

Estimation of demographic population parameters using non-invasive methods in woodland
caribou (*Rangifer tarandus caribou*)

by

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Abstract

Demographic population parameters of woodland caribou were estimated using non-invasive fecal sampling. Fecal pellet sampling occurred in several woodland caribou populations from 2004 to 2013. Samples were amplified at 10 microsatellite loci and unique individuals identified. We used fecal pellet reproductive hormones levels and pellet dry weight to differentiate calf from adult age-classes. Results demonstrate that pellet weight, pregnane, and testosterone were able to identify age-classes which were used in capture-mark-recapture analysis in program MARK. In addition, we estimated effective population size of woodland caribou using linkage disequilibrium, sibship assignment and temporal methods. Results demonstrate that the linkage disequilibrium method produces precise estimates, and a reduced number of loci or number of unique genotypes produced precise estimates. This study demonstrates the power of non-invasive fecal sampling for determining age-classes and estimating demographic population parameters of woodland caribou, with potential for use in other populations or species.

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A Note to the Reader

When reading this thesis, you will find the use of “we” opposed to “I” in describing the study objectives, methods, results and discussion. Although I am the primary author of this thesis, without the help of my entire committee and other members of the caribou lab, this thesis would not have been possible. From thinking of ideas, lab work, the multitude of research that was done previous to my thesis, and the countless hours of advice and guidance from my committee, this thesis is better represented by using “we” in describing the various parts of this thesis.

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General Introduction

Woodland caribou (*Rangifer tarandus caribou*) are a subspecies of caribou that has experienced extensive population declines and diminished ranges across Canada, due to predation, habitat fragmentation and alteration from anthropogenic activities (Thomas and Gray 2002, McLoughlin et al. 2003, Environment Canada 2012, COSEWIC 2014). Predation is the main limiting factor in woodland caribou, being a paramount threat for Central Mountain and Boreal populations and the main proximate cause of mortality in woodland caribou (Stuart-Smith et al. 1997, Rettie and Messier 1998, Rettie and Messier 2000, Thomas and Gray 2002). As a result of declining populations, woodland caribou are listed under the Species at Risk Act (SARA), as well as under provincial legislations (Manitoba Conservation 2005, Cichowski 2010). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has identified twelve designatable units of caribou: Arctic Archipelago, Dolphin-Union, Barren-ground, Eastern Migratory, Newfoundland, Boreal, Northern Mountain, Central Mountain, Southern Mountain, Torngat Mountain, Atlantic-Gaspésie, and Dawson's caribou (COSEWIC 2011). The increasing isolation of woodland caribou populations is a major consequence of habitat fragmentation, which may result in restricted gene flow, decreases in genetic diversity, and inbreeding accumulation (Couvet 2002, Ball et al. 2010, Hettinga et al. 2012, Robinson and Moyer 2013). Decreased gene flow and genetic diversity has been identified in several populations of woodland caribou (Courtois et al. 2003, Ball 2010, Galpern et al. 2012b, Hettinga et al. 2012, Flasko 2014). Reductions in population size and effective population size is mainly due to habitat loss and exploitation, habitat modification, and altered spatial population dynamics (Frankham et al. 2002, England et al. 2010). Knowing the effective population size is crucial for species of conservation interest, such as woodland caribou.

The age-structure of populations changes over time, and knowledge of these changes is important for understanding trends in population parameters such as recruitment, population growth, survival, and reproductive status (Gaillard et al. 1998, Reilly 2002, Festa-Bianchet et al. 2003). Survival is often age-specific, with woodland caribou calves having significantly lower survival probabilities than adults and this can have a significant impact on population parameters (Gustine et al. 2006, Pinard et al. 2012, Leblond et al. 2013, Traylor-Holzer 2015). Many behaviours are limited to older animals, such as dispersal events and breeding; not distinguishing between calves and juveniles when determining population reproductive rates can lead to inaccurate estimates of genetic structure and dispersal patterns (Ball 2010).

Woodland caribou are elusive by nature and extremely sensitive to anthropogenic disturbances, making caribou a difficult species to observe and study (Brown et al. 2000, Festa-Bianchet et al. 2011). Traditional methods of monitoring wild caribou populations, such as radio-collaring (Rettie and Messier 1998), cause stress and harm to the animal under study (Valkenburg et al. 1983, Cattet et al. 2008, Omsjoe et al. 2009), collars can be unreliable (Johnson et al. 2002), extremely costly (Valkenburg et al. 1983), and the data collected are often female-biased (Seip 1992, Rettie and Messier 1998, McLoughlin et al. 2004). Non-invasive methods of monitoring wildlife populations have been developed as an alternative to traditional sampling methods (Waits and Paetkau 2005, Ball et al. 2007, Ball 2010, Morden et al. 2011b). Fecal DNA and fecal hormones contain a multitude of information that traditional collaring methods cannot provide, and can be extracted from fecal pellets to identify unique individuals, identifying pregnancy status, estimating population sizes, determining age-structure, monitoring population productivity, and phylogeographic analyses (Eggert et al. 2003, Kühl et al. 2009, Ball 2010, Morden et al. 2011a, Morden et al. 2011b, Flagstad et al. 2012, Hettinga et al. 2012). In

particular, estimates of population size often play a critical role in wildlife studies, and collecting consecutive yearly estimates of population size can allow the growth rate of sampled populations to be obtained, providing information on overall population health (Mills 2007).

Research Problem

The purpose of this study was to estimate demographic population parameters of woodland caribou using non-invasive methods.

Objectives:

- 1) Develop methods of estimating an age-class for woodland caribou populations for use in capture-mark-recapture studies;
- 2) Develop methods to estimate effective population sizes and the ratio of effective to census population size of woodland caribou populations.

The studied populations consist of the Tonquin, Brazeau and Maligne herds (hereafter referred to as South Jasper) in Jasper National Park, Alberta; the North Interlake and The Bog herds in Manitoba; The Prince Albert Greater Ecosystem (PAGE) herd in Saskatchewan; and the Slate Islands herd in Ontario. The South Jasper herds are considered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) to be part of the Central Mountain Designatable Unit (DU), whereas the other study populations are part of the Boreal DU (COSEWIC 2011). The South Jasper and North Interlake herds have been studied extensively for several years and have an extensive capture history which can be used for capture-mark-recapture analyses and effective population size to census population size ratios.

General Methods

To meet our objectives, we analyzed microsatellite data and reproductive hormone levels from woodland caribou fecal pellets from samples collected over sampling events from 2004 to 2013. Samples were genotyped at ten microsatellite loci: RT5, RT6, RT9, RT24, RT30, BM888, Map2C, BM848, BMS1788, and RT7 (Bishop et al. 1994, Wilson et al. 1997, Cronin et al. 2005). An additional eight loci were run for the South Jasper population: RT1, RT13, RT27, BM4513, BM6506, OHEQ, NVHRT16 and FCB193 (Stone et al. 1995, Røed and Midthjell 1998, Jones et al. 2000). Caribou-specific Zfx/Zfy primers were used for sex identification.

All samples per population were clustered into genotypes and a capture history was built for use in analyses. Building a capture history allows for the identification of new individuals that enter into a population, and can help differentiate between adults and calves. We also weighed pellets after being dried and measured the levels of testosterone (in males) and pregnane (in females) to help determine the age-classes of individual caribou (Ball 2010, Morden et al. 2011a, Morden et al. 2011b).

Capture-mark-recapture analyses were run in program MARK to estimate demographic population parameters including survival, recruitment, and population size. Age-class data were included to determine differences between calf and adult population parameter rates. Effective population size estimates were determined through several methods, including linkage disequilibrium (Hill 1981, Waples 2006), sibship assignment (Wang 2009), and the temporal method (Nei and Tajima 1981).

Justification of Research

Information on demographic population parameters such as survival, recruitment, population size and effective population size are critical for conserving wild populations (Reilly 2002, Festa-Bianchet et al. 2003, Kühl et al. 2009). Information on population parameters is crucial for species that have experienced widespread population decline, such as in woodland caribou, as the loss of individuals can affect the local population. Our research will advance methods of estimating population parameters in wild populations by the incorporation of age-classes for age-specific population parameters, as well as advance our understanding of effective population size and effective to census population size ratios in woodland caribou. These non-invasive genetic sampling techniques will give the necessary tools for monitoring and conserving wild ungulate populations.

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Chapter One: Fecal pellet analysis as a method to estimate age-class to determine age-specific demographic population parameters

Abstract

As all designatable units of woodland caribou are of conservation concern as per the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), and listed as a protected species under the Federal *Species at Risk Act* (SARA), monitoring population sizes and trends is crucial. Non-invasive fecal sampling provides a mean for collecting data without the need to come into contact with the species. Woodland caribou calves have significantly lower survival rates than adults, and determining age-specific population parameters such as survival can provide more information about endangered populations. The objectives of this chapter are to determine if a combination of hormonal levels in fecal pellets, pellet size and pedigree analysis can help to more confidently identify calves in the dataset; and if we can identify calves, does the survival rate of calves differ from that of adults, and if this difference is similar across time. Non-invasive fecal sampling occurred in Jasper National Park from 2006 to 2013. We analysed fecal pellets from the Tonquin, Maligne and Brazeau woodland caribou herds for testosterone and pregnane content for males and females, respectively, and found the dry fecal pellet weight. The hormone content along with the fecal pellet weight and individual capture history was used to determine the age-classes (calves or adults) of all individuals in the populations. The age-classes results for the Tonquin herd were then used in capture-mark-recapture analyses to determine whether or not separating adults from calves would impact apparent survival estimates. We were able to successfully classify 200 out of 208 individuals

from the Tonquin herd using a combination of hormone content, fecal pellet weight and capture history, with the 8 remaining individuals in the capture history having no available pellets to be analysed. Capture-mark-recapture results indicate that there was no significant difference between calf and adult survival rates in the Tonquin herd. As woodland caribou calves give birth in May or June, and our sampling occurred in Jasper National Park in fall (October to January) each year, calves were roughly six months old at the time sampling occurred. Although we can successfully determine the age-class of individuals, first sampling may have occurred too late after calving (6-7 months) to capture the period of greatest differences in calf and adult survival rates.

Introduction

The knowledge of demographic and population parameters are crucial for the effective management and conservation of any wild population (Kühl et al. 2009, Morden et al. 2011b, DeCesare et al. 2012). The age structure of populations changes over time, and knowledge of these changes is important for understanding trends in population growth, survival, net rate of change, and reproductive status (Gaillard et al. 1998, Reilly 2002, Festa-Bianchet et al. 2003, Morden et al. 2011b). A key demographic component of population growth is age-specific survival (Chaloupka and Limpus 2005) and is necessary for understanding and exploring trends in net rate of change, population growth, mortality, and reproductive status of a population (Gaillard et al. 2000, Morden et al. 2011a, Morden et al. 2011b, Hervieux et al. 2013). Many behaviours are limited to older animals, such as dispersal events and breeding (Ball 2010). If younger individuals are not identified in a data-set, non-breeding and non-dispersing young remain included in the estimates, and can lead to inaccurate genetic structure and dispersal patterns of a population (Ball 2010). While adult female survival is often considered to be the main demographic parameter that affects population growth rate in caribou populations, the variation in calf survival is what drives yearly changes in the dynamics of the population (Gaillard et al. 1998, Gaillard et al. 2000, Hervieux et al. 2013). Hervieux et al. (2013) found that caribou population declines across Alberta were the result of both low adult survival as well as low calf survival, and not just one vital rate. In the À la Pêche population in west-central Alberta, both adult female survival and calf recruitment were responsible for driving declines in the population (DeCesare et al. 2012, Hervieux et al. 2013). Calf survival is highly variable from year to year, potentially due to many causes such as predation, drought, harsh winters, late parturition, low birth rate and early growth rates, poor calving areas, and genetic factors (Fancy

et al. 1994, Singer et al. 1997, Gaillard et al. 1998, Hervieux et al. 2013). Although adult mortality is affected by the same factors, adult survival is less variable than calf survival. Calf survival is highly sensitive to limiting factors, whereas adult female survival is not as affected by limiting factors (Gaillard et al. 1998). Hatter and Janz (1994) found that highly variable calf survival played a bigger role than adult survival in explaining changes in the population size of Columbian black-tailed deer (*Odocoileus hemionus columbianus*). Elk (*Cervus elaphus*) calf survival in the Rocky Mountain region accounted for 75% of the variation in population growth rates (Raithel et al. 2007). Numerous ungulate studies have found that calf survival is significantly lower than adult survival, and has an affect on population growth rates (Stenhouse et al. 1995, Smith and Anderson 1998, Albon et al. 2000, Stubsjoen et al. 2000, Raithel et al. 2007, Harris et al. 2008).

Non-invasive fecal sampling (NFS) has been used as a method for identifying unique individuals through DNA extraction, identifying pregnancy status, estimating population sizes, determining age-structure, monitoring population productivity, and phylogeographic analyses (Eggert et al. 2003, Kühl et al. 2009, Morden et al. 2011a, Morden et al. 2011b, Flagstad et al. 2012, Hettinga et al. 2012). Determining age-classes using NFS can be used to predict population structure, and is a growing area of research (Reilly 2002, Morrison et al. 2005, Nowak et al. 2009, Morden et al. 2011a, Flagstad et al. 2012, Hedges et al. 2013). Age-classes can be identified using fecal pellet morphometrics (the physical measuring of the morphological shape of the fecal pellet). For species that produce solid, semi-uniformly shaped pellets, there is potential to assign age-classes based on pellet size, as the size of the pellet increases with body weight, which should increase with age until adulthood (Ball 2010). By measuring the size of the elephant bolus, Flagstad et al. (2012) and Hedges et al. (2013) were able to determine the age-

structure of wild Asian elephants (*Elephas maximus*), and Nowak et al. (2009) and Morrison et al. (2005) determined the age-structure of African elephants (*Loxodonta africana*); while Reilly (2002) was able to determine the age-structure of Sumatran elephants (*Elephas maximum sumatranus*) using the diameter of dung bolus along with shoulder height and forefoot circumference. Ball (2010) was able to show differences in woodland caribou (*Rangifer tarandus caribou*) calf, juvenile and adults using fecal pellet lengths. By combining fecal pellet length, width and depth, Morden et al. (2011a) determined the age-classes of Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and Sanchez-Rojas (2004) classified age-classes in mule deer (*Odocoileus hemionus*).

Reproductive hormones have also been used to determine age-class in a number of species (Lynch et al. 2002, Rooney et al. 2004, Castro and Sousa 2005, Seraphin et al. 2008, Beehner et al. 2009, Morden et al. 2011b). Seraphin et al. (2008) found that fecal testosterone levels increased with age in male eastern chimpanzees (*Pan troglodytes schweinfurthii*), as did Beehner et al. (2009) in yellow baboons (*Papio cynocephalus* or *P. hamadryas cynocephalus*), chacma baboons (*Papio ursinus* or *P. h. urinus*) and geladas (*Theropithecus gelada*). Lynch et al. (2002) found that mean testosterone levels in male tufted capuchin monkeys (*Cebus apella*) were significantly higher than in sub-adults. Fecal androgen levels in male common marmosets (*Callithrix jacchus*) have been shown to differ between juveniles and adults (Castro and Sousa 2005). Male american alligator (*Alligator mississippiensis*) hatchlings have significantly lower testosterone plasma levels than male adults (Lance 1989, Rooney et al. 2004), and female hatchlings have significantly lower estrogen plasma levels than female adults (Crain et al. 1997, Guillette et al. 1997).

Female reindeer in Finland were found to have significant differences in fecal progesterone metabolite levels between calves and yearlings/adults (Morden et al. 2011b). Progesterone levels in female caribou and reindeer can serve as an accurate indicator of pregnancy (Bubenik et al. 1997, Ropstad et al. 2005, Shipka et al. 2007, Morden et al. 2011b). In pregnant females, progesterone levels rise after conception in early fall, remaining at consistent high levels throughout the length of the gestation period and declining shortly before parturition in the spring (Bubenik et al. 1997, Shipka et al. 2007). In non-pregnant females, progesterone levels increase and decrease several times before peaking in spring (Bubenik et al. 1997, Shipka et al. 2007).

Genetic composition and variability within a population can be determined by knowing how individuals are related (Pemberton 2008, Cope et al. 2014). The study of familial relationships between individuals began on controlled, experimental populations (Anderson 1974, Milkman and Zeitler 1974, Cope et al. 2014). However, complete data on mating events and social interactions in wild populations is uncommon, as long-term studies of populations are necessary to acquire this information. For cryptic or secretive species, sightings and sampling can be rare and difficult. The application of highly variable genetic markers (such as microsatellite DNA markers) allowed for the study of direct familial relationships between individuals based on genotypes to be applied to wild populations (Pemberton 2008, Cope et al. 2014). Microsatellite markers are well-suited for the study of familial relationships, as they provide highly polymorphic, heritable genetic identity data for individuals. Combined with appropriate statistical techniques, microsatellites can infer parentage or sibling relationships among individuals (Selkoe and Toonen 2006, Pemberton 2008, Cope et al. 2014).

There are several approaches to constructing pedigrees, including field observation, parentage assignment and sibship inference. In parentage assignment, offspring are assigned to a finite list of candidate parents, based on their shared alleles (Pemberton 2008, Cope et al. 2014). Using exclusion with associated exclusion probabilities calculated from allele frequencies is the simplest method of providing statistical support for this approach; however, most natural populations do not have complete candidate sampling, marker panels may not be powerful enough to exclude all but one candidate parent, and genotyping errors can cause false exclusion of a true parent, so few studies use an exclusionary approach (Pemberton 2008, Riester et al. 2009). The likelihood approach is the most common approach in wild populations, as it makes better use of genotype data and uses allele frequencies as well (Pemberton 2008). Several programs use the likelihood approach to reconstructing pedigrees, such as COLONY 2.0 (Jones and Wang 2010a) and CERVUS (Kalinowski et al. 2007). Most parentage assignment programs support datasets of two generations; taking an offspring list and a list of candidate mothers and fathers, and generating a list of possible parents. However, many populations have multi-generational pedigrees, in which the offspring and candidate mothers and fathers have overlapping generations. As the age data for the individuals in the population is absent, the ordering of genotypes into generations is not known, and has to be estimated from the genotype data only (Almudevar 2003, Pemberton 2008). If using a program (such as COLONY) that only supports two generations, all possible parentage combinations are not permitted as valid pedigrees (Pemberton 2008). Riester et al. (2009) created a new algorithm for pedigree reconstruction when using multi-generational, overlapping populations, with the option of including prior knowledge such as age, sex, sampling locations, sub-pedigrees and allele frequencies in creating a pedigree. Complexity arises when parental or candidate parental

information is missing from the available data. Sibship reconstruction attempts to infer sibling relationships by partitioning a single-generation population into full-sibling and half-sibling groups (Wang 2004). Individuals in the full-sib and half-sib groups share on average one-half or one-quarter of their alleles, respectively. Individually, parentage assignment and sibship inference are useful tools for pedigree reconstruction, but by combining the two approaches, more complete pedigrees can be constructed (Pemberton 2008, Cope et al. 2014). Coltman et al. (2005) combined these two methods in a study of bighorn sheep (*Ovis canadensis*). Candidate sires were only partially sampled, and the paternity of 235 individuals was assigned using CERVUS (Kalinowski et al. 2007). COLONY (Jones and Wang 2010a) was used after paternity analysis to identify half-sibling relationships among the unassigned offspring, identifying 38 clusters of 167 paternal half-siblings (Coltman et al. 2005). DiBattista et al. (2009) combined CERVUS and COLONY for pedigree reconstruction of lemon sharks (*Negaprion brevirostris*). Morrissey et al. (2012) reconstructed the pedigree of the red deer (*Cervus elaphus*) by using MasterBayes (Hadfield et al. 2006), a Bayesian framework program, and using COLONY to identify sibling relationships among the unassigned offspring. In all instances, combining sibship inference with parentage assignment substantially increased the number of pedigree links available for analysis.

Capture-Mark-Recapture Analysis

Capture-mark-recapture (CMR) analyses are a key method for estimating population parameters of a studied species (Mills 2007). Traditional CMR methods involve the use of physical markers, such as leg bands, to mark individuals (Luikart et al. 2010). In the initial capture session, individuals are marked and released, and in subsequent recapture sessions, the proportion of marked (recaptured) individuals is quantified, and the census population size (N) is

estimated from the ratio of marked to unmarked individuals (Luikart et al. 2010). CMR assumes that all individuals are randomly mixed and sampled, and have an equal capture probability (Luikart et al. 2010). Genetic CMR is an alternative to more traditional CMR methods by combining non-invasive genetic sampling with CMR in order to collect critical data about populations without the need to handle, capture, or even observe individuals (Waits and Paetkau 2005, Coster et al. 2011). This method has many advantages over traditional CMR methods, such as higher capture probabilities, reduced tag loss, and a simple study design (Coster et al. 2011). Non-invasive genetic sampling (NGS) allows the marking of individuals by using DNA from collected feces (Morden et al. 2011b, Hettinga et al. 2012, Hedges et al. 2013), hair (Boulanger et al. 2002), urine (Valiere and Taberlet 2000), feathers (Hogan et al. 2008), sloughed skin (Valsecchi et al. 1998), or other tissue samples (Waits and Paetkau 2005, Luikart et al. 2010).

Closed CMR models assume there are no additions or subtractions of individuals during the study period. It is assumed that births, deaths, emigration, and immigration are not occurring during or between sampling occasions (Luikart et al. 2010). Open CMR models differ from closed models in that they allow for changes in N , and can provide survival and net rate of change estimates (Luikart et al. 2010). Open models require larger sample sizes and longer study times than closed models. Closed models are more commonly used than open models because they can provide reasonably precise and accurate estimates of N with less data than open models (Luikart et al. 2010). Closed models can model variation in capture probability, which is an advantage over open models, where researchers must use robust design to account for capture variation (Luikart et al. 2010).

The Cormack-Jolly-Seber (CJS, Cormack 1964, Jolly 1965, Seber 1965) model is the most commonly used model for open populations. It allows year-specific estimates of apparent

survival (ϕ), and p , capture probability. Only apparent survival can be estimated in CJS, as $1-\phi$ represents both individuals that died and individuals that emigrated out of the population.

Estimates of ϕ from the CJS model are robust to individual heterogeneity in capture probability (Lukacs and Burnham 2005). True survival can be underestimated using a CJS model if animals permanently emigrate from the study area; however the South Jasper population is a closed population, with very little emigration out of, and immigration into, the population.

Population size (N) estimates can be calculated using K-sample models for survey years with more than two sampling occasions. The Darroch estimator is a K-sample method that estimates N for closed populations. Each animal has the same probability of capture on any given sampling occasion, but capture probabilities are allowed to vary from one sampling occasion to the next (Williams et al. 2002). K-sample models assume that the population is closed to additions and losses of individuals between sampling occasions; marks are neither lost nor overlooked; and capture probabilities are appropriately modeled (Williams et al. 2002).

Robust design modelling was proposed by Pollock (1982) as a sampling scheme that combines features from both closed and open population models to deal with the issues of bias in CJS models (such as individual heterogeneity and behavioural responses to trapping and handling). Robust design reduces bias caused by unequal catchability and provides estimates for parameters that are unidentifiable by the CJS method (Pollock 1982, Kendall et al. 1995, Kendall et al. 1997). The robust design model consists of k primary periods, with each primary period having at least 2 secondary samples, and assumes it is a closed population within each primary period (Kendall et al. 1995). Robust design estimates population size by applying the appropriate closed population model to data from the secondary samples within each primary sampling period. It also estimates survival, as survival estimates are less sensitive to heterogeneity in

capture probabilities (Kendall et al. 1995). The secondary sample occasions are close together in time (within each primary sampling period) allowing for the assumption that no mortality or emigration occurs. For each trapping session (i), the probability of first capture (p_{ij}), probability of recapture (c_{ij}), and the population size (N_i) are estimated (j indicates the number of trapping occasions after the first in the session). The probability of survival (S_i), the probability of not being captured during the primary trapping session i given the individual was present during the primary trapping session $i - 1$ (γ''_i), and the probability of not being captured during the primary trapping session i given the individual was not present during the primary trapping session $i - 1$ (γ'_i) are estimated for the intervals between trapping sessions (Kendall et al. 1997). Program MARK includes an option for Pradel formulation in its robust design models. Pradel's λ is a measure of the rate of change of the age class that the encounter histories were derived from, or the estimate for the population rate of growth (Pradel 1996, Cooch and White 2012). Pradel's λ is dependent on the animal under study being seen at some point during the experiment.

Incorporating age-class into CMR studies can impact population parameters. Chaloupka and Limpus (2005) looked at age-class-specific survival probabilities of a population of green sea turtles (*Chelonia mydas*) by assigning an age-class classification to each turtle at first capture. Using age-classes allowed the authors to detect a decreasing age-class-specific natural mortality rate for the population (Chaloupka and Limpus 2005). Marescot et al. (2015) found strong evidence for age-specific survival of black-tailed deer (*Odocoileus hemionus columbianus*). Since large herbivores, such as caribou, have strong age-structured populations with markedly iteroparous life histories, different vital rates (such as age-specific survival and reproduction) will respond differently to various limiting factors (Jorgenson et al. 1997, Gaillard et al. 1998). The age-structure of the population affects estimates of adult survival, as yearly

juvenile survival is more varied than adult survival, due to limiting factors such as predation, harsh winters, poor calving areas, late parturition, and low birth rates (Jorgenson et al. 1997, Gaillard et al. 1998, DeCesare et al. 2012). Juvenile survival, highly sensitive to limiting factors, determines net rate of change in a population, and is an important factor in explaining changes in population size (Gaillard et al. 1998).

The objectives of this chapter are:

- Develop methods of estimating an age-class for woodland caribou populations for use in capture-mark-recapture studies;
- Use the developed age-classes to determine age-specific population parameter rates for the Tonquin herd of woodland caribou using capture-mark-recapture analysis.

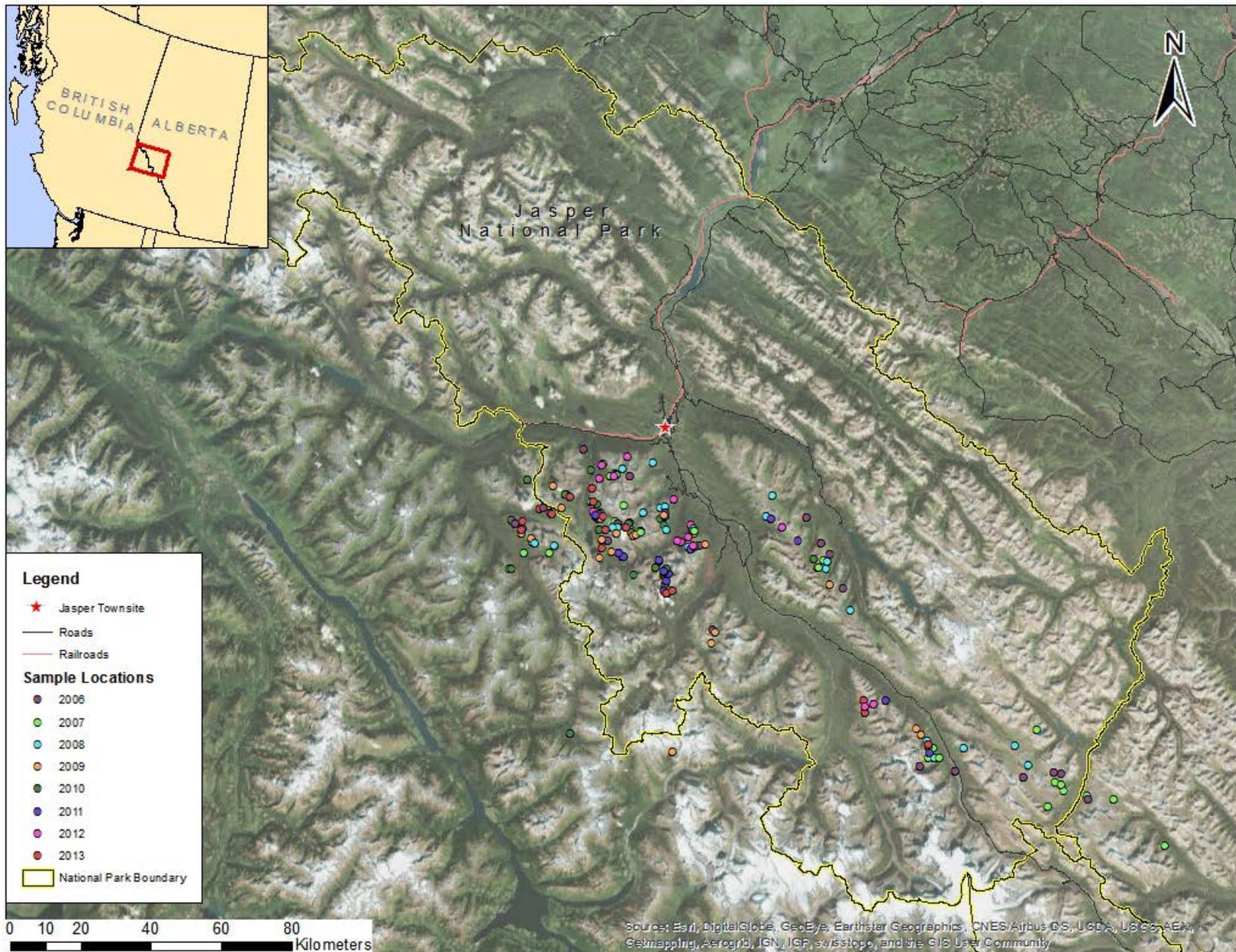
Methods

Study Area

Data were collected from the Tonquin, Maligne, and Brazeau woodland caribou herds (collectively referred to as South Jasper), and the North Interlake woodland caribou herd. The South Jasper herds are located in Jasper National Park (52°23'–52°84' N, 116°81'–118°45' W), Alberta, Canada (Map 1). The South Jasper herds belong to the Central Mountain Population (DU9), as assigned by COSEWIC, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2011). South Jasper caribou are listed as threatened under Canada's *Species at Risk Act* (Environment Canada 2014) and by the provincial Wildlife Act (Cichowski 2010). The Central Mountain population's range has decreased by up to 40%, 13 of 19 herds are in decline, and the Banff herd was extirpated in 2009 (Environment Canada 2014). Central Mountain caribou herds are small, increasingly isolated, and subject to multiple developments (Hebblewhite et al. 2007, Bradley and Neufeld 2012, Weckworth et al. 2013, Environment Canada 2014). The isolated populations in southern Jasper National Park are smaller compared to the A la Pêche population in northern Jasper National Park (Environment Canada 2014). South Jasper caribou migrate seasonally, cratering for terrestrial lichens in winter by descending into alpine and subalpine regions in search of arboreal lichens. They use valley bottoms and lower slopes in early winter, moving to upper slopes and ridge tops after the snow pack deepens and hardens, surviving for 6-8 months feeding on arboreal lichens (Thomas and Gray 2002). Moose (*Alces alces*), white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), elk (*Cervus canadensis*), bighorn sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus*), grizzly bears (*Ursus arctos*), black bears (*Ursus americanus*), cougars (*Puma concolor*), coyotes

(*Canis latrans*), wolves (*Canis lupus*) and wolverines (*Gulo gulo*) also occur in the same region as the South Jasper herds.

