%); 8 samples were from grizzly bear, 11 samples were from black bear, and one sample (B118) was a mixed sample of grizzly and black bear. We obtained DNA for species identification from 12 of the 13 wolf samples (93%); and all were identified as (*Canis lupus*).

Individual Identification: We obtained high quality DNA from 7 of the 8 grizzly bear hair samples, allowing us to conduct individual identification using our microsatellite panel. Four unique individuals were identified. One individual (Grizzly _6) was a re-capture of a bear identified from hairs collected in 2009 (both detections were from the "B3 corral"). Three new grizzly bears were identified: "Grizzly _14" was detected from two sites and multiple dates, while "Grizzly_15" and "Grizzly _16" were each detected once. We obtained high quality DNA from 9 of the 11 (82%) hairs identified as black bear allowing us to conduct individual identification. Nine individuals were detected. One black bear (Black_2) was a re-capture of a bear identified in 2009 (both detections were from the "B2 corral"). Eight new black bears were identified, all detected once. We obtained quality DNA for individual identification from 9 of the

12 (75%) wolf hair samples. Five individuals were detected, 3 were re-captures of individuals detected in 2009 and two were new individuals. Three wolves (denoted as Wolf_4, Wolf_7, and Wolf_10) were detected from multiple sites and/or dates. Two other individuals were each detected once from a single sample. Combining the data from 2009 and 2010, this study identified 41 unique individuals: 16 grizzly bears, 13 black bears and 12 wolves (Table 3 and 4).

Table 3. Wolf samples identified with DNA analysis by species (*Canis lupus*) and individual in 2009 and 2010 in Atlin, British Columbia.

sample ID	Species ID	Individual ID	2010 recapture	location	Date
W8	Wolf	Wolf_1		W7	7/24/2009
W17	Wolf	Wolf_1		W11 snare	8/12/2009
W24	Wolf	Wolf_1		W11 snare	8/18/2009
W10	Wolf	Wolf_2		B7 corral	8/7/2009
W10	Wolf	Wolf_2		B7 corral	8/7/2009
W15	Wolf	Wolf_3		W3 snare	8/11/2009
W22	Wolf	Wolf_3		W3 snare	8/17/2009
W12	Wolf	Wolf_4		B6 corral	8/7/2009
W106	Wolf	Wolf_4	**yes**	B7 corral	7/20/2010
W112	Wolf	Wolf_4	**yes**	B7 corral	8/16/2010
W13	Wolf	Wolf_5		W8 snare	8/7/2009
W6	Wolf	Wolf_6		W7	7/18/2009
W7	Wolf	Wolf_7		W9	7/24/2009
W102	Wolf	Wolf_7	**yes**	W21	7/7/2010
W104	Wolf	Wolf_7	**yes**	B20 corral	7/9/2010
W113	Wolf	Wolf_7	**yes**	B20 corral	8/16/2010
W21	Wolf	Wolf_8		B7 corral	8/15/2009
W25	Wolf	Wolf_9		B3 corral	8/15/2009
W26	Wolf	Wolf_10		W6	7/20/2009
W103	Wolf	Wolf_10	**yes**	W2	7/8/2010
W107	Wolf	Wolf_10	**yes**	W9	8/3/2010
W114	Wolf	Wolf_11	no	W22	8/17/2010
W116	Wolf	Wolf_12	no	40..4	7/17/2010
BW3	Wolf	poor DNA		B6 corral	8/7/2009
W16	Wolf	poor DNA		W15 snare	8/11/2009
W20	Wolf	poor DNA		W7 snare	8/15/2009
W109	Wolf	poor DNA		B6 corral	8/12/2010
W110	Wolf	poor DNA		W21	8/14/2010
W111	Wolf	poor DNA		W11	8/14/2010
W11	poor DNA			W7 snare	8/7/2009
W101	poor DNA			W6	7/7/2010

Table 4. Bear samples identified with DNA analysis by species (*Ursus arctos* or *Ursus americanus*) and individual in 2009 and 2010 in Atlin, British Columbia.

sample ID	Species ID	Individual ID	2010 recapture	location	Date
B13	Black bear	BLACK_1		25..1	7/8/2009
B9	Black bear	BLACK_2		B2 corral	7/8/2009
B125	Black bear	BLACK_2	**yes**	B2 corral	8/17/2010
B25	Black bear	BLACK_3		B8 corral	7/16/2009
B33	Black bear	BLACK_4		8..1	7/19/2009
B53	Black bear	BLACK_5		27..2	7/30/2009
B102	Black bear	BLACK_6	no	B7 rub tree	7/4/2010
B103	Black bear	BLACK_7	no	B20 corral	7/9/2010
B108	Black bear	BLACK_8	no	B2 corral	7/24/2010
B115	Black bear	BLACK_9	no	B6 corral	8/12/2010
B123	Black bear	BLACK_10	no	B20 corral	8/16/2010
B124	Black bear	BLACK_11	no	B6 corral	8/16/2010
B127	Black bear	BLACK_12	no	B3 corral	8/22/2010
B128	Black bear	BLACK_13	no	B3 corral	8/22/2010
B106	Black bear	poor DNA		22..4	7/10/2010
B107	Black bear	poor DNA		B7 rub tree	7/20/2010
B55	Grizzly bear	GRIZZLY_1		BR3	7/31/2009
B60	Grizzly bear	GRIZZLY_1		B7 rub tree	7/31/2009
B65	Grizzly bear	GRIZZLY_1		B7 rub tree	8/7/2009
B51	Grizzly bear	GRIZZLY_2		5..2	7/30/2009
B66	Grizzly bear	GRIZZLY_2		B5 corral	8/12/2009
B19	Grizzly bear	GRIZZLY_3		5..1	7/11/2009
B21	Grizzly bear	GRIZZLY_4		11..1	7/11/2009
B45	Grizzly bear	GRIZZLY_4		5..1	7/27/2009
B47	Grizzly bear	GRIZZLY_5		BR5	7/28/2009
B56	Grizzly bear	GRIZZLY_6		B3 corral	7/31/2009
B112	Grizzly bear	GRIZZLY_6	**yes**	B3 corral	8/5/2010
B63	Grizzly bear	GRIZZLY_7		B6 corral	8/7/2009
B67	Grizzly bear	GRIZZLY_8		20..2	8/15/2009
B69	Grizzly bear	GRIZZLY_8		17..2	8/15/2009
B70	Grizzly bear	GRIZZLY_9		B3 corral	8/15/2009
B75	Grizzly bear	GRIZZLY_10		BR5 rub tree	8/17/2009
B36	Grizzly bear	GRIZZLY_11		25..1	7/20/2009
B39	Grizzly bear	GRIZZLY_12		8..1	7/23/2009
B73	Grizzly bear	GRIZZLY_13		B10 corral	8/16/2009
B101	Grizzly bear	GRIZZLY_14	no	B3 rub tree	7/4/2010
B110	Grizzly bear	GRIZZLY_14	no	B7 rub tree	8/3/2010
B113	Grizzly bear	GRIZZLY_14	no	B3 rub tree	8/5/2010
B119	Grizzly bear	GRIZZLY_14	no	B7 rub tree	8/12/2010
B104	Grizzly bear	GRIZZLY_15	no	B7 rub tree	7/9/2010
B121	Grizzly bear	GRIZZLY_16	no	B3 rub tree	8/13/2010
B77	Grizzly bear	poor DNA		14 .. 1	6/23/2009
B5	Grizzly bear	poor DNA		23..1	7/2/2009
B15	Grizzly bear	poor DNA		B7 rub tree	7/10/2009
B27	Grizzly bear	poor DNA		8..1	7/17/2009
B132	Grizzly bear	poor DNA		McDonal Lk rd	6/28/2010
B118	MIX B&G	poor DNA		B6 corral	8/12/2010
B126	poor DNA			B10 corral	8/17/2010
B129	poor DNA			B3 corral	8/22/2010

