Knowledge of population demographics serves as a critical baseline for evaluating and understanding factors leading to population declines. However, because moose are solitary, prefer densely vegetated habitats, and are present at low densities, collecting population data is challenging. Traditional methods of studying moose that require capture, radio-collaring, and aerial surveys are costly, sometimes produce unreliable results, can be harmful to the study animal, and are discouraged in some jurisdictions such as national parks. Non-invasive sampling, the collection of data without having to capture, handle, or in any manner physically restrain study animals, has proven to be a valuable tool for acquiring accurate population data from free-ranging ungulates when using traditional methods is neither feasible nor practical.

In December 2013, we initiated a three-year non-invasive moose population study in YNP with the main objective to estimate population demographics of NR moose. For three consecutive winters we will be systematically collecting fecal pellets from the extent of NR moose wintering habitat. We are extracting DNA from epithelial cells on the pellet surface and through genetic testing will be able to identify individual moose and their genders. Female pellet samples will be analyzed for pregnancy hormone concentrations to make inferences on pregnancy rates. Because fecal pellet size is directly related to moose size, and therefore to moose age, we will use various pellet measurements to differentiate between age classes. These data will be used in capture-recapture modeling to estimate population abundance and vital rates.

✦ STUDY OBJECTIVES

The ultimate goal of our study is to demonstrate the use of non-invasive methods to estimate population abundance and age and sex specific vital rates of northern YNP moose. To this end, we have the following objectives:

1. Systematically survey NR wintering moose habitat over three winter field seasons to collect fecal pellets from as many individual moose as possible as a source of DNA and pregnancy hormones.

2. Determine the genotype of individual moose using microsatellite analysis of fecal DNA for estimating minimum population size.

3. Identify individual moose gender through PCR-based fecal DNA analysis to identify females for analysis of pregnancy hormone concentrations. After genotypes are determined, gender will be used to estimate population sex ratios and sex specific vital rates.

4. Determine pregnancy hormone concentrations in female moose using enzyme-immunoassay. Comparing our data to thresholds for pregnancy of known-pregnant moose from Wyoming and Montana we will be able to make inferences on study population pregnancy rates.

5. Explore the use of moose pellet morphometry to differentiate between calf, yearling, and adult age classes. Age class data will be used to estimate age-specific vital rates.

6. Use robust design capture-recapture (CR) analysis of microsatellite genotypes to estimate population parameters including census population size and gender- and age-specific rates of recruitment, survival, and population change.

Moose in Barronette willows, Soda Butte drainage, December 22, 2013. Moose abandoned this area only weeks later when snow depth exceeded 110 cm (~43 inches).

Cow moose and bull calf on Blacktail Deer Plateau. Note cropped ear on cow, an external sign of possible artery worm infection. Yellowstone Wolf Project photo.

✦ BACKGROUND

Moose in Yellowstone National Park

When YNP was established in 1872, moose sightings were extremely rare and moose may not have appeared on the NR until the early 1900's. In 1925, the U.S. Forest Service conducted surveys in several northern YNP drainages and reported a count of 65 moose. By 1936, an estimated 193 moose were found in the Hellroaring, Buffalo Fork, and Slough Creek drainages. In 1945, the Montana Fish and Game Department issued 40 permits to hunt moose in the area just north of YNP because of concern that willows were being over-utilized by wintering ungulates including moose. Northern range estimated spring maximums were recorded of 292 moose in 1968, 385 in 1969, and 383 in 1970. Aerial counts of moose were conducted between 1968 and 1992 and range from a high of 100 in 1972 to only four observed in 1986. Since the counts were not closely correlated with other index data, the flights did not produce complete survey data, and the cost of survey flights could not be justified, aerial censuses for moose in the Park were stopped after 1992. The most recent moose research in YNP was conducted by Dan Tyers between 1987 and 2001. His work focused on moose winter ecology on the NR and the use of population indices for monitoring demographic trends. He documented the importance of mature and old growth conifer forests for wintering moose. He also had the opportunity to study YNP moose before and after the devastating 1988 fires. He demonstrated that the 30% reduction of mature and old growth conifer forest caused a significant decline in moose numbers in the Park and that effect is still evident today. Currently, there is no population data for the NR moose population.

Non-invasive sampling

Estimating reliable population parameters is essential for the conservation and effective management of wildlife species. However, for species such as moose that are present at low density, occupy large home ranges, are solitary, and prefer heavy cover, acquiring such information can be challenging.

The most common methods of gathering moose population data include aerial surveys, physical capture, and the use of radio collars. However, these methods have limitations that can affect both their use and the quality of the data that are gathered as they are weather dependent, time consuming, logistically challenging, and often cost prohibitive. In addition, these methods are often discouraged in national parks because they are likely to alter the visitor's experience of observing animals in their natural environment.

Physical capture and the restraint of moose for processing, as well as the subsequent collection of biological data, can be extremely invasive and can cause injury and physiological stress to the animals, which in turn can lead to capture-induced death. Aerial surveys, which require the use of sightability correction factors, can sometimes produce biased estimates of abundance as their accuracy depends on many factors including snow cover, type of aircraft, moose density, forest canopy closure, topography, and observer experience.

An alternative to these traditional methods is non-invasive sampling, the collection of biological data without having to capture, handle, or in any manner physically restrain study animals. Such methods are useful and often preferred in wildlife studies because they reduce costs while at the same time produce dependable population data, and they provide researchers with a tool to study animals that cannot otherwise be studied using traditional methods. Biological samples collected in this manner include hair, feces, urine, and saliva. Non-invasive genetic sampling (NGS) was introduced in 1992 to obtain genetic data from rare European brown bears and has emerged as one of the most accurate, efficient, and versatile tools for monitoring wildlife populations and for studying population demographics. Using DNA extracted from non-invasively collected samples, researchers can determine a study animal's individual genotype and gender, and from these, estimate population parameters using capture-recapture (CR) models.

One of the most common sources of DNA for NGS studies is feces. DNA is extracted from epithelial

cells found on the surface of fecal pellets that have been sloughed off from the intestinal wall. The sampling of feces is not only easy and inexpensive, but also allows for the collection of large sample sizes.

