Factors Affecting Moose Population Declines in British Columbia

2019 Progress Report: February 2012–May 2019

by

G. Kuzyk, C. Procter, S. Marshall, H. Schindler,
H. Schwantje, M. Scheideman, D. Hodder

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Authors' Addresses

Gerald Kuzyk, British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 205 Industrial Road G, Cranbrook, BC. V1C 7G5

Chris Procter, British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 1259 Dalhousie Drive, Kamloops, BC. V2C 5Z5

Shelley Marshall, British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2000 S Ospika Boulevard, Prince George, BC. V2N 4W5

Heidi Schindler, British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 3726 Alfred Avenue, Smithers, BC. V0J 2N0

Helen Schwantje, British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2080 Labieux Rd, Nanaimo, BC. V9T 6J9

Matt Scheideman, British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2000 S Ospika Boulevard, Prince George, BC. V2N 4W5

Dexter Hodder, John Prince Research Forest, P.O. Box 2378, Fort St. James, BC. V0J 1P0

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EXECUTIVE SUMMARY

In 2013, the B.C. government initiated a research project to determine the factors affecting Moose population change in central B.C. by evaluating a landscape change hypothesis proposed by Kuzyk and Heard (2014). This report provides preliminary results and interpretation of the data collected from February 2012 to May 2019. It is preceded by four annual reports: Kuzyk et al. (2015, 2016, 2017, 2018a) and follows the recently revised research design for this project (Kuzyk et al. 2019). This research was initiated because Moose abundance in some areas of central British Columbia (B.C.) had declined since the early 2000s, causing concern with First Nations and stakeholders. Much of the decline happened concurrently with a Mountain Pine Beetle (MPB) outbreak that killed a large proportion of mature pine trees and resulted in increased salvage logging and road building. In response to the Moose decline, a 5-year provincially-coordinated Moose research project was initiated by the B.C. Ministry of Forests, Lands and Natural Resource Operations (FLNRO) [as of 2017, the Ministry name changed to Ministry of Forests, Lands, Natural Resource Operations and Rural Development (FLNRORD)]. In February 2012, a Moose study with similar objectives began on the Bonaparte Plateau and was integrated with this project. The primary research objective of this project is to evaluate a landscape change hypothesis, which states that Moose declines coincided with a Mountain Pine Beetle outbreak where habitat changes and increased salvage logging and road building resulted in greater vulnerability to Moose from hunters, predators, nutritional constraints, age/health and environmental conditions. It assumes Moose survival will increase when: a) forest cutblocks regenerate to the point where vegetation obstructs the view of predators and hunters; b) resource roads created for logging are rendered impassable; and c) Moose become more uniformly dispersed on the landscape. We evaluated that hypothesis by identifying causes and rates of cow Moose mortality and examining factors that contributed to their vulnerability. To assess the causes and rates of calf mortality, an important research gap previously identified at the outset of this project, Moose calves that were 8-months old were collared in Bonaparte and Prince George South in the winters of 2016/17, 2017/18, and 2018/19. This progress report provides data and a preliminary interpretation of the results from 28 February 2012 to May 2019 from five study areas in central B.C.: Bonaparte; Big Creek; Entiako; Prince George South; and the John Prince Research Forest.

Since this project was initiated in 2012, we fitted GPS radio collars on a total of 577 individual Moose: 478 cows and 99 8-month old calves. Twenty-nine cow Moose were recaptured to replace collars (total GPS radio collars = 507). Since 2016/17, we have collared 99 8-month old calf Moose in the Bonaparte (n = 60) and Prince George South (n = 39) study areas. Three configurations of GPS radio collars were used on cow Moose: those programmed for one fix/day (n = 149); 2 fixes/day (n = 109); and >2 fixes/day (n = 249). All calf collars collected 2 fixes/day. As of 30 April 2019, 189 GPS collars were active on cow Moose, 167 censored (i.e., dropped at end of battery life, stopped collecting data or slipped from Moose), and 122 were associated with Moose that died.

One hundred and twenty-two collared cows have died during this study period. We identified the probable proximate cause of death for 115 cow mortalities as 71 predation (54 Grey Wolf, 12 bear, 5 Cougar), 17 hunting (2 licensed, 15 unlicensed), 24 health-related (11 apparent starvation, 3 failed predation attempt, 1 chronic bacterial infection, 1 peritonitis, 1 pleuritis, 1 prolapsed uterus, 1 bacterial septicemia, 5 unknown health-related), and 3 natural accident. The cause of death remains unknown for 7 cows. There were 29 collared calf mortalities and 8 yearling mortalities; yearling survival was monitored from surviving collared calves. Proximate probable cause of calf mortality was 20 predation (14 wolf, 2 Cougar, 4 bear), 8 health-related (4 apparent starvation, 2 apparent starvation/tick, 1 failed predation attempt, 1 gastro-intestinal infection) and 1 vehicle collision. Proximate probable cause of yearling mortality was 3 predation (1 wolf, 2 bear) and 5 hunting (3 licensed, 2 unlicensed).

The majority of cow and calf Moose were in good body condition at the time of capture. A standard set of biological samples were collected that included age and body condition estimates. Seven-year average pregnancy rates observed in this study ranged from 67–93%, with the lowest observed in the Bonaparte (67%) and Prince George South (78%) study areas. Average rates in the remaining study areas were 85–93%. Parturition rates (determined by analyzing cow movement rates) and pregnancy rates may vary from each other in the same year, but one metric is not consistently higher than the other.

The landscape change hypothesis assumes cow survival to be the primary driver influencing Moose population change because declines in some areas occurred rapidly. Our overall cow survival rates were inconsistent with this hypothesis as they were within the range reported from other stable Moose populations (i.e., >85%). The Bonaparte, Big Creek and John Prince study areas had cow survival >85% in all years, whereas Entiako was below 85% in the last four years and Prince George South below 85% in three years.

Bone-marrow-fat analysis from cow Moose mortalities (n = 83) showed 49% in good body condition (>70% marrow fat), 28% in poor body condition (20–70% marrow fat), and 23% with acute malnutrition (<20% marrow fat). The majority of mortalities involving cows with acute malnutrition and poor body condition occurred between April and June, while mortalities in the remainder of the years typically involved cows in good body condition. Serological screening and ancillary testing did not demonstrate substantial exposure to pathogens (i.e., pathogens that would likely have increased a Moose's likelihood of death); however, some cows were emaciated at death with no apparent additional cause(s) of death determined to date.

With cow survival rates mostly above 85% has led to the increasing importance of evaluating Moose calf survival in relation to population declines. Our data on calf survival have determined a wide variation in the late winter survival of collared Moose calves to recruitment at age one. It has ranged from 45 % (± 22%) in 2017, 75 % (± 13%) in 2018, and 76 % (± 14%) in 2019.

Analyses on habitat selection patterns of radio-collared Moose were completed in July 2018 at the University of Northern British Columbia (UNBC) and are currently underway at the University of Victoria and John Prince Research Forest. An analysis was completed in the spring of 2019 at UNBC on how habitat use affects cow survival across study areas. This includes management recommendation aimed to improve Moose survival rates in the Province. Final manuscripts for this survival analysis are under development. Two new PhD students started work on the project in 2019: a student at the University of Victoria started in January and will be focusing on Moose calf survival while another started in September at UNBC and will be focusing on Moose movement ecology and interactions with forest management.

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1. INTRODUCTION

Moose are a valued species by the citizens of British Columbia (B.C.) for consumptive and non-consumptive purposes. Moose populations in some areas of the province have declined by 50–70% since the early 2000s, while Moose populations in other areas of the province were stable or increasing (Kuzyk 2016; Kuzyk et al. 2018b). Moose declines within central B.C. coincided with a Mountain Pine Beetle (*Dendroctonus ponderosae*; MPB) outbreak which resulted in increased salvage logging of beetle-killed timber, associated road building and increased levels of mortality of pine trees >30 years old (Alfaro et al. 2015). These large-scale alterations to the landscape may have influenced the distribution and abundance of Moose, hunters and predators (Janz 2006; Ritchie 2008). In response to these Moose declines and ongoing concerns from the public, a provincially coordinated Moose research project was initiated in 2013 by the Province of B.C. (Ministry of Forests, Lands, Natural Resource Operations and Rural Development; FLNRORD) and its partners (Kuzyk and Heard 2014). A Moose study with similar objectives on the Bonaparte Plateau north of Kamloops began in February 2012 and was integrated into this project. We also collaborated with other Moose studies in B.C. (i.e., Sittler 2019) and other jurisdictions as part of the Canadian Wildlife Directors Moose subcommittee established in July 2018.

A landscape change hypothesis was developed to evaluate Moose population change (Kuzyk and Heard 2014). The landscape change hypothesis states that Moose declines coincided with a Mountain Pine Beetle (MPB) outbreak because habitat change, increased salvage logging and associated road building resulted in greater vulnerability to Moose from hunters, predators, nutritional constraints, age/health and environmental conditions. The primary assumptions of the landscape change hypothesis are Moose survival will increase when: a) forestry cutblocks regenerate to the point where vegetation obstructs the view of predators and hunters; b) resource roads created for logging are rendered impassable due to deactivation or forest ingrowth; and c) Moose become more uniformly dispersed on the landscape (Kuzyk and Heard 2014). Because the declines occurred over a relatively short time period, it was assumed cow Moose survival would have a greater proportional effect on population growth than calf survival (Gaillard et al. 1998). In order to evaluate the landscape change hypothesis, we determined cow survival rates and probable causes of mortality through monitoring survival of at least 30 GPS radio-collared cow Moose in each of five study areas (n = 150 annually) for five years (i.e., December 2013 to March 2018, Kuzyk and Heard 2014). We determined mortality rates, causes and contributing factors (Mumma and Gillingham 2019) in comparison to the predictions of the landscape change hypothesis.

Since the outset of this project, we acknowledged that calf survival could be a substantial contributing factor to Moose population declines, either in conjunction with declining cow survival or on its own, but financial and logistical constraints limited our initial focus to cow survival monitoring (Kuzyk and Heard 2014). To begin filling the knowledge gap of the influence of calf survival on population trends (Kuzyk and Heard 2014), we GPS radio-collared twenty 8-month old calves in one study area (Bonaparte) in January and February of 2017. We radio-collared approximately forty 8-month old calves in two study areas (Bonaparte and Prince George South) in 2017/18 and 2018/19. The objective is to continue to radio-collar 8-month old calves in these two study areas to measure their survival and causes of mortality until they are recruited into the population at one year of age, which is when survival rates of calves appear to align with adult survival rates (Hickey 1955). In January 2019, we partnered with University of Victoria

for an in-depth analysis of calf survival in relation to landscape change. We plan to continue assessing survival rates of calves through calf surveys of radio-collared cows or general composition surveys in some study areas, where calves are not being collared, for the duration of this project.

This report describes the fieldwork and preliminary results from February 2012–May 2019 (see Appendix A for all products related to this project). UNBC recently completed a complementary analysis of habitat selection (see Scheideman 2018) and survival analysis (Mumma and Gillingham 2019) of radio-collared cow Moose in this study. In addition, researchers at UNBC and JPRF have concluded analyses on migratory movements of Moose in that study area (Chisholm et al. 2019). The overall research design for the project has been updated in a separate document to reflect the current and future direction of the project (Kuzyk et al. 2019). We continue to actively engage with First Nations and stakeholders on the status and future direction of this project. This research project will be continued indefinitely and new components will be incorporated to help understand Moose population change and enable sound management recommendations (Kuzyk et al. 2019).

2. STUDY AREA

This study area description is like that provided in Kuzyk et al. (2018a) as there was little annual variation in biotic or abiotic features within study areas between years. This research project occurred in five study areas across the Interior Plateau of central B.C.: Bonaparte, Big Creek, Entiako, Prince George South (PG South), and John Prince Research Forest (JPRF; Figure 1, Table 1, Figure 2). Most of the plateau lies between 1200–1500 m above sea level, and was characterized by rolling terrain with a mosaic of seral stages, coniferous forest and wetland areas. The climate is generally continental, with warm, dry summers and cold winters with complete snow coverage. Dominant ecological zones of the interior include Sub-Boreal Spruce (SBS) and Engelmann-Spruce Subalpine Fir (ESSF) in the north, and Sub-Boreal Pine-Spruce (SBPS) and Interior Douglas-Fir (IDF) in the south (Meidinger and Pojar 1991). The study areas, delineated using the cumulative distribution of radio-collared Moose locations in each of the study areas, ranged from 6700 km^2 to >18000 km^2 (Table 1).

Logging was the primary resource land use in all study areas, with an increase in salvage logging after the large-scale MPB outbreak in the early 2000s (Alfaro et al. 2015). The degree of pine tree mortality, associated salvage logging and access development varied among study areas resulting from the natural variation in the dominant forest types, severity of the MPB attack, and the extent of reserve areas that did not allow logging. The proportion of the Timber Harvesting Land Base sprayed with herbicide to promote regrowth of harvestable tree species in each study area (to 2018) ranged from 0% (Big Creek) to 4.5% (Prince George South) with most herbicide application occurring after the year 2000. Access for recreational use, such as hunting, all-terrain vehicle (ATV) use and hiking, was primarily through resource roads created for logging. Free-ranging cattle (*Bos taurus*) are common in the Bonaparte and Big Creek, and to a lesser extent in PG South and Entiako study areas, and feral horses (*Equus caballus*) also occur in the Big Creek study area.

In addition to Moose, the Interior Plateau supports other large mammals: Elk (*Cervus canadensis*); Mule Deer (*Odocoileus hemionus*); White-tailed Deer (*O. virginianus*); Caribou (*Rangifer tarandus*); Grey Wolf (*Canis lupus*); Grizzly Bear (*Ursus arctos*); Black Bear (*U. americanus*); and Cougar (*Puma concolor*), all of which occur at varying densities and distributions (Shackleton 1999; Mowat et al. 2013; Kuzyk and Hatter 2014). All study areas contain multi-prey, multi-predator species assemblages (Table 1).

Moose, however, were the primary wild ungulate in all study areas except the Bonaparte study area, where Mule Deer are also abundant.

Moose hunting by First Nations for food, social and ceremonial needs, and licensed hunting by B.C. residents and non-residents occurred in all study areas. Licensed Moose hunting in B.C. is regulated through sex- and age-specific General Open Season (GOS) or Limited Entry Hunting (LEH) opportunities, with harvest type and seasons generally managed at the Wildlife Management Unit (WMU) scale. Within their traditional territories, First Nations have the right to harvest any number of Moose for food, social and ceremonial needs without season, sex or age restrictions. Given widespread concerns for Moose populations, licensed cow harvests were substantially reduced in all areas of the province for the 2016 season. All antlerless permit numbers were reduced to one permit annually per unit or zone overlapping the PG South, JPRF and Bonaparte study areas, which has significantly reduced licensed cow harvest in

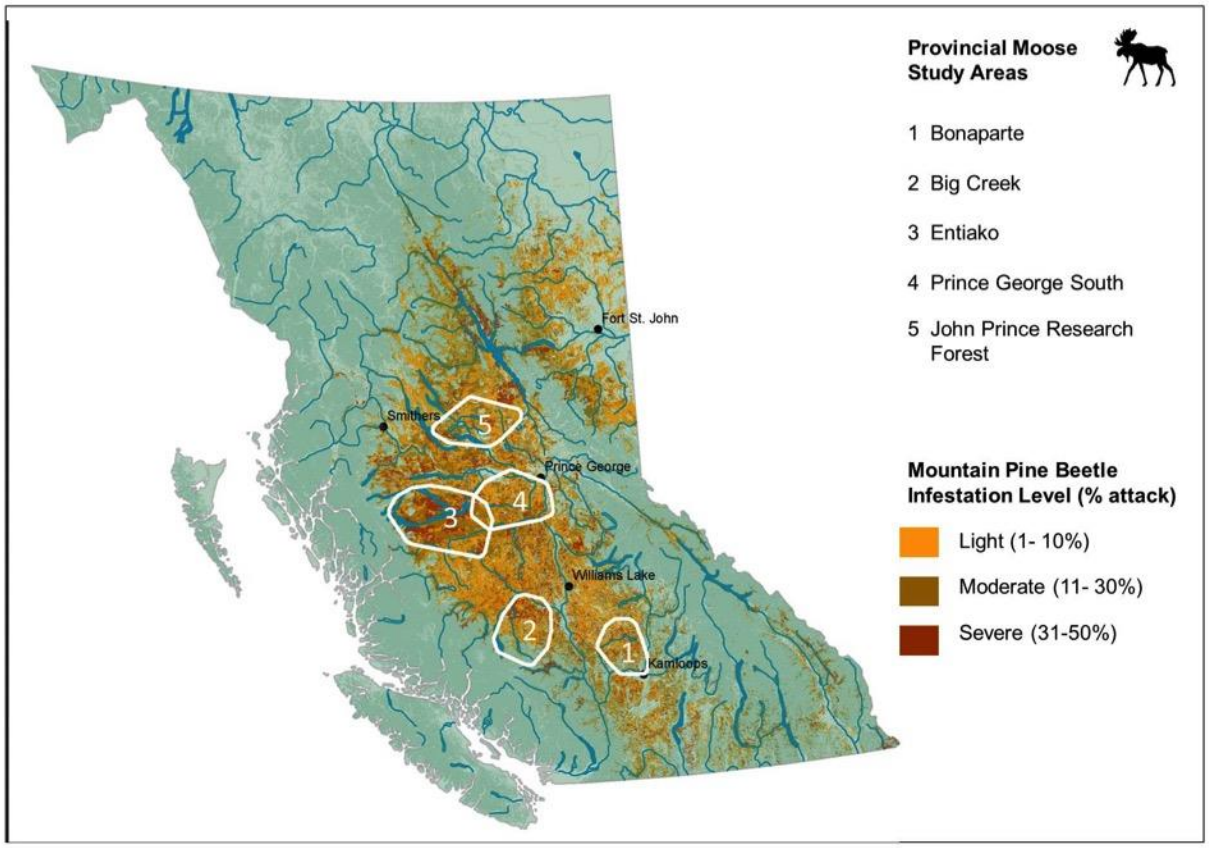

Figure 1. Moose research study areas in central British Columbia, where cow Moose survival has been monitored in the Bonaparte study area since February 2012 and in the other four study areas since December 2013, overlaid on Mountain Pine Beetle Infestation spatial data layer (2016). The study areas were selected to encompass a range of land cover types and disturbance levels. Study area boundaries are described by minimum-convex polygons around locations of all collared cow Moose in each study area.

Table 1. Description of relevant anthropogenic, biotic and abiotic characteristics in five Moose research study areas in central B.C. where cow Moose survival has been monitored.

Study Area/Region/ Management Unit/ Landform	Landscape Feature Prevalence[1]		BEC Zones[3]	Relative Abundance of Potential Predators[4]	Wild Ungulates and Relative abundance[4]	Domestic/ Feral Ungulates and Relative Abundance[4]
Bonaparte 6800 km^2, Region 3 (Thompson), 3-29, 3-30B, Interior Plateau	MPB: L/P Logging: P Roads: P Wildfire (<30yrs): R Herbicide by THLB[2]: 0.02%	Provincial Park: R Agriculture: S Crown Cattle Range: P Mining: R	IDF: 33% SBPS: 23% MS: 22% ESSF: 8% SBS: 7% BG/PP: 7%	Wolves: M Black Bears: M/H Cougars: M/H Grizzly Bears: N	Mule Deer: H White-tailed Deer: M Elk: L Caribou: N	Cattle: H Domestic Sheep: L Feral Horses: N
Big Creek 9800 km^2, Region 5 (Cariboo), 5-04, Interior Plateau/ Coast Mountains	MPB: L/P Logging: P Roads: P Wildfire (<30yrs): S Herbicide by THLB[2]: 0.00%	Provincial Park: R Agriculture: R Crown Cattle Range: L Mining: N	SBPS: 48% IDF: 36% MS: 12% ESSF: 3% AT/BG: <1%	Wolves: M Black Bears: M Cougars: L/M Grizzly Bears: M	Mule Deer: L/M White-tailed Deer: L Elk: N Caribou: N	Cattle: H Domestic Sheep: L Feral Horses: H
Entiako 18,000 km^2 Region 6 (Skeena), 6-01, 6-02, Interior Plateau/ Coast Mountains	MPB: P Logging: S Roads: S Wildfire (<30yrs): S Herbicide by THLB[2]: 0.24%	Provincial Park: L Agriculture: N Crown Cattle Range: N Mining: N	SBS: 48% ESSF: 32% SBPS: 12% AT: 4% MH: 2% CWH: 1% MS: <1%	Wolves: M/H Black Bears: M/H Cougars: L Grizzly Bears: M	Mule Deer: L White-tailed Deer: N Elk: L Caribou: L/M	Cattle: L Domestic Sheep: N Feral Horses: N
Prince George South 11,000 km^2 Region 7A(Omineca) 7-10 to 7-12, Interior Plateau	MPB: P Logging: P Roads: P Wildfire (<30yrs): R Herbicide by THLB[2]: 4.47%	Provincial Park: R Agriculture: S Crown Cattle Range: L Mining: N	SBS: 93% ESSF: 7%	Wolves: M Black Bears: M/H Cougars: L Grizzly Bears: L	Mule Deer: L White-tailed Deer: L Elk: L Caribou: N	Cattle: L Domestic Sheep: N Feral Horses: N
John Prince Research Forest 9600 km^2, Region 7A (Omineca), 7-14, 7-25, Interior Plateau	MPB: L Logging: L Roads: P Wildfire (<30yrs): N Herbicide by THLB[3]: 0.13%	Provincial Park: R Agriculture: N Crown Cattle Range: N Mining: N	SBS: 95% ESSF: 5%	Wolves: M Black Bears: H Cougars: N Grizzly Bears: M	Mule Deer: L White-tailed Deer: L Elk: L Caribou: N	Cattle: N Domestic Sheep: N Feral Horses: N

[1]Proportion of landscape affected: Pervasive (P) = 71–100%, Large (L) = 31–70%, Small (S) = 11–30%, Restricted (R) = 1–10%, Negligible (N) = <1%. Pine abundance varies.

[2]Proportion of timber harvest land base (THLB) to which herbicide has been applied current to 2018. Earliest date of herbicide application was in 1986.

[3]Biogeoclimatic Ecosystem Classification (BEC): Interior Douglas Fir (IDF), Sub-Boreal Pine and Spruce (SBPS), Montane Spruce (MS), Engelmann Spruce Sub-alpine Fir (ESSF), Montane Spruce (MS), Sub-boreal Spruce (SBS), Bunchgrass (BG), Ponderosa Pine (PP), Alpine Tundra (AT), Mountain Hemlock (MH), and Coastal Western Hemlock (CWH).

