

Sociogenetics: Exploring fine-scale social structure of woodland caribou (*Rangifer tarandus caribou*)

By

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A Thesis Submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfilment of the requirements of the degree of

MASTER OF NATURAL RESOURCES MANAGEMENT

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ABSTRACT

The sociogenetic structure of woodland caribou was explored using non-invasive fecal sampling. Fecal pellet sampling occurred in South Jasper during the fall of (2006 to 2012) and in North Interlake during the winter (2004 to 2010). Samples were amplified at 10 microsatellite loci and unique individuals identified. We used fecal pellet morphometrics and measured fecal reproductive hormone levels to distinguish calf from adult age-classes of woodland caribou. In addition, we conducted pedigree analysis of South Jasper caribou using the COLONY 2.0 program. Results demonstrated that pellet morphology, pregnane, and testosterone were able to differentiate age-class. Additionally, South Jasper caribou herds exhibited a polygynous mating system whereby few males dominated the reproductive output (only 20%) and female reproductive output was evenly distributed (39%). This study demonstrates the ability of non-invasive fecal methods to answer important questions pertaining to the age-class, mating system and fitness of woodland caribou.

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A NOTE TO THE READER

As you read this dissertation, you will find the use of “we” instead of “I” in describing the study hypotheses, design, results and conclusions. Although I am the primary author of this document, the few years of hard work that brought this study together was a collaborative effort from me and all my committee members. From brainstorming of research ideas, to field sampling, lab work, and the many, many hours of helpful advice and guidance, this study would not have been completed without the valuable input and guidance from my committee. As a result, I felt it was better represented by using “we” in describing the various phases of this thesis.

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GENERAL INTRODUCTION

Habitat loss and fragmentation are some of the most influential causes of species decline today and in the past (Fahrig 2002, Faulkner 2004, Sole and Montoya 2006, Herrera and Doblas-Miranda 2013). There are numerous consequences of fragmentation but one of the most significant is that of the breaking apart of populations and the accompanying effects of genetic deterioration due to lack of gene flow (Couvét 2002). In addition, observable changes in the probability of persistence, home-range overlap, territoriality, group size, and mating system have been shown in animals of varying species (Banks et al. 2007). Woodland caribou (*Rangifer tarandus caribou*) are a subspecies of caribou that have experienced extensive population declines throughout their range as a result of habitat fragmentation and alteration due to anthropogenic activities (Environment Canada 2011). As a result, woodland caribou have been designated as threatened under the Species at Risk Act (SARA). In many cases, decreases in gene flow and genetic diversity in local populations of woodland caribou have been quantified (Courtois et al. 2003a, Ball et al. 2010, Galpern et al. 2012b, Hettinga et al. 2012). Less understood are the potential consequences of a drastically changing landscape and consequent population declines on the fine-scale social structure and mating system of local caribou populations. It is essential to first understand these aspects of woodland caribou life history in order to later identify how they may be affected by population declines and subsequently mitigate for these effects.

Examining genetic relationships across individuals of a local population can provide valuable information on group dynamics, social behaviour and spatial distribution of the animal under study (Pérez-Espona 2010). In addition, determining the

age-structure of populations can further reveal trends in recruitment, population growth, and reproductive status (Reilly 2002) and is necessary for use in cohort analysis. Age-structure of populations may change over time and recognizing these changes in conjunction with exploring genetic relationships can help to identify trends in population growth, group composition, and reproductive activity (Festa-Bianchet et al. 2003). Exploring gender-based measures of dispersal and the reproductive contribution of males and females across local populations can also help reveal underlying processes of population dynamics. Building pedigrees via genetic relationships, rather than observed relationships, are critically important in conservation biology as they can provide information on inbreeding (Richard et al. 2009), heritability (Charmantier et al. 2006), and gene flow (Zeyl et al. 2009). In particular, studying relationships across a landscape at a finer scale can provide insight into potential movement of animals between herds.

With their elusive nature and sensitivity to anthropogenic disturbance, caribou can be a rather difficult animal to observe and study. Various methods to monitor caribou populations have been developed by researchers, including aerial surveys and radiocollaring of animals (Rettie and Messier 1998, Wittmer et al. 2005). These commonly used techniques may not only cause harm and stress to the animal under study (Côté et al. 1998, Cattet et al. 2008, Omsjoe et al. 2009), they are extremely costly (Haigh 1979, Valkenburg et al. 1983), often have female biases associated with the data collected (Seip 1992, McLoughlin et al. 2003, Sorensen et al. 2008), and recently there has been criticism of the ethicality of invasive methods (Jewell 2013). Consequently, a great deal of effort has been invested in non-invasive research for *Rangifer*, using fecal pellet

samples to gather data (Ball et al. 2007, Ball 2010, Petersen et al. 2010, Morden 2011, and Hettinga et al. 2012).

Research Problem

The purpose of this study was to investigate the fine-scale sociogenetic structure of woodland caribou.

Objectives:

- 1) Develop methods for estimating an age-class for wild woodland caribou populations using fecal morphometrics and fecal hormones;
- 2) Analyze genetic and fecal hormone data to investigate the mating system and individual fitness of woodland caribou (sociogenetics);

As there is limited information on the fine-scale sociogenetic structure and dynamic of woodland caribou, we developed a series of *a priori* expectations:

Objective 1:

- Since fecal pellet morphometrics have been shown to help determine the age-class of known age caribou (Ball 2010) and captive reindeer (Morden et al. 2011a), we expect that measuring fecal pellets will also produce age-class estimates for wild populations of woodland caribou.
- Since measures of fecal reproductive hormones have been shown to differ between age-class in a number of species (Lynch et al. 2002, Rooney et al. 2004, Castro and Sousa 2005, Morden et al. 2011b), we expect that fecal hormone levels will differ between the reproductive age-classes (i.e. calf from adult) of woodland caribou.

Objective 2:

- Since the most commonly found mating system in social mammalian species is polygyny (Banfield 1975, Hirth 1977, Holand et al. 2005, Holand et al. 2007, Archie et al. 2008), we expect to find the same in woodland caribou and that relatively few male woodland caribou will dominate reproductive output;
- Since female reindeer have been shown to exhibit social rank (Banfield 1975, Holand et al. 2004a, Holand et al. 2004b), and social rank has been shown to affect fecundity of captive female reindeer (Holand et al. 2004b), we expect that some female woodland caribou will have comparatively higher fitness and produce more offspring relative to other females.

The studied populations consist of the Tonquin herd, Brazeau herd and Maligne herd (hereafter referred to as South Jasper) in Jasper National Park (JNP) and the North Interlake herd in central Manitoba, Canada. Herds from JNP and Manitoba are considered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as two different Designatable Units (DU), JNP herds being part of the Central Mountain DU and Manitoban herds being part of the Boreal DU (COSEWIC 2011). While these herds are of different sizes and of different demographic status, they have been monitored extensively over the years and therefore have an extensive capture history to assist in the first stages of delineating putative age-classes for sampled individuals. Studying the fine-scale social structure of South Jasper's three herds will also provide valuable insight into individual reproductive output and highlight any potential movement of caribou that may have occurred between herds.

General Methods

To meet our objectives, we analyzed microsatellite data and reproductive hormone levels from fecal pellets and measured fecal pellet morphology from samples collected over sampling events ranging from 2004 to 2012.

Measuring Genetic Relatedness

Samples are routinely genotyped at ten microsatellite loci and include RT5, RT6, RT9, RT24, RT30, BM888 (Wilson et al. 1997), Map2C, BM848 (Bishop et al. 1994) BMS1788 and RT7 (Cronin et al. 2005). Parentage and sibship assignment was carried out using maximum likelihood methods implemented in COLONY 2.0 (Jones and Wang 2009). Other pedigree software consider relationships from pairs of individuals (i.e. dyads), but COLONY uses a maximum likelihood method to simultaneously determine parental and sibling relationships among individuals from their multi-locus genotypes. This method is not only more flexible in terms of allowing for a variety of input parameters, it is also more robust and accurate in defining true familial relationships (Wang and Santure 2009, Wang 2013a).

Developing an Age-Class

Building on an existing data set, we have clustered all samples into genotypes and have prepared a capture history for analysis. Building a capture history is a useful tool for identifying new individuals that have entered a local population and can help differentiate between probable calves and adults. For a more thorough approach to determine the age of different caribou in local populations (calf or adult), we measured the morphology of fecal pellets (Ball 2010, Morden et al. 2011a) and measured levels of progesterone, estrogen and testosterone derived from fecal pellets.

Justification of Research

Information on population parameters such as age-structure and reproductive status are critical for conservation of any wild population (Reilly 2002, Festa-Bianchet et al. 2003, Mysterud and Østbye 2006, Kuhl et al. 2009). Information on population structure is especially important in cases where a species has experienced population decline, as the loss of individuals may have an effect at the local population level. Our research will not only advance our understanding of fine-scale population dynamics and mating system of woodland caribou, it will help complete the non-invasive toolkit developed by Drs Wilson and Manseau to study and monitor wildlife populations. Effective management of a species can greatly benefit from research encompassing that species' ecology, biology, life history, and sociogenetic structure and dynamic. Although there is much information in the literature on caribou for the first three research fields, there remains a gap on the latter specific to woodland caribou. Scientists have made progress in these research areas for semi-domesticated reindeer (Holand et al. 2004a, Holand et al. 2004b, Holand et al. 2005, Weladji et al. 2009), yet there is still a considerable lack of information on the social structure and mating system of North American woodland caribou. These new non-invasive genetic sampling techniques are giving us the necessary tools to investigate key conservation questions in wild, large ungulate populations.

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**CHAPTER ONE: Fecal hormones analysis as a non-invasive tool to estimate age-class of
woodland caribou (*Rangifer tarandus caribou*)**

ABSTRACT

Fecal pellet morphometrics and hormone content were measured to distinguish calf from adult woodland caribou within the South Jasper and North Interlake herds. Fecal pellet collections occurred in the fall from 2006 to 2011 for South Jasper and in the winter from 2004 to 2010 for North Interlake. All samples were amplified at 10 microsatellite loci, and unique individuals in each population identified. A capture history was used to identify samples from adults, observed in at least three previous capture years; and putative calves, individuals first observed in later years. Fecal pellets were measured for length, width, and depth, dry weighed, and analyzed for levels of progesterone, estrogen, and testosterone content. Results showed a significant difference in fecal pellet size and weight between putative calves and adults of both males and females for both South Jasper (fall) and North Interlake (winter). Progesterone levels were significantly higher in South Jasper (fall) adult females and North Interlake (winter) adult-pregnant females. Furthermore, testosterone was significantly higher and estrogen was significantly lower in South Jasper (fall) adult males compared to putative calves. No clear difference in hormone levels was found among North Interlake (winter) male age-classes. This study shows the potential of non-invasive fecal sampling for use in cohort analysis and in combination with capture-mark-recapture methods may provide valuable insight on the age-structure of woodland caribou populations.

INTRODUCTION

Information on population parameters such as age-class and reproductive status are critical for conservation and effective management of any wild population (Kuhl et al. 2009, Morden et al. 2011b). Age-structure of populations may change over time and recognizing these changes can help managers identify trends in population growth, group composition, and reproductive activity (Festa-Bianchet et al. 2003). In addition, determining an age-class can be valuable when investigating a species' social structure as age has been shown to indicate dominance, behaviour and fitness in populations of ungulate species (Clutton-Brock et al. 1982, Aycrigg and Porter 1997, Banks et al. 2005, Holand et al. 2005, Weladji et al. 2006, Weladji et al. 2009), has been linked to the matriarchal social ranking of reindeer (*Rangifer tarandus tarandus*) (Banfield 1975, Holand et al. 2004a, Holand et al. 2004b), and has also been linked to the social hierarchy of male caribou (*Rangifer tarandus*) (Barrette and Vandal 1986).

Due to its informative value, researchers often spend substantial time and money capturing and tagging live animals for age determination (Hudy et al. 2010). In the past, more invasive methods have been used for determining age-class including tooth sectioning, molar tooth wear, eye lens weight, body measurements, advancement of lumbar epiphyseal fusion, and body weight (Hudy et al. 2010). More recently, there has been some criticism on the ethicality of using invasive methods for monitoring wild populations and the reliability of the data collected (Jewell 2013). Visual identification of an animal's age has also been used but this may lead to inaccurate estimations, requires suitable environmental conditions and may potentially be fraught with errors (Miller 2003, Lacy 2012). Non-invasive methods for determining age have been proposed as

better alternatives since wildlife can be difficult and risky to capture (Haig and Ballou 2002). Southgate (2005) suggested the measuring of fecal pellets as an alternative to traditional age classification methods as pellets are relatively easy to find and collect. Non-invasive fecal sampling (NFS) is growing in popularity among researchers and has been used to identify unique individuals through DNA extraction, estimate population sizes, identify pregnancy status, determine age-structure, monitor population productivity, and even for phylogeographical analyses (Eggert et al. 2003, Kuhl et al. 2009, Morden et al. 2011a, Morden et al. 2011b, Flagstad et al. 2012, Hettinga et al. 2012, Kluetsch et al. 2012). Consequently, NFS may be a useful alternative to more invasive methods for determining the age-class of individuals.

Utilizing NFS for identifying age-class is an emergent field of research yet has shown some success (Reilly 2002, Sanchez-Rojas et al. 2004, Southgate 2005, Morden et al. 2011a, Flagstad et al. 2012). Specifically, fecal morphometrics, the physical measuring of a fecal pellet's morphological shape, has been used to identify age-class on its own. Flagstad et al. (2012) was able to group individuals of Asian elephants (*Elephas maximus*) into three age-classes (calves or juveniles, sub-adults, and adults) by measuring the bolus size of elephant dung. Ball (2010) was able to show differences in woodland caribou calf, juvenile, and adult fecal pellet lengths and Morden et al. (2011a) found that age-classification of reindeer could be determined by combining fecal pellet length, width, and depth with an accuracy of 91%. In addition to fecal morphometrics, the validity of measuring levels of reproductive hormones to determine age-class has been investigated for a variety of species (Lynch et al. 2002, Rooney et al. 2004, Castro and Sousa 2005, Seraphin et al. 2008, Morden et al. 2011b). Fecal androgen levels have been

shown to differ between adult and juvenile male common marmosets (*Callithrix jacchus*) (Castro and Sousa 2005). Estrogen (E₂) and testosterone (T) plasma levels of American alligator (*Alligator mississippiensis*) hatchlings are significantly lower than in adult female estrogen (Crain et al. 1997, Guillette Jr. et al. 1997) and adult male testosterone plasma levels (Lance 1989, Rooney et al. 2004). Mean testosterone levels of sub-adult male tufted Capuchin monkeys (*Cebus apella*) have similarly revealed significantly lower levels than in adult male Capuchins (Lynch et al. 2002). Seraphin et al. (2008) was also able to show that testosterone levels, extracted from feces, increased with age in male eastern chimpanzees (*Pan troglodytes schweinfurthii*). Furthermore, Morden et al. (2011b) found significant differences in fecal progesterone metabolite levels between female reindeer calves and yearling/adult reindeer in Finland. Though studies conducted on fecal hormones analysis between age groups are rare, these studies still illustrate their capability in delineating age-class estimates. Morden et al. (2011a, 2011b) emphasize the potential of combining fecal morphometrics with fecal hormone analysis to yield more accurate results in age-class determination for reindeer and caribou populations. Thus, fecal hormone analysis paired with fecal morphometrics may reveal more accurate results in age-classification of woodland caribou by non-invasive means.

For species at risk, determining the age-class of individuals at the local population level is particularly important as the loss of individuals can likely affect the overall age-structure and recruitment rate. Woodland caribou (*Rangifer tarandus caribou*) are a subspecies of caribou that have experienced extensive population declines throughout their range and consequently have demanded much attention from wildlife researchers and conservationists (Environment Canada 2012). NFS is especially important for these

elusive animals as they are highly sensitive to disturbance and can be difficult to observe and study due to their occurrence at low densities across extensive areas (Courtois et al. 2003b). Here we combine fecal morphometrics with measures of progesterone, estrogen, and testosterone fecal content to assess the validity of this non-invasive method in estimating an age-class for two wild populations of woodland caribou. Since fecal pellet morphometrics have been shown to help determine the age-class of known age caribou (Ball 2010) and captive reindeer (Morden et al. 2011a), we expect that measuring fecal pellets will also produce age-class estimates for wild populations of woodland caribou. In addition, because measures of reproductive hormones have been shown to differ between age-class in a number of species (Lynch et al. 2002, Rooney et al. 2004, Castro and Sousa 2005, Morden et al. 2011b), we expect that fecal hormone levels will differ between the reproductive age-classes (i.e. calf from adult) of woodland caribou. To identify putative calves, we used non-invasive genetic sampling of pellets to identify unique genotypes, determine their sex, and then build a capture history for both study populations.

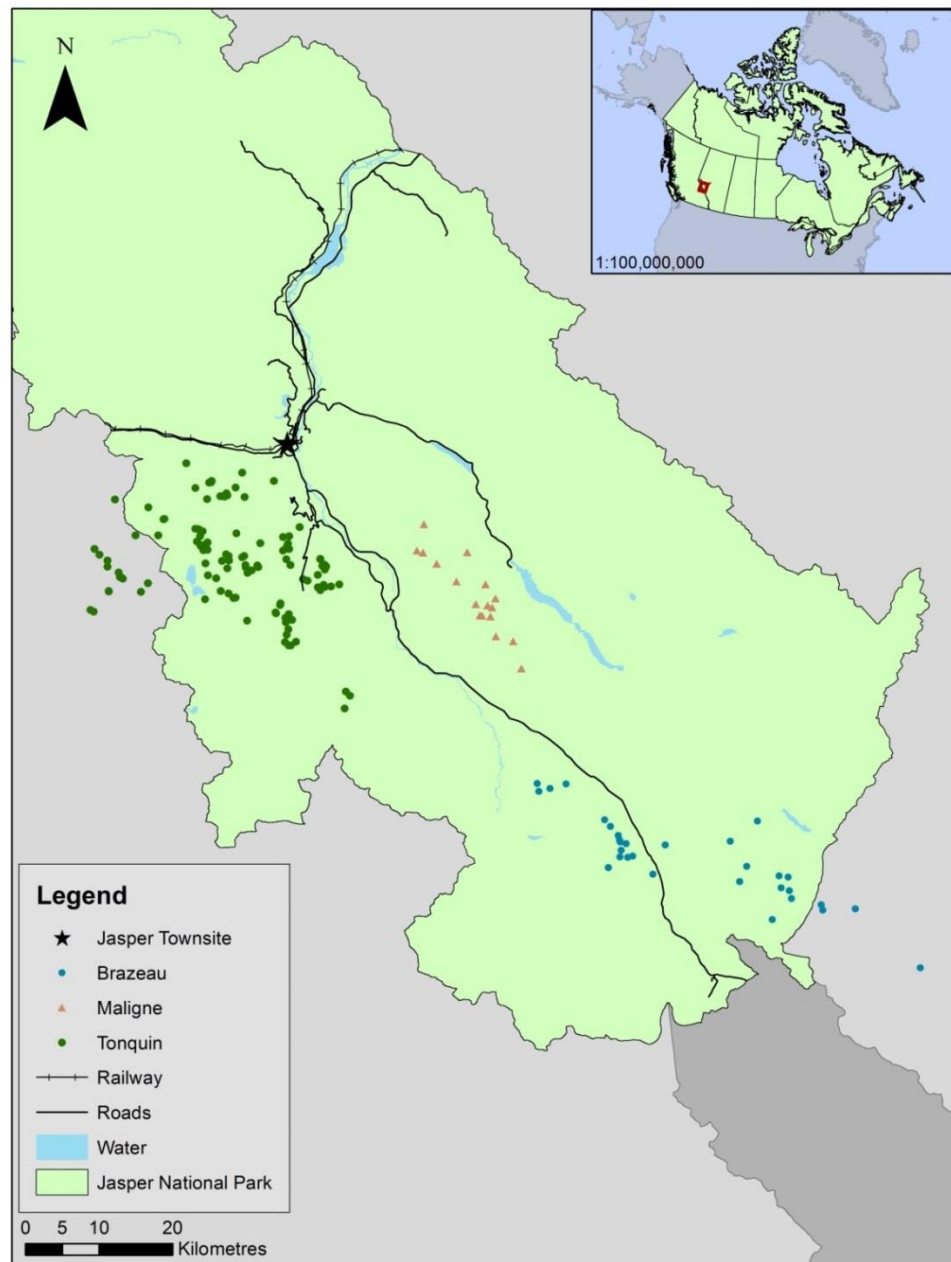
METHODS

Study Area

We used data collected from two woodland caribou population ranges, the Tonquin, Brazeau, and Maligne (hereafter referred to as South Jasper) woodland caribou herds and the North Interlake woodland caribou herd. The South Jasper herds are located in Jasper National Park (52°23'–52°84' N, 116°81'–118°45 W), Alberta, Canada (Figure 1). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assigned the South Jasper herds to the Central Mountain population (DU8) (COSEWIC 2011) and the last reported population estimate was 130 caribou in 2009 (Hettinga 2010).