Map 1: Map of the South Jasper herds based on fecal pellet collection from 2006 to 2013.



Pellet Collection

Two fecal pellet collections occurred during the fall (October to December) from 2006 to 2013 for the South Jasper herds (Table 1); an additional collection occurred during the winter of 2007 (January 2008). Most individuals in the South Jasper range have been sampled, with a total of 1,768 samples collected (225 unique genotypes). Table 2 shows the summary of capture history results for the Tonquin herd, one of the South Jasper woodland caribou herds. Fecal pellets were collected in the snow, placed in sterile bags, and kept frozen until lab analysis.

Table 1: Summary of South Jasper capture history results

Sampling Time	Number of Samples Successfully Scored	Number of Unique Genotypes	Number of Genotypes Observed in Previous Capture Event	Proportion
Oct-06	152	74	-	-
Nov-06	97	38	12	0.32
Oct-07	119	53	-	-
Nov-07	107	49	27	0.55
Jan-08	37	15	6	0.40
Oct-08	124	72	-	-
Nov-08	114	46	22	0.48
Oct-09	134	62	-	-
Nov-09	118	56	39	0.70
Oct-10	121	56	-	-
Nov-10	99	40	26	0.65
Oct-11	86	40	-	-
Nov-11	126	50	28	0.56
Oct-12	105	33	-	-
Nov-12	71	24	14	0.58
Oct-13	95	40	-	-
Nov-13	63	24	18	0.75
TOTAL	1768	772	192	-

Table 2: Summary of Tonquin capture history results

Sampling Time	Number of Samples Successfully Scored	Number of Unique Genotypes	Number of Genotypes Observed in Previous Capture Event	Proportion
Oct-06	123	61	-	-
Nov-06	35	16	8	0.50
Oct-07	76	37	-	-
Nov-07	63	29	14	0.48
Jan-08	37	15	6	0.40
Oct-08	106	61	-	-
Nov-08	81	34	20	0.59
Oct-09	113	52	-	-
Nov-09	101	49	33	0.67
Oct-10	98	42	-	-
Nov-10	99	40	26	0.65
Oct-11	74	34	-	-
Nov-11	115	44	28	0.63
Oct-12	82	22	-	-
Nov-12	50	17	11	0.65
Oct-13	64	28	-	-
Nov-13	63	24	18	0.75
TOTAL	1380	605	164	-

Genetic Analysis

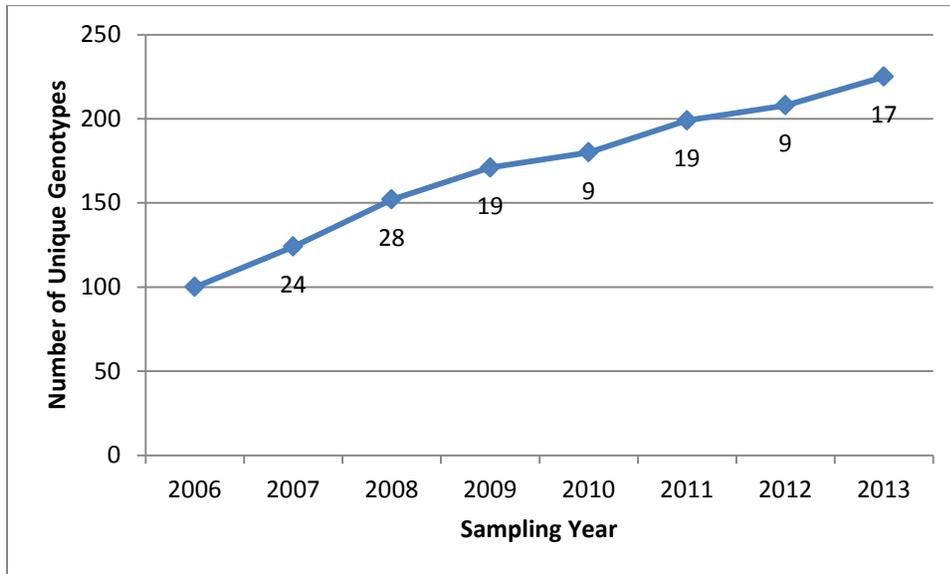
DNA extraction occurred by removing the mucosal layer of cells that coats the fecal pellets and following the extraction protocol outlined in (Ball et al. 2007). Eighteen polymorphic microsatellite loci were used (RT1, RT5, RT6, RT7, RT9, RT13, RT24, RT27, RT30, Map2C, BM4513, BM6506, BM848, BM888, BMS1788, OHEQ, NVHRT16 and FCB193) (Bishop et al. 1994, Stone et al. 1995, Wilson et al. 1997, Røed and Midthjell 1998, Jones et al. 2000, Cronin et al. 2005), along with caribou-specific Zfx/Zfy primers for sex identification.

Scoring took place using the program GeneMarkerTM after DNA was extracted and the target microsatellite markers were amplified. Two to three independent scorers examine the DNA profiles and then agree on a final score, following a documented protocol. Scoring results were uploaded to a shared database where results can be compared, and any discrepancies between scorers were flagged and discussed until a consensus was reached. If a consensus could not be reached, the sample was re-profiled to confirm the score, or removed from any further analyses and concluded to be a problematic sample.

Creating a Capture History

The capture history is created by identifying unique genotypes seen at each sampling period and clustering groups of samples that have the same unique genotypes. With eight years of sample collection (2006 to 2013), a large number of samples have been collected for the same animals, resulting in a large dataset with multiple samples belonging to the same individuals. Clustering of genotypes from samples collected over subsequent years and multiple sampling locations allowed individuals to be followed through time and space, which can be used in developing an age-class. The ALLELEMATCH program (Galpern et al. 2012a) identifies unique genotypes by clustering multilocus genotype data as well as identifying potential genotyping errors.

Figure 1: Cumulative number of unique genotypes observed through time for the South Jasper woodland caribou (*Rangifer tarandus*) herd. Data labels indicate the number of new individuals identified each year.



Hormone Analysis and Fecal Pellet Analysis

All hormone analysis took place at the Toronto Zoo's Endocrinology Lab. Three pellets per sample were selected for analysis. Pellets were dried overnight to remove any excess water, and weighed to the nearest 0.01 gram, and the average weight of the three pellets was taken to determine the average pellet weight per sample. Hormone content was measured in three individual pellets per fecal sample, with pellets homogenized for even distribution of hormones. Extraction followed the protocol of Morden et al. (2011b). Fecal pregnane and testosterone metabolite levels were quantified within the extracts using enzyme immunoassays (Morden et al. 2011b).

Age-Class Determination

Fecal pellet dry weight and hormone content per pellet (testosterone for males and pregnane for females), were used to determine the age-classes of South Jasper caribou. Pellets were chosen from the first occasion an individual was observed. Individuals were assigned to

two age-classes, either calves or adults. The threshold level between age-classes was set as the calf mean + two standard deviations for all three variables (fecal pellet dry weight, testosterone [males] and pregnane [females]) based on Flasko et al. (2015)'s thresholds. Hormone levels and weight of each individual were plotted against Flasko et al.'s thresholds to start classifying calves and adults. As individuals were categorized into calves or adults, the threshold for our data changed. For each individual, both the hormone content and pellet dry weight were used together to determine what age-class it belonged to. If both values were below the threshold, the individual was classified as a calf. If both values were above the threshold, it was classified as an adult. For those individuals where only one metric was above the threshold level (either hormone content or pellet weight), they were also classified as an adult. This process continued until the threshold level stabilized and all individuals were in the appropriate age-class. Individuals that were first observed in later sampling years (2011 and 2012) that did not have hormone or pellet data available were classified as calves for pedigree purposes. The South Jasper herd is a closed population that has been extensively sampled since 2006, so new genotypes that are identified in later capture years have a high probability of being calves, and not new individuals that have migrated from surrounding areas, or individuals who have not been sampled in previous years.

Pedigree Analysis

Using 18 loci for the pedigree analysis, only nearly complete unique genotypes with few missing loci were selected. Program FRANz (Riester et al. 2009) was used for parentage assignment, and COLONY 2.0 (Jones and Wang 2010a) was used for sibship assignment. FRANz uses an algorithm to construct pedigrees of natural populations by making use of prior information such as the age and sex of individuals, and statistical confidence is estimated by Markov Chain Monte Carlo (MCMC) sampling. FRANz has been used in a number of studies

(Ford et al. 2012, Miyamoto et al. 2013, Moore et al. 2014, Anderson et al. 2015, Ford et al. 2015).

Age-class and sex information was included in the analysis for the South Jasper herd of woodland caribou. FRANz uses date of birth to determine the age of individuals, so the date of birth of calves was given as the year of capture (i.e. a calf captured in 2006 has a year of birth of 2006). Individuals that were identified as adults were born previous to the first sampling year (2006), so the date of birth of these individuals was set to a year pre-2006. The age of first possible reproduction was set to 2 years of age for both sexes, as caribou can breed as young as two years of age (Eloranta and Nieminen 1986, Adams and Dale 1998, Miller 2003). FRANz identified all parents from the sampled population, but did not infer genotypes of missing parents that were not sampled. In order to find the missing parents that were not sampled, COLONY was used for inferring sibling relationships and identifying parental genotypes of non-sampled individuals. COLONY determines parental and sibling relationships among individuals from their multi-locus genotypes using a maximum likelihood method. COLONY can determine sibling relationships (full-siblings or half-siblings) even if the mother and/or father were not sampled and genotyped. The program provides an inferred genotype for that individual, and can determine how many offspring are attributed to that inferred parent. COLONY was used for inferring the missing sibling relationships that FRANz did not supply, creating a complete pedigree, with both a mother and a father for all individuals.

COLONY is widely used for pedigree analysis (Wang 2004, Wang 2009, Wang and Santure 2009, Walling et al. 2010, Jones and Wang 2010a, Karaket and Poompuang 2012, Wang 2012, Wang 2013a, Wang 2013b), however it was not chosen as the primary software for the pedigree analysis, as it does not incorporate prior information such as ages of individuals

sampled. Since the primary focus of this pedigree reconstruction was to use the age-class of individuals in South Jasper, FRANz was chosen for the primary pedigree reconstruction. The COLONY analysis was set up to allow polygamous mating in both sexes, the full-likelihood model was selected at medium precision, and a sibship prior was not set. Only parent-offspring and sibling relationships that had a probability of ≥ 0.9 were included for subsequent analyses. CraneFoot (Mäkinen et al. 2005) was used to build a visual pedigree-derived network of the parent-offspring relationships.

Capture-Mark-Recapture Modeling

Two different analyses were run on the same data for the Tonquin herd – the four groups (adult females, adult males, calf females and calf males), hereon referred to as the age-class analysis; and two groups (males and females), hereon referred to as the two-group analysis.

Age-Class Analysis

Individuals were separated into four groups: adult females, adult males, calf females, and calf males using results from the age-class determination. Those individuals where age-class could not be determined (8 individuals in total) were excluded from the analysis. Program MARK (White and Burnham 1999) was used for capture-mark-recapture analyses. Cormack-Jolly-Seber (CJS) models were run on the four groups to determine apparent survival (ϕ) and recapture probability (p) for models including age-class as a parameter. We ran sub-optimal models for the age-class data because we wanted to obtain age-specific estimates of survival for calves and adults. More models could have been run after removing age as a parameter, and likely would have been better fitting models for the data, but that is beyond the scope of what we were trying to achieve for this chapter. Survival parameters were fixed at 1.0 within years for the top CJS models to reflect closure in order to determine ϕ . Without fixing the within-year

sampling occasions to 1.0, MARK would give estimates for ϕ for each sampling occasion (two to three estimates of ϕ per year), whereas we want the ϕ estimate for each year overall. The first sampling occasion (the first two in 2007 as there were three sampling occasions) in each year was fixed so that only the last sampling occasion produced an estimate in order to better compare survival and recapture rates among the four groups. Likelihood-ratio tests (LRTs) were run for the top models (with parameters fixed) to determine if there were significant differences between models, based on structural differences in the models. LRTs compares a pair of models, one being the more restricted ‘nested’ model, and the other the full model.

Two-group Analysis

Individuals were separated into males and females. Robust design with Pradel models were chosen in Program MARK to get estimates of net rate of change (λ), survival (ϕ), and population size. The best fitting model for net rate of change (λ) found with Program MARK was used, and similar models with different λ (i.e. $\lambda(g*t)$, $\lambda(t)$) to get group- and time-specific rates of change.

Table 3: Parameter constraints used in population modeling of the Tonquin woodland caribou herd.

Parameter Estimates	Parameter Constraints
Apparent Survival - ϕ	(.)
	t
	a
	s
	a*s
	a*t
	s*t
	a*s*t
	F()
	M()
Recapture Probability – p (CJS)	(.)
	t
	a
	s

Table 3: Parameter constraints used in population modeling of the Tonquin woodland caribou herd.

Parameter Estimates	Parameter Constraints
	a*s a*t s*t a*s*t F() M()
Net rate of change - λ	(.) t g g*t
Capture probability - p (Robust Design)	(.) t g g*t pM(a+bE+off)
Recapture probability - c (Robust Design)	g*t c=p2

(.) – null model; no variation for estimated parameter value

t – time effect; parameter estimate varies based on capture interval

a – age effect; parameter estimate varies based on age-class sampled

s – sex effect; parameter estimate varies based on sex sampled

a*s – age effect, sex effect, interaction between age effect and sex effect; parameter estimates will vary by age and sex sampled, as well as between an interaction of age and sex terms

a*t – age effect, time effect, interaction between age effect and time effect; parameter estimates will vary by age and time sampled, as well as between an interaction of age and time terms

s*t – sex effect, time effect, interaction between sex effect and time effect; parameter estimates will vary by sex and time sampled, as well as between an interaction of sex and time terms

a*s*t – age effect, sex effect, time effect, interaction between group age effect, sex effect and time effect; parameter estimates will vary by age, sex, and time sampled, as well as between an interaction between these three terms

F() – parameter estimate for females only; all of the above models can be used for just females

M() – parameter estimate for males only; all of the above models can be used for just males

pM(a+bE+off) – effort model for males with offset for 2010 sampling year

c=p2 – recapture probability is equal to recapture probability

Population Size

K-sample Darroch models (Otis et al. 1978) were used in program CAPTURE (White et al. 1978) to estimate population size for all sampling years. N was found for the population as a whole, and separately for both females and males to look at sex-specific population size estimates. Estimates of N from robust design models were included to compare against the N estimates found from the Darroch models. We were unable to run robust design in program MARK on the Tonquin herd using the four groups (adult females and males, calf females and

males), as numerical convergence was never reached. In order to compare methods, previous results using only two groups (males and females) were used (Arnason 2015). Visual survey (telemetry) N results were also used to compare estimates of N . Radio collars and program NOREMARK (White 1996) were used to estimate N and precision (Bradley et al. 2013).

Results

Age-Class Determination

Using hormone content per pellet, pellet weight, and capture history, I was able to determine the age-class of 200 individuals out of 208, or 96.2% of the sampled population (Figure 2, Figure 3). Flasko et al. (2015) found an error rate of 15% for known adults and 7% for known putative calves in the South Jasper population, successfully classifying 85% of adults and 93% of calves. I used the same data and method as Flasko et al. (2015), expanding upon it by including all individuals of the population. The 8 individuals that could not be assigned an age-class did not have hormone data or fecal pellet dry weight data available, as the samples for these individuals could not be located, and were from early years in the capture history, so their age could not be inferred by their capture date. 76 calves and 124 adults were identified, and these age-classes were used along with the 8 individuals of unknown age in pedigree reconstruction.

Figure 2: South Jasper female pellet weight (g) versus pregnane (ng/g). Black dashed line represents calculated calf threshold (mean + two SD). Red dashed line represents calculated calf threshold from (Flasko et al. 2015).

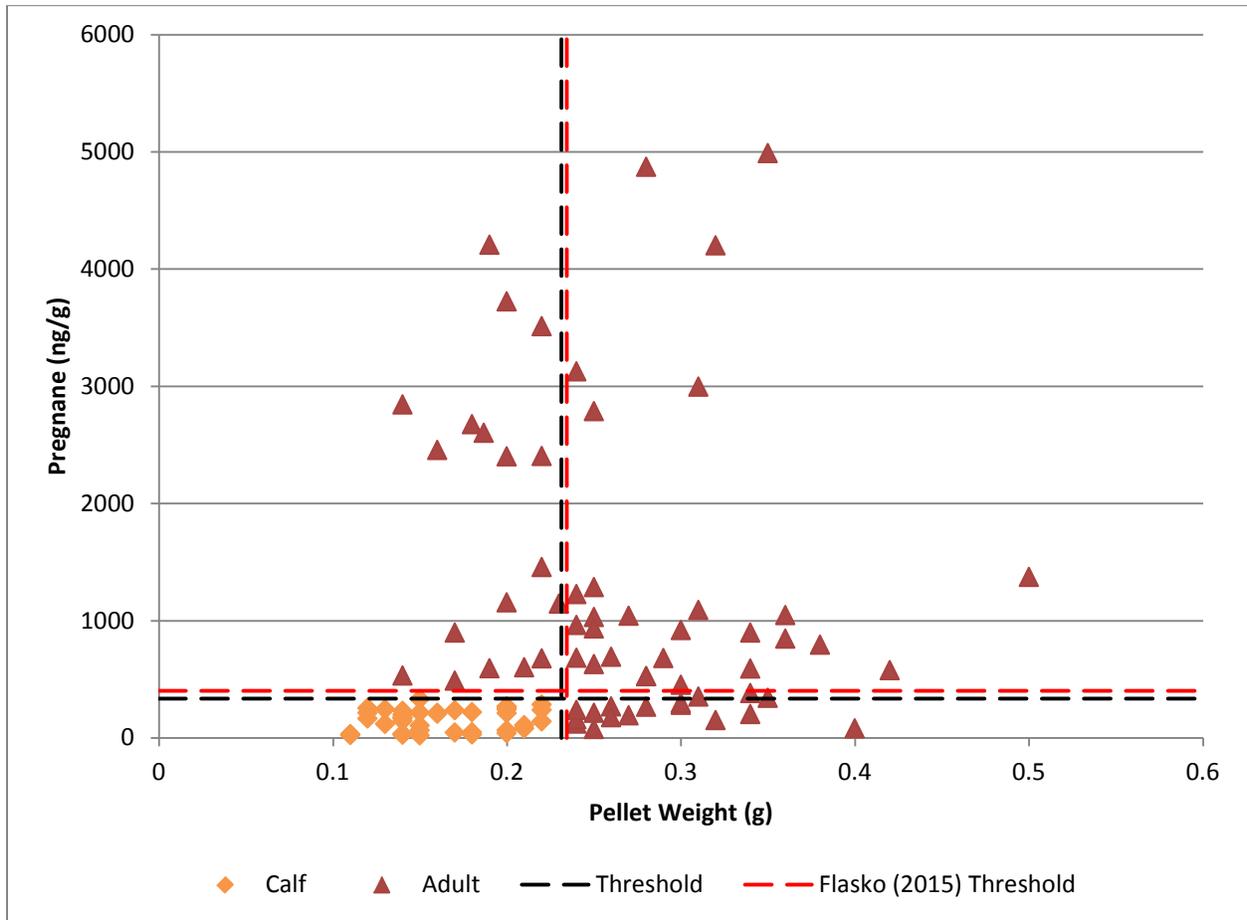
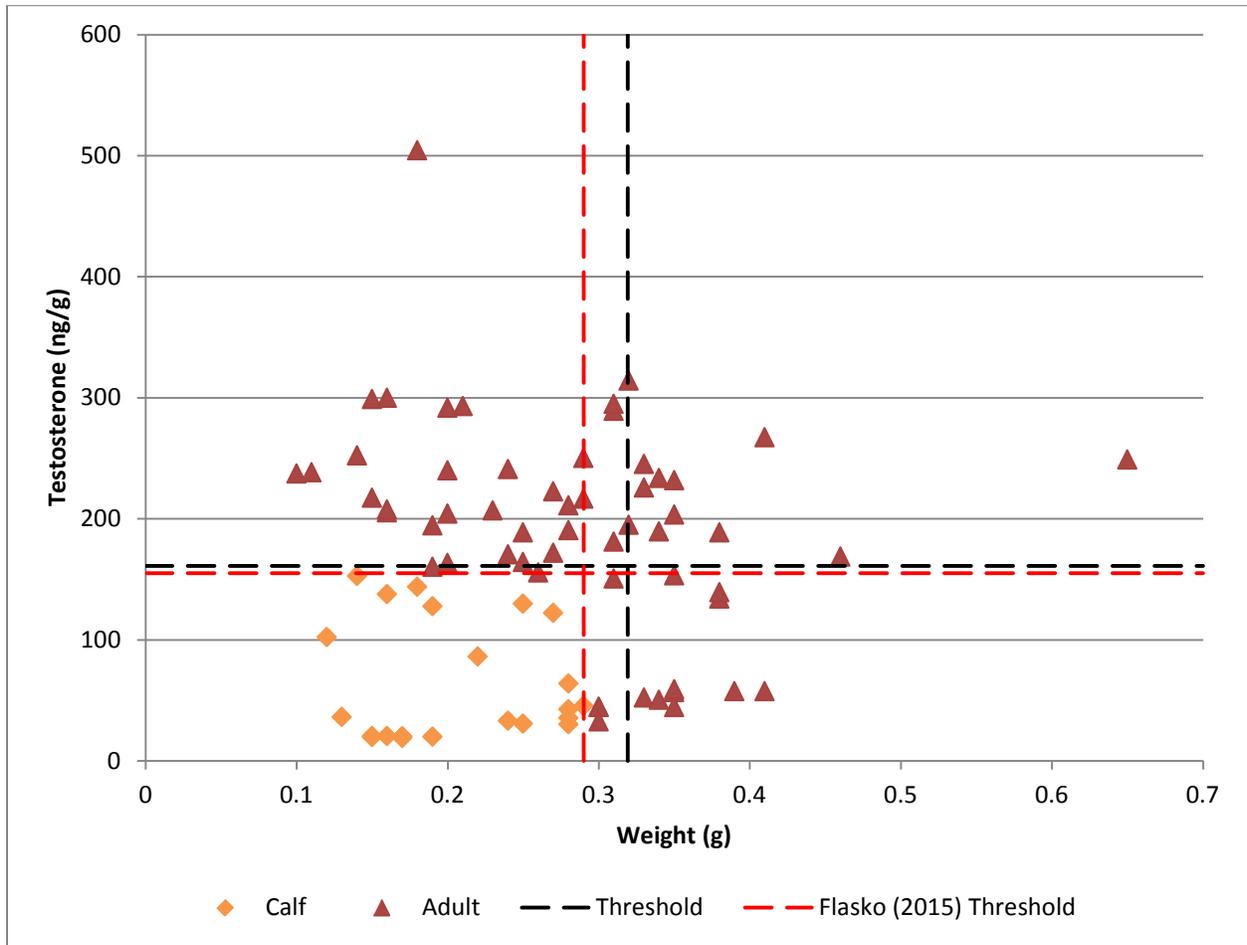


Figure 3: South Jasper male pellet weight (g) versus testosterone (ng/g). Black dashed line represents calculated calf threshold (mean + two SD). Red dashed line represents calculated calf threshold from (Flasko et al. 2015).

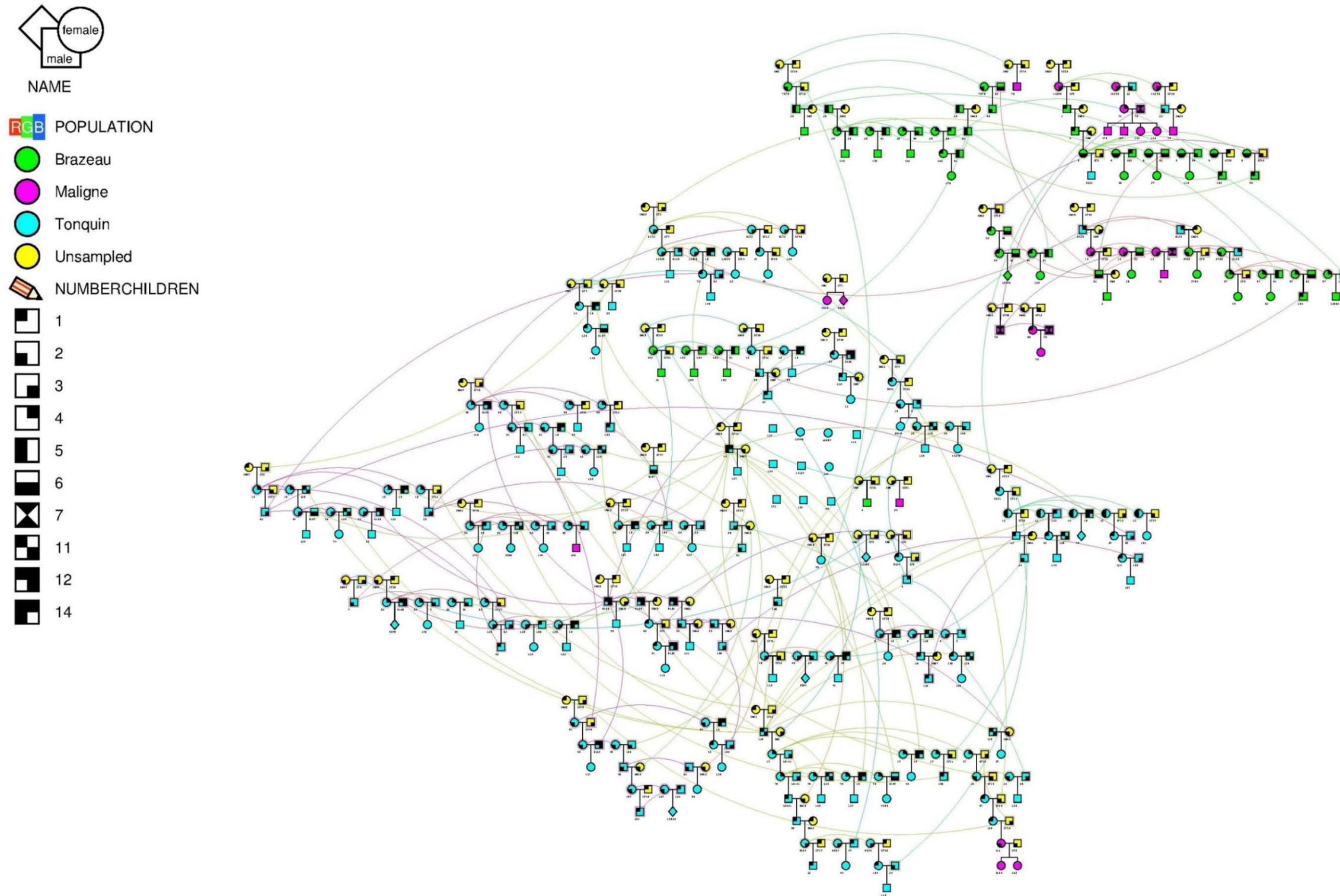


Pedigree Reconstruction

I used a total of 208 individuals in the pedigree analysis (107 females, 93 males and 8 unknown). Of these female genotypes, 59 produced offspring, equating to 55.1% of females successfully producing offspring that survived to their first fall. Of the male genotypes, 31 produced offspring, equating to 33.3% of males successfully reproducing offspring that survived to their first fall. FRANz was able to identify 52.4% of the fathers and 50.9% of the mothers, identifying a total of 64.9% percentage of parents. Combined with COLONY, 100% of the mothers and fathers were genetically assigned or reconstructed. COLONY inferred 29 fathers and 24 mothers, for a total of 60 unique fathers and 83 unique mothers.

The full pedigree for the South Jasper population is shown in Figure 4. Appendix A includes more detailed figures of portions of the pedigree.

Figure 4: Pedigree of South Jasper woodland caribou herd.



Capture-Mark-Recapture Analysis

In total, 61 adult females, 56 adult males, 42 female calves and 26 male calves were included in the age-class CMR analysis, for a total of 183 capture histories. For the sex-effect results, 85 females and 71 males were included in the CMR analysis.

Likelihood-Ratio Tests (Age-Classes)

Likelihood-ratio tests (LRTs) were run for the top ten models identified by MARK (Table 6). Figure 5 shows the relationship of the top ten models and the results of the LRTs between nested models. The top ten models identified by MARK were closely related models; 12 models are shown in Figure 5, with only two additional models needed to run LRTs between the models. One effect was removed between each nested model (identified by an arrow in Figure 5); the effect removed and the LRT results are listed in Table 4. Results in black text in Figure 5 indicate there were no significant differences between nested models. The reduced models are a better choice of model than the more general model, as statistically they fit the data equally well, but require fewer parameters to do so. Two LRTs produced significant differences between nested models, both involving removing the male recapture time effect.

Figure 6 demonstrates the lack of an age effect in the South Jasper population. There were no significant effects between any of the LRTs (Table 5). The reduced models with age effects removed are better model choices than the more general model, as they statistically fit the data equally as well, but require fewer parameters to do so.

Figure 5: Likelihood-ratio tests for top ten CJS models for the Tonquin herd of woodland caribou from 2006-2013. Φ = apparent survival, p = recapture probability, a = age effect, s = sex effect, t = time effect. χ^2 results followed by (degrees of freedom). P represents significance value.