✦ STUDY DESIGN

Study area

Much of the NR (Figure 1) landscape below 2,000 meters (m) is open, treeless and covered by sagebrush and grassland vegetation. Mid-elevations are dominated by Douglas fir and lodgepole pine forests and upper elevations, between 2,200 m and 3,000 m, are dominated by Engelmann spruce, subalpine fir and whitebark pine. Willow occurs along streams and rivers and in wet forested areas. Scattered aspen stands, which represent <2% of the area, dot the landscape at mid elevations.

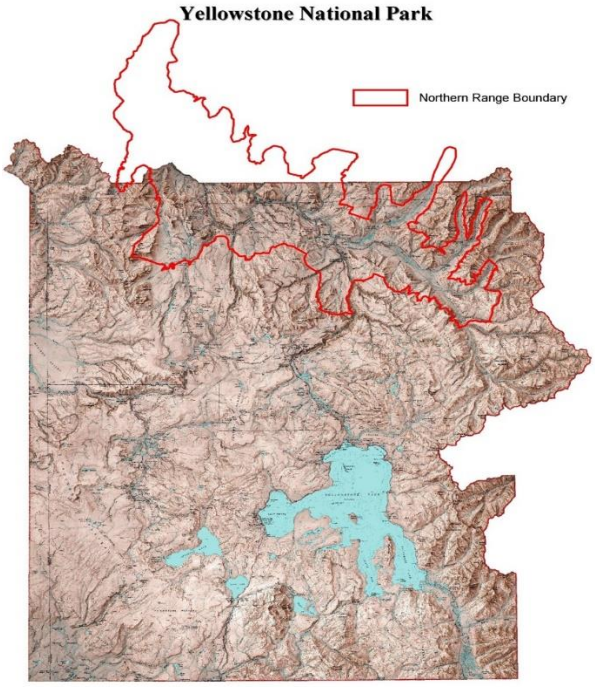


Figure 1. Yellowstone's Northern Range

Research conducted between 1987 and 1991 in northern YNP found that the cover types most used by wintering moose were lodgepole pine and Engelmann spruce/subalpine fir forests over 300 years old, and concentrations of tall and low willow species. One-hundred to three-hundred year-old conifer forests with associated willow were also important. Most moose moose were found in riparian habitats that supported late-winter, and in higher elevation mature and old- growth conifer forests that supported regenerating subalpine fir during mid-winter. Much of this mid-winter habitat on the NR was lost when the fires of 1988 burned approximately 30% of the mature conifer forest.

Our study area encompasses the portion of the Northern Yellowstone Elk Winter Range, located inside YNP, as well as some contiguous creek drainages located north of the Park (Figure 2). Specifically, moose winter habitat within our study includes those drainages containing willow, and willow associated with mature conifer forests. These include Glen, Fawn, Panther, Blacktail Deer, Oxbow, Geode, Hell Roaring, Elk, Lost, Tower, Slough, Crystal, Cache, Pebble, Amphitheater, and Soda Butte Creeks. In addition, we are sampling from portions of the Gardiner, Lamar and Yellowstone rivers. The drainages north of the Park in the Gallatin National Forest include the upper Hellroaring, Coyote, Buffalo, and Slough Creeks. Our study area covers approximately 1,000 km^2.

Sampling design

Our sampling design fits the requirements of Robust Design CR analysis (see 'Capture-Recapture Analysis'). We will sample for three consecutive winters; 2013, 2014, and 2015 (called sampling *periods*), and collect samples during two distinct intervals from December 15 - January 15 and April 1 - April 30. These are termed 'early-winter' and 'late-winter' sampling *sessions*. Sampling during each of these two sessions will require 25 to 28 days or *occasions*. Assuming there are approximately 100 moose in our study area, we will need to collect 125 samples during each sampling *session* in order to generate reliable population parameter estimates.

We chose the sampling session dates for several reasons: to allow comparison between all early- and late-winter data; to have snow on the ground, which is helpful for tracking moose and finding samples, and to allow for efficient ski travel; to ensure that temperatures are below freezing to preserve DNA in samples; to sample moose at times of the year when they are most concentrated; and to allow for initial assessment and reassessment of moose pregnancy status.

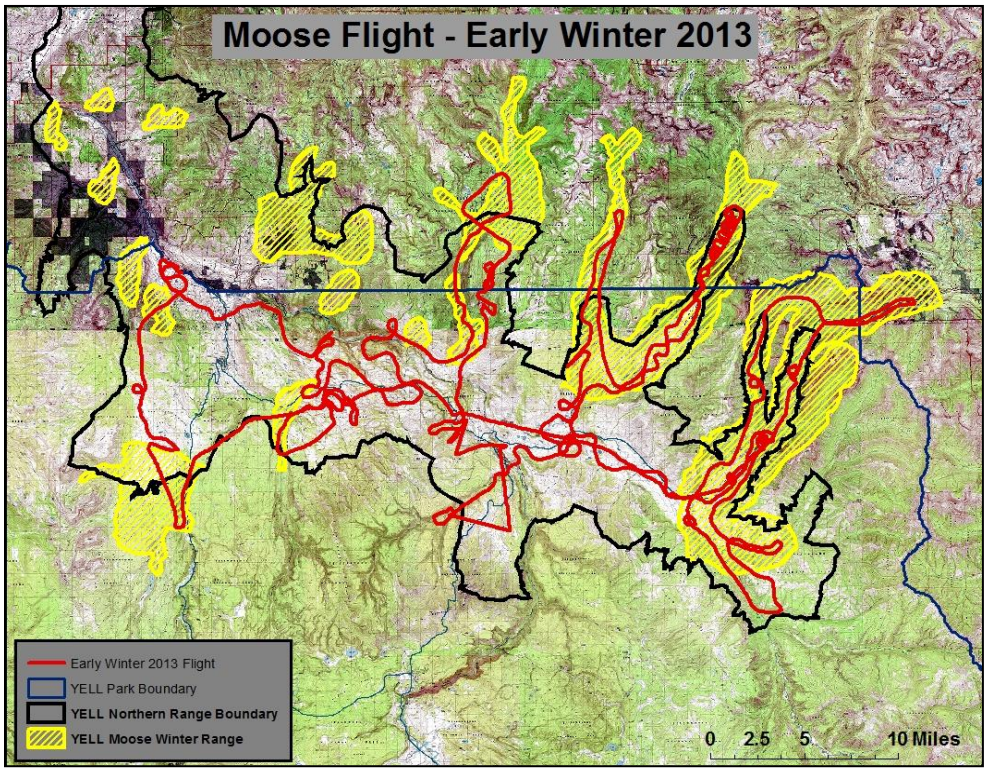


Figure 2. Study area showing early winter moose survey flight and historic moose winter range**.**

