Use of Fecal DNA to Estimate Population Demographics of the Boreal and Southern Mountain Ecotypes of Woodland Caribou

By

Peter N. Hettinga

A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfilment of the Requirements
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MASTER OF NATURAL RESOURCES MANAGEMENT

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Abstract

This study looked at the efficacy of using woodland caribou fecal pellets as a source of DNA to identify sampled animals and estimate population demographics. Fecal pellet samples were collected using systematic surveys of woodland caribou ranges in Jasper National Park, Alberta and the North Interlake region, Manitoba. Collection of pellet samples took place when snow was present to allow for tracking and location of caribou cratering areas and to obtain good quality DNA. DNA was amplified at ten polymorphic loci and one sex-specific primer. To estimate population size ($\widehat{N}$) and population growth rate (λ), mark-recapture models were used. Model assumptions were evaluated and tested by stratifying available samples based on herd and gender information. In using the Mh (jackknife) model, the population sizes for south Jasper National Park were estimated at 125 animals in 2006-2007 (95% CI: 114, 143), 91 animals in 2007-2008 (95% CI: 83, 105) and 134 animals in 2008-2009 (95% CI: 123, 152); comparable to the mark-resight population estimates calculated over the same sampling periods. Genetic diversity indices for the different herds in Jasper National Park presented a lower genetic diversity for the smaller Maligne and Brazeau herds when compared to the larger Tonquin and A La Peche herds. Use of population assignment tests on samples collected in Jasper National Park indicated considerable admixture between the different herds despite earlier telemetry work demonstrating strong herd fidelity. The North Interlake population was estimated at 134 animals (95% CI: 122,151) in 2006-2007 and 106 animals (95% CI: 97, 121) in 2007-2008. Using data collected between 2005 and 2008, population growth rate for North Interlake was estimated at 0.83 (90% confidence interval: 0.65, 1.02). As a λ below 1 indicates a declining population, continue monitoring of the North Interlake herd

is highly recommended. This studied clearly showed that the sampling of fecal DNA is a reliable and noninvasive alternative to monitoring woodland caribou population sizes and trends in the boreal and mountain regions.

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Chapter 1 Study Context

1.1 Introduction

The range and occurrence of woodland caribou, *Rangifer tarandus caribou*, has diminished substantially within Canada and the United States (Thomas and Gray 2002, McLoughlin et al. 2003). This is particularly evident at lower latitudes and is attributed to factors ranging from human disturbance to climate change (Thomas and Gray 2002). The resulting retraction of woodland caribou northwards has strongly affected sedentary 'forest-dwelling' woodland caribou relative to migratory 'forest-tundra' woodland caribou (Thomas and Gray 2002). Subsequent mention of 'woodland caribou' in this paper will be in reference to forest-dwelling woodland caribou only.

Under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), five populations of woodland caribou are identified based on geographic area to guide regional monitoring and management strategies (Thomas and Gray 2002). These populations and their status (in brackets), are the: Northern Mountain (special concern), Southern Mountain (threatened), Boreal (threatened), Atlantic-Gaspésie (endangered) and the Newfoundland (not at risk) populations (COSEWIC 2002).

Under COSEWIC the term 'population' is reserved for usage with these five aforementioned groups which can be broken down into 'local populations' or 'herds' based on geography (COSEWIC 2002). Use of alternate terms like 'subpopulation' or 'metapopulation' should be avoided as the ability of researchers to discern these levels of population structuring is often limited (Thomas and Gray 2002). However, as many

publications on woodland caribou do not to conform to these terms, I will use the same terminology within cited sources to avoid inadvertently changing any intended meanings.

1.1.1 Population surveying

The collection of woodland caribou demographic information often occurs through aerial survey supplemented by the placement of radiocollars on select, usually female, animals (eg. Edmonds 1988, Seip 1992, Stuart-Smith et al. 1997, Rettie and Messier 1998). This allows researchers to estimate population size, calf mortality, geographic distribution, population age-sex structure, etc. Attaining these estimates often requires supplemental training and sampling methods including the sexing and ageing of animals by sight, collecting tissue samples for lab work and in estimating population size using sightability corrections and mark-recapture models (Seip 1992, Stuart-Smith et al. 1997, Rettie and Messier 1998).

In particular, estimates of population size often play a critical role in wildlife studies (Lancia et al. 1996) and is the basis for population viability analysis (PVA) which can indicate, on a temporal scale, the likelihood of a population going extinct (Morris and Doak 2002). By estimating population size over subsequent sampling periods, the growth rate of sampled populations can also be calculated and used to inform on overall population health (Morris and Doak 2002, Mills 2007).

In studying the effect of population size on population growth rates of mountain ecotype woodland caribou, Wittmer et al. (2005b) revealed that an inverse density dependent relationship exists where smaller size ‘subpopulations’ have lower per capita growth rates. Accordingly, recovery strategies for woodland caribou should focus on a high number of individual animals being present to form a local population, as facilitated

through functional landscape connectivity to enable higher growth rates and recovery (Wittmer et al. 2005b).

1.1.2 Jasper National Park woodland caribou

Jasper National Park (JNP) woodland caribou are part of the Southern Mountain population listed as threatened (COSEWIC 2002). The size and distribution of woodland caribou 'subpopulations' in JNP, particularly in south JNP, have declined considerably over the past 30 years (Whittington et al. 2005, Parks Canada 2006). This is similar to what has been observed elsewhere in Alberta (Edmonds 1988, Dzus 2001).

There are four woodland caribou 'subpopulations' in JNP: Brazeau, Maligne, Tonquin, and A La Peche (Neufeld and Bradley 2007). While the northernmost 'subpopulation,' A La Peche has a relatively stable population (Alberta Woodland Caribou Recovery Team 2005), the Maligne, Tonquin and Brazeau are declining in population size and are the central focus of the South Jasper National Park Caribou Research Project (Whittington et al. 2005). In south Jasper, it has been determined that the development of roads and trails has contributed to the isolation of 'subpopulations' and has resulted in increased interactions with other species; notably humans and wolves (Whittington et al. 2005, Hebblewhite et al. 2007). Currently JNP biologists are interested in assessing alternate means of estimating population size which may reduce direct interaction with studied caribou without compromising the ability to effectively monitor them and assess population growth trends (Bradley, pers. comm.).

1.1.3 North Interlake woodland caribou

The North Interlake region of Manitoba lies between Lakes Winnipeg and Manitoba in central Manitoba. Habitat type is predominately boreal forest and a provincial highway and high yield power-line right-of-way bisect the area on a north-south axis. Using population assignment techniques, Ball et al. (2010) demonstrated that two distinct genetic clusters exist in the North Interlake; now referred to as 'Upper' and 'Lower' North Interlake. Recent work delineating landscape connectivity in the North Interlake has shown low levels of landscape connectivity between Upper and Lower North Interlake (Fall et al. 2007). A previously published population size estimate for the number of woodland caribou in the North Interlake was 50-75 animals (Manitoba Conservation 2005).

1.1.4 Noninvasive genetic sampling

Noninvasive genetic sampling (NGS) can provide an alternate means of estimating population demographics that does not require direct interaction between researcher and study species (Kendall and McKelvey 2008). Using NGS, genotype information from processed samples can serve as a unique mark for mark-recapture analysis (Kohn et al. 1999, Woods et al. 1999). Notably, the use of bear hair-snares to snag hair from black bear, *Ursus americanus*, and grizzly bear, *Ursus arctos*, populations has been used to estimate population size using closed mark-recapture models, as well as survival and population rate of change parameters using open population models (Woods et al. 1999, Paetkau 2003, Boulanger et al. 2008, Kendall et al. 2009).

The extraction and amplification of DNA from caribou feces has been studied and techniques have been developed to ensure the accuracy of genotype information for use

in analysis (Ball et al. 2007). Using sampled caribou feces as a source of DNA has not yet been incorporated into mark-recapture modelling but has enabled studies identifying sampled levels of genetic diversity (Ball et al. 2010) , levels of gene flow between sampled herds (Ball et al. 2010), and the difference between historical and contemporary genealogical lineages (Petersen et al. 2010).

The use of genetic sampling techniques is of importance in assessing the shared genetic heritage of sampled local populations and identifying the extent of migration occurring between them. In the sampling of fecal pellets from North Interlake woodland caribou 2004-2006, Ball et al. (2010), using population assignment tests, found two distinct genetic clusters existing in this area (an 'Upper' and 'Lower' cluster). This was in contrast to the commonly held view that there was only a single herd in the North Interlake (Manitoba Conservation 2005). The use of similar genetic sampling and testing techniques could also be used with caribou herds elsewhere to identify if cryptic population structure exists. This would have particular relevance in determining the potential for movement of animals between herds and the creation of management units.

1.2 Objectives

The analysis of woodland caribou fecal DNA can provide an alternate means of estimating population demographics. The objectives of this study are to:

1 Apply closed mark-recapture models to estimate population size ($\hat{N}$) with noninvasively sampled fecal pellets from woodland caribou herds in Jasper National Park and the North Interlake region of Manitoba,

2 Apply open mark-recapture models for the purpose of estimating population rate of growth (λ) with sampled genetic information from the North Interlake region of Manitoba,

3 Assess sampled genetic characteristics of woodland caribou herds in Jasper National Park using estimates of genetic diversity and gene flow between herds, and;

4 Make informed recommendations on the continued collection of fecal pellet samples from Jasper National Park and the North Interlake region of Manitoba.

Chapter 2 Literature Review

2.1 Woodland Caribou Ecology

2.1.1 Taxonomy

As cervids, caribou (*Rangifer tarandus*), are identifiable by a number of characteristics: a four chambered stomach, no gall bladder attached to the liver, short tails, relatively large ears, glands located in the preorbital pits, and four mammae (on females) (Pattie and Hoffman 1990). Some characteristics distinct to caribou include: antlers on both sexes, long metapodial bones, well-marked tarsal and interdigital glands and relatively simple crests on the cheek teeth (Banfield 1974, Miller 2003).

Some of the other taxonomic characteristics unique to caribou are adaptations to living in colder, more northerly environments where snowfall is abundant. This includes shovel like hooves which are used to dig through snow and to disperse weight when traveling on snowpack (Thomas and Gray 2002, Miller 2003) and hollow 'guard hairs,' present over winter season and trap body heat otherwise lost through convection (Miller 2003).

Physically, with the exception of being somewhat larger, woodland caribou (*Rangifer tarandus caribou*), do not differ substantially in appearance from other caribou subspecies (Miller 2003). The size of an adult woodland caribou varies seasonally from 100-250 kg with males weighing 10 – 50% more than females (Miller 2003). Maximum body weight is typically reached in males and females at 6 and 4.5 years, respectively (Miller 2003).

Adult pelage length and colour varies seasonally with the summer coat brown with white areas on the ventral mane, underside of tail and hoof edges. The winter coat, consisting primarily of guard hairs, grows from the summer coat and turns adult pelage white with brown on the anterior limb surfaces and on the upper portions of the head and back (Pattie and Hoffman 1990, Miller 2003). Calves are born brown with a dark stripe running dorsally with undersides of white or light gray (Miller 2003).

2.1.2 Diet

Woodland caribou are herbivorous with their diet primarily consisting of lichen (Miller 2003). Terrestrial lichens are varied in distribution and located in alpine and tundra areas, as well as on the forest floor in climax forests whereas arboreal lichens grow off old and mature coniferous stands (Thomas and Gray 2002). There is some variation between woodland caribou local populations in their foraging strategies for lichen (Thomas and Gray 2002). However, consumption of arboreal lichens typically takes place in areas where deep snow prevents successful cratering behaviour; where caribou paw and dig for available lichens (Rettie et al. 1997, Thomas and Gray 2002).

Various grass, sedge and shrub species also form part of the diet of woodland caribou (Rettie et al. 1997, Thomas and Gray 2002); particularly sprouting shoots during the early spring (Rettie et al. 1997). It has been noted that woodland caribou are able to meet their nutritional requirements over the winter when little lichen is available (Bergerud 1974). This is thought possible through an alternate reliance on other forage species (Bergerud 1974), individuals alternately living off fat reserves (Thomas and Gray 2002) and lower seasonal energy requirements (Miller 2003).

2.1.3 Reproduction

The onset of the breeding season, 'the rut,' is initiated by decreasing photoperiod in the fall (Ropstad 2000). On congregating, intrasexual competition begins to establish breeding hierarchies where dominant males, typically older and exhibiting seasonally high testosterone levels, breed with harems until sexually mature females are impregnated (Bubenik et al. 1997). Cows exhibit polyestrous behaviour where if not initially impregnated hormone cycling will continue, with fertilizations in wild reindeer populations occurring as late as February with calves born in late summer (Ropstad 2000).

If bred during the rut, females give birth to a single calf in late-spring after a gestation period of 225 - 235 days (Messier et al. 1990). Parturition rate in impregnated females is approximately 85% (Stuart-Smith et al. 1997, Rettie and Messier 1998). Within large herbivore species, while adult mortality rates remain relatively stable from year to year, calf mortality is quite variable (Gaillard et al. 1998). This is observable in woodland caribou 'subpopulations' where predation is the most frequent cause of mortality for calves and adults alike (Bergerud 1974;1988, Seip 1992).

2.1.4 Distribution and habitat use

There are a number of groupings within woodland caribou, *Rangifer tarandus caribou*, based on geography, genetic lineage, behaviour, diet, conservation status, etc. (Thomas and Gray 2002). One such distinction is between forest-dwelling and forest-tundra woodland caribou (Thomas and Gray 2002). In comparison to forest-tundra woodland caribou, forest-dwelling woodland caribou show less of a tendency to migrate,

with migrations < 100 km seasonally (Thomas and Gray 2002), or to congregate; particularly during the post-calving period (Miller 2003).

The average distribution of woodland caribou, east of the Cordilleran mountains, is 1 – 4 caribou/100 km^2 with habitat selection tending towards old and mature forest stands which are lichen rich and peatlands (Rettie and Messier 2000, Thomas and Gray 2002). Increased range sizes are apparent in the winter season (when there is snow) and is attributed to more exhaustive foraging behaviour (Stuart-Smith et al. 1997). In the summer season (when there is no snow), range size is smaller, particularly for calving females, and is attributed to abundant forage availability (Edmonds 1988), multiyear site fidelity (Shoesmith 1977) and as a means of avoiding conspecific species (Seip 1992).

Woodland caribou are niche specialists that preferentially select low-productivity habitats (Thomas and Gray 2002). By avoiding productive areas where more forage material is available, and alternately selecting areas where lichen is available, woodland caribou avoid apparent competition with other cervid species such as moose (*Alces alces*), whitetail deer (*Odocoileus virginianus*) and elk (*Cervus canadensis*) and , in turn, avoid predators wolf (*Canis rufus*), cougar (*Puma concoler*) and grizzly bear (*Ursus arctos*) (Bergerud and Elliot 1986, Rettie and Messier 1998, Mahoney and Virgil 2003).

Summer habitat selection by woodland caribou is seen as a predator avoidance strategy rather than forage driven (Bergerud 1974, Rettie and Messier 1998, Wittmer et al. 2005b). This is especially apparent in the summer season, when predation is higher, with the selection of islands and alpine areas, particularly by calving mothers, where there is an apparent lack of forage material (Ferguson et al. 1988, Seip 1992).

2.1.5 Mountain caribou

Woodland caribou described as ‘mountain caribou’ or ‘mountain ecotype’ woodland caribou show distinctive summer season habitat selection behaviour. The habitat selection strategy of mountain caribou, in the summer season, involves migration to alpine areas and, as with most habitat selection by woodland caribou, particularly over the calving season, is considered a predator avoidance strategy (Edmonds 1988, Seip 1992, Thomas and Gray 2002).

For mountain caribou there are documented differences in winter foraging strategies between local populations (Thomas and Gray 2002) which has hampered past relocation efforts (Warren et al. 1996). While some mountain caribou in British Columbia, during the winter season, feed on arboreal lichen species in sub-alpine areas; often through utilizing snow packs and steep gradients, the forage response by Alberta’s mountain caribou is more similar to the ‘northern mountain’ ecotype of woodland caribou, found in west-central BC, in that it will crater for terrestrial lichens despite arboreal lichens being available (Warren et al. 1996, Thomas and Gray 2002).

2.1.6 Threats

The fragmentation of habitat associated with natural events, such as fire and flooding, and anthropogenic activities, such as recreational and industrial developments, often limit the distribution of woodland caribou over a landscape (Bergerud 1988, Dyer et al. 2001, McLoughlin et al. 2003, Miller 2003). Woodland caribou residing at lower latitudes are particularly susceptible to landscape changes through anthropogenic land development and other landscape uses (Bergerud 1988, Thomas and Gray 2002).

Predation of woodland caribou is the proximal cause of mortality with predation rates highest during the summer season when calving occurs (Seip 1992, Wittmer et al. 2005a, Wittmer et al. 2005b). Habitat fragmentation often creates a matrix habitat suited to alternate prey species such as moose and whitetail deer which in turn attract predator species (Bergerud and Elliot 1986, Rettie and Messier 1998, Mahoney and Virgil 2003). Indeed predation of woodland caribou is higher in fragmented landscapes (Kinley and Apps 2001). The migration of whitetail deer to areas inhabited by woodland caribou is also potentially fatal to caribou through transmission of the parasites *Elaphostrongylus tenuis* and *Pneumostrongylus tenuis* (Miller 2003).

Within already diminished populations, decreases in population size further limit the chances of population growth through inverse density dependence, or 'Allee' effects (Allee et al. 1949, Courchamp et al. 1999). Wittmer et al. (2005b) demonstrated an inverse density dependent relationship exists within woodland caribou 'subpopulations' whereby smaller `subpopulations' have lower per capita growth rates; despite similar per capita pregnancy rates. This in particular does not bode well for 'subpopulations' at lower latitudes where landscape fragmentation has an isolating effect and caribou herds are effectively cut off from one another (James and Stuart-Smith 2000, Dyer et al. 2001).

2.1.7 Conservation status

In Canada, under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), woodland caribou have been placed into National Ecological Areas (NEA) based on geographic location. The five populations and there conservation status are: the Boreal population (threatened), the Southern Mountain population (threatened), the

Northern Mountain population (special concern), the Newfoundland population (not at risk) and the Atlantic-Gaspésie population (endangered) (COSEWIC 2002).

Parks that intercede one of the NEA designations are bound to protect resident woodland caribou populations. Many of these conservation strategies involve minimizing habitat fragmentation and maintaining connectivity (Thomas and Gray 2002). Other techniques that have been applied include relocation of woodland caribou to areas with shrinking 'subpopulations' (Warren et al. 1996) and predator reduction programs (Bergerud 1988). The utility of such strategies is largely based on the results of population demographics studies which monitor changes in population size and distribution to assess and guide management decisions.

2.1.8 Caribou monitoring strategies

In monitoring woodland caribou and estimating population demographic information aerial surveying methods are often used. The use of a sightability index can allow biologists to estimate the probability of observing cervid species during aerial surveys based on environmental and situational factors (Samuel et al. 1987). The environmental and situational factors that apply to woodland caribou could include survey location (alpine vs. boreal), prevailing ground conditions (i.e. snow-covered or not), group size and the spatial isolation of animals in relation to yearly breeding cycle (Thomas and Gray 2002, Miller 2003).

Use of radiocollars to track caribou is often used in conjunction with aerial surveys (Seip 1992, Rettie and Messier 1998, Mahoney and Virgil 2003, Whittington et al. 2005). Researchers, by discerning the proportion of radiocollared animals observed during a survey, can derive population demographic estimates including population size

using mark-resight modelling techniques (Bartmann et al. 1987, White and Garrott 1990). Recruitment rates can also be assessed using radiocollars by observing adult female animals after calving and monitoring calf survival over time (Rettie and Messier 1998, Wittmer et al. 2005a).

The genetic sampling of woodland caribou has taken place through the collection of blood and tissue samples from animals and has been used to assess gene flow patterns between herds (McLoughlin et al. 2004, Boulet et al. 2007, McDevitt et al. 2009), to distinguish ecotypes (Cronin et al. 2006, McDevitt et al. 2009), and identify landscape barriers to movement (McLoughlin et al. 2004, Boulet et al. 2007). In acquiring woodland caribou DNA using noninvasive genetic sampling, the sampling of fecal pellets has proven to be a suitable and reliable source of DNA provided precautions are taken to use samples identified as being of good quality (Ball et al. 2007) and has been used to assess gene flow between herds (Ball et al. 2010, Petersen et al. 2010).

2.2 Noninvasive Genetic Sampling

2.2.1 Background

Noninvasive genetic sampling (NGS) of wildlife species can provide an alternate means of monitoring wildlife species that minimizes direct interaction between researcher and study species (Kendall and McKelvey 2008). NGS can be used to delineate population structure through the analysis of gene frequencies and gene flow (Paetkau and Strobeck 1994) as well as to provide a basis for estimating population size (Kohn et al. 1999). In particular, with NGS, the use of mark-recapture models is facilitated through the potential for many samples to be available relative to direct sampling techniques

which require individual animals to be captured and physically marked or tagged (Lukacs and Burnham 2005b). Generally, however, it has been found that noninvasive tissue sources provide lower quantity and quality DNA than that extracted using more conventional blood and tissue sampling techniques (Taberlet et al. 1996, Paetkau 2003).

2.2.2 Molecular ecology

Use of extracted DNA to analyze differences between individuals is made largely possible through polymerase chain reaction (PCR) techniques, which can effectively replicate a single strand of DNA *ad infinitum* (Taberlet et al. 1996). By cycling a DNA sample through the PCR process, with specific DNA primer sequences, select DNA segments can be replicated and isolated to form a multi-locus genotype for each sample. Genotype mapping techniques can then read the amino acid composition at a given locus, including by size and weight or through the placement of radioactive or fluorescent amino acids into PCR gene products (Templeton 2006).

