A noninvasive and integrative approach for improving density and abundance estimates of moose

Article *in* Journal of Wildlife Management · March 2022

DOI: 10.1002/jwmg.22200

6 authors, including:

Ky Koitzsch
K2 Consulting, LLC
4 PUBLICATIONS **22** CITATIONS
SEE PROFILE

Colby Anton
Montana Fish Wildlife and Parks
5 PUBLICATIONS **36** CITATIONS
SEE PROFILE

Received: 20 March 2021 | Revised: 6 December 2021 | Accepted: 18 December 2021

DOI: 10.1002/jwmg.22200

RESEARCH ARTICLE

THE JOURNAL OF WILDLIFE MANAGEMENT | THE WILDLIFE SOCIETY

A noninvasive and integrative approach for improving density and abundance estimates of moose

Ky B. Koitzsch[1] | Colby B. Anton[2] | Lisa O. Koitzsch[1] | Tessa L. Tjepkes[3] | Abby C. Schumann[3] | Jared L. Strasburg[3]

[1]K2 Consulting, LLC, PO Box 953, Waitsfield, VT 05673, USA

[2]Yellowstone Center for Resources, National Park Service, Yellowstone National Park, WY 82190, USA

[3]Department of Biology, University of Minnesota-Duluth, 207 Swenson Science Building, 1035 Kirby Drive, Duluth, MN 55812, USA

Correspondence

Ky B. Koitzsch, K2 Consulting, LLC, PO Box 953, Waitsfield, VT 05673, USA.
Email: kkoitzsch@gmavt.net

Present address

Colby B. Anton, Montana Cooperative Wildlife Research Unit, University of Montana, Missoula, MT 59801.

Tessa L. Tjepkes, Wild Beginnings Nature Play School, 4575 McComber Road, Duluth, MN 55803.

Abby C. Schumann, Minnesota Poultry Testing Laboratory, PO Box 126, Willmar, MN 56201.

Funding information

R. and J. Playter; Camp Fire Club of America; A. B. Heath; University of Minnesota-Duluth Biology Department; R. B. and K. B. Koitzsch; University of Wyoming/NPS Small Grant Program; S. and C. Heppel

Abstract

Acquiring demographic data for moose (*Alces alces*) can be difficult because they are solitary in nature, they prefer densely vegetated and mountainous habitats, and they often occur at low density. Such data, however, are essential for long-term population monitoring, evaluating management practices, and effective conservation. Winter aerial surveys are the standard method for estimating moose population parameters, but they can be logistically challenging, expensive, and subject to sightability correction, which necessitates the capture of study animals for initial model development. Herein, we demonstrate a noninvasive alternative approach for estimating population parameters of moose in northern Yellowstone National Park, where aerial surveys were attempted but proved ineffective. We determined individual moose genotype and sex using microsatellite polymerase chain reaction amplification of DNA extracted from fecal pellets, integrated ancillary pellet sample data (i.e., metadata) in genotype analysis to aid in the identification of matching genotypes, and used spatially explicit capture-recapture (SECR) modeling to estimate sex-specific density and abundance. We collected 616 samples over 3 consecutive winters (Dec 2013–Apr 2016) and within 2 sampling occasions each winter. We recorded 514 captures of 142 individual moose (69 males, 73 females). Overall density ranged between 0.062 moose/km^2 and 0.076 moose/km^2 and averaged 0.034/km^2 for females and 0.033/km^2 for males. Abundance estimates were 150 moose in 2013 (female = 76,

95% CI = 55–105; male = 74, 95% CI = 54–103), 186 in 2014 (female = 95, 95% CI = 63–142; male = 91, 95% CI = 60–138), and 160 in 2015 (female = 79, 95% CI = 58–108; male = 81, 95% CI = 59–110). Average population sex ratio was 0.99 males/female. We demonstrate that SECR analysis of fecal DNA genotypes, using metadata in genotype analysis to help identify matching moose genotypes, is a promising alternative method for estimating sex-specific density and abundance of a low-density moose population in a mountainous and forested landscape.

KEYWORDS

abundance, *Alces alces*, density, fecal DNA, integrative, metadata, moose, noninvasive, spatially explicit capture-recapture, Yellowstone National Park

Estimating reliable population parameters is essential for the effective management and conservation of wildlife species (Rosenberg et al. 1995, Mondol et al. 2009, Brøseth et al. 2010) and serves as an important baseline for evaluating and understanding factors leading to population changes (DelGiudice et al. 2011). This is particularly important in North America where moose (*Alces alces*) populations are declining across their southern distribution (Murray et al. 2006, Monteith et al. 2015, Jones et al. 2017, Nadeau et al. 2017, Arsenault et al. 2019). Acquiring accurate demographic data for moose is difficult, however, because it is a wide-ranging species that often occupies densely forested and mountainous habitats, frequently exists at low density, and infrequently congregates in groups (Timmermann and Buss 1997, Toweill and Vecellio 2004).

Winter aerial surveys using fixed- or rotary-wing aircraft remain the standard method for estimating moose population parameters (Gasaway et al. 1986, Timmermann 1993) despite documented shortcomings (Boyce et al. 2012, Wald and Nielson 2014). Most problematic, aerial surveys are subject to sightability bias (LeResche and Rausch 1974, Gasaway et al. 1985) and require sightability correction (Peterson and Page 1993, Giudice et al. 2012). While sightability correction models have been developed for many habitats, researchers who apply existing models to their data sets often find that these produce disparate population estimates (Harris et al. 2015). Consequently, new models must be created for their specified study area, which requires invasive and potentially dangerous moose capture for radio-collaring or marking during initial model development (Anderson and Lindzey 1996). Other aerial survey methods, such as mark-resight (Bowden and Kufeld 1995), mark-recapture distance sampling (Oyster et al. 2018), and infra-red thermal imaging (Millette et al. 2011), share similar limitations and challenges. In addition, capture, handling, and marking of animals is sometimes discouraged in areas such as national parks because of concern for the animals' welfare and intrusion on visitor experiences (Berger et al. 1999, Mech and Barber 2002).

Non-aerial methods for making population inferences include analysis of moose hunter harvest statistics (Boyce et al. 2012, DeCesare et al. 2016) and sighting rates (Kantar and Cumberland 2013, Boyce and Corrigan 2017). While data collected using these methods are relatively easy and inexpensive to gather and require no direct population monitoring, they often produce imprecise population estimates and are best suited for tracking population changes over time (Rönnegård et al. 2008, Kantar and Cumberland 2013). Considering the limitations of existing methods for monitoring moose populations, managers continue to explore safer, less invasive, more reliable, and more cost-effective alternatives (Boyce et al. 2012, DeCesare et al. 2014, Oyster et al. 2018).

Noninvasive genetic sampling (NGS) has emerged as an important tool for estimating wildlife abundance (Lukacs and Burnham 2005, Waits and Paetkau 2005). Noninvasive genetic sampling methods are safer for researchers and study animals and can be used in habitats where sightability is poor and in study areas where aerial methods are not practical, feasible, or permitted (Berger et al. 1999, Schwartz et al. 2007, Goode et al. 2014). Feces are one of the most widely used sources of DNA for NGS studies (Fernando et al. 2003) and are commonly used for ungulates because their fecal pellets are abundant, are easy to collect, and allow for the collection of large sample sizes (Brinkman and Hundertmark 2009).

Studies using fecal DNA genotypes with closed population capture-recapture (CR) methods to estimate population parameters are common and have been applied to a variety of mammals (Banks et al. 2003, Eggert et al. 2003, Bellemain et al. 2005). They have also been widely used for studies of ungulate species including woodland caribou (*Rangifer tarandus caribou*; Hettinga et al. 2012), argali (*Ovis ammon*; Harris et al. 2010), mountain goat (*Oreamnos americanus*; Poole et al. 2011), Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*; Schoenecker et al. 2015), and various deer species (*Odocoileus* spp.; Brinkman et al. 2011, Lounsberry et al. 2015; roe deer [*Capreolus capreolus*]; Ebert et al. 2012). These more traditional CR methods, however, have limitations because they do not accommodate the ecological processes that influence animal detections or how data is collected (Royle et al. 2013).

To overcome these limitations, CR methodology has been developed to make ecological processes explicit in models by including detection locations in individual encounter histories (Efford 2004, Royle and Young 2008). These spatially explicit capture-recapture (SECR) methods have many advantages over traditional CR methods as they 1) provide for direct estimates of density that are less biased by edge effects and incomplete detection, 2) relate population density and size estimates to specified geographical regions, 3) accommodate unstructured sampling designs, 4) are more robust to violations of geographic closure assumptions, and 5) allow for variation in sampling effort across a study area (Efford 2004, Obbard et al. 2010, Thompson et al. 2012, Efford et al. 2013).

Consequently, ungulate studies applying SECR modeling of fecal DNA genotypes to estimate population parameters have become more common (Goode et al. 2014, Brazeal et al. 2017, Furnas et al. 2018, Ebert et al. 2021). Whereas numerous moose studies have used genotyping of fecal DNA to address a variety of topics (Cronin et al. 2001, Schmidt et al. 2009, Wasser et al. 2011, Kretser et al. 2016, Rea et al. 2016), none have applied SECR analysis to fecal DNA genotypes to estimate moose population parameters.

Shiras moose (*Alces alces shirasi*) were first reported in northern Yellowstone National Park (YNP) in the early 1900s (Houston 1982) and their numbers increased rapidly as they exploited abundant riparian willow. Barmore (2003) estimated spring maximum population size approaching 400 moose between 1967–1970 from data collected incidentally while censusing elk (*Cervus canadensis*). The 1988 forest fires destroyed large tracts of moose wintering habitat across YNP, which led to a 75% decline in moose (Tyers 2006). The first attempt to directly census moose in northern YNP was made between 1988 and 1992 using aerial surveys (Tyers 2003). Because flights did not produce complete survey data and aerial counts did not closely correlate with other existing indices of population size, their cost could not be justified, and they were discontinued. Tyers (2003) concluded that even with more efficient searching from a low-flying helicopter, accurate moose population estimates would not be feasible because of low moose density and limited visibility due to heavily forested habitats. Despite concerns that moose are declining across the Greater Yellowstone Ecosystem, specifically in northern YNP, no current population data exists. Park officials estimated from anecdotal evidence that approximately 200 moose inhabited northern YNP at the time of our study.

We initiated a 3-year noninvasive population study to address the challenges of estimating moose population parameters in northern YNP. Our specific objectives were to demonstrate the utility of SECR modeling of fecal DNA genotypes as a tool for estimating sex-specific moose density and abundance; assess the efficacy of including ancillary pellet sample data (i.e., metadata) into genotype analysis to help identify moose genotypes, their matches, and genotyping errors; and provide rigorous estimates of sex-specific moose density and abundance for the northern YNP area. We predicted that our methods would be a viable alternative for estimating moose density and

abundance in heavily forested and mountainous landscapes and would confirm that moose in northern YNP exist at low density.

STUDY AREA

We conducted our study in a 1,100-km^2 portion of northern YNP and the adjacent southern reaches of the Custer-Gallatin National Forest in the central Rocky Mountains of North America, USA (44°57′N, 110°20′W; Figure 1). Our study area approximated the boundary of the Northern Yellowstone elk winter range as described by Houston (1982) but included some adjacent high-elevation mature conifer forests and excluded low-elevation developed areas north of YNP along the Yellowstone River. A plowed road between Mammoth, Wyoming and Cooke City, Montana, USA, bisected the study area and allowed reasonable access for winter surveys. The study area was approximately 75 km east to west, 35 km north to south, and bordered by the Gallatin Mountains to the west, the Absaroka Mountains to the north and east, and the Washburn Range to the south. The climate was characterized by long, cold winters (Dec–Mar) and short, cool summers (Jun–Sep; Houston 1982) with drought conditions becoming more common over the past half century (McMenamin et al. 2008). Average snow depth and daily minimum and maximum temperatures varied from 52 cm and −14°C to −4°C in early-winter (10 Dec–18 Jan) to 35 cm and −5°C to 8°C in late-winter (Apr; National Centers for Environmental Information 2018).