Stable Isotope Analysis

Stable isotope analysis was performed on samples from 15 moose, 26 caribou, 7 mountain goats, 5 Stone's sheep, 4 beaver, 11 snowshoe hare, 4 least chipmunk, 5 northern red backed vole, 96 plants, 13 black bear (one individual had samples from 2009 and 2010), 17 grizzly bear (one individual had samples from 2009 and 2010) and 22 wolf (12 hair samples from rub pads and 10 pelts) at the UC Davis Stable Isotope Facility. Data on stable isotope analysis are preliminary. Results obtained in April 2011 indicate that there is substantial separation between prey species (Figure 5). However, overlap between Stone's sheep, caribou, beaver and mountain goats suggests that it may not be possible to determine differences in assimilated predator diet between these species. Further analysis of the data is required to determine which species will be maintained in Bayesian isotopic mixing models.

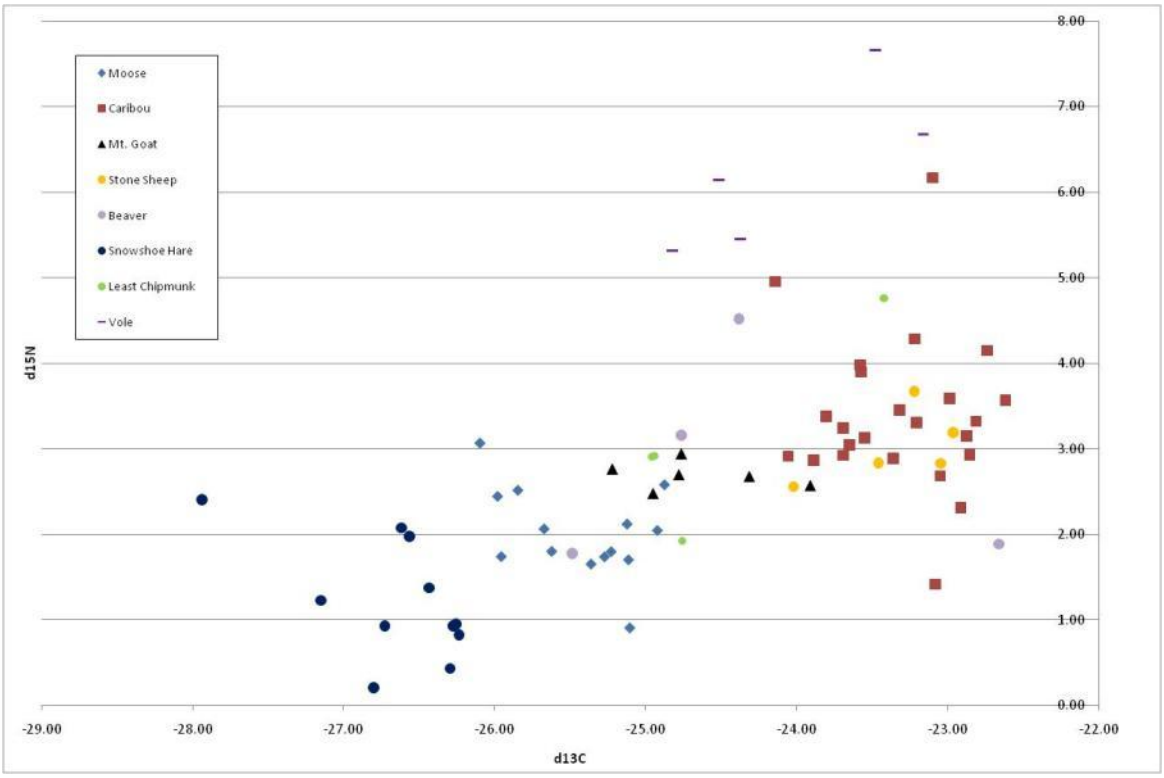


Figure 5. The mixing space with $\delta^{15}N$ and $\delta^{13}C$ values of potential wolf and bear prey species in northwestern British Columbia.

Discussion

In this study we were able to collect non-invasive hair samples from a range of species across a large study area. We successfully obtained wolf hair samples from non-invasive rub pads. Since the method was successful in both Montana and northern Canada it appears to be a robust approach that can effectively encompass many different habitats and species assemblages. We recommend the use of the Forsyth wolf gland as a lure for the wolf rub pads and found that its application led to the highest success in acquiring hair samples. We also obtained wolf hair samples from bear hair corrals which was an unexpected result given wolves general avoidance of human structures especially in areas where they are trapped. Some of our snares failed to collect any hair samples, suggesting that they were located in areas of low or no wolf activity. Prior knowledge of pack distribution, for example in other study areas where a proportion of wolves are collared, would allow more effective placement of snares to maximize data acquisition.

