

A Non-Invasive Population Study of Moose in Northern Yellowstone National Park

2014 ANNUAL REPORT



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Report written by Ky and Lisa Koitzsch

Summary

In 2013, we initiated a three-year moose population study in northern Yellowstone National Park (YNP). Our primary objective is to demonstrate and evaluate the use of non-invasive genetic and hormonal methods for estimating and monitoring moose population size and vital statistics. For three consecutive winters, and twice during each winter, we are systematically surveying all wintering moose habitat within northern YNP in order to collect moose fecal pellets as a source of DNA and progestagen hormones for genetic and pregnancy analyses. Genetic methods are used to identify individual moose and their genders using DNA extracted from epithelial cells on the pellet surfaces. Pellet samples from females are further analyzed to determine concentrations of pregnancy hormones to make inferences about pregnancy status and population pregnancy rates. Since fecal pellet size is directly correlated to moose body size, and therefore age, we are also exploring the use of pellet morphometrics to differentiate between calf, yearling, and adult age-classes. These data will be used in future capture-recapture modeling to estimate population abundance, rate of population change, and other age- and gender-specific vital rates.



Bull moose in Round Prairie – Soda Butte Creek.

This study will generate important moose population data for YNP, the Custer-Gallatin National Forest, Montana Fish, Wildlife and Parks, and regional moose biologists and managers within the Greater Yellowstone Ecosystem. It is also our hope that we can demonstrate the use of these non-invasive methods as an accurate, efficient, and cost effective alternative for long-term moose population monitoring throughout the species' range.

This report summarizes field data and results from gender and pregnancy analyses of our second year (2014) samples as well as genotype analysis of our first year (2013) samples. It also presents a minimum population count derived from 2013 genotypes. Early-winter (EW) sampling began on December 13, 2014 and ended on January 17, 2015. Late-winter (LW) sampling was conducted from April 1 to April 16, 2015. Typically, the LW sampling period ends on April 30th but we had to shorten it due to lack of snow. We also collected pellet samples opportunistically during mid-winter (MW) from January 18 to March 31, 2015.

Study Area

Our 1,100 square kilometer study area encompasses all moose wintering habitat in northern YNP. It includes major portions of every river and creek drainage in this part the Park as well as the headwaters of the Hellroaring, Buffalo, Slough, and Soda Butte Creeks that are located north of the Park in the Custer-Gallatin National Forest. At its widest points, our study area stretches approximately 75 km from the foot of the Gallatin Mountains in the west to the historic mining town of Cooke City to the east, and 35 km from the southern reaches of the Absaroka Mountains north of the Park border to the slopes of the Washburn Range to the south.

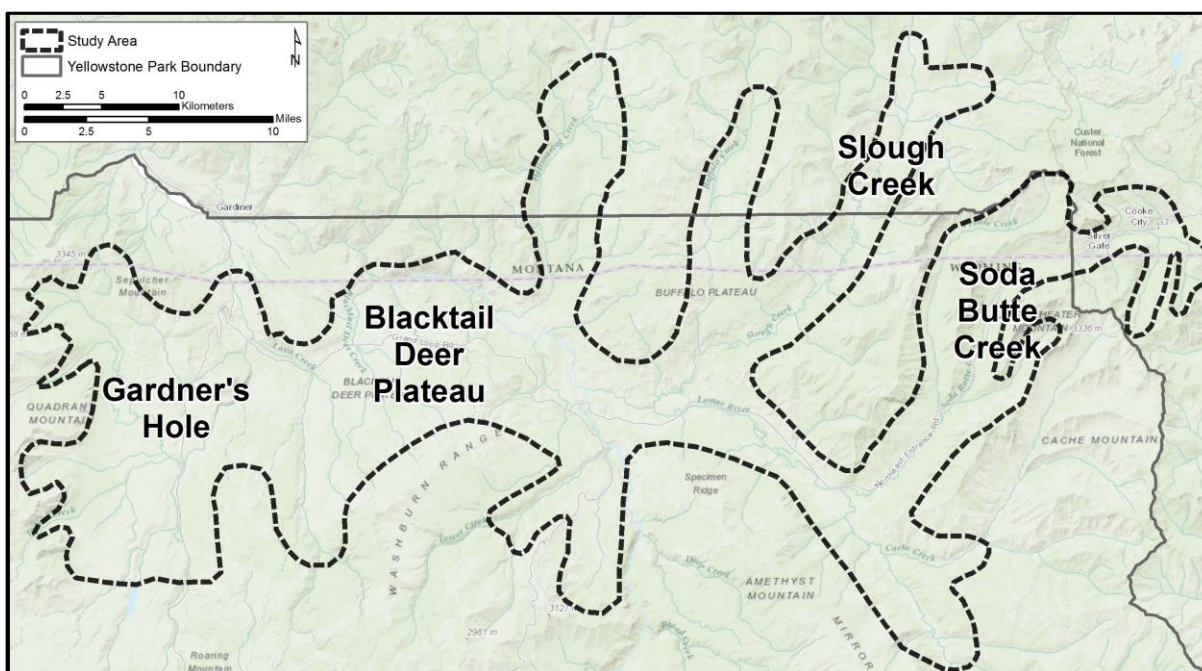
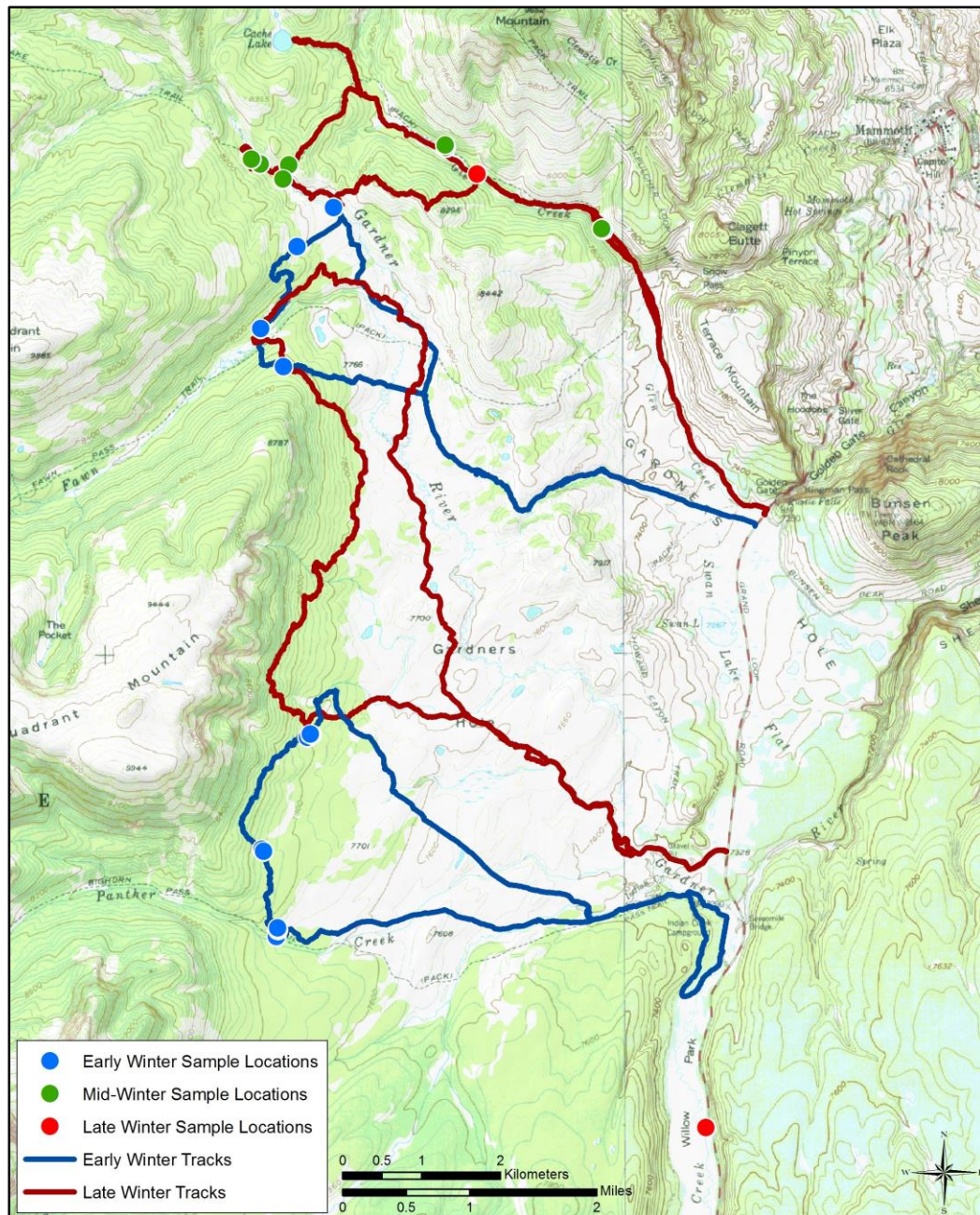