The study area was composed of sagebrush-steppe vegetation at elevations below 2,000 m and mature conifer forests at higher elevations. Douglas-fir (*Pseudotsuga menziesii*) predominated at elevations below 2,300 m, lodgepole pine (*Pinus contorta*)-Engelmann spruce (*Picea engelmannii*)-subalpine fir (*Abies lasiocarpa*) forests between

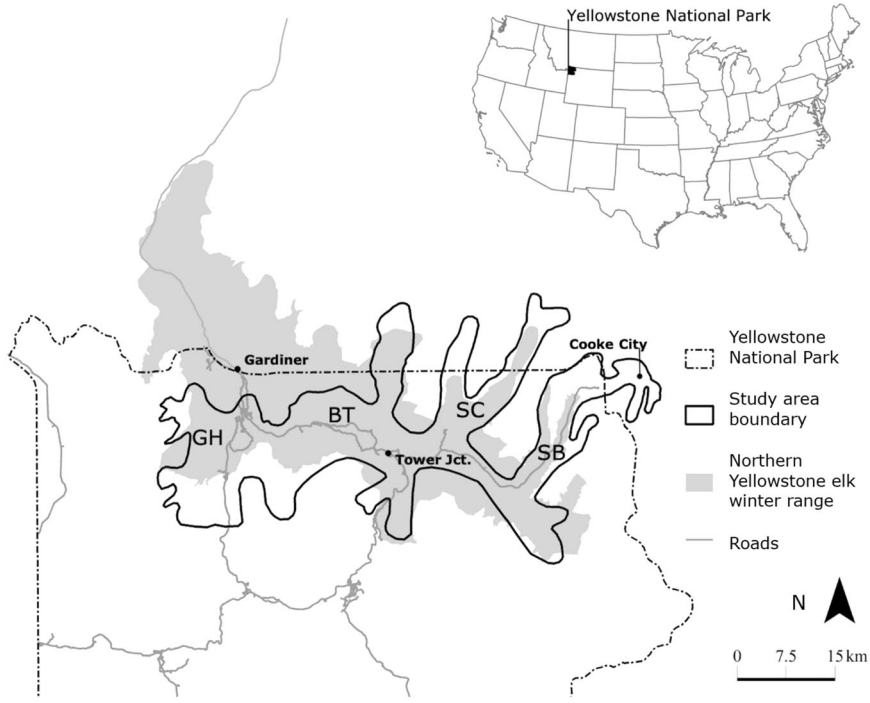

FIGURE 1 The northern Yellowstone National Park, USA, study area for non-invasive genetic monitoring of moose (Dec 2013–Apr 2016). Also, shown are 4 distinct sampling regions: Gardner's Hole (GH), Blacktail Deer Plateau (BT), Slough Creek (SC), and Soda Butte Creek (SB)

2,300 m and 2,560 m, and whitebark pine (*Pinus albicaulis*) at higher elevations (Renkin and Despain 1992, Yellowstone National Park 1997). Approximately 30% of mature conifer forests burned in 1988 and now exist as regenerating lodgepole pine forests (Tyers 2006). Small quaking aspen (*Populus tremuloides*) stands were scattered at lower elevations and willows (*Salix* spp.) were present in most riparian corridors and some wet forested areas.

Moose shared the study area with 5 native ungulates, white-tailed deer (*O. virginianus*), mule deer (*O. hemionus*), elk, bighorn sheep, and bison (*Bison bison*), and 1 introduced species, the mountain goat. Four large carnivores that prey on moose were present including grizzly bear (*Ursus arctos*), American black bear (*U. americanus*), gray wolf (*Canis lupus*), and mountain lion (*Puma concolor*).

Primary moose wintering habitat included river and stream drainages that contained willow and mature lodgepole pine-Engelmann spruce-subalpine fir forests. The most important forests for moose were those >300 years old (Tyers 2003). From December to early January, moose concentrated in riparian areas and fed primarily on willow. In late January when increasing snow depths impeded their mobility and covered low willow species, moose retreated to the protective cover of higher elevation conifer forests and fed primarily on regenerating subalpine fir and deciduous shrubs (Tyers 2003). Moose returned to lower elevation riparian areas to feed on willow after snow melted in late winter. Peek (1974) reported similar patterns of winter habitat use by Shiras moose. In regions where mature conifer forests burned in 1988, moose moved between creek drainages and scattered Douglas-fir stands where they fed on willow and deciduous shrubs and moved downstream towards the Yellowstone River when snow accumulated in upper drainages (Tyers and Irby 1995).

METHODS

Sampling

We collected moose fecal pellet samples during 2 occasions each within 3 consecutive winter sessions (2013–2015). This robust design sampling scheme allowed estimation of within session variation in moose detection probability and space use (Pollock 1982). We collected samples from 10 December to 18 January and during April, hereafter referred to as early-winter (EW) and late-winter (LW) sampling periods, respectively. We considered samples collected from December 2013 to April 2014, December 2014 to April 2015, and December 2015 to April 2016 as 2013, 2014, and 2015 samples, respectively. We chose sampling dates to match when moose were concentrated in lower elevation willow ecosystems where they were more accessible for sampling (Tyers 2003), and for increasing the likelihood of cold temperatures for preserving pellet DNA and the snowpack required for efficient ski travel to track moose and locate samples. Sampling in EW and LW also reduced temporal replication of spatial samples.

To improve precision of parameter estimates, we systematically surveyed all known and potential moose wintering habitat in the study area and used a high sampling effort to maximize sample size (i.e., the number of unique individuals sampled) and the number of recaptures of individuals (Marucco et al. 2011, Royle et al. 2013). Weather permitting, we conducted a study area survey flight prior to each sampling occasion to locate moose and moose sign. We established survey routes based on knowledge acquired from flights and the spatial behavior and wintering ecology of moose in northern Yellowstone (Tyers 2003, Ebert et al. 2010). Routes paralleled creek and river corridors and along topographic contours through mature conifer forests to elevations of 2,600 m, the approximate maximum elevation used by wintering moose in northern YNP. We designed routes 9–16 km long to allow field technicians to complete ≥1 route/day.

We divided our study area into 4 geographically distinct sampling regions: Gardner's Hole, Blacktail Deer Plateau, Slough Creek, and Soda Butte Creek (Figure 1). Between 6–11 survey routes were required to thoroughly sample all moose wintering habitat within a region. We completed all survey routes in one region before moving to the next. The order in which we sampled regions depended on moose seasonal habitat use and snow conditions.

For example, in EW we focused on the Slough and Soda Butte Creek regions first because moose were more concentrated there, and in LW we sampled the lower elevation Slough Creek and Blacktail Deer Plateau regions first because they were prone to earlier snow melt. Sampling effort varied between study occasions based on weather, which ultimately determined the number of days, and which routes, we sampled. For example, we did not survey during heavy snowstorms or along routes where snow had melted in late-April. We considered our sampling design as unstructured in that we established survey routes *a priori*, but sampling varied along their length to follow fresh moose tracks (Russell et al. 2012, Thompson et al. 2012, Kretser et al. 2016). We recorded kilometers traveled for each survey route with Garmin STC 62 global positioning system units (Garmin, Olathe, KS, USA) as a measure of sampling effort.

A pair of technicians skied parallel to one another at a distance that varied between 100–400 m to ensure thorough route coverage until encountering moose tracks. Following Brinkman et al. (2010*a*) who reported greater genotyping success using fresh pellets, we collected pellets deposited within 24 hours or that were older but had remained frozen. We determined sample age by the crispness of the associated moose track edge, or the amount of time elapsed since the last snow event. Like other northern temperate zone ungulate species, moose are in relatively poor nutritional condition during winter, so to minimize disturbing them, we followed tracks backwards until we found pellets that met our criteria. To avoid mixing pellet samples from different moose, we collected pellets only from isolated tracks or bedding sites. To maintain independence between samples and maximize the number of individual moose sampled, after collecting samples, we continued along the route for ≥400 m, or until encountering a different sized track or moose group, before searching for the next sample. To reduce bias associated with sampling along the length of potentially elongated moose home ranges (Efford 2019), we searched all tracks that intersected our routes, which ensured a 2-dimensional survey.

We collected 30 pellets from each pellet group using single-use nitrile gloves to prevent cross-contamination of DNA between samples: 10 each for genetic, morphometric, and pregnancy hormone analyses. We collected pellets haphazardly from within pellet groups because steroid hormones can be unevenly distributed within feces (Millspaugh and Washburn 2003). We collected tissue samples opportunistically from moose carcasses discovered during sampling. We placed samples in doubled zipper storage bags labeled with a field identification number and Universal Transverse Mercator (UTM) coordinates and stored them frozen at −20°C (Hettinga et al. 2012, Flasko et al. 2017).

Genotyping

We assigned a sample identification number (ID) to all pellet samples and extracted DNA using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA). We modified the manufacturer's protocol to minimize plant secondary compounds that could inhibit polymerase chain reaction (PCR) amplification and lead to poor genotyping results (Monteiro et al. 1997, Wehausen et al. 2004). To minimize inhibitor quantity in DNA extracts, we used only intact pellets with no exposed inner material and washed the epithelial cells from their surfaces by gently agitating them in lysis buffer (Flagstad et al. 1999, Maudet et al. 2004, Luikart et al. 2008). We extracted tissue DNA using the Thermo Scientific GeneJET Genomic DNA Purification kit (Thermo Fisher Scientific, Pittsburgh, PA, USA) following a modified protocol (Tjepkes 2015).

We determined sex and genetic identity for all DNA samples and optimized PCR conditions for sex markers and microsatellites following Tjepkes (2015). First, we tested 2 sex primer pairs, KY1/KY2 and SE47/SE48 (Brinkman and Hundertmark 2009) that amplified alleles on the amelogenin gene. We chose the SE47/SE48 pair because it more consistently provided higher PCR success rates, produced more distinct gel bands, and accurately determined sex for 100% of 44 known-sex samples based on visual identification or obvious behavioral clues (e.g., antler rubbing on trees). Because of these qualities, we required only a single definitive result to determine sex. We attempted amplifications up to 3 times for each sample, the first 2 with DNA diluted 1:1 with 1X TE buffer, and the

third using undiluted DNA. We electrophoresed PCR products and visualized them on a 1.5% agarose gel stained with 10 mg/mL of ethidium bromide. For samples collected <24 hours after deposition that did not produce a definitive result after 3 PCRs, we reextracted DNA from a second pellet and repeated PCRs in triplicate as previously described. We removed those that did not produce a definitive result after the initial 3 PCRs and that were collected >48 hours after deposition from further analysis.

We chose our suite of microsatellite markers following a pilot study of 27 used in other studies of moose (Table S1, available in Supporting Information). We compared PCR success rates and discriminatory characteristics of markers including number of alleles, expected (H_E) and observed (H_O) heterozygosity, and polymorphic information content (PIC) using Cervus 3.0.7 (Field Genetics, London, United Kingdom). Based on these data and following Paetkau (2003) who suggested using ≥6 microsatellites when mean H_E was <0.7 (ours averaged <0.5), we chose a suite of 9 that exhibited the highest combined PCR success rates and genetic variability: BM2830, BM848, Cervid14, CRFA, IGF-1, KCSN, NVHRT03, RT1, and RT30 (Table 1). All microsatellite markers were used previously in studies of moose in North America (Wilson et al. 1997, 2003; Cronin et al. 2001; Schmidt et al. 2009; Tjepkes 2015).