[4]Relative abundance/density: H = high, M = moderate, L = Low, N = nil or negligible.

all areas. In the Bonaparte study area, the 3-year average annual antlerless harvest prior to 2016 was 9 and the 3-year annual average since was 0.3, a reduction of approximately 97% by licensed hunters. In the PG South study area, the 3-year average annual antlerless harvest prior to 2016 was 5 and the 3-year annual average since was 1, a reduction of approximately 80% by licensed hunters. In the JPRF study area, the 3-year average annual antlerless harvest prior to 2016 was 5 and the 3-year annual average since was 0, a reduction of approximately 100% by licensed hunters.

3. METHODS

3.1 Capture and Handling

Details of the field methods were originally presented in Kuzyk and Heard (2014) and certain methodologies have been updated and presented in Kuzyk et al. (2015, 2016, 2017, 2018). Current methods are generally the same as those presented in Kuzyk et al. (2018) as they have become standardized over the course of the project. The current capture form and handling protocol are included in Appendix A.

Captures were conducted in accordance with the British Columbia *Wildlife Act* under permit CB17-277227 (see Figures 3 – 9 for different stages of handling process). Cows have been captured annually since 2012 as part of this project, and winter of 2016/17 was the first season that included radio-collaring twenty 8-month old calf Moose. Generally, we captured cow and calf Moose between December and March, using chemical immobilization by aerial

Figure 2. Aerial view of Stuart Lake in the John Prince Research Forest study area, February 2019.

darting; aerial net gunning was used to immobilize cow Moose in early project years. Aerial darts were remotely delivered with either a Pneudart or Dan-Inject darting system. Some captures of calf Moose used a combination of net gunning and aerial darting.

Carfentanil citrate (1.4 mL at 3 mg/mL; Chiron Compounding Pharmacy Inc, Guelph, ON) and xylazine hydrochloride (0.5 mL at 100 mg/mL; Chiron Compounding Pharmacy Inc, Guelph, ON) were combined in one dart to immobilize Moose in early years of the project, with naltrexone hydrochloride (9 mL at 50 mg/mL; Chiron Compounding Pharmacy Inc, Guelph, ON) as a reversal agent. BAM II (Chiron Compounding Pharmacy Inc, Guelph, ON), a premixed combination of butorphanol (27.3 mg/mL), azaperone (9.1 mg/mL) and medetomidine (10.9 mg/mL), has been used as the sole immobilizing agent for cows and calves on this project since the winter of 2016/17. After refinement of BAM II dose testing (Thacker et al. 2019), 3.5 mL and 2.0 mL of BAM II were considered effective for predictably and safely immobilizing cows and 8-month old Moose, respectively. Naltrexone hydrochloride (1 mL at 50 mg/mL; Chiron Compounding Pharmacy Inc, Guelph, ON) and atipamezole hydrochloride (7 mL at 25 mg/mL; Chiron Compounding

Figure 3. Wildlife Biologist Conrad Thiessen preparing to deploy a dart containing immobilization drug BAM II during capture of a cow Moose in the Entiako study area, February 2019.

Pharmacy Inc, Guelph, ON) were used to reverse the effects of these standard doses of BAM II upon completion of handling and sampling.

We fitted each cow Moose with a GPS radio collar that collected 1-2 fixes per day (Vectronic Aerospace VERTEX Survey Globalstar radio collars, Berlin) or >2 fixes per day (Advanced Telemetry Systems G2110E radio collars, Isanti, MN or Vectronic Aerospace VERTEX Survey Iridium radio collars, Berlin). At the outset of the project, we chose radio collars with one or two positional fixes daily to facilitate survival monitoring for up to five years. We deployed radio collars capable of collecting >2 fixes daily when funds were available to begin addressing other objectives, including calving rates and fine scale habitat use, as well as to improve fix rate success. Moose calves were fitted with expandable collars that collected six fixes per day (Vectronic Aerospace VERTEX Survey Iridium radio collars, Berlin). Calf collars expanded from an initial size of 50 cm to 80 cm (average neck circumference of an adult female Moose) using protected expandable material. Collared calves will be monitored for collar sizing as they grow to yearlings and beyond, and collars will be removed if growth rates exceed collar sizing and collars do not rot off. Cotton spacers designed to rot-off within one year were put on collars deployed on bull calves because they could rapidly exceed the maximum expansion capable with these collars. Cotton spacers designed to rot off within two years were put on collars deployed on female calves, as their neck growth is not as rapid as bulls.

Figure 4. Moose sample kit prepared by the B.C. Wildlife Health Unit and used by wildlife biologists to take biological samples while processing captured Moose Samples include pellets, blood, hair and a tissue biopsy, August 2019.

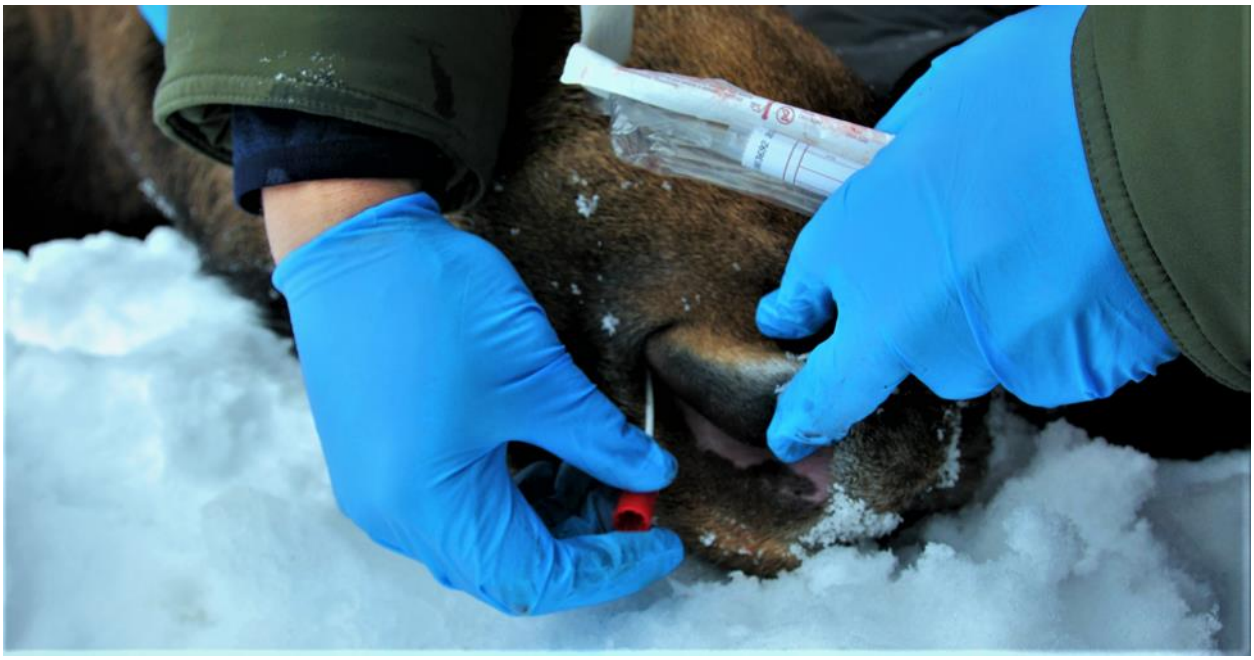

Figure 5. Wildlife Biologist Conrad Thiessen nasal swabbing cow Moose during capture in the Entiako study area, February 2019.

Figure 6. Wildlife biologists processing a cow Moose after capture: Morgan Anderson (foreground) using an ultrasound machine to estimate rump fat for determining body condition and Matt Scheideman (background) taking blood for pregnancy and serological testing, December 2018.

Some calves were weighed, to the nearest kilogram, in a body blanket lifted by a helicopter where the capture location and conditions were conducive to do so. Key morphological measurements (i.e., chest girth, total length, shoulder height, hind-foot length) were taken on Moose calves to assist in estimating weight when direct weighing was not possible. Using those data, we regressed actual weight with individual and combined morphometric measurements (i.e., total length, chest girth, total length + chest girth, etc.) to assess the relationship between those morphometric measurements and actual weight and whether measurements alone could be reliably used to estimate calf weight. We used the best regression to estimate weight of all captured calves and compared calf weights by sex, study area and year.

3.2 Biological Samples

We examined and sampled captured Moose according to a standard protocol (Appendix A) that assessed for: 1) age class using tooth eruption, staining and wear as an index (Passmore et al. 1955); 2) body condition, using an index simplified from Franzmann (1977); 3) external parasite presence and prevalence (e.g., ticks); and 4) presence of calves. Tick transect protocols began in 2018/19 following Bergeron and Pekins (2014) methods to count two 10x10 cm plots (upper edge of shoulder blade and on rump midway between hipbone and base of tail). In each plot, we counted ticks on 4 parallel, 10 cm transects spaced 2 cm apart by parting the hair down to the skin to count all ticks visible (Sine et al. 2009). In Bonaparte and PG South, we measured maximum rump fat of cows (i.e., MAXFAT) using portable ultrasounds (Sonosite M-Turbo and Ibex Pro), and used those measurements to estimate percent total ingesta-free body fat (IFBF, Stephenson et al. 1998). We compared body fat levels between cows with and without calves present, and between study areas.

All Moose were sampled according to a standard protocol that is updated annually. It included drawing 20–35 ml of blood using an 18 gauge x

Figure 7. Wildlife Biologist Mark Wong ear-tagging a cow Moose in the Entiako study area, February 2019.

Figure 8. Wildlife biologist Heidi Schindler preparing immobilization reversal drugs in Entiako study area, February 2019.

Figure 9. Newly radio-collared cow Moose recovering following administration of immobilization reversal drugs in the John Prince Research Forest study area, February 2019.

1.5 inch needle and distributing it in a number of blood tubes. Each tube was carefully handled and protected from temperature extremes. After capture sessions, the tubes were spun using portable centrifuges for serum, buffy coat and plasma harvesting. These were decanted into cryovials (Figure 10) and held at -20 C. Serum was submitted for pregnancy testing using pregnancy-specific protein B levels (PSPB). Past comparison of results from dual pregnancy tests using progesterone and PSPB resulted in the preference for PSPB testing only (Thacker et al. 2019). Serological testing focused on exposure to pathogens considered of high priority for impacts on survival and reproduction of wild ungulate populations, utilizing the experience of other research programs, including the B.C. Boreal Caribou Health Program. Serum was screened for antibodies for Johne's disease, *Neospora caninum*, Bovine Viral Diarrhea virus, and Parainfluenza 3 virus. Serum from a subset of cow Moose was submitted for testing for exposure to *Erysipelothrix rhusipathiae* and *Toxoplasma gondii.* Serum from blue top vials was assessed for trace mineral levels (manganese, iron, cobalt, copper, zinc, selenium, and molybdenum). Whole blood collected for plasma and buffy coat harvesting was archived at -80 C. For 2018/19, whole blood in a RNA buffer was frozen for gene transcription assessment. Each animal had nasal swabbing done for *Mycoplasma ovipneumoniae* PCR.

We obtained fecal samples for parasitological assessment; key parasites for investigation were *Parelaphostrongylus tenuis* (meningeal worm), *Fascioloides magna* (giant liver fluke), and *P. odocoilei* (and other gastrointestinal nematodes). Analyses for fecal parasites has not occurred in all years due to lack of laboratory access, but feces have been archived for further work if needed. A 6-mm punch biopsy of the ear for the application of an ear tag was air-dried and archived for genetics. We collected at least 100 hairs with roots from between the shoulders for cortisol testing.

3.3 Survival Rates

The radio-collars were programmed to send a mortality alert via email and text message if no movement was detected via the internal tip switch for 8 hours (previous years had mortality sensors set between 4 and 24 hours). In some cases, collars remained in sufficient motion post-mortality to prevent the mortality signal from being triggered, particularly for predation events where the collar was frequently moved when predators were feeding.

Figure 10. Wildlife Health Biologist Maeve Winchester using pipette to withdraw serum following spinning blood tube in centrifuge in Nanaimo, August 2019.

Annual survival rates were calculated for cow Moose from 28 February 2012–30 April 2019. The biological year started on 1 May to coincide with the time immediately prior to the average time of parturition for Moose in northern (Gillingham and Parker 2008) and southern British Columbia (Poole et al. 2007). All cow Moose were assumed to be random individuals and representative of the population with equal risk of mortality (i.e., no cow Moose were assumed to be predisposed to predation due to giving birth or the presence of a calf). We calculated survival rates by pooling individual cow Moose across all study areas and for each study area. Survival analysis and mortality results included only cow Moose that lived >5 days post-capture to avoid the potential bias or effects of capture-related stresses and physiological changes on survival (Neumann et al. 2011). Cow survival rates were calculated weekly and summarized by biological year (1 May–30 April) using a Kaplan-Meier estimator (Pollock et al. 1989).

Calf survival rates were calculated from date of capture (at about 8 months) to 22 May of the same year, the average date of their first birthday, also using a Kaplan-Meier estimator (Pollock et al. 1989). Survival analysis and mortality results included only calf Moose that lived >5 days post-capture (Neumann et al. 2011). For surviving calves, we also calculated yearling survival rates from their first to second birthdays (i.e., 22 May of their first year to 22 May of their second year). We considered calves recruited into the population at their first birthday (Bender 2006), as that is likely when survival rates begin to align with adult survival rates (Hickey 1955). Data from this project indicate yearling survival rates are approaching rates observed with adults, although sample sizes are relatively low (see Section 4.3).

3.4 Mortality Causes

Following receipt of a collar mortality notification, we conducted mortality site investigations according to a standardized protocol as soon as logistically feasible, typically within 24–48 hours. Ground telemetry techniques were sometimes used to determine the mortality location when concealed by thick vegetation or snow cover. We determined the probable proximate (i.e., direct) cause of mortality following a standardized protocol (Kuzyk and Heard 2014), and we continually refined the definitions for probable proximate cause of mortality as new circumstances arose (Appendix B). Ultimate (i.e., indirect) causes of mortality that were not evident during mortality investigation are determined later through testing of biological samples and assessing results (Mumma and Gillingham 2019). The mortality investigation form undergoes periodic updates for continual improvement (Appendix C).

Site investigation included standardized scene photography for context and evidence recording. Samples from cow and calf Moose were collected during mortality site investigations to understand the proximate and ultimate cause of death (Figure 11, Appendix C). Samples available for collection varied depending largely on proximate cause of death (e.g., Grey Wolf kills typically have bones but no soft tissues remaining, while health-related mortalities often have all sample materials available). For each mortality, we collected at least one long bone, usually the femur, or if none was available, the jaw, to assess body condition through bone marrow fat analysis (Neiland 1970). Marrow fat is the last fat store to be used as body condition deteriorates, therefore high dry weight proportions do not necessarily represent individuals in good body condition, but low scores are a definitive indicator of poor nutritional status (Mech and Delgiudice 1985). We considered animals with a marrow dry weight <70% to be in poor body condition and those with <20% to have been experiencing malnutrition that would lead to mortality from starvation (Sand et al. 2012). Bones were bagged and frozen as soon as practical to maintain representation of marrow when the Moose was alive. Marrow was

removed from an approximately 10-cm long section from the center of each bone, dried in an oven at 60 C, and weighed daily until the weight stabilized, indicating all moisture had been evaporated. The final dry weight divided by the initial wet weight was the index of body condition. When available, an incisor was extracted during mortality site investigations to determine the age of the Moose. Cementum aging was conducted by Matson's Lab (Manhattan, MT). A variety of frozen and fixed (in formalin) tissue samples from mortality site investigations were collected when available (see Appendix C) and were archived or sent for analysis at the Animal Health Centre (B.C. Ministry of Agriculture, Abbotsford, BC) or other laboratories as appropriate to provide health-related information baselines such as trace minerals and help interpret ultimate cause of death.

3.5 Calf Production, Survival, and True Recruitment

Calf parturition rates and dates were calculate-ed by assessing daily cow movement rates through the parturition period (DeMars et al. 2013; McGraw et al. 2014; Severud et al. 2015; Obermoller 2017). Calving movements are generally classified by a long-distance movement followed by a reduction in movements due to low mobility of calves

Figure 11. Wildlife Biologist Morgan Anderson filling blood tube with blood collected from dead Moose at mortality site for health testing, June 2019.

immediately post-birth. We used the first day that a reduction in movement rates was observed as the estimated birth date (Severud et al. 2015). Data from estimated calf parturition dates in Bonaparte and PG South, the study areas with the most high-fix rate collars, were averaged annually from 2014–2019 to determine the mean birth date. Mean birth-date was 22 May ± 0.9 days (95% CI) and that date was used to calculate calf survival rates to their average first birthday. Given variability in movement patterns and associated uncertainty in determining if parturition occurred, we removed animals from the analysis when there was uncertainty whether calving occurred. We used parturition rates to establish minimum calf/cow ratios (number of calves/100 cows) at birth and to compare with pregnancy rates estimated by blood serum analyses on captured cows in the Bonaparte and PG South study areas. Aerial surveys were conducted approximately 3-4 weeks post-birth in Bonaparte and PG South study areas by locating collared cows and assessing calf presence; this information contributes to information on early calf survival and an in-depth analysis of calf survival. We estimated summer calf survival y estimating calf ratios at birth from collared cows and comparing those ratios to mid-winter calf ratios measured from aerial composition surveys.

When funding was available, we located collared cow Moose to assess calf survival of uncollared calves in the late winter (mid-February – late March) for those: 1) that were determined to be pregnant the previous winter; 2) that had a calf present when collared earlier in the winter; 3) for which there was uncertainty regarding whether or not they had a calf present when collared earlier in the winter because they were in a mixed group of cows and calves; 4) that were collared in previous years; or 5) whose fine-scale movement data (if available) suggested that they were parturient in the previous spring/summer months. The most recent GPS locations of cows were mapped prior to the survey to facilitate efficient search times in locating collared cows. Survey crews in a helicopter radio-tracked collared cows and determined if calves were present. Estimates of tick prevalence through hair loss were assessed for cows and calves. We developed a standardized calf survey data form in June 2017 (Appendix D).

To calculate true calf recruitment rates (to age of 1), we first completed aerial composition surveys to estimate calf ratios that would be comparable to typical survey-based mid-winter calf ratios generally used by biologists. As these mid-winter ratios are currently used as the recruitment index to inform Moose population management. We then corrected those mid-winter calf ratios with survival rates of collared 8-month old calves to their average first birthday. We assume that cow deaths are too few to substantially alter the cow/calf ratios between mid-winter and recruitment of calves to one year of age. To understand the effect of true recruitment on Moose population trend, we calculated the rates of population change using cow survival rates, the mid-winter recruitment index and true recruitment at age 1, assuming half the calves were female and using the equation developed by Hatter and Bergerud (1991);

$$\lambda = \frac{S}{1 - R}$$

where S = survival from 8 months to age 1 as a proportion and R = the proportion of female calves in the female population, i.e., cows + female calves.

3.6 Density Surveys

Moose density estimates were developed for each study area near the initiation and the 5-year point in the study. Density surveys were typically conducted in 5–7 consecutive days in December–March using stratified random blocks

Table 2. Number and status of all GPS radio collars (*n* = 507) deployed on Moose (*n* = 478 i.e., 29 recollars) in all study areas in central B.C. from February 2012– 30 April 2019.

Study Year	Deployed Collars*	Individuals Collared**	Mortalities	Censored Collars	Active Collars***
2012	9	9	0	0	9
2012-2013	29	29	2	0	36
2013-2014	131	131	7	28	132
2014-2015	69	69	11	14	176
2015-2016	100	100	32	24	220
2016-2017	52	49	22	34	213
2017-2018	26	15	25	16	187
2018-2019	91	76	23	51	189
Totals	507	478	122	167	189

*Includes recaptures where the original collar was replaced by a new collar
**number of individual cows collared
***Derived by modifying the number of collars active at the end of the previous year by the number of new collars deployed and lost through mortalities or censoring

that could be remeasured to detect population trends 5–7 years later (Gasaway et al. 1986). Certain surveys were modified to include habitat-based stratification (Heard et al. 2008). All survey types produced comparable density estimates. A sightability correction factor developed in central B.C. (Quayle et al. 2001) was used to account for detection probability. These surveys followed established standards for accuracy and precision (90% CI) with allowable error from ±15–25% of the estimated population size (RISC 2002).

4. RESULTS

4.1 Capture and Handling

From February 2012–30 April 2019, we captured and radio-collared 507 cow Moose of which 29 were recaptured to replace collars with dead batteries or close to anticipated battery end life (Tables 2 and 3). There were 372 cows captured by aerial darting and 135 captured by aerial net gunning. Twenty calf Moose (12 female, 8 male) were captured and fitted with GPS radio collars in the Bonaparte study area in January and February 2017. In January and February 2018, 20 calf Moose (6 female, 14 male) were collared in Bonaparte and 20 calf Moose (11 female, 9 male) in Prince George South study areas. In January and February 2019, 20 calf Moose (9 female, 11 male) were collared in Bonaparte and 19 calf Moose (7 female, 12 male) in PG South study areas.

Of the 507 GPS radio collars deployed on cows, there were 249 collars that collected more than two position fixes/day (range 4-16 fixes/day), 109 collars that collected two fixes/day and 149 collars that collected one fix/day (Table 4). We censored collars (*n* = 167) when they ceased tracking Moose movements due to low battery voltage, collar malfunctions, or when they physically slipped from Moose. All calf collars deployed were programmed to collect six fixes per day.

4.2 Biological Samples

Of the 478 cow Moose captured to date, 474 were assessed for age via tooth eruption, staining and wear patterns (Figure 12), with 85% (*n* = 404) classified as adults (4.5–7.5 years old), 11% (*n* = 51) as aged (>8.5), and 4% (*n* = 19) as young (1.5– 3.5 years old). Body condition for the 429 animals assessed at capture showed that

Table 3. Number and status of all GPS radio collars (n = 507) deployed on Moose (n = 478 i.e., 29 recollars) in each study area in central B.C. from February 2012–30 April 2019.

Study Area	Study Year	Deployed* Collars	Individuals** Collared	Mortalities	Censored Collars	Active*** Collars
Bonaparte	2012	9	9	0	0	9
	2012-2013	29	29	2	0	36
	2013-2014	14	14	3	28	19
	2014-2015	30	30	2	6	41
	2015-2016	36	36	7	6	64
	2016-2017	20	17	5	28	48
	2017-2018	7	7	1	3	51
	2018-2019	19	17	5	9	54
	Totals	164	159	25	80	54
Big Creek	2013-2014	40	40	0	0	40
	2014-2015	13	13	3	8	42
	2015-2016	5	5	6	2	39
	2016-2017	6	6	4	0	41
	2017-2018	3	1	4	2	36
	2018-2019	14	14	4	19	27
	Totals	81	79	21	31	27
Entiako	2013-2014	45	45	1	0	44
	2014-2015	9	9	4	0	49
	2015-2016	17	17	10	16	40
	2016-2017	4	4	9	1	34
	2017-2018	10	2	6	3	27
	2018-2019	13	13	5	7	28
	Totals	98	90	35	27	28
PG South	2013-2014	17	17	1	0	16
	2014-2015	17	17	2	0	31
	2015-2016	16	16	6	0	41
	2016-2017	15	15	2	5	49
	2017-2018	6	5	12	6	36
	2018-2019	20	19	9	10	36
	Totals	91	89	32	21	36
JPRF	2013-2014	15	15	2	0	13
	2014-2015	0	0	0	0	13
	2015-2016	26	26	3	0	36
	2016-2017	7	7	2	0	41
	2017-2018	0	0	2	2	37
	2018-2019	25	13	0	6	44
	Totals	73	61	9	8	44

*Includes recaptures where the original collar was replaced by a new collar
**Total number of independent cows collared
***Derived by modifying the number of collars active at the end of the previous year by the number of new collars deployed and lost through mortalities or censoring

Table 4. Programmed fix schedule for GPS radio collars (n = 507; 478 new captures and 29 recollars) deployed on cow Moose in each study area in central B.C. from February 2012–30 April 2019.