Two types of DNA are available from animal samples for genotyping: mitochondrial DNA and nuclear DNA. Genotyping of each DNA type, through use of specific primers during PCR, will provide alternate insights into sampled individuals (Templeton 2006). Analysis of mitochondrial DNA will yield genetic information useful in identifying individuals to the species and subspecies level. Alternately, extraction and analysis of nuclear DNA can be used in finding varying microsatellite markers and is useful in discerning individual identity and genetic differences (Kohn et al. 1999, Templeton 2006).

2.2.3 Amplification errors

Through following certain protocols during the collection of woodland caribou fecal samples and extraction of DNA, it has been shown that genotyping errors can be largely avoided (Ball et al. 2007). Firstly, the collection of fecal pellets when there is a layer of snow on the ground is beneficial in the cold preserving the DNA present in the mucosal coat surrounding fecal pellet samples (Maudet et al. 2004). Second, use of a wiping protocol to remove sloughed mucosal cells without fecal matter increases the concentration of usable template DNA for amplification (Ball et al. 2007).

Reviews of the problems encountered with NGS revolve around its characterization as a source of low-target and low-template DNA (Waits et al. 2001, Lukacs and Burnham 2005b). These refer to instances where low quantities and/or qualities of DNA increase the propensity for amplification errors. Two of the amplification errors that resound in genotyping studies include allele dropout and false alleles (Taberlet et al. 1996, Morin et al. 2001, Waits et al. 2001). Allele dropout occurs when, subsequent to genotype mapping, individuals heterozygous for an allele appear as homozygous because of one allele failing to be amplified (Taberlet et al. 1996) Alternately, false alleles has the opposite effect where homozygous individuals appear as heterozygous because of DNA artefacts created during the PCR process (Taberlet et al. 1996).

The incorporation of erroneous genotypes in the calculation of demographic parameters using mark-recapture models can result in estimates being positively bias with increased variability (Creel et al. 2003). Other errors encountered in genotype analysis includes the ‘shadow effect,’ where an insufficient number of microsatellite markers are

used to differentiate individuals, resulting in parameter estimates that are negatively biased with reduced variability (Mills et al. 2000).

The multiple tubes approach is a technique used to control for erroneous genotypes created through allele dropout and false alleles amplification errors. By dividing available genetic material from an NGS source into multiple tubes and doing repeated PCR analysis, samples can be assessed multiple times as to the consistency of genotyping results with less dependable samples retested and possibly discarded (Taberlet et al. 1996). As running multiple PCR amplifications is costly in terms of time and resources, different methods are used to genotype samples which require fewer amplifications (Miller et al. 2002, Schwartz et al. 2006, Adams and Waits 2007).

A number of techniques have also been adopted to prevent amplification errors prior to the DNA amplification process. The collection of fecal samples in optimal environments can allow increased success in amplifying DNA quantities (Maudet et al. 2004). By quantifying the amount of DNA present in each sample, prior to amplification, it is also possible to cull poor quality samples (Taberlet et al. 1997). Pilot studies can also be used to identify which microsatellite markers are more error prone and should be discarded prior to use for monitoring and management (Piggott et al. 2006).

2.2.4 Gene flow

The examination of sampled genetic material from sampled populations allows comparison between them using Wright's (1951) F-statistics. Estimation of the inbreeding coefficient (F_{IS}) compares alleles sampled at an individual level to the larger subpopulation where low F_{IS} values are indicative of an unlikely hereditary relationship between the individual and the sampled subpopulation (Templeton 2006). Alternately,

estimation of F_{ST} calculates the level of genetic relatedness between sampled subpopulations and is useful in discerning historical range boundaries and landscape level barriers to migration and geneflow (Allendorf and Luikart 2007).

A disadvantage of calculating F_{IS} and F_{ST} is that it entails subpopulation structure to be known *a priori* (Balloux and Lugon-Moulin 2002, Coulon et al. 2006). As a method of discerning gene flow between populations, assignment tests use sampled genotype information to group sampled individuals based on genetic similarities (Paetkau et al. 1995). The use of assignment tests to group individuals frequently incorporates population-wide genetic assumptions including Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) (Pritchard et al. 2000). Alternately, Bayesian statistics are also used as a basis for population assignment tests though applying priors in grouping genetic identities: including sampling location and the number of *a priori* groupings identified (Pritchard et al. 2000, Coulon et al. 2006).

2.2.5 Population size estimates

Use of NGS to estimate population size can be done in a number of ways. As each individual animal, with the exception of monozygotic twins, triplets, etc., is identifiable by its unique genetic code, these can be counted to yield census estimates. However, as it is unlikely that all individuals in a population can be successfully sampled, the use of mark-recapture models can be alternately applied (White and Burnham 1999, Lukacs and Burnham 2005b). The use of sampled genetic information has not been demonstrated in previous studies as a method of estimating population demographics for woodland caribou; although these techniques have been implemented with other wildlife species

notably black bears (*Ursus americanus*) and grizzly bears (*Ursus arctos*) (Taberlet et al. 1997, Kohn et al. 1999, Piggott et al. 2006, Valiere et al. 2007).

2.3 Mark-recapture modeling

2.3.1 Closed population models

Using mark-recapture analysis population size estimates, $\widehat{N}$, can be calculated using a Lincoln-Peterson (LP) approach, with only a single post-collaring recapture event, or K-sample models, where multiple post-collaring recapture events are conducted (Mills 2007). Calculated population size estimates are subject to meeting a number of assumptions to ensure calculations are representative of the sampled population (Otis et al. 1978, White et al. 1982). Notably, 'population closure' is important and entails demographic closure i.e. no births or deaths between sampling periods and 'geographic closure' i.e. no migration of individuals to or from the study population in the duration of sampling (Lancia et al. 1996). Failure to meet the assumption of population closure often entails use of alternate mark-recapture models for 'open' populations where estimates for apparent survival must also be parameterized and used in modelling population parameters.

2.3.2 Open population models

A mark-recapture model for use with populations where births, death or migration is occurring ('open' populations), is the Jolly-Seber (JS) model (Seber 1982). In the use of the JS model, there are a number of alternate derivations available and useful in examining sampled biological characteristics from sampled populations. The relatively simple Cormack-Jolly-Seber (CJS) model is solely used to estimate apparent survival, Φ,

and capture probability, p, while the more complex Pradel (1996) model can be used to estimate population rate of growth, λ. An important factor in the use of open population models is the inestimability of certain parameters where several years of sampling information is needed to yield biologically meaningful data (Lebreton et al. 1992).

Chapter 3 Robust design DNA mark-recapture methods to assess population size estimates and population growth rates of Woodland Caribou, *Rangifer tarandus*, in the North Interlake region of Manitoba

Abstract

The North Interlake woodland caribou herd in Manitoba is part of the boreal woodland caribou population listed as threatened under COSEWIC (2002) and the provincial and federal Species at Risk Acts (SARA). To facilitate monitoring and management of the North Interlake herd, the purpose of this study was to develop and test a noninvasive method to estimate population size and population growth rate using fecal DNA. Through the sampling of woodland caribou feces over multiple sampling times for use in mark-recapture modelling, 'captured' and 'recaptured' animals were identified based on sampled genetic information. Assumptions for mark-recapture models were validated for both open and closed population models and sampling information was used to estimate population parameters for two identified genetic clusters in the North Interlake as well as for the entire North Interlake region. Using sampled genetic information, parameter estimates were also calculated specific to sampled gender information. Between 2004 and 2008, a total of 841 samples were collected and 166 unique genotypes were obtained (95 female and 71 male). MARK software was used to derive population estimates using model averaging and the Mh (jackknife) model. For the entire North Interlake, the population was estimated at 124 and 103 animals using model averaging in 2007 and 2008, respectively, and 134 and 109 animals when using the Mh (jackknife) model. Simulations done using the Mh (jackknife) model indicated calculated population estimates becoming negatively bias as the number of samples used in analysis was reduced. For open population modelling, using samples collected between 2005 and 2008, a population growth rate (λ) of 0.83 was obtained (90% CI: 0.65, 1.02). These results indicate a declining population of woodland caribou in the North Interlake and it is advised that action be taken to assess the potential causes of the population decline in this region and continue monitoring animals through the systematic collection of fecal pellet samples.

3.1. Introduction

The boreal population of woodland caribou is listed as threatened in Canada by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2002) and the federal and provincial Species at Risk Acts (SARA). The current range of woodland caribou in Canada extends from Newfoundland to British Columbia and is negatively affected by habitat fragmentation, predation and climate change (Dyer et al. 2001, Thomas and Gray 2002). Generally, southerly ranging boreal woodland caribou herds face a relatively high risk of extirpation and are often considered an indicator species for a healthy boreal forest ecosystem (Thomas and Gray 2002, McLoughlin et al. 2003).

In facilitating the growth of woodland caribou herds, estimates of population size and population growth rate are critical in guiding management decisions (Thomas and Gray 2002, Environment Canada 2008) and assessing herd health and longevity (Wittmer et al. 2005b, Hebblewhite et al. 2007). In the monitoring of woodland caribou herds, adult female animals are often selected and collared and used as indicators of animal health through assessment of habitat use patterns, survival rates, calf recruitment rates and general physical condition (Edmonds 1988, Seip 1992, Rettie and Messier 1998, Courtois et al. 2003b, McLoughlin et al. 2003, Sorensen et al. 2008).

While providing demographic information essential to monitoring woodland caribou, the use of radio-collars is perceived as an invasive technique: particularly in the capturing techniques used when collars are deployed on ungulate species (Murray and Fuller 2000). In the ethical treatment of wildlife species, it is thought biologists should reduce the impact of monitoring studies when possible (Murray and Fuller 2000). There are reported instances of collars being lost or damaged or causing damage to studied

animals (Krausman et al. 2005). Some ungulates have also demonstrated changes in dispersal behaviour following capture events (Brooks et al. 2008, Morellet et al. 2009) and have demonstrated changes in calf survival rates (Côté et al. 1998). As population growth in ungulate populations is largely sensitive to changes in the mortality rate of adult female animals (Gaillard et al. 1998), it is particularly important that any sampling aimed at these animals be done carefully; particularly in studying already declining herds (Courchamp et al. 1999).

3.1.1 Noninvasive genetic sampling

The noninvasive genetic sampling (NGS) of animal tissues done using sampled hair and fecal material can provide a substantive DNA source for use in identifying sampled animals (Taberlet et al. 1996, Kohn and Wayne 1997). Through identifying sampled animals, NGS provides a means of obtaining population demographic information on cryptic and rare species using mark-recapture modelling (Palsboll et al. 1997, Kohn et al. 1999, Woods et al. 1999). NGS has been particularly successful in monitoring black bear (*Ursus americanus)* and grizzly bear (*Ursus arctos)* populations in Canada and the United States using sampled hair (Woods et al. 1999, Paetkau 2003) and fecal samples (Bellemain et al. 2005) to estimate population size. Other species where NGS has been used in estimating population demographics has included coyotes (*Canis latrans*) (Kohn et al. 1999), wolves (*Canis lupus*) (Creel et al. 2003) and badgers (*Meles meles*) (Frantz et al. 2003).

As well as estimating population demographics, NGS has been applied to a range of other wildlife monitoring initiatives (Kendall and McKelvey 2008). Notably, the sampling of genetic information from neighbouring animal communities can be used to

infer geneflow patterns and genetic structure where the amount of migration and shared genetic lineage between populations is inferred (Paetkau et al. 1998, Cegelski et al. 2003). Quantifying the amount of genetic diversity in a population and comparing it to historical samples or those from other sampled populations has also been used to assess relative population health (Paetkau and Strobeck 1994). This approach has particular merit in assessing the impact of reduced population sizes through the calculation of effective population size (Nm) relative to commonly used estimates of population size ($\widehat{N}$) (Luikart et al. 2010).

A major trade-off in the use NGS relative to direct sampling of DNA sources i.e. blood and tissue biopsies is that the quality of sampled genetic information is sometimes compromised (Taberlet et al. 1996, Pompanon et al. 2005). Accordingly, serious consideration has been given to using NGS and various methods have been developed to increase the quality of sampled genotype information (reviewed in Waits and Paetkau 2005). Commonly, the rerunning of a subset of samples is used in calculating error rates which will inform on the general success of sampling and amplification methods through a pairwise comparison of genotyped samples (Pompanon et al. 2005).

Some precautionary measures incorporated in the use of NGS to decrease calculated error rate levels includes the improvement and refinement of laboratory protocols (Taberlet et al. 1996, Paetkau 2003, McKelvey and Schwartz 2004), improved handling and storage protocols (Piggott 2004, Roon et al. 2005), more selective sample collection i.e. based on season (Maudet et al. 2004) and statistical calculations to account for sampling biases or genotyping errors (Lukacs and Burnham 2005a, Knapp et al. 2009). The absence of robust protocols to ensure correct genotyping information can

result in costly laboratory work to mitigate poor sampling habits, the loss of data and the misidentification of individual animals (Taberlet et al. 1996, McKelvey and Schwartz 2004, Roon et al. 2005).

The collection of woodland caribou genotype information using fecal pellet samples has had its own unique trajectory. In using fecal samples as a source of DNA, Ball et al. (2007) documented an improved method where sloughed intestinal epithelial cells are wiped from the surface of fecal pellets with the amount of available target DNA quantified. To this extent the winter-collected pellets from woodland caribou produce a large quantity of target DNA for use in amplification (Ball 2007). High amplification rates have also been obtained from winter collected fecal pellets from other ungulate species including the Iberian ibex (*Capra ibex*) and the Corsican mouflon (*Ovis mouflon*) (Maudet et al. 2004).

3.1.2 Mark-recapture models

The use of NGS has been successfully applied to mark-recapture methods to estimate population demographics for a number of wildlife species (reviewed in Lukacs and Burnham 2005b). Population size is often estimated with closed population models using software programs CAPTURE (White et al. 1978) and MARK (White and Burnham 1999). Estimates of population size are important when managing wildlife populations as they provide baseline data for monitoring population changes and predicting when studied populations may become extinct (Morris and Doak 2002, Mills 2007).

Closed population models

'Closed' population modelling is applied in cases where the sampled population is not affected by emigration, immigration, natality and mortality (Otis et al. 1978, White et al. 1982). More specifically, closed population models are based on the following assumptions:

1) the population is closed so the number of animals being sampled remains constant and there are no losses through births, deaths or migration,

2) there is no loss of tags and previously captured animals can be correctly distinguished,

and;

3) all animals have the same capture probability at each sampling period (Otis et al. 1978, Thompson et al. 1998).

Violation of model assumptions can serve to bias calculated population size estimates (Otis et al. 1978, White et al. 1982). Assumption #1 is best considered in applying an appropriate sampling design where between sampling times the sampled population is not changing due to natural phenomena i.e. births, deaths or migration. In a management context, the area sampled should also be inclusive of all animals considered as part of the same population so estimates are reflective of actual management units (Mills 2007). Assumption #2 can be validated through proper marking techniques where previously sampled and marked animals are easily identified i.e. through the use of permanent tags which are clear and readable (Otis et al. 1978, White et al. 1982).

Assumption #3 is often relaxed as a strict sampling requirement through the incorporation of alternate models where capture probability is parameterized. Alternate parameterizations of capture probability often include: sampling time, trap effects, individual heterogeneity and sampling group characteristics (male or female, adult of juvenile, etc). Failure to account for varying capture probabilities can lead to biases in calculated parameter estimates (Otis et al. 1978, Thompson et al. 1998, Williams et al. 2002) and misinterpretation of sampled biological phenomena (White et al. 1982).

The use of erroneous and false genotyping information is problematic in the application of mark-recapture models to estimate population size (Waits and Leberg 2000). Notably, in the estimation of population size, the inclusion of erroneous genotypes can serve to positively bias calculated estimates (Creel et al. 2003). Another problem which can bias population size estimates is through using an insufficient number of loci to discern the identity of sampled animals i.e. the shadow effect (Mills et al. 2000). In these instances, calculated estimates will be negatively bias; particularly in the estimation of population size for large populations (Mills et al. 2000).

Open population models

Alternate to the use of 'closed' population models, 'open' population models are used to estimate changes in a sampled population over time. Through violating the assumption that the sampled population is closed to births, deaths and migration, the additional parameterization of apparent survival (Φ) as well as capture probability (p) is required (Seber 1982, Williams et al. 2002). Using open population models, estimated apparent survival (Φ) takes into account animal survival between sampling times and

also whether animals are remaining in the sampling area. These factors limit the use of the apparent survival in its applicability to estimating actual animal survival; although can be used as a lower bound estimate (Sandercock 2006).

Open population models, which parameterize Φ and p, can be used to assess changes in sampled populations through parameterization of population losses due to emigration and deaths and population gains through births and immigration (Seber 1982, Lebreton et al. 1992). Using derivations of the Jolly-Seber open population model (Seber 1982), more complex models with additional parameters can be used to calculate population growth rate (λ) using the temporal symmetry approach (Pradel 1996).

In using open population models, there are a number of assumptions that should be followed to increase estimator precision and accuracy. These consist of:

1) no loss of tags and previously captured animals can be correctly distinguished,
2) all emigration of animals from the sampling area is permanent,
3) animals are independently sampled,
4) equal capture probability (p) at each sampling time,

and;

5) equal survival (Φ) between sampling times (Seber 1982, Williams et al. 2002).

Similarly to closed population modelling assumptions, assumption #1 highlights the need to correctly identify captured animals based on an accurate reading of permanent tags. When an animal ID is incorrectly read or lost the animal may be incorrectly thought removed from a studied population (Lebreton et al. 1992). In meeting assumption #2,

some open population models do not account for temporary emigration whereas other open population models have been parameterized to do so (Pollock et al. 1990).

Assumption #3 requires that animals are independently sampled to correctly assess all sampled animals rather than a subset which is more prone to being sampled i.e. those animals part of a larger more easily sampled group. One common way of assessing for independence in sampling is through testing for overdispersion (Anderson et al. 1994). This remains a practical way of assessing model fit when considering the application of the Cormack-Jolly-Seber model to parameterize capture probability (p) and apparent survival (Φ) but has not been appropriately tested in estimating lambda (λ) with the Pradel (1996) model (Sandercock 2006).

Meeting assumptions #4 and #5 are typically dealt with in a similar nature as to the handling of alternate parameterizations of capture probability (p) in closed population modelling. With open population models however alternate parameterizations of apparent survival (Φ) are also done to model the sampled population changing over time. Models used in forming alternate parameterizations of p and Φ take into account sampling covariates including sampling time, group information or any other sampling data collected in tandem with sampling events (Lebreton et al. 1992).

Robust design

Robust design modelling incorporates a sampling design where a population is sampled for the purpose of calculating both open and closed population parameters (Pollock 1982). In using robust design all assumptions used in open and closed population models, as described above, apply (Williams et al. 2002). Sampling times

used in forming capture histories for open population modelling are ‘primary intervals’ and the sampling times within each primary interval are ‘secondary intervals’ and take place over a relatively short time frame. In studying grizzly bears, Stetz et al. (2010) used an NGS based robust design to estimate population demographics through a combination of open population modelling, to estimate λ, and closed population modelling, to estimate $\widehat{N}$.

3.1.3 Study objectives

The objective of this study is to assess the use of NGS in identifying individual animals across multiple sampling times for the purpose of mark-recapture modelling. Through mark-recapture modelling, both open and closed population models will be applied to yield estimates of population rate of growth (λ) and population size ($\widehat{N}$), respectively. To further examine the efficacy of using sampled genetic information to estimate population demographics, available samples will be stratified based on sampled genetic information including sampled genetic cluster and gender. Based on the perceived success of calculated estimates, recommendations will be made on the further use of NGS to monitor woodland caribou in the North Interlake.

3.2 Methods

3.2.1 Study area

The North Interlake region of Manitoba lies between Lake Winnipeg and Lake Manitoba in central Manitoba, an area of approximately 4000 km^2 (Fig 1). Habitat type is predominately boreal forest and can be characterized by a mix of treed muskeg and old jack pine dominated stands, black spruce and poplar-dominated upland areas (Manitoba

Conservation 2005). A provincial highway and high yield hydro transmission corridor bisect the area on a north-south axis. Other large mammal species in the area include white-tailed deer (*Odocoileus virginianus*), moose (*Alces alces*), wolves (*Canis lupus*) and black bears (*Ursus americanus*).

Using population assignment techniques, Ball et al. (2010) demonstrated that two distinct genetic clusters exist in the North Interlake; now referred to as 'Upper' and 'Lower' North Interlake. Ball et al. (2010) also documented more gene flow between Upper North Interlake and caribou ranges to the north than between Upper and Lower North Interlake. Recent work modelling landscape connectivity in the North Interlake has also shown potential fragmentation between Upper and Lower North Interlake (Fall et al. 2007, Manseau, unpublished data). While not nominally referred to as separate herds, the Upper and Lower genetic clusters are increasingly being considered as discrete units for both management and monitoring purposes.

