DISSERTATION

EVALUATION OF POPULATION MONITORING STRATEGIES FOR GREATER SAGE-GROUSE (*CENTROCERCUS UROPHASIANUS*) IN NORTHWESTERN COLORADO

Submitted by

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ABSTRACT

EVALUATION OF POPULATION MONITORING STRATEGIES FOR GREATER SAGE-GROUSE (*CENTROCERCUS UROPHASIANUS*) IN NORTHWESTERN COLORADO

Population monitoring programs are essential for the proper management of wildlife species but, despite recent advances in methodologies, generating accurate and defensible estimates of population size and trend remains a key challenge for wildlife biologists and managers and effective monitoring programs generally require considerable resources, effort and funding. For this reason, managers often turn to the use of population indices to monitor species. The greater sage-grouse (*Centrocercus urophasianus*) is a species of conservation concern throughout its range in western North America. Since the 1950s, high counts of males at leks have been used as an index for monitoring populations and are often assumed to represent overall population trend. However, the relationship between the lek-count index and true population size is unclear, resulting from a reliance on numerous untested assumptions, and the reliability of these counts for monitoring population trend has been questioned. In addition, lek-count data do not provide information about the female population, a crucial component for assessing a population's growth potential. There is a need for development and evaluation of alternative methods to obtain reliable estimates of population trend and test assumptions underlying the lek-count index. We tested two novel methods for monitoring a small greater sage-grouse population in Northwest Colorado. We found that a large and variable proportion of the lekking male population was missing from lek-count data each year when not all leks were known and counted, the lek-count index poorly represented true annual male abundance in small

populations, and the possibility of large annual variation in male-to-female sex ratio should be considered when extrapolating female abundance from male count data. Our results suggest that, while lek-count data may be useful for detecting large changes in the abundance of lekking males over time, observations of trend based on annual lek-count index data may misrepresent true population trend in relatively small populations.

DEDICATION

This dissertation is dedicated to my parents who have always supported me in the pursuance of my dreams, and to my husband Patrick for his love and encouragement as I close this chapter in my life and open the door to several others. Without you this completion of this dissertation project would not have been possible. Thank you for being there for me through all the tough times, supporting me despite my failures, encouraging (and satisfying) my thirst for adventure, and always celebrating my successes, no matter how small.

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INTRODUCTION

Population monitoring programs are essential for the proper management of wildlife species. Well-designed monitoring strategies allow researchers to determine the status and temporal trends of the focal species; these are often keystone, umbrella, threatened or endangered, candidate, game, and invasive species. Information gained from wildlife monitoring programs allows managers to adjust land-use strategies, species status under federal or state law, hunting regulations, and mitigation plans in response to population trends. Monitoring programs also allow researchers to identify key factors such as disease, human land use, or natural disturbances that influence population dynamics. Additionally, species' status assessments under the Endangered Species Act (ESA) require rigorous, quantitative methods for assessing population size and trend (NRC 1995). Scientifically rigorous abundance estimation and monitoring methods are therefore critical in determining conservation status of species and populations. To provide the information needed to evaluate the status of a population and inform management decisions, monitoring efforts should provide accurate and defensible estimation of population size and trend.

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Since the 1970s, significant progress has been made in wildlife population estimation driven by the practical need to estimate abundance and monitor populations over time (Burnham 2004). Advancements in survey methods, statistical models that account for imperfect detectability, and technology have contributed significantly to improvements in population size estimation and monitoring. Methods used to estimate wildlife population size have expanded to include stratified and cluster sampling, capture-mark-recapture, occupancy, dual-frame sampling, adaptive cluster sampling, and distance sampling, among others. The development of technologies such as radio telemetry, satellite telemetry, global positioning systems (GPS), geographic information systems (GIS), genetic analysis, and advanced open-source computer analysis programs (e.g., MARK, PRESENCE, DISTANCE, etc.) represent major advances that contribute to improved monitoring strategies for wildlife species. Progress has also been made in the development of non-invasive (or minimally invasive) sampling methods that reduce or eliminate disturbance to wildlife species, such as genetic mark-recapture, re-sighting, camera trapping, and track surveys. Additionally, innovations in the size and design of radio and satellite transmitters have resulted in reduced impact to study animals, allowed researchers to evaluate habitat use and management in a greater variety of species, and reduced the cost of monitoring per individual.

Despite recent progress, generating accurate and defensible estimates of population size and trend remains a key challenge for wildlife biologists and managers, and the accurate estimation of wildlife population density requires a considerable investment of resources and time (Witmer 2005). This is particularly true for species that are rare or elusive, have low population densities, or occupy remote or difficult-to-access areas. Researchers investigating these populations face numerous challenges, such as logistical problems resulting from work in

remote areas, low population densities, clustering of animals, imperfect detectability, and limited funding. These challenges often force investigators to turn to population indices to estimate abundance or monitor population trends because they are significantly cheaper and easier to obtain. Indices use indirect evidence of the presence of animals (i.e. nest or den sites, territorial markings, fecal deposits, tracks), or counts of a detectable subset of the population (i.e. bat roost counts, avian point counts, aerial ungulate surveys, lek counts, etc.) to measure relative abundance. However, indices often rely on untested assumptions and their relationship to the true population is usually unclear (Witmer 2005). This has resulted in extensive criticism of indices in wildlife monitoring dating back to the early 1980s (Burnham 1981, Johnson 1995, Thompson et al. 1998, Nichols et al. 2000, Applegate 2000, Anderson 2001, Yuccoz et al. 2001, Williams et al. 2001, Pollock et al. 2002, Ellingson & Lukacs 2003, Walsh et al. 2004, Walsh et al. 2010). A key criticism of indices is that they only represent the portion of the population that is detected, which may vary across time, space and observer, diminishing their value (Johnson 2008). As a result, the true relationship between indices and population abundance is poorly understood and potentially biased (Walsh et al. 2004, Witmer 2005, White 2005). Indices may therefore be inadequate when estimates of abundance and trend are required to determine proper management of wildlife populations.

Greater sage-grouse (*Centrocercus urophasianus*) populations are typically monitored using counts of males attending leks as an index to population size and trend, but the method has faced substantial criticism due to its dependence on several untested assumptions (Beck & Braun 1980, Applegate 2000, Walsh et al. 2004, Walsh et al. 2010). Despite extensive availability of literature on methods to monitor greater sage-grouse populations, there remain large gaps in our knowledge that create challenges for effective conservation of the species. We have identified

these gaps as a need to: 1) investigate untested assumptions underlying population indices based on high counts of lekking males, and 2) evaluate potential alternatives to traditional methods for estimating population abundance and trend. In the chapters in this dissertation, we evaluate two novel monitoring methods (dual-frame lek surveys and non-invasive genetic mark-recapture) to estimate abundance in greater sage-grouse populations, make recommendations for their use in small populations, and investigate key assumptions underlying current lek-based monitoring methods. We also discuss implications of our results for the use of the lek-count index for estimating population size and monitoring trend in a small, isolated population of greater sage-grouse in northwestern Colorado.

BACKGROUND

Greater Sage-Grouse Conservation

The greater sage-grouse (*Centrocercus urophasianus*) is a species of conservation concern that currently occupies 11 U.S. states and 2 Canadian provinces (Schroeder et al. 2004). The species has experienced range-wide declines in abundance and distribution throughout the past century (Hornady 1916, Girard 1937, Patterson 1952, Rogers 1964, Autenrieth 1981, Connelly & Braun 1997, Schroeder et al. 1999, Schroeder et al. 2004, Aldridge et al. 2008, Garton et al. 2015). These declines are partly attributed to habitat loss and human land-use conflicts associated with oil and gas development (Walker et al. 2007, Doherty et al. 2008, Braun et al. 2002, Copeland et al. 2009, Naugle et al. 2011) and have resulted in repeated petitions for federal listing of the species under the U.S. Endangered Species Act (ESA). In 2010, the species was designated by the United States Fish and Wildlife Service as warranted for protection under the ESA, but precluded due to higher priorities (USFWS 2010). In 2015, the agency revisited

that decision and concluded the species was not warranted for federal protection, stating that the species "remains relatively abundant and well-distributed across the species' 173-million acre range and does not face the risk of extinction now or in the foreseeable future." (USFWS 2015). Despite this decision, it is likely that the species will continue to be the subject of future listing petitions.

To better understand the status of this species, the USFWS requires unbiased and precise population estimates based on defensible monitoring techniques. Such approaches aid in understanding the current status and future trends of sage-grouse populations and abundance across the species range and guide management practices. However, current monitoring strategies for greater sage-grouse only provide information on relative population trends of male sage-grouse that attend leks (Connelly et al. 2004), which have been shown to potentially misrepresent both population size and trend (Walsh et al. 2004). For this reason, the development of innovative sampling and analysis methods for estimating greater sage-grouse abundance is critical.

Lek-Count Index

During the spring breeding season, male greater sage-grouse gather to display on traditional strutting grounds known as leks (Patterson 1952, Wiley 1978). Lekking behavior in the spring provides a unique opportunity to observe and count grouse that are otherwise highly cryptic, and seasonal high counts of males detected on leks are typically used as an index of male population size. Lek counts can be used to obtain estimates of relative population trends and changes in species distribution, and to identify key locations on the landscape for management

and conservation efforts. Historically, lek counts have been considered to be the most reliable, if not the only, means for monitoring populations of sage-grouse over large areas, and these counts are currently used by state wildlife agencies throughout the western United States (Connelly et al. 2004, WAFWA 2015). Since the early 2000s, lek counts have been based on standard sampling protocols intended to reduce the influence of temporal and weather-related variation in male lek attendance (Connelly et al. 2003). They are relatively cheap and easy to conduct and are assumed to provide information on relative population size and trend. However, lek counts are subject to numerous sources of sampling bias and therefore may not generate rigorous population estimates required for protection and management of species (Walsh et al. 2010). Even though the procedures for obtaining these counts have been standardized to improve consistency in collection of count data, the counts are still subject to sources of bias, and reliable inference rests on several untested assumptions (Johnson & Rowland 2007). While some aspects of the lek-count index have been investigated previously (e.g. daily timing of counts [Monroe et al. 2016] and the importance of scale and repeated counts within a season [Fedy & Aldridge 2011]), other spatial and temporal aspects relating to variation in detectability either have not been assessed, or the extent to which they vary among populations and over time is unknown. When developing the objectives of my dissertation research, we identified six potential sources of bias that may influence lek-count data and its relationship to true population abundance: imperfect detection probability; the proportion of leks known and counted; variable attendance at leks by males; variation among observers making lek counts; the frequency of inter-lek movements by males; and male-to-female sex ratio (Figure 1).

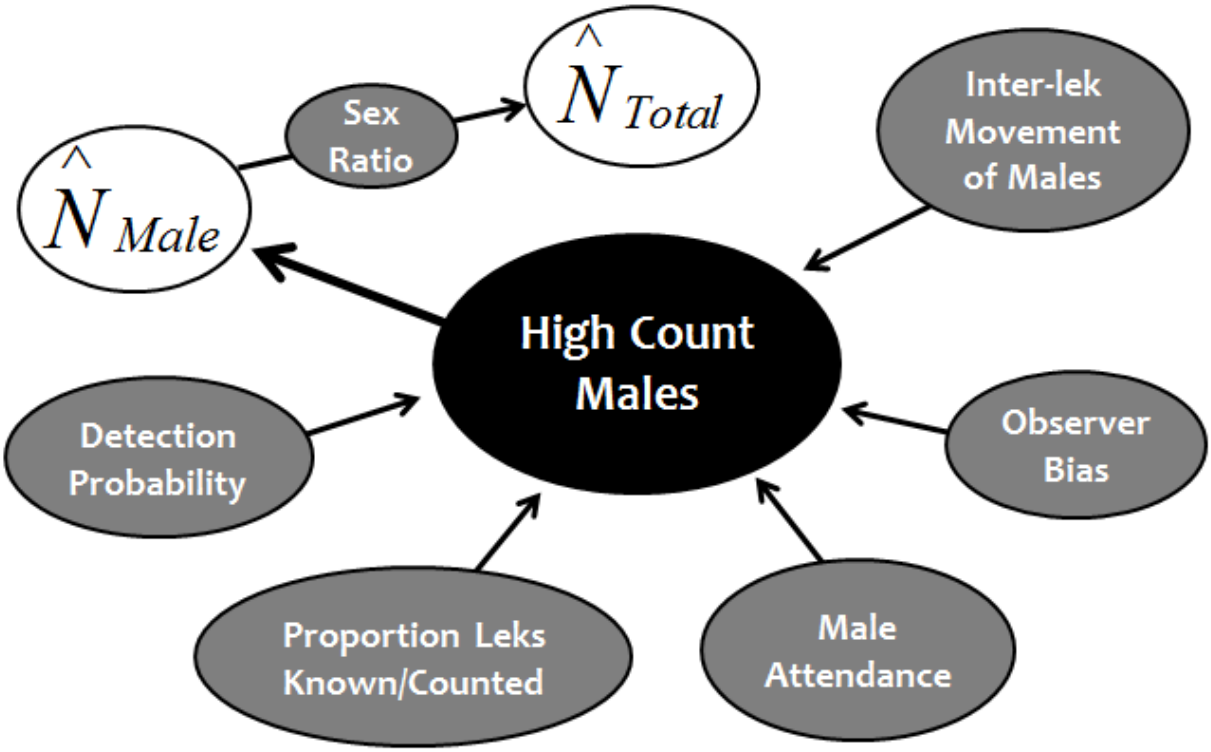

Figure 1. Sources of bias and uncertainty (gray bubbles) in the relationship between high count of males and population abundance.

Detection probability: Historically, wildlife biologists used raw counts of animals, assumed that counts were reliable indices of population size, and largely ignored the possibility of imperfect detectability (White 2005). It is now widely recognized that index methods often rest on critical and unrealistic assumptions concerning probability of detection (Anderson 2001), and this source of bias should not be ignored if population monitoring results are used to make management and conservation decisions. Imperfect detection of male sage-grouse attending leks has been demonstrated (Gibson et al. 2014, Fremgen et al. 2016) and may be influenced by numerous factors, including variation in the age, behavior, or posture of individual birds, lek

size, and lek characteristics such as vegetative cover, aspect, bare ground, and snow cover (Fremgen et al. 2016).

In the past, wildlife monitoring efforts have relied on counts (C) of animals with the assumption that those counts are equal to abundance (N) (Anderson 2001, Pollock et al. 2002) or that a constant, but unknown, proportion of the population is counted across time and space. However, it is now widely acknowledged that counts are biased estimators of abundance (i.e., $C \neq N$), that detectability varies widely in space and time (Conn et al. 2004), and that population estimates must account for imperfect detectability (p). In reality, counts must be adjusted for detection probability to obtain unbiased estimates of the true population size ($N = C/p$). Concerning greater sage-grouse lek monitoring, a range of factors such as lek size, vegetation on and around leks, and visibility may impact our ability to detect male sage-grouse, attending a lek. Estimating detection probability requires repeat observations and, in theory, can be incorporated into lek-based monitoring if lek-count data collection is standardized to require multiple visits to known lek sites.

Proportion of leks known and counted: Despite increased count effort, and in some cases, systematic searches for new leks, some leks remain unknown, particularly those that are small in size or that occur in remote areas (Johnson & Rowland 2007). There may be a sampling bias in favor of large leks because they are more easily detected (WAFWA 2015). Violation of assumptions underlying tools to prioritize habitat conservation and management is also a possibility if a substantial proportion of leks is unknown (Coates at al. 2013), potentially resulting in a serious effect on lek-based management. Despite the traditional nature of leks as annual usage sites, new lek sites can be established, and existing leks may become abandoned over time. Other than the loss of lek sites due to land use and land cover changes, the

environmental drivers of changes in lek location and status are still poorly understood (Connelly et al. 2011). Because unknown leks cannot be counted, any males attending these leks are excluded from counts and do not contribute information to assess population status, size, or trend. For this reason, information on the proportion of leks that are known and counted in a population, and how much that proportion varies annually, is critical for monitoring long-term trend based on male lek-count data.

Male lek attendance: Imperfect lek attendance by males has been well-documented, with numerous studies reporting daily and seasonal variation in attendance among males (Dalke et al. 1960, Eng 1963, Hartzler 1972, Wiley 1973, Jenni & Hartzler 1978, Emmons & Braun 1984, Dunn & Braun 1985, Walsh et al. 2004, Blomberg et al. 2013, Sadoti et al. 2016). Evidence suggests that attendance of males may be much lower than 100%. For example, a study by Walsh et al. (2004) estimated that only 42% of marked adult males and 19% of yearling males attended leks per sighting occasion. Blomberg et al. (2013) estimated the probability of males attending at least one study lek in a season to range from 0.56-0.87. Lek attendance of males may be influenced by weather, presence of females, or time of day or season. It is also affected by survival of males during the breeding season since a male must remain alive in order to attend leks. Lek attendance also differs between adult and yearling males (Walsh et al. 2004). Yearling males typically attend at lower rates than adults and often do not begin attending leks until later in the breeding season (Jenni & Hartzler 1978, Emmons & Braun 1984). Imperfect male attendance can influence lek-count data and may have a greater effect on estimates of population trend if maximum lek attendance by males does not align with survey times. Additionally, inconsistent attendance may significantly affect estimates of population trend if it results in annual variation in attendance rates. Attempts to estimate male abundance from lek-count data

require reliable estimates of, or critical assumptions, regarding attendance of adult and yearling males (and therefore, also the ratio of adult to yearling males in the population).

Inter-lek movement of males: Attendance of males at >1 lek within a single breeding season is also problematic for lek-based population monitoring. Standardized monitoring strategies throughout the species' range require multiple visits to known lek sites. For this reason, biases can occur when males move between leks because individuals may be included in the maximum male count at one, two, or multiple leks, or missed on counts altogether. This bias could increase if lek-count data include counts from additional visits (>3) and are not adjusted for effort. A potential solution to this is the use of "lek routes" wherein all leks in an area are surveyed in a single morning for a combined high count (Connelly et al. 2003), however, most lek monitoring programs currently use high counts at individual leks rather than pooling the data. Some researchers have reported that inter-lek movement of males is rare (Gibson et al. 2014), but males in other studied populations are known to attend multiple leks within a season (Fremgen et al. 2017; Colorado Parks and Wildlife, unpublished data). Overall, the frequency and extent of this behavior and is still poorly understood. For example, Fremgen et al. (2017) found that inter-lek movement of males occurred more frequently than previously reported, and those movements would bias breeding season lek counts.

Inter-lek movement of males warrants further investigation because the behavior may significantly affect lek-count data. If male movement patterns are constant across years then the impact on population trend may be minimal or irrelevant, however, if the rate of inter-lek movement varies among years, it may bias lek-count data and result in an index trend that is not representative of true population trend. More research is clearly needed to estimate the extent

and frequency of inter-lek movement among males and the impact those movements may have on lek-count data and assessment of population trend.

Observer bias: Potential biases resulting from observer variability in the collection of lek-count index data should be acknowledged. An observer's ability to detect, correctly determine sex of, and accurately count multiple sage-grouse at leks is dependent on experience, quality of equipment (e.g., optics), proper training, and inherent ability. Methods exists for researchers to reduce or measure observer bias; such as using designated observation viewpoints for lek counts to minimize variation between observers, the use of double-observer protocols to estimate observer-specific detection probabilities (Nichols et al. 2000), or the use of covariates (MacKenzie et al. 2002) for observer identification when modeling lek detection probabilities.

Sex ratio: Lek-count data provide information on the population of lekking males and fail to represent the female population; a critical component that directs total population abundance (Johnson & Rowland 2007). Greater sage-grouse females primarily attend leks to assess male fitness and mate with males during a brief period prior to, and during, nesting but otherwise do not regularly attend leks during the breeding season. Females are smaller, more cryptic, and more difficult to detect and count on leks than males. As a result, lek counts are typically only used to provide information about the number of males attending leks. If only male abundance is estimated, then estimates of sex ratio (males per female) are also needed to obtain estimates of total population abundance.

Sex ratios of greater sage-grouse populations are generally female-biased but estimates vary considerably throughout the species' range (Connelly et al. 2011), ranging from 1 to 3 females per male (Patterson 1952, Rogers, 1964, Beck 1977, Autenrieth 1981, Walsh et al. 2004,

Atamian 2007, Broms 2007). In Colorado, estimates of male-to-female sex ratios based on harvested birds were; 1:1.9 (Rogers 1964); spring breeding populations were 1:2.3-1:3 (Walsh et al. 2004), and winter populations were 1:1.6 (Beck 1977). State wildlife agencies often assume a ratio of 1 male per every 2 females (PPR-GSGWG 2008). Data on sex ratio is available from several states. Unfortunately, because many are based on data from hunter-harvested birds (Connelly et al. 2011), they are subject to bias since vulnerability to hunter harvest likely varies by sex (Connelly et al. 2000, Wik 2002). Other sex ratio data from observations of birds attending leks (Patterson, 1952, Keller et al. 1941), band-recovery data (e.g., Zablan et al. 2003), or non-random visual sampling of mixed sex flocks (e.g. Beck 1977) may also be subject to sampling biases. Thus, we lack reliable estimates of sex ratio based on random sampling of sage-grouse populations.

Each of the six potential sources of bias discussed above affects the accuracy of greater sage-grouse abundance estimates to an unknown degree and may vary over time and among populations. As a result of these unknowns, the true relationship between the lek-count index and population abundance is poorly understood. Lek-count indices continue to be the primary metrics for monitoring changes in greater sage-grouse populations over time. However, their ability to determine population trend has been questioned due to sampling biases in favor of larger leks (WAFWA 2015), and their utility for providing defensible estimates of population size is limited (Walsh et al. 2010). Long-term lek-count datasets are, however, widely available and are often the only data that allow inference to historic population trends and assessment of population changes across the species' range (Connelly 2004).

While indices, such as traditional lek counts, may detect large-scale changes in population size for large populations, this may not be true for changes in the size of small

populations. Biological populations change proportionately over time as a consequence of population growth being a multiplicative process. The use of indices to detect the direction of population change and temporal trend (i.e., the population increasing or decreasing over time) is defensible only if a number of critical assumptions have been met. The most significant of these is that the relationship between the index and true abundance is linear (Skalski et al. 2005). If the rate of change is large, indices may correctly reflect the direction of change in large populations, however, this may be unreliable for small populations. For example, with populations of different sizes (e.g., 1,000 vs. 100 individuals) that are declining at the same rate (e.g., $\lambda = 0.9$), the absolute change in abundance will be an order of magnitude greater in the larger population, than in the smaller one. The power to detect changes in population size is strongly affected by the coefficient of variation in abundance and can be thought of in terms of effect size, or the magnitude of change in abundance that a monitoring program has the power to detect (Gerrodette 1987). For large populations, a sample size of N = n may be sufficient to detect a decline of 10% per year because the effect size is large. In contrast, detecting the same percentage decline in smaller populations requires being able to detect a much smaller effect size and a larger sample would be needed. For indices such as those used to monitor greater sage-grouse, large changes in abundance are much more likely to be detected than small changes in abundance. Therefore, drawing inferences to trend of small populations from change (or lack of change) in the lek-count index needs to be done cautiously.

For sage-grouse, lek-count biases may also be greater in small populations. For example, detectability of leks may decrease and be more variable with smaller lek sizes, leading to higher variance in the estimate of population abundance (Thompson 2012). If the imprecision of lek index estimates exceeds the potential magnitude of annual change in the populations, power to

detect change will be effectively zero. These limitations of the lek index highlight the need to better understand what apparent trends in lek index data may indicate about true population trend, and develop more robust estimation methodologies required for monitoring small populations. Investigations into the reliability of lek-count data for estimating and monitoring changes in population size, and the development of alternative population monitoring methods, are key research priorities for greater sage-grouse management (Naugle & Walker 2007). There is a great need for researchers to: 1) quantify and test assumptions underlying the lek-count index to learn how these factors influence lek-count data and assessments of status, abundance, and trend; and 2) develop and evaluate new techniques for more rigorously estimating population abundance and trend.

Several alternative methods to traditional lek-count indices are being explored. For example, occupancy analyses using spatially-based sample units (MacKenzie et al. 2006) to detect presence of leks (Johnson & Rowland 2007), N-mixture models to provide more defensible estimates of population trends based on lek-count data (McCaffrey et al. 2016), and integrated population models that incorporate population count and demographic data (Aldridge & Boyce 2007). Other promising alternatives for monitoring sage-grouse populations that have not been evaluated include dual-frame sampling (Haines & Pollock 1998) modified to account for imperfect detection, and genetic mark-recapture based on non-invasive sampling of fecal pellets.

Study Area

The Parachute-Piceance-Roan (PPR) is one of seven recognized greater sage-grouse populations in Colorado (Figure 2). The species' occupied range in the PPR is characterized by the rolling terrain of the Roan Plateau with numerous broad ridge tops formed where the Plateau drops off into canyons and drainages formed by creeks and tributaries. Vegetation on the ridge tops and plateaus is dominated by mountain big sagebrush (*Artemisia tridentata vaseyana*),

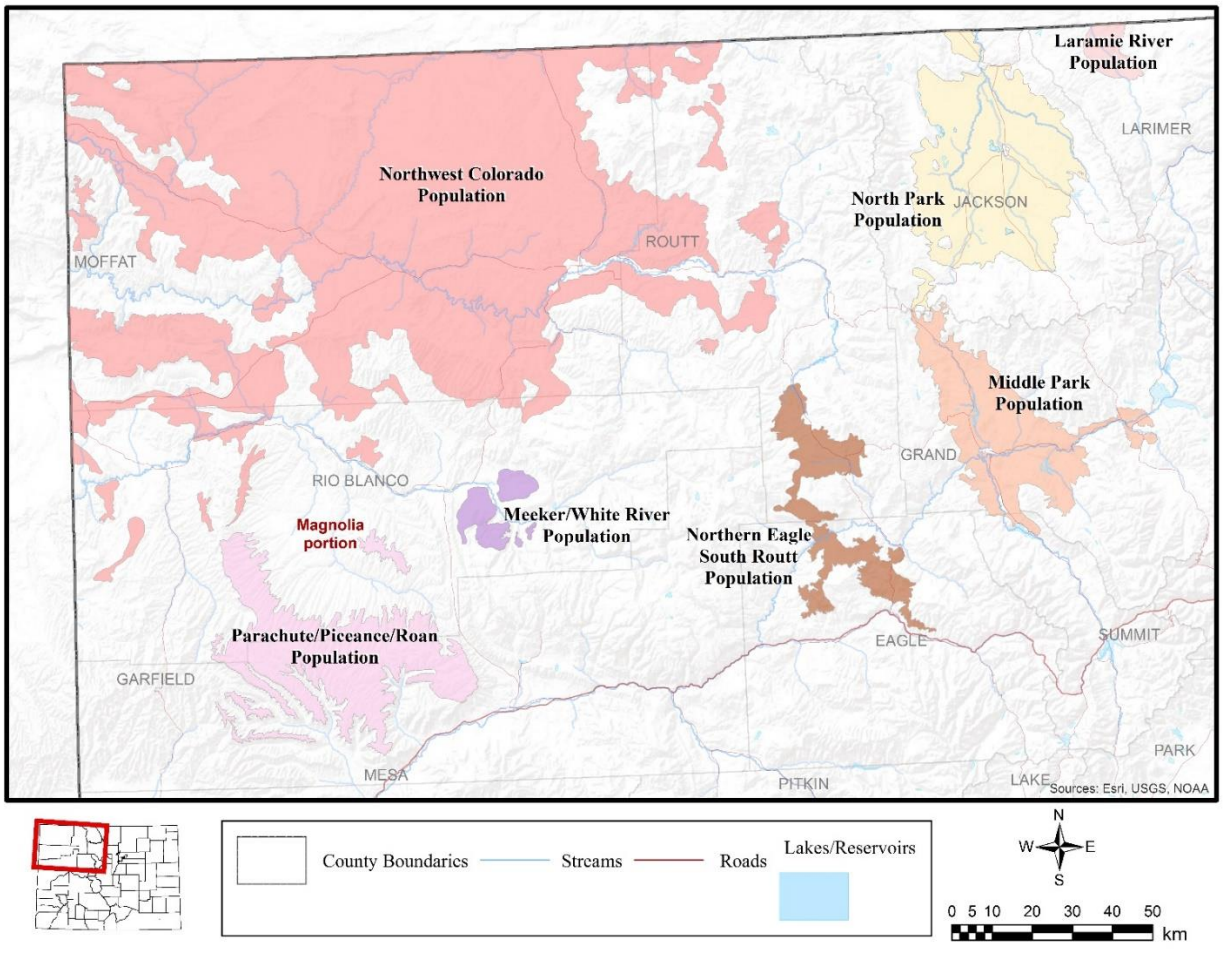

Figure 2. Current greater sage-grouse range and populations in Colorado (Source: Colorado Park and Wildlife).

mixed sagebrush-mountain shrub habitat and pinyon-juniper (*Pinus edulis, Juniperus spp.*) woodlands with patches of aspen (*Populus tremuloides*). Mixed sagebrush-mountain shrub habitat is primarily comprised of mountain sagebrush and serviceberry (*Amelanchier spp.*) with Gambel oak (*Quercus gambelii*), snowberry (*Symphoricarpus spp.*), antelope bitterbrush (*Purshia tridentata*), and mountain mahogany (*Cercocarpus spp.*). In the PPR, greater sage-grouse are largely restricted to sagebrush-dominated ridge tops and plateaus at higher elevations from 2,150–2,750m in elevation (Krager 1977, Hagen 1999, CGSSC 2008, Walker et al. 2016). Approximately 35% of the species range is owned and managed by state or federal agencies with the remaining privately owned by energy companies and ranches.

The PPR population represents approximately 4% of the greater sage-grouse population in Colorado (CGSSC 2008, PPR-GSGWG 2008). Two specific threats faced by greater sage-grouse in the PPR include rapidly increasing natural gas development and pinyon-juniper encroachment into sagebrush (PPR-GSGWG 2008). Hunting for sage-grouse has been closed in the PPR since 1995 due to low male counts. The PPR population may be especially vulnerable due to its small size (the population is thought to fluctuate somewhere between 500 and 1,200 birds) and reductions in suitable habitat resulting from ongoing changes in land cover, particularly rapidly expanding natural gas extraction (PPR-GSGWG 2008). Understanding how the population responds to these changes, as well as mitigation efforts, is important for management of the population. For this reason, obtaining reliable baseline estimates of abundance and evaluating the reliability of the lek-count index for monitoring is critical to assessing future impacts to the population.

The first lek-count data available for the PPR population is for the 1976 and 1977 breeding season, after which no consistent, standardized monitoring of leks occurred until 2005

(PPR-GSGWG 2008). A high male count of approximately 234 males was recorded at 27 active leks from fixed-wing aircraft in 1976-1977 (Krager 1977), and high male count data from fixed-wing (2005 only) and helicopter lek surveys are available from 2005-2016 (Figure 3). Colorado

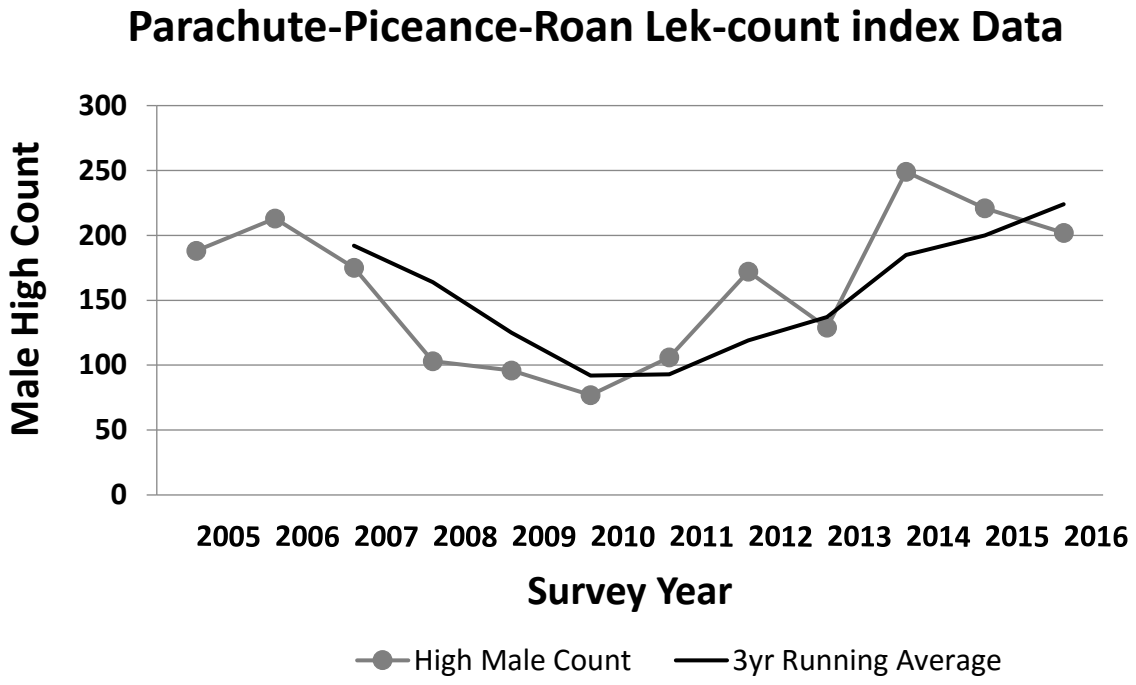

Figure 3. Lek-count index data showing summed high counts and a three-year running average for lekking males across all leks surveyed from 2005-2016 in the Parachute-Piceance-Roan, courtesy of Colorado Parks and Wildlife.

Parks and Wildlife typically employs a three-year running average used to smooth variations in annual male counts arising from factors, such as weather conditions, that may affect male attendance and behavior (PPR-GSGWG 2008; Figure 3).

Available data for the PPR do not allow rigorous inferences regarding population size and trend. Historical monitoring data for the PPR are based exclusively on high male counts at

leks, and there are no independent estimates of abundance against which to compare count data. Colorado Parks and Wildlife recognizes the limitations of using lek counts as an index of abundance and currently uses trends in the three-year running average of annual male high counts to inform management decisions without attempting to generate either male-specific or overall annual population estimates. However, information on how lek-count index data relates to true population abundance and trend would be extremely valuable as a baseline against which to compare historical and future estimates.

Monitoring methods that provide unbiased estimates of population size and trend are needed for proper management, especially for low-density populations. There are no scientifically defensible, long-term datasets to assess the status and trend of the PPR population. Evaluating alternative monitoring strategies for this population was the primary goal of my dissertation research. Our study evaluated the use of two novel methods to estimate of the number of lekking males and total population abundance not previously applied to greater sage-grouse populations. We also used these methods to evaluate other key lek-count index assumptions regarding the proportion of leks known and counted, detection probability of leks, and assumed male-to-female sex ratio. The results discussed in this dissertation provide valuable information to improve management of greater sage-grouse in the PPR and insights for applying dual-frame surveys and genetic mark-recapture methods to other sage-grouse populations.

PROJECT OBJECTIVES

1) Estimate the proportion of active leks and abundance of greater sage-grouse males attending leks in the Parachute-Piceance-Roan population, over three consecutive lekking seasons, using a dual-frame survey methodology that accounts for imperfect detectability.

2) Estimate pre-breeding abundance of greater sage-grouse in the Parachute-Piceance-Roan population during two consecutive winter seasons using genetic mark-recapture methods.

3) Estimate pre-breeding sex ratio and winter flock composition of greater sage-grouse in the Parachute-Piceance-Roan population during two consecutive winter seasons using genetic sampling.

LITERATURE CITED

Aldridge, C. L., Nielsen, S. E., Beyer, H. L., Boyce, M. S., Connelly, J. W., Knick, S. T. and M. A. Schroeder. 2008. Range-wide patterns of greater sage-grouse persistence. Diversity and Distributions 14: 983-994.

Aldridge, C. L. and M. S. Boyce. 2007. Linking occurrence and fitness to persistence: habitat-based approach for endangered greater sage-grouse. Ecological Applications 17:508-526.

Anderson, D. R. 2001. The need to get the basics right in wildlife field studies. Wildlife Society Bulletin (1973-2006), 29:1294-1297.

Applegate, R. D. 2000. Use and misuse of prairie chicken lek surveys. Wildlife Society Bulletin. 28:457-248.

Atamian, M. T. 2007. Brood ecology and sex ratio of greater sage-grouse in East-Central Nevada. M.S. Thesis, University of Nevada, Reno, NV, USA.

Autenrieth, R. E. 1981. Sage grouse management in Idaho. Idaho Department of Fish & Game No. 9.

Beck, T. D. I. 1977. Sage-grouse flock characteristics and habitat selection in winter. Journal of Wildlife Management 41:18-26.

Beck, T. D. I. and C. E. Braun. 1980. The strutting ground count: variation, traditionalism, and management needs. Proceedings of the Western Association of Fish and Wildlife Agencies 60:558-566.

Blomberg, E. J., Sedinger, J. S., Nonne, D. V., and M. T. Atamian. 2013. Annual male lek attendance influences count-based population indices of greater sage-grouse. Journal of Wildlife Management 77:1583-1592.

Braun, C. E., Oedekoven, O. O., and C. L. Aldridge. 2002. Oil and gas development in western North America: effects on sagebrush steppe avifauna with particular emphasis on sage grouse. Transactions of the North American Wildlife and Natural Resources Conference 67:337-349.

Broms, K. 2007. Small game population reconstruction: model development and applications. Thesis, University of Washington, Seattle, WA, USA.

Burnham, K. P. 1981. Summarizing remarks: environmental influences. Studies in Avian Biology 6:324-327.

Burnham, K. P. 2004. Foreword. Pages xi – xiii *in* W.L. Thompson, editor. Sampling rare or elusive species: concepts, designs and techniques for estimating population parameters. Island Press, Washington, DC.

Coates, P. S., Casazza, M. L., Blomberg, E. J., Gardner, S. C., Espinosa, S. P., Yee, J. L., Wiechman, L., and B. J. Halstead. 2013. Evaluating greater sage-grouse seasonal space use relative to leks: implications for surface use designations in sagebrush ecosystems. Journal of Wildlife Management 77:1598-1609.

Colorado Greater Sage-Grouse Steering Committee (CGSSC). 2008. Colorado greater sage-grouse conservation plan. Colorado Division of Wildlife, Denver, Colorado, USA.

Conn, P. B., Bailey, L. L., and J. R. Sauer. 2004. Indexes as surrogates to abundance for low-abundance species. Pages 59-74 *in* W.L. Thompson, editor. Sampling rare or elusive species: concepts, designs, and techniques for estimating population parameters. Island Press, Washington, DC.

Connelly, J. W. and C. E. Braun. 1997. Long-term changes in sage grouse (*Centrocercus urophasianus*) populations in western North America. Wildlife Biology 3:229-234.

Connelly, J. W., Apa, A. D., Smith, R. B., and K. P. Reese. 2000. Effects of predation and hunting on adult sage grouse *Centrocercus urophasianus* in Idaho. Wildlife Biology 6:227-232.

Connelly, J. W., Reese, K. P., and M. A. Schroeder. 2003. Monitoring of greater sage-grouse habitats and populations. University of Idaho, College of Natural Resources Experiment Station Bulletin 80:1-47. Moscow, ID, USA.

Connelly, J. W., Knick, S. T., Schroeder, M. A., and S. J. Stiver. 2004. Conservation assessment of greater sage-grouse and sagebrush habitats. Western Association of Fish and Wildlife Agencies, Cheyenne, WY, USA.

Connelly, J. W., Hagan, C. A., and M. A. Schroeder. 2011. Characteristics and dynamics of greater sage-grouse populations. Pages 53-67 *in* S.T. Knick & J.W. Connelly, editors. Greater sage-grouse: ecology and conservation of a landscape species and its habitats. Studies in Avian Biology 38, University of California Press, Berkley, CA, USA.

Copeland, H. E., Doherty, K. E., Naugle, D. E., Pocewicz, A., and J.M. Kiesecker. 2009. Mapping oil and gas development potential in the US Intermountain West and estimating impacts to species. PloS One 4:e7400.

Dalke, P. D., Pyrah, D. B., Stanton, D. C., Crawford, J. E., and E. F. Schlatterer. 1963. Ecology, productivity and management of sage grouse in Idaho. Journal of Wildlife Management 27:811-841.

Doherty, K. E., Naugle, D. E., Walker, B. L., and J. M. Graham. 2008. Greater sage-grouse winter habitat selection and energy development. Journal of Wildlife Management 72:187-195.

Dunn, P. O. and C. E. Braun. 1985. Natal dispersal and lek fidelity of sage grouse. Auk 102:621-627.

Ellingson, A. R. and P. M. Lukacs. 2003. Improving methods for regional landbird monitoring: a reply to Hutto and Young. Wildlife Society Bulletin 31:896-902.

Emmons, S. R. and C. E. Braun. 1984. Lek attendance of male sage grouse. Journal of Wildlife Management 48:1023-1028.

Eng, R. L. 1963. Observations on the breeding biology of male sage grouse. Journal of Wildlife Management 27:841-846.

Fedy, B. C. and C. L. Aldridge. 2011. The importance of within-year repeated counts and the influence of scale on long-term monitoring of sage-grouse. Journal of Wildlife Management 75:1022-1033.

Fremgen, A. L., Hansen, C. P., Rumble, M. A., Gamo, R.S., and J. J. Millspaugh. 2016. Male greater sage-grouse detectability on leks. Journal of Wildlife Management 80:266-274.

Fremgen, A. L., Rota, C. T., Hansen, C. P., Rumble, M. A., Gamo, R. S., and J. J. Millspaugh. 2017. Male greater sage-grouse movements among leks. Journal of Wildlife Management doi:10.1002/jwmg.21208.

Garton, E. O., Wells, A. G., Baumgardt, J. A., and Connelly, J. W. 2015. Greater sage-grouse population dynamics and probability of persistence. Final report to Pew Charitable Trusts.

Gerrodette, T. 1987. A power analysis for detecting trends. Ecology 68:1364-1372.

Gibson, D., Blomberg, E. J., Atamian, M. T., and J. S. Sedinger. 2014. Lek fidelity and movement among leks by male greater sage-grouse *Centrocercus urophasianus*: a capture-mark-recapture approach. Ibis 156:729-740.

Girard, G. L. 1937. Life history, habits and food of the sage grouse, *Centrocercus urophasianus* Bonaparte. University of Wyoming, Committee on Research, Laramie, USA.

Hagen, C. A. 1999. Sage grouse habitat use and seasonal movements in a naturally fragmented landscape, northwestern Colorado. Thesis, University of Manitoba, Winnipeg, Canada.

Haines, D. E. and K. H. Pollock. 1998. Estimating the number of active and successful bald eagle nests: an application of the dual frame method. Environmental and Ecological Statistics 5:245-256.

Hartzler, J. E. 1972. An analysis of sage grouse lek behavior. Dissertation. University of Montana, Missoula, MT, USA.

Hornady, W. T. 1916. Save the sage grouse from extinction, a demand from civilization to the western states. New York Zoological Bulletin 5:179-219.

Jenni, D. A. and J. Hartzler. 1978. Attendance at a sage grouse lek: implications for spring censuses. Journal of Wildlife Management 42:46-52.

Johnson, D. H. 1995. "Point counts of birds: What are we estimating?" Monitoring bird populations by point counts. General Technical Report, PSW-GTR-149. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station.

Johnson, D. H. 2008. In defense of indices: the case of bird surveys. Journal of Wildlife Management 72:857-868.

Johnson, D. H. and M. M. Rowland. 2007. The utility of lek counts for monitoring greater sage-grouse. Pages 15-24 *in* K.P. Reese and R.T. Bowyer, editors. Monitoring populations of greater sage-grouse: proceedings of a symposium at Idaho State University. College of Natural Resources Experiment Station Bulletin 88. Moscow, ID, USA.

Keller, R. J., Shepherd H. R., and R. N. Randall. 1941. Survey of 1941: North Park, Jackson County, Moffat County, including comparative data of previous seasons. Colorado Game and Fish Commission Sage Grouse Survey 3. Denver, CO, USA.

Krager, R. 1977. Survey of sage grouse strutting ground complexes and seasonal use areas within the Piceance Basin Wildlife Habitat Area. Unpublished progress report. Colorado Division of Wildlife, Fort Collins, USA.

MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S., Royle, J. A., and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83:2248-2255. Academic Press.

MacKenzie, D. I., Nichols, J. D., Royle, J. A., Pollock, K. H., Bailey, L. L., and J. E. Hines. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Elsevier, San Diego, CA, USA.

McCaffrey, R., Nowak, J. J., and P. M. Lukacs. 2016. Improved analysis of lek-count index data using N-mixture models. Journal of Wildlife Management 80:1011-1021.

Monroe, A. P., Edmunds, D. R., and C. L. Aldridge. 2016. Effects of lek count protocols on greater sage-grouse population trend estimates. Journal of Wildlife Management 80:667-678.

National Research Council (NRC) Committee on Scientific Issues in the Endangered Species Act. 1995. Science and the Endangered Species Act. National Academy Press, Washington D.C.

Naugle, D. E. and Walker, B. L. 2007. A collaborative vision for integrated monitoring of greater sage-grouse populations. Pages 57-62 *in* K.P. Reese and R.T. Bowyer, editors. Monitoring populations of greater sage-grouse: proceedings of a symposium at Idaho

State University. College of Natural Resources Experiment Station Bulletin 88. Moscow, ID, USA.

Naugle, D. E., Doherty, K. E., Walker, B. L., Holloran, M. J., and H. E. Copeland. 2011. Energy development and greater sage-grouse. Pages 489-504 *in* S.T. Knick & J.W. Connelly, editors. Greater sage-grouse: ecology and conservation of a landscape species and its habitats. Studies in Avian Biology 38, University of California Press, Berkley, CA, USA.

Nichols, J. D., Hines, J. E., Sauer, J. R., Fallon, F. W., Fallon, J. E., and P. J. Heglund. 2000. A double-observer approach for estimating detection probability and abundance from point counts. Auk, 117:393-408.

Parachute-Piceance-Roan Greater Sage-Grouse Work Group (PPR-GSGWG). 2008. Parachute-Piceance-Roan (PPR) greater sage-grouse conservation plan. Colorado Division of Wildlife, Denver, CO, USA.

Patterson, R. L. 1952. The sage grouse in Wyoming. Wyoming Game and Fish Commission and Sage Books, Inc., Denver, CO, USA.

Pollock, K. H., Nichols, J. D., Simons, T. R., Farnsworth, G. L., Bailey, L. L., & J. R. Sauer. 2002. Large scale wildlife monitoring studies: statistical methods for design and analysis. Environmetrics 13:105-119.

Rogers, G. E. 1964. Sage grouse investigations in Colorado. Technical Publication Number 16, Game Research Division, Colorado Game, Fish and Parks Department.

Sadoti, G., Johnson L., and T. P Albright. 2016. Modeling environmental and survey influences on lek attendance using long-term survey data. Ibis 158:821-833.

Schroeder, M. A., Young J. R., and C. E. Braun. 1999. Sage-grouse (*Centrocercus*

urophasianus). Page 28 *in* A. Poole and F. Gill, editors. The Birds of North America, No. 425. The Birds of North America, Inc., Philadelphia, PA, USA.

Schroeder, M. A., Aldridge, C. L., Apa, A. D., Bohne, J. R., Braun, C. E., Bunnell, S. D., Connelly, J. W., Deibert, P. A., Gardner, S. C., Hilliard, M. A., Kobriger, G. D., and C. W. McCarthy. 2004. Distribution of sage-grouse in North America. Condor 106:363-376.

Skalski, J. R., Ryding K. E., and J. J. Millspaugh. 2005. Wildlife demography: analysis of sex, age, and count data. Elsevier Academic Press, San Diego, CA, USA.

Thompson, S. K. 2012. Sampling (third edition). Wiley & Sons Inc., Hoboken, New Jersey, USA.

Thompson, W. L., White, G. C., and C. Gowan. 1998. Monitoring vertebrate populations. Academic Press, San Diego, CA, USA.

United States Fish and Wildlife Service (USFWS). 2010. 12-month finding for petitions to list the greater sage grouse (*Centrocercus urophasianus*) as threatened or endangered. Federal Register 75:13909-14014.

United States Fish and Wildlife Service (USFWS). 2015. Endangered and threatened wildlife and plants; 12-month finding on a petition to list greater sage-grouse (*Centrocercus urophasianus*) as an endangered or threatened species. Federal Register 80:59857-59942.

Walker, B. L., Apa, A. D., and K. Eichhoff. 2016. Mapping and prioritizing seasonal habitats for greater sage-grouse in northwestern Colorado. Journal of Wildlife Management 80:63-77.

Walker, B. L., Naugle, D. E., and K. E. Doherty. 2007. Greater sage-grouse population response to energy development and habitat loss. Journal of Wildlife Management 71:2644-2654.

Walsh, D. P., White, G. C., Remington, T. E., and D. C. Bowden. 2004. Evaluation of the lek-count index for greater sage-grouse. Wildlife Society Bulletin, 32:56-68.

Walsh, D. P., Stiver, J. R., White, G. C., Remington, T. E., and A. D. Apa. 2010. Population estimation techniques for lekking species. Journal of Wildlife Management 74:1607-1613.

Western Association of Fish and Wildlife Agencies (WAFWA). 2015. Greater sage-grouse population trends: an analysis for lek-count databases 1965-2015. Report. Western Association of Fish and Wildlife Agencies, Cheyenne, WY, USA.

White, G. C. 2005. Correcting wildlife counts using detection probabilities. Wildlife Research 32:211-216.