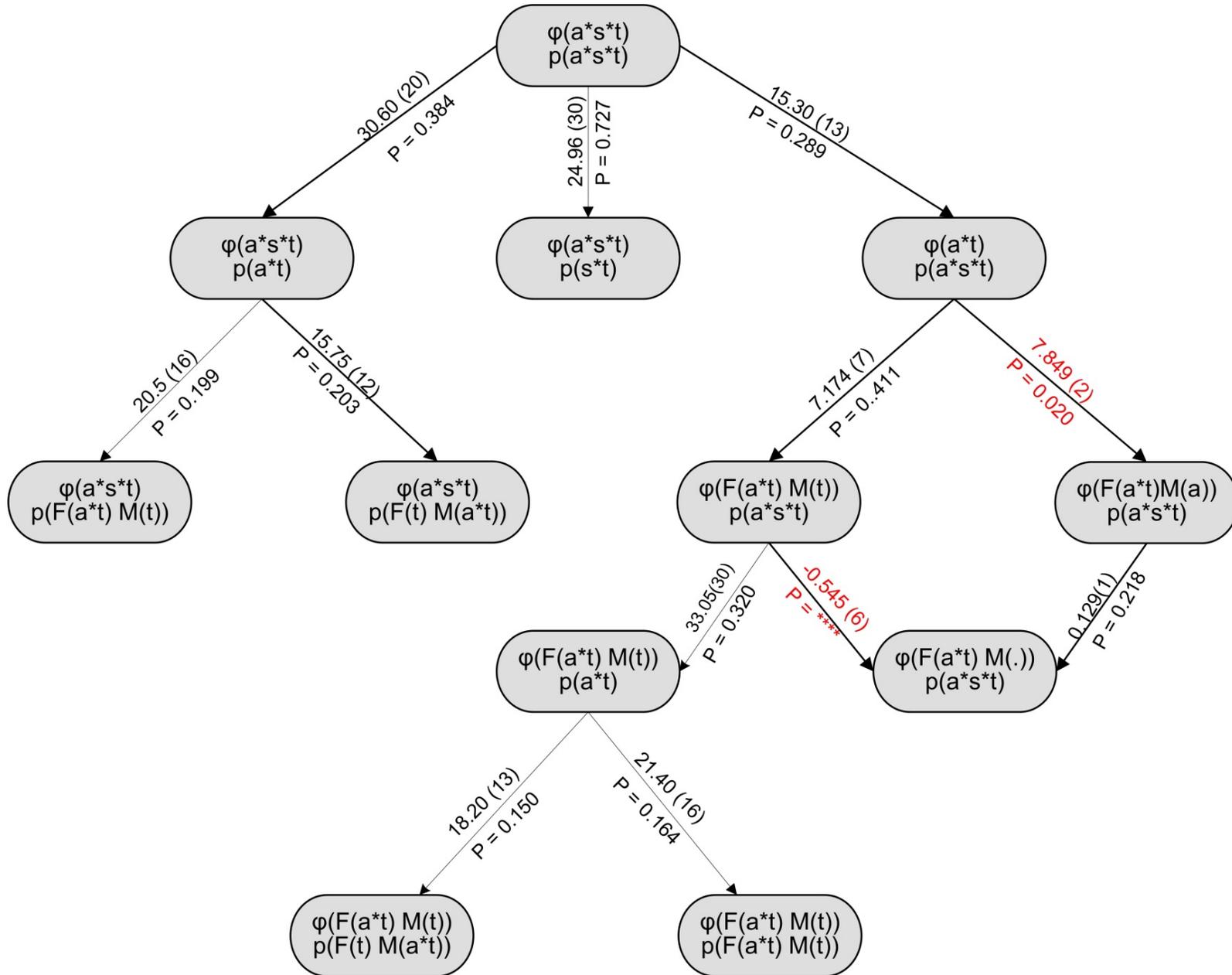


Table 4: Likelihood-ratio test results for the top ten CJS models and associated nested models. Significant values in italics.

Reduced Model	General Model	χ^2	df	Prob.	Effect removed
$\varphi(a^*t) p(a^*s^*t)$	$\varphi(a^*s^*t) p(a^*s^*t)$	15.3	13	0.289	φ sex effect
$\varphi(a^*t) p(a^*s^*t)$	$\varphi(F(a^*t) M(a)) p(a^*s^*t)$	7.849	2	<i>0.0198</i>	φ male time effect
$\varphi(a^*t) p(a^*s^*t)$	$\varphi(F(a^*t) M(t)) p(a^*s^*t)$	7.174	7	0.411	φ male age effect
$\varphi(F(a^*t) M(.)) p(a^*s^*t)$	$\varphi(F(a^*t) M(a)) p(a^*s^*t)$	0.129	1	0.7192	φ male age effect (φ held constant)
$\varphi(F(a^*t) M(.)) p(a^*s^*t)$	$\varphi(F(a^*t) M(t)) p(a^*s^*t)$	-0.545	6	*****	φ male time effect (φ held constant)
$\varphi(a^*s^*t) p(a^*t)$	$\varphi(a^*s^*t) p(a^*s^*t)$	30.602	29	0.3844	p sex effect
$\varphi(a^*s^*t) p(a^*t)$	$\varphi(a^*s^*t) p(F(a^*t) M(t))$	20.501	16	0.1985	p male age effect
$\varphi(a^*s^*t) p(a^*t)$	$\varphi(a^*s^*t) p(F(t) M(a^*t))$	15.747	12	0.2031	p female age effect
$\varphi(a^*s^*t) p(s^*t)$	$\varphi(a^*s^*t) p(a^*s^*t)$	24.956	30	0.7271	p age effect
$\varphi(F(a^*t) M(t)) p(a^*t)$	$\varphi(F(a^*t) M(t)) p(a^*s^*t)$	33.053	30	0.3202	p sex effect
$\varphi(F(a^*t) M(t)) p(a^*t)$	$\varphi(F(a^*t) M(t)) p(F(a^*t) M(t))$	21.403	16	0.1635	p male age effect
$\varphi(F(a^*t) M(t)) p(a^*t)$	$\varphi(F(a^*t) M(t)) p(F(t) M(a^*t))$	18.198	13	0.1501	p female age effect

Figure 6: Likelihood-ratio tests for the most parameterized model, $\varphi(a^*s^*t) p(a^*s^*t)$, removing age effects for both φ and p . Φ = apparent survival, p = recapture probability, a = age effect, s = sex effect, t = time effect. χ^2 results followed by (degrees of freedom). P represents significance value.

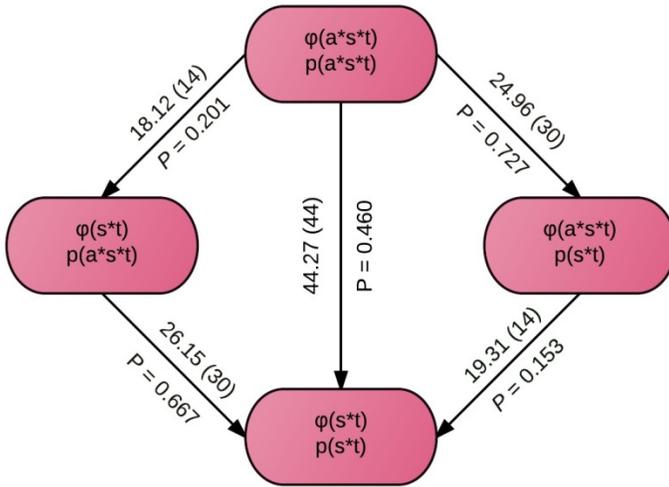


Table 5: Likelihood-ratio test results for the most parameterized model, $\varphi(a^*s^*t) p(a^*s^*t)$, removing age effects for both φ and p . Significant values in italics.

Reduced Model	General Model	χ^2	df	Prob.	Effect removed
$\varphi(a^*s^*t) p(s^*t)$	$\varphi(a^*s^*t) p(a^*s^*t)$	24.956	30	0.7271	p age effect
$\varphi(s^*t) p(a^*s^*t)$	$\varphi(a^*s^*t) p(a^*s^*t)$	18.119	14	0.2014	φ age effect
$\varphi(s^*t) p(s^*t)$	$\varphi(a^*s^*t) p(a^*s^*t)$	44.27	44	0.4602	φ and p age effect
$\varphi(s^*t) p(s^*t)$	$\varphi(a^*s^*t) p(s^*t)$	19.314	14	0.1533	φ age effect
$\varphi(s^*t) p(s^*t)$	$\varphi(s^*t) p(a^*s^*t)$	26.151	30	0.6674	φ p age effect

Apparent Survival (φ)

Age-class Analysis

The top ten CJS models for the Tonquin herd are presented in Table 6. All ten models have an age and time effect for females, while the males have an age effect in six models and a time effect in eight models. The best model is $\varphi(a^*s^*t) p(F(a^*t) M(t))$, the fourth model in Table

6. The first three models have very low number of parameters, and MARK is likely fixing parameters that are close to 0, underestimating the number of parameters. Model $\phi(a*s*t)$ $p(a*s*t)$ was the most parameterized model, with age, sex and time effects for both apparent survival and recapture probability. However, apparent survival estimates for the four groups (adult females, adult males, calf females, and calf males) had very wide overlapping 95% confidence intervals (Figure 7, Table 7), with no significant differences in survival rates for calves, adults, males or females. The final year with survival estimates (2012) had poor results, with three groups (adult females, calf female and males) not producing estimates, and adult males producing an estimate, but with a very large confidence interval. This is the result of the last sampling year (2013) having very few samples included in the analysis – only 20 in October 2013 and 1 in November 2013.

Table 6: Top CJS model results from Program MARK for the Tonquin herd from 2006-2013 with 2 age-classes (calves and adults).

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Number of Parameters	Deviance
$\phi(F(a*t) M(t)) p(a*s*t)$	1674.8	0	0.999	1	5	1044.3
$\phi(F(a*t) M(a)) p(a*s*t)$	1698.2	23.368	0	0	7	1063.6
$\phi(F(a*t) M(.)) p(a*s*t)$	1701.2	26.372	0	0	7	1066.6
$\phi(a*s*t) p(F(a*t) M(t))$	1707.7	32.840	0	0	21	1043.9
$\phi(a*s*t) p(F(t) M(a*t))$	1712.0	37.152	0	0	17	1056.6
$\phi(F(a*t) M(t)) p(F(t) M(a*t))$	1723.93	49.1176	0	0	22	1058.0
$\phi(a*s*t) p(s*t)$	1726.6	51.784	0	0	20	1064.9
$\phi(F(a*t) M(t)) p(F(a*t) M(t))$	1726.8	51.972	0	0	25	1054.4
$\phi(a*s*t) p(a*s*t)$	1728.1	53.296	0	0	32	1040.5
$\phi(a*t) p(a*s*t)$	1732.3	57.475	0	0	28	1053.4

Figure 7: Apparent survival (ϕ) for the model $\phi(a*s*t) p(a*s*t)$ for the Tonquin herd of woodland caribou from 2006-2013. AF = adult female; CF = calf female; AM = adult male; CM = calf male.

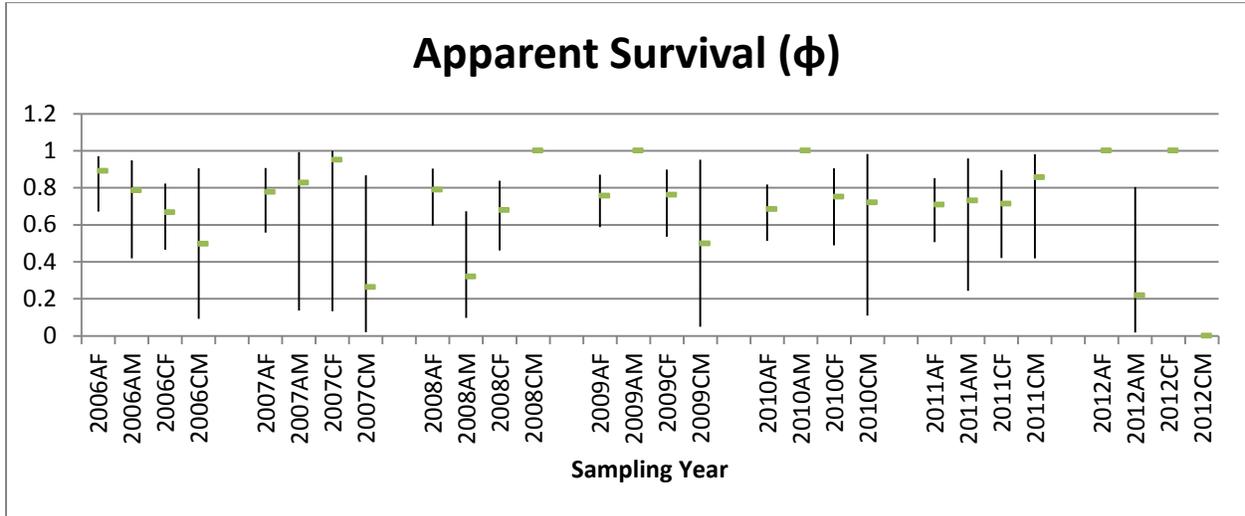


Table 7: Real parameter estimates for ϕ for the model $\phi(a*s*t) p(a*s*t)$ for the Tonquin herd of woodland caribou from 2006-2013.

	Year	Parameter Estimate	95% LCI	95% UCI
Adult Female	2006	0.89042	0.671589	0.969959
	2007	0.77756	0.557877	0.906401
	2008	0.788695	0.596756	0.903975
	2009	0.756686	0.587922	0.871447
	2010	0.684771	0.513998	0.816912
	2011	0.708616	0.506763	0.851989
	2012	1	0.999967	1.000033
Adult Male	2006	0.785141	0.418119	0.948936
	2007	0.826353	0.137051	0.993036
	2008	0.319709	0.096533	0.673956
	2009	1	1	1.000001
	2010	1	0.999997	1.000003
	2011	0.730897	0.244222	0.958034
	2012	0.217687	0.018469	0.804494
Calf Female	2006	0.667651	0.465882	0.822275
	2007	0.951642	0.13396	0.999601
	2008	0.678998	0.462657	0.838619
	2009	0.761399	0.536353	0.897987
	2010	0.751986	0.489939	0.9054

Table 7: Real parameter estimates for ϕ for the model $\phi(a*s*t) p(a*s*t)$ for the Tonquin herd of woodland caribou from 2006-2013.

	Year	Parameter		
		Estimate	95% LCI	95% UCI
Calf Male	2011	0.714159	0.421293	0.895557
	2012	1	0.999945	1.000055
	2006	0.496555	0.091469	0.906215
	2007	0.263534	0.019278	0.866914
	2008	1	0.999999	1.000001
	2009	0.498756	0.04835	0.95119
	2010	0.72128	0.109652	0.981942
	2011	0.857143	0.419389	0.98033
	2012	0	0	0

Two-group Analysis

We were unable to run robust design in program MARK on the age-class data, as numerical convergence was never reached. Population parameter estimates were found for the two-group data. The top ten robust design models are in Table 8. Nine of the top ten models have a time effect for female capture probability, and a male offset for sampling effort for capture probability. The top model is $\phi(t) \lambda(t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$, the second model in Table 8.

Table 8: Top Robust Design model results from Program MARK for the Tonquin herd from 2006-2013 for males and females.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Number of Parameters	Deviance
$\phi(.) \lambda(.) pF(t) pM(a+bE+off) c(g*t) N(g*t)$	181.4	0	0.679	1	45	83.7
$\phi(t) \lambda(t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$	183.7	2.355	0.209	0.308	57	57.2
$\phi(t) \lambda(g) pF(t) pM(a+bE+off) c(g*t) N(g*t)$	187.7	6.276	0.029	0.043	54	68.4
$\phi(t) \lambda(.) pF(t) pM(a+bE+off) c(g*t) N(g*t)$	188.7	7.313	0.018	0.026	53	71.9
$\phi(.) \lambda(g) pF(t) pM(a+bE+off) c(g*t) N(g*t)$	188.8	7.434	0.017	0.024	48	84.0
$\phi(t) \lambda(g+t) p(g*t) c(g*t) N(g*t)$	190.0	8.585	0.009	0.014	69	33.2
$\phi(t) \lambda(g*t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$	190.5	9.142	0.007	0.010	64	46.5
$\phi(g) \lambda(g) pF(t) pM(a+bE+off) c(g*t) N(g*t)$	191.2	9.793	0.005	0.008	49	84.0

Table 8: Top Robust Design model results from Program MARK for the Tonquin herd from 2006-2013 for males and females.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Number of Parameters	Deviance
$\phi(t) \lambda(g) pF(\cdot) pM(a+bE+pff) c=p2$ $N(g^*t)$	191.4	10.000	0.005	0.007	43	98.4
$\phi(t) \lambda(m(g+t) pF(t) pM(a+bE+off)$ $c(g^*t) N(g^*t)$	191.7	10.296	0.004	0.006	60	57.7

Apparent survival was similar between females and males, with all years having overlapping 95% confidence intervals. Survival was highest in 2007 ($F\phi = 0.93$, $M\phi = 0.95$), decreasing in 2008 and remaining relatively constant until 2012, the last year of estimates ($F\phi = 0.69$, $M\phi = 0.57$).

Figure 8: Sex-specific yearly apparent survival (ϕ) estimates for the Tonquin herd of woodland caribou from 2006-2012 found using Robust Design model $\phi(g^*t) \lambda(g^*t) pF(t) pM(a+bE+off) c(g^*t) N(g^*t)$. Error bars indicate 95% confidence intervals.

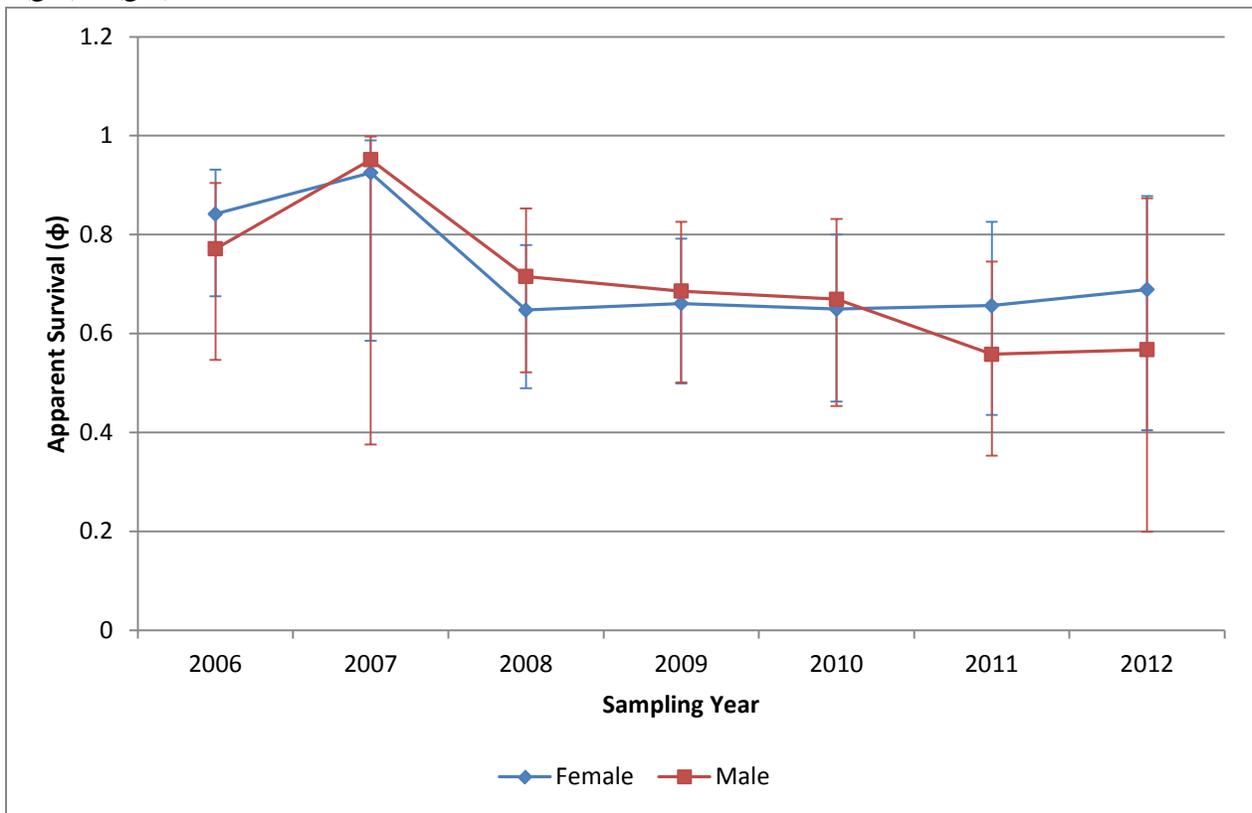
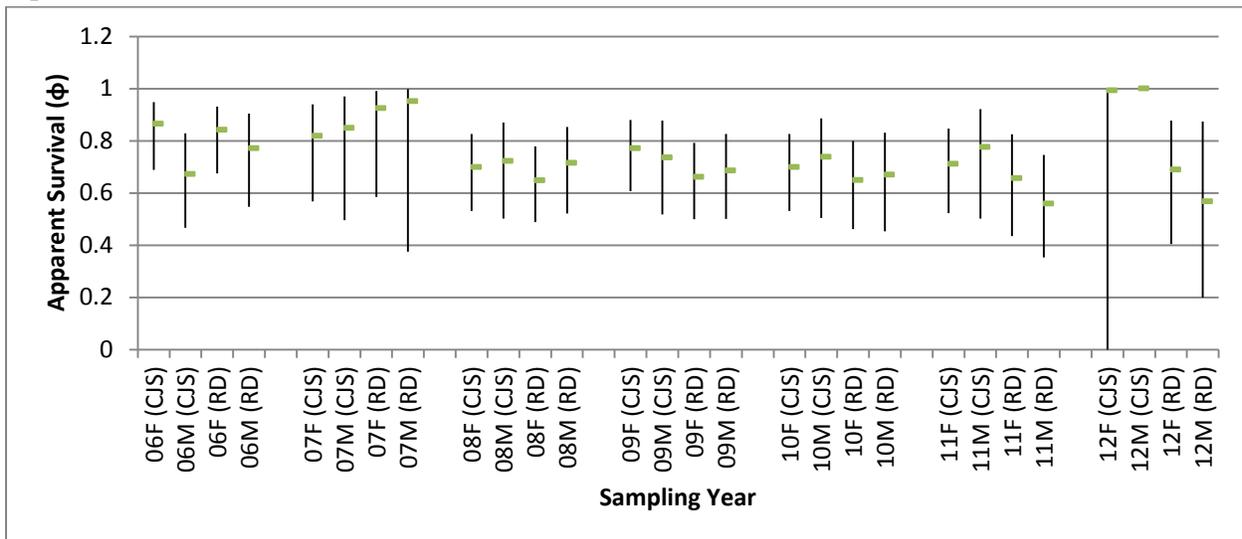


Table 9: Sex-specific yearly apparent survival estimates for the Tonquin herd of woodland caribou from 2006-2012 found using Robust Design model $\phi(t) \lambda(t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$. 95% confidence intervals in brackets.

Year	Females	Males
2006	0.84 (0.68, 0.93)	0.77 (0.55, 0.90)
2007	0.93 (0.59, 0.99)	0.95 (0.38, 1.00)
2008	0.65 (0.49, 0.78)	0.71 (0.52, 0.85)
2009	0.66 (0.50, 0.79)	0.69 (0.50, 0.83)
2010	0.65 (0.46, 0.80)	0.67 (0.45, 0.83)
2011	0.66 (0.44, 0.83)	0.56 (0.35, 0.75)
2012	0.69 (0.40, 0.88)	0.57 (0.20, 0.87)

When comparing survival estimates between the age-class data and the two-group data, they produced similar estimates (Figure 9). For the CJS models, 2012 did not produce estimates with confidence intervals, as there were very few samples included for 2013 for estimating survival for the year prior (this is due to the remove of the 8 samples that did not have hormone or pellet data available, which were all from 2013).

Figure 9: Comparison of sex-specific yearly apparent survival estimates for the Tonquin herd of woodland caribou from 2006 to 2012 using age-class data (CJS) and two-group data (RD). Lines represent 95% confidence intervals.



Population Net Rate of Change

Population net rate of change (λ) was found using the model $\phi(t) \lambda(t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$ from the robust design analysis. From 2006 to 2011, the Tonquin population had a declining trend in λ for both males and females with λ estimates decreasing below 1, indicating negative net rate of change (Figure 10, Table 10). In 2012, net rate of change estimates increased, with λ for both males and females above 1, indicating positive net rate of change from the previous year. The population size results also indicate there is a positive trend in net rate of change from 2012 to 2013 (Figure 11, Table 12).

Figure 10: Plot of λ by year and sex for the Tonquin herd of woodland caribou from 2006-2012 found using Robust Design model $\phi(t) \lambda(g*t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$. F = female λ ; M = male λ ; * = constant λ for both sexes. Line separates positive and negative net rate of change ($\lambda = 1$).

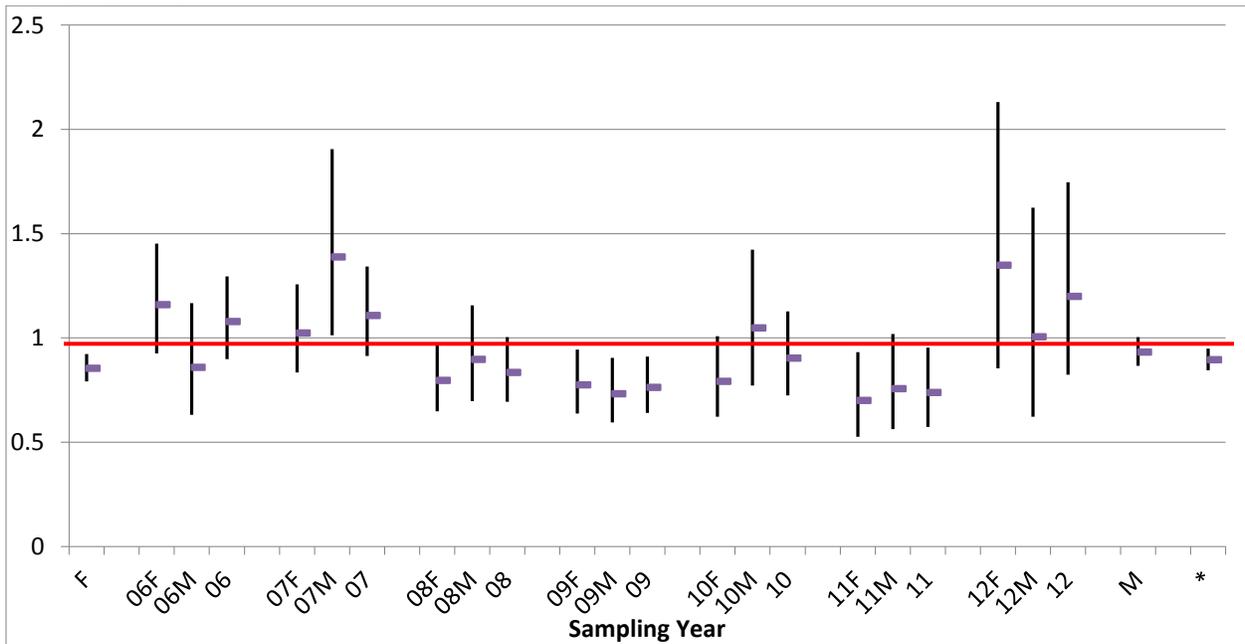


Table 10: Sex-specific yearly net rate of change estimates for females and males in the Tonquin herd of woodland caribou from 2006-2013 using Robust Design model $\phi(t) \lambda(t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$. 95% confidence intervals in brackets.

Year	Female	Male	Constant (both sexes)
2006	1.15 (0.93, 1.45)	0.86 (0.63, 1.17)	1.08 (0.90, 1.30)
2007	1.02 (0.83, 1.26)	1.39 (1.01, 1.91)	1.11 (0.91, 1.34)
2008	0.80 (0.65, 0.98)	0.90 (0.70, 1.16)	0.83 (0.69, 1.00)
2009	0.78 (0.64, 0.94)	0.73 (0.59, 0.90)	0.76 (0.64, 0.91)
2010	0.79 (0.62, 1.01)	1.05 (0.77, 1.42)	0.90 (0.72, 1.13)
2011	0.70 (0.53, 0.93)	0.76 (0.56, 1.02)	0.74 (0.57, 0.95)
2012	1.35 (0.85, 2.13)	1.01 (0.62, 1.63)	1.20 (0.82, 1.75)
2006-2012	0.85 (0.79, 0.92)	0.93 (0.87, 1.00)	0.90 (0.85, 0.95)

Population Size

Sex-specific population size estimates were found using the model $\phi(t) \lambda(t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$ from the robust design analysis. From 2006 to 2012, there is a negative trend in population size, decreasing from 60 females and 36 males in 2006, to 17 females and 17 males in 2012 (Figure 11, Table 11). In 2013 there was a slight increase in N , with the number of females increasing to 22 and the number of males increasing to 20. As sampling in 2012 began a month later than in other sampling years (in November and December, compared to October and November in all other sampling years; Table 1), and individuals had likely already moved out of the study area during this time.

The estimates for total population size in Figure 11 were found by adding the female and male population size estimates. The confidence intervals for total population size were found using $Total\ Population \pm 1.96\ SE$, where $Total\ population\ SE = \sqrt{var(F) + var(M)}$. This formula is correct when the covariance is 0, and the correct formula is $Total\ population\ SE = \sqrt{var(F) + var(M) + 2cov(F, M)}$. However the female and male estimates are formed from the same dataset and are likely positively correlated. The confidence intervals used here for the

total population size estimates are thus likely narrower than the true confidence intervals, as we have a correlation between females and males.

Figure 11: Sex-specific population size (N) estimates for males and females in the Tonquin herd of woodland caribou from 2006-2013 using Robust Design model $\phi(t) \lambda(t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$. Error bars indicate 95% confidence intervals.