Study Area	>2 Fixes/Day	2 Fixes/Day	1 Fix/Day
Bonaparte	128	36	0
Big Creek	17	11	53
Entiako	38	21	39
PG South	41	16	34
JPRF	25	25	23
Totals	**249**	**109**	**149**

66% (*n* = 286) were in good body condition, 17% (*n* = 75) were in excellent body condition, 12% (*n* = 52) were in fair body condition, 3% (*n* = 16) were in poor body condition, and 1% (*n* = 3) were emaciated (Figure 13). Body condition assessments found poorer body condition overall in 2016/17 and in PG South (Figures 14 and 15). Body condition of calves was assessed for 94 individuals and 62% (*n* = 58) were in good condition, 37% (*n* = 35) were in fair condition and 1% (*n* = 1) were in poor condition. Calves were present at capture with 37% of cows (Figure 16).

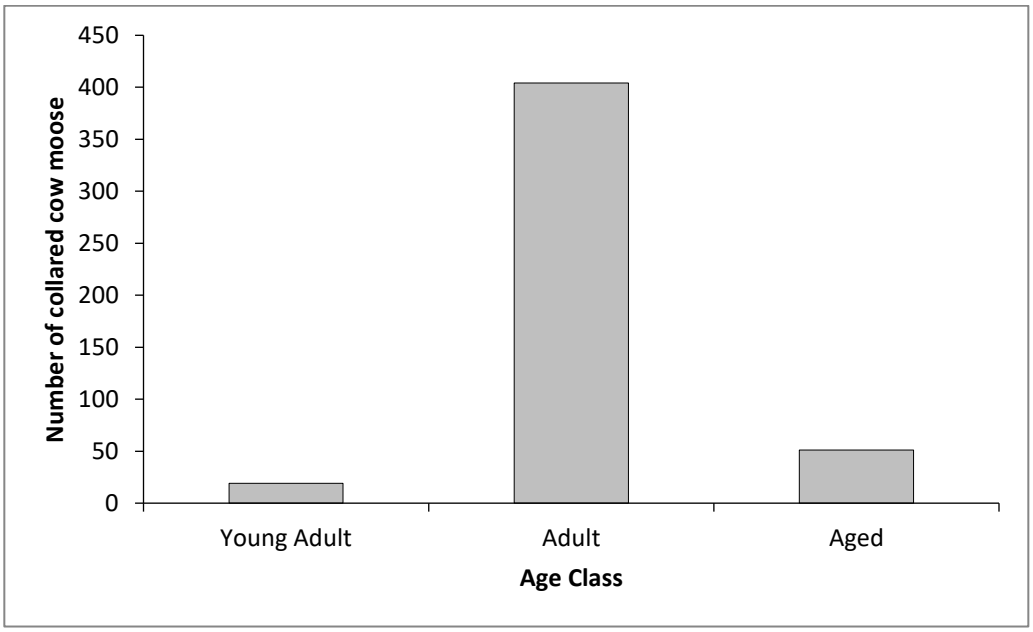

Figure 12. Age class summary of 474 cow Moose radio-collared in central B.C. from February 2012–30 April 2019 with ages estimated by tooth wear patterns at capture. Young Adult Moose were estimated to be 1.5–3.5 years old, Adults as 4.5–7.5 years old, and Aged as >8.5 years old.

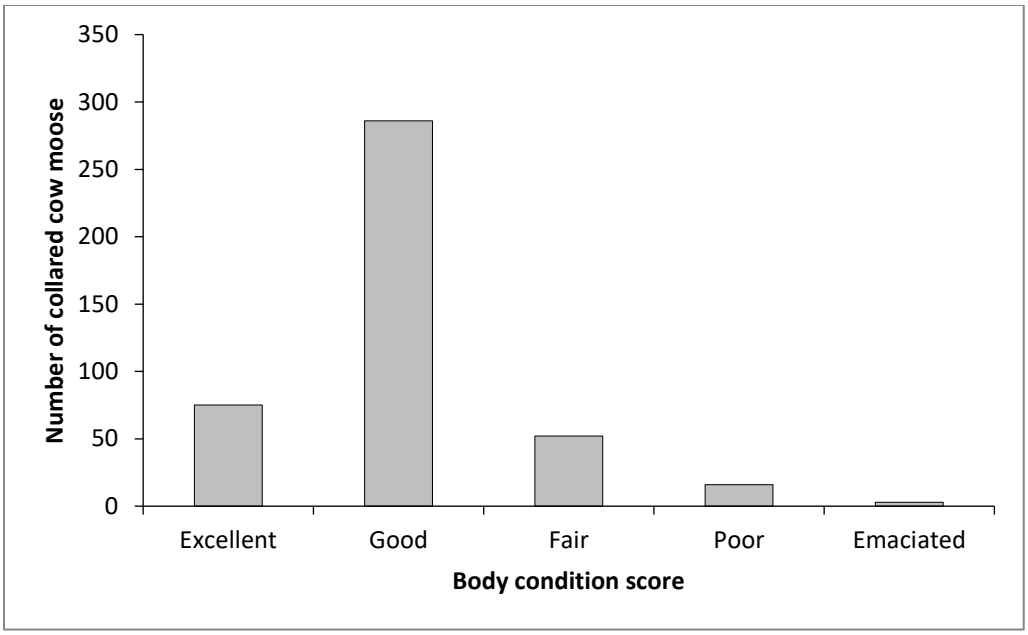

Figure 13. Body condition scores of 432 cow Moose radio-collared in central B.C. from February 2012–30 April 2018. Condition scores were assessed at capture using external physical traits modified from Franzmann (1977).

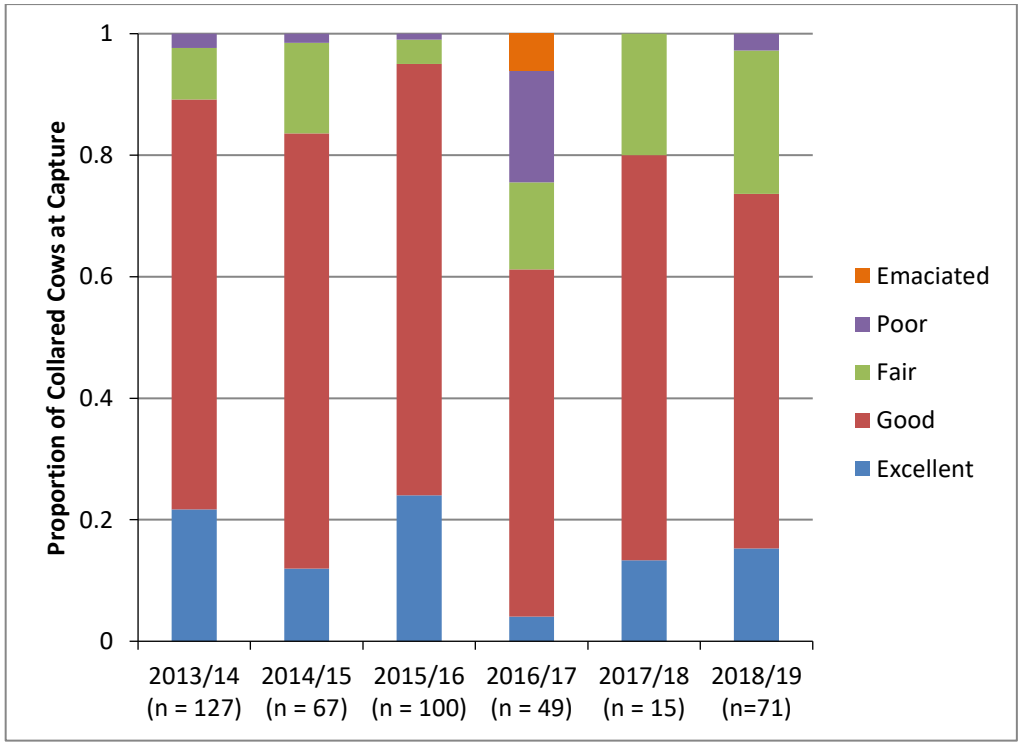

Figure 14. Annual body condition scores of 432 cow Moose radio-collared in central B.C. from February 2012-30 April 2019. Condition scores were assessed at capture using external physical traits modified from Franzmann (1977).

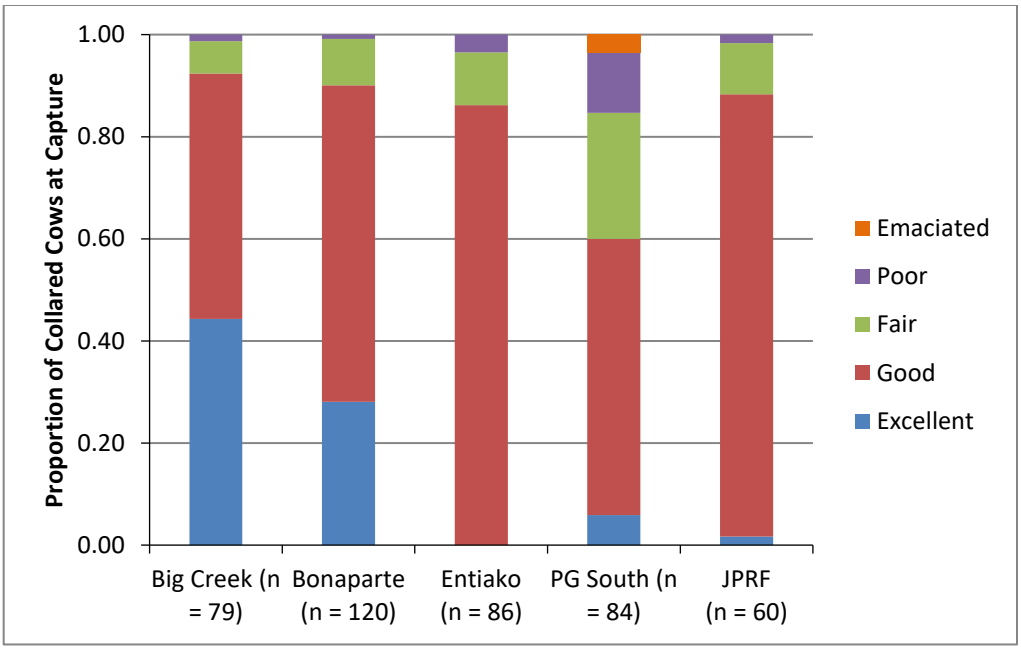

Figure 15. Study area specific body condition at time of capture scores of 432 cow Moose radio-collared in central B.C. from February 2012-30 April 2019. Condition scores were assessed using external physical traits modified from Franzmann (1977).

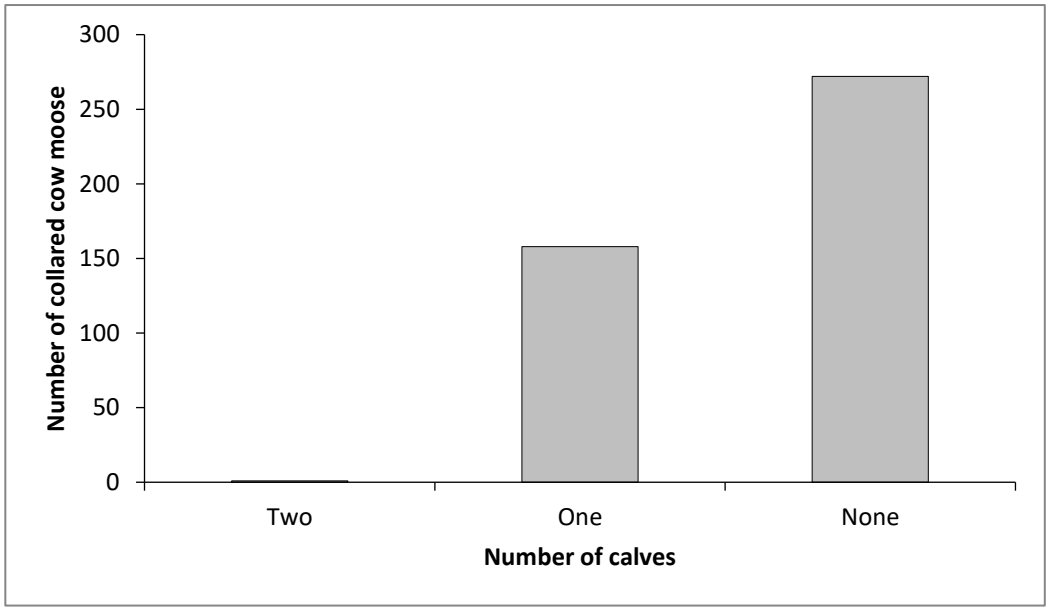

Figure 16. Calf status of 431 radio-collared cow Moose at capture in central B.C. from February 2012–30 April 2019.

The number of ticks were highly variable between individuals. The study area with the greatest amount of ticks was Big Creek (9.4 ± 3.0 ticks), and the lowest number of ticks observed was in JPRF (2.1 ± 0.7). Calves had higher tick loading than cows; in Bonaparte, tick transects averaged 7.7 (± 4.2), and PG South had an average of 19.6 (± 6.4) ticks in 2019.

Of the 61 cows assessed with ultrasound, mean MAXFAT was 16.4 mm (± 2.9 mm [95% CI]), and 19.2 mm (± 4.5 mm [95% CI]) in Bonaparte and PG South, respectively, which corresponded to an estimated average % IFBF of 8.8% (± 0.8%, 95% CI) and 9.5% (± 0.9%, 95% CI) in the Bonaparte and PG South study areas, respectively (Figure 17). Differences between study areas were not significant, however, the skinniest Moose were observed in the Bonaparte study area (range 2.7 – 11.3% body fat) and the fattest Moose were observed in the PG South study area (range 6.4 – 15.9% body fat; Figure 17). Cows without calves were generally fatter than those that had calves present and the difference was significant in the Bonaparte study area but not in the PG South study area (Figure 18). Of the 431 cow Moose where we recorded calf presence at capture, 63% (n = 272) were unaccompanied by a calf, 37% (n = 158) had one calf and <1% (n = 1) had twins (Figure 14). This excludes the calf status of the cows selectively collared to facilitate the calf-collaring program in Bonaparte and PG South.

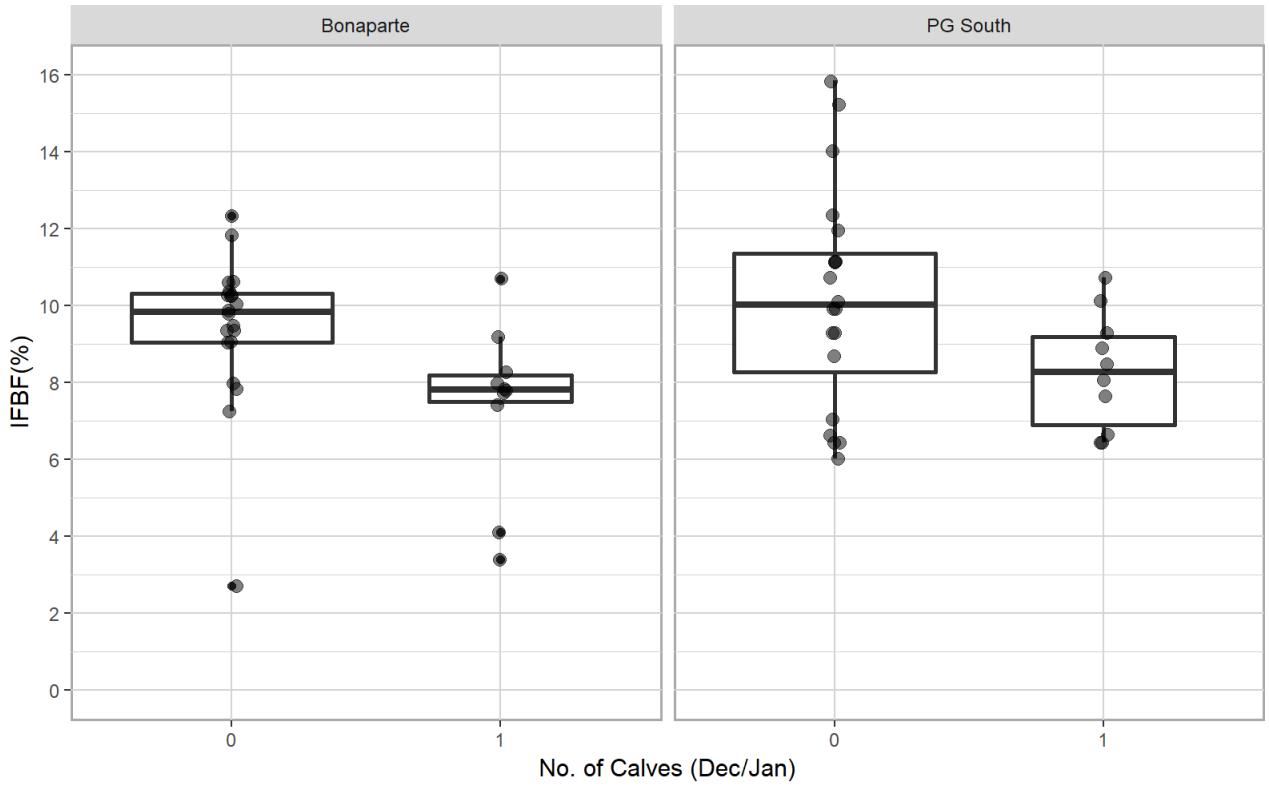

Figure 17. Boxplots of ingesta free body fat (IFBF, %) of adult female Moose estimated from ultrasound rump fat measurements (i.e., MAXFAT; Stephenson et al. 1998) at time of capture in the Bonaparte and Prince George South study areas, December - January 2019.

The average observed weight of calves in the Bonaparte was 179 kg (± 8 kg, 95% CI; *n* = 29) with an average annual weight of 182 kg (± 15 kg, *n* = 8) in 2017, 183 kg (± 9 kg, *n* = 11) in 2018 and 173 kg (± 17 kg, *n* = 10) in 2019 (Figure 19). The average observed weight of calves in PG South was 185 kg (± 9 kg, *n* = 12) with an average annual weight of 201 kg (± 6 kg, *n* = 4) in 2018 and 176 kg (± 7 kg, *n* = 8) in 2019 (Figure 19). The average combined observed weight of calves in both study areas was 180.6 (± 6 kg, n = 42).

Total length of Moose calves, using the Bonaparte data only (n=30), was found to best predict calf weight ($y = 1.7688x - 209.1920$; $r^2 = 0.72$, $p < 0.001$). Combining total length and chest girth was a comparable predictor and could also be used to estimate calf weight ($r^2 = 0.70$, $p < 0.001$). We used the total length regression to predict weight of all captured Moose calves where the total length measurement existed (n = 102; Bonaparte n = 59, Prince George South n = 43). Estimated calf weights were similar by sex, study area and year, and averaged 172 kg (± 4 kg, 95% CI). Moose calves from 2018 in the Prince George South study area were observed to be, on average, smaller than previous years and those calves from the Bonaparte study area ,but the difference was not significant (Figure 19).

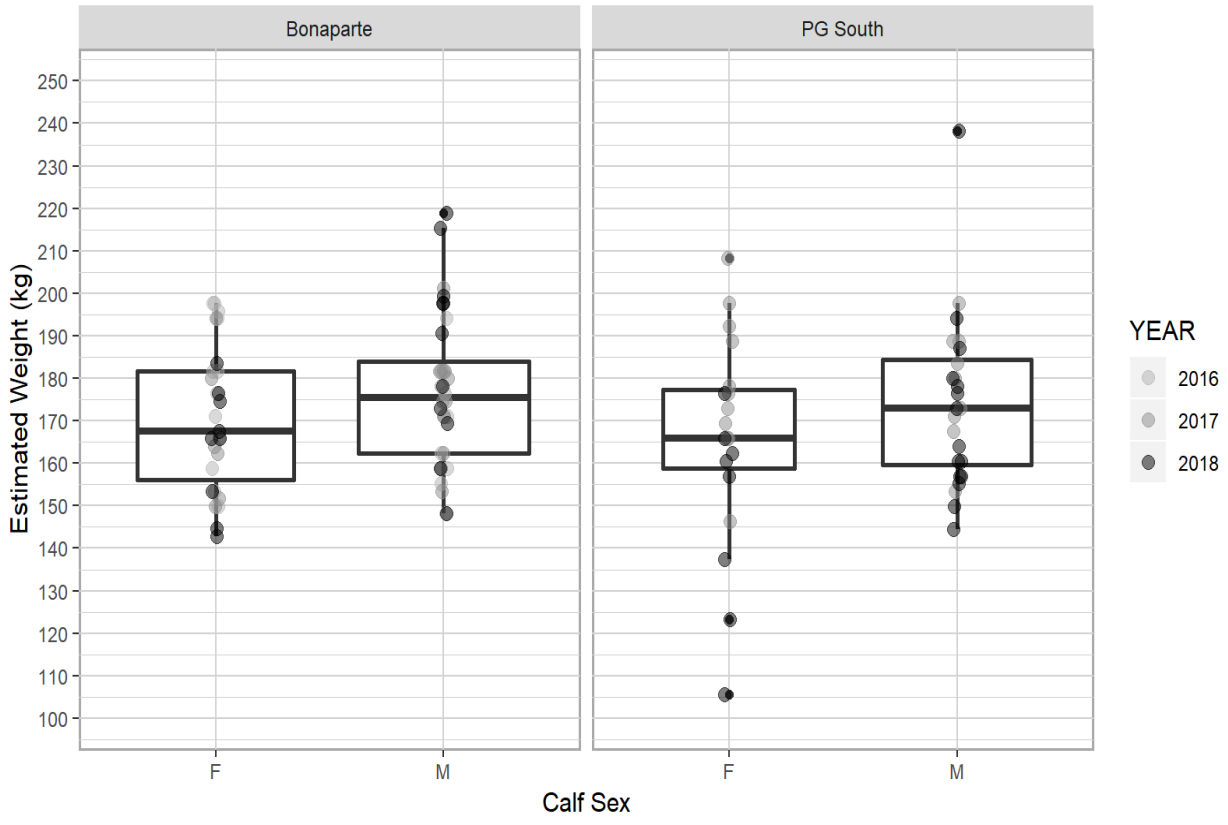

Figure 18. Boxplots of estimated weights of male and female Moose calves at time of capture in the Bonaparte and Prince George South study areas in January – February 2016, 2017 and 2018.