Figure 1: Northern YNP study area showing locations of four wintering moose subpopulations.

Moose wintering habitat in our study area includes stream drainages containing willow, especially those with adjacent mature lodgepole pine-Engelmann spruce-subalpine fir forests. Willow and regenerating subalpine fir are the most important browse species for wintering northern YNP moose. Consequently, willowy drainages flanked by mature conifer forests provide the best moose wintering habitat. Aspen and choke cherry browse, *Ribes* spp. (gooseberry and other currants), and old man's beard (arboreal lichen) are also important wintering food sources. Figure 2 shows the Gardner's Hole Region that has all of these winter habitat components. Also depicted are transects we surveyed and samples collected in 2014.



Flight Survey

If weather and plane availability allows, a moose survey flight is taken before each winter study period to help inform and focus field survey efforts. In 2014 we completed a LW survey flight on March 29th with veteran pilot Rodger Stradley from Gallatin Flying Service (Figure 3). During this 524 km flight we observed six moose: two cows and their female calves, a lone cow, and a lone bull, all in upper Slough Creek. Just as

importantly, we were able to eliminate the need to survey Buffalo Creek as well as some of the upper reaches of the Gardner's Hole drainages, where we saw no sign of moose. Due to poor flying conditions and conflicts with other research flights we were not able to take an EW survey flight.

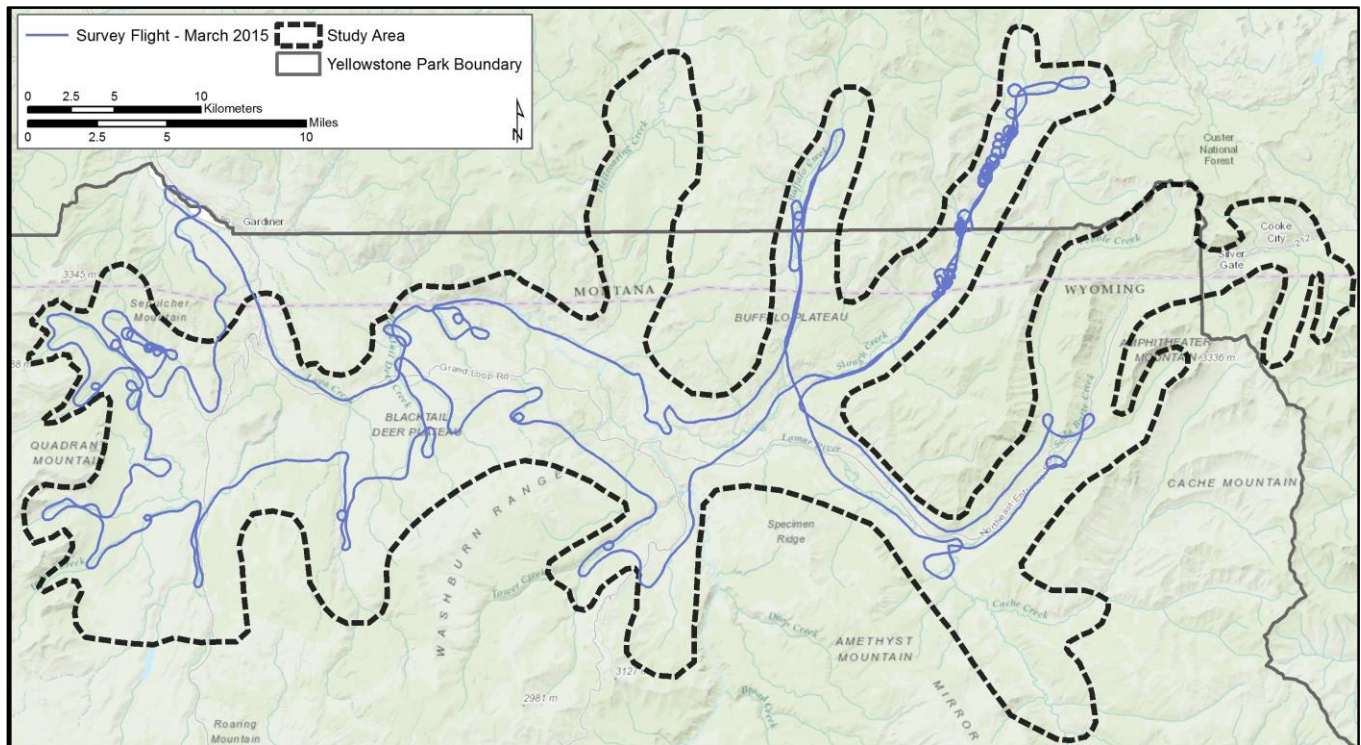


Figure 3: Study area with March 2015 survey flight.

Data Collection

The winter of 2014 was characterized by warmer temperatures and lesser amounts of snow than during our 2013 field season. Average daily temperature, as recorded at the Tower Junction weather station located near the center of our study area, was -9.1 degrees Celsius in EW and 3.8 degrees Celsius in LW. Average snow depth for EW 2014 was 40.9 cm, while in LW it was 0.4 cm. Total monthly snowfall for EW was 68 cm and in LW it was 12.2 cm. Very little snow accumulated after the first week of February and daily high temperatures exceeded 0 degrees Celsius for 28 days in March. Consequently, much of our study area that lies below 2,200 m was snow-free by the middle of April.