South Jasper caribou are listed as threatened by both the provincial Wildlife Act (Alberta Sustainable Resource Development and Alberta Conservation Association 2010) and

Figure 1. Map of the location of the South Jasper herds based on field pellet collection sites.

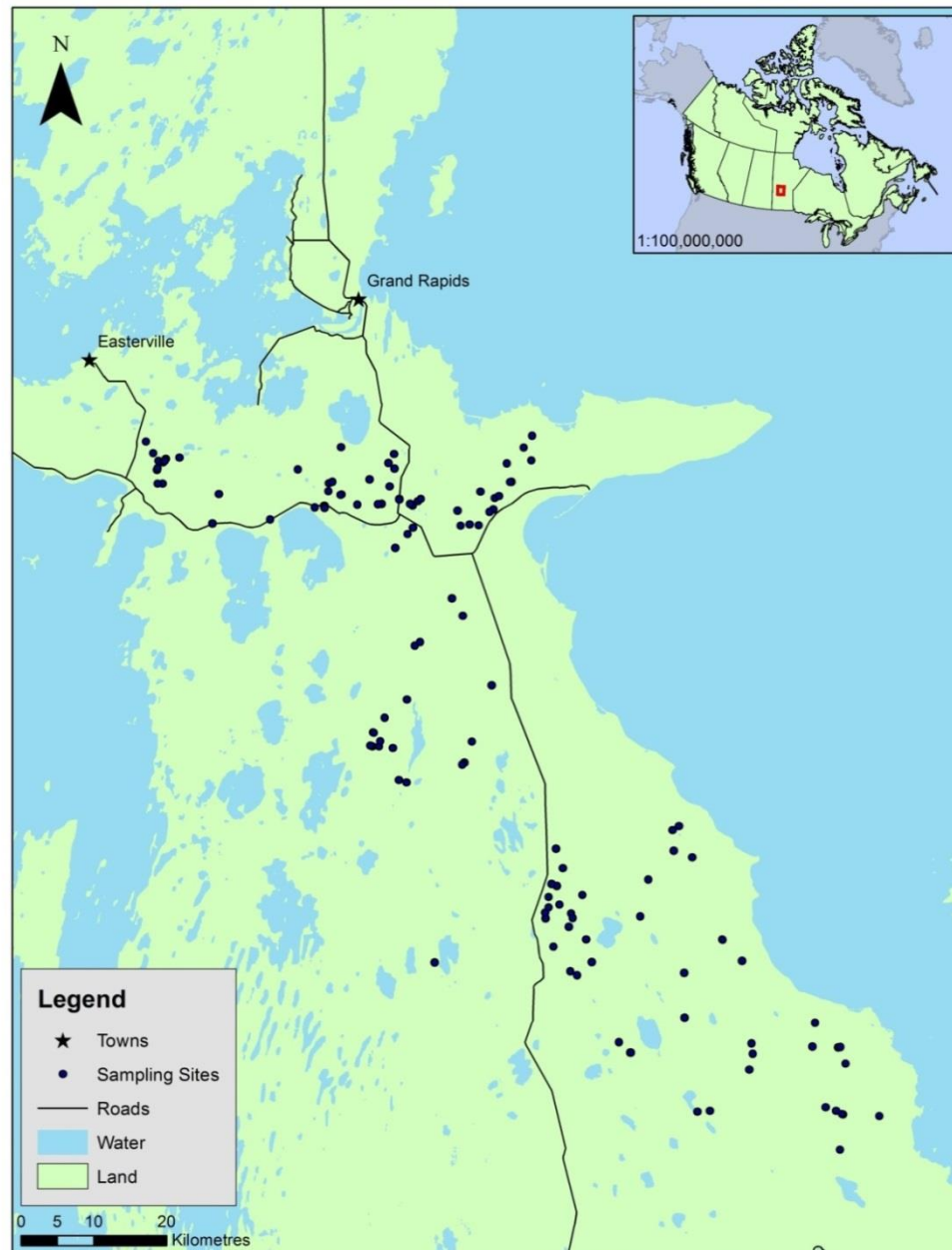


federal Species at Risk Act (SARA) (Environment Canada 2012). Minimal movement has been observed between these three herds, however telemetry data showed some cases of movement between the Maligne and Brazeau herds (Whittington et al. 2005a) and genetic capture-mark-recapture (CMR) surveys occurring in the Fall since 2006 have shown four cases of male caribou movements between all three herds. The South Jasper caribou were once estimated to have approximately 450 animals in the 1960s. Since then their population size has decreased considerably and has become more fragmented likely due to apparent competition brought on by management practices and anthropogenic disturbance (Whittington et al. 2005a, Hebblewhite et al. 2007, Bradley and Neufeld 2012). Since the extirpation of Banff National Park's woodland caribou in 2009 (Hebblewhite et al. 2010), the South Jasper caribou herds are Alberta's most southerly ranging caribou. They migrate altitudinally with the seasons, descending from mountain peaks into valley bottoms as winter progresses and snow depth increases. Here they reside for 6-8 months preferentially foraging on terrestrial lichen (Warren et al. 1996, Thomas and Gray 2002), remaining in the alpine for spring calving through to the end of the rut (COSEWIC 2011). Other large mammals and predatory species that occur in the area are moose (*Alces alces*), mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), bighorn sheep (*Ovis canadensis*), elk (*Cervus canadensis*), mountain goats (*Oreamnos americanus*), grizzly bears (*Ursus arctos*), black bears (*Ursus americanus*), cougars (*Puma concolor*), coyotes (*Canis latrans*), wolverines (*Gulo gulo*), and wolves (*Canis lupus*).

The North Interlake herd is located between Lake Winnipeg and Lake Winnipegosis (52°13'–53°02' N, 98°22'–99°70 W), Canada (Figure 2). COSEWIC

assigned the North Interlake herd to the Boreal Woodland population (DU6) (COSEWIC 2011) and the last population estimate was at 100 caribou in 2009 (Hettinga et al. 2012).

Figure 2. Map of the location of the North Interlake herd based on field pellet collection sites.



Boreal Woodland caribou are listed as threatened by both COSEWIC (COSEWIC 2002) and under SARA (Environment Canada 2012). Boreal forest dominates the North Interlake's range, consisting of peat lands, treed muskeg, black spruce, old jack pine stands and tamarack-dominated upland areas (Manitoba Conservation 2005). The ecoclimate is characterized by short warm summers and long cold winters with annual snowfall rates sufficient for tracking caribou from the end of December to the end of March (Hettinga et al. 2012). Various other large mammals and predatory species are found in the area and include moose, white-tailed deer, wood bison (*Bison bison athabasca*), black bears, coyotes, and wolves. Hydro transmission corridors and two provincial highways intersect the area, which may contribute to the fragmenting of the herd previously shown by examining the population genetic structure of the region (Ball et al. 2010). Ball et al. (2010) found 2 distinct genetic clusters in the North Interlake via individual-based clustering methods and defined them as the Upper and Lower North Interlake (Ball et al. 2010, Hettinga et al. 2012). The Upper North Interlake was shown to have more gene flow with neighbouring western caribou herds (i.e. The Bog herd) and the North Interlake as a whole has shown evidence of isolation-by-distance (Ball et al. 2010, M. Manseau, Parks Canada/University of Manitoba, unpublished data). Additionally, Hettinga et al. (2012) found that the Lower North Interlake exhibited higher population estimates with a higher proportion of females than the Upper North Interlake. The results of a six-year survey period (2005-2010) showed a significant and constant decline ($\lambda = 0.92$) (Hettinga et al. 2012, M. Manseau, Parks Canada/University of Manitoba, pers. comm.). The impact of a local road network may be adding to the fragmentation of the Upper and Lower North Interlake, and the North Interlake as a

whole has been shown to have little connectivity to other areas due to Lake Winnipeg to the west and Lake Manitoba plus others to the east, and due to a hydro reservoir in the north (Manseau et al. 2002, Fall et al. 2007). The North Interlake and South Jasper caribou herds are of different sizes and of different demographic status which will be useful for comparisons across different landscapes.

Fecal Pellet Collection and Lab Analysis

Two fecal pellet collections took place per year during the fall (October to December) of 2006 to 2012 for South Jasper herds and during the winter months of 2004 to 2010 (January or February to March) for the North Interlake herd. In addition, we conducted fecal pellet collections for South Jasper during the month of March between 2007 and 2009 and again in 2012 during the recruitment surveys; and an added collection occurred in January of 2008. Most of the caribou across the South Jasper and North Interlake ranges have been sampled at least once, with a total of 1,732 samples collected equalling 214 unique genotypes in South Jasper and 1,135 samples collected equalling 216 unique genotypes in North Interlake.

We collected fecal pellets in the snow, placed them in sterile bags, and kept them frozen at -20°C until lab analysis. For more information on sampling design, see Hettinga et al. (2012). We completed DNA extraction first by removing the mucosal sheath of cells that coats fecal pellets, and followed the extraction protocol outlined in Ball et al. (2007). We used ten polymorphic microsatellite loci (RT5, RT6, RT9, RT24, RT30, BM888, Map2C, BM848, BMS1788 and RT7; Bishop et al. 1994, Wilson et al. 1997, Cronin et al. 2005) along with caribou-specific Zfx/Zfy primers for sex identification.

Once we extracted DNA from fecal pellets and amplified the target microsatellite markers, scoring of alleles took place using the program GeneMarker™. Our lab protocol requires that two to three independent scorers examine DNA profiles and that all agree on a final score. Scoring follows a documented protocol that provides details on allele peak morphologies for the amplified loci, including procedures for dealing with difficult allele morphologies, and the strength of alleles in relative fluorescence units (rfu). Peaks found below 200 rfu were never scored due to low confidence in these scores being clear alleles. Results from the independent scorers were uploaded to a shared database where scorers compared results and discrepancies were automatically flagged. If a scoring discrepancy was found, then a discussion took place among scorers until a consensus was reached. Otherwise the sample was re-profiled to confirm a score or the sample was removed from further analysis and was concluded to be a problematic sample. We followed this procedure for all alleles found among all loci.

Building a Capture History

We developed a capture history by identifying unique genotypes seen at each sampling period to cluster groups of samples together belonging to unique genotypes (Figure 8, Appendix A). Since collections have started, we have accrued samples from the same animal repeatedly, resulting in a large dataset of multiple samples belonging to one individual (Table 1, Table 2). By clustering multiple samples of the same genotype, captured over subsequent years and at multiple locations, individuals can be followed through time and space and can be used as a first step in building an age-class (Figure 3). To accomplish this, we used the ALLELEMATCH program (Galpern et al. 2012a) to identify unique genotypes. ALLELEMATCH was designed to cluster multilocus

genotype data to determine unique individuals and to identify potential genotyping errors.

In addition, ALLELEMATCH has the ability to cope with genotyping errors and can handle large datasets containing multiple samples of individuals, much like the current data set for the South Jasper, and North Interlake.

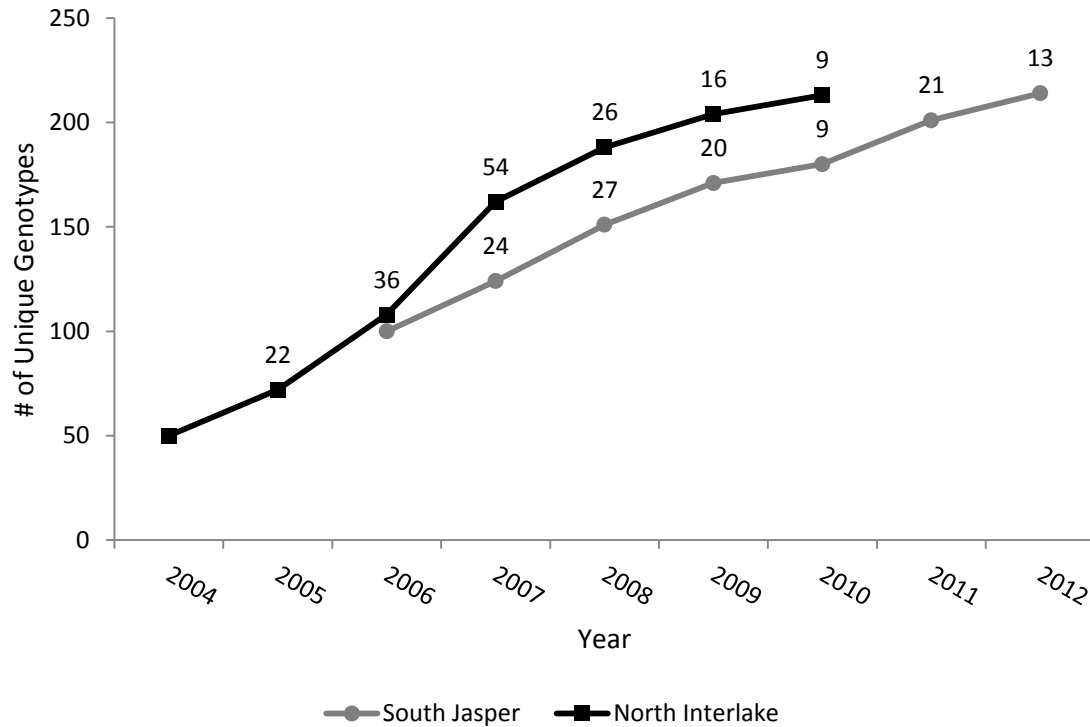
Table 1. Summary of South Jasper capture history results.

Sampling Time	# Samples Successfully Scored	# of Unique Genotypes	# Genotypes Observed in Previous Capture Event	Proportion
Oct 2006	173	74	-	-
Nov 2006	97	38	12	0.32
Oct 2007	134	53	-	-
Nov 2007	117	49	27	0.55
Jan 2008	44	15	6	0.40
Oct 2008	127	72	-	-
Nov 2008	125	46	22	0.48
Oct 2009	142	62	-	-
Nov 2009	132	56	39	0.70
Oct 2010	113	56	-	-
Nov 2010	110	40	26	0.65
Oct 2011	88	41	-	-
Nov 2011	148	50	28	0.56
Oct 2012	108	34	-	-
Nov 2012	74	26	14	0.54
Total	1732	712	174	-

Table 2. Summary of North Interlake capture history results.

Sampling Time	# Samples Successfully Scored	# of Unique Genotypes	# Genotypes Observed in Previous Capture Event	Proportion
Jan 2004	94	50	-	-
Feb 2005	82	33	-	-
Feb 2006	103	53	-	-
Feb 2007	170	74	-	-
Mar 2007	118	47	23	0.49
Jan 2008	116	55	-	-
Mar 2008	173	55	28	0.51
Jan 2009	122	45	-	-
Feb 2009	95	30	11	0.37
Jan 2010	37	17	-	-
Feb 2010	25	13	5	0.38
Total	1135	472	67	-

Figure 3. Cumulative number of unique genotypes observed through time for South Jasper and North Interlake caribou herds. Data labels indicate the number of new individuals identified each year.



Age-Class Determination

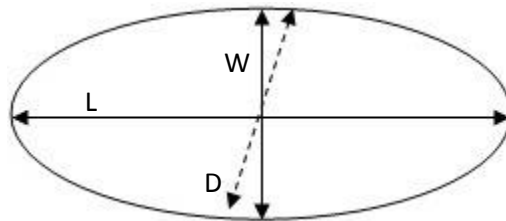
We compared fecal pellet morphometrics and estrogen, progesterone, and testosterone levels for putative calves, adult males, and adult females of the South Jasper and North Interlake woodland caribou herds. We selected adult fecal samples from individuals that had been observed in our capture history in at least 3 previous capture years, preferring individuals that first appeared in the earliest years (i.e. as early as 2006 to 2008 for South Jasper and 2004 to 2006 for North Interlake) and using the sample collected from the individual in its third year. We selected putative calf samples from individuals that first appeared in our capture history in later years (i.e. preferring individuals first observed in 2009, 2010, or 2011 for South Jasper, and 2008, 2009, or

2010 for North Interlake). The South Jasper herds and North Interlake herd are closed populations that have been sampled extensively over the years and thus new genotypes identified in later years have a high probability of being calves and not individuals that have migrated from elsewhere. Accordingly, two North Interlake female putative calf samples were selected from individuals that first appeared in 2007. Furthermore, female pregnane levels are a known indicator of pregnancy status (Morden et al. 2011b) and we consequently moved 5 of the 11 North Interlake putative calves to the adult age-class as they were found to be pregnant.

Fecal Pellet Morphometrics

We selected five apparently complete and whole pellets per sample for measurement. Our methods followed that of Morden et al. (2011a) and included measuring each pellet for its maximum length (L), maximum width (W), and depth (D) at 90° rotation from W (Figure 4) and an approximated volume index was calculated from the product of each measurement (hereafter referred to only as volume, mm³). We used digitized calipers to measure pellets to the nearest 0.01 of a millimetre and each pellet was measured by the same person to avoid inter-observer bias. In addition, we weighed three fecal pellets per sample to the nearest 0.01 gram after drying the samples overnight.

Figure 4. Fecal pellet dimensions measured for pellet morphometrics. L = length, W = width, and D = depth at 90° rotation from width.



Hormone Extraction and Analysis

All hormones analysis took place at the Toronto Zoo's Endocrinology Lab. We kept fecal samples frozen at -20°C until hormone analysis began. We first dried feces overnight at approximately 75° C to remove any excess water. We measured hormone content in three individual pellets per fecal sample. Each pellet was homogenized for even distribution of the hormones and extraction was then done following the protocol of Morden et al. (2011b). In brief, we extracted fecal pellets using 80% methanol:water (v:v) at a ratio of 0.04 g/ml and rotating overnight. We quantified fecal estrogen, progesterone and testosterone metabolite levels within the extracts using enzyme immunoassays (Kummrow et al. 2011, Morden et al. 2011b).