Wik, P. A. 2002. Ecology of the greater sage-grouse in south-central Owyhee County, Idaho. Thesis, University of Idaho, Moscow, ID, USA.

Wiley, R. H. 1973. The strut display of male sage grouse: a “fixed” action pattern. Behavior 47:129-152.

Wiley, R. H. 1978. The lek mating system of the sage grouse. Scientific American 238:114-125.

Williams, B. K., Nichols, J. D., and M. J. Conroy. 2001. Analysis and management of animal populations. Academic Press, San Diego, CA, USA.

Witmer, G. W. 2005. Wildlife population monitoring: some practical considerations. Wildlife Research 32:259-263.

Yoccoz, N. G., Nichols, J. D., and T. Boulinier. 2001. Monitoring of biological diversity in space and time. Trends in Ecology and Evolution 16:446-453.

Zablan, M. A., Braun, C. E., and G. C. White. 2003. Estimation of greater sage-grouse survival in North Park, Colorado. Journal of Wildlife Management 67:144-154.

CHAPTER 2: USING DUAL-FRAME SURVEYS OF LEKS TO IMPROVE MONITORING OF GREATER SAGE-GROUSE (*CENTROCERCUS UROPHASIANUS*) POPULATIONS IN NORTHWESTERN COLORADO[2]

SUMMARY

Effective wildlife population monitoring programs are critical for ensuring proper management of species but often require substantial resources, effort and funding. For this reason, managers often turn to the use of indices to monitor populations. However, indices have been heavily criticized because they rely on untested assumptions. For this reason, it is important to both evaluate more rigorous methods of population estimation and to test key assumptions underlying indices. The lek-count index is used to monitor populations of greater sage-grouse (*Centrocercus urophasianus*), a species of conservation concern, throughout its range in the western U.S. and Canada. The proportion of leks that are known and counted is critical information for managers to have when interpreting population trend data based on counts of lekking males. However, wildlife management agencies often have little to no quantitative information on the number of leks that exist in a population, and therefore, how many are included in, or excluded from, lek-based trend analyses and management efforts. We used dual-frame surveys of leks, in combination with occupancy analysis to adjust for imperfect detection, to estimate the total number of active leks, the total number of males attending leks, and the

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proportion of active leks that are known and counted on standard lek counts, during three consecutive breeding seasons in a small, low-density population in northwestern Colorado. We estimated that lek count and management efforts that rely exclusively on known lek locations may overlook as many as 26-55% of active leks and 22-57% of males attending leks each year. Our results suggest that a large proportion of the lekking male population in the PPR is missing from annual lek-count index data, and because this proportion varies annually, it may have a substantial impact on trend analyses. Managers need to recognize this potential source of bias in lek-count data and, if possible, account for it in trend analyses and management efforts. We recommend mapping potential lek habitat and conducting dual-frame surveys in conjunction with occupancy analyses over multiple years to quantify the proportion of active leks being missed by standard lek counts, as well as annual variation in this proportion, in specific populations of interest.

INTRODUCTION

Population Monitoring

Wildlife population monitoring is critical for ensuring proper management of species, but it is challenging and requires considerable time and effort (Witmer 2005). For this reason, wildlife managers often turn to population indices, which are cheaper and easier to obtain, to estimate population size and monitor trend. Indices measure variables thought to correlate with abundance or density of species in an area (Caughley 1977) and can be based on a variety of metrics, such as the presence of animals, hair, feathers, scat, tracks, nests, or camera trap detections. Examples of commonly used indices include avian point counts (Johnson 2008), pellet surveys for ungulates (Rowland et al. 1984, Fuller 1991), and lek counts for greater sage-

grouse (Connelly et al. 2003, Walsh et al. 2004, Johnson & Rowland 2007, WAFWA 2015). However, indices often rely on untested assumptions about their relationship to the true population (Witmer 2005), and may only represent changes in a subset of the population being sampled rather than the entire population (Johnson 2008). Assumptions of indices frequently include 1) constant probability of detection and 2) that a constant proportion of the true population is counted. For species of conservation concern, there is a critical need to test these assumptions. This can be accomplished by quantifying associated variables to determine their potential impact on index data and the extent to which they vary annually.

The greater sage-grouse (*Centrocercus urophasianus*) is a species of conservation concern that has experienced historical population declines and substantial contraction of its pre-settlement distribution (Connelly & Braun 1997, Connelly et al. 2004, Schroeder at al. 2004). These declines, in combination with habitat loss and human land use conflicts (Knick et al. 2003, Connelly et al. 2004, Holloran et al. 2005, Walker et al. 2007) have resulted in petitions for federal listing of the species under the Endangered Species Act (USFWS 2010, USFWS 2015). The greater sage-grouse is often regarded as an umbrella species whose protection is likely to offer conservation benefits for other sagebrush-obligate species (Rowland et al. 2006) and also has economic value as a game species and recreational birding attraction during the lekking season.

Management and conservation of greater sage-grouse populations should be based on accurate and defensible estimates of population size and trend over time. Historically, sage-grouse populations have been monitored by counting males during the spring breeding season as they gather to display on traditional strutting grounds known as leks (Patterson 1952, Connelly et al. 2003). Lek counts are currently considered the only practical means of monitoring

populations of lek-breeding grouse over large areas, and they remain widely used by state wildlife agencies throughout the western United States (Applegate 2000, Connelly et al. 2004). Managers use lek-count data to determine conservation status of populations, set harvest limits, establish land management and restoration guidelines, and monitor impacts of anthropogenic land use and disturbance on populations. Lek locations are also used to identify and prioritize sage-grouse habitat in state conservation plans (CGSSC 2008, PPR-GSGWG 2008). Lek counts typically follow standardized protocols that specify season dates, recommended number of visits per lek per season, time of day, and weather conditions suitable for counting strutting males (Connelly et al. 2003). Standardized protocols are intended to reduce bias due to temporal variation in male attendance and increase the reliability of lek counts for documenting changes in relative abundance over time (Connelly et al. 2004, WAFWA 2015).

The use of the lek-count index to estimate population size and trend relies on several untested assumptions and, as a result, their reliability for estimating population abundance, and even trend, has been criticized (Beck & Braun 1980, Applegate 2000, Walsh et al. 2004, Walsh et al. 2010). This in turn, creates challenges for establishing effective policies to manage and conserve populations. Current index-based monitoring strategies for greater sage-grouse only provide information on relative population trends (Connelly et al. 2004) and may actually misrepresent both population size and trend (Walsh et al. 2004). For these reasons, investigations into the reliability of lek-count data for monitoring changes in population size and development of innovative methods to estimate population size and trend are a research priority for greater sage-grouse (Naugle & Walker 2007).

A key assumption underlying the use of lek counts for monitoring and management is that the proportion of leks that are known and counted during annual monitoring efforts is either

relatively high (i.e., most if not all leks are known) or at least constant over time (CGSSC 2008). This assumption will have consequences for population assessment if the set of leks that are known and counted differs greatly from the true set of leks in the population (which is typically unknown) or if the proportion of leks known and counted varies annually. The use of lek locations and count data to prioritize areas for conservation assumes that the majority of leks are known and available estimates of population size and trend are unbiased. However, if lek counts are biased, for example, because many lek locations are unknown (Coates et al. 2013) or detectability is low and variable (Walsh et al. 2004), then areas prioritized for conservation (e.g., Doherty et al. 2010) based on lek data may be incomplete or inadequate.

Dual-frame surveys provide an opportunity to estimate the abundance of lekking males, the total number of active leks, and the proportion of leks in a population that are currently known (and presumably counted). In dual-frame sampling methodologies, surveys are conducted within two sampling frames, the list frame and the area frame (Hartley 1962). The list frame is comprised of a list of known point locations (e.g., bald eagle nests, Haines & Pollock 1998) that serve as list-frame sample units while the area frame is comprised of area-based sample units that are surveyed to locate previously unknown point locations. This approach allows each frame to effectively offset weaknesses of the other (Kott & Vogel 1995); the list frame provides information on all known units of interest, while the area frame provides information on the completeness of the list frame. The method is most often used in public and business surveys (Hartley 1962) but, to date, the only published use of dual-frame surveys to monitor wildlife populations was to estimate the number of active and successful bald eagle nests in the United States (Haines & Pollock 1998, USFWS 2007). Dual-frame surveys are thought to be most useful for monitoring breeding populations for species that have highly visible and stable

breeding locations (Haines & Pollock 1998), making it a potential candidate for improving monitoring of sage-grouse leks.

We investigated the use of dual-frame surveys to improve population monitoring of greater sage-grouse in a small, low-density population in northwestern Colorado. We employed dual-frame lek surveys over three consecutive spring lekking seasons from 2012-2014 to estimate: 1) the total number of active greater sage-grouse leks, 2) the proportion of the total number of leks in the population that are known and counted, and 3) the number of males attending leks each year. We also examined trade-offs between cost and precision of estimates under different sampling strategies that vary the proportion of list-frame points and area-frame cells surveyed.

Study Area

We studied greater sage-grouse in the Parachute-Piceance-Roan (PPR) population in Rio Blanco and Garfield counties in northwestern Colorado (Figure 4, see Supplement A for details). Sage-grouse in the PPR occupy mountain big sagebrush (*Artemisia tridentata vaseyana*) and mixed sagebrush-mountain shrub habitats on ridges, plateaus, and the upper ends of drainages between 2,150–2,750 m in elevation (Krager 1977, Hagen 1999, CGSSC 2008, Walker et al. 2016). Areas of suitable habitat are naturally fragmented and separated by steep drainages, cliffs, patches of aspen (*Populus tremuloides*), conifers, and non-sagebrush shrubs such as serviceberry (*Amelanchier* sp.) and Gambel oak (*Quercus gambelii*). Leks primarily occur within areas of bare ground or low-growing vegetation along the tops of sagebrush-dominated ridges and are often in close proximity to each other. Typical lek sites include meadows or other clearings,

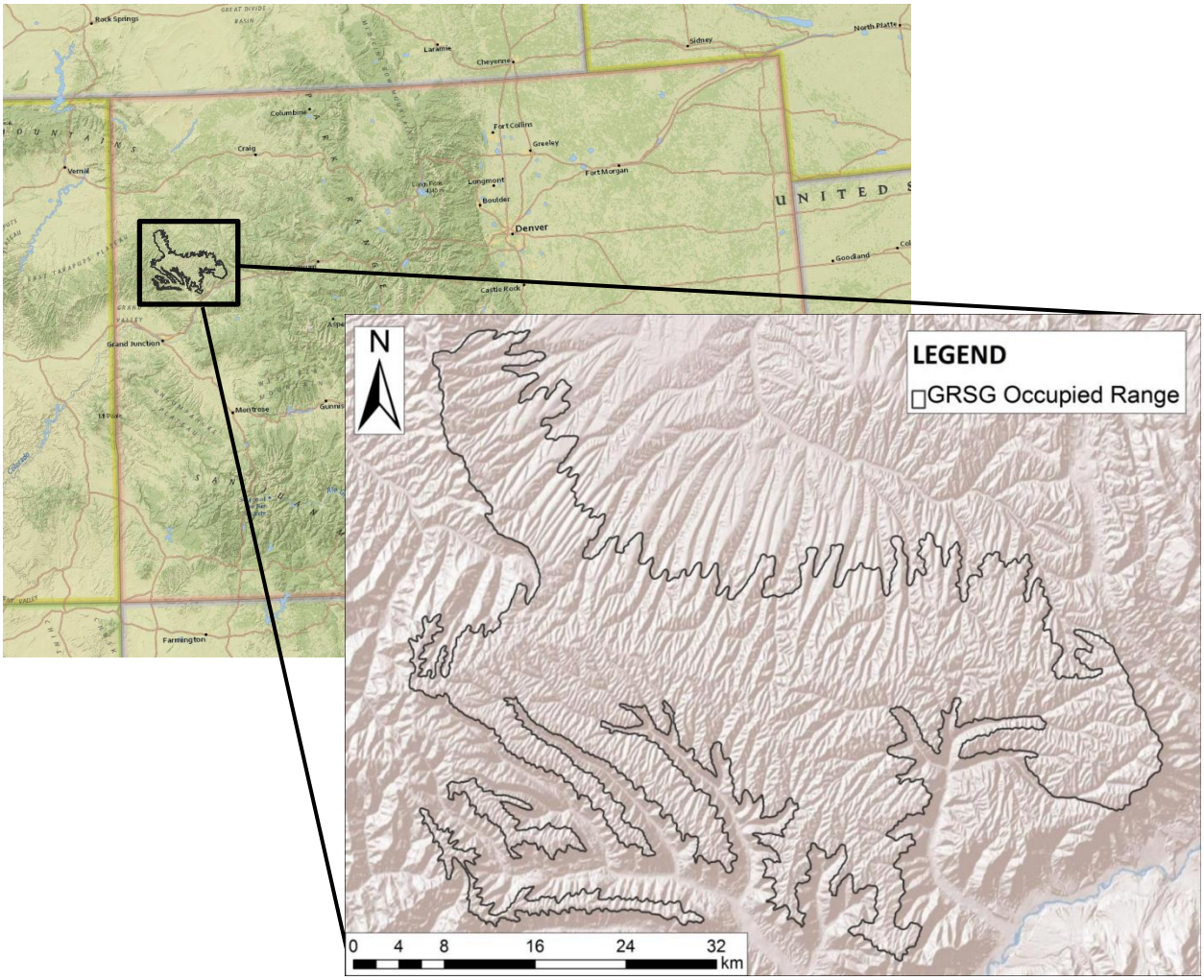


Figure 4. Map of the Parachute-Piceance-Roan greater sage-grouse core population in northwestern Colorado with shaded topographic relief and the current (2012) occupied range boundary.

areas of livestock use (i.e. open areas around stock ponds and mineral licks; Figure 5), grassy openings on ridges, and occasionally disturbed areas such as well pads and pipeline cuts.

Leks in the PPR typically support relatively few males, compared to other larger GRSG populations, with an average size of 4-9 males per active lek and annual total high counts of males ranging from 77–249 males from 2005-2016 (Colorado Parks and Wildlife [CPW],

Figure 5. A typical lek site in the Parachute-Piceance-Roan. Leks are often located in open areas along sagebrush-dominated ridges.

unpublished data). The rate of use of known lek sites (i.e., those with males observed in the past 5 years) by breeding males is apparently low, with 28-71% observed to have displaying males between 2012 and 2016 (CPW, unpublished data). High annual variation in lek use suggests either poor detection probability of lekking males, low lek-site fidelity of males, or relatively high rates of annual lek establishment and abandonment (i.e. turnover), all of which may influence apparent trend in lek-count data over time, particularly if not all active lek locations are known. The objectives of this study were to obtain improved estimates of the abundance of active leks and lekking males and to quantify what proportion of leks and males are being missed on standard lek counts and in lek-based management efforts in the PPR.

METHODS

Definitions

We defined a "known lek site" as any open area on the ground, represented by a central waypoint, where ≥ 1 males have been observed strutting on ≥ 2 occasions (i.e., visits) in the last 5 years during the March-May breeding season, and an "active lek" as any known lek site with ≥ 1 strutting males observed in the survey year of interest. This definition is intended to restrict leks to just those locations where males regularly strut by excluding temporary or aberrant strutting locations. We assigned all newly discovered strutting locations a status of "potentially active" until strutting males had been confirmed on ≥ 2 occasions (i.e., visits). However, our dual-frame survey design required the use of lek status definitions specific to a single year to determine sample units and summarize annual survey results and estimates. For this reason, all potentially active strutting locations were included in our list of known lek sites and used to determine sampling units prior to each survey season (see Supplement B for details). Using potentially active locations to identify survey cells was appropriate because detectability of leks is imperfect and these locations are sometimes only confirmed as active leks by a second observation in a subsequent year.

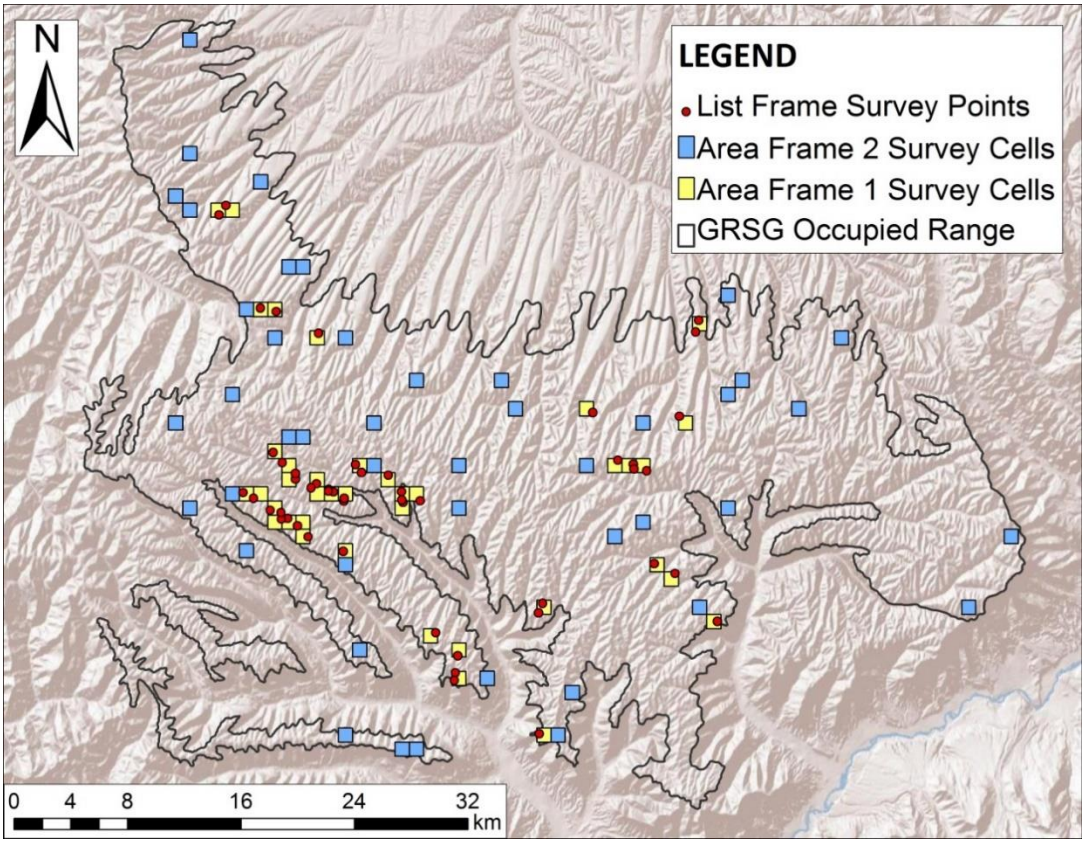
Dual-Frame Surveys

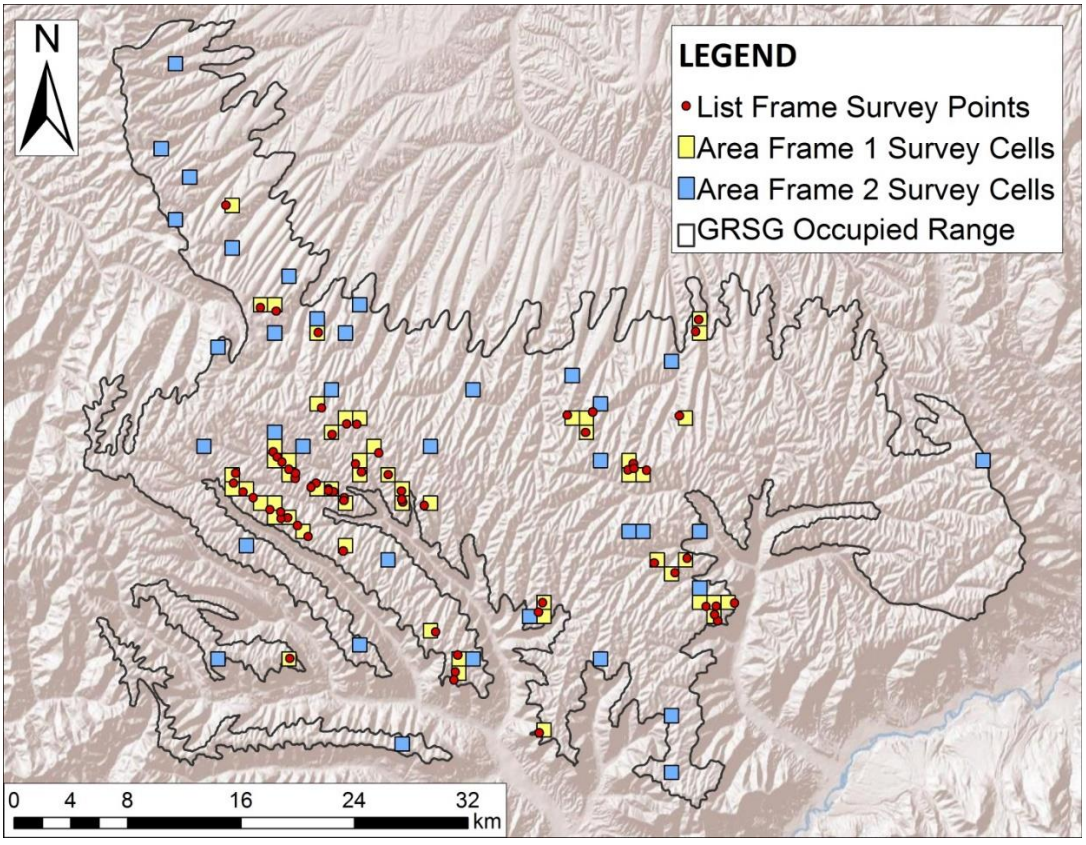
We used an overlapping dual-frame survey methodology wherein point-based sample units in the list frame (i.e., known lek sites) occur within area-based sample units (i.e., cells) in the area frame (Haines & Pollock 1998). When data from known list-frame points are excluded from area-frame cells (a process known as unduplication; Haines & Pollock 1998, Otto and Sauer 2007), the remaining data can be used to estimate the number of unknown points not

currently in the list frame (i.e., unknown leks). Unduplication allows estimators for the total number of points and their associated variances in each frame to be independent of each other and therefore, they can be combined to estimate the total number of points (i.e., total number of leks) in the entire study area (Otto and Sauer 2007). These estimators are referred to as screening estimators because points included in the estimator for each frame are excluded, or screened, from the other frame (Hartley 1962, Haines & Pollock 1998).

Helicopter Surveys

We used dual-frame surveys to estimate the number of active leks in the PPR for three consecutive spring breeding seasons from 2012-2014. We compiled a list of known lek sites in the PPR from CPW's statewide lek database and used that list as our initial list frame (LF) in the first survey season in 2012 (Figure 6). We then overlaid the study area (i.e., the area frame [AF]) with a grid of 1-km^2 cells such that the entire study area was captured within the AF cell grid and all AF cells were either partially or completely within the study area boundaries. An advantage of dual-frame surveys is the AF can be stratified based on habitat type, geography or other attributes (Haines & Pollock 1998). We divided the AF into two strata, area frame 1 (AF_1) and area frame 2 (AF_2). Area $frame_1$ included all AF cells that overlapped ≥ 1 known lek sites in the LF. AF_2 consisted of all remaining cells in the study area (i.e., those that did not overlap a LF lek). We used the reversed randomized quadrant-recursive raster (RRQRR) in ArcGIS (Theobald et al. 2001) to select a spatially balanced random sample of AF_2 cells with equal inclusion probabilities each year and updated LF, AF_1, and AF_2 prior to conducting surveys in the following year to incorporate new leks discovered the previous year (Figure 6). Sampling


N
LEGEND
List Frame Survey Points
Area Frame 2 Survey Cells
Area Frame 1 Survey Cells
GRSG Occupied Range
0 4 8 16 24 32
km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List Frame Survey Points
Area Frame 1 Survey Cells
Area Frame 2 Survey Cells
GRSG Occupied Range
0 4 8 16 24 32
km

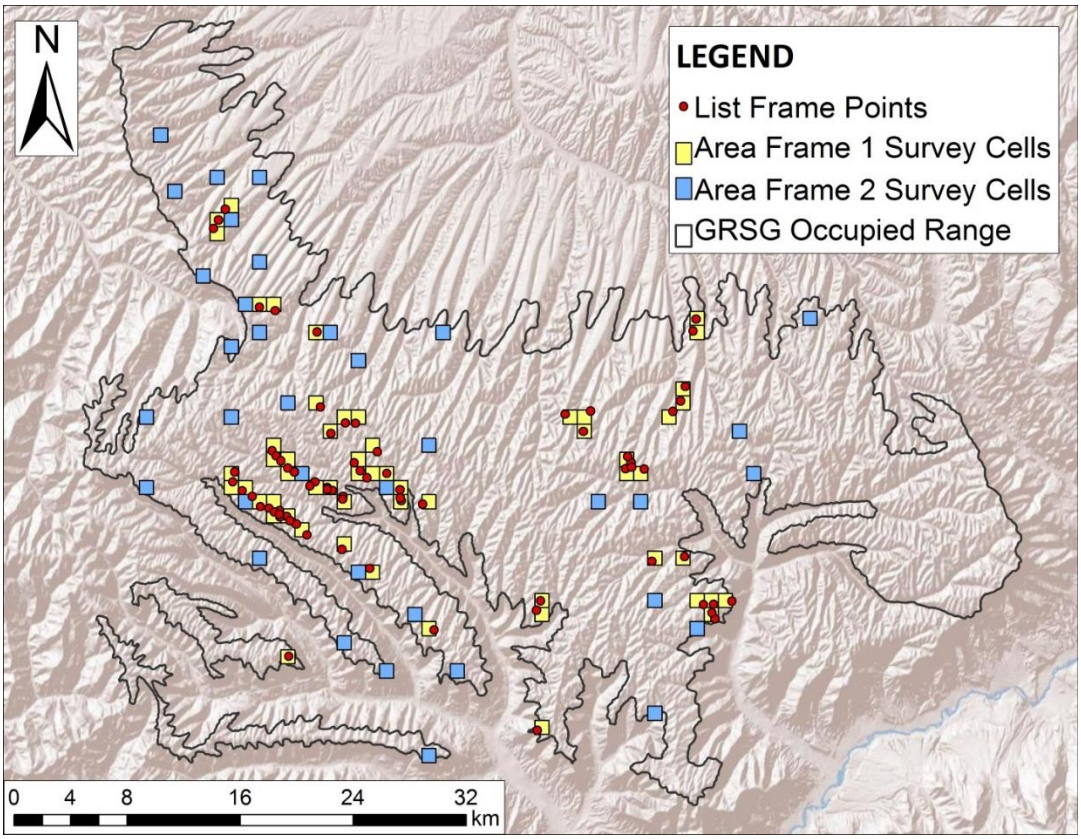

Figure 6. Dual-frame sample units for greater sage-grouse leks in the Parachute-Piceance-Roan population in northwestern Colorado in 2012 (top), 2013 (middle), and 2014 (bottom).

units in these frames had an inclusion probability of 1 because we surveyed all LF points and AF_1 cells each year. We surveyed the maximum possible number of AF_2 cells each year within the constraints imposed by flight logistics and funding.

We searched for and counted strutting males at all known lek sites in the LF, in all AF_1 cells, and in our sample of AF_2 cells by helicopter on three sampling occasions (on average once every 6 days) within each season. Surveys were conducted between 17 April and 4 May each year to capture the period of peak male attendance in the PPR (CPW unpublished data) and followed CPW's standard lek count protocols (restricting counts to 0.5 hours before sunrise to

1.5 hours after sunrise on days with little or no precipitation and wind speeds < 15 mph). We divided the study area into five sections with pre-determined flight paths to maximize survey efficiency and minimize flight distance. Consecutive surveys on each route were flown in the opposite direction to reduce any influence of time of day on counts and areas of obviously unsuitable habitat (e.g., cliffs and conifer forest) were not surveyed (see Supplement C for details).

ANALYSIS

Revision of Potential Lek Habitat

Some habitat areas within AF_2 were found to be unsuitable for sage-grouse leks (e.g., steep, thickly-vegetated hillsides, patches of forest, etc.). Therefore, to increase the precision of dual-frame survey estimates, we excluded portions of AF_2 with little or no potential to support a lek. Following the conclusion of dual-frame surveys, CPW produced and validated a resource selection model to identify greater sage-grouse breeding habitat (i.e., pre-nesting, lekking, nesting, and early brood-rearing habitat) in the PPR (Walker et al. 2016). Lek sites represent a subset of breeding habitat, and, in the PPR, leks only occur on ridges with relatively gentle slopes. We used the boundaries of known lek arenas to determine minimum thresholds for breeding resource selection function (RSF) scores, topographic position index, and slope, and then applied those thresholds across the study area to develop a potential lek habitat layer (Figure 7). We then identified the minimum proportion of potential lek habitat in an AF_2 cell known to support a lek and, prior to analysis, removed all AF_2 cells (both surveyed and unsurveyed) below that minimum threshold.

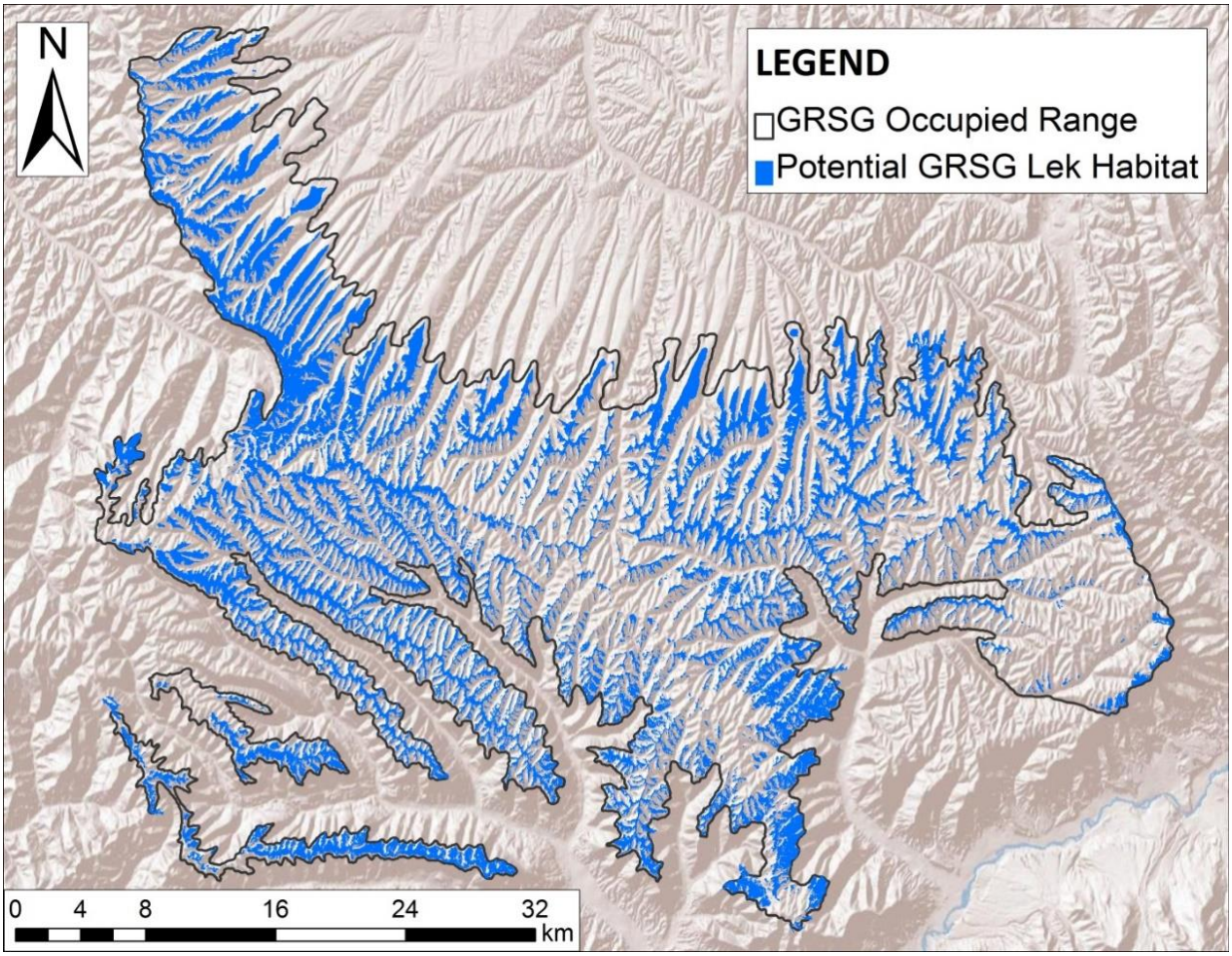

Figure 7: Predicted potential greater sage-grouse lek habitat in the Parachute-Piceance-Roan population in northwestern Colorado (CPW unpublished data).

We excluded new leks discovered incidentally (i.e. those discovered from helicopter while traveling between survey cells) from occupancy analysis in the year they were first discovered. This was necessary because these leks occurred outside our sampling frames and were typically visited < 3 times that year. However, we included high male counts at incidental leks for calculating average males per area-frame lek to improve point estimates of the total number of males attending area-frame leks. We included incidental leks when truncating lower confidence interval values for the estimated total number of active leks in a given year.

Occupancy Analysis

We used "Occupancy Estimation with Detection < 1" models in Program MARK (White and Burnham 1999) to estimate the proportion of active leks in the list frame ($\hat{\psi}_{LF}$) and the probability of detecting an active lek in the list frame ($\hat{p}_{LF}$) and in each area-frame stratum ($\hat{p}_{AF1}$, $\hat{p}_{AF2}$). Assumptions for occupancy models included: 1) constant within-season occupancy of each sample unit, and 2) independence of sample units. We used attribute groups to distinguish the three sampling frames and analyzed occupancy data for each year separately. We ran a total of five models, estimating occupancy ($\hat{\psi}$) by group (sampling frame) and allowing detection probability (*p*) to vary over time and by group, for each year's analysis (Table 1).

Table 1. Occupancy models for dual-frame surveys of greater sage-grouse leks in the Parachute-Piceance-Roan population in northwestern Colorado, 2012-2014.

Model
p(.) Psi(g)
p(g) Psi(g)
p(t) Psi(g)
p(g+t) Psi(g)
p(g*t) Psi(g)

We used model averaging based on Akaike's Information Criteria values adjusted for small sample size (AIC_c) to obtain parameter estimates in program MARK. We were unable to estimate $\hat{p}_{AF2}$ due to the low incidence of leks in AF_2. We assumed detection probability of all new leks in both area frames was the same and set $\hat{p}_{AF1} = \hat{p}_{AF2}$ for all group-varying models. This is a reasonable assumption because all newly discovered leks during the study were small in size (1-9 males) and should therefore have similar detection probabilities.

Estimators

We used Hartley's screening estimator (Hartley 1962, Haines & Pollock 1998), modified to account for imperfect detectability, to estimate the total number of active leks in the population ($\hat{T}$) and its associated variance ($Var(\hat{T})$). In our analysis, occupancy in the LF refers to the proportion of LF leks that were active, and occupancy for AF_1 and AF_2 refers to the proportion of 1-km^2 cells containing ≥ 1 active leks (excluding known lek sites already in the LF).

Following Haines & Pollock (1998), we estimated the total number of active leks in the LF total using the equation,

$\hat{t}_{LF} = N_{LF}\bar{y}_{LF}$ where,

N_{LF} = the number of lek sites on the current LF and,

$\bar{y}_{LF}$ = a proportion equal to the number of LF lek sites that were active (n_O) divided by the number of lek sites sampled from the list frame (n_{LF})

Importantly, this estimator is unbiased only when detection probability, $p = 1$ as assumed by Haines & Pollock (1998). This proportion is equivalent to the naïve estimate of occupancy in an occupancy analysis (MacKenzie et al. 2006). We used the encounter history information from multiple surveys to estimate the per visit detection probability and adjusted the estimate for the proportion of lek sites that were active for imperfect detectability. This is equivalent to an adjusted estimate of an occupancy rate in an occupancy analysis (MacKenzie et al. 2006). For our adjusted estimate of the proportion of active leks in the LF, we use the symbol ψ as in an

occupancy analysis. Therefore, the total number of active leks in the LF was estimated using the equations,

$\hat{\bar{y}} = n_0 / n_{LF} = \psi_{naive}$, following Haines & Pollock (1998) screening estimator with,

$\hat{\bar{y}}_{adj} = \left(n_0 / n_{LF} \right) / \, p^* = \psi_{LF}$, to adjust for imperfect detectability and,

$\hat{t}_{LF} = N_{LF} \hat{\psi}_{LF}$, to estimate of the total number of active leks in the LF with variance,

$$\text{var}(\hat{\psi}_{LF}) = N_{LF}^2 \left[\frac{\hat{\psi}_{LF}(1-\hat{\psi}_{LF})}{n_{LF}} + \frac{\hat{\psi}_{LF}(1-\hat{p}^*_{LF})}{n_{LF}\hat{p}^*_{LF}} \right] \text{ where,}$$

$\hat{t}_{LF}$ = the estimated number of active leks in the LF

N_{LF} = total number of known lek sites in the current LF

n_{LF} = number of LF lek sites surveyed

n_0 = number of sites from the n_{LF} sampled sites that had active leks

$\hat{p}_{LF}$ = per sample occasion detection probability

k = number of surveys

$\hat{p}^*_{LF} = (1-(1-\hat{p}_{LF})^k)$ = estimated probability of detecting ≥ 1 males on ≥ 1 occasion at a LF lek site in a single season with *k* sampling occasions (based on the probability of ≥ 1 detection, MacKenzie et al. 2006)

We estimated the number of active leks in AF_1 using the equation,

$\hat{t}_{AF1} = N_{AF1}\bar{y}_{AF1}$ where,

$\bar{y}_{AF1} = \frac{1}{n_{AF1}}\sum_{i=1}^{n_{AF1}} y_{i_{AF1}}$ = sample mean number of active leks detected in AF_1

N_{AF1} = number of 1-km^2 cells in AF_1

n_{AF1} = number of AF_1 cells surveyed

$y_{i_{AF1}}$ = number of active leks detected in AF_1 sample cell i

The probability of detection for active leks was unknown, so we derived an estimate of the per-visit detection probability $\hat{p}_{AF1}$ and $\hat{\text{var}}(\hat{p}_{AF1})$ from the AF_1 encounter histories using program MARK using the equation,

$\hat{t}_{AF1} = \frac{N_{AF1}\bar{y}_{AF1}}{\hat{p}^*_{AF1}}$, (Thompson 2012) with variance,

$$\text{var}(\hat{t}_{AF1}) = \frac{N^2_{AF1}}{\hat{p}^{*2}_{AF1}}\left[\left(\frac{1-\hat{p}^*_{AF1}}{N_{AF1}}\right)\bar{y}_{AF1} + \frac{\bar{y}^2_{AF1}}{\hat{p}^{*2}_{AF1}}\text{var}(\hat{p}^*_{AF1})\right] \text{ where,}$$

$\hat{p}^*_{AF1} = (1-(1-\hat{p}_{AF1})^k)$ = estimated probability of detecting ≥ 1 males on ≥ 1 occasion at an active AF_1 lek in a single season with *k* sampling occasions

The variance equation for AF_1 does not include a finite correction factor $(N-n)/N$, because all cells within this frame were sampled.

We estimated the number of active leks in AF_2 using the equation,

$\hat{t}_{AF2} = N_{AF2}\bar{y}_{AF2}$ where,

$\bar{y}_{AF2} = \frac{1}{n_{AF2}}\sum_{i=1}^{n_{AF2}} y_{i_{AF2}}$ = sample mean number of leks detected in AF_2

N_{AF2} = number of 1-km^2 cells in AF_2

n_{AF2} = number of AF_2 cells surveyed

$y_{i_{AF2}}$ = number of active leks detected in AF_2 sample cell i

As with AF_1, detection of probability for active leks in AF_2 was unknown, but we derived estimates as before using the equation,

$\hat{t}_{AF2} = \frac{N_{AF2}\bar{y}_{AF2}}{\hat{p}^*_{AF2}}$ with variance,

$\text{var}(\hat{t}_{AF2}) = \frac{N_{AF2}^2}{\hat{p}^{*2}_{AF2}}\left[\left(\frac{N_{AF2}-n_{AF2}}{N_{AF2}}\right)\frac{s^2}{n_{AF2}} + \left(\frac{1-\hat{p}^*_{AF2}}{N_{AF2}}\right)\bar{y}_{AF2} + \frac{\bar{y}^2_{AF2}}{\hat{p}^{*2}_{AF2}}\text{var}(\hat{p}^*_{AF2})\right]$ (Thompson 2012) where,

$\hat{p}^*_{AF2} = (1-(1-\hat{p}_{AF2})^k)$ = estimated probability of detecting ≥ 1 males on ≥ 1 occasion at an active AF_2 lek in a single season with *k* sampling occasions and,

$s^2 = \frac{1}{n_{AF2}-1}\sum_{i=1}^{n_{AF2}}(y_{i_{AF2}} - \bar{y}_{AF2})^2$ = sampling variance for mean number of active leks per cell in AF_2

We estimated the total number of active leks in the study area using the combined estimator equation,

$\hat{T} = \hat{t}_{LF} + \hat{t}_{AF1} + \hat{t}_{AF2}$ with variance,

$Var(\hat{T}) = \text{var}(\hat{t}_{LF}) + \text{var}(\hat{t}_{AF1}) + \text{var}(\hat{t}_{AF2})$

We estimated the proportion of active leks known and counted using the number of active leks detected in the LF divided by our estimates for the total number of active leks in all three frames. Known lek sites in the LF represent those that would have been surveyed during standard lek monitoring efforts by CPW, based on a total of 3 helicopter visits with no additional lek survey efforts to search for unknown leks. We truncated 95% lower confidence intervals for our estimates using the total number of active leks actually observed during dual-frame survey efforts each year, including incidental leks, as these values represent the minimum number of known active leks in the PPR. We also calculated the proportion of males attending leks that were known and counted in the PPR.

We estimated the number of males attending leks in the PPR using the average high male count for active leks detected in the LF and AF strata multiplied by the estimated number of active leks in each frame or stratum. For each lek, we used the within-year maximum number of males detected over the 3 survey occasions to estimate the mean number of males per lek in the study area. We then estimated the number of males in the LF using the equation,

$\hat{m}_{LF} = \hat{t}_{LF}\overline{x}_{LF}$ where,

$\hat{m}_{LF}$ = the estimated number of males attending active leks in the LF

$\hat{t}_{LF}$ = total number of active leks in the LF

$\bar{x}_{LF}$ = the estimated mean number of males per active lek in the LF, based on the within-year maximum male count with variance,

$$\text{var}(\hat{m}_{LF}) = \text{var}(\hat{t}_{LF})(\bar{x}_{LF})^2 + \text{var}(\bar{x}_{LF})(\hat{t}_{LF})^2 + \text{var}(\hat{t}_{LF})\,\text{var}(\bar{x}_{LF})$$ (Goodman 1962)

Equations to estimate the number of males attending leks were the same for both AF strata with,

$\hat{m}_{AFi} = \hat{t}_{AFi}\bar{x}_{AFi}$ where,

$\hat{m}_{AFi}$ = the estimated number of males attending active leks in AF stratum *i*

$\hat{t}_{AFi}$ = total number of active leks in AF stratum *i*

$\bar{x}_{AFi}$ = the estimated mean number of males per active lek in AF stratum *i* based on the maximum male count, with variance,

$$\text{var}(\hat{m}_{AF}) = \text{var}(\hat{t}_{AFi})(\bar{x}_{AFi})^2 + \text{var}(\bar{x}_{AFi})(\hat{t}_{AFi})^2 + \text{var}(\hat{t}_{AFi})\,\text{var}(\bar{x}_{AFi})$$

To estimate the total number of males attending leks in the study area we used the combined estimator,

$\hat{M} = \hat{m}_{LF} + \hat{m}_{AF1} + \hat{m}_{AF2}$ with variance,

$$Var(\hat{M}) = \text{var}(\hat{m}_{LF}) + \text{var}(\hat{m}_{AF1}) + \text{var}(\hat{m}_{AF2})$$

We conducted two separate occupancy analyses to address uncertainty over whether to include or exclude potentially active leks because a failure to designate these sites as active leks may be the result of imperfect detection or imperfect attendance of males at leks. Results from our primary analysis (see RESULTS below) used only data from confirmed active lek sites each year, and our secondary analysis also included data from potentially active lek sites that were never confirmed by a second observation (see Supplement D and Appendix A).

RESULTS

We surveyed LF points and AF cells for lekking males from mid-April to early May in three consecutive years, 2012-2014. We surveyed 49-66 LF points, 39-57 AF_1 cells, and 36-45 AF_2 cells during each study year. The number of unsurveyed AF_2 cells in each year ranged from 587-598 out of a total of 631-634 available cells.

The total number of active leks we detected differed between our sampling frames, with the most leks detected in the LF and the fewest in AF_2. Total leks detected per frame were similar across years with 17-23 detected in the LF, 3-4 in AF_1 and 0-1 in AF_2. Between-year turnover in observed use of known lek sites with data for consecutive years was 26.9% (14/52) from 2012-2013 and 25.0% (16/64) from 2013-2014.

We discovered a total of 21 new leks during dual-frame surveys during the three survey seasons, including observations in the AF and incidental leks (Table 2). We observed a total of 3-4 AF_1 cells with ≥ 1 new leks in each year and one AF_2 cell with ≥ 1 leks in 2012 and 2014; we observed no active leks in AF_2 in 2013 (Table 3). A total of two new leks (both discovered incidentally in 2013) were identified as historical lek locations that had been inactive for more than 10 years prior to the 2012 season. In addition to leks newly discovered during surveys,

Table 2. Summary of new leks detected in the area frame by year in the Parachute-Piceance-Roan greater sage-grouse population, 2012-2014.

	2012	2013	2014
Area Frame 1 (dual-frame surveys)	3	4	4
Area Frame 2 (dual-frame surveys)	1	0	1
Incidental Leks* (dual-frame surveys)	2	3	3
Total New Leks Detected (dual-frame surveys)	6	7	8
Total New Leks Detected (other CPW surveys)	4	1	4
Total New Leks Detected (all surveys combined)	10	8	12

* Leks detected in the area frame during dual frame surveys but outside sampled units (i.e. while travelling between sample units)

Table 3. Total sampling units with ≥ 1 active greater sage-grouse leks detected in each sampling frame by year in the Parachute-Piceance-Roan, 2012-2014.

	2012	2013	2014
List Frame	22	17	23
Area Frame 1	3	3*	4
Area Frame 2	1	0	1

*One AF1 cell in 2013 had 2 new leks detected

additional leks were discovered each year during the course of field work for other CPW research projects. The number of additional leks discovered was 4 in 2012, 1 in 2013, and 4 in 2014. The number of new leks discovered each year as a result of all research efforts in the PPR, including dual-frame surveys, was 10 in 2012, 8 in 2013, and 12 in 2014; a total of 30 over the three-year period.

Estimates of the total number of males for all combined leks across all sample frames, based on the within-year maximum number of males detected at each lek across all three visits, were similar in 2012 and 2013 but increased by more than a factor of two in 2014 (Table 4). The

Table 4. Estimates of the total number of greater sage-grouse males for all combined leks by year, based on the high count of males detected per lek across all 3 visits, in the Parachute-Piceance-Roan, 2012-2014.

	2012	2013	2014
List Frame	69	63	148
Area Frame 1	22	12	14
Area Frame 2*	8	5	16
Total All Frames	99	80	178

*Includes incidental leks found in the frame to improve estimates.

mean high male count per active lek (all lek sites with ≥ 1 males observed in a given year) and per known lek site (all lek sites with ≥ 1 males in the past 5 years) varied among years and sampling frames (Tables 5 and 6). The number of males per new lek for combined years

Table 5. Mean high count of greater sage-grouse males per active lek by year in the Parachute-Piceance-Roan, 2012-2014.

	2012	2013	2014
List Frame	3.14	3.71	6.43
Area Frame 1	7.33	3.00	3.50
Area Frame 2*	2.67	1.67	4.00
Total All Frames	3.54	3.33	5.74

*Includes incidental leks found in the frame to improve estimates.

Table 6. Mean high count of greater sage-grouse males per known lek site by year in the Parachute-Piceance-Roan, 2012-2014.

	2012	2013	2014
List Frame	1.41	1.03	2.21
Area Frame 1	7.33	3.00	3.50
Area Frame 2*	2.67	1.67	4.00
Total All Frames	1.8	1.21	2.37

*Includes incidental leks found in the frame to improve estimates.

ranged from 1-9 with a mean of 3.7 (N=21, SE 2.44). The mean number of new leks detected per AF cell, for combined years, was 1.1 for AF1 and 1.0 for AF2.

Our top occupancy estimation model varied by year, with group (g) and constant (.) detection probability (p) models receiving the most support given our data (Table 7). Model-averaged estimates of the proportion of known leks in the LF that were active (ψ_{LF}) varied by year from 0.29-0.57 during the three-year study period (Table 8). Model-averaged detection probability estimates for leks in the list frame were lowest in 2012 ($\hat{p}_{LF}$ = 0.39-0.41) and similar

Table 7. Program MARK model summaries by year for dual-frame survey analysis of greater sage-grouse leks in the Parachute-Piceance-Roan population.