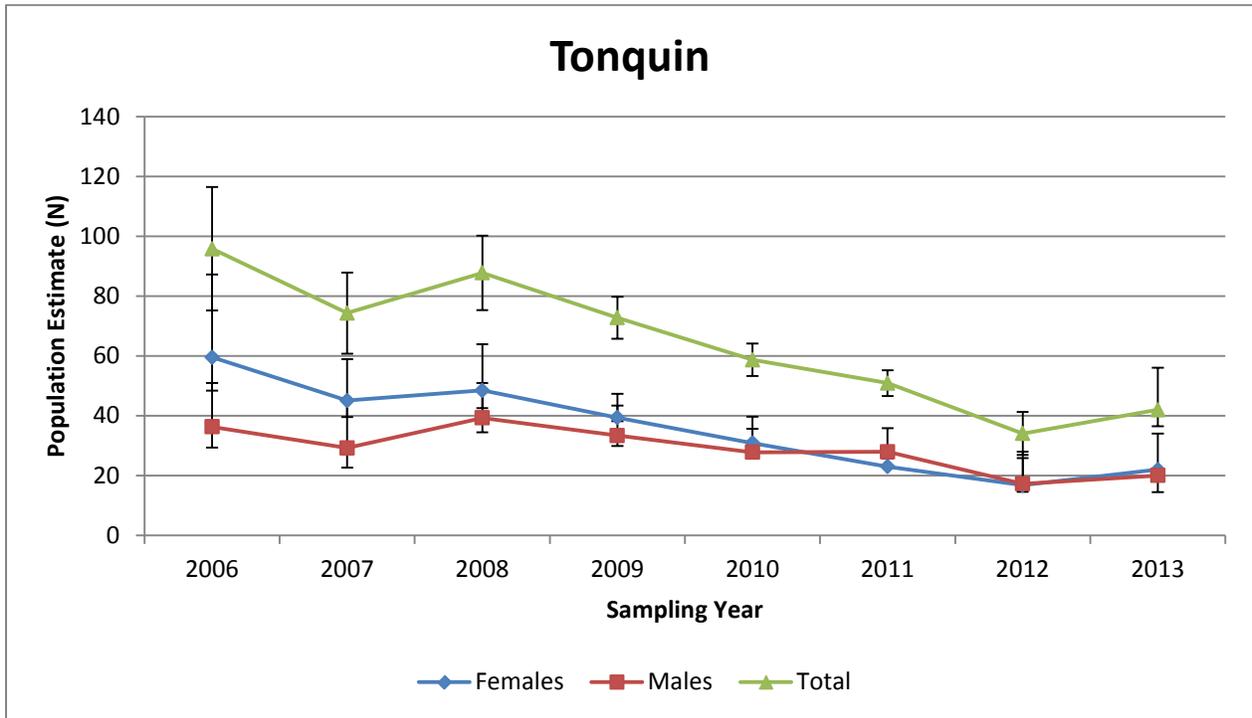


Table 11: Sex-specific yearly population size estimates for females and males in the Tonquin herd of woodland caribou from 2006-2013 from Robust Design model $\phi(t) \lambda(t) pF(t) pM(a+bE+off) c(g*t) N(g*t)$. 95% confidence intervals in brackets.

Year	Females	Males
2006	59.54 (48.44, 87.17)	36.29 (29.32, 50.97)
2007	45.07 (39.59, 58.91)	29.23 (22.70, 44.86)
2008	48.47 (42.60, 63.92)	39.28 (34.44, 50.96)
2009	39.41 (38.21, 47.30)	33.36 (29.86, 43.42)
2010	30.90 (29.34, 39.63)	27.78 (26.33, 35.64)
2011	23.00 (23.00, 23.00)	27.94 (25.80, 35.84)
2012	16.81 (14.56, 28.03)	17.28 (14.91, 25.91)
2013	22.00 (22.00, 22.02)	20.1 (14.53, 34.01)

Yearly population size estimates for the Tonquin population from the Darroch, robust design, and visual survey methods are shown in Table 12. Overall, there appears to be a

declining population size from year to year with all methods of estimating N . When comparing Darroch to the robust design and visual survey estimates, it produces comparable yearly estimates, except for the first sampling year, 2006 (Figure 12). 2006 was the first year of sampling for the Tonquin herd, with the first sample occurring in October, and there was very low recapture probability in November 2006, with only 17% recapture rate (Appendix A, Table 14). Low numbers of animals being captured affects the population size estimate, as there are less individual capture histories for that sampling occasion, causing the 2006 population size to be overestimated with very large confidence intervals ($N = 221 (158, 349)$, Table 14) and very large standard error ($SE = 46.6$). The robust design estimates were taken from the model $\phi(t) \lambda m(t) p F(t) p M(a+bE+off) c=p2 N(g*t)$, and the N estimates for both sexes were summed together for a total population size estimate. Sex-specific population size estimates for Darroch and robust design are shown in Table 13. Similar to results from the entire Tonquin population, both methods produced comparable yearly estimates for all years, except for females 2006 (Figure 13 and Figure 14). Males produced comparable results in 2006, with Darroch producing a lower estimate than robust design (Darroch = 25 (24, 39); RD = 36 (29.3, 51.0)), whereas Darroch overestimated N for females in 2006, producing a very large confidence interval (Darroch = 82 (54, 173); RD = 60 (48.4, 87.2)). It is likely that some degree of trap response occurred during the first year of sampling (2006) that is causing the estimate to be overestimated. Table 17 in Appendix A shows the number of individuals that were captured and recaptured at each sampling occasion for each year. Although 50 individuals were captured during the first sampling occasion (X_{10}), only 7 of these individuals were recaptured during the second sampling occasion as well (X_{11}), and only 7 new individuals were captured during the second sampling occasion that weren't captured during the first (X_{01}). In total, only 14 individuals were captured

during the second sampling occasion in 2006, compared to 57 captured during the first sampling occasion; of all the sampling years, 2006 had the lowest number of individuals seen during the second occasion compared to the first (Table 17, Appendix A).

Figure 12: Population size estimates using Darroch (D), visual survey (V), and robust design (R) for the Tonquin herd of woodland caribou. Lines represent 95% confidence intervals.

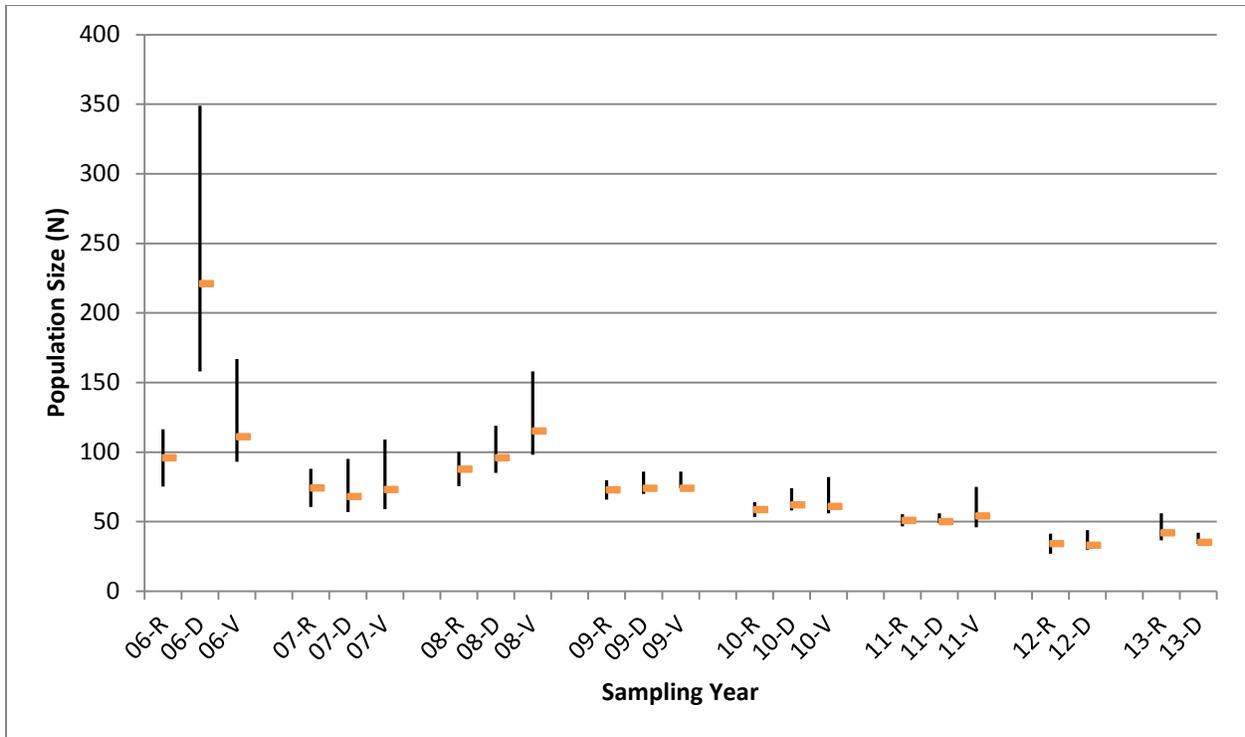


Table 12: Yearly population size estimates for the Tonquin herd of woodland caribou from 2006-2013.

Year	Darroch	Robust Design	Visual Survey
2006	221 (158, 349)	96 (75.2, 116.4)	111 (93, 167)
2007	68 (57, 95)	74 (60.7, 87.9)	73 (59, 109)
2008	96 (85, 119)	88 (75.3, 100.2)	115 (98, 158)
2009	74 (70, 86)	73 (65.8, 79.8)	74 (74, 86)
2010	62 (58, 74)	59 (53.2, 64.1)	61 (56, 82)
2011	50 (49, 56)	51 (46.6, 55.2)	54 (46, 75)
2012	33 (30, 44)	34 (26.9, 41.3)	-
2013	35 (34, 42)	42 (37, 56)	-

Figure 13: Comparison of female yearly population size estimates using Darroch (D) and robust design (R) for the Tonquin herd of woodland caribou. Lines represent 95% confidence intervals.

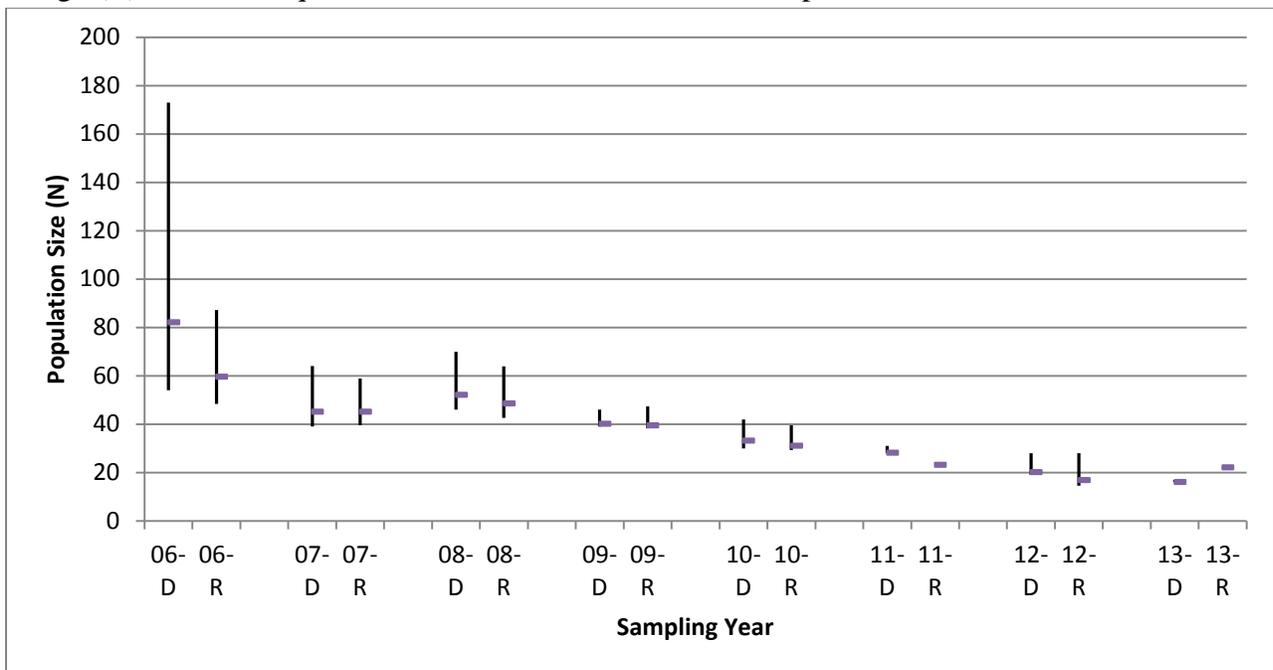


Figure 14: Comparison of male yearly population size estimates using Darroch (D) and robust design (R) for the Tonquin herd of woodland caribou. Lines represent 95% confidence intervals.

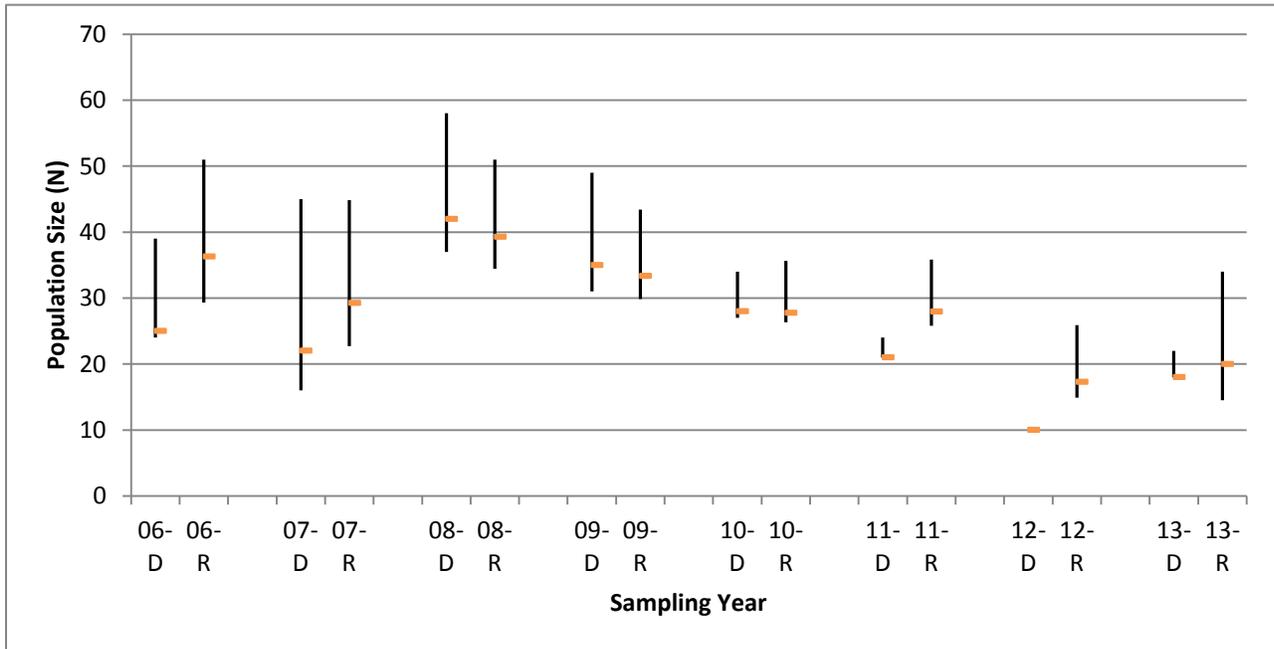


Table 13: Sex-specific yearly population size estimates for the Tonquin herd of woodland caribou from 2006-2013.

Sex	Year	Darroch	Robust Design
Females	2006	82 (54, 173)	60 (48.4, 87.2)
	2007	45 (39, 64)	45 (40.0, 58.9)
	2008	52 (46, 70)	48 (42.6, 63.9)
	2009	40 (39, 46)	39 (38.2, 47.3)
	2010	33 (30, 42)	31 (29.3, 39.6)
	2011	28 (28, 31)	23 (23, 23)
	2012	20 (19, 28)	17 (14.6, 28.0)
	2013	16 (16, 17)	22 (22, 22)
Males	2006	25 (24, 39)	36 (29.3, 51.0)
	2007	22 (16, 45)	29 (22.7, 44.9)
	2008	42 (37, 58)	39 (34.4, 51.0)
	2009	35 (31, 49)	33 (29.9, 43.4)
	2010	28 (27, 34)	28 (26.3, 35.6)
	2011	21 (21, 24)	28 (25.8, 35.8)
	2012	10 (10, 10)	17 (14.9, 25.9)
	2013	18 (18, 22)	20 (14.5, 34.0)

Discussion

Age-Class Determination

Our results show that pregnane levels in female caribou can be a useful first step in identifying age-classes in South Jasper by separating calves and non-pregnant females from females in oestrous. The high variation in pregnane levels of adult females indicate that many females were in oestrous or early stages of pregnancy. Caribou are a polyestrous species, with oestrous cycling occurring continually until fertilization occurs (Bubenik et al. 1997, Shipka et al. 2007). Average peak levels of progesterone in captive pregnant female caribou and reindeer are higher than in non-pregnant females, and single samples have been frequently used to infer pregnancy (Flood et al. 2005, Ropstad et al. 2005, Shipka et al. 2007). Progesterone levels in non-pregnant females fluctuate throughout the breeding season, peaking in spring (Bubenik et al. 1997, Shipka et al. 2007). Peak levels of progesterone in non-pregnant females vary between individual caribou (Shipka et al. 2007). This makes separating female calves and non-pregnant adult females challenging, as non-pregnant individuals may have been sampled at a point in their ovulation cycle where pregnane levels are low. Using two variables together (hormone levels and fecal dry pellet weight) gives more information about the individual than just hormone levels or pellet weight alone. Body mass has been linked to fecal pellet size in many species, with calves having significantly smaller pellets than adults (Coe and Carr 1983, Chapman 2004, Sanchez-Rojas et al. 2004, Ball 2010, Morden et al. 2011a). We were able to successfully separate male calves and adults in South Jasper using testosterone levels along with fecal pellet dry weight. Testosterone levels in male caribou peak in August and September during the rutting season (Whitehead and McEwan 1973, Stokkan et al. 1980, Bubenik et al. 1997). Testosterone levels have been shown to increase with age in reindeer, with significant differences between male

calves and adults (Leader-Williams 1979, Flasko 2014). The South Jasper woodland caribou herds have been closely studied for several years, with a nearly complete capture history of the entire population. Samples were collected from the first occasion an individual was sampled for the majority of the population, but it was not known beforehand whether that individual was a calf or adult, instead relying solely on hormone content and fecal pellet weight to determine age-classes of individuals. Our threshold results were very similar to Flasko et al. (2015)'s thresholds for males and females (Figure 2 and Figure 3). We built off of Flasko et al.'s methods, using the same data and methods, expanding it to include all members of the population. Flasko et al. was able to classify 85% of adults and 93% of calves correctly, with only 15% and 7% misclassified, respectively. Using these methods, we classified 96.2% of all individuals.

Capture-Mark-Recapture

Apparent Survival

Results from the likelihood-ratio tests (LRTs) on the CJS models show that there are no significant age effects in survival for both calf and adult females and males. Although the top model, $\phi(F(a*t)) M(t) p(a*s*t)$, has an age effect for females in the model, plotting the yearly survival estimates for adult females and calf females shows that the 95% confidence intervals overlap for all groups (Figure 7). Figure 6 clearly shows that there is no age effect for our study population, as the less parameterized models (with the age effect removed) are not significantly different from the most parameterized model, $\phi(a*s*t) p(a*s*t)$, which has an age effect for both apparent survival and recapture probability. The IUCN SSG Conservation Breeding Specialist Group (CBSG) modeled calf and adult survival rates of captive and wild woodland caribou and found that calf survival was significantly lower than adult survival rates. Mortality of calves is highest within the first week after birth, with 18% of deaths occurring on the first day of birth,

53% during the first week, 67% within the first month, and 90% of all calf deaths occurring within the first six months after birth (Traylor-Holzer 2015). Gustine et al. (2006) reported survival rates of 54% and 79% in 2002 and 2003 of woodland caribou calves in the first 56 days after birth in northern British Columbia, with survival higher through the calving season (88%; May 25th – June 14th) compared to the summer season (69%; June 15th – July 31st). Pinard et al. (2012) found that only 46% of woodland caribou calves in Québec survived during the first 50 days following birth, and survival rates were low during the first two or three weeks of life. Another study of forest-dwelling woodland caribou in Québec found that only 41% of calves survived their first year after birth (Leblond et al. 2013). Although no significant differences were found between calf and adult survival for the South Jasper population, age-classes can be used to estimate different survival rates for calves and adults. Since our first sampling occasion took place in the fall (October for all years, except for November in 2012), and calves are born in May or June (Bergerud 1975, Eloranta and Nieminen 1986, Adams and Dale 1998, Miller 2003), our calves in Tonquin were five- to six-months old at the time of first sampling, with the second sampling occasion occurring roughly a month after the first sampling occasion. Since we are not sampling calves until they are six months of age, we are less likely to see a difference between adult and calf survival, since 90% of calf deaths occur within the first six months (Traylor-Holzer 2015). When comparing the estimates of apparent survival for the age-class data (using CJS models) and the two-group data (using Robust Design models), they produced similar estimates of survival for all years (Figure 9).

Two-group Analysis

Robust Design analyses showed that net rate of change decreased from 2006 to 2011, and increased in 2012 (Figure 10). In 2012, sampling occurred a month later than in all other years

(November and December in 2012, compared to in October and November in other years), and individuals in the Tonquin herd had left the survey area at this time. The Tonquin herd belongs to the Central Mountain population, which are found in steep, mountainous habitats with deep snowfall, and winter at high elevations to feed on arboreal lichens (Terry et al. 2000, COSEWIC 2011). During the sampling in 2012, individuals in the survey area had likely already migrated to higher elevations, which depressed survival and net rate of change estimates for that year, and the overall net rate of change as well (Arnason pers. comm.). Net rate of change increased in 2012 when sampling occurred in October and November, and net rate of change was over 1, indicating positive net rate of change.

Population Size

The sex-specific population size estimate indicates that there are more females in the population than males, with the sex ratio becoming more equal from 2010 to 2013 (Figure 11). Overall, the population size has decreased significantly, from 96 individuals in 2006 to only 42 in 2013. However, there was a slight increase from 2012 to 2013 (34 to 42 individuals), which aligns with the net rate of change results, which showed that the population had a positive net rate of change rate from 2012 to 2013. The decrease in net rate of change and population size may be due to the late sampling that occurred during 2012.

Comparing the Robust Design results to the closed population model results and visual survey results shows that all methods produce comparable yearly estimates of population size (Figure 12). There appears to be some degree of trap response and sex-based capture heterogeneity that occurred during the first sampling year (2006). A trap response is a situation where marking or capturing an individual causes a change in its capture or survival probability for some time after it is initially captured (Nichols et al. 1984). For all three methods of

estimating population size (Darroch, robust design, and visual), especially the Darroch estimator, have upwardly biased estimates of N , with very large confidence intervals (Figure 12). When looking at the sex-specific estimates of N using the Darroch method and the robust design method, it appears that the trap response is occurring primarily in the females (Figure 13, Figure 14). A “trap-shy” response (where individuals avoid the trap or sample location) can seriously affect estimates of N , causing N to be overestimated (Nichols et al. 1984). With a low number of captures occurring during the second sampling occasion in 2006 (Table 17 Appendix A), something may have occurred during the first sampling occasion that caused individuals not to return to the sample location, or there was a change between the first and second sampling occasions that lowered the number of individuals available for sampling. The Darroch estimator only uses data from one year of sampling to estimate N for that year, whereas robust design produces yearly estimates of N from all of the data together, which may explain why the Darroch estimate is much more biased than the robust design estimate for 2006. Using sequential population size estimates from closed population models, like the Darroch method, are a useful alternative of assessing population growth when open population modeling cannot be done. As we were unable to run robust design on the Tonquin herd for the age-class data, we could not get an estimate for the population rate of growth (λ), so the yearly N estimates from the closed population Darroch estimator allows population growth to still be assessed.

Conservation and Management Implications

The stratification of available samples based on age and sex provided a better method of determining the pedigree and parents of the South Jasper population. Knowing the age of individuals and the birth year of calves allows parents to be assigned based on age and not solely

on genetic relatedness alone, ensuring that calves are correctly assigned to adult parents, and calves are not considered to be parents when they are too young to have produced offspring.

If managers are interested in determining calf survival rate through non-invasive sampling methods, sampling should occur closer to the calving period in May or June. With up to 90% of calf mortality occurring in the first six months after birth (Traylor-Holzer 2015), survival rate is likely higher by the time sampling occurs in the fall. Sampling earlier means a larger number of calves can be sampled. Sampling multiple times per year (two or three times) while sampling closer to the calving period can enable managers to detect within-year changes in survival. With large changes in calf survival from the first week and the first few months after birth, sampling a population more than once per year can enable managers to determine these changes in calf mortality rates.

As woodland caribou are listed as an endangered species, management efforts should focus on monitoring population growth for identifying signs of negative population growth. For small populations or populations with several groups included in the models (such as sex and age groups), using sequential population size estimates from closed population models can give an alternative method of assessing population growth with less data available.

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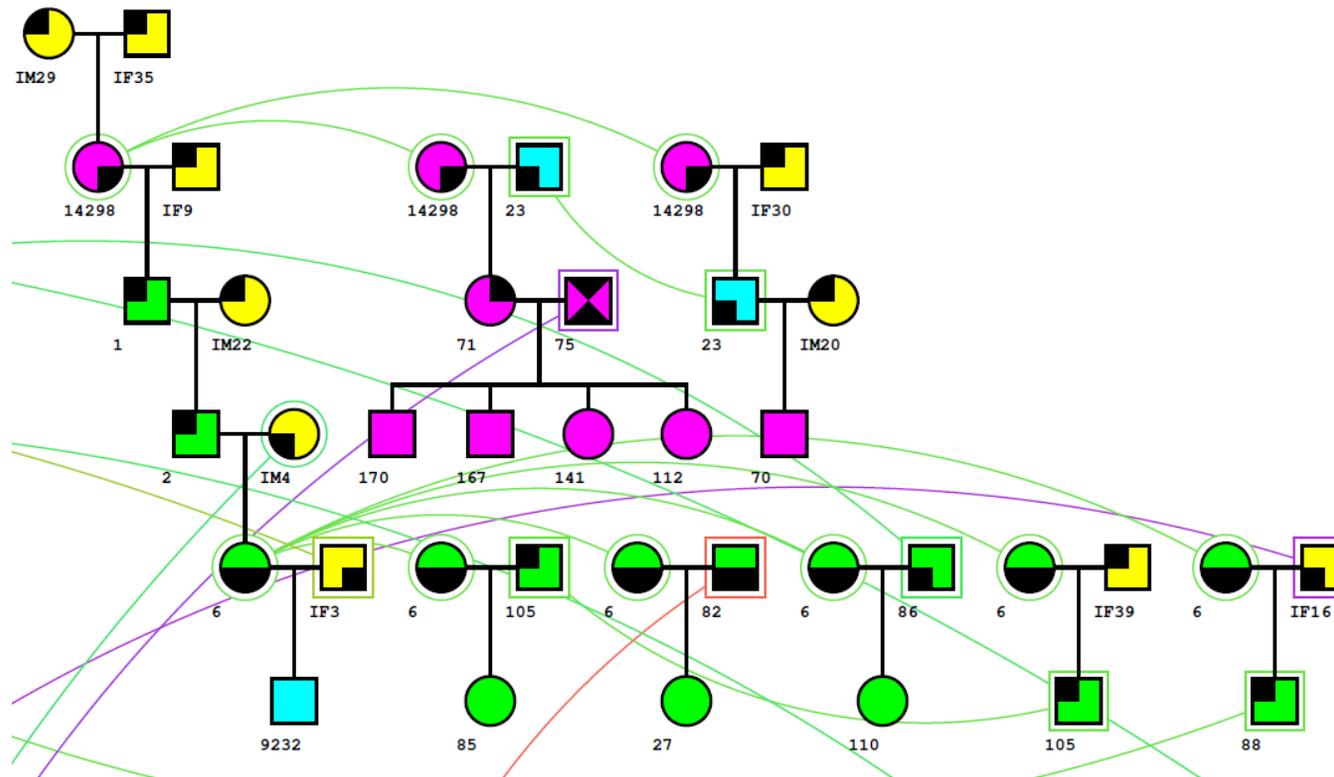
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Appendix A

Figure 15: Close-up of full pedigree detailing the different types of familial relationships in the South Jasper population of woodland caribou.

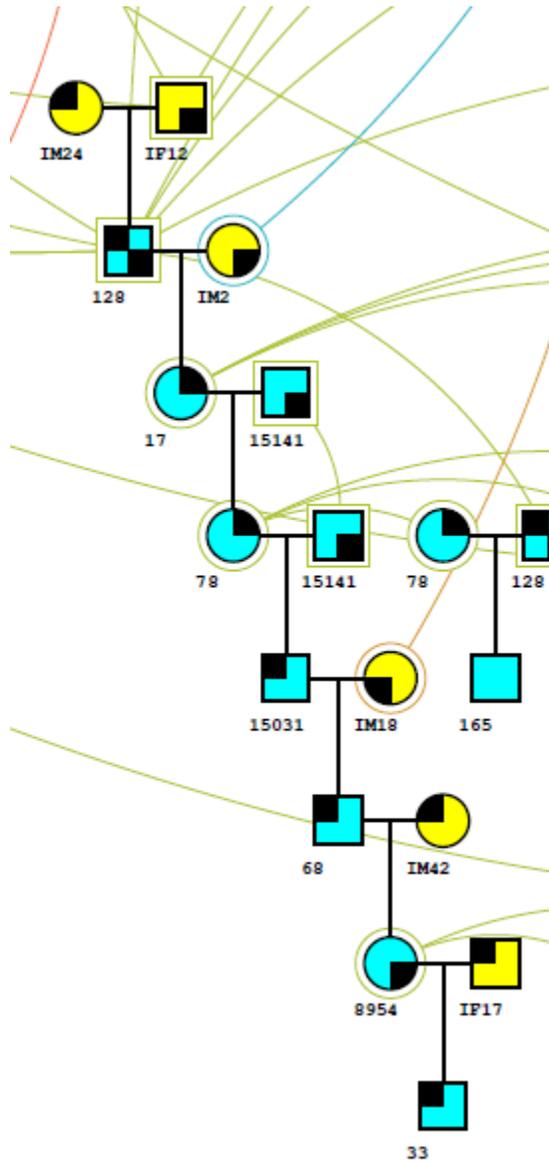


Green represents Brazeau, blue represents Tonquin, and magenta represents Maligne; yellow represents unsampled individuals who are inferred parents. Circles are females and squares are male. Leader lines indicate other familial relationships that are drawn elsewhere in the population, with the colour corresponding to the herd the offspring belongs to. The numbers are the individual cluster identifier.

An example of a full family is individuals 71 and 75, parents with four full-offsprings (individuals 170, 167, 141, and 112), and all belong to Maligne.

An example of a mixed-parent family is shown by female 6 from Brazeau, who has six offspring but with 6 different fathers. These are drawn side-by-side when possible. With the amount of polygyny in woodland caribou, there are few instances in the South Jasper population where a male and female mate more than once with each other, leading to a lot of half-siblings and a very difficult to draw pedigree.