Statistical Analysis

We used a generalized linear model (GLM) (PROC GENMOD using the SAS 9.4 software; SAS Institute Inc. 2013) to examine the relationship between age-class and each of the five test variables, except for female pregnane and fall female estrogen. We omitted these variables from the analysis because we were not able to achieve normal distributions, even after transformations. This may have been caused by the effect of both seasons (adult females exhibiting polyestrous behaviour during the fall and potentially pregnant in winter) and thus it was not necessary to include pregnane or fall estrogen in the analysis. To achieve normality of our remaining data, we log-transformed only winter female pellet volume and estrogen, and winter male estrogen. All other variables were normally distributed without requiring transformation. Four independent analyses were completed: South Jasper females and males (fall), North Interlake females and males (winter). We sampled all individuals only once and therefore we did not include

individual as a random variable in our model. In addition, we conducted a non-parametric test for significant differences in pregnane levels between South Jasper (fall) and North Interlake (winter) female age-classes. This included a Kruskal-Wallis test between North Interlake (winter) female age-classes and a Wilcoxon Rank Sum test between South Jasper (fall) female age-classes (PROC NPAR1WAY using the SAS 9.4 software; SAS Institute Inc. 2013). We accomplished all analyses by using SAS 9.4 software (SAS Institute Inc. 2013) and we accepted a 5% significance level.

RESULTS

In total we selected 38 females (9 putative calves, 29 adults) and 28 males (13 putative calves, 15 adults) from South Jasper (fall) for use in our study as well as 26 females (6 putative calves and 20 adults) and 27 males (13 putative calves, 14 adults) from North Interlake (winter).

Results from the South Jasper (fall) female GLM showed significant differences between putative calf and adult pellet weight and volume (Table 3, $p < 0.0001$; Figure 5. A). In addition, results from the North Interlake (winter) female GLM also showed significant differences between putative calf and adult pellet weight and volume (Table 4, $p < 0.05$; Figure 5. B). However, no significant difference was found between putative calf and adult fall female testosterone (Table 3, $p = 0.7898$; Figure 7. A), or winter female testosterone (Table 4, $p = 0.5614$; Figure 7. B) and estrogen (Table 4, $p = 0.2404$; Figure 7. B).

Female pregnane was not included in the GLM but demonstrated highly significant differences between South Jasper (fall) age-classes and North Interlake (winter) age-classes, except for putative calf and adult non-pregnant (North Interlake

putative calf mean pregnane = 472.9 ng/g, SD = 199.8 ng/g, adult non-pregnant mean pregnane = 423.0 n/g, SD = 7.7 ng/g) (Figure 6). Examining levels of fecal pregnane can accurately identify pregnancy (Morden et al. 2011b) and thus explains the considerably high levels of pregnane in North Interlake females, which were subsequently moved to the “Adult Pregnant” age-class. The high levels of pregnane in South Jasper’s females may not only indicate the presence of some potentially pregnant females, but is an indication of female adults in oestrous as this is the time of year when the caribou are in rut (Ropstad 2000). See Figure 9 (Appendix A) for a detailed breakdown of the varying hormone levels in South Jasper females.

Table 3. South Jasper (fall) female generalized linear model examining the relationship between age-class and pellet weight (a), pellet volume (b), and testosterone (c).

Significant values are in italics.

	<i>b</i>	SE	χ^2 (df = 36)	<i>p</i> -value
a) Pellet Weight (g)				
Intercept	0.167	0.020	70.60	<0.0001
Age-class: Adult – Calf	0.098	0.023	18.76	<0.0001
b) Pellet Volume (mm³)				
Intercept	903.542	151.935	35.37	<0.0001
Age-class: Adult – Calf	700.506	173.920	16.22	<0.0001
c) Testosterone (ng/g)				
Intercept	131.665	11.495	131.21	<0.0001
Age-class: Adult – Calf	3.508	13.158	0.07	0.7898

Results from the South Jasper (fall) male GLM showed significant differences between putative calf and adult pellet weight and volume (Table 5, $p < 0.0001$; Figure 5. C) and testosterone (Table 5, $p = 0.0064$; Figure 7. C). Estrogen also showed a significant difference between South Jasper (fall) male putative calf and adult age-classes, however estrogen was observed to be lower in adults than in calves (Table 5, $p = 0.0012$; Figure 7. C). Estrogen also appeared to occur in overall higher levels than testosterone for both

Table 4. North Interlake (winter) female generalized linear model examining the relationship between age-class and pellet weight (a), log pellet volume (b), testosterone (c), and log estrogen (d). Significant values are in italics.

	<i>b</i>	SE	χ^2 (df = 24)	<i>p</i> -value
a) Pellet Weight (g)				
Intercept	0.148	0.012	143.60	<i><0.0001</i>
Age-class: Adult – Calf	0.033	0.014	5.36	<i>0.0206</i>
b) Pellet Volume (log mm³)				
Intercept	2.812	0.044	4093.48	<i><0.0001</i>
Age-class: Adult – Calf	0.113	0.050	5.04	<i>0.0248</i>
c) Testosterone (ng/g)				
Intercept	227.630	20.215	126.80	<i><0.0001</i>
Age-class: Adult – Calf	13.385	23.049	0.34	<i>0.5614</i>
d) Estrogen (log ng/g)				
Intercept	2.669	0.048	3091.87	<i><0.0001</i>
Age-class: Adult – Calf	0.064	0.055	1.38	<i>0.2404</i>

putative calves and adults, and was negatively correlated with testosterone between age-classes (Figure 7. C). Lastly, results from the North Interlake (winter) male GLM showed significant differences between putative calf and adult pellet weight and volume (Table 6,

$p < 0.001$; Figure 5. D). However, no significant differences were found between North Interlake (winter) male putative calf and adult hormone levels (Table 6, Figure 7. D). Yet male fecal hormones appear to occur in higher levels and are more variable in North Interlake (winter) than in the South Jasper (fall) (e.g. South Jasper putative calf mean testosterone = 122.9 ng/g, SD = 16.1 ng/g, adult mean testosterone = 146.3 ng/g, SD = 28.4 ng/g; North Interlake putative calf mean testosterone = 275.1 ng/g, SD = 103.5 ng/g, adult mean testosterone = 258.8 ng/g, SD = 91.1 ng/g) (Figure 7).

Table 5. South Jasper (fall) male generalized linear model examining the relationship between age-class and pellet weight (a), pellet volume (b), testosterone (c), pregnane (d), and estrogen (e). Significant values are in italics.

	<i>b</i>	SE	χ^2 (df = 26)	<i>p</i> -value
a) Pellet Weight (g)				
Intercept	0.185	0.014	169.44	<i><0.001</i>
Age-class: Adult – Calf	0.133	0.020	46.92	<i><0.001</i>
b) Pellet Volume (mm³)				
Intercept	937.761	109.684	73.10	<i><0.001</i>
Age-class: Adult – Calf	1261.962	149.857	70.92	<i><0.001</i>
c) Testosterone (ng/g)				
Intercept	122.852	6.293	381.17	<i><0.001</i>
Age-class: Adult – Calf	23.453	8.597	7.44	<i>0.0064</i>
d) Pregnanone (ng/g)				
Intercept	243.113	20.723	137.66	<i><0.001</i>
Age-class: Adult – Calf	-8.074	28.310	0.08	<i>0.7755</i>
e) Estrogen (ng/g)				
Intercept	270.151	14.73	336.32	<i><0.001</i>
Age-class: Adult – Calf	-65.429	20.126	10.57	<i>0.0012</i>

Table 6. North Interlake (winter) male generalized linear model examining the relationship between age-class and pellet weight (a), pellet volume (b), testosterone (c), pregnane (d), and log estrogen (e). Significant values are in italics.

	<i>b</i>	SE	x^2 (df = 25)	<i>p</i> -value
a) Pellet Weight (g)				
Intercept	0.162	0.012	174.25	<0.001
Age-class: Adult – Calf	0.064	0.017	14.05	0.0002
b) Pellet Volume (mm³)				
Intercept	784.480	75.426	108.17	<0.001
Age-class: Adult – Calf	465.155	104.746	19.72	<0.001
c) Testosterone (ng/g)				
Intercept	275.082	25.947	112.40	<0.001
Age-class: Adult – Calf	-16.250	36.033	0.20	0.6520
d) Pregnane (ng/g)				
Intercept	378.001	28.398	177.19	<0.001
Age-class: Adult – Calf	72.240	39.437	3.36	0.0670
d) Estrogen (log ng/g)				
Intercept	2.695	0.045	3622.66	<0.001
Age-class: Adult – Calf	0.024	0.062	0.15	0.6945

Figure 5. Mean (\pm SD) fecal pellet volume (mm^3) and weight (g) of both age-classes for South Jasper (fall) females (A), North Interlake (winter) females (B), South Jasper (fall) males (C), and North Interlake (winter) males (D). Data labels indicate sample size. In all cases, differences between age-classes are significant ($p < 0.05$).

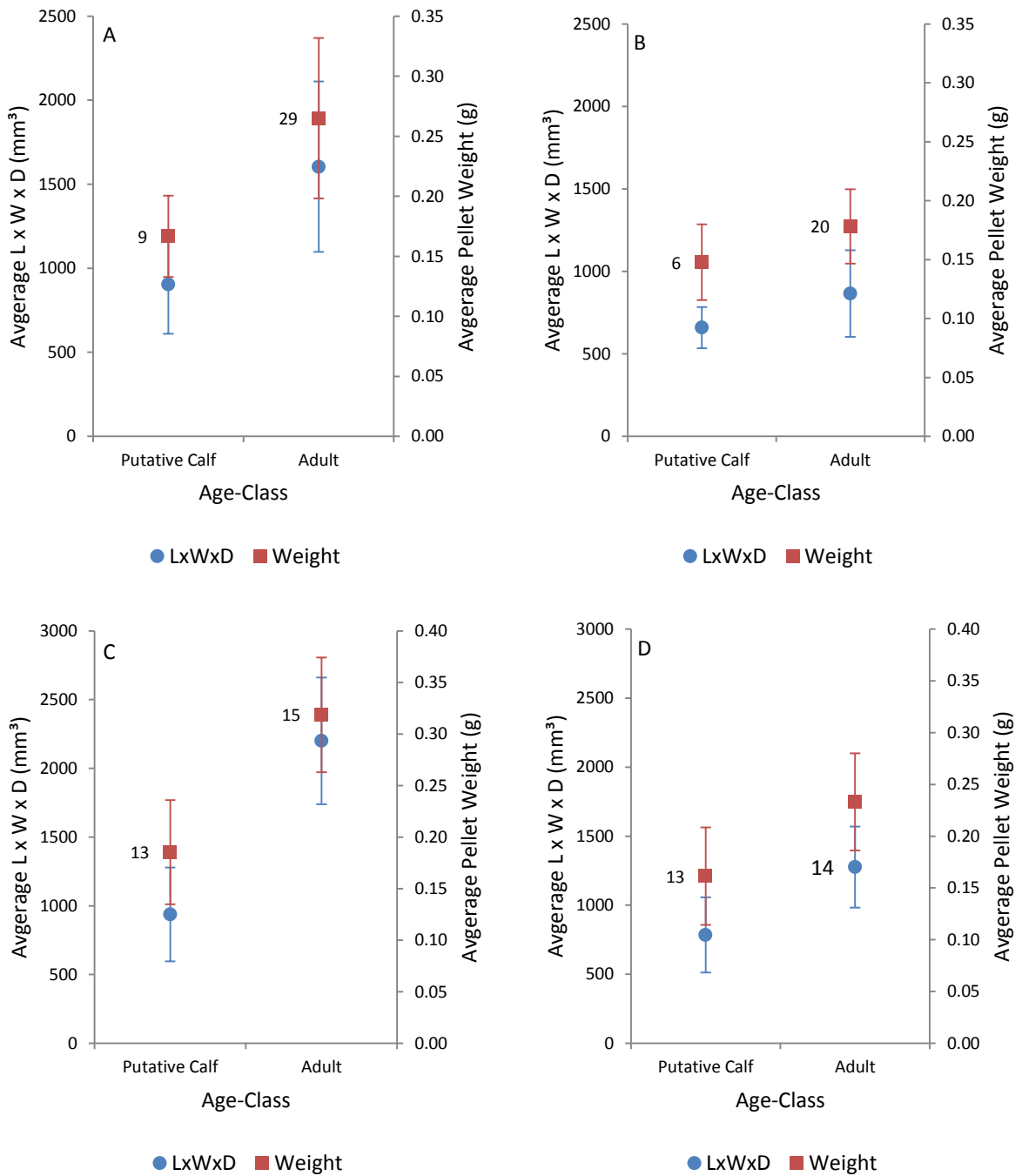


Figure 6. Mean (\pm SD) pregnane content (ng/g) of each age-class for South Jasper (fall) females (A), and North Interlake (winter) females (B). Data labels indicate sample numbers. Differences between all age-classes are significant ($p < 0.001$); except for putative calf and adult non-pregnant (B).

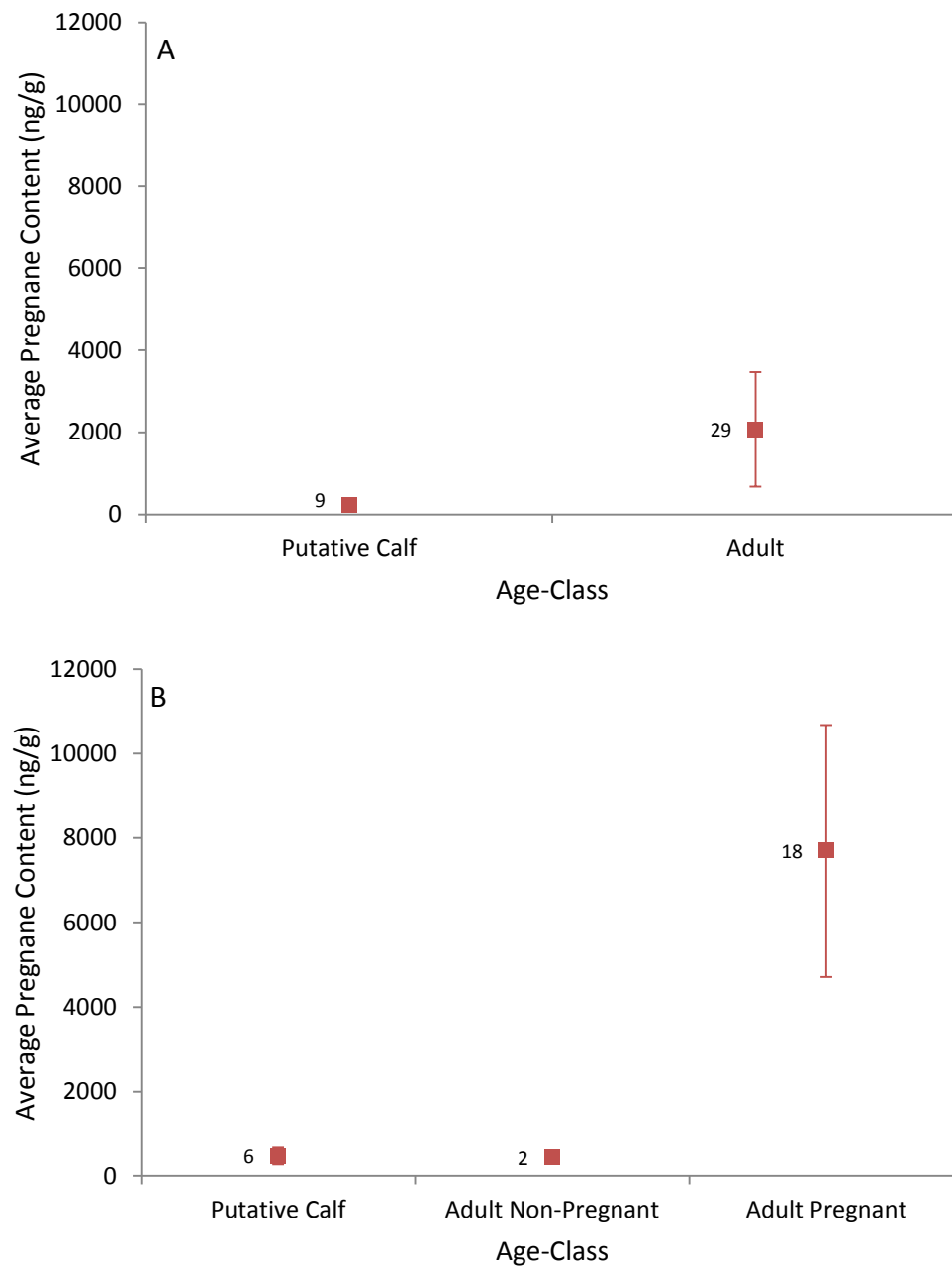
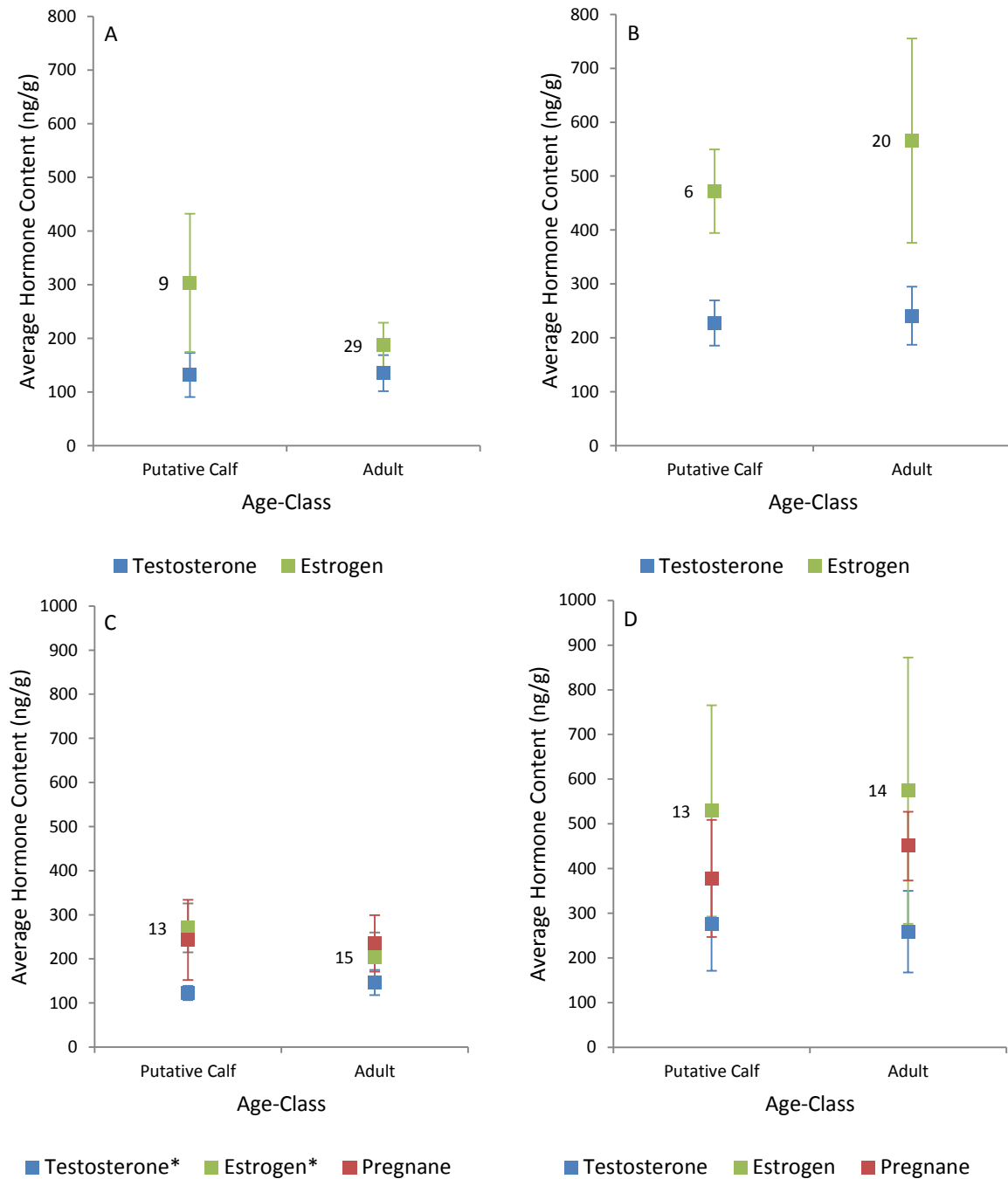


Figure 7. Mean (\pm SD) fecal hormone content (ng/g) of each age-class for South Jasper (fall) females (A), North Interlake (winter) females (B), South Jasper (fall) males (C), and North Interlake (winter) males (D). Data labels indicate sample numbers. Asterisks indicate significant differences ($p < 0.05$).