Year and Model	AICc	Delta AIC_c	AIC_c Weights	Model Likelihood	Num. Param.	Deviance
2012						
p(.) Psi(g)	205.94	0.00	0.580	1.000	4	10.96
p(g) Psi(g); $p_{AF1}=p_{AF2}$	207.62	1.68	0.251	0.432	5	10.48
p(t) Psi(g)	209.19	3.24	0.114	0.197	6	9.86
p(g+t) Psi(g); $p_{AF1}=p_{AF2}$	210.94	5.00	0.048	0.082	7	9.37
p(g*t) Psi(g); $p_{AF1}=p_{AF2}$	214.82	8.88	0.007	0.012	9	8.69
2013						
p(g) Psi(g); $p_{AF1}=p_{AF2}$	179.89	0.00	0.272	1.000	5	12.24
p(.) Psi(g)	180.11	0.22	0.244	0.898	4	14.60
p(g+t) Psi(g); $p_{AF1}=p_{AF2}$	180.47	0.58	0.204	0.749	7	8.45
p(t) Psi(g)	180.68	0.79	0.183	0.674	6	10.86
p(g*t) Psi(g); $p_{AF1}=p_{AF2}$	181.96	2.07	0.097	0.356	9	5.45
2014						
p(g) Psi(g); $p_{AF1}=p_{AF2}$	236.85	0.00	0.573	1.000	5	15.34
p(.) Psi(g)	238.15	1.29	0.300	0.524	4	18.76
p(g+t) Psi(g); $p_{AF1}=p_{AF2}$	240.92	4.06	0.075	0.131	7	15.05
p(t) Psi(g)	242.16	5.31	0.040	0.070	6	18.48
p(g*t) Psi(g); $p_{AF1}=p_{AF2}$	244.76	7.90	0.011	0.019	9	14.42

Table 8. Model-averaged estimates of the proportion of known greater sage-grouse lek sites that were active in the List Frame (ψ_{LF}) by year, with standard error (SE) and 95% upper (UCI) and lower (LCI) confidence intervals, in the Parachute-Piceance-Roan, 2012-2014.

Proportion active leks (ψ_{LF}) by year	Estimate	SE	95% LCI	95% UCI
2012	0.57	0.11	0.36	0.76
2013	0.29	0.06	0.19	0.43
2014	0.37	0.06	0.25	0.50

to detection probabilities in the area frame that year ($\hat{p}_{AF}$ = 0.43-0.45); detection probabilities in the area frame were relatively constant across all survey years (see Appendix B). Average detection probabilities per year for the sampling frames were 0.40, 0.62, and 0.64 in the LF for 2012, 2013 and 2014, respectively; with 0.44, 0.42, and 0.42 for AF_1 and AF_2. Estimates of p^* (the probability of detecting an active lek at least once across 3 visits during a season) were lowest in the first year (2012) in the LF at 0.78, then increased to 0.95 in 2013 and 2014; estimates for p^* in the AF varied from 0.80-0.82 across years (Figure 8).

Estimates of the proportion of leks previously known and counted (i.e. surveyed) each year by standard lek flights were 0.45 in 2012, 0.74 in 2013 and 0.45 in 2014, and the estimated proportion of males known and counted were 0.43, 0.78, and 0.57, respectively for the three years (Table 9). These results suggest that, during our study, lek counts based exclusively on monitoring known lek sites (i.e., leks in the list frame) over 3 visits would have failed to survey 26-55% of the total number of leks and 22-57% of males attending leks in the PPR (Table 9). Results from the supplemental analysis (see Appendix A) were similar to those of the primary analysis, concluding that standard lek counts would have failed to include 32-55% of the total number of active leks and 26-55% of males attending leks in the PPR.

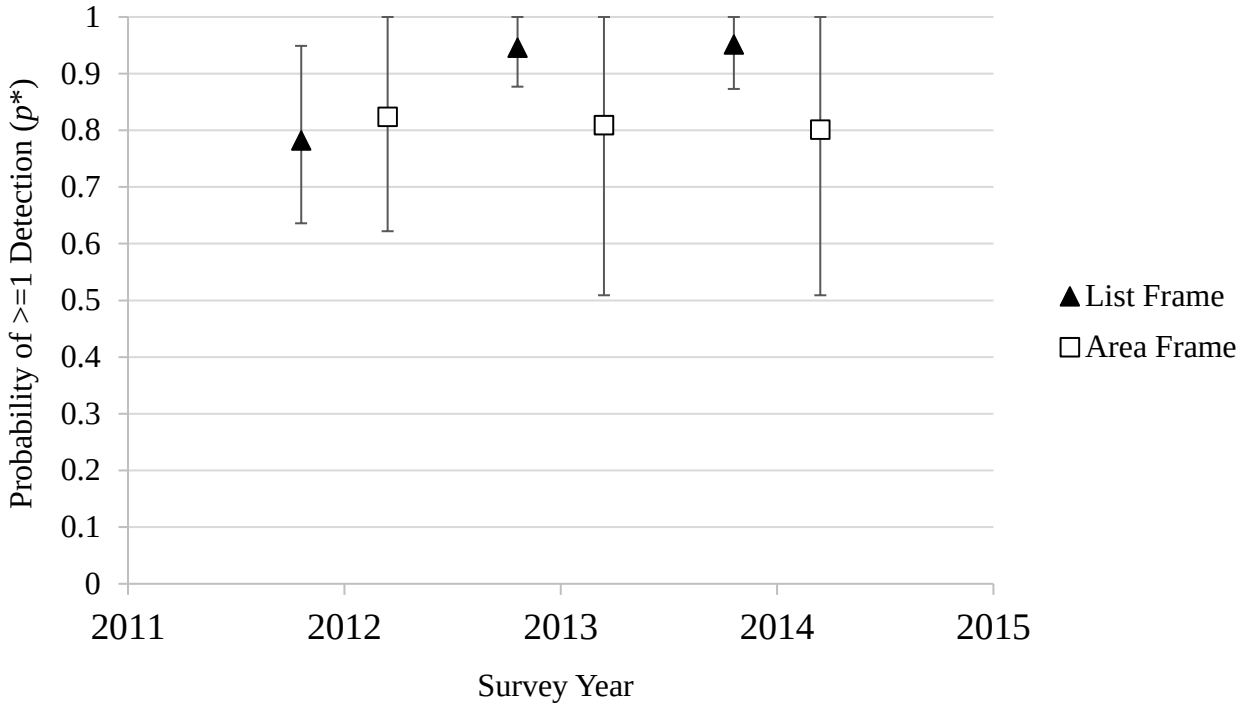

Figure 8. Estimated probability of detecting ≥ 1 greater sage-grouse males at a lek (List Frame) or ≥ 1 males at ≥1 leks (Area Frame) across three sampling occasions (p^*), with 95% confidence intervals, from dual-frame lek surveys in the Parachute-Piceance-Roan, 2012-2014.

Table 9. Total number of detected and estimated active leks, proportion of active leks known and counted, and total estimated number of males attending leks from dual-frame lek surveys in the Parachute-Piceance-Roan greater sage-grouse population, 2012-2014.

Survey Year	2012			2013			2014		
Total Leks Detected (List Frame)	22			17			23		
Total Males Detected (List Frame)	69			63			148		
	Est. /No.	95% LCI	95% UCI	Est. /No.	95% LCI	95% UCI	Est. /No.	95% LCI	95% UCI
Estimated No. Active Leks (List Frame)	28.1	22	36.9	17.9	17	25.2	24.2	23	32.2
Estimated No. Active Leks (Area Frame 1)	3.6	3	5.7	5.0	4	8.4	5.00	4	8.3
Estimated No. Active Leks (Area Frame 2)	17.0	1	49.8	0.0	0.0	0.0	21.9	1	65.1
No. Incidental Leks Observed		2			3			3	
Total No. Estimated Active Leks	48.8	28*	82.8	22.9	24*	30.9	51.1	31*	95.1
Total Estimated High Count Males	160	99*	360	81	80*	203	260	178*	665
Est. Proportion Active Leks Known & Counted	0.45	0.79*	0.27	0.74	0.71*	0.55	0.45	0.74*	0.24
Est. Proportion Males Known & Counted	0.43	0.70*	0.19	0.78	0.79*	0.31	0.57	0.83*	0.22

* The LCI was truncated to the number of active leks/high count males observed during dual frame surveys, including incidental lek locations.

DISCUSSION

Our results suggest that, if standard lek monitoring flights only monitor known and potentially active lek sites in the PPR, as many as 26-55% of active leks and 22-57% of males attending leks may be missing from count records each year. These findings are supported by the number of new leks discovered during this study; a total of 30 leks across the three-year study period and an additional 8 leks during the 2015-2017 lekking seasons, resulting from continued CPW survey efforts. Failure to survey unknown lek sites, even if they only support a relatively small number of males each (< 10 males), may result in substantial underestimation of the total abundance of lekking males and may affect assessment of population trend, particularly if the proportion of leks and males missed varies among years, as suggested by our results. At best, based on 95% confidence limits, a minimum of 20-30% of active leks and 17-30% of lekking males in the PPR may go unsurveyed in any given year. Although new leks are occasionally discovered during standard flight surveys, a concerted effort to survey for new leks may be required in the PPR.

We estimated the number of leks in the PPR greater sage-grouse population using dual-frame survey estimators from Haines & Pollock (1998) with two key differences: 1) we conducted repeated surveys of sample units to estimate detection probability to account for imperfect detection of leks, and 2) we stratified area-frame cells into two frames based on the presence of known leks from the list frame. Estimating detection probability allowed us to account for variation in factors known to affect detectability of males on leks, including lek size, environmental conditions, male behavior, and daily, seasonal, or age-related lek attendance (Fremgen et al. 2016). Even if males regularly attend leks during the breeding season, they may be absent at the time of a helicopter survey; for example, if males were flushed by a predator or

vehicle prior being visited by the helicopter or if they left the lek early because no females were present. Our use of two area-frame strata was based on observations that the majority of new leks in the PPR are discovered in close proximity to known leks and, for that reason, cells including known leks were expected to have a higher likelihood of supporting additional leks. Encounters of new leks not on the LF supported defining two area-frame strata because AF_1 cells consistently contained more previously unknown leks than AF_2. Approximately 85% of leks discovered in the area frame were found in AF_1 cells.

Estimates of detection probability estimates in this study may be specific to the PPR population, and it is unknown if they are representative of other greater sage-grouse populations. The estimated number of active leks in the PPR was lowest in 2013 when total maximum male counts were also the lowest. Even though the population was estimated to have more active leks in 2012, the average number of males per occupied lek in 2014 was more than twice that of 2012, resulting in a much larger total maximum male count in 2014. Annual turnover in apparent use of leks by males (i.e., not corrected for detection probability <1), based on leks surveyed in consecutive years, was observed in ~25% of known lek sites. The cause of turnover between years in the PPR is unknown, but may be related to within or between year inter-lek movement caused by low male or female population density, rapidly changing population density, highly fragmented and limited available breeding habitat (Hagen 1999, Walker et al. 2016), or changing availability of potential lek arenas over time due to natural or anthropogenic disturbance (e.g., fires, construction of well pads and pipelines). We suspect that larger populations with more stable lek dynamics and those inhabiting more stable landscapes will have higher rates of lek occupancy and detectability, and lower rates of lek turnover among years, but dual-frame lek surveys in such populations are needed to test those ideas.

In theory, as the number of leks on the list frame increases, surveys should eventually include almost all existing active leks. Our list frame increased in size each year as newly discovered leks were added based on detections in the area frame in the previous year. However, if new leks continue to form, obtaining a complete list frame may not be possible. During our study (2012-2014), and in the years that followed (2015-2017), survey efforts in the PPR continued to discover new leks. However, nearly all new leks discovered during the study period were near (<1 km from) previously known leks. In populations with more stable leks, the discovery of new leks should increase the proportion of leks that are known (and counted) each year and the sampling frame may be nearly complete after several survey seasons. The applicability of our findings regarding detectability and proximity of lek sites will need to be evaluated in larger or higher density populations, or those with more contiguous habitat.

In dual-frame surveys, the area frame can be stratified based on attributes of interest to increase sampling efficiency (Haines & Pollock 1998). We divided the area frame into two strata to take advantage of the patchy distribution of breeding habitat in the PPR and clustering of leks within those areas. Indeed, nearly all new leks discovered during the study period were in the vicinity of previously known leks. For this reason, stratification of the area frame may be especially important in populations where potential lek habitat is restricted and naturally fragmented, like in the PPR. Additionally, the use of a potential lek habitat layer to better define the sampling frame is important for maximizing sampling efficiency and obtaining accurate point estimates from dual-frame lek surveys. Our original definition of AF_2 included all 1-km^2 cells that intersected occupied range and we originally allocated survey effort under the assumption that all AF_2 cells could support a lek. However, we found that some portions of occupied range had vegetation and topographic characteristics inconsistent with sage-grouse leks (e.g., steep

slopes, patches of forest), so our original designation of AF_2 included numerous cells with little or no potential to support a lek. Including data from those cells would have substantially overestimated the total number of active leks in AF_2 each year, and the variance of those estimates. Restricting sampling of AF_2 cells using a potential lek habitat layer allowed us to improve dual-frame estimates by appropriately restricting AF_2 cells to just those that would reasonably support a lek. We removed both available and sampled AF_2 cells from analyses under the assumption that they should have not been surveyed to begin with. This adjustment was important to avoid overestimating the total number and variance of occupied leks in both AF_2 and in the population as a whole. We recommend that any future dual-frame lek survey efforts delineate potential lek habitat prior to determining the area-frame strata to maximize survey efficiency and minimize estimator variance.

We pooled data for AF_1 and AF_2 to estimate detection probabilities by setting $\hat{p}_{AF1}$ equal to $\hat{p}_{AF2}$ in each of our models that allowed p to vary with sampling frame (i.e., "group"). We had insufficient data to estimate $\hat{p}_{AF2}$ because so few leks were detected in AF_2. This may be a limitation of dual-frame lek surveys when monitoring either low-density or well-studied populations where few new leks are expected. Our decision to pool data across area-frame strata assumed that detection probability of new leks was equal in all area-frame cells, regardless of proximity to known lek sites. We this was defensible because lek size is thought to be a major influence on lek detectability (WAFWA 2015) and all new leks detected in either area-frame stratum were of similar size (fewer than 10 males per lek with an average of <4). Dual-frame estimators were sensitive to sparse data in the area frame. We detected no leks in AF_2 in 2013. For that reason, it is likely that we underestimated the total number of leks in AF_2 that year. This conclusion is supported by the fact that our point estimate for the number of leks in 2013 was

below the number of leks we actually observed (Table 9) after accounting for incidentally discovered leks. This effect may be diminished in future dual-frame lek surveys by focusing area-frame sampling in areas with greater potential to support leks.

Estimated detection probabilities for leks in area-frame strata were relatively constant throughout the study (0.34-0.50). However, estimated detection probabilities for leks in the list frame were lower in 2012 (0.39-0.41) and similar to those in the area-frame strata that year (0.43-0.45). The reason for lower detection probability in the list frame the first year is unknown, however, it is possible that observers (and pilots) unfamiliar with the location of known leks were less likely to detect strutting males if they flushed from the lek prior to being detected. Familiarizing observers with the location of known lek sites prior to surveys may lessen this effect in subsequent years. For this reason, managers that implement dual-frame surveys (especially from aircraft) should consider familiarizing observers (and pilots) with all list-frame leks prior to dual-frame surveys to eliminate this possibility.

Our estimates of the detectability of leks relied on two assumptions: (1) that within-season occupancy of sample units did not change, and (2) that sample units were independent. These assumptions may be violated if inter-lek movement of males (Fremgen et al. 2017) causes some leks to be abandoned or reoccupied during the sampling period. Because dual-frame surveys require multiple observations per sample unit, inter-lek movement of males between sampling occasions would violate these assumptions. This would result in underestimation of lek detection probability and the proportion of leks known and counted, and overestimation of the total number of leks and males. If the majority of leks in a population are known, inter-lek movement would cause any analysis method based on based on summed high male counts across leks to overestimate the number of lekking males in the population. Additional information is

needed on the frequency of inter-lek movements by males to quantify its effects on detection probability of leks and on high male counts to better understand how it affects results of dual-frame lek surveys and other lek-based monitoring methods. In addition to potential bias from movement of males, dual-frame estimates of the total number of leks and maximum number of males do not account for observer bias or variation in detection probability of individual males, lek attendance, or inter-lek movement and estimates are still subject to these potential sources of bias. Dual-frame sampling also does not address other key assumptions regarding the use of lek-count data for monitoring populations, such as variation in count effort or sex ratio, and information on annual variation in sex ratio is required if trying to extrapolate male count data to estimate the female population or the entire population.

An important consideration when planning dual-frame surveys is how to allocate sampling effort among sampling frames to balance the cost of surveys (e.g., the number and length of helicopter flights, survey effort by ground crews) with precision of estimates. We examined various scenarios for how to optimize allocation of area-frame surveys in relation to estimator precision given budget limitations (Table C1, Appendix C). We recommend sampling the area-frame strata as proportionally as possible (i.e., sampling the same proportion of total available sample units in each strata) to minimize expected standard errors while also attempting to ensure that neither area-frame effort results in occupancy values of zero. For the PPR, optimizing sampling by more proportionally surveying the two area-frame strata (i.e. surveying 20 AF_1 and 140 AF_2 cells) is expected to reduce the magnitude of the standard error for estimated total number of leks by nearly 40% (Table C1, Appendix C) compared to our results. If enough resources and funding are available, increasing the number of sampling occasions from three to four can reduce the standard error by approximately 45%. Still, decisions about sampling

allocation also need to consider other potential trade-offs specific to the study population. For example, if the study area is large in size or leks are widely dispersed within a population, it may make the most sense from a logistical standpoint to sample AF_1 cells around all sampled LF leks to increase survey efficiency and save both fuel and time during flights. Increasing the number of sampling occasions, and therefore, the duration of the sampling period, may also exacerbate any bias caused by inter-lek movement of males.

MANAGEMENT IMPLICATIONS

State wildlife agencies often have little or no quantitative information about how many greater sage-grouse leks exist in specific populations, and therefore, how many are included in (or excluded from) assessments of population size and trend used to assess population status and inform management actions. This is a primary criticism of lek-based monitoring strategies and a major concern for wildlife managers. Dual-frame surveys, when accompanied by estimates of detection probability, may be useful, particularly when combined with lek habitat models, to obtain baseline estimates of the proportion of leks being surveyed. This would enable managers to better assess the need for additional lek survey efforts or to adjust lek-count index data based on high male counts to better represent population size and trend of lekking males. The method may also help identify specific areas where managers should focus additional efforts to survey for new leks.

A key advantage of dual-frame lek surveys is that they are compatible with, and easily incorporated into, existing lek-count monitoring efforts, particularly for populations already monitored via aircraft. Standard lek counts already require multiple visits to all, or a subset of

known leks (i.e., the list frame) during a specified sampling period, and count protocols for dual-frame surveys and standard counts are identical. However, dual-frame surveys require additional effort to define, stratify, and survey for leks within area frames, preferably from aircraft to increase survey efficiency.

Dual-frame surveys conducted over multiple years would also be useful to quantify annual variation in the proportion of leks that are known and counted in specific populations of interest. Our data suggest that this proportion may vary substantially over time in small, low-density populations. In populations with stable lek dynamics, it may be possible to obtain a nearly complete list frame after just a few years of surveys, particularly in more well-studied populations.

We encourage managers to use caution when interpreting data from any monitoring efforts that rely on male high count data collected over multiple visits to assess trend in populations. Quantifying the frequency, extent, and annual variation in male detectability on leks, male lek attendance, and inter-lek movements in greater sage-grouse populations should be a priority for further research to assess the potential impacts on lek-count data and trend analysis. For dual-frame surveys conducted by helicopter, it may be possible to partially offset the impacts of male movement by pooling male counts for all leks surveyed on a single day (i.e. the total male count from all leks surveyed in a region) to determine seasonal high counts, rather than using high counts from each lek individually. If males visit neighboring leks more often than distant leks, this would reduce the chance that those males would inflate the high male count for each lek.

LITERATURE CITED

Applegate, R. D. 2000. Use and misuse of prairie chicken lek surveys. Wildlife Society Bulletin. 28:457-248.

Beck, T. D. I. and C. E. Braun. 1980. The strutting ground count: variation, traditionalism, management needs. Proceedings of the Western Association of Fish and Wildlife Agencies 60:558-566.

Caughley, G. 1977. Analysis of vertebrate populations. Wiley, London, United Kingdom.

Coates, P. S., Casazza, M. L., Blomberg, E. J., Gardner, S. C., Espinosa, S. P., Yee, J. L., Wiechman, L., and B. J. Halstead. 2013. Evaluating greater sage-grouse seasonal space use relative to leks: implications for surface use designations in sagebrush ecosystems. Journal of Wildlife Management 77:1598-1609.

Colorado Greater Sage-Grouse Steering Committee (CGSSC). 2008. Colorado greater sage-grouse conservation plan. Colorado Division of Wildlife, Denver, Colorado, USA.

Connelly, J. W. and C. E. Braun. 1997. Long-term changes in sage grouse (*Centrocercus urophasianus*) populations in western North America. Wildlife Biology 3:229-234.

Connelly, J. W., Knick, S. T., Schroeder, M. A., and S. J. Stiver. 2004. Conservation assessment of greater sage-grouse and sagebrush habitats. Western Association of Fish and Wildlife Agencies, Cheyenne, Wyoming, USA.

Connelly, J. W., Reese, K. P., and M. A. Schroeder. 2003. Monitoring of greater sage-grouse habitats and populations. University of Idaho, College of Natural Resources Experiment Station Bulletin 80. Moscow, Idaho, USA.

Doherty, K. E., Naugle, D. E., and Walker, B. W. 2010. Greater sage-grouse nesting habitat: the importance of managing at multiple scales. Journal of Wildlife Management 74:1544-1553.

Fremgen, A. L., Hansen, C. P., Rumble, M. A., Gamo, R. S., and J. J. Millspaugh. 2016. Male greater sage-grouse detectability on leks. Journal of Wildlife Management 80:266-274.

Fremgen, A. L., Rota, C. T., Hansen, C. P., Rumble, M. A., Gamo, R. S., and J. J. Millspaugh. 2017. Male greater sage-grouse movements among leks. Journal of Wildlife Management doi:10.1002/jwmg.21208.

Fuller, T. K. 1991. Do pellet counts index white-tailed deer numbers and population change? Journal of Wildlife Management 55:393-396.

Goodman, L.A. 1962. Variance of the product of two random-variables. Journal of the American Statistical Association 57:54-60.

Haines, D. E. and K. H. Pollock. 1998. Estimating the number of active and successful bald eagle nests: an application of the dual frame method. Environmental and Ecological Statistics 5:245-256.

Hagen, C. A. 1999. Sage grouse habitat use and seasonal movements in a naturally fragmented landscape, northwestern Colorado. Thesis, University of Manitoba, Winnipeg, Canada.

Hartley, H. O. 1962. Multiple-frame surveys. The Proceedings of the Social Statistics Section, American Statistical Association 19:203-206.

Holloran, M. J. and S. H. Anderson. 2005. Greater sage-grouse population response to natural gas development in western Wyoming: are regional populations affected by relatively localized disturbances? Transactions of the North American Wildlife and Natural Resources Conference 70:160-170.

Johnson, D. H. 2008. In defense of indices: the case of bird surveys. Journal of Wildlife Management 72:857-868.

Johnson, D. H. and M. M. Rowland. 2007. The utility of lek counts for monitoring greater sage-grouse. Pages 15-24 *in* K.P. Reese and R.T. Bowyer, editors. Monitoring populations of greater sage-grouse: proceedings of a symposium at Idaho State University. College of Natural Resources Experiment Station Bulletin 88. Moscow, ID, USA.

Knick, S. T., Dobkin, D. S., Rotenberry, J. T., Schroeder, M. A., Vander Haegen, M. A., and C. Van Riper. 2003. Teetering on the edge or too late? Conservation and research issues for avifauna of sagebrush habitats. Condor 105:611-634.

Kott, P. S. and F. A. Vogel. 1995. Multiple-frame business surveys. Business Survey Methods 185-203. John Wiley and Sons: New York, New York, USA.

Krager, R. A. 1977. Survey of sage grouse strutting ground complexes and seasonal use areas within the Piceance Basin Wildlife Habitat Area. Colorado Division of Wildlife, Fort Collins, Colorado, USA.

MacKenzie, D. I., Nichols, J. D., Royle, J. A., Pollock, K. H., Bailey, L. L., and J. E. Hines. 2006. Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence. Academic Press: San Diego, California, USA.

Otto, M. C. and J. R. Sauer. 2007. Contiguous 48 states bald eagle pair survey design. Appendix to the draft bald eagle monitoring plan, United States Fish and Wildlife Service Bald Eagle Monitoring Team.

Parachute-Piceance-Roan Greater Sage-Grouse Work Group (PPR-GSGWG). 2008. Parachute-Piceance-Roan (PPR) greater sage-grouse conservation plan. Colorado Division of Wildlife, Denver, Colorado, USA.

Patterson, R. L. 1952. The sage grouse in Wyoming. Wyoming Game and Fish Commission and Sage Books, Inc., Denver, Colorado, USA.

Naugle, D. E. and B. L. Walker. 2007. A collaborative vision for integrated monitoring of greater sage-grouse populations. Pages 57-62 *in* Reese, K. P. and R.T. Bowyer, editors. Monitoring populations of greater sage-grouse: proceedings of a symposium at Idaho State University. College of Natural Resources Experiment Station Bulletin 88. Moscow, Idaho, USA.

Rowland, M. M., White, G. C., and E. M. Karlen. 1984. Use of pellet-group plots to measure trends in deer and elk populations. Wildlife Society Bulletin 12:147-155.

Rowland, M. M., Wisdom, M. J., Suring, L. H., and C. W. Meinke. 2006. Greater sage-grouse as an umbrella species for sagebrush-associated vertebrates. Biological Conservation 129:323-335.

Schroeder, M. A., Aldridge, C. L., Apa, A. D., Bohne, J. R., Braun, C. E., Bunnell, S. D., Connelly, J. W., Deibert, P. A., Gardner, S. C., Hilliard, M. A., Kobriger, G. D., and C. W. McCarthy. 2004. Distribution of sage-grouse in North America. Condor 106:363-376.

Theobald, D. M., Stevens. Jr., D. L., White, D., Urquhart, N. S., Olsen, A. R., and J. B. Norman. 2007. Using GIS to generate spatially balanced random survey designs for natural resource applications. Environmental Management 40:134–146.

Thompson, S. K. 2012. Sampling. 3rd Edition. Wiley: Hoboken, New Jersey, USA.

United States Fish and Wildlife Service (USFWS) Bald Eagle Monitoring Team. 2007. Draft post-delisting monitoring plan for the bald eagle (*Haliaeetus leucocephalus*).

United States Fish and Wildlife Service (USFWS). 2010. 12-month finding for petitions to list the greater sage grouse (*Centrocercus urophasianus*) as threatened or endangered. Federal Register 75:13909-14014.

United States Fish and Wildlife Service (USFWS). 2015. Endangered and threatened wildlife and plants; 12-month finding on a petition to list greater sage-grouse (*Centrocercus urophasianus*) as an endangered or threatened species. Federal Register 80:59857-59942.

Walker, B. L., Apa, A. D., and K. Eichhoff. 2016. Mapping and prioritizing seasonal habitats for greater sage-grouse in Northwestern Colorado. Journal of Wildlife Management 80:63-77

Walker, B. L., Naugle, D. E., & K. E. Doherty. 2007. Greater sage-grouse population response to energy development and habitat loss. Journal of Wildlife Management 71:2644-2654.

Walsh, D. P., Stiver, J. R., White, G. C., Remington, T. E. and A. D. Apa. 2010. Population estimation techniques for lekking species. Journal of Wildlife Management 74:1607-1613.

Walsh, D. P., White, G. C., Remington, T. E., and D. C. Bowden. 2004. Evaluation of the lek-count index for greater sage-grouse. Wildlife Society Bulletin 32:56-68.

Western Association of Fish and Wildlife Agencies (WAFWA). 2015. Greater sage-grouse population trends: an analysis for lek-count databases 1965-2015. Report. Western Association of Fish and Wildlife Agencies, Cheyenne, WY, USA.

White, G. C. and K. P. Burnham. 1999. Program Mark: survival estimation from populations of marked animals. Bird Study 46:120-139.

Witmer, G. W. 2005. Wildlife population monitoring: some practical considerations. Wildlife Research 32: 259-263.

CHAPTER 3: NON-INVASIVE GENETIC MARK-RECAPTURE ANALYSIS OF FECAL PELLETS TO ESTIMATE PRE-BREEDING ABUNDANCE OF GREATER SAGE-GROUSE (*CENTROCERCUS UROPHASIANUS*) IN NORTHWESTERN COLORADO[3]

SUMMARY

The greater sage-grouse (*Centrocercus urophasianus*) is a species of conservation concern throughout its range. Since the 1950s, high counts of males at leks have been used as an index for monitoring populations and are often assumed to represent overall population trend. However, the relationship between the lek index and true population size is unclear and the reliability of these counts for monitoring populations has been questioned. In addition, lek-count data do not provide useful information about the female population, a crucial demographic component to assess a population's growth potential. There is a need to develop and evaluate alternative methods to obtain reliable estimates of population trend and to test key assumptions underlying the lek-count index. We used non-invasive genetic mark-recapture to estimate pre-breeding abundance in a small, low-density population of greater sage-grouse in northwestern Colorado during two consecutive winter seasons in 2012-2013 and

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2013-2014. We estimated population size as 335 (95% C.I. 287-382) in the first winter and 745 (95% C.I. 627-864) in the second, an apparent doubling of population size during the two-year study period. The change in male abundance estimates from genetic mark-recapture paralleled the trend observed in high male count data from lek observations, but the lek-count index poorly represented estimated annual male abundance. Our data suggest that, while lek counts may be useful for detecting relatively large changes in population size over time, they may not sufficiently detect significant changes in the size of small populations. Unbiased estimates of population size are essential to inform management decisions, particularly for small populations and populations experiencing rapid, large-scale land-use change.

INTRODUCTION

Lek-Count Index

Defensible estimates of population abundance are essential for effective management of wildlife species. They are critical for determining federal and state conservation status, establishing land-use strategies or regulations to manage species and their habitats, and assess the effectiveness of management and conservation efforts. This is particularly true for species of conservation concern, such as the greater sage-grouse (*Centrocercus urophasianus*; hereafter "GRSG"), which have experienced recent population declines and significant reductions in geographic range (Connelly & Braun 1997, Connelly et al. 2004, Schroeder et al. 2004, Aldridge et al. 2008, Garton et al. 2015). These declines, in combination with ongoing habitat loss, degradation, and human land-use conflicts, have prompted repeated petitions for federal listing of the species under the Endangered Species Act. A major concern when evaluating listing proposals is the methods used to estimate population trend and the utility of the underlying data.

The primary monitoring state variable for sage-grouse is the annual lek-count index data, based on the maximum number of males observed at a lek over multiple surveys (Connelly et al. 2004). Use of this index dates back to the 1950s, and the index is still widely used by state wildlife agencies throughout the western United States to detect evidence of population trends (WAFWA 2015).

Despite its frequent application for population assessment and management decisions, the use of the lek-count index for making reliable inference to population trend has been extensively criticized (Beck & Braun 1980, Applegate 2000, Walsh et al. 2004, Walsh et al. 2010). The lek-count index is based on seasonal high counts of males detected at leks. However, detectability of males is affected by many factors and the index may only represent the subset of males that attend leks in their respective population. This has led to questions regarding the use of count data as an index of population abundance and the relationship between the lek-count index and the true number of males attending leks remains unclear. Attempts to infer trend or extrapolate total abundance from lek-count data rely on untested assumptions regarding detectability of males, the number of leks known and counted, rates of male lek attendance and inter-lek movement of males during the count period, observer variability, and sex ratio (when attempting to estimate total population abundance). The extent to which many of these factors affect count data remains unknown for sage-grouse populations. For these reasons, the development of innovative methods to test assumptions underlying the lek-count index is critical for attaining unbiased estimates of population abundance and trend.

In recent years, attempts have been made to evaluate and improve lek-count protocols for GRSG to generate unbiased estimates of population abundance and trend (Connelly et al. 2003, Walsh et al. 2004, Naugle & Walker 2007, Walsh et al. 2010, Fedy & Aldridge 2011, Blomberg

et al. 2013, Monroe et al. 2016, McCaffrey & Lukacs 2016, McCaffrey et al. 2016). In addition to research evaluating the reliability of standard lek counts, alternatives to traditional ground and aerial lek monitoring strategies, such as the use of unmanned aircraft and infrared imagery to locate and count leks, are being investigated (Gillette et al. 2013, Christie et al. 2016). Emphasis on survey methods that reduce disturbance to birds is important for improvement, and development, of methods.

Genetic Mark-Recapture

Non-invasive genetic monitoring is a promising tool for assessing the status and trend of wildlife populations and can provide information often unattainable using traditional monitoring methods (Schwartz et al. 2006). Genetic mark-recapture (GMR) techniques take advantage of the unique genetic fingerprint of every individual in a population, which allows the determination of individual identities from samples obtained in the environment, and therefore replace invasive capture techniques and the need for physical tags or markers. Recent advances in non-invasive GMR techniques using passive collection of DNA sources such as scat, feathers and hair have created new opportunities to apply mark-recapture theory (Lukacs & Burnham 2005a). Genetic mark-recapture has been used to estimate population size in a variety of vertebrate taxa including grizzly and brown bears (Mowat & Strobeck 2000, Boulanger et al. 2004, Bellemain et al. 2005), black bears (Coster et al. 2011), northern pike (Miller et al. 2001), northern goshawks (Bayard de Volo et al. 2005), capercaillie (Rosner et al. 2014), Gunnison sage-grouse (Oyler-McCance and St. John 2010, unpublished report) and humpback whales (Palsboll 1997). The use of fecal DNA in GMR studies was first attempted in coyotes (Kohn et al. 1999).

While genetic mark-recapture techniques have previously been used to estimate the number of sage-grouse individuals attending specific lek breeding sites (Oyler-McCance and St. John, unpublished report), the technique has yet to be used to estimate total population abundance. The use of GMR methods to estimate GRSG abundance is appealing since the methods are largely non-invasive and have the potential to yield precise estimates of demographic parameters not attainable from lek-count data. However, challenges remain because DNA from passively collected genetic samples, such as feces and shed feathers, is often of low quality (Tablerlet et al. 1996, Buchan et al. 2005, Broquet et al. 2007, Hogan et al. 2007, Panasci et al. 2011) and quality can be highly variable among samples (Miquel et al. 2006). Problems arising from poor quality DNA samples include amplification failure, allelic dropout, and mutation during amplification (Lukacs & Burnham 2005a, Buchan et al. 2005), each of which may result in genotyping error. Such error violates a key assumption of mark-recapture models that "marks" are correctly identified and recorded. Lukacs & Burnham (2005b) showed that genotyping error can result in biased abundance estimates from closed mark-recapture models. However, it is possible to address many of the challenges of low quality DNA by altering field collection methods and using laboratory protocols that minimize the occurrence, and therefore the impact, of low genotyping success rates and genotyping error in GMR studies (Paetkau 2003, Panasci et al. 2011, Marucco et al. 2011, Lampa et al. 2013).

Despite the logistical challenges of genetic sampling during the winter, the advantages for monitoring species such as the GRSG are promising. In most studies, GRSG exhibit relatively low mortality during the winter, with survival estimates ranging from 0.82 to 1.0 (Robertson 1991, Wik 2002, Hausleitner 2003, Beck et al. 2006, Batazzo 2007). Low mortality rates minimize the risk of violating the assumption of demographic closure and allow for the use

of more robust closed mark-recapture models. Additionally, because GRSG are primarily ground-dwelling, winter snow cover facilitates detection of flocks and sampling of genetic samples left behind by birds as they forage.

We evaluated the use of a non-invasive, GMR method based on GRSG fecal (and feather) samples collected in winter to estimate total pre-breeding population abundance in a small, geographically isolated, low-density population in Colorado. Specifically we, 1) estimated pre-breeding abundance of male and female greater sage-grouse using primarily non-invasively collected fecal pellet and feather samples from winter flock locations, and 2) investigated the sampling effort required to obtain desired levels of precision for abundance estimates from non-invasive sampling of winter flocks.

Study Area

The Parachute-Piceance-Roan (PPR) population of GRSG is located in northwestern Colorado (Figure 9), is one of seven currently recognized populations in the state (CGSSC 2008). This low-density population constitutes approximately 4% of Colorado's total GRSG population based on lek-count data (CGSSC 2008). Approximately 35% of sage-grouse occupied range in the PPR is owned and managed by state or federal agencies with the remaining 65% privately owned by energy companies and ranches (PPR-GSGWG 2008).

The PPR is characterized by diverse terrain with broad ridgetops that drop off into steep drainages and canyons on the western and southern extents. Vegetation on ridgetops and plateaus is dominated by a mosaic of mountain big sagebrush (*Artemisia tridentata vaseyana*), mixed

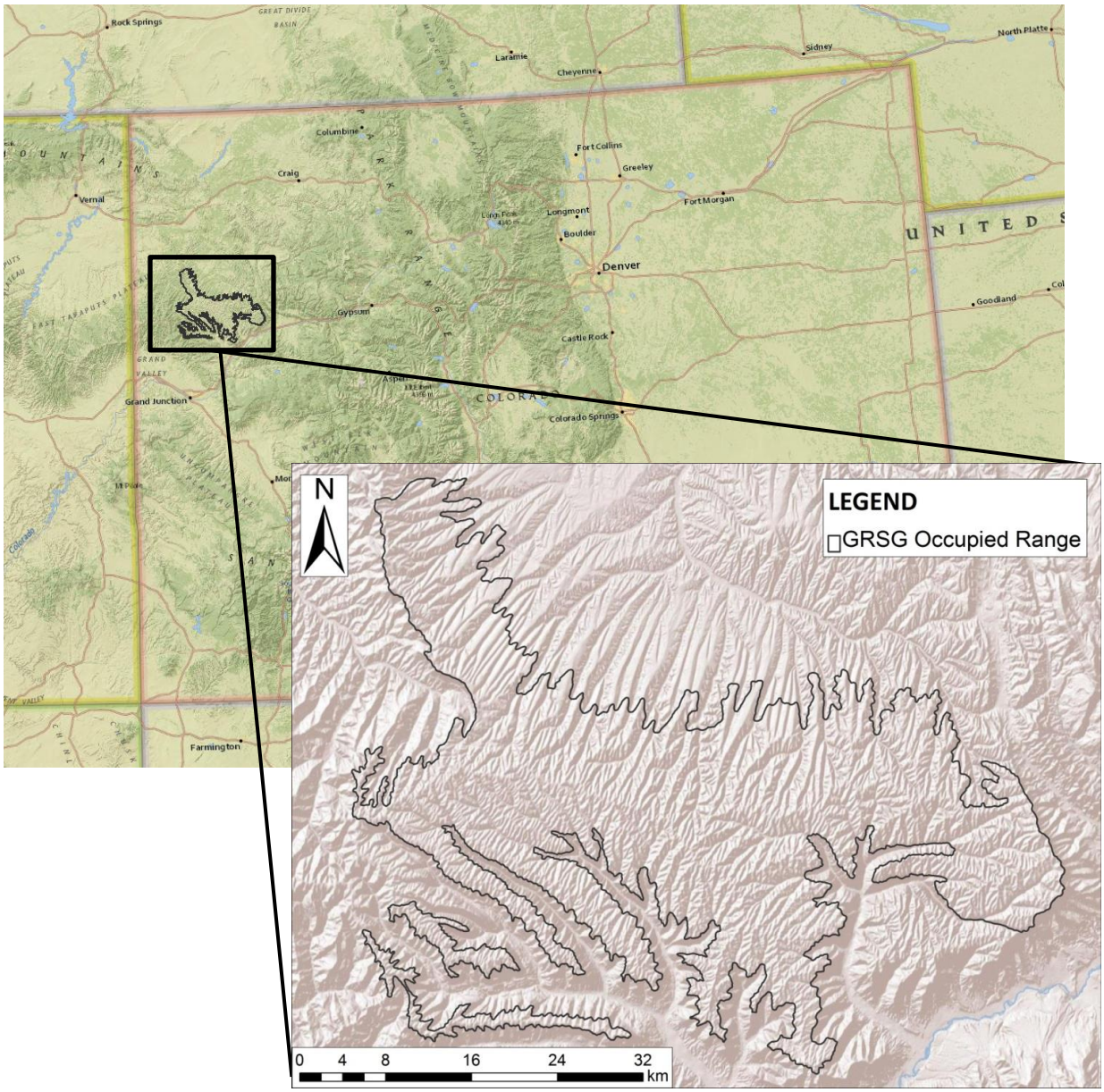

Figure 9. Study area map showing the current (2012) occupied range boundary for the core Parachute-Piceance-Roan greater sage-grouse population in northwestern Colorado, with shaded relief to illustrate topography.

sagebrush-mountain shrubs, and pinyon-juniper (*Pinus edulis*, *Juniperus spp.*) woodlands with scattered patches of aspen (*Populus tremuloides*) and other conifers.

In the PPR, GRSG are largely restricted to sagebrush, sagebrush-grassland, and mixed sagebrush-mountain shrub on ridge tops, plateaus, and shallow drainages (Figure 10) at elevations from 2,150–2,750m in elevation (Krager 1977, Hagen 1999, CGSSC 2008, Walker et al. 2016). Winters in the PPR are generally characterized by deep snow and sage-grouse habitat use is restricted by the availability of exposed sagebrush cover. The study area is covered by a network of developed oil and gas access roads and undeveloped ranch two-tracks along ridgetops and drainages that facilitate access. The population is geographically isolated, but not genetically distinct, from other Colorado populations (PPR-GSGWG 2008).

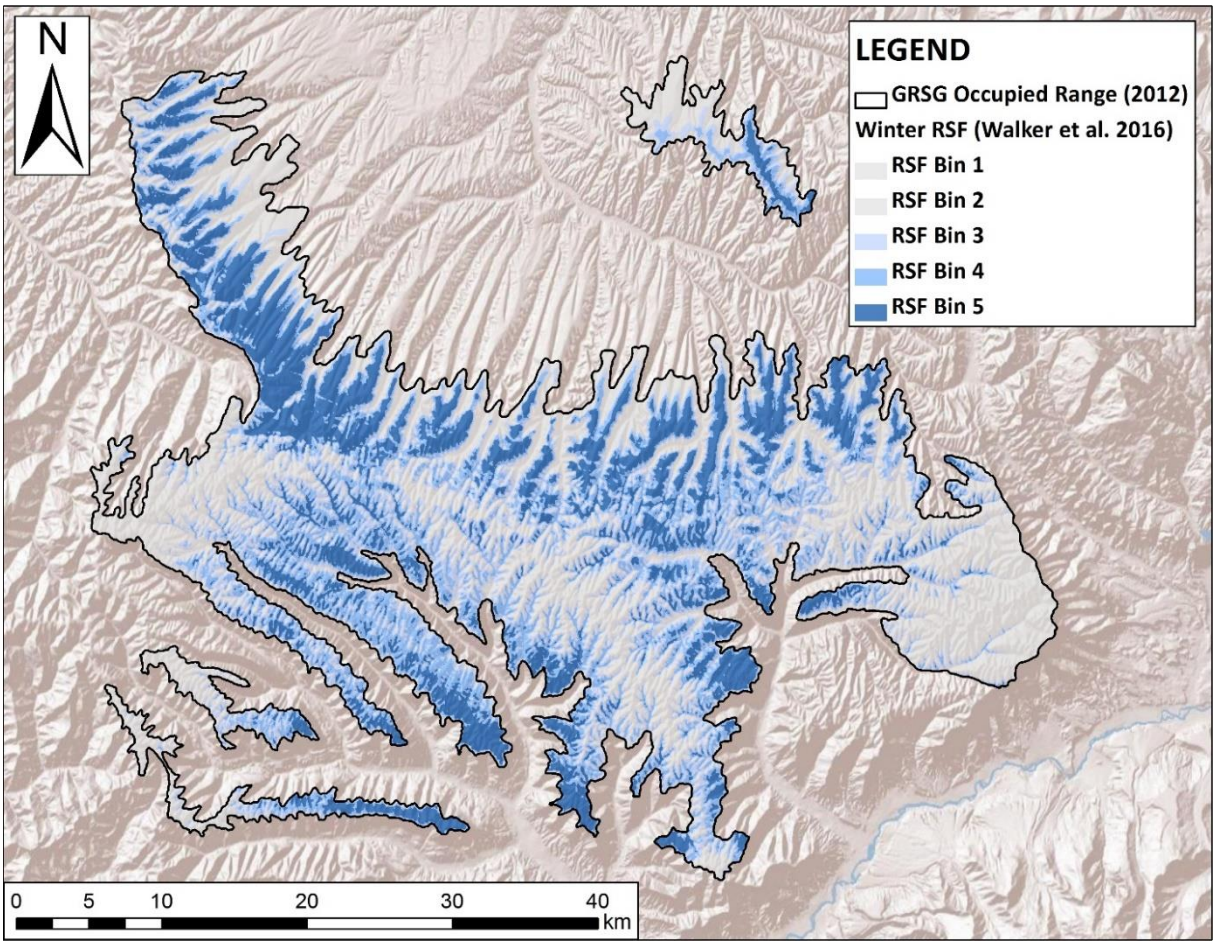

Figure 10. Winter resource selection function map showing areas of greater predicted relative intensity of use by greater sage-grouse within the Parachute-Piceance-Roan occupied range boundary (Walker et al. 2016).

Located on the southern edge of the species' range in Colorado, the PPR population was monitored irregularly until 2005 when managers recognized the potential for impacts from increasing energy development. As a result, the population lacks a rigorous long-term lek-count dataset for assessing population status and trend. Colorado Parks and Wildlife (CPW) lek-count data from 2005-2016 (Figure 11), show a fluctuating pattern in annual high male counts. These

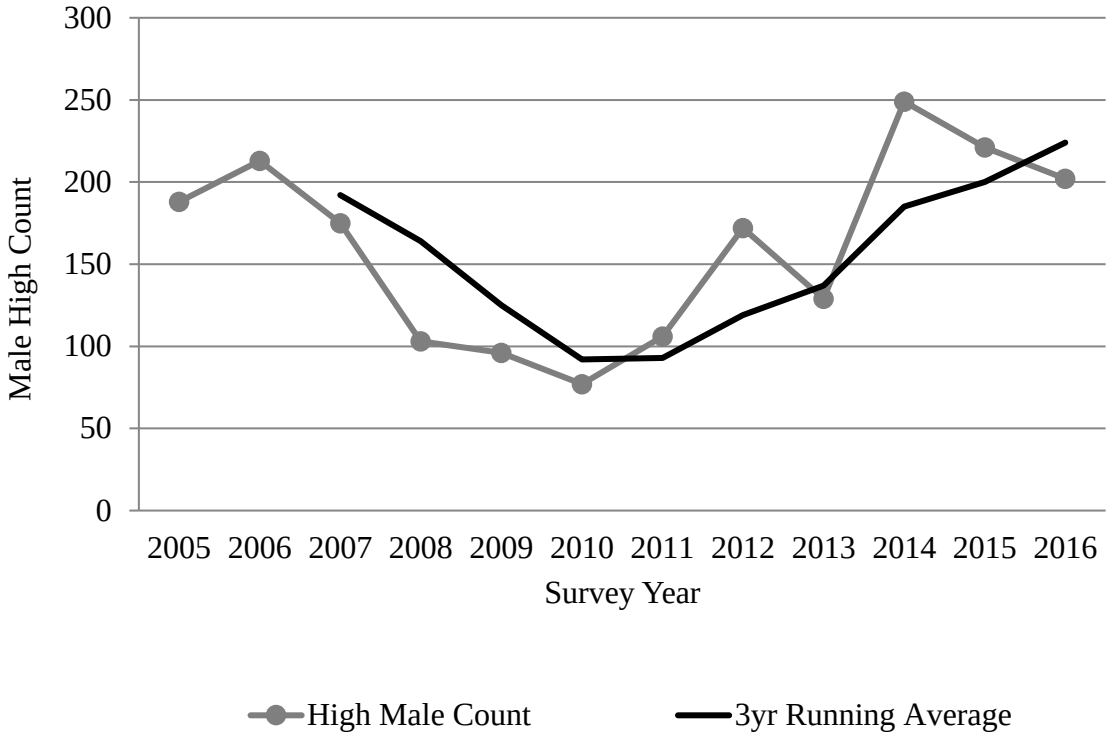

Figure 11. Colorado Parks and Wildlife lek-count data showing high male counts summed across leks and the three-year running average in the Parachute-Piceance-Roan greater sage-grouse population in northwestern Colorado from 2005-2016.

data suggest a population drop in 2013 and a strong rebound in 2014, the two years corresponding to our study (see Supplement A for details).

METHODS

Sage-Grouse Captures and Survival Monitoring

We trapped and marked GRSG in the study area from mid-July through early November in 2012 and 2013 to estimate winter survival. Colorado Parks and Wildlife's (CPW, USDA Registration #84-R-0045) Animal Care and Use Committee approved all trapping, handling and marking methods with Colorado State University (CSU) inter-agency approval (#07-2011 and #08-2012; Appendices E-I). We trapped birds using bumper-mounted and stationary net launchers (Giesen et al. 1982), night-time spotlight trapping and hoop-netting (Wakkinen et al. 1992), drop-nets, and hand-held, compressed-air net guns (Wildlife Capture Services, Flagstaff, AZ) from trucks, all-terrain vehicles, and on foot.