There is uncertainty in diagnosing pregnancy in cow Moose via serum progesterone when progesterone levels are low (Kuzyk et al. 2017). Therefore, we compared pregnancy status from progesterone and PSPB assessments and determined that PSPB was the best indicator of Moose pregnancy rates and is now our standard method used to assess pregnancy. All pregnancy results reported in Table 6 are from PSPB analyses. Estimated pregnancy rates ranged from 47–100% (Table 5). Differences between parturition (determined by analysing cow movement rates) and pregnancy rates estimated in the Bonaparte study area varied from 4–29% with the largest difference occurring when PSPB sample size was lowest (i.e., 2017/18, $n = 6$). No obvious trend existed. Given some probability of abortion, we expected estimated parturition rates to be lower than pregnancy rates, however, parturition rates exceeded pregnancy rates for the three of the six years. Overall, average parturition rates across the seven year period was similar to the average pregnancy rate and the difference was not substantial.

Initial serological screening of cow Moose indicated minimal exposure to a suite of pathogens selected for assessment at the early stages of the project. Additional assessments have been added and serum samples are now divided for archiving to use for future health analyses as warranted. Trace nutrient requirements and metabolism are not well characterized for Moose; however, some nutrient levels appear to be sub-optimal in some Moose, with variation observed between study areas.

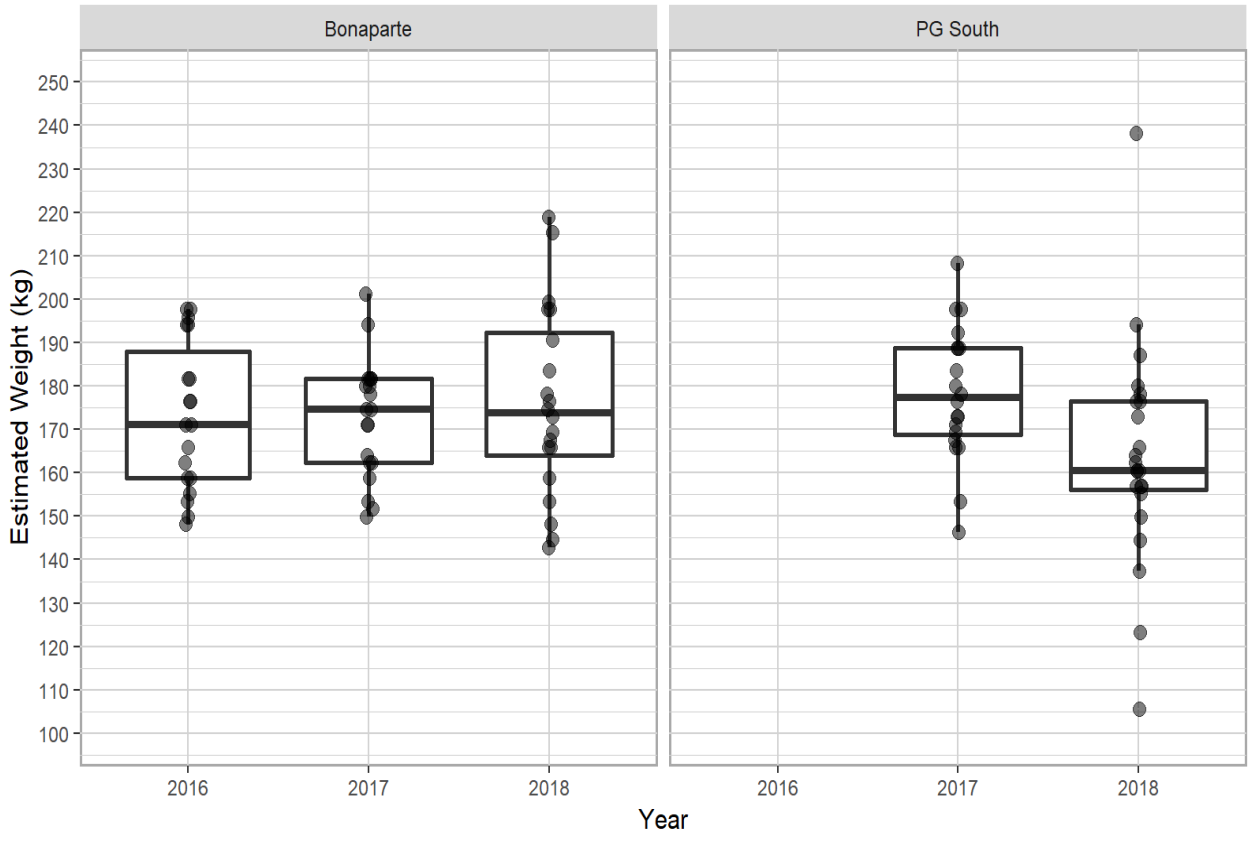

Figure 19. Boxplots of estimated Moose calf weights by year and study area at time of capture in the Bonaparte and Prince George South study areas, January-February 2016, 2017 and 2018.

Table 5. Pregnancy rates of radio-collared cow Moose (indicated by PSPB analysis) in central B.C. from February 2012–30 April 2019.

Study Area	Pregnancy (± 95% CI)							
	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18	2018-19	Mean
Bonaparte	72 ± 17% (n = 25)	85 ± 19% (n = 13)	71 ± 17% (n = 24)	47 ± 16% (n = 36)	68 ± 18% (n = 22)	50 ± 3% (n = 6)	77 ± 15% (n = 30)	67 ± 7% (n = 156)
Big Creek	n/a	89 ± 10% (n = 38)	75 ± 26% (n = 8)	100 - 22% (n = 5)	100 - 24% (n = 4)	67 ± 37% (n = 3)	79 ± 20% (n = 14)	86 ± 8% (n = 72)
Entiako	n/a	86 ± 10% (n = 43)	63 ± 28% (n = 8)	83 ± 17% (n = 18)	100 - 24% (n = 4)	90 ± 19% (n = 10)	92 ± 16% (n = 13)	85 ± 7% (n = 96)
PG South	n/a	86 ± 18% (n = 14)	64 ± 22% (n = 14)	75 ± 20% (n = 16)	87 ± 17% (n = 15)	50 ± 31% (n = 6)	83 ± 13% (n = 29)	78 ± 8% (n = 94)
JPRF	n/a	100 - 10% (n = 15)	n/a	88 ± 12% (n = 26)	100 - 18% (n = 7)	n/a	91 ± 12% (n = 23)	93 ± 6% (n = 71)

Table 6. Survival rates of radio-collared cow Moose in central B.C. from February 2012–30 April 2019.

Year	Survival Estimate (± 95% CI)	Number of Collars
2012	100 ± 0%	9
2012/13	95 ± 7%	38
2013/14	90 ± 9%	167
2014/15	92 ± 5%	201
2015/16	85 ± 5%	276
2016/17	89 ± 7%	272
2017/18	89 ± 4%	228
2018/19	89 ± 5%	271

Health-related factors were identified as the probable cause of death in several Moose mortalities (further described in Thacker et al. 2019). Preliminary evaluation of health data from capture and mortality samples suggested that the occurrence and potential impact of selected health determinants, including viral and bacterial pathogens, ectoparasites, endoparasites, and non-infectious measures (e.g., body condition, pregnancy rates, long-term stress and trace nutrient levels) can vary between study areas. Although most health determinants evaluated to date are within ranges reported in Moose populations elsewhere, there is evidence that some determinants (e.g., gastrointestinal parasitism in calves) may be sporadically killing some age classes of Moose in some study areas. No single factor, however, can be identified as the cause of apparent differences in the overall health status and/or performance of populations in these study areas at the present time. Likewise, the scope of this current Moose health monitoring cannot adequately evaluate the potential sub-lethal or cumulative effects of various health determinants on the fitness of individual Moose or the performance of Moose populations in these study areas. Thacker et al.
(2019) contains a detailed assessment of Moose health results from this project, providing the first comprehensive baseline herd health assessment of Moose populations in British Columbia.

4.3 Survival Rates

From 2012–2019, the annual survival rate from all radio-collared cow Moose pooled across all study areas varied from 85–100 % (Table 6, Figure 20). Cow survival rates varied across study areas (Figure 20) and were lowest in the Entiako and PG South study areas. Survival rates in some years in PG South and consistently in Entiako in recent years are below the 85% threshold typically used to assess for population stability (see Section 5.2). All survival rates in

other study areas are consistently above 85%, though confidence intervals sometimes extend below 85%. Survival of calves from age 8 months to recruitment (1 year of age) varied from 45% to 85% (Figure 21, Table 7) and

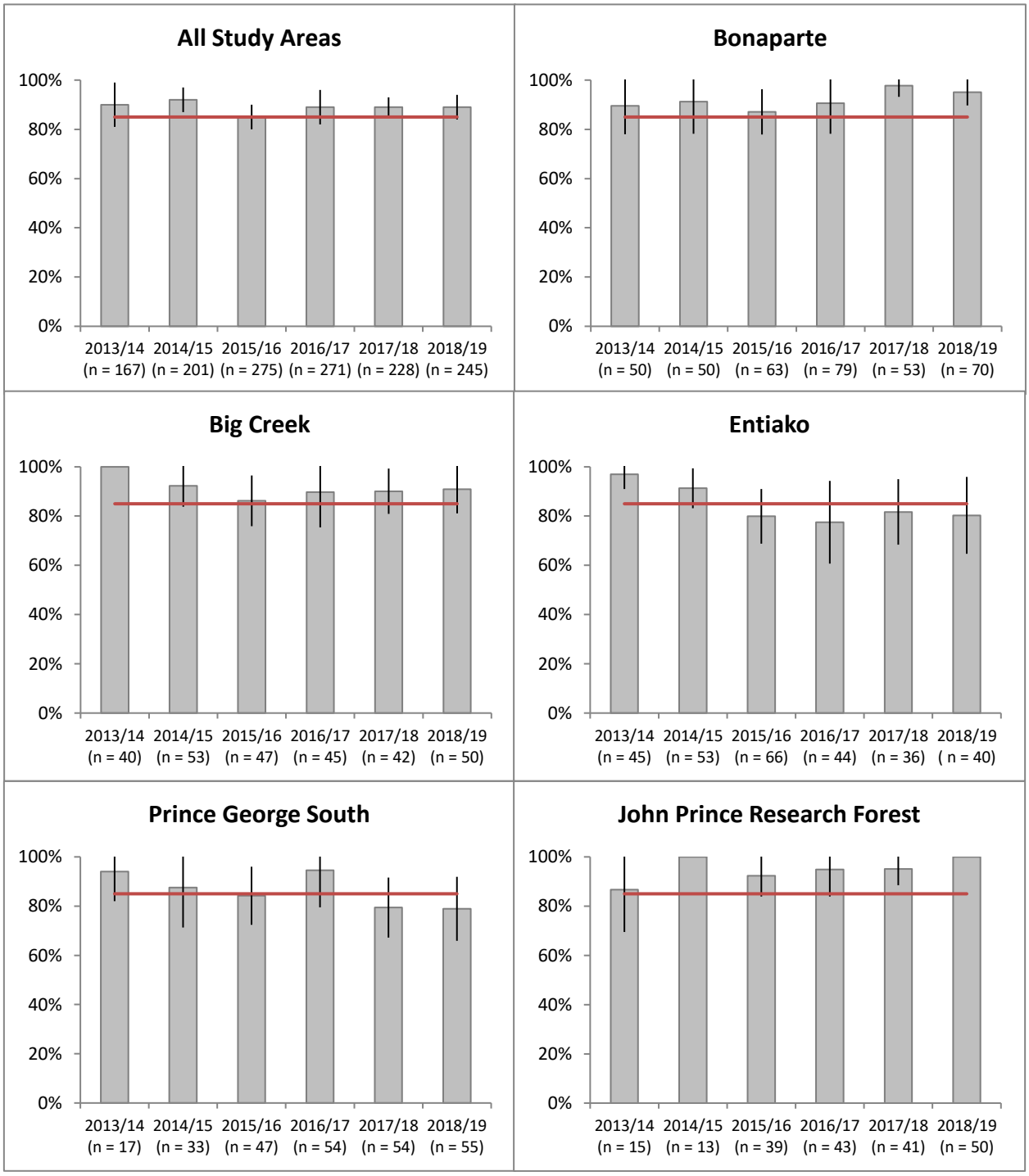

Figure 20. Survival rates of radio-collared cow Moose for all study areas combined and separated by study area, 1 May 2012 – 30 April 2019. Red line indicates survival rate of 85%, which is generally indicative of a stable population.

Table 7. Survival rates of radio-collared calf Moose (8-12 months) and those that survived to be yearlings (age 1- age 2) in central B.C. from January 2017–22 May 2018.

Year	Study Area	Age Class	Survival Estimate (± 95% CI)	Maximum Number of Active Collared Moose
2016/17	Bonaparte	8-12 months	45 ± 22%	20
2017/18	Bonaparte and PGS	8-12 months	78 ± 13%	40
2017/18	Bonaparte	8-12 months	85 ± 16%	20
2017/18	PGS	8-12 months	70 ± 20%	20
2017/18	Bonaparte	age 1- age 2	78 ± 27%	9
2018/19	Bonaparte and PGS	8-12 months	76 ± 14%	39
2018/19	Bonaparte	8-12 months	80 ± 18%	20
2018/19	PGS	8-12 months	74 ± 20%	19
2018/19	Bonaparte	age 1- age 2	80 ± 20%	16
2018/19	PGS	age 1- age 2	85 ± 19%	14

survival of yearlings (age 1 to age 2) varied between 78% and 85%. The sample size for cows in 2012 (n = 9) and calves in all years was small, warranting caution when interpreting those survival estimates.

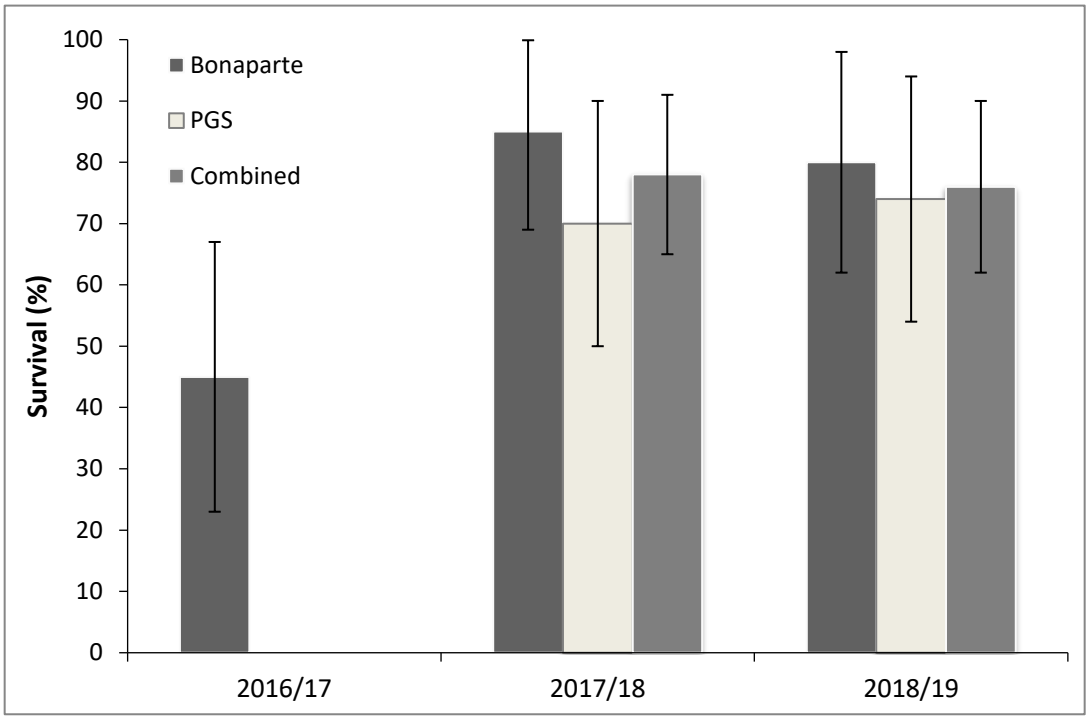

Figure 21. Late winter survival rates (i.e., time of capture to age 1) of radio-collared calf Moose in central B.C. from January 2017–22 May 2018.

4.4 Mortality Causes

Mortalities occurred on 122 of the 478 radio-collared cow Moose between February 2012 and 30 April 2019 (see examples in Figures 22 – 25). Probable proximate causes of death (see Appendix C) were 58% from predation, 20% from health-related causes, 14% from hunting, 2% natural accident, and 6% unknown (Table 8; Figure 26 and 27). We classified mortalities as unknown when there was minimal evidence available at the mortality site to reliably assign a cause of death; these instances occurred when mortality site investigations were significantly delayed due to radio collar malfunctions or predators moving the collar post-mortality such that a long delay occurred between the mortality event and the initiation of the mortality signal or the collar being positioned underneath the dead Moose thus limiting its transmission success. Cow mortalities peaked in spring with 57% of mortalities occurring between March and June (Figure 28, n = 122).

Figure 22. A mortality site investigation of a collared cow Moose mortality within the Prince George South study area. The proximate cause of death was health-related, February 2019.

Figure 23. A mortality site investigation of a collared calf Moose mortality within the Prince George South study area. The proximate cause of death was bear predation, May 2019.

Figure 24. Wildlife biologist Matt Scheideman skinning a collared cow Moose as part of a mortality site investigation within the John Prince Research Forest study area. The proximate cause of death was health-related, July 2019.

Figure 25. **Wildlife biologists Heidi Schindler and Jennifer Atkins examining wounds on a collared cow Moose a part of a mortality site investigation within the Entiako study area. The proximate cause of death was Grizzly Bear predation, June 2018.**

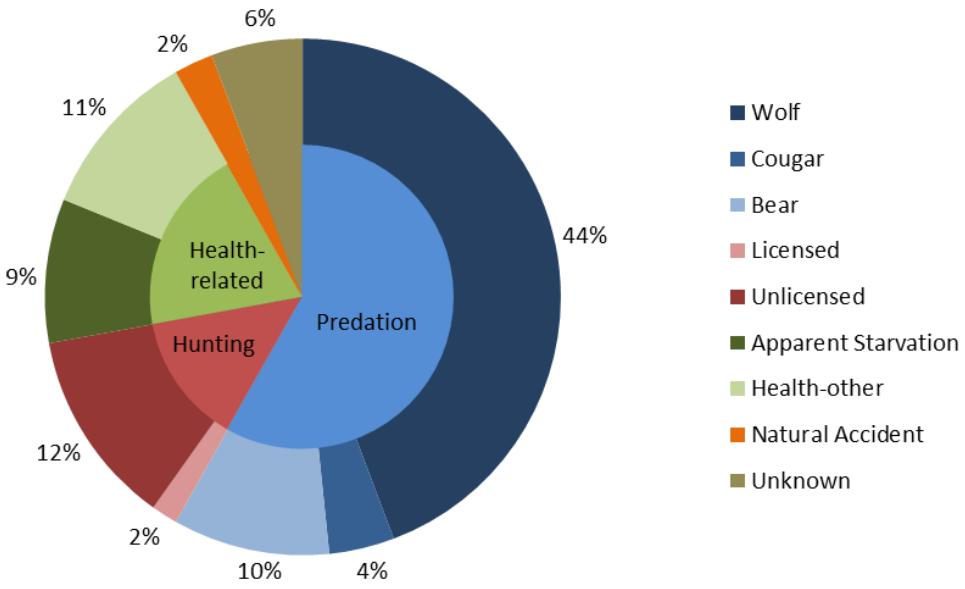

Figure 26. Probable proximate cause of death of radio-collared cow Moose (n = 122) in central B.C. from February 2012–30 April 2019. Cause of death proportions do not sum to 100% due to rounding.

Table 8. Number of mortalities and probable proximate cause of death of radio-collared cow Moose in central B.C. from February 2012 – 30 April 2019.

Study Area	Mortalities	Probable Proximate Cause of Death
Bonaparte	25	8 predation (6 wolf, 2 Cougar), 7 hunting (1 licensed, 6 unlicensed), 10 health-related (3 apparent starvation, 2 failed predation attempt, 1 chronic bacterial infection, 4 unknown health-related)
Big Creek	21	11 predation (8 wolf, 1 Cougar, 2 bear), 5 hunting (unlicensed), 4 health-related (1 bacterial septicemia*, 1 apparent starvation, 1 failed predation attempt, 1 peritonitis**), 1 natural accident
Entiako	35	26 predation (21 wolf, 5 bear), 2 health-related (1 prolapsed uterus, 1 unknown health-related), 2 natural accident, 5 unknown
PG South	32	21 predation (14 wolf, 2 Cougar, 5 bear), 3 hunting (1 licensed, 2 unlicensed), 8 health-related (7 apparent starvation, 1 pleuritis***)
JPRF	9	5 predation (wolf), 2 hunting (unlicensed), 2 unknown
Totals	122	71 predation (54 wolf, 5 Cougar, 12 bear), 17 hunting (2 licensed, 15 unlicensed), 24 health-related (11 apparent starvation, 3 failed predation attempt, 1 chronic bacterial infection, 1 peritonitis, 1 pleuritis, 1 bacterial septicemia, 1 prolapsed uterus, 5 unknown health-related), 3 natural accident, 7 unknown

***Bacterial Septicemia:** The presence of infective agents or their toxins in the bloodstream, sometimes called blood poisoning, characterized by elevated body temperature, chills, and weakness. Generally there is a primary site of infection that serves as the source of the pathogen. This is a serious condition that must be treated promptly otherwise the process of infection leads to circulatory collapse, profound shock and death.

****Peritonitis:** The inflammation of the peritoneum, the lining of the peritoneal cavity, or abdomen, by an infectious agent, usually bacteria but may be fungi or even a virus. The initiating cause may be a puncture of an organ, intestinal tract or the abdomen wall for entry of a pathogen. Left untreated, peritonitis can rapidly spread into the blood (sepsis) and to other organs, resulting in multiple organ failure and death.

*****Pleuritis**: The inflammation of the pleura, the lining of the thoracic cavity or chest by an infectious agent, usually bacteria but may be fungi or even a virus. The initiating cause may be a puncture of the thorax through the hide for entry of a pathogen, a severe infection of the respiratory tract (lungs) or a systemic infection with extension to the parietal (lining over the ribs) or visceral (lining over the lungs) pleura. Pleuritis is extremely painful and may result in fibrous adhesions attaching the lungs to the ribcage. In chronic cases, these can become scarred and walled off to persist over long time periods.