During the 2014 field season, we surveyed most of the drainages within our study area including: Glen, Fawn, Panther, Indian, Obsidian, Lava, Blacktail Deer, Oxbow, Geode, Elk, Yancey, Lost, Tower, Antelope, Slough, Pebble, Soda Butte, Warm, and Republic Creeks. In addition, we sampled from portions of the Gardner, Lamar, and Yellowstone Rivers. While conducting surveys, our field technicians spent 24 days on skis in EW covering 488 km in order to survey 304 km of study transect. During the LW period, they spent 12 days on skis and covered 372 km to survey 280 km of study transect (Table 1).

Table 1: 2014 field work summary.

2014	EW	MW	LW	Total
Samples Collected	105	35	45	185
Transect Surveyed (km)	304	NA	280	584
Days in Field	24	NA	12	36
Effort (km skied)	488	NA	372	860

In 2014, we collected a total of 185 fecal pellet samples; 105 during EW, 35 during LW, and 45 during MW (Figure 4). Mid-winter samples were collected to make inferences about winter home range size of moose, track changes in female pregnancy hormone concentrations over the winter, and allow us to sample moose we have not yet sampled during our regular sampling periods. In MW, we were able to investigate other potential moose wintering areas in Amethyst and Hellroaring Creeks and the saddle between Pebble and Warm Creeks. We did find old wintering sign but found no recent evidence of their presence.

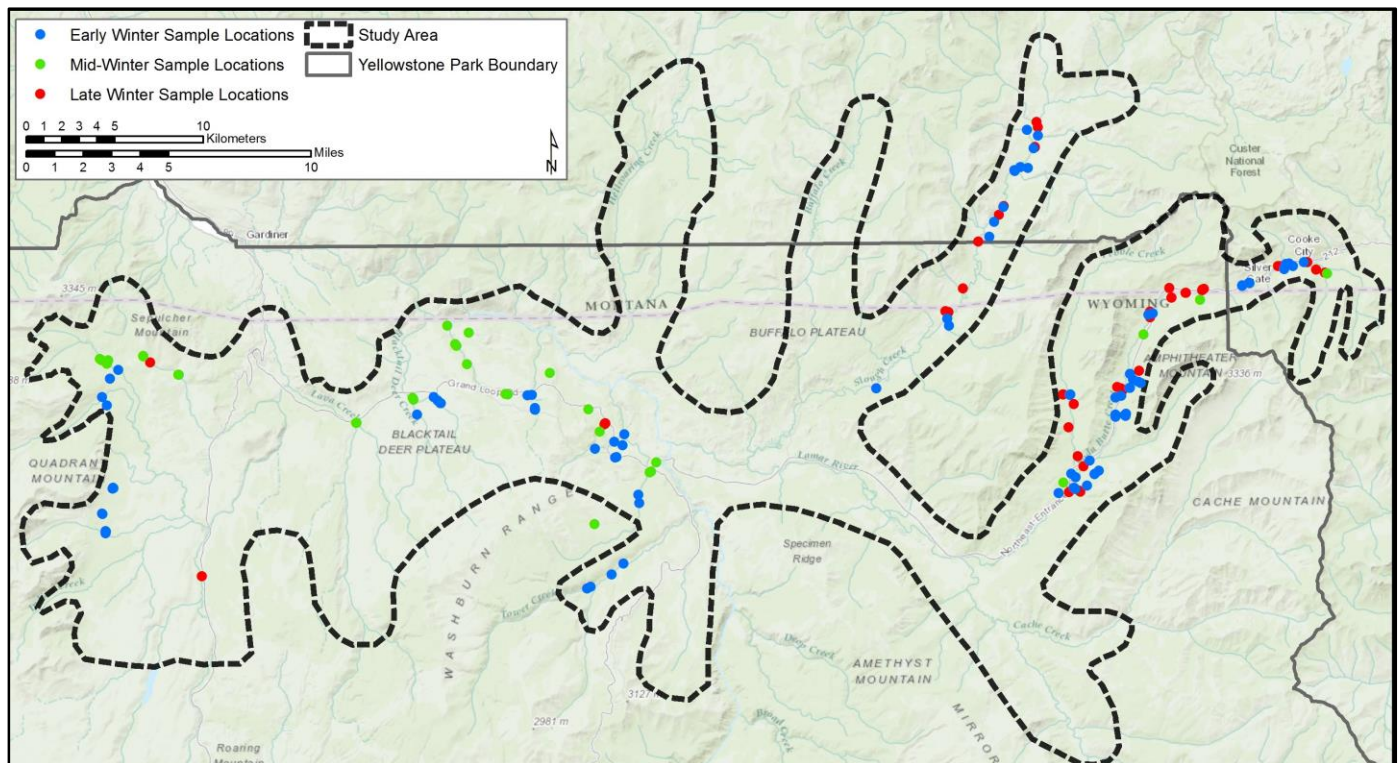


Figure 4: Study area with early-winter (blue), mid-winter (green), and late-winter (red) sample locations.

Pellets were located by following fresh moose tracks. Consistent with ethical data collection, moose were back-tracked so as not to disturb the animal. Thirty pellets were collected for each individual moose; 10 each for DNA, pregnancy hormone, and age-class analyses. The three samples of 10 pellets were double-bagged with a label displaying the field identification number in the outside bag and frozen at -20°C until they could be analyzed. Data collected from each sample included 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. Spatial data including transects travelled and sample locations were recorded on Garmin GPSMap 62stc hand-held GPS units.



**Ky Koitzsch collecting pellets from a moose bed on the banks of Fawn Creek in Gardner's Hole.
(Photo courtesy of Ronan Donovan)**

Genetic Analysis

We continue to work with the genetics lab at the University of Minnesota-Duluth to determine genders and individual identities (or genotypes) for our samples. We have completed gender analysis for 2013 and 2014 samples and we have completed preliminary genotyping for 271 samples from 2013. We are currently reanalyzing questionable genotypes from 2013 and working on preliminary genotype analysis of 185 samples from 2014. Both should be completed by May 2016.

Genetic analysis begins with the removal of intestinal cells from the fecal pellet surface that were sloughed off during pellet deposition. DNA is then extracted from these cells using the QIAamp DNA Stool Mini Kit (Qiagen Inc. Valencia, CA). Following extraction, DNA is amplified using polymerase chain reaction (PCR), a process that is used to increase the quantity of short segments of repeating DNA called microsatellites. Because microsatellites can be species specific and can differ between individuals, they can be used to determine genders and genotypes from our pellet samples. We used PCR of a pair of sex-linked microsatellites to determine gender and PCR of a suite of autosomal (not sex-linked) microsatellites to determine genotype.

Microsatellite primers are used in PCR to amplify specific microsatellites. Primers can identify different forms of a microsatellites called alleles and each individual moose has two alleles for each microsatellite, one contributed by each parent. The unique combination of alleles in the DNA from each pellet sample determines the gender and genotype of the moose that deposited them.

Gender

Following methodology previously used on moose and other ungulate species, genders are determined using the SE47/SE48 primer pair to amplify the X- and Y-linked alleles of the amelogenin gene (Brinkman and Hundertmark 2009). The amplified products are then analyzed using gel electrophoresis to determine gender. A single product band identifies a female and a double band identifies a male (Figure 5).