We aged and sexed captured birds using plumage characteristics, molt patterns, weight, tarsal length, and head length. Colorado Parks and Wildlife (CPW) affixed adult and yearling males with 30g rump-mounted, solar-powered global positioning system (GPS) platform transmitter terminal (PTT) satellite transmitters (Northstar Science and Technology, King George, VA) as part of an ongoing collaborative project. Leg loop attachments for GPS transmitters were based on the method B design described in Bedrosian and Craighead (2007). We equipped adult and yearling females with 22-g, battery-powered, VHF necklace collars equipped with mortality sensors (Advanced Telemetry Systems, Isanti, MN). Uniquely numbered, state-issued, aluminum leg identification bands were attached to all captured birds. We collected a feather sample for genetic analysis from the flanks of each bird using a sterile nitrile glove and stored the sample in Whirlpak© bags or paper envelopes. A small number of additional samples used in our study were collected from birds captured by CPW during the lekking season.

We monitored collared birds daily (GPS-marked males) or bi-weekly (VHF-collared females) to detect potential mortalities that occurred throughout our winter sampling seasons. Females were primarily monitored using truck-mounted omnidirectional and Yagi hand-held antennas to detect mortalities. We used fixed-wing aircraft to confirm the status of females just prior to the start and end of our winter sampling periods and to locate missing females as needed in 2012-2013. More intensive aerial monitoring to confirm status and obtain location data on females was conducted every 2-4 weeks during the 2013-2014 winter season. This allowed monitoring movement of females into, and out of, areas crews could not access. Suspected mortalities were investigated on the ground ensure lack of movement was not the result of a slipped transmitter and to confirm cause of mortality.

Non-Invasive Genetic Sampling

We conducted non-invasive genetic sampling of GRSG fecal pellets and shed feathers during two consecutive winter seasons from early November to mid-March in 2012-2013 and 2013-2014. We divided the study area into 200 x 200 m plots and selected a spatially-balanced random sample of approximately 1,000 plots using a reversed randomized quadrant-recursive raster (RRQRR) algorithm (Theobald et al. 2007, Figure 12). Plots were pre-stratified using resource selection function (RSF) values representing predicted relative intensity of use by GRSG during the breeding season (Walker 2010, Figure 13). Stratification allowed us to focus sampling in habitats with higher probability of GRSG use and to increase capture probability (*p*). We used breeding RSF values to inform sampling because winter RSF layers were not available when our study began and GRSG select habitats with similar vegetative and topographic features during breeding and winter (Walker et al. 2016). We divided plots into sets consisting of 30 plots

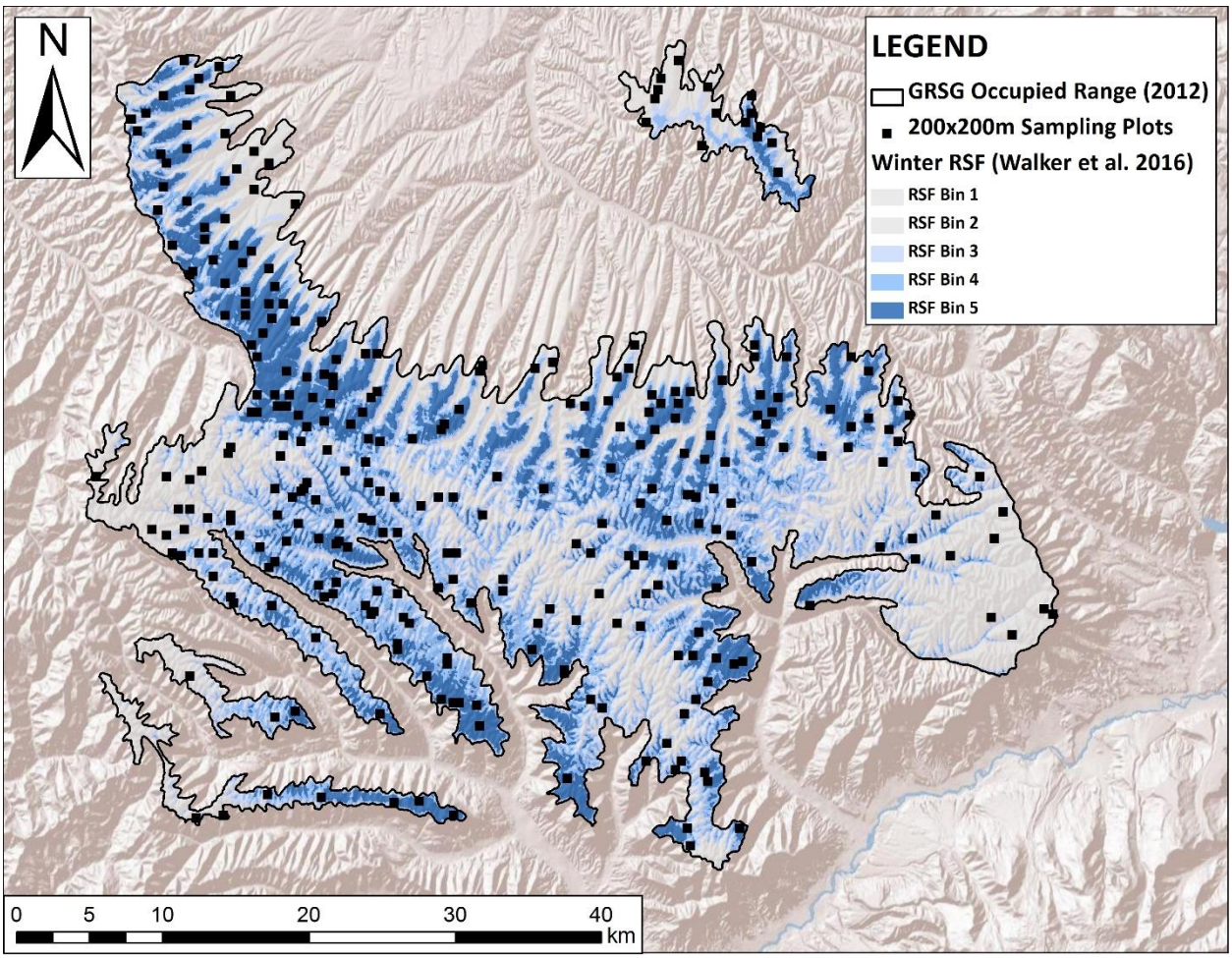

Figure 12. A subset of spatially-balanced random sample plots used for genetic mark-recapture sampling during winter seasons in 2012-2013 and 2013-2014, shown here with winter resource selection function maps, showing predicted relative intensity of use by greater sage-grouse from Walker et al. (2016).

per set based on the order in which they were selected by RRQRR. We were initially concerned that high winter site fidelity of GRSG might result in few recaptures and low detection probability if new sample plots were surveyed each occasion, so we surveyed plot sets in a rotating panel to increase recapture probability and balance this concern with spatially representative sampling of the population. We selected 60 plots (two sets) to sample during each sampling occasion in a rotating fashion (i.e., occasion 1 included sets 1 and 2, occasion 2

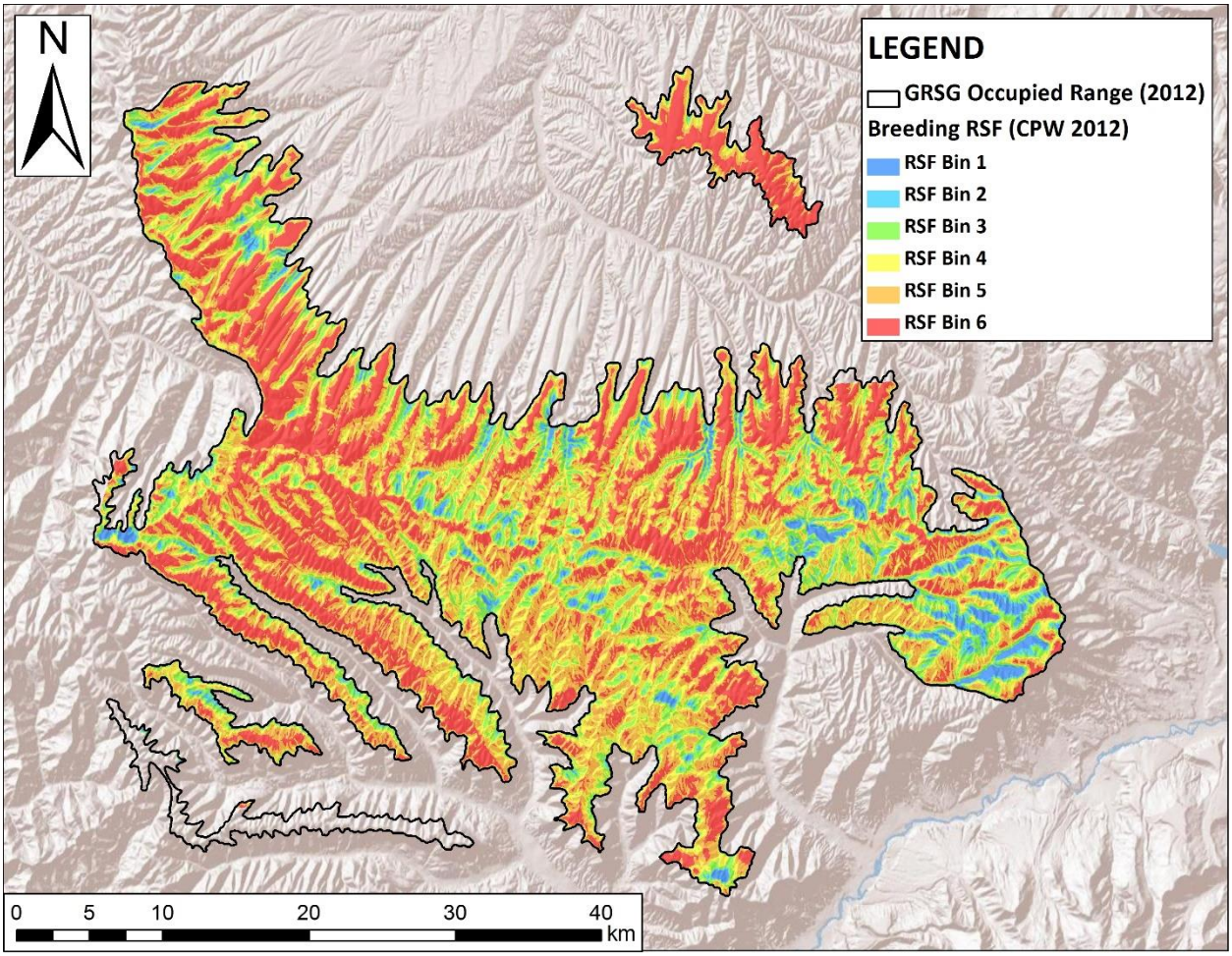

Figure 13. Breeding habitat map, showing areas of greater predicted relative intensity of use by greater sage-grouse, used to pre-stratify genetic mark-recapture sampling plots within the Parachute-Piceance-Roan occupied range boundary (Walker et al. 2010).

included sets 3and 4, occasion 3 included sets 1 and 5, occasion 4 included sets 2 and 6, and so on) so that no plot was sampled on more than 2 occasions. Sampling occasions were allowed to overlap temporally but not spatially, to facilitate more intensive sampling during periods of good weather and snow conditions and to reduce commute times to distant or difficult to access portions of the study area.

To collect fecal pellet and feather samples for analysis, we accessed plots by truck or snowmobile and surveyed on foot/snowshoe to locate sites used by flocks. Plot surveys involved walking transects sufficiently close together (approximately 50 meters) to allow full visibility of the area between transects; binoculars were used to scan plots and adjacent habitats for signs of use by GRSG including tracks, pellets, feathers, and live birds. Transects varied to ensure full coverage of each plot given variation in terrain, vegetation, snow, and weather conditions that influenced visibility of, and therefore the probability of detecting, bird sign. Plot areas characterized as non-habitat, such as pinyon-juniper woodlands and cliffs, were not surveyed. We avoided conducting surveys during adverse weather (e.g. during precipitation or winds sufficient to cover bird tracks) and surveys were delayed for a minimum of 24 hrs following a wind or snow event to allow birds time to utilize habitats. Time since the last snow or wind event was tracked to balance the total number of days allowed for accumulation of tracks and other sign prior to surveys in different regions of the study area. We were unable to sample some plots in the southern half of the study area due to restrictions on private property.

Because of the low density of birds in our study area, we surveyed additional GRSG habitat on foot and used binoculars to add detections while traveling to and from random plots. All sampled incidental flocks were assigned the sample occasion number of the associated plot. Location data for GPS and VHF-marked birds was withheld from crews conducting field surveys and telemetry searches were performed separately from random surveys to avoid sampling bias associated with prior knowledge of GRSG use locations.

When a flock was detected, technicians searched the area for evidence of a roost site, estimated the number of birds in the flock, and marked the locations of individual pellets and roost piles with colored pin flags. For flock sites where roost piles were not detected and track

density suggested a large number of birds had been present, we flagged off a 20 ft length of the track paths and collected all pellet and feather samples within the area. This track distance was based on field observations that individual birds generally defecate at least once every 20 ft while foraging. We collected pellets and feathers using fresh nitrile gloves for each sample to avoid cross-contamination. We removed snow and debris from each fecal pellet sample without scraping the pellet surface to avoid accidentally removing DNA. We collected a total of 8-10 pellets from each roost pile and collected caecal samples only when other sources of DNA were not available. We placed samples in 4-oz Whirlpak© bags (eNasco, Fort Atkinson, WI) with 1-gram silicone desiccant packets (Fisher Scientific, Houston, TX) to absorb excess moisture. We packed samples in snow until we transferred them to a -20° F, manual defrost freezer for temporary storage, and later to a -80° F freezer until analysis. We avoided thawing, re-freezing, and exposing samples to UV radiation to maintain DNA integrity. We combined individual pellets in the same sample bag only if they came from the same roost pile or were otherwise known to be from the same bird because GRSG in the same flock often use the same path. Feathers located at flock-use sites were always packaged separately.

We attempted to collect at least one fecal or feather sample from each individual bird present at each flock-use site. This was possible due to the relatively small size of flocks in the population. Because GRSG often cross paths and walk on top of each other's tracks while foraging, making identification of pellets from a single bird difficult in most cases, we targeted roost piles (piles of pellets deposited by a single individual while roosting) (Figure 14) for sampling to minimize the number of samples required for collection. When roost piles were not present, sampling often required collecting more pellets than birds present to obtain a representative sample from each bird. Roost piles are produced by a single bird, so sampling

Figure 14. Greater sage-grouse fecal pellet roost pile and caecal dropping (upper left).

from roost piles reduces the likelihood of individual birds being represented multiple times (Baumgardt et al. 2013) and provides confidence that all birds present at a flock site were represented in the samples collected.

Power Analysis

We performed preliminary data simulations for closed mark-recapture models in program MARK (White and Burnham 1999) prior to our study by manipulating values for *N* (true

population size), *p* (capture probability), heterogeneity in *p* with varied mixture proportions (*pi*), and the number of sampling occasions. Simulations indicated that we would need to achieve capture probabilities $\geq p = 0.1$ for a minimum of four sampling occasions to obtain unbiased estimates of abundance given a true population size of 500-1,000 individuals (≥ 95% of simulations with confidence intervals including the true population size; Appendix J). Simulations also indicated that high heterogeneity in *p* among individuals would significantly lower the precision of abundance estimates (Appendix K).

Following the conclusion of this study, we re-ran our analysis using modified input files based on our data to simulate variation in sampling effort to make recommendations to the minimum number of sampling occasions required to obtain desired levels of precision for abundance estimates. We performed these simulations using data from completed sampling occasions in this study with variation in the number of completed sampling occasions (4, 6, or 8) in each season (see Appendix L).

ANALYSIS

Genetic Analysis of Samples

We isolated DNA from fecal pellet and caecal samples following the Qiagen (Hilden, Germany) protocol from Human Stool DNA isolation using QIAmp DNA Stool Mini Kits, QIAmp Fast DNA Mini Kits, or in 96-well plates using the DNeasy 96 Blood and Tissue Kit (Qiagen) following the Animal Tissues protocol. Fecal pellet extractions were performed under a ventilation hood dedicated to low-quality DNA extractions. DNA from fecal pellets was obtained by cutting fecal tissue from the surface of larger pellets, or using a lengthwise section of smaller

pellets, that maximized the inclusion of the pellet surface. DNA extractions from feather samples were performed using DNeasy Blood and Tissue Kits (Qiagen) following a user-developed protocol for purification of DNA from nails, hair, or feathers. DNA from feather samples was obtained by removing the proximal tip of the rachis. Extraction negatives were included in each set of extractions. QIAmp fast DNA Mini Kits performed poorly for fecal samples and ~250 samples extracted using these kits were re-extracted using the DNeasy 96 Blood and Tissue Kits with greater success.

We used seven microsatellite loci to identify individual GRSG, including six polymorphic loci (BG6; Piertney and Hoglund 2001, SGMS06.6 and MSP11 (Oyler-McCance and St. John 2010) and SG29, SG36, and SG39 (Fike et al. 2015)) and one sexing locus (a region of the CDH gene using the primers 1237L (GAGAAACTGTGCAAAACAG) and 1272H (TCCAGAATATCTTCTGCTCC; Kahn et al. 1998)). These microsatellite loci were known to be the most polymorphic and reliable based on previous work on Gunnison sage-grouse (Oyler-McCance and St. John 2010). Loci were amplified using a multiplex pre-amplification method (Piggott et al. 2004). Because DNA from non-invasively collected sources, particularly fecal DNA, are typically low in quality and at increased risk for genotyping error (e.g. allelic dropout during PCR), we repeated the amplification process to confirm results for all samples. We amplified all samples twice with the aim of obtaining two complete matching multi-locus genotypes. In addition, we ran positive and negative controls within each set to maximize quality and consistency of genotyping. Samples with unsuccessful amplification of most loci were re-extracted and re-amplified at least twice. Detailed protocols for all modified extractions and PCR steps are described in Appendix M.

Repeated amplifications generated multiple multi-locus genotypes for each sample. We compared corresponding genotypes and generated a consensus genotype for each sample. As is common with low quality DNA, some genotypes did not match across all amplification attempts. In these cases, we re-amplified mismatching loci to confirm a consensus genotype with matching scores. If there was still a mismatch after two rounds of re-amplification, we determined genotypes conservatively, and scored individuals as heterozygous at a locus if they were heterozygous at least once with a homozygous match for one of the alleles in that heterozygote. Loci were scored as "no data" if the genotypes were complete mismatches for each amplification attempt or could not otherwise be confirmed. Two rounds of review were performed on sample pairs with genotypes that differed by a single locus, by referencing all genetic analysis results and collection data, to ensure correct assignment of scores.

To be included in our data analysis, samples were required to have a minimum of six out of seven successful loci determinations. Unique individuals and their capture histories were determined using Dropout Utility 1.2 & Dropout 2.3.1 (McKelvey & Schwartz 2005). Dropout analysis was performed a total of three times using separate data for the two winter sampling seasons, and combined data to identify individuals detected in consecutive seasons. Feather samples from captures of radio-collared birds that died prior to the start of each winter sampling season were excluded from the data. We included feather samples from captures of juveniles in our analysis for seasons they were known to be alive, either by detection in our non-invasive samples or a later recapture event. In order to use data from captured juveniles with unknown fate (those with no recapture events) we needed to determine how many would be expected to survive until the start of the winter season. We assigned a juvenile survival rate of 0.76 for the period between the end of the trapping period and the start of winter sampling efforts each year.

This estimate was based on apparent survival estimates from our radio-collared adult females during that time period and was similar to previously recorded mean adult GRSG survival of 0.75 for the months of September and October (Thompson 2012). We first accounted for all juveniles with known fates, then randomly selected juveniles of unknown fate to achieve inclusion of the number of capture histories equal to the number of juveniles expected to have survived until the start of each winter sampling period. We excluded data from a total of 2 juveniles of unknown fate in 2012-2013 and 3 in 2013-2014.

Mark-Recapture Analysis

Estimates of pre-breeding abundance were obtained using "Huggins' Closed Mark-Recapture" models (Program MARK; White and Burnham 1999) with initial capture probability equal to subsequent captures ($p=c$). Sex was used as a group covariate to derive separate estimates of abundance and detection for males and females. A spatial (region) covariate ("North" or "South"; Figure 15) was used to model possible heterogeneity in capture probabilities between individuals in the southern part of the study area, where winter access was not permitted on private property, and individuals in the northern part of the study area where access was comparatively unrestricted. Assignment of covariate values to individuals was based on the location of their first detection. Restricted access in the South resulted in unequal sampling effort for that region. Models for various combinations of group and time effects (Table 10) were fitted and model results averaged based on Akaike's Information Criteria **(**AIC_c) values. Feather sample data from captures constituted our first GMR sampling occasion with the remainder of sampling occasions based on non-invasive sampling of fecal and feather samples.

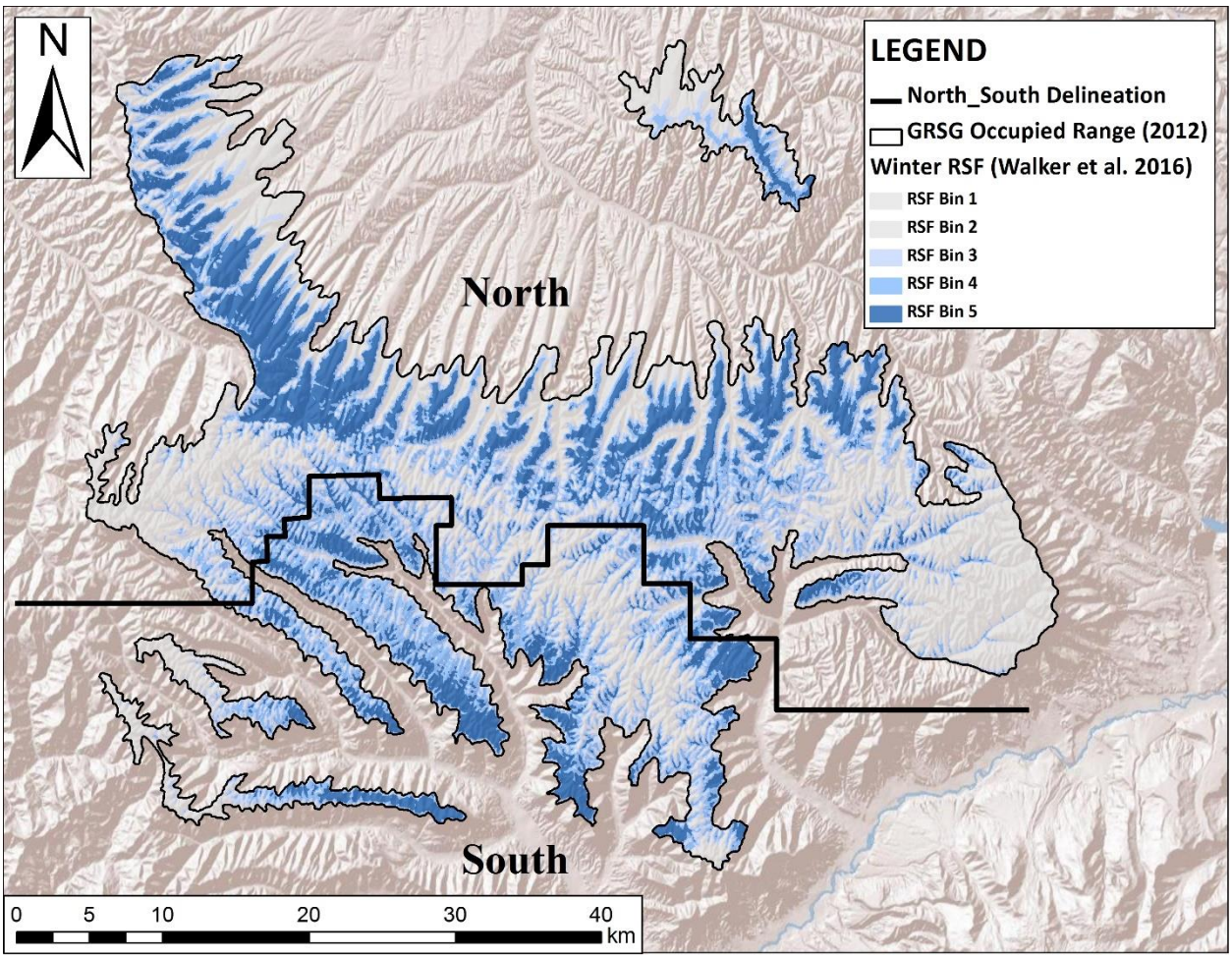

Figure 15. Map of the Parachute-Piceance-Roan greater sage-grouse occupied range with line delineating "North" and "South" regions of the study area.

Table 10. List of closed *p=c* mark-recapture models, varying by time (t) and group (g) effect, with regional covariate "region" used in genetic mark-recapture analysis of greater sage-grouse winter genetic sample data from the Parachute-Piceance-Roan, 2012-2014.

Model Name
p(t) = c + region
p(g+t) = c + region
p(g*t) = c + region
p(.) = c + region
p(g) = c + region

Model Assumptions

Closed-population mark-recapture models have four key assumptions: (1) demographic closure during the sampling period, (2) no individual heterogeneity in detection probability, (3) unique IDs are correctly recorded (genotypes are assigned correctly), and (4) no markers are lost. First, we assumed demographic and geographic closure of the population requiring no births, deaths, immigration, or emigration. We assumed that there was no unexplained heterogeneity in the probability of detection and capture (collection of DNA samples) of individuals at flock-use sites. We assumed that genotypes used in our analysis were correctly determined. In addition, we assumed that differences in DNA extraction and amplification success from passively-collected genetic samples were the result of random environmental conditions that affect DNA quality and not differences between individual birds. Loss of markers is not relevant to genetic-mark recapture because there is no use of a physical tag that can become damaged or lost.

RESULTS

We collected 116 feather samples during two seasons of GRSG captures and used 91 samples from telemetered adults and non-telemetered juveniles in our mark-recapture analysis (Table 11). Colorado Parks and Wildlife deployed a total of 22 rump-mounted PTT collars on adult and yearling males in 2012 and 6 additional males in 2013, 16 of which survived until the start of the winter sampling season in 2012 and 11 (including males collared the previous year) survived until the start of the 2013 winter sampling season. We deployed VHF necklace collars on 34 adult and yearling females in 2012 and 13 additional females in 2013. Twenty-four females survived until the start of the first winter sampling season and 23 females (including several surviving from the previous season) survived until the start of the 2013 winter season.

Table 11. Feather collection summary from greater sage-grouse captures in the Parachute-Piceance-Roan for sampling season one (2012-2013) and season two (2013-2014).

	Adult/Yearling Males	Adult/Yearling Females	Juvenile Males	Juvenile Females	Total
Season 1 (2012-2013)					
No. Samples Collected	22	36	7	8	73
Season 2 (2013-2014)					
No. Samples Collected	6	14	15	8	43
No. Samples Used in Analysis	22	36	18	15	91

Naïve estimates of overwinter survival of males from 1 November through 15 March was 0.875 (14/16) in 2012-2013 (hereafter season one) and 0.909 (10/11) in 2013-2014 (hereafter season two), and for females 0.958 (23/24) in season one and 0.913 (21/23) in season two. Four of six winter mortalities occurred near the end of the sampling seasons in late February and early March. This suggests that 95% of collared adult and yearling birds in season one and 97% of birds in season two were available for sampling for the majority of the season.

We sampled random plots and incidental flock-use sites from 11 November to 14 March in season one and 4 November – 14 March in season two. We collected non-invasive genetic samples from 120 flock-use sites in season one and 146 flock-use sites in season two. Flock site detections were based on tracks (65%), roost piles (19%), and the presence of birds (13%) with the remaining 3% from other sign including fecal pellets on bare ground, feathers, and caecal piles. We assumed most non-invasive samples were deposited since the last wind or snow event and were ≤ 9 days old in season one and ≤ 14 days old in season two. Fecal pellet samples from 2 flocks in season one and 11 flocks in season two were assumed to be old and we were unable to predict the age of these samples. As we expected, detection of GRSG sign in sample plots was low, with evidence of use only detected in 2.5% of plots sampled in season one and 3.5% in

season two. The majority of flock-use sites (95.5%; n = 266) were sampled as incidental flock-use sites.

Collection locations for all genetic samples used in this study, including captures and flock-use sites, are shown in Figure 16. Based on large-scale area use patterns from all available GPS and VHF location data (Figure 17), our genetic samples generally occurred in the same areas of use as marked birds.

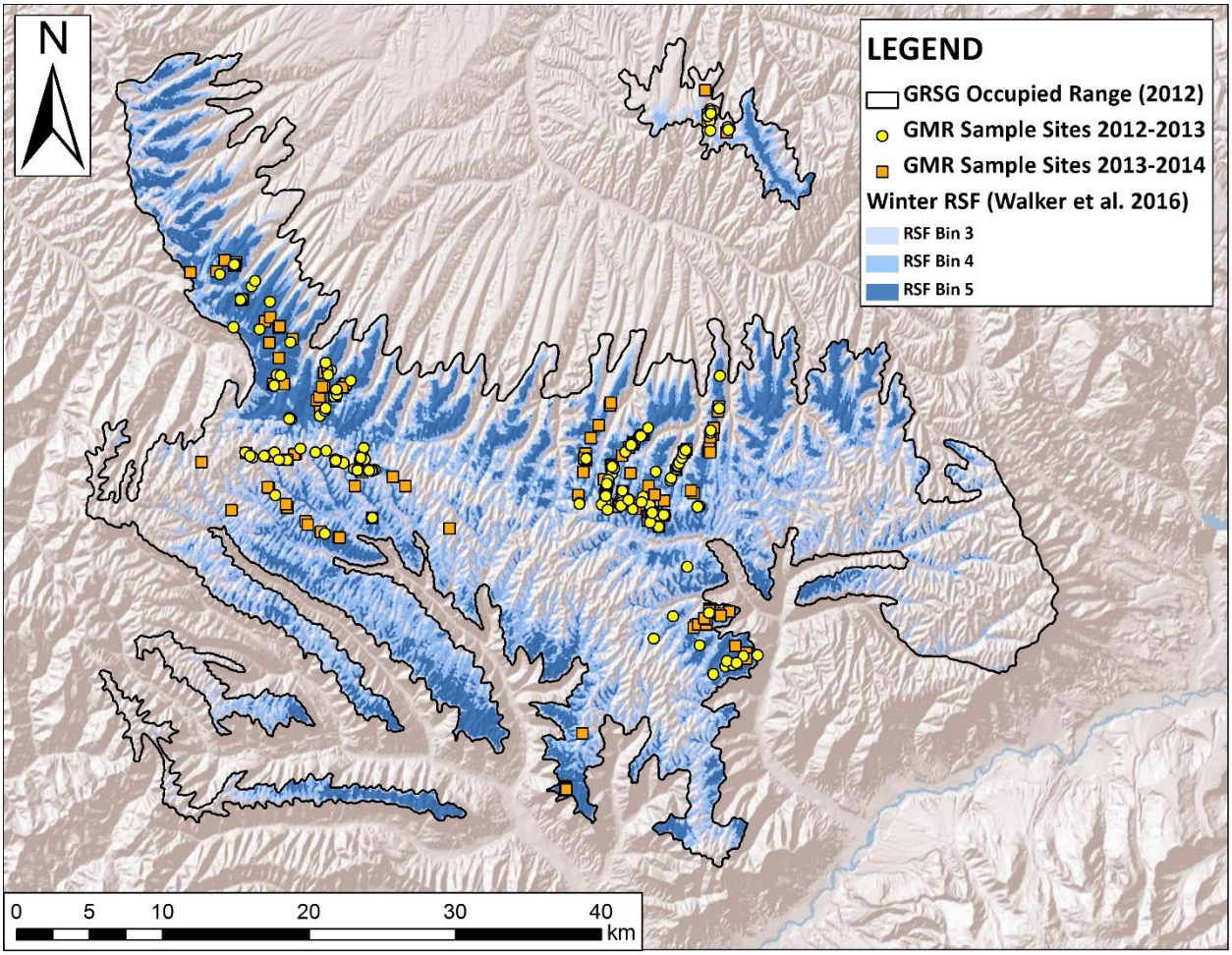

Figure 16. Greater sage-grouse genetic mark-recapture sample locations in season one (2012-2013) and season two (2013-2014), with winter resource selection function map layer, in the Parachute-Piceance-Roan population.

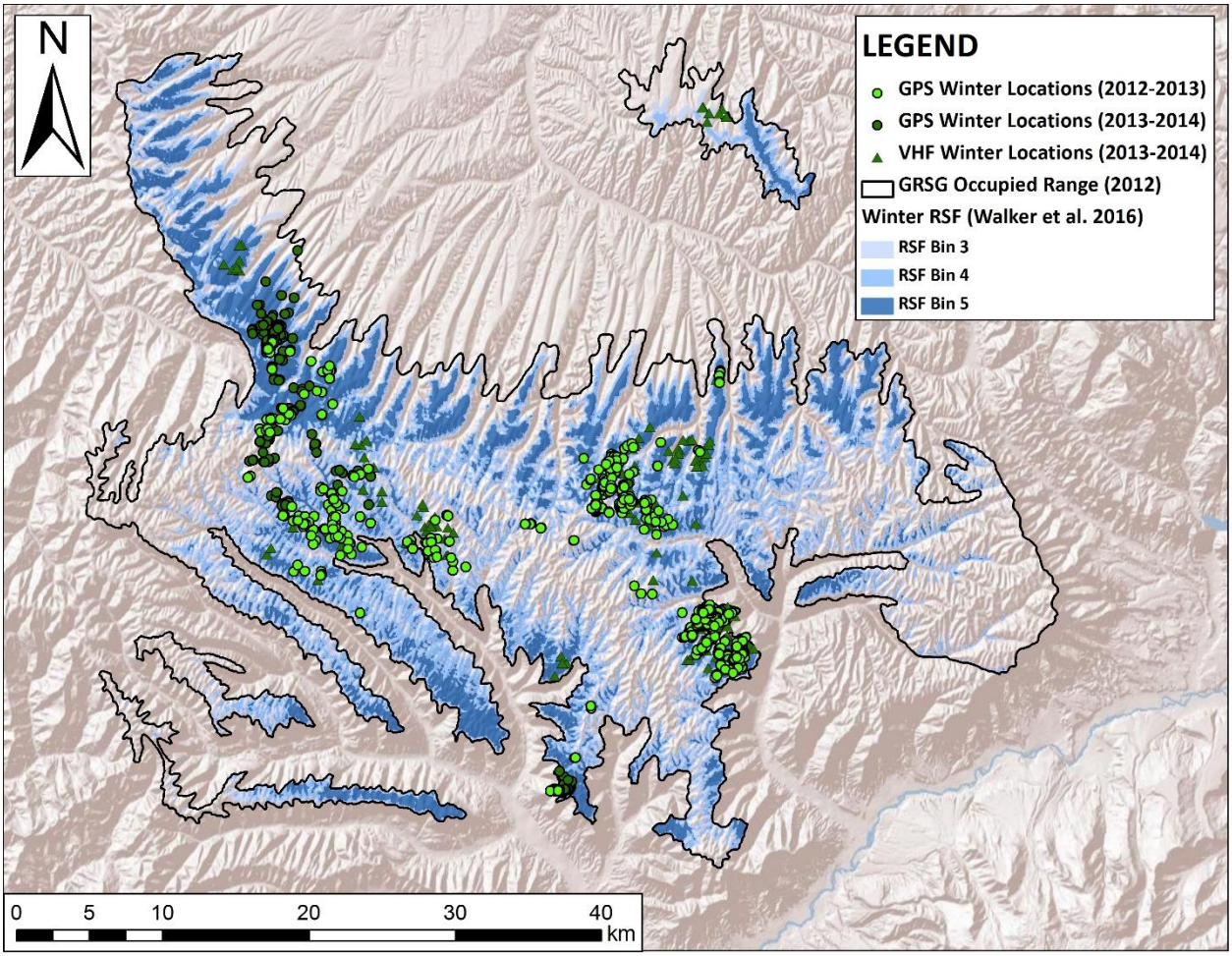

Figure 17. Greater sage-grouse winter locations of GPS-marked male and VHF-marked female locations, with winter resource selection function map layer, in the Parachute-Piceance-Roan for combined winter seasons, 1-November through 14 March.

We collected 1089 genetic samples from multiple source types across seven sampling occasions in season one (2012-2013) and 1,268 across eight occasions in season two for a total of 2,357 samples during the two-year study period (Table 12). Roost piles were discovered at 40% (48/120) of flock-use sites sampled in season one and 52% (76/146) of sites in season two. We terminated the last sampling occasion in both seasons early (on 14 March) to avoid potential sampling bias caused by birds (esp. males) moving toward leks at the start of the breeding

season. Sampling in occasion seven in season two was also incomplete because sampling efforts were hindered by adverse weather conditions.

Table 12. Summary of samples collected and used in genetic mark-recapture analysis by sample type and year in the Parachute-Piceance-Roan greater sage-grouse population in northwestern Colorado in two winter seasons.

	Fecal Pellets	Capture Feathers	Flock Feathers	Caecum	Total
Season 1 (2012-2013)					
No. Samples Collected	1,003	52	34	0	1,089
No. Samples used in Analysis	929	52	15	0	996
Season 2 (2013-2014)					
No. Samples Collected	1,198	39	29	2	1,268
No. Samples used in Analysis	906	39	14	0	959
Seasons 1 and 2 combined					
No. Samples Collected	2,201	91	63	2	2,357
No. Samples used in Analysis	1,835	91	29	0	1,955

DNA from capture feathers had the highest rate of success for extraction and amplification with 100% (n = 91) of samples successfully analyzed (≥ 6 loci successfully analyzed), followed by DNA from fecal pellets (83%, n = 2,201), and feathers collected at flock-use sites (46%, n = 63). Caecal samples had 0% success (n=2), and were not included in the analysis. Pellet samples constituted our largest data source (n = 2,201 of 2,357 total samples) and the majority (1,835) were successfully analyzed (Figure 18). QIAmp Fast Mini Stool Kits performed poorly for fecal samples and ~250 samples extracted using these kits were re-extracted using the DNeasy 96 Blood and Tissue Kits with greater success.

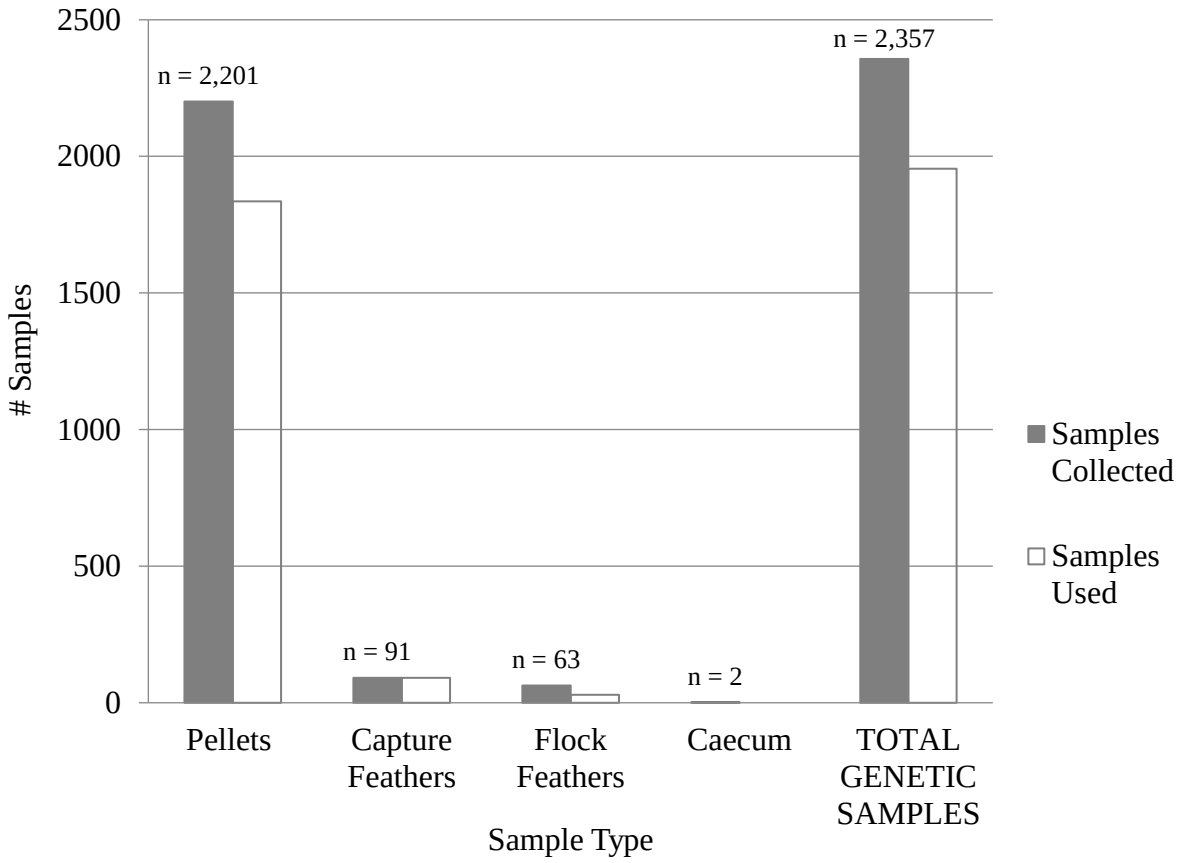

Figure 18. Number of genetic samples collected and successfully genotyped (≥ 6 loci successfully analyzed) and used in genetic mark-recapture analysis, by sample type, collected at winter flock-use sites of greater sage-grouse in the Parachute-Piceance-Roan population in northwestern Colorado, 2012-2013 and 2013-2014.

Each microsatellite loci used in our analyses performed well for fecal pellet samples with success rates > 90% for the sexing, SG29, SG36 and SG39 loci (Figure 19). Success rates for DNA from feathers collected at flock sites were much lower but with the same relative success rate for each locus. Capture feathers had the highest percentage of samples with seven successfully analyzed loci, followed by fecal pellet samples (77%) and flock site feathers (38%) (Figure 20). We found the MSP11 locus to be the most polymorphic with the greatest number of unique alleles (n=16), followed by SGMS06.6 and WY BG6 (n=11), SG29 and

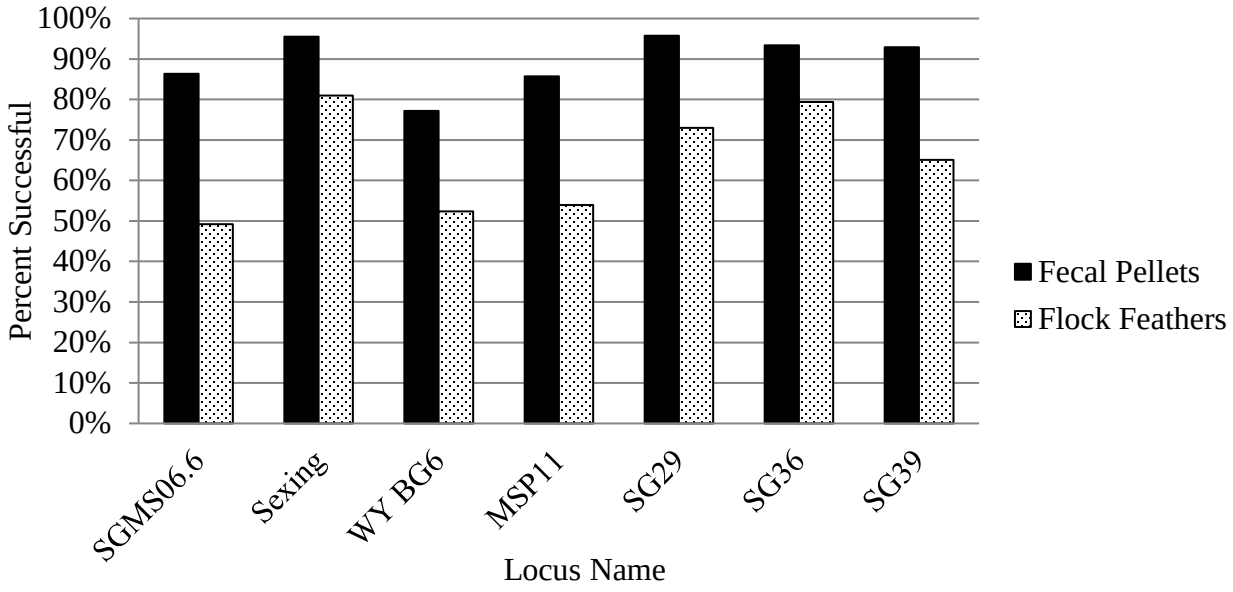

Figure 19. Extraction and amplification success rates by microsatellite locus for non-invasively collected fecal pellets and feather samples collected at winter flock-use sites of greater sage-grouse in the Parachute-Piceance-Roan population in northwestern Colorado, 2012-2013 and 2013-2014.

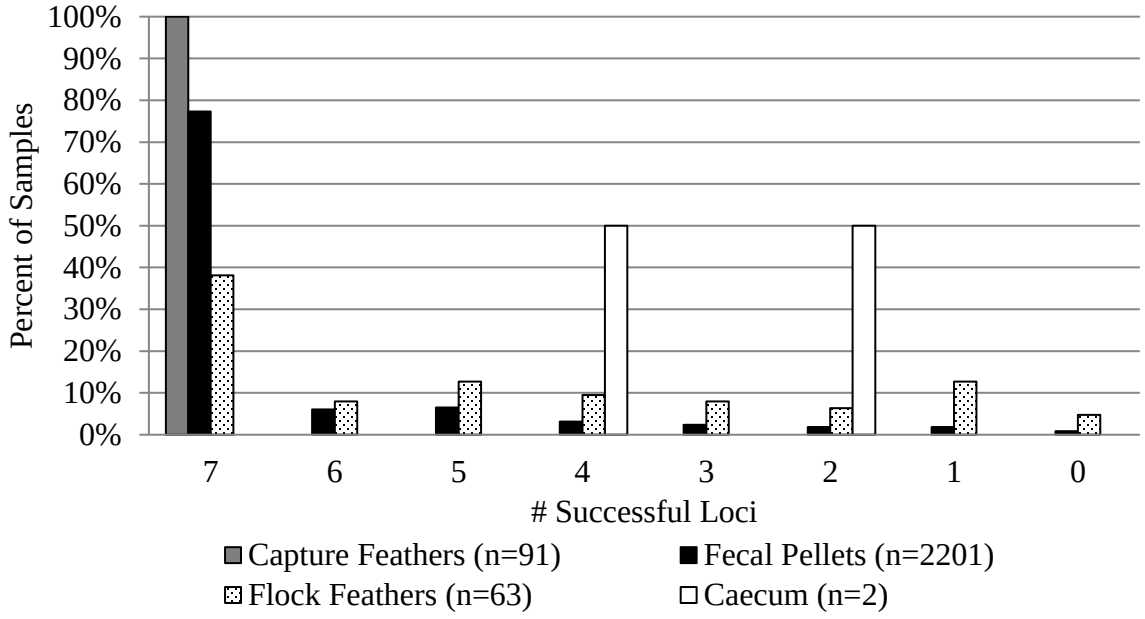

Figure 20. Percent of genetic samples with 0-7 successfully analyzed loci by sample type, collected at winter flock-use sites of greater sage-grouse in the Parachute-Piceance-Roan population in northwestern Colorado, 2012-2013 and 2013-2014.

SG39 (n=9), and SG36 (n=5). Observed heterozygosity for each locus was 0.69 (SGMS06.6), 0.64 (Sexing), 0.77 (WY BG6), 0.79 (MSP11), 0.69 (SG29), 0.62 (SG36), and 0.66 (SG39).

We identified 543 unique individual GRSG during the two sampling seasons (Table 13). The majority of individuals identified during our study (57% in season one and 76% in season two) had only one capture event in a single season (Figure 21), with the maximum of five captures for one individual in each of the winter sampling seasons.

Table 13. Number of unique individual greater sage-grouse detected in each of two winter sampling seasons in the Parachute-Piceance-Roan population in northwestern Colorado.

	Season 1 (2012-2013)	Season 2 (2013-2014)	Combined Seasons*
Males	62	154	195
Females	173	236	348
Total	235	390	543

*Some individuals were detected in both seasons.

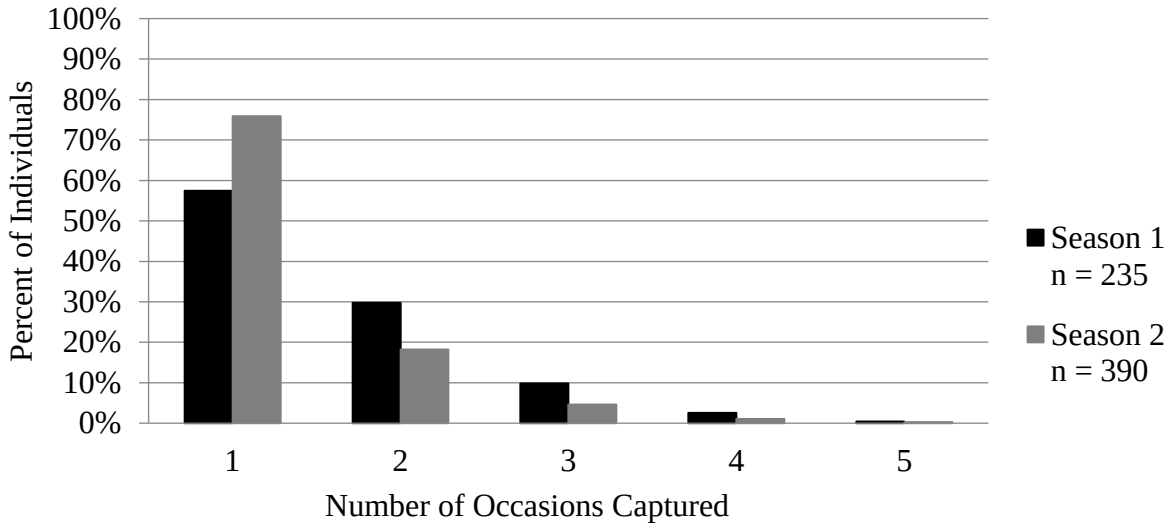

Figure 21. Frequency of capture events per unique individual greater sage-grouse identified during winter genetic mark-recapture sampling efforts in the Parachute-Piceance-Roan population in season one (2012-2013) and season two (2013-2014).