We also will collect pellets opportunistically between January 16 and March 31, which we term our 'mid-winter' sampling *session*. In combination with snow depth measurements, genotype data from these samples will be useful in evaluating temporal and spatial shifts within winter habitat as well as evaluate individual winter home range sizes. All mid-winter samples will be used for morphometric analysis, female samples will be analyzed for pregnancy hormone concentration, and all genotypes will be used to estimate minimum population size.

Effective sampling requires that encounter rates with moose be maximized. To ensure this, we are sampling from specific habitat types used by wintering moose. In addition, at the beginning of each sampling session, we fly the extent of the study area in a Piper Super-Cub to locate concentrations of moose and moose sign. These flights aid in focusing our field efforts and in eliminating the need to search drainages with no moose or moose sign. Based on flight information and YNP willow and mature conifer cover type maps, we have divided our study area into sampling transects by creek drainage. Transects are sampled once during each sampling session by a field crew of two technicians.

Data collection

Fecal pellets were located by following fresh moose tracks (determined by the age of the tracks as compared to the date of the most recent snowfall).

Collecting moose pellets from track

Consistent with ethical data collection, moose were back-tracked so as not to disturb the animal. To ensure sterile sample collection, disposable gloves were worn to collect pellets. Thirty pellets were collected for each individual moose: 10 each for DNA analysis, pregnancy hormone analysis, and pellet morphometrics. The three samples of 10 pellets were double-bagged with a label displaying the field identification number in the outside bag, stored in a quart-sized bag containing the field ID number, and frozen at -20°C until they could be analyzed. Frozen pellet samples were delivered to the University of Minnesota-Duluth (UMD) for genotype and gender analysis and to the Smithsonian Conservation Biology Institute in Front Royal, Virginia, for pregnancy testing. K2 Consulting in Waitsfield, Vermont analyzed morphometrics and will conduct future CR analyses.

Ky Koitzsch, pilot Steve Ard, and his Super-Cub on Christmas Day

Pellets for DNA, pregnancy, and age-class analyses

Data collected from each sample includes field identification number, date and time collected, GPS location, habitat type and forest age class, snow depth, estimated age of sample, and age and gender of the moose if known. Transects travelled and sample locations were recorded on Garmin GPSMap 62 hand-held GPS units. These spatial data were converted to shape files in DNR-GPS and then mapped using the ArcMap extension of ArcGIS.

✦ ANALYSES

DNA extraction and amplification

The first steps of our genetic analysis are the extraction of DNA from epithelial cells on the surface of fecal pellets and the amplification of DNA using a process called polymerase chain reaction or PCR. To overcome the challenges of using low quality and quantity DNA common in fecal samples, we have tested and optimized each step of our genetic methodology, from field sampling to PCR conditions. Overall, the results from this first year have been very positive, and the time and resources spent this year will improve the efficiency and success of the study moving forward. Different extraction kits and protocols resulted in varying levels of DNA extraction success and DNA purity. Therefore, we tested multiple extraction kits and protocols to find the pairing that produced the best results. From these tests we have chosen to extract DNA using the QIAamp DNA Stool Mini Kit.

From the 270 samples collected, including 36 samples that resulted in poor (< 2.0 ng/µl DNA product) or failed extractions, DNA concentrations average 21.11 ng/µl, which is well over what is required for PCR amplification for genotyping. Many of the 'poor' or 'failed' extractions were from older pellet samples which were collected purposely to determine what age pellets still contained viable DNA. We found difficulty extracting DNA from pellets older than ~4-7 days, particularly if the pellets were collected in direct sunlight and had dried up, and when late-winter pellets were saturated with water or had undergone repetitive freezing and thawing.

PCR amplification of the SE primer pair (SE47/SE48) is done immediately post-extraction and is used to verify extraction success and determine gender. Even with successful DNA extractions, we found our initial PCR amplifications to be inconsistent, likely because of PCR inhibitors common in fecal material. To remedy this we refined our PCR process by adding bovine serum albumin (BSA), which has been shown to limit the effects of PCR inhibitors. For a comparison of visualized PCR product with and without the inclusion of BSA see Figure 3.

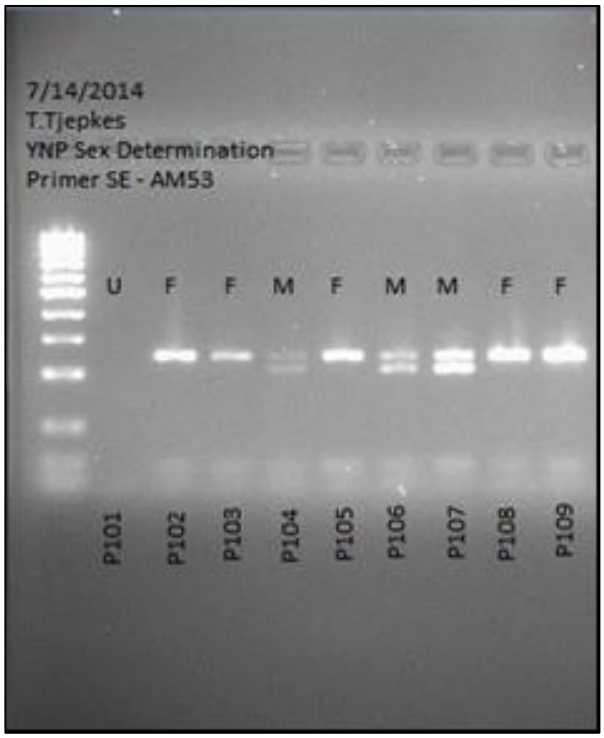


Figure 3. Improvement in visualization with BSA. Note brighter upper bands on right**.**

In addition to including BSA, we have modified other PCR conditions for optimal amplification of each microsatellite (Table 1). Finally, we have included a multiplex pre-amplification step which is designed to increase the quality and quantity of the desired DNA template. In this step, an initial large-volume PCR containing all primers for the loci to be genotyped is conducted. Product from this pre-amplification step is then used in individual PCRs for each locus or smaller subsets of loci. This method requires additional effort and is not beneficial for all loci; therefore it is only used for loci that are not amplifying consistently using traditional single step PCR (Table 1). Refinement of our genotyping process has resulted in an increased genotyping success rate from 58% to 88%, after removal of non-working and monomorphic microsatellites. Continued modification will still be attempted to increase this rate, and any missing data can be filled in by data replication.