One of the challenges of stable isotope analysis are assumptions regarding the growth period and molt of both prey and predator hairs. Prey diet is likely to change significantly with seasons (for example, caribou depend on lichen in the winter) and this could alter isotopic values throughout the year (Stotyn 2008). It was difficult to determine what season to attribute to prey species hair in our study area and this has the potential to create variation in our results. Ideally a mix of prey hair, blood and other tissues would allow for precise estimates of prey isotopic body content at different times of the year. Further, there is little information about the timing of bears hair growth and molt. The body condition of bears likely has a large impact on coat growth. If nutrition is limited, molt can be delayed until late season foods like berries become plentiful (Jacoby et al. 1999). In some situations, bear hair may not start growing until late June or July. This would make it difficult to detect bear predation on ungulate calves during their first few weeks of life when they are most vulnerable (Stotyn et al. 2007). Similarly, the assumptions about wolf coat growth are based on only two references (Young and Goldman 1944, Mech 1974). There is potential for guard hairs to grow throughout the winter and at different rates in different locations on the body. A controlled feeding study by J. Derbridge at the Wildlife Science Center, Columbus, MN with captive wolves over the summer of 2011 has the potential to provide known values for hair growth period and diet-tissue fractionation, and consequently improve the reliability of stable isotope analysis for wolf diet in our study.

By examining seasonal predator diet with hair sections from wolves, grizzly bears and black bears, our study will be one of the first examples of a stable isotope analysis to specifically tests important assumptions about wolf and bear predation on ungulate calves. Young animals may have different isotope levels than their mothers since they are essentially one trophic step higher when nursing. Jenkins et al. (2001) did not find any isotopic differences between moose mothers and calves, but did find differences between caribou calves and their mothers during their first 70 days. If this is true in our study area, we might be able to examine predation of caribou claves by adding 2.0 to the $\delta^{15}N$ value of adult caribou.

5. LICHEN SAMPLING

Jean Polfus, Hannah Tannebring and Kate Shlepr

Functional habitat loss associated with avoidance of habitat close to human development is a growing threat to caribou and reindeer (*Rangifer tarandus)* populations across their circumpolar range (Weclaw and Hudson 2004, Sorensen et al. 2008). Caribou and reindeer have been shown to reduce use of areas within 5 km of infrastructure and human activity by 50-95% (Vistnes and Nellemann 2008). Avoidance of areas near anthropogenic features has been observed for caribou in response to roads, seismic lines, oil well sites, human settlements, tourist resorts and cabins, power lines, hydroelectric developments, mine sites, logging clearcuts, and recreational snowmobile traffic (Dyer et al. 2001, Nellemann et al. 2001, Nellemann et al. 2003, Schaefer and Mahoney 2007, Seip et al. 2007, Polfus et al. *in review*). Lichens can make up 50-75% of caribou winter diet (Scotter 1964, Gaare and Skogland 1975, Boertje 1984, Arseneault et al. 1997, Moen et al. 2007, Gilichinsky et al. 2011). Because of their slow growth (average rate of 4-6mm per year) lichen height and biomass can be used to measure grazing pressure (Skogland 1989, Arseneault et al. 1997, Collins et al. 2011). Other authors have also used lichen biomass as an indirect measure for caribou and reindeer avoidance of human infrastructure (Nellemann and Cameron 1996, Nellemann et al. 2000, Nellemann et al. 2001, Vistnes et al. 2004, Dahle et al. 2008). These studies found that lichen height and biomass decreased with increasing distance to roads, resorts and power lines in Norway.

We measured lichen height and biomass to test the predictions of habitat models developed for the Atlin herd of northern mountain woodland caribou (*Rangifer tarandus caribou*). Models developed in 2010 with locations for GPS collared caribou (section 1. Polfus 2010) indicated that caribou avoid multiple human features in the Atlin area. Location data was used to generate zones of influence (ZOI) buffers around different types of human developments (roads, mines, the town of Atlin and cabins and hunting camps) that represented the area affected by human disturbance. ZOI buffers are especially important when used to measure cumulative effects, mitigate impacts or inform population models (Sorensen et al. 2008), but differences in methods can lead to controversies about buffer widths and significance (Gunn et al. 2011). In the context of resource selection, avoidance does not indicate that caribou never occurred near human developments, but rather, areas near developments were used less than expected. Thus,

we predicted that there would be a negative correlation between the height or biomass of lichen and distance to roads across the study area because caribou would overgraze lichen in areas far from human disturbance and grazing pressure would be lowest near roads in areas within the winter ZOI. We also predicted that presence of scat would increase in areas outside the winter ZOI.

Methods

In 2009 we estimated lichen biomass to build a biomass specific regression equation to convert percent lichen cover and lichen height to biomass. At each site we established five $10m^2$ plots. To randomize the location of the plots at each site, one person was spun in a circle and then threw a stick over one of their shoulders (Dahle et al. 2008). The location of the stick indicated the position of the central lichen plot which determined the location of the four other plots (Figure 7). We recorded the number of piles of caribou scat within each $10m^2$ circle to determine caribou presence or absence. We recorded the percent cover and species of overstory trees (recorded as <1, 1-10, 11-25, 26-50, 51-75, 76-95, >95% cover) and took descriptive photos at each site. To estimate the lichen biomass (g/m^2) we clipped all *Cladina* and *Cladonia spp.* greater than 2 cm (to simulate the available lichen to caribou cropping) within 0.5 x 0.5 m wooden frames within each $10m^2$ plot (Figure 6; Hebblewhite 2006). Weight (g) was recorded in the field with 100g and 1500g Pesola scales after air drying the samples.

Figure 6. Lichen biomass plot where lichen was clipped in a 0.25 m^2 box, dried and weighed.