We estimated flock sizes using the number of unique individuals identified at flock-use sites from genetic data. Winter flock sizes in the PPR ranged from 1-25 birds with an average of 7.67 birds per flock in season one and 7.39 birds per flock in season two (Figure 22). The

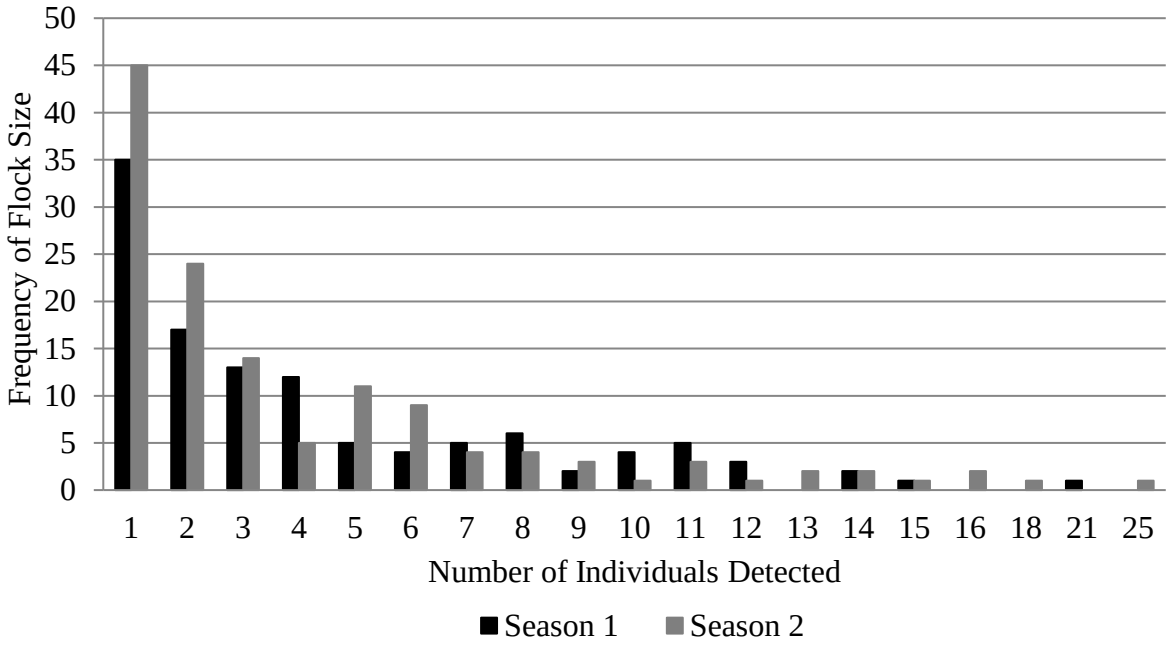

Figure 22. Observed winter flock sizes of greater sage-grouse in the Parachute-Piceance-Roan population in northwestern Colorado based on the number of unique individuals detected at flock-use sites in season one (2012-2013) and season two (2013-2014).

majority of flocks were small with 73% of flocks having ≤ five unique individuals, 17% with 6-10 individuals, and only 10% of flocks with > 10 individuals. We were unable to successfully analyze genetic samples from five flocks in season one and 12 flocks in season two due to poor quality DNA. As a result, those flock sites contributed no information to flock size statistics. Estimates of the number of birds present, based on track observations and the number of samples collected, suggest that those flocks were small, consisting of only 1-2 birds each.

For flocks with sample extraction and amplification success rates < 25% (n=20), one consisted of a single down feather sample and one a single caecal sample. Of the other 18 flocks, four consisted of single fecal pellet samples and ≥ 10 had fecal pellets that were found in melted snow or were waterlogged, windblown, or appeared old (i.e. desiccated).

We collected and analyzed repeat fecal pellet samples for some individuals at flock-use sites in both winter seasons. While a large number of flock sample sets contained no repeat fecal pellet samples, a total of 75 flocks (63%) had ≥ 1 repeat sample in season one and 65 flocks (45%) in season two (Figure 23). An average of 28% (SD 0.27) of fecal pellet samples per flock

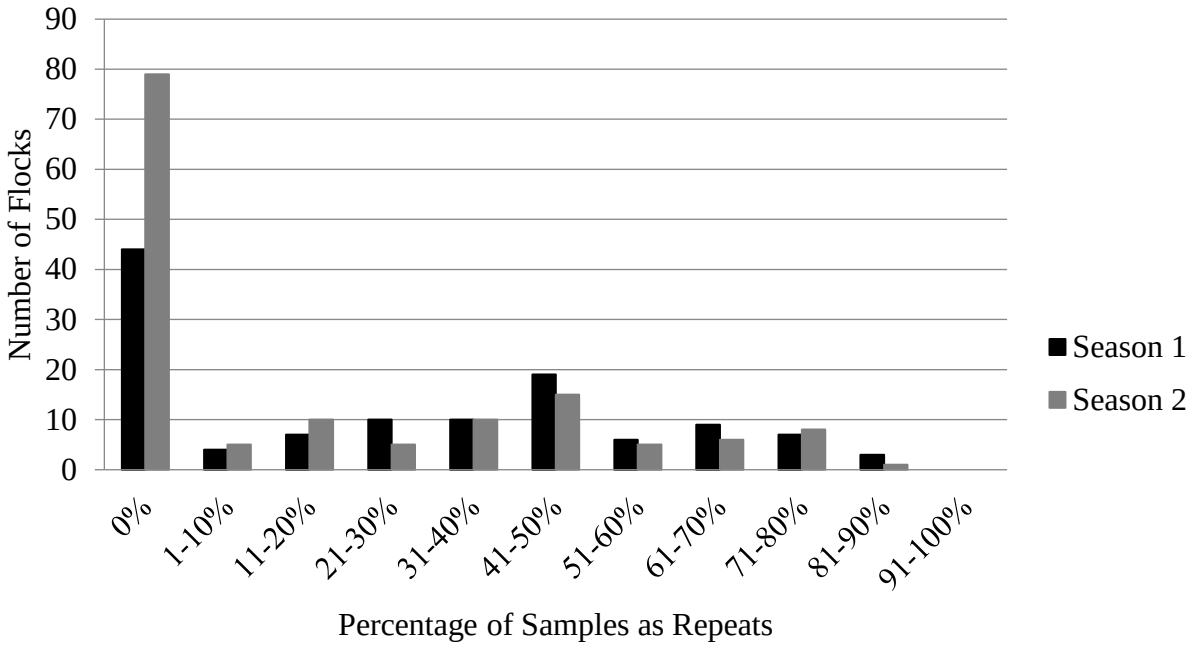

Figure 23. Frequency of repeat greater sage-grouse fecal pellet samples for unique individuals collected and analyzed at the same flock location in season one (2012-2013) and season two (2013-2014).

were repeats in season one and 19% (SD 0.26) were repeats in season two. The majority of feather samples from flock-use sites were repeat collections from individuals also identified by

fecal pellet samples; only 2 feather samples in season one and 3 feather samples in season two resulted in identification of unique individuals not already detected by fecal DNA.

Based on AIC_c rankings, we found the greatest support for our interactive group (i.e., sex) and time effect model (p[g*t]) in season one, and our time varying model (p[t]) in season two (Table 14). These results suggest a difference in detection probability between the two sexes in season one but not in season two.

Table 14. Table of model-averaged AIC_c results for Huggins' Closed Mark-Recapture models in Program MARK, season one (2012-2013) and season two (2013-2014).

Model Name	AIC_c	Delta AIC_c	AIC_c Weights	Model Likelihood	No. Param.	Deviance
Season 1 (2012-2013)						
p(g*t) = c + region	1,618	0.00	0.736	1.00	14	1,590
p(g+t) = c + region	1,621	2.50	0.211	0.287	8	1,605
p(t) = c + region	1,624	5.26	0.053	0.072	7	1,610
p(g) = c + region	1,646	27.07	0.00	0.00	2	1,642
p(.) = c + region	1,648	29.80	0.00	0.00	1	1.646
Season 2 (2013-2014)						
p(t) = c + region	2,077	0.00	0.727	1.00	8	2,061
p(g+t) = c + region	2,079	1.98	0.271	0.373	9	2,061
p(g*t) = c + region	2,089	11.85	0.002	0.003	16	2,057
p(.)= c + region	2,471	394.26	0.000	0.000	1	2,469
p(g) = c + region	2,473	396.23	0.000	0.000	2	2,469

Model-averaged detection probabilities ranged from 0.100-0.326 for males and 0.085-0.225 for females in season one (Table 15) and 0.00-0.168 for males and 0.00-0.167 for females in season two (Table 16). Based on model-averaged estimates of p, probability of being captured at least once during a sampling season (p*) was 0.797 for males and 0.674 for females in season one, and 0.525 for males and 0.522 for females in season two.

Table 15. Table of model-averaged capture probability parameter (*p*) estimates for Huggins' Closed Mark-Recapture models by attribute group (sex), season one (2012-2013).

Capture Probability (*p*) by Sex				
Males	Estimate	SE	LCI	UCI
Occasion 1	0.220	0.052	0.134	0.339
Occasion 2	0.228	0.049	0.145	0.338
Occasion 3	0.214	0.056	0.124	0.345
Occasion 4	0.157	0.045	0.088	0.265
Occasion 5	0.162	0.042	0.095	0.262
Occasion 6	0.326	0.059	0.223	0.450
Occasion 7	0.100	0.048	0.037	0.242
Females				
Occasion 1	0.128	0.023	0.089	0.181
Occasion 2	0.200	0.028	0.150	0.261
Occasion 3	0.107	0.022	0.070	0.159
Occasion 4	0.085	0.019	0.055	0.129
Occasion 5	0.142	0.024	0.102	0.195
Occasion 6	0.225	0.030	0.171	0.289
Occasion 7	0.141	0.026	0.098	0.200

Table 16. Table of model-averaged capture probability parameter (*p*) estimates for Huggins' Closed Mark-Recapture models by attribute group (sex), Season two (2013-2014).

Capture Probability (*p*) by Sex				
Males	Estimate	SE	LCI	UCI
Occasion 1	0.039	0.008	0.026	0.058
Occasion 2	0.158	0.019	0.124	0.198
Occasion 3	0.066	0.011	0.048	0.091
Occasion 4	0.168	0.020	0.133	0.210
Occasion 5	0.129	0.016	0.100	0.165
Occasion 6	0.131	0.017	0.102	0.167
Occasion 7	0.000	0.000	0.000	0.000
Occasion 8	0.001	0.001	0.000	0.004
Females				
Occasion 1	0.039	0.008	0.026	0.057
Occasion 2	0.156	0.018	0.125	0.194
Occasion 3	0.066	0.010	0.048	0.089
Occasion 4	0.167	0.018	0.134	0.207
Occasion 5	0.128	0.016	0.101	0.162
Occasion 6	0.130	0.016	0.102	0.164
Occasion 7	0.000	0.000	0.000	0.000
Occasion 8	0.001	0.001	0.000	0.004

Pre-breeding abundance estimates were similar for all fitted models within each year (77-88 males and 243-261 females in season one (Table 17); 290-310 males and 451-487 females in season two (Table 18)). Model-averaged estimates of total pre-breeding abundance were 335 (78

Table 17. Huggins' Closed Mark-Recapture pre-breeding abundance estimates by model for male and female greater sage-grouse in the Parachute-Piceance-Roan with SEs and 95% lower and upper confidence intervals in season one (2012-2013).

Model Name	Est. N Males	SE	LCI	UCI
p(g*t) = c + region	77	5.92	69	94
p(g+t) = c + region	78	6.03	70	94
p(t) = c + region	87	6.86	77	105
p(g) = c + region	78	6.20	70	96
p(.) = c + region	88	7.03	78	106
	Est. N Females			
p(g*t) = c + region	257	17.63	229	300
p(g+t) = c + region	258	17.67	230	300
p(t) = c + region	243	13.79	221	276
p(g) = c + region	261	18.18	232	304
p(.) = c + region	246	14.17	223	279

Table 18. Huggins' Close Mark-Recapture pre-breeding abundance estimates by model for male and female greater sage-grouse in the Parachute-Piceance-Roan with SEs and 95% lower and upper confidence intervals in season two (2013-2014).

Model Name	Est. N Males	SE	LCI	UCI
p(t) = c + region	294	23.46	255	348
p(g+t) = c + region	291	30.38	243	364
p(g*t) = c + region	290	30.31	242	363
p(.) = c + region	314	26.05	271	374
p(g) = c + region	310	33.88	256	392
	Est. N Females			
p(t) = c + region	451	32.76	396	526
p(g+t) = c + region	455	39.53	390	547
p(g*t) = c + region	455	39.57	390	547
p(.) = c + region	483	36.58	421	565
p(g) = c + region	487	44.23	414	590

males and 257 females) in season one and 745 (293 males and 452 females) in season two (Tables 19-20).

Table 19. Model-averaged estimates of pre-breeding abundance ($\hat{N}$) for male and female greater sage-grouse in the Parachute-Piceance-Roan with SEs and 95% lower and upper confidence intervals in season one (2012-2013).

Sex	Estimate ($\hat{N}$)	SE	LCI	UCI
Male	78	6.39	65	90
Female	257	17.75	222	292
Total	335		287	382

Table 20. Model-averaged estimates of pre-breeding abundance ($\hat{N}$) for male and female greater sage-grouse in the Parachute-Piceance-Roan with SEs and 95% lower and upper confidence intervals in season two (2013-2014).

Sex	Estimate ($\hat{N}$)	SE	LCI	UCI
Male	293	25.59	243	344
Female	452	34.79	384	520
Total	745		627	864

Our estimates suggest that pre-breeding abundance increased by 410 individuals (an approximate doubling of population size) from winter season one (2012-2013) to winter season two 2013-2014) (Tables 19-20). Males showed a greater increase in population size (376%) than females (176%). The change in pre-breeding abundance estimates of males from GMR analyses between winter 2012-2013 and winter 2013-2014 paralleled CPW lek-count index data between spring 2013 and spring 2014, with both showing a steep increase (Figure 24).

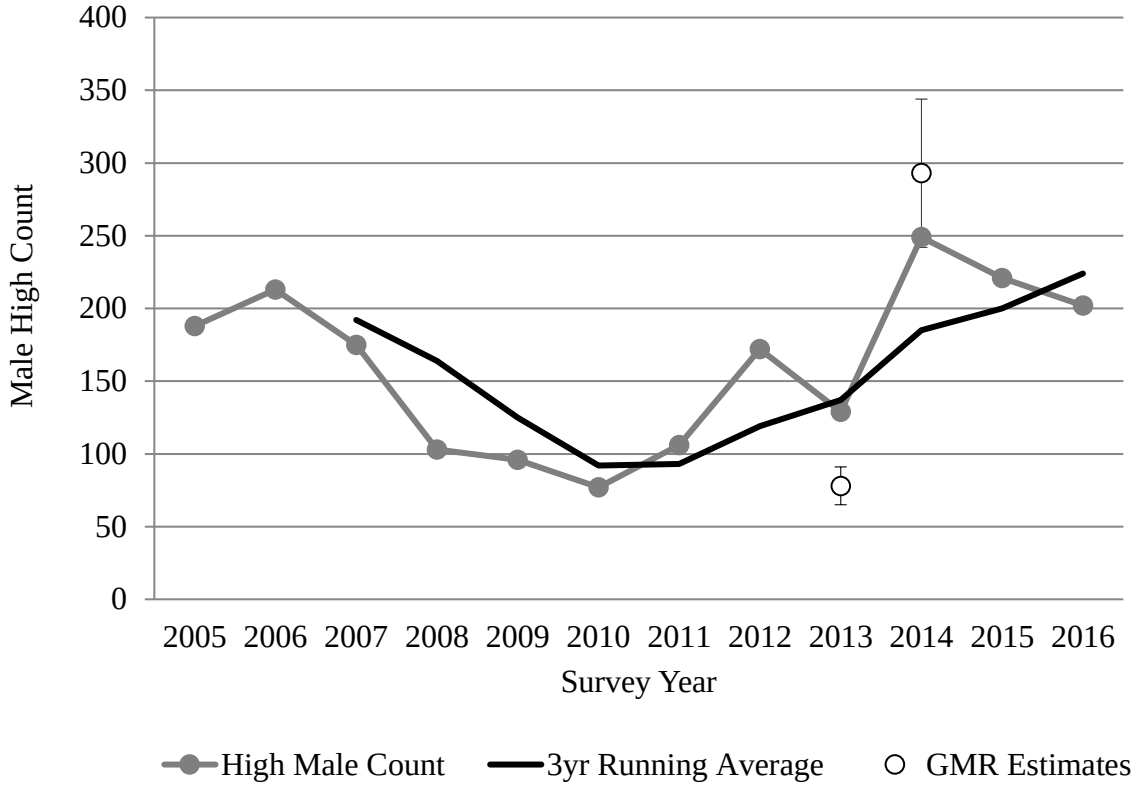

Figure 24. Graph of Colorado Parks and Wildlife lek-count index data showing summed high male counts across leks vs. estimates of male abundance from genetic mark-recapture analysis, including 95% confidence intervals, for the Parachute-Piceance-Roan greater sage-grouse population in northwestern Colorado.

Based on our power analysis, we found that reducing the number of sampling occasions from six to four would increase SEs of our estimates by a factor of 2-3 for season one estimates and 1.5-1.7 for season two estimates. Even if precision of estimates for a smaller number of sampling occasions are acceptable, we still need to be cautious of potential bias in our estimates when using data from ≤ five sampling occasions with potential detection probabilities of ≤ 0.1, as was suggested by the results from our preliminary power analysis. Increasing the number of complete occasions to eight would have reduced SEs by a factor of 0.47-0.59 in season one and 0.62-0.66 in season two.

DISCUSSION

We were able to obtain precise estimates of abundance for a small, low-density population of GRSG using non-invasive winter sampling of fecal pellets (and to a lesser extent, feathers) at flock-use sites. We estimated more than a doubling in pre-breeding abundance between the two sampling seasons. Our GMR estimates paralleled data from the lek-count index and the results collectively suggest that the GRSG population in the PPR was at a relative low point in 2013 and rebounded strongly in 2014.

Population dynamics of GRSG are similar to other ground-nesting, upland game birds in that they often exhibit large annual fluctuations in population size due to environmental factors (Connelly et al. 2011, Fedy & Doherty 2011), most likely driven by annual variation in productivity (Holloran 2005, Huwer et al. 2008, Taylor et al. 2012). Large fluctuations can be either attributed to local recruitment, resulting from high (and possibly correlated) annual variation in key vital rates that drive population growth, including nest success, chick and juvenile survival, and female survival (Taylor et al. 2012), or movement of animals between populations (i.e., immigration and emigration). Large annual fluctuations in lek-counts have been observed throughout the species' range (Rich 1985, Fedy & Aldridge 2011, WAFWA 2015). However, based on life-stage simulation analyses (Walker 2008, Taylor et al. 2012), the magnitude of increase in abundance we observed (female-based lambda = 1.75; 95%, C.I. 1.40-2.12) represents a fairly extreme population growth rate requiring high rates of nest initiation, nest success, and juvenile survival , if based solely on local recruitment. Because demographic and geographic contributions to the population were not monitored during this study, it is unknown whether the increase in population size can be attributed solely to an excellent year for recruitment or immigration from neighboring populations. There has been no evidence of marked

birds emigrating from the PPR population in more than 10 years of intensive study (CPW, unpublished data), and the PPR is a geographically isolated population at the southern edge of the species' range in Colorado, so high rates of immigration are unlikely. However, rates of immigration into the PPR from other larger, neighboring populations to the north are currently unknown.

We propose that the apparent large increase in population size in the PPR was likely the result of extensive recruitment of juveniles following the 2013 breeding season. Our estimates of male and female population size increased by a factor of 3.77 and 1.76, respectively, between winter 2012-2013 and winter 2013-2014. Because mortality rates of juvenile males increase as they mature and enter the breeding population (Zablan et al. 2003), the disproportionate increase in males in the population in season two can be explained by low abundance of males in the previous year combined with the fact that we estimated sex ratio prior to high breeding-season mortality of males. The result would be a significant increase in the proportion of juvenile males in the population in the second year of our study. Several studies estimated male-to-female sex ratio at hatch to be 1:1 (Atamian and Sedinger 2010, Guttery et al. 2013) with male and female juveniles shown to have no difference in survival from September through March (Beck et al. 2006). If true for most populations, any contribution to the population from recruitment is expected to be approximately the same for males and females through the end of their first winter. Because male numbers in the PPR were estimated to be much lower than females in season one, a subsequent year of high productivity and juvenile survival would result in a much larger proportional increase in the male population.

While the magnitude of increase for our GMR estimates and CPW's lek-count index data were similar, high male counts at leks did not closely match male abundance estimates in either

year. Based on our current understanding of the PPR population, we are uncertain which factors most influence high male counts, though we suspect that an interaction of factors may be responsible for the differences. In 2013, the lek-count index high male count was 129, compared to our estimates of 78 males (95% C.I. 65-90). We speculate that the higher number of males counted on leks may be due to inter-lek movement of males in the population during the count period, resulting in some males being counted at > 1 lek. Some researchers have reported that inter-lek movement of males is rare (Gibson et al. 2014), but males in other studies have been shown to attend multiple leks within a season (Fremgen et al. 2017). In support of this possibility, a concurrent CPW study of the same population revealed that some males attended multiple leks during our sampling period (B. Walker, CPW, unpublished data). The frequency of these movements and whether they are consistent among years is unclear. Multiple counts of individual males are more likely to occur, rather than males being missed altogether, because CPW's lek monitoring protocols require multiple visits to each lek within a season. Moreover, the number of lek-count surveys increased during this study as a result of more intensive survey efforts from combined field research crew surveys, early-season survey flights and dual-frame flights that resulted in ≥ six visits to some lek sites. In contrast, CPW's high male count in 2014 (249) was lower than our GMR estimates (293; 95% C.I. 243-344), though still contained within the 95% confidence interval. While there was also evidence of inter-lek movement of males in 2014, lower attendance rates of yearling males may have been a larger factor, particularly if a large increase in the proportion of yearlings occurred prior to the 2014 breeding season. Yearling males exhibit lower lek attendance rates than adult males (Walsh et al. 2004), a factor that may have reduced lek-count index values in 2014. The three-year running-average used by CPW to smooth out uncontrolled annual environmental variation was even further from male GMR

estimates than raw annual lek counts during the two years of our study, suggesting that these averages more poorly correspond to true population size.

Genetic mark-recapture methods may be useful for larger populations if methods are adjusted to reduce costs (e.g., by randomly selecting a subset of samples to analyze from each flock). However, these cost savings may come at the expenses of lower precision of estimates. Sampling only roost piles would also minimize (or perhaps eliminate) the probability of sampling individuals multiple times at flock sites and the cost associated with analyzing those additional samples. In our study, we attempted to collect ≥ 1 genetic sample from each individual present at each flock-use site. This was possible due to the relatively small size of flocks (≤ 25 individuals) in this population. Sampling of roost piles, when available, was the most efficient and cost effective strategy. However, roost piles were only found at 40% of flock locations in season one and at 52% in season two, so sampling only roost piles would have substantially reduced our sample size and precision of estimates. In the absence of roost piles, sampling of individual pellets (i.e., those found along tracks) resulted in a number of repeat samples being analyzed for the same individuals at some flock locations; ~25% of fecal pellet samples collected were repeat samples from individuals already detected in the same flock. We recommend collecting samples from roost piles when possible. We also recommend collecting individual pellets from flock-use sites, then selecting a random sample from each site for genetic analysis if necessary. This strategy would require little additional effort in the field, and provides the option to analyze additional samples later if the project budget allows or additional funding becomes available. The collection and analysis of feather samples from flock-use sites offered little benefit to our mark-recapture datasets due to small sample sizes and lower success rates for DNA analysis. In our study, flock feathers contributed only 5 unique identifications for

individuals not already identified using DNA from fecal pellets and may not warrant the additional cost of genetic analysis.

We believe we adequately met assumptions of population closure, both demographic and geographic, required for unbiased mark-recapture estimates of abundance. First, the PPR population of GRSG is geographically isolated from neighboring populations, which reduces the likelihood of immigration or emigration in winter. In GRSG, dispersal occurs primarily among yearlings in spring (Thompson 2012). Second, unlike other populations in Colorado, there is no evidence of marked birds moving into or out of the population during our study or indeed, during winter or any other season from 2006-2016 (B. Walker, CPW, unpublished data). Third, sampling in winter ruled out any possibility of local recruitment from births. Fourth, mortality likely had little impact on our abundance estimates. We observed high overwinter survival of both GPS and VHF-marked birds during our study, with 95-97% of marked adults and yearlings alive and available for sampling throughout the majority of our winter sampling seasons.

The majority of birds were sampled non-invasively using fecal pellets as a DNA source, resulting in little disturbance to the population. Unlike traditional mark-recapture studies that involve invasive trapping, marking, and either recapture or resighting of animals, non-invasive sampling largely eliminates concerns about negative impacts on individual or population-level behavior or survival and concerns about animals becoming trap-happy or trap-shy. Only a small number of birds in our sample were physically captured and marked and this was done primarily to test the closed-population assumptions of our models or as part of other research.

Based on previous work on Gunnison sage-grouse pellets (Oyler-McCance and St. John, unpublished report), we anticipated a low rate of success in amplification of GRSG DNA from

fecal samples. In that pilot study, fecal pellets were collected on leks during the spring when temperatures were generally warmer and more variable, which may have contributed to faster degradation of DNA. Factors that contributed to our high amplification success rates likely included: (1) sample handling and storage methods that minimized thawing, refreezing and exposure to UV radiation; (2) sampling in winter means most samples became frozen, or remained cold, in snow after defecation, reducing degradation of DNA; (3) sampling only pellets deposited since the last wind of snow event which resulted in most fecal pellet samples being relatively fresh (recently deposited since the last snow or wind event), (4) sampling pellets (e.g., from the middle of roost piles) which were relatively protected from UV radiation and absorption of excess moisture from contact with snow; and (5) removal of excess snow and the use of desiccant packets to reduce excess moisture. Baumgardt et al. (2013) suggest that DNA from GRSG fecal pellets have slow rates of degradation in the winter, based on samples with ≤ 34 exposure days—the majority of our samples were exposed for ≤ 14 days. Fecal pellet samples from flocks with low success rates for genetic analysis suggest that environmental factors (e.g., UV exposure, fluctuating temperatures, desiccating winds), and thawing (e.g., warm daily temperatures that caused melting snow and allowed pellets to thaw, then refreeze when temperatures dropped overnight), impacted the quality of DNA in fecal pellet samples. Previous studies suggest an effect of diet on amplification success of some fecal DNA (Murphy et al. 2007, Panasci et al. 2011) including success rates for GRSG (Baumgardt et al. 2013). Exclusive consumption of sagebrush by GRSG may have influenced the success rate of DNA analysis (e.g. by reducing the presence of extraction inhibitors in our samples) but this possibility needs to be investigated. Each of these factors potentially contributed to our ability to maintain the integrity of DNA quality and successfully extract and amplify DNA. Our two-step amplification process

was also designed to increase success rates for low quality DNA obtained from passively-collected sources. Low analysis success rates of feather samples collected at flock sites was not surprising because those samples consisted of small down feathers for which a very small amount of quality DNA was present at the tip of the rachis. As with any project involving the use of genetic materials, particularly those involving low quality sources of DNA, proper training and supervision of field and laboratory technicians is also critical for obtaining high-quality results and preventing cross-contamination of samples.

We observed relatively low detection probabilities that differed by sex in one of our two sampling seasons. We suspect that segregation of birds by sex in winter, and possibly also male age composition, may have influenced these detection probabilities. Male and female GRSG are reported to segregate during winter months with male and female-dominated flocks using slightly different habitats (Beck 1977). To address this possibility, we modeled our data using sex as a grouping covariate to account for heterogeneity in capture probabilities resulting from variation in habitat use or flock size between the sexes. We found strong support for a sex effect in season one, but not in season two. Two factors may have contributed to lower detectability in 2013-2014. First, more frequent winter storms in the latter part of the season may have resulted in fewer detections because tracks and pellets were more often covered by wind or fresh snowfall. For example, estimated capture probabilities were extremely low in sampling occasions seven and eight in season two (0.000 and 0.001, respectively), a consequence of adverse weather conditions that largely inhibited sampling. Second, an increase in the abundance of GRSG in season two may also have lowered capture probabilities because the presence of more birds, and presumably more flocks, meant we were likely unable to sample the same proportion of flock-use locations with the same amount of survey effort.

The use of a spatial covariate was necessary to model heterogeneity in capture probabilities between individuals residing in different regions of the study area. Winter access restrictions for some private property in the southern half of the study area prevented equal sampling effort in all areas. While we did observe frequent movements of marked GRSG between accessible and inaccessible areas, birds that remained in inaccessible areas were expected to have lower capture probabilities. Two areas in the southwest portion of the study area, 4A Mountain and Kimball Mountain, also were not sampled due to logistical and safety issues in winter. However, we believe these areas support few, if any, GRSG during breeding and winter due to limited and poor quality habitat and extremely few observations of sage-grouse in these areas in the past decade (based on extensive helicopter surveys). If these areas did host a small number of birds, failing to account for them may have led to a small negative bias in our GMR abundance estimates. Additionally, the majority of our pellet samples were collected from incidental flock locations detected while traveling along roads to and from random plot locations. It is unknown if this introduced any biases to our estimates, however, we believe this is unlikely given that nearly all GRSG habitat in the PPR contains access roads and we sampled a large number of random plots to ensure adequate spatial coverage of the study area.

While we found that genetic mark-recapture can provide robust estimates of abundance in small populations, both field data collection and laboratory analyses were expensive and labor intensive, likely making genetic mark-recapture analyses impractical for annual monitoring of GRSG populations. Cost per sample for DNA analysis will depend on multiple factors including costs for laboratory analysis, volume of samples analyzed, number of microsatellite loci amplified, and number of samples requiring repeated analysis. In general, our analysis costs were ~$100 per fecal pellet sample and slightly less for feather samples. Multiple amplification rounds

on each of our samples to confirm genotypes added to analysis costs. However, costs may be reduced if researchers elect to rely on methods to test for and model genotyping error rates (e.g., blind samples) rather than repeating analysis for each individual sample to obtain a consensus genotype. Field sampling costs are associated with multiple factors including study area size, difficulty of winter access, sampling strategy, and number of sampling occasions. Despite the high cost of these methods, GMR may be justified in years when precise estimates of population size are critical for management decisions, to provide baseline estimates of abundance prior to changes in land-use or experimental habitat treatments, or at regular intervals to calibrate less robust estimates of abundance such as the lek-count index.

Our abundance estimates provide insights into the relationship between the lek count and true population size in the PPR. During our period of study, we estimated an approximate doubling of the male population, an increase in population size reflected in both the GMR estimates and the lek-count index. However, it is unclear if these two monitoring methods would lead to similar inference in years with a smaller magnitude of change in population size.

MANAGEMENT IMPLICATIONS

Application of genetic mark-recapture methods using winter fecal pellet samples can provide accurate baseline estimates of pre-breeding abundance of GRSG for small populations that are crucial for assessing both current and future status of GRSG populations. However, the methodology is expensive, time consuming and logistically challenging to employ. In contrast, the lek-count index, widely used to monitor sage-grouse populations, is cheaper and easier to use but the relationship between those counts and true population size is largely unknown.

Additionally, the lek index has no associated estimates of precision, provides no insights to the abundance of females, and is subject to several unmeasured sources of sampling bias.

Our estimates suggest that the lek-count index (and especially use of a three-year moving average) may mask actual fluctuations in population size rather than controlling for unmeasured biases. In contrast to GMR estimates, the lek-count index is subject to numerous factors that affect the index including inconsistent attendance of leks by males, inter-lek movement of males leading to over-counting, and an unknown number of leks that are not surveyed. Collectively, not accounting for these factors may significantly undermine the utility of the lek-count index as a reliable method to infer status and trend of GRSG populations in the PPR.

LITERATURE CITED

Aldridge, C. L., Nielsen, S. E., Beyer, H. L., Boyce, M. S., Connelly, J. W., Knick, S. T. and M. A. Schroeder. 2008. Range-wide patterns of greater sage-grouse persistence. Diversity and Distributions 14: 983-994.

Atamian, M. T., and J. S. Sedinger. 2010. Balanced sex ratio at hatch in a greater sage-grouse (*Centrocercus urophasianus*) population. Auk 127:16-22.

Applegate, R. D. 2000. Use and misuse of prairie chicken lek surveys. Wildlife Society Bulletin 28:457-248.

Batazzo, A. M. 2007. Winter survival and habitat use by female greater sage-grouse (*Centrocercus urophasianus*) in south Phillips County, Montana. Thesis, University of Montana, Missoula, USA.

Baumgardt, J. A., Goldberg C. S., Reese, K. P., Connelly, J. W., Musil, D. D., E. Garton, E.O., and L. P. Waits. 2013. A method for estimating population sex ratio for sage-grouse using noninvasive genetic samples. Molecular Ecology Resources 13:393-402.

Bayard de Volo, S., Reynolds, R. T., Topinka, J. R., May B., and M. F. Antolin. 2005. Population genetics and genotyping for mark-recapture studies of northern goshawks (*Accipiter gentilis*) on the Kaibab Plateau, Arizona. Journal of Raptor Research 39:285-295.

Beck, T. D. I. 1977. Flock characteristics and habitat selection in winter. Journal of Wildlife Management 41:18-26.

Beck, J. L., Reese, K. P., Connelly, J. W., and M. B. Lucia. 2006. Movements and survival of juvenile greater sage-grouse in southeastern Idaho. Wildlife Society Bulletin 34:1070-1078.

Beck, T. D. I. and C. E. Braun. 1980. The strutting ground count: variation, traditionalism, management needs. Proceedings of the Western Association of Fish and Wildlife Agencies 60:558-566.

Bedrosian, B. and D. Craighead. 2007. Evaluation of techniques for attaching transmitters to common raven nestlings. Northwestern Naturalist 88:1-6.

Bellemain, E., Swenson, J. E., Tallmon, D., Brunberg, S., 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.

Blomberg, E. J., Sedinger, J. S., Nonne, D. V. and M. T. Atamian. 2013. Annual male lek attendance influences count-based population indices of greater sage-grouse. Journal of Wildlife Management 77:1583-1592.

Boulanger, J., McLellan, B. N., Woods, J. G., Proctor, M. F., and C. Strobeck. 2004. Sampling design and bias in DNA-based capture-mark-recapture populations and density estimates of grizzly bears. Journal of Wildlife Management 68:457-469.

Broquet, T., Menard, N., and E. Petit. 2007. Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conservation Genetics 8:249-260.

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

Christie, K. S., Gilbert, S. L., Brown, C. L., Hatfield, M., and Hanson, L. 2016. Unmanned aircraft systems in wildlife research: current and future applications of a transformative technology. Frontiers in Ecology and the Environment 14:241-251.

Colorado Greater Sage-Grouse Steering Committee (CGSSC). 2008. Colorado greater sage-grouse conservation plan. Colorado Division of Wildlife, Denver, Colorado, USA.

Connelly, J. W. and C. E. Braun. 1997. Long-term changes in sage grouse *Centrocercus urophasianus* populations in western North America. Wildlife Biology 3:229-234.

Connelly, J. W., Reese, K. P. and M. A. Schroeder. 2003. Monitoring of greater sage-grouse habitats and populations. University of Idaho, College of Natural Resources Experiment Station Bulletin 80. Moscow, ID, USA.

Connelly, J. W., Knick, S. T., Schroeder, M. A., and S. J. Stiver. 2004. Conservation assessment of greater sage-grouse and sagebrush habitats. Western Association of Fish and Wildlife Agencies, Cheyenne, WY, USA.

Connelly, J. W., Hagen, C. A., and M. A. Schroeder. 2011. Characteristics and dynamics of greater sage-grouse populations. Pages 53-67 *in* S. T. Knick and J. W. Connelly, editors. Greater sage-grouse: ecology and conservation of a landscape species and its habitats. Studies in Avian Biology 38, University of California Press, Berkeley, USA.

Coster, S. S., Kovach, A. I., Pekins, P. J., Cooper, A. B., and A. Timmins. 2011. Genetic mark-recapture population estimations in black bears and issues of scale. Journal of Wildlife Management 75:1128-1136.

Fedy, B. C. and C. L. Aldridge. 2011. The importance of within-year repeated counts and the influence of scale on long-term monitoring of sage-grouse. Journal of Wildlife Management 75:1022-1033.

Fedy, B. C. and K. E. Doherty. 2011. Population cycles are highly correlated over long time series and large spatial scales in two unrelated species: greater sage-grouse and cottontail rabbits. Oecologia 165:915-924.

Fike, J. A., Oyler-McCance, S. J., Zimmerman, S. J., and T. A. Castoe. 2015. Development of 13 microsatellites for Gunnison Sage-grouse (*Centrocercus minimus*) using next-generation shotgun sequencing and their utility in greater sage-grouse (*Centrocercus urophasianus*). Conservation Genetics Resources 7:211-214.

Fremgen, A. L., Rota, C. T., Hansen, C. P., Rumble, M. A., Gamo, R. S., and J. J. Millspaugh. 2017. Male greater sage-grouse movements among leks. Journal of Wildlife Management doi:10.1002/jwmg.21208.

Garton, E. O., Wells, A. G., Baumgardt, J. A., and Connelly, J.W. 2015. Greater sage-grouse population dynamics and probability of persistence. Final report to Pew Charitable Trusts.

Gibson, D., Blomberg, E. J., Atamian, M. T. and J. S. Sedinger. 2014. Lek fidelity and movement among leks by male greater sage-grouse *Centrocercus urophasianus*: a capture-mark-recapture approach. Ibis 156:729-740.

Giesen, K. M., Schoenberg, T. J., and C. E. Braun. 1982. Methods for trapping sage grouse in Colorado. Wildlife Society Bulletin 10:224-231.

Gillette, G. L., Coates, P. S., Petersen, S., and J. P. Romero. 2013. Can reliable sage-grouse lek counts be obtained using aerial infrared technology? Journal of Fish and Wildlife Management 4:386-394.

Guttery, M. R., T. A. Messmer, E. T. Thacker, N. Gruber, and C. M. Culumber. 2013. Greater sage-grouse sex ratios in Utah: implications for reporting population trends. Journal of Wildlife Management 77:1593–1597.

Hagen, C. A. 1999. Sage grouse habitat use and seasonal movements in a naturally fragmented landscape, northwestern Colorado. Thesis, University of Manitoba, Winnipeg, Canada.

Hausleitner, D. 2003. Population dynamics, habitat use and movements of greater sage-grouse in Moffat County, Colorado. Thesis, University of Idaho, Moscow, ID, USA.

Hogan, F. E., Cooke, R., Burridge, P., and J.A. Norman. 2007. Optimizing the use of shed feathers for genetic analysis. Molecular Ecology Resources 8:561-567.

Holloran, M. J. 2005. Greater sage-grouse (*Centrocercus urophasianus*) population response to natural gas field development in western Wyoming. Dissertation, University of Wyoming, Laramie, USA.

Huwer, S. L., Anderson, D. R., Remington, T. E., and G. C. White. 2008. Using human-imprinted chicks to evaluate the importance of forbs to sage-grouse. Journal of Wildlife Management 72:1622-1627.

Kahn, N. W., St. John, J., and T. W. Quinn. 1998. Chromosome-specific intron size differences in the avian CHD gene provide an efficient method for sex identification in birds. Auk 115:1074-1078.

Kohn, M. H., York, E. C., Kamradt, D. A., Haught, G., Sauvajot, R. M., and R. K. Wayne. 1999. Estimating population size by genotyping faeces. Proceedings of the Royal Society B 266: 657-663.

Krager, R. A. 1977. Survey of sage grouse strutting ground complexes and seasonal use areas within the Piceance Basin Wildlife Habitat Area. Colorado Division of Wildlife, Fort Collins, Colorado, USA.

Lampa, S., Henle, K., Klenke, R., Hoehn, M., and B. Gruber. 2013. How to overcome genotyping errors in non-invasive genetic mark-recapture population size estimation: a review of available methods illustrated by a case study. Journal of Wildlife Management 77:1490-1511.

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

Lukacs, P. M. and K. P. Burnham. 2005b. Research Notes: estimating population size from DNA-based closed capture-recapture data incorporating genotyping error. Journal of Wildlife Management. 69:396-403.

Marucco, F., Boitani, L., Pletscher, D.H., 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.

McCaffrey, R. and P. M. Lukacs. 2016. A generalized integrated population model to estimate greater sage-grouse population dynamics. Ecosphere 7:1-14.

McCaffrey, R., Nowak, J. J. and P. M. Lukacs. 2016. Improved analysis of lek-count index data using N-mixture models. Journal of Wildlife Management 80:1011-1021.

McKelvey, K. S., and M. K. Schwartz. 2005. Dropout: a program to identify problem loci and samples for noninvasive genetic samples in a capture-mark-recapture framework. Molecular Ecology Notes 5:716-718.

Miquel, C., Bellemain, E., Poillot, C., Bessiere, J., Durand, A., and P. Taberlet. 2006. Quality indexes to assess the reliability of genotypes in studies using noninvasive sampling and multiple-tube approach. Molecular Ecology Notes 6:985-988.

Miller, L. M., Kallemeyn, L., and W. Senanan. 2001. Spawning-site and natal-site fidelity by northern pike in a large lake: mark-recapture and genetic evidence. Transactions of the American Fisheries Society. 130: 307-316.

Monroe, A. P., Edmunds, D. R., and C. L. Aldridge. 2016. Effects of lek count protocols on greater sage-grouse population trend estimates. Journal of Wildlife Management 80:667-678.

Mowat, G. and C. Strobeck. 2000. Estimating population size of grizzly bears using hair capture, DNA profiling, and mark-recapture analysis. Journal of Wildlife Management 64:183-193.

Naugle, D. E. and B. L. Walker. 2007. A collaborative vision for integrated monitoring of greater sage-grouse populations. Pages 57-62 *in* Reese, K. P. and R. T. Bowyer, editors. Monitoring populations of greater sage-grouse: proceedings of a symposium at Idaho State University. College of Natural Resources Experiment Station Bulletin 88. Moscow, ID.

Oyler-McCance, S. J. and J. St. John. (Unpublished Report). Final report for "Pilot study to assess the effectiveness of DNA extraction from Gunnison sage-grouse feces for use in population estimation studies." Unpublished Report. United States Geological Survey.

Oyler-McCance, S. J. and J. St. John. 2010. Characterization of small microsatellite loci for use in non-invasive sampling studies of Gunnison sage-grouse (*Centrocercus minimus*). Conservation Genetics Resources 2:17-20.

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

Palsboll, P. J., Allen, J., Berube, M., Clapham, P. J., Feddersen, T. P., Hammond, P. S., Hudson, R. R., Jorgensen, H., Katona, S., Larsen, A. H., Larsen, F., Lien, J., Mattila, D. K., Sigurjonsson, J., Sears, R., Smith, T., Sponer, R., Stevick, P., and N. Oien. 1997. Genetic tagging of humpback whales. Nature 388: 767-769.

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

Parachute-Piceance-Roan Greater Sage-Grouse Work Group (PPR-GSGWG). 2008. Parachute-Piceance-Roan (PPR) greater sage-grouse conservation plan. Colorado Division of Wildlife, Denver, USA.

Piertney, S. B. and J. Hoglund. 2001. Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). Molecular Ecology Resources 1:303-304.

Piggot, M. P., Bellemain, E., Taberlet, P., 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.

Rich, T. 1985. Sage grouse population fluctuations: evidence for a 10-year cycle. Idaho BLM Technical Bulletin 85-1.

Robertson, M. D. 1991. Winter ecology of migratory sage grouse and associated effects of prescribed fire in southern Idaho. Thesis, University of Idaho, Moscow, USA.

Rösner, S., Brandl, R., Segelbacher, G., Lorenc, T., and J. Müller. 2014. Noninvasive genetic sampling allows estimation of capercaillie numbers and population structure in the Bohemian forest. European Journal of Wildlife Resources 60:789-801.

Schroeder, M. A., Aldridge, C. L., Apa, A. D., Bohne, J. R., Braun, C. E., Bunnell, S. D., Connelly, J. W., Deibert, P. A., Gardner, S. C., Hilliard, M. A., Kobriger, G. D., and C. W. McCarthy. 2004. Distribution of sage-grouse in North America. Condor 106:363-376.

Schwartz, M. K., Luikart, G., and R. S. Waples. 2006. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22:25-33.

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

Taylor, R. L., Walker, B. L., Naugle, D. E., and L. S. Mills. 2012. Managing multiple vital rates to maximize greater sage-grouse population growth. Journal of Wildlife Management. 76:336-347.

Theobald, D. M., Stevens Jr., D. L., White, D., Urquhart, N. S., Olsen, A. R., and J. B. Norman. 2007. Using GIS to generate spatially balanced random survey designs for natural resource applications. Environmental Management 40:134-146.

Thompson, T. R., 2012. Dispersal ecology of greater sage-grouse in northwestern Colorado: evidence from demographic and genetic methods. Dissertation. University of Idaho, Moscow, ID, USA.

Wakkinen, W. L., Reese, K. P., and J. W. Connelly. 1992. Sage grouse nest locations in relation to leks. Journal of Wildlife Management 56:381-383.

Walker, B. L. 2010. Interim Progress Report: greater sage-grouse research in the Parachute-Piceance-Roan region of western Colorado Part II: multi-scale habitat selection and seasonal habitat mapping. Unpublished progress report. Colorado Division of Wildlife, Grand Junction, USA.

Walker, B. L., Apa, A. D., and K. Eichhoff. 2016. Mapping and prioritizing seasonal habitats for greater sage-grouse in northwestern Colorado. Journal of Wildlife Management 80:63-77.

Walsh, D. P., White, G. C., Remington, T. E., and D. C. Bowden. 2004. Evaluation of the lek-count index for greater sage-grouse. Wildlife Society Bulletin 32:56-68.

Walsh, D. P., Stiver, J. R., White, G. C., Remington, T. E. and A. D. Apa. 2010. Population estimation techniques for lekking species. Journal of Wildlife Management 74:1607-1613.

Western Association of Fish and Wildlife Agencies (WAFWA). 2015. Greater sage-grouse population trends: an analysis for lek count databases 1965-2015. Report. Western Association of Fish and Wildlife Agencies, Cheyenne, WY, USA.

White, G. C. and K. P. Burnham. 1999. Program Mark: survival estimation from populations of marked animals. Bird Study 46:120-139.

Wik, P. A. 2002. Ecology of greater sage-grouse in south-central Owyhee County, Idaho. Thesis, University of Idaho, Moscow, ID, USA.

Zablan, M. A., Braun, C. E., and G. C. White. 2003. Estimation of greater sage-grouse survival in North Park, Colorado. Journal of Wildlife Management 67:144-154.

CHAPTER 4: ESTIMATING WINTER SEX RATIO AND FLOCK COMPOSITION OF GREATER SAGE-GROUSE (*CENTROCERCUS UROPHASIANUS*) FROM NON-INVASIVE, GENETIC MARK-RECAPTURE ANALYSIS OF FECAL PELLETS IN NORTHWESTERN COLORADO[4]

SUMMARY

A population's sex ratio, and the extent to which it varies over time, is an important factor for management and conservation of wildlife species. However, this population metric can be difficult to quantify in species where the sexes are not equally detectable. The greater sage-grouse (*Centrocercus urophasianus*) is a species of conservation concern throughout its range in North America due to historical range-wide declines in abundance and distribution. Sage-grouse populations are primarily monitored using counts of males at traditional breeding grounds, or "leks", in the spring as an index of population size and trend. Unfortunately, lek counts provide little information about female abundance, a key driver of sage-grouse population growth. Available estimates of sex ratio for many sage-grouse populations are thought to be biased due to the sampling methodologies used. There is a need for managers to obtain reliable estimates of

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sex ratio, and to understand the extent to which it varies annually, to estimate effective population size and likelihood of persistence. We estimated pre-breeding sex ratio during two consecutive winter seasons using data from a mark-recapture study in a small, low-density population of greater sage-grouse in northwestern Colorado. Sex ratio varied markedly between the two years, with male-to-female estimates of 1:3.29 in winter 2012-2013 and 1:1.54 in 2013-2014. We found evidence of segregation of males and females in winter flocks in 2012-2013 but not in 2013-2014, a year of much higher overall abundance. Wildlife agencies should consider the potential for large annual variation in sex ratio of sage-grouse populations if extrapolating female or total abundance from male lek-count data.

INTRODUCTION

Population Sex Ratio

A population's sex ratio, and the extent to which it varies annually, is an important factor for management and conservation of wildlife species. For species of conservation concern, reliable information on sex ratios are required to estimate effective population sizes and as inputs to population viability analyses (Frankham 1995, Lens et al. 1998, Brook et al. 2000 Saether 2004). Effective population size provides key insights into a population's persistence likelihood (Lande and Barrowclough 1987, Frankham 1995). However, adult sex ratios, and their patterns of variation, are often poorly understood and quantifying these metrics can be challenging, particularly if one sex is more difficult to detect (Donald 2007). In these cases, information on population sex ratio is required to make inference to total abundance.