The outcomes for the effectiveness of pellet morphology and pellet hormone levels in delineating age-class for both males and females are summarised in Table 7.

Table 7. Summary of results for confidently identifying age-class. Details in brackets () provide brief explanation for outcome.

	South Jasper (Fall)		North Interlake (Winter)	
	Female	Male	Female	Male
Pellet morphology	Yes	Yes	Yes	Yes
Pregnane	Maybe (oestrous)	No	Maybe (pregnant)	No
Testosterone	No	Yes	No	No
Estrogen	No	Maybe	No	No

We conclude that pellet morphology is the most effective tool to differentiate putative calves from adults; however differences were clearer for South Jasper (fall) caribou than North Interlake (winter) caribou, particularly when sampling males (Figure 5). Fecal pregnane may be used to identify adult females in the luteal phase when sampling in the fall or, more accurately, adult females that are pregnant when sampling in winter (Figure 6). Following this, only pellet morphology may be used to further differentiate remaining females that are clearly not in the luteal phase (fall) or are non-pregnant adults (winter) (Figure 5. A, B). Male age-classes may first be distinguished by pellet morphology, and in South Jasper (fall) may be further differentiated by assessing differences in fecal testosterone levels, and possibly estrogen levels. North Interlake (winter) male age-

classes may be distinguished by pellet morphology only, as there were no clear differences in fecal hormone levels found.

DISCUSSION

For South Jasper and for North Interlake, we found significant differences between putative calf and adult age-classes in pellet weight and volume for female and male caribou. However, fecal hormone results were less clear. Pregnane was significantly higher in female adults for both South Jasper (fall) and North Interlake (winter) sampling, while only fecal testosterone and estrogen showed significant differences between South Jasper (fall) male age-classes. Absolutely no differences were found in fecal hormone levels for North Interlake (winter) male age-classes. These varying fecal hormone results may be due to the effect of season and may illustrate herd differences in fecal hormone content, or a combination of these two factors. These two caribou populations are found on different landscapes, where diet may differ at the local population level (Miller 2003), thus having implications for both fecal pellet size and hormone content. Morden et al. (2011a) also found that sampling month led to differences in pellet dimensions of Svalbard reindeer (*Rangifer tarandus platyrhynchus*). While we did find a difference in pellet weight and volume between age-classes, South Jasper (fall) caribou appeared to have larger pellets than North Interlake (winter) caribou (Figure 5). In addition, woodland caribou are also known to lower their seasonal energy requirements (Miller 2003) and live off fat reserves (Thomas and Gray 2002) during the winter months, all of which may have implications for pellet size and hormone content. Furthermore, our results may be impacted by the smaller female putative calf sample size, particularly for North Interlake. Perhaps if we had more samples the difference between putative calf and adult female

pellet weight and volume would be more pronounced or tighter, as seen in the male pellet weight and volume results. Nevertheless, our expectation that measuring fecal pellets as a means to differentiate calf and adult woodland caribou was supported, having shown clear differences between age-class in males and in females, despite the small female sample size. Our findings are in line with other studies as Ball (2010) was able to distinguish woodland caribou calf and yearling age-classes from adult woodland caribou using pellet length, and was able to distinguish calf age-class using pellet width. Morden et al. (2011a) found that adult reindeer had longer pellets than calves, and adults and yearlings had wider pellets than calves. Similarly, differences in fecal morphometrics have been shown to indicate age-class in various other species, including the greater bilby (*Macrotis lagotis*) (Southgate 2005), Sumatran elephants (*Elaphus maximus*) (Reilly 2002), and mule deer (Sanchez-Rojas et al. 2004). While Southgate (2005) only found moderate success in differentiating between age-classes of the greater bilby, Sumatran elephant dung bolus diameter was found to be highly correlated with age using the Von Bertalanffy growth curve (Reilly 2002); and mule deer adult male, adult female, and yearling age-classes were distinguishable via measures of pellet length, width, length-to-width, and volume (Sanchez-Rojas et al. 2004).

Our results showed that pregnane may be a useful first step in separating calf from adult female caribou by identifying South Jasper (fall) females sampled while in oestrous and pregnant females sampled in North Interlake (winter). The variation in pregnane levels found in South Jasper (fall) adult females, including peaks of high pregnane content, revealed females that were in oestrous or perhaps in early stages of pregnancy. Caribou and reindeer are a polyestrous species whereby oestrous cycling occurs

continually until fertilization (McEwan and Whitehead 1972, Ropstad 2000, Shipka et al. 2007). During oestrous, high peaks of progesterone are observable and while these progesterone peaks may not be as high as when a female is pregnant, they are still distinctly high enough to conclude that the individual is in oestrous and thus is of reproductive age (Ropstad et al. 1995, Shipka et al. 2007). Consequently, these progesterone peaks that are observable in oestrous females would not be observable in calves, seeing as they are not of reproductive age. This explains the significantly higher concentrations of pregnane we found in our South Jasper (fall) adult female age-class when compared to the putative calf age-class. When sampling in the winter, high progesterone levels are known to serve as accurate indicators of pregnant female caribou and reindeer (Bubenik et al. 1997, Ropstad et al. 2005, Shipka et al. 2007, Morden et al. 2011b). Progesterone levels rise after conception and remain at high levels consistently throughout the gestation period, until just prior to parturition whereby progesterone levels drop significantly (Bubenik et al. 1997, Shipka et al. 2007). At the same time, estrogen levels are at baseline and rise as progesterone drops before parturition (Bubenik et al. 1997, Shipka et al. 2007), occurring in the last trimester of pregnancy when estrogen levels are markedly higher (Messier et al. 1990). Similar results have been shown to occur in elk (Stoops et al. 1999). This may explain for the estrogen hormone being the least effective in differentiating calf from adult in South Jasper (fall) females but exhibiting some difference between age-classes in North Interlake (winter) females.

Testosterone also exhibited highly significant differences between putative calf and adult age-classes for South Jasper (fall) males. Not surprisingly, testosterone levels peak during the rutting season for adult male caribou and reindeer (Whitehead and

McEwan 1973, Stokkan et al. 1980, Bubenik et al. 1997). Additionally, testosterone levels have been shown to increase with age in wild reindeer while studying the antler cycles of males (Leader-Williams 1979). Bubenik et al. (1997) also reported that males sampled with the highest testosterone peaks were behaviourally the most aggressive bulls and had the largest antlers. This finding was also found in white-tailed deer whereby the highest testosterone levels were found in the most aggressive bucks (Bubenik and Schams 1986). Furthermore, Stokkan et al. (1980) found that testosterone levels were correlated with fighting rank in reindeer. Pampas deer (*Ozotoceros bezoarticus* *bezoarticus*) have also been found to exhibit reproductive behaviour when testosterone levels were high via fecal sampling (Pereira et al. 2005). In addition, dominant males of the Père David's deer (*Elaphurus davidianus*), who were most often observed in control of female harems, were found to have higher secretions of testosterone (Li et al. 2004). These findings can explain the presence of high testosterone in adult males sampled in South Jasper (fall), particularly those individual males with exceptionally high testosterone content, and may have the potential to specify which males are likely to dominate the reproductive output relative to their conspecifics.

In reindeer bulls, estrogen levels have been shown to peak during rut in mid-August and decrease slowly over the following months (Bubenik et al. 1997). We observed significantly lower levels of estrogen in South Jasper (fall) adult male caribou compared to putative calves, which was puzzling. However it may be that we are sampling South Jasper males in the months following peak rut, when estrogen levels begin to drop. Additionally, estrogen has been demonstrated as an important hormone in male longitudinal bone development, following its conversion from testosterone by the

aromatase enzyme (Riggs et al. 2002). Perhaps estrogen is found at such high levels in male calves because they are quickly growing and developing. Furthermore, estrogen has been shown to significantly inhibit antler growth and function in adult male ungulates as Goss (1968) found that injected estrogen slowed regeneration of antlers and caused premature velvet shedding and ossification. It would therefore be harmful for adult male caribou to have high levels of estrogen at this time of year, when the presence of antlers plays an important role in dominating females (Barrette and Vandal 1986, Hirotani 1994). However these explanations are all speculative for a result that was unexpected, and simply may be due to our sample size. Perhaps with more sampling, these differences would disappear.

In lieu of our hormones analysis results, we find our hypothesis on fecal hormone levels differing between the reproductive age-classes (i.e. calf from adult) of woodland caribou only moderately supported. While a few hormones were able to differentiate putative calves from adults (i.e. female pregnane and South Jasper male testosterone and estrogen) there were no clear differences in fecal content of any hormone for North Interlake (winter) male caribou or in fecal estrogen and testosterone content for females. Pregnane may only be useful in differentiating females during the luteal phase in fall and pregnant females from calves in winter. Beyond this, testosterone and estrogen may be the only hormones used to differentiate calf from adult males, and perhaps only when sampling in the fall. Lastly, our sample size for female calves in winter was smaller than anticipated and there were only a couple adult non-pregnant females for comparison.

While our results demonstrate the potential of pellet morphology and reproductive hormones in defining an age-class for the wild populations of caribou studied here, this

does not necessarily translate to other herds. These particular populations have been closely monitored over the years, allowing for a near-complete capture history from which to select putative calves. This provided our study with the advantage of identifying clear adults and putative calves with confidence beforehand, a circumstance that is not likely when initiating NFS in a new population. It may take multiple capture events before identifying new individuals entering the study population and determining whether it is open or closed. This could have implications for identifying putative calves, as new individuals in an open population may be calves or migrants from elsewhere. In addition, these differences in pellet size and fecal hormone levels may not be clear across all populations. Diet can vary across a species range depending on the habitat of the local population, and resource availability, and this in turn can play a large role in pellet size and hormone content (Thomas and Gray 2002, Miller 2003, Ninnes et al. 2010, Morden et al. 2011a, Morden et al. 2011b). Furthermore, these methods may not work across all species, as demonstrated in the inability for pellet size to differentiate between juvenile and adult age-classes of European wild rabbits (*Oryctolagus cuniculus*) (Delibes-Mateos et al. 2009, Rouco et al. 2009). Nevertheless, our fecal pellet morphology expectation was supported and fecal hormone content expectation was moderately supported, illustrating that while these parameters may vary by sex, population, or season, they still reveal the potential of this non-invasive method. Thus, our study demonstrates the feasibility of implementing fecal pellet morphometrics and fecal hormones analysis, via NFS, as a viable method to identify age-class in wild populations of caribou.

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APPENDIX A

Figure 8. Number of unique genotypes observed each sample year for South Jasper's three caribou herds (A) and the North Interlake herd (B).

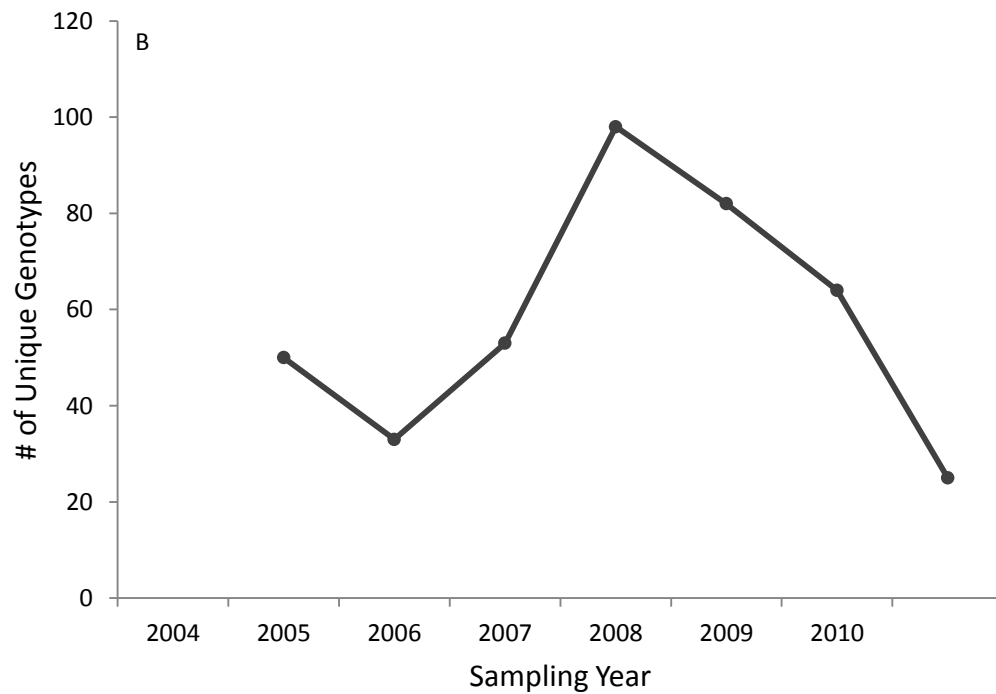
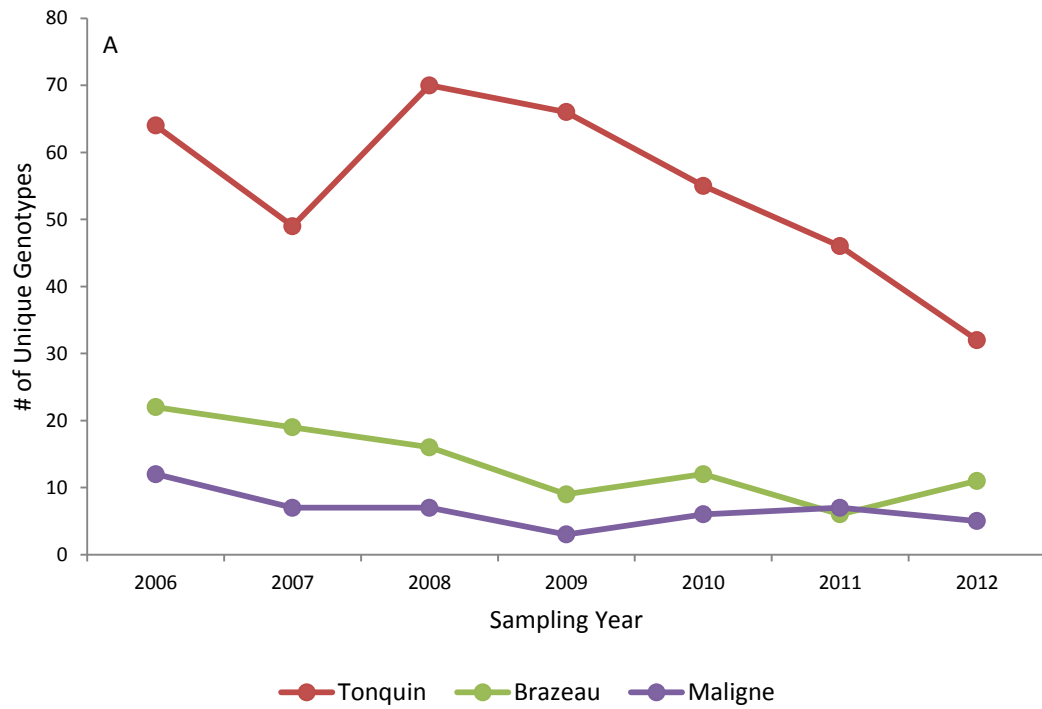
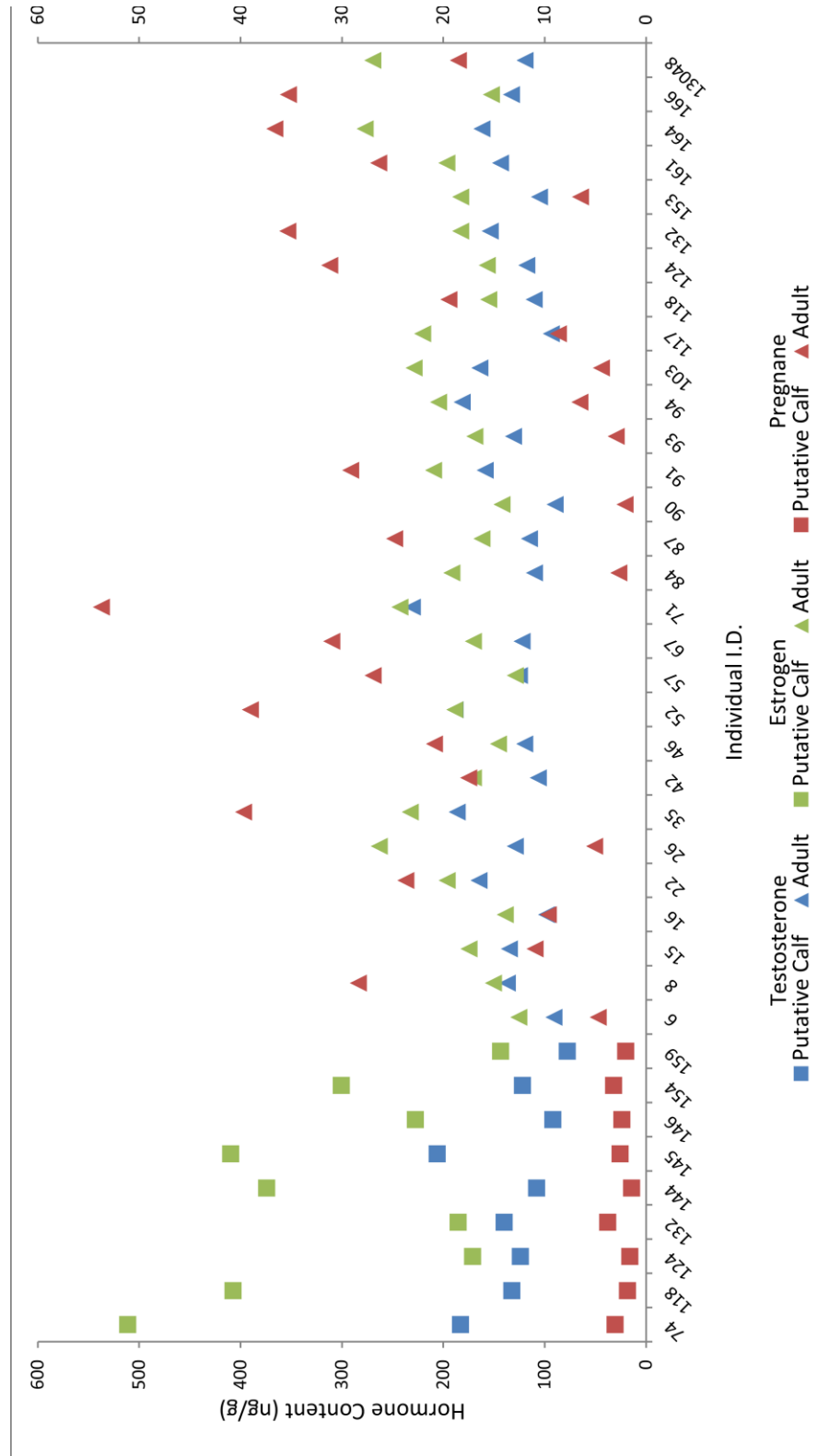


Figure 9. Hormone content (ng/g) of individual South Jasper females belonging to both age-classes.