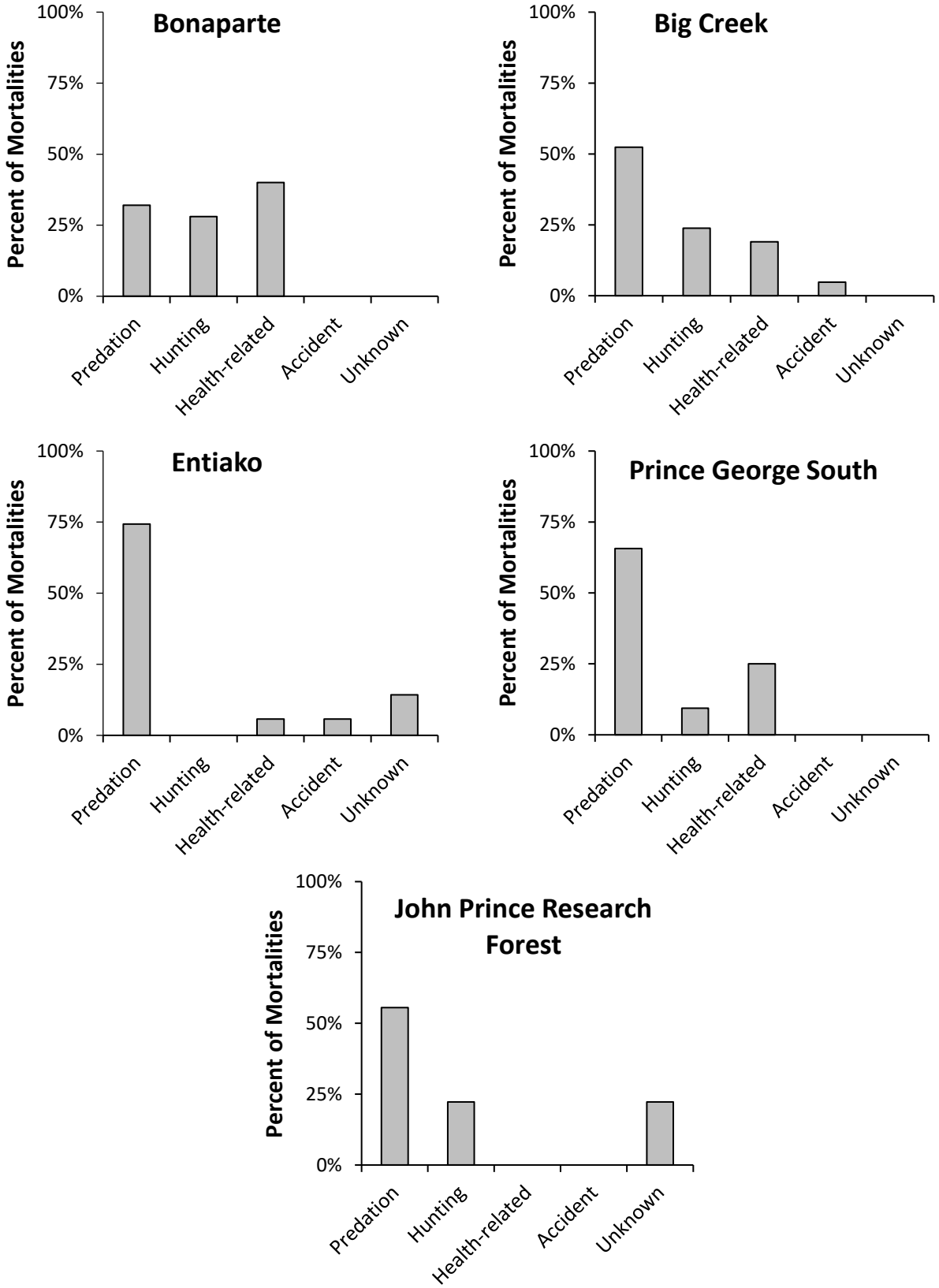

Figure 27. Probable proximate cause of death of radio-collared cow Moose (n = 122) by study area in central B.C. from February 2012–30 April 2018.

Table 9. Number of mortalities and probable proximate cause of death of radio-collared calf Moose (8 – 24 months) in central B.C. from January 2017 – 22 May 2019.

Study Area	Age Class	Mortalities	Probable Proximate Cause of Death
Bonaparte	Calf	18	Female: 5 predation (2 wolf, 2 Cougar, 1 bear), 4 health-related (2 apparent starvation, 1 apparent starvation/tick, 1 failed predation attempt), 1 vehicle collision Male: 5 predation (wolf), 3 health-related (2 apparent starvation, 1 gastro-intestinal infection)
PG South	Calf	11	Female: 1 health-related (apparent starvation) Male: 10 predation (7 wolf, 3 bear)
Bonaparte	Yearling	6	Female: 0 Male: 2 predation (1 wolf, 1 bear), 4 hunting (2 Licensed, 2 Unlicensed)
PG South	Yearling	2	Female: 0 Male: 1 predation (bear), 1 hunting (Licensed)
Totals		37	Female: 5 predation (2 wolf, 2 Cougar, 1 bear), 5 health-related (3 apparent starvation, 1 apparent starvation/ticks, 1 failed predation attempt), 1 vehicle collision Male: 18 predation (13 wolf, 5 bear), 5 hunting (3 Licensed, 2 Unlicensed), 3 health-related (2 apparent starvation, 1 gastro-intestinal infection)

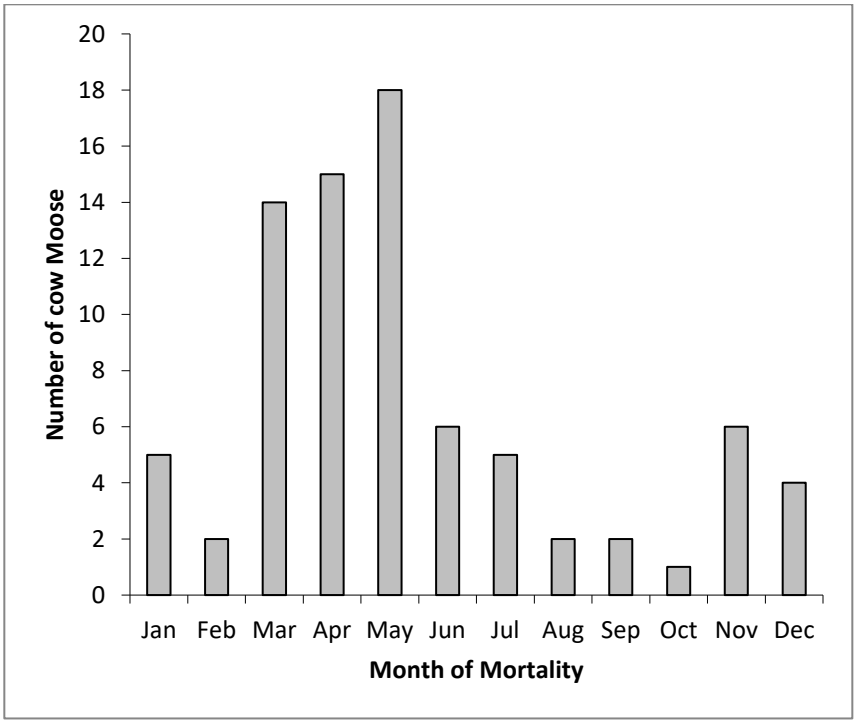

Figure 28. Month of death for radio-collared cow Moose (n = 122) in central B.C. from February 2012–30 April 2019.

Table 10. Body condition at time of death (as indexed by marrow fat) and probable proximate cause of death for collared cow Moose that died in central B.C. from February 2012–30 April 2019.

Probable Proximate Cause of Death	*n*	Average Marrow Fat % (±95% CI)	Marrow Fat % Range
Predation - all	58	59.6 (7.2)	5 – 89
Predation – wolf	41	66.4 (7.4)	5 – 89
Predation – bear	12	40.7 (18.0)	7 – 84
Predation - Cougar	5	49.2 (26.3)	6 – 76
Apparent Starvation	8	6.6 (1.1)	5 – 9
Health - Other	8	30.6 (22.3)	7 – 85
Hunting	6	77.3 (14.2)	43 – 88
Natural Accident	1	5.0	5

Of the 99 calf Moose radio-collared in winter of 2016/17, 2017/18, and 2018/19, there were 29 calf mortalities and 8 yearling mortalities (Table 9). Proximate probable cause of calf mortality was 20 predation (14 wolf, 2 Cougar, 4 bear), 8 health-related (4 apparent starvation, 2 apparent starvation/tick, 1 failed predation attempt, 1 gastro-intestinal infection) and 1 vehicle collision. We recorded a significantly higher proportion of health-related, particularly apparent starvation, mortalities (i.e., 45%) in 2016/17. Proximate probable causes of mortality for yearling were 5 from hunting (3 licensed, 2 unlicensed), and 3 from predation (1 wolf, 3 bear).

When we examined the relationships between cow body condition and age and causes of mortality, we found that bone marrow fat analysis conducted on cow Moose mortalities (n = 83) showed 49% in good body condition (>70% marrow fat), 23% in poor body condition (20–70% marrow fat) and 28% with acute malnutrition (<20% marrow fat). The majority of mortalities involving cows with acute malnutrition and poor body condition occurred between April and June while mortalities in the remainder of the year typically involved cows in good body condition (Figure 29). Mortality causes associated with cow Moose in good body condition included predation, health (excluding apparent starvation) and hunting. Mortality causes associated with Moose in poor condition and acute malnutrition included predation, apparent starvation, health-other, hunting and natural accident (Table 10). All hunting kills, except for one, were of cow Moose in good condition and all apparent starvation mortalities had marrow fat levels <10%. Sixty-two percent of predation kills were of cow Moose in good condition, and 25, 83, and 100% of direct mortalities by wolves, bears and Cougar, respectively, were of cow Moose in states of poor condition or malnutrition.

Calf marrow was assessed in Bonaparte and PG South which were all classified as being either in poor body condition or with acute malnutrition. Fifteen marrows were assessed from Bonaparte; 10 were < 20% and five were between 20-70% marrow fat. In Prince George South, 10 marrows were assessed; seven were <20% and three between 20-70% marrow fat.

Average age of cow Moose at death was 10. Age at death ranged from 2 to 18 years, and differences by probable proximate cause of death were not significant (Table 11). There was no apparent trend associated with age and probable proximate cause of death, but those killed by predators and health-related factors tended to be slightly older.

Table 11. Average and range of age at death for collared cow Moose by probable proximate cause of death for collared cow Moose that died in central B.C. from February 2012–30 April 2019.

Probable Proximate Cause of Death	n	Average Age (±95%CI)	Age Range
Predation - all	52	11 (1.2)	2 - 18
Predation - wolf	39	11 (1.4)	2 - 18
Predation - bear	10	10 (2.5)	2 - 16
Predation - Cougar	3	14	14
Apparent Starvation	10	9 (3.2)	2 - 15
Health - Other	9	11 (2.6)	3 - 17
Hunting	6	9 (2.4)	5 - 12
Natural Accident	2	10 (0.4)	9 - 11

4.5 Calf Production, Survival and True Recruitment

In both study areas, we observed significant variation across years in calf production, calf survival and true recruitment at age 1 (Table 12). Due to mortality of calves in the late winter period, true recruitment when calves turn 1 year of age was consistently lower, regardless of year or study area, than recruitment indices measured in mid-winter from aerial surveys by an average (± SE) of 29.2 ± 6.6%. Although based on only three years of data thus far, the data suggest that when calf production is higher, so is calf survival (in both summer and winter) and true recruitment. More data are required to assess whether that trend persists.

Figure 28. Body condition (as indexed by marrow fat) for each individual collared cow Moose mortality shown by month of mortality (n = 83) in central B.C. from February 2012–30 April 2019. Acute malnutrition is associated with marrow fat <20% (below red line), poor body condition is associated with marrow fat between 21% and 70% (between orange and purple lines), and good body condition is associated with marrow fat >70% (above purple line).

Table 12. Calf production, summer calf survival and true calf recruitment in the Bonaparte and Prince George South study areas from May 2016 – June 2018. Estimates of error are 95% confidence intervals. Sample size (n) is the number of cows the estimate is derived from.

Year	Study Area	Minimum No. Calves/100 Cows at Birth[1]	No. Calves/100 Cows Mid-June[2]	No. Calves/100 Cows Mid-winter [3]	Maximum Calf Pre-Winter Survival (%)[4]	No. Calves/100 Cows Mar. 31[2]	True Recruitment Rate (No. Calves/100 Cows at age 1)[5]
2016/17	Bonaparte	59 (46 – 72) (*n* = 59)	n/a	13 (7 – 19) (*n* = 184)	22% (15 – 26)	16 (*n* = 32)	6 (3 - 9)
2017/18	Bonaparte	76 (64 – 88) (*n* = 46)	64 (*n* = 47)	32 (23 – 41) (*n* = 194)	42% (36 – 47)	38 (*n* = 40)	27 (20 – 35)
2017/18	PG South	79 (62 – 96) (*n* = 24)	n/a	34 (29 – 39) (*n* = 280)	43% (39 - 46)	26 (*n* = 35)	24 (20 – 27)
2018/19	Bonaparte	80 (68 - 92) (*n* = 41)	n/a	28 (17 - 39) (n = 116)	35% (25 – 42)	n/a	22 (14 - 31)
2018/19	PG South	48 (30 - 66) (*n* = 31)	65 (44 - 86) (*n* = 20)	31 (21 - 41) (n = 128)	65% (62 – 70)	n/a	23 (16 - 30)

[1] Estimated from movement analyses for collared cows and assumes all cows had only 1 calf (i.e., no twinning)
[2] Estimated from aerial searches of collared cows and their calves
[3] Estimated from aerial composition surveys in respective study areas
[4] Estimated by comparing survey-based calf ratio mid-winter to estimated calf ratio at birth; maximum calf survival estimate as twinning rate at birth not known
[5] True recruitment = mid-winter calf ratio x calf survival from mid-winter to age 1 (estimated from collared calves — see Table 7)

Calf production, survival and recruitment parameters were similar between Bonaparte and Prince George South study areas in 2017/18 and 2018/19. We will continue to monitor annual variation between study areas as calf monitoring continues over the years.

Differences between mid-winter recruitment indices and what we defined as true recruitment (i.e., the number of calves that survived to age 1) reduced estimates of population rate of change by approximately 4% (range 3%-5%; n=3; Table 13). Higher population growth rate in Bonaparte in 2017/18 resulted from higher cow and calf survival that year, while a negative population trend in Prince George South resulted from a relatively low 2017/18 cow survival.

From 2014–2019, we conducted 22 late winter (February and March) surveys across the five study areas to assess the survival of calves associated with radio-collared cows. Results varied among study areas with calf/cow ratios ranging from 8–40 calves/100 cows (Table 14).

4.6 Density Surveys

At the initiation of the study, Moose densities ranged from 170–770 Moose/1000 km^2 among study areas and have largely experienced population declines (Table 15).

Table 13. Comparison of Moose population rate of change (lambda) estimated using recruitment indices during mid-winter surveys and survival rates from collared cows and calves to recruitment at age 1. Lambda was calculated as S/(1-R) where S is cow survival and R is female calf/cow ratio (Hatter and Bergerud 1991).

Year	Study Area	Lambda – Survey-based Mid-winter (95% CI)	Lambda – True Recruitment Age 1 (95% CI)
2016/17	Bonaparte	0.98 (0.82 – 1.07)	0.93 (0.78 – 1.01)
2017/18	Bonaparte	1.14 (1.06 – 1.19)	1.11 (1.03 – 1.16)
2017/18	PG South	0.92 (0.79 – 1.04)	0.88 (0.75 - 1.01)
2018/19	Bonaparte	1.08 (0.96 - 1.17)	1.05 (0.94 - 1.13)
2018/19	PG South	0.95 (0.82 - 1.06)	0.92 (0.79 - 1.04)

Table 14. Calf surveys to determine presence of calves with radio-collared cow Moose in central B.C. from March 2014–March 2019. The number of collared cows observed is presented parenthetically.

Study Area	# Calves/100 cows in Late Winter					
	2014	2015	2016	2017	2018	2019
Bonaparte	-	25 (40)	26 (68)	16 (32)	38 (40)	-
Big Creek	28 (41)	37 (43)*	33 (43)	27 (41)	32 (37)	34 (29)
Entiako	-	-	14 (44)	9 (35)	15 (26)	27 (30)
PG South	-	39 (18)	27 (44)	40 (49)	26 (35)	-
JPRF	-	8 (13)*	17 (36)	40 (42)	37 (38)	-

*Indicates the survey was completed in February, all others occurred in March of associated year.

Table 15. Moose density and trend estimates resulting from aerial surveys in five Moose research study areas in central B.C., where cow Moose survival has been monitored.

Study Area	Moose Density at Project Start ± 90% CI (winter year)[1]	Moose Density near End of First 5 Years ± 90% CI (winter year)[1]	Annual Population Trend
Bonaparte	296 ± 18/1000 km^2 (2012/13)	254 ± 41/ 1000 km^2 (2017/18)	-2.8 %
Big Creek	170 ± 39/1000 km^2 (2011/12)	220 ± 38/ 1000km^2 (2016/17)	+5.9%*
Entiako	267 ± 45/1000 km^2 (2012/13)	217 ± 46/ 1000 km^2 (2018/19)	-3.1%
PG South	630 ± 102/1000 km^2 (2011/12)	400 ± 78/1000 km^2 (2016/17)	-7.3%
JPRF	770 ± 93/1000 km^2 (2011/12)	490 ± 84/1000 km^2 (2016/17)	-7.3%

*Survey conditions in Big Creek in the 2011/12 survey were not ideal; temperatures were >0C with sparse snow cover in more than half the survey blocks.

5. DISCUSSION

5.1 Capture, Handling and Biological Samples

As of April 2019, we have monitored survival of 478 cows and ninety-nine calves collared at 8-months old. At the time of capture, the majority of cow Moose were predominately mid-aged adults (i.e., only 11% classed as old and 4% young) and were assessed using a body condition score as being in fair to excellent body condition, with only 3% in poor condition and 1% emaciated. However, condition varied by year and study area, with a higher proportion of Moose in poor condition at capture in 2016/17 and in PG South. Of the ninety-four 8-month old calves assessed for body condition, 99% were in good and fair body condition and 1% in poor condition. Although a standard condition evaluation protocol exists, it is possible that observer bias during captures has some degree of influence over body condition assessments between study areas. Given these methods and results, Moose populations overall in these study areas do not seem to be in poor condition; however, these subjective measures of body condition may not be sufficiently sensitive to detect variation in condition that may influence the fitness of individual Moose. To address those issues, this is the first year we measured body fat on cow Moose with ultrasonography to help better characterize condition of these Moose populations. We observed average rump fat measurements of 16.4 mm and 19.2 mm in the Bonaparte and PG South study areas, respectively, which corresponded to estimated body fat levels of 8.8% and 9.5%. We consider these estimates to be reflective of summer conditions and characterizing the condition of adult females entering winter (peak condition) for 2018. Some cows were captured in January, a little later than desired to estimate summer condition, due to less than ideal capture conditions in early December; however, given the mild winter conditions (very little snow) through December and January, we assume cows did not appreciably lose fat during that time. Cows that had calves present were generally thinner than cows without calves, due to the energy expense associated with lactation through the summer (Cook et al. 2013). Data reflective of the summer condition of Moose are scarce. Much of the data that exists is collected much later in the winter and therefore estimates are reflective of not only their condition entering winter, but also reflective of the variable winter conditions experienced in that year. Testa and Adams (1998) observed rump fat on female Moose in 1994-95 prior to winter in Alaska, and found that reproductive females had average rump fat of 29 mm and non-reproductive females had average rump fat of 42 mm - nearly twice that observed to date in this study. This indicates that Moose in our study areas could be much fatter than they were observed to be in 2018. Interestingly, body fat levels observed in this study, at the very start of winter, were similar to those observed in a declining Moose population in Minnesota late in the winter (DelGiudice et al. 2011), where average % body fat of adult females was found to be 9.8% in late February and early March. DelGiudice et al. (2011) do not believe that body condition alone explains population declines observed in Minnesota. Rather, they hypothesized that chronic summer and autumn nutritional limitation might be contributing minor effects on survival and reproduction, but when combined with occasional severe winters, the effects on survival and reproduction becomes more significant in those years. Data over multiple years are required to assess how Moose condition varies on an annual basis, and how it might be contributing to Moose population change in B.C.

Seven-year average pregnancy rates observed in this study ranged from 67 – 93%, with the lowest observed in the Bonaparte (67%) and PG South (78%) study areas; average rates in the other three study areas were 85–93%. Boer (1992) reported an average of 84% pregnancy rates from various studies around North America though they can vary due to differences in

nutritional condition of the females. Pregnancy rates for several populations of Moose in parts of Alaska were 76–97% (Schwartz 1998) whereas another study determined pregnancy rates that varied from 60–100% in accordance with nutritional status (Gasaway et al. 1992). In North Dakota during a period of Moose population growth Jensen et al. (2018) reported a pregnancy rate of over 95% while Ruprecht et al. (2016) reported an average pregnancy rate of 74% along the southern edge of Moose distribution in Utah. In Minnesota, chronically low pregnancy rates, between 38 and 59%, were reported in a nutritionally-stressed Moose population (Murray et al. 2006). These data from Minnesota may indicate some Moose populations in British Columbia with low pregnancy rates may be experiencing nutritional limitations. Using data provided by Testa and Adams (1998), probability of pregnancy varies with the condition of Moose entering winter such that 10.4% body fat is required by female Moose to maintain an 85% probability of being pregnant. We used 85% as a threshold to evaluate as it approximates the North American average reported by Boer (1992). Average % body fat in both study areas was below this threshold, which indicates that the annual condition of Moose likely explains low and variable pregnancy rates. However, other factors (e.g., age) can also influence pregnancy rates for Moose (Heard et al. 1997; Murray et al. 2006). Age data from our study suggest Moose populations are trending toward older age distributions due to lower recruitment rates. There are many health-related factors that can also influence Moose pregnancy, parturition and calf survival rates, and ultimately recruitment (Thacker et al. 2019).

Sixty-two percent of collared Moose calves were judged to be in good body condition at time of capture, 37% were in fair condition and 1% (n = 1) were in poor condition. We developed a regression equation to estimate the weight of Moose calves based on their total length using data from 30 calves in the Bonaparte study area where we observed their actual weight. The average estimated weight of all calves 8-9 months of age in both the Bonaparte and PG South study areas was 172 ± 4kg. This is in the lower end of the range reported in Alaska over several years for 9–10 month old calves (167.5–191.4 kg, Keech et al. 2011) but larger than average weights of 9–10 month old calves reported elsewhere in Alaska (148.9 kg, Keech et al. 1999; 157–170 kg, Boertje et al. 2007) and less than the average weight of 7 month old calves reported in North Dakota (196 kg, Jensen et al. 2013). Keech et al. (1999) attribute their low average weight of 9–10 month old calves to poor nutritional status of their study Moose population due to high Moose densities. Similarly, Jensen et al. (2013) attribute their higher average weight of calves to high nutritional status of their Moose population arising from use of high quality forage in agricultural areas. Boertje et al. (2007) also indicated their average weights varied with nutritional status, and suggested that average calf weights of >190 kg are predictive of high nutritional status. Thus, calves in this study appear to be smaller on average than those from populations characterized by high nutritional status, which aligns with other indications (e.g., low pregnancy rates, observations of low average body fat) that some Moose may not be in great condition and further highlights the need to continue to objectively characterize the body condition of cow Moose at capture and how it varies over time. As calf weights in the literature are reported at different ages (i.e., 7–10 months) and during a period of time when Moose are generally losing weight (i.e., early to late winter), caution is required in interpreting these data as the time of the year calves were weighed may introduce variation that is reflective of the time of year as opposed to true differences in the weights of calves. We plan to continue monitoring the size of calves as an index to the nutritional status of Moose populations and to allow investigation into relationships between the size of calves and their probability of survival.