The greater sage-grouse (*Centrocercus urophasianus*) (hereafter "sage-grouse") is a species of conservation concern that has experienced range-wide declines in population abundance and distribution throughout the past century, (Autenrieth 1981, Connelly & Braun 1997, Schroeder et al. 1999, Schroeder et al. 2004, Aldridge et al. 2008, Garton et al. 2015). Sage-grouse populations are commonly monitored using high counts of males during the spring breeding season as they gather to display on traditional strutting grounds known as leks (Patterson 1952, Connelly et al. 2003). Lek counts typically follow standardized protocols that specify season dates, recommended number of visits per lek per season, time of day, and weather conditions suitable for counting strutting males (Connelly et al. 2003). These counts serve as an index for populations over large areas and are widely used by state wildlife agencies throughout the western United States (Connelly et al. 2004). However, because female sage-grouse are more difficult to detect and do not regularly attend leks, lek counts provide little information about female abundance or population trend. For this reason, many wildlife agencies either do not record female counts or consider data on female detections unreliable. However, female population size is a critical metric for management because it directly reflects the growth potential of a population, and is particularly relevant for polygamous species for which not all males successfully breed in a given year (Johnson & Rowland 2007).

A key untested assumption of monitoring programs based on the lek-count index is that sex ratio remains constant across years, however, few reliable data are available to test that assumption (Walsh et al. 2004, Connelly et al. 2011, Sedinger 2007). Available estimates of male-to-female sex ratios favor females, and wildlife agencies often assume a ratio of approximately 1:2 (PPR-GSGWG 2008, CGSSC 2008, Atamian and Sedinger 2010, USFWS 2010, Garton et al. 2011). However, using lek-count data to estimate female and total population

size is questionable if sex ratios have not been validated (CGSSC 2008, Guttery et al. 2013). Estimating this metric is difficult because sex ratios of sage-grouse populations are known to vary seasonally (Shyvers 2017, Chapter 3) as a result of differential survival of males and females, with male mortality increasing as birds age (Patterson 1952, Braun 1984, Swenson 1986, Zablan et al. 2003). In addition, sage-grouse show substantial annual and geographic variation in survival that affects both sex ratio and population age structure (Connelly et al. 2011, Taylor et al. 2012).

Variation in sex ratio estimates among sage-grouse populations throughout the species' range have been documented (Connelly et al. 2011). Unfortunately, no studies to date have investigated annual variation in sex ratio, and many available estimates may not be applicable to spring breeding populations. Studies have estimated a male-to-female sex ratio of ~1:1 at hatch (Bush 2005, Atamian and Sedinger 2010, Guttery et al. 2013) and at 42 days of age (Guttery et al. 2013). However, because of differential mortality of juvenile and adult males, and variation in annual rates of juvenile mortality, these ratios are not useful for estimating sex ratio of breeding populations the following spring. Sex ratios have been estimated in several states using data from hunter-harvested birds (Connelly et al. 2011). These estimates, obtained from an analysis of birds harvested in the fall using wing characteristics to classify birds to sex and age class (Eng 1955, Crunden 1963, Braun et al. 2015), were generally greater than 1:2, but varied markedly among years and locations (Connelly et al. 2011, Braun et al. 2015). While estimates from fall harvest data suggest that sex ratios are consistently female-biased, they are unlikely to accurately reflect breeding-season sex ratio due to annual variation in fall and winter mortality rates for different age and sex classes, selective harvest of specific age and sex classes, and differential vulnerability of certain age or sex classes to harvest (Connelly et al. 2000, Wik 2002).

Sex ratio estimates for pre-breeding (winter) populations may accurately represent sex ratio of breeding populations if they are based on unbiased sampling methods. Overwinter survival of sage-grouse is typically high (Robertson 1991, Wik 2002, Hausleitner 2003, Beck et al. 2006, Batazzo 2007, Connelly et al. 2011), so an estimate of winter sex ratio should be representative of sex ratio at the start of the breeding season. Visual estimates of the sex composition of winter flocks yielded male-to-female sex ratio estimates of 1:1.56 to 1:1.63 in Colorado (Beck 1977). However, visual estimates may be biased by misidentification of the sex of juvenile birds (which can be difficult to distinguish from females in the field at a distance), lower detection probability of small flocks that may have a different sex composition, or missing data (e.g., ~15% of 5,105 birds on winter counts in Colorado could not be correctly sexed; Beck 1977). Estimates of sex ratios from spring counts at leks (Keller et al. 1941, Patterson 1952) are not considered reliable because of high temporal variation in both female and male lek attendance (Patterson 1952, Johnson & Rowland 2011). More recent studies using mark-resight estimates of male and female abundance estimated spring male-to-female sex ratios for greater and Gunnison sage-grouse to be less than 1:2 (Walsh 2004, Stiver et al. 2008). However, these studies were not based on random population sampling and did not investigate annual variation within the study populations.

Genetic Sampling

Recently, several authors have suggested that genetic analysis of winter fecal samples would provide more reliable estimates of pre-breeding sex ratio (Garton et al 2011, Baumgardt et al. 2013). Sex determination of birds from DNA samples, using amplification of the CHD gene, has proven to be useful in several studies (Lens et al. 1998, Whittingham and Dunn 2000,

Baumgardt et al. 2013). Non-invasive genetic sampling using non-invasively collected sources of DNA (e.g., fecal pellets or shed feathers) is a promising tool for conservation and management of wildlife species and can provide valuable information that cannot be obtained using traditional monitoring approaches (Schwartz et al. 2006). However, challenges arise because passively collected genetic samples, such as feces and shed feathers, are often characterized by low quality DNA and frequently result in genotyping errors (e.g., allelic dropout and PCR-generation of false alleles) (Tablerlet et al. 1996, Panasci et al. 2011, Buchan et al. 2005, Broquet et al. 2007, Hogan et al. 2007, Baumgardt et al. 2013) that can be highly variable among samples (Miquel et al. 2006). Fortunately, it is possible to overcome challenges of low quality DNA with improved field collection methods and laboratory protocols that minimize the occurrence of low genotyping success rates and genotyping error (Paetkau 2003, Panasci et al. 2011, Marucco et al. 2011, Lampa et al. 2013).

We used data from a genetic mark-recapture study (Chapter 3) to estimate pre-breeding sex ratio during two consecutive winter seasons in a geographically isolated greater sage-grouse population in northwestern Colorado. Specifically, we: 1) estimated annual variation in sex ratio based on sex-specific population estimates derived from genetic analysis of fecal pellets and feathers (Chapter 3); 2) quantified sex composition of winter flocks; and 3) investigated sample size requirements for obtaining accurate sex ratio estimates.

Study Area

The Parachute-Piceance-Roan (PPR) population is one of seven recognized greater sage-grouse populations in northwestern Colorado (Figure 25; CGSSC 2008). This small, low-density

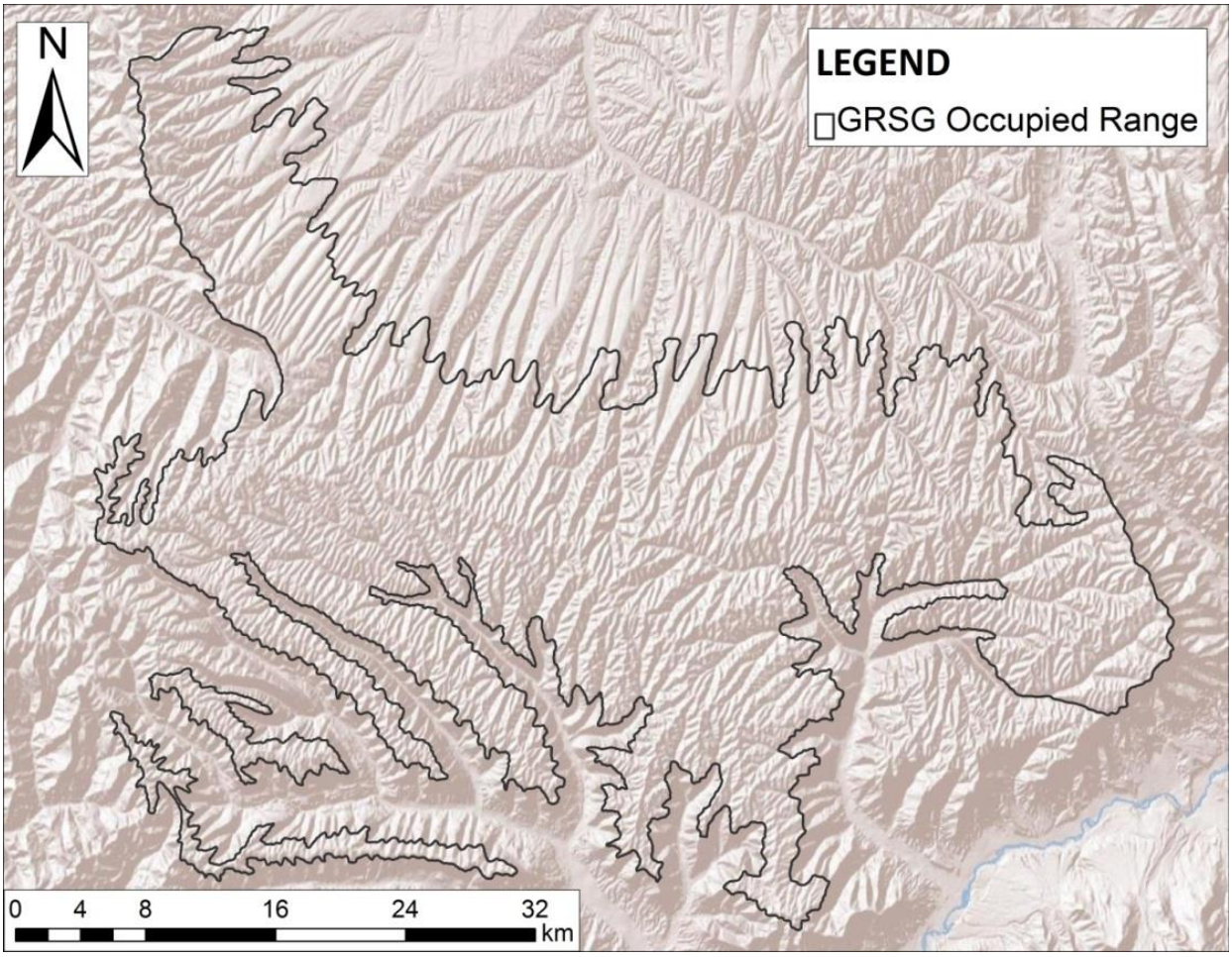

Figure 25. Study area map of the current (2012) occupied range boundary for the core Parachute-Piceance-Roan greater sage-grouse population in northwestern Colorado, with shaded relief.

population constitutes approximately 4% of Colorado's total population based on lek-count data (CGSSC 2008, PPR-GSGWG 2008). The PPR is characterized by diverse terrain with broad ridge tops that drop off into steep drainages and canyons on the western and southern extents. Greater sage-grouse in this population are largely restricted to sagebrush, sagebrush- grassland, and to a lesser extent, mixed sagebrush-mountain shrub on ridge tops, plateaus, and shallow drainages (Figure 26) at elevations from 2,000 – 2,750 m. (PPR-GSGWG 2008, Walker at al. 2016).

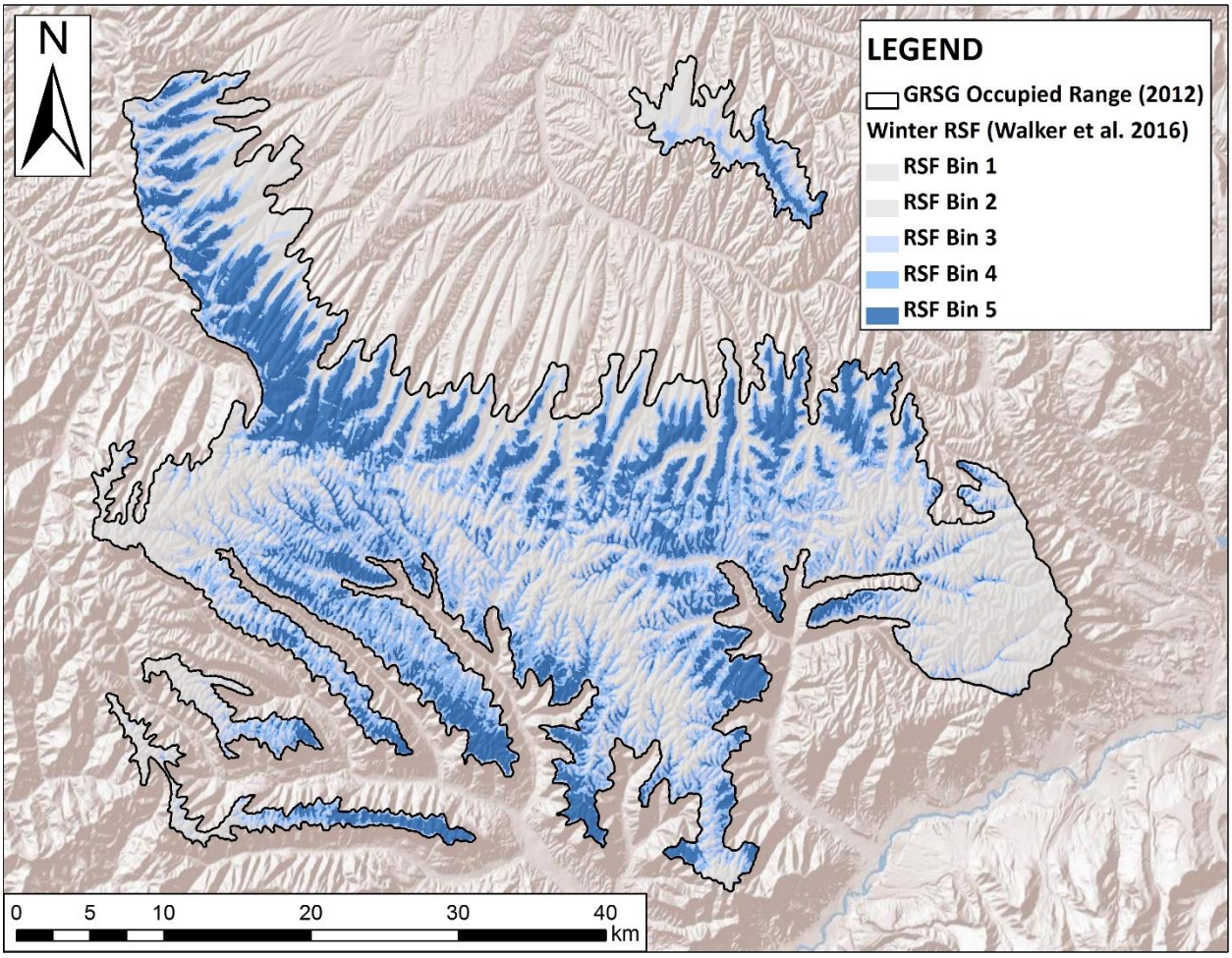

Figure 26. Winter resource selection function map showing areas of greater predicted relative intensity of use within the Parachute-Piceance-Roan greater sage-grouse occupied range boundary (Walker et al. 2016).

Vegetation on ridge tops is dominated by a mosaic of mountain big sagebrush (*Artemisia tridentata vaseyana*), mixed sagebrush and mountain shrubs, and pinyon-juniper (*Pinus edulis*, *Juniperus spp.*) woodlands with scattered patches of aspen (*Populus tremuloides*) and conifers. Winters in the PPR are generally characterized by deep snow and sage-grouse habitat use is restricted by the availability of exposed sagebrush cover. The area is covered by a network of developed oil and gas access roads and undeveloped two-tracks along ridge tops and drainages

that facilitate access. The PPR is geographically isolated, but not genetically distinct, from other Colorado populations (PPR-GSGWG 2008). While male-to-female sex ratio for the breeding population in Middle Park, Colorado was estimated in one year (e.g., 1:2.2 in 2001, Walsh 2002, Walsh et al. 2004), there are currently no estimates of sex ratio available for other populations, including the PPR.

METHODS

Sage-Grouse Captures and Survival Monitoring

We trapped and marked GRSG in the study area from mid-July through early November in 2012 and 2013 to estimate winter survival. Colorado Parks and Wildlife's (CPW, USDA Registration #84-R-0045) Animal Care and Use Committee approved all trapping, handling, and marking methods and we obtained inter-agency approval from Colorado State University (CSU) (#07-2011 and #08-2012; Appendices E-I). We aged and sexed captured birds using plumage characteristics, molt patterns, weight, tarsal length, and head length, then banded all captured birds with uniquely numbered aluminum leg bands. Non-juvenile males were affixed with 30-g, rump-mounted, solar-powered GPS PTT satellite transmitters (Northstar Science and Technology, King George, VA) or 22-g, battery-powered, and juvenile males and females VHF necklace collars equipped with mortality sensors (Advanced Telemetry Systems, Isanti, MN). We collected a feather sample for genetic analysis from the flanks of each captured bird using a sterile nitrile glove, and stored samples in Whirlpak© bags or paper envelopes. A small number of additional samples were collected from birds captured by CPW during the lekking season. See METHODS in Chapter 3 for trapping, collar-attachment, and monitoring details.

Non-Invasive Genetic Sampling

We performed non-invasive genetic sampling of greater sage-grouse fecal pellets and shed feathers during two consecutive winter seasons, early November to mid-March in 2012-2013 and 2013-2014. We divided the study area into 200 x 200 m plots and selected a spatially-balanced random sample of approximately 1,000 plots using a reversed randomized quadrant-recursive raster (RRQRR) algorithm (Theobald et al. 2007) and divided plots into sets of 30; a subset of which was sampled (Figure 27). Plots selection was stratified using values from breeding habitat resource selection function layers (Walker 2010).

We were initially concerned that high winter site fidelity of greater sage-grouse might result in few recaptures and low detection probability if new sample plots were surveyed each occasion. We surveyed plot sets in a rotating panel to balance this concern with sufficient sampling of the population. This was accomplished by selecting two sets (60 plots total) in a rotating fashion (i.e. occasion 1 included sets 1 and 2, occasion 2 included sets 3 and 4, occasion 3 included sets 1 and 5, occasion 4 included sets 2 and 6, and so on) so that no plot was sampled on more than 2 occasions. Sampling occasions were allowed to overlap temporally but not spatially to facilitate more intensive sampling during good weather and snow conditions and to reduce overall commute times to distant or difficult-to-access parts of the study area.

To collect fecal pellet and feather samples for analysis, we accessed plots by truck or snowmobile and surveyed on foot/snowshoe to locate sites used by flocks. Survey transects varied in width to ensure full coverage of each plot given variation in terrain, vegetation, snow, and weather conditions that influenced visibility of bird sign. We did not survey areas of non-habitat, such as pinyon-juniper woodlands and cliffs, and we did not conduct surveys during

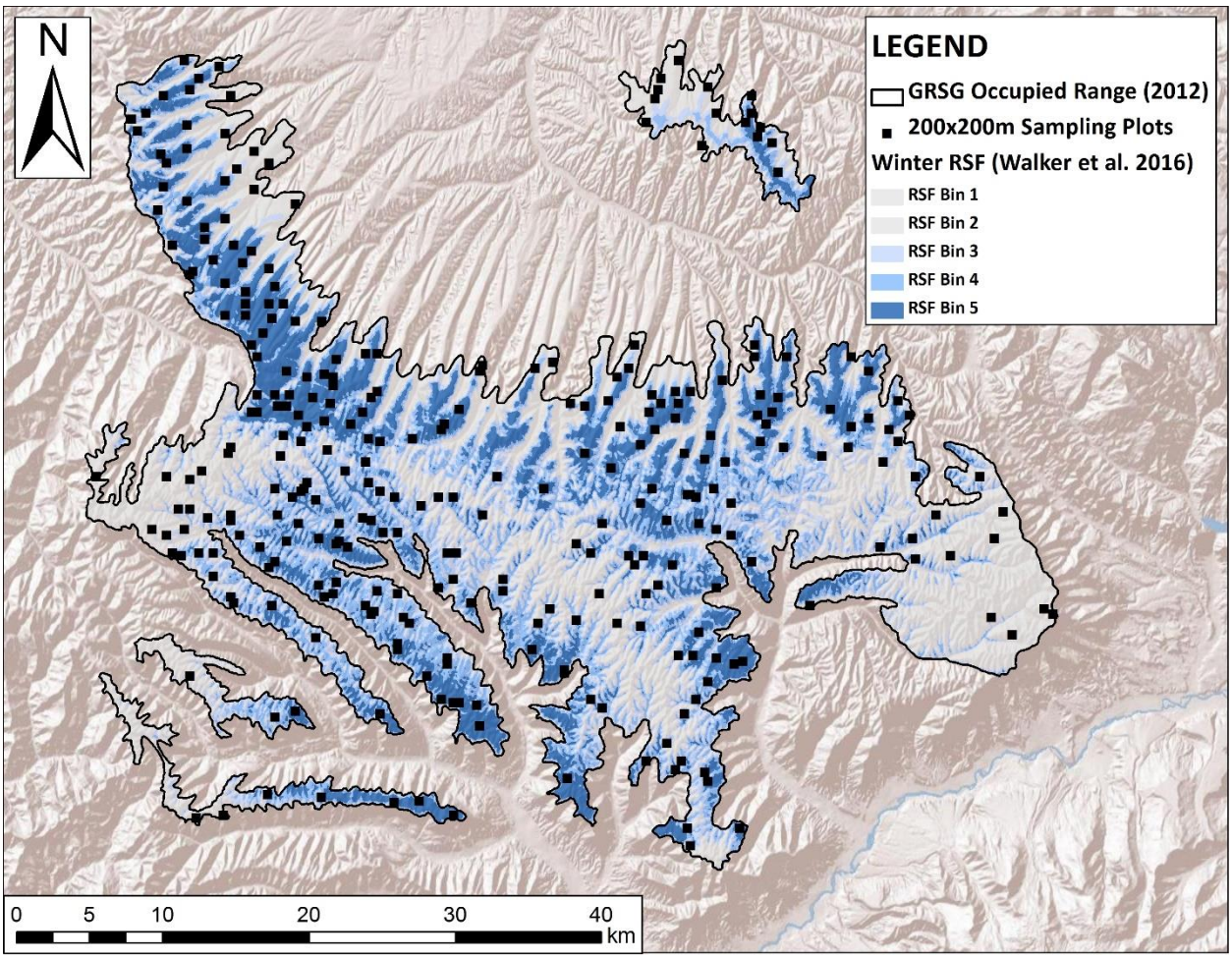

Figure 27. A subset of a spatially-balanced random sample of plots used for sex ratio sampling during winter seasons in 2012-2013 and 2013-2014, with winter resource selection function maps from Walker at al. 2016.

adverse weather conditions (i.e., heavy snow or wind) that would substantially reduce our detection probability. Surveys were delayed for a minimum of 24 hrs following a wind or snow event to allow birds time to leave tracks and deposit pellets. Because of the low density of birds in our study area, we surveyed additional suitable habitat on foot and used binoculars to add detections while traveling to and from random plots. All incidental flocks sampled were assigned the sample occasion number of the associated plot. We withheld location data for GPS and VHF-

collared birds from crews conducting field surveys, and telemetry searches were performed separately from random surveys to avoid sampling bias associated with prior knowledge of marked bird use locations.

When a flock was detected, we searched the area for evidence of a roost site, estimated the number of birds in the flock, and marked the locations of individual pellets and roost piles with colored pin flags. For flock sites where roost piles were not detected and track density suggested a large number of birds had been present, we marked a 20 ft length of the track paths with flagging and collected all pellet and feather samples within the marked area. Pellets and feathers were collected using fresh nitrile gloves for each sample to avoid cross-contamination and carefully removed snow and debris from each fecal pellet sample without scraping the pellet surface to avoid accidental removal of DNA. We collected a total of 8-10 pellets from each roost pile. Individual pellets were combined in the same sample bag only if they came from the same roost pile or were otherwise known to be from the same bird. Feathers located at flock-use sites were always packaged separately. Caecal samples were collected only when other sources of DNA were not available. Samples were placed in 4-oz Whirlpak© bags (eNasco, Fort Atkinson, WI) with 1-g silicone desiccant packets (Fisher Scientific, Houston, TX) to absorb excess moisture, packed in snow until transferred to a -20° F, manual defrost freezer for temporary storage. Samples were later transferred to a -80° F freezer until analysis. We avoided thawing, re-freezing, and exposure of samples to UV radiation to maintain DNA integrity.

We attempted to collect at least one fecal or feather sample from each individual bird present at each flock-use site. We targeted roost piles (Figure 28) for sampling to minimize the number of samples required for collection. Because they are produced by a single bird, sampling from roost piles reduces the likelihood of individual birds being represented multiple times

(Baumgardt et al. 2013) and provides confidence that all birds present at a flock site were represented in the samples collected. When roost piles were not present, sampling often required collecting more pellets than birds present to ensure we obtained ≥ 1 sample from each bird.

Figure 28. Example of a greater sage-grouse fecal pellet roost pile (center), targeted for genetic sampling, and caecal dropping (upper left). Photo credit: J. Shyvers

ANALYSIS

Genetic Analysis of Samples

We isolated DNA from fecal pellet and caecal samples following the Qiagen (Hilden, Germany) protocol from Human Stool DNA isolation using QIAmp DNA Stool Mini Kits, QIAmp Fast DNA Mini Kits, or in 96-well plates using the DNeasy 96 Blood and Tissue Kit (Qiagen) following the Animal Tissues protocol. Fecal pellet extractions were performed under a ventilation hood dedicated to low-quality DNA extractions. DNA extractions from feather samples were performed using DNeasy Blood and Tissue Kits (Qiagen) following a user-developed protocol for purification of DNA from nails, hair, or feathers.

We used 7 microsatellite loci to identify individual GRSG, including 6 polymorphic loci (BG6; Piertney and Hoglund 2001, SGMS06.6 and MSP11 (Oyler-McCance and St. John 2010) and SG29, SG36, and SG39 (Fike et al. 2015)) and one sexing locus (a region of the CDH gene using the primers 1237L (GAGAAACTGTGCAAAACAG) and 1272H (TCCAGAATATCTTCTGCTCC; Kahn et al. 1998)). These microsatellite loci were known to be the most polymorphic and reliable based on previous work on Gunnison sage-grouse by Oyler-McCance and St. John (2010). Loci were amplified using a multiplex pre-amplification method (Piggott et al. 2004). Because DNA from non-invasively collected sources, particularly fecal DNA, are typically low in quality and at increased risk for genotyping error (e.g. allelic dropout during PCR), we repeated the amplification process to confirm results for all samples. We amplified all samples twice with the aim of obtaining two complete matching multi-locus genotypes. In addition, we ran positive and negative controls within each sample set to maximize quality and consistency of genotyping. Samples with unsuccessful amplification of most loci

were re-extracted and re-amplified at least twice. Detailed protocols for all modified extractions and PCR steps are described in Appendix M.

We compared corresponding genotypes and generated a consensus genotype for each sample. We defined a successful locus determination as a score confirmed by one or more rounds of amplification (see Appendix M for details). To be included in our data analysis, samples were required to have a minimum of 6 of 7 successful loci determinations. Unique individuals and their capture histories were determined using Dropout Utility 1.2 & Dropout 2.3.1 (McKelvey & Schwartz 2005). Feather samples from captures of radio-collared birds that died prior to the start of each winter sampling season were excluded from the data. Inclusion of data from juveniles with unknown fates was determined using survival estimates from the literature (see ANALYSIS in Chapter 3 for details on mark-recapture analysis).

Sex Ratio Analysis Models

Estimates of pre-breeding abundance of both males and females (i.e., naïve estimates of sex ratio) were obtained using "Huggins' Closed Mark-Recapture" models (Program MARK; White and Burnham 1999) with initial capture probability equal to that of subsequent captures ($p=c$). Sex was used as a group covariate to derive separate estimates of abundance and detection for males and females. A spatial (region) covariate ("North" or "South"; Figure 29.) was used to model possible heterogeneity in capture probabilities between individuals in the southern part of the study area, where winter access was restricted, and individuals in the northern part of the study area where access was comparatively unrestricted. Assignment of covariate values to individuals was based on the location of their first detection. Restricted access in the South

resulted in unequal sampling effort for that region. Models for various combinations of group and time effects (Table 21) were fitted and model results averaged based on Akaike's Information Criteria adjusted for small population size **(**AIC_c) values. Feather sample data from captures constituted our first GMR sampling occasion with the remainder of sampling occasions based on non-invasive sampling of fecal and feather samples.

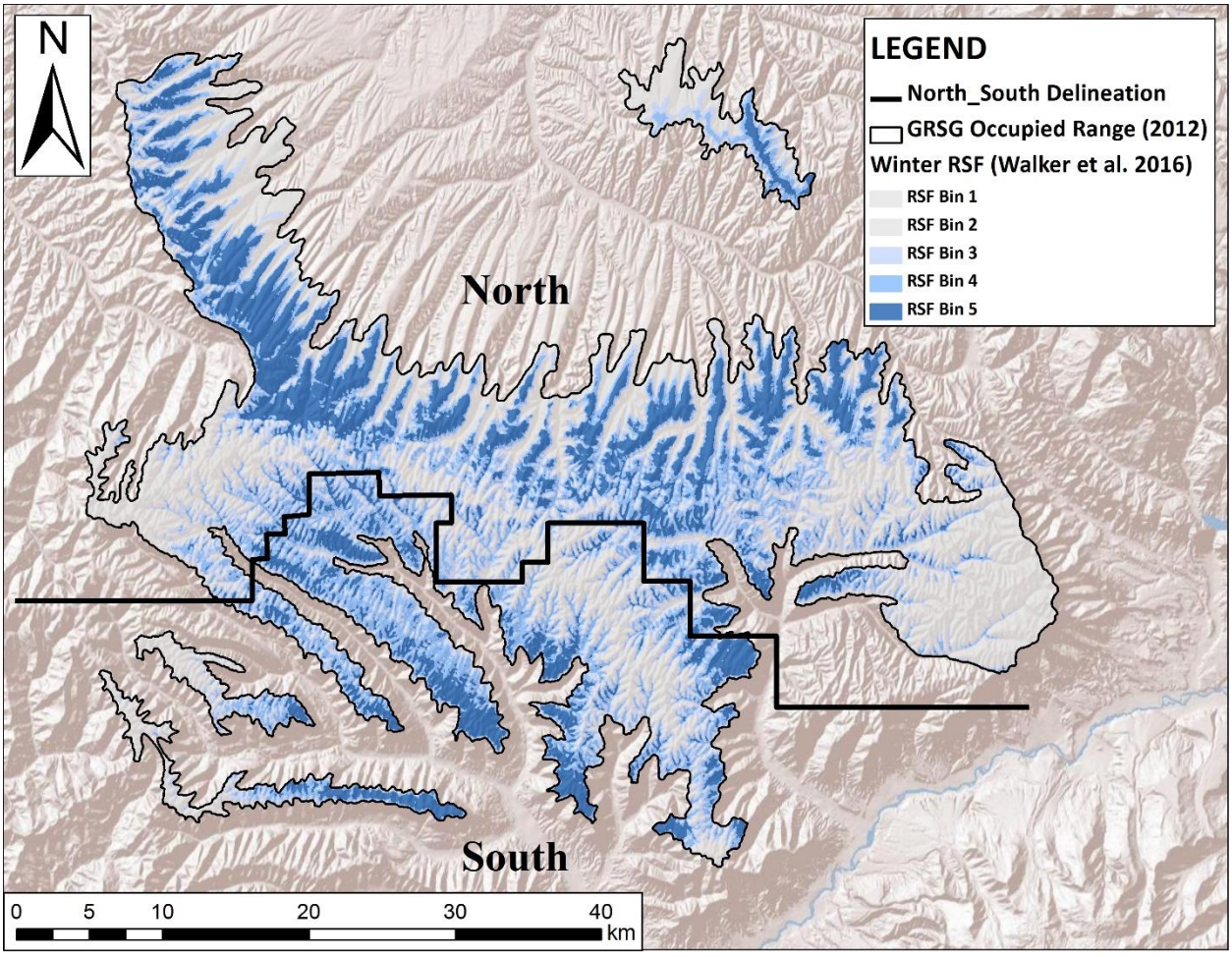

Figure 29. Map of the Parachute-Piceance-Roan greater sage-grouse occupied range with winter resource selection function layer (Walker et al. 2016) and line delineating "North" and "South" regions of the study area.

Table 21. List of closed *p=c* mark-recapture models, varying by time (t) and group (g) effect, with regional covariate "region" used in genetic mark-recapture analysis of greater sage-grouse data from the Parachute-Piceance-Roan, 2012-2014.

Model Name
p(t) = c + region
p(g+t) = c + region
p(g*t) = c + region
p(.) = c + region
p(g) = c + region

We re-ran the same models using Closed Robust Design Multi-State data types with "Huggins' p and c with state probabilities" with *p = c* to estimate omega (Ω), the probability of being a male, equivalent to the estimated percentage of males in the population. We used a dummy primary occasion (representing a second season with two sampling occasions consisting of all zeros) to enable the robust design model to estimate this parameter using data from only one season (Kendall et al. 2012). This analysis provided us with confidence intervals for the omega parameter that are not provided by other closed mark-recapture models in program MARK.

We investigated whether male and female grouse segregated in winter flocks in both study years by testing for equality of mean proportions of males in observed flocks compared to expected proportions of males in the population based on our estimates of Ω (Zar 1999). We assumed that the proportion of males in observed flocks would be approximately equal to the proportion of males in the population if individuals from each sex were randomly distributed among flocks.

Sample Size Requirements

To determine the minimum effort required to achieve precise and unbiased estimates of sex ratio with different population sizes, we investigated the impact of sampling effort on precision of estimates. We ran closed mark-recapture models with data type full p & c simulations (n=500) in Program MARK using detection probability $p_{male} = 0.115$ & $p_{female} = 0.114$ for population sizes of 300, 600, and 1,000. We calculated the percentage of simulations that included the true proportion of males in the 95% confidence interval to assess bias of estimates, the coefficient of variation (CV) for male and female abundance estimates, and the range of estimates for proportion of males. We re-ran "Huggins' p and c Closed Robust Design Multi-State models" using modified input files with variation in the number of sampling occasions to determine the level of precision achieved.

RESULTS

We collected feather samples during two seasons of sage-grouse capture efforts between April and November, and non-invasive winter samples from random plots and opportunistic encounters with flocks from 11 November 2012 – 14 March 2013 (season one) and from 4 November 2013 – 14 March 2014 (season two). Our analyses were based on genetic data from a total of 116 captures, and non-invasive genetic samples from a total of 120 flock use sites in season one and 146 flock use sites in season two (Figure 30). We non-invasively collected a total of 1,089 genetic samples from multiple source types across 7 sampling occasions in season one (2012-2013) and 1,268 across 8 occasions in season two, for a combined total of 2,357 samples collected during the two-year study period.

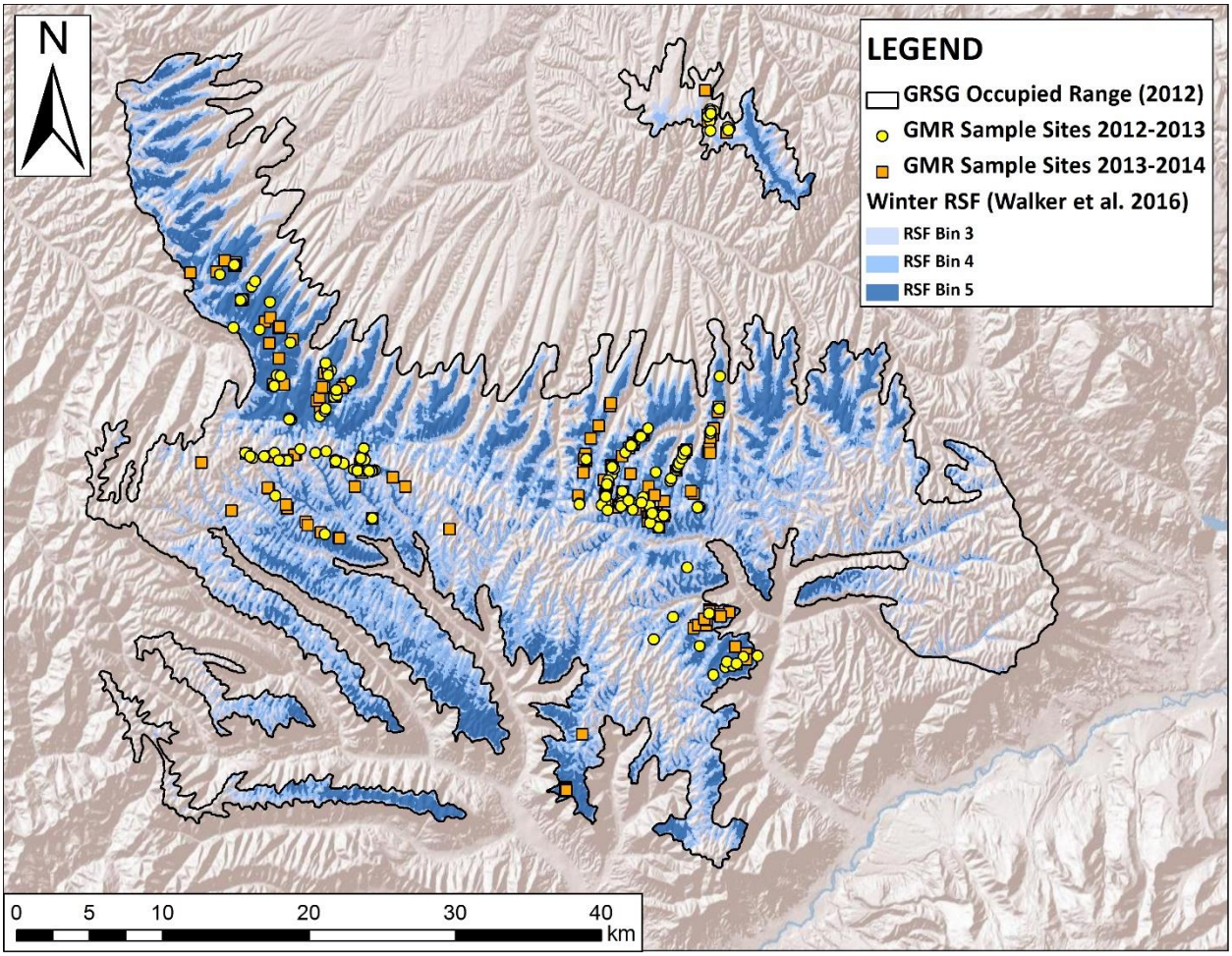

Figure 30. Greater sage-grouse genetic sample locations in season one (2012-2013) and season two (2013-2014) overlaid with a winter resource selection function layer from Walker at al. 2016 in the Parachute-Piceance-Roan population.

DNA from capture feathers produced the highest rate of success for analyzing the CDH sexing locus (100%; n = 91), followed by DNA from fecal pellets (95.5%; n = 2,201), feathers collected at flock use sites (81%; n = 63), and caecal samples (50.0%; n=2). The CDH locus was successfully analyzed and confirmed by at least two amplifications in 95.3% of all genetic samples. Pellet samples constituted the largest proportion of the data used in our analysis with 83.4% of all samples collected (n = 2,201) successfully analyzed (≥6 loci successfully analyzed).

We detected a total of 235 unique sage-grouse individuals in 2012-2013 and 390 in 2013-2014 (Table 22). Correcting for the number of birds detected in both winter seasons (82 individuals; 21 males and 61 females), the total number of unique individuals detected during our study period was 543. Naïve estimates of sex ratio, based on the number of unique male and female sage-grouse detected without correction for differences in detectability between sexes, were 1:2.79 in season one (2012-2013) and 1:1.53 in season two (2013-2014) with an average sex ratio of 1:1.89 for both seasons combined (Table 22).

Eighty percent of winter sage-grouse flocks contained females in season one and 70% contained females in season two. In season one, 41% of flocks consisted of only females, 23% consisted of only males, and 39% were mixed-sex flocks. Flock composition was more evenly distributed in season two, with 39% of flocks consisting of only females, 30% consisting of only

Table 22. Number of unique individual greater sage-grouse detected and naïve sex ratio estimates in each of two winter sampling seasons in the Parachute-Piceance-Roan population in northwestern Colorado.

	Season 1 (2012-2013)	Season 2 (2013-2014)	Combined Seasons*
Males	62	154	195
Females	173	236	348
Total	235	390	543
Naïve Sex Ratio (Male-to-Female)	1.2.79	1:1.53	1:1.78

*Some individuals were detected in both seasons.

males, and 31% mixed-sex (Figure 31). We found strong evidence of non-random distribution of males and females within winter flocks in season one ($p = 0.0337$) but not in season two ($p = 0.1681$). The majority of single-sex flocks were small, with 62/70 (89%) containing ≤ 5 birds in season one and 80/92 (87%) in season two (Figures 32-33). On average, mixed flocks of all sizes had more females than males, with females constituting 66% of all mixed flock birds in season one and 53% in season two (Figure 34).

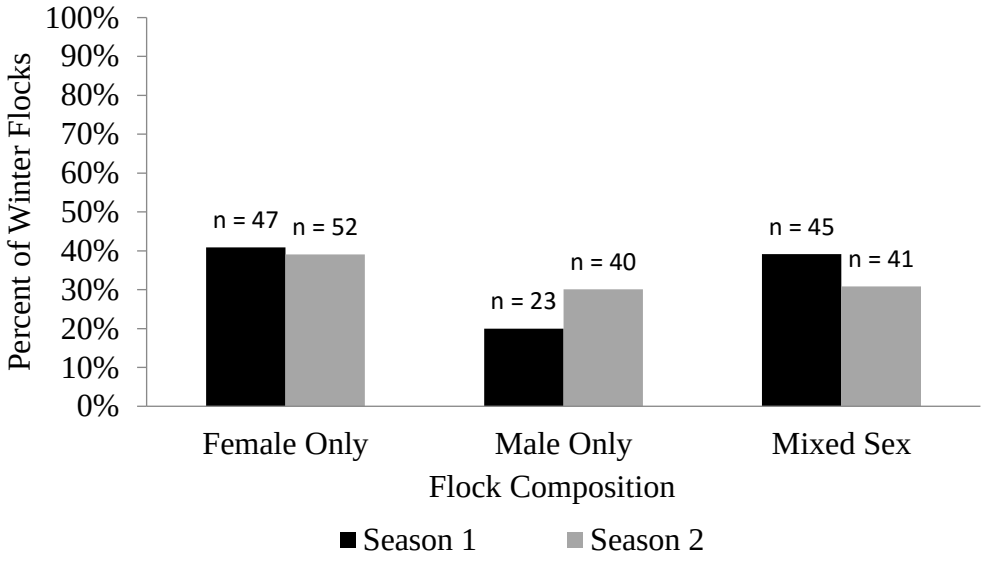

Figure 31. Percent of winter greater sage-grouse flocks that were female-only, male-only, and mixed-sex in the Parachute-Piceance-Roan in season one (2012-2013) and season two (2013-2014).

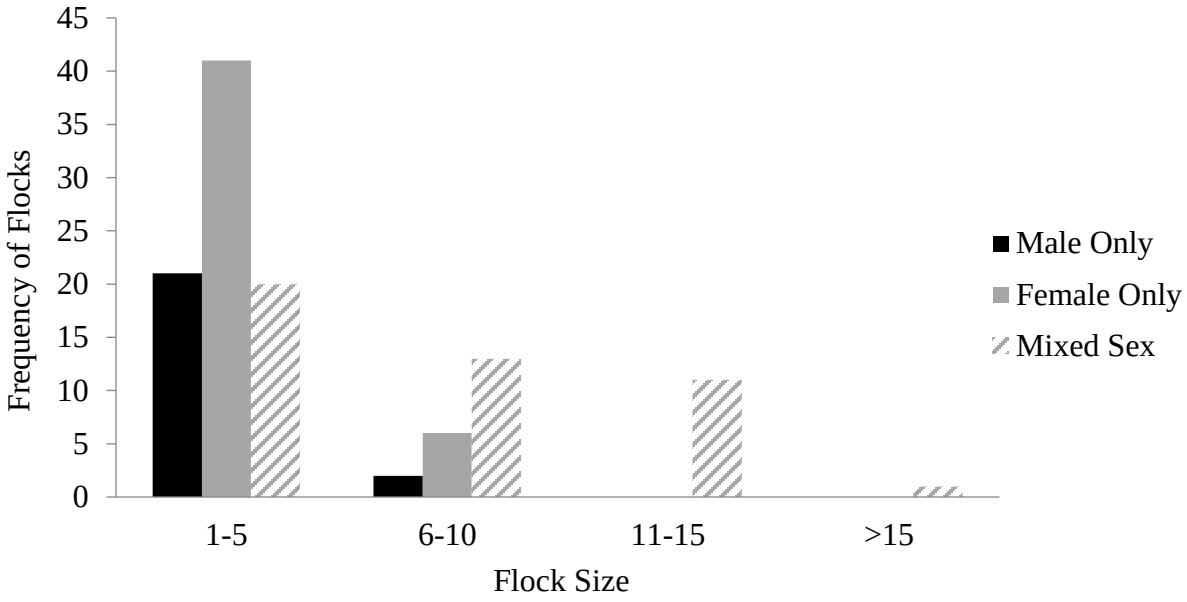

Figure 32. Frequency of winter greater sage-grouse flocks of different size by composition; female-only, male-only and mixed-sex in the Parachute-Piceance-Roan, season one (2012-2013).

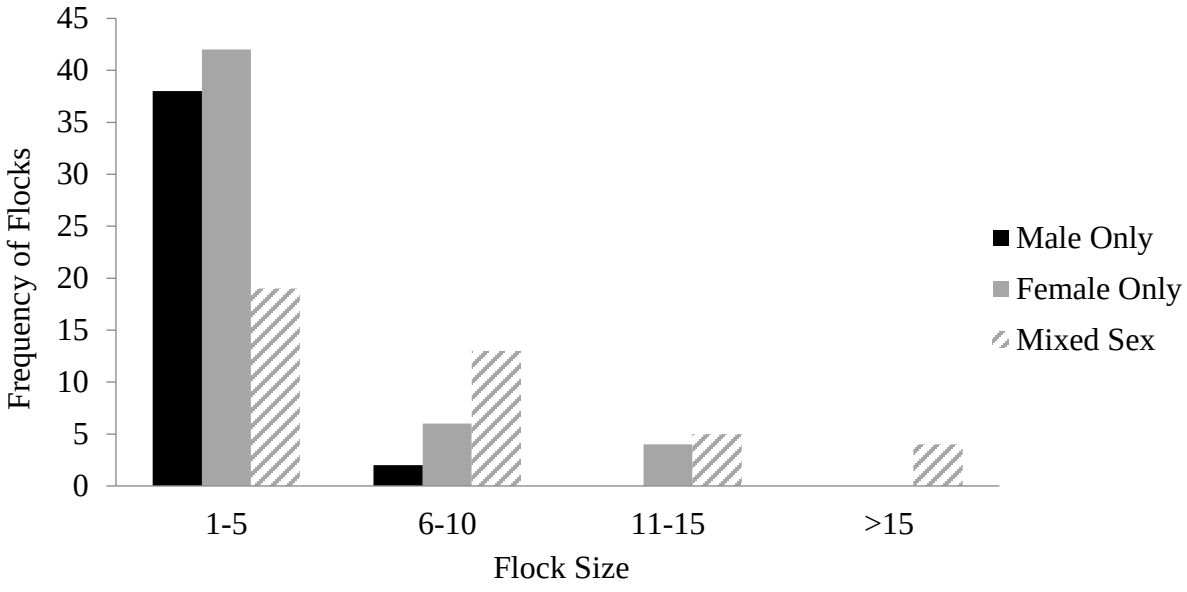

Figure 33. Frequency of winter greater sage-grouse flocks of different size by composition; female-only, male-only, and mixed-sex in the Parachute-Piceance-Roan, season two (2013-2014).

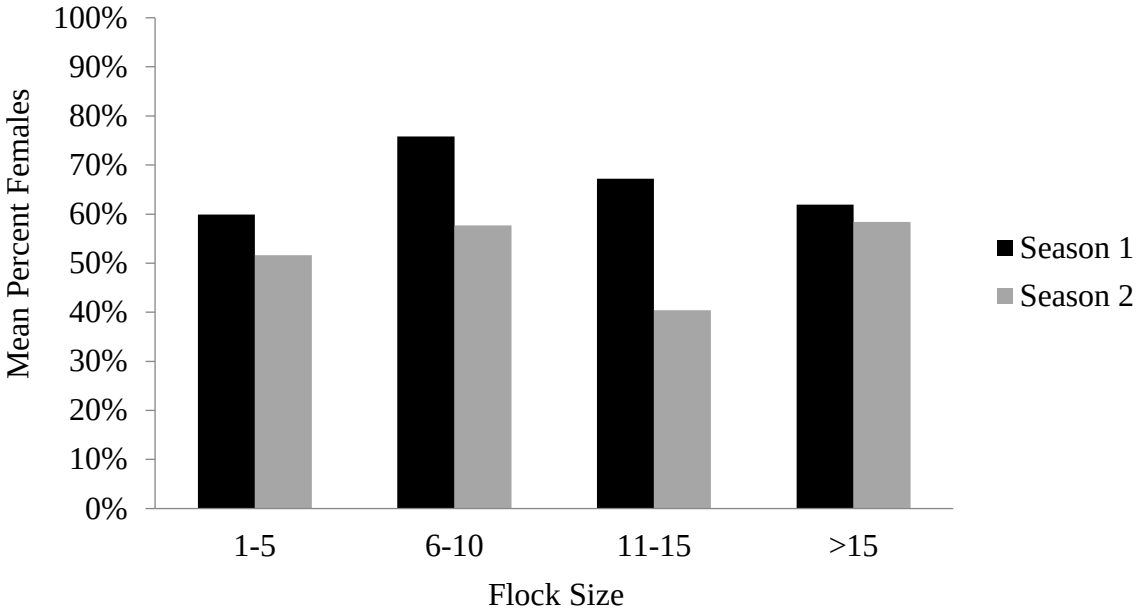

Figure 34. Average percent females in greater sage-grouse winter flocks of varying sizes in the Parachute-Piceance-Roan in season one (2012-2013) and season two (2013-2014).