In 2010, we measured lichen height rather than biomass at each site. To select sites, we used GIS mapping software (ArcGIS 9.3.1 ESRI, Redlands, CA) to select lodgepole pine stands within high quality potential habitat (developed with habitat models described in section 1) within 3 km of roads. Random locations within these sites were generated with Hawth's Tools extension for ArcGIS 9.3.1. When we reached a site, location of the center plot was randomized in the same way as 2009. We recorded the same general information as in 2009. Lichen cover

was determined within the 0.5 x 0.5 m wooden frame by quadrants (recorded as <1, 1-10, 11-25, 26-50, 51-75, 76-95, >95% cover). Lichen height was measured at 9 locations each separated by 12.5 cm within the frame. Height was measured by placing a stick into the lichen which was pushed down until reaching resistance. Height of lichen within 0.5 cm of the stick was recorded by genus. We recorded UTM locations at each site which were used to find the distance to roads using ArcGIS 9.3.1.

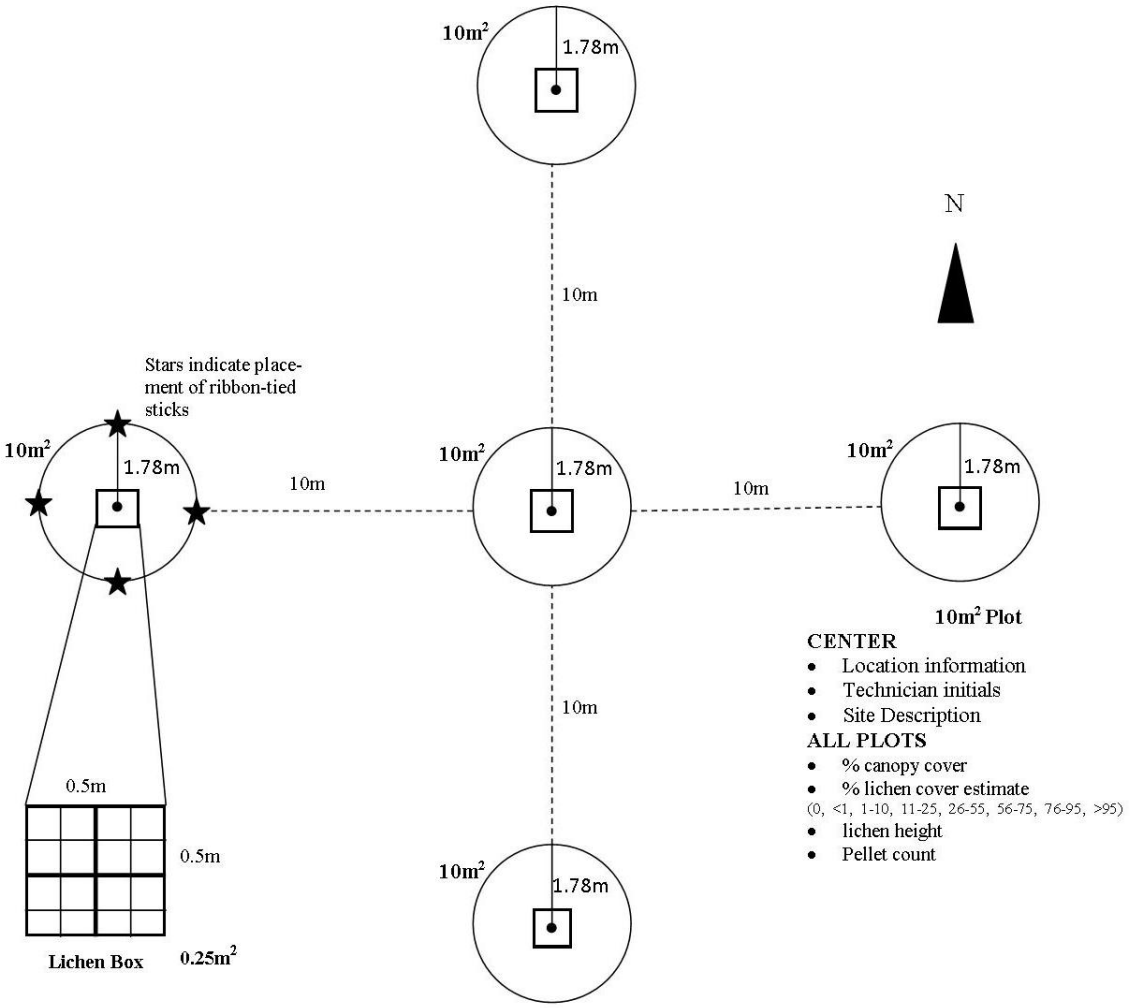


Figure 7. Lichen site diagram showing the five different plots where lichen height, lichen % cover and pellet counts were recorded.