CHAPTER TWO: Exploring fine-scale social structure of woodland caribou (*Rangifer tarandus caribou*)

ABSTRACT

The social structure of woodland caribou was investigated in the Tonquin, Brazeau and Maligne herds of Jasper National Park's central mountain caribou through pedigree analysis. Non-invasive genetic sampling of caribou fecal pellets occurred over six consecutive years, from 2006 to 2012. A total of 1,732 samples were amplified at 10 microsatellite loci resulting in 200 unique genotypes (105 females, 95 males) and used in our analysis. Parentage assignment was determined via the maximum likelihood method implemented in COLONY 2.0 and a pedigree was constructed. Results showed that fewer males produced more offspring (20% of males reproduced, with a maximum of 13 offspring by a single male), while more females produced fewer offspring (39% of females reproduced, with a median of 3), thus representing a polygynous mating system. In addition, parent-offspring and sibling relationships were found between individuals belonging to different herds indicating that some movement of caribou has occurred between herds. This study demonstrates the potential of non-invasive genetic sampling as a method to assess the reproductive fitness of animals in a population, the social structure of caribou and its innovative potential for monitoring caribou movements within and between populations.

INTRODUCTION

Understanding and monitoring the effects of landscape changes on species ecology and population parameters has been greatly studied and quantified (Bender et al. 1998, Foster 1999, Foster and Endler 1999, Stutchbury 2007, Herrera and Doblas-

Miranda 2013). Yet far less research effort has been invested in understanding the influence of population declines on the life-history and behaviour of species (Foster 1999, Stutchbury 2007). In particular, examining the impact of a small population size or low population density on a species' social structure and mating system has been only minimally studied and is even less understood (Banks et al. 2005, Banks et al. 2007, Stutchbury 2007). Sociality is a quintessential characteristic of many mammalian species including elephants (*Loxodonta spp.* Douglas-Hamilton 1972, Wittemyer et al. 2005, Archie et al. 2008), felids (Packer 1986), canids (Mech 1970, Davidar 1975, Frame et al. 1978, Macdonald 1979), numerous dolphins and whales (Tyack 1986, Mann et al. 2000), and various ungulates (Banfield 1975, Emlen and Oring 1977, Barrette and Vandal 1986, Aycrigg and Porter 1997, Cross et al. 2005, Sundaresan et al. 2007). Understanding how sociality may change and adapt to decreased and fragmented populations is essential to knowing how to mitigate for potentially harmful effects.

The most commonly occurring mammalian social structure is that which exhibits stable social group composition, male-biased dispersal, moderate permanent breeding associations between male and female groups, and polygynous mating; whereby all females in a group mate with the same few males (Hirth 1977, Archie et al. 2008, Bonnot et al. 2010). The characteristics outlined above have been referred to as the 'breeding group paradigm' by Archie et al. (2008). Populations that follow this paradigm more likely have high co-ancestry among group members, significantly high genetic differentiation between groups, and especially heterozygous offspring relative to the high co-ancestry within groups (van Staaden 1995, Sugg et al. 1996, Dobson et al. 1998, Storz 1999, Archie et al. 2008). The breeding group paradigm has been documented in a

variety of mammalian species and include, but is not necessarily limited to, primates (Turner 1981, Melnick et al. 1984, De Jong et al. 1994, Pope 1998), rodents (Schwartz and Armitage 1981, Chesser 1983, van Staaden et al. 1996, Dobson et al. 1998, 2004), few carnivore species (Spong et al. 2002), few bat species (Wilkinson 1985), rabbits (Surridge et al. 1999), and rock-wallabies (Hazlitt et al. 2004, Hazlitt et al. 2006). In these circumstances, fine-scale genetic structure is more likely to occur as a result of non-random patterns of mating and dispersal (Archie et al. 2008). However not all social mammals follow all of these characteristics strictly (e.g. elephants, Douglas-Hamilton 1972, Wittemyer et al. 2005; lions, *Panthera leo*, Packer 1986; and *Canidae spp.* Fox 1975), and different environments may constitute alternative social structure between and within populations (Hirth 1977, Molvar and Bowyer 1994, Kie 1999). For many ungulate species, habitat characteristics play an influential role in shaping the size and dynamic of groups or herds, and subsequently have implications for ungulate social structure (Hirth 1977, Molvar and Bowyer 1994, Kie 1999).

Investigating social structure can be particularly difficult when trying to observe rare or elusive species, or species that are especially sensitive to anthropogenic disturbances. Under these circumstances, use of non-invasive methods may be a priority. For wild populations where social relationships cannot be constructed by direct observation, familial relationships can be identified by use of molecular markers (Emery et al. 2001, Duchesne et al. 2005, Jones and Wang 2009). While parentage and sibship analysis has traditionally been used for management of captive populations, it has become increasingly popular in the study of wild populations (Haig and Ballou 2002). Studying familial relationships from genetic data has the potential for being especially

valuable in wildlife research as it can help determine evolutionary relationships (Pamilo and Crozier 1982), presence of inbreeding (Richard et al. 2009), heterozygosity, gene flow between populations and individuals (Zeyl et al. 2009), founder contribution, kinship, and overall fitness contribution and structure of a population (Haig and Ballou 2002, Jones and Wang 2010), all without having to directly observe the animal. Comparison of familial relationships across populations may also identify the effect of habitat and landscape characteristics (Pemberton 2008). Studying the sociogenetic structure (i.e. the social structure derived from genetic data) and dynamic of species is not entirely new and has been used in the past to investigate the social structure of wild populations. The promiscuous mating behaviour observed in cheetahs (*Acinonyx jubatus*) was once thought to be primarily exhibited in males (Gottelli et al. 2007). However genetic relatedness analysis of cheetahs revealed that females were also highly promiscuous, having litters of offspring from more than one male. This was concluded to be an inbreeding avoidance strategy and provided critical insight on the mating system of cheetahs (Gottelli et al. 2007). The same insight was found in the Eurasian badger (*Meles meles*), where a marker-based pedigree revealed that Eurasian badgers displayed high levels of extra-group matings as a strategy to avoid inbreeding (Carpenter et al. 2005). Furthermore, Charpentier et al. (2006) utilized genetic based familial relationships to examine the influence of inbreeding on the life history traits of mandrills (*Mandrillus sphinx*), and found that female inbreeding was associated with smaller body size and first conception at a young age. Inferring familial relationships from genetic data has also been used to show social structure (Amos et al. 1993), mating patterns (Clapham and Palsbøll 1997, Jones and Avise 1997), kinship (Fontaine and Dodson 1999) and to

determine reproductive success (Rico et al. 1992, Jones et al. 1998). These studies emphasize the potential of sociogenetics, particularly in cases where population decline is suspected to have an effect on a local population's social structure.

Woodland caribou are found all across Canada, in different habitats and landscapes, and therefore may exhibit different behaviours across their wide geographic distribution (Foster 1999). However, little is conclusively known about their overall social structure and mating system, and how this may be subsequently influenced by population decline. The consequent loss of individuals and their genetic diversity in a population, may have implications for the social organization of local herds as has been demonstrated across a diversity of species. (Bender et al. 1998, Banks et al. 2007, Beckmann 2011). Though there is limited information available regarding the social structure of woodland caribou (Banfield 1975, Barrette and Vandal 1986), there is some information available on the population structure and reproductive behaviours of domesticated and semi-domesticated reindeer (*R. t. tarandus*) in northern Europe (Røed et al. 2002, Holand et al. 2004a, Holand et al. 2004b, Holand et al. 2005, Røed et al. 2005, Holand et al. 2007, Tennenhouse et al. 2010, Tennenhouse et al. 2012). Since the information on reindeer pertains to smaller, more sedentary populations of domesticated animals, results from studies of population structure may not be directly applicable to North American caribou. Woodland caribou in particular are found on far different landscapes than European reindeer, are in different habitats, encounter different environmental pressures, and may arguably occupy different niches altogether. Additionally, differences in species' social interaction have been observed in populations found in environments that differ by geography and demography (Banks et al. 2007).

However, the consequences of small population numbers on reindeer social structure are worth consideration. Holand et al. (2007) showed that inbreeding avoidance did not occur in smaller herds of captive reindeer, which may negatively affect the genetic structure of some herds. With this lack of inbreeding avoidance found in captive herds of reindeer (Holand et al. 2007), along with the relatively low level of recruitment in *Rangifer* (Banfield 1975), this may have serious implications for isolated populations of woodland caribou. Consequently, our study investigated the mating system, reproductive output, and individual fitness via sociogenetics of a woodland caribou population in decline, the South Jasper caribou herds (Brazeau, Maligne, and Tonquin) located in Jasper National Park. Since the most commonly found mating system in social mammalian species is polygyny (Banfield 1975, Hirth 1977, Holand et al. 2005, Holand et al. 2007, Archie et al. 2008), we expected to find the same in woodland caribou and that only few select male woodland caribou would dominate reproductive output; and since female reindeer have been shown to exhibit social rank (Banfield 1975, Holand et al. 2004a, Holand et al. 2004b), and social rank has been shown to affect fecundity of captive female reindeer (Holand et al. 2004b), we expected that a few select female woodland caribou would have comparatively higher fitness and produce more offspring relative to other females. Before sociogenetic analysis could begin, we identified unique individuals and determined their sex, by way of non-invasive genetic sampling of caribou fecal pellets and subsequently built a capture history.

METHODS

Study Area

Please refer to ‘Study Area’ Chapter 1, for details on the study area of South Jasper caribou.

Fecal Pellet Collection and Lab Analysis

Please refer to ‘Fecal Pellet Collection and Lab Analysis’ Chapter 1, for details on the fecal pellet collection and lab analysis of South Jasper caribou samples.

Building a Capture History

Please refer to ‘Building a Capture History’ Chapter 1, for details on the capture history built for South Jasper caribou.

Pedigree Analysis

Prior to doing pedigree analysis, we selected only near complete unique genotypes using 10 loci. We used all genotypes with ≤ 1 loci missing, only 2 genotypes with ≤ 2 loci missing, and 3 genotypes with ≤ 3 loci missing. We excluded unique genotypes with > 3 loci missing from the analysis to ensure more accurate parentage assignments. We excluded putative calves (see Chapter One) from subsequent analyses of reproductive output (i.e. percent successfully reproducing individuals). For each locus, we calculated allelic drop-out and error rates (i.e. false alleles) across all of South Jasper (considering all three herds) and calculated allelic diversity, expected (H_E) and observed (H_O) heterozygosities for South Jasper and for each herd individually (Tonquin, Brazeau, and Maligne). In addition, we tested for departures from Hardy-Weinberg equilibrium (HWE) and probability of linkage disequilibria for each herd using the Markov chain method (1000000 iterations) implemented in GENEPOP 4.2 (Rousset 2008).

We used the COLONY 2.0 (Jones and Wang 2009) software for parentage assignment and to infer sibling relationships. COLONY uses a maximum likelihood method to simultaneously determine parental and sibling relationships among individuals from their multi-locus genotypes and differs from other programs that consider

relationships from pairs of individuals (i.e. dyads). This software has been tested and utilized extensively for pedigree analysis (Wang 2004, Wang 2009, Wang and Santure 2009, Jones and Wang 2010, Walling et al. 2010, Karaket and Poompuang 2012, Wang 2012, 2013b, Wang 2013a) and therefore was chosen as the primary software for our analysis as we were most confident with its capability compared to other available methods (e.g. CERVUS, Kalinowski et al 2007, Karaket and Poompuang 2012; and MasterBayes, Hadfield et al. 2006, Walling et al. 2010). COLONY 2.0 can tolerate loci that are not in HWE or are in linkage disequilibria and also allows for the input of additional information pertinent to your study population, such as allelic dropout and error rates, which further assists in parentage assignment (Wang 2004). In our analysis, we allowed for polygamous mating in both sexes, selected the full-likelihood model at medium precision and did not set a sibship prior. We used only those parent-offspring and sibling relationships that were found to be of a probability ≥ 0.9 for subsequent analyses. Lastly, we built a pedigree-derived network of the parent-offspring relationships surrounding the male and female genotypes that dominated the reproductive output using CYTOSCAPE 3.0.1. (Shannon et al. 2003). Further explanation regarding the validity and reliability of our data is provided in the corresponding paragraph of our General Conclusions.

RESULTS

The microsatellite loci exhibited moderate allelic diversity in South Jasper (considering all three herds), containing 6 to 11 alleles per locus (mean = 8.8) and had expected and observed heterozygosities ranging from 0.57 to 0.83 (mean = 0.75) and 0.54 to 0.78 (mean = 0.71), respectively (Table 8). Allelic dropout was almost negligible for

all loci (≤ 0.01) and error rates/false alleles were at extremely low levels as well (≤ 0.05).

Of the three herds, the Tonquin showed the most allelic diversity, containing 5 to 8 alleles per locus (mean = 6.8), while the Brazeau showed the least at 3 to 5 alleles per locus (mean = 5.1) (Table 9, Table 10, Table 11). However, the Maligne showed the most expected and observed heterozygosities ranging from 0.65 to 0.83 (mean = 0.74) and 0.61 to 0.94 (mean = 0.76), respectively (Table 11). Two out of 10 loci deviated from HWE ($p < 0.05$) in the Tonquin and the global Hardy-Weinberg estimate also deviated from HWE

Table 8. Summary of South Jasper genetic diversity statistics for each locus (200 unique genotypes). *A*, number of alleles; *H_E*, expected heterozygosity; *H_O*, observed heterozygosity; ADO, allelic dropout; FA, false alleles.

Locus	<i>A</i>	<i>H_E</i>	<i>H_O</i>	ADO	Error Rate/FA
BM848	6	0.75	0.72	0.00	0.050
BM888	10	0.76	0.71	0.00	0.002
BMS1788	9	0.79	0.75	0.00	0.001
MAP2C	11	0.80	0.73	0.01	0.003
RT24	7	0.71	0.68	0.00	0.001
RT30	9	0.83	0.78	0.00	0.001
RT5	10	0.79	0.72	0.00	0.003
RT6	7	0.57	0.54	0.00	0.003
RT7	9	0.68	0.72	0.01	0.041
RT9	10	0.79	0.75	0.00	0.001
Mean	8.8	0.75	0.71	0.00	0.001

($p < 0.05$), while only one locus deviated from HWE ($p < 0.05$) in Brazeau, and no loci deviated from HWE in Maligne; neither of the Brazeau and Maligne global Hardy-Weinberg estimates deviated from HWE (Table 9, Table 10, Table 11). Lastly, linkage disequilibrium between loci was observed in 47% of pairwise comparisons between Tonquin loci, 18% of pairwise comparisons between Brazeau loci, and 27% of pairwise comparisons between Maligne loci (data not shown).

Table 9. Summary of Tonquin genetic diversity statistics for each locus (151 unique genotypes). A , number of alleles; H_E , expected heterozygosity; H_O , observed heterozygosity; HWE, Hardy-Weinberg test for heterozygote deficiency [GENEPOP 4.2]. Significant deviation from HWE are italicized.

Locus	A	H_E	H_O	HWE
BM848	5	0.74	0.71	0.6631
BM888	7	0.74	0.74	0.0683
BMS1788	6	0.77	0.73	0.1882
MAP2C	8	0.78	0.71	<i>0.0366</i>
RT24	7	0.68	0.67	<i>0.0014</i>
RT30	7	0.82	0.78	0.2005
RT5	8	0.77	0.74	0.4166
RT6	5	0.56	0.54	0.4972
RT7	7	0.68	0.70	0.7224
RT9	8	0.82	0.83	0.3456
Mean	6.8	0.74	0.71	<i>0.0085*</i>

Table 10. Summary of Brazeau genetic diversity statistics for each locus (37 unique genotypes). A , number of alleles; H_E , expected heterozygosity; H_O , observed heterozygosity; HWE, Hardy-Weinberg test for heterozygote deficiency [GENEPOP 4.2]. Significant deviation from HWE are italicized.

Locus	A	H_E	H_O	HWE
BM848	5	0.77	0.78	0.7604
BM888	4	0.68	0.54	<i>0.0199</i>
BMS1788	7	0.74	0.84	0.9336
MAP2C	7	0.81	0.76	0.2684
RT24	5	0.70	0.68	0.3431
RT30	5	0.73	0.68	0.2537
RT5	5	0.57	0.62	0.3115
RT6	3	0.47	0.51	0.8767
RT7	5	0.63	0.76	0.9723
RT9	5	0.41	0.46	0.7819
Mean	5.1	0.65	0.66	0.6932*

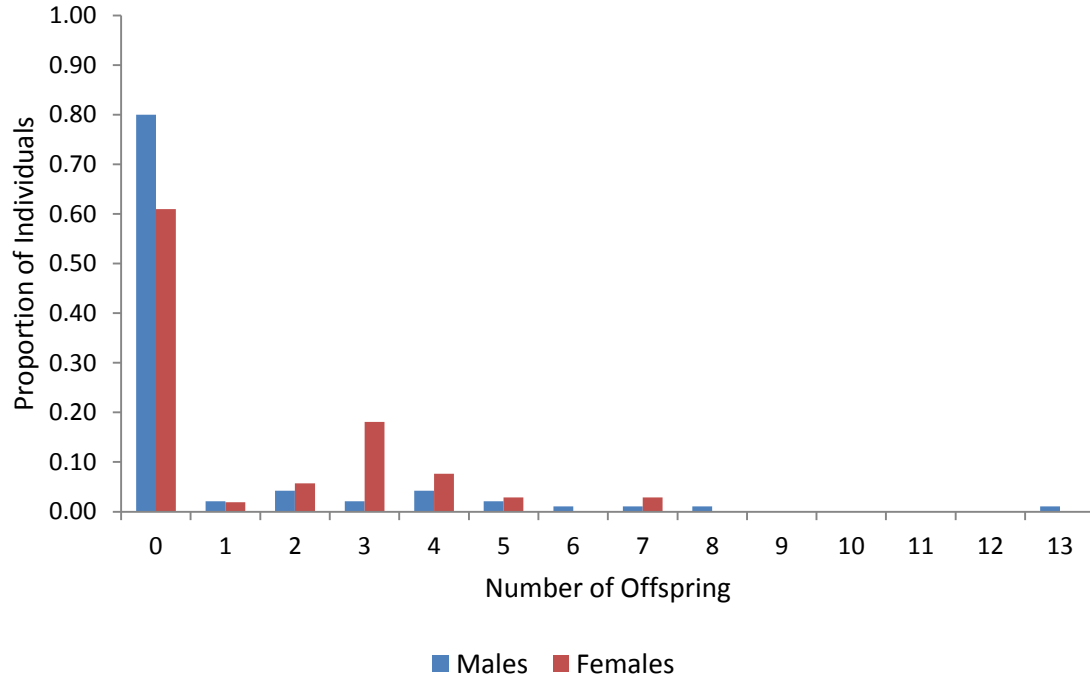
We used a total of 105 unique female genotypes and 95 unique male genotypes in our pedigree analysis. Of these female genotypes, 41 produced offspring, totalling 139 mother-offspring relationships within all of South Jasper. This equated to 39% of females successfully producing offspring that survived to at least their first fall, when our sampling occurs. Of the total male genotypes, 19 produced offspring, equalling 86 father-offspring relationships within all of South Jasper, and resulting in 20% of males

successfully reproducing offspring that survived to at least their first fall. There were therefore a higher proportion of non-reproducing males than females, and females most often produced 3 offspring per individual (Figure 10).