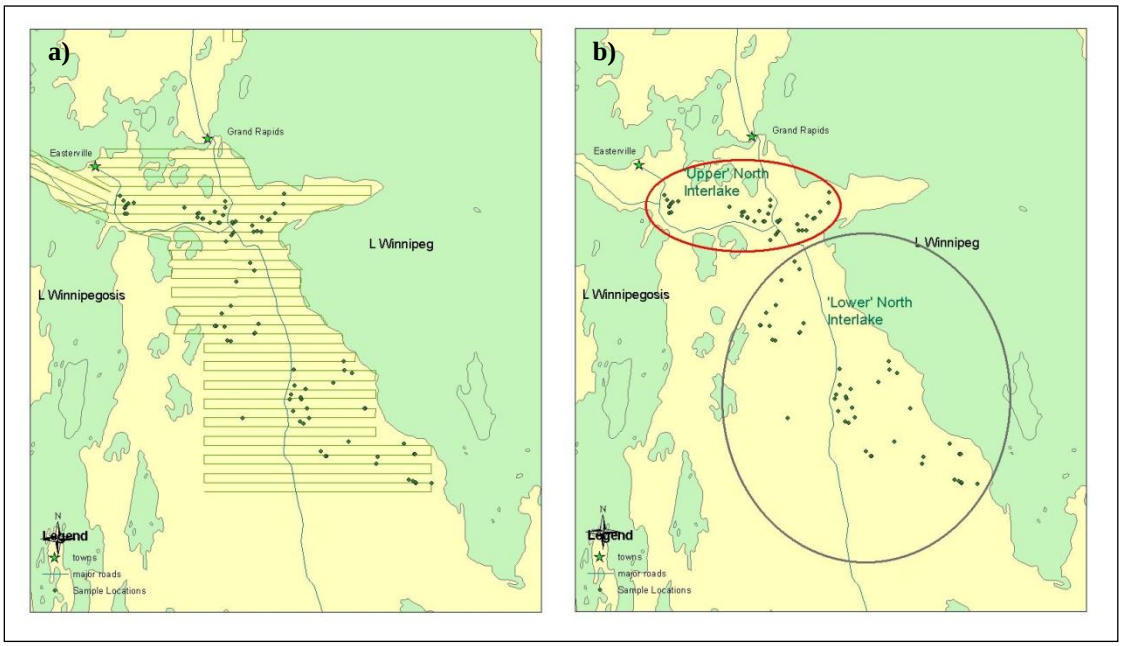

Figure 1 a) Flight lines flown at 3 km intervals over the North Interlake study area and b) delineated Upper and Lower North Interlake sampling areas with sample collection sites 2004-2008

3.2.2 Sampling techniques

The study area was flown systematically by fixed-wing aircraft (Cessna 185 and 337) in winter (between January and March). Sampling began in 2004 until 2008 and consisted of two collections in 2007 (January and February) and 2008 (February and March) and one collection in the February of 2004, 2005 and 2006. Transect lines were flown at three km apart and two observers recorded and mapped the location of cratering sites. Using a helicopter (Bell 206 or Robinson R44), those located cratering sites were sampled for pellet samples.

Multiple protocols were put in place to collect high quality samples for use in genetic analyses. Typically a minimum of ten pellets were collected per sample and, to reduce the potential of collecting pellets from multiple animals in each sample, pellets selected were often those frozen together as 'pucks' (rather than loosely dispersed pellets). An effort was also made to collect fresh pellet samples (not buried in snow) where possible. Disposable wooden sticks were used to collect samples and samples were placed in sterile bags with digitally produced labels.

At each cratering site, the number of samples collected was approximately 1.4 times the number of woodland caribou thought to have been present at each site. This was determined through an investigation of tracks at each sampling area and, where possible, sightings of actual caribou. All collected samples were frozen at -20°C and sent to the Natural Resources DNA Forensics and Profiling Centre at Trent University in Peterborough, Ontario for genetic analysis.

3.2.3 Genetic analysis

Eight fecal pellets per sample were used for DNA analysis. The protocols used to extract and amplify the DNA from the collected pellet samples are in part outlined in Ball et al. (2007) where the mucosal coat surrounding each fecal pellet was removed. Following quantification of 5ng of target caribou DNA from each sample, the DNA was amplified using nine polymorphic, fluorescent-labelled microsatellite markers. The ten microsatellite loci used in analysis included RT5, RT6, RT9, RT24, RT30, BM888 (Wilson et al. 1997), Map2C, BM848 (Bishop et al. 1994) BMS1788 and RT7 (Cronin et al. 2005). In amplifying available DNA quantities, microsatellite markers were split into four multiplexes; Multiplex 1- RT6, RT9 and RT24, Multiplex 2 – Map2C and BM848, Multiplex 3 – Bms1788, RT7 and Multiplex 4 – BM888, RT5 and RT30.

All polymerase chain reaction (PCR) trials to amplify the 5ng of template DNA were conducted in a 10 µl volume containing 1X PCR buffer, 2.0mM MgCl; 0.02 µg/ml Bovine Serum Albumin, 0.4 µM of each the forward and reverse primers for those listed above, 0.2 µM of each dinucleotide triphosphate and 0.5 unit of Taq polymerase (Invitrogen Life Technologies). The thermocycling process for multiplexes run consisted of 94°C for 5 min, 29 cycles of 94°C for 30 sec, 56°C for multiplexes 1 and 3 (or 60°C for multiplexes 2 and 4) for 30 sec and 72°C for 20 sec followed by a final extension time of 60°C for 45 min.

Genotyping of microsatellite loci following amplification was done independently by three scorers. Identification of alleles was done using GeneMarker® with the assistance of documented scoring protocols, designed by the scorers *a priori*, which included details on expected allele peak morphologies for microsatellite loci amplified,

expected strength of alleles in relative fluorescence units (rfu) and protocols for how to score certain hard-to-score allele morphologies.

When scoring, samples with missing allele information at a locus were scored as a -99. Loci with allele scores of less than 100 rfu or for any reason remaining unscored were given a -99 designation. Scoring results from all three scorers were entered into an online database where differences in the scoring of samples were automatically flagged. Samples flagged for differences at a locus were further reviewed by scorers to determine if consensus could be reached, including in possibly giving the sample a -99 designation. If agreement could not be reached on a questionable locus it also resulted in a -99 score being given. If a sampled genotype retained 3 or more -99 scores it was considered a low quality sample and excluded from further analysis.

3.2.4 Individual identification

Genotype information from the scored samples was compiled in GeneCap (Wilberg and Dreher 2004) and organized into capture histories using a $P_{(ID)sib}$ cutoff of 0.05 for determining matches between genotyped samples (Woods et al. 1999). Samples identified as not having another genetic match after allowing for one or two allele mismatches were reamplified. Novel genotypes mismatching by one or two alleles to another genotype were considered a match in meeting a $P_{(ID)sib}$ cut off of 0.05. A comparison of sampled gender information for matching samples was also done to ensure accuracy in assigned gender information.

3.2.5 Closed population modelling

To estimate population size ($\widehat{N}$) for 2007 and 2008, K-sample models (Otis et al. 1978) were used in program MARK (White and Burnham 1999). In calculating an

estimate of population size for 2007 the February and March 2007 sampling times were incorporated in forming capture histories. Alternately, for the 2008 estimate, the January and March 2008 sampling times were used in forming capture histories.

In calculating population size, two alternate methods were used: model averaging and the Mh(jackknife) model. Using model averaging, three models were tested in MARK and were ranked based on AICc calculations (Burnham and Anderson 2002). The three models tested used different parameterizations of capture probability to determine model fit (Otis et al. 1978). The null model (Mo) uses a single parameter to model capture probability (p) and is constant across all sampling times. The behavioural model (Mb) uses two parameters to model capture probability for animals sampled a single time (p) and those animals subsequently sampled afterwards (c). Lastly, the time effects model (Mt) parameterizes capture probability to be distinct at each sampling time (regardless of an animal's previous capture history). In the use of model averaging to calculate population size, calculated $\widehat{N}$ estimates using these three models were averaged and standard error and 95% confidence intervals were calculated using MARK's unconditional standard error estimate output.

Use of the Mh(jackknife) model (Otis et al. 1978) was done using the CAPTURE application in MARK. The Mh(jackknife) model has been demonstrated in studies utilizing noninvasive genetic sampling as robust to sampling heterogeneity where animals are sampled an unequal number of times and have different capture probabilities (Mills et al. 2000, Frantz et al. 2003). While the Mh(Chao) model is similarly considered robust to this form of sampling variation (Chao 1987), its use has been documented as often too

imprecise; particularly in considering small sample sizes (Frantz et al. 2003, Tredick et al. 2007).

Calculation of population size estimates for 2007 and 2008 were done for the entire North Interlake study area as well as the Upper and Lower North Interlake genetic clusters. Estimates were calculated for these areas when gender information was pooled (to attain a population-wide estimate) as well as for each sampled gender. Coefficient of variation (CV) for calculated population size estimates was done through dividing estimated standard error by calculated $\hat{N}$ values (White et al. 1982).

To examine estimator accuracy in the use of the Mh(jackknife) model, simulations were done where estimates were calculated with reduced numbers of samples. This analysis was done for the entire North Interlake area and simulations were done using 90, 80, 70, 60 and 50% of available samples in calculating estimates for both 2007 and 2008. For each level of randomization applied, 10 sets of simulated data were run with the results averaged. Randomizations of available samples took place using a custom constructed JAVA program where an equal percentage of samples were removed from each sampling time based on the quantity of samples available (and not as a constant number of samples reduced from both sampling times).

3.2.6 Open population modelling

The estimation of population parameters using open population models often follows use of the Jolly-Seber model which parameterizes apparent survival (Φ) and capture probability (p) (Seber 1982, Williams et al. 2002). The Pradel (1996) model uses a modified version of the Jolly-Seber model to estimate population rate of growth (λ)

based on parameterizations of apparent survival (Φ) and seniority (γ) (a reverse derivation of Φ) and follows the formula:

$$\lambda_{rea} = \Phi_t / \gamma_{t+1}$$ Pradel (1996),

While calculation of apparent survival (Φ) using mark-recapture models is only of limited biological significance, calculation of population growth rate (λ) is accurate relative to other means of estimating λ, including through use of count data and projection matrices (Nichols and Hines 2002, Sandercock and Beissinger 2002). In the use of the Pradel (1996) model to estimate λ, capture effects have been found to produce biased estimates (Hines and Nichols 2002); indicating the use of NGS may be ideal as capture effects are largely avoided. It has also been shown that estimates of λ using the Pradel (1996) model are not biased by capture heterogeneity given a sufficient number of sampling times, however, variation in calculated capture probability and apparent survival parameters will bias calculated λ estimates (Hines and Nichols 2002).

Through the use of open population models in this study, different parameterizations of apparent survival (Φ) and capture probability (p) were applied in estimating population rate of growth, λ. λ estimates calculated were particular to the entire North Interlake, the Upper and Lower North Interlake genetic clusters and based on gender. Estimates for Upper and Lower North Interlake were calculated separately, instead of using covariate parameters for sampling locations, due to the different number of sampling times done for each.

In fitting sampling data to models, eight different sets of parameter constraints were applied in the estimation of survival (Φ) and capture probability (p) parameters. Population rate of growth (λ) was only constrained to be constant (.) or based on

sampling group i.e. male or female (g). For each alternate constraint placed on λ, 64 models (8^2) were tested. The different parameterizations used in modelling open population parameters and their potential biological meanings are presented in Table 1.

Calculation of population parameters using model averaging was done in MARK to account for model selection uncertainty (Burnham and Anderson 2002). Ranking of models used in constraining λ was done using Akaike Information Criterion for small sample sizes (AICc) (Burnham and Anderson 2002). Calculation of standard errors and 95% confidence intervals were also done using unconditional standard error estimates in MARK (White and Burnham 1999, Burnham and Anderson 2002).

Data from seven sampling times between 2004 and 2008 were used to calculate Φ, p and λ for Upper North Interlake and data from six sampling times between 2005 and 2008 were used in calculating these parameters for North Interlake and Lower North Interlake (due to their absence in being sampled in 2004 when only the Upper North Interlake area was surveyed).

Table 1 Parameter constraints used in open population modelling of woodland caribou herds in the North Interlake, Manitoba

Parameters Estimates	Parameter Constraints
Apparent Survival Φ	(.) t g g*t T g*T g+T g+t
Capture Probability p	(.) t g g*t T g*T g+T g+t
Population Growth Rate λ	(.) g

g – group effect; parameter estimate varies based on group sampled
t – time effect; parameter estimate varies based on capture interval
(.) – null model; no variation for estimated parameter value
g*t – group effect, time effect, interaction between group effect and time effect; parameter estimates will vary by group and time sampled as well between an interaction of these two terms
g+t – group effect, additive time difference; test for time and group effects; estimates for each group calculated using linear slope with different intercept values, no interaction value tested
T – linear time effect; using only an intercept and slope to determine time effect instead of parameter values at each sampling time i.e. the time effects (t) model
g*T – group effect, linear time effect, interaction between group and linear time effect
g+T – group effect, additive linear time effect

3.3 Results

3.3.1 Sampling results

Between 2004 and 2008, 841 samples were collected over seven collection times and of these 791 (94%) were successfully genotyped and used to develop capture histories for sampled animals (Table 2). With the exception of the March 2004 collection,

which was only of the Upper North Interlake area, the number of sites sampled for each collection ranged between 10 (February 2005) and 18 (February 2007). In total, 166 unique genotypes were observed (95 females, 71 males). For each collection time, each animal was sampled an average of 1.9 – 2.8 times; although most animals were sampled only a single or two times in both 2007 (Fig 2) and 2008 (Fig 3).

Table 2 Scoring status following amplification of North Interlake samples

Sampling time	# sites sampled	# samples collected	#scored samples	#unique genotypes			#scored samples / unique genotype
				all	male	female	
March 2004	5	44	43	17	5	12	2.5
February 2005	10	87	85	32	16	16	2.7
February 2006	15	105	105	56	34	22	1.9
February 2007	18	182	165	73	37	36	2.3
March 2007	11	121	114	49	18	31	2.3
January 2008	15	127	118	55	19	36	2.1
March 2008	14	174	153	55	19	36	2.8
TOTAL	88	841	791				

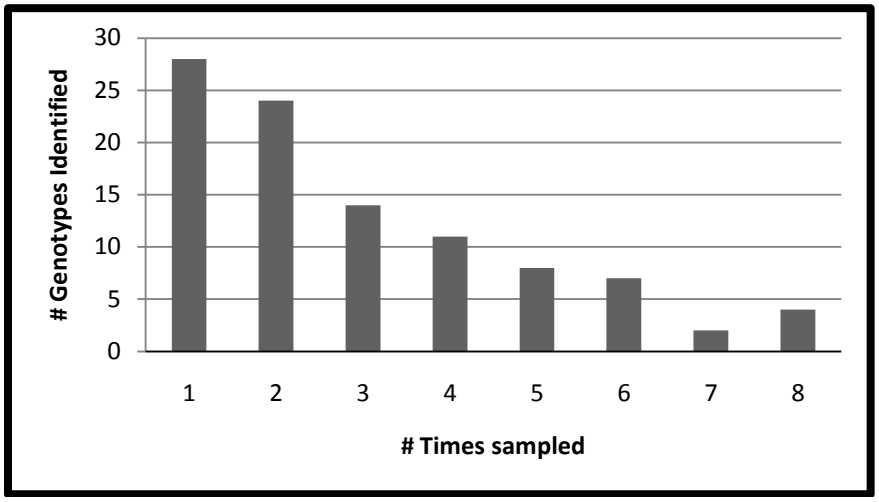

Figure 2 Sampling frequency for genotypes sampled in 2007 from North Interlake

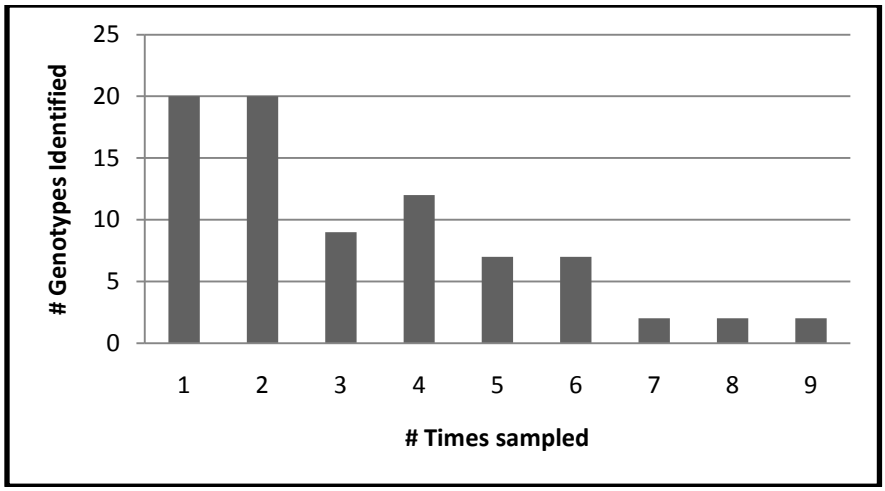

Figure 3 Sampling frequency for genotypes sampled in 2008 from North Interlake

With each sampling time, the proportion of genotypes that had been observed in previous sampling times increased (Fig. 4). An exception to this is the January 2008 sampling time where the number of genotypes resampled was comparable to the number seen in March 2007. In the last sampling time: March 2008, 76% of the unique genotypes obtained had been sampled previously.

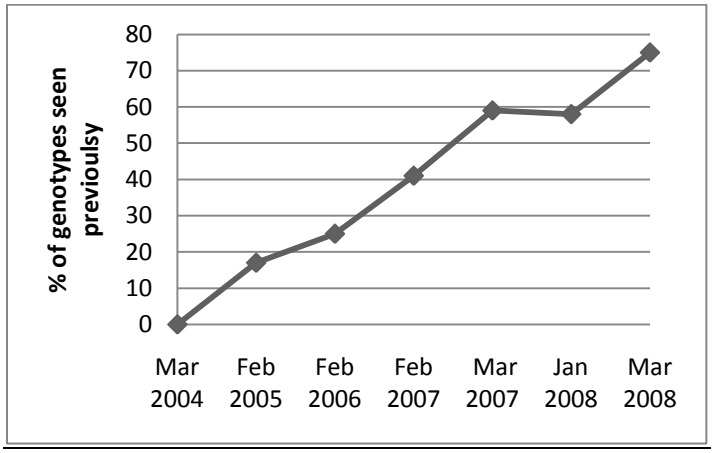

Figure 4 Proportion of previously seen genotypes at each sampling time

3.3.2 Closed population modelling

Population size estimates for North Interlake in 2007 and 2008 varied based on the application of either model averaging or the Mh (jackknife) model (Table 3). Using model averaging, the 2007 and 2008 estimates were 124 (95% CI: 104, 209) and 103 (95% CI: 90, 133), respectively (Table 3). Alternately the Mh (jackknife) models yielded estimates of 134 animals (95% CI: 122, 151) for 2007 and 106 (95% CI: 97, 121) for 2008. The increased variation in the 2007 estimate using model averaging is due to the high degree of differentiation between models tested where 75.5% of the unconditional standard error was due to model variation; compared to 3.82% in 2008.

Table 3 North Interlake woodland caribou population estimates for 2007 and 2008

Sampling Information					Population Estimates							
Year	Sampling group	# unique genotypes			Mh (jackknife)				Model Averaging			
		Time 1	Time 2	# Matches	$\widehat{N}$	SE	95% CI	CV	$\widehat{N}$	SE	95% CI	CV
2007	**All North Interlake**											
	All samples	73	49	24	134	7.5	(122,151)	0.06	124	22.12	(104,209)	0.18
	Males only	37	18	12	57	4.8	(51, 70)	0.08	46	5.7	(44, 78)	0.13
	Females Only	36	31	12	75	5.7	(67, 89)	0.08	92	18.1	(66,145)	0.21
	Lower North Interlake											
	All samples	53	29	13	96	6.5	(86,111)	0.07	88	22.7	(72, 191)	0.26
	Males only	26	10	8	37	3.9	(32, 48)	0.11	31	3.8	(29, 44)	0.12
	Females Only	27	19	5	58	5.2	(51,71)	0.09	80	36.2	(50,225)	0.22
	Upper North Interlake											
	All samples	20	20	11	37	3.7	(33,47)	0.10	35	4.8	(30,108)	0.14
	Males only	11	8	4	19	2.9	(17,29)	0.15	19	5.1	(16,44)	0.28
	Females Only	9	12	7	16	2.3	(15,25)	0.14	15	2.3	(15,29)	0.16
2008	**All North Interlake**											
	All samples	55	55	29	106	6.3	(97, 121)	0.06	103	9.9	(90, 133)	0.10
	Males only	19	19	8	40	4.1	(35, 51)	0.10	43	9.2	(34, 76)	0.22
	Females Only	36	36	21	65	4.7	(59, 77)	0.07	61	5.8	(54, 80)	0.10
	Lower North Interlake											
	All samples	32	37	16	70	5.3	(63,83)	0.08	75	16.3	(59,134)	0.22
	Males only	11	11	4	24	3.2	(21,34)	0.13	27	8.7	(20,81)	0.32
	Females Only	21	26	12	45	4.2	(40,56)	0.09	47	11.7	(36,227)	0.25
	Upper North Interlake											
	All samples	23	18	13	34	2.7	(31,44)	0.08	30	2.7	(29,44)	0.09
	Males only	8	8	4	15	2.4	(13,24)	0.16	15	3.2	(13,30)	0.23
	Females Only	15	10	9	17	2.3	(17,27)	0.14	17	1.1	(17,23)	0.07

For the calculated number of males, using model averaging, calculated $\widehat{N}$ estimates were similar in 2007 with 46 males (95% CI: 44, 78) and in 2008 with 43 males (95% CI: 34, 76). However, using the Mh (jackknife) model, the estimated $\widehat{N}$ varied significantly where, in 2007, 57 animals were estimated (95% CI: 51, 70) compared to 40 animals (95% CI: 35, 51) in 2008.

In estimating $\widehat{N}$ for the number of females in North Interlake using model averaging, the 2007 estimate was much larger at 92 females and was more variable (95% CI: 50, 225) than that for females in 2008 where 61 animals were estimated (95% CI: 54, 80). This added variation in the calculation of $\widehat{N}$ for females in 2007 is likely due to their low recapture rate in February 2007. The $\widehat{N}$ estimate for females using the Mh (jackknife) model was 75 (95% CI: 67, 89) in 2007 and 65 (95% CI: 59, 77) in 2008.