Determining gender

Identification of individual gender is required for estimating population sex ratio and sex-specific population parameters, and for monitoring gender-related population trends. For our study, it will also be necessary for separating female from male pellet samples for pregnancy analysis. The most common method for determining gender in moose is the identification of sexually dimorphic characteristics. Males are identified by the presence of antlers or pedicels on adults or antler buds on calves, and females by the presence of a white vulva patch. However, when sightability of moose is limited, these methods are not useful. In the last 15 years, PCR analysis of fecal DNA has become an important tool for studying gender in moose.

Determining gender in mammals involves the identification of X- and Y-chromosome-specific DNA sequences. The method we chose, validated for a number of North American ungulates including moose, uses the SE47/SE48 primer pair to amplify common X- and Y-linked gene markers on the amelogenin gene. Amplified products are short, which is important when

Table 1. Characteristics of 1 sex-linked (SE47/SE48) and 27 autosomal microsatellites. They are either PCR amplified using single step PCR (S) or pre-amplified (P) as indicated in PCR plex column, along with specific multiplex. Also shown are the fluorescently labeled primers (M13 label) and optimal annealing temperatures.

PCR plex	Locus	Size range (bp)	M13 label	Ann. Temp. (^{o}C)
S,Uniplex	SE47/SE 48	224, 259	-	53
S,MP1	RT23	160-170	VIC	54
S,MP1	RT5	151-161	FAM	54
S,MP2	BM2830	83-88	PET	50
S,MP2	BM4513	122-142	VIC	50
S,MP3	Map2C	NA	PET	50
S,MP3	IGF-1	110-112	FAM	54
S,MP4	BM848	215-227	FAM	54
S,MP4	RT9	118-132	PET	54
S,MP4	RT24	230-252	PET	54
S,MP4	RT30	196-214	VIC	54
P1,MP1	BL42	232-238	FAM	53,49
P1,MP1	NVHRT03	111-113	PET	53,54
P2,MP2	NVHRT24	140-146	NED	50,47
P2,MP2	RT1	232-234	PET	50,47
P2,MP2	BM888	173-183	FAM	50,50
P2,MP2	BM1225	227-251	FAM	50,50
P2,MP2	KCSN	190-192	PET	50,50
P2,MP3	Cervid2	105-113	NED	50,50
P2,MP3	IRBP	141-145	NED	50,50
P3,MP3	NVHRT21	153-169	VIC	54,54
P3,MP3	CRFA	253-255	VIC	54,54
P3,MP4	Cervid14	207-221	FAM	54,54
P2,MP3	NVHRT01	178-190	PET	50,50
MP1	RT27	149-159	NED	53
MP1	FCB193	NA	NED	51
MP3	INRA003	178-199	FAM	50
MP4	NVHRT34	124-126	FAM	50

working with low quality and quantity of DNA, and differ in length so they can be easily separated using gel electrophoresis. Electrophoresis displays a single band for females and a double band for males (Figure 4).

We determined definitive genders, based on distinct X- and Y-linked product bands using gel electrophoresis, for 234 (86%) of the 270 samples. Of those, 111 (47%) were female and 123 (53%) were male. Of the remaining 36 samples, 23 (8%) showed no PCR product and 13 (5%) showed questionable results in that the bands were not distinctive. Of the 23 that showed no PCR products, 19 were collected in late-winter when snow was melting, snow events were less frequent, and the age of the samples became difficult to determine. Some of these samples were aged at one week or older and others were collected assuming they were mid-winter samples just to see if the DNA was still viable.

We collected pellets from 43 known-sex moose based on visual identification and identified 100% of the genders correctly. These results helped to validate our gender determining methodology using the PCR-amplified SE primer pair.

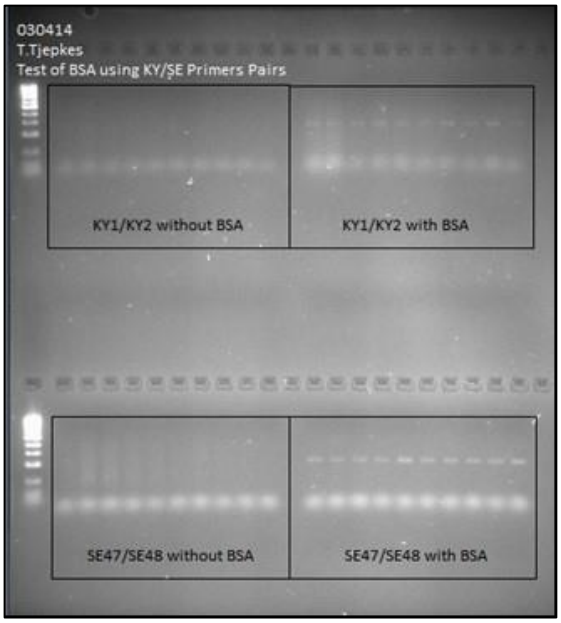


Figure 4. Gender signature on agarose gel showing a single band for females and double band for males

Determining genotype

Individual genotypes are required to estimate population abundance and vital rates and are determined through the analysis of DNA microsatellite markers. A number of genetic markers have already been developed for North American and European moose populations and at least 30 variable microsatellite markers have been characterized for moose in the Molecular Ecology Resources Primer database (http://tomato.bio.trinity.edu/). Many more have been developed for other ungulate species that also amplify in moose.