Table 11. Summary of Maligne genetic diversity statistics for each locus (18 unique genotypes). A , number of alleles; H_E , expected heterozygosity; H_O , observed heterozygosity; HWE, Hardy-Weinberg test for heterozygote deficiency [GENEPOP 4.2]. Significant deviation from HWE are italicized.

Locus	A	H_E	H_O	HWE
BM848	5	0.70	0.67	0.7325
BM888	7	0.81	0.78	0.1152
BMS1788	6	0.73	0.72	0.2253
MAP2C	8	0.82	0.83	0.6774
RT24	5	0.65	0.78	0.9383
RT30	6	0.79	0.94	0.9583
RT5	7	0.83	0.83	0.1037
RT6	5	0.65	0.61	0.0461
RT7	7	0.71	0.78	0.7792
RT9	9	0.75	0.67	0.4952
Mean	6.5	0.74	0.76	0.5296*

Figure 10. Proportion of males and females producing offspring. $N = 95$ males and 105 females.



The number of offspring produced per individual female and male is represented in Figure 11 and Figure 12 respectively. Of the females that produced offspring, three in particular (17, 77, and 13048) produced the maximum number of offspring (7 total each) among females and clearly dominated the female reproductive output (hereafter referred to as ‘super-females’) all of which belonged to the Tonquin herd. The maximum number of offspring produced by a single male was 13, followed by 10, 8, and 7 offspring by male’s 18, 14242, 6155, and 75 respectively (hereafter referred to as ‘super-males’). Given that comparatively few males produced offspring in South Jasper, these four males clearly dominated the reproductive output. This is especially true for super-male 18 who produced the most offspring in all of South Jasper (13 offspring), and super-male 75 (7 offspring) who was the only male belonging to the Maligne herd to produce offspring.

Figure 11. Number of offspring produced per individual female for each herd. N = 105.

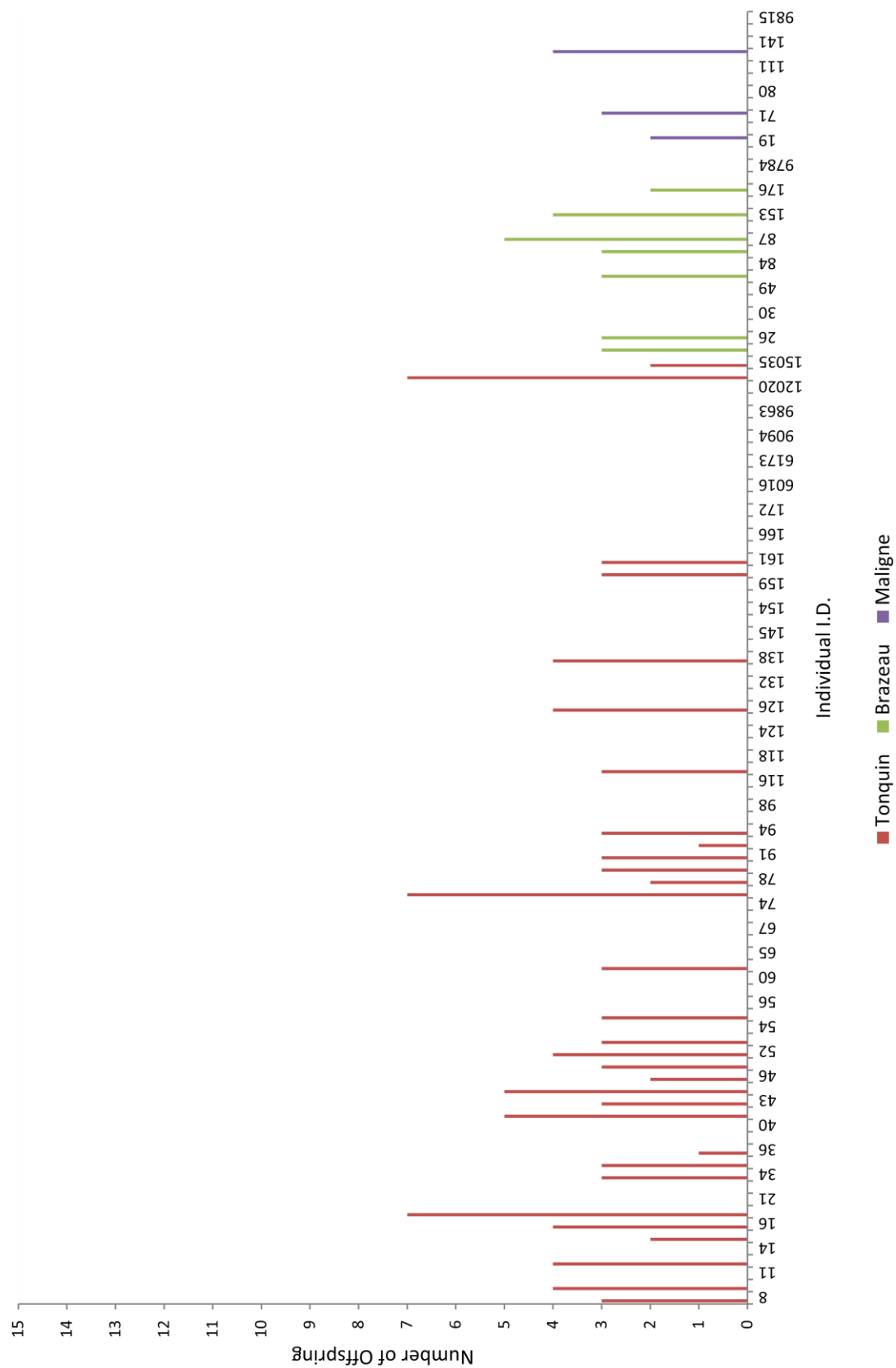
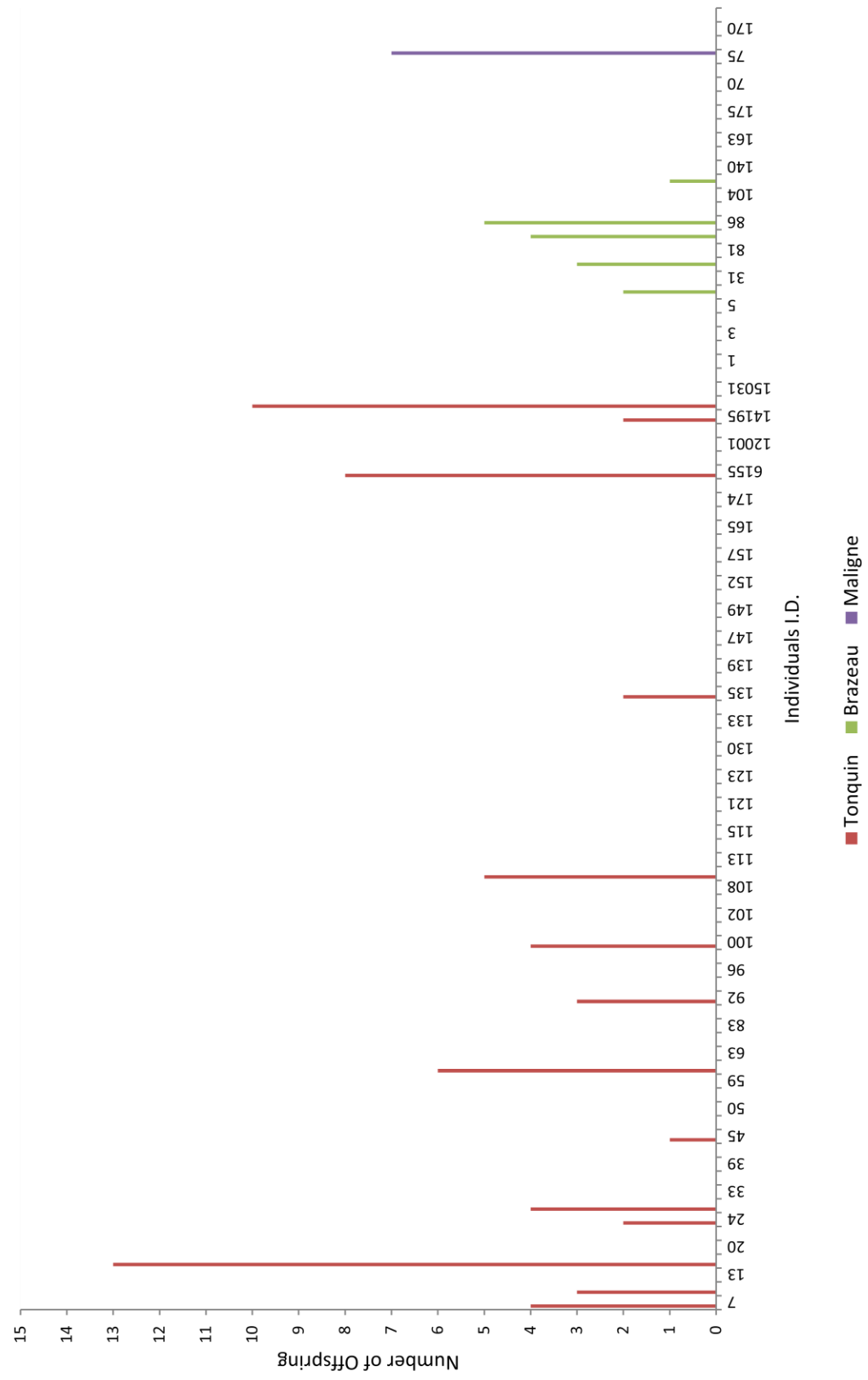
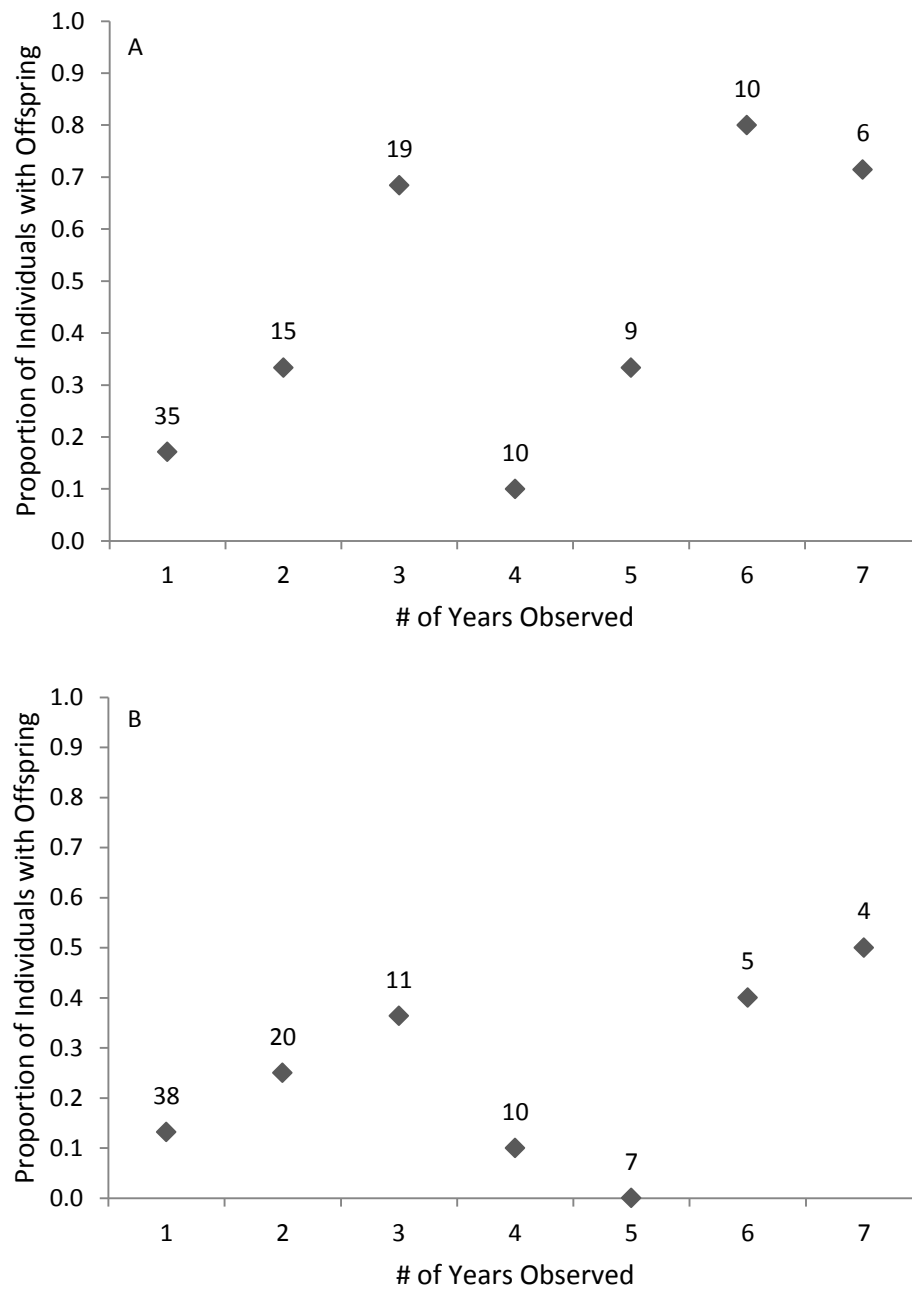


Figure 12. Number of offspring produced per individual male for each herd. N = 95.



The age, and thus the number of years these super-females and super-males have been reproducing, may have an influence on how many offspring they produce. Figure 13 represents the proportion of females and males that have produced offspring based on the number of years they have been observed across our capture history (from 2006 to 2012). It is important to emphasise that these years do not represent age explicitly, but represent the minimum number of years these animals are known to have been alive based on when they were captured. For example, an animal that is '4 Years Observed' is at least 4 years of age, but possibly older, as they have been captured in 4 consecutive sampling events, or in ≥ 2 capture events that have spanned 4 years (e.g. female 156 was captured in 2006 and in 2009 and is therefore at least 4 years of age). For both females and males, an increasing proportion of individuals produced offspring from 1 to 3 years observed, whereas in 3 years observed there were more females that produced offspring than females that did not (70% of females had ≥ 1 offspring). A drop in the proportion of individuals producing offspring appears in 4 and 5 years observed, particularly for 5 years observed where 0% of males had offspring. Six and 7 years observed show the highest proportion of individuals producing offspring for both females and males. However, few individuals were found in 6 and 7 years observed years while more individuals were found in earlier observed years. Super-females 17, 77, and 13048 have been observed over 2, 5, and 1 years respectively, while super-males 18, 75, 6155, and 14242 have been observed over 3, 7, 1, and 1 years respectively. For a more detailed breakdown of the number of individuals producing offspring relative to the number of years they have been known alive, see Figure 17 and Figure 18 in Appendix B.

Figure 13. The proportion of females (A) and males (B) with ≥ 1 offspring relative to the number of years they have been observed across our capture history (2006-2012). This includes individuals that have been captured in consecutive sampling years and individuals that have demonstrated their presence in the population for more than just the years that they have been captured. Data labels indicate number of individuals.



Across South Jasper, we found 17 full-sibling relationships and 790 half-sibling relationships (Figure 14 and Figure 15). One of these full-sibling relationships was mothered by super-female 17, two were mothered by super-female 77, and three full-sibling relationships are a result of matings between super-male 75 and female 71. Overall, 20 full-sibling and 774 half-sibling relationships were found among females, while 8 full-sibling and 714 half-sibling relationships were found among males. When considering full- and half-sibling relationships together, females had a maximum of 17 and median of 6 sibling relationships while males had a maximum of 16 and median of 7 sibling relationships.

Parent-offspring relationships, sibling relationships and mating relationships were primarily maintained within each of the three South Jasper herds; however there were some exceptions (Table 12). One father-offspring relationship was found between Tonquin and Maligne and another between Brazeau and Maligne; while three mother-offspring relationships were shared between Tonquin and Brazeau, one between Tonquin and Maligne, and two between Brazeau and Maligne (Table 12. A, B). Numerous half-sibling relationships were shared between all three South Jasper herds, while only one Tonquin-Maligne and one Brazeau-Maligne full-sibling relationships were found (Table 12. C, D). Finally, one mating relationship (i.e. parents of offspring) was found between each herd (Table 12. E).

Figure 14. Number of sibling relationships per individual female for each herd. N = 105.