We PCR amplified microsatellite loci in a series of singleplex reactions using the multitube approach where we compared resulting genotypes to confirm consensus genotypes and identify genotyping errors (Navidi et al. 1992, Taberlet et al. 1996, Frantz et al. 2003, Brinkman et al. 2010*a*). We chose this method because singleplex reactions were simpler to design and optimize than multiplex reactions. We used diluted 1:1 or undiluted DNA, depending on which concentration was successful for sexing. We added bovine serum albumin to all PCR mixtures, including those for sexing, to further relieve interference from amplification inhibitors (Kreader 1996, Kohn and Wayne 1997). We implemented a 2-step pre-amplification method to improve amplification success and reduce genotyping error by increasing the quality and quantity of the DNA template (Piggott et al. 2004, Hedmark and Ellegren 2006).

TABLE 1 Measures of genetic variability for 9 autosomal microsatellite markers including number of alleles (N), allele length in base pairs (length), expected heterozygosity (H_E), observed heterozygosity (H_O), polymorphic information content (PIC), and polymerase chain reaction success rate (success) used to genotype moose in northern Yellowstone National Park, USA (Dec 2013–Apr 2016). We provide the study references in which markers were used. We generated data from 514 genotypes included in genetic analysis using Cervus 3.0.7 (Field Genetics, London, United Kingdom) from samples of moose fecal pellets collected in Yellowstone National Park, USA, 2013–2016

Marker	N	Length	H_E	H_O	PIC	Success	References[a]
BM848	7	362–382	0.78	0.62	0.75	69	2,4,6,7,8
BM2830	3	127–131	0.39	0.38	0.32	97	1,4,5
Cervid14	3	235–239	0.42	0.37	0.34	97	2,4
CRFA	2	272–274	0.34	0.21	0.28	44	3,4
IGF-1	2	125–127	0.41	0.44	0.32	93	2,3,4
KCSN	3	210–216	0.41	0.30	0.34	46	3,4
NVHRT03	3	130–136	0.67	0.65	0.59	97	4
RT1	2	253–255	0.40	0.43	0.32	61	4,5,9
RT30	2	216–218	0.40	0.34	0.32	92	2,4,5,6,8,9,10,11
$\bar{x}$	3		0.47	0.42	0.40	77	

[a]References: [1]Broders et al. (1999), [2]Wilson et al. (2003), [3]Cronin et al. (2001), [4]Tjepkes (2015), [5]Schmidt et al. (2009), [6]Murray et al. (2012), [7]Finnegan et al. (2012), [8]Rea et al. (2016), [9]Wilson et al. (1997), [10]Wasser et al. (2011), [11]Kretser et al. (2016).

We used this method for 4 microsatellites for which we found it to increase PCR success rates: CRFA, KCSN, NVHRT03, and RT1. We ran PCRs for individual markers in triplicate and visualized products using an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA, USA). A single technician scored genotypes based on fragment lengths using GeneMarker software (version 2.6.0, Softgenetics LLC, State College, PA, USA). We identified a heterozygote at a locus if both alleles appeared at least twice, and a homozygote when 1 allele appeared in all successful repeats (Ebert et al. 2012). We conducted no further analysis for loci that showed consensus genotypes and repeated PCRs in triplicate for loci that produced either ambiguous alleles or no PCR product. We combined consensus loci genotypes to generate full consensus genotypes for each sample.

We calculated individual microsatellite PCR success rates as the proportion of times the microsatellite appeared in the consensus genotypes of all samples (n = 616). We report PCR success rates of individual microsatellites for all sessions, average PCR rate of all sessions, and compared rates between EW and LW samples in all sessions. We calculated per-locus genotyping error rates following the methods of Pompanon et al. (2005). Specifically, we defined the per-locus genotyping error rate as the proportion of single locus genotypes with one or both alleles scored incorrectly as compared to a reference genotype. We report per-locus error rates for all microsatellites for all sessions.

Genotype analysis

We used the R package ALLELEMATCH version 2.5 (Galpern et al. 2012) to identify unique genotypes, match genotypes across sampling occasions and sessions, and screen for genotyping errors. Critical to our methodology, ALLELEMATCH accommodated genotyping errors and missing allele data, and allowed the inclusion of metadata with genotypes. Metadata were ancillary attributes of pellet samples that appeared with each genotype in output files and served as non-genetic markers for comparing between genotypes. Many have cited the utility of metadata in genotype analysis to test for incongruities in genetic data, identify genotyping errors, and verify the geographic consistency of genetic results (Bellemain et al. 2005, Marucco et al. 2011, Bonner and Holmberg 2013, Augustine et al. 2018). Because of relatively low genetic variation in our study population (Table 1), metadata also helped us discern between different moose that had closely matching or identical genotypes.

We included 4 pieces of metadata, chosen for their discriminatory characteristics: geographic region where samples were collected (sampling region), average sample pellet volume (pellet volume), sample progestagen concentration (progestagen concentration), and date the sample was collected (sample date).

We divided the study area into 4 geographically distinct sampling regions (Figure 1) between which moose had a low probability of moving because of topographic features such as high mountains and deep river canyons, vast stretches of non-suitable moose wintering habitat, and deep snow at high elevations that separated them. Specifically, from a moose's perspective, Gardner's Hole was separated from regions to the east by vast stretches of regenerating lodgepole pine and sage brush, and deep snow at elevations >2,200 m; Blacktail Deer Plateau was separated from eastern regions by the Yellowstone River canyon, large expanses of sagebrush, and a high-elevation windswept and treeless plateau; and Slough and Soda Butte Creeks were separated by one another by a steep snow-covered mountain range reaching elevations of 3,000 m. In addition, moose have a high fidelity to their winter home ranges (Bailey and Franzmann 1983, Van Dyke et al. 1995). We assigned a sampling region to each sample collected. Because moose were essentially restricted to their chosen wintering habitats, we used sampling region to verify the geographic consistency of our genetic results under the assumption that an individual moose could only be sampled from a single sampling region during a given sampling occasion or session.

Pellet volume has been used to differentiate between age- and sex-classes of mule deer (Sánchez-Rojas et al. 2004), caribou (Ball 2010), reindeer (*R. tarandus platyrhynchus*; Morden et al. 2011), and moose (MacCracken and Van Ballenberge 1987). Pellet volume is determined by measuring dried pellets to the nearest 0.01 cm for maximum length, maximum width, and depth, and then calculating a pellet volume index (cm^3; MacCracken and Van

THE JOURNAL OF WILDLIFE MANAGEMENT

THE WILDLIFE SOCIETY

Ballenberge 1987, Morden et al. 2011). We strove to assign age class (calf or adult) to our samples based on pellet volume. To establish that volume differed between calf and adult age classes, we first determined pellet volume for 208 known-age samples, based on cementum annuli analysis and visual identification, from an on-going moose study (N. J. DeCesare, Montana Fish, Wildlife & Parks, personal communication). We oven-dried the most intact 8 of 10 pellets from each sample at 60°C for 48 hours and calculated the average pellet volume. For consistency, the same pair of technicians measured all pellets. We found that mean (± SD) pellet volume was greater ($P < 0.001$) for females (yearling and older; 7.6 ± 1.59 cm^3, $n = 176$) than for calves (4.9 ± 0.86 cm^3, $n = 32$; Figure 2A). Next, using

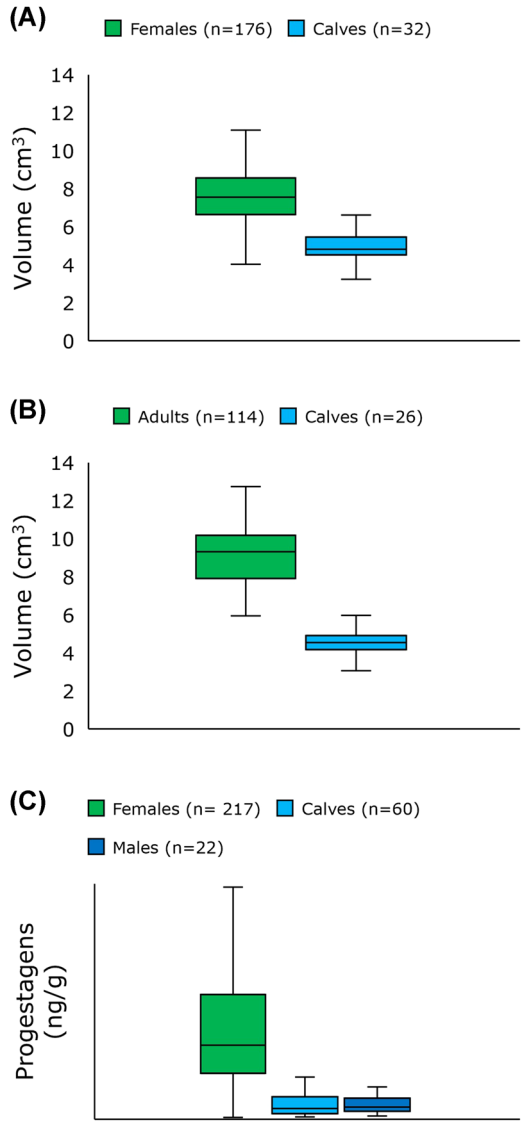

FIGURE 2 Variation in pellet volume (cm^3) and progestagen concentration (ng/g) between moose age and sex classes (Dec 2013–Apr 2016). A) Difference in pellet volume between adult females and calves, Montana, USA; B) difference in pellet volume between either-sex adults and either-sex calves, northern Yellowstone National Park, USA; and C) difference in progestagen concentration between adult females (yearling or older), either-sex calves, and males, northern Yellowstone National Park

the same methods we determined volume for 140 known-age samples, based on visual identification, from this YNP study and found similar differences. Mean pellet volume was greater ($P < 0.001$) for either-sex adults (yearling or older; 9.1 ± 1.67 cm^3, $n = 114$) than for either-sex calves (4.6 ± 0.81 cm^3, $n = 26$; Figure 2B).

Based on the 2 analyses, we concluded that pellets with volumes <6.0 cm^3 (the approximate mid-point between mean adult and calf pellet volume) were most likely deposited by calves and those >6.0 cm^3 were deposited by adults. Finally, we determined pellet volume for all measurable YNP study pellet samples ($n = 568$) and designated them as either calf or adult. We then used pellet volume to help discern between similar genotypes or confirm them as matches. We also noted a trend during the study where pellet volumes were generally smaller in LW as compared to EW, which we hypothesized was due to a switch of diet from willow to upland conifer vegetation for many of the study moose or a reduction in forage intake. We also considered this trend during genotype analysis that an individual moose would commonly have larger pellets in EW as compared to LW.

Progestagens are steroid hormones that function to maintain pregnancy in mammals and in pregnant individuals rise during the pregnancy cycle and remain elevated until parturition in cervids (Asher 2011) including moose (Schwartz et al. 1995). We determined fecal progestagen concentration (ng/g dry weight) using enzyme-immunoassay at the Smithsonian Conservation Biology Institute in Front Royal, Virginia, following methods of Scarlata et al. (2011) for all YNP female (older than a calf) samples and a random sampling from either-sex calves and males (older than a calf). We previously determined sample sex through genetic testing and assignment of age class designation from morphometric pellet analysis. We also visually confirmed some sex and age classes. Analysis of 299 pellet samples indicated that progestagen concentrations differed between sex and age classes of moose. The mean concentration (±SD) was higher for females (3,591 ± 2,188.19 ng/g, $n = 217$) than for males ($P < 0.001$, 677 ± 526.18 ng/g, $n = 22$), and for either-sex calves ($P < 0.001$, 645 ± 521.69 ng/g, $n = 60$; Figure 2C). Based on these variations, we used progestagen concentration to help discern between similar genotypes or confirm them as matches. Specifically, the more similar the progestagen concentrations were between 2 closely matching genotypes, the more confidently we could conclude that they were from the same moose. Progestagen concentration would also help us differentiate between female and male moose genotypes in instances where genetic sexing was inconclusive.