The number of microsatellites needed for genetic determination of NR moose was established in a pilot study. We tested 27 autosomal primers for microsatellites previously developed for moose on 14 pellet samples (Table 1). We have since discarded five of these, four of which did not amplify consistently (INRA003, RT27, FCB193, and NVHRT34), and one that was monomorphic in the YNP moose population (NVHRT01) and thus provided no information for genetic differentiation. We found low genetic diversity in our pilot study, which requires more loci to be considered for genotyping, so we chose to use all of the remaining 22 microsatellites for further analysis.

For the 16 microsatellites that have been analyzed thus far, we found 2-6 alleles per locus and calculated expected and observed heterozygosity, two measures of the amount of genetic variation in the NR moose population (Table 2). In comparison, a genetic study of Alaskan moose, using many of the same microsatellites, showed the number of alleles per locus to range between three and 12. The microsatellite locus (RT 30) containing 12 different alleles in the Alaskan study only showed three allelic variants in our study population so far. We also observe both lower and higher number of alleles in YNP moose at other loci compared to similar studies on moose, although some of these differences could be due to varying sample size. Data from additional sampling seasons will help clarify this question.

The total number of unique individuals sampled in the first year will be determined by creating a genotypic fingerprint for each sample using data from the 22 microsatellites. The number of unique fingerprints will represent the minimum number of individuals sampled in
the population. Samples collected in following years will be used to improve the minimum population size estimate and to estimate census population size (N_C) using the mark-recapture method and Program MARK. Genotype determination for the first year is expected to be completed by the end of 2014, after which the minimum number of individuals will be determined.

Table 2. Number of alleles (N_A) and heterozygosity (expected H_E and observed H_O) at 16 autosomal microsatellite loci.

Locus	N_A	H_E	H_O
RT23	6	0.617	0.88
RT5	2	0.074	0.077
BM2830	2	0.406	0.355
IGF-1	2	0.378	0.313
BM848	6	0.781	0.66
RT9	3	0.071	0.02
RT24	6	0.68	0.617
RT30	3	0.372	0.33
BL42	5	0.74	0.769
NVHRT03	4	0.663	0.633
RT1	5	0.635	0.432
BM888	5	0.573	0.514
Cervid2	3	0.411	0.538
NVHRT21	4	0.74	0.818
CRFA	3	0.392	0.5
Cervid14	4	0.434	0.361

Estimating pregnancy rates

Knowledge of the reproductive status of individuals in a population can provide information about age specific fertility, population reproductive potential, and trends in population size. Traditional methods of assessing pregnancy, which are all invasive and require capture, include drawing blood, performing trans-rectal ultrasound, and palpation of the reproductive tract. In the past two decades, however, non-invasive methods of assessing pregnancy using analysis of fecal hormones have been developed and used for moose. The method we are using, and the one most commonly used in ungulates, is enzyme-immunoassay (EIA) of progestagen hormones. Progestagens are a group of hormones that function to maintain pregnancy in mammals and increase throughout the pregnancy cycle.

Pregnancy is determined based on a threshold level of progestagen concentration. Researchers in Alaska found concentrations above 7,000 ng/g for captive pregnant moose and in the southern Greater Yellowstone Ecosystem, researchers reported fecal progestagen concentrations >10,600 ng/g for 21 known-pregnant moose and <2,600 ng/g for four non-pregnant moose. Where pregnancy rates for moose have averaged ~84% across North America, researchers of declining moose populations have reported much lower pregnancy rates. In on-going studies of Shiras moose, pregnancy rates ranging from 48% to 74% have been reported for Wyoming's Sublette moose herd and 75% to 80% for Montana moose.

It is important to note when making inferences about pregnancy rates of free ranging populations using non-invasive methods that pregnancy hormone concentrations can vary between regions and populations and that pregnancy rates can also vary for a number of reasons including habitat quality, moose density, and population age structure.

Progestagen concentrations for our early winter samples varied from 168 ng/g to 5,227 ng/g (N=60) and 13 of the lowest 21 concentrations were from calves. We submitted calf samples (based on visual observation and/or pellet, track and bed size) to determine baseline hormone concentrations as compared to adults, which is useful for differentiation between calf and yearling age-classes. Concentrations of our late winter samples varied from 131 ng/g to 9,191 ng/g (N=42). With this batch we included three known males in order to get baseline concentrations, which will help to differentiate between cows and bulls. Whereas these males did not have the lowest three concentrations, they did fall in the lowest 14 values ranging between 197 ng/g and 467 ng/g.

We compared our concentrations to mid- and late-winter samples of known pregnant-moose from on-going studies in Montana and Wyoming that identified thresholds for pregnancy of 1,130 ng/g and 2,100 ng/g, respectively (Figure 5). Because pregnancy hormone concentrations increase throughout the pregnancy cycle, we used our late-winter concentrations in comparison to generate a more reliable estimation of pregnancy rate. After removing the three known bulls (N=39) from our late winter samples, 25 (64%) were above the Montana threshold and 24 (62%) were above the Wyoming threshold. Assuming threshold concentrations for pregnancy of northern Yellowstone moose are similar to those of Shiras moose in neighboring regions, it would suggest that between 62% and 64% of our late-winter samples were from pregnant females.