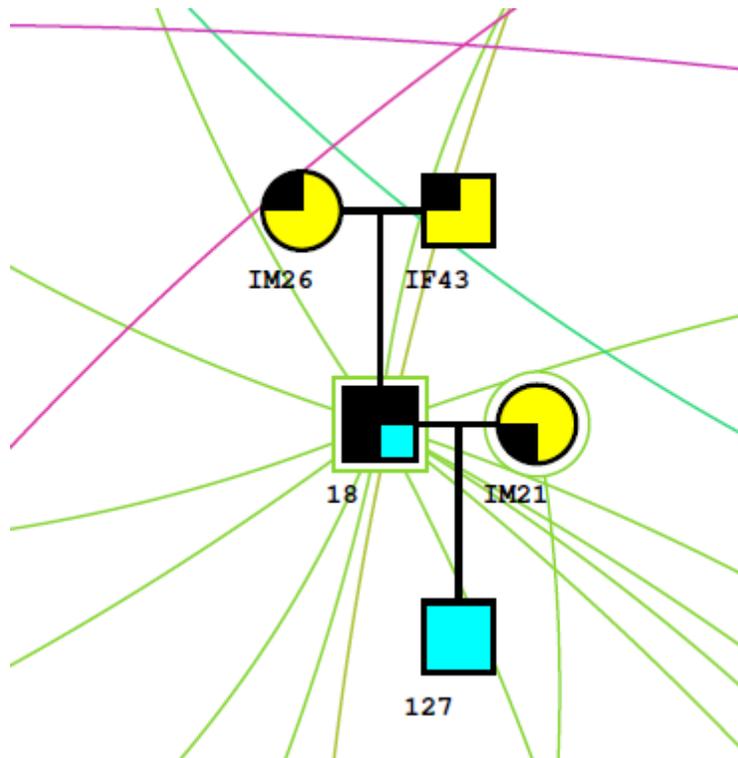
Figure 16: Close-up of full pedigree detailing a lineage of individuals in the South Jasper population of woodland caribou.



This figure shows a lineage of related individuals from the Tonquin population. It shows 8 levels of parents, including the parents, grandparents, and great-grandparents of individual 33.

Individual 33 is from the Tonquin population, and his mother is individual 8954, and his father is inferred (because that individual was not captured during sampling).

Figure 17: Example of a male with numerous offspring from the South Jasper population of woodland caribou.



Male 18 is an example of an individual with many offspring, and many leader lines leading to other areas of the drawn pedigree. Male 18 is the father of 13 individuals, while only one offspring (male 127) is shown in this location in the pedigree.

Table 14: Yearly Darroch estimate results for the Tonquin herd from 2006-2013.

Year	# animals captured	\hat{p} occasion 1	\hat{p} occasion 2	\hat{p} occasion 3	Population estimate	SE	95% LCI	95% UCI
2006	97	0.32	0.17		221	46.598	158	349
2007	49	0.53	0.40		68	9.071	57	95
2008	75	0.16	0.60	0.33	96	8.377	85	119
2009	66	0.69	0.64		74	4.090	70	86
2010	55	0.66	0.64		62	3.904	58	74
2011	48	0.66	0.86		50	1.645	49	56
2012	28	0.67	0.52		33	3.422	30	44
2013	33	0.77	0.68		35	1.831	34	42

Table 15: Female yearly Darroch estimate results for the Tonquin herd from 2006-2013.

Year	# animals captured	\hat{p} occasion 1	\hat{p} occasion 2	\hat{p} occasion 3	Population estimate	SE	95% LCI	95% UCI
2006	41	0.42	0.12		82	26.893	54	173
2007	35	0.6	0.42		45	5.981	39	64
2008	42	0.13	0.65	0.33	52	5.740	46	70
2009	38	0.78	0.73		40	1.747	39	46
2010	29	0.58	0.71		33	2.694	30	42
2011	27	0.69	0.87		28	0.886	28	31
2012	18	0.81	0.40		20	1.982	19	28
2013	16	0.44	0.93		16	0.250	16	17

Table 16: Male yearly Darroch estimate results for the Tonquin herd from 2006-2013.

Year	# animals captured	\hat{p} occasion 1	\hat{p} occasion 2	\hat{p} occasion 3	Population estimate	SE	95% LCI	95% UCI
2006	23	0.86	0.16		25	3.0136	24	39
2007	14	0.41	0.37		22	6.4141	16	45
2008	33	0.19	0.57	0.36	42	5.1692	37	58
2009	28	0.58	0.52		35	4.3997	31	49
2010	26	0.79	0.61		28	1.7113	27	34
2011	21	0.66	0.89		21	0.5116	21	24
2012	10	0.6	0.90		10	0.0053	10	10
2013	17	0.73	0.73		18	1.062	18	22

Table 17: Individual capture history results per year for the Tonquin herd of woodland caribou.

Year	X ₁₀	X ₀₁	X ₁₁	Total captured during first occasion		Total captured during second occasion		Proportion of individuals seen during second compared to first		
2006	50	7	7	57		14		0.25		
2007	22	13	14	36		27		0.75		
2008	Three sampling occasions; see below									
2009	19	15	32	51		47		0.92		
2010	15	14	26	41		40		0.98		
2011	5	15	28	33		43		1.30		
2012	11	6	11	22		17		0.77		
2013	9	6	18	27		24		0.89		
Three Sampling Occasions								Captured during first occasion	Captured during second occasion	Captured during third occasion
X ₁₁₁	X ₁₁₀	X ₁₀₁	X ₀₁₁	X ₀₀₁	X ₁₀₀	X ₀₁₀				
2008	4	3	3	16	9	5	35	15	58	32

X₁₁ – individual captured at both sampling occasions
 X₁₀ – individual captured at first sampling occasion, not captured at second sampling occasion
 X₀₁ – individual not captured at first sampling occasion, captured at second sampling occasion
 X₁₁₁ – individual captured at all three sampling occasions
 X₁₁₀ – individual captured at first two sampling occasions, not captured at third sampling occasion
 X₁₀₁ – individual captured at first sampling occasion, not captured at second sampling occasions, and recaptured at third sampling occasion
 X₀₁₁ – individual not captured at first sampling occasion, and captured at second and third sampling occasions
 X₀₀₁ – individual not captured at first two sampling occasions, captured at third sampling occasion
 X₁₀₀ – individual captured at first sampling occasion, not captured at second and third sampling occasions
 X₀₁₀ – individual not captured at first sampling occasion, captured at second sampling occasion, and not captured at third sampling occasion

Chapter Two: Using non-invasive fecal sampling to estimate effective population size of woodland caribou (*Rangifer tarandus caribou*)

Abstract

Effective population size is one of the most important parameters in conservation biology, but can be very difficult to estimate in wild populations. For species at risk, such as woodland caribou (*Rangifer tarandus*), obtaining enough demographic data to calculate N_e is challenging. This has given rise to the use of genetic methods to provide estimates of N_e , in which the animal under study does not need to be captured in order to obtain data. Non-invasive fecal sampling has been used to obtain genetic data from woodland caribou for use in the estimation of population parameters, such as N_e . The objectives of this chapter are to determine the minimum number of loci and the minimum number of unique genotypes needed to estimate effective population size; and to determine how the precision of effective population size to census population size ratio varies between the Tonquin and North Interlake woodland caribou herds. The effective population size of woodland caribou was investigated in the Tonquin, Prince Albert Greater Ecosystem (PAGE), North Interlake, The Bog and Slate Island herds. Non-invasive genetic sampling occurred in the Tonquin herd from 2006 to 2013; in the North Interlake from 2004 to 2010; in The Bog from 2003 to 2012 (only samples from 2004 to 2007 were included in this analysis); in the PAGE from 2005 to 2008; and in 2009 for the Slate Islands herd. Samples were amplified at 18 microsatellite loci for South Jasper and 10 microsatellite loci for all other herds, and used in effective population size analysis. Effective population size

estimates were determined via the linkage disequilibrium method and the temporal method implemented in NeEstimator V2, and the sibship assignment method implemented in COLONY 2.0. Simulation results showed that a reduction in the number of loci used or the number of unique genotypes used in N_e estimation led to an increase in the variance of N_e , but results were still precise when only 6 loci or 30 unique genotypes were used. N_e estimates for all populations were precise when using the linkage disequilibrium method and the N_e/N ratio varied between 16% and 54% between populations. This study demonstrates the potential of using one-sample N_e estimators for determining the yearly N_e of woodland caribou populations, allowing managers to detect early signs of genetic erosion and inbreeding, with the potential of using N_e/N ratios to assess the degree to which populations are able to avoid local extirpation. Finally, we examined different hypotheses to explain the observed differences in N_e/N ratio among populations and the conservation implications of these findings.

Introduction

Effective population size (N_e) is the size of an idealized population experiencing the same rate of random genetic drift or change as the real population under consideration, giving rise to the same variance of gene frequency or rate of inbreeding; it is the number of breeders contributing genes across generations in a population (Frankham 1995, DeYoung and Honeycutt 2005, Palstra and Ruzzante 2008, Luikart et al. 2010, Weckworth et al. 2013). Demographic and genetic parameters, such as sex ratio, variance of reproductive success among individuals, mating system, and mode of inheritance, affect the N_e of a population (Wang 2005). N_e is one of the most important population parameters in conservation biology, but can be very difficult to estimate in wild populations (Jorde and Ryman 1995, Wang 2005, England et al. 2006, Waples and Yokota 2007, Skrbinek et al. 2012). Genetic methods of collecting the demographic data necessary for calculating N_e have been used for species that are difficult to sample (Nunney and Elam 1994, Frankham 2005, Waples and Do 2010). N_e can help explain patterns of genetic variation in natural populations, and can describe the loss of genetic variation and the rate of inbreeding accumulation; inbreeding depression and the loss of genetic diversity impact the extinction risk of wild populations (Robinson and Moyer 2013). Reductions in population size and N_e from population decline and fragmentation is increasingly threatening populations around the world due to habitat loss and exploitation, habitat modification, and altered spatial population dynamics (Frankham et al. 2002, England et al. 2010). Because of this, estimating N_e can be critical for species of conservation concern. Woodland caribou is a threatened species, and is difficult to observe and study due to their elusive nature (Thomas and Gray 2002). With the advancement of non-invasive research methods, it has become possible to obtain large amounts

of data from caribou fecal samples (Ball et al. 2007, Ball 2010, Petersen et al. 2010, Morden et al. 2011a, Morden et al. 2011b, Hettinga et al. 2012, Flasko 2014).

Genetic derived estimates of N_e can be obtained from four approaches: linkage disequilibrium, temporal variance in allele frequencies, heterozygote excess, and the amount of standing genetic diversity present in natural populations (Wang 2005, Robinson and Moyer 2013). Different methods of estimating N_e are available (Nei and Tajima 1981, Waples 2005, Waples and Do 2008, Zhdanova and Pudovkin 2008, Wang 2009), with each method applying to different time frames in a population's demographic history (Waples 2005, Robinson and Moyer 2013) and use different definitions of N_e (Crandall et al. 1999, Robinson and Moyer 2013). Comparing estimates of N_e becomes difficult even when produced from data collected from the same population. Linkage disequilibrium (LD) and the temporal method both estimate contemporary N_e but apply to different time periods, with the temporal method requiring at least two samples while LD only requires one (Hill 1981, Nei and Tajima 1981, Williamson and Slatkin 1999, Waples 2006). Estimates from LD primarily reflect the inbreeding effective size of the preceding generation (Hare et al. 2011) while the temporal method estimates the harmonic mean N_e over the period between the two samples (Waples 2005, Robinson and Moyer 2013). A disadvantage of two-sample estimators is that one may have to wait a generation or more to collect a second sample, which can be prohibitive in long-lived species such as caribou, making one-sample estimators advantageous, since only a single sample is required (Tallmon et al. 2008). The use of one-sample methods for estimating N_e may not be appropriate for continuously distributed populations that are not panmictic (Neel et al. 2013). If breeding only occurs in relatively small local areas, or if the scale of the global population is significantly larger than the

parent-offspring dispersal distance, the global N_e will be underestimated regardless of how much of the geographic range of the population is sampled (Neel et al. 2013).

Table 18 shows the different N_e estimators used in this study, and compares the different methods, assumptions, strengths and limitations of each method. See Luikart et al. (2010) for a complete list of all N_e estimators, including estimators not used in this study.

Table 18: Comparison of N_e estimators used in this study

N_e estimator	Method	Assumptions	Strengths	Limitations	Software & References
Linkage disequilibrium	estimate N_e from a single sample by analysing random linkage disequilibrium that occurs by change in each generation in finite populations	<ul style="list-style-type: none"> • Closed to immigration • Random mating • Random sampling • Discrete generations • Markers selectively neutral and independent 	Only requires one sample	Could be strongly biased by substructure, admixture, age structure and small samples	LDNe; Waples and Do (2008)
Sibship assignment	infer N_e from the sibship frequencies estimated from a sibship assignment analysis, using the multilocus genotypes of a sample of offspring taken at random from a single cohort in a population	<ul style="list-style-type: none"> • Discrete generations • Random sampling • Closed to immigration • Markers selectively neutral and independent 	<ul style="list-style-type: none"> • Does not require the assumption of random mating • Only requires one sample 	Estimates biased downward when N_e is large, sample size much smaller than N_e , and number of loci is low	Colony 2.0; Wang (2009)
Temporal	estimate N_e from the standardized variance in allele frequency change	<ul style="list-style-type: none"> • Discrete generations • Random mating • Random sampling • Markers selectively neutral and independent 	estimates long-term N_e	Requires two samples spaced a generation or more apart; prohibitive in long-lived species	NeEstimator; Do et al. (2014)

Most estimators of N_e have the assumption that the population under study has discrete generations, an assumption that is commonly violated in natural populations, such as woodland caribou (Palstra and Ruzzante 2008, Petersen et al. 2010, Neel et al. 2013). Using estimators that are designed for discrete generations on populations with overlapping generations can introduce substantial bias, causing N_e estimates to be substantially higher (Palstra and Ruzzante 2008, Robinson and Moyer 2013). For iteroparous species with overlapping generations, such as woodland caribou, N_e is related to the effective number of breeders in one reproductive cycle (N_b). N_e is an important factor in shaping long-term evolutionary processes, but many evolutionary processes (such as sexual selection) are more short-term processes, and are defined by seasonal bouts of reproduction, making N_b a more relevant parameter than N_e (Waples et al. 2013, Waples and Antao 2014, Waples et al. 2014). N_b is an easier parameter to estimate and monitor in species with long generation times, such as in woodland caribou, as data from only one breeding season is needed (Waples et al. 2013, Waples et al. 2014). Female woodland caribou give birth to one calf per breeding season, and some females are excluded from reproducing in a season, and both of these factors influence N_b (Flasko 2014, Waples et al. 2014).

The sibship assignment (SA) method outlined by Wang (2009) estimates N_e in discrete, non-overlapping generations. This assumption is violated with woodland caribou, as they have overlapping generations, which can introduce substantial bias (Palstra and Ruzzante 2008, Robinson and Moyer 2013). When this method is used on samples of individuals taken at random from a single cohort of a population with overlapping generations, SA gives an estimate of the N_b that produced the cohort. The LD method (Waples and Do 2008) estimates different parameters depending on the samples used in the analysis. Estimates based on single-cohort

samples reflects N_b , whereas using mixed-age samples reflects N_e (Waples and Antao 2014). The woodland caribou samples included in this analysis were collected at random from the population as a whole, including individuals of various ages. Waples and Do (2010) suggested that if the samples included enough cohorts to approximately represent one generation, the estimate resulting from LD would be close to N_e per generation, and was tested in a number of iteroparous species by Waples et al. (2014). The amount of LD in a closed population (assuming selective neutrality) with discrete generations is the product of two processes: new LD generated by reproduction of a finite number of individuals, and the residual LD from previous generations that was not broken down by recombination between loci (Hill and Robertson 1968, Waples et al. 2014). If the population size changes, the residual LD and the newly generated LD represent different effective sizes. In breeders from a single time period, LD is a function of N_b , while the residual LD from the population as a whole is a function of N_e (Waples 2005, Waples et al. 2014).

The temporal method requires at least one generation to pass between samples, but in practice the time period must be significantly longer than one generation to produce unbiased estimates, especially in species with overlapping generations (Waples and Yokota 2007, Skrbinek et al. 2012). It can become heavily biased when used in populations with overlapping generations (Waples and Yokota 2007, Skrbinek et al. 2012). This makes the temporal method very difficult to apply in a monitoring framework. Jorde and Ryman (1995) proposed a method that provides approximately unbiased results for overlapping generations, but this method requires detailed demographic information and many loci, limiting the usefulness of the method to the exploration of historical change in N_b (Skrbinsek et al. 2012).

N_e can be compared to the adult census population size (N) to determine the ratio of effective to census population size (Frankham 1995, Palstra and Ruzzante 2008). N_e and N are key in determining the degree to which caribou populations are able to avoid extirpation or extinction from demographic, environmental, or genetic random events, such as environmental catastrophes, inbreeding depression, temporary recruitment failures, or losses of genetic diversity at low population sizes (Luikart et al. 2010, Palstra and Fraser 2012, Weckworth et al. 2013). Population extirpation is accelerated in small populations, due to the positive feedback between reduced population size and inbreeding depression (Pusey and Wolf 1996, Schwartz et al. 1998, Keller and Waller 2002, O'Grady et al. 2006, Weckworth et al. 2013).

The objectives of this chapter are:

- Assess the effects of 1) the number of loci and 2) sample size on the precision of the effective population size estimates;
- Determine 1) how the effective population size to census population size ratio varies between the Tonquin and North Interlake herds and 2) determine if the population genetics of a given herd (e.g. isolation by distance) may be correlated with the obtained N_e/N ratio.

Methods

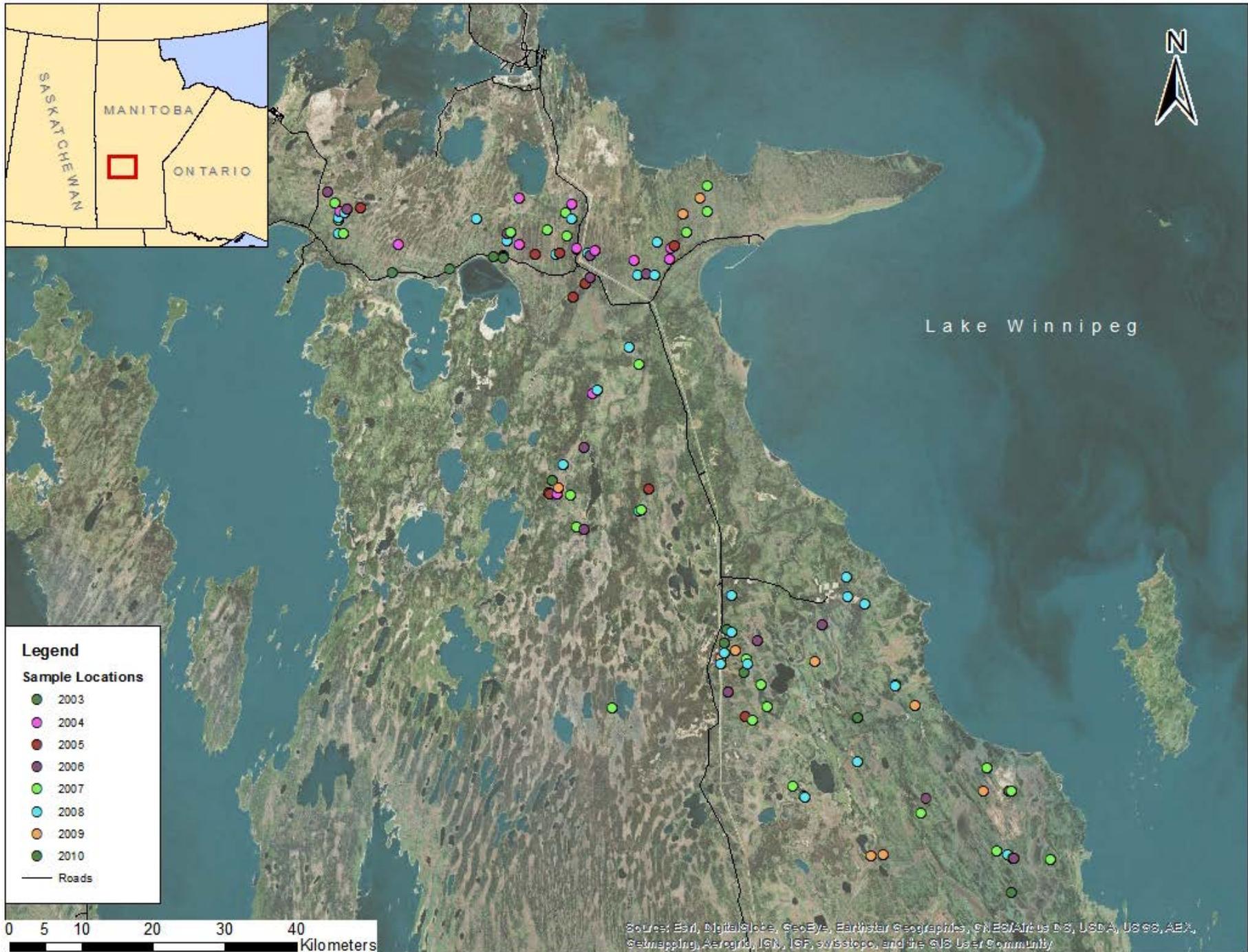
Fecal pellet collection occurred from 2004 to 2010 for North Interlake, and from 2006 to present for the Tonquin herd, where at least one entire generation of caribou has been sampled. With a minimum of seven years of data available, it is possible to use two-sample estimators on the North Interlake, Jasper National Park, as the two-sample estimators of N_e require the second

sample to be collected a generation later than the first collection event (Tallmon et al. 2008). Fecal pellet collection occurred in 2009 for the Slate Islands population, from 2004 to 2007 for The Bog herd, and in 2007 for the PAGE herd. One-sample estimators were run on the North Interlake, The Bog, PAGE and Slate Islands populations.

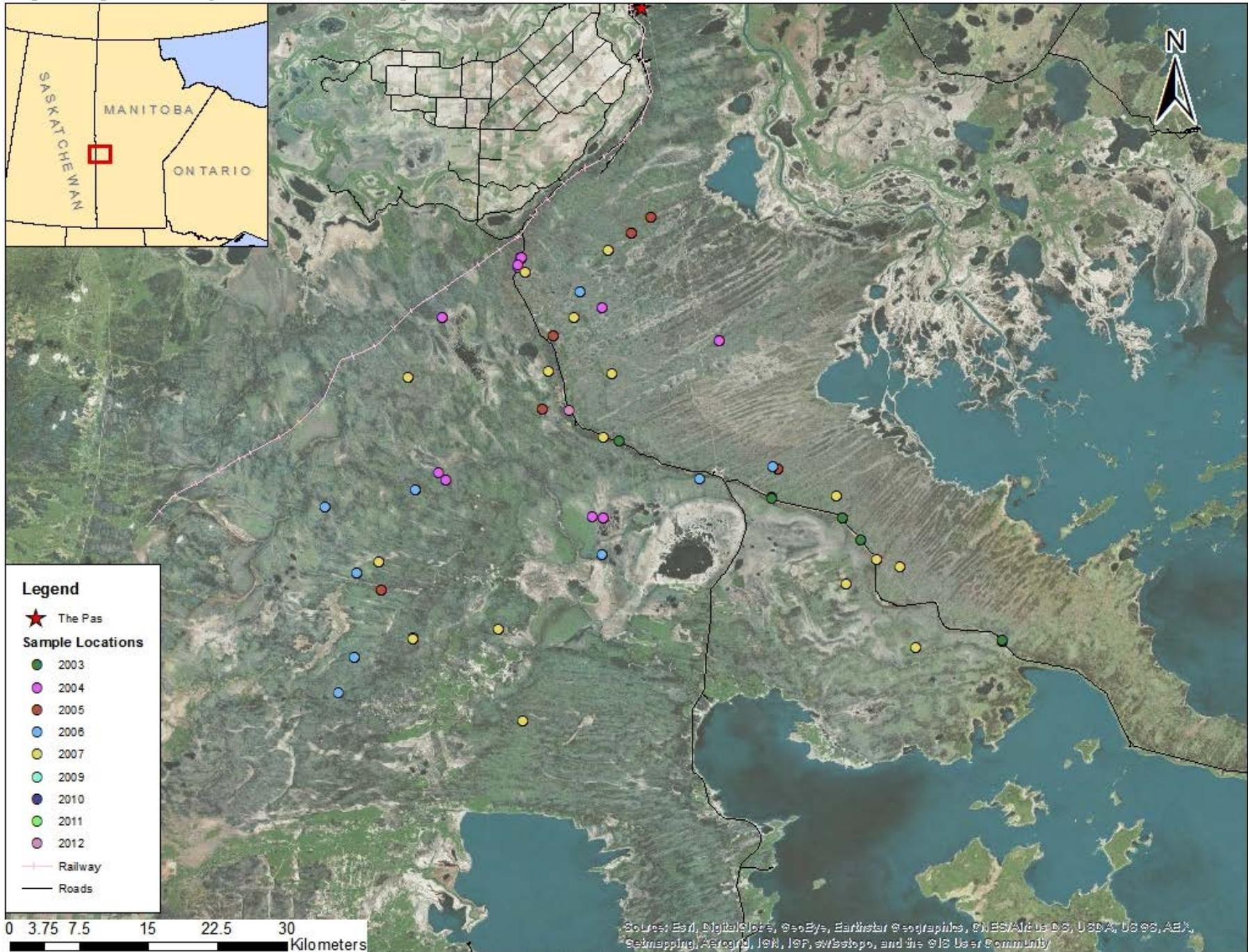
Study Area

Please refer to ‘Study Area’ in Chapter 1 for details on the study areas.

Map 2: Map of the North Interlake herd based on fecal pellet collection from 2003 to 2010.



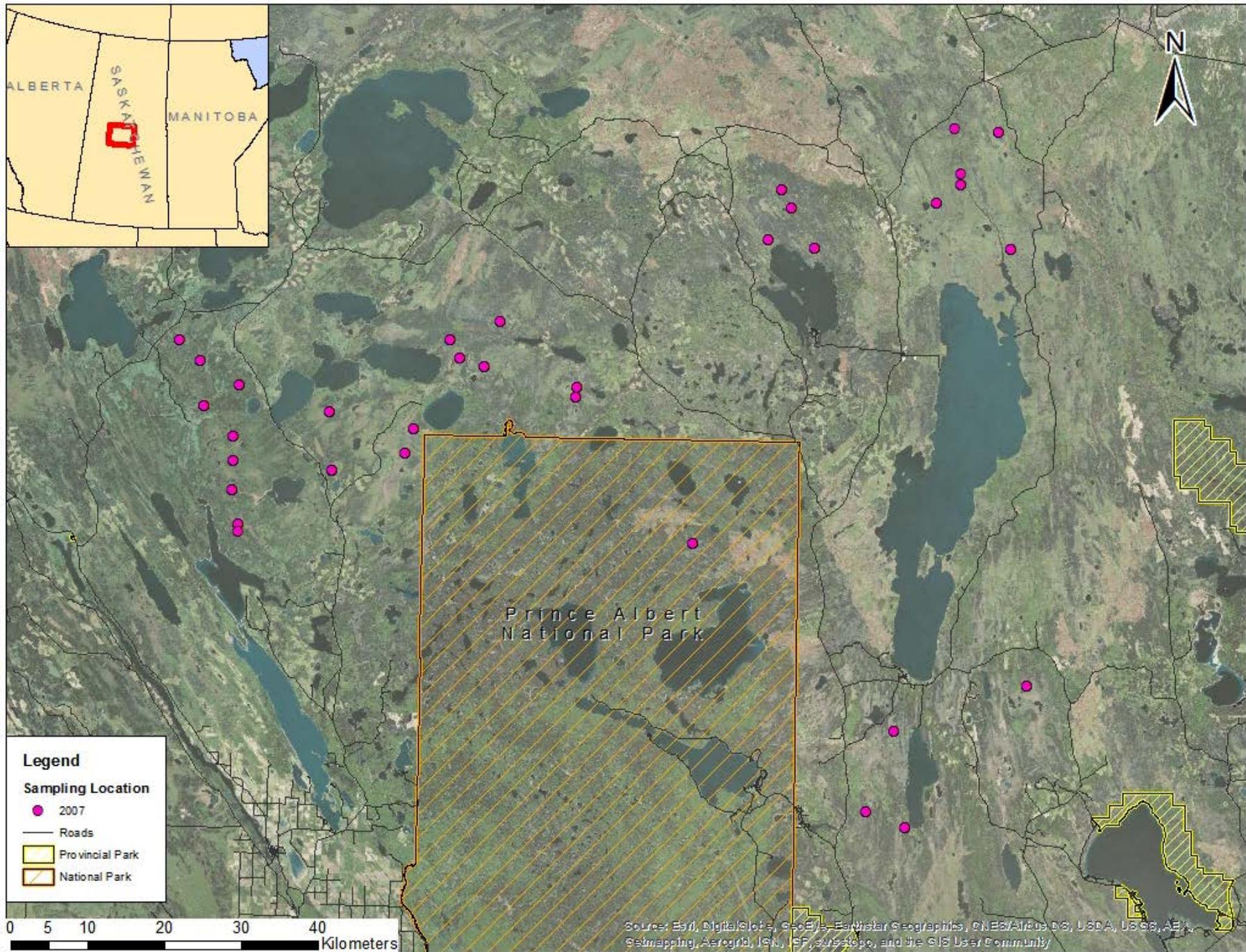
Map 3: Map of The Bog herd based on fecal pellet collection from 2003 to 2012.



Map 4: Map of the Slate Island herd based on fecal pellet collection in 2009.



Map 5: Map of the PAGE herd based on fecal pellet collection in 2007.



Pellet Collection

Please refer to ‘Pellet Collection’ in Chapter 1 for details on the fecal pellet collection of caribou samples.

Genetic Analysis

Please refer to ‘Genetic Analysis’ in Chapter 1 for details on the fecal pellet genetic analysis of caribou samples.

Creating a Capture History

Please refer to ‘Creating a Capture History’ in Chapter 1 for details on creating a capture history for woodland caribou.