Calculations of $\widehat{N}$ using model averaging and the Mh (jackknife) model indicated that the Lower North Interlake has a larger population of woodland caribou than the Upper North Interlake. Using model averaging the estimated $\widehat{N}$ for animals in Lower North Interlake in 2007 was 88 (95% CI: 72, 191) compared to the 2008 estimate of 75 (95% CI: 59, 134). Using the Mh (jackknife) model to calculate estimates for Lower North Interlake in 2007, an $\widehat{N}$ estimate of 91 was calculated (95% CI: 86, 111) and for 2008 an $\widehat{N}$ estimate of 70 was calculated (95% CI: 63, 83). This indicates the calculated $\widehat{N}$ estimate for 2008, using the Mh (jackknife) model, as significantly less than calculated for 2007; indicating a potential population decline taking place over this period.

In calculating $\widehat{N}$ for Upper North Interlake, using model averaging, an estimate of 35 was calculated (95% CI: 30, 108) whereas, in 2008, an estimate of 30 was calculated

(95% CI: 29, 44). Calculations of $\widehat{N}$, in calculating estimates using the Mh (jackknife) model for Upper North Interlake in 2007 and 2008, had estimates of 37 (95% CI: 33, 47) and 34 (95% CI: 31,44), respectively.

A comparison of calculated estimates using model averaging and the Mh (jackknife) to detect population trends between 2007 and 2008 indicated a generally declining population trend for all sampled groups (Table 4). An exception to this is in the estimation of females in Upper North Interlake using both model averaging and the Mh (jackknife) model.

Table 4 Comparison of 2007 and 2008 calculated population size estimates, $\widehat{N}$

Sampling group		Model Averaging			Mh(jackknife)		
Sampling region	Group	2007	2008	2008/2007	2007	2008	2008/2007
All North Interlake	All samples	124	103	0.83	134	106	0.79
	Males only	46	43	0.93	57	40	0.70
	Females Only	92	61	0.66	75	65	0.87
Lower North Interlake	All samples	88	75	0.85	96	70	0.73
	Males only	31	27	0.87	37	24	0.65
	Females Only	80	47	0.59	58	45	0.78
Upper North Interlake	All samples	35	30	0.86	37	34	0.92
	Males only	19	15	0.79	19	15	0.79
	Females Only	15	17	1.13	16	17	1.06

Calculated $\widehat{N}$ estimates done using the Mh (jackknife) model where reduced sample sizes were simulated through a reduction of available samples by 10 – 50%, indicated a growing negative bias in $\widehat{N}$ estimates relative to the number of samples used (Fig 5a). 95% confidence intervals calculated for simulations indicated that a reduction of samples by 40% or 50% could lead to significantly different results with confidence limits negatively bias to originally produced point estimates. In calculating an estimate for all animals in 2007, when only 60% of available samples were used the original point estimate of 134 was not included in calculated 95% confidence intervals. This was

similarly seen in the 2007 estimation of males and females when 50% and 60% of available samples were used, respectively.

Simulations done in calculating $\widehat{N}$ for the number of males and females in 2008 using reduced sample sizes also indicated a reduction in estimator accuracy as sample size decreased (Fig 5b). In estimating $\widehat{N}$ for males, reducing available samples by 50% resulted in the calculated 95% confidence intervals not including the original point estimate of 43. Simulations done for females and both genders combined indicated that when 60% of available samples were used, calculated 95% confidence intervals did not include the original point estimates of 65 and 106 animals, respectively.

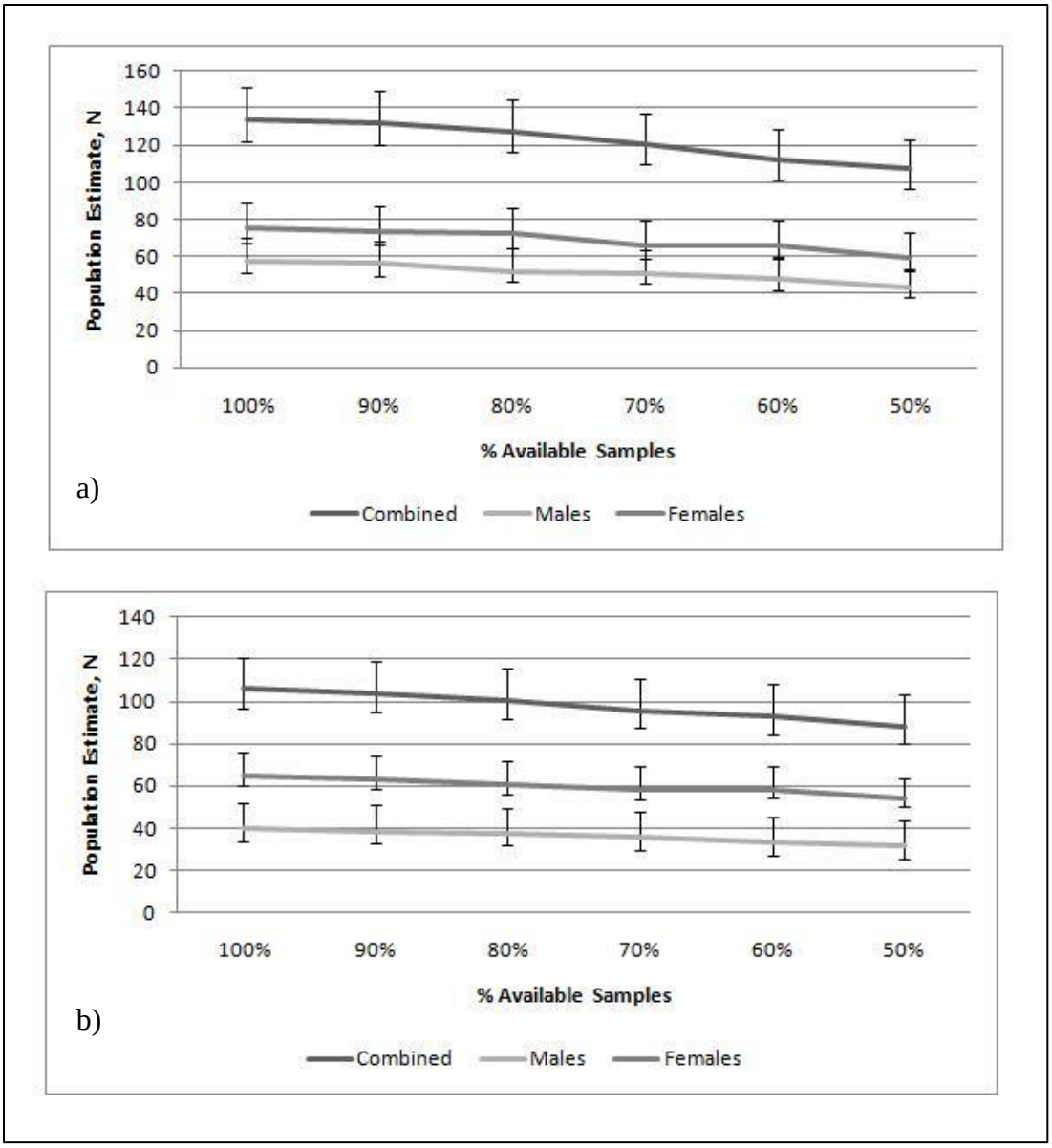

Figure 5 North Interlake population estimate simulations where samples used in analysis were randomly reduced in calculating a) 2007 and b) 2008 estimates

3.3.3 Open population modelling

The top models selected in parameterizing apparent survival (Φ), capture probability (p) and estimated rate of population growth (λ) values showed some consistency in models selected for North Interlake (Table5), Lower North Interlake (Table 6) and Upper North Interlake (Table 7).

Table 5 Top 10 models selected using Pradel (1996) model in program MARK for North Interlake woodland caribou sampling data 2005-2008

Models tested where λ = .				Models tested where λ = g			
Model	AICc	ΔAICc	AICc Weight	Model	AICc	ΔAICc	AICc Weight
Φ(.) p(g*t) λ (.)	1141.431	0	0.20514	Φ(.) p(t) λ (g)	1140.887	0	0.28088
Φ(.) p(g+t) λ (.)	1141.639	0.2076	0.18492	Φ(g) p(t) λ (g)	1142.423	1.5358	0.13032
Φ(.)p(t) λ (.)	1141.779	0.3478	0.1724	Φ(.) p(g+t) λ (g)	1142.631	1.7439	0.11744
Φ(g) p(g+t) λ (.)	1143.12	1.6888	0.08817	Φ(.) p(g*t) λ (g)	1142.9	2.0136	0.10263
Φ(g) p(g*t) λ (.)	1143.184	1.7524	0.08541	Φ(T) p(t) λ (g)	1143.005	2.1186	0.09738
Φ(T) p(g*t) λ (.)	1143.48	2.0488	0.07365	Φ(g) p(g+t) λ (g)	1143.49	2.6037	0.07641
Φ(T) p(g+t) λ (.)	1143.742	2.3106	0.06461	Φ(g+T) p(t) λ (g)	1144.569	3.6823	0.04456
Φ(T) p(t) λ (.)	1143.893	2.4621	0.0599	Φ(T) p(g+t) λ (g)	1144.777	3.8905	0.04015
Φ(T) p(g*T) λ (.)	1144.477	3.0459	0.04474	Φ(T) p(g*t) λ (g)	1144.94	4.0529	0.03702
Φ(g+T) p(t) λ (.)	1145.984	4.5528	0.02106	Φ(T) p(g*T) λ (g)	1144.952	4.0648	0.0368

Table 6 Top 10 models selected using Pradel (1996) model in program MARK for Lower North Interlake woodland caribou sampling data 2005-2008

Models tested where λ = .				Models tested where λ = g			
Model	AICc	ΔAICc	AICc Weight	Model	AICc	ΔAICc	AICc Weight
Φ(.) p(t) λ (.)	696.5702	0	0.1959	Φ(.) p(t) λ (g)	695.852	0	0.3445
Φ(.) p(g+t) λ (.)	696.6834	0.1132	0.18512	Φ(g) p(t) λ (g)	697.5253	1.6733	0.14922
Φ(.) p(g*t) λ (.)	697.3048	0.7346	0.13568	Φ(T) p(t) λ (g)	697.8514	1.9994	0.12677
Φ(g) p(g+t) λ (.)	697.9429	1.3727	0.09862	Φ(.) p(g*t) λ (g)	698.1536	2.3016	0.10899
Φ(T) p(g+t) λ (.)	698.1951	1.6249	0.08694	Φ(g+T) p(t) λ (g)	698.7155	2.8635	0.0823
Φ(T) p(t) λ (.)	698.546	1.9758	0.07295	Φ(T) p(g*t) λ (g)	699.1297	3.2777	0.0669
Φ(g) p(t) λ (.)	698.7709	2.2007	0.06519	Φ(T) p(g+t) λ (g)	699.3923	3.5403	0.05867
Φ(g+T) p(g+t) λ (.)	698.8861	2.3159	0.06154	Φ(g) p(g*t) λ (g)	700.4634	4.6114	0.03434
Φ(g) p(g*t) λ (.)	699.1059	2.5357	0.05513	Φ(g+T) p(g*t) λ (g)	701.2272	5.3752	0.02344
Φ(T) p(g*t) λ (.)	699.6065	3.0363	0.04293	Φ(t) p(t) λ (g)	704.3732	8.5212	0.00486

Table 7 Top 10 models selected using Pradel (1996) model in program MARK for Upper North Interlake woodland caribou sampling data 2004-2008

Models tested where λ = .				Models tested where λ = g			
Model	AICc	ΔAICc	AICc Weight	Model	AICc	ΔAICc	AICc weight
Φ(.) p(T) λ (.)	515.3496	0	0.18894	Φ(.) p(T) λ (g)	517.0055	0	0.30597
Φ(t) p(.) λ (.)	515.4865	0.1369	0.17644	Φ(g*t) p(T) λ (g)	518.11	1.1045	0.17613
Φ(g*t) p(T) λ (.)	515.775	0.4254	0.15274	Φ(g) p(T) λ (g)	518.8692	1.8637	0.1205
Φ(g) p(T) λ (.)	516.7854	1.4358	0.09216	Φ(.) p(g*T) λ (g)	519.3417	2.3362	0.09514
Φ(T) p(T) λ (.)	517.0753	1.7257	0.07972	Φ(t) p(T) λ (g)	519.4488	2.4433	0.09018
Φ(t) p(T) λ (.)	517.3367	1.9871	0.06996	Φ(T) p(.) λ (g)	519.6389	2.6334	0.08201
Φ(.) p(g+T) λ (.)	517.4977	2.1481	0.06454	Φ(.) p(g+T) λ (g)	520.8079	3.8024	0.04571
Φ(g+t) p(.) λ (.)	517.5501	2.2005	0.06288	Φ(g+T) p(.) λ (g)	521.5908	4.5853	0.0309
Φ(t) p(g) λ (.)	517.6565	2.3069	0.05962	Φ(T) p(g) λ (g)	521.8144	4.8089	0.02763
Φ(g*t) p(g+T) λ (.)	517.8912	2.5416	0.05302	Φ(g+t) p(g) λ (g)	521.95	4.9445	0.02582

The weighting of alternate models tested for North Interlake and Upper and Lower North Interlake indicated there not being a single parsimonious model with 0.34 observed as the highest weighting of a tested model. For the entire North Interlake study area, examination of models selected in fitting capture history information indicated that the best fit model varied based on constraints on λ where the Φ (.) p(g*t) model was selected when λ was constrained to be constant (.) and the Φ (.) p(t) model was selected when λ was constrained to give gender specific estimates (g). Alternately, Upper and Lower North Interlake best fit models had the same parameterizations when λ was alternately constrained with the Φ (.) p(T) and Φ (.) p(t) models selected, respectively.

In the use of model averaging to estimating capture probability, p, calculations for North Interlake (Fig 6a), Lower North Interlake (Fig 6b) and Upper North Interlake (6c) varied based on the interpretation of male and female capture histories. For Upper North Interlake calculated point estimates increased at each sampling time and were similar between males and females with calculated confidence intervals only varying slightly. The calculated capture probability estimate for females in North Interlake in February 2005 was 0.17 (90% CI: 0.02, 0.32) significantly less than estimated capture probability values in February 2007 of 0.46 (90% CI: 0.34, 0.58), January 2008 of 0.52 (90% CI: 0.41, 0.64) and February 2008 of 0.54 (90% CI: 0.41, 0.67). Similarly, capture probability for females in Lower North Interlake in March 2005 varied significantly at the above listed sampling times also.

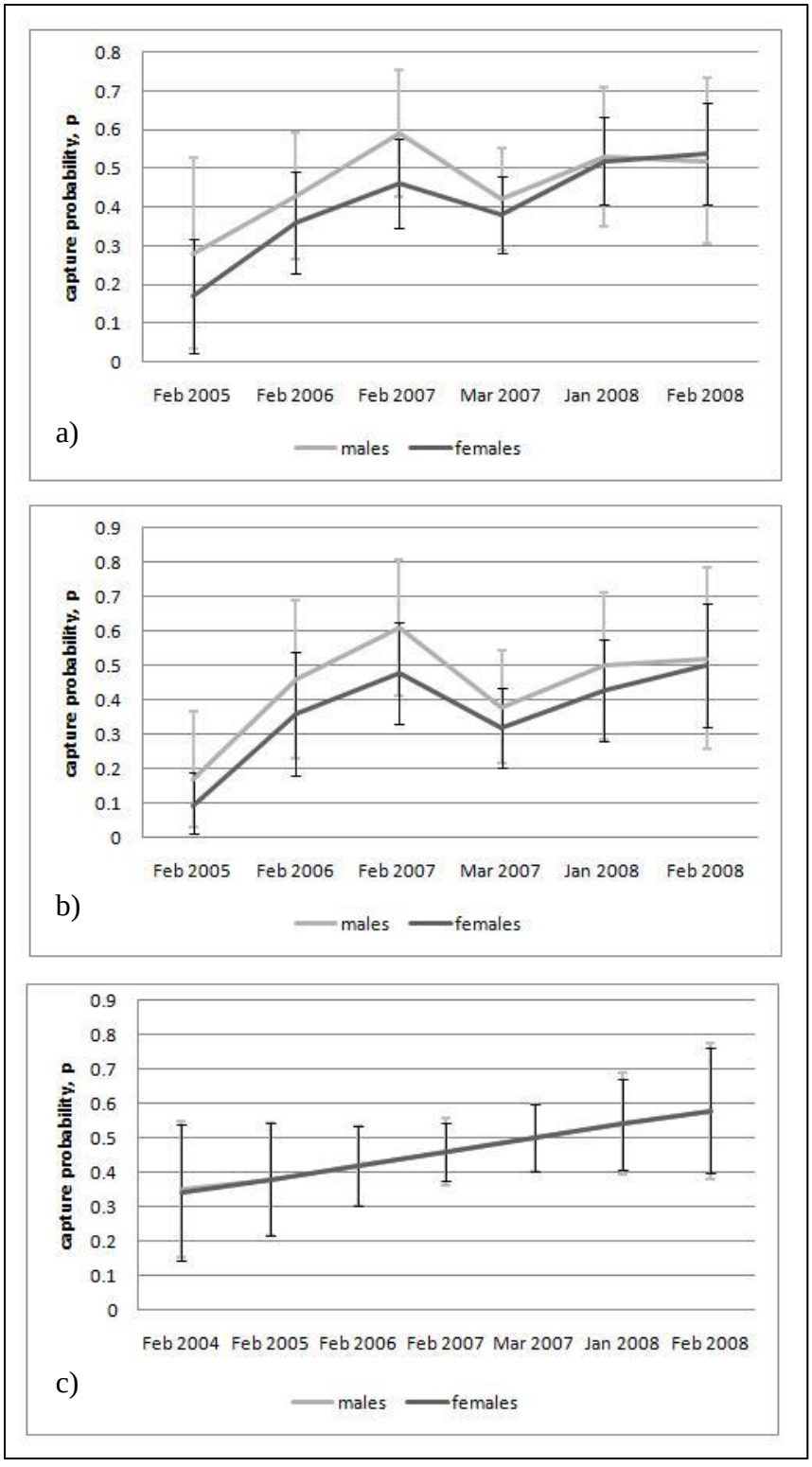

Figure 6 Capture probability estimates with 90% confidence intervals for sampled woodland caribou herds in a) North Interlake b) Lower North Interlake and c) Upper North Interlake

Calculated estimates of apparent survival, Φ, indicated varying estimates for male and female animals in North Interlake (Fig 7a), in Lower North Interlake (Fig 7b) and Upper North Interlake (7c). Calculated values of apparent survival appeared much less variable when calculated for North Interlake relative to Lower North Interlake and Upper North Interlake. For Lower North Interlake although calculated apparent survival estimates did not significantly vary based on sampling times, the estimates for March 2007 and 2008 were highly variable. Apparent survival estimates for Upper North Interlake were also highly variable, particularly in the estimation of the apparent survival for males in March 2007 and for females in February 2008.

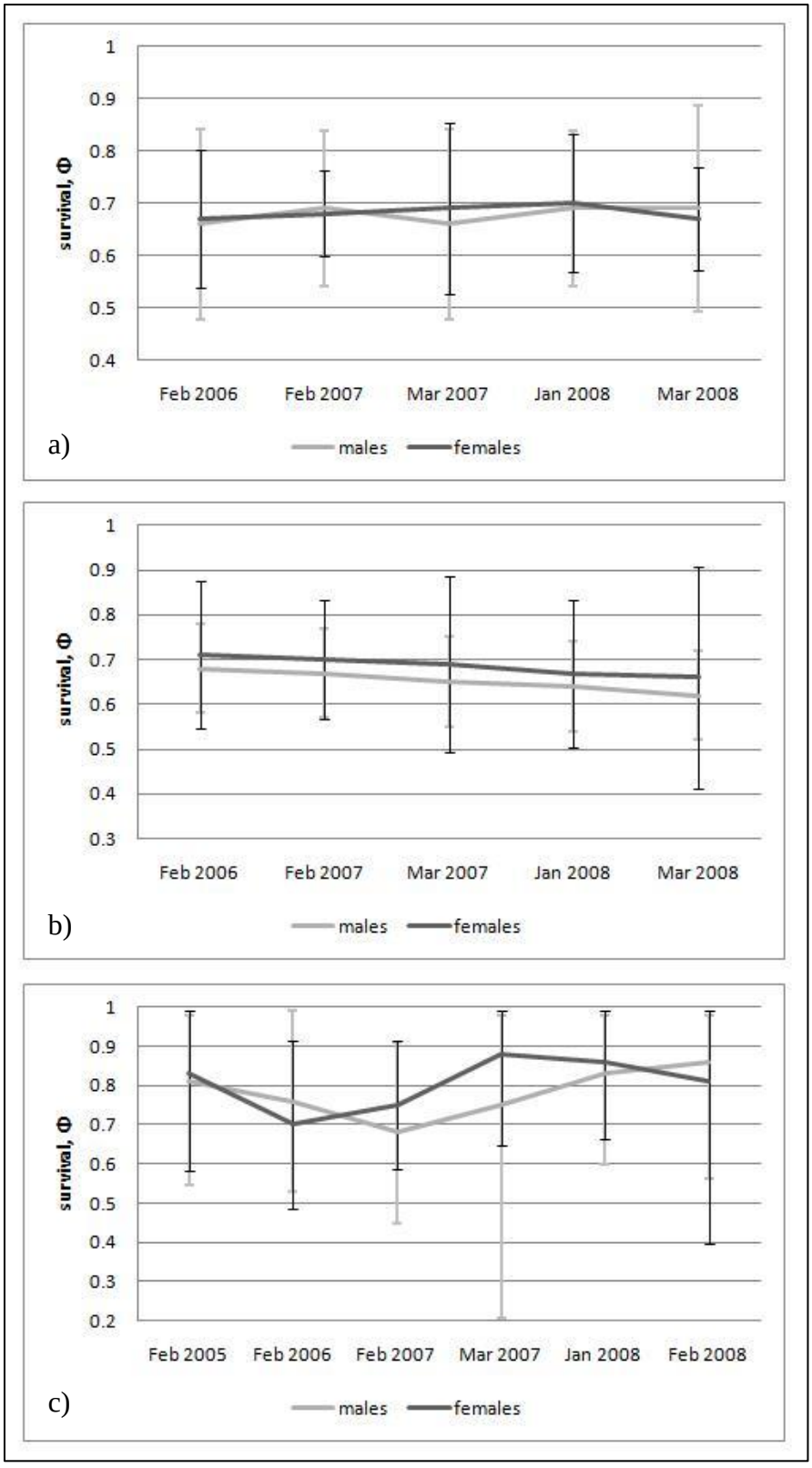

Figure 7 Apparent survival estimates with 90% confidence intervals for sampled woodland caribou herds in a) North Interlake b) Lower North Interlake and c) Upper North Interlake

Model averaging was used in calculating λ and estimates varied based on sampling area and in the calculation of gender-specific estimates (Table 8). Calculated λ estimates for North Interlake indicated a declining population where λ = 0.83 (90% CI: 0.65, 1.02) for all animals, 0.80 for males (90% CI: 0.54, 1.06) and 0.86 for females (90% CI: 0.67, 1.06). For Lower North Interlake, λ was estimated at 0.87 (90% CI: 0.62, 1.12), the estimate for females was 0.93 (90% CI: 0.64, 1.22) and for males was 0.82 (90% CI: 0.45, 1.20). Alternately, for Upper North Interlake, λ was estimated at 0.90 (90% CI: 0.73, 1.06), the estimate for females was 0.86 (90% CI: 0.70, 1.02) and for males was 0.93 (90% CI: 0.71, 1.15).