We used sample date (year, month, day) to verify the temporal consistency of our genetic analysis under the assumptions that 2 samples collected on the same day at a distance apart exceeding that which a moose could reasonably move in a single winter day could not have been deposited by the same moose, samples collected on the same day in different sampling regions could not have been deposited by the same moose, and genotypes from calf samples collected in one year could not match genotypes from adult samples collected in previous years. Because ALLELEMATCH accommodated only 1 metadata column, we combined metadata into a single data string for each sample genotype. For example, metadata for sample ID 21 (Soda Butte Creek, 8.4 cm^3, 2,948 ng/g, 131221) was entered as SB_8.4_2948_131221.

We included moose sex in the genotypes for each sample to increase the discriminatory power of our suite of microsatellites to 10 genetic markers (Brinkman et al. 2010*a*, Galpern et al. 2012). We included in ALLELEMATCH analysis all samples that successfully genotyped at ≥6 markers plus those that were successful at 5 markers and for which we had all 4 pieces of metadata (Data File S1_Allelematch Input, available in Supporting Information). Of the latter group, males and calves were not required to have progestagen concentration. We considered all other samples as poor quality and removed them from further analysis following Paetkau (2003). Removing genotypes within a given threshold of missing data also reduces ambiguity in the dataset when performing genotype analysis (Galpern et al. 2012).

We identified unique genotypes and their matches in a 4-step process by manually reviewing ALLELEMATCH.html output generated using 4 preset alleleMismatch parameters (a criterion of dissimilarity that determines the maximum number of alleles that can mismatch for 2 genotypes to be declared identical; Galpern et al. 2012): 0, 1, 2, and 3. Reviewing this range of output enabled us to find the matches for the multipleMatch genotypes (those that matched >1 unique genotype; Galpern et al. 2012) that existed in our dataset because of missing allele data, residual genotyping error, and low genetic diversity of the study population. We generated

match probability (P_{sib}) values for all genotypes even if they were missing allele data using the doP_{sib} = all command (Galpern et al. 2012). We reviewed all matching genotype groups from HTML output tables in order from 0 to 3 alleleMismatch analyses. We accepted genotypes as matching based on a combination of the P_{sib} value and whether metadata were consistent with the match as previously described.

We accepted matches from 0 alleleMismatch analysis when $P_{sib} \leq 0.1$ and metadata were consistent with the match. We chose this more conservative P_{sib} threshold to accommodate comparison between genotypes with multiple missing or mismatched alleles. We put matching genotypes from this analysis into groups. We accepted matches from 1 alleleMismatch analysis using the 0 alleleMismatch criteria and either combined them with an existing 0 alleleMismatch group in which ≥1 of the 1 alleleMismatch matches already existed or assigned matches as a new matching group. We accepted matches from 2 and 3 alleleMismatch analyses using the same criteria. We classified genotypes that did not match any others based on these criteria as singleton genotypes. We also compared the number of individual genotypes identified using our methodology with metadata to the number identified by ALLELEMATCH without metadata. See Supporting Information for an example of how metadata are used in genotype analysis (Using Metadata in Genotype Analysis.pdf).

Statistical analysis

We used the secr package (Efford 2020) in program R (R Core Team 2020) to estimate sex-specific moose density, baseline detection probability (g0), and scaling parameter (σ), an approximation of space use. Models in secr employ a maximum likelihood-based approach to select the best models by comparing Akaike's Information Criterion corrected for small sample size (AIC_c; Burnham and Anderson 2002) and have been used for numerous noninvasive studies of mammal populations (Sarmento et al. 2014, Morehouse and Boyce 2016, Keiter et al. 2017), including deer (Goode et al. 2014, Brazeal et al. 2017, Furnas et al. 2018, Ebert et al. 2021). We started the analysis by discretizing the sampling area using a *post hoc* grid cell size of 2.25 km^2 (the square of half the mean maximum distance moved), resulting in 270 proximity-type traps (Figure 3). Although smaller than previously reported home range sizes for moose in the study area (Tyers 2003), we chose this grid cell size to ensure that even the smallest moose home ranges would include a grid cell center (Wallace et al. 2003), allow for heterogeneity in the spatial organization of individuals (Thompson et al. 2012), and maximize both the number of unique individuals sampled and the number of spatial recaptures of individuals (Royle et al. 2013). This discretization of the study area had been implemented in other unstructured spatial sampling study designs where encounters did not arise from stationary trap locations (Russell et al. 2012, Thompson et al 2012). We buffered the trapping grid by 6 km (2 times the full mean maximum distance moved) to generate a 2,439-km^2 statespace (Figure 3). Considering knowledge from past distribution surveys of moose in northern YNP (Barmore 2003, Tyers 2003), the buffer was sized so that the resulting statespace was large enough to include all moose with a non-negligible probability of being detected (Royle et al. 2013). Additionally, this buffered area approximated the maximum extent of available winter habitat used by moose in northern YNP. We built session- and occasion-specific moose encounter histories by assigning genotypic detections to the center point of the grid cell within which they were collected and designated samples into 1 of 2 sampling occasions (EW or LW) within 1 of 3 sessions (years; Data File S2_Encounter History, available in Supporting Information). We assigned females and their dependent calves as separate individuals. We included genotypes from samples (either pellet or tissue) recovered from the carcasses of dead moose to effectively restrict detection probability to zero for these individuals in subsequent sampling occasions.

We developed biologically relevant models to estimate sex-specific moose density and sex- and occasion-specific g0 and σ. We modeled each session separately and estimated parameters as a function of occasion, sex, or as constant (1). We estimated density of each sex independently and then combined estimates for total density (Morehouse and Boyce 2016). We included sex as a model covariate because sex ratios within moose populations can be highly variable (Van Ballenberghe and Ballard 2007) and because known differences in winter home range

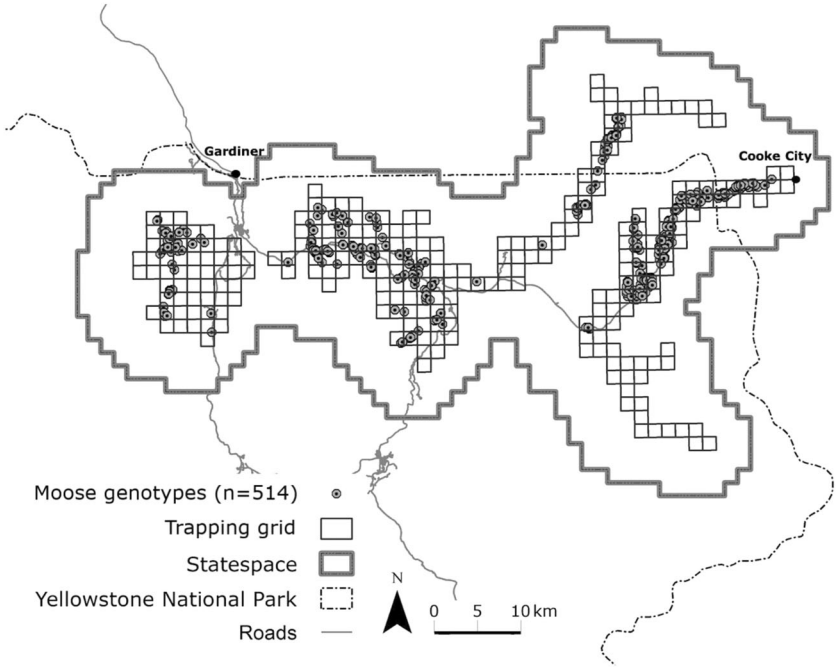

FIGURE 3 The northern Yellowstone National Park, USA, study area showing the trapping grid, moose sample locations (n = 514), and statespace (Dec 2013–Apr 2016)

sizes between female and male moose in the study area (Tyers and Irby 1995) would likely reflect differences in g0 and σ between sexes. We used the exponential function to explain the decomposition of g0 as estimated activity centers are mapped farther from trap centers (Efford 2004, Royle et al. 2013). The exponential detection function takes the form

$$g(d) = g0\, e^{\left(\frac{-d}{\sigma}\right)},$$

where $g0$ represents baseline detection probability, σ is the spatial scaling parameter, and d is defined as the distance between an individual's activity center and a trap. Further, we incorporated spatial variation in sampling by assigning occasion-specific sampling effort values to each grid cell using the kilometers traveled during sampling surveys (Russell et al. 2012, Thompson et al. 2012, Efford et al. 2013; Data File S3_Trap Locations and Survey Effort, available in Supporting Information). We obtained estimates of density (moose/km^2), g0, and σ by averaging models within Δ10 AIC$_c$ of the top model using the model.average function in the package (Burnham and Anderson 2002, Efford 2020). We estimated session- and sex-specific moose abundances by multiplying densities by the area of the statespace. We calculated annual and average population sex ratios (males/female), which included calves, from sex-specific abundance estimates.

RESULTS

Over the 6 sampling occasions, a pair of technicians averaged 22 field days and skied 15.5 km per day to survey 341 km of survey transect and totaled 130 field days and 2,047 km of transect surveyed over the entire study (Table S2, available in Supporting Information). We collected 616 genetic samples including 614 fecal pellet samples and 2

tissue samples. Of the 616 samples, 82% (507) amplified at ≥6 out of 10 genetic markers and 20% (124) at all 10 (Figure 4). We included 514 of the 616 sample genotypes in ALLELEMATCH analysis. Of 514 genotypes, 495 (96%) were successful at ≥6 of 10 markers and 19 (4%) at 5 markers. We detected 71 individuals in 2013 (34 males, 37 females), 63 in 2014 (29 males, 34 females), and 76 in 2015 (39 males, 37 females; Table S3, available in Supporting Information). We identified 142 individual moose (69 males, 73 females) over the course of the study (See Data File S4_142 Moose, available in Supporting Information, to see how 514 sample IDs were assigned to 142 moose groups). We sampled individuals an average of 3.6 times with 32.4% (n = 46) detected once, 53.5% (n = 76) detected 2–6 times, and 6.3% (n = 9) detected ≥11 times (Figure S1, available in Supporting Information). Discriminatory statistics for individual microsatellites, based on 514 sample genotypes included in ALLELEMATCH, suggested relatively low genetic diversity within the study population with an average of 3 alleles, mean H_E of 0.47, mean H_O of 0.42, and mean PIC of 0.40 (Table 1).

Top models differed across years (Table S4, available in Supporting Information). Model averaging yielded sex-specific moose density estimates averaging 0.034 females/km^2 and 0.033 males/km^2 with a combined density ranging from 0.062–0.076 moose/km^2 (Table 2). Abundance estimates were 150 moose in 2013, 186 in 2014, and 160 in 2015 (Table 2). Detection probability (g0) varied by occasion with EW probabilities greater than LW probabilities by a factor of 2.9, 5.9, and 1.6 in 2013, 2014, and 2015, respectively, but not by sex (Table 2). Scaling parameter (σ) did not vary by sex but varied by year, with values greater in LW as compared to EW in 2013 and 2014 and greater in EW as compared to LW in 2015 (Table 2). The population sex ratio (males/female), as derived from sex-specific population estimates (Table 2), was 0.97, 0.96, and 1.03 in 2013, 2014 and 2015, respectively and averaged 0.99. See Table S5 (available in Supporting Information) for moose density, g0, and σ estimates for the top 5 models from each session.