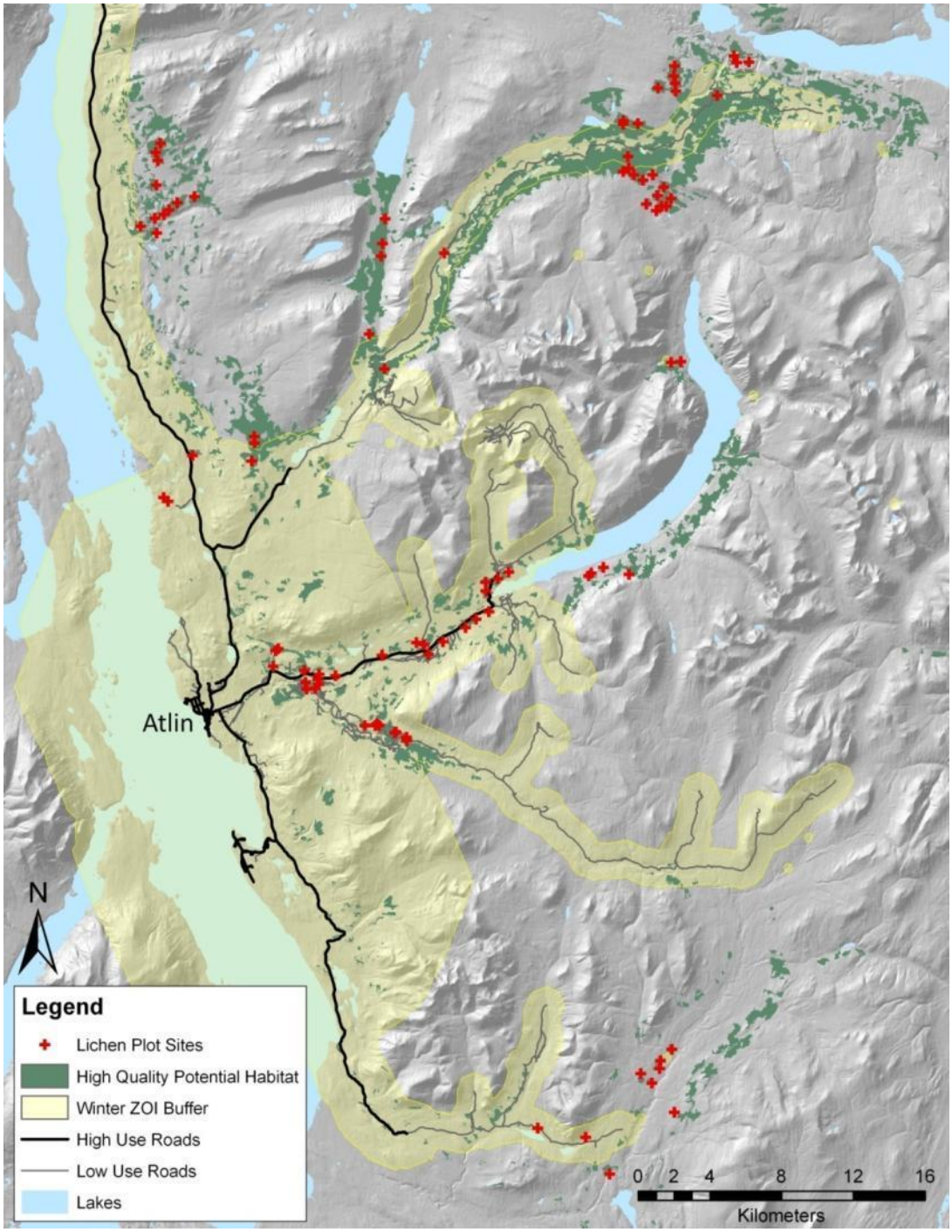


Figure 8. Sites of the 94 lichen plots conducted in 2009 and 2010 inside and outside of the winter zone of influence (ZOI) buffer found to be avoided by the Atlin herd of northern woodland caribou.

Results

In 2009, biomass of *Cladina* and *Cladonia spp.* was recorded at 15 sites. This information was used to produce a regression between the average height of lichen (*Cladina* and *Cladonia spp*.) * lichen cover which was collected in 2010 (Figure 9). The regression ($R^2 = 0.3145$) was used to produce estimates of lichen biomass for the 2010 sites: $y = 0.041x + 11.216$ where y is the predicted biomass and x is the average height of *Cladina* and *Cladonia spp*. * lichen cover.

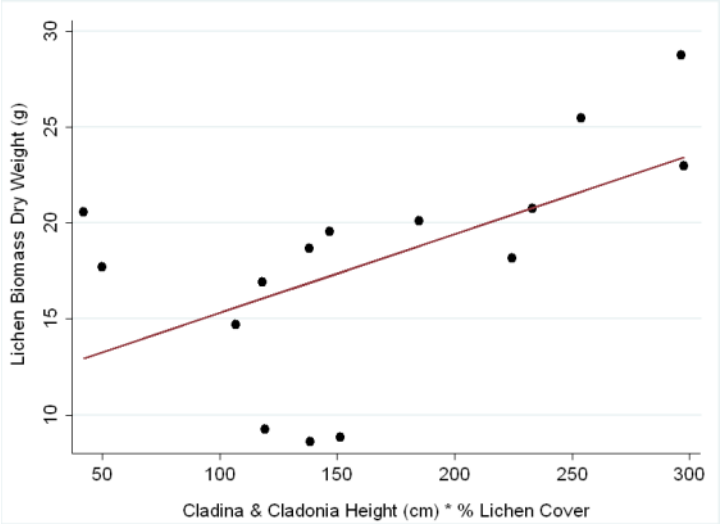


Figure 9. Regression between lichen height *cover and biomass.

In 2010, lichen height and cover was recorded at 94 sites (Figure 8) of which 41 fell within the winter ZOI buffer and 53 fell outside the ZOI buffer. We found no relationship between lichen height (average for *Cladina* and *Cladonia spp.*), lichen cover or the predicted biomass values and distance to roads ($R^2 = 0.0049$, $R^2 = 0.0445$ and $R^2 = 0.0156$, Figure 10). There was also no difference in the average height of *Cladina* and *Cladonia spp.* inside or outside of the ZOI buffer (Figure 11). However, we did detect the presence of caribou scat more often outside the ZOI buffer (30 of 53 sites) than inside the ZOI buffer (16 of 25 sites, Chi-square test p-value = 0.0087). The probability of detecting caribou scat also increased with increasing lichen cover (logistic regression $\beta = 0.054$, SE = 0.01698).