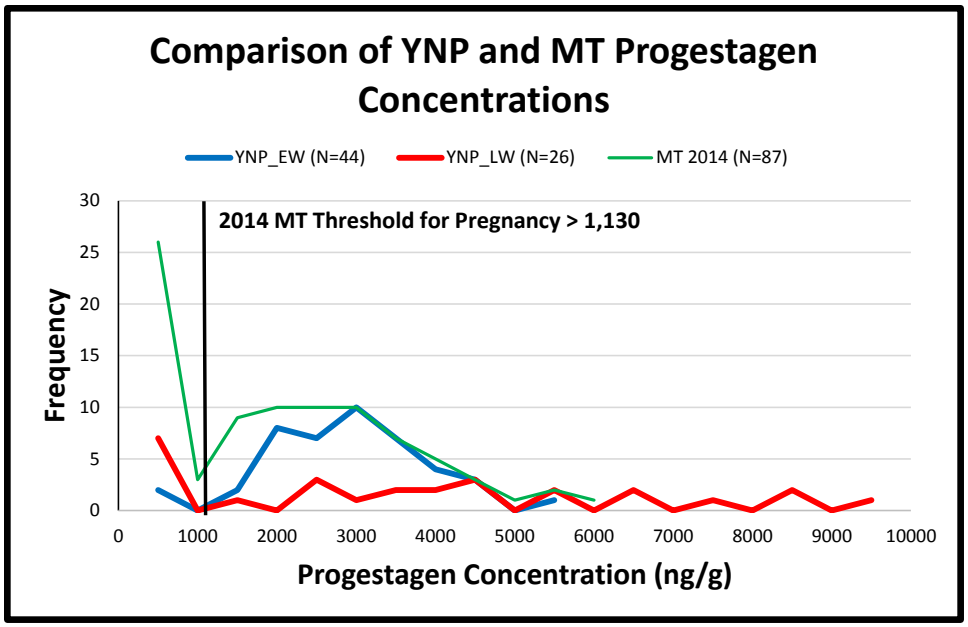


Figure 5. Comparison of early and late winter YNP progestagen concentrations to known pregnant Montana moose. Threshold for pregnancy for Montana moose was 1,130 ng/g.

Age class determination

Ages of study animals are used to determine the age structure of wildlife populations, which in turn are used to estimate population growth rates and for estimating age-specific vital rates. Common methods of aging cervids include counting cementum annuli on extracted teeth, analyzing tooth wear or replacement, and weighing and measuring the animal, all of which entail physical capture. A non-invasive alternative to these traditional methods is fecal pellet morphometric analysis in which the age class of animals is determined by pellet size. Application of these analyses requires a positive relationship between animal live-weight, age, and pellet size.

Researchers studying reindeer in Norway differentiated between calf, yearling, and adult age classes of females. They found adult pellets were longer than calf pellets, and adult and yearling pellets were wider than calf pellets. In addition, they showed that pellet volume, based on pellet length, width, and depth measurements, could differentiate between calf, yearling and adult age classes 91% of the time. Researchers in Denali National Park correctly identified 91% of known-age and -sex moose based on pellet volume estimates. Combining pellet age class data with other non-invasive genetic and hormonal techniques will improve population monitoring of ungulate species.

Our goal in conducting morphometric analysis of moose pellets is to differentiate between calf, yearling, and adult age classes. In each group of pellets we collected, ten to twelve pellets were oven dried at 60° C in a convection oven for 48 hours. After drying, partial pellets and equal numbers of the largest and smallest pellets were discarded from each sample, leaving the eight most representative in size and shape of the original sample. Using digital calipers with a precision of 0.01 mm, each pellet was measured for maximum length (L_p), width (W_p), and depth (D_p) at 90° rotation from W_p. Using the product of the mean values of the three measurements, we calculated a volume index for each sample; $V_s = L_p \times W_p \times D_p$. Volume index was then converted to cm^3.

Two-hundred and twenty samples of both genders combined, 120 from early winter and 100 from late winter, were measured. Volume indices, and pellet average widths and lengths ranged from 3.2 cm^3 to 11.6 cm^3 (Figure 6), 12.4 mm to 20.9 mm, and 19.2 mm to 33.5mm, respectively**.** Using the same methodology we also measured 57 known-age pellet samples from female moose provided by Montana Fish Wildlife & Parks from an ongoing study. Montana samples were collected during mid- and late-winter moose capture and late-winter/early-spring ground surveys. Average pellet volume for each age class is shown in Figure 7. We used one-way ANOVAs to identify variation in pellet measurement means between age classes. We found significant differences in pellet mean volume between calves and yearlings ($P=0.002$) and between calves and adults (age 2.5-12.5) ($P=0.00002$) but no difference between yearlings and adults. We also found significant differences in pellet mean width between calves and yearlings ($P<0.001$) and between calves and adults.

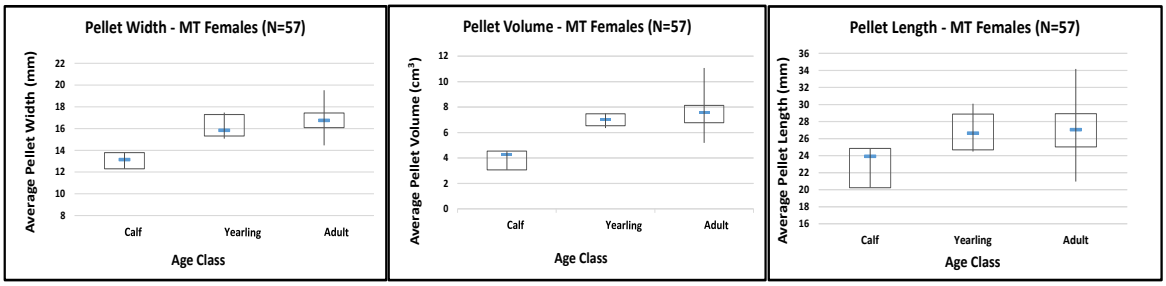


Figure 8. Box plots showing average pellet width, volume, and length of known-age MT moose. There were no significant differences in pellet length between age classes.

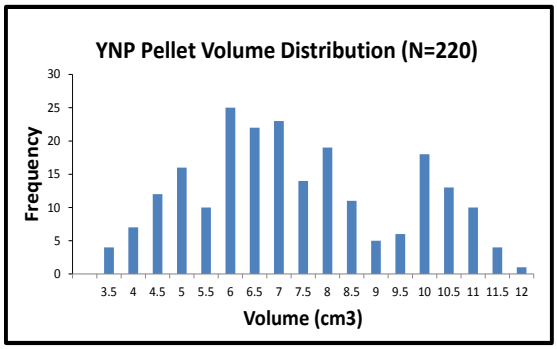


Figure 6. Frequency distribution of YNP pellet volume (P<0.001) but no difference between yearlings and adults.