Individual microsatellite PCR success ranged from 44–97% and averaged 77% for the 514 samples included in genotype analysis (Table 1). Success of the SE47/SE48 sex primer pair was 93%. Average PCR success combining microsatellites with the sex primer pair (10 total markers) was 79%. Average PCR success rate of 9 autosomal microsatellites (n = 616 samples) was greater in 2013 (85%) as compared to 2014 (54%) and 2015 (59%; Table 3). This reduction in 2014 and 2015 was a result of reduced success of 4 microsatellites (BM848, CRFA, KCSN, and RT1), which averaged 86% in 2013 but 56% and 60% in 2014 and 2015, respectively. Success rates also varied by occasion for all sessions with LW rates lower than EW rates. Success rates dropped from EW to LW: 93% to 78% in 2013, 61% to 44% in 204, and 67% to 50% in 2015. Average per-locus genotyping error rates varied by session increasing from 8% in 2013 to 19% in 2015 (Table S6, available in Supporting Information). In addition, we found

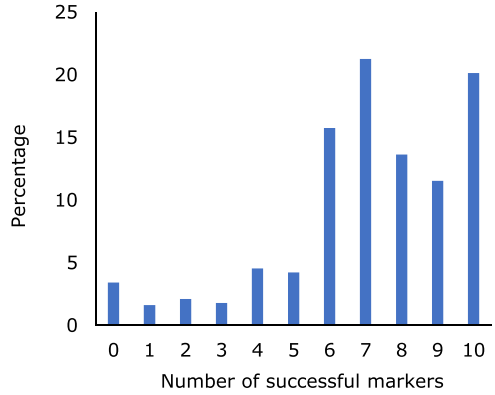

FIGURE 4 Percentage of 616 moose genetic samples from northern Yellowstone National Park, USA, that were successfully genotyped at 0 to 10 microsatellite markers (Dec 2013–Apr 2016)

TABLE 2 Population parameter estimates for moose in northern Yellowstone National Park, USA, for 3 winter sampling sessions (2013–2015) including sex-specific density (moose/km^2) and abundance (*N*), and sex- and occasion-specific detection probability (g0) and scaling parameter (σ) in kilometers. Occasions were early-winter (EW) and late-winter (LW). We derived abundance by multiplying density by 2,439 km^2 (statespace). We obtained estimates by averaging models within 10 Akaike's Information Criterion of the top model using the model.average function in the R package secr (Efford 2020)

Session	Sex	Density (SE)	95% CI	*N* (SE)	95% CI	Occasion	g0 (SE)	95% CI	σ (SE)	95% CI
2013	Female	0.031 (0.005)	0.022–0.043	76 (13)	55–105	EW	0.091 (0.033)	0.044–0.180	2.2 (0.4)	1.5–3.1
						LW	0.032 (0.012)	0.015–0.068	3.5 (0.8)	2.3–5.3
	Male	0.030 (0.005)	0.022–0.042	74 (12)	54–103	EW	0.090 (0.035)	0.041–0.185	2.2 (0.4)	1.5–3.3
						LW	0.031 (0.012)	0.015–0.065	3.6 (0.7)	2.4–5.3
	Total	0.062 (0.010)	0.044–0.085	150 (25)	109–207					
2014	Female	0.039 (0.008)	0.026–0.058	95 (20)	63–142	EW	0.094 (0.033)	0.047–0.180	2.5 (0.5)	1.7–3.6
						LW	0.016 (0.009)	0.005–0.048	3.1 (0.9)	1.7–5.5
	Male	0.037 (0.008)	0.025–0.057	91 (20)	60–138	EW	0.089 (0.030)	0.045–0.168	2.5 (0.5)	1.7–3.6
						LW	0.015 (0.008)	0.005–0.045	3.1 (0.9)	1.8–5.5
	Total	0.076 (0.016)	0.051–0.115	186 (40)	123–280					
2015	Female	0.033 (0.005)	0.024–0.044	79 (13)	58–108	EW	0.082 (0.021)	0.049–0.133	2.8 (0.4)	2.1–3.6
						LW	0.052 (0.024)	0.021–0.125	2.1 (0.5)	1.4–3.3
	Male	0.033 (0.005)	0.024–0.045	81 (13)	59–110	EW	0.084 (0.021)	0.051–0.135	2.7 (0.4)	2.1–3.6
						LW	0.053 (0.025)	0.021–0.129	2.1 (0.5)	1.3–3.3
	Total	0.066 (0.010)	0.048–0.089	160 (26)	117–218					

TABLE 3 Per session (2013–2015) and average microsatellite polymerase chain reaction (PCR) success rates for 616 genetic samples from moose in northern Yellowstone National Park, USA (Dec 2013–Apr 2016). Success rates were based on 241 consensus genotypes from 2013, 161 from 2014, and 214 from 2015

	PCR success rates			
Marker	2013	2014	2015	Average
BM848	0.88	0.59[a]	0.28[a]	0.58
BM2830	0.98	0.81	0.90	0.90
Cervid14	0.93	0.80	0.86	0.86
CRFA	0.78	0.01[a]	0.20[a]	0.33
IGF-1	0.78	0.81	0.88	0.82
KCSN	0.83	0.19[a]	0.08[a]	0.37
NVHRT03	0.91	0.84	0.95	0.90
RT1	0.80	0.20[a]	0.46[a]	0.49
RT30	0.84	0.77	0.82	0.81
$\bar{x}$	0.86	0.56[a]	0.60[a]	0.67

[a]Notable reduction in PCR success following the first year.

longer microsatellite fragments were more prone to amplification failure than shorter ones (Figure S2, available in Supporting Information).

DISCUSSION

Our density estimates of moose in northern YNP were similar to other Shiras moose populations such as the Jackson herd (0.057–0.085 moose/km^2), which has declined precipitously in the last 2 decades (A. B. Courtemanch, Wyoming Game and Fish Department, unpublished report), and some Utah, USA, populations (0.010–0.075 moose/km^2) that are known to occupy marginal habitat (Wolfe et al. 2010). Our estimates were lower than those of expanding populations in eastern Washington, USA (0.33–0.67 moose/km^2; Oyster et al. 2018), and North Park in Colorado, USA (0.12 moose/km^2; Yost 2008). They were also similar to estimates for moose in northern YNP generated by Barmore (2003), 0.03–0.20 moose/km^2, between 1967–1970 from data collected while censusing elk. Caution should be used, however, when comparing our density estimates to others because they were generated using different methods and so may not be directly comparable. Our findings of low population density of moose in northern YNP were expected because the availability of suitable habitat is limited. Much of the region is dominated by sagebrush-steppe vegetation and scattered stands of Douglas-fir, making it more suitable for elk, bison, and deer. Mature and old-growth conifer forests, the most important component of mid- and late-winter moose habitat, had been greatly reduced by the 1988 and successive forest fires and will not regenerate for centuries (Tyers 2003). Furthermore, large populations of elk and bison will likely persist on the landscape and continue to limit available willow forage for moose (Singer et al. 1994, Painter and Ripple 2012). Under these conditions, moose in northern YNP will likely persist at low density. Our abundance estimates were consistent with anecdotal northern YNP population estimates of approximately 200 moose.

Because our study population was considered naturally regulated, we expected a balanced population sex ratio similar to that reported for adult moose in a naturally regulated population on Isle Royale (Peterson 1977). This balanced sex ratio should benefit moose in northern YNP because high or stable adult moose sex ratios are

positively associated with population growth (Arsenault et al. 2019). Whereas our methods were not designed to estimate population growth rates, greater abundance estimates in both 2014 and 2015 compared to 2013 suggest an increasing trend in moose population size in northern YNP and support anecdotal evidence of population increase as reported moose sightings in the study area have become more commonplace over the last 2 decades.

We found detection probabilities for both sexes in all sessions were greater in EW occasions compared to LW occasions. Probabilities did not vary significantly between sexes within sessions. These findings were consistent with 2 observations: moose concentrated in lower elevation riparian willow ecosystems during late-December and early-January making them more accessible to EW sampling and increasing snow depths starting in late-January compelled moose to move into the protective cover of mature conifer forests or to lower elevations along the Yellowstone River and made them less accessible to LW sampling. Additionally, melting snow conditions in LW made locating moose and moose pellets for sampling more difficult.

Findings of greater detection probabilities in EW were consistent with σ estimates from 2015, which were also greater in EW as compared to LW. Presumably, technicians were more likely to cross tracks and collect samples from moose exhibiting greater space use in EW than in LW when moose were occupying high-elevation forests and presumably smaller home ranges. Conversely, σ estimates from 2013 and 2014 were greater in LW compared to EW. It is plausible that the 2014 LW estimates were higher because an early snow melt allowed moose to return to lower elevation habitats sooner, increasing their chance of being sampled. This same reasoning, however, does not explain greater estimates in LW 2013 when snow remained throughout much of the study area until the end of April. Despite research that has documented smaller home ranges for females than for male moose (Tyers and Irby 1995, Hundertmark 2007), we found similar σ values between sexes. We hypothesize that the greater space use of barren adult females and presumably yearling females without calves, compared to females with calves that experienced restricted mobility in deep snow conditions, may have contributed to these more similar σ values between sexes.

Because we sampled in the winter and because moose show high wintering habitat fidelity, our study population was subjected to minimal violations of closure assumptions. Violations of geographic closure (no emigration and immigration) were minimal because topographic features such as high mountains and deep snow surrounding most of the study area restricted moose movement to and from the study area. The only corridor where winter movement was possible was along the Yellowstone River north of the park, where lower elevation ranch land and developed residential areas offered poor wintering habitat. Violations of demographic closure were minimal because no moose were added by births or lost to hunting. In addition, because wintering moose in northern YNP often occupied higher elevations with deeper snow than the wolves' preferred prey species (elk and deer), moose were more spatially removed from predation risk. We did, however, document the mortality of 3 moose: 2 were killed by wolves and 1 drowned in a lake. Future studies should consider the effects of natural mortality and predation on demographic closure, especially in predator-rich study areas.

Supporting our assertion that moose had a low probability of moving between sampling regions, we found based on genetic results that 3 of 142 moose (2%) were detected in multiple sampling regions over the course of the study. Only 1 of these moose (<1%) was identified in different regions within the same winter occasion. Over the course of 11 days, this moose traveled 23 km between adjacent regions. While it seems unlikely that moose would travel these distances during the winter in such a short time interval, similar mid-winter movements have been documented following moose encounters with wolves (R. O. Peterson, Michigan Technological University, personal communication).

When included with each sample genotype in ALLELEMATCH, metadata proved to have great discriminating power and were critical for genotype analysis. The number of unique genotypes identified by ALLELEMATCH compared to the number identified using our methods with metadata, demonstrated this. ALLELEMATCH identified 456, 425, 326, and 208 unique genotypes, from 0, 1, 2, and 3 alleleMismatch analyses, respectively. After careful review of HTML output tables from these analyses using our methodology with integrated metadata, we identified 142 unique genotypes. Our methods using metadata were particularly useful for discerning between similar

genotypes that would be assigned as matches based on ALLELEMATCH genotype analysis alone but were from different individuals. Such matches were possible because of the low genetic diversity of our study population. For example, in one instance the genotype from sample ID 95 perfectly matched at all 10 markers with genotypes from 3 other samples collected in the same sampling region and during the same occasion (Table S7, available in Supporting Information). We initially scored these 4 as matches based on our 0 alleleMismatch criteria; however, a 50% greater pellet volume and 3-fold larger progestagen concentration of sample ID 95 confirmed that it was deposited by a pregnant female. The other 3 samples were from a female calf. Although our methodology was labor intensive, it allowed us to confidently identify unique genotypes and their matches and to include sample genotypes with as few as 5 of 10 successful genetic markers in genotype analysis.

Our average 2013 PCR success rate was comparable to those from other winter fecal-based studies of ungulates (Maudet et al. 2004, Luikart et al. 2008, Ebert et al. 2012), but our 2014 and 2015 rates were lower. Per-locus genotyping error rates for all microsatellites across all sessions were higher than other reported winter rates for ungulates (Maudet et al. 2004, Luikart et al. 2008, Hettinga et al. 2012).