Table 8 Lambda calculations for sampled woodland caribou herds in North Interlake and Lower North Interlake (2005-2008) and Upper North Interlake (2004-2008)

Sampling Area	Population	Population Rate of Growth, λ			
		Estimate	SE	90% CI	CV
North Interlake	combined	0.83	0.11	(0.65,1.02)	0.14
	males	0.80	0.16	(0.54,1.06)	0.20
	female	0.86	0.12	(0.67,1.06)	0.14
Lower North Interlake	combined	0.87	0.15	(0.62,1.12)	0.17
	males	0.82	0.16	(0.45,1.20)	0.15
	females	0.93	0.12	(0.67,1.06)	0.14
Upper North Interlake	combined	0.90	0.10	(0.73, 1.06)	0.11
	males	0.93	0.10	(0.71,1.15)	0.10
	females	0.86	0.10	(0.70,1.02)	0.12

3.4 Discussion

This study utilized a robust-design sampling approach (Pollock 1982) where population demographic estimates were calculated using closed and open population models. In the use of mark-recapture models, following model assumptions reduces bias in calculated estimates and increases their integrity for use in wildlife monitoring and management settings (White et al. 1982, Williams et al. 2002).

In examining the applicability of demographic estimates calculated in this study to monitoring woodland caribou herds, careful consideration was paid to how model assumptions were met. Population rate of growth, λ, and population size estimates, $\widehat{N}$, calculated using open and closed population models, respectively, indicated that, as of 2008, North Interlake had approximately 106 woodland caribou and was experiencing negative population growth.

3.4.1 Closed population modelling results

Closed population modelling assumptions – population closure

As a general indicator of population closure, the use of genetic clustering techniques (Ball et al. 2010) and landscape modelling work (Manseau et al. 2002, Fall et al. 2007) indicated genetic and landscape barriers to movement for caribou in the North Interlake. Movement between clusters defined in Ball et al. (2010) is increasingly unlikely as the extent of habitat fragmentation in the North Interlake area through fires, logging and human development continues (Manseau, unpublished data). In examining sampled genotypes from Upper and Lower North Interlake 2004-2008, there were only two instances of animals captured in both areas; two animals that were originally sampled in Upper North Interlake subsequently sampled in Lower North Interlake. This lack of movement between Upper and Lower North Interlake may be due to the intersection of two major highways in the area which could reduce movement rates between these areas. Movement between the Upper North Interlake animals and the more northerly Bog population remains a possibility (Ball et al. 2010) although is considered limited; particularly between sampling times used in estimating $\widehat{N}$.

In examining the potential for mortality of animals to occur between sampling times, there remains a distinct possibility that some animals may die between sampling times and therefore be removed from sampling. This could serve to positively bias population estimates and increase variability in calculated confidence intervals through a loss of 'marked' animals. However research on woodland caribou has shown low woodland caribou mortality rates in January and February relative to other months (Seip 1992, McLoughlin et al. 2003), indicating that if mortalities were occurring the amount of bias produced would be less than if sampling was occurring at other times of the year.

Closed population modelling assumptions – no tag loss or misreading of tags

Using NGS with mark-recapture models avoids problems associated with tag loss where a marked animal may lose its tag or have its tag misread. However, some consideration should be given to the possibility of adding animals into a population through the creation of false genotypes/marks which could introduce bias into calculated $\hat{N}$ estimates (Mills et al. 2000, Waits and Leberg 2000, Creel et al. 2003). The amplification and scoring protocols used in this study in acquiring genetic identities is robust to this possibility through quantification of available DNA (Ball et al. 2007), the reamplification of genotypes which only appear once in sampling, the scoring of unique genotypes multiple times and the culling of low quality samples (Paetkau 2003).

Closed population modelling assumptions – equal catchability

Equal catchability of animals is an assumption of closed population models which can be mitigated by the use of alternate models where capture probability is alternately

parameterized based on sampling covariates (Otis et al. 1978, White et al. 1982). This is likely required in this study as, in conducting the surveys over 2007 and 2008, the number of caribou successfully 'marked' and 'recaptured' was quite variable and not directly related to the number of samples collected.

For the January and March 2008 sampling times, 55 and 54 unique genotypes were obtained from 118 and 172 samples, respectively. Alternately, for the February and March 2007 sampling times, 65 and 49 unique genotypes were obtained from 178 and 122 samples, respectively. As it is an assumption that all animals have the same capture probability, it is ideal that roughly equal numbers of genotypes will be sampled at each sampling time. While this did occur in 2008, it did not occur in 2007 and may precipitate the need for alternate models to account for variation in capture probability in the sampling of woodland caribou in North Interlake.

Model use

In the calculation of $\widehat{N}$ in this study when recapture rates were high, such as in 2008 in the North Interlake, model averaging methods produced estimates that could be considered viable in a management context in studying large ungulates (calculated CV of 0.10) (Murray and Fuller 2000). Alternately, the calculated 2007 $\widehat{N}$ estimate, using model averaging, had a high calculated CV value (0.18) and is likely due to poor recapture rates. Alternately, $\widehat{N}$ estimates calculated using the Mh (jackknife) model had calculated CV values of 0.06 for 2007 and 2008; despite poor recapture rates in 2007. Simulations done in this study also indicated that estimates calculated using the Mh (jackknife) model were relatively accurate when available samples were reduced by up to 40%.

The reason for the relative imprecision of calculated estimates using model averaging is the variation between modelling results where alternate models were tested. For 2007 the behavioural model, Mb, produced estimates with a very high degree of precision but with point estimates considerably different than the null, Mo, and time effects, Mt, models. This deviation between calculated $\widehat{N}$ estimates and associated 95% confidence intervals led to a large amount of added in variation when $\widehat{N}$ estimates were averaged as per the model averaging formula used. Alternately, in 2008, all three models yielded roughly equivalent $\widehat{N}$ estimates, with the null model (Mo) selected as the best-fit model, where no considerable added in variation was observed after model averaging was applied (Hettinga, unpublished results).

Sample stratification

The relative sampling success apparent in the sampling of animals in estimating $\widehat{N}$ over a large area can be inferred through repeated analysis following the stratification of various sampling groups (Boulanger et al. 2004, Mulders et al. 2007, Harris et al. 2010). In this way, variance components can be more closely assessed based on stratified groups instead of sole consideration of a larger 'everything-in' type estimate. Stratification of available samples can also be used in finding estimates particular to sampled groups which may also be of use in monitoring and management scenarios (Mulders et al. 2007, Harris et al. 2010).

The division of samples based on gender was an intuitive method of dividing samples which has been done in other studies utilizing NGS to estimate population parameters (Boulanger et al. 2004, Mulders et al. 2007). In estimating the number of

females in North Interlake in 2007 and 2008, when considering model averaging, a high CV value of 0.18 was calculated; identical to when all animals were included in analysis. For the estimation of $\widehat{N}$ for males, however, the calculated CV value was considerably lower at 0.13. This difference in calculated CV values based on gender groupings highlights the possibility of monitoring population demographics through looking at specific groups when sampling of the population as a whole has been less successful.

Similarly, using model averaging with samples stratified by gender, in the estimation of population size for 2008, the calculated CV value tied to the estimation of males was high (0.22) relative to females (0.10) and all animals combined (0.10). As females comprise a larger portion of the North Interlake population, as indicated by calculated estimates, the use of this sole demographic in monitoring overall population health has validity. In particular, an estimate of the number of females is important in assessing the health of ungulate populations where changes in population growth are sensitive to the number of adult female animals (Gaillard et al. 1998). This also corresponds to typical monitoring efforts used in the study of woodland caribou where it is predominately female animals that are radiocollared and studied.

Dividing available samples based on genetic cluster also had useful monitoring applications. Using model averaging to estimate population size for the Lower and Upper North Interlake regions in 2007, it was observed that calculated CV values varied based on sampling group where CV for Lower North Interlake was high (0.26) relative to Upper North Interlake (0.14) and combined North Interlake (0.18). As Upper North Interlake is the smaller of the two genetic clusters, based on calculated $\widehat{N}$ estimates, the use of this estimate has validity in assessing demographic changes in what is the more

'at-risk' grouping due to inverse density dependent effects (Wittmer et al. 2005b). In the estimation of $\widehat{N}$ in 2008, using model averaging, this trend was again repeated where calculated CV for Upper North Interlake was lower (0.09) than that estimated for Lower North Interlake (0.22) and the combined North Interlake (0.10). The relative ability of biologists to sample this area could be due to its smaller geographic size and their familiarity with it; which would also be indicated by a calculated linear increase in capture probability occurring 2004-2008.

A combination of using both sampled gender and genetic cluster information provides another method of stratifying sampling information to assess sampling success and monitor demographic changes. In assessing $\widehat{N}$ estimates for females in Lower North Interlake in 2007 a high CV of 0.45 was calculated; beyond that of other stratified sampling groups. Similarly in Lower North Interlake in 2008 $\widehat{N}$ for females and males were high at 0.25 and 0.32, respectively. This would indicate the relative difficulties in sampling the Lower North Interlake where low recapture rates may affect the precision of calculated $\widehat{N}$ estimates. This may be a result of the geographically large sampling area or because of variation in environmental conditions where sampling is prohibitive i.e. not enough snow at certain sampling times making caribou tracks and cratering areas difficult to find.

Calculated $\widehat{N}$ estimates for males in Upper North Interlake having calculated CV values higher than for females in 2007 (0.28 vs. 0.16) and 2008 (0.23 vs. 0.07) may be due to the relative difficulties in sampling male animals in this area. Notably, there being a low number of male animals (< 20) may make use of mark-recapture models prohibitive due to demographic stochasticity where small changes in sampling frequency

are attributable to large changes in variability (Morris and Doak 2002). In the estimation of $\widehat{N}$, instances of demographic stochasticity may preclude the use of mark-recapture models where instead other methods, such as population census techniques, could be alternately considered.

3.4.2 Open population modelling

In the estimation of population parameters using open population models, the violation of model assumptions will bias calculated estimates and undermine their use for monitoring sampled populations. Violations which bias calculations from open population models includes equal catchability, tag loss or misreading of identification tags, approximately equal chances of survival between sampling times, all emigration being permanent, sampling as instantaneous and all animals being sampled independently (Williams et al. 2002). How modelling assumptions were met in this study was reviewed in detail.

Open model assumptions – equal catchability

Similarly to closed population modelling, the assumption of equal catchability is important in ensuring that all animals have the same chance of being sampled at each sampling time. Stratification of available samples based on sampled genetic cluster and gender information indicated that the Upper North Interlake genetic cluster had higher recapture rates than Lower North Interlake and males tended to have higher recapture rates than females; particularly in Lower North Interlake. As it has been indicated that unequal capture probability rates can affect calculated λ estimates, violation of this assumption was a concern in this study (Hines and Nichols 2002).

Open model assumptions – equal chance of survival between sampling times

The estimation of apparent survival is indicative of losses to the population through both deaths and emigration with the assumption that 'survival' should not deviate between samples times. As there is little movement occurring to and from our sampling area (Ball et al. 2010), a large part of this assumption is being met *a priori* where estimates of apparent survival should closely match actual survival. While there would be expected to be higher mortality rates between sampling times occurring over longer sampling intervals i.e. February 2007 to January 2008 than sampling times closer together i.e. January 2008 to February 2008 the intervals are weighted. Variation in calculated apparent survival estimates can lead to the calculation of biased λ estimates and was a concern in this study (Hines and Nichols 2002).

Open model assumptions – no tag loss or misreading of tags

Similar to the use of closed population models, the importance of reliably identifying sampled animals is important in reducing bias surrounding the calculation of population estimates. In the sampling of animals using NGS, particularly over the number of sampling times used in this study, animals were often sampled multiple times with strict protocols used in ensuring animals were correctly identified. In addition the lab protocol of reamplifying any genotype encountered only a single time also ensured those animals sampled and identified was being done correctly.

Open model assumptions – emigration is permanent

As there is expected to be little migration occurring between North Interlake and neighbouring caribou populations to the north and northeast (Fall et al. 2007, Ball et al. 2010), this assumption was largely met *a priori*. Also sampling of the North Interlake was done such that the extent of the range was systematically surveyed. A good example of this is the area west of provincial highway #6 in the area close to Lake Manitoba and the southern extent of the surveyed range where, despite no animals being observed in this area, it was continually surveyed for caribou signs and potential sampling opportunities.

Open model assumptions – sampling is instantaneous

Within this study there was the risk of sampling older fecal pellets as part of collections occurring. However sampling protocols dictated that a fresh layer of snow is present on the ground to allow biologists to survey sites of recent caribou activity for pellet samples used in analysis. The sampling protocol of also sampling fecal pellets on the surface of the snow, rather than buried underneath, would have also ensured the sampling of 'fresh' pellet samples as opposed to older ones which could have existed in the area and been sampled at earlier sampling times.

Open model assumptions – all animals are sampled independently

Animals that tend to travel and get sampled together tend not to have independent fates (Williams et al. 2002). The minimum time between sampling times of a month (in the case of 2007) or two months (2008) should be considered adequate in allowing a

population to mix. Use of the Pradel (1996) model to estimate λ has been found to be robust to capture heterogeneity (Hines and Nichols 2002); although because few sampling times were used to estimate λ in this study, the Pradel (1996) model may not be as robust compared to if more sampling times were used (Hines and Nichols 2002).

Open population modelling - estimates

Estimates of population rate of growth, λ, in this study indicated a declining population trend where the threat of extirpation should be considered relatively high. While all calculated λ estimates were not significantly less than 1.00, in the calculation of 90% confidence intervals, there was only a single instance of a calculated point estimate being more than 1.00 (for females in Upper North Interlake). If the calculated λ rates in this study are indicative of actual population rate of growth, they are among the lowest seen in estimates calculated for nine populations in Alberta (range 0.88-1.03) (Alberta Woodland Caribou Recovery Team 2005) and seventeen populations in British Columbia (range 0.82 – 1.03) (Wittmer et al. 2005a). A reason for this may be the inclusion of calves and juvenile animals in this study where other recorded estimates solely considered adult females.

Use of the Pradel (1996) model to estimate λ has been found to be positively biased in its estimation over sampling periods when the number of sampled unmarked animals exceeds those that are marked (Hines and Nichols 2002). In this study it was demonstrated by the fourth sampling time (of six) that sampling frequencies were such that above 50% of animals sampled had been previously genotyped. This highlights the need for increasing the number of sampling times in use of the Pradel (1996) model to

estimate λ. Accordingly, calculated λ estimates for Upper North Interlake may be more accurate than those calculated for Lower North Interlake and the entire North Interlake as more sampling times were used in its calculation.

Variation in calculated capture probability and apparent survival parameters have been demonstrated as potential sources of bias for calculated λ estimates using the Pradel (1996) model (Hines and Nichols 2002). This is in contrast to use of the Cormack-Jolly-Seber model which is more robust to this form of variation as use of the temporal symmetry approach, followed in use of the Pradel (1996) model, requires additional consideration of unmarked as well as marked animals including entry times as calculated through estimation of a seniority (γ) parameter (Nichols and Hines 2002). In this study, as it was demonstrated that calculated capture probability and apparent survival parameters varied substantially based on sampling time, it could be inferred that this was a source of bias for calculated λ estimates in this study.

3.4.3 Management implications

The sampling of woodland caribou fecal pellets and extraction of available DNA for use in mark-recapture modelling is a novel approach in estimating population demographics. Through sampling information on the unique genetic identities of woodland caribou in the North Interlake, a range of mark-recapture models were applied to allow for the estimation of population size and population rate of growth using closed and open population models, respectively. This study indicated that while challenges exist in meeting model assumptions, the use of NGS to collect information on woodland caribou may be a vital management alternative that does not rely on direct sampling techniques.

The stratification of available samples based on gender information and genetic cluster aided in providing group specific estimates and to assess sampling methods. In 2007, where use of model averaging to calculate population size was limited through poor recapture rates, it was determined that viable estimates could still be derived for portions of the sampled population and could be useful in informing on rates of population change when overall sampling success is limited. In the use of the Mh (jackknife) model estimates retained a high degree of accuracy and precision in relation to model averaging methods also applied in this study. However it is recommended that use of the Mh (jackknife) model be done cautiously as it may underestimate the number of animals in a population (Frantz et al. 2003) and model fit cannot be assessed relative to other tested models in MARK.

As woodland caribou are listed as endangered, management of them ought to focus on monitoring population growth. In the continued monitoring of woodland caribou in the North Interlake using NGS, the addition of more sampling years in the formation of capture histories would further decrease the variability in calculated λ estimates for use in monitoring and management. However, the use of sequential population size estimates, using closed population models, may be an alternate way of assessing population growth where fewer model assumptions need to be met and where population growth can still be assessed.

Chapter 4 Use of fecal DNA from woodland caribou herds in Jasper National Park to examine genetic characteristics and estimate population size

Abstract

This study utilized noninvasive genetic sampling of woodland caribou herds in Jasper National Park to provide a means of estimating population sizes. As woodland caribou are listed as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and under the Federal Species at Risk Act (SARA), monitoring population sizes and trends is critical. To test the assumption of population closure and correctly delineate population boundaries, population assignment tests were run to assess the degree of genetic relatedness between sampled herds. Mark-recapture models were applied to samples collected over multiple sampling times from south and north Jasper National Park and for each *a priori* defined herd in the Park. Observed heterozygosity and allele richness estimates were also calculated and compared between herds in the park and to other herds using published results. Genetic diversity was lower for the smaller *a priori* defined herds, Brazeau and Maligne, when compared to the larger Tonquin and A La Peche herds. Population assignment tests indicated a level of migration and geneflow between herds beyond that reported in telemetry studies. Population size estimates calculated for south Jasper National Park, over three sampling years, were similar to those calculated using mark-resight surveys; indicating the potential of this approach in future monitoring efforts. The continued sampling of fecal pellets from woodland caribou populations is recommended as a noninvasive alternative to estimating population size and trends and as a means of studying genetic diversity and gene flow.

4.1 Introduction

The range and occurrence of woodland caribou (*Rangifer tarandus caribou)* has diminished substantially within Canada and the United States (COSEWIC 2002). This is particularly evident at lower latitudes and is attributed to factors including predation (Bergerud 1988, Wittmer et al. 2005b), anthropogenic disturbances (James and Stuart-Smith 2000, Dyer et al. 2001) and climate change (Vors and Boyce 2009). This has prompted management initiatives to further monitor existing caribou populations and the listing of boreal and southern mountain ecotypes of woodland caribou as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2002) and protected under the Species at Risk Act (SARA).

The collection of demographic information on woodland caribou populations is of substantial importance to management and recovery efforts (Thomas and Gray 2002). In particular, assessing population size can be used to infer the overall likelihood of survival for sampled populations as mountain ecotype woodland caribou have demonstrated inverse density dependence where smaller isolated populations have faster rates of population decline (Wittmer et al. 2005b).

While the use of radiocollars to monitor sampled populations can be used as a basis for calculating population size estimates, this technique is not adequate for smaller populations where stochastic factors limit the estimability of parameters (Thompson et al. 1998). Also, as only female animals are collared in monitoring studies, calculated estimates may be biased by sex-biased dispersal; particularly those related to breeding period in the timing of sampling events (Côté et al. 2002).

Genetic information has been used as an alternate means of monitoring wildlife species. In particular, the genetic sampling of woodland caribou has been used to assess genetic diversity levels (McLoughlin et al. 2004, Boulet et al. 2007, McDevitt et al. 2009), examine hybridization between caribou ecotypes (Cronin et al. 2006, McDevitt et al. 2009) and in demonstrating probable landscape barriers to dispersal and geneflow (McLoughlin et al. 2004, Boulet et al. 2007). In addition, in the use of genetic material sampled from noninvasively collected fecal pellets, Ball et al. (2010) was able to detect a previously unclassified population in central Manitoba using population assignment techniques.

4.1.1 Noninvasive genetic sampling

Noninvasive genetic sampling (NGS) is an alternate means of monitoring wildlife populations that minimizes interaction between researcher and study species (Taberlet et al. 1996, Kohn and Wayne 1997). NGS uses DNA present in sampled hair and feces and has been used to estimate population sizes and trends, gene flow, relatedness and population bottlenecks in various species (Kendall and McKelvey 2008). Most of these measures are derived from microsatellite markers where small amounts of sampled DNA are successfully amplified to yield amounts required for analysis while also providing gender information on sampled individuals (Shaw et al. 2003).