We used model averaging to obtain pre-breeding estimates of male and female abundance and omega (Ω, the proportion of males in the population). We estimated that the pre-breeding abundance of sage-grouse increased by ~410 individuals, an approximate doubling in population size, from season one (2012-2013; total $N_1 = 335$) to season two (2013-2014; total $N_2 = 745$) (lambda = 2.22; Table 23). We estimated a much greater increase in the size of the male population (lambda = 3.76) between years than females (lambda = 1.76). Our estimated sex ratios, based on model averaging, were 1:3.29 in season one and 1:1.54 in season two with a combined average for both seasons of 1:2.42.

Table 23. Model-averaged estimates of pre-breeding abundance ($\hat{N}$) for male and female greater sage-grouse with standard errors and 95% lower and upper confidence intervals, omega (Ω; the proportion of males in the population) with 95% confidence intervals, and estimated sex ratio in the Parachute-Piceance-Roan in season one (2012-2013) and season two (2013-2014).

	Sex	Estimate ($\hat{N}$)	SE	LCI	UCI	Omega (Ω)	LCI	UCI	Sex Ratio (M:F)
Season 1 (2012-2013)									
	N (Male)	78	6.387	65	90	0.233	0.179	0.298	
	N (Female)	257	17.745	222	292	0.767	0.702	0.821	
	N (Total)	335		287	382				1:3.29
Season 2 (2013-2014)									
	N (Male)	293	25.592	243	344	0.393	0.339	0.451	
	N (Female)	452	34.786	384	520	0.607	0.549	0.661	
	N (Total)	745		627	864				1:1.54

We investigated the sample size required to achieve desired precision of sex ratio estimates needed for population conservation and management. Simulation options for the parameter Ω were not available, so we were unable to account for co-variances arising from non-independence of male and female population proportions in our simulated confidence intervals for Ω. For that reason, we report approximate estimates (Figure 35). Using conservative estimates for capture probability, sex ratio estimates obtained from a minimum of two sampling occasions for population sizes 300-1,000 will be both unbiased and precise (Figure 36), however, precision decreases with lower sampling effort (Figures 37-38). Based on our study, we found that a minimum of 4-5 full sampling occasions were required to obtain sex ratio

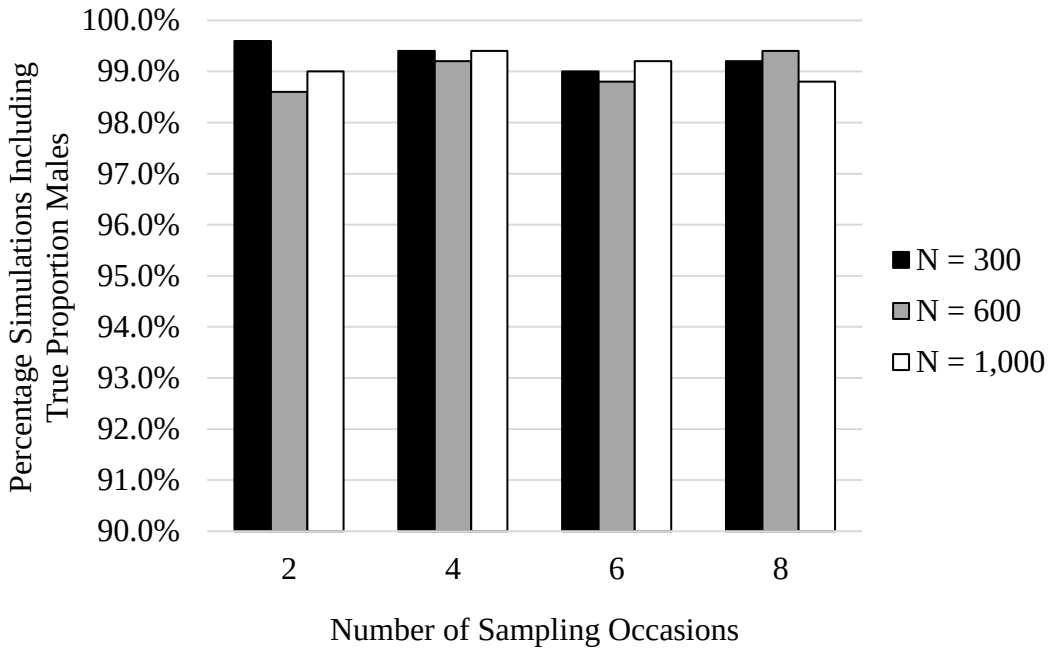

Figure 35. Closed mark-recapture model simulation results (500 repetitions): percentage of simulations including the true proportion of males (0.25) with detection probability $p_{male} = 0.115$ and $p_{female} = 0.114$; and population sizes 300, 600 and 1,000.

estimates with levels of precision (coefficient of variation ≤ 0.15) but more than 6 occasions offered little additional benefit (Figure 38).

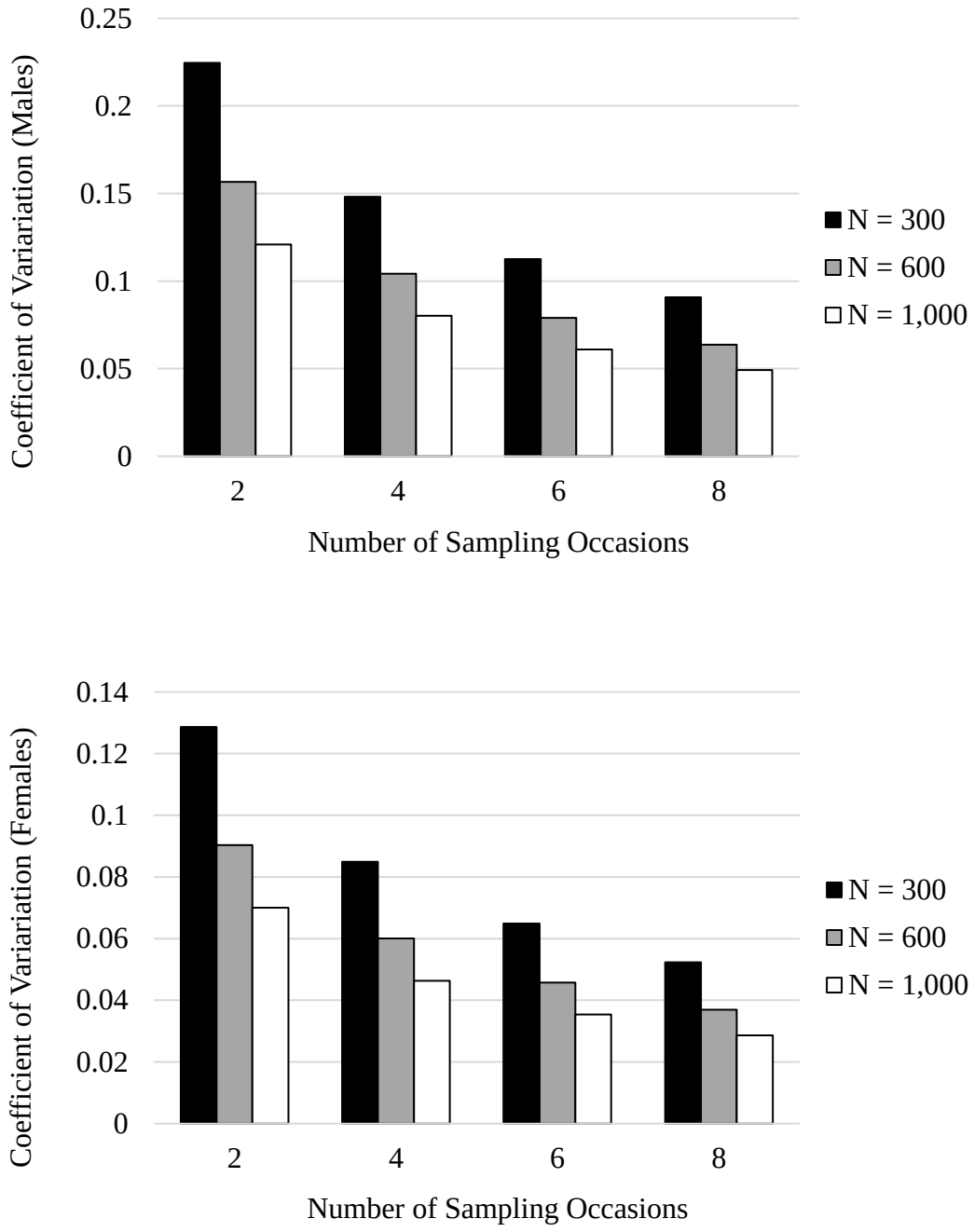

Figure 36. Closed mark-recapture model simulation results (500 repetitions): coefficient of variation for simulated male (top) and female (bottom) abundance estimates for 2, 4, 6, and 8 full sampling occasions with detection probability p_{male} = 0.115 & p_{female} = 0.114; and population sizes 300, 600 and 1,000.

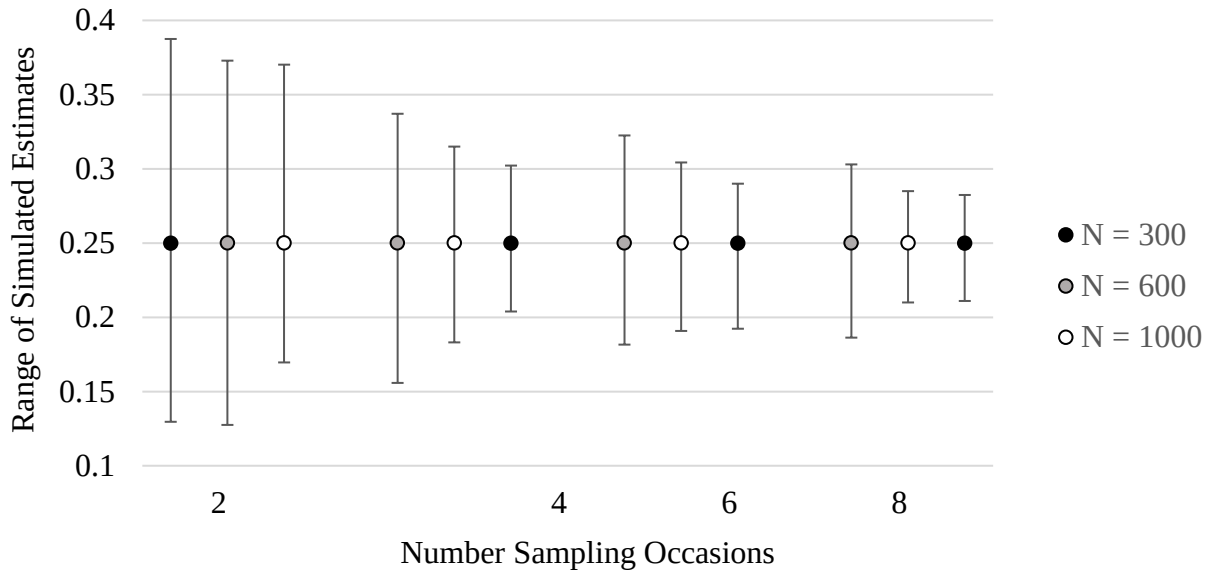

Figure 37. Closed mark-recapture model simulation results (500 repetitions): range of simulated estimates for number of full sampling occasions vs. estimated proportion males; with true proportion males = 0.25, detection probability p_{male} = 0.115 and p_{female} = 0.114; and population sizes 300, 600 and 1,000.

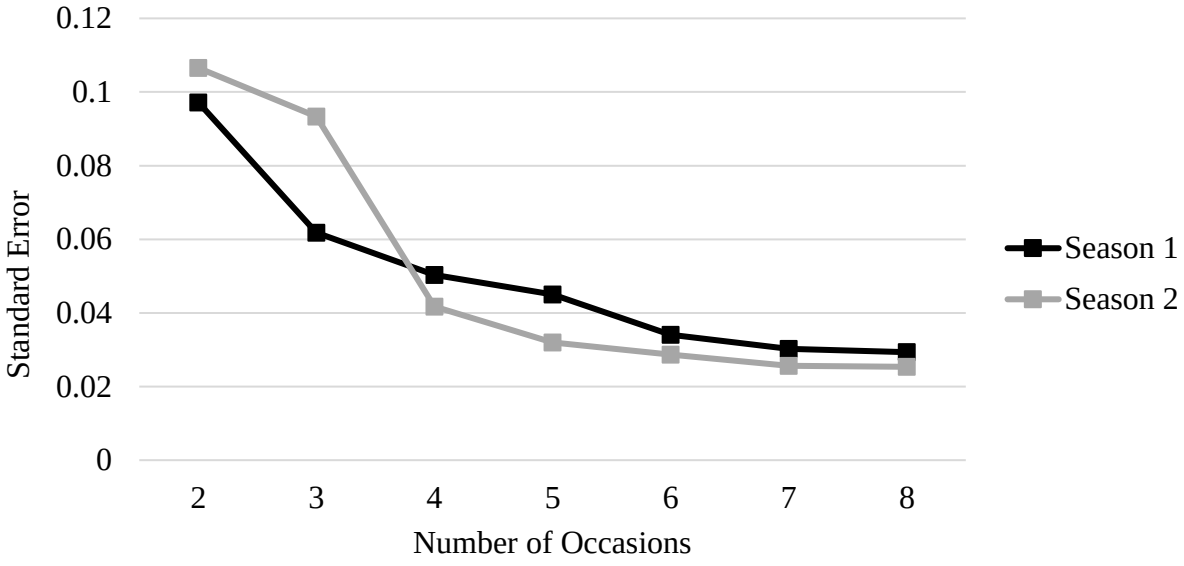

Figure 38. Sampling effort vs. precision, results of simulations using data from this study to determine the number of sampling occasions needed to achieve specific SE values.

DISCUSSION

We provide the first pre-breeding sex ratio estimates for a greater sage-grouse population based on genetic sampling of winter flock use locations. As in other studies, we observed strongly female-biased sex ratios. However, we also observed a significant change in male-to-female sex ratio estimates during a two-year period that corresponded with a large increase in estimated population size. We speculate that the increase in the male-to-female sex ratio between seasons one and two, from 1:3.29 to 1:1.54, was due to the combination of a year of low male abundance in winter 2012-2013 combined with high reproductive success and juvenile recruitment in 2013. Male-to-female sex ratios of winter (and breeding) sage-grouse populations with female-biased adult sex ratios are expected to increase (i.e., shift closer to 1:1) following years of high reproductive success because of a 1:1 sex ratio at birth coupled with a large influx of juvenile males with higher survival, compared to adult males (Patterson 1952, Braun 1984, Swenson 1986, Zablan et al. 2003), results in a relatively larger increase in the male population compared to females. The lower the male population size in the preceding year, the larger the magnitude of increase will be in male abundance in a subsequent year following a period of high reproductive success.

The two-year average sex ratio estimate from our study (1:2.42) was similar to the values often assumed by wildlife agencies for estimating total population size (1:2). However, our results demonstrate that sex ratio can vary substantially among years. Our sex ratio estimates were higher than those based on fall harvest data ranging from 1:1.15-1:2.4 (Rogers 1964, Swenson 1986, Broms 2007, CGSSC 2008, Guttery et al. 2013, Braun et al. 2015), but closer to results obtained using mark-recapture estimates of abundance in spring (1:2.3 to 1:3.2) for greater sage-grouse (Walsh 2002) and 1:2.1 for Gunnison sage-grouse (Stiver et al. 2008). Lower

sex ratio estimates from our study, compared to those from birds harvested in the fall, may be the result of annual or seasonal variation in true sex ratio, or they may reflect a bias in harvest data due to differential harvest of males and females (Connelly et al. 2000, Wik 2002).

We found evidence of sex-based segregation of sage-grouse in winter-flocks in season one when population abundance was relatively low, but no evidence of segregation in season two. These results reflected in pronounced differences in male and female capture probabilities during season one (Chapter 3). If male and female-dominated flocks use slightly different habitats in the winter, as previously observed (Beck 1977), the result may be differences in detection probability between flocks dominated by either males or females. We suspect that if juvenile males remain in flocks with females during their first winter, segregation of sexes may be more evident in years with older male age structure (e.g., following ≥ 1 years of low reproductive success or recruitment) but may be masked in years following high recruitment (Chapter 3). Unfortunately, genetic data provide no information on age composition of winter flocks, so we were unable to determine whether males in mixed flocks were primarily juveniles or adults. It is possible that adult males segregate from females to a greater extent than yearling sage-grouse, but this needs to be further investigated.

We believe our study methods adequately met assumptions of closed-population mark-recapture models (Chapter 3). We made an additional assumption to accompany laboratory methods that assign sex based on non-invasively collected genetic samples; we assumed that successful DNA analysis of fecal pellet and feather samples did not differ between individuals or sexes. A previous study investigating the potential for use of field-collected fecal pellet samples to identify sex ratio of greater sage-grouse found that success rates for extraction of DNA differed between males and females, but the resulting bias was minimal particularly if based on

relatively fresh pellet samples (≤ 34 days of age; Baumgardt et al. 2013). We believe we met the assumption of unbiased genetic analysis for several reasons: 1) nearly all fecal pellet samples used in this study were known to be ≤ 14 days old, 2) we used very few samples of unknown age; 3) we used a pre-amplification PCR method (Piggot et al. 2004) to improve microsatellite amplification and error rates; 4) we observed success rates > 95% for our sexing locus, and 5) we confirmed genotyping scores for ≥ 6 loci using ≥ 2 repetitions of PCR amplification to avoid misidentification of individual birds.

MANAGEMENT IMPLICATIONS

Accurate estimates of pre-breeding population sex ratio, and its annual variability, are required to extrapolate male-based lek-count data to abundance of females and to overall population size. Reliable estimates of pre-breeding population sex ratio can be obtained using non-invasive genetic data for mark-recapture analyses. Our findings support the conclusions of Baumgardt et al. (2013) that the use of fecal DNA for estimating sex ratio has great potential and the method may be feasible for estimating sex ratio in populations throughout the species' range. Our study demonstrates that applications of genetic mark-recapture sampling in the field can provide robust estimates of sex ratio for small, low-density populations.

Variability of sex ratio estimates between the first and second years of our study suggests that managers should exercise caution when using an average sex ratio estimate (e.g., 1:2) to extrapolate female population size from male lek-count data due to the potential for annual variation in population sex ratio. Managers should also consider the potential for sex ratio to vary in relation to age structure of males in the population. Age structure of the male population will

be biased toward juveniles following years of high reproductive success, and those years are likely to produce increases in annual lek counts. We recommend against using sex ratios based on fall harvest data to estimate female population size from lek-count data because they appear to underestimate the proportion of males in spring breeding populations.

LITERATURE CITED

Aldridge, C. L., Nielsen, S. E., Beyer, H. L., Boyce, M. S., Connelly, J. W., Knick, S. T., and M. A. Schroeder. 2008. Range-wide patterns of greater sage-grouse persistence. Diversity and Distributions 14: 983-994.

Atamian, M. T., and J. S. Sedinger. 2010. Balanced sex ratio at hatch in a greater sage-grouse (*Centrocercus urophasianus*) population. Auk 127:16–22.

Autenrieth, R. E. 1981. Sage grouse management in Idaho (No. 9). Idaho Department of Fish & Game.

Batazzo, A.M. 2007. Winter survival and habitat use by female greater sage-grouse (*Centrocercus urophasianus*) in south Phillips County, Montana. Thesis, University of Montana, Missoula, MT, USA.

Baumgardt, J. A., Goldberg C. S., Reese, K. P., Connelly, J. W., Musil, D. D., E. Garton, E.O., and L. P. Waits. 2013. A method for estimating population sex ratio for sage-grouse using noninvasive genetic samples. Molecular Ecology Resources 13:393-402.

Beck, T. D. I. 1977. Sage grouse flock characteristics and habitat selection in winter. Journal of Wildlife Management 41: 18–26.

Beck, J. L., Reese, K. P., Connelly, J. W., and M. B. Lucia. 2006. Movements and survival of juvenile greater sage-grouse in southeastern Idaho. Wildlife Society Bulletin 34:1070-1078.

Braun, C. E. 1984. Attributes of a hunted sage grouse population in Colorado, U.S.A. International Grouse Symposium 3:148-162.

Braun, C. E., Budeau, D. A., and M. A. Schroeder. 2015. Fall population structure of sage-grouse in Colorado and Oregon. Wildlife Technical Report, pages 1-68. Oregon Department of Fish and Wildlife, Salem, OR, USA.

Brook, B. W., Burgman, M. A., and R. Frankham. 2000. Differences and congruencies between PVA packages: the importance of sex ratio for predictions of extinction risk. Ecology and Society 4.

Broms, K. 2007. Small game population reconstruction: model development and applications. Thesis. University of Washington, Seattle, WA, USA.

Broquet, T., Menard, N., and Petit, E. 2007. Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conservation Genetics 8:249-260.

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

Bush, K. L., Vinsky, M. D., Aldridge, C. L., and C. A. Paszkowski. 2005. A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater sage-grouse (*Centrocercus urophasianus*). Conservation Genetics 6:867-870.

Colorado Greater Sage-Grouse Steering Committee (CGSSC). 2008. Colorado greater sage-grouse conservation plan. Colorado Division of Wildlife, Denver, Colorado, USA.

Connelly, J. W. and C. E. Braun. 1997. Long-term changes in sage grouse (*Centrocercus urophasianus*) populations in western North America. Wildlife Biology 3:229-234.

Connelly, J. W., Reese, K. P. and M. A. Schroeder. 2003. Monitoring of greater sage-grouse habitats and populations. University of Idaho, College of Natural Resources Experiment Station Bulletin 80. Moscow Idaho.

Connelly, J. W., Knick, S. T., Schroeder, M. A., and S. J. Stiver. 2004. Conservation assessment of greater sage-grouse and sagebrush habitats. Western Association of Fish and Wildlife Agencies, Cheyenne, WY, USA.

Connelly, J. W., Apa, A. D., Smith, R. B., and K. P. Reese. 2000. Effects of predation and hunting on adult sage grouse *Centrocercus urophasianus* in Idaho. Wildlife Biology 6:227-232.

Connelly, J. W., Hagan, C. A., and M. A. Schroeder. 2011. Characteristics and dynamics of greater sage-grouse populations. Pages 53-67 *in* S. T. Knick & J. W. Connelly, editors. Greater sage-grouse: ecology and conservation of a landscape species and its habitats. Studies in Avian Biology 38, University of California Press, Berkley, CA.

Crunden, C. W. 1963. Age and sex of sage grouse from wings. Journal of Wildlife Management 27:846–850.

Donald, P. F. 2007. Adult sex ratios in wild bird populations. Ibis 149:671-692.

Eng, R. L. 1955. A method for obtaining sage grouse age and sex ratios from wings. Journal of Wildlife Management 19:267–272.

Fike, J. A., Oyler-McCance, S. J., Zimmerman, S. J., and T. A. Castoe. 2015. Development of 13 microsatellites for Gunnison Sage-grouse (*Centrocercus minimus*) using next-generation shotgun sequencing and their utility in Greater Sage-grouse (*Centrocercus urophasianus*). Conservation Genetics Resources 7:211-214.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genetics Research 66:95-107.

Garton, E. O., Connelly, J. W., Horne, J. S., Hagen, C. A., Moser, A., and M. A. Schroeder. 2011. Greater sage-grouse population dynamics and probability of persistence. Pages 293-382 *in* S. T. Knick and J. W. Connelly, editors. Greater sage-grouse: ecology and conservation of a landscape species and its habitats. Studies in Avian Biology 38, University of California Press, Berkeley, CA, USA.

Garton, E. O., Wells, A. G., Baumgardt, J. A., and Connelly, J. W. 2015. Greater sage-grouse population dynamics and probability of persistence. Final report to Pew Charitable Trusts.

Guttery, M. R., Messmer, T.A., Thacker, E. T., Gruber, N., and C. M. Culumber. 2013. Greater sage-grouse sex ratios in Utah: implications for reporting population trends. Journal of Wildlife Management 77:1593–1597.

Hausleitner, D. 2003. Population dynamics, habitat use and movements of greater sage-grouse in Moffat County, Colorado. Thesis, University of Idaho, Moscow, ID, USA.

Hogan, F. E., Cooke, R., Burridge, P., and J. A. Norman. 2007. Optimizing the use of shed feathers for genetic analysis. Molecular Ecology Resources 8:561-567.

Johnson, D. H. and M. M. Rowland. 2007. The utility of lek counts for monitoring greater sage-grouse. Pages 15-24 *in* K.P. Reese and R.T. Bowyer, editors. Monitoring populations of greater sage-grouse: proceedings of a symposium at Idaho State University. College of Natural Resources Experiment Station Bulletin 88. Moscow, ID, USA.

Kahn, N. W., St. John, J., and T. W. Quinn. 1998. Chromosome-specific intron size differences in the avian CHD gene provide an efficient method for sex identification in birds. Auk 115:1074-1078.

Keller, R. J., Shepherd, H. R., and R. N. Randall. 1941. Survey of 1941: North Park, Jackson County, Moffat County, including comparative data of previous seasons. Colorado Game and Fish Commission Sage Grouse Survey 3, Denver, CO, USA.

Kendall, W. L., White, G. C., Hines, J. E., Langtimm, C. A., and J. Yoshizaki. 2012. Estimating parameters of hidden Markov models based on marked individuals: use of robust design data. Ecology 93:913-920.

Lampa, S., Henle, K., Klenke, R., Hoehn, M., and B. Gruber. 2013. How to overcome genotyping errors in non-invasive genetic mark-recapture population size estimation: a review of available methods illustrated by a case study. Journal of Wildlife Management 77:1490-1511.

Lande, R. and G. F. Barrowclough. 1987. Effective populations size, genetic variation, and their use in population management. Pages 87-124 *in* M.E. Soule, editor. Viable populations for conservation. Cambridge University Press.

Lens, L., Galbusera, P., Brooks, T., Waiyaki, E., and T. Schenck. 1998. Highly skewed sex ratios in critically endangered Taita thrush as revealed by CHD genes. Biodiversity and Conservation 7:869-873.

Marucco, F., Boitani, L., Pletscher, D. H., 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.

McKelvey, K. S., and M. K. Schwartz. 2005. Dropout: a program to identify problem loci and samples for noninvasive genetic samples in a capture-mark-recapture framework. Molecular Ecology Notes 5:716-718.

Miquel, C., Bellemain, E., Poillot, C., Bessiere, J., Durand, A., and P. Taberlet. 2006. Quality indexes to assess the reliability of genotypes in studies using noninvasive sampling and multiple-tube approach. Molecular Ecology Notes 6:985-988.

Oyler-McCance, S. J. and J. St. John. 2010. Characterization of small microsatellite loci for use in non-invasive sampling studies of Gunnison sage-grouse (*Centrocercus minimus*). Conservation Genetics Resources 2:17-20.

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

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

Patterson, R. L. 1952. The Sage Grouse in Wyoming. Wyoming Game and Fish Commission and Sage Books, Inc., Denver, Colorado, USA.

Parachute-Piceance-Roan Greater Sage-Grouse Work Group (PPR-GSGWG). 2008. Parachute-Piceance-Roan (PPR) greater sage-grouse conservation plan. Colorado Division of Wildlife, Denver, CO, USA.

Piertney, S. B. and J. Hoglund. 2001. Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). Molecular Ecology Resources 1:303-304.

Piggot, M. P., Bellemain, E., Taberlet, P. 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.

Robertson, M. D. 1991. Winter ecology of migratory sage grouse and associated effects of prescribed fire in southern Idaho. Thesis, University of Idaho, Moscow, ID.

Rogers, G. E. 1964. Sage grouse investigations in Colorado. Colorado Game, Fish and Parks Department Technical Publication Number 16.

Saether, B. E., Engen, S., and R. Lande. 2004. Time to extinction in relation to mating system and type of density regulation in populations with two sexes. Journal of Animal Ecology 73:925-934.

Sedinger, J.S. 2007. Pages 15-24 *in* K.P. Reese and R.T. Bowyer, editors. Monitoring populations of greater sage-grouse: proceedings of a symposium at Idaho State University. College of Natural Resources Experiment Station Bulletin 88. Moscow, ID, USA.

Schroeder, M. A., Young, J. R., and C.E. Braun. 1999. Sage-grouse (*Centrocercus urophasianus*). Page 28 *in* A. Poole and F. Gill, editors. The Birds of North America, No. 425. The Birds of North America, Inc., Philadelphia, PA, USA.

Schroeder, M. A., Aldridge, C. L., Apa, A. D., Bohne, J. R., Braun, C. E., Bunnell, S. D., Connelly, J. W., Deibert, P. A., Gardner, S. C., Hilliard, M. A., Kobriger, G. D., and C. W. McCarthy. 2004. Distribution of sage-grouse in North America. Condor 106:363-376.

Schwartz, M. K., Luikart, G., and R. S. Waples. 2006. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22:25-33.

Stiver, J. R., Apa, A. D., Remington, T. E., and R. M. Gibson. Polygyny and female breeding failure reduce effective population size in the lekking Gunnison sage-grouse. Biological Conservation 141:472-481.

Swenson, J. E. 1986. Differential survival by sex in juvenile sage grouse and gray partridge. Ornis Scandinavica 17:14-17.

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

Taylor, R. L., Walker, B. L., Naugle, D. E., and L. S. Mills. 2012. Managing multiple vital rates to maximize greater sage-grouse population growth. Journal of Wildlife Management. 76:336-347.

United States Fish and Wildlife Service (USFWS). 2010. 12-month finding for petitions to list the greater sage grouse (*Centrocercus urophasianus*) as threatened or endangered. Federal Register 75:13909-14014.

Walker, B. L. 2010. Interim Progress Report: Greater sage-grouse research in the Parachute-Piceance-Roan region of western Colorado Part II: multi-scale habitat selection and seasonal habitat mapping. Colorado Division of Wildlife unpublished report.

Walker, B. L., Apa, A. D., and K. Eichhoff. 2016. Mapping and prioritizing seasonal habitats for greater sage-grouse in northwestern Colorado. Journal of Wildlife Management 80:63-77.

Walsh, D. P. 2002. Population estimation techniques for greater sage-grouse. Thesis, Colorado State University, Fort Collins, CO.

Walsh, D. P., White, G. C., Remington, T.E., and D. C. Bowden. 2004. Evaluation of the lek-count index for greater sage-grouse. Wildlife Society Bulletin 32:56-68.

White, G. C. and K. P. Burnham. 1999. Program Mark: survival estimation from populations of marked animals. Bird Study 46:120-139.

Whittingham, L. A. and P. O. Dunn. 2000. Offspring sex ratios in tree swallows; females in better condition produce more sons. Molecular Ecology 9:1123-1129.

Wik, P. A. 2002. Ecology of the greater sage-grouse in south-central Owyhee County, Idaho. Thesis, University of Idaho, Moscow, ID, USA.

Zablan, M. A., Braun, C. E., and G. C. White. 2003. Estimation of greater sage-grouse survival in North Park, Colorado. Journal of Wildlife Management 67:144-154.

Zar, J. H. 1999. Biostatistical Analysis, 4th edition. Prentice-Hall Inc., New Jersey, USA.

SUPPLEMENTAL MATERIALS

Supplement A

Colorado Parks and Wildlife (CPW) uses lek-count index data, based on seasonal high counts of males attending leks, to monitor greater sage-grouse in this population and to guide habitat management and prioritization efforts. Located on the southern edge of the species' range in Colorado, the PPR was not closely monitored until 2005 when managers recognized the potential for impacts from increasing energy development. As a result, managers lack a rigorous long-term dataset for assessing the status and trend of the population. Colorado Parks and Wildlife (CPW) lek-count data dating back to 2005 (Figure S1) demonstrates a fluctuating pattern in annual high male counts. These data suggest the population dropped in 2013 and then rebounded in 2014, two of the years corresponding to our study. CPW is aware of the limitations of the lek-count index and currently uses trend in annual male high counts to inform management decisions, without attempting to generate either male-specific or overall population estimates. A three-year running average is used by CPW to control for factors that may impact attendance of males and annual variation in detectability. Still, supplemental information on how lek-count index data relate to true population abundance and trend would be extremely valuable to the agency. The PPR population faces several challenges to persistence, specifically habitat loss and disturbance resulting from widespread energy development and pinyon-juniper encroachment into sagebrush (PPR-GSGWG 2008). Due to its small size, the population is considered vulnerable and subject to adverse impacts from rapidly expanding oil and natural gas extraction activities in the area that involve exploration, increased vehicle traffic, increased number of roads, well pad construction, and associated pipelines, powerlines and buildings

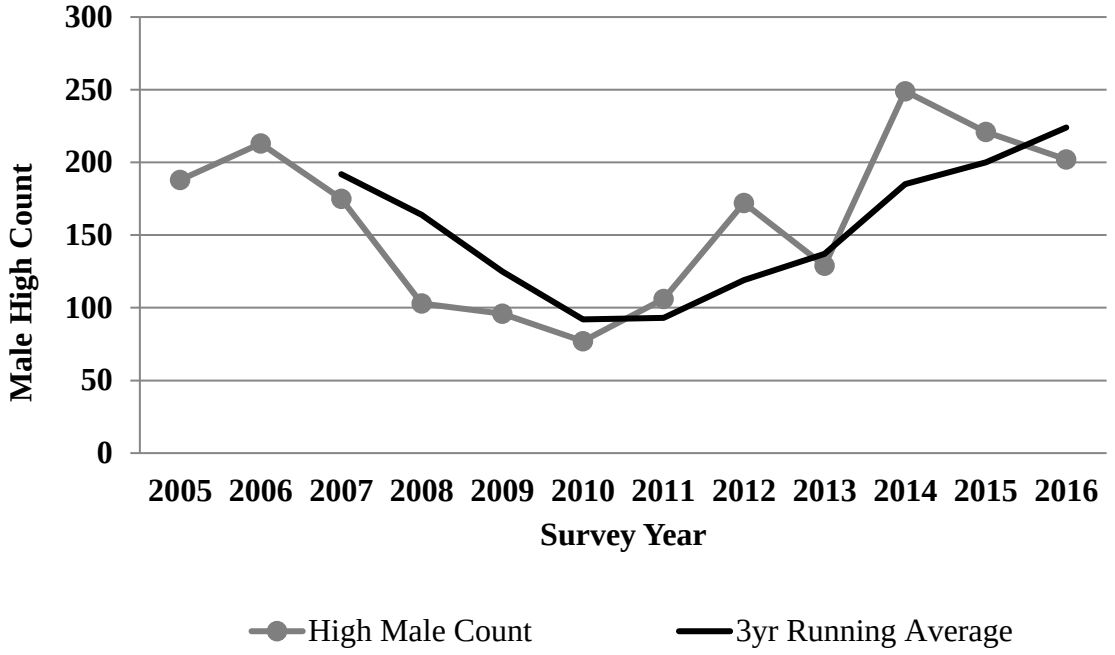

Figure S1. Lek-count data showing high male counts summed across leks and the three-year running average in the Parachute-Piceance-Roan greater sage-grouse population in northwestern Colorado from 2005-2016 (data courtesy of Colorado Parks and Wildlife).

(PPR-GSGWG 2008). Managers currently have a critical need to assess the status of this population and better understand how lek count trend data relate to true population size.

We defined the study area as the CPW 2012 occupied range boundary for the core PPR population, excluding the Magnolia area. This population supports approximately 4% of the total greater sager-grouse males counted on leks in Colorado (CGSSC 2008). Disturbance associated with major anthropogenic land uses (e.g., livestock grazing, oil and gas development) may influence lek turnover in this population by increasing the number and distribution of openings within sagebrush habitat that are suitable for strutting, or by influencing strutting activity at leks over time.

Colorado Parks and Wildlife uses lek-count data based on seasonal high counts of males attending leks to monitor populations of greater sage-grouse and to guide habitat management and prioritization efforts. Annual total high counts of males from CPW lek counts ranged from 77–249 males at 55–92 known leks per year from 2005-2016 (Colorado Parks and Wildlife, unpublished data). The population was monitored inconsistently from the 1970's through 2005 due to the logistical difficulties of accessing high-elevation areas in early spring. Intensive, standardized monitoring of leks from helicopter began in 2006. The recent increase in monitoring efforts to obtain baseline monitoring data in the PPR was motivated by the need to assess impacts of future land use changes in the area given that the Piceance Basin is currently experiencing widespread development from oil and gas extraction that are expected to increase considerably in the next 20-30 years (CGSSC 2008). Despite the increase in monitoring, there are currently no defensible estimates of population abundance or trend for the population as a result of untested assumptions. CPW recognizes the limitations of using lek-count data as an index of abundance (CGSSC 2008), however, there is a lack of information to assess potential sources of bias in lek-count data (i.e., the number of leks known and counted). This creates challenges for determining conservation status and implementing proper management strategies for the population.

Supplement B

Our study was based on lek definitions for small populations in the Colorado greater sage-grouse conservation plan (CGSSC 2008). CPW defines a "lek" (i.e., a lek site or lek arena) as any open area on the ground (represented in the statewide database as a central point location) where ≥ 1 males have been observed strutting on ≥ 2 occasions (i.e., visits) within, or across

years, during the March-May breeding season. Lek arenas typically must be >150 m apart and separated by otherwise unsuitable topography or vegetation to be considered separate leks in CPW's database. The agency defines the current status of a lek as "Active" if it has had ≥ 1 males observed on ≥ 2 visits in the previous 5 years. This definition is intended to restrict leks to just those locations where males regularly strut by excluding temporary or aberrant strutting locations. All newly discovered strutting locations are assigned a status of "Unknown" (referred to in Chapter 2 as "potentially active") until strutting males have been observed on ≥ 2 occasions (i.e., visits). As a result, locations with "Unknown" status in the CPW database sometimes require more than one year of visits to document males on ≥ 2 occasions and confirm the location as an "Active" lek. Our dual-frame study design required the use of lek definitions that were specific to a single year to enable determination of sample units and summarize annual survey results and estimates, so we used slightly modified version of CPW's terminology. For this study, we defined a "known lek site*"* as any location with a CPW lek status of "Active" or "Unknown" and an "active lek*"* as any known lek with ≥ 1 males observed in the survey year of interest. Our inclusion of "Unknown" leks (i.e., "potentially active") leks was necessary for designating sample units in the year following their discovery; their inclusion, or exclusion, in our final analysis was based on subsequent confirmation of these sites as leks per CPW's definitions and will be discussed further in our methods section.

Supplement C

We typically flew 15-70m above the ground to facilitate visibility. Whenever we detected a lek, we typically hovered ≥ 100m away (horizontally) and counted the number of males and females present. The number of observers remained the same for each flight and consisted of the

pilot and one primary observer as the passenger. Observers used binoculars as needed to help distinguish non-strutting and yearling males from females and minimize flushing of birds. Any birds of unknown sex that flushed from the lek were followed until we determined number and sex. After counting, we increased altitude to ≥100m to avoid disturbing birds as much as possible, flew over the center of the lek, and recorded its location with a global positioning system (GPS) unit.

Supplement D

We conducted two separate occupancy analyses to address uncertainty over whether to treat "Unknown" (i.e., "potentially active") status locations as known leks. First, we analyzed data excluding counts from locations categorized as "Unknown" status during and after the study (based on CPW data thorough 2016) so our analysis was consistent with the lek definition for small populations in the Colorado State Plan (CGSSC 2008). Subsequently, we conducted a supplementary analysis that included counts from "Unknown" status locations to see if their inclusion significantly changed our results (Appendix A).

Supplement E

Prior to this study, CPW had no estimates for this proportion specific to the PPR, though they assumed the percentage of active leks that were not known to be approximately 10% for other larger populations in northwestern Colorado (CGSSC 2008). During surveys, we incidentally discovered males strutting at two locations later determined to be "Historic" lek sites where males had not been observed for at least 10 years. These locations were not included in

CPW's list of active and unknown status leks that comprised our list frame strata in 2012 and 2013 and were therefore not included in the proportion of known leks prior to their discovery. The proportion of leks that are unknown and therefore missing from lek-count data may be further reduced if CPW also monitors all leks of "Historic" or "Inactive" status (defined as a previously known lek site with < 2 detections in the previous 5 years) on a regular basis as part of the list frame.

Leks in the PPR generally have few males and may be more dynamic than in many other populations, and detection probabilities are known to be imperfect, which poses difficulties for determining the status of leks over time. Our supplementary analysis (Appendix A) employed a different definition of the term "lek" than is currently used by CPW for small populations. However, occupancy and detectability estimates in the supplementary analysis were similar to those in our original analysis and did not meaningful change our conclusions.

APPENDICES

Appendix A: Supplemental dual-frame analysis, including data from all active and potentially active lek locations.

We conducted dual frame flights to survey list frame points and area frame cells during the lekking season in the PPR from mid-April to early May in three consecutive years from 2012-2014. We surveyed 50-72 LF points, 39-57 AF_1 cells, and 36-45 AF_2 cells during each study year (Table 1A).

Table 1A. Supplemental analysis: Summary of dual-frame survey effort for greater sage-grouse leks in the Parachute-Piceance-Roan, 2012-2014.

	2012	2013	2014
List Frame Points Surveyed	50	66	72
Area Frame 1 Cells Surveyed	39	54	57
Area Frame 2 Cells Surveyed	45	36	36
Area Frame 2 Cells Not Surveyed	587	598	595
Total Available Area Frame 2 Cells	632	634	631

The total number of leks we detected differed between our sampling frames, with the most leks detected in the LF and the fewest in AF_2. Total leks detected per frame were relatively stable across years with 17-23 detected in the LF, 3-4 in AF_1 and 0-1 in AF_2 (Table 3A). Between-year turnover in observed use of known lek sites with data for consecutive years was 26.9% (14/52) from 2012-2013 and 25.0% (16/64) from 2013-2014.

We discovered a total of 25 new leks during the three survey seasons of, including observations in the area frame and incidental leks (Table 3A). We observed a total of 3-5 AF_1 cells with ≥ 1 leks in each year and one AF_2 cell with ≥ 1 leks in 2012 and 2014; we observed no active leks in AF_2 in 2013 (Table 2A). In addition to leks newly discovered during surveys, additional leks were discovered each year during the course of field work for other CPW research projects. The number of additional leks discovered was 8 in 2012, 1 in 2013, and 4 in 2014. The total number of new leks discovered each year as a result of all research efforts in the PPR, including dual frame sampling, were 15 in 2012, 10 in 2013, and 13 in 2014; a total of 38 over the three-year period.

Table 2A. Supplemental analysis: Total sampling units with ≥ 1 active leks detected in each sampling frame by year.

	2012	2013	2014
List Frame	22	17	23
Area Frame 1	3	4*	4
Area Frame 2	1	0	1

*One AF1 cell in 2013 had 2 new leks detected

Table 2A. Supplemental analysis: New greater sage-grouse leks detected in the area frame by year during dual frame sampling in the Parachute-Piceance-Roan, 2012-2014.

	2012	2013	2014
Area Frame 1 (Dual Frame)	3	5	4
Area Frame 2 (Dual Frame)	1	0	1
Incidental Leks* (Dual Frame)	3	4	4
Total New Leks Detected (Dual Frame)	7	9	9
Total New Leks Detected (Other CPW Surveys)	8	1	4
Total New Leks Detected (Dual Frame + other Surveys)	15	10	13

* Leks detected in the area frame during dual frame surveys but outside the sampled units (i.e. while travelling between sample units)

Estimates of the total number of males for all combined leks across all sample frames, based on the maximum number of males detected at a given lek across for all three visits, were similar in 2012 and 2013 but increased by more than a factor of two in 2014 (Table 4A). The mean maximum number of males per active lek (all lek sites with ≥ 1 male observed in a given year) and males per known lek site (all lek sites with ≥ 1 males in the past 5 years) varied between year and sampling frame (Tables 5A and 6A). The maximum count of males per new lek for combined years ranged from 1-9 males with a mean of 3.24 (N=25, SE 2.44). The mean number of leks detected per occupied area frame cell, for combined years, was 1.09 for AF1 and 1.0 for AF2.

Table 4A. Supplemental analysis: Estimates of the total number of greater sage-grouse males for all combined leks by year, based on the high count males detected per lek across all three visits, in the Parachute-Piceance-Roan, 2012-2014.

	2012	**2013**	**2014**
List Frame	69	63	148
Area Frame 1	22	13	14
Area Frame 2*	9	6	17
Total Max Count Males	100	82	179

*Includes incidental leks found in the frame to improve estimates.

Table 5A. Supplemental analysis: Mean high count of greater sage-grouse males per lek by year in the Parachute-Piceance-Roan, 2012-2014.

	2012	2013	2014
List Frame	3.14	3.71	6.43
Area Frame 1	7.33	2.60	3.50
Area Frame 2*	2.25	1.50	3.40
Total All Frames	3.33	3.15	5.59

*Includes incidental leks found in the frame to improve estimates.

Table 6A. Supplemental analysis: Mean high count of greater sage-grouse males per known lek site by year in the Parachute-Piceance-Roan, 2012-2014.

	2012	2013	2014
List Frame	1.39	0.95	2.03
Area Frame 1	7.33	2.60	3.50
Area Frame 2*	2.25	1.50	3.40
Total All Frames	1.75	1.09	2.18

*Includes incidental leks found in the frame to improve estimates.

Models were used to estimate the proportion of known leks in the LF that were active in a given year (ψ_{LF}) and the probability of detection for leks in all sampling frames (p). Our top estimation model varied by year, with group (g), group/time additive (g+t), and constant (.) detection probability (p) models receiving the most support given our data (Table 7A)

Table 7A. Supplemental analysis: Estimation model ranking by year for dual frame data, determined using Akaike Information Criteria (AIC_c) rankings and weights, based on finite sample size in Program MARK.

Year and Model	AICc	Delta AIC_c	AICc Weights	Model Likelihood	Num. Param.	Deviance
2012						
p(.) Psi(g)	207.11	0	0.580	1	4	10.96
p(g) Psi(g), $AF_1(p)=AF_2(p)$	208.79	1.68	0.251	0.432	5	10.48
p(t) Psi(g)	210.36	3.25	0.114	0.197	6	9.86
p(g+t) Psi(g), $AF_1(p)=AF_2(p)$	212.11	4.99	0.048	0.083	7	9.38
p(g*t) Psi(g), $AF_1(p)=AF_2(p)$	215.98	8.87	0.007	0.012	9	8.69
2013						
p(g+t) Psi(g), $AF_1(p)=AF_2(p)$	190.53	0	0.347	1	7	8.35
p(g) Psi(g), AF1(p)=AF2(p)	191.15	0.62	0.255	0.735	5	13.32
p(g*t) Psi(g), $AF_1(p)=AF_2(p)$	192.11	1.57	0.158	0.455	9	5.45
p(t) Psi(g)	192.39	1.86	0.137	0.395	6	12.40
p(.) Psi(g)	192.97	2.43	0.103	0.2965	4	17.28
2014						
p(g) Psi(g), AF1(p)=AF2(p)	241.71	0	0.573	1	5	15.34

p(.) Psi(g)	243.01	1.30	0.300	0.523	4	18.76
p(g+t) Psi(g), $AF_1(p)=AF_2(p)$	245.76	4.05	0.076	0.132	7	15.05
p(t) Psi(g)	247.01	5.30	0.041	0.071	6	18.48
p(g*t) Psi(g), $AF_1(p)=AF_2(p)$	249.59	7.87	0.011	0.020	9	14.42

Our model-averaged estimates of the proportion of known lek sites that were active, or potentially active, in the list frame (ψ_{LF}) varied by year from 0.29 – 0.576 during the three-year study period (Table 8A).

Table 8A. Supplemental analysis: Modeled-averaged estimates of the proportion of known greater sage-grouse lek sites that were active in the List Frame (ψ_{LF}) by year, with standard error (SE) and 95% upper (UCI) and lower (LCI) confidence intervals, in the Parachute-Piceance-Roan, 2012-2014.

Proportion of active leks (ψ_{LF}) by year	Estimate	SE	95% LCI	95% UCI
2012	0.56	0.11	0.35	0.75
2013	0.27	0.06	0.17	0.40
2014	0.34	0.06	0.23	0.46

Model-averaged detection probability estimates for leks in the list frame were lowest in 2012 and similar to detection probabilities in the area frame that year; detection probabilities in the area frame were relatively constant across all survey years (Table 9A). Average detection probabilities per year for the sampling frames were 0.40, 0.62, and 0.64 in the LF for 2012, 2013 and 2014, respectively; with 0.44, 0.32, and 0.42 for AF_1 and AF_2. Estimates of p^* (the probability of detecting a lek at least once during a season) were lowest in the first year (2012) in

the list frame at 0.78, then increased to 0.95 in 2013 and 2014; estimates for p^* were 0.69 – 0.82 in the area frame (Figure 1A).

Table 9A. Supplemental analysis: Model-averaged probability of detection (*p*) estimates, by year, with SE and 95% upper (UCI) and lower (LCI) confidence intervals, for greater sage-grouse in the Parachute-Piceance-Roan, 2012-2014.