Nucleic acids are prone to natural degradation as they undergo chemical changes and strands become fragmented (Lindahl 1993, Buś and Allen 2014). Environmental conditions such as moisture and warm temperatures can accelerate degradation by promoting the growth of DNA-degrading bacteria and fungi (Regnaut et al. 2006, Thacker et al. 2006, Buś and Allen 2014). Rain can wash DNA from pellet surfaces and cause pellets to dissolve (Brinkman et al. 2011). The greater PCR failure and genotyping error that occurred for LW samples as compared to EW samples during all sessions, even for pellets that were deposited within 24 hours, was likely attributable to greater DNA-degrading conditions in LW. Numerous studies using fecal DNA have similarly documented lower PCR success and higher genotyping error rates under such degrading conditions (Murphy et al. 2007, Brinkman et al. 2010*b*). Recordings of high average maximum temperatures and low average snow depths in LW occasions (8°C, 35 cm) compared to EW occasions (−4°C, 52 cm) over the course of the study, is consistent with these findings (data from Silvergate, MT weather station; National Centers for Environmental Information [NCEI] 2018). We also observed considerably lower PCR success of 2014 and 2015 LW samples compared to 2013 LW samples. We hypothesize that higher average daily maximum temperatures and lower average snow depths in April of 2014 (9°C and 3 cm) and 2015 (9°C and 18 cm) compared to 2013 (5°C and 84 cm) contributed to these differences (NCEI 2018).

Variation in the qualities of seasonal forage available to ungulates affects the amount of intestinal cells sloughed off on pellet surfaces, and in turn the quantity of DNA in the sample extracts. It is assumed that more coarse, abrasive, and higher-cellulose-content winter forage passes through the intestine more slowly than forage available in other seasons and so promotes a greater accumulation of intestinal cells on pellet surfaces (Maudet et al. 2004). Consequently, researchers have found higher PCR success and lower genotyping error rates using ungulate fecal samples collected in colder months (Maudet et al. 2004, Luikart et al. 2008, Harris et al. 2010), as did Rea et al. (2016) with moose. Indeed, prior studies of northern Yellowstone moose diets found higher crude fiber content in willow compared to subalpine fir (Tyers 2003). From tracking moose, we determined that EW diets were composed predominately of coarse willow browse, whereas LW diets were composed more of regenerating subalpine fir, upland deciduous shrubs, and arboreal lichen. We hypothesize that this seasonal difference in diet may also have contributed to greater EW PCR success rates compared to LW rates.

Other factors can affect PCR amplification and genotyping error after samples are collected. Fecal DNA is subject to degradation if samples are not preserved properly (Panasci et al. 2011). Our preservation method of storing pellets at −20°C was suitable for samples that we collected frozen and stored in the freezer the same day but not for pellets that we collected unfrozen and that remained unfrozen for several days. This occurred in LW during multi-day back country sampling trips. We found that DNA in many of these samples, even if collected <24 hours after deposition and formed of a coarse willow diet, failed to amplify. Under such conditions we recommend storing pellets in 95% ethanol following Fernando et al. (2003) and others who have had success with this method for ungulate pellets (Luikart et al. 2008, Harris et al. 2010, Brinkman et al. 2011). Research has also shown that

amplification success decreases with time of storage (Roon et al. 2003). Our 2014 and 2015 samples were subjected to longer storage periods in our lab and had greater PCR amplification failure and genotyping error than our 2013 samples. To reduce potential problems associated with extended storage, we suggest extracting DNA within 6 months. Because we observed, like Buchan et al. (2005), that longer microsatellite fragments were more prone to amplification failure than shorter ones, we recommend that researchers focus on using microsatellites with the shortest possible amplicon lengths.

We also found evidence that pre-amplification of 3 microsatellites (CRFA, KCSN, and NVHRT03) may have contributed to lower PCR success rates (Table 3). We hypothesize that the overall higher concentration of DNA (both template and non-template) in extracts following pre-amplification may have led to DNA binding magnesium chloride to stabilize itself and thereby reducing its availability to the Taq polymerase, a magnesium-dependent enzyme (Lorenz 2012). This higher concentration of DNA could also have led to the primers being exhausted by template DNA before enough copies were amplified. Pre-amplification may also have caused non-specific amplification patterns making scoring alleles difficult. Our results, however, cannot explain why a fourth microsatellite (RT1), also subjected to pre-amplification, suffered no significant reduction in PCR amplification success, and that BM848, not subjected to pre-amplification, had greatly reduced PCR success after the first year. As others have questioned the value of pre-amplification (De Barba and Waits 2010), we suggest researchers use caution when considering its incorporation into lab protocols.

Based on our experience and findings, especially that detection probability of moose and PCR rates were higher in all EW sampling occasions compared to LW occasions, we recommend that pellet samples be collected during early-winter when pellets remain frozen and are less susceptible to DNA degradation; higher cellulose diets promote the accumulation of intestinal cells on pellet surfaces; persistent snow conditions allows for tracking, locating pellets, and determining time since pellet deposition; and moose are more concentrated at lower elevations and therefore more accessible to sampling. When establishing sampling dates, researchers should also consider that continued global warming trends will likely contribute to narrower winter sampling windows. While we did not find sex-specific variation in parameter estimates as expected based on documented difference in behavior and winter habitat use between female and male moose (Tyers and Irby 1995, Bubenik 2007), we support the use of sex-specific models for future studies of moose as parameter estimates are likely to differ between sexes in other study areas. Because annual variation in winter snowfall and temperature at times limited our ability to locate moose tracks and collect samples, and density estimates varied annually, we recommend applying these methods over multiple years. Given the physical demands and challenging winter conditions of the field work, and the high sampling effort required to guarantee a high recapture rate (Marucco et al. 2012), we recommend enlisting self-reliant and physically fit technicians who are competent back-country travelers. Technicians should be knowledgeable about moose winter ecology, experienced wildlife trackers, and familiar with the study area. Furthermore, we recommend that researchers select similar suites of microsatellite markers as other moose studies in their regions, which will promote cooperative refinement and optimization of PCR protocols for individual microsatellites and ultimately result in reduced lab costs for moose genetic studies, allow for genetic comparison between study populations, and promote collaboration between researchers.

CONSERVATION IMPLICATIONS

Robust population density and abundance estimates are important for effective moose conservation and management, particularly in North America where moose populations are experiencing wide-spread decline. These results show that SECR analysis of genotypes derived from noninvasively collected fecal pellets, coupled with the inclusion of metadata in genotype analysis, is a viable alternative method for estimating winter population parameters of moose, especially in areas where dense forests and mountainous terrain make aerial survey methods ineffective. Additionally, our methods can improve population estimates in genetic studies where appreciable

genotyping error is expected and are applicable to population studies of other ungulates that are difficult to monitor. These methods are most suited for long-term monitoring of moose populations that occupy discrete wintering habitats that are a permanent part of the landscape as opposed to those using transient winter habitats associated with forest fires and timber harvest.

ACKNOWLEDGMENTS

We thank D. W. Smith, K. A. Gunther, D. R. Stahler, E. A. Stahler, M. C. Metz, K. A. Cassidy, B. J. Cassidy, S. Sigler, R. A. Renkin, and K. Loveless for advice and logistical help; YNP Wolf and Cougar Project crews, N. Christensen, G. Glynn, E. McCain, and Ecology Project International for help with sample collection; D. B. Tyers for support and guidance; N. J. DeCesare for his collaboration; B. Chan, T. Schwartz, G. Gassman, K. Dooley, R. Feigley, W. Allen, and J. Zimmer for help with backcountry logistics; N. Boisseau, J. Brown and C. Chamberlin for their expertise; T. Pinky and A. K. Throop for GIS assistance; L. and D. Lyman for their hospitality; and pilots R. Stradley and S. Ard for safe flying. We also thank R. O. Peterson, D. W. Smith, D. R. Stahler, N. J. DeCesare, and S. R. Hoy for manuscript review and comments. Funding was provided by the University of Minnesota-Duluth Biology Department, the University of Wyoming/NPS Small Grant Program, the Campfire Club of America, S. and C. Heppel, A. B. Heath, R. and J. Playter, and R. B. and K. B. Koitzsch.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICS STATEMENT

Sampling protocols for Study #YELL-06000 were reviewed and approved by the National Park Service.

DATA AVAILABILITY STATEMENT

Data available in article supplementary material.

REFERENCES

Anderson, C. R, Jr., and F. G. Lindzey. 1996. Moose sightability model developed from helicopter surveys. Wildlife Society Bulletin 24:247–259.

Arsenault, A. A., A. R. Rodgers, and K. Whaley. 2019. Demographic status of moose populations in the boreal plain ecozone of Canada. Alces 5:43–60.

Asher, G. W. 2011. Reproductive cycles of deer. Animal Reproduction Science 124:170–175.

Augustine, B. C., J. A. Royle, M. J. Kelly, C. B. Satter, R. S. Alonso, E. E. Boydston, and K. R. Crooks. 2018. Spatial capture–recapture with partial identity: an application to camera traps. Annals of Applied Statistics 12:67–95.

Bailey, T. N., and A. W. Franzmann. 1983. Mortality of resident vs. introduced moose in confined populations. Journal of Wildlife Management 47:520–523.

Ball, M. C. 2010. Faecal pellet size can be used to differentiate age-classes in caribou: implications for non-invasive genetic studies. Conservation Genetics Resources 2:239–241.

Banks, S. C., S. D. Hoyle, A. Horsup, P. Sunnucks, and A. C. Taylor. 2003. Demographic monitoring of an entire species (the northern hairy-nosed wombat, Lasiorhinus krefftii) by genetic analysis of non-invasively collected material. Animal Conservation 6:101–107.

Barmore, W. J. 2003. Ecology of ungulates and their winter range in northern Yellowstone National Park: research and synthesis, 1962–1970. Yellowstone Center for Resources, Mammoth Hot Springs, Wyoming, USA.

Bellemain, E., J. E. Swenson, D. Tallmon, S. Brunberg, and P. Taberlet. 2005. Estimating population size of elusive animals with DNA from hunter-collected feces: four methods for brown bears. Conservation Biology 19:150–161.

Berger J., J. W. Testa, T. Roffe, and S. L. Monfort. 1999. Conservation endocrinology: a non-invasive tool to understand relationships between carnivore colonization and ecological carrying capacity. Conservation Biology 13:980–989.

Bonner, S., and J. Holmberg. 2013. Mark-recapture with multiple non-invasive marks. Biometrics 69:766–775.

Bowden, D. C., and R. C. Kufeld. 1995. Generalized mark-sight population size estimation applied to Colorado moose. Journal of Wildlife Management 59:840–851.

Boyce, M. S., P. W. Baxter, and H. P. Possingham. 2012. Managing moose harvests by the seat of your pants. Theoretical Population Biology 82:340–347.

Boyce, M. S., and R. Corrigan. 2017. Moose survey app for population monitoring. Wildlife Society Bulletin 41:125–128.

Brazeal, J. L., T. Weist, and B. N. Sacks. 2017. Noninvasive genetic spatial capture-recapture for estimating deer population abundance. Journal of Wildlife Management 81:629–640.

Brinkman, T. J., and K. J. Hundertmark. 2009. Sex identification of northern ungulates using low quality and quantity DNA. Conservation Genetics 10:1189–1193.

Brinkman, T. J., D. K. Person, F. S. Chapin, W. Smith, and K. J. Hundertmark. 2011. Estimating abundance of Sitka black-tailed deer using DNA from fecal pellets. Journal of Wildlife Management 75:232–242.

Brinkman, T. J., D. K. Person, M. K. Schwartz, K. L. Pilgrim, K. E. Colson, and K. J. Hundertmark. 2010*a*. Individual identification of Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) using DNA from fecal pellets. Conservation Genetics Resources 2:115–118.

Brinkman, T. J., M. K. Schwartz, D. K. Person, K. L. Pilgrim, and K. J. Hundertmark. 2010*b*. Effects of time and rainfall on PCR success using DNA extracted from deer fecal pellets. Conservation Genetics 11:1547–1552.

Broders, H. G., S. P. Mahoney, W. A. Montevecchi, and W. S. Davidson. 1999. Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces*, in Canada. Molecular Ecology 8:1309–1315.