We continue to evaluate and monitor our capture methods and protocols to ensure use of the most humane and effective methods for capturing Moose, while maximizing opportunities to collect biological samples when animals are immobilized or restrained. The B.C. wild ungulate herd health assessment model (FLNRORD, unpublished data) is building a baseline on this species, using established methods and investigating new measures of Moose herd health. The identification of the impacts of cumulative effects such as disturbance and habitat changes, winter ticks, nutrition and other factors influencing overall health has allowed collaborative research aimed at furthering understanding of their importance and how widespread these factors, and others, may be. Assessing and monitoring Moose herd health, as well as standardization of procedures and increased experience and consistency in capture and mortality site investigation crews, has resulted in improved field methods and documentation.

5.2 Survival Rates

The landscape change hypothesis states Moose declines coincided with a MPB outbreak where habitat changes and increased salvage logging and road building resulted in greater vulnerability to Moose from hunters, predators, nutritional constraints, age, health and environmental conditions (Kuzyk and Heard 2014). We determined cow survival rates as the first evaluation of the landscape change hypothesis because cow survival has a greater proportional effect on population change than does calf survival (Kuzyk and Heard 2014). Monitoring survival rates of 478 cow Moose from 2012-2019 (n = 8 years) is sufficient to evaluate this hypothesis. Annual cow survival rates for all study areas combined was greater than 85%, which is within the range reported from stable Moose populations, i.e., >85% (Bangs et al. 1989; Ballard et al. 1991; Bertram and Vivion 2002). These combined cow survival rates were higher than those estimated for cow Moose in studies from the Northwest Territories (85%, Stenhouse et al. 1995) and northern Alberta (75–77%, Hauge and Keith 1981). On an individual study area basis, Bonaparte, Big Creek and JPRF study areas were characterized by cow survival above 85% in all years. In comparison, Entiako was below 85% in the last four of six years, which is indicative that cow survival is contributing to population decline in this study area. PG South was below 85% in three of five years, with the most recent two years being below 85% (Figure 15). When all study areas were combined, survival rates over these seven years were not indicative of Moose population declines and were inconsistent with the cow survival rate component of the landscape change hypothesis, suggesting that calf survival and recruitment is the primary factor influencing Moose population change.

5.3 Mortality Causes

Determining mechanisms influencing vulnerability of cow survival was the second evaluation of the landscape change hypothesis (Kuzyk and Heard 2014). Over the last six years, over half (58%) of cow Moose died from predation (proximate cause of death), with the majority of those killed by wolves. Predation by wolves occurred in all study areas, and predation by bears occurred in three study areas. Predation by bears and Cougar occurred only in Bonaparte, PG South and Big Creek. Of note, JPRF had predation of cow Moose by only wolves. The second most frequent proximate cause of death of cow Moose was health-related issues (20%) from a variety of causes (Thacker et al. 2019). Proximate cow Moose mortalities from hunting were 14%. Hunting was initially assumed to be one of the main factors, along with predation, that would influence Moose population change, as increased number of roads and reduced visual cover from cutblocks would make Moose more vulnerable to hunters (Kuzyk and Heard 2014). Determination of the role of landscape features in influencing differential causes of cow mortality

by study area is currently in process (Mumma and Gillingham 2019) and is also being examined on the study area scale.

Our study is steadily increasing the sample size of bone marrow fat samples from radio-collared cows (n=83). Nearly half these cow Moose were in good body condition; however, more than a quarter of them were in a state of acute malnutrition. Mortalities from a state of acute malnutrition mainly occurred between April and June and may simply reflect the annual cycle of body condition of Moose, since their poorest body condition occurs naturally during late winter/early spring (Franzmann and Arneson 1976; Fong 1981; Ballard 1995). However, as hypothesized to explain Moose declines in Minnesota, DelGiudice et al. (2011) suggested variation in the condition of Moose entering winter may also be affected by winter conditions in some years, such that Moose are not able to accumulate sufficient reserves to survive to green-up the following spring. If this were true, we would expect variation on an annual basis in the frequency of death by apparent starvation, which is consistent with our observations in this study. For example, in the Bonaparte and Prince George study areas, apparent starvation in the late winter appears to have been a more important factor in a couple years of the study but not all, which may indicate Moose may have been in poorer condition in certain late winter/early spring periods of some years. We recognize the limitations of using marrow fat as an index to body condition as individuals with high levels of marrow fat may still not be in good condition. Marrow fat is the last fat storage to be mobilized, and it is possible for individuals to lose all other fat stores and still have high levels of marrow fat. Marrow fat indicates animals in poor condition but may not reliably indicate an individual in good condition (Mech and DelGiudice 1985). Marrow fat levels may vary with the specific bones selected for analysis (Spears et al. 2003). We tried to maintain consistency with the bones selected for analysis but if desired bones were not available, we used bones that were. Marrow fat levels may also be overestimated due to weather conditions and length of exposure prior to field collection and freezing, or from samples being frozen too long. Ballard (1995) suggests inferring body condition of the larger Moose population (not just those that are dead) by comparing the condition of those dead by natural and unnatural causes. We have explored this option though we did not have sufficient samples of unnatural mortalities to do this. Our results suggest that, based on marrow fat levels, most calf Moose were in poor condition at time of death. However, it is important to recognize that young developing Moose are naturally characterized by low marrow fat levels, because energy intake is invested in body growth and maintenance as opposed to fat storage and thus does not reliably indicate their habitat condition or nutritional status (Fong 1981). Spears et al. (2003) also found calves to be characterized by lower marrow fat than adults.

Age at death varied between 2–18 years of age, and there appeared to be no differences in proximate cause of death by age. The ages at death observed in this study suggest that cow Moose died at an older age, regardless of cause, which indicates we randomly captured and monitored older Moose, possibly because individuals in the population were older, or that older Moose are more vulnerable to all causes of mortality (Peterson 1977; Montgomery et al. 2014). This seems unlikely given that some mortality factors operate randomly relative to age (Ericsson and Wallin 2001). An alternative explanation is that random Moose captures were biased toward older individuals, which also seems unlikely. A recent analysis of Moose ages determined from hunter-harvested Moose from 1982-2003 and ages from mortalities from this study found the average age of random cows harvested from 1982-2003 (n = 2,016; age = 3.84, SD = 3.03) to be much younger than the small sample of cows (n = 47; age = 10.93, SD = 3.72) dying from all causes (i.e., hunter harvest, health, predation, natural accident) during this study

(Kuzyk et al. in prep/press). This indicates Moose populations prior to this research may have been characterized by a younger age distribution.

5.3 Calf Production, Survival and True Recruitment

Late winter survival rates of calves from 8-12 months (age 1) varied from 45% to 85% across years and study areas. Our results found an average of 29% lower recruitment of Moose calves at age 1 relative to recruitment indices observed during aerial surveys in mid-winter. This is important since true recruitment is capable of changing population trends on an annual basis, especially in years where lower calf recruitment may interact with years of lower cow survival. Data collected to date in this study suggest mid-winter recruitment ratios generated from aerial surveys better reflect early calf survival than recruitment to age 1, and that mid-winter recruitment ratios need to be corrected to account for late winter and early spring mortality in order to better understand the influence of recruitment on Moose populations. Variation in recruitment observed to date in this study is consistent with relatively constant adult survival and large annual variation in juvenile recruitment observed in many other ungulate populations (Gaillard et al. 1998; Gaillard et al. 2000). Maintaining long-term monitoring of calf survival and recruitment is important to capture longer-term variation in this parameter, and will enable a better understanding of the role of these factors in Moose population change. Survival rates of yearlings (age 1 to age 2) varied between 78% and 85%, which, although based on a relatively small sample size, are approaching survival rates observed for adult females in this study, and provide some support for assumptions described earlier in defining true recruitment as the age at which survival rates of calves approximate adults.

Since 2016/17, of the 99 calves collared, there were 29 calf mortalities, all of which occurred after 11 March and before the calves turned age one. Proximate probable causes of mortality of calves were 20 predation (14 wolf, 2 Cougar, 4 bear), 8 health-related (4 apparent starvation, 2 apparent starvation/tick, 1 failed predation attempt, 1 gastro-intestinal infection) and 1 vehicle collision. About half of total mortalities were from wolves, with a higher proportion of male calves (n=12) than females (n=2). We recorded a significantly higher proportion of health-related mortalities (particularly apparent starvation) in 2016/17 (i.e., 45%) in the Bonaparte study area than has been seen since (25% and 0% in 2017/18 and 2018/19, respectively). The lack of apparent starvation mortalities in late winter/early spring 2018 and 2019, relative to 2017, is of interest as it may relate to our work on maternal body condition as a potential driver of calf survival, and it may provide support to the idea that Moose were in poorer condition in the recent past than they have been recently, as discussed above. In the Prince George South study area, the number of overall calf mortalities has been higher than in Bonaparte in 2017/18 and 2018/19 (n=11 vs. n=8) but similar rates of predation and health-related causes were observed. Predation and health-related causes were responsible for 91% and 9% and 88% and 12% of calf mortalities in PG South and Bonaparte, respectively, over those two years. The proportion of predation mortalities observed in this study on older Moose calves is similar to that observed in Minnesota (i.e., 84%) for neonate calves (Severud et al. 2019), and similarly, wolves were responsible for a high proportion of predation mortalities in both areas, 70% in this study and 77% in Minnesota (Severud et al. 2019). Causes of neonate calf mortality are not known in our study, but we have observed maximum survival calf rates, from birth through their first summer, that average 41%, ranging from 22-65% (Table 13), which indicates that there are important mortality factors influencing early calf survival and contributing to observed recruitment rates. Indeed, all studies of neonate Moose calf mortality in North America of which we are aware have consistently identified predation as the most important mortality factor

for neonate calves (e.g., Ballard et al. 1981, Larsen et al. 1989, Ballard et al. 1991, Osborne et al. 1991, Testa et al. 2000, Keech et al. 2011, Patterson et al. 2013, Severud et al. 2019), although the dominant predator, whether it is wolves or bears or the species of bear, varies by study area.

5.4 Density Surveys

We now have density estimates from all study areas, from the beginning of the study to five-six years later (Table 2). Most study areas experienced population declines ranging from 2.8% to 7.3% annually, with a reported increase in one study area which may be an artifact of poor survey conditions during the initial survey (Table 2). As discussed above, cow survival rates have generally been sufficient to maintain stable to increasing populations through the research period, and continued declines in these study areas indicate that calf survival and recruitment is the primary driver of Moose population change, although there is evidence that lower female survival rates in Entiako and PG South in some years are contributing to population change.

6. MANAGEMENT RECOMMENDATIONS

Several theses and publications are arising from this research that will provide management recommendations. Analyses of habitat selection of radio-collared cow Moose were completed at UNBC for the Big Creek, Entiako and PG South study areas (Scheideman 2018), and are ongoing at the University of Victoria for the Bonaparte study area (Francis et al., in prep.). Mumma and Gillingham (2019) undertook an analysis of cow Moose mortality in relation to landscape disturbance features related to salvage logging. A study investigating seasonal migrations of collared cows and fine-scale winter occupancy patterns at the John Prince Research Forest has been completed (Chisholm et al. 2019). Thacker et al. (in prep.) are completing an analysis of Moose health using biological samples collected at time of Moose capture and mortality,

Scheideman (2018) quantified seasonal home range selection, home range size and daily movements, and within home range selection of GPS radio-collared cow Moose in the Big Creek, Entiako and PG South study areas. Individual variation among cow Moose was evident at both home range and within home range scales. Collared cow Moose selected lodgepole pine-leading stands at both spatial scales despite the die-off of pine due to MPB. Clear-cuts following the MPB outbreak were avoided in drier locations, and there were trade-offs between cover and browse evident where disturbance due to salvage logging was highest. Generally, MPB salvage logging reduced Moose habitat, and thereby influenced selection by female Moose (see Scheideman 2018 for details). Key recommendations from this research are:

- Reduce road densities by sufficiently rehabilitating unused forestry roads to hinder predator and human travel, replant corridors with deciduous and crop species to restore to a productive site.
- Increase local landscape heterogeneity by creating smaller clearings with greater proportions of intact forest between stands.
- Reduce herbaceous stand-tending where regeneration is slow and incorporate deciduous species in re-stocking post-harvest in dry sites, focusing palatable species near edge.
- Retain remaining pine-leading stands in relationship to suitable Moose habitat as these sites post-MPB still contain high stocking standards, heterogeneity in vertical and horizontal structure, and increased diversity of understory species.

The Habitat Conservation Trust Foundation supported a comprehensive cow-survival analysis with UNBC, which was completed in April of 2019 (Mumma and Gillingham 2019). The analysis used a cause-specific approach to

understand the spatial and temporal influence of key landscape disturbance features on risk to Moose. Key findings were: 1) risk of wolf predation increased for Moose that used areas with lower road densities over the previous 365 days; 2) risk from human harvest was increased for Moose that used areas with higher road densities on any given day and a greater proportion of young cutblocks (aged 1-8) during the previous seven days; and 3) risk of apparent starvation increased for Moose that used areas with higher road densities over the previous 365 days and higher proportions of young cutblocks (aged 1-8) over the previous 180 days. Manuscripts are currently being developed for peer-reviewed journals that will interpret these results and provide management recommendations.

Chisholm et al. (2019) used GPS radio-collared data from cow Moose in the John Prince Research Forest study area to investigate patterns of seasonal migration. Of the 45 collared individuals, 53% had migratory trajectories across all study years, 18% had resident trajectories across all study years, and switching between migratory and resident trajectories was observed in 29%. The median annual home range size for migratory trajectories was 257 km^2, while that for resident trajectories was 68 km^2. Collared cow Moose that exhibited migratory behavior had fewer calves (31.3% had calves, range: 18% – 40%) present then those that were resident (42.6% had calves, range: 22% - 46%). Recommendations generated include:

- Timing of population estimate surveys should be in later winter (February) to keep counts consistent between years, or a year-specific correction factor could be applied to account for inter-annual variation in spatial distribution of Moose;
- Migratory corridors should be identified and protection measures explored; and
- An investigation into the mechanisms driving this partial migration should be pursued and impacts on recruitment studied.

Francis et al. (in prep.) examined seasonal habitat selection of female Moose in response to landscape change in the Bonaparte study area, and examined specific hypotheses to test whether habitat selection patterns were a function of forage availability, mortality risk or the cumulative effects of salvage logging. The cumulative effects of forage availability and risk best predicted habitat selection of female Moose in all seasons, with the exception of calving and fall seasons, where risk, measured by response to linear features, better defined habitat selection patterns. Moose were found to be using intensively logged areas and declining within them, which suggests these areas may be ecological traps and require a nuanced approach to addressing the issue. Changes in how the landscape functions to support Moose, as opposed to habitat loss in these disturbed landscapes, likely explain Moose population declines. The key recommendations at this point in the research are:

- Restore roads, especially adjacent to cutblocks and key habitat features, to limit potential for increased mortality risk associated with roads.
- Management should address how these disturbed areas function to potentially contribute to increased mortality. Management options could include establishing screening cover around cutblocks and key habitat features (e.g., riparian areas) for visual obstruction and leaving coarse woody debris on cutblocks to aid moose in escaping predation. Alternatively, management could consider removing the resource subsidy drawing moose into disturbed areas, though that is neither practical or socially palatable.

Using biological samples collected from collared Moose at capture and during mortality site investigations, Thacker et al. (in prep.) developed a baseline of Moose herd health, assessed for variations in Moose health geographically,

identified specific health issues affecting Moose, and determined if health may be contributing to Moose population trends. This manuscript is in progress, and results with management recommendations are being developed.

7. FUTURE RESEARCH DIRECTION

This project is currently in its seventh year. Our research to date has provided a better understanding of factors affecting cow Moose survival and initial insights into the importance of calf survival and recruitment, and variation in that parameter, in the B.C. interior. An updated research design was completed in summer of 2019 (Kuzyk et al. 2019) that provides details of important areas on which to focus future research direction. These important research areas should be investigated to broaden our understanding of factors influencing Moose population dynamics and facilitate the development of management recommendations to benefit Moose populations in the province. We will continue to work with our academic partners to refine and develop research projects that will inform future management recommendations.

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APPENDIX A. MOOSE RESEARCH PROJECT PRODUCTS

Provincial Moose Research Project: Research Design 2014

http://a100.gov.bc.ca/pub/eirs/viewDocumentDetail.do?fromStatic=true&repository=BDP&documentId=12090

Provincial Moose Research Project: Progress Report 2015

http://a100.gov.bc.ca/pub/eirs/viewDocumentDetail.do?fromStatic=true&repository=BDP&documentId=12431

Provincial Moose Research Project: Progress Report 2016

http://a100.gov.bc.ca/pub/eirs/viewDocumentDetail.do?fromStatic=true&repository=BDP&documentId=12520

Provincial Moose Research Project: Progress Report 2017

http://a100.gov.bc.ca/pub/eirs/viewDocumentDetail.do?fromStatic=true&repository=BDP&documentId=12720

Provincial Moose Research Project: Progress Report 2018

http://a100.gov.bc.ca/pub/eirs/viewDocumentDetail.do?fromStatic=true&repository=BDP&documentId=12800

Provincial Moose Research Project: Updated Research Design 2019

http://a100.gov.bc.ca/pub/eirs/viewDocumentDetail.do?fromStatic=true&repository=BDP&documentId=12850

Habitat Use and Selection, Scheideman 2018

http://web.unbc.ca/~michael/Pubs/Scheideman_MSc_Thesis.pdf

Survival Analysis, Mumma and Gillingham 2019

http://web.unbc.ca/~michael/Mumma_and_Gillingham_2019.pdf

Provincial Moose Research Project Video: 2019

https://youtu.be/xH_epWSjMEo

APPENDIX B. MOOSE RESEARCH CAPTURE FORM AND SAMPLING PROTOCOL FOR CAPTURED MOOSE IN CENTRAL B.C.

MOOSE RESEARCH CAPTURE FORM

Study Area:_____

Date:_____ Time:_____ Chase Time:_____

Personnel:_____

General Location:_____

Waypoint:_____ UTM: E_____N_____

Lat_____Long_____

Capture Method: ☐ Net Gun ☐ Net + Sedation ☐ Immobilization

Mother ID:_____

WLH ID:_____ Sex: _____

Collar type:_____

Frequency:_____

Collar ID/ser. No.:_____

Ear Tag:_____ Right Ear

Age Class

Young AD ☐

Adult ☐

Aged ☐

Calf ☐

Calf Count **0** **1** **2** **3**

Lactating? Y / N

Measurements

Weight Range: ☐ 3-400 Kg ☐ 4-500 Kg ☐ 5-600 Kg

Ultrasound (mm):_____ image: Y/N

Neck Circ:_____cm

Underbite Distance*:_____mm

*(front of premaxilliary pad to tip of middle incisor)

Body Condition

Excellent ☐

Good ☐

Fair ☐

Poor ☐

Emaciated ☐

Samples

Hair: Y / N

Ear Biopsy: Y / N

Blood: Y / N

Fecal: Y / N

Parasites Y / N

Calves

Weight (kg):

Total Length (cm):

Chest Girth (cm):

Shoulder Height (cm):

L Hind Foot Length (cm):

Ticks? ☐ Number of ticks (Shoulder) ☐ Number of ticks (Rump)

Photos? Y / N Photo of underbite? Y / N

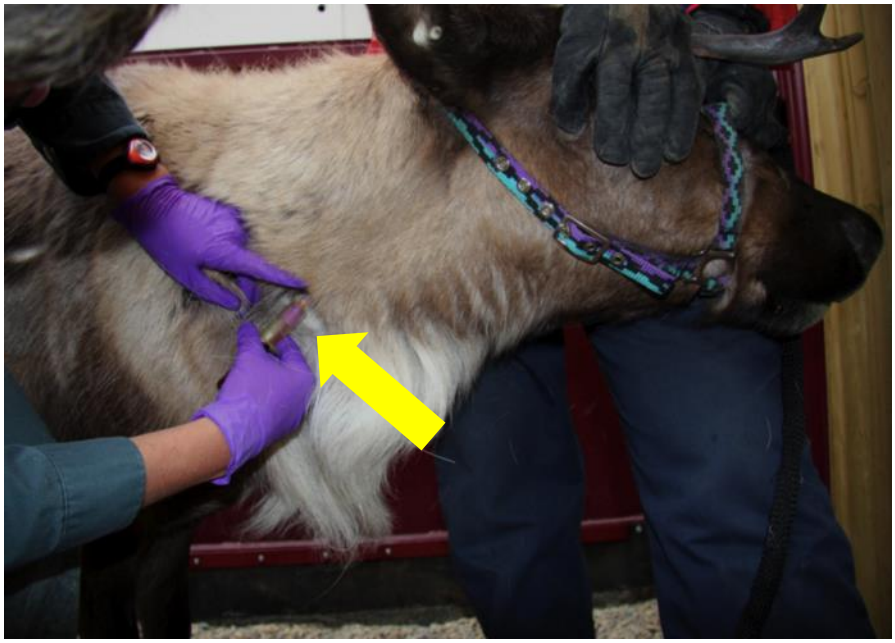
Abnormalities or Comments:_____

_____

MOOSE SAMPLING PROTOCOL

Blood Collection

- **Blood is the most important sample to collect from each Moose.**
- **Training and experience are required to collect blood. The most experienced team member should oversee blood collection by new staff and should take blood when handling times are limited.**
- Ensure Moose are well restrained before blood collection. Head control and proper positioning (head and neck not bent or twisted) are especially important if taking blood samples from the jugular vein.
- Each kit has all supplies and blood tubes for completing the collection.
- Ensure the blood collection tubes are at 18-25ºC prior to use. **NOTE**: all blood tubes are best at this temperature as it avoids temperature shock to the blood cells.
- Blood is collected with needle and syringe from **EITHER** the jugular vein, the cephalic vein (front leg), or the saphenous vein (hind leg).