Detection Probability Parameter (p)	Estimate	SE	95% LCI	95% UCI
2012				
List Frame, Occasion 1	0.41	0.08	0.26	0.58
List Frame, Occasion 2	0.40	0.08	0.26	0.57
List Frame, Occasion 3	0.39	0.08	0.24	0.56
Area Frame, Occasion 1	0.45	0.13	0.22	0.70
Area Frame, Occasion 2	0.44	0.13	0.22	0.70
Area Frame, Occasion 3	0.43	0.13	0.21	0.68
2013				
List Frame, Occasion 1	0.59	0.11	0.38	0.78
List Frame, Occasion 2	0.73	0.13	0.43	0.91
List Frame, Occasion 3	0.55	0.12	0.32	0.76
Area Frame, Occasion 1	0.31	0.23	0.05	0.79
Area Frame, Occasion 2	0.43	0.29	0.07	0.89
Area Frame, Occasion 3	0.22	0.21	0.02	0.76
2014				
List Frame, Occasion 1	0.64	0.07	0.49	0.76
List Frame, Occasion 2	0.63	0.07	0.48	0.76
List Frame, Occasion 3	0.64	0.07	0.49	0.77
Area Frame, Occasion 1	0.42	0.20	0.13	0.78
Area Frame, Occasion 2	0.41	0.20	0.13	0.77
Area Frame, Occasion 3	0.42	0.20	0.13	0.78

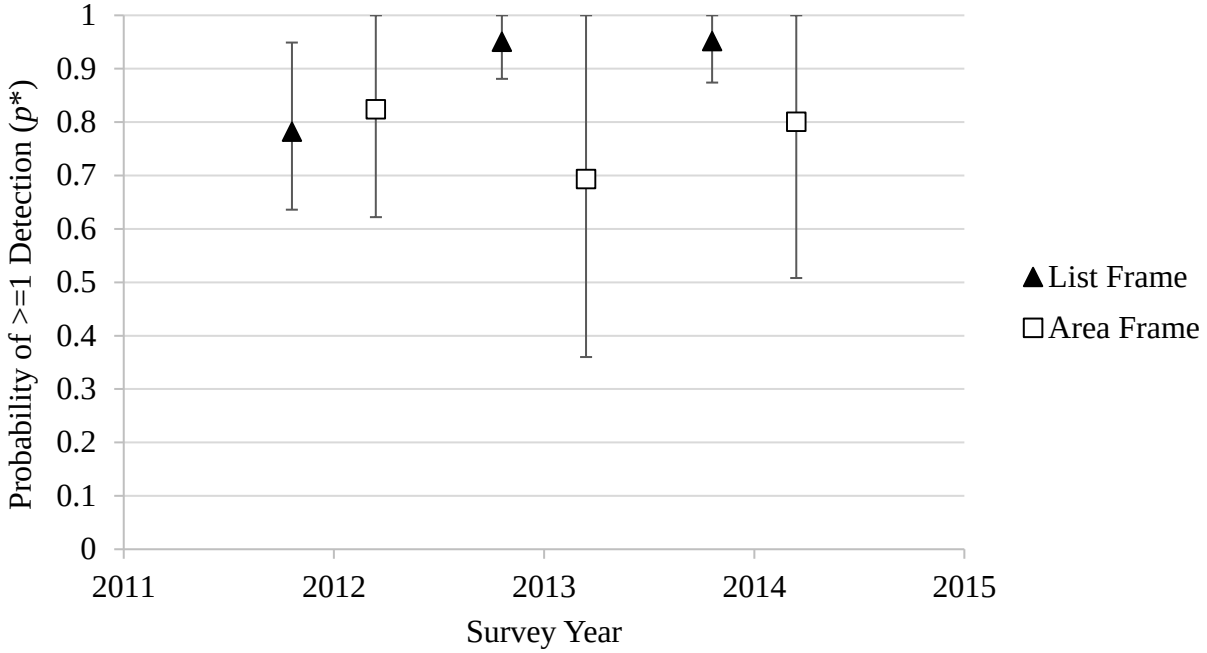

Figure 1A. Supplemental analysis: Estimated probability of detecting ≥ 1 greater sage-grouse males at a lek (List Frame) or ≥ males at ≥1 leks (Area Frame) across three sampling occasions (*p**), with 95% confidence intervals, from dual-frame lek surveys in the Parachute-Piceance-Roan, 2012-2014.

The estimate of the proportion of leks previously known and surveyed each year by standard lek flights was 0.45 in 2012, 0.68 in 2013 and 0.45 in 2014, and the proportion of males known and counted to be 0.45, 0.74, and 0.60, respectively for the three years (Table 11A). These results suggest that, during our study, lek counts based exclusively on monitoring known lek sites (i.e., leks in the list frame) would have failed to survey 32-55% of the total number of leks and 26-55% of males attending leks in the PPR.

Table 10A. Supplemental analysis: Total detected and estimated active leks, proportion of active leks known and counted, and total estimated number of males attending leks from dual-frame lek surveys in the Parachute-Piceance-Roan population of greater sage-grouse, 2012-2014.

Survey Year	2012			2013			2014		
Total Leks Detected (List Frame)	22			17			23		
Total Males Detected (List Frame)	69			63			148		
	Est. /No.	95% LCI	95 % UCI	Est. /No.	95% LCI	95% UCI	Est. /No.	95% LCI	95% UCI
Estimated No. Active Leks (List Frame)	28.1	22	36.9	17.9	17	25.2	24.2	23	32.3
Estimated No. Active Leks (Area Frame 1)	3.6	3	5.7	7.2	5	14.4	5.00	4	8.3
Estimated No. Active Leks (Area Frame 2)	17.0	1	49.8	0.0	0.0	0.0	21.9	1	65.1
No. Incidental Leks Observed		3			4			4	
Total No. Estimated Active Leks	48.8	29*	82.8	25.1	26*	35.3	51.1	32*	95.2
Estimated Total No. Males	153	100*	349	85	82*	209	247	179*	642
Est. Proportion Active Leks Known & Counted	0.45	0.75*	0.27	0.68	0.65*	0.48	0.45	0.72*	0.24
Est. Proportion Males Known & Counted	0.45	0.69*	0.20	0.74	0.77*	0.30	0.60	0.83*	0.23

* LCI truncated to the number of occupied leks observed by during dual frame sampling, including incidental lek locations.

Appendix B: Additional dual frame results summary tables.

Table B1. Summary of dual-frame survey effort for greater sage-grouse leks in the Parachute-Piceance-Roan, 2012-2014.

	2012	2013	2014
List Frame Points Surveyed	49	61	66
Area Frame 1 Cells Surveyed	39	54	57
Area Frame 2 Cells Surveyed	45	36	36
Area Frame 2 Cells Not Surveyed	587	598	595
Total Available Area Frame 2 Cells	632	634	631

Table B2. Model-averaged probability of detection (*p*) estimates, by year, with SEs and 95% upper (UCI) and lower (LCI) confidence intervals for greater sage-grouse in the Parachute-Piceance-Roan, 2012-2014.

Detection Probability Parameter (p)	Estimate	SE	95% LCI	95% UCI
2012				
List Frame, Occasion 1	0.41	0.08	0.26	0.58
List Frame, Occasion 2	0.40	0.08	0.26	0.57
List Frame, Occasion 3	0.39	0.08	0.24	0.56
Area Frame, Occasion 1	0.45	0.13	0.22	0.70
Area Frame, Occasion 2	0.44	0.13	0.22	0.70
Area Frame, Occasion 3	0.43	0.13	0.21	0.68
2013				
List Frame, Occasion 1	0.60	0.10	0.40	0.77
List Frame, Occasion 2	0.69	0.12	0.42	0.87
List Frame, Occasion 3	0.56	0.11	0.34	0.76
Area Frame, Occasion 1	0.42	0.25	0.09	0.84
Area Frame, Occasion 2	0.50	0.27	0.11	0.89
Area Frame, Occasion 3	0.34	0.24	0.06	0.81
2014				
List Frame, Occasion 1	0.64	0.07	0.49	0.76
List Frame, Occasion 2	0.63	0.07	0.48	0.76
List Frame, Occasion 3	0.64	0.07	0.49	0.77
Area Frame, Occasion 1	0.42	0.20	0.13	0.78
Area Frame, Occasion 2	0.41	0.20	0.13	0.77
Area Frame, Occasion 3	0.42	0.20	0.13	0.78

Table B3. Model-averaged probability of detecting ≥ 1 males at a lek (List Frame) or ≥ 1 males at ≥ 1 leks (Area Frame) across three sampling occasions within a single season (p^*), with SEs and 95% upper (UCI) and lower (LCI) confidence intervals for greater sage-grouse in the Parachute-Piceance-Roan, 2012-2014.

Probability of ≥1 Detection (p*)	Estimate	SE	95% LCI	95% UCI
2012				
List Frame	0.78	0.08	0.64	0.95
Area Frame	0.82	0.12	0.62	1.00
2013				
List Frame	0.95	0.03	0.88	1.00
Area Frame	0.81	0.23	0.51	1.00
2014				
List Frame	0.95	0.04	0.87	1.00
Area Frame	0.80	0.20	0.51	1.00

Appendix C: Sampling Effort vs Precision

Table C1: Table of Estimated Standard Error (SE) given variations in the number of Area Frame cells sampled per stratum and number of sampling occasions, for the Parachute-Piceance-Roan greater sage-grouse population.

Plots Sampled				Plots Available			Standard Error (SE)			
LF	AF1	AF2	Total Plots Sampled	LF	AF_1	AF_2	LF	AF_1	AF_2	SE ($\hat{T}$)
Sampling Occasions = 2										
75	60	100	160	75	60	630	4.80	2.84	14.00	15.07
75	40	120	160	75	60	630	4.80	3.70	12.84	14.20
75	20	140	160	75	60	630	4.80	5.54	11.94	14.01
75	60	60	120	75	60	630	4.80	2.84	17.90	18.75
75	40	80	120	75	60	630	4.80	3.70	15.58	16.71
75	20	100	120	75	60	630	4.80	5.54	14.00	15.80
75	60	40	100	75	60	630	4.80	2.84	21.82	22.52
75	40	60	100	75	60	630	4.80	3.70	17.90	18.90
75	20	80	100	75	60	630	4.80	5.54	15.58	17.21
Sampling Occasions = 3										
75	60	100	160	75	60	630	4.45	1.85	11.08	12.08
75	40	120	160	75	60	630	4.45	2.69	10.09	11.35

75	20	140	160	75	60	630	4.45	4.32	9.32	11.19
75	60	60	120	75	60	630	4.45	1.85	14.37	15.16
75	40	80	120	75	60	630	4.45	2.69	12.42	13.46
75	20	100	120	75	60	630	4.45	4.32	11.08	12.70
75	60	40	100	75	60	630	4.45	1.85	17.65	18.29*
75	40	60	100	75	60	630	4.45	2.69	14.37	15.28
75	20	80	100	75	60	630	4.45	4.32	12.42	13.88
Sampling Occasions = 4										
75	60	100	160	75	60	630	4.33	1.42	9.85	10.85
75	40	120	160	75	60	630	4.33	2.26	8.94	10.19
75	20	140	160	75	60	630	4.33	3.8	8.23	10.04
75	60	60	120	75	60	630	4.33	1.42	12.87	13.65
75	40	80	120	75	60	630	4.33	2.26	11.08	12.11
75	20	100	120	75	60	630	4.33	3.8	9.85	11.41
75	60	40	100	75	60	630	4.33	1.42	15.85	16.49
75	40	60	100	75	60	630	4.33	2.26	12.87	13.76
75	20	80	100	75	60	630	4.33	3.8	11.08	12.49

Input values for simulations were based on average occupancy (ψ_{LF}=0.41, ψ_{AF1}=0.08, ψ_{AF2}=0.02), detection probability (p_{LF}=0.63, p_{AF}=0.43), and variance estimates (var($\hat{p}^{*}_{AF}$)=0.032, s^2_{AF}=0.02) obtained from this study.

*****Equivalent to sampling effort used in this study.

Appendix D: Preliminary Power Analysis

The power of a statistical test is the probability that the test will reject the null hypothesis (e.g. a wildlife population is stable or growing) when the null hypothesis is false. I conducted an analysis to investigate the statistical power to detect a 5%, 7.5% and 10% change in occupancy (the proportion of sample units containing one or more leks) based on lek activity observed during dual-frame surveys. Simulations were conducted in Program Mark (White and Burnham 1999) using input parameter values expected to represent the true population occupancy and anticipated sampling effort for the list and area frames. Capture histories were simulated based on input data (expected occupancy rate, detection probability and number of sample units surveyed) and analyzed in Program Mark to obtain standard errors (Runge et al. 2007). Power

calculations were generated using Program R (Table D1). Results indicate that with expected sampling effort, power to detect a minimum of 7.5% annual rate of decline in occupancy will be approximately 0.95 with 15 years of surveillance.

Runge, J.P., Hines, J.E., and J.D. Nichols. 2007. Estimating species-specific survival and movement when species identification is uncertain. Ecology 88:282-288.

Table D1: Dual-frame surveys, power to detect percent change in population over time, Parachute-Piceance-Roan greater sage-grouse population.

Assumed Occupancy					
	Plots	Occupied	Psi List	Psi Area	p
Total	2000	100	0.90	0.05	0.90

Number of plots		List	Area	Total		Power to detect a 5% annual decline				Power to detect a 7.5% annual decline				Power to detect a 10% annual decline			
List Frame	Area Frame	E[SE(ψ)]	E[SE(ψ)]	E[SE(ψ)]	E[CV]	5 years	10 years	15 years	20 years	5 years	10 years	15 years	20 years	5 years	10 years	15 years	20 years
30	100	0.07	0.02	0.02	0.45	0.32	0.72	0.85	0.91	0.41	0.83	0.94	0.97	0.51	0.92	0.98	0.99
40	100	0.06	0.02	0.02	0.44	0.32	0.72	0.86	0.91	0.41	0.85	0.94	0.98	0.52	0.92	0.98	0.99
50	100	0.05	0.02	0.02	0.40	0.33	0.71	0.87	0.92	0.41	0.87	0.95	0.98	0.51	0.93	0.98	1.00
60	100	0.04	0.02	0.02	0.40	0.34	0.74	0.87	0.92	0.44	0.84	0.95	0.98	0.53	0.94	0.99	1.00

Appendix E: Colorado Parks and Wildlife Animal Care and Use Committee animal use proposal approval letter #07-2011

COLORADO PARKS AND WILDLIFE
1313 Sherman Street, Room 618 • Denver, Colorado 80203
Phone (303) 866-3437 • FAX (303) 866-3206
wildlife.state.co.us • parks.state.co.us

Colorado Division of Parks and Wildlife
Animal Care and Use Committee

Date: September 30, 2011

To: Brett Walker

Re: Project File Number # **07-2011**. Evaluating Lek-Based Monitoring and Management Strategies for Greater Sage-Grouse in the Parachute-Roan Population in Northwestern Colorado.

Your animal care and use protocol for the project shown above was reviewed by the Colorado Division of Wildlife and has received final approval.

This project was originally submitted for review on: September 14, 2011
The original approval date for this project is: September 30, 2011
The project may be continued by annual updates submitted each September using the CDOW ACUC Renewal Form until: September 30, 2021

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first ten years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the tenth anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Bob Davies, CDPOW ACUC Chair
Colorado Division of Parks and Wildlife
317 W. Prospect Rd.
Fort Collins, CO 80526
(970) 472-4416

STATE OF COLORADO
John W. Hickenlooper, Governor • Mike King, Executive Director, Department of Natural Resources
Rick D. Cables, Director, Colorado Parks and Wildlife
Parks and Wildlife Board: David R. Brougham • Gary Butterworth, Vice-Chair • Chris Castilian
Dorothea Farris • Tim Glenn, Chair • Allan Jones • Bill Kane • Gaspar Pericone • Jim Pribyl • John Singletary
Mark Smith, Secretary • Robert Streeter • Lenna Watson • Dean Wingfield
Ex Officio Members: Mike King and John Salazar

Appendix F: Colorado Parks and Wildlife Animal Care and Use Committee animal use proposal approval letter #08-2012

COLORADO PARKS & WILDLIFE

317 W. Prospect Rd. • Fort Collins, Colorado 80526
Phone (970) 472-4300 • FAX (970) 472-4457
wildlife.state.co.us • parks.state.co.us

Colorado Parks and Wildlife
Animal Care and Use Committee

Date: June 26, 2012

To: Brett Walker

Re: Project File Number # **08-2012**. Evaluation of Alternative Population Monitoring Strategies for Greater Sage-Grouse in Northwestern Colorado.

Your animal care and use protocol for the project shown above was reviewed by the Colorado Division of Wildlife and has received final approval.

This project was originally submitted for review on: June 18, 2012
The original approval date for this project is: June 26, 2012
The project may be continued by annual updates submitted each September using the CPW ACUC Renewal Form until: June 26, 2021

Federal laws and guidelines require that institutional animal care and use committee's review ongoing projects annually. For the first ten years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the tenth anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Mat Alldredge, CPW ACUC Acting Chair
Colorado Parks and Wildlife
317 W. Prospect Rd.
Fort Collins, CO 80526
(970) 225-6189

STATE OF COLORADO
John W. Hickenlooper, Governor • Mike King, Executive Director, Department of Natural Resources
Rick D. Cables, Director, Colorado Parks and Wildlife
Parks and Wildlife Commission: David R. Brougham • Gary Butterworth, Vice-Chair • Chris Castilian
Dorothea Farris • Tim Glenn, Chair • Allan Jones • Bill Kane • Gaspar Perricone • Jim Pribyl • John Singletary
Mark Smith, Secretary • Robert Streeter • Lenna Watson • Dean Wingfield
Ex Officio Members: Mike King and John Salazar

Appendix G: Colorado State University Institutional Animal Care and Use Committee inter-agency proposal approval letter #07-2011

Institutional Animal Care and Use Committee
Inter-institutional Agreement

This form should be completed in cases where animal work is to take place at an institution other than Colorado State University (CSU) where CSU is funding the animal use activity (either directly or through the sub-contracting of a sponsored project), or where CSU representatives are directly participating in the work involving live vertebrate animals. Federal animal use regulations allow institutions to develop methods to ensure the appropriate regulatory compliance without requiring duplicative review of animal care and use protocols when two or more institutions are collaborating in research, testing or teaching involving the use of live vertebrate animals. This Inter-institutional Agreement provides assurance that the collaborative animal use has received Institutional Animal Care and Use Committee (IACUC) review.

Name of Collaborating Institution Providing IACUC Review (Collaborating Institution): Colorado Division of Parks and Wildlife

Collaborating Institution USDA Registration #: 84-R-0045

Collaborating Institution Animal Welfare Assurance (AWA) #: na

AAALAC Accreditation Status: na

The Officials signing below agree that ***Colorado State University*** may rely on the designated IACUC of Colorado Division of Parks and Wildlife for the review and continuing oversight of its research involving animals described below: (*check all that apply*)

This agreement covers the following specific protocol(s):

Name of Research Project (grant/contract title): Evaluation of Population Monitoring Strategies for Greater Sage-Grouse in Western Colorado.
Name of Principal Investigator: Dr. Brett L. Walker
Sponsor or Funding Agency, if any: Colorado Division of Parks and Wildlife via subcontract by CSU of Exxon grant funds.
Sponsor's Award Number, if any: na
IACUC Protocol Title: Evaluating Lek-Based Monitoring and Management Strategies for Greater Sage-Grouse in the Parachute-Piceance-Roan Population in Northwestern Colorado.
IACUC Protocol Approval #: 07-2011
IACUC Protocol Approval Date: 09-30-2011

☐ The animals involved in the activity will be owned by Colorado State University, but housed at the Collaborating Institution.

☐ The animals involved in the activity will be owned by the Collaborating Institution.

X Other (describe): Animals involved in the activity will be wild caught and released at the location of capture immediately following processing.

The following documents are attached:

Approved: February 22, 2011

X *Official documentation of approval by the IACUC*

The CSU IACUC requests that the collaborating institution provide, as applicable:

- Documentation of IACUC approval for modifications to the approved protocol as well as triennial reviews of the protocols.
- Notification of review and reporting of any incidents of non-compliance with PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, or any suspension of this activity by the IACUC
- Additionally, CSU requests that the collaborating institution provide notification of change in PHS Assurance status or AAALAC, International Accreditation status.

Colorado State University remains responsible for ensuring compliance with the IACUC's determinations and with the Terms of its OLAW-approved Animal Welfare Assurance. This document must be kept on file by both parties and provided to OLAW upon request. Completion of this document provides assurance that the review performed by the Collaborating Institution's IACUC meets animal welfare requirements prescribed in the institution's OLAW-approved Animal Welfare Assurance.

Signature of Signatory Official for Collaborating Institution:

Date: 2-8-2012

~~Michael W. Miller, D.V.M., Ph.D.~~ Jeffrey M. VerSteeg, Assistant Director
(Type or Print Institutional Signatory Name)

Senior Wildlife Veterinarian
(Institutional Signatory Title)

Signature of Signatory Official for *Colorado State University*:

Date: 7-17-2012

William H. Farland
Vice President for Research and Institutional Official
Colorado State University

This information should be submitted to:
Institutional Animal Care and Use Committee
Research Integrity and Compliance Review Office
321 General Services Building
2011 Campus Delivery
Colorado State University
Fort Collins, CO 80523-2011
Fax: 970-491-2293 (Attn: IACUC Coordinator) or bill.moseley@colostate.edu

Colorado State University Animal Welfare Assurance #: A3572-01
Colorado State University USDA Registration #: 84-R-0003

Appendix H: Colorado State University Institutional Animal Care and Use Committee inter-agency proposal approval letter #08-2012

Institutional Animal Care and Use Committee
Inter-institutional Agreement

This form should be completed in cases where animal work is to take place at an institution other than Colorado State University (CSU) where CSU is funding the animal use activity (either directly or through the sub-contracting of a sponsored project), or where CSU representatives are directly participating in the work involving live vertebrate animals. Federal animal use regulations allow institutions to develop methods to ensure the appropriate regulatory compliance without requiring duplicative review of animal care and use protocols when two or more institutions are collaborating in research, testing or teaching involving the use of live vertebrate animals. This Inter-institutional Agreement provides assurance that the collaborative animal use has received Institutional Animal Care and Use Committee (IACUC) review.

Name of Collaborating Institution Providing IACUC Review (Collaborating Institution): Colorado Division of Parks and Wildlife

Collaborating Institution USDA Registration #: 84-R-0045

Collaborating Institution Animal Welfare Assurance (AWA) #: na

AAALAC Accreditation Status: na

The Officials signing below agree that ***Colorado State University*** may rely on the designated IACUC of Colorado Parks and Wildlife (Collaborating Institution name) for the review and continuing oversight of its research involving animals described below: (*check all that apply*)

This agreement covers the following specific protocol(s):

Name of Research Project (grant/contract title): Evaluation of Population Monitoring Strategies for Greater Sage-Grouse (*Centrocercus urophasianus*) in Western Colorado.
Name of Principal Investigator: Dr. Brett L. Walker
Sponsor or Funding Agency, if any: Colorado Parks and Wildlife and Colorado State University by Exxon grant funds.
Sponsor's Award Number, if any: na
IACUC Protocol Title: Evaluation of Alternative Population Monitoring Strategies for Greater Sage-Grouse in Northwestern Colorado.
IACUC Protocol Approval #: 08-2012
IACUC Protocol Approval Date: 06-26-2012

☐ The animals involved in the activity will be owned by Colorado State University, but housed at the Collaborating Institution.

☐ The animals involved in the activity will be owned by the Collaborating Institution.

X Other (describe): Animals involved in the activity will be caught and released at the location of capture immediately following processing.

Approved: February 22, 2011

The following documents are attached:
X *Official documentation of approval by the IACUC*

The CSU IACUC requests that the collaborating institution provide, as applicable:

- Documentation of IACUC approval for modifications to the approved protocol as well as triennial reviews of the protocols.
- Notification of review and reporting of any incidents of non-compliance with PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, or any suspension of this activity by the IACUC
- Additionally, CSU requests that the collaborating institution provide notification of change in PHS Assurance status or AAALAC, International Accreditation status.

Colorado State University remains responsible for ensuring compliance with the IACUC's determinations and with the Terms of its OLAW-approved Animal Welfare Assurance. This document must be kept on file by both parties and provided to OLAW upon request. Completion of this document provides assurance that the review performed by the Collaborating Institution's IACUC meets animal welfare requirements prescribed in the institution's OLAW-approved Animal Welfare Assurance.

Signature of Signatory Official for Collaborating Institution:

Date: 6/26/2012

Mat Alldredge, Ph.D.
(Type or Print Institutional Signatory Name)

Mammals Researcher, CPW ACUC Chair
(Institutional Signatory Title)

Signature of Signatory Official for *Colorado State University*:

Date: 9-17-2012

William H. Farland
Vice President for Research and Institutional Official
Colorado State University

This information should be submitted to:
Institutional Animal Care and Use Committee
Research Integrity and Compliance Review Office
321 General Services Building
2011 Campus Delivery
Colorado State University
Fort Collins, CO 80523-2011
Fax: 970-491-2293 (Attn: IACUC Coordinator) or bill.moseley@colostate.edu

Colorado State University Animal Welfare Assurance #: A3572-01

Appendix I: Colorado Division of Wildlife (Colorado Parks and Wildlife) Animal Care and Use Committee addendum to ongoing research

COLORADO DIVISION OF WILDLIFE ANIMAL CARE AND USE COMMITTEE (CDOWACUC) ADDENDUM TO ONGOING, APPROVED WILDLIFE RESEARCH PROJECTS

Note: This form may not be used for new wildlife research projects.

1. CDOW ACUC File #**07-2011**

2. Principal Investigator(s): **Brett Walker** Phone: **970-255-6125**

3. Title of project: **Evaluating Lek-Based Monitoring and Management Strategies for GRSG in the Parachute-Roan Population in Northwestern Colorado**

4. Fiscal year of this project's initiation: **FY11-12**

5. List names of all new personnel associated with this project: **No new personnel**

6. Are sample sizes or numbers of animals to be used the same as originally described with no substantive changes (i.e., 10% of original proposal)? If sample sizes or animal numbers are substantially greater or less than described in original study plan, provide a justification for this change: **I propose to deploy up to 45 VHF transmitters (15/year) on females and juvenile males over three years to help field crews to locate and capture adult/yearling males in the field. Juvenile males will be recaptured as yearlings in spring and redeployed with GPS transmitters. VHF females can later be used to augment sample sizes for an upcoming PhD project at CSU (a proposal for the CSU project will be submitted for approval this winter).**

7. Is the animal component of the project the same as originally described with no substantive changes (e.g., anesthesia, analgesia, capture methods, euthanasia, species, surgical procedures, etc.)?

Yes _____ No **X**_____. *If No, describe changes:* **We have been unable to capture any yearling/adult males along roads using net launchers or spotlighting (as originally proposed) after two weeks of field work. This suggests that we need to attempt additional capture methods. One capture method we've used successfully in the past is the "Judas bird" method, in which we track and go in on birds with VHF transmitters, and use a CODA shoulder-mounted net gun during the day or hoop net at night to capture other birds in the same flock. I propose to deploy standard 21-g VHF necklace collars on females and on juvenile males in summer and fall, track and go in on those birds to help us find males, and use shoulder-mounted net guns (day) or hoop nets (night) to capture yearling/adult males that are with VHF birds. All juvenile males with VHF transmitters in fall will be recaptured as yearlings as soon as possible the following spring and outfitted with GPS transmitters (per my original proposal). Any females encountered with functioning or non-functioning VHF transmitters will be recaptured as soon as possible after the nesting season in the final year of the study (summer 2014) and transmitters will be removed. Hoop nets are standard for use with spotlighting and have already been approved as part of the original proposal. Few data are available on injury rates with CODA shoulder-mounted net guns. In previous field work, 2 of 9 females captured with net guns were injured during capture, but what part of the capture process (netting, securing, or handling) caused the injury wasn't clear. One injured bird died within a week of capture, the other died within a month of capture (unpub. data). Because such small sample sizes are not conclusive, we will keep track of injury rates for shoulder-mounted net gun captures and report those to ACUC for review. We will discontinue use of the shoulder-mounted net gun (and only conduct hoop-netting at night) if net guns cause > 2 major injuries (abrasions that cause excessive bleeding, lacerations, broken wings or legs, etc.) among the first 10 males captured.**

8. Will the foregoing changes result in greater levels of pain, suffering, stress, discomfort, deprivation, etc., experienced by experimental animals than those originally described and approved?

Yes ____ No_**X**__

If answered yes, attach detailed justification and indicate here the date of search, source of literature search, date range searched, and key words an combination of key words searched to document the lack of alternative methods:

9. Will additional pain and suffering be controlled? Yes _____ No _____ N/A _**X**___

If answered no, attach a detailed justification.

If answered yes, attach a detailed description of how pain and suffering will be controlled.

10. If required, was the attending veterinarian consulted when planning these changes?

Yes ____ No_**X**__

11. Does the proposed project now include planned euthanasia of animals? Yes ____ No_**X**__

Date: _____ Signed: _____

Principal Investigator

Date: _____ Signed: _____

ACUC Attending Veterinarian

Date: _____ Signed: _____

Chairperson (or designee), CDOW ACUC

Updated 9/13/2007, M. Michaels

Appendix J: Preliminary results from closed mark-recapture simulations in Program MARK using manipulated values for N (true population size), p (capture probability), and number of sampling occasions with no heterogeneity in p with 500 repetitions.

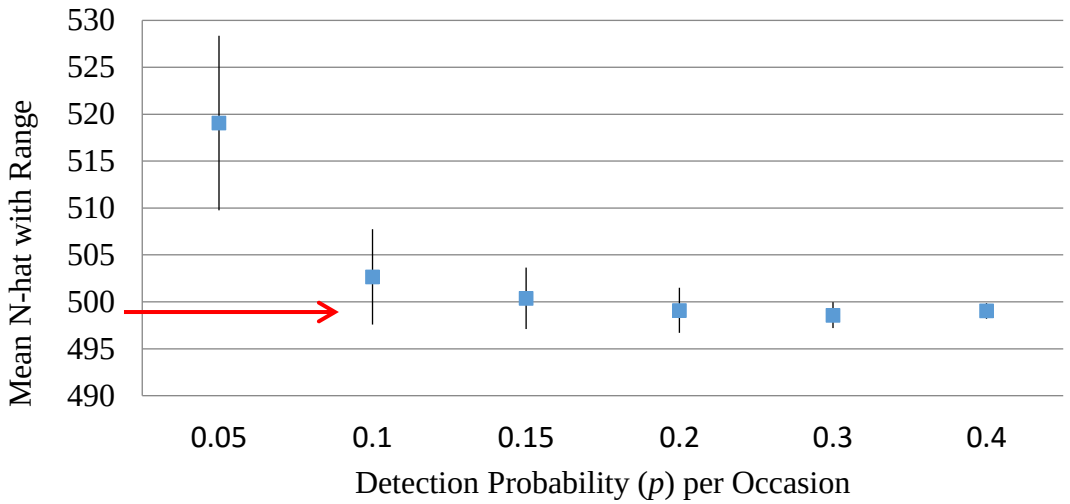

Figure J1: Simulation results for true population size (N)=500 (red arrow) with 5 sampling occasions and no heterogeneity.

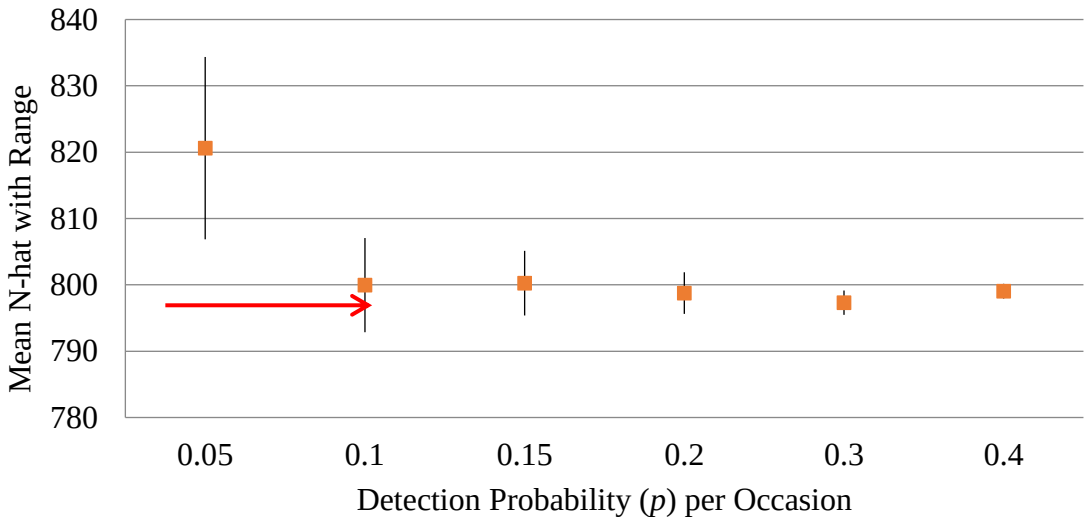

Figure J2: Simulation results for true population size (N)=800 (red arrow) with 5 sampling occasions and no heterogeneity.

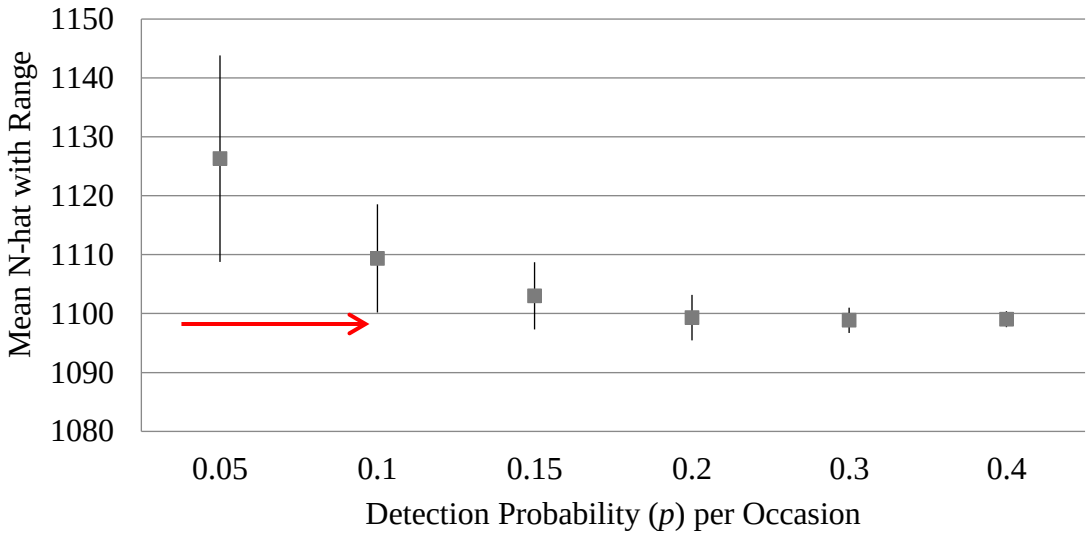

Figure J3: Simulation results for true population size (N)=1,000 (red arrow) with 5 sampling occasions and no heterogeneity.

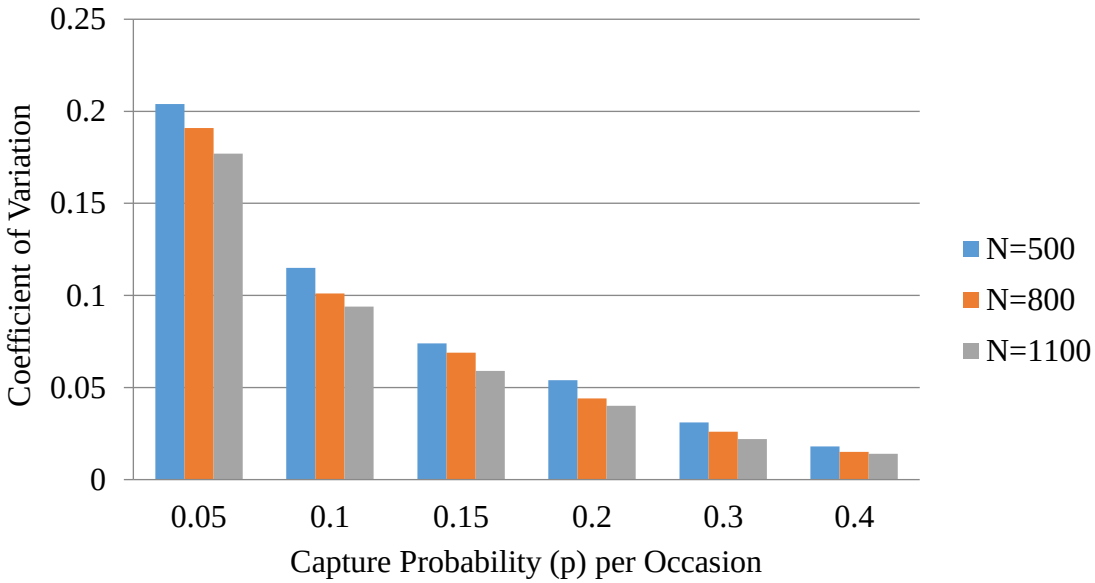

Figure J4: Simulation results for coefficient of variation (CV) with varied values for true population abundance (*N*) and capture probability (*p*), with 5 sampling occasions and no heterogeneity.

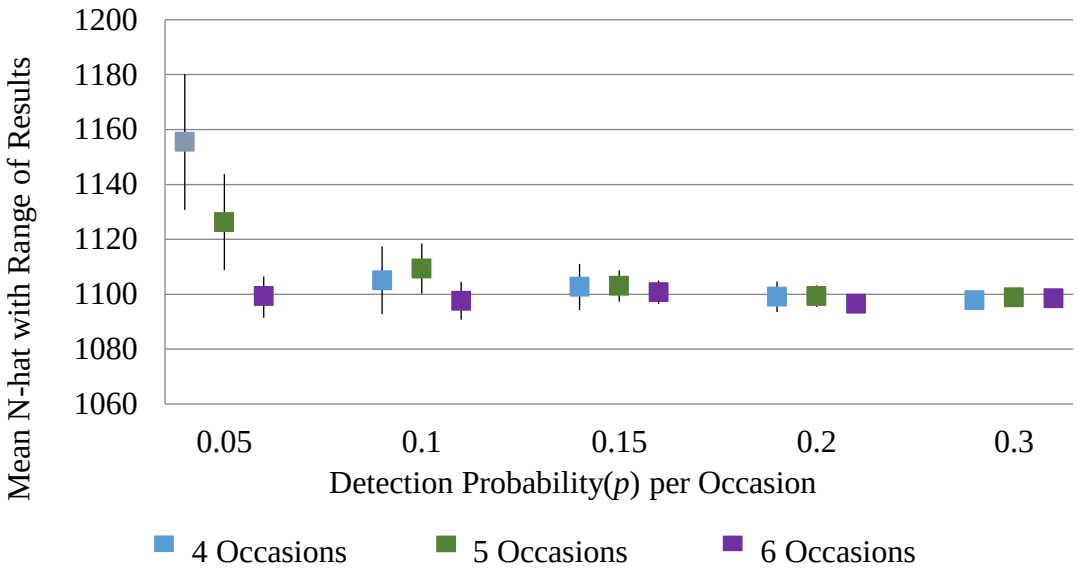

Figure J5: Graph of simulation results with varied number of sampling occasions and capture probability (p) for true population size N = 1,100; no heterogeneity.

Appendix K: Preliminary results from closed mark-recapture simulations in Program MARK accounting for heterogeneity in capture probabilities (p).

Table K1: Table of simulation results using mixture proportions (*pi*) to model heterogeneity in, and varying values of, capture probability (*p*), with true population size *N* = 500. Simulation results with coefficient of variation (CV) values greater than 0.20 are (orange).

		Mixture A					Mixture B							Prop Sims w/
Occ.	pi	p1	p2	p3	p4	p5	p1	p2	p3	p4	p5	Real Est.	CV	True in C.I.
5	0.5	0.14	0.8	0.8	0.8	0.8	0.14	0.5	0.5	0.5	0.5	503.587	0.019638	0.96
5	0.5	0.14	0.8	0.8	0.8	0.8	0.14	0.3	0.3	0.3	0.3	523.516	0.093703	0.99
5	0.5	0.14	0.8	0.8	0.8	0.8	0.14	0.2	0.2	0.2	0.2	575.656	0.45857	0.984
5	0.5	0.14	0.8	0.8	0.8	0.8	0.14	0.1	0.1	0.1	0.1	24488.06	9.960782	0.964
5	0.5	0.14	0.6	0.6	0.6	0.6	0.14	0.3	0.3	0.3	0.3	520.832	0.171583	0.92
5	0.5	0.14	0.6	0.6	0.6	0.6	0.14	0.2	0.2	0.2	0.2	608.133	2.19945	0.918
5	0.5	0.14	0.6	0.6	0.6	0.6	0.14	0.1	0.1	0.1	0.1	6396.776	6.862889	0.878
5	0.5	0.14	0.6	0.6	0.6	0.6	0.14	0.05	0.05	0.05	0.05	3156.625	7.900767	0.783
5	0.5	0.14	0.4	0.4	0.4	0.4	0.14	0.2	0.2	0.2	0.2	502.538	0.119967	0.81
5	0.5	0.14	0.4	0.4	0.4	0.4	0.14	0.1	0.1	0.1	0.1	1170.979	8.514337	0.724
5	0.5	0.14	0.4	0.4	0.4	0.4	0.14	0.05	0.05	0.05	0.05	1017.566	6.511615	0.624
5	0.5	0.14	0.3	0.3	0.3	0.3	0.14	0.1	0.1	0.1	0.1	531.584	0.886059	0.658
5	0.5	0.14	0.3	0.3	0.3	0.3	0.14	0.05	0.05	0.05	0.05	1654.069	8.428267	0.754
5	0.5	0.14	0.2	0.2	0.2	0.2	0.14	0.05	0.05	0.05	0.05	4777.701	16.77231	0.758

Appendix L: Post-analysis simulations based on collected data. Predicted variation in standard error of abundance estimates with 4, 6 and 8 complete sampling occasions with standards error.

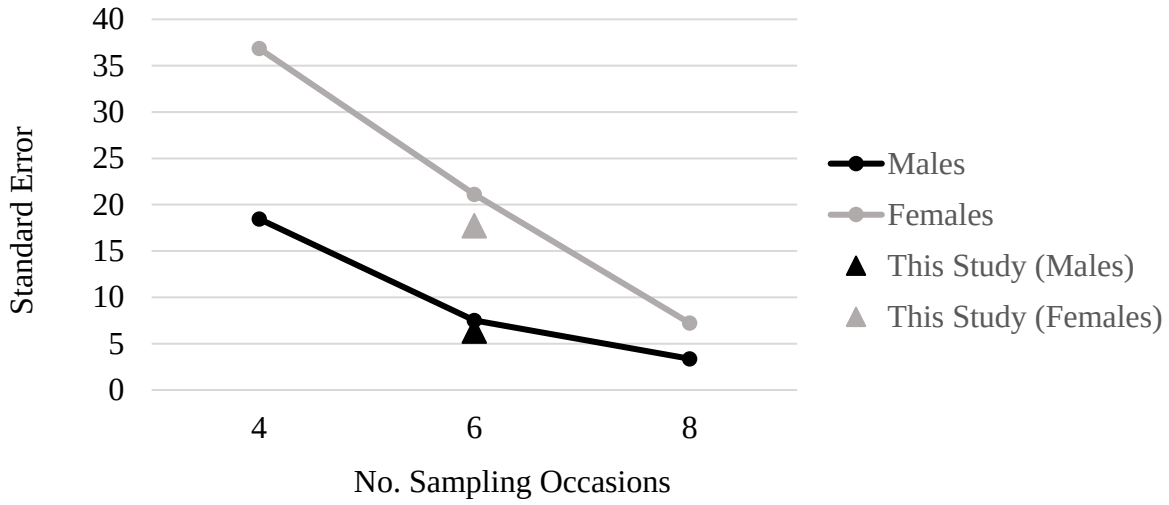

Figure L1: Simulated results for standard errors with varied sampling efforts (4, 6, or 8 sampling occasions), season one (2012-2013).

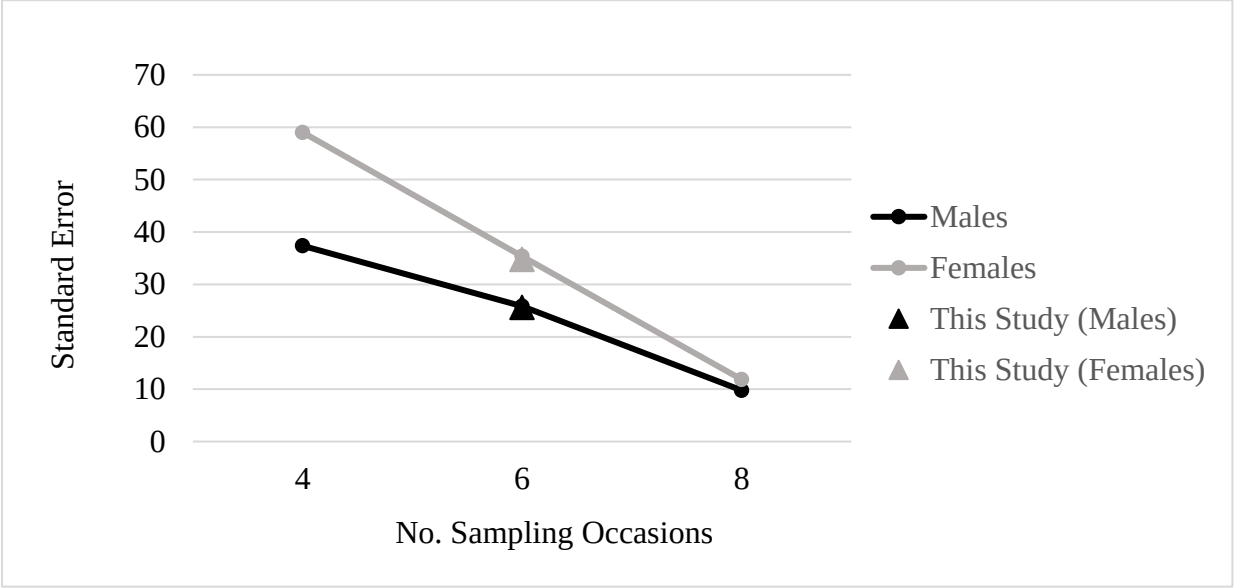

Figure L2: Simulated results for standard errors with varied sampling efforts (4, 6, or 8 sampling occasions), season two (2013-2014).

Appendix M: Detailed protocols for extraction and genotype scoring of DNA from feather, fecal pellet samples, and caecal samples.

Protocols for the Stool Mini Kits used a modified elution step using 80µL Buffer AE after five minutes of incubation at room temperature with extractions performed by hand or with steps automated on a QiaCube (Qiagen). Protocols for the DNeasy 96 kits were modified to include an overnight digestion consisting of 900 µL Buffer ATL, 20µL Proteinase K, and 20µL 1M DTT and an elution in 80µL Buffer AE after five minutes of incubation at room temperature. Protocols for the DNeasy Blood and Tissue Kits modified the elution step for samples to be eluted in 120 µL Buffer AE after five minutes of incubation at room temperature.

The multiplex pre-amplification method is a two-step procedure that requires an initial PCR using a pool of primer pairs with a final concentration of 0.01 µM. This initial step involved a primer pool consisting of unlabeled primers for the 7 loci and was performed following the conditions outlined in Piggott et al. (2004) with the exception of using 10 µL fecal DNA as the template for the 50 µL reaction. The 2nd step used 3 µl of the PCR product produced in the 1st step as template for 12.5 µL reactions containing 0.2 mM of each dNTP, 1X GoTaq Flexi Buffer (Promega, Madison, WI), 1.5mM $MgCl_2$, 1X BSA, 0.5 µM of each primer (dye-labeled forward), and 1 U of *Taq* DNA polymerase (Promega). The amplification conditions for the 2nd step were: 94^{o}C for 2 min, then 94^{o}C for 30 sec, annealing temp (52°C for MSP11, 57°C for SGMS06.6, BG6, and sexing, and 60°C for SG29, SG36, and SG39) for 30 sec, 72^{o}C for 30 sec for 40 cycles, then 60^{o}C for 45 min and a final extension at 72^{o}C for 10 min. PCR products were multi-loaded based on product size and primer label, combined with GeneScan LIZ 600 internal lane size standard (Applied Biosystems, Foster City, CA), and electrophoresed through a

capillary gel matrix using an AB3500 Automated DNA Sequencer (Applied Biosystems). Allele sizes were determined for each locus using GeneMapper v5 software (Applied Biosystems).

Repeated amplifications generated multiple multi-locus genotypes for each sample. We compared corresponding genotypes and generated a consensus genotype for each sample. As is common with low quality DNA, some genotypes did not match across all amplification attempts. In these cases, we re-amplified mismatching loci to confirm a consensus genotype with matching scores. If there was still a mismatch after two rounds of re-amplification, we determined genotypes conservatively, and scored individuals as heterozygous at a locus if they were heterozygous at least once with a homozygous match for one of the alleles in that heterozygote. Loci were scored as "no data" if the genotypes were complete mismatches for each amplification attempt or could not otherwise be confirmed. Two rounds of review were performed on sample pairs with genotypes that differed by a single locus, by referencing all genetic analysis results and collection data, to ensure correct assignment of scores.