Brøseth, H., Ø. Flagstad, C. Wärdig, M. Johansson, and H. Ellegren. 2010. Large-scale noninvasive genetic monitoring of wolverines using scats reveals density dependent adult survival. Biological Conservation 143:113–120.

Bubenik, A. B. 2007. Behavior. Pages 173–221 *in* A. W. Franzmann and C. C. Schwartz, editors. Ecology and management of the North American moose. Second edition. University Press of Colorado, Boulder, USA.

Buchan, J. C., E. A. Archie, R. C. Van Horn, C. J. Moss, and S. C. Alberts. 2005. Locus effects and sources of error in noninvasive genotyping. Molecular Ecology Notes 5:680–683.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel interference: a practical information-theoretic approach. Second edition. Springer-Verlag, New York, New York, USA.

Buś, M. M., and M. Allen. 2014. Collecting and preserving biological samples from challenging Environments for DNA analysis. Biopreservation and Biobanking 12:17–22.

Cronin, M. A., J. C. Patton, R. Courtois, and M. Crête. 2001. Genetic variation of microsatellite DNA in moose in Québec. Alces 37:175–187.

De Barba, M., and L. P. Waits. 2010. Multiplex pre-amplification for noninvasive genetic sampling: is the extra effort worth it? Molecular Ecology Resources 10:659–665.

DeCesare, N. J., J. R. Newby, V. Boccadori, T. Chilton-Radandt, T. Thier, D. Waltee, K. Podruzny, and J. A. Gude. 2016. Calibrating minimum counts and catch per unit effort as indices of moose population trend. Wildlife Society Bulletin 40:537–547.

DeCesare, N. J., T. D. Smucker, R. A. Garrott, and J. A. Gude. 2014. Moose status and management in Montana. Alces 50: 35–51.

DelGiudice, G. D., B. A. Sampson, M. S. Lenarz, M. W. Schrage, and A. J. Edwards. 2011. Winter body condition of moose (*Alces alces*) in a declining population in northeastern Minnesota. Journal of Wildlife Diseases 47:30–40.

Ebert, C., F. Knauer, I. Storch, and U. Hohmann. 2010. Individual heterogeneity as a pitfall in population estimates based on non-invasive genetic sampling: a review and recommendations. Wildlife Biology 16:225–240.

Ebert, C., J. Sandrini, B. Spielberger, B. Thiele, and U. Hohmann. 2012. Non-invasive genetic approaches for estimation of ungulate population size: a study on roe deer (*Capreolus capreolus*) based on faeces. Animal Biodiversity and Conservation 35:267–275.

Ebert, C., J. Sandrini, B. Welter, B. Thiele, and U. Hohmann. 2021. Estimating red deer (*Cervus elaphus*) population size based on non-invasive genetic sampling. European Journal of Wildlife Research 67:1–13.

Efford, M. G. 2004. Density estimation in live-trapping studies. Oikos 106:598–610.

Efford, M. G. 2019. Non-circular home ranges and the estimation of population density. Ecology 100:e02580.

Efford, M. G. 2020. secr: spatially explicit capture-recapture models. R package version 4.3.3. https://CRAN.R-project.org/package=secr

Efford, M. G., D. L. Borchers, and G. Mowat. 2013. Varying effort in capture–recapture studies. Methods in Ecology and Evolution 4:629–636.

Eggert, L. S., J. A. Eggert, and D. S. Woodruff. 2003. Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana. Molecular Ecology 12:1389–1402.

Fernando, P., T. N. C. Vidya, C. Rajapakse, A. Dangolla, and D. J. Melnick. 2003. Reliable noninvasive genotyping: fantasy or reality? Journal of Heredity 94:115–123.

Finnegan, L. A., P. J. Wilson, G. N. Price, S. J. Lowe, B. R. Patterson, M.-J. Fortin, and D. L. Murray. 2012. The complimentary role of genetic and ecological data in understanding population structure: a case study using moose (*Alces alces*). European Journal of Wildlife Research 58:415–423.

Flagstad, Ø., K. Røed, J. E. Stacy, and K. S. Jakobsen. 1999. Reliable noninvasive genotyping based on excremental PCR of nuclear DNA purified with a magnetic bead protocol. Molecular Ecology 8:879–883.

Flasko, A., M. Manseau, G. Mastromonaco, M. Bradley, L. Neufeld, and P. Wilson. 2017. Fecal DNA, hormones, and pellet morphometrics as a noninvasive method to estimate age class: an application to wild populations of Central Mountain and Boreal woodland caribou (*Rangifer tarandus caribou*). Canadian Journal of Zoology 95:311–321.

Frantz, A. C., L. C. Pope, P. J. Carpenter, T. J. Roper, G. J. Wilson, R. J. Delahay, and T. Burke. 2003. Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Molecular Ecology 12:1649–61.

Furnas, B. J., R. H. Landers, S. Hill, S. S. Itoga, and B. N. Sacks. 2018. Integrated modeling to estimate population size and composition of mule deer. Journal of Wildlife Management. 82:1429–1441.

Galpern, P., M. Manseau, P. Hettinga, K. Smith, and P. Wilson. 2012. ALLELEMATCH: an R package for identifying unique multilocus genotypes where genotyping error and missing data may be present. Molecular Ecology Resources 12: 771–778.

Gasaway, W. C., S. D. Dubois, and S. J. Harbo. 1985. Biases in aerial transect surveys for moose during May and June. Journal of Wildlife Management 49:777–784.

Gasaway, W. C., S. D. DuBois, D. J. Reed, and S. J. Harbo. 1986. Estimating moose population parameters from aerial surveys. University of Alaska, Institute of Arctic Biology, Fairbanks, USA.

Giudice, J. H., J. R. Fieberg, and M. S. Lenarz. 2012. Spending degrees of freedom in a poor economy: a case study of building a sightability model for moose in northeastern Minnesota. Journal of Wildlife Management 76:75–87.

Goode, M. J., J. T. Beaver, L. I. Muller, J. D. Clark, F. T. Van Manen, C. A. Harper, and P. S. Basinger. 2014. Capture-recapture of white-tailed deer using DNA from fecal pellet groups. Wildlife Biology 20:270–278.

Harris, R. B., M. Atamian, H. Ferguson, and I. Keren. 2015. Estimating moose abundance and trends in northeastern Washington State: index counts, sightability models, and reducing uncertainty. Alces 51:57–69.

Harris, R. B., J. Winnie, Jr., S. J. Amish, A. Beja-Pereira, R. Godinho, V. Costa, and G. Luikart. 2010. Argali abundance in the Afghan Pamir using capture-recapture modeling from fecal DNA. Journal of Wildlife Management 74:668–677.

Hedmark, E., and H. Ellegren. 2006. A test of the multiplex pre-amplification approach in microsatellite genotyping of wolverine faecal DNA. Conservation Genetics 7:289–293.

Hettinga, P. N., A. N. Arnason, M. Manseau, D. Cross, K. Whaley, and P. J. Wilson. 2012. Estimating size and trend of the North Interlake woodland caribou population using fecal-DNA and capture-recapture models. Journal of Wildlife Management 76:1153–1164.

Houston, D. B. 1982. The northern Yellowstone elk: ecology and management. Macmillan, New York, New York, USA.

Hundertmark, K. J. 2007. Home range, dispersal and migration. Pages 303–335 *in* A. W. Franzmann and C. C. Schwartz, editors. Ecology and management of the North American moose. Second edition. University Press of Colorado, Boulder, USA.

Jones, H., P. J. Pekins, L. E. Kantar, M. O'Neil, and D. Ellingwood. 2017. Fecundity and summer calf survival of moose during 3 successive years of winter tick epizootics. Alces 53:85–98.

Kantar, L. E., and R. E. Cumberland. 2013. Using a double-count aerial survey to estimate moose abundance in Maine. Alces 49:29–37.

Keiter, D. A., A. J. Davis, O. E. Rhodes, F. L. Cunningham, J. C. Kilgo, K. M. Pepin, and J. C. Beasley. 2017. Effects of scale of movement, detection probability, and true population density on common methods of estimating population density. Scientific Reports 7:1–12.

Kohn, M. H., and R. K. Wayne. 1997. Facts from feces revisited. Trends in Ecology & Evolution 12:223–227.

Kreader, C. A. 1996. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. Applied and Environmental Microbiology 62:1102–1106.

Kretser, H., M. Glennon, A. Whitelaw, A. Hurt, K. Pilgrim, and M. Schwartz. 2016. Scat-detection dogs survey low density moose in New York. Alces 52:55–66.

LeResche, R. E., and R. A. Rausch. 1974. Accuracy and precision of aerial moose censusing. Journal of Wildlife Management 175–182.

Lindahl, T. 1993. Instability and decay of the primary structure of DNA. Nature 362:709–715.

Lorenz, T. C. 2012. Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. Journal of Visualized Experiments 63:e3998.

Lounsberry, Z. T., T. D. Forrester, M. T. Olegario, J. L. Brazeal, H. U. Wittmer, and B. N. Sacks. 2015. Estimating sex-specific abundance in fawning areas of a high-density Columbian black-tailed deer population using fecal DNA. Journal of Wildlife Management 79:39–49.

Luikart, G., S. Zundel, D. Rioux, C. Miquel, K. A. Keating, J. T. Hogg, B. Steele, K. Foresman, and P. Taberlet. 2008. Low genotyping error rates and noninvasive sampling in bighorn sheep. Journal of Wildlife Management 72:299–304.

Lukacs, P. M., and K. P. Burnham. 2005. Review of capture-recapture methods applicable to noninvasive genetic sampling. Molecular Ecology 14:3909–3919.

MacCracken, J. G., and V. Van Ballenberge. 1987. Age- and sex-related differences in fecal pellet dimensions of moose. Journal of Wildlife Management 51:360–364.

Marucco, F., L. Boitani, D. H. Pletscher, and M. K. Schwartz. 2011. Bridging the gaps between non-invasive genetic sampling and population parameter estimation. European Journal of Wildlife Research 57:1–13.

Marucco, F., L. M. Vucetich, R. O. Peterson, J. R. Adams, and J. A. Vucetich. 2012. Evaluating the efficacy of non-invasive genetic methods and estimating wolf survival during a ten-year period. Conservation Genetics 13:1611–1622.

Maudet, C., G. Luikart, D. Dubray, A. Von Hardenberg, and P. Taberlet. 2004. Low genotyping error rates in wild ungulate faeces sampled in winter. Molecular Ecology Notes 4:772–775.

McMenamin, S. K., E. A. Hadly, and C. K. Wright. 2008. Climatic change and wetland desiccation cause amphibian decline in Yellowstone National Park. Proceedings of the National Academy of Sciences 105:16988–16993.

Mech, L. D., and S. M. Barber. 2002. A critique of wildlife radio-tracking and its use in national parks: a report to the U.S. National Park Service. U.S. Geological Survey, Reston, Virginia, USA.

Millette, T. L., D. Slaymaker, E. Marcano, C. Alexander, and L. Richardson. 2011. AIMS-Thermal-A thermal and high-resolution color camera system integrated with GIS for aerial moose and deer census in northeastern Vermont. Alces 47:27–37.

Millspaugh, J. J., and B. E. Washburn. 2003. Within-sample variation of fecal glucocorticoid measurements. General and Comparative Endocrinology 132:21–26.

Mondol, S., K. U. Karanth, N. S. Kumar, A. M. Gopalaswamy, A. Andheria, and U. Ramakrishnan. 2009. Evaluation of non-invasive genetic sampling methods for estimating tiger population size. Biological Conservation 142:2350–2360.

Monteiro, L., D. Bonnemaison, A. Vekris, K. G. Petry, J. Bonnet, R. Vidal, J. Cabrita, and F. Mégraud. 1997. Complex polysaccharides as PCR inhibitors in feces: *Helicobacter pylori* model. Journal of Clinical Microbiology 35:995–998.

Monteith, K. L., R. W. Klaver, K. R. Hersey, A. A. Holland, T. P. Thomas, and M. J. Kauffman. 2015. Effects of climate and plant phenology on recruitment of moose at the southern extent of their range. Oecologia 178:1137–1148.