Estimating N_e

Linkage Disequilibrium

The linkage disequilibrium (LD) method (Hill 1981, Waples 2006, Waples and Do 2008) is a method of estimating N_e from a single sample by analysing random linkage disequilibrium that happens by chance in each generation in finite populations (Hill 1981, Waples and Do 2010). LD can provide comparable precision in estimates to the temporal method, if a realistic number of loci and alleles are used (10 alleles/locus) (Waples 2006). Linkage disequilibrium between alleles at two gene loci is referred to as the difference between the observed frequency of a two-locus gamete, and its expected frequency based on random association and population allele frequencies (Waples 2006). LD can be directly estimated from gametic frequencies, but most natural populations only have genotypic data available; in these instances, Burrows’ Δ is used for estimating LD as it is easy to calculate, does not depend on the assumption of random mating, and does not require haplotype data, which is not usually available for natural

populations (Waples 2006, Waples and Do 2008). When sample size is less than the true (unknown) N_e , the standard LD method (Hill 1981) was shown to be biased; however, Waples (2006) developed an empirical correction that minimizes bias from LD estimates. The computer program LDNe (Waples and Do 2008) implements the bias-correction method proposed by Waples (2006), and can accommodate an unlimited number of populations, individuals, alleles, and loci.

NeEstimator V2 (Do et al. 2014) was used for estimating N_e using the LD method. This program includes an updated version of LDNe (Waples and Do 2008) to incorporate methods for handling missing data proposed by Peel et al. (2013). LDNe uses Burrows' Δ for estimating LD, which can be standardized to adjust for the effects of allele frequencies. This yields a correlation coefficient, r_Δ , which is the basis for estimating N_e . Separate values for r_Δ are calculated for each pair of alleles at each pair of loci, and the estimator of r_Δ is used to compare alleles at different loci (Waples 2006, Waples and Do 2008):

$$\hat{r}_\Delta = \frac{\hat{\Delta}}{\sqrt{[\hat{p}(1 - \hat{p}) + (h_i - \hat{p}^2)][\hat{q}(1 - \hat{q}) + (h_j - \hat{q}^2)]}}$$

Where h_i and h_j are the observed frequencies of AA and BB homozygotes at loci i and j , respectively, and \hat{p} and \hat{q} are sample frequencies of alleles A and B. N_e is estimated from the overall mean \hat{r}_Δ^2 averaged across multiple loci and alleles.

LDNe can handle missing data by adjusting the effective size for each sampling to account for the missing data. For each pair of loci i and j , the sample size $S_{i,j}$ is computed as the number of individuals with scored genotypes for both loci (Waples and Do 2008). The overall effective sample size is computed as the weighted harmonic mean of $S_{i,j}$ with weights proportional to $n_{i,j}$, and this weighted-harmonic-mean sample size is used in the following

formula to estimate N_e for randomly mating populations with sample sizes greater than 30, which is the case in the Tonquin and North Interlake herds (Waples and Do 2008):

$$E(\hat{r}_{sample}^2) = 1/S + 3.19/S^2$$

$$\hat{N}_e = \frac{1/3 + \sqrt{1/9 - 2.76\hat{r}^{2'}}}{2\hat{r}^{2'}}$$

Peel et al. (2013) proposed an improved method for dealing with missing data. When sample sizes vary across loci, the sampling error can be underestimated, causing a downward bias in N_e . The methods suggested by Peel et al. (2013) were incorporated into the newer version of LDNe, included in the program NeEstimator V2 (Do et al. 2014). This program was used for all LD estimates to ensure missing data was accounted for.

Since allele frequencies close to 0 or 1 can affect \hat{r}_{Δ}^2 and therefore \hat{N}_e (Waples 2006, Waples and Do 2010), LDNe returns separate estimates for three default critical values ($P_{crit} = 0.05, 0.02, 0.01$) in which all alleles with frequencies less than the P_{crit} are excluded from the estimate. Waples and Do (2010) recommended selecting a P_{crit} that is the larger of 0.02 or a value screens out alleles that only occur in one copy (when sample size (S) > 25 : $P_{crit} = 0.02$; when $S \leq 25$: $1/(2S) < P_{crit} \leq 1/S$). Allele frequencies for all populations are found in Table 36 to Table 39. The Bog and North Interlake caribou herds both had sampling occasions with $S \leq 25$, so alternate critical values were selected (Table 19).

Ten loci were used for estimating N_e using LDNe. Estimates were calculated for each sampling year (Tonquin = 2006 to 2013; North Interlake = 2004 to 2010; The Bog = 2004 to 2007; Slate Islands = 2009) as well as an overall N_e for the entire sampling period. LDNe returned 95% confidence intervals.

Table 19: Number of unique genotypes per sampling year used for estimating N_e using the LD method for the Tonquin, North Interlake, The Bog, Slate Islands and PAGE woodland caribou herds.

Sampling year	Sampling Year									
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Tonquin	-	-	64	56	76	67	55	49	28	34
North Interlake	50	32	56	97	82	63	25	-	-	-
The Bog	16	41	29	55	-	-	-	-	-	-
Slate Islands	-	-	-	-	-	80	-	-	-	-
PAGE	-	46	31	82	39	-	-	-	-	-

Sibship Assignment Method

The sibship assignment (SA) method (Wang 2009) derives estimates for N_e from the frequencies that a pair of offspring randomly taken from the population would be siblings sharing one or two of the same parents. Each individual in the sample population is genotyped and the data used in sibship analysis to calculate half-sib and full-sib probabilities, which estimates the N_e of a random-mating population (Wang 2009). The assumption of random mating is not required for the SA method because non-random mating is taken into account in the analysis and the accuracy of sibship assignments is little affected by non-random mating. N_e is underestimated by the SA method when populations are polygamous, but generally have small bias, with most estimates falling within a relatively narrow range of N_e (Wang 2009).

Remote relations are not accounted for using the SA method. Offspring that do not share any common parents may be related through common ancestors such as grandparents or earlier generations, and first cousins sharing a common grandparent are not inferred in sibship analysis or used in estimating N_e (Wang 2009).

The SA method assumes that a sample taken of the population is taken at random from a single discrete generation of the population (Wang 2009). The following equation can be used to estimate N_e from the multilocus genotypes of a sample of individuals that are taken at random from a single cohort in a population:

$$\frac{1}{N_e} = \frac{1 + 3\alpha}{16} (P_{1,11} + 2P_{1,12} + P_{1,22} + P_{2,11} + 2P_{2,12} + P_{2,22}) - \frac{\alpha}{2} \left(\frac{1}{N_1} + \frac{1}{N_2} \right)$$

Two randomly selected individuals (sex denoted as s and r) may be non-sibs (sharing no parents), paternal half-sibs (sharing the father), maternal half-sibs (sharing the mother) or full-sibs. The probabilities of the pair of individuals being paternal half-sibs, maternal half-sibs and full-sibs are denoted as $Q_{1,SR}$, $Q_{2,SR}$ and $Q_{3,SR}$. N_e can be estimated by the probabilities of a pair of offspring being half-sibs and full-sibs with the following equation:

$$\frac{1}{N_e} = \frac{1 + 3\alpha}{16} \left(2(Q_{3,11} + 2Q_{3,12} + Q_{3,22}) + \sum_{i=1}^2 (Q_{i,11} + 2Q_{i,12} + Q_{i,22}) \right) - \frac{\alpha}{2} \left(\frac{1}{N_1} + \frac{1}{N_2} \right)$$

When there is no difference between survival of male and female offspring regarding within and between family selections, $Q_{i,11} \equiv Q_{i,12} \equiv Q_{i,22} \equiv Q_i$ for $i = 1, 2, 3$, which reduces the above equation to:

$$\frac{1}{N_e} = \frac{1 + 3\alpha}{4} (Q_1 + Q_2 + 2Q_3) - \frac{\alpha}{2} \left(\frac{1}{N_1} + \frac{1}{N_2} \right)$$

Which means that N_e can be estimated by the probabilities that a randomly selected pair of offspring (of any sex) are half-sibs and full-sibs (Wang 2009). Unlike the linkage disequilibrium and heterozygote excess methods, this method does not require the assumption of random mating, because the above equations take non-random mating into account.

Sibship assignment estimation is run through program COLONY (Jones and Wang 2010a). A dataset including the multilocus genotypes of a single cohort sample of individuals is run through COLONY, and N_e is estimated from the predictive equations listed above (Wang 2009). Multilocus genotype datasets from the Tonquin herd in Alberta, North Interlake and The Bog herds in Manitoba, and the Slate Island herd in Ontario were run through COLONY to estimate N_e using the SA methods. Table 19 lists the sampling years of multilocus genotypic data that was used for the estimation of N_e for various woodland caribou herds across Canada.

Temporal Method

The temporal method (Nei and Tajima 1981, Williamson and Slatkin 1999) is a two-sample estimator of N_e and requires at least two samples from the population spaced a generation apart. It uses the F -statistic, which is a measure of the standardized variance of gene frequency changes that estimates the variance effective population size from temporal samples of allele frequency data (Nei and Tajima 1981, Williamson and Slatkin 1999). The variance of allele frequency between generations is $P(1 - P)/(2N_e)$, where P is the population frequency in the initial generation, and the effects of initial allele frequency can be compensated for by using some variation of Wright's standardized variance (F) (Waples 1989). It assumes discrete generations, no selection, and the population is isolated with no migration (Nei and Tajima 1981, Pollak 1983, Waples 1989). It estimates the harmonic mean N_e over the period separating the estimates (Robinson and Moyer 2013).

The temporal method has been extended for use with populations that have overlapping generations where the assumption of complete isolation cannot be held valid (Jorde and Ryman 1995, Waples and Yokota 2007, Palstra and Ruzzante 2008). Jorde and Ryman (1995) proposed

a modified temporal method for iteroparous species that takes age-classes into account. When generations overlap, changes in the magnitude of allele-frequency is not only determined by N_e and the sampling interval, but also by age-specific survival and birth rates (Jorde and Ryman 1995). The Jorde-Ryman method requires detailed demographic information and the ability to assign individuals to an age-class.

The temporal method was run using program NeEstimator (Do et al. 2014). NeEstimator can produce three different estimates of the standardized variance in allele frequency, F : F_e (Nei and Tajima 1981), F_k (Pollak 1983) and F_s (Jorde and Ryman 2007).

Nei and Tajima's (1981) measure of allele frequency change, F_c :

$$F_c = \frac{1}{a} \sum_{i=1}^a \frac{(x_i - y_i)^2}{z_i - x_i y_i}$$

Pollak's (1983) measure of allele frequency change, F_k :

$$F_k = \frac{1}{a-1} \sum_{i=1}^a \frac{(x_i - y_i)^2}{z_i}$$

Jorde and Ryman's (2007) measure of allele frequency change, F_s :

$$F_s = \frac{\sum_{i=1}^a (x_i - y_i)^2}{\sum_{i=1}^a z_i (1 - z_i)}$$

Where a is the number of alleles at the locus and x_i and y_i are the observed frequencies of the i th allele in the two samples, respectively, with an unweighted mean of z_i (Jorde and Ryman 2007).

Pollak (1983) extended Nei and Tajima's (1981) analysis to samples taken at more than two sampling occasions. Jorde and Ryman's estimator differs from the other two temporal estimators

in that the numerator and denominator are estimated separately, by summing over all alleles before dividing, which is equivalent to weighing each allele i by

$$\omega_i = \frac{z_i(1 - z_i)}{\sum_{i=1}^a z_i(1 - z_i)}$$

Which was recommended by (Reynolds et al. 1983, Weir and Cockerham 1984) when estimating spatial genetic differentiation (F_{st}).

There are two sampling plans recognized under the temporal method, depending on how the samples were drawn from the population (Nei and Tajima 1981, Waples 1989). Under plan I, individuals are sampled after reproduction, or sampled non-destructively and returned to the population before reproduction begins. Under plan II, individuals are sampled before reproduction and not returned to the population. The sampling for woodland caribou herds in Tonquin, North Interlake, followed a plan I sampling design. All three temporal method estimators were run in NeEstimator (Do et al. 2014).

N_e/N Ratio – Census Population Size (N)

The census population size (N) was used for determining the N_e/N ratio for the Tonquin and North Interlake caribou herds. N was determined through capture-mark-recapture analysis, using robust design modeling. Robust design modeling (Pollock 1982) combines features from both closed and open population models into a single model. The robust design model consists of k primary periods, with each primary period having at least 2 secondary samples, and assumes it is a closed population within each primary period (Kendall et al. 1995). Robust design estimates population size by applying the appropriate closed population model to data from the secondary samples within each primary sampling period. Census population size (N) was estimated by

using the robust design method, implemented in Program MARK (White and Burnham 1999). Please see Chapter 1 for more information on robust design. The effective population size estimate was compared to the census population size estimate from the previous year. Samples in generation i are produced by the effective breeders in generation $i - 1$, so the estimate applies to N in the previous generation ($i - 1$) (Waples 2005, Palstra and Ruzzante 2008). The N_e/N ratio could not be determined for the PAGE and Slate Islands populations. Sampling occurred in the Slate Islands population in 2007 and 2009, two years apart, meaning N_e and N could not be compared to determine the N_e/N ratio. For the PAGE population, the sampling was only designed for capture-mark-recapture in 2007, so there is no second year to get the N_e/N ratio.

Isolation by distance (IBD) was tested using program GenAlEx 6.5 (Peakall and Smouse 2012). IBD was tested in all study populations to see if IBD was present in the populations, and whether or not this had an impact on the N_e or N_e/N results.

Simulations

Simulations were run to determine if the number of loci and the number of unique genotypes used in each population were enough to produce good estimates of N_e . The linkage disequilibrium method was chosen for the simulations. The sample year 2008 from the Tonquin population was chosen because the Tonquin population had 18 loci available, and 2008 was the year with the highest number of unique genotypes. In order to simulate the effect of the number of loci included in estimating N_e , simulations were run from 18 loci (the maximum number of loci available) to 5 loci. In order to simulate the effect of the number of unique genotypes on N_e estimates, simulations were run from 20 unique individuals up to 76 unique individuals (all the available individuals for the study population). A random number generator was used in order to

select which loci or which samples to remove before running the simulation. For each simulation, a new random number was generated, and each level (number of loci or number of unique genotypes) was run ten times to ensure the results were robust. The estimates were then averaged, and the standard deviation was calculated for each level.

Results

Simulations

Simulation results for the number of loci included in N_e estimation using the linkage disequilibrium (LD) method are shown in Figure 18 and Table 20. The average estimate did not change greatly with a reduced number of loci (ranging from 34.3 individuals at 9 loci to 37.2 individuals at 5 loci), but the standard deviation increased with each reduction in the number of loci. 18 loci had a standard deviation of 0 because it includes all loci, and all 10 runs had the same result. $CV(N_e)$ declined with an increase in the number of loci used in analysis (Figure 18 and Table 20). Using a minimum of 6 loci produced N_e estimates with good precision [$CV(N_e) < 0.2$], as outlined by Waples and Do (2010).

Simulation results did not differ greatly when either 10 loci or 18 loci were used for analyses (Figure 19 and Table 21). The average N_e estimate did not change greatly when the number of unique genotypes used in analysis was reduced from 76 samples (all samples available) to only 30 samples, but the standard deviation increased with a reduction in the number of unique genotypes. Using only 20 samples for analysis had increased estimates for N_e , as well as large standard deviations. $CV(N_e)$ increased when less samples were used; using 76 to 30 samples produced estimates with good precision [$CV(N_e) < 0.2$], and CV was very similar whether 18 or 10 loci were used for analyses (Figure 19 and Table 21).

Figure 18: Effects of the number of loci used on the precision of N_e estimates for the 2008 sampling year of the Tonquin herd.

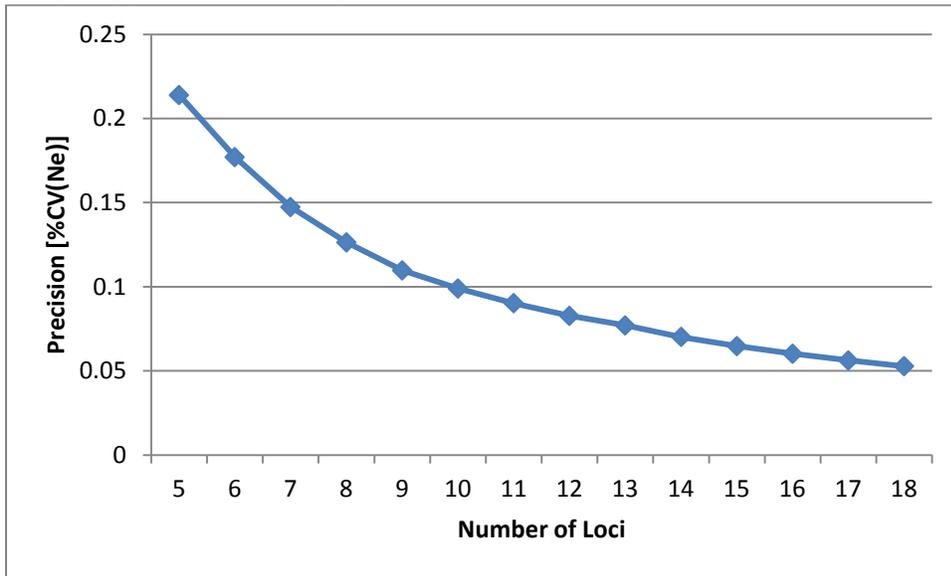


Table 20: Loci simulation results for the 2008 sampling year for the Tonquin herd. Simulations with good precision in italics.

Number of loci	Average N_e Estimate	Standard Deviation	% Coefficient of Variation (CV) of N_e
18	35.5	0	5.3
17	35.37	1.29	5.6
16	35.26	2.09	6.0
15	35.91	2.97	6.5
14	36.37	3.97	7.0
13	36.57	4.69	7.7
12	35.66	5.16	8.3
11	35.2	6.08	9.0
10	35.07	7.4	9.9
9	34.32	7.54	11.0
8	35.08	9.95	12.6
7	36.2	12.73	14.7
6	37.02	12.09	17.7
5	37.24	17.44	21.4

Figure 19: Effects of the number of unique genotypes used on the precision (coefficient of variation) of N_e estimates for the 2008 sampling year of the Tonquin herd.

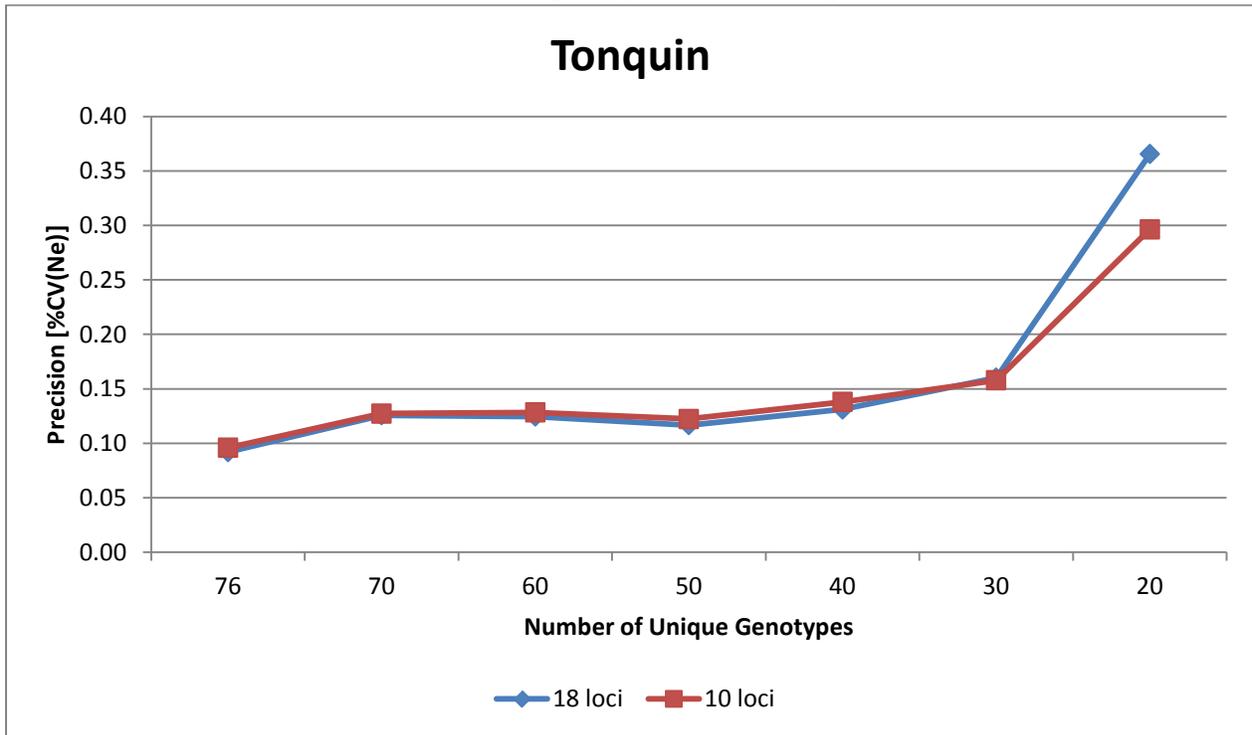


Table 21: Number of unique genotypes simulation results for the 2008 sampling year for the Tonquin herd. Simulations with good precision in italics.

Number of Unique Genotypes	18 loci			10 loci		
	Average N_e Estimate	Standard Deviation	% Coefficient of Variation (CV) of N_e	Average N_e Estimate	Standard Deviation	% Coefficient of Variation (CV) of N_e
76	32.7	0	9.2	30.2	0	9.6
70	32.98	3.15	<i>12.6</i>	31.62	3.03	<i>12.7</i>
60	31.69	3.68	<i>12.5</i>	29.64	3.76	<i>12.8</i>
50	34.37	7.44	<i>11.7</i>	33.48	5.86	<i>12.2</i>
40	33.13	4.65	<i>13.1</i>	32.54	7.32	<i>13.8</i>
30	34.28	12.5	<i>16.0</i>	30.18	8.21	<i>15.8</i>
20	53.14	55.94	36.6	39.68	16.06	29.6

Closed populations

Table 22 outlines the number of unique genotypes per sex included in the effective population size analyses, as well as the number of loci used for each population. The same ten loci were used for each population. Enough loci were used (10) and sample sizes per year were large enough (minimum 25) to create unbiased estimates for the LDNe method (see simulation results in Table 20 and Table 21).

The linkage disequilibrium (LD) and the sibship assignment (SA) methods gave similar yearly estimates for the Tonquin (Figure 20), North Interlake (Figure 22), Slate Islands (Figure 24), and PAGE (Figure 25) herds with overlapping confidence intervals. (Schenker and Gentleman 2001). The LD and SA methods produced similar results for most years for The Bog, but did not have overlapping confidence intervals for all years (Figure 23).

Table 22: Detailed sampling information of unique genotypes included in effective population size analysis.

Population	Females	Males	Unknown	# of loci used
Tonquin	85	70	6	10
North Interlake	118	88	7	10
The Bog	113	53	12	10
Slate Islands	32	36	12	10
PAGE	56	23	3	10

Figure 20: Yearly linkage disequilibrium and sibship assignment N_e estimates for the Tonquin herd of woodland caribou (*Rangifer tarandus*). Error bars represent the 95% confidence intervals.

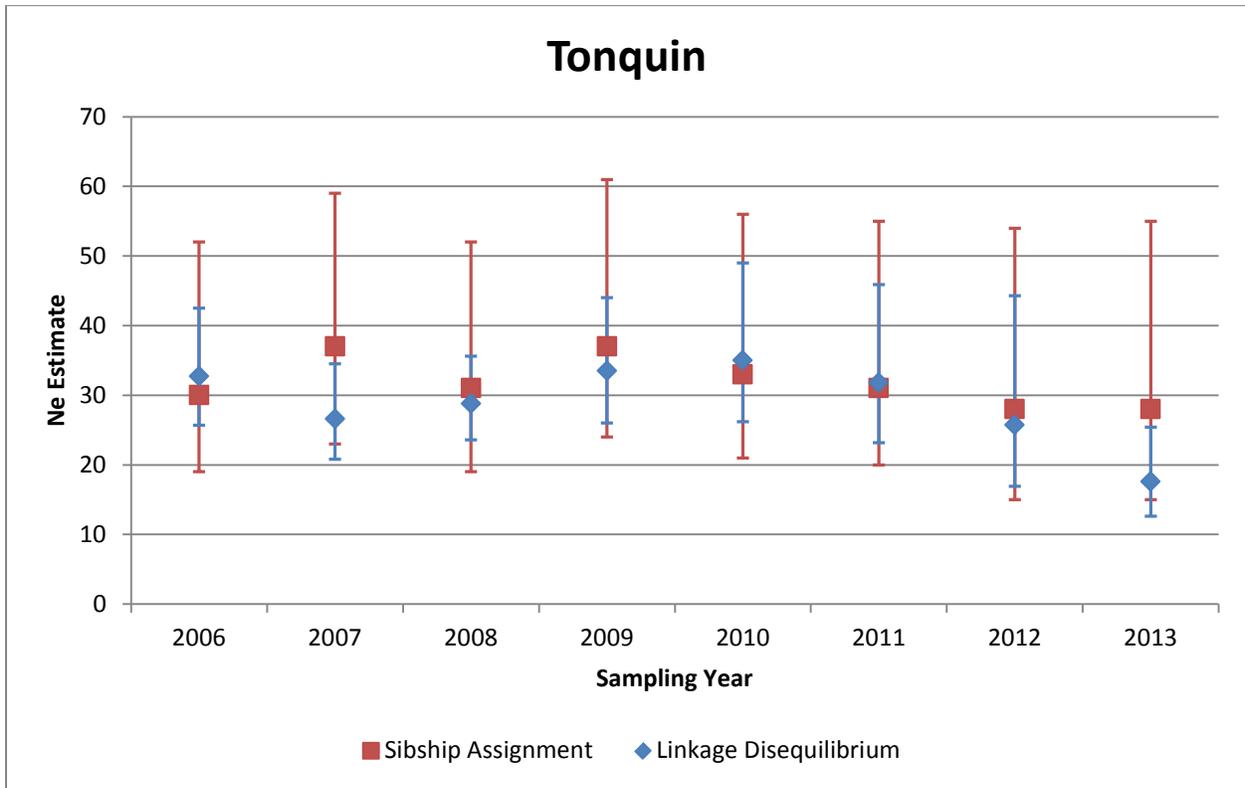


Table 23: Linkage Disequilibrium and Sibship Assignment N_e estimates for the Tonquin population of woodland caribou (*R. tarandus*)

Sampling Year	Linkage Disequilibrium			Sibship Assignment				
	Sample size	Estimated N_e	LCI	UCI	Sample Size	N_e estimate	95% LCI	95% UCI
2006	64	32.7	25.7	42.5	63	30	19	52
2007	56	26.6	20.8	34.5	55	37	23	59
2008	76	28.8	23.6	35.6	76	31	19	52
2009	67	33.5	26	44	66	37	24	61
2010	55	35	26.2	49	55	33	21	56
2011	48	31.8	23.2	45.9	47	31	50	55
2012	28	25.7	16.9	44.3	26	28	15	54
2013	34	17.6	12.6	25.4	20	28	15	55

Yearly results for ten loci and 18 loci are shown in Figure 21. The ten loci included in these analyses were the ten loci used for all populations of woodland caribou included in this study (see Table 36 to Table 39 for list of loci). All yearly estimates provided similar estimates, however 18 loci had tighter confidence intervals than the 10 loci estimates.

Figure 21: Yearly N_e results for the Tonquin herd of woodland caribou, 10 loci vs. 18 loci, using the linkage disequilibrium method. Error bars indicate 95% confidence intervals.

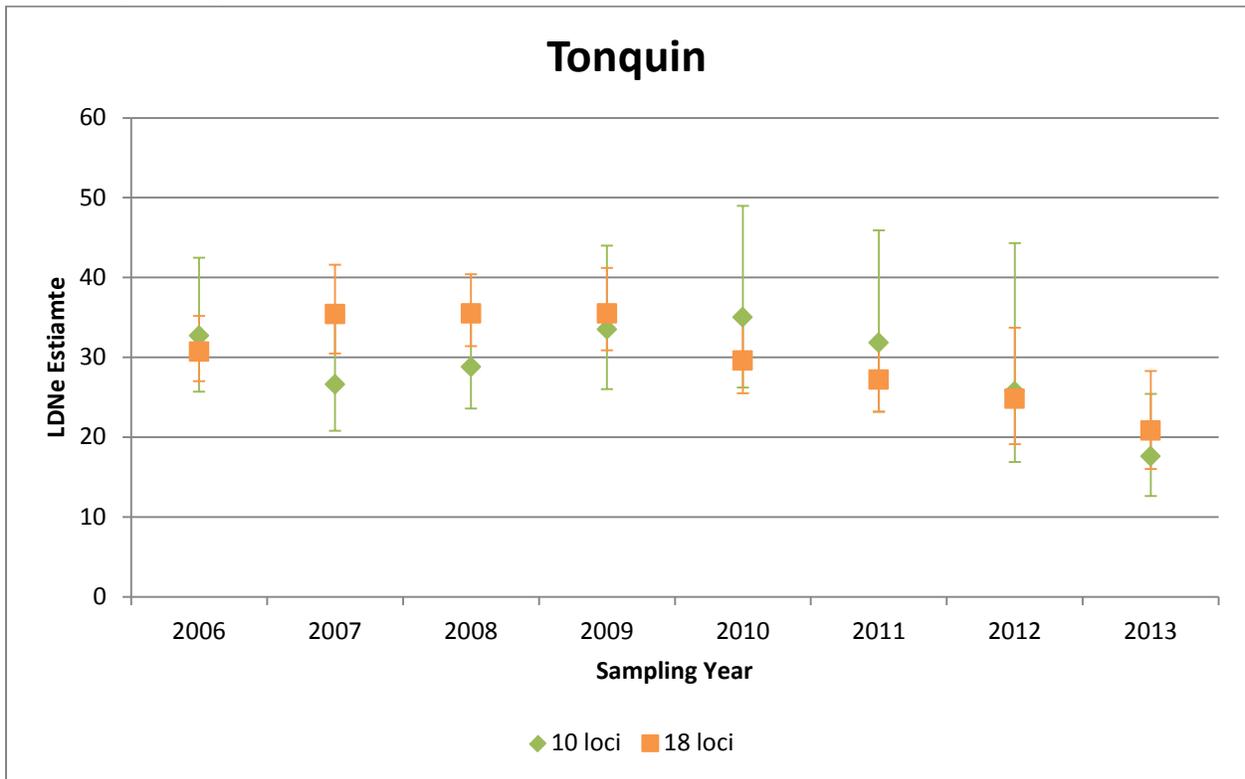


Table 24: Yearly N_e results for the Tonquin herd of woodland caribou, 10 loci vs. 18 loci, using the linkage disequilibrium method. 95% confidence intervals in brackets.