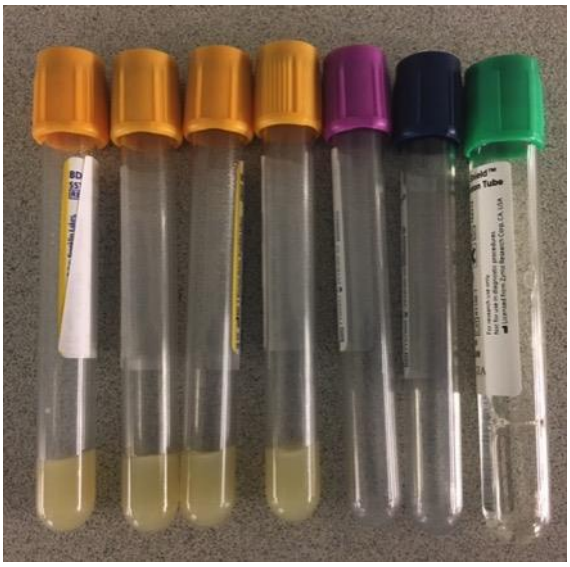
Location for blood sampling from the jugular vein (yellow arrow). Picture from: University of Calgary, Faculty of Veterinary Medicine, *Rangifer* Anatomy Project.

- The vein must be held off by a hand or tourniquet to build up pressure and locate the vein.
- Hold the needle with the bevel up and insert carefully beside or above the vein and puncture the vein. Once the vein is punctured blood is seen in the needle hub and the blood

can then be pulled into the syringe. Slowly pull past the 35 ml mark to ensure that enough blood is collected to fill all tubes.

- There is an extra needle in each kit. Please secure used needles in a crush proof, puncture proof sharps container and syringes without needles can be disposed of in a sealed garbage bag.

Blood Transfer to Sample Collection Tubes



- The blood collection tubes are in the kit in a BUNDLE with an elastic band. They contain a variety of fluids or compounds and are all under negative pressure.
- To prevent hemolysis (the rupture of red blood cells turning serum pink or red), do not squirt/force blood into collection tubes. Instead, once the syringe is full, carefully insert the needle through the end and negative pressure will passively draw the blood from the needle/syringe into the collection tube.
- If the vacuum has been compromised, blood can be gently and slowly injected along the sides of the tube.
- **START FILLING THE YELLOW TOP TUBES, FOLLOWED BY THE BLUE, PURPLE AND THE GREEN TOP TUBE LAST.**
- **GENTLY INVERT BACK AND FORTH ALL THE TUBES IN THEIR BUNDLE IMMEDIATELY AFTER COLLECTION TO ENSURE THE BLOOD AND CONTENTS ARE WELL MIXED (30 seconds to 1 minute). THIS IS ESPECIALLY FOR PURPLE AND GREEN TOPS TO MIX THEIR CONTENTS TO PRESERVE BLOOD CELLS.**
- Each blood tube type is designed to collect the specific samples required for pregnancy determination, health and disease surveillance, trace nutrient testing, etc.
- The quality of data obtained from blood samples will be compromised by improper collection, handling, processing, and storage. Please ensure blood protocols are followed.
- **ONCE FILLED, HANDLE BLOOD TUBES WITH CARE: PLACE UPRIGHT IN A COOLER IN THE HELICOPTER, PROTECT FROM SHAKING, ROUGH HANDLING, DIRECT SUNLIGHT, FREEZING, AND HEAT.**

Blood Post Field Processing and Storage

Supplies needed: From the Wildlife Health Program

- **Cryovials - 2 ml volume**
- **Preprinted labels with: Species, WLH ID, Blood Sample Type (i.e. serum, plasma, buffy, trace, RNA)**
- **Transfer pipettes - disposable plastic**
- **Small Ziploc bag to keep all blood samples grouped together by individual WLH ID#.**

Gold Top (SST) Serum Tubes

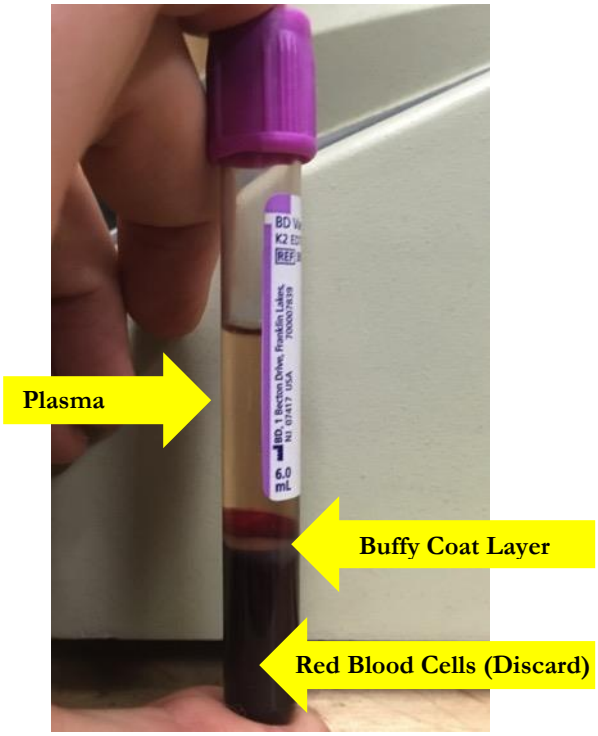
- Once back from the field, centrifuge gold top tubes for 15 minutes after blood has clotted and within 12 hours of collection.
- After centrifuging, serum (clear, yellowish liquid) will be separated from clotted blood by the gel plug. If the gel does not separate the serum you may need to re-spin.
- Decant serum into labelled cryovials using a disposable transfer pipette.
- **Use a new transfer pipette for each type of tube and use transfer pipettes to process samples from each Moose.**
- If a transfer pipette becomes contaminated with gel, blood from the clot, other debris, etc. discard and use a new pipette.
- Fill each cryovial with 1 ml, with a maximum of 2 ml serum.
- Please do not use cryovials > 2 ml. Freeze/thaw can degrade serum samples and is required for sub sampling if larger cryovials are used.
- Ensure each cryovial is labelled with: WLH ID **(NOT COLLAR FREQUENCIES)**, study area, species, **SERUM**, and date. **USE PREPRINTED LABELS**
- Store serum from gold top tubes frozen (minimum -20 ºC).
- **Recap gold top tube and retain one with clot (frozen, minimum -20 ºC).**

Royal Blue Top (Trace Nutrient) Serum Tube

- Centrifuge for 15 minutes once blood has clotted and within 12 hours of collection.
- **Royal blue top tubes do not have separating gel, so try not to disturb the clot after centrifuging and while processing.**
- **Royal blue top tubes are also more easily affected by hemolysis (red blood cell rupture). Please note (in data sheet comments section) if the serum sample from a royal blue top tube appears red as hemolysis may influence interpretation of results.**
- Decant serum into two labelled cryovials. Label cryovials with WLH ID, study area, species, **TRACE NUTRIENTS**, and date. **USE PREPRINTED LABELS**
- Store serum from the royal blue top tube frozen (minimum -20 ºC).

- Discard the clot and the royal blue top tube.

Purple Top (EDTA) Whole Blood Tube



- **Remember this tube MUST be mixed immediately after collection**.
- Centrifuge the purple top tube for 15 minutes as with other tubes.
- **CAUTION AFTER SPINNING**. The blood cell and plasma layers in the purple top tube are in a liquid state (not clotted). Do not bump or disturb the red blood cell layer and buffy coat (the opaque white blood cell layer between plasma and red cells) before sampling.
- **RE-CENTRIFUGE IF LAYERS ARE ACCIDENTALLY DISTURBED.**
- Collect plasma (clear/yellow layer) into cryovials using a new pipette.
- Fill each cryovial with a maximum of 2 ml plasma.
- Label each cryovial with WLHID, study area, species, **PLASMA**, and date. **USE PREPRINTED LABELS**
- Store plasma from the purple top tube frozen (minimum -20 ºC).
- Collect the buffy coat (opaque middle layer) into a **SEPARATE** cryovial.
- Label this vial with WLH ID, herd, species, BUFFY, and date. USE PREPRINTED LABELS
- The buffy coat sample will appear red as some red blood cells will be sucked up with the white blood cell layer. **Try to minimize this as much as possible.**
- Store buffy from the purple top tube coat frozen (minimum -20 ºC).
- Discard the remaining red blood cell layer and the purple top tube.

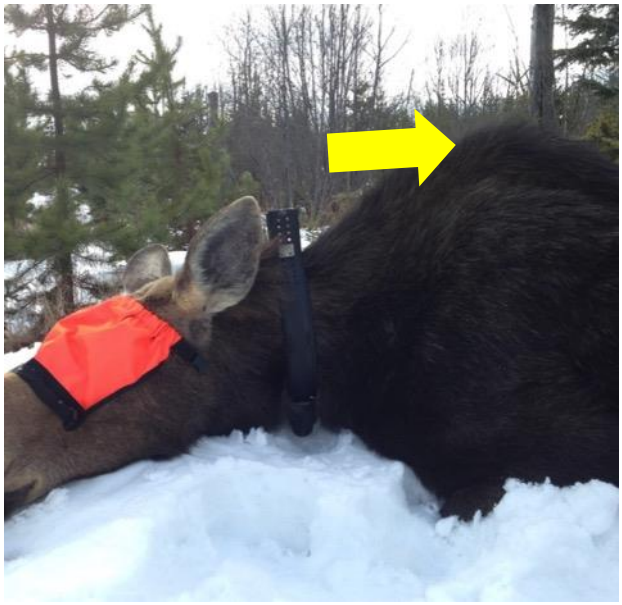
Green Top DNA/RNA Blood Tube NEW****

- The DNA/RNA blood tube should be the **last tube that blood is placed into.**
- **Remember this tube MUST be mixed immediately after collection.**
- **Do not spin the green top tube or draw off serum.**
- The blood is stabilized in this tube and can be sent to Wildlife Health in the original collection tube.
- Green top tubes do not need to be frozen, they can be kept at room temperature.

Skin Biopsy

- Use the 6 mm biopsy punch to place holes for each ear tag. Use the same punch for both ears if two tags are used.
- Avoid large blood vessels in the ear.
- Punch blade is very sharp. Use an old piece of radio collar belt or folded paper placed on the back of the ear to protect your fingers.
- Transfer each ear biopsy into the **SMALLER PAPER ENVELOPE** provided in the kit.
- Record the number of biopsies collected.
- Air dry (in envelope) at room temperature.
- Ensure biopsy sample envelope is labelled with: WLH ID, study area, species, body site of collection, and date.
- Store skin biopsies at room temperature, protected from heat, light, and moisture.
- **DO NOT FREEZE SKIN BIOPSIES.**
- Dispose of used punches in a crush proof, puncture proof sharps container.

Hair



- **PLUCK** hair from the **TOP OF THE SHOULDER** (yellow arrow) where skin is as dry and as free of contaminants (blood, dirt etc.) as possible.
- Use needle nose pliers, hemostats or a Leatherman to obtain undamaged, intact hairs with roots.
- Place hair (more is better) in the **LARGE PAPER ENVELOPE** provided. **ENSURE ENVELOPE IS WELL FILLED**
- Ensure hair samples are dry before long-term storage.
- Wet or damp hair samples should be gently blotted (not wiped) with paper towel immediately on return from the field then air dried before transferring to a new envelope for long-term storage.
- Air dry wet or damp hair samples out of direct sunlight and protected from heat (i.e. NOT near a wood stove, hot windowsill, on a truck dashboard etc.).
- Ensure hair samples are labelled with: WLH ID, study area, species, body site of collection, and date. Also note on labels if samples were collected from wet or dirty animals.

- For long-term storage keep Moose hair samples at room temperature in a dry, white, paper envelope protected from heat, light, and moisture.
- Silica desiccant can be kept in the same general storage container (i.e. if storing many envelopes containing hair in a larger Rubbermaid etc.).
- **DO NOT FREEZE HAIR SAMPLES**

Feces

- Using the glove provided, collect a "palm full" of fecal pellets per rectum (or from the ground/snow).
- If collecting from the rectum, be careful to prevent tissue damage.
- Place pellets in the Whirl-pak (**NO ZIPLOCS**) provided, remove as much air as possible and avoid crushing pellets.
- Fold the tabs, sealing the bag and store the fecal sample frozen (minimum -20 ºC).
- **AVOID FREEZING/THAWING.**

External Parasites

- Collect a sample of any external parasites (e.g. different life stages if present) if noted.
- **10+ winter ticks should be collected from any infested Moose.**
- In the field, ectoparasites can be temporarily placed in any small container if well sealed.
- Back at the lab, transfer specimens into cryovial(s) or screw-top specimen containers with 70% ETOH (Ratio of 10 parts ethanol:1-part parasite).
- Label containers with WLH ID, study area, species, parasite type, body location recovered, and date.
- Store 70% ETOH at room temperature, protected from heat and light.

SHIPPING INSTRUCTIONS

ALL KITS and SAMPLES MUST BE RETURNED TO:

Helen Schwantje, Wildlife Health Program
Forests, Lands and Natural Resource Operations
2080 Labieux Road
Nanaimo BC V9T 6J9

This includes sample kits that were not used for this season. Please do not keep unused kits for "extra" sampling supplies. Return unused kits so that we can keep track of WLH IDs.

SHIPPING CHECKLIST:

- **Serum (FROZEN) in multiple cryovials from 4 Gold Top SST Tubes.**
- **Plasma (FROZEN) in multiple cryovials from 1 Purple Top EDTA Tube.**
- **Buffy Coat (FROZEN) in SEPARATE cryovial from 1 Purple Top EDTA Tube.**
- **Serum (FROZEN) in two cryovials from 1 Royal Blue Trace Nutrient Tube.**
- **Green Top DNA/RNA Blood Tube – NOT spun or sub-sampled into cryovials**
- **1 x whirl-pack with 10-20 fecal pellets (FROZEN).**
- **1 x small envelope with skin biopsy (ROOM TEMPERATURE, DRY, PROTECT FROM HEAT LIGHT AND MOISTURE, DO NOT FREEZE).**
- **1 x large envelope with plucked hair from shoulder (ROOM TEMPERATURE, DRY, PROTECT FROM HEAT LIGHT AND MOISTURE, DO NOT FREEZE).**
- **Parasites in cryovial(s) if collected (70% ETOH, ROOM TEMPERATURE)**
- **1 x completed CAPTURE FORM**

AGE CLASS ESTIMATE (Tooth wear from Passmore et al. 1955)		
AGE CLASS	**AGE EST**	**DESCRIPTION OF TOOTH WEAR**
YOUNG ADULT	1 ½	Permanent teeth in place. Cheek teeth are visible in lower jaw. Third premolar may still have 3 cusps.
	2 ½	Third premolar has 2 cusps. Third molar has erupted. All premolars and molars show slight wear and stain. Outer canine teeth in final position. Incisors with little wear or staining.
	3 ½	Lower jaw has now elongated. Last cusp of third molar no longer cradled in lower jaw. Dentine now wider than enamel.
ADULT	4 ½	Wear on lingual crest and cupping of molars becomes increasingly pronounced.
	5 ½	
	6 ½	
	7 ½	
AGED	8 ½	Pit (infundibula) of 1st molar completely worn.
	9 ½	
	10 ½	
	11 ½	
	12 ½	Pit (infundibula) of 3rd premolar completely worn.
	13 ½	
	14 ½	

BODY CONDITION SCORING SYSTEM		
Body Condition	SCORE (Franzmann 1977)	PHYSICAL DESCRIPTION (Franzmann 1977)
	10	Prime, fat animal with thick, firm rump fat by sight. Well fleshed over back and loin. Shoulders and rump round and full.
	9	Choice, fat Moose with evidence of rump fat by feel. Fleshed over back and loin. Shoulders round and full.
Excellent	8	Good, fat Moose with slight evidence of rump fat by feel. Bony structures of back and loin not prominent. Shoulders well fleshed.
Good	7	Average Moose with no evidence of rump fat, but well fleshed. Bony structures of back and loin evident by feel. Shoulders with some angularity.
Fair	6	Moderately-fleshed Moose beginning to demonstrate one of the following conditions: (A) definition of neck from shoulders; (B) upper foreleg (humerus and musculature) distinct from chest; or (C) rib cage prominent.
Poor	5	Two of the characteristics listed in 6 are evident.
Emaciated	4	All Three of the characteristics in 6 are evident.
	3	Hide fits loosely about neck and shoulders. Head carried at a lower profile. Walking and running postures appear normal.
	2	Signs of malnutrition. Outline of the scapula evident. Head and neck low and extended. Walks normally but trots and paces with difficulty, cannot canter
	1	Point of no return. Generalized appearance of weakness. Walks with difficulty; cannot trot, pace or canter.
	0	Dead.

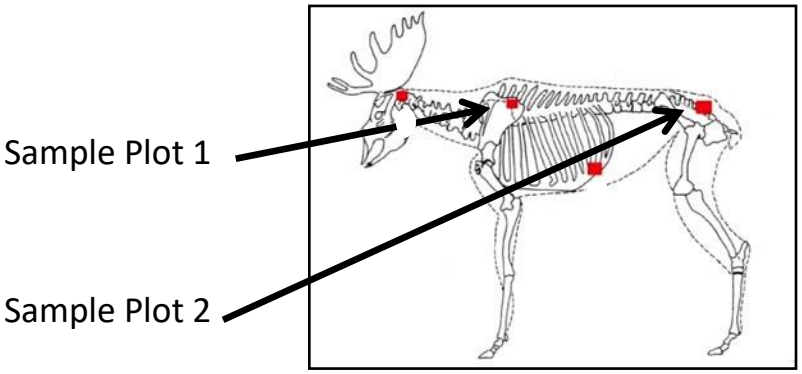
Winter Tick Sampling:

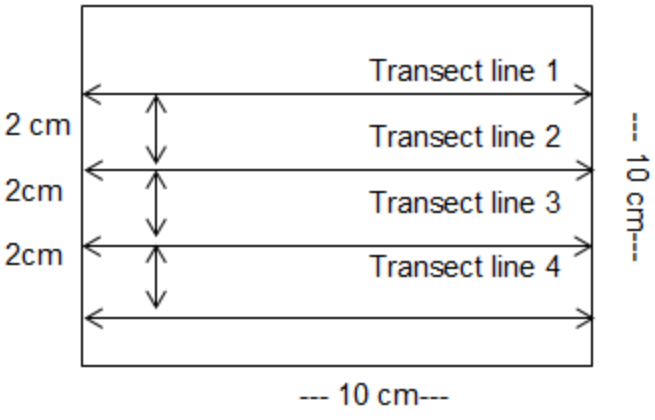
Winter ticks will be counted in two 10 x 10 cm sampling plots:

1. the upper edge of the shoulder blade, and

2. the rump midway between the hipbone and the base of the tail.

- In each plot, ticks will be counted on 4 x parallel transect lines 10 cm long and spaced 2 cm apart.
- Part the hair with a comb to the skin and count all visible ticks along each transect line.
- Record the <u>total number of ticks</u> from each sampling plot (add all four transect lines) and record the total on the data sheet in the bottom left of the first page.





APPENDIX C. DEFINITIONS OF PROBABLE PROXIMATE CAUSES OF MOOSE MORTALITY IN CENTRAL B.C.

- **Hunting:** Moose killed by humans for recreation, food, social or ceremonial purposes.
    - **Licensed hunting:** Moose killed by licensed hunters in accordance with hunting regulations.
    - **Unlicensed hunting:** Moose killed by hunters not in accordance with hunting regulations.
- **Predation:** Moose that have been killed by a predator.
- **Health-related:** Moose that died of an underlying health-related cause (starvation, parasitism, mineral deficiency, non-infectious disease, etc.) or pathogen (i.e., infectious disease) as identified through carcass field necropsy and/or subsequent pathology, or no other clear causes of mortality was evident.
    - **Apparent starvation:** Moose that have died in very poor condition and are emaciated as evidenced by extreme gross examination (lack of bone marrow fat and lack of visible body fat). Bony structures of shoulders, back, loins, ribs and hips are visually evident. No other clear causes of mortality are obvious or found.
    - **Failed predation attempt**: Moose that have died from a failed predation attempt. Causes of death may include shock associated with blood loss, trauma and pain, dehydration, septicemia and other sequelae of extreme exertion such as myopathy.
    - **Chronic bacterial infection**: A bacterial infection of more than several days duration of subcutaneous and deeper tissues.
    - **Peritonitis**: The inflammation of the peritoneum, the lining of the peritoneal cavity, or abdomen, by an infectious agent, usually bacteria but may be fungi or even a virus. The initiating cause may be a puncture of an organ, intestinal tract or the abdomen wall for entry of a pathogen. Left untreated, peritonitis can rapidly spread into the blood (sepsis) and to other organs, resulting in multiple organ failure and death
    - **Bacterial septicemia**: The presence of infective agents or their toxins in the bloodstream, sometimes called blood poisoning. It is characterized by elevated body temperature, chills, and weakness. Generally, there is a primary site of infection that serves as the source of the pathogen. This is a serious condition that must be treated promptly otherwise the process of infection leads to circulatory collapse, profound shock and death.
    - **Pleuritis**: The inflammation of the pleura, the lining of the thoracic cavity or chest by an infectious agent, usually bacteria but may be fungi or a virus. The initiating cause may be a puncture of the thorax through the hide for entry of a pathogen, a severe infection of the respiratory tract (lungs) or a systemic infection with extension to the parietal (lining over the ribs) or visceral (lining over the lungs) pleura. Pleuritis is extremely painful and may result in fibrous adhesions attaching the lungs to the ribcage. Chronic cases can become scarred and walled off to persist over long time periods.
    - **Prolapsed Uterus**: The uterus is everted (inside out) from the abdominal cavity through the pelvic canal during a complicated parturition or calving, due to a misrepresentation or severe straining from other reasons.
    - **Unknown health-related:** Moose that were definitively not killed by predation, hunting or natural accident and no underlying health-related cause or pathogen was detected.

- **Natural accident:** Moose that have died naturally from a cause that was accidental in nature (i.e., drowning, mired in mud, avalanche, etc.).
- **Vehicle Collision:** Moose that have died as a direct result of a motor vehicle strike.
- **Unknown:** Moose that have died and no clear cause of death was identified, which in most cases is due to lack of evidence at mortality site.

APPENDIX D. MORTALITY SITE INVESTIGATION FORM USED TO ASSESS CAUSE OF MORTALITY FOR MOOSE IN CENTRAL B.C. (REVISED JUNE 2019).

V2.2 June 2019

BC Moose Research Program - Mortality Site Investigation Form

LOCATION

Date of site visit (DD/MM/YYYY): _____ Date mort. signal received: _____ Date of death _____

General location: _____ Personnel: _____

Waypoint: _____ UTM Zone: _____ E: _____ N: _____ or Lat: _____ Long _____

ANIMAL IDENTIFICATION

WLH ID: _____ OTHER ID: _____ Sex: M / F Study Area: _____

Found dead ☐ Euthanized ☐ Euth. method: _____ If Euth. collect blood 2 x gold top

State of decomposition (Circle): Fresh / Bloated / Active decay (maggots) / Desiccated / Bones Collar only

Ear tag(s) (number and colour): Left: _____ Right: _____

Collar recovered: Yes / No Freq.: _____ Ser. No.: _____ Circle: Functional / Damaged / Destroyed / N/A

MORTALITY SITE DESCRIPTION

Describe mortality site and document with photos/video, especially for nonpredated "drop dead" animals.

Photos should include wide angle, medium and close-up views with a scale reference.