In the use of NGS techniques, careful attention must be paid to the quality of sampled genetic material. In the use of DNA from hair and fecal samples, erroneous genotypes can be created due to environmental degradation, contamination between samples, small tissue samples and a lack of robust laboratory protocols (Mills et al. 2000, Waits and Leberg 2000, Paetkau 2003). The creation of erroneous genotypes can have

considerable repercussions in studies involving mark-recapture modelling (Mills et al. 2000, Creel et al. 2003). In particular, the inclusion of erroneous genotypes in mark-recapture modelling, for the purpose of calculating population size, can result in drastic overestimates if left unchecked (Waits and Leberg 2000, Creel et al. 2003).

To protect against potential errors in the genotyping process, robust protocols have been developed to ensure the quality of sampled genotype data (Taberlet et al. 1996, Paetkau 2003). In acquiring woodland caribou DNA using NGS, the sampling of fecal pellets has proven to be a suitable and reliable source of DNA provided precautions are taken to use samples identified as having sufficient amounts of template DNA; which reduces problems associated with allelic dropout and PCR inhibition (Ball et al. 2007). The amplification of DNA from winter-collected fecal pellets of caribou has resulted in higher amplification success rates when compared to summer-collected samples (Ball 2007); results similar to those shown when comparing summer versus winter collected fecal samples from other ungulate species (Maudet et al. 2004).

4.1.2 Mark-recapture modelling

The systematic collection of identity information from sampled animals over multiple sampling times allows for the use of mark-recapture models to estimate various population attributes (Seber 1982). For the purpose of estimating population size, closed population mark-recapture models are often used and require certain model assumptions be met (Otis et al. 1978, White et al. 1982). The most notable assumption is that of population closure accounting for demographic closure, where the population remains unaffected by births and deaths over all sampling times, and geographic closure, where no animals are moving in or out of the study area (Otis et al. 1978, Williams et al. 2002).

These assumptions generally require that sampling done for the purpose of mark-recapture modelling take place over a relatively short temporal scale (Williams et al. 2002).

Stratification of sampling areas is often done to measure the contribution of a portion of available samples to overall estimates and indicate if a portion of the sampled population is not meeting model assumptions (Boulanger et al. 2002, Boulanger et al. 2004, Mulders et al. 2007). Through stratifying available samples based on gender or sampling information estimates of population size can be derived for a portion of a sampled population where the population as a whole does not conform to meeting model assumptions (Boulanger et al. 2004, Mulders et al. 2007, Harris et al. 2010). Through stratifying samples it can also be determined if sampling protocols are suited to continued monitoring efforts or should be revised accordingly (Boulanger et al. 2004).

4.1.3 Population assignment techniques

Sampled genetic information has been used to study dispersal as an alternative to direct sampling techniques where a portion of animals in a population are tracked using radiocollars. Common analyses used to assess dispersal using sampled genetic information are population assignment tests (Berry et al. 2004, Goossens et al. 2005). Using assignment tests, animals are grouped based on genetic similarities (Paetkau et al. 1995). Using population assignment techniques researchers have been able to differentiate populations into distinct genetic clusters that were otherwise considered admixed (Rueness et al. 2003) and, in some cases, demonstrate the extent of migration when interbreeding is occurring (Sacks et al. 2005).

In testing the assumptions of geographic closure used in closed population modelling, the extent of dispersal between populations has important implications. The correct delineation of management units is of interest in defining biological groupings where management efforts can be aimed (Mills 2007). Wrongly identifying a sampled group as being a single large population, where several isolated clusters exist, could lead to the erroneous conclusion that a population is healthy. This is of particular relevance in the study of mountain ecotype woodland caribou where small populations have inverse density dependence effects and are adversely affected by small population sizes (Wittmer et al. 2005b).

4.1.4 Study objectives

The objective of this study is to determine the efficacy of using noninvasive fecal sampling as a means of estimating population size through closed capture-recapture modelling. The use of population assignment tests will be done as an attempt to investigate the extent of dispersal, if any, occurring between the four *a priori* defined herds in Jasper National Park: the Tonquin, Maligne, Brazeau and A La Peche. Various genetic attributes will be measured and compared between herds as well as to those provided in other studies of woodland caribou in Canada. Results from genetic based mark-recapture work will be compared to estimates calculated using mark-resight surveys over the same time periods to test modelling results. Based on study findings recommendations will be made on the continued monitoring and management of woodland caribou herds in Jasper National Park using NGS.

4.2 Methods

4.2.1 Study area

Woodland caribou in Jasper National Park are part of the Southern Mountain woodland caribou population which is listed as threatened by the COSEWIC (2002) and under the Species at Risk Act (SARA). The A La Peche population resides partly in the northern part of Jasper National Park (Edmonds 1988, Dzus 2001, McDevitt et al. 2009) and is considered stable with a calculated rate of population change of 1.07 (95% CI: 1.033-1.118) based on five years of sampling information (Alberta Woodland Caribou Recovery Team 2005).

Currently, it is thought that no movement occurs between the A La Peche herd in the north and the Tonquin, Maligne and Brazeau herds in the south based on telemetry studies and disjunct distributions (Bradley pers. comm.). Within South Jasper National park, using radiocollaring from adult females between 2006 and 2009, no movement has been recorded between the Tonquin population, on the west side of highway 93, with the Maligne and Brazeau populations, on the east side. There has been some recorded instances of animal movement between the Maligne and Brazeau using this same telemetry data (Whittington et al. 2005).

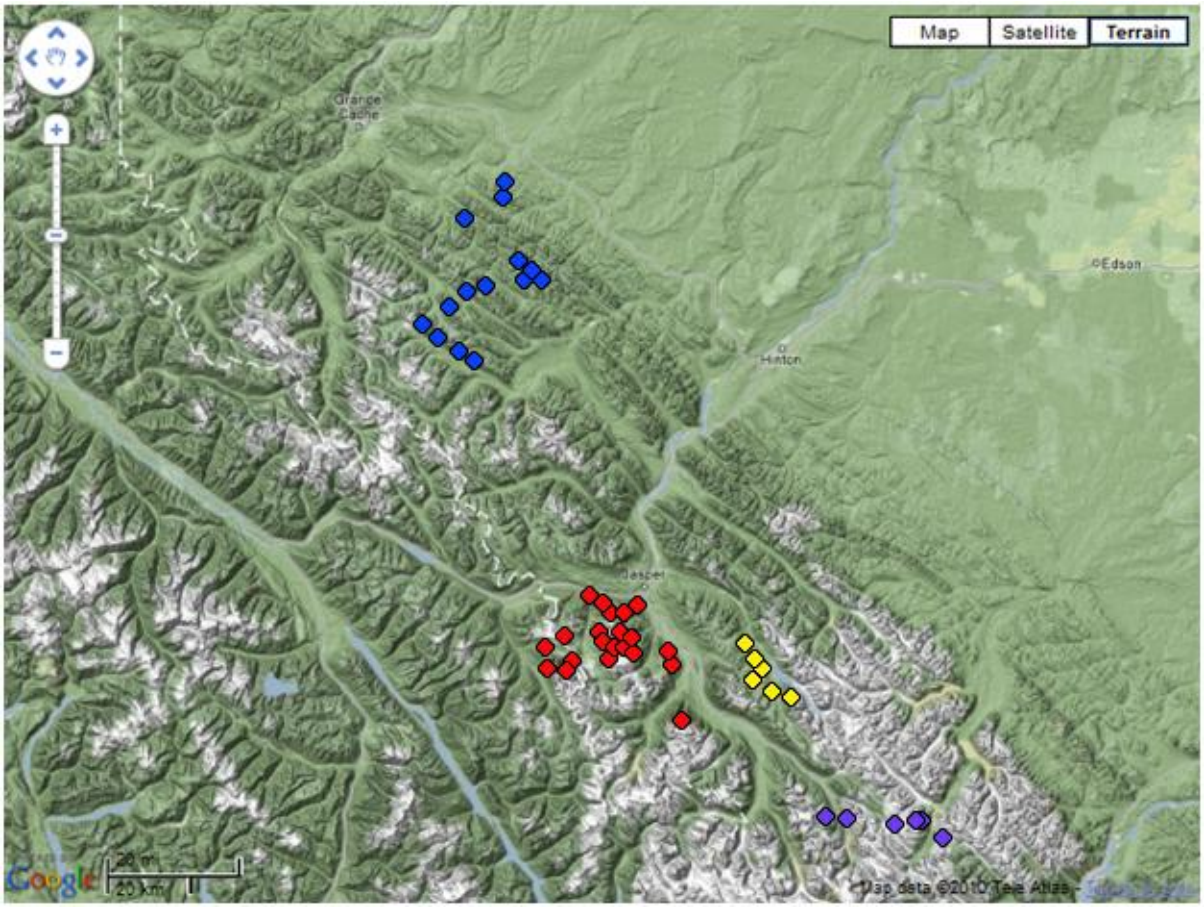

Figure 8 Jasper National Park Sampling Points 2006-2008. Coloured points represent sampled herds Blue – A La Peche, Red – Tonquin, Yellow – Maligne, Purple – Brazeau

Source: ©2010 Google - Map data©2010 Tele Atlas. Copy right information accessed at http://www.google.com/permissions/geoguidelines.html on August 18/2010

Within Jasper National Park it is though that anthropogenic disturbances, habitat loss, and increased predation have caused the fragmentation and decline of resident woodland caribou populations (Dzus 2001, Smith 2004, Whittington et al. 2005, Hebblewhite et al. 2007). Within south Jasper National Park, there are thought to be two distinct subpopulations with little migration and gene flow occurring between them: Tonquin and Maligne-Brazeau (Hebblewhite et al. 2007). More recently, Maligne and Brazeau have come to be considered separately also (Neufeld and Bradley 2007).

Caribou herds in South Jasper National Park have shrunk considerably over the past 40-50 years (Whittington et al. 2005, Parks Canada 2006). In the 1960s estimated population sizes were approximately 450 animals where today's estimates are closer to 100 (Whittington et al. 2005). Following the extirpation of caribou from Banff National Park (Hebblewhite et al. 2009), the south Jasper National Park woodland caribou herds are the most southerly ranging in Alberta.

4.2.2 Sample collection

Collection of fecal pellet samples from the Tonquin, Brazeau and Maligne herds occurred twice in 2006-2007 and 2007-2008; except for the Tonquin herd which was sampled three times in 2007-2008. Sampling of the A La Peche herd in north Jasper National Park occurred twice in 2007-2008. Initial flightlines used in surveying were based on resource selection function mapping done to determine high-quality woodland caribou habitat areas in the Park (Whittington et al. 2005). These areas were surveyed using a Bell 206 Jet Ranger helicopter, with the same flightlines used as a guide in subsequent sampling times.

Each sampling time in the Maligne, Brazeau, Tonquin and A La Peche ranges occurred between October and January and lasted 1-2 days. Exact scheduling of flight-days was based on snow depth and weather conditions. Having sufficient snow on the ground was essential to track caribou and locate cratering areas. Visual observations of caribou during surveys frequently resulted in landing in proximity to them to collect fresh pellet samples; however whether this was done was often based on landing conditions and the availability of samples elsewhere.

To collect pellet samples researchers split off in different directions after landing to collect a predetermined number of samples. The number of samples collected at each sampling location was 1.4x the number of animals suspected to have been at each sampling site and follows previous research done on identifying optimal sampling levels (Manseau pers. comm.).

In collecting pellet samples, sterile disposable tongue depressors were used as scoops to leverage frozen fecal pellet samples into sterilized pre-labelled sampling bags. Pellet samples frozen together as ‘pucks’ or ‘patties’ were preferred to scattered pellets to reduce the potential for collecting pellets from multiple animals in a single sample. Newer samples on the surface of the snow were also preferred to older samples that were buried in snow. After collection, samples remained frozen and were stored at -20°C until DNA extraction.

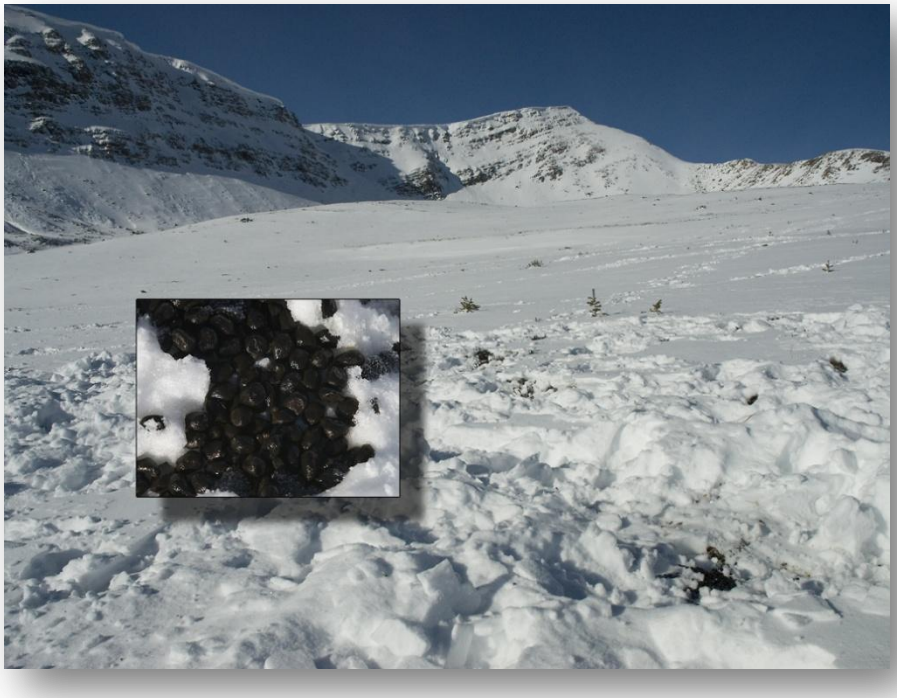

Plate 1 Collection of pellet samples from Maligne range

Used with permission of Mark Bradley on July 27, 2010

Samples were sent to the Natural Resources Profiling and Forensic Centre at Trent University for extraction and amplification of DNA. DNA was extracted from pellet samples through the swabbing of sloughed mucosal cells surrounding each pellet. Extraction and amplification of DNA from fecal pellets took place as per Ball et al. (2007). Typically eight pellets from each sample were used as stock DNA for future genotyping work where reamplification was required.

Following quantification of 5ng of target DNA, each sample was amplified using ten polymorphic, fluorescent-labeled microsatellite markers. The ten microsatellite loci used in analysis included RT5, RT6, RT9, RT24, RT30, BM888 (Wilson et al. 1997), Map2C, BM848 (Bishop et al. 1994), BMS1788 and RT7 (Cronin et al. 2005). In amplifying target DNA, microsatellite markers where split into four multiplexes; Multiplex 1- RT6, RT9 and RT24, Multiplex 2 – Map2C and BM848, Multiplex 3 – Bms1788 and RT7 and Multiplex 4 – RT5, BM888 and RT30.

All polymerase chain reaction (PCR) trials to amplify the 5ng of template DNA were conducted in a 10 µl volume containing 1X PCR buffer, 2.0mM MgCl; 0.02 µg/ml Bovine Serum Albumin, 0.4 µM of each the forward and reverse primers for those listed above, 0.2 µM of each dinucleotide triphosphate and 0.5 unit of Taq polymerase (Invitrogen Life Technologies). The thermocycling process for multiplexes run consisted of 94°C for 5 min, 29 cycles of 94°C for 30 sec, 56°C for multiplexes 1 and 3 (or 60°C for multiplexes 2 and 4) for 30 sex and 72°C for 20 sec followed by a final extension time of 60°C for 45 min.

Scoring of alleles at each loci was done using GeneMarker^{TM} where three people scored all alleles and met to discuss differences in scoring which resulted in either a)

consensus being reached on the sampled alleles or, b) if the sample in question was consistently difficult to score, removal of the sample from downstream analysis. Samples were matched using GeneCap (Wilberg and Dreher 2004) where a $P_{(ID)sib}$ cutoff of 0.05 was implemented (Woods et al. 1999, Waits et al. 2001). Genotypes only identified once were subsequently reamplified at all ten loci to confirm the presence of unique genotypes. Matches between the first and second amplification resulted in the inclusion of the sample as a unique genotype.

4.2.4 Genetic diversity and dispersal indices

To assess the sampled genetic characteristics of sampled herds varying genetic diversity measures were calculated. Allele frequencies and estimates of observed heterozygosity, H_o, and expected heterozygosity, H_e (Hartl and Clark 1989) were calculated using GenAlEx (Peakall and Smouse 2006). $F_{IS,}$ a measure of deviation between H_{obs} and H_{exp} (Weir and Cockerham 1984), was done for each sampled herd using FSTAT (Goudet 1995) where Weir and Cockerham's F_{ST} (1984) was also calculated to show the extent of genetic divergence between sampled herds.

Calculations to determine if sampled genotypes were in Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) were done in GenePop (Raymond and Rousset 1995). To test HWE values, when considered significant at the $p = 0.01$ level, a sequential Bonferroni test was used to validate calculated p-values (Rice 1989).

Structure 2.3 is a software program developed to use a Bayesian clustering algorithm to define K, the number of sampled genetic clusters, which are grouped to maximize HWE (Pritchard et al. 2000, Falush et al. 2003). In the use of program

Structure, Evanno et al. (2005) advised the use of ΔK to inform on the number of genetic clusters instead of interpreting maximum likelihood calculations for calculated K values.

In running Structure 2.3, various settings were applied in accordance with published instructions regarding the analysis of genetic data. Settings used followed the F-model as proposed by Falush et al. (2003) where admixture between populations was assumed as well as correlated allele frequencies. In running Structure 2.3 a burn-in length of 20 000 steps was used in optimizing calculated parameter values where 15 000 additional steps were used in the estimation of parameter values. Ten replications were done of each tested K value where the number of *a priori* defined clusters was set to range from 1 to 10 in accordance with the Evanno et al. (2005) ΔK method.

Using GeneClass 2 (Piry et al. 2004), the Bayesian approach of Rannala and Mountain (R&M) (1997), the frequentist approach of Paetkau (1995) and the calculation of first-generation migrants using the approach of Paetkau et al. (2004) were compiled. GeneClass 2.0 uses algorithms different than those in Structure and does not assign K number of clusters but instead uses *a priori* defined sampling groupings. Using GeneClass 2, sampled allele frequencies are required to be in LD but not in HWE (Paetkau et al. 1995, Rannala and Mountain 1997). Settings used in the application of GeneClass were set with an assignment threshold of $p < 0.01$ and a default frequency default of 0.01 for missing alleles; following the Paetkau et al. (1995) approach.

4.2.5 Population estimation

Population size estimates were calculated for South Jasper National Park by park staff using mark-resight methods (Bartmann et al. 1987, White and Garrott 1990). As this

effort took place in tandem with the collection of fecal pellet samples for use in NGS based mark-recapture estimates, results were compared between the two.

To estimate population size using fecal DNA, the closed-captures option in program MARK was used (White 1996) where the Mh (jackknife) model was selected from the CAPTURE (White et al. 1978) application. Heterogeneity models are generally recommended for use with noninvasive genetic sampling where capture probability varies between sampled animals (Mills et al. 2000, Mowat and Strobeck 2000, Boulanger and McLellan 2001, Frantz et al. 2003). Coefficient of variation (CV) estimates were calculated using SE/$\widehat{N}$ as an indicator of estimator precision (White et al. 1982).

Population size estimates for south Jasper National Park were also calculated, through the stratification of available samples, to yield estimates specific to the *a priori* defined herds (Maligne, Brazeau, Tonquin) and by gender. These calculations were also done using the Mh (jackknife) estimator through the CAPTURE application in MARK. As Tonquin in 2007-2008 was sampled on three occasions estimates were calculated twice: first in considering two sampling times and second in considering three sampling times. Population size for A La Peche was calculated separately from those in South Jasper National Park and was only done in 2007-2008.

4.3 Results

A larger number of samples were collected for Tonquin and A La Peche relative to Maligne and Brazeau (Table 9). There were a number of instances where variability was encountered in the number of samples collected from each herd; particularly for Maligne (2006-2007) where 12 and 38 samples were collected in the October and

December sampling times and for Tonquin (2006-2007) where 132 and 36 samples were collected from the October and November sampling times. In addition, of the 12 samples collected from Maligne in October 2006 only 3 of these could be successfully amplified. For Tonquin (2008-2009) the number of samples was reduced to equal 1.4x the number of samples for animals suspected to have been at each sampling site (in accordance with predefined sampling instructions).

Table 9 Sampling information for sampled woodland caribou herds in Jasper National Park 2006-2009

Sampling Period	Herd	Sampling Time	# sites sampled	# samples collected	#samples used	#unique genotypes		
						All	Male	Female
2006-2007	**Maligne**	Oct 11	2	12	3	2	1	1
		Dec 1	3	38	36	11	5	6
	Brazeau	Oct 17	4	31	20	10	6	4
		Dec 7	3	24	15	10	5	5
	Tonquin	Oct 21	9	132	108	53	21	32
		Nov 30	5	36	29	13	3	10
2007-2008	**Maligne**	Oct 5	2	9	7	3	0	3
		Nov 8	1	12	12	5	2	3
	Brazeau	Oct 4-5	8	45	38	15	8	7
		Nov 20	4	41	37	16	9	7
	Tonquin	Oct 10	5	82	64	29	5	24
		Nov 19	6	64	62	28	8	20
		Jan 23	4	44	41	15	8	7
	A La Peche	Dec 6-7	10	102	97	47	21	26
		Jan 1	8	88	88	40	12	28
2008-2009	**Maligne**	Oct 18	2	7	7	6	2	4
		Nov 7	2	13	12	5	3	2
	Brazeau	Oct 19	1	13	13	6	0	6
		Nov 19	3	22	22	10	6	4
	Tonquin	Oct 21	11	146	106	63	27	36
		Nov 6	8	104	73	31	16	14

The number of unique genotypes identified in 2006-2007 for Maligne, Brazeau and Tonquin was 11, 17, and 60, respectively. The number of unique genotypes identified in 2007-2008 in Maligne, Brazeau, Tonquin and A La Peche was 6, 20, 50 and 71,

respectively. For 2008-2009 the number of unique genotypes was 8, 14 and 75 for the Maligne, Brazeau and Tonquin herds, respectively.