We were able to determine definitive genders for 150 (81%) of our 185 pellet samples. Of the remaining 35, four were identified as questionable males and four as questionable females, in that the PCR product bands on the gel were visible but not distinct. Twenty-seven showed no result, in that no product bands were visible, and were labeled as ‘unknown’ (Figure 6). Of our 185 samples, 25 were collected from known-gender moose, animals we identified with 100% confidence either visually or from a combination of other field evidence. In the lab, we were able to correctly assign genders to 21 (84%) of these. Of the remaining four, one was a male identified as a female and three were unknowns.

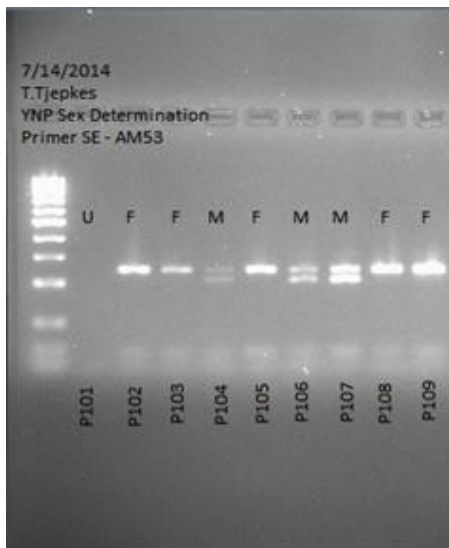


Figure 5: Gel image showing male and female signatures.

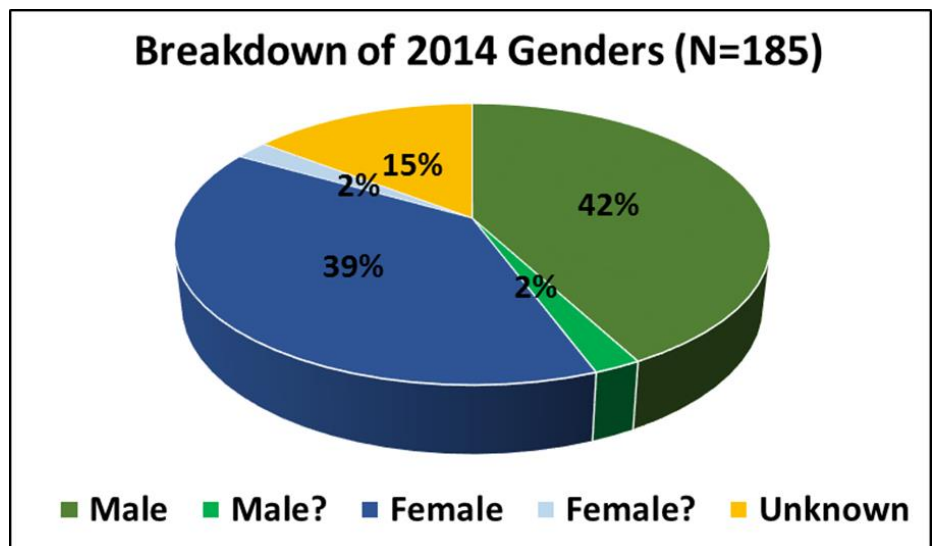


Figure 6: Percentage breakdown of genders for 185 pellet samples.

We are fairly confident that our lab protocol is not the reason for getting unknown results since we run our gender test up to six times per sample and then re-extract DNA and re-run gender tests if we do not get results after this. Pellet exposure to UV radiation, freeze/thaw cycles, and moisture that can promote the growth of bacteria, can all be attributed to the degradation of DNA. Also, DNA that was extracted from the pellet surface may not contain the sequences targeted by the SE47/SE48 primer pair.

Genotype

Individual microsatellites have different discriminating power to differentiate between genotypes. Polymorphic Information Content (PIC) and heterozygosity are measures of informativeness whose values range between zero and one. The higher the number, the greater the discriminating power of the microsatellite. The number of alleles for each microsatellite and its success rate (% of time the microsatellite is successfully identified using PCR) are also important measures. The greater the number of alleles and the higher the success rate the better. Researchers choose a suite of microsatellites that have the greatest combined discriminating power for identifying individual genotypes in a population. After pilot testing 27 microsatellites to determine success rates and using Cervus 3.0.7 software (Field Genetics Ltd, London, England) to determine their number of alleles, observed heterozygosity (Ho), expected heterozygosity (He), and PIC, we settled on a final suite of eight; BM2830, BM848, Cervid14, RT30, RT1, NVHRT03, KCSN and CRFA (Table 2). Average success rate of these was 86.4% and average number of alleles per marker, Ho, He, and PIC were 6.38, 0.41, 0.51, and 0.44, respectively.

Table 2: Discriminatory characteristics of our eight microsatellite markers.

Marker	# Alleles	Success Rate	Ho	He	PIC
BM2830	3	97.78	0.332	0.398	0.320
BM848	10	88.56	0.579	0.798	0.766
Cervid14	9	91.88	0.361	0.425	0.356
RT30	4	82.65	0.344	0.449	0.354
RT1	5	81.18	0.459	0.448	0.361
NVHRT03	8	90.04	0.689	0.674	0.602
KCSN	6	83.76	0.339	0.486	0.416
CRFA	6	75.27	0.181	0.377	0.330
Average	6.38	86.39	0.411	0.507	0.438

Following PCR of the 8 microsatellites, genotypes were assigned for each pellet sample using GeneMarker software (v.2.6.0, Softgenetics LLC, State College, PA). GENEAP analytical software with a sibling probability of identity of 0.05 was then used to distinguish between unique genotypes (Wilberg and Dreher 2004). GENEAP compares each individual genotype to all other genotypes in the data set to determine if DNA from pellet sample X matches that of sample Y. If so, they originated from the same moose (Table 3).

We input our 271 genotypes from 2013 and after the removal of 48 problem genotypes (those that matched two other genotypes that did not match one another), 223 genotypes remained in the analysis. From these 223, GENEAP identified pairs of matching genotypes. Some were exact matches, so were presumably the same moose, and some differed by only one allele and were labeled 'matches with one allele difference'. Through GENEAP analysis, 129 unique individuals were identified. However, these

'matches with one allele difference' must still be subjected to PCR reanalysis of mismatched allele pairs before we are able to determine the final number of individuals which we predict will be between 80 and 90 individuals.

Table 3: Matching genotypes from two samples collected in the Soda Butte drainage from the same pregnant female moose. Location, pellet volume index (PVI) and hormone concentration (HC) correlate well between samples. Note that 16 alleles, two for each marker, are used to identify each study moose.

ID#	BM2830		BM848		Cervid14		RT30		RT1		NVHRT03		KCSN		CRFA		Location	PVI	HC
21F	129	131	378	382	239	239	216	216	251	253	130	132	212	212	270	272	Soda Butte	8.44	2948
41F	129	131	378	382	239	239	216	216	251	253	130	132	212	212	270	272	Soda Butte	8.21	3000

In the meantime, we used additional data including locations where pellets were collected, pregnancy hormone concentrations of adult female moose, and pellet volumes of all moose to compare these 'matches with one allele difference'. Through this process we were able to accept or reject these matches as being from the same moose and in doing so identified 82 individual moose, our minimum population count. While we expect the number of individuals to be similar to this after PCR reanalysis of mismatched allele pairs, we think the use of this additional data for comparing genotypes will allow us to more accurately identify individuals than by Genecap analysis of genotypes alone.