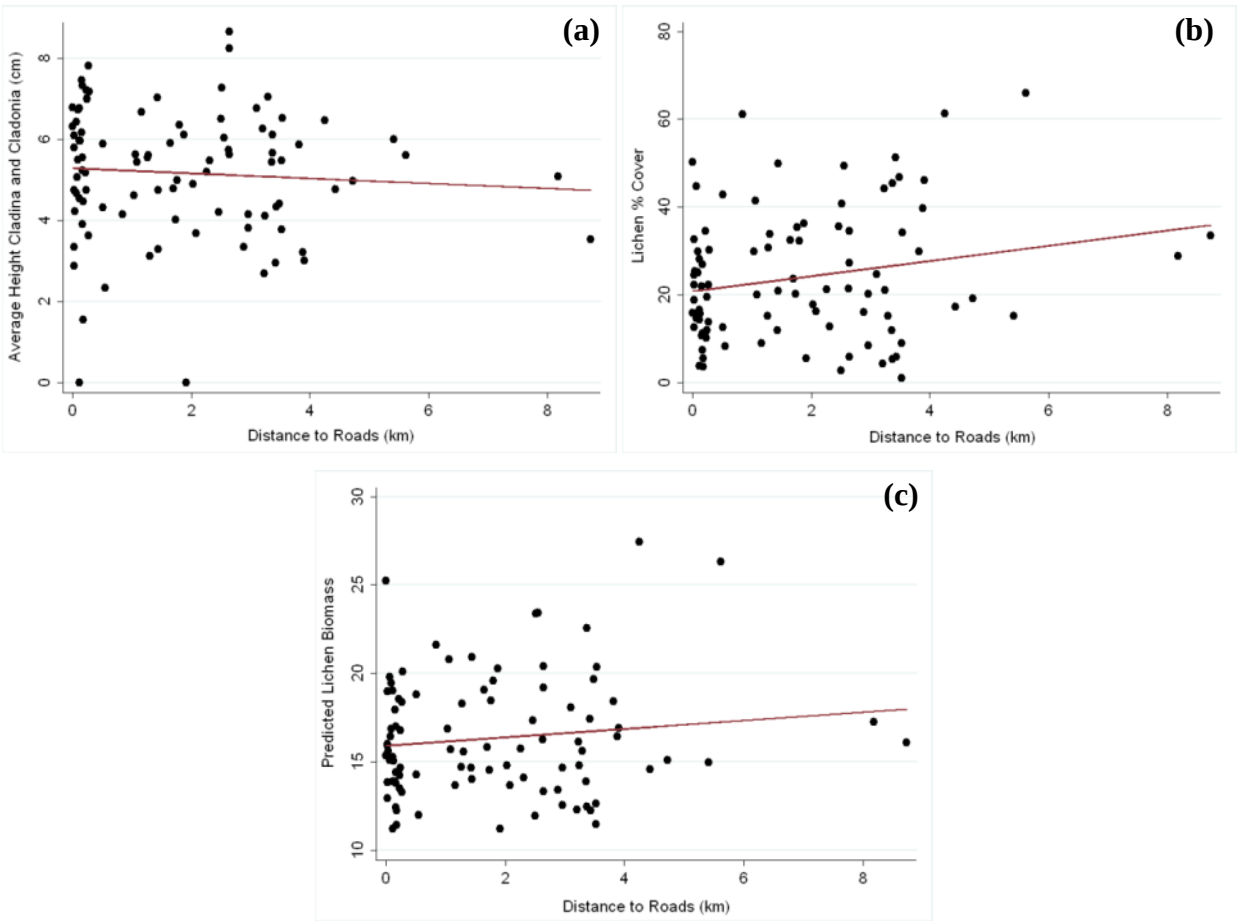


Figure 10. Average height of *Cladina* and *Cladonia spp.* (a), lichen percent cover (b) and lichen biomass (c) in relation to distance to roads.

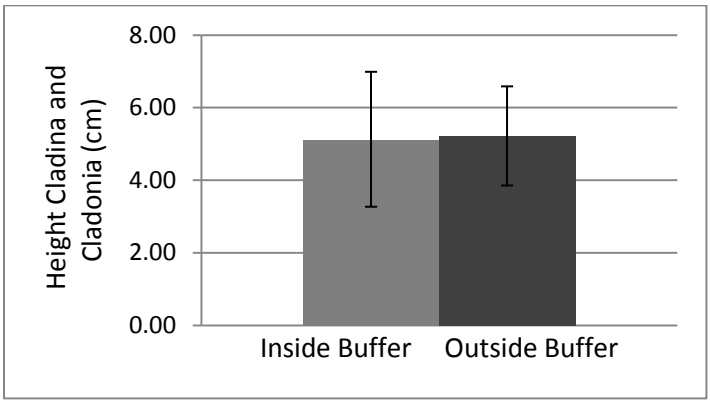


Figure 11. Average height of *Cladina* and *Cladonia spp.*within and outside the zone of influence avoidance buffer.

Discussion

Our study does not seem to support the results of previous research which indicate that lichen height/biomass decreases with increasing distance from human disturbance and is indicative of caribou grazing pressure (Nellemann and Cameron 1996, Nellemann et al. 2000, Nellemann et al. 2001, Vistnes et al. 2004, Dahle et al. 2008). This could be associated with a number of key assumptions. First, it is unlikely that lichen height/biomass is related to caribou foraging and trampling alone in our study area. Lichen growth is undoubtedly dependent on a number of ecological and climatic conditions such as canopy cover, tree species, hydrology and historic fires (Coxson and Marsh 2001, Dunford et al. 2006, Joly et al. 2010). Second, studies that did find a relationship between lichen growth and caribou presence occurred in areas with much higher densities of caribou/reindeer than in the Atlin area where population estimates are between 500-700 caribou. The density of caribou in the Atlin herd might not be enough to produce detectable effects on lichen at the regional level since forage is not commonly considered a limiting factor for northern mountain woodland caribou (Hegel et al. 2010). However, antidotal evidence within the study area does suggest that in certain key wintering areas lichen is over-grazed. However, due to the distribution of lichen and caribou winter range these patterns might not be apparent when scaled-up to the entire study region. Finally, the patchy distribution of lichen in the study area might have made measuring a representative sample of lichen height and biomass difficult (see Figure 12). Other studies have measured lichen abundance in areas where lichen carpets occur. Areas of full lichen carpets were rare in our study area, making generalities about cover and biomass dependent on small lichen patches that were not statistically significant. Caribou are able to smell for lichen through the snow and thus these patches are potentially important habitats during the winter (Pruitt 1959, Johnson et al. 2000).

Interestingly, we did find a difference between the presence of caribou scat within and outside the ZOI buffer. In our study area, caribou scat may be more indicative of caribou presence than lichen height/biomass. Thus, scat transects may be an efficient way of monitoring the Atlin caribou herd in the future. Specifically, perpendicular scat transects that radiate away from roads might be used to test the appropriateness of the winter ZOI buffer.

Figure 12. Lichen sampling sites representative of the patchy nature of lichen cover across the study area.

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APPENDIX A. STABLE ISOTOPE ANALYSIS HAIR PREPARATION PROTOCOL

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Step-by-step guide for preparation of hairs for stable isotope analysis

Step 1: Grounding the work station

Some hair is particularly charged (it appears the lighter colored the hair the worse the static charge), making working and moving individual hairs into vials a difficult process. Grounding the work station helps reduce static electricity.