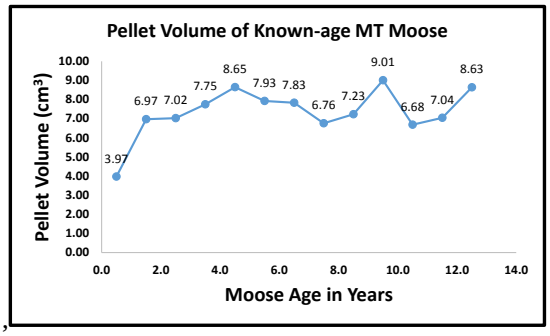


Figure 7. Average pellet volume of female Montana moose by age (N=57)

No differences in mean pellet length were detected between age-classes (Figure 8). As our data set builds we hope to be able to separate between yearling and adult cohorts.

These data will be used for future estimation of age-groups for our female samples using discriminant function analysis. We are also exploring the use of cluster analysis to group our male samples into age-classes. Cluster analysis will be particularly useful for our study of free-ranging moose because unlike discriminant function analysis, it does not require a priori age-class data.

Capture-recapture analysis

Capture-Recapture (CR) analysis compares the relative proportions of marked to unmarked animals in successive samples to estimate population abundance. In the last 15 years, the use of fecal DNA-based CR analysis of microsatellite genotypes to estimate population parameters and trends has become common place. In addition to population abundance, such analysis can provide estimated rates of fecundity, recruitment, survival, temporary emigration and immigration, and population change.

We will use Robust Design models in Program MARK, a Windows-based computer program to estimate census population size and gender- and age-specific rates of recruitment, survival, and population change. Robust Design models analyze two levels of sampling (primary and secondary) in order to generate estimated population parameters. We chose MARK because it is commonly used and highly versatile and it offers a full complement of Robust Design models. MARK has also been used in recent population studies of Sitka black-tailed deer, mountain goats, and caribou. Once individual genotypes have been determined we will begin creating capture histories of each moose which are the input data for our CR analysis. Capture-recapture estimates of NR moose vital rates will be compared to those from other populations of Shiras moose.

✦ FIELD SUMMARY

Pellet samples and sampling effort

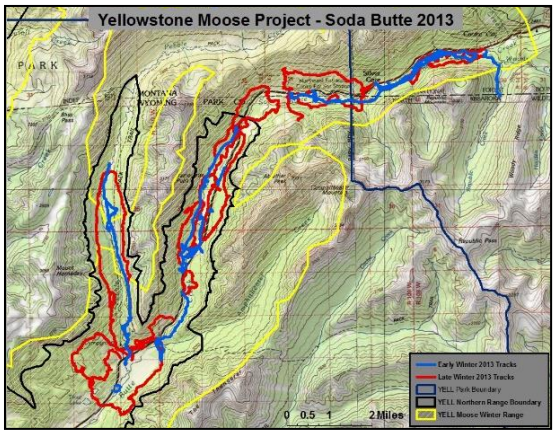
Over the course of our 2013 field season (December 15, 2013 to April 30, 2014) we collected 270 fecal pellet samples: 125 early-winter, 114 late-winter, and 31 mid-winter. Pellet samples were collected in all study area drainages with the exceptions of Hellroaring,

Coyote, Buffalo, Panther, Glen, Crystal, and Cache Creeks where we did not find any moose.

During our early-winter sampling, our field crew, Ky and Lisa Koitzsch, skied 687 km to survey 304 km of study transect over 29 field days and conducted one moose survey flight of 569 km. Two multi-day trips of five and three days were taken to sample moose in the Frenchy's Meadow area of upper Slough Creek and Miller Creek, respectively. During our late-winter sampling, the field crew skied 553 km to survey 357 km of transect over 20 field days (Figure 9). No multi day trips or survey flights were taken during the late-winter session due to deteriorating snow conditions and chronically poor flying conditions (wind and/or poor visibility). Sampling effort was directly correlated to daily weather. We did not sample during snow storms because moose tracks and pellets were covered, visibility was poor, and avalanche conditions were potentially dangerous. The state of the snow pack also dictated how much area we could cover. When snow was deep and unconsolidated it took more effort to break trail which slowed our progress and when the snow was crusted and supported our weight we could sample more efficiently.

Habitat use

Similar to earlier studies of northern YNP moose, we found two different patterns of habitat use by moose on the NR depending on the occurrence of mature conifer forests. In much of the western and central portion of our study area, such as the Blacktail Deer Plateau, where most of the mature conifer was burned in the 1988 fires, moose spent the early-winter in upper creek drainages feeding on willow. As snow depths increased, thereby making willow unavailable, moose moved downstream towards the Yellowstone River where snow depths were moderate and there they fed on small pockets of willow in steep drainages. By late winter, some moose were found at ~1,740 m (~5,600 ft.) elevation along the Yellowstone River. In contrast, moose in the eastern



The Soda Butte Creek drainage supports the highest densities of moose on the NR because of the abundant willow and mature conifer forests.

portion of our study area, such as the Soda Butte Creek drainage, fed on willow in the early winter but moved to higher elevations when snow depths made this food source unavailable. Here, the mature conifer canopy offered thermal cover, lesser snow depths allowing easier travel, and available browse primarily in the form of regenerating subalpine fir. We sampled from one moose that had been feeding and bedding at 2,430 m (~8,000 ft.). We found the highest concentrations of wintering moose in the Soda Butte Creek drainage because of its abundant willow stands and mature lodgepole pine/Engelmann spruce/subalpine fir forests.

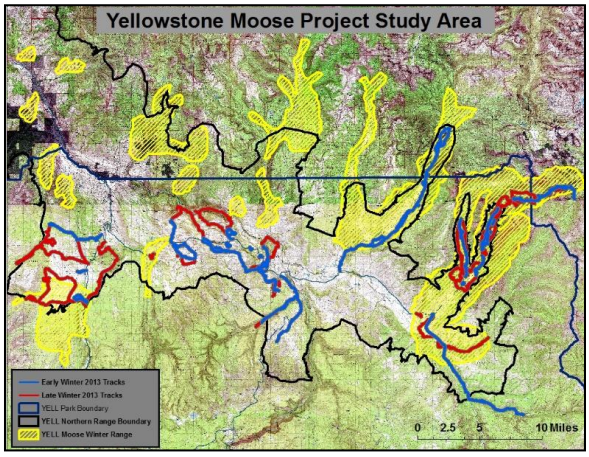
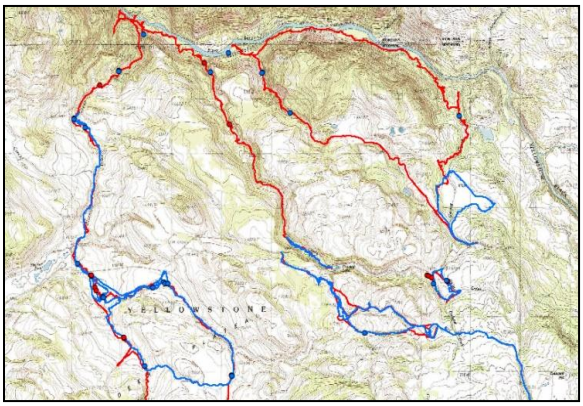