Combined Data

By combining our additional data and genders from 82 individual moose we were able to classify them based on gender and age. The breakdown was 25 male adults, 10 male calves, 37 female adults, 7 female calves, and 3 moose of unknown gender and age (Figure 7). These data provide a population sex ratio of 67 bulls/100 cows and a calf-cow ratio of 46 calves/100 cows.

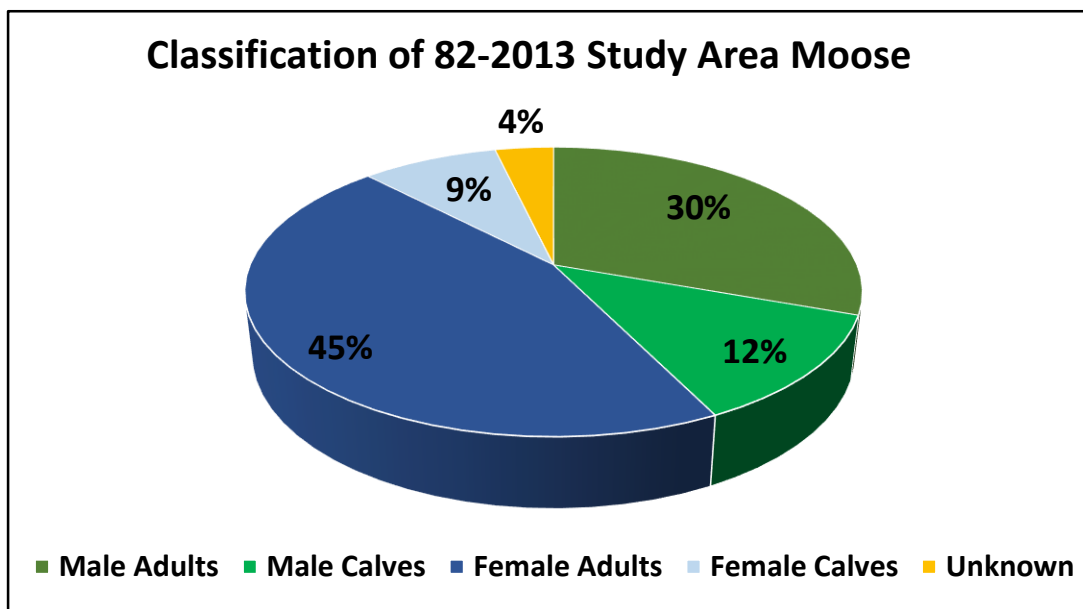


Figure 7: Gender and age classification of 82 study area moose.

Pregnancy

It is well known that concentrations of some pregnancy hormones increase throughout the pregnancy cycle of mammals, and that the pregnancy status of moose can be determined through analysis of these hormones (Schwartz et al. 1995). Progestagens, a class of steroid hormones, are involved in reproduction and can be found in moose fecal pellets. Concentration analysis of these is being conducted at the Smithsonian Conservation Biology Institute using enzyme-immunoassay and follows methods by Scarlata et al. 2012. In 2014, we sent 52 samples from yearling and adult females, 39 EW and 14 LW, for pregnancy



Adult female moose in Oxbow Creek during our late-winter survey.

testing. We also submitted six samples from calves (five female and one male) and one from an adult male in order to continue to establish baseline progestagen levels for these groups. In the future, these baseline levels will provide additional information to help differentiate between calves, bulls and cows.

Progestagen concentrations of our 52 female moose ranged between 351 and 12,450 nanograms per gram (ng/g). By comparing these concentrations to those of known-pregnant moose from an ongoing Montana Fish, Wildlife and Parks (MFWP) study (DeCesare and Newby 2015), we can make inferences about the pregnancy status of YNP moose assuming concentrations are similar between study areas.

MFWP determined that the threshold for pregnancy of 86 adult cow moose captured between January and April 2013-2014 and aged ≥ 2.5 years was 1,575 ng/g with a predicted probability of pregnancy of 95%. Pregnancy status was based on analysis of pregnancy specific protein B within blood serum (Huang et al. 2000). We applied this threshold to our 52 female moose from 2014 (40 EW and 12 LW). We determined that 32 of 52 (62%) samples were above the Montana threshold suggesting that these samples were from pregnant moose. All 12 LW samples exceeded the Montana threshold by an average of 378% (Figure 8).

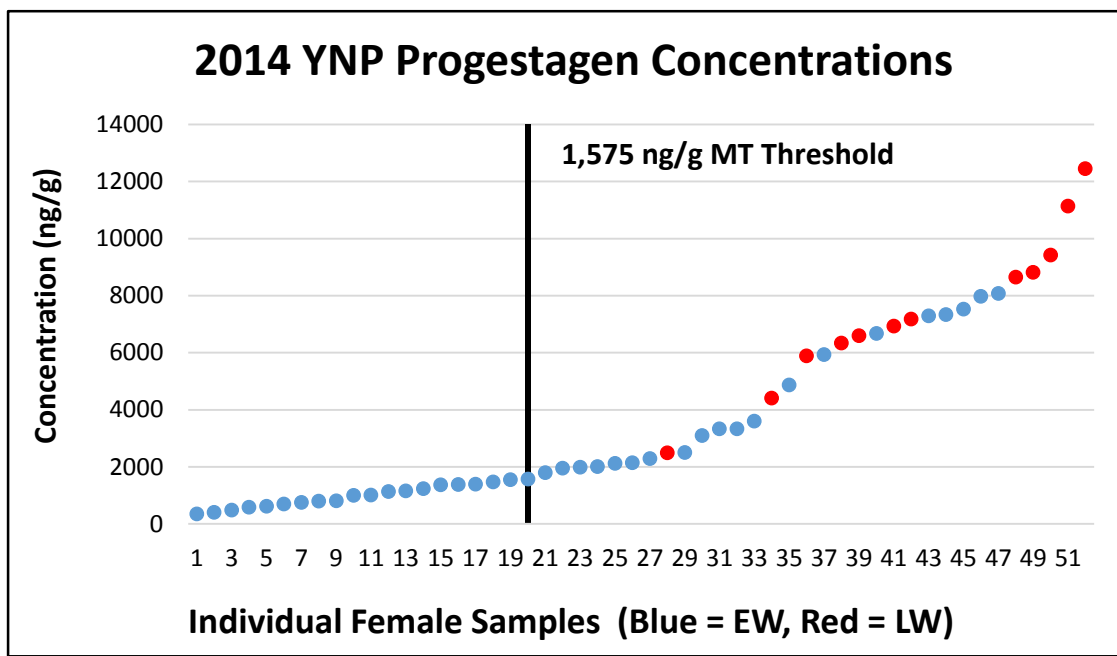


Figure 8: 2014 Progesterone concentrations from 52 female samples compared to the Montana pregnancy threshold. Note that all LW samples are above the threshold.