Morden, C. C., R. B. Weladji, E. Ropstad, E. Dahl, and O. Holand. 2011. Use of faecal pellet size to differentiate age classes in female Svalbard reindeer *Rangifer tarandus platyrhynchus*. Wildlife Biology 17:441–448.

Morehouse, A. T., and M. S. Boyce. 2016. Grizzly bears without borders: spatially explicit capture-recapture in southwestern Alberta. Journal of Wildlife Management 80:1152–1166.

Murphy, M. A., K. C. Kendall, A. Robinson, and L. P. Waits. 2007. The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. Conservation Genetics 8:1219–1224.

Murray, D. L., E. W. Cox, W. B. Ballard, H. A. Whitlaw, M. S. Lenarz, T. W. Custer, T. Barnett, and T. K. Fuller. 2006. Pathogens, nutritional deficiency, and climate influences on a declining moose population. Wildlife Monographs 166: 1–30.

Murray, D. L., K. F. Hussey, L. A. Finnegan, S. J. Lowe, G. N. Price, J. Benson, K. M. Loveless, K. R. Middel, K. Mills, D. Potter, et al. 2012. Assessment of the status and viability of a population of moose (*Alces alces*) at its southern range limit in Ontario. Canadian Journal of Zoology 90:422–434.

Nadeau, M. S., N. J. DeCesare, D. G. Brimeyer, E. J. Bergman, R. B. Harris, K. R. Hersey, K. K. Huebner, P. E. Matthews, and T. P. Thomas. 2017. Status and trends of moose populations and hunting opportunity in the western United States. Alces 53:99–112.

National Centers for Environmental Information [NCEI]. 2018. National Oceanic and Atmospheric Administration, Climate Data On-line. Data from the Silver Gate, MT Weather Station, USC00247603. http://www.ncdc.noaa.gov/cdo-web/. Accessed Mar 2018.

Navidi, W., N. Arnheim, and M. Waterman. 1992. A multiple-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. American Journal of Human Genetics 50:347–59.

Obbard, M. E., E. J. Howe, and C. J. Kyle. 2010. Empirical comparison of density estimators for large carnivores. Journal of Applied Ecology 47:76–84.

Oyster, J. H., I. N. Keren, S. J. Hansen, and R. B. Harris. 2018. Hierarchical mark-recapture distance sampling to estimate moose abundance. Journal of Wildlife Management 82:1668–1679.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology 12: 1375–1387.

Painter, L. E., and W. J. Ripple. 2012. Effects of bison on willow and cottonwood in northern Yellowstone National Park. Forest Ecology and Management 264:150–158.

Panasci, M., W. B. Ballard, S. Breck, D. Rodriguez, L. D. Densmore, III, D. B. Wester, and R. J. Baker. 2011. Evaluation of fecal DNA preservation techniques and effects of sample age and diet on genotyping success. Journal of Wildlife Management 75:1616–1624.

Peek, J. M. 1974. On the nature of winter habits of Shiras moose. Canadian Naturalist 101:131–174.

Peterson, R. O. 1977. Wolf ecology and prey relationships on Isle Royale. National Park Service Scientific Monograph Series 11:1–210.

Peterson, R. O., and R. E. Page. 1993. Detection of moose in midwinter from fixed-wing aircraft over dense forest cover. Wildlife Society Bulletin 21:80–86.

Piggott, M. P., E. Bellemain, P. Taberlet, and A. C. Taylor. 2004. A multiplex pre-amplification method that significantly improves microsatellite amplification and error rates for faecal DNA in limiting conditions. Conservation Genetics 5: 417–420.

Pollock, K. H. 1982. A capture-recapture design robust to unequal probability of capture. Journal of Wildlife Management 46:752–757.

Pompanon, F., A. Bonin, E. Bellemain, and P. Taberlet. 2005. Genotyping errors: causes, consequences, and solutions. Nature Reviews Genetics 6:847–859.

Poole, K. G., D. M. Reynolds, G. Mowat, and D. Paetkau. 2011. Estimating mountain goat abundance using DNA from fecal pellets. Journal of Wildlife Management 75:1527–1534.

R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rea, R. V., C. J. Johnson, B. W. Murray, D. P. Hodder, and S. M. Crowley. 2016. Timing moose pellet collections to increase genotyping success of fecal DNA. Journal of Fish and Wildlife Management 7:461–466.

Regnaut, S., F. S. Lucas, and L. Fumagalli. 2006. DNA degradation in avian faecal samples and feasibility of non-invasive genetic studies of threatened capercaillie populations. Conservation Genetics 7:449–453.

Renkin, R. A., and D. G. Despain. 1992. Fuel moisture, forest type, and lightning-caused fire in Yellowstone National Park. Canadian Journal of Forest Research 22:37–45.

Rönnegård, L., H. Sand, H. Andren, J. Månsson, and Å. Pehrson. 2008. Evaluation of four methods used to estimate population density of moose *Alces alces*. Wildlife Biology 14:358–371.

Roon, D. A., L. P. Waits, and K. C. Kendall. 2003. A quantitative evaluation of two methods for preserving hair samples. Molecular Ecology Notes 3:163–166.

Rosenberg, D. K., W. S. Overton, and R. G. Anthony. 1995. Estimation of animal abundance when capture probabilities are low and heterogeneous. Journal of Wildlife Management 59:252–261.

Royle, J. A., R. B. Chandler, R. Sollmann, and B. Gardner. 2013. Sampling design. Pages 277–303 *in* Spatial capture-recapture. Academic Press, Cambridge, Massachusetts, USA.

Royle, J. A., and K. V. Young. 2008. A hierarchical model for spatial capture–recapture data. Ecology 89:2281–2289.

Russell, R. E., J. A. Royle, R. Desimone, M. K. Schwartz, V. L. Edwards, K. P. Pilgrim, and K. S. McKelvey. 2012. Estimating abundance of mountain lions from unstructured spatial sampling. Journal of Wildlife Management 76:1551–1561.

Sánchez-Rojas, G., S. Gallina, and M. Equihua. 2004. Pellet morphometry as a tool to distinguish age and sex in the mule deer. Zoo Biology 23:139–146.

Sarmento, P., J. Cruz, C. Eira, and C. Fonseca. 2014. A spatially explicit approach for estimating space use and density of common genets. Animal Biodiversity and Conservation 37:23–33.

Scarlata, C. D., B. A. Elias, J. R. Godwin, R. A. Powell, D. Shepherdson, L. A. Shipley, and J. L. Brown. 2011. Characterizing gonadal and adrenal activity by fecal steroid analyses in pygmy rabbits (*Brachylagus idahoensis*). General and Comparative Endocrinology 171:373–380.

Schmidt, J. I., K. J. Hundertmark, R. T. Bowyer, and K. G. McCracken. 2009. Population structure and genetic diversity of moose in Alaska. Journal of Heredity 100:170–180.

Schoenecker, K. A., M. K. Watry, L. E. Ellison, M. K. Schwartz, and G Luikart. 2015. Estimating bighorn sheep (*Ovis canadensis*) abundance using noninvasive sampling at a mineral lick within a national park wilderness area. North American Naturalist 75:181–191.

Schwartz, C. C., S. L. Monfort, P. H. Dennis, and K. J. Hundertmark. 1995. Fecal progestagen concentration as an indicator of the estrous cycle and pregnancy in moose. Journal of Wildlife Management 59:580–583.

Schwartz, M. K., G. Luikart, and R. S. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22:25–33.

Singer, F. J., L. C. Mark, and R. C. Cates. 1994. Ungulate herbivory of willows on Yellowstone's northern winter range. Journal of Range Management 47:435–443.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L. P. Waits, and J. Bouvet. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24:3189–3194.

Thacker, C. R., C. Oguzturun, K. M. Ball, and D. S. Court. 2006. An investigation into methods to produce artificially degraded DNA. International Congress Series 1288:592–594.

Thompson, C. M., J. A. Royle, and J. D. Garner. 2012. A framework for inference about carnivore density from unstructured spatial sampling of scat using detector dogs. Journal of Wildlife Management 76:863–871.

Timmermann, H. R. 1993. Use of aerial surveys for estimating and monitoring moose populations-a review. Alces 29:35–46.

Timmermann, H. R., and M. E. Buss. 1997. Population and harvest management. Pages 559–615 *in* A. W. Franzmann and C. C. Schwartz, editors. Ecology and management of the North American moose. University Press of Colorado, Boulder, USA.

Tjepkes, T. 2015. Genetic analysis of moose populations from Minnesota and Yellowstone National Park. Thesis, University of Minnesota-Duluth, Duluth, USA.

Toweill, D. E., and G. Vecellio. 2004. Shiras moose in Idaho: status and management. Alces 40:33–43.

Tyers, D. B. 2003. Winter ecology of moose on the northern Yellowstone winter range. Dissertation, Montana State University, Bozeman, USA.

Tyers, D. B. 2006. Moose population history on the northern Yellowstone winter range. Alces 42:133–149.

Tyers, D. B., and L. R. Irby. 1995. Shiras moose winter habitat use in the upper Yellowstone River Valley prior to and after the 1988 fires. Alces 31:35–43.

Van Ballenberghe, V., and W. Ballard. 2007. Population dynamics. Pages 223–245 *in* A. W. Franzmann and C. C. Schwartz, editors. Ecology and management of the North American moose. Second edition. University Press of Colorado, Boulder, USA.

Van Dyke, F., B. L. Probert, and G. M. Van Beek. 1995. Moose home range fidelity and core area characteristics in south-central Montana. Alces 31:93–104.

Waits, L. P., and D. Paetkau. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 69:1419–1433.

Wald, E. J., and R. M. Nielson. 2014. Estimating moose abundance in linear subarctic habitats in low snow conditions with distance sampling and a kernel estimator. Alces 50:133–158.

Wallace, R. B., H. Gomez, G. Ayala, and F. Espinoza. 2003. Camera trapping for jaguar (*Panthera onca*) in the Tuichi Valley, Bolivia. Journal of Neotropical Mammalogy 10:133–139.

Wasser, S. K., J. L. Keim, M. L. Taper, and S. R. Lele. 2011. The influences of wolf predation, habitat loss, and human activity on caribou and moose in the Alberta oil sands. Frontiers in Ecology and the Environment 9:546–551.

Wehausen, J. D., R. R. Ramey, and C. W. Epps. 2004. Experiments in DNA extraction and PCR amplification from bighorn sheep feces: the importance of DNA extraction method. Journal of Heredity 95:503–509.

Wilson, G. A., C. Strobeck, L. Wu, and J. W. Coffin. 1997. Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. Molecular Ecology 6:697–699.

Wilson, P. J., S. Grewal, A. Rodgers, R. Rempel, J. Saquet, H. Hristienko, F. Burrows, R. Peterson, and B. N. White. 2003. Genetic variation and population structure of moose (*Alces alces*) at neutral and functional DNA loci. Canadian Journal of Zoology 81:670–683.

Wolfe, M. L., K. R. Hersey, and D. C. Stoner. 2010. A history of moose management in Utah. Alces 46:37–52.

Yellowstone National Park. 1997. Yellowstone's Northern Range: complexity and change in a wildland ecosystem. National Park Service, Mammoth Hot Springs, Wyoming, USA.

Yost, J. A. 2008. Moose data analysis unit plan DAU M-1, North Park Game Management Units 6, 16, 17, 161, 171. Colorado Division of Wildlife, Steamboat Springs, USA.

Associate Editor: Michael Wisdom.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Koitzsch, K. B., C. B. Anton, L. O. Koitzsch, T. L. Tjepkes, A. C. Schumann, and J. L. Strasburg. 2022. A noninvasive and integrative approach for improving density and abundance estimates of moose. Journal of Wildlife Management e22200. https://doi.org/10.1002/jwmg.22200