Figure 9. Early winter (blue) and late winter (red) sampling transects



Blacktail Deer Plateau. All of the drainages shown (Blacktail Deer, Oxbow, Geode Creeks) flow north into the Yellowstone River.

Subalpine fir saplings in understory of mature lodgepole forest (above). Heavily browsed subalpine fir (below).

Field observations

From our observations it appears that northern Yellowstone moose are relatively healthy. Of the 43 animals we observed closely enough to determine gender, we did not notice any tick induced hair loss, abnormal behavior, or unhealthy looking animals. Over the course of the winter we also did not find any ticks in the hundreds of beds we examined. Other than two individuals with cropped ears, we didn't see any other physical evidence that would suggest that artery worm is having a significant impact on YNP moose. Perhaps because moose in northern YNP occupy higher elevation habitats than other populations of Shiras moose, the associated prolonged winters make them more immune to tick infestations and a relatively lower mule deer density keeps them isolated from chronic artery worm infection.

Cow moose that drowned in Trout Lake, YNP. Ky Koitzsch pulling a tooth for aging by counting cementum annuli.

There appeared to be a fair number of recruited calves in the population based on our observations over the course of the winter. In addition, we observed one pair of twin calves, one pair was reported to us, and another was identified based on tracks and beds during sampling.

We recovered two moose carcasses in our study area that we had likely collected fecal pellets from. One female calf was killed by wolves in March and an adult cow drowned in a lake in October. From these carcasses we were able to obtain tissue samples for DNA genotype analysis that we will compare to genotypes of previously sampled moose. These 'dead recoveries' of moose are important for aspects of our CR modeling and for validation of our genotyping and gender methodology.

We observed a difference in pellet size and form between early winter and late winter and between the two habitat types used by wintering moose. In general, moose that fed primarily on willow deposited pellets that were large in volume, oval in shape, light in

color, and sawdust-like in consistency. Those moose that utilized old growth conifer deposited pellets that were smaller in volume, angular in shape, black in color, and smooth in consistency. Those moose that utilized both habitats deposited pellets intermediate in both size and form. In general, early-winter pellets were larger than late-winter pellets.

We believe these differences were due to variation in forage quantity and quality between the two habitat types. The more abundant and coarser woody stems of willow contributed to the larger oval pellets while the less coarse and less abundant understory twigs, buds, and leaves of subalpine fir and *Ribes* spp. (among others) contributed to smaller, angular pellets. We also noted an increase in late winter consumption of arboreal lichen (Old Man's Beard) within the mature conifer forests. Numerous studies have documented moose eating arboreal lichen during the winter as a supplementary food source and some researchers believe moose may use it as a source of free water during the cold and dry winter months. Because moisture content of the forage is often the most important factor in determining pellet form and shape, it seems likely that those moose ingesting large volumes of arboreal lichen would deposit less formed, smoother, and moister pellets. Future microhistological analysis of YNP moose pellets may provide insights into these differences.

✦ FUNDING AND SUPPORT

We greatly appreciate all those who have helped fund and support our project. We received funding from a UW-NPS Research Grant, the Campfire Club of America Conservation Fund, Andrew B. Heath, and Dick and Karen Koitzsch. We thank Yellowstone National Park for providing housing and the Yellowstone Center for Resources for daily logistical support. We especially thank the University of Minnesota-Duluth for providing funding for genetic analyses.

✦ ACKNOWLEDGEMENTS

We would like to thank Doug Smith, Kerry Gunther, Stacey Gunther, Dan Stahler, Matt Metz, Kira Cassidy, Erin Stahler and U.S. Park Rangers Brian Chan, Gabe Gassman, and Tom Schwartz from Yellowstone National Park; Dan Tyers from the U.S. Forest Service for his pioneering contributions to moose research in YNP and for sharing his knowledge with us; Rachel Feigley and Walt Allen from the Gallatin National Forest; Nick DeCesare and Karen Loveless from Montana Fish, Wildlife and Parks; Brett Jesmer from the University of Wyoming and the Wyoming Cooperative Fish and Wildlife Research Unit; and Nicole Presley and Janine Brown from the Smithsonian Conservation Biology Institute for all of their help and guidance. Thank you to Laurie and Dan Lyman for their generous hospitality. We also thank Steve Ard of Tracker Aviation for his safe flying. Special thanks to Tessa Tjepkes for her persistence and dedication on the genetics side of this project and Carol Chamberlin for her help organizing and compiling our data.

✦ PROJECT STAFF

Ky and Lisa Koitzsch own K2 Consulting, LLC and live in Waitsfield, Vermont. They are wildlife biologists who have been specializing in winter studies of predators and their prey. They have recently been working on the Isle Royale Wolf/Moose Project and the Yellowstone Wolf Project. Ky received a Master's degree from the University of Vermont where he quantified moose habitat suitability of Vermont Wildlife Management Units. He is especially interested in moose winter ecology. Lisa has a background in avian studies and is currently working on a study of wolf predation in YNP using GPS cluster analysis to locate wolf kills.

Jared Strasburg is an assistant professor at the University of Minnesota-Duluth who specializes in evolutionary and population genetics. He works with a range of organisms, including moose, wolves and other carnivores, and sunflowers.

Tessa Tjepkes is a graduate student at the University of Minnesota-Duluth who is conducting our genetic analysis under the direction of Jared Strasburg. In addition to her work applying non-invasive techniques to study Northern Yellowstone moose genetics she is also studying population genetics of northeastern Minnesota moose.