We also looked at the pregnancy status of a subset of our 2013 females. We applied the Montana threshold to 17 adult females from within the Soda Butte Region of our study area (Figure 9). For 12 of them, we only had data from one sampling period. We found that 14 out of 17 were above the threshold suggesting that 82% were pregnant.

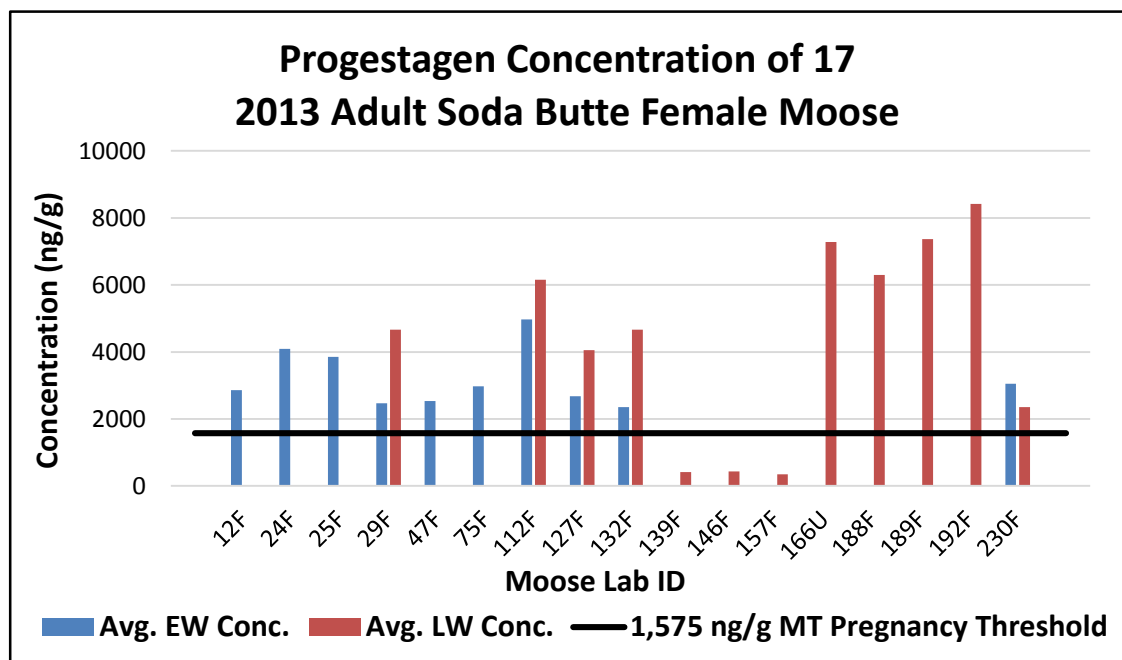


Figure 9: YNP hormone concentration compared to Montana threshold level for 17 adult female moose from the Soda Butte Creek drainage.

Age-Class

Research has shown that pellet morphometry can be used as a tool to differentiate between age-classes in captive moose (MacCracken and Van Ballenberge 1987) and that it can be used in conjunction with non-invasive genetic sampling when age-specific data is required (Ball 2010). By analyzing pellet measurements from known-aged cow moose provided by MFWP, we are hopeful to be able to classify YNP moose into calf, yearling and adult age-classes using discriminant function analysis. With this comparison we make the assumption that because of the close geographic proximity of YNP and Montana study areas both populations of moose produce similarly sized pellets at different age-classes. Since we don't have any morphometric pellet data for known-aged adult male moose, we will explore the use of cluster analysis for differentiating between bull age-classes.



Calipers are used to measure pellets to the hundredth of a millimeter. In our study area, this brown pellet suggests to us that the moose was feeding on willow. A black pellet is more indicative of a diet of subalpine fir and old man's beard lichen.

Pellets are dried in a convection oven at 65 degrees Celsius for 48 hours and then measured to determine their average diameter and average length. A volume index is calculated where volume equals average diameter x average depth (measured at 90 degree rotation from diameter) x average length. Eight pellets are measured from each sample. In 2014 we measured 177 YNP pellet samples and 85 known-aged Montana samples. This brought our total sample sizes up to 437 for YNP and 142 for Montana. Average pellet volume index, diameter, and length for 437 YNP moose ranged from 3.06 cm³ to 13.11 cm³, 12.38 mm to 21.87 mm, and 17.70 mm to 37.26 mm, respectively. A distribution between pellet volumes from male and female YNP moose is shown in Figure 10 and average pellet volumes of known-aged Montana moose is shown in Figure 11.

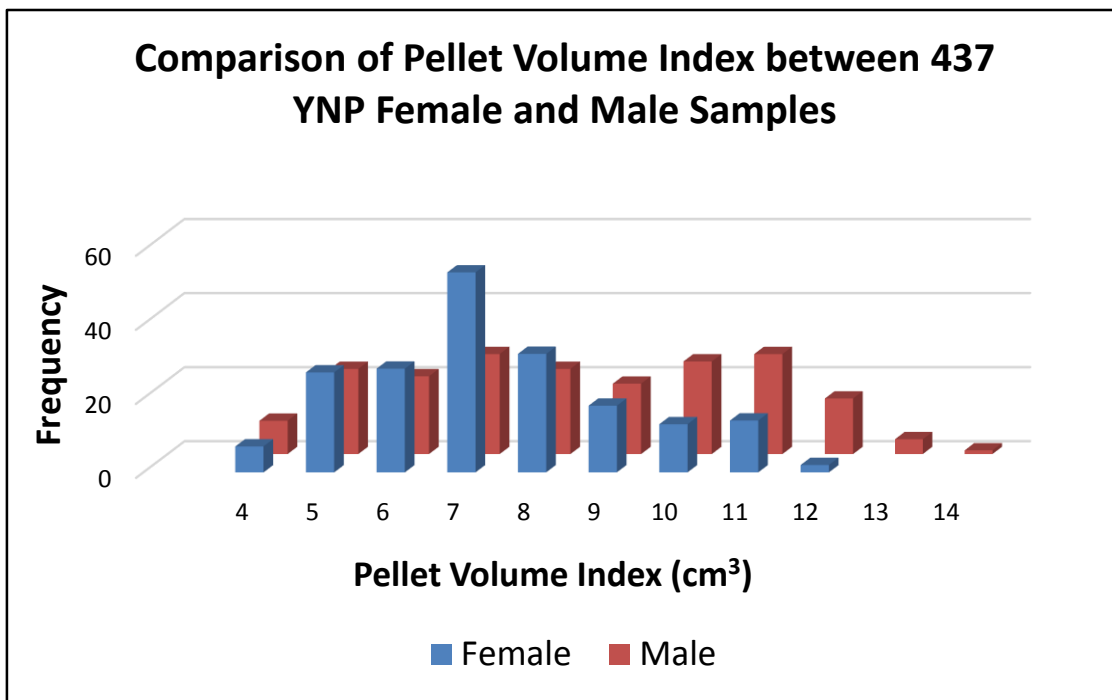


Figure 10: Frequency distribution of pellet volume between male and female YNP moose. The greater proportion of larger pellets 10 cm³ and greater are from males.