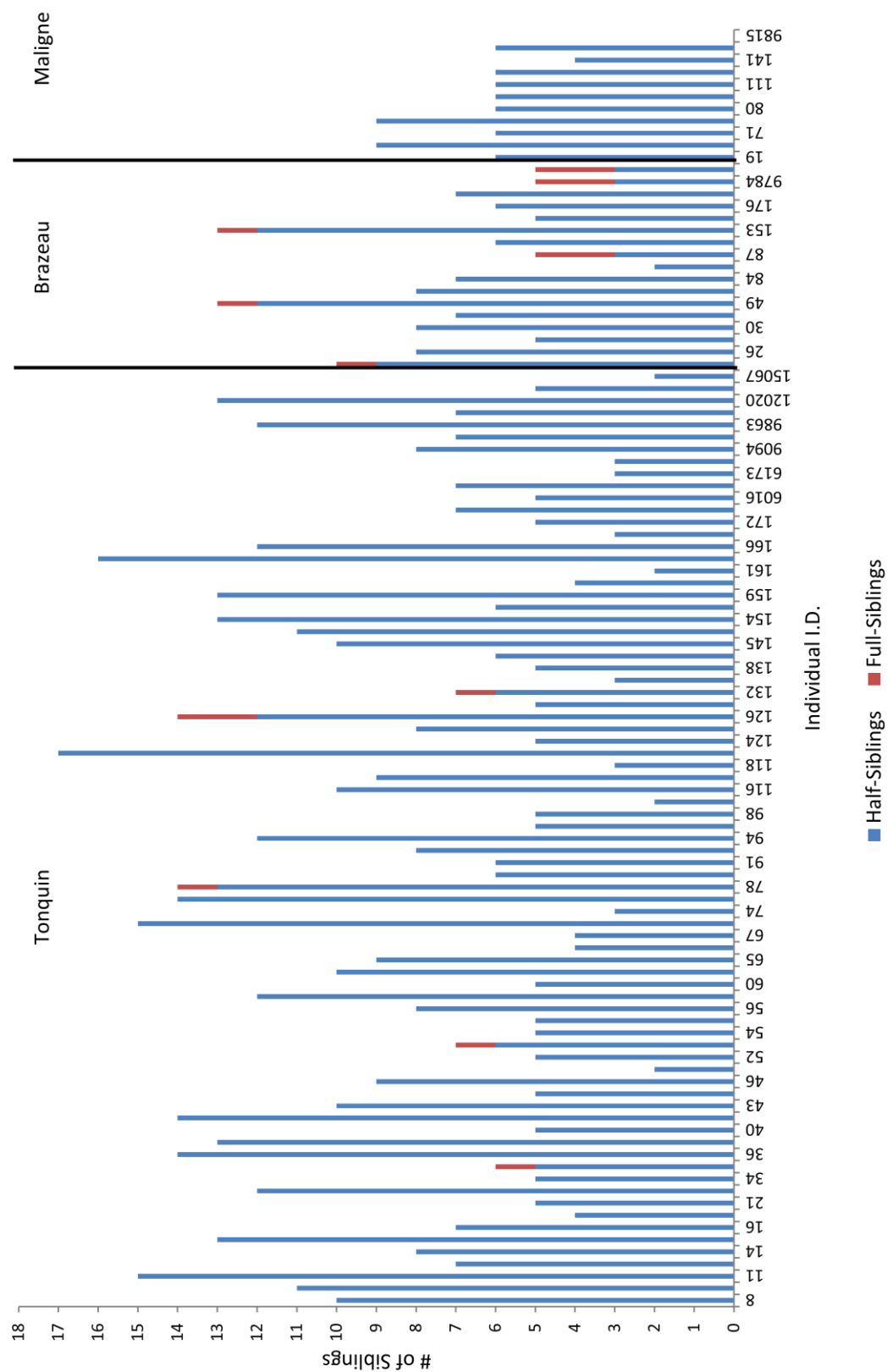


Figure 15. Number of sibling relationships per individual male for each herd. N = 95.

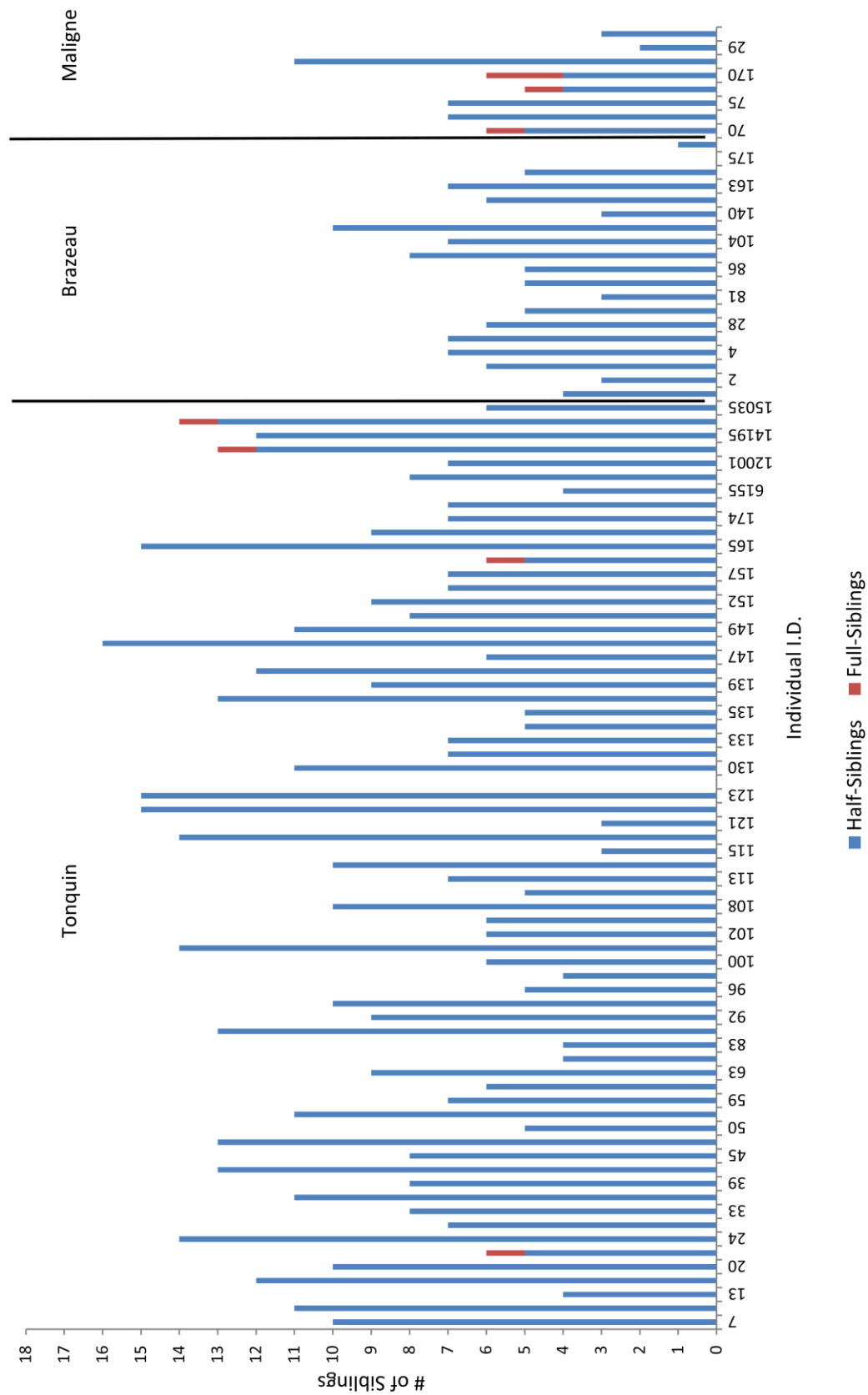


Table 12. Distribution of father-offspring relationships by herd (A), mother-offspring relationships by herd (B), half-sibling relationships by herd (C), full-sibling relationships by herd (D), and the number of mating relationships by herd (E). These values only depict mating relationships between known individuals in our capture history and not unknown parents identified by COLONY 2.0.

A)	Tonquin	Brazeau	Maligne
Tonquin	66	0	1
Brazeau		12	1
Maligne			6

B)	Tonquin	Brazeau	Maligne
Tonquin	105	3	1
Brazeau		18	2
Maligne			7

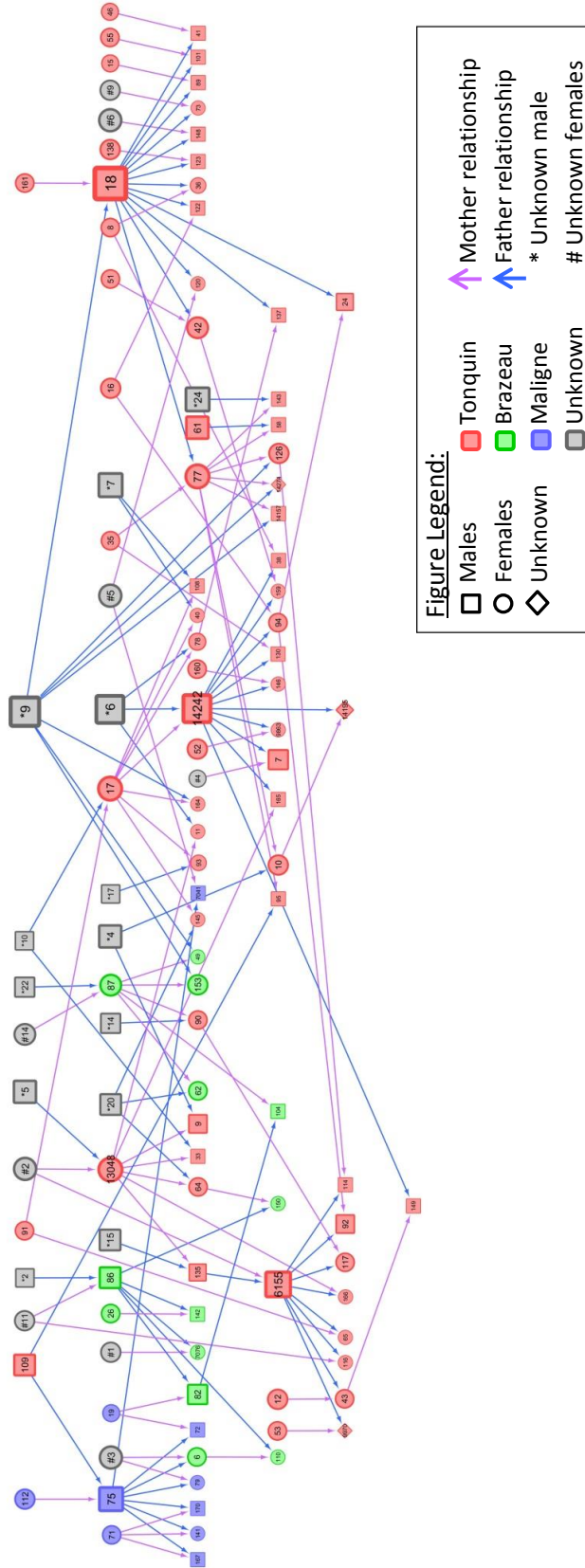
C)	Tonquin	Brazeau	Maligne
Tonquin	570	67	40
Brazeau		66	23
Maligne			24

D)	Tonquin	Brazeau	Maligne
Tonquin	6	0	1
Brazeau		4	1
Maligne			5

E)	Tonquin	Brazeau	Maligne
Tonquin	52	1	1
Brazeau		8	1
Maligne			4

A pedigree derived network representing the parent-offspring relationships surrounding the three super-females (17, 77, and 13048) and four super-males (18, 75, 6155, and 14242) is represented in Figure 16. From this pedigree, the many relationships shared between these super-females and super-males appear complex but are discernable.

Figure 16. Pedigree derived network of parent-offspring relationships surrounding the 3 super-females (individuals 17, 77, and 13048) and 4 super-males (individuals 18, 75, 6155, and 14242). Node size and border width correspond to number of offspring that individual has produced (min = 0 offspring, max = 13 offspring).



Super-female 17 is the parent of super-male 14242, super-male 18 is the parent of super-female 77, and super-female 13048 is the grandparent of super-male 6155. Mother of super-female 17, female 91 (with 3 offspring) mated with super-male 6155 and produced female 65 (with no offspring) and thus super-female 17 and female 65 are half-siblings. Super-male 18 produced female 42 (5 offspring) who subsequently mated with super-male 14242 to produce female 159 (no offspring). In almost all cases, parents of super-females and super-males are also successful reproducers, as denoted by the large nodes of the parents to these super-females and -males. In addition, all super-females and -males have produced ≥ 1 offspring that are also successful reproducers. Super-female 17 produced female 160 (3 offspring) in addition to super-male 14242; super-female 77 produced female 10 (4 offspring) and female 126 (4 offspring); super-female 13048 produced male 135 (2 offspring), female 64 (3 offspring), and male 9 (3 offspring); super-male 18 produced female 42 mentioned earlier (5 offspring), in addition to super-female 77; super-male 75 produced female 6 (3 offspring); super-male 6155 produced female 43 (3 offspring), female 117 (3 offspring), and male 92 (3 offspring); and super-male 14242 produced male 7 (4 offspring) and female 94 (3 offspring).

Furthermore, the pedigree reveals some of the relationships shared between herds. Male 109 (5 offspring) belongs to the Tonquin herd but is the father of super-male 75 who belongs to the Maligne herd. Super-male 75 also has an offspring belonging to Brazeau, female 6. Additionally, female 64 (offspring of super-female 13048) produced an offspring belonging to the Brazeau herd, female 150 (no offspring); and female 87 (5 offspring) produced an offspring belonging to Tonquin, female 90 (3 offspring). Additionally, male 109 mated with super-female 77 and reproduced male 95 (no

offspring) and thus super-male 75 and male 95 are half-siblings belonging to different herds. Maligne male 72 (offspring of super-male 75) is the half-sibling of male 82 who belongs to the Brazeau herd. Though this pedigree depicts the relationships surrounding the most prominent reproducers in South Jasper, it is only a subset of the myriad of familial relationships that are present within and between the South Jasper caribou herds.

DISCUSSION

This study revealed the mating system and reproductive output of male and female South Jasper woodland caribou via sociogenetics. Though only ten loci were used in our study, the diversity estimates of the ten loci were more than sufficient to identify numerous familial relationships and an overall understanding of the relatedness of these caribou was achieved. These caribou clearly exhibited a polygynous mating system as demonstrated by the unequal distribution of reproductive output by males and relatively equal distribution of reproductive output by females. Fewer males produced many offspring, which included many males that did not produce any offspring; 3 offspring per reproducing female was most common, with more females producing fewer offspring overall. Polygyny is arguably the most common mating system among ungulate species (Banfield 1975, Murray 1982, Mloszewski 1983) and is particularly relevant in larger ungulate species where male-to-male competition plays a larger role in monopolizing females (Emlen and Oring 1977, Geist and Bayer 1988) and as a driver of antler and body size (Plard et al. 2011). Polygyny has been expressed in semi-domesticated populations of reindeer (Holand et al. 2004b, Holand et al. 2005, Holand et al. 2007, Tennenhouse et al. 2010, Tennenhouse et al. 2012) and its presence has been mentioned

in North American caribou (Bergerud 1974). Accordingly, it is not unexpected to find that the woodland caribou of South Jasper also exhibit a polygynous mating system.

The implication of age showed that older individuals were more likely to have produced offspring and thus the longer an individual was present, the greater the chance she/he had at producing calves that at least survived to the fall and therefore were more fit. However, the clear drop in fitness of both females and males at 4 and 5 years observed is puzzling. Furthermore, the influence of age on the reproductive output of male reindeer has been studied but with varying conclusions. L'Italien et al. (2012) found that male reindeer social rank played a more significant role in a male's access to mating opportunities than did age-structure alone, although male reindeer of high social rank were most often larger and older. Tennenhouse et al. (2012) found that reproductive effort was higher in older dominant male reindeer, who were also better at timing their reproductive energetic costs with female peak oestrous than younger dominant males. In contrast, Roed et al. (2005) found that some yearling males were more successful in reproducing than older males, which may explain why we found that some of our super-males were observed in years 1 or 3 and were thus potentially younger in age. But perhaps these super-males simply are not producing enough fecal pellets for frequent capture in our sampling, as polygynous mating is recognized as having negative consequences on male reindeer body condition (Barboza et al. 2004) and may have implications for eating habits and consequently the number of pellets males produce. The same might be true for the super-females as they are more preoccupied with defending their offspring than eating (Brown and Kotler 2004, Panzacchi et al. 2010). However, Weladji et al. (2006) did find that older female reindeer had more successful breeding

attempts and were better at rearing offspring. Nevertheless the exact age of these animals is not yet known, although these methods are being developed (Flasko, Chapter 2); at this time we can only speculate about the apparent impact of age on the reproductive output of the South Jasper caribou.

The considerable number of half- and full-sibling relationships found across South Jasper caribou indicates that this population is composed of highly related individuals, likely a consequence of the population's small size and isolation. In small populations, after only a few generations all individuals will be related to some extent (Frankham et al. 2002). The herds of South Jasper are extremely small, and the population in its entirety was last estimated in 2009 as 130 animals (Hettinga 2010), although there are more recent estimates to suggest that the population is now about 51 caribou (Bradley pers comm). When populations are this small, there is an elevated risk of inbreeding, genetic drift, and a subsequent loss of overall genetic diversity. The high level of sibship shared between individuals in South Jasper might reveal that breeding between like individuals (i.e. individuals who share alleles that are identical by descent and not identical by chance) is occurring to some extent. However, loss of heterozygosity is also an indication of inbreeding (Höglund 2009), and our population's heterozygosity was not especially low. In addition, inbreeding itself does not always equate to inbreeding depression, where the effects of inbreeding become harmful (Höglund 2009). Yet, the many relationships that were found across herds also demonstrates some movement of individuals between herds which might indicate dispersal or, more speculatively, inbreeding avoidance (Greenwood 1980, Linklater and Cameron 2009). This finding is arguably more surprising as these three herds are divided by major

highways, all of which are considered to be major barriers to animal movement and dispersal (Oberg et al. 2002, Whittington et al. 2005, Hervieux et al. 2013). Regardless, the South Jasper population has undergone significant decline over recent years (Whittington et al. 2005a, Hebblewhite et al. 2007) and the impact of inbreeding is a legitimate concern that deserves more attention. The small population size and high level of relatedness in South Jasper as shown by the considerably high presence of sibship cumulatively may allude to inbreeding as a potential factor in the population's structure or at least may indicate that it will be a factor in the immediate future.

The high reproductive output demonstrated by South Jasper's super-females and super-males shows that these individuals are comparatively more fit than their conspecifics and hence may have some heritable traits or learned behaviours that support their reproductive success. Social rank is known to occur in captive female reindeer (Banfield 1975, Holand et al. 2004a, Holand et al. 2004b) and dominance in caribou and reindeer bulls has also been demonstrated (Stokkan et al. 1980, Barrette and Vandal 1986, 1990, Hirotsu 1994, Bubenik et al. 1997, Røed et al. 2002, L'Italien et al. 2012, Tennenhouse et al. 2012). Holand (2004a) showed that female body mass, age and antler size as well as their combined effect determined the social rank of a female in a captive herd of reindeer. Additionally, Holand (2004b) showed that maternal rank was linked to fecundity. Higher-ranked females had higher fecundity and birthed sooner than lower-ranked females and pre-weaning growth rate increased with a female's social rank (Holand et al. 2004b). Furthermore, Barrette and Vandal (1986) were able to show a dominance hierarchy in a group of 20 woodland caribou from Parc des Grands-Jardins in Quebec. They found that a male caribou's social rank was highly related to its access to

resources, whereby males with larger antlers were more dominant and subordinates with smaller antlers were more submissive towards them (Barrette and Vandal 1986). Body mass, antler size and age seem to be the traits that are most crucial to the success of male reproduction in *Rangifer* (Bergerud 1974, Reimers 1983, Skogland 1989, L'Italien et al. 2012), while body condition, weight and age play an important role in the success of female *Rangifer* to reproduce (Clutton-Brock 1984, Tveraa et al. 2003, Weladji et al. 2006). The super-females and super-males identified in our study may be like the dominant individuals described in these studies. Or more generally, the super-males identified by our study may simply be the males that are better at tending to oestrous females or at defending harems (Bergerud 1974, Kojola and Nieminen 1986). These super-females and -males also tended to share relationships among themselves and with other successful reproducers, indicating that the traits or learned behaviours that make these individuals so successful are being passed onto their relatives. Genetic relatedness and kinship are important factors that influence social structure among various social mammalian species (Archie et al. 2008, Beisner et al. 2011) and it is evident that this is the same for South Jasper woodland caribou.