In comparing genotypes from each herd only one genotype (out of 190) was observed in multiple herds. This occurred with the sampling of a male animal in Maligne in October 2006 which was later seen in Brazeau in November 2007.

4.3.1 Genetic diversity

The ten microsatellite loci amplified and scored had between 3 and 12 alleles each (Table 10). The average number of alleles for all sampled loci for each herd was 5 for Brazeau, 5.4 for Maligne, 6.2 for Tonquin and 10.4 for A La Peche.

Table 10 Allele richness estimates for sampled woodland caribou herds in Jasper National Park

		Herd				
		Brazeau	**Maligne**	**Tonquin**	**A La Peche**	**Combined**
Locus	**BM848**	5	5	5	11	12
	BM888	4	6	7	13	14
	BMS1788	5	6	6	11	11
	MAP2C	6	6	7	10	12
	RT5	5	6	7	12	12
	RT6	3	3	5	9	9
	RT7	4	5	5	8	9
	RT9	6	6	8	11	12
	RT24	5	4	6	10	11
	RT30	7	7	6	9	11
Average		5	5.4	6.2	10.4	

HWE calculations indicated sampled allele frequencies for the Maligne, Brazeau and Tonquin herds being in equilibrium for all loci examined. A La Peche, however, significantly deviated from HWE at four loci at the $p \leq 0.01$ level (Table 11). However, after using a Bonferroni correction (Rice 1989), no loci were found to deviate significantly from HWE at the $p = 0.01$ level.

Calculations done to determine if sampled loci were in linkage disequilibrium indicated multiple pair of loci as linked when all populations were pooled. BMS1788 and

RT24, BMS1788 and RT7, BMS1788 and RT6 and BM888 and RT30 were determined to be linked at a highly significant level ($p < 0.001$).

Table 11 Calculated P-values for Hardy-Weinberg equilibrium calculations for sampled woodland caribou herds in Jasper National Park

		Herd				
		Brazeau	**Maligne**	**Tonquin**	**A La Peche**	**All Herds**
Locus	**BM848**	0.20	0.22	0.37	0.09	0.20
	BM888	0.18	0.53	0.28	0.08	0.18
	BMS1788	0.93	0.92	0.61	0.05	0.93
	MAP2C	0.86	0.74	0.77	0.50	0.86
	RT5	0.43	0.06	0.94	0.73	0.43
	RT6	0.28	0.82	0.26	0.34	0.28
	RT7	0.81	0.39	0.75	< 0.01	0.81
	RT9	0.37	0.49	0.71	< 0.01	0.37
	RT24	0.43	0.63	0.99	< 0.01	0.43
	RT30	0.09	0.41	0.29	0.11	0.09
Average		0.43	0.65	0.89	< 0.01	0.43

Calculations of expected heterozygosity, $H_{e,}$ ranged between 0.67 - 0.81 for Maligne, Brazeau, Tonquin and A La Peche (Table 12). A similar range was also reported for observed heterozygosity values, H_o , (0.69 – 0.82). In comparing H_e and H_o values, the Maligne herd had a corresponding F_{IS} estimate of -0.111. The F_{IS} values for Brazeau, Tonquin and A La Peche herds were -0.021, -0.001 and 0.050, respectively. Calculated $P_{ID(sibs)}$ values for the four populations were below the $p < 0.01$ threshold and ranged from 3.7×10^{-4} (Brazeau) to 3.9×10^{-5} (A La Peche)

Table 12 Genetic characteristics of sampled woodland caribou herds in Jasper National Park. Columns in table including: number of sampled animals (N) allelic diversity (Na) observed heterozygosity (H_{obs}) expected heterozygosity (H_{exp}) probability of identity calculations ($P_{(ID)}$ and $P_{(ID)sibs}$) and inbreeding coefficient (F_{IS})

Herd	**N**	**Na**	**H_{obs}**	**H_{exp}**	**P_{ID}**	**$P_{ID(sibs)}$**	**F_{IS}**
Brazeau	26	5	0.69	0.67	5.7×10^{-9}	3.7×10^{-4}	-0.021
Maligne	12	5.4	0.82	0.71	7.7×10^{-10}	1.9×10^{-4}	-0.111
Tonquin	79	6.2	0.74	0.73	2.0×10^{-10}	1.4×10^{-4}	-0.001
A La Peche	71	10.4	0.77	0.81	4.7×10^{-13}	3.9×10^{-5}	0.050
TOTAL	188	6.8	0.76	0.73	2.7×10^{-12}	5.3×10^{-5}	

The use of indirect methods to estimate gene flow between herds, using F_{ST} estimates, indicated moderate levels of genetic differentiation between Brazeau and

Tonquin (0.069) and Brazeau and A La Peche (0.079) and the lowest between Maligne and Tonquin (0.043) and Maligne and A La Peche (0.044) (Table 13).

Table 13 F_{ST} calculations for sampled woodland caribou herds in Jasper National Park

		Herd			
		Brazeau	Maligne	Tonquin	A La Peche
Herd	Brazeau	-	0.052	0.069	0.079
	Maligne	-	-	0.043	0.044
	Tonquin	-	-	-	0.054
	A La Peche	-	-	-	-
Global F_{ST} – 0.059					

4.3.2 Population assignment

Calculation of ΔK using the method applied by Evanno et al. (2005) with calculated parameter values from Stucture 2.3 indicated the presence of three peaks where population structuring exists when K was optimized for values between 1 and 10. The first of these three peaks (at K=2) indicates an initial "contact zone," where broader scale population structuring exists, and at subsequent levels (K=4, 9) where finer scale population structure is present (Fig. 9).

The relevance of using these clusters to inform on dispersal between sampled populations indicates the extent of movement occurring between them. In the case of K =2 (Fig 10) the clusters are consistent with south Jasper National Park (consisting of Maligne, Brazeau and Tonquin) and north Jasper National Park (consisting of A La Peche only). In examining K =4 the genetic clusters did not match those of the four *a priori* defined herds and indicates a considerable amount of admixture occurring between them.

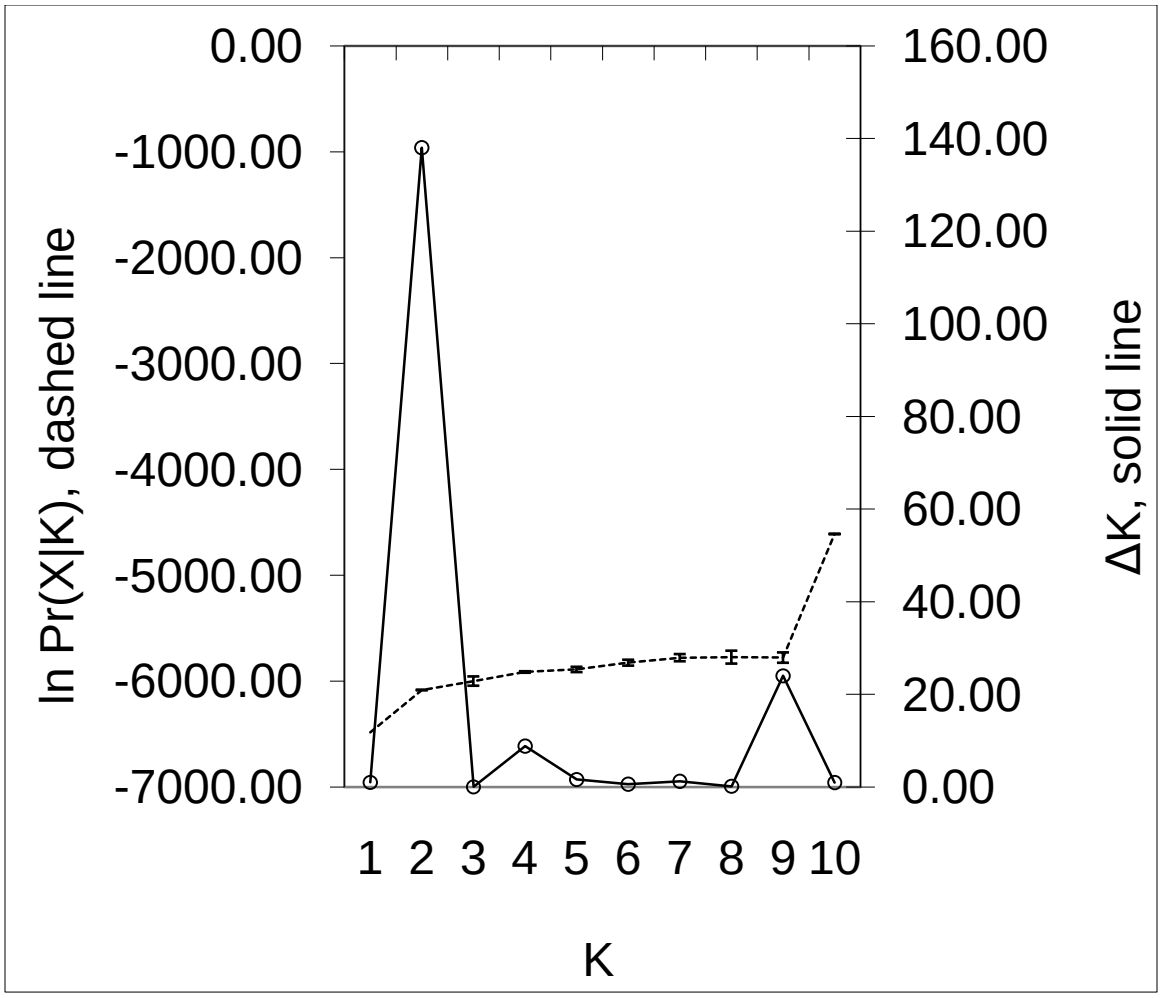

Figure 9 Calculated K and ΔK values for calculated maximum likelihood scores using Structure 2.3 when interpreting sampled genetic data from Jasper National Park 2006-2008

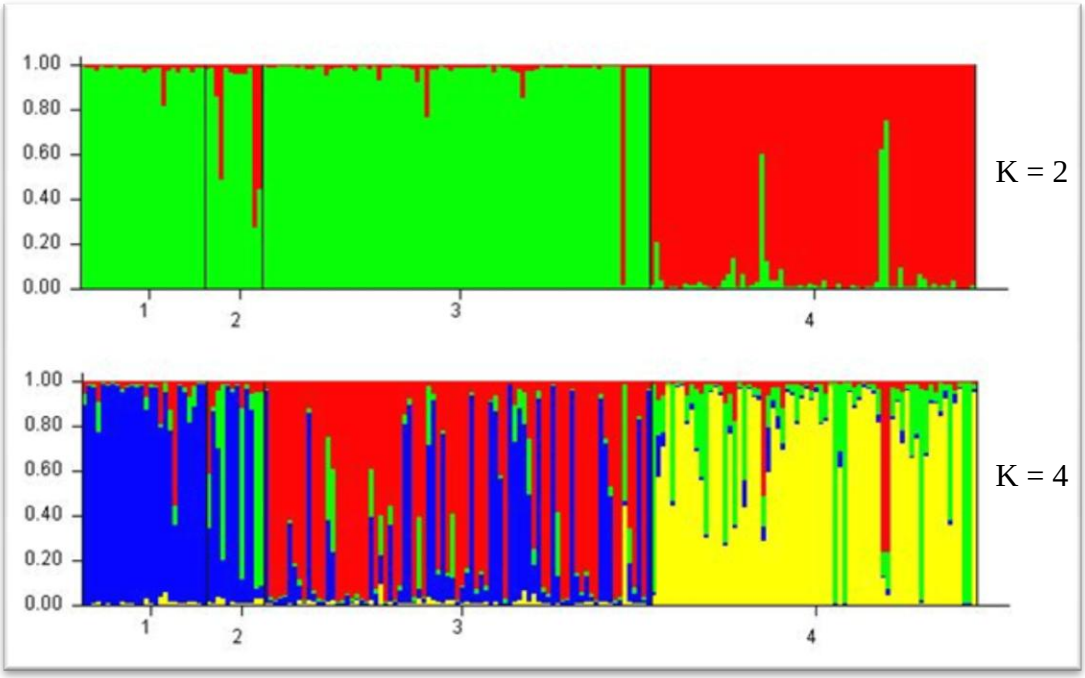

Figure 10 Population assignment results using Structure 2.3 with sampled genetic data from woodland caribou herds in Jasper National Park where 1 - Brazeau, 2 - Maligne, 3 - Tonquin and 4 - A La Peche

Further investigation of calculated gene flow levels using GeneClass 2 identified considerable admixture between herds. The use of population assignment algorithms outlined in Rannala and Mountain (R&M) (1997) and Paetkau et al. (1995) calculated 12 and 15 individuals (out of 189), respectively, as being genetically closer to other herds than those they were originally sampled in (Table 14). All 12 sampled genotypes flagged for reassignment using the R&M approach were also flagged using the Paetkau et al. (1995) approach. The three sampled genotypes flagged using the Paetkau et al. (1995) approach, not selected using the R&M approach, were from A La Peche and closely assigned to Tonquin. Using a threshold of 80% in the individual assignment of animals to populations, as per Ball (2007), using the R&M and Paetkau et al. (1995) methods, the number of reassigned samples dropped to four and seven, respectively.

Table 14 Quantification of gene flow between sampled woodland caribou herds in Jasper National Park using assignment test results of Rannala and Mountain (1997) and Paetkau et al. (1995). Sampled genotypes with a high level of assignment (≥ 0.80) to alternate herds are in bold

				Rannala and Mountain					Paetkau et al.					
ID	Sampled Herd	# Loci	sex	ASSIGNED	Brazeau	Maligne	Tonquin	ALP	ASSIGNED	Brazeau	Maligne	Tonquin	ALP	Agreement
9136	Brazeau	10	m	Maligne	15.8	**84.2**	0.0	0.0	Maligne	3.7	**96.2**	0.1	0.0	yes
6100	Tonquin	9	f	Maligne	27.6	42.4	30.0	0.0	Brazeau	68.5	17.2	14.3	0.0	no
6119	Tonquin	8	m	Maligne	22.7	68.0	8.9	0.4	Maligne	18.2	75.2	6.2	0.3	yes
6120	Tonquin	10	m	Brazeau	55.4	8.2	36.3	0.0	Brazeau	54.6	9.5	35.8	0.0	yes
6172	Tonquin	10	m	Brazeau	78.6	10.7	10.6	0.0	Brazeau	78.7	12.0	9.4	0.0	yes
8988	Tonquin	7	f	Maligne	0.0	58.9	41.0	0.1	Maligne	0.1	68.5	31.4	0.0	yes
9034	Tonquin	10	f	A La Peche	0.0	11.0	5.1	**84.0**	A La Peche	0.1	15.3	0.3	**84.3**	yes
9094	Tonquin	9	f	Maligne	2.4	**83.3**	14.3	0.0	Maligne	2.2	**85.5**	12.3	0.0	yes
9096	Tonquin	10	m	Brazeau	37.1	29.3	33.6	0.0	Maligne	34.7	36.7	28.6	0.0	no
8730	A La Peche	6	m	Brazeau	46.0	8.8	0.4	44.8	Brazeau	75.7	6.9	2.1	15.3	yes
8778	A La Peche	10	m	A La Peche	0.0	0.0	22.9	77.1	Tonquin	0.0	0.0	**83.7**	16.3	no
8832	A La Peche	10	f	A La Peche	0.0	0.0	45.7	54.3	Tonquin	0.0	0.0	**93.0**	6.9	no
8833	A La Peche	10	f	Tonquin	0.0	0.0	**89.8**	10.2	Tonquin	0.0	0.0	**99.9**	0.0	yes
8861	A La Peche	10	f	A La Peche	0.0	0.3	0.0	99.7	Tonquin	2.6	0.1	**94.1**	3.3	no

Detection of first-generation migrants using the Paetkau et al. (2004) approach identified 20 individuals as originating from herds other than those originally sampled (Table 15). The genotypes identified as migrants using the Paetkau et al. (2004) and Paetkau et al. (1995) methods differed by 5 individuals, of which all were from Brazeau or Maligne and were assigned to each other. This in contrast to both the R&M and Paetkau et al. (1995) methods which only identified one potential migrant between these two herds.

Table 15 Number of first generation migrants detected between Jasper National Park woodland caribou herds using Paetkau et al. (2004) method

		N	Herd assigned to			
			Brazeau	**Maligne**	**Tonquin**	**A La Peche**
Herd Sampled	**Brazeau**	26	23	3	-	-
	Maligne	12	3	9	-	-
	Tonquin	79	3	5	70	1
	A La Peche	71	1	2	2	66

4.3.3 Population estimates

Estimation of population size, $\widehat{N}$, for South Jasper National Park using the Mh(jackknife) model indicated population sizes of 125 animals in 2006-2007, 91 animals in 2007-2008 and 98 animals in 2008-2009 (Table 16). These trends in calculated $\widehat{N}$ values match those seen in the use of mark-resight models by park biologists in Jasper over the same sampling periods (Fig 11). This would indicate that the use of NGS is a relatively accurate measure of population size when the Mh (jackknife) model is applied.

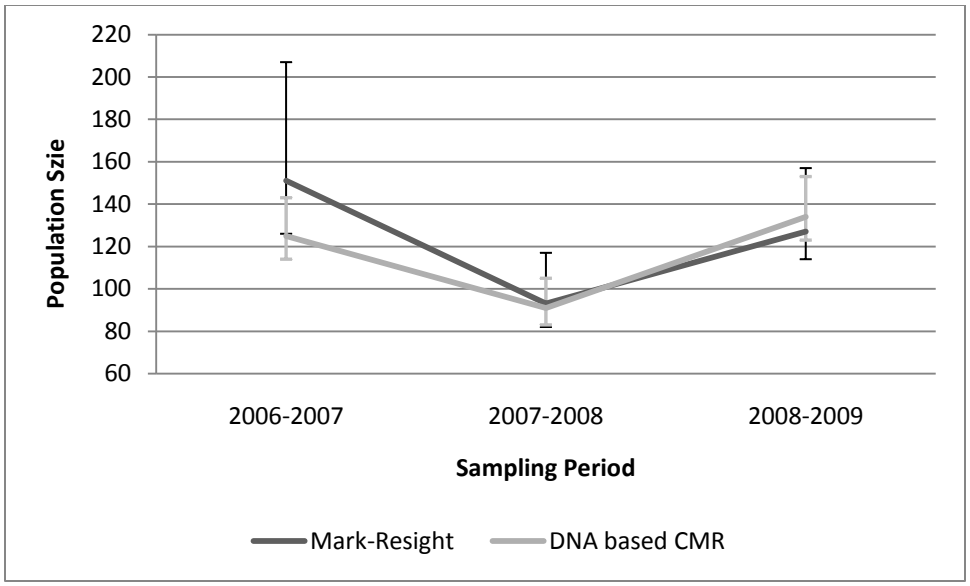

Figure 11 Comparison of population estimates calculated for south Jasper National Park using mark-resight and DNA based mark-recapture methods

Further examination of population estimates broken down by gender (Table 16) indicates the number of sampled females is generally higher than the number of males. The estimated number of males in 2007-2008 was significantly lower than the estimated number of males in 2006-2007 and 2008-2009 (the females were also lower, but not significantly). This may provide the rationale behind the lower calculated population estimates in 2007-2008, calculated using both mark-resight and genetically based mark-recapture models, indicating that males may have moved out of the sampling area or were otherwise unavailable for sampling during this sampling period.

Population size estimates calculated for *a priori* defined herds and based on gender were done to more closely assess population trends for these groups and the ability to sample them. Calculations were done for 2006-2007 (Table 17), 2007-2008 (Table 18), and 2008-2009 (Table 19).

Over the 2006-2007 sampling period, 11, 17, and 60 unique genotypes were sampled and identified as unique individuals in the Maligne, Brazeau, and Tonquin herds. Estimated population size ($\widehat{N}$) was 15, 23, and 85, respectively (Table 17). High coefficient of variation (CV) calculations for Maligne and Brazeau indicate estimator imprecision as higher than that considered ideal for use in management contexts with ungulate species (< 10%) (Murray and Fuller 2000). The estimate for the number of animals in Maligne is likely confounded by variation in the number of animals sampled in the first (2) and second (10) sampling times. Similarly, the estimated number of male animals in Tonquin may be similarly confounded due to the relatively low number of animals sampled in the second sampling interval (3) relative to the first (22).

Over 2007-2008 the number of unique genotypes identified was 6, 20, 56 and 71 for the Maligne, Brazeau, Tonquin and A La Peche herds (Table 18). Calculated population estimates were 9, 21, and 112 for Maligne, Brazeau, and A La Peche. The population estimate for Tonquin varied depending on the number of sampling times used in closed population modelling. Using two sampling times, an estimate of 58 animals was calculated whereas using three sampling times the estimated number of animals was 75.