Circle: No snow / Snow / Fluffy Snow depth (cm):_____ Ice crust ☐ Circle: Light/Moderate/Heavy Sinking depth (cm):_____

Pictures ☐
Video ☐

EXTERNAL EXAM

Select all that apply. Describe any abnormal findings and 'Other' in comments section on page 3.

Carcass Location		Carcass State		Body Condition		Body/Skin/Hair		Eyes		Ears/Nose	
In open	☐	**Fresh #**	☐	Excellent	☐	Hair loss	☐	Clear	☐	Ear crusting	☐
In water	☐	Frozen	☐	Good	☐	Ticks/parasites	☐	Swollen	☐	Ulcers/sores	☐
In cover	☐	Decomposed	☐	Fair	☐	Lumps/warts	☐	Cloudy	☐	**Discharge ***	☐
Buried	☐	**Intact #**	☐	Poor	☐	Wounds	☐	**Discharge ***	☐	Other	☐
On roadside	☐	Disarticulated	☐	Emaciated	☐	Other	☐	Other	☐		
Collar only	☐	Scattered	☐	Unknown	☐			One / L / R / Both			
Other	☐	Scavenged	☐								

Oral Cavity		Teeth		Bones and Joints		Hooves		Feces		Reproductive	
Ulcers/sores	☐	Teeth worn	☐	Fracture(s)	☐	Excessive wear	☐	No feces	☐	Lactating	☐
Rumen content	☐	Teeth irregular	☐	Joints swollen	☐	Abnormal wear	☐	Diarrhea	☐	Udder abnormal	☐
Blood	☐	Teeth broken	☐	Joint fluid clear/pus/blood	☐	Overgrown	☐	Fecal staining mild/mod/extr.	☐	**Vaginal discharge ***	☐
Other	☐	Feed impacted	☐			Infection	☐				
		Other	☐	Antler deform.	☐	Other	☐	Blood	☐	Abortion	☐
				Retained velvet	☐			Rectal prolapse	☐	Testes abnorm.	☐
				Other	☐			Other	☐	**Penis discharge ***	☐
										Other	☐

* Discharge Clear / Cloudy / Purulent (Pus) / Blood Collect samples/swabs ☐

 # Consider slinging out/removing intact and fresh carcasses for necropsy by a project veterinarian

If calf/fetus present:

Aborted fetus: Single ☐ Twin ☐ Male ☐ Female ☐ Collect whole aborted fetus(es) ☐ No fetus(es) ☐

Calf: Alive ☐ Dead ☐ Single ☐ Twin ☐ Male ☐ Female ☐ Age: _____ Cause of death:_____ No Calf ☐

Winter ticks: No / Yes **Collect 10+ engorged and not engorged in 70% ETOH ☐ Collect 10+ engorged and not engorged NO ETOH ☐**

Winter tick count (in 2 locations) Number of ticks - sample 1(shoulder): _____ Number of ticks - sample 2 (rump): _____

Hair Loss: None / Mild (5-20%) / Moderate (20-40%) / Severe (40-80%) / Extreme (>80%)

INTERNAL EXAM

Before sampling, take pictures of opened chest (showing heart and lungs) and abdominal cavities (Left side: showing intact gastrointestinal tract, liver, spleen). Take pictures of and describe all abnormal findings in comments section.

1) EXAMINE	Normal	Abnormal
Mouth/Tongue/Larynx/Esophagus	☐	☐
Trachea and large airways	☐	☐
Lungs (front, middle, back lobes)	☐	☐
Heart (chambers/valves/blood vessels)	☐	☐
Liver	☐	☐
Left and right kidneys	☐	☐
Spleen	☐	☐
Lymph nodes (under skin, in abdomen)	☐	☐
Rumen	☐	☐
Glandular stomach (abomasum)	☐	☐
Small intestine (several sections)	☐	☐
Large intestine/colon (several sections)	☐	☐
Skull/spine	☐	☐
Reproductive tract (female and male)	☐	☐
Other	☐	☐

2) EVALUATE INTERNAL FAT RESERVES – Circle appropriate

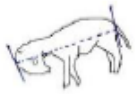
Subcutaneous:	Plentiful/Moderate/Scant/None
Heart:	Plentiful/Moderate/Scant/None
Mesentery/Omentum:	Plentiful/Moderate/Scant/None
Kidney:	Plentiful/Moderate/Scant/None
Marrow:	Red-Runny/Pink-Semi-Solid/Firm-Creamy

Do not crack bones *unless multiple long bones are available.*

3) IF PREGNANT

Crown- Rump Length Fetus(es) 1:_____ cm 2: _____ cm

Fetal Weight 1:_____ g 2: _____ g



Evidence of abortion: Yes / No

Evidence of fetal or placental abnormalities: Yes / No / Unk

CALL PROGRAM VET FROM FIELD IF EVIDENCE OF ABORTION OR ABNORMAL; COLLECT FETUS AND PLACENTA ☐

4) IDENTIFY PROXIMATE CAUSE OF DEATH (for health related – include details in comments on next page)

COD		Confidence		Species		Confidence	
Predation	☐	Defin.	☐	Wolf	☐	Defin.	☐
Hunter	☐	Prob.	☐	Grizzly Bear	☐	Prob.	☐
Collision	☐	Poss.	☐	Black Bear	☐	Poss.	☐
Natural Accident	☐			Unk. Bear	☐		
				Cougar	☐		
Health	☐			Other	☐		
Unknown	☐						
Other	☐						

COMMENTS FOR EXTERNAL EXAM, INTERNAL EXAM, SUSPECTED PROXIMATE vs. ULTIMATE CAUSE OF DEATH If animal is found alive, describe symptoms (e.g. lying down, circling, vocalizing, aggressive, dull, etc.)
Questions in the field or the lab? Contact Dr. Helen Schwantje 250-751-3234 or cell 250-361-7619

MOOSE TISSUE SAMPLES TO COLLECT IN THE FIELD (AS AVAILABLE) Samples MUST be processed ASAP when back at the office. Post-field sub-sampling described on the following processing sheet.			
Head (or obex and RPLN in field for CWD)	☐	Spleen (palm size piece)	☐
Pictures of jaw and incisors#	☐	Lymph nodes (if abnormal)	☐
Teeth (2 incisors, one to age and one to archive)	☐	Intestine (if abnormal + fresh) - Open and assess a few sections of large and small intestines and abomasum. Collect parasites if found.	☐
Ear tip x 2	☐		
Hair (100+ intact from top shoulder preferred)	☐	Rumen contents (palm full)	☐
Intact Long bone (femur or humerus)	☐	Feces (10-20 pellets from colon)	☐
Skeletal muscle (from leg, palm size piece)	☐	Fetus and placenta - if abnormal or aborted	☐
Lung front lobe (palm size piece, right)	☐	Uterus and ovaries - if abnormal or abortion	☐
Lung middle lobe (palm size piece, left and right)	☐	Calf (if newborn and dead)	☐
Lung back lobe (palm size piece, left and right)	☐	Cysts and tumors (if unknown cause, include adjacent normal tissue)	☐
Heart (full cross section of atria and both ventricles)	☐	Winter ticks (see above) 20+ all life stages, engorged and not engorged)	☐
Blood (heart/jugular in 2 x gold top)	☐	PREDATION SAMPLES	
Whole left kidney + fat	☐	* Fill out and attach predator ID data form if swabs collected*	
Whole right kidney (keep separate from left kidney)	☐	DNA (hide/collar punctures/bite/rake wounds; swab in field is best)	☐
Liver (palm size piece x 3 in separate bags)	☐	Predator hair	☐
		Predator scat	☐
		Other	☐

Take three photos of teeth and jaw – one from each side and one from the front showing the incisors

ALL SAMPLES IN SEPARATE WHIRL PAK BAGS, EACH LABELLED WITH:
WLH ID, SPECIES, STUDY AREA, SEX, SAMPLE TYPE, DATE

Moose Mortality Sample Processing and Storage

SAMPLE	PROCESSING	STORAGE
Intact head	Double heavy garbage bag/seal well	Frozen [a]
Obex and retropharyngeal lymph nodes (RPLNs)	Collect and subsample as per CWD sampling protocols if experienced. Otherwise bring out head and freeze	a) Whole obex, ½ of each RPLN: Fixed [b] b) ½ of each RPLN: Frozen
Teeth	Place in non-manila envelope (air dry)	Room temperature (to PG office)
2 x Ear tips	Place in 2 separate non-manila envelopes (air dry)	Room temperature [c, d]
Hair x 100	Separate non-manila envelope per body region collected if required to get a large enough sample (air dry)	Room temperature
Long bone x 1	Place in bone bag/seal well	Frozen (for PG office)
Skeletal muscle	Place in whirl-pak /seal well	Frozen
Lung front lobe Right	Subsample at office [e, f] - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) 2, 1 cm thick sections (1 from each lobe, if abnormal take up to 4 per lobe): Fixed b) Remaining tissue: Frozen
Lung middle lobe Right	Subsample at office - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) 2, 1 cm thick sections (1 from each lobe, if abnormal take up to 4 per lobe): Fixed b) Remaining tissue: Frozen
Lung back lobes Left and Right	Subsample at office - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) 2, 1 cm thick sections (1 from each lobe, if abnormal take up to 4 per lobe): Fixed b) Remaining tissue: Frozen
Heart	Subsample at office - Fixed portions in 10% formalin	2, 1 cm thick sections: Fixed
Heart blood	Place blood tubes in whirl-pak/seal well	Frozen
Left kidney + fat	Place in whirl-pak/seal well	Frozen (for PG office)
Right kidney	Subsample at office - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) 1-2, 1 cm thick cross sections: Fixed b) Remaining tissue, divided into two separate whirl-paks: Frozen
Liver	Subsample at office - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) 1-2, 1 cm thick cross sections: Fixed b) Remaining tissue, divided into three separate whirl-paks: Frozen
Spleen	Subsample at office - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) 1-2, 1 cm thick cross sections: Fixed b) Remaining tissue, divided into two separate whirl-paks: Frozen
Lymph nodes	Subsample at office - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) 1-2, 1 cm thick cross sections: Fixed b) Remaining tissue: Frozen
Various intestine	Subsample at office - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) 1-2, 1 cm thick cross sections but only if fresh! Fixed b) Remaining tissue: Frozen
GI parasites	70% ETOH in well-sealed container	Room temperature
Rumen contents	Place in whirl-pak/seal well	Frozen
Feces	Place in whirl-pak/seal well	Frozen
Fetus and placenta	Place in a bone bag/seal well	Frozen
Uterus	Subsample at office, if abnormal - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) If abnormal, 1-3, 1 cm thick cross sections: Fixed b) Palm size part: Frozen
Ovaries	Intact ovaries fixed in 10% formalin	Fixed

SAMPLE	PROCESSING	STORAGE
Calf	Double heavy garbage bag/seal well	Frozen
Abscesses, cysts and tumors	Subsample at office - Fixed portions in 10% formalin - Frozen in separate whirl-pak/seal well	a) 1-2, 1 cm thick cross sections: Fixed b) Remaining tissue: Frozen
Winter ticks[g]	Subsample at office - Half in 70% ETOH in well-sealed container (i.e. CWD container) - Half frozen in separate cryovial or whirl pak ***without*** ETOH	a) Room temperature for sample with ETOH (10:1 of ethanol:ticks) b) Frozen for sample without ETOH
Predator scat[h]	Place in whirl-pak/seal well	Frozen (to PG office)
Predator DNA	Collect as per double swab protocol[i]	Room temperature (to PG office)
Predator hair	Non-manila paper envelope (air dry)	Room temperature (to PG office)

NOTES ON HANDLING, STORING, SHIPPING SAMPLES and TISSUES

Note: Most supplies are provided by the Wildlife Health Program. Contact us before you run out.

a) Frozen tissue samples must be stored and shipped at minimum -20˚C. **For long-term storage, only freeze tissue samples in whirl-paks (or similar). Do not use Ziplocs. Avoid freeze/thaw.**

b) Fixed tissue samples in 10% Neutral Buffered Formalin. Fixed tissue must be stored at room temperature in a leak proof, puncture-proof container with a 10:1 formalin: tissue ratio. **Fixed tissue *must not be frozen***. In addition, **fixed tissue *must not be shipped in the same box/cooler as frozen samples***, as formalin fumes can kill live pathogens which limit the efficacy of tissue culture and other diagnostics.

c) Air dry samples at room temperature in an area protected from excessive heat (i.e. not near a stove, heater, or on a truck dashboard), light, and moisture. **If samples or envelopes are wet when initially collected in the field, transfer to a fresh, dry envelope immediately on return to the lab and before leaving to air dry. Be sure to label the new envelope.**

d) Hair and tissue samples stored at room temperature must always be protected from heat, light, and moisture. Envelopes can be stored in a cardboard box and sent to the WLH program lab. Please do NOT stockpile dry samples.

e) Subsampling usually requires collection of both fixed (in 10% formalin) and frozen samples.

f) Collecting fixed tissue in 10% formalin:

- **Tissues must be fixed as soon as possible after collection to preserve for microscopic exams.**
- **To ensure proper penetration of formalin, tissue samples must be ≤ 1 cm thick.**
- To reduce artefact, always trim tissues to size using a sharp knife or scalpel on a plastic cutting board. Handle tissues carefully. Use forceps and do not crush or squeeze. Handle from the edge.
- If lesions are found, collect and fix several sections of the abnormal area. Include the edge of where abnormal meets normal tissue.
- **All tissue to be fixed must be placed into a leak-proof, puncture-proof, container(s) with a 10:1 ratio of formalin: tissue.**
- With the exception of intestines and CWD samples (obex and RPLNs), which should be placed in their own containers, different tissues can be fixed in the same container.
- Please record the types of fixed tissues on the container's label and also in a separate Excel file.
- **REMEMBER: FORMALIN IS TOXIC. DO NOT BREATHE IT AND USE ONLY WHERE THERE IS GOOD VENTILATION. ALWAYS WEAR GLOVES AND EYE PROTECTION.**

g) Winter tick - tick associated hair loss scoring in moose

HAIR LOSS CATEGORY	PATTERN
None (No Picture)	No hair loss or breakage
Mild (Picture 1)	Few small to medium sized patches of broken hair or hair loss
Moderate (Picture 2)	Several or large patches broken hair or hair loss - NO EXPOSED SKIN
Severe (Picture 3)	Several or large patches broken hair or hair loss with 1-2 small areas exposed skin
Extreme (Picture 4)	Several or large patches broken hair or hair loss with large or > 2 areas of exposed skin

***Note degree of tick associated hair loss observed in moose is not always correlated with infestation burden.**

Tick burden assessment

- Part the hair along the upper edge of the shoulder blade with a comb or ruler.
- Count the number of ticks observed along a single 10 cm x 2 cm transect.
- Part the hair along the rump.
- Count the number of ticks observed along a single 10 cm x 2 cm transect.
- If there is significant hair loss on the shoulder perform the assessment only on the rump.


Photo: H. Schindler

Photo: M. Scheideman

- Collect a representative sample (e.g. various life stages, engorged, not engorged) of ticks.
- Record WLH ID, date of collection, management unit, and host (i.e. moose)

For Identification Purposes:

- Store **half of the ticks** in a cryovial with alcohol (ethanol, 70-95%), with collection information on an external label written in pencil (or ink that is not affected by alcohol (indelible). Be sure to use enough ethanol in the cryovial, especially for engorged ticks, which will dilute the alcohol content (minimum 10:1 ratio; ethanol: ticks).
- Ensure the cryovials are well-sealed to prevent evaporation.
- Keep at room temperature, protected from heat and light.

For DNA Isolation and/or Pathogen Detection:

- Store **the other half of the ticks** frozen (-20C), ***without*** **ethanol** in a cryovial with collection data recorded on the outside of the cryovial as above.
- Keep in -20C freezer and ship to Wildlife Health Lab in Nanaimo, ensuring they stay frozen.

Frozen ticks and ticks in alcohol must be stored and shipped separately.

h) CAUTION: THERE IS A ZOONOTIC DISEASE RISK FROM PREDATOR SCAT - *Echinococcus* spp. tapeworms from wolf, coyote, and fox feces.

- Always wear gloves and coveralls when doing necropsies and if collecting scat.
- Collect carnivore feces with a stick or disposable utensil.
- Do not contaminate clothing, field or laboratory equipment, helicopters, trucks etc.
- Predator DNA is best obtained from the outside of scat samples. To maintain accuracy, do not crush scat (i.e. try to maintain the sample's original shape) and collect in whirl-pak(s) significantly larger than the sample itself.

i) PREDATOR DNA SWAB PROTOCOL

This protocol can be used when the predator species is unclear (i.e. predator hair and/or scat were not available to collect for DNA analysis).

Equipment needed for double swab protocol:

- Sharp knife and scalpel (with multiple, disposable blades) or disposable scalpels
- Nitrile gloves
- Sterile swabs - successful identification may be decreased depending on type of swab used. Prefer individually packaged, fine tipped, cotton or poly swabs with plastic handle.
- Paper envelopes

- Whirl-paks
- Silica desiccant
- Sharpie
- Stapler and staples
- Small ethanol tubes for swab collection
- Large ethanol tube for sterilizing knife
- Kleenex/paper towel
- Lighter
- ** 95% ETOH is recommended as the best wetting agent for collecting swabs. Other wetting agents (i.e. denatured alcohol, isopropyl alcohol) or sterile water can be used if 95% ETOH is not available however, there is potential for decreased success.

If carcass present:

- Put on new nitrile gloves.
- Carefully examine the carcass for killing wounds as identified by the presence of haemorrhage.
- Take pictures of the wound (wide angle and close-up perspectives) without disturbing the wound site.
- Dip swab in the **SMALL** ethanol tube.
- Swab wound (~10 seconds).
- Place swab in non-Manila envelope and snap off shaft.
- Staple envelope shut and label envelope with Wildlife Health ID and swab number using a sharpie (e.g. xx-xxxx - **1A**).
- Place envelope in a whirl-pak with silica desiccant.
- Fill out Predator Identification Data Form. Attach a copy of this form to the Caribou Mortality Site Data Form
- Repeat process on the same wound with a second swab (Identified as xx-xxxx - **1B**)
- Change gloves.
- Identify other killing wounds or feeding wounds [defined below] (Identified as xx-xxxx - **2A**, xx-xxxx - **2B**, etc.) and repeat the double swab process.

If only bones and/or collar present:

- Perform the double swab procedure on any remains (including the collar) that appear to have been chewed on by predators.

Considerations: Avoid cross-contaminating wounds

- Small amounts of DNA can easily be transferred between wounds.
- Do not touch multiple wounds with the same gloves during wound identification.

Identification of wounds

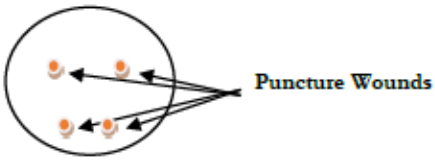
- Carefully skin the animal trying to preserve puncture marks and determining areas of hemorrhage.
- Sterilize knife after examining each wound - wipe knife with new Kleenex or paper towel - dip knife blade in the **LARGE** ethanol filled tube, carefully, burn ethanol off blade using lighter.

Bite description

- **Killing wound** is a wound that caused death or that occurred before death as indicated by hemorrhage.
- **Feeding wound** is any other wound on a carcass such as bites or chewing with no hemorrhaging or an area of the carcass that has been consumed.

Swabbing Technique

- **AVOID DRENCHING THE SWAB IN BLOOD.**
- **For killing wound** - swab entire area around puncture wounds.
- **For feeding wound** - concentrate swabbing on areas that appear to have been bitten or chewed.



Predator Identification Data Form

Attach Copy to Moose Mortality Site Data Form

Date (DD/MM/YYYY): _____

Summary: Predator species 1: _____ species 2: _____ species 3: _____
Unknown ☐

WLH ID (for the mortality): _____

Swab No. 1 Check boxes as swabs are completed. A ☐ B ☐	**Wound type** (Circle) Killing Feeding Other _____	**Wound Location and Description** Pictures ☐
Swab No. 2 Check boxes as swabs are completed. A ☐ B ☐	**Wound type** (Circle) Kill Feed Other _____	**Wound Location and Description** Pictures ☐
Swab No. 3 Check boxes as swabs are completed. A ☐ B ☐	**Wound type** (Circle) Kill Feed Other _____	**Wound Location and Description** Pictures ☐
Swab No. 4 Check boxes as swabs are completed. A ☐ B ☐	**Wound type** (Circle) Kill Feed Other _____	**Wound Location and Description** Pictures ☐

V2.2 June 2019

THE MORTALITY INVESTIGATION FORM AND ALL SAMPLES, WITH THE EXCEPTION OF THOSE IDENTIFIED FOR PRINCE GEORGE, MUST BE RETURNED TO:

Wildlife Health Program
Attention: Dr. H. Schwantje
Ministry of Forests, Lands, Natural Resource Operations and Rural Development
2080 Labieux Road
Nanaimo BC, V9T 6J9

Phone Numbers:
Helen: (250) 751-3234 Cait: (250) 751-3219 Lab: (250) 751 7246

Shipping:

- Frozen samples MUST remain frozen during transport or their use is compromised.
- Appropriate insulated shipping containers and icepacks and can be purchased at low cost from ULINE.ca or contact the Wildlife Health Program.
- **Formalin/fixed samples must be shipped separately from frozen samples**. If tissues are appropriately trimmed and have been fixed for > 36 hours, excess formalin can be drained off prior to shipping (leave samples covered by a piece of paper towel wetted with a small amount of formalin).
- When shipping tissue samples in formalin ensure they are in leak-proof, puncture-proof containers and double bagged with ample absorbent material (paper towel etc.) in case of leaks.
- **Please notify** the Wildlife Health Lab in Nanaimo **BEFORE samples are shipped**
  Shari Willmott, (250) 751-7246 Shari.Willmott@gov.bc.ca
  Maeve Winchester, (250) 751-7246 Maeve.Winchester@gov.bc.ca
  Cait Nelson, (250) 751 3219 Cait.Nelson@gov.bc.ca
  or Helen Schwantje, (250) 751-3234 Helen.Schwantje@gov.bc.ca
- **Try to ship samples on Monday or Tuesday, never past Wednesday.**

APPENDIX E. CALF SURVEY FORM USED DURING LATE-WINTER MOOSE SURVEYS TO MONITOR CALF/COW RATIOS.

BC Moose Research Study – Winter Calf Survival Survey													
Study Area:					Personnel:								
Survey Date(s)					Weather Conditions (Temperature, Cloud cover, Precipitation, Snow coverage)								Survey Time (hours)
#	Frequency	SN	WLHID	Last Fix Date	GPS WPT #	UTM Zone	Easting or Latitude	Northing or Longitude	Cow Located (Y/N)	Calf Present (#/No)	Ticks' (Cow)	Ticks' (Calf)	Comments/Cow Condition/ Incidental Observations
1													
2													
3													
4													
5													
6													
7													
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27													
'Hair loss classes: None; Mild (5-20%); Moderate (20-40%); Severe (40-80%); Ghost (>80%)													