Items and Equipment:

Aluminum foil (or some sort of conductor, e.g. a metal plate)
Three prong replacement electrical cord (can be found at hardware store) **Be sure to tape off the two wires that are not the green/ground wire.
Wire with an alligator clip at each end

Set up:

Tear an approximate 1.5 ft x 1.5 ft piece of foil and place it on the work space. Next, clamp one of the alligator clips onto the exposed "ground" wire on the three-prong cord (be sure the "hot" wire and the "neutral" wire are taped off and only the "ground" wire is exposed). Plug the three-prong cord into an outlet or extension cord. Fold one of the upper corners of the foil over to prevent tearing. Clamp the other alligator clip onto the folded corner of the foil. These steps are important to reduce the static electricity. Glass vials also reduce static electricity. Avoid using plastic vials

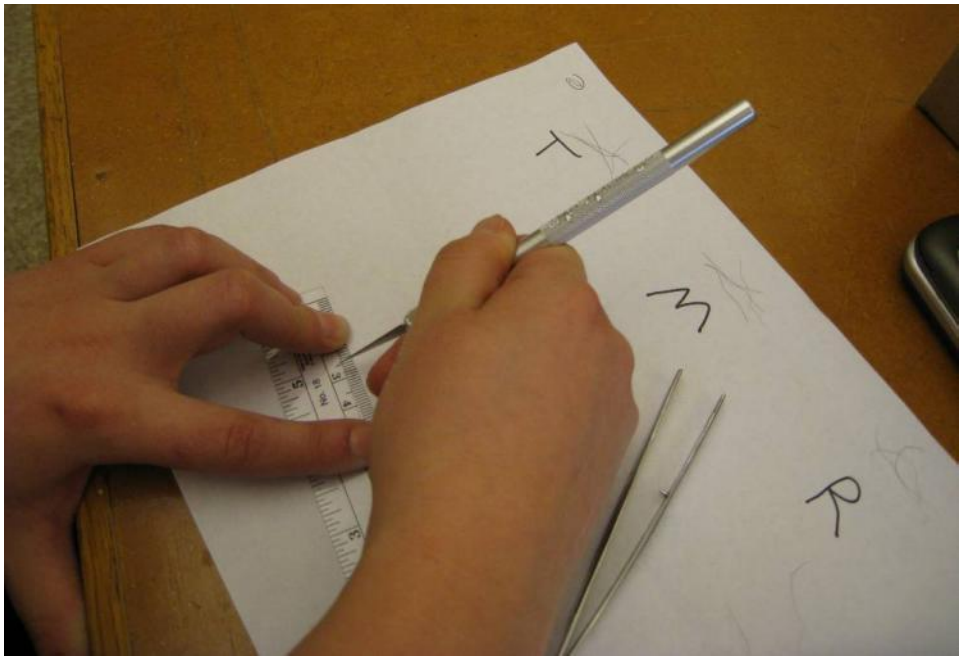
Foil work station set up.

Step 2: Selecting hairs

Prey species hair: Put the animal hair on the grounded foil. Some species' hair is not as charged and it can be easier to work on a piece of paper that contrasts the color of the hair. Use forceps or tweezers to find hairs that have a tip and a root. The UC Davis stable isotope facility needs between **1.00-1.25 mg** of hair. Try to select more than this to allow for a small loss of hair during the washing or grinding/cutting stages. For ungulates the number of hairs depends on the size of the hair and remember that ungulate hairs are hollow. Small mammals require more hairs. Do your best to remove unwanted hair fragments and under fur. Place the hairs into a small glass vial with a cap.

Predator hairs: Our project assessed the diet of grizzly bears, black bears and wolves. The predator hairs were cut into 3 equal sections (root, middle, tip). We were very careful to select FULL hairs, with roots and tips. Each hair was measured and cut with a xacto knife into three equal sections. Each section represents the diet during the growth of the hair. Root = fall, middle = summer and tip = spring. More than 10 full hairs is usually needed to make a minimum sample when each hair is cut into three pieces. Remember you need **1.00-1.25 mg** of hair for each sample. The weight of each sample will depend on the length of each hair that is being sectioned.

A dissection scope can be used to make finding roots and tips easier.