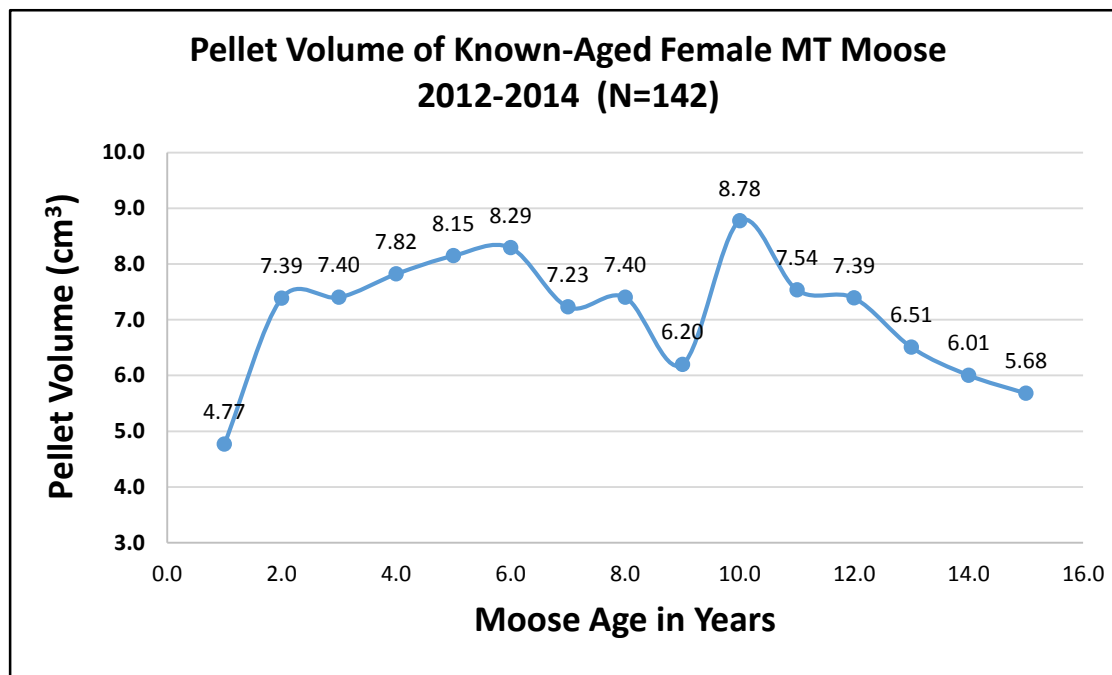


Figure 11: Pellet volume index (PVI) for 142 known-aged female MT moose.

After statistical analysis of 142 female Montana pellet samples we found significant differences in pellet volume, diameter, and length between calf and yearling/adult age-classes but have not been able to differentiate between yearlings and adults using any of these measurements. We are hoping that as our Montana data set builds we will be able to show this differentiation.

Winter Subpopulations

It has been documented that moose in some regions have a high affinity for specific wintering habitats and have smaller winter ranges than during the rest of the year because their winter forage is spatially limited and deep snow inhibits their mobility. We find that northern YNP moose abandon upper stream drainages, where they feed for much of the year, when snow depths exceeded 80 cm. In addition, geographic barriers such as river canyons and mountain ranges, and large areas of poor wintering habitat, including vast tracts of sagebrush steppe and large stands of regenerating lodgepole pine, seem to isolate wintering moose.



Skiing transects along the Soda Butte Creek between Silver Gate and Cooke City, MT during a late-winter survey. This region contains abundant riparian willow and adjacent mature conifer forests.

Genotype data from 271 first year pellet samples suggest that during the winter of 2013 there were four distinct subpopulations of wintering moose in our study area. This is based on the fact that we have found no genetic evidence that any animals moved between these four geographic regions. The regions that these four subpopulations occupy are Gardner's Hole, Blacktail Deer Plateau, Soda Butte Creek, and Slough Creek. Vast stands of regenerating lodgepole pine forests, extensive stretches of sagebrush, and the steep canyon of the Gardner River likely separate the Gardner's Hole moose from the Blacktail Deer Plateau moose. The Yellowstone River corridor and poor quality wintering moose habitat to its east could potentially separate Blacktail Deer Plateau moose from those in the Slough and Soda Butte Creek regions. A mountainous ridge and the accompanying deep snow and steep rocky terrain that stretches between Slough and Soda Butte Creeks likely acts as a barrier that keeps these moose populations separated for the winter.

Gardner's Hole

In addition to the upper Gardner River, this region includes the drainages of Glen, Fawn, Panther, Indian, and Obsidian Creeks located between and on the eastern flanks of Electric, Quadrant and Antler Peaks. This region is characterized by sparsely-willowed creek bottoms, large expanses of sagebrush steppe, and broad swaths of mature subalpine fir, Engelmann spruce, and lodgepole pine forests.



Mature conifer forests (the most important winter habitat for moose in the Gardner's Hole Region) blanket the foothills of Electric Peak above the willowy banks of Fawn Creek.

Moose in early-winter are found on the fringes of mature conifer forests taking advantage of available willow forage along nearby streams. By mid-winter when snow covers the willow and restricts movement, moose retreat to the protective cover of the forests where they feed mostly on regenerating subalpine fir browse, *Ribes* spp., and old man's beard. When snow depths subside in the late-winter and spring, moose move back into the willow drainages.

Blacktail Deer Plateau

This region encompasses all drainages flowing from the Blacktail Deer Plateau including Lava, Blacktail Deer, Oxbow, Geode, Elk, Yancey, Lost, and Tower Creeks. Antelope Creek to the south of the Plateau is also part of this region. Most creeks in this area flow directly into the Yellowstone River. This region contains large expanses of non-forested upland with its associated shrub-steppe grass and sagebrush vegetation, pockets of mature Douglas fir and regenerating lodgepole pine forests, and riparian willow. Willow is found in greater abundance in the upper reaches of the drainages and is less abundant in the steep creeks as they get closer to the Yellowstone River.



Blacktail Deer Creek on a frigid morning survey. We have seen the same cow moose with a right cropped ear in this drainage for the last four years.

On the Plateau, moose move freely between drainages feeding mostly on willow while supplementing their diet with chokecherry, *Ribes* spp., aspen, red-osier dogwood where available, and occasionally alder. As snow depths increase, moose move down the creeks where snow depths are more moderate. Some moose eventually end up along the southern bank of the Yellowstone River browsing on willow.

Soda Butte Creek

This region includes Soda Butte Creek and its tributaries from the confluence of Soda Butte Creek and the Lamar River, upstream to Cooke City. It also includes Cache Creek and the stretch of the Yellowstone River between its confluences with Soda Butte Creek and Cache Creeks. Tributaries of Soda Butte Creek providing suitable winter habitat include Pebble, Amphitheatre, Warm, Woody, and Republic Creeks. This region contains the majority of mature conifer forests left in northern YNP following the expansive forest fires of 1988 except for the Cache Creek drainage where most of its forests were almost completely burned. Soda Butte Creek contains pockets of willow and mature conifer forest along its entire length with large expanses of willow at its confluences with Pebble Creek and the Lamar River. This mix of abundant willow and adjacent mature conifer makes this region the most suitable for wintering moose and, not surprisingly, supports over half the wintering moose in northern YNP.