Table 16 Results of closed population modelling to estimate population size for South Jasper National Park over 3 sampling years

Year	Sampling Area	# unique genotypes				Population Estimates			
		Total	Time 1	Time 2	# Recaptures	$\widehat{N}$	SE	95% CI	CV
2006-2007	All samples	88	66	33	11	125	7.60	(114,143)	6%
	Males only	37	29	13	5	52	4.90	(45,64)	9%
	Females Only	51	37	20	6	72	5.81	(64,87)	8%
2007-2008	All samples	70	47	49	26	91	6.30	(83,105)	7%
	Males only	25	13	19	7	33	3.67	(29,43)	13%
	Females Only	45	34	30	19	54	5.05	(47,67)	9%
2008-2009	All samples	98	75	46	23	134	7.50	(123,152)	6%
	Males only	43	29	25	11	58	3.77	(51,70)	7%
	Females Only	54	46	20	12	74	5.61	(66,88)	8%

Table 17 Population size estimates for Maligne, Brazeau and Tonquin herds 2006-2007

Year	Sampling Area	# unique genotypes				Population Estimates			
		Total	Time 1	Time 2	# Recaptures	$\widehat{N}$	SE	95% CI	CV
2006	**Maligne**								
	All samples	11	2	10	1	15	2.73	(13,24)	18%
	Males only	6	1	5	0	-	-	-	-
	Females Only	5	1	5	1	6	1.73	(6,15)	29%
	Brazeau								
	All samples	17	10	10	3	23	3.24	(20,33)	14%
	Males only	9	6	5	2	11	2.29	(10,20)	21%
	Females Only	8	4	5	1	10	2.29	(9,19)	23%
	Tonquin								
	All samples	60	54	13	7	85	6.30	(76,100)	7%
	Males only	22	22	3	3	30	3.77	(26,41)	13%
	Females Only	38	32	10	4	54	5.05	(47,67)	9%

Table 18 Population size estimates for Maligne, Brazeau, Tonquin and A La Peche herds 2007-2008

Year	Sampling Area	# unique genotypes				Population Estimates			
		Total	Time 1	Time 2	# Recaptures	$\widehat{N}$	SE	95% CI	CV
2007	**Maligne**								
	All samples	6	3	5	2	8	1.94	(8,17)	31%
	Males only	2	0	2	0	-	-	-	-
	Females Only	4	3	3	2	5	1.22	(5,10)[1]	18%
	Brazeau								
	All samples	20	15	16	11	23	2.60	(21,32)	8%
	Males only	11	8	9	6	12	1.94	(12,22)	9%
	Females Only	9	7	7	5	10	1.73	(10,20)	10%
	Tonquin (2 sampling times considered)								
	All samples	44	29	28	13	58	4.80	(47,86)	8%
	Males only	12	5	8	1	16	2.87	(14,26)	18%
	Females Only	32	24	20	12	41	3.87	(36,52)	9%
	Tonquin(3 sampling times considered)								
	All samples					75	7.62	(65,95)	10%
	Males only					28	5.04	(23,43)	18%
	Females Only					45	5.39	(39,61)	12%
	A La Peche								
	All samples	71	47	30	16	97	6.42	(88,113)	7%
	Males only	27	21	12	6	36	3.97	(32,47)	11%
	Females Only	44	26	28	10	60	5.05	(53,73)	8%

Estimator precision was generally good in the calculation of population estimates in 2007-2008, with the exception of Maligne, where a majority of CV estimates were over 10%. Estimation of the number of male animals in Tonquin, when using calculations including two or three sampling intervals, indicated relatively high calculated CV values (18%).

In calculating population size for the number of animals sampled in 2008-2009, 8, 14 and 75 unique individuals were identified for the Maligne, Brazeau and Tonquin herds. Population size for these herds was estimated at 9, 19 and 102 animals respectively. Calculated CV values for the both the Maligne and Brazeau herds were high (> 10%) indicating estimates that are not ideal for monitoring purposes (Murray and Fuller 2000).

Table 19 Population size estimates for Maligne, Brazeau and Tonquin herds 2008-2009

Year	Sampling Area	# unique genotypes				Population Estimates			
		Total	Time 1	Time 2	# Recaptures	N	SE	95% CI	CV
2008	**Maligne**								
	All samples	8	6	5	3	9	1.94	(9,19)	21%
	Males only	3	2	3	2	-	-	-	-
	Females Only	5	4	2	1	6	1.73	(6,15)	29%
	Brazeau								
	All samples	14	6	10	2	19	3.0	(16,28)	16%
	Males only	6	0	6	0	8	2.12	(7,17)	28%
	Females Only	8	6	4	2	10	2.12	(9,19)	21%
	Tonquin								
	All samples	75	63	30	18	102	6.53	(93,118)	6%
	Males only	34	27	16	9	45	4.33	(40, 57)	10%
	Females Only	41	36	14	9	56	4.9	(49, 68)	9%

4.4 Discussion

The calculation of population size estimates using sampled genetic information is a potentially valuable way of assessing population size in woodland caribou herds where the capture and direct handling of animals can be avoided. This study looked at ways of assessing the accuracy of calculated population estimates for caribou herds in Jasper National Park where samples used in analysis were also considered based on *a priori* defined herds sampled and gender information.

4.4.1 Genetic diversity

In assessing the genetic characteristics of woodland caribou in Jasper National Park it was demonstrated that woodland caribou herds of relatively small size (Maligne and Brazeau) were less genetically diverse than the larger ones (Tonquin and A La Peche). This is a similar to other studies of woodland caribou where smaller or isolated herds become more genetically distinct through the processes of genetic drift and alleles becoming fixed (Cronin et al. 2006, Boulet et al. 2007). In this study Maligne deviated from this pattern in calculations of allele richness and expected heterozygosity (H_e) by having estimates above those estimated for Brazeau. Higher genetic diversity estimates for Maligne could be caused by small population size biasing calculated estimates i.e. demographic stochasticity or this herd having not yet gone through a population bottleneck following a rapid reduction in population size (Frankham et al. 2002).

While estimates of expected heterozygosity were comparatively low for Maligne and Brazeau in comparison to Tonquin and A La Peche, they were within range of other levels calculated for woodland caribou populations in west-central Alberta (0.74-0.79) (McLoughlin et al. 2004), Manitoba (0.68-0.76) (Ball 2007), Quebec (0.63-0.68) (Courtois et al. 2003a) and the Yukon (0.74-0.82) (Zittlau et al. 2000). Relative to these other populations A La Peche had a high calculated expected heterozygosity (0.81); likely due to its relatively large population size.

4.4.2 Population assignment

The identification of a single animal moving between the Maligne and Brazeau populations corresponds to past monitoring efforts involving radiocollaring where potential movement between these two herds was detected (Whittington et al. 2005). This

sole instance of an animal being directly sampled in two herds over three sampling years indicates that movement between herds is likely not common. As it is typically adult female animals that are radiocollared and in some studies male animals have shown a greater tendency to disperse (Côté et al. 2002), the use of alternate methods to assess movement beyond known herd boundaries is important.

Results of population assignment tests indicated that substantial gene flow was occurring between herds. This is a relative improbability based on the lack of movement between the A La Peche and South Jasper National Park populations based on published findings (Edmonds 1988, Dzus 2001) and radiocollar work (Whittington et al. 2005). Instances of movement between Maligne and Tonquin are more probable based on the relative short distance between these two herds, although, there is a major highway between them which could act as a deterrent to movement (James and Stuart-Smith 2000, Dyer et al. 2001).

It has been demonstrated that admixture models can results in the miss-assignment of individuals to genetic clusters (Francois and Durand 2010). In particular there are recorded instances of population assignment tests of woodland caribou being unable to successfully differentiate caribou populations where, while population assignment tests were able to correctly identify to which side of a river sampled caribou belonged, animals were incorrectly clustered at a finer geographic scale (McLoughlin et al. 2004). In this study, while Structure 2.3 differentiated the north and south Jasper animals at K = 2, at K = 4 the assignment of individuals demonstrated migration and genetic mixing beyond that considered reasonable. While this has been a problem associated with relatively small F_{ST} values in other woodland caribou studies (Boulet et

al. 2007) those values in this study are in excess of those required to correctly differentiate genetic clusters using program Structure (Pritchard et al. 2000).

A reason for the incorrect interpretation of herd boundaries by assignment tests is that while measurements are instructive of the shared genetic traits between herds there is no differentiation between past or present gene flow patterns (Whitlock and McCauley 1999). As it is thought that many more caribou once inhabited south Jasper National Park (Whittington et al. 2005), it can be assumed that there were genetic relationships between these herds prior to them becoming smaller and more fragmented; particularly in south Jasper National Park where the Brazeau and Maligne herds have only recently become considered as different units.

4.4.3 Population estimates

The population estimates for South Jasper National Park calculated in this study were compared to those done using mark-resight techniques. Interpretation of NGS based population size estimates indicated that the decline in population size in 2007-2008 was caused by a lower number of males in Tonquin. This provides some validity in the use of NGS in monitoring woodland caribou herds at a relatively advanced and dependable level to those approaches now used i.e. radiocollaring based mark-resight analysis.

In the collection of fecal pellet samples, it was often noted that lower numbers of samples were collected in the second or third sampling times. This was particularly evident within the Tonquin. Stratification of available samples for Tonquin based on gender indicated a change in population dynamics in 2007-2008 where the ratio of females to males decreased from 4.8:1 in the first collection to 2.5:1 in the second collection and to 1:1 in the third collection. This would indicate a notable change in herd

dynamics and likely corresponds to yearly breeding behaviour where animals leave the subalpine sampling area following successful breeding events.

While differences in capture probability based on sampling time can be modeled using the Mt models from Otis et al. (1978), they could not be used effectively in this study (Hettinga, unpublished data). This was particularly evident in the calculation of population size for south Jasper National park in 2006-2007 and 2008-2009 when recapture rates were relatively low. This indicates the relative importance of maintaining sampling success between sampling times. This may be best achieved by coordinating sampling times to be earlier in the fall and closer together so herds, particularly Tonquin, is equivalently sampled throughout.

Mark-recapture modelling through use of sampled genotypes was a better analysis tool for estimating population size in Tonquin and A La Peche as opposed to Brazeau and Maligne. The small size of the Brazeau and Maligne herds made use of mark-recapture models difficult because of situations where all or no animals were recaptured between sampling periods; particularly in calculating sex-specific estimates. This occurred in 2006-2007 in Maligne where no males were seen and only one female was recaptured and in Brazeau where only two males and one female were recaptured. For Maligne in 2007-2008, an estimate for males could again not be calculated due to no males being recaptured. That the monitoring of smaller herds is problematic is a problem associated with demographic stochasticity; where smaller populations are more difficult to sample and model mathematically (Morris and Doak 2002).

The alternate use of census information as a means of estimating population size for Maligne and Brazeau has some merit in that there are no associated error rates or

confidence intervals and only one sampling time would be required. While sampling of the Maligne and Brazeau ranges over two sampling times ensures herds are sampled more thoroughly, the use of additional flights to guarantee this information could be considered extraneous. This is validated through all genotypes sampled in Brazeau in October 2009 having been previously seen and the observation of only a single new genotype in Maligne in October 2009.

4.4.4 Conclusions

Monitoring of genetic diversity and gene flow within sampled woodland caribou herds is an informative process that has been reliably used to quantify the effects of reduced population size, population fragmentation and the occurrence of population bottlenecks. However, the long life span and the low reproductive rate of woodland caribou makes them less than an ideal study species for demonstrating the loss of genetic diversity over the short-term. In considering large mammal species it is generally expected that small populations will not be adversely affected by a lack of genetic diversity, but rather due to low effective population sizes where they are more susceptible to environmental factors (Courtois et al. 2003a) and population catastrophes (Morris and Doak 2002).

Accordingly, a management priority in the monitoring of woodland caribou rests on the reliable collection of population size information to aid in recovery efforts so management successes and setbacks can be detected. This is particularly important as smaller herds are notoriously hard to manage due to demographic stochasticity associated with smaller sample sizes. This creates something of a vicious cycle as when populations

are most in need of monitoring and management (if there is still a hope they can recover) they become more difficult to manage effectively (Hebblewhite et al. 2009).

NGS based mark-recapture analysis provides a noninvasive alternative to monitoring and managing woodland caribou which is a good reason for its continued use. As the handling of animals through direct sampling techniques (e.g. radiocollaring) has demonstrated adverse effects for other studied ungulate species (Côté et al. 1998, Swenson et al. 1999, Morellet et al. 2009), it is best that any negative effects to already small, declining populations be avoided (Murray and Fuller 2000). Where no direct evidence exists to relate the adverse effects of direct capture techniques on woodland caribou, common sense and the precautionary principle should prevail.

Chapter 5 Research Summary

5.1 Management Implications

Monitoring techniques used in studying wildlife populations are often assessed based on the treatment of sampled animals (Murray and Fuller 2000) in which standards have changed over time (Arnemo et al. 2006). Early studies involving woodland caribou where mortality rates were as high as 11% were considered justifiable (Barrett et al. 1982) but today would be considered too high as capture related moralities rates of less than 2% are recommended (Spraker 1993, Arnemo et al. 2006). While few studies report on capture mortalities involving the sampling of woodland caribou (with Rettie et al. (1998) being a notable exception), in the net-gunning of 3350 whitetail deer, Webb et al. (2008) indicated 1.6% of captures resulting in mortalities; meeting the goal of sampling mortality rates of less than 2% but still yielding a relatively high number of mortalities (54 animals).

Where the capture of ungulate species may not always result in immediate mortalities, studies have shown other notable effects associated with the capture and handling of the animals. Côté et al. (1998) demonstrated that chemical immobilization of mountain goats led to decreased fertility rates and an increase in the rate of abandonment by mothers of calves. Swenson et al. (1999) demonstrated that ear placed radio-transmitters significantly increased the rate of mortality for sampled moose calves. Krausman et al. (2005) recorded instances of caribou mortalities due to bacterial infections of lesions caused by radio-collar placement. Often, assessing the risks of capture techniques is difficult as there is no way to obtain a control group because of the necessity of first sampling a population to acquire scientific information (including the

effects of capturing) (Murray and Fuller 2000). While measurements of the incidence of mortality for sampled ungulate species is instructive, it may not provide the whole picture on sampling effects and the role they play on animal survival and longevity.

In conservation sciences, the precautionary principle is often applied to ensure that management techniques error on the side of caution in protecting animals and the environment from situations which, while not having been proved to cause undue harm, *could* cause undue harm and where a lack of scientific knowledge is apparent. When monitoring wildlife species, the long-term consequences of sampling techniques should also be assessed with less invasive techniques alternately considered and developed (Murray and Fuller 2000). Since many woodland caribou herds are declining and small population sizes precipitate further herd declines, i.e. inverse density dependence (Wittmer et al. 2005b), the precautionary principle approach should apply to research and monitoring efforts to ensure adverse effects are not occurring through monitoring efforts being used.

This study investigated the sampling of woodland caribou fecal pellets as an alternate noninvasive method of monitoring woodland caribou through calculating population demographics which can then be used in assessing herd health and status. In the studying of noninvasive sampled DNA from caribou, it was demonstrated as a successful method for characterizing sampled genetic information and for estimating population demographics using mark-recapture models. The specific objectives of this study were to:

1 Evaluate the success of closed mark-recapture models to estimate population size ($\widehat{N}$) with noninvasively sampled fecal pellets from woodland caribou herds in Jasper National Park and the North Interlake region of Manitoba

2 Evaluate success of open mark-recapture models for the purpose of estimating population rate of growth (λ) with sampled genetic information from the North Interlake region of Manitoba

3 Assess sampled genetic characteristics from woodland caribou herds in Jasper National Park using estimates of genetic diversity and gene flow between herds

4 Make informed recommendations on the continued collection of fecal pellet samples from Jasper National Park and the North Interlake region of Manitoba

5.2 Study Conclusions

5.2.1 Success of closed population modelling to estimate population size, $\widehat{N}$

This study benefitted through the alternate estimation of population size using mark-resight models. This allowed estimates calculated using sampled fecal pellets to be compared with those of an approach which has been readily used and is considered accurate. In this study, it was found that there was a close parallel in the use of the heterogeneity Mh (jackknife) model to estimates calculated using mark-resight models. This validated the sampling approach used in this study as well as the selection of the heterogeneity model in the estimation of population size with sampled fecal pellets. With simulations done in this study it was also demonstrated that the Mh (jackknife) model is robust to limited numbers of samples being available in the estimation of population size for mid size groups of approximately 50 – 150 animals.

The method of assessing sampling success used in this study was through the stratification of available samples based on sampling area and gender. In this study, stratification of available samples indicated that the smaller herds i.e. Maligne and Brazeau were more difficult to successfully sample for mark-recapture purposes when considered separately from the south Jasper National Park population. Difficulties were also apparent in calculating gender-specific estimates for male and female animals in Tonquin, Maligne and Brazeau. While the calculation of gender specific estimates was likely hindered in Maligne and Brazeau by the small herd sizes, for the Tonquin it was because of changing population dynamics where animals were moving away from the rutting grounds as sampling times progressed later into the year.

In the sampling and estimation of population size in the North Interlake, stratification of available samples was also useful in demonstrating overall sampling success and exploring the utility of calculated mark-recapture estimates. In the calculation of population size estimates for 2007, stratifying available samples indicated that poor recapture rates of females in Lower North Interlake was a source of added variability in calculated estimates. This would indicate the relative ability for managers to use sample stratification to yield estimates for monitoring purposes when difficulties are encountered in sampling populations as a whole.

5.2.2 Success of open population modelling to estimate population rate of growth, λ

The use of open population models for the purpose of estimating population rate of change, λ, indicated negative growth rates for sampled caribou in North Interlake. This finding should be closely considered by provincial wildlife officials as previous reports had considered this population as stable (Manitoba Conservation 2005). However, there

are a number of factors that could serve to dampen our conclusions including the small number of sampling times, the variability surrounding calculated estimates and violation of model assumptions where stable capture probability and apparent survival estimates are ideal. To this extent, while additional sampling periods can help mitigate the deficiencies of calculated λ estimates through more accurate parameterizations, the alternate use of closed population models to assess demographic changes has merit in requiring less model assumptions be met.

5.2.3 Use of sampled genetic information to inform on herd health and dispersal between herds

Evaluation of sampled genetic diversity levels between studied herds using expected heterozygosity, H_e, and allele richness, Na, estimates affirmed what has been demonstrated in previous studies where smaller populations have lower calculated genetic diversity levels (Frankham et al. 2002). Calculated F_{ST} estimates indicated a genetic relationship between Maligne and A La Peche and between Tonquin and A La Peche comparable to those estimated for boreal ecotype woodland caribou studied elsewhere. F_{ST} estimates also indicated a closer genetic relationship between Maligne and A La Peche (0.044) than between Maligne and Brazeau (0.052), the latter of which were up until recently nominally referred to as a single 'subpopulation.'

Similarly to calculated F_{ST} estimates, the use of population assignment tests to differentiate populations and test dispersal indicated sampled caribou populations in south and north Jasper National Park having considerable admixture; in particular between the sampled Maligne and A La Peche herds. Other studies of woodland caribou, however, have demonstrated erroneous findings through the use of population assignment tests in correctly identifying population of origin (McLoughlin et al. 2003, Boulet et al.

2007). Because of the relatively low recruitment rates and long life-spans of caribou, they are a less than optimal study species to study intergenerational population trends compared to short-lived highly reproductive species (Frankham et al. 2002).

5.2.4 Sampling considerations

The estimation of population size using mark-recapture models are based on a number of assumptions (Williams et al. 2002). Notably, high initial capture rates and high recapture rates in subsequent sampling events play an important role in decreasing the variability of calculated estimates (White et al. 1982). When low recapture rates are encountered they can be offset using a variety of methods. Most notably the use of alternate models to fit capture history information can reduce the variability in population size estimates when compared to a null model which assumes no changes in capture probability (White et al. 1982). In using multiple models, a rationale is also provided for why recapture rates are low i.e. because of time effects or behavioural effects where adjustments in sampling methods can then subsequently be made (Otis et al. 1978).

In applying varying models to capture information on sampled animals, the accuracy of estimates produced largely depends on the robustness of collected data (Otis et al. 1978, White et al. 1982). To determine how capture probability varies with time, it is important to have sampling information representative of a number of sampling times. In this study a limitation was in the low number of sampling times used to test alternate models in describing varying capture probabilities. While a range of models are available using mark-recapture software, their applicability with capture histories derived from only two sampling times is limited. In the sampling of Tonquin, it was apparent that animals moved out of the sampling range after the rut and that capture heterogeneity was

present. These conditions would infer use of a model which applies differences in capture probability based on both sampling time and heterogeneity effects i.e. the Mth model. In addition, the sampling of covariates in the estimation of population size, including use of the Mh(jackknife) model, is usually reserved for instances where there is a minimum of three sampling times.

The use of NGS in monitoring wildlife species has been extensively reviewed in how genotyping errors can impact calculated estimates; including in the use of mark-recapture models and population assignment techniques. This study benefitted from having strict protocols for the scoring of genetic data using multiple scorers. By having a consensus-based approach, an emphasis was placed on keeping only "good-quality" samples where those that were problematic were carefully considered and often culled. Additional precautions could also be taken however in further increasing the quality of genetic data, particularly in following those procedures set out in previous studies (Frantz et al. 2003, Hansen et al. 2008). These studies often rely on the amplification of sampled fecal pellets multiple times with comparisons between runs to ensure amplified and scored genetic data is accurate (Pompanon et al. 2005). This is of particular importance in the use of population assignment techniques where it has been demonstrated that one erroneous or miss-assigned genotype can bias estimates of sampled genetic clusters (Morin et al. 2009).

5.3 Future Directions

As laboratory procedures have been developed around the use of sampled woodland caribou fecal pellets to estimate diet quality (Barten et al. 2001) and hormone

levels (Messier et al. 1990), there is potential for fecal sampling to be a standalone substitute for the use of radiocollars. This is in keeping with the development of sampling tools to ensure that capture effects on sampled animals are minimized and an effort is made to use the least harmful approach available in studying wildlife species; particularly those considered rare or endangered (Murray and Fuller 2000)

The results of this study demonstrated that DNA from sampled woodland caribou fecal pellets can be used within a mark-recapture framework to estimate population demographics. This was demonstrated in the calculation of population size for the North Interlake and Jasper National Park woodland caribou herds. Open population models were also used in the estimation of population rate of growth estimates for the North Interlake caribou herds and various genetic attributes were used to measure genetic diversity and dispersal for sampled Jasper National Park herds. It is the belief of the author that genetic sampling of woodland caribou herds can provide a noninvasive alternative to estimating population demographics but that changing population demographics of sampled herds should be closely monitored to ensure violations to modelling assumptions are known with sampling methods adapted accordingly.

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