Here, we provide empirical evidence to support that woodland caribou indeed follow a polygynous mating system. Our results showed that fewer males dominated the reproductive output, with four super-males in particular disproportionately contributing offspring to the population. Consequently, our expectation that few select male woodland caribou would dominate the reproductive output in South Jasper was supported. Furthermore, seeing as few select females dominated the reproductive output of South Jasper caribou as demonstrated by the three super-females, our expectation that few

select female woodland caribou would have comparatively higher fitness relative to other females was also supported. This study demonstrates the potential of using sociogenetics via non-invasive genetic sampling to explore the fine-scale relationships that make up the social structure of populations.

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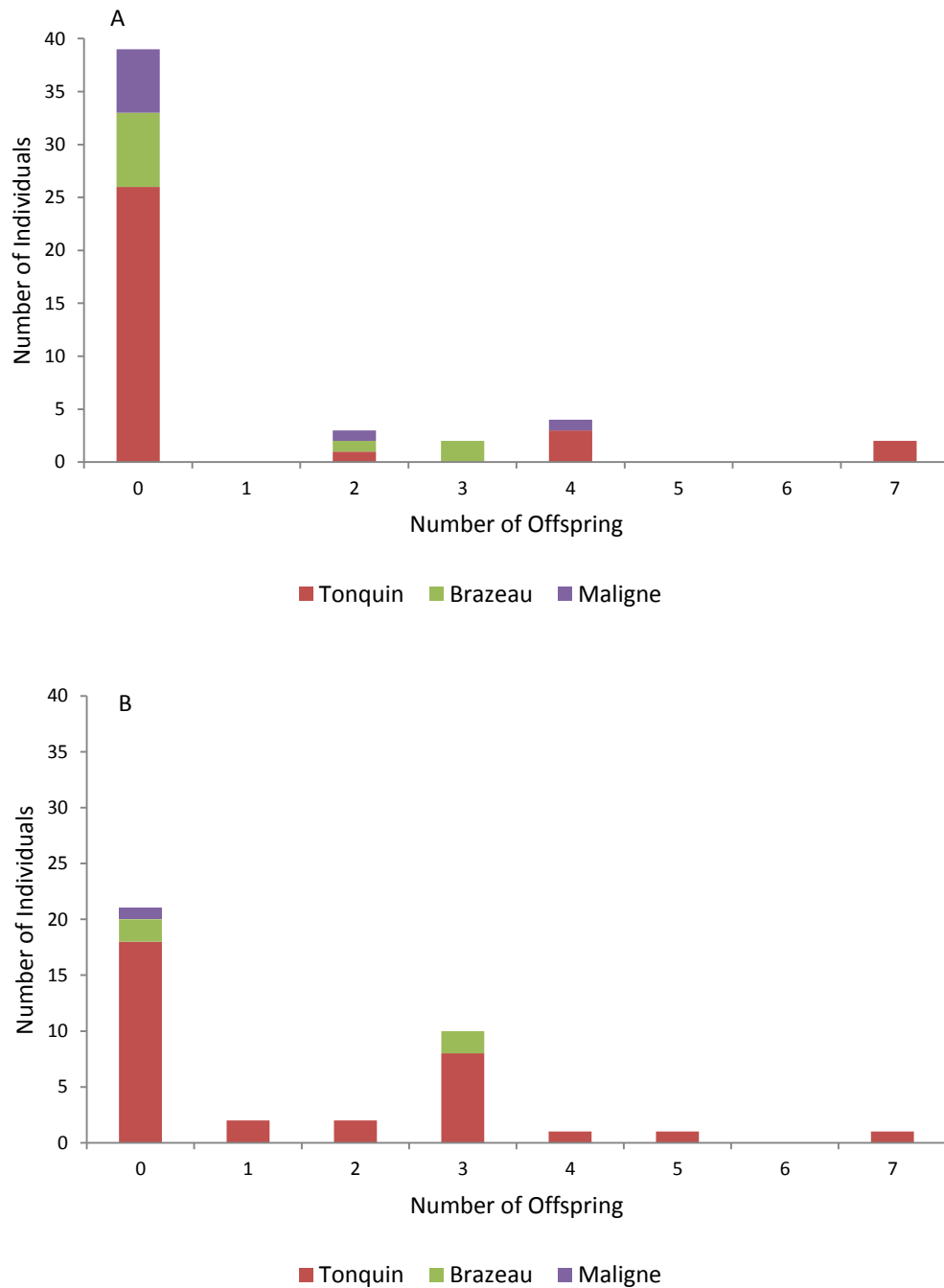
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APPENDIX B

Figure 17. Number of females producing offspring relative to 1-2 years known alive (A), 3-5 years known alive (B), and 6-7 years known alive (C).



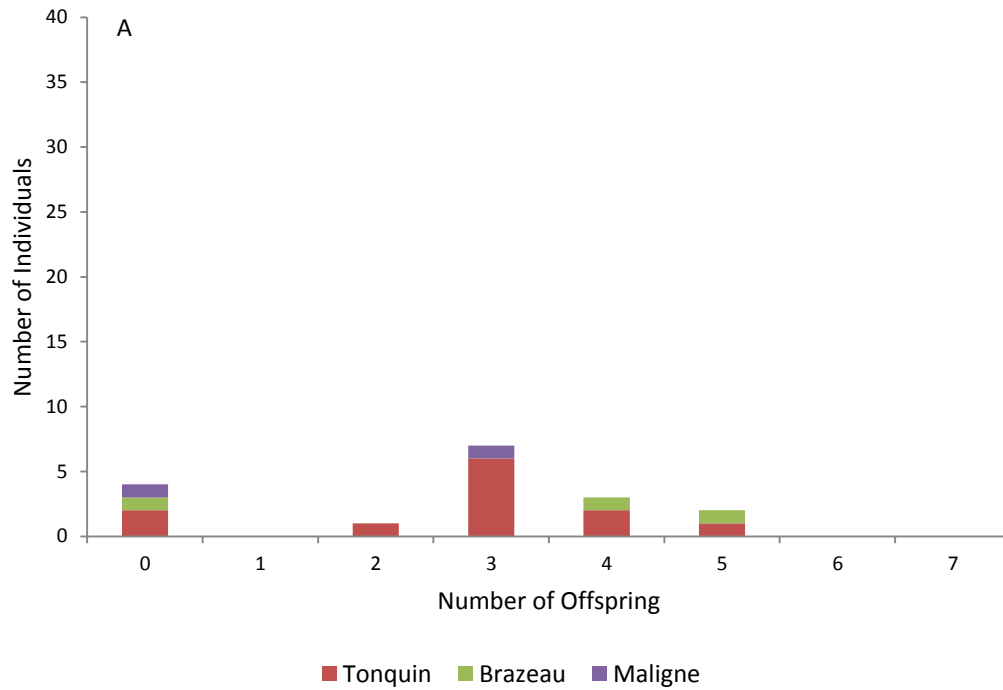
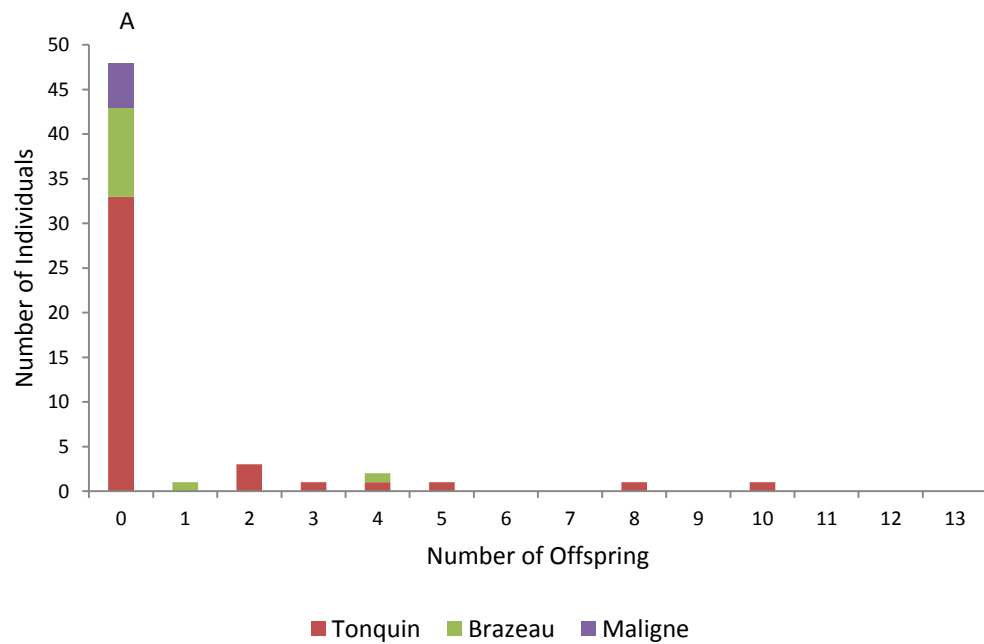
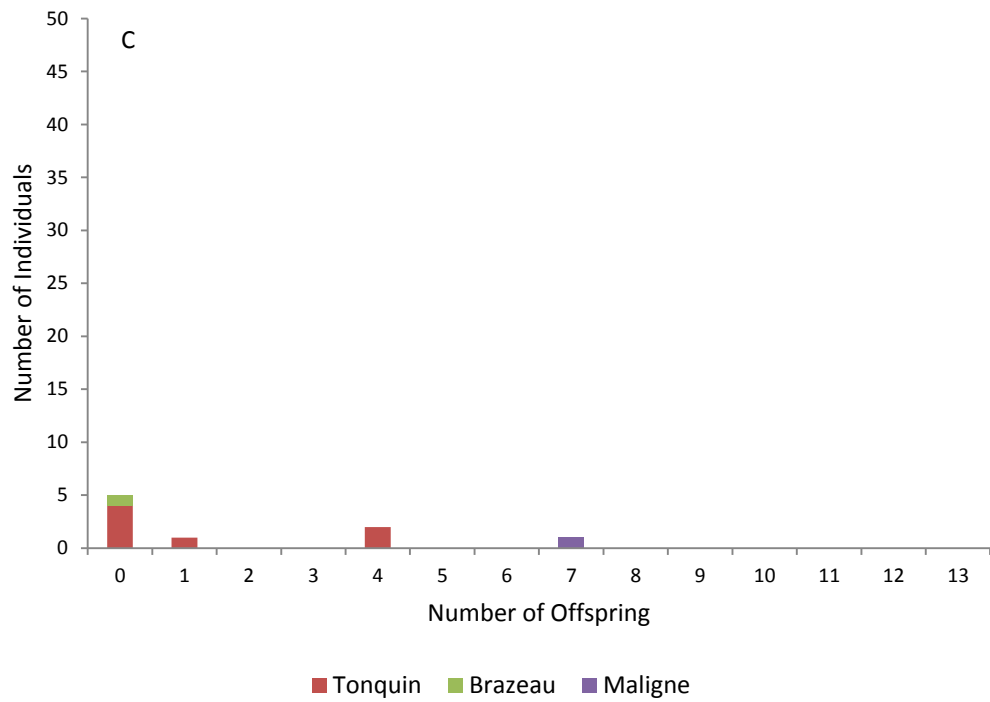
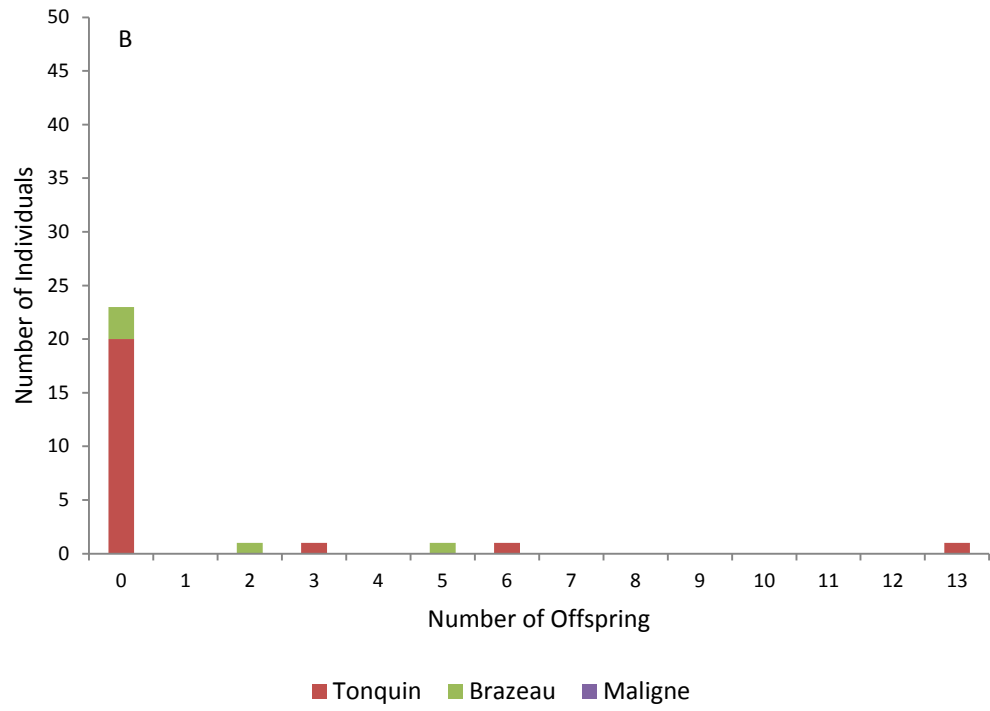


Figure 18. Number of males producing offspring relative to 1-2 years known alive (A), 3-5 years known alive (B), and 6-7 years known alive (C).





GENERAL CONCLUSION

The methods expressed in this thesis to determine age-class and to explore the sociogenetics of woodland caribou may only have been feasible due to our highly monitored study populations. The manner in which we identified putative calves and the relatively high number of identified familial relationships in our pedigree analysis was due to our established capture history. Both South Jasper and North Interlake have been sampled annually, most often more than once a year and over a ≥ 6 year period. This allowed us to observe when new individuals entered the population (i.e. potential offspring), allowed us to observe them through time (i.e. via multiple capture events), and enabled us to comprehensively sample a majority of individuals from both populations. Consequently, in order to implement these methods in other populations or species, a similar sampling situation might be necessary to at least make the desired outcome feasible. In addition, these populations are relatively small, closed populations. These methods may not be sufficient enough when applied to large-scale, open populations of caribou, such as the larger more migratory herds. Caribou vary in size, behaviour, morphology, and diet across their range (Thomas and Gray 2002, Miller 2003, COSEWIC 2011) which may have implications for pellet morphology, hormone content, and even social structure and mating system. Tundra reindeer found in large herds have been observed to exhibit more promiscuous behaviour, where bulls move through herds and mate opportunistically (Banfield 1975) while sedentary populations of caribou are recognized as being more differentiated than migratory populations (Boulet et al. 2007) and hence likely demonstrate more stable social organization than migratory populations, as was shown in our study. Due to this diversity of characteristics across the *Rangifer*

species, distinguishable calf and adult thresholds in pellet morphology and reproductive hormone levels are likely not achievable. However, it may be possible to establish thresholds that are local population specific. For example, South Jasper male putative calf pellet approximated volume index did not exceed 1279.5 mm^3 at the upper confidence limit while adult pellet size did not fall below 1738.5 mm^3 at the lower confidence limit. This leaves a buffer of 459.0 mm^3 between age-classes and thus may be later used to define a threshold. Though Ball (2010) may have been presumptuous in providing thresholds to separate age-class via pellet length, he did show that calves aged 4-7 months significantly differed in both pellet length and width from all older age-classes. The results by Ball (2010) along with ours demonstrate that pellet morphology may be used to confidently distinguish calf from adult age-classes only. Nevertheless, replicating this study in other local populations and analysing more putative calf and adult samples of our study populations is necessary to further validate our results and to establish more precise thresholds. Following this, age-class of individuals may be incorporated into CMR research for cohort analyses.

Our sociogenetics study revealed the potential of pedigree analysis via non-invasive genetic sampling, but accurate pedigree analysis is dependent upon the amount of data available, the type of genetic data being utilized to infer relatedness, and the generational time the data spans. The most important factors to consider when planning to use any form of genetic data to assign kin relationships is the number of loci being used and their allelic diversity (Smouse and Chevillon 1998, Bernatchez and Duchesne 2000). Bernatchez and Duchesne (2000) emphasise the importance of both these factors, however they explain that the number of loci is the more important factor of the two. The

most commonly preferred genetic marker for inferring relatedness has been microsatellites due to their single locus information, their codominance, high variability, and their short DNA fragments (Parker et al. 1998, Jones and Ardren 2003, Pemberton 2008). Generally, Bernatchez and Duchesne (2000) recommend that loci spanning 6 to 10 alleles per locus be used for assigning parentage based on allelic diversity, which is in line with our South Jasper allele frequency results. Additionally, Bernatchez and Duchesne (2000) provide a summary table (see table in Appendix 1, Bernatchez and Duchesne 2000) based on their model predictions that may help researchers determine the minimum amount of loci required to determine parentage for a given population size. Based on their model, and our use of 95 South Jasper males and 105 South Jasper females, Bernatchez and Duchesne (2000) recommend a use of 7 to 12 loci to derive parentage with a 90% probability of success. Although our study's use of 10 microsatellite loci falls in line with this model, it would be wise to replicate the study using more microsatellite loci to validate the inferred relationships. Pedigree analysis from non-invasive genetic sampling is a new method that is not well established in the literature and this validation via more loci is therefore essential even though overall results are predicted to remain unchanged.

The time between when an animal enters the population (i.e. offspring) and their fecal pellets are picked up is arguably the most notable limitation of our study. Though we can say with confidence that nearly all individuals in these populations have been sampled based on our high sampling effort and well established capture history, there is still some uncertainty as to whether we are capturing new individuals in their first year. This is a legitimate concern as there are a few examples in our capture history of an

animal being captured in one year and then not re-captured until years later (Table 8 and Table 9 Appendix A). For South Jasper, a few of the super-females and super-males and a number of the prominent breeders of both sexes were only observed once or twice in our capture history. One possible explanation for this is that bull males are more preoccupied with copulating and defending females than they are with eating and subsequently producing pellets. Similarly, females with calves may be more concerned with defending these calves than they are with foraging for themselves. Male reindeer body condition is known to be negatively affected during polygynous mating (Barboza et al. 2004) which may translate to neglecting to forage appropriately and therefore few pellets are produced. At the same time, mothers of ungulate species have a recognized cost between foraging and offspring survival (Brown and Kotler 2004, Panzacchi et al. 2010). Consequently, if animals are not producing enough pellets for researchers to frequently collect in the field, animals are thus not being captured in a timely manner. The number of fecal pellets being produced is beyond our control and the best way to mitigate this problem is to ensure thorough sampling. Fortunately, our sampling effort has been occurring over ≥ 6 years and we are assured that nearly all individuals in these populations have been captured, if only once.

The research objectives and findings expressed in this study are only a few of the numerous opportunities and research avenues that can be explored when using non-invasive genetic sampling. The methods used to define age-class may be furthered by sampling more putative calves and adults in South Jasper and North Interlake and later implemented in other study populations. Sociogenetics may be further used to estimate levels of inbreeding, effective population size and for comparisons in mating system and

social structure in other local caribou populations found across landscapes. Capture histories, pedigree results and age-class analysis may all be incorporated together to better define age-class and to follow relationships through time. These avenues for future research are just a few of the diversity of questions that stem from our results and supported by these non-invasive methods. It is therefore of benefit to scientists and the species they study to consider incorporating non-invasive genetic research methods as we demonstrate here their ability to answer research questions previously considered too difficult to pursue.

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