Moose use this habitat like those in the Gardner's Hole region do, feeding predominantly on willow when available in EW then moving to higher elevation mature conifer forests in MW. Here moose have a high

preference for both regenerating subalpine fir, *Ribes* spp., and old man's beard. Because of the close proximity of willowed creek bottoms to the forest, moose tend to move back and forth between habitat types frequently and therefore can be found in isolated pockets of willow along streams throughout the winter.



Lisa Koitzsch breaking trail along Soda Butte Creek. Similar to the Gardner's Hole region, some moose winter here at over 2,400 meters (8,000 feet) on the steep slopes of surrounding mountains.

Slough Creek

This region includes the entire length of Slough Creek and its tributaries from its confluence with the Lamar River to its upper reaches north of YNP in the Custer-Gallatin National Forest. Here it is joined by Bull, Abundance, Frenchy, Wolverine, and Lost Creeks in an area called Frenchy's Meadow. Other tributaries that provide wintering habitat for moose include Buffalo, Hornaday, Elk Tongue, and Cutoff Creeks. This region contains four vast stretches of riparian willow along the Slough Creek drainage and small pockets of willow in its tributaries. The Slough Creek is flanked by mature Douglas fir-Engelmann spruce stands and broad swaths of regenerating lodgepole pine. Prior to the fires of 1988, based on moose biologist Dan Tyer's estimation, this region provided some of the best year-round habitat for moose. Today, while some mature forests still exist along the lower Slough Creek drainage, most of the upper reaches have been burned and so regenerating lodgepole pine forests are most abundant. Consequently, habitat suitability for wintering moose is much lower than in other regions.



Frenchy's Meadow in the upper Slough Creek drainage. After the devastating 1988 fires, moose populations in this region declined precipitously with the loss of critical winter habitat. Regenerating forests on both sides of the meadow still bear the scars from the fires.

We have observed moose using this area in much the same way in both EW and LW, spending a large portion of their winter browsing on riparian willow and periodically moving up into adjacent tributaries or slopes searching for regenerating subalpine fir, aspen browse, and *Ribes* spp. Here we have found the most browsing of regenerating lodgepole pine than anywhere else in our study area.

Field Observations

We continue to find that moose in northern YNP seem very healthy. All moose had full coats and we did not observe any emaciated animals. We saw no visible signs of tick infestation or tick induced hair loss, and have not found a single tick in over 1,000 moose beds that we have examined. Over the first two years of our study we have seen two different adult cow moose with cropped ears, an external sign of possible artery worm infection, but both moose otherwise appeared normal. Each moose was seen with a calf in at least two of the last three years.

It seems that the northern YNP moose population is fecund based on our documentation of numerous calves in 2013 and 2014 and relatively higher pregnancy hormone concentrations than in neighboring Montana moose populations. Low population density, the availability of adequate quality and quantity of year-round habitat, relatively low predation from wolves and bears, and a lack of obvious infection by parasites may all contribute to this. After analysis of our 2014 genotypes we will be able to see which females that we assumed were pregnant in 2013 actually had calves with them in 2014.



**Cow with twin calves in Soda Butte Creek near Footbridge parking area.
(Photo courtesy of Doug McLaughlin)**

We performed a necropsy on a bull moose presumably killed by wolves in Glen Creek during the last week of March. We collected a tissue sample for DNA analysis, a section of femur for bone marrow fat analysis, and an incisor for aging by counting cementum annuli. A marrow fat content of 71% showed that the eight-year-old bull was in poor nutritional condition. Genetic analysis of DNA will determine if we have previously collected samples from this moose.

In the Future

Capture-Recapture Analysis

Once genotype analysis is completed for all three years and we have generated capture histories for individual moose, we will explore the use of two different types of capture-recapture modes for generating estimates of population size, rate of population change, and sex- and gender-specific vital rates. We originally designed our sampling methodology to use the robust design analyses in Program

MARK (White and Burnham 1999) that requires two secondary sampling periods per primary sampling; our early- and late-winter sampling periods being the secondary periods within the winter primary sampling period. Many recent non-invasive population studies of North American ungulates have used this program (Brinkman et al. 2011, Poole et al. 2011, Hettinga et al. 2012). We are also exploring the use of spatial capture-recapture models. These may be better suited for our study design because they take into account the spatial pattern of where individual moose are sampled from or encountered. If time and resources permit we may compare the use of both types of models.

Winter Range Size and Movement Patterns

Based on multiple locations from individuals throughout the winter we will determine minimum winter range sizes for moose. By collecting pellets in EW, MW and LW and mapping sample locations, we can also document winter movement patterns of individuals.

Urine Patterns for Determining Gender

We continue to explore the use of snow urine patterns for assigning genders to moose. We are finding that the most important characteristics of a urine pattern are its location in relation to the hind feet and the shape of the urine hole in the snow. Males typically urinate at or forward of the hind feet and produce an elongated hole where females tend to urinate at or behind the feet and produce a rounder hole. We have observed bulls rub urinating when agitated with feet close together while rubbing their hocks and urinating on them. The pattern seen from this is of the hind feet close together and splattered urine. We have found that females spray urinate when startled, spraying a fan of urine behind their back legs. We have seen this mostly when we accidentally jump a cow from her bed. We have yet to determine if females rub their hind legs together while doing this. We commonly observe bulls dribbling urine while walking and we have not observed this with females.



Male urine pattern on the left and female urine pattern on the right.

Funding and Support

We greatly appreciate all those who have helped fund and support our project. We received funding this year from the Campfire Club of America's Conservation Fund and we thank Andrew B. Heath for his help in securing this grant. We are grateful to Scott and Carolyn Heppel and to Rob and Joy Playter for their generous donations and their interest in our project. We thank Douglas W. Smith and Yellowstone National Park for providing housing and for daily logistical support. We especially thank the Department of Biology at the University of Minnesota-Duluth for providing funding for genetic analyses.

Acknowledgements

We would like to thank Douglas Smith, Kerry Gunther, Daniel and Erin Stahler, Kira Cassidy, Stacy Sigler, Nels Christensen, and the Yellowstone Cougar Project crew for their advice, support, field, and logistical help; U.S. Park Rangers Tom Schwartz, Kevin Dooley, Brian Chan, and Gabe Gassman for all their help in backcountry logistics; Dan Tyers, U.S. Forest Service biologist, for his pioneering contributions to moose research in YNP and for sharing his knowledge with us; Walt Allen and Jeremy Zimmer from the Gallatin National Forest for their continued support; Nick DeCesare, moose biologist with Montana Fish, Wildlife and Parks for his collaboration and efforts on our behalf; Karen Loveless from Montana Fish, Wildlife and Parks, and Nicole Boisseau and Janine Brown from the Smithsonian Conservation Biology Institute for all of their professional help and guidance. We are very grateful for the generous hospitality offered to us by Dan and Laurie Lyman during our Soda Butte Creek surveys. We thank pilot Roger Stradley of Gallatin Air Service for his safe flying and in depth knowledge of northern YNP. Special thanks to Abby Schuman at the University of MN - Duluth for all her hard work and dedication with our genetic analyses, and to Carol Chamberlin for her generous help in organizing our data.



And of course, this study would not be possible without the generous contributions from the YNP moose! (Photo courtesy of Peter Murray)

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