Sampling Year	10 loci	LCI	UCI	18 loci	LCI	UCI
2006	32.7	25.7	42.5	30.7	27.0	35.2
2007	26.6	20.8	34.5	35.4	30.5	41.6
2008	28.8	23.6	35.6	35.5	31.4	40.4
2009	33.5	26	44	35.5	30.9	41.2
2010	35	26.2	49	29.6	25.5	34.7
2011	31.8	23.2	45.9	27.2	23.2	32.3

Table 24: Yearly N_e results for the Tonquin herd of woodland caribou, 10 loci vs. 18 loci, using the linkage disequilibrium method. 95% confidence intervals in brackets.

Sampling Year	10 loci	LCI	UCI	18 loci	LCI	UCI
2012	25.7	16.9	44.3	24.8	19.1	33.7
2013	17.6	12.6	25.4	20.8	16.0	28.3

Figure 22: Yearly linkage disequilibrium and sibship assignment N_e estimates for the North Interlake herd of woodland caribou (*Rangifer tarandus*). Error bars represent the 95% confidence intervals.

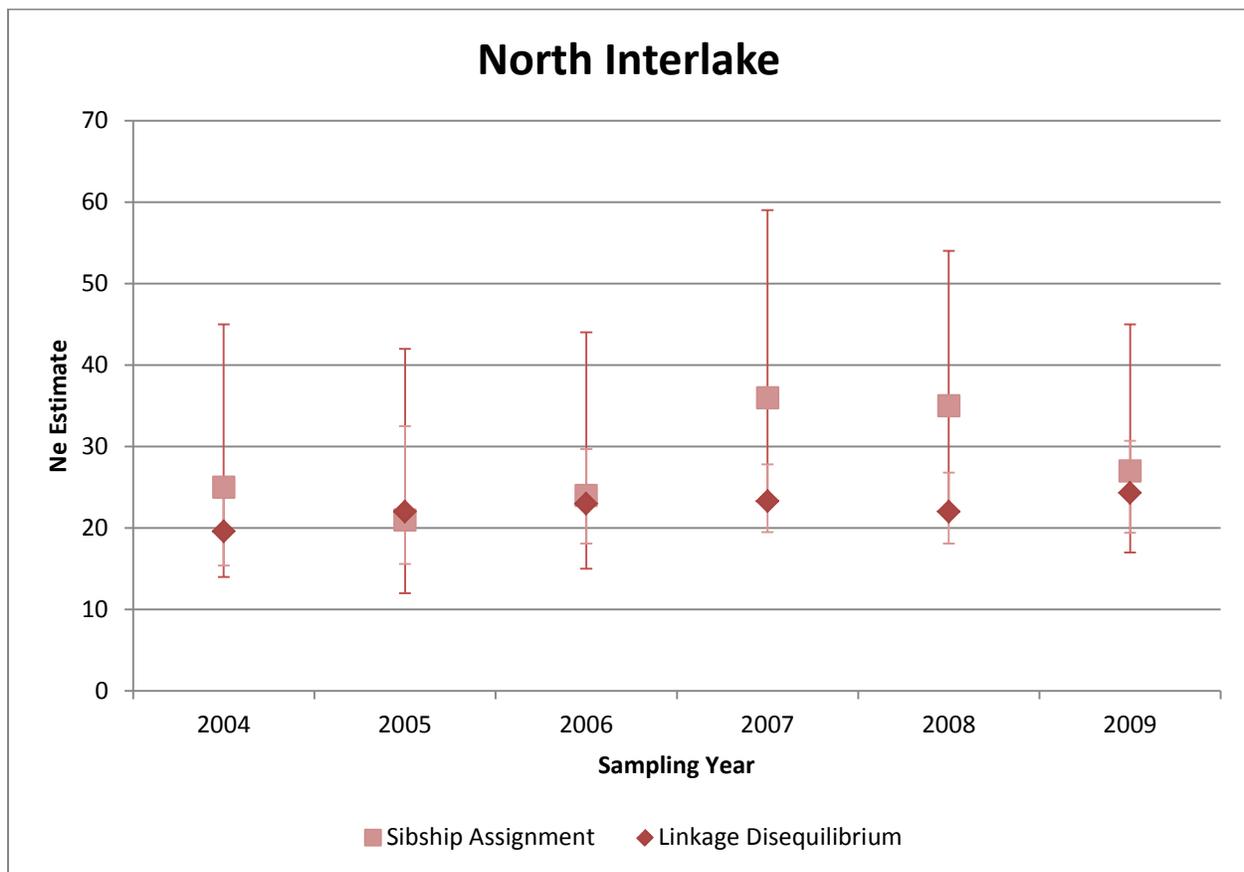


Table 25: Linkage Disequilibrium and Sibship Assignment N_e estimates for the North Interlake herd of woodland caribou (*R. tarandus*)

Linkage Disequilibrium					Sibship Assignment			
Sampling Year	Sample size	Estimated N_e	LCI	UCI	Sample Size	N_e estimate	95% LCI	95% UCI
2004	50	19.6	15.4	25.4	50	25	14	45
2005	32	22	15.6	32.5	32	21	12	42
2006	56	23	18.1	29.7	56	24	15	44
2007	97	23.3	19.5	27.8	97	36	23	59
2008	82	22	18.1	26.8	147	35	22	54
2009	63	24.3	19.4	30.7	63	27	17	45
2010	25	25.3	14.8	55.2	25	16	8	38

Figure 23: Yearly linkage disequilibrium and sibship assignment N_e estimates for The Bog herd of woodland caribou (*Rangifer tarandus*). Error bars represent 95% confidence intervals.

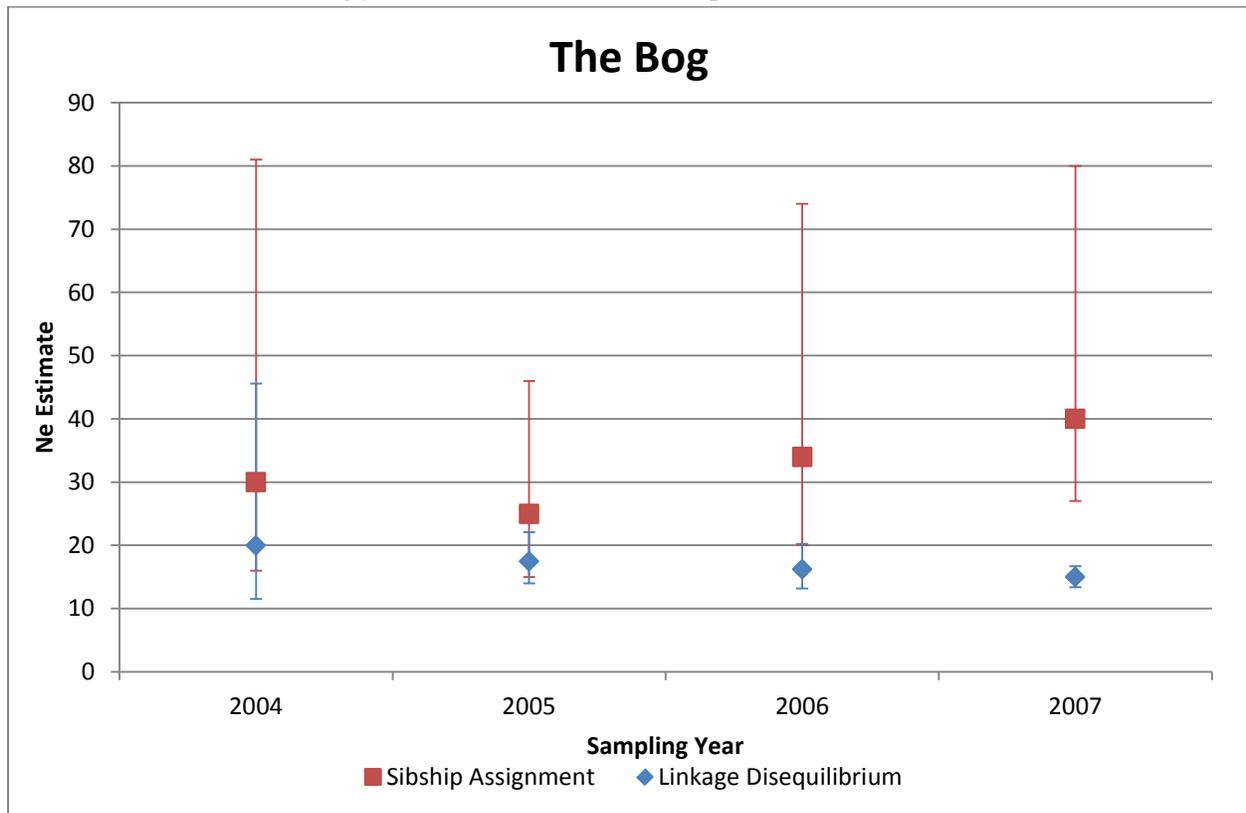


Table 26: Linkage Disequilibrium and Sibship Assignment N_e estimates for The Bog herd of woodland caribou (*R. tarandus*).

Sampling Year	Sample size	Linkage Disequilibrium			Sibship Assignment		
		Estimated N_e	LCI	UCI	N_e estimate	95% LCI	95% UCI
2004	16	20	11.5	45.6	30	16	81
2005	41	17.5	14	22.1	25	15	46
2006	29	16.2	13.2	20.2	34	20	60
2007	60	15	13.4	16.7	40	27	67

Figure 24: Yearly linkage disequilibrium and sibship assignment N_e estimates for the Slate Islands herd of woodland caribou (*Rangifer tarandus*). Error bars represent the 95% confidence intervals.

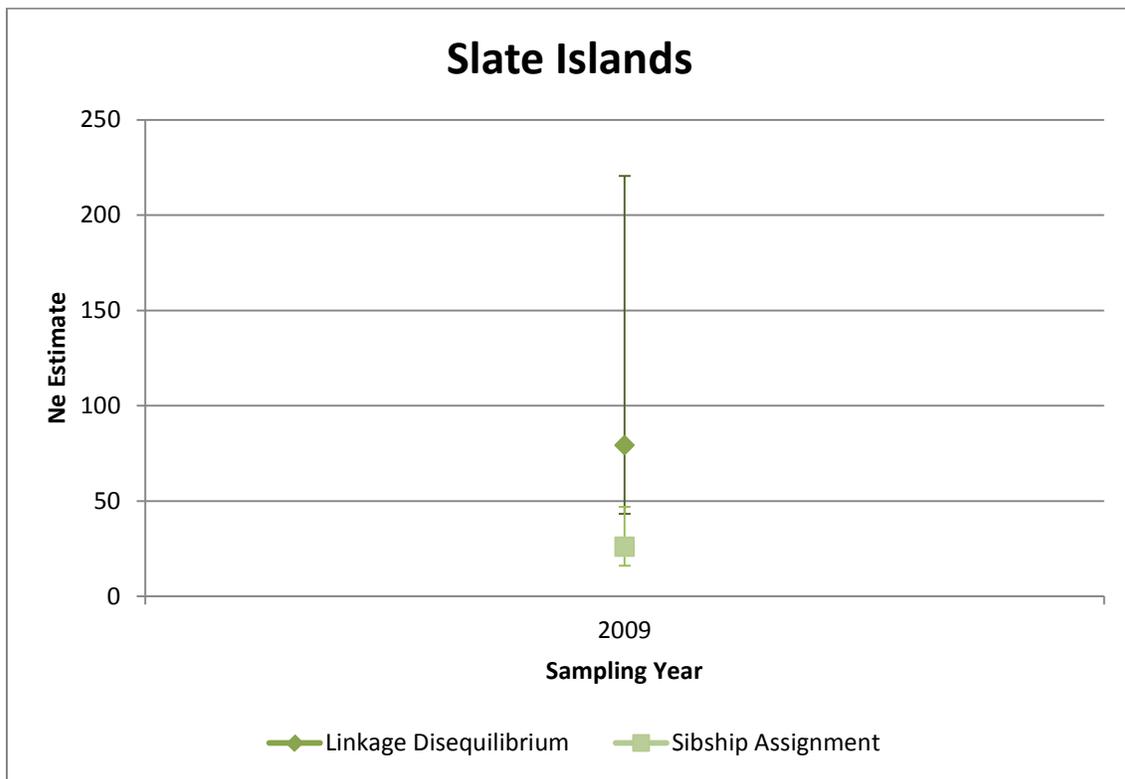


Table 27: Linkage Disequilibrium and Sibship Assignment N_e estimates for the Slate Islands herd of woodland caribou (*R. tarandus*)

Linkage Disequilibrium					Sibship Assignment		
Sampling Year	Sample size	Estimated N_e	LCI	UCI	N_e estimate	95% LCI	95% UCI
2009	80	79.2	43.2	220.6	26	16	47

Figure 25: Yearly linkage disequilibrium and sibship assignment N_e estimates for the PAGE herd of woodland caribou (*Rangifer tarandus*). Error bars indicate 95% confidence intervals.

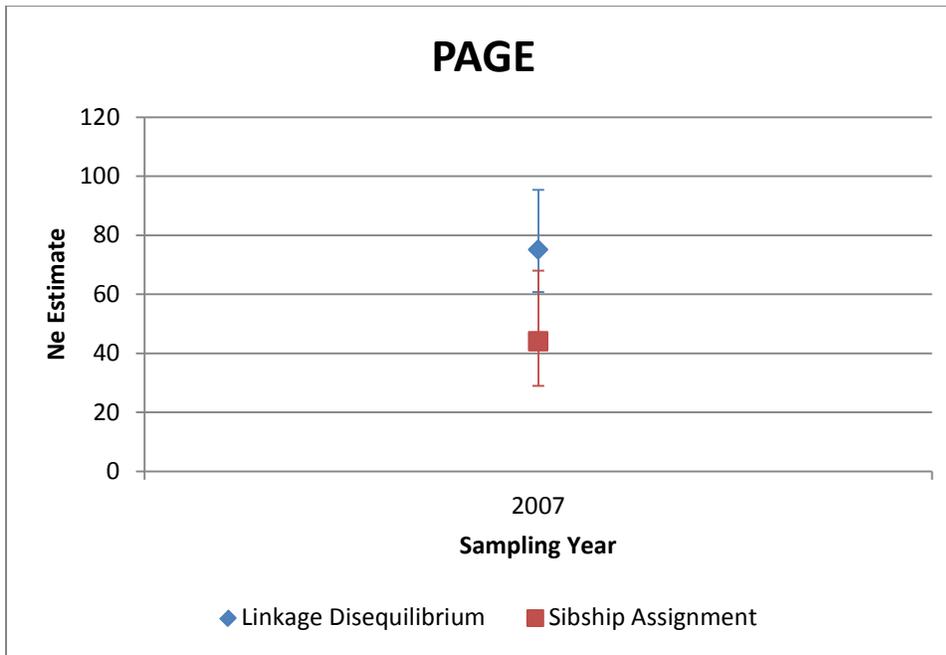


Table 28: Linkage Disequilibrium and Sibship Assignment N_e estimates for the PAGE herd of woodland caribou (*R. tarandus*).

Linkage Disequilibrium					Sibship Assignment		
Sampling Year	Sample size	Estimated N_e	LCI	UCI	N_e estimate	95% LCI	95% UCI
2007	82	75.1	60.8	95.4	44	29	68

The temporal method (TM) was used for the Tonquin and North Interlake herds (Figure 26). Data collected from the first and last year of sampling were used for the TM method to

represent sampling once per generation (Tonquin: 2006 and 2013; North Interlake: 2004 and 2010).

Figure 26: Two-sample N_e estimates for the Tonquin and North Interlake herds of woodland caribou (*Rangifer tarandus*). Error bars indicate 95% confidence intervals.

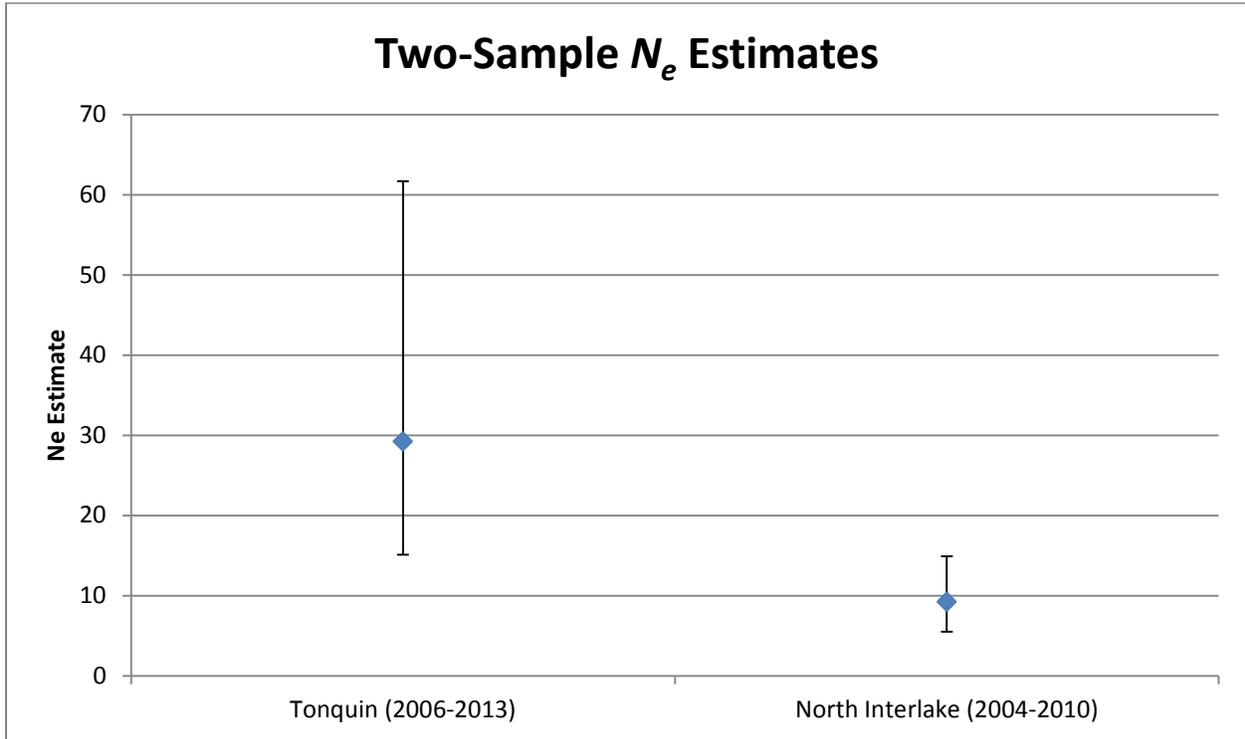


Table 29: Two-sample N_e estimates for the Tonquin, and North Interlake populations of woodland caribou (*Rangifer tarandus*) using the Jorde and Ryman (1995) temporal method.

Population	N_e Estimate	LCI	UCI	# of samples
Tonquin (2006-2013)	29.2	15.1	61.7	98
North Interlake (2004-2010)	9.2	5.5	14.9	75

N_e/N

The N_e/N ratio for the Tonquin herd did not remain constant from sampling years 2006 to 2013 (Figure 27, Table 33). The linkage disequilibrium method was used for the N_e estimates and census N estimates were found using the best model from capture-mark-recapture modeling

(see Chapter 2). The N_e/N ratio increased overall from 2006 to 2013, with the highest ratio occurring in 2010. The N_e/N ratio increased slightly for the North Interlake year from 2005 to 2008 (Figure 28, Table 34). For both populations, the N_e/N ratio increased with a decrease in N . N_e remained relatively constant throughout the sampling period, with confidence intervals overlapping each year throughout the sample years. Isolation by distance (IBD) results show that slight IBD was found in the Tonquin herd only in 2008 ($r_{xy} = 0.01$). IBD was found in the North Interlake in all years except for 2009, and three of four sampling years in the PAGE. The PAGE population only had one sampling year available to compare effective population size to census population size, with a N_e/N ratio of 32.9% for the year 2007. R_{xy} values were lower in the Tonquin herd compared to the other populations.

Table 30: Isolation by distance (IBD) results of the Tonquin herd of woodland caribou. Significant values are in italics.

Year	Mantel's R_{xy}	P
2006	0.136	0.050
2007	0.062	0.150
2008	0.111	<i>0.010</i>
2009	0.094	0.110
2010	0.033	0.270
2011	0.030	0.250
2012	0.064	0.150
2013	0.031	0.250

Table 31: Isolation by distance (IBD) results of the North Interlake herd of woodland caribou. Significant values are in italics.

Year	Mantel's R_{xy}	P
2004	0.337	<i>0.010</i>
2005	0.214	<i>0.010</i>
2006	0.268	<i>0.010</i>
2007	0.232	<i>0.010</i>

Table 31: Isolation by distance (IBD) results of the North Interlake herd of woodland caribou. Significant values are in italics.

Year	Mantel's R_{xy}	P
2008	0.252	<i>0.010</i>
2009	-0.029	0.420
2010	0.326	<i>0.010</i>

Table 32: Isolation by distance (IBD) results of the PAGE herd of woodland caribou. Significant values are in italics.

Year	Mantel's R_{xy}	P
2005	0.234	<i>0.010</i>
2006	0.105	0.080
2007	0.123	<i>0.010</i>
2008	0.129	<i>0.030</i>

Figure 27: Yearly N_e vs. N estimates for the Tonquin herd of woodland caribou (*R. tarandus*). Linkage disequilibrium estimates used for N_e . Error bars indicate 95% confidence intervals. Yearly population size estimates (N) from capture-mark-recapture models. The line graph represents the N_e/N ratio.

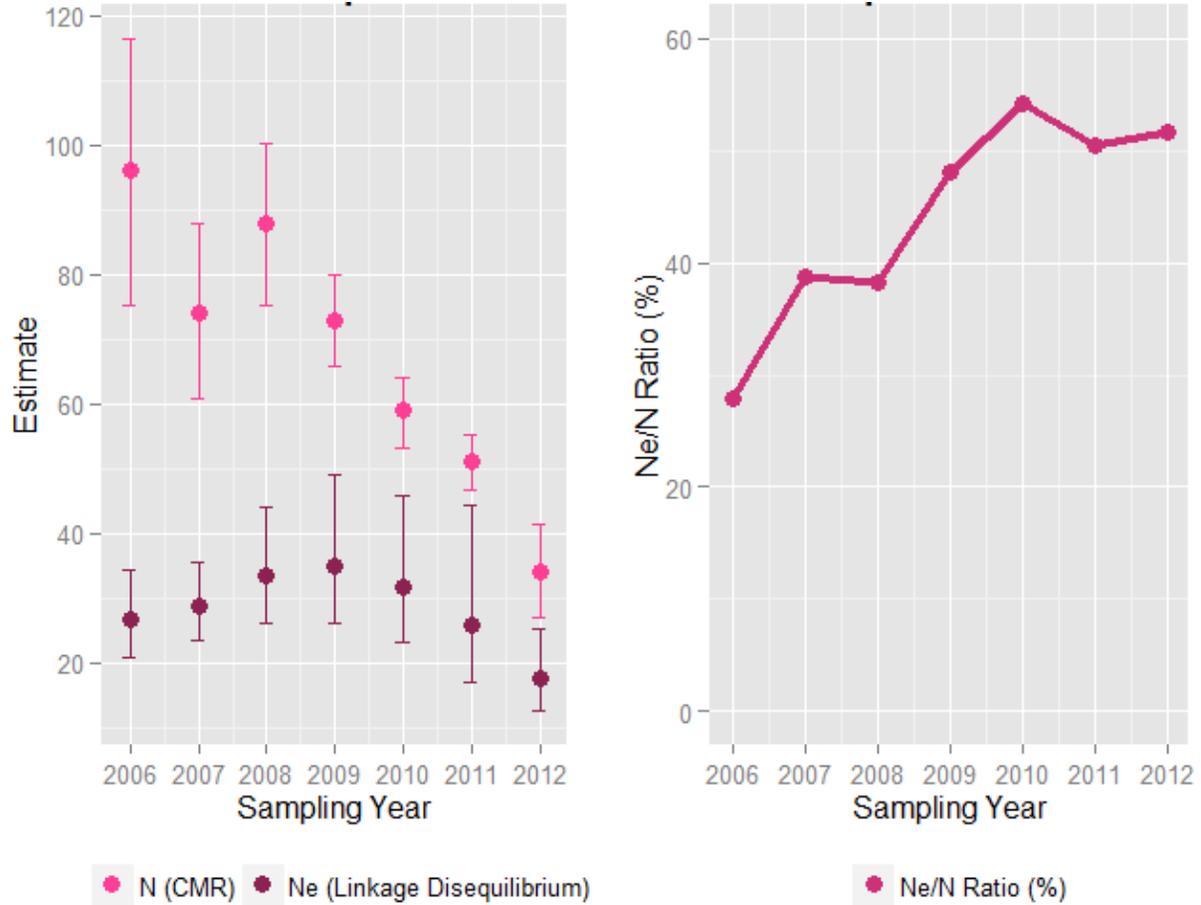


Table 33: Yearly N_e vs. N estimates for the Tonquin herd of woodland caribou (*R. tarandus*). Brackets indicate 95% confidence intervals.

Sampling Year	N estimate (CMR)	N_e estimate (LD)	N_e/N ratio
2006	95.8 (75.2, 116.4)	26.6 (20.8, 34.5)	0.278
2007	74.3 (60.7, 87.9)	28.8 (23.6, 35.6)	0.388
2008	87.8 (75.3, 100.2)	33.5 (26, 44)	0.382
2009	72.8 (65.8, 79.8)	35 (26.2, 49)	0.481
2010	58.7 (53.2, 64.1)	31.8 (23.2, 45.9)	0.542
2011	50.9 (46.6, 55.2)	25.7 (16.9, 44.3)	0.505
2012	34.1 (26.9, 41.3)	17.6 (12.6, 25.4)	0.516

Figure 28: Yearly N_e vs. N estimates for the North Interlake herd of woodland caribou (*R. tarandus*). Linkage disequilibrium estimates used for N_e . Error bars indicate 95% confidence intervals. Yearly population size estimates (N) from capture-mark-recapture models. The line graph represents the N_e/N ratio.

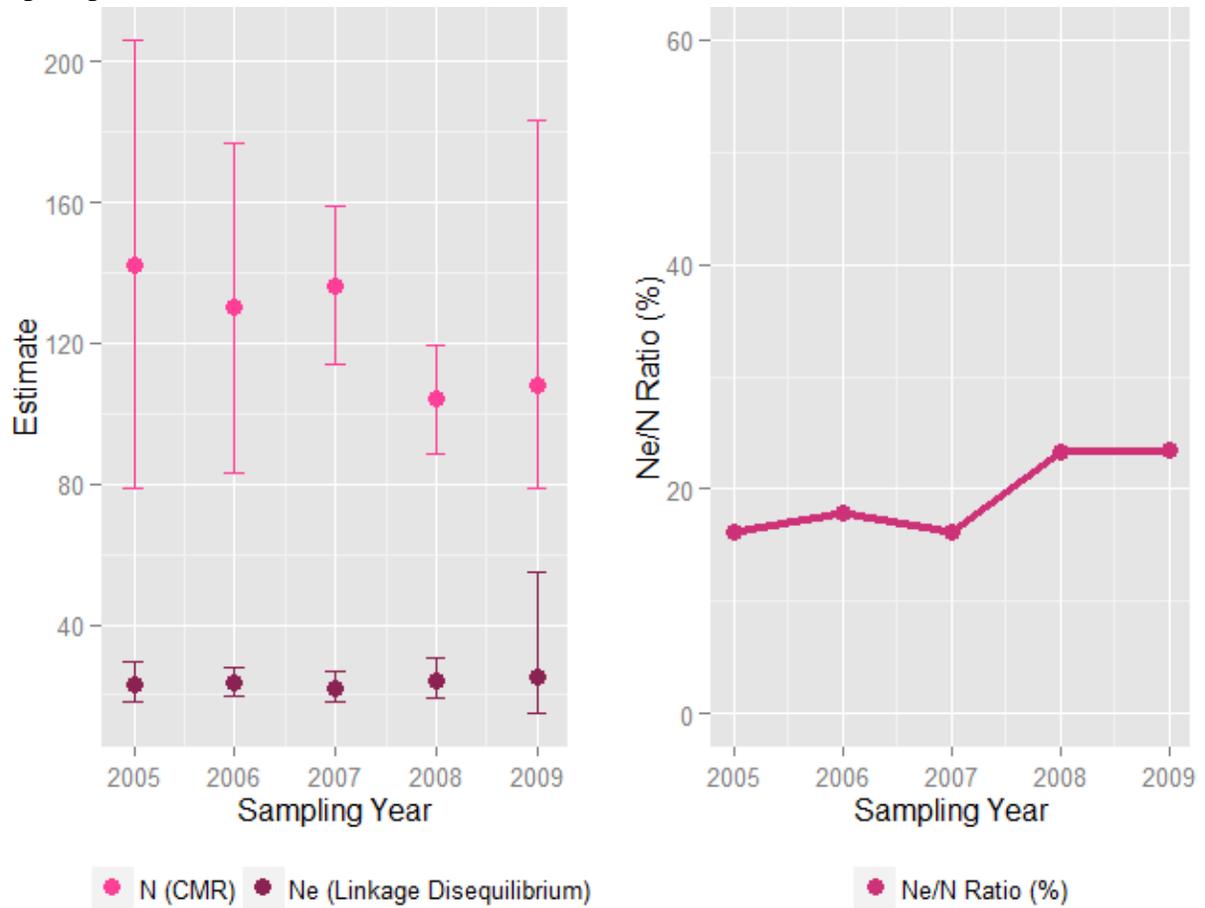


Table 34: Yearly N_e vs. N estimates for the North Interlake herd of woodland caribou (*R. tarandus*). Brackets indicate 95% confidence intervals.

Sampling Year	N estimate (CMR)	N_e estimate (LD)	N_e/N ratio
2005	142 (78.5, 206.1)	23 (18.1, 29.7)	0.162
2006	130 (83.4, 176.6)	23.3 (19.5, 27.8)	0.179
2007	136 (113.8, 158.9)	22 (18.1, 26.8)	0.161
2008	104 (88.6, 119.4)	24.3 (19.4, 30.7)	0.234
2009	108 (78.9, 183.6)	25.3 (14.8, 55.2)	0.235

Figure 29: 2007 N_e vs. N estimates for the PAGE herd of woodland caribou (*R. tarandus*). Linkage disequilibrium estimate used for N_e . Error bars indicate 95% confidence intervals. Population size estimate (N) from capture-mark-recapture models.