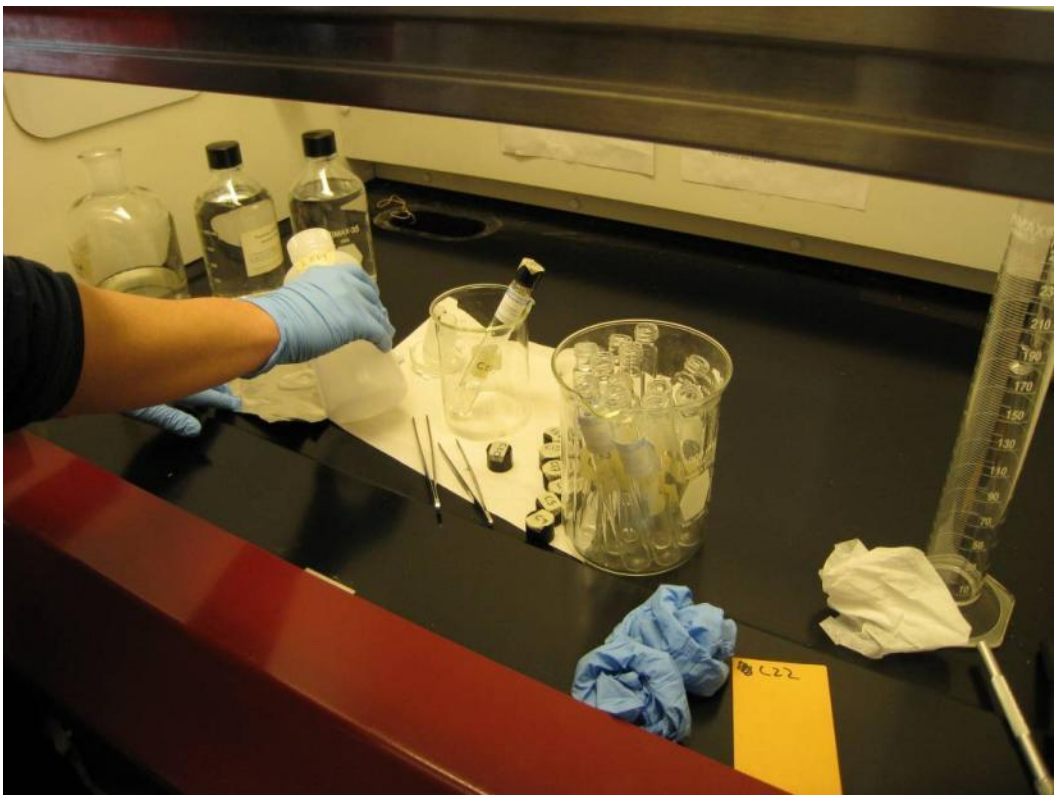


Cutting predator hair into 3 equal sections

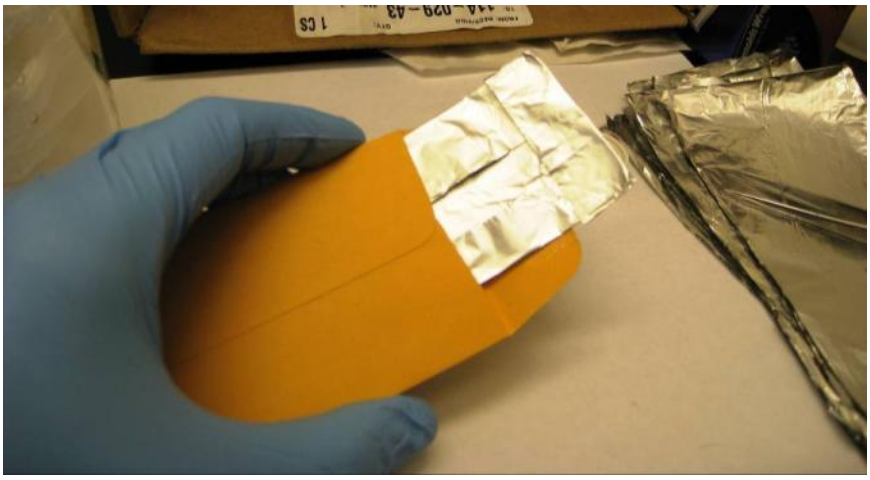
Step 3: Chloroform-Methanol wash

Items and Equipment:
Work in a ventilation hood
Gloves
Large forceps
Beaker ~300mL
Solvent wash bottle
Kimwipes
Glass waste bottle (clearly labeled “chloroform/methanol waste”)

C-M wash: Mix a 2:1 chloroform/methanol solution. Be careful to make sure that all fumes are contained in the fume hood and wear gloves. Make sure the solution is adequately mixed. Use the wash bottle to add the C-M solution to each vial with hair in it, completely covering the hairs. Swirl the vial to make sure all hairs are covered with solution. **Allow the hairs to soak for 24 hours in the fume hood.**



Removing hairs: Carefully prepare ~8x15 cm pieces of aluminum foil while wearing gloves. Squirt a small amount of the C-M solution in the wash bottle onto the top of the foil strip. Use a kimwipe to wipe the solution around, cleaning off the foil. Use a tweezers to remove a sample of hair from a vial. Fold the foil in half and insert the hair into the foil, squeezing the foil shut around the hair and carefully removing the tweezers[2]. After the sample has been placed back into the envelope, closely examine the C-M solution in the vial to make sure there are no hairs left behind. Fold the aluminum foil around the hair sample and place in a labeled coin envelope. Dry in a drying oven at low heat for 24 hours.



[2] Note: Because of the air movement caused by the ventilation hood, hairs tend to blow away, so work carefully.

Step 4: Grinding/cutting hairs

We sent our hair samples to the UC Davis Analytical Lab to be cut into small pieces, weighed and loaded into tin boats.

Nikki Schwab
Management Services Officer
UC Davis Analytical Lab
224 Hoagland Hall
University of California, Davis 95616

phone: 530-754-6594
fax: 530-752-9892
email: nkschwab@ucdavis.edu
website: http://anlab.ucdavis.edu

Guidelines for this process:

- Samples need to be handled with gloves to prevent contamination after the C-M wash.
- Be careful not to cross contaminate between samples.
- Homogeneous sub samples are required for the stable isotope analysis. The most efficient way to homogenize the sample would be to grind the hairs. However, cutting the hairs into small enough pieces to fit into the tin capsules is also possible. **To ensure a homogeneous sample, please try to include the full length of several hairs, rather than root only or tips only**.
- Hair samples will be sensitive to static electricity and to slight breeze.
- The UC Davis stable isotope facility recommends measuring between **1.00-1.25 mg** of material into the tin capsules. The less variation between sample weights the better. Samples can be between 0.05 and 1.5 mg (some of the predator hair might only have 0.05 per sample).
- Weight must be recorded for each sample.
- One tray includes 96 well sites –the stable isotope facility requires all wells be filled.
- It is absolutely essential that no hairs are sticking out of the capsules.

Step 5: Stable isotope analysis

Our samples were taken to the UC Davis stable isotope facility to be analyzed for ^{13}C and ^{15}N.

UC Davis Stable Isotope Facility
Department of Plant Sciences
One Shields Avenue, Mail Stop 1
Davis, CA 95616

E-mail: sif@ucdavis.edu
Phone: 530-754-7517
Fax: 530-752-4361 (ATTN: Stable Isotope Facility)
Website: http://stableisotopefacility.ucdavis.edu

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APPENDIX B. ADDITIONAL RESOURCES

Jacob's Industries in Whitehorse
Dry Ice for shipping frozen samples for pregnancy tests
867-667-7606
$8.50 for 1 lb blocks

Toronto Zoo
Fecal sample processing and hormone analysis. The cost is $12 per sample for dry fecal analysis and $10 per sample for wet fecal analysis.
Contact Info:
Gabriela Mastromonaco
Reproductive Physiology
Toronto Zoo
361A Old Finch Avenue
Scarborough, ON M1B 5K7
Phone: 416-392-5951
Fax: 416-392-4979
Email: gmastromonaco@torontozoo.ca

Wildlife Genetics International
Working with a sample size of 70 or more, costs (US dollars): $10.73 for extraction, $8.81 for gender, $35.26 for individual ID (or $39.67 if gender and individual ID are run at the same time).

Contact Info:
David Paetkau, PhD, President
Box 274 (post)
Suite 200, 182 Baker Street (courier)
Nelson, BC V1L 5P9
Phone: 250/877-352-3563x222
FAX: 250-352-3567 www.wildlifegenetics.ca