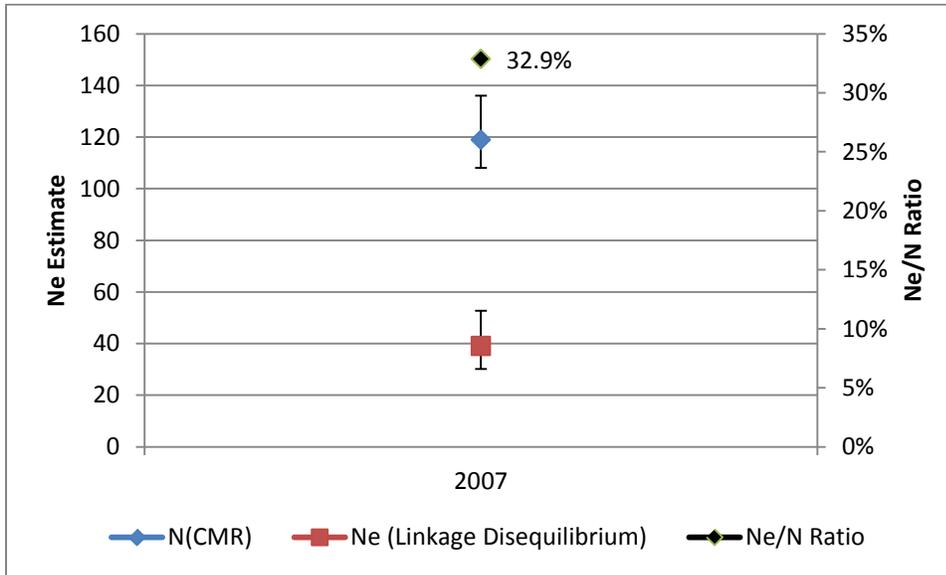


Table 35: 2007 N_e vs. N estimate for the PAGE herd of woodland caribou (*R. tarandus*). Brackets indicate 95% confidence intervals.

Sampling Year	N estimate (CMR)	N_e estimate (LD)	N_e/N ratio
2007	119 (108, 136)	39.1 (30.2, 52.7)	0.329

Discussion

Simulations

Simulation results for the Tonquin herd show a slight decrease in precision with each reduction in the number of loci used and the number of unique genotypes used for estimation of N_e . However, most simulations still had good precision [$CV(N_e) < 0.2$], with both a minimum of 6 loci, as well as using 30 genotypes producing results with good precision (Waples and Do 2010). $CV(N_e)$ (coefficient of variation of N_e) declined (and increased precision) with more loci being included and more samples being included. $CV(N_e)$ is an increasing function of N , as variance is higher and precision is lower in populations that have a large effective population size (Waples and Do 2010).

There was little difference in the precision between using 10 loci or 18 loci. Even when only using 30 samples at 10 loci, N_e estimation results were comparable to using 18 loci with the same number of unique genotypes (Table 21 and Figure 19). The Tonquin herd is a small population, with estimates of $N < 100$ individuals (Table 33). Precision is strongly affected by the interaction between the sample size and the effective size of a population. Waples and Do (2010) found that if N is only 100 individuals, even small samples of 25 individuals analysed at 20 loci is adequate enough for producing adequate N_e estimates; but if the same number of unique genotypes are taken for larger populations ($N \approx 1000$ individuals), the results are much less accurate. Since all populations of woodland caribou used in this study are small populations estimated at less than 100 individuals, small sample sizes (around 25 samples) can provide meaningful information about N_e for these populations. Separately, increasing the number of loci

used and increasing the sample size of individuals both lead to gains in precision when using the linkage disequilibrium (LD) method (England et al. 2010, Waples and Do 2010).

Waples and Do (2010) found that the LD method has little or no bias for $P_{\text{crit}} \geq 0.05$. Estimates become slightly biased upwards when lower frequency alleles are allowed into the analysis, which is more pronounced in small sample sizes. Selecting a lower P_{crit} value leads to N_e estimates that are more precise, but potentially more biased (Waples 2006, Waples and Do 2010). However, Waples and Do (2010) found that the number of loci used in analysis did not have an appreciable effect on bias. Choosing a P_{crit} that is the larger of 0.02 or a value that screens out alleles only occurring once is a practical guideline for balancing the trade-off between precision and bias, and leads to largely unbiased estimates of N_e with high precision (Waples and Do 2010).

The results from the simulations indicate that the number of loci used and the number of unique genotypes used for the remaining populations (North Interlake, The Bog, PAGE and Slate Islands) is enough to produce unbiased estimates of N_e . These populations all had 10 loci used in the analysis, and had large enough sample sizes per year to produce unbiased estimates of N_e .

Closed Populations

For closed populations (Tonquin, North Interlake, The Bog, Slate Islands and PAGE), both methods (LD and SA) determined yearly estimates of N_e . However, the 95% confidence intervals for these estimates were very large in some instances, and therefore did not produce similar results. For most years in all studied populations, the LD method had narrower confidence intervals compared to the SA method. For example, the LD method for The Bog population produced much smaller estimates of N_e for all four sampling years (2004 to 2007) and

much smaller confidence intervals compared to the SA method. Some estimates were more than double the LD method for the same year (15 individuals for LD compared to 40 for SA in 2007, Figure 23). The confidence interval for the SA method in 2004 had a range of 65 individuals, significantly larger than the N_e estimate of 30. The very large confidence intervals on most of the estimates means that although the intervals overlap, it does not mean the estimates had comparable results (Knezevic 2008). Mean annual calf recruitment rates from telemetry data in 2010 and 2011 indicate very low calf recruitment of 13 calves per 100 cows in 2010 and 6 calves per 100 cows in 2011 (JORO Consultants 2012).

At small sample sizes, the SA method can provide estimates that are biased low, while the LD method should be robust at these sample sizes (Wang 2009, Skrbinek et al. 2012). For the North Interlake population, the lowest N_e estimates from the SA method occurred in 2005 and 2010, which correspond with the years with the lowest number of unique genotypes collected (2005 = 32; 2010 = 25 [Table 19]). The SA method showed great variation in N_e per year, initially decreasing in 2005, increasing up until 2007, and then decreasing dramatically in the final year of sampling. The LD method, however, provided a steadier yearly N_e estimate from 2004 to 2010, with a slight increase in N_e each year. If the small sample sizes are taken into account, this can explain the low estimates in 2005 and 2010 for the SA method, because the SA method is biased at low sample sizes, whereas the LD method is not biased at low sample sizes (2010, Skrbinek et al. 2012).

When the sample size is significantly smaller than the actual N_e , the information that can be used by the SA method is very limited (Wang 2009). SA N_e estimates for The Bog were consistently higher than those from the LD method. The number of unique genotypes collected

increased from 2004 to 2007, from 16 samples in 2004 to 60 in 2007. The SA method shows a decrease in N_e from 2004 to 2005, while increasing up until 2007. Since the SA method is biased at low sample sizes, this explains the lower estimates in 2004 and 2005 compared to 2006 and 2007. The LD method showed a slight decrease in N_e , gradually decreasing from 2004 to 2007. The LD confidence interval became narrower from 2004 to 2007, which corresponds with an increase in the number of unique genotypes collected during the same time period (Table 26). 60 samples were collected in 2007, the most collected out of any sampling year, and had the narrowest confidence interval ($N_e = 15$; 95% CI = 13.4, 16.7). The SA confidence intervals remained relatively constant throughout the sampling years, and were significantly wider than the LD confidence intervals for the same years (Figure 23). The difference between the LD and SA estimates can be due to the different methods of estimating N_e . The LD method estimates N_e by measuring gametic disequilibrium, or analysing the random linkage disequilibrium that occurs by chance at each generation, while the SA method estimates N_e by estimating the frequencies that a pair of offspring taken randomly from a population would be half- or full-siblings (Hill 1981, Waples 2006, Wang 2009). Open populations can affect results from the SA method. If sampling takes place after immigration has occurred, the sample contains individuals whose parents are not members of the target population, which in turn causes the sibship frequencies to be underestimated, and thus causes N_e to be overestimated. If sampling occurs before migration has occurred, the N_e estimates should not be affected by migration (Wang 2009). Little migration occurs in forest-dwelling caribou in the summer and winter, with most movement occurring in the spring before calving occurs, and in the fall before the rut begins (Thomas and Gray 2002). Calving occurs in May or early June, with most of the PAGE females calving by mid-May (Thomas and Gray 2002, Arsenault and Manseau 2011).

N_e/N

The relationship between N_e and N can be challenging to accurately estimate. If the N_e estimate is not matched with the appropriate estimate of N , it can result in biologically misleading conclusions (Waples 2005). Genetic and demographic data cannot be directly related, even when collected from exactly the same time period, unless population size is constant (Palstra and Fraser 2012). Both N_e and N estimates used in my analyses came from genetic data collected from the same time period, and therefore the N_e/N ratio can be calculated for the Tonquin and North Interlake populations. Assuming constant population size for a population that does not have constant population size can yield upwardly biased N_e/N ratios (Palstra and Fraser 2012). There can be large variation in estimates of N_e/N ratios, depending on the life history characteristics of the species being studied. Species with high fecundity may have lower N_e/N ratios due to high variance in family sizes, and potentially have greater fluctuations in population size from generation to generation (Frankham 1995). Values as low as 10^{-6} have been reported for Pacific oysters (*Crassostrea gigas*) (Hedgecock et al. 1992) and as high as 0.994 in humans (*Homo sapiens*) (Frankham 1995). The variation in N_e/N ratios is due to whether unequal sex-ratio, variance in family size, and fluctuations in population size were included, and different variations of N , such as adult census population size or total census population size, were used (Frankham 1995). Frankham (1995) found that fluctuations in population size was the most important variable that affected N_e/N ratios. Polygamous species are expected to have lower N_e/N ratios than monogamous species, due to an unequal sex-ratio and the high variance of male reproductive contribution (Frankham 1995). Woodland caribou are a polygamous species. In small, isolated populations, Flasko (2014) found that super-males dominate the reproductive output, and the male reproductive contribution is highly variable.

One-sample estimators, such as the LD method, provide an estimate of the effective number of parents that produced the progeny from which the sample population is drawn (Waples 2005). Samples in generation i are produced by the effective breeders in generation $i - 1$, so the estimate applies to N in the previous generation ($i - 1$) (Waples 2005, Palstra and Ruzzante 2008). Therefore, the estimate for N in 2006 was compared to the N_e estimate for 2007 in order to get the appropriate N_e/N ratio. The N_e/N ratio for the Tonquin herd increased overall during the sampling period, from 0.278 in 2006 to 0.516 in 2012 (Table 33). N estimates declined significantly overall during the sampling period, from 96 individuals in 2004 to a low of 34 in 2012, roughly a two-thirds reduction in population size. Contemporary single-sample N_e estimates are not likely to be seriously affected by population declines. If N_e in the population is small, LD will detect that small size, regardless of whether or not the population was larger in previous generations (Waples 2005).

The North Interlake herd had significantly lower N_e/N ratios compared to the Tonquin herd. The N_e/N ratio increased slightly from 2005 to 2008, from 0.162 to 0.234 (Table 34). N estimates declined during this period, from 142 individuals in 2005 to 108 individuals in 2009, while N_e remained relatively constant during this time period, explaining the increase in the N_e/N ratio. The N_e/N ratio decreases as N increases, with large populations having a proportionally smaller N_e/N ratio than small populations (Ardren and Kapuscinski 2003, Palstra and Ruzzante 2008, Luikart et al. 2010). The North Interlake population has a larger estimated N than Tonquin (2009 estimates were 108 individuals for North Interlake and 72 for Tonquin). The North Interlake woodland caribou range is in a highly fragmented landscape, surrounded by lakes, with a major highway running through the middle of the range, and Mantel results showed that isolation by distance (IBD) is a factor for this population (Table 31). However, the Tonquin

population, also a closed population, is not affected by IBD for most years; IBD was only present in 2008 ($r_{xy} = 0.111$), with a lower regression coefficient than all of the North Interlake years (lowest $r_{xy} = 0.214$ in 2005), indicating that IBD is not as much of a factor in Tonquin compared to North Interlake. This may explain why the North Interlake population had significantly lower N_e/N ratios compared to Tonquin; increased difficulty in breeding with individuals geographically farther away may cause a lower number of individuals to produce offspring in the population. Alternatively, boreal woodland caribou populations (North Interlake, PAGE) may have stronger harem-like behaviour compared to mountain woodland caribou populations (Tonquin). The PAGE population had a N_e/N ratio of 32.9% in 2007, slightly higher than North Interlake, and comparable to the lowest N_e/N ratio for the Tonquin herd. IBD was a factor for the PAGE population in three of the four years sampling occurred (2007 $r_{xy} = 0.123$).

The N_e/N ratio for both Tonquin and North Interlake increased from year to year with a decrease in N estimates. Ardren and Kapuscinski (2003) termed the inverse relationship between N_e/N and N the ‘genetic compensation’ effect; when fewer individuals are present in a population, there is a higher proportion of individuals apparently reproducing successfully. This has been documented in salmonids (Ardren and Kapuscinski 2003, Araki et al. 2007, Fraser et al. 2007), amphibians (Jehle et al. 2005, Beebee 2009), butterflies (Saarinen et al. 2010), insects (Watts et al. 2007), and leopards (Sugimoto et al. 2014). Genetic compensation could explain the increase in the N_e/N ratio for the Tonquin and North Interlake woodland caribou herds.

Many factors can influence the N_e/N ratio. Threatened species often exhibit lower (neutral) genetic diversity, and therefore may be expected to exhibit relatively low effective population sizes (Palstra and Ruzzante 2008). Populations that are affected by deterministic

factors that contribute to census declines generally have lower N_e/N ratios, as these factors act to reduce N_e below biological expectations. However, if environmental stochasticity is the factor driving population declines, it may increase N_e/N ratios, since all individuals in a population are affected equally, thereby reducing individual reproductive variance (Palstra and Ruzzante 2008). Environmental stochasticity is the temporal fluctuations in reproductive rates and mortality that affect all individuals of a population, including such factors as food availability, parasites, competitors, rainfall, fire, and temperature (Caughley 1994, Carroll and Fox 2008). Woodland caribou are highly sensitive to habitat fragmentation, anthropogenic activities, and other environmental factors (Thomas and Gray 2002, Weladji et al. 2002, Environment Canada 2014).

Understanding N_e/N ratios can reveal what factors drive N_e below N , which can facilitate more effective conservation and management decision-making (Palstra and Fraser 2012). For genetic management of a population, it is not enough to just know N_e . In order to conserve populations with small N_e , the factors that are causing the reduction in N_e need to be identified in order to create the optimal genetic management strategy (Wang 2009). Management strategies to increase N_e would differ depending on the factors influencing N_e ; an unbalanced sex ratio, large variance in family size in males and/or females, or a small census size would all require different management plans in order to increase N_e (Wang 2009). [SM1]

Temporal method

The temporal method requires at least one generation to pass in between sampling occasions (Nei and Tajima 1981, Pollak 1983, Waples 1989, Jorde and Ryman 2007). The estimates from the temporal method depend on the sampling plan that was used, either Plan I (individuals sampled after reproduction or sampled non-destructively, and returned to the

population), or Plan II (individuals sampled before reproduction and not returned to the population). The samples used in my analyses follow a Plan I sampling, as samples are sampled non-invasively and individuals are not removed from the population (Waples 1989). The temporal method assumes discrete generations, and using this method on overlapping generations can introduce substantial bias. Bias caused by overlapping is difficult to predict and complex, as it depends on many factors, such as the species-specific survivorship pattern, the age classes sampled, and the sampling interval (Luikart et al. 2010). The Jorde and Ryman (1995) model for the temporal method showed that the temporal change in allele frequencies depends on N_e as well as age structure and age-specific birth and survival rates. It provides unbiased estimates of N_e when age-specific survival and birth rates are available. However, in order to get unbiased estimates, it is generally recommended that more than one generation of sampling is necessary. Bias could be small when using the temporal method on overlapping generations if the elapsed time between samples is long enough that the genetic drift signal strongly dominates sampling considerations (Jorde and Ryman 1995, Waples and Yokota 2007, Palstra and Ruzzante 2008).

One-sample and two-sample estimates cannot be directly compared, because they never estimate N_e in exactly the same generations (Waples 2005). The one-sample methods estimate the inbreeding effective size, and is associated with transmission of genetic material from one generation to the next, while the temporal method is a function of the variance effective size (Waples 2005). The inbreeding effective size is related to the rate of increase in inbreeding or loss of heterozygosity, and the variance effective size is related to the rate of allele frequency change (Waples 2005, Luikart et al. 2010).

The Tonquin population has eight years of sampling (2006 to 2013), and the North Interlake have seven years of sampling (2004 to 2010), and theoretically the temporal method can be used for these populations, as the generation length for woodland caribou is roughly seven years (Thomas and Gray 2002). One generation of sampling is generally too short of a time period to get unbiased estimates of N_e (Jorde and Ryman 1995, Waples and Yokota 2007, Palstra and Ruzzante 2008). The two-sample N_e results differed greatly between study populations, with significant differences between 95% confidence intervals; Tonquin was estimated at 29 individuals over an eight-year period, whereas North Interlake was only estimated at 10 individuals over a seven-year period. Woodland caribou are a long-lived species, and waiting a generation or more to collect samples for estimating N_e is impractical. Many populations of woodland caribou are declining across Canada, and using the temporal method is not useful for conservation management of a species. One-sample estimators have the benefit of only needing one year of sampling data to get an estimate of N_e , and yearly fluctuations in N_e can also be estimated.

Conservation and Management Implications

We were able to estimate yearly N_e for closed woodland caribou populations across western Canada using the methods expressed in this chapter. One-sample N_e estimators are superior to two-sample estimators for monitoring woodland caribou populations, as one-sample estimators can detect yearly changes in N_e (Waples and Do 2008, Wang 2009) that allow managers to detect early signs of loss of genetic variation and inbreeding in populations (Frankham 1995, Palstra and Ruzzante 2008), while using a two-sample estimator and pooling the data from several years can disguise subtle patterns of population structure and affect the

estimation of N_e (Watts et al. 2010). It also provides N_e/N ratios when used in conjunction with capture-mark-recapture (Frankham 1995, Palstra and Fraser 2012) that can help determine the degree to which populations are able to avoid extirpation and extinction from demographic, environmental, or genetic random events (Luikart et al. 2010, Palstra and Fraser 2012, Weckworth et al. 2013). It is a powerful tool in detecting decreases in N_e for closed populations of woodland caribou, as yearly changes in N_e were detected across all study populations.

The linkage disequilibrium method was able to produce estimates of N_e even at low sample sizes or few loci. Results from the linkage disequilibrium method show that even small amounts of data can provide adequate estimates; using a minimum of 30 unique genotypes when analysing 10 loci produces results with good precision; increasing the number of loci (up to 18 loci) did not increase the precision of the estimates. Therefore, even with a low number of unique genotypes, good estimates of N_e can be obtained. These results clearly show that one-sample N_e estimators are advantageous over two-sample N_e estimators for monitoring and conservation of woodland caribou populations in Canada.

The populations in this study are relatively small, closed populations, and these methods may not be adequate enough when applied to large-scale, continuous populations of caribou. Most genetic-based approaches of estimating N_e assume a closed population, and have not been used to a great extent on open populations. N_e should be estimated in larger herds or continuous populations of woodland caribou to see if the methods used in this study can be extended to other populations.

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Appendix B

Table 36: Allele frequencies for the Tonquin woodland caribou herd.

Locus	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9
BM4513	111	119	123	125	131	133			
Frequency	0.093	0.037	0.111	0.352	0.185	0.222			
BM6506	197	201	203	205	209				
Frequency	0.303	0.197	0.318	0.121	0.061				
BM848	362	364	372	374	376				
Frequency	0.278	0.200	0.044	0.156	0.322				
BM888	164	170	174	176	184	210			
Frequency	0.173	0.378	0.163	0.163	0.092	0.031			
BMS1788	105	107	109	113	115	117	119		
Frequency	0.100	0.100	0.180	0.310	0.010	0.290	0.010		
FCB193	94	96	102	104	106	108			
Frequency	0.083	0.117	0.017	0.567	0.033	0.183			
MAP2C	91	101	103	105	109	113	115		
Frequency	0.122	0.194	0.112	0.133	0.031	0.398	0.010		
NVHRT16	151	173	183	185	189				
Frequency	0.385	0.212	0.135	0.115	0.154				
OHEQ	257	263	271	277	279	283	291	295	
Frequency	0.125	0.054	0.036	0.071	0.232	0.179	0.018	0.286	
RT1	234	236	238	240	242	244	250		
Frequency	0.406	0.047	0.031	0.078	0.109	0.250	0.078		
RT13	296	298	300	304	306	308	310		
Frequency	0.056	0.185	0.185	0.148	0.093	0.278	0.056		

Table 36: Allele frequencies for the Tonquin woodland caribou herd.

Locus	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9
RT24	213	215	217	219	227				
Frequency	0.082	0.143	0.163	0.459	0.153				
RT27	132	134	136	138	144	146	148	150	156
Frequency	0.273	0.318	0.023	0.023	0.114	0.045	0.091	0.091	0.023
RT30	183	191	193	195	199	201	219		
Frequency	0.114	0.148	0.182	0.227	0.102	0.182	0.045		
RT5	92	104	106	108	110	114			
Frequency	0.010	0.367	0.102	0.173	0.245	0.102			
RT6	102	104	106	112					
Frequency	0.041	0.541	0.398	0.020					
RT7	208	218	226	228	234				
Frequency	0.031	0.316	0.133	0.388	0.133				
RT9	114	116	118	120	122	124	128		
Frequency	0.130	0.250	0.300	0.030	0.060	0.140	0.090		

Table 37: Allele frequencies for the North Interlake woodland caribou herd.

Locus	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9	Allele 10	Allele 11	Allele 12
BM848	362	364	368	372	374	380						
Frequency	0.553	0.338	0.010	0.002	0.087	0.010						
BM888	162	164	170	172	174	176	178	180	182	184	194	
Frequency	0.007	0.403	0.163	0.240	0.002	0.083	0.063	0.017	0.015	0.002	0.005	
BMS1788	105	107	113	115	117	119	121	123	125	127	129	131
Frequency	0.003	0.051	0.005	0.107	0.048	0.480	0.048	0.081	0.005	0.091	0.003	0.079
MAP2C	91	95	101	103	107	109	113					
Frequency	0.035	0.179	0.017	0.637	0.005	0.125	0.002					
RT24	205	215	217	219	221	225	233	237				
Frequency	0.215	0.003	0.018	0.646	0.083	0.008	0.003	0.025				
RT30	183	189	191	193	197							
Frequency	0.060	0.007	0.147	0.519	0.266							
RT5	92	98	102	104	108	110	112	114	116	118		
Frequency	0.324	0.101	0.129	0.045	0.002	0.272	0.028	0.061	0.035	0.002		
RT6	92	96	98	102	104	106	108	110	112			
Frequency	0.045	0.152	0.055	0.104	0.007	0.175	0.448	0.012	0.002			
RT7	218	220	226	228	234	236						
Frequency	0.130	0.002	0.509	0.059	0.206	0.092						
RT9	102	114	116	118	122	124						
Frequency	0.009	0.002	0.623	0.009	0.268	0.088						

Table 38: Allele Frequencies for The Bog woodland caribou herd.

Locus	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9	Allele 10	Allele 11
BM848	360	362	364	370	372	374	378	380			
Frequency	0.010	0.604	0.299	0.003	0.003	0.013	0.023	0.044			
BM888	162	164	170	172	174	176	178	180	182	184	188
Frequency	0.017	0.509	0.188	0.051	0.080	0.031	0.023	0.028	0.051	0.020	0.003
BMS1788	107	113	115	117	119	121	123	125	127	129	
Frequency	0.020	0.055	0.081	0.064	0.410	0.046	0.075	0.017	0.173	0.058	
MAP2C	91	95	97	101	103	105	107	109			
Frequency	0.070	0.285	0.004	0.144	0.374	0.011	0.048	0.063			
RT24	205	213	215	217	219	221	225	235	237		
Frequency	0.119	0.003	0.055	0.116	0.503	0.073	0.110	0.003	0.018		
RT30	183	191	193	195	197	201	205				
Frequency	0.158	0.101	0.557	0.021	0.140	0.003	0.021				
RT5	92	98	102	104	106	110	112	114	116	118	
Frequency	0.163	0.071	0.320	0.014	0.003	0.240	0.123	0.057	0.003	0.006	
RT6	92	96	98	102	104	106	108				
Frequency	0.028	0.104	0.135	0.006	0.035	0.135	0.557				
RT7	212	218	222	224	226	228	232	234	236	238	
Frequency	0.012	0.064	0.043	0.052	0.462	0.130	0.003	0.182	0.043	0.009	
RT9	100	102	112	114	116	118	120	122	124	128	
Frequency	0.009	0.103	0.041	0.013	0.406	0.025	0.003	0.294	0.097	0.009	

Table 39: Allele frequencies for the Slate Islands woodland caribou herd.

Locus	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6
BM848	362	364	370	372	374	
Frequency	0.487	0.280	0.007	0.080	0.147	
BM888	164	178	180			
Frequency	0.472	0.285	0.243			
BMS1788	109	115	119	121		
Frequency	0.317	0.106	0.387	0.190		
MAP2C	91	101	103	105		
Frequency	0.373	0.317	0.218	0.092		
RT24	209	217	219	221		
Frequency	0.229	0.136	0.350	0.286		
RT30	183	191	193	195	197	201
Frequency	0.080	0.145	0.304	0.167	0.123	0.181
RT5	92	98	100	102	104	
Frequency	0.122	0.051	0.026	0.756	0.045	
RT6	92	106	108			
Frequency	0.100	0.100	0.800			
RT7	226	228	234			
Frequency	0.449	0.221	0.331			
RT9	116	122				
Frequency	0.541	0.459				

General Conclusion

The methods used in this thesis to assign age-classes to woodland caribou may have only been possible due to the study population being highly monitored for several years. Yearly sampling efforts are on-going in the South Jasper woodland caribou population beginning in 2006, with sampling occurring two to three times a year in the fall, creating an established capture history. Having an eight year capture history has allowed us to detect when new individuals entered the population (which are potential offspring), detect changes in population parameters over time, and has allowed us to sample nearly all individuals from the population. In order to use the age-classification methods described here in other populations or species, it may be necessary to undertake a similar sampling design, to get as many individuals in the population sampled, and to be able to detect yearly changes in population parameters. Subspecies of caribou vary in size, morphology, behaviour, and diet across their range (Thomas and Gray 2002, Miller 2003, COSEWIC 2011), leading to differences in pellet morphology and hormone content. Although differences in characteristics across caribou and other ungulate populations mean that the thresholds determined in this thesis may not be applicable, it may be possible to establish thresholds that are specific to local populations, using the methodology described in this thesis.

In contrast, the effective population size methods described in this chapter were shown to be applicable to a number of woodland caribou populations across Canada, populations with different life histories, behaviour, and habitat. The linkage disequilibrium method was effective for a number of woodland caribou populations, including both central mountain and boreal woodland caribou. Central mountain caribou occur in British Columbia and west-central Alberta, occupying ranges with diverse topography, terrain, and environmental conditions, using high

elevation forests in mid- and late-winter to forage on arboreal lichens, moving to lower elevations in spring (Thomas and Gray 2002, Environment Canada 2014). Boreal caribou are distributed across Canada in seven provinces and two territories, distributed broadly throughout the boreal forest, occupying areas of old-growth forests with an abundance of lichens, muskegs, peat lands, and upland or hilly areas (Thomas and Gray 2002, Environment Canada 2012). Our simulation results show that these methods are useful when there is less data available, either fewer loci analyzed or fewer samples collected, and can potentially be used in other populations. Linkage disequilibrium is a widely-used method that has been used on numerous other species (Ardren and Kapuscinski 2003, Watts et al. 2007, Skrbinek et al. 2012, Pfahler and Distl 2015), and several other ungulates such as horses (Corbin et al. 2010, Hreidarsdottir et al. 2014), cattle (Thevenon et al. 2007, Kim and Kirkpatrick 2009, Flury et al. 2010), pigs (Nsengimana et al. 2004, Uimari and Tapio 2011), and sheep (Garcia-Gamez et al. 2012, Zhao et al. 2014). Weckworth et al. (2013) estimated effective population size and the effective population size to census population size ratio for 8 populations of woodland caribou in the Rocky Mountains of British Columbia and Alberta, including populations from the central mountain, southern mountain, and boreal caribou designatable units. However, only one estimate of effective population size estimates was found for each population, as the sampling period was smaller than the sampling period for our study. Our results show that the effective population size and the effective population size to census population size ratio can vary greatly from year to year, which can be more pronounced in declining populations. A similar sampling design to the study presented in this thesis may be necessary to detect yearly changes in effective population size.

The time period between when an animal enters the population (i.e. offspring births) and when their fecal pellets are collected is likely the most notable limitation of our study. Although

we can say with confidence that almost all individuals in the South Jasper population have been sampled, based on eight (and still on-going) years of high sampling effort, there is still some uncertainty whether these new individuals being captured are in their first year (calves), or individuals that had not been sampled yet. The time between when a calf is born and when their fecal pellets are collected also reduces the ability of detecting differences between calf and adult population parameters. Our results show that there were no significant differences between calf and adult survival rates for the South Jasper population; however, with our fecal pellet collection occurring in the fall, calves were five to six months old at the time of collection, and calf mortality is greatest during the first six months after birth (Gustine et al. 2006, Pinard et al. 2012, Leblond et al. 2013, Traylor-Holzer 2015). If managers are interested in obtaining age-specific population parameters such as survival, it is likely that sampling will need to occur closer to the calving season (May or June; Bergerud 1975, Eloranta and Nieminen 1986, Adams and Dale 1998, Miller 2003) in order to detect changes in calf survival when mortality is highest.

The research objectives and findings in this study illustrate only a few of the many opportunities researchers and managers can explore when using non-invasive genetic sampling. The methods used to assign age-classes can be implemented into other study populations or other species. Age-class may be further used in capture-mark-recapture studies if sampling is done during the period when calf mortality is the highest, and determine the changing survival rate of calves during this uncertain time period. The methods used for estimating effective population size can be extended for use in populations that have low amounts of data available for analysis. Non-invasive genetic sampling is extremely versatile, and our results illustrate only a few of the avenues of research available using this method of sampling. It is therefore beneficial for scientists and managers to consider using non-invasive genetic sampling as a research method, as

we have demonstrated in this study the ability of non-invasive genetic sampling to answer numerous research questions regarding wild populations.

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