Federal Aid in Wildlife Restoration Grant W-176-R

Annual interim report, September 2020

Pronghorn Movement and Population Ecology

Dr. Joshua Millspaugh, Professor, University of Montana, Forestry 108, Missoula, MT 59812, Joshua.millspaugh@mso.umt.edu

Jesse DeVoe, Research Associate, Montana Wildlife Research Cooperative Unit, Natural Sciences Room 205, Missoula, MT 59812, jessedevoe@gmail.com

Dr. Kelly Proffitt, Research Biologist, Montana Fish, Wildlife & Parks, 1400 S. 19th Ave, Bozeman, MT 59715, kproffitt@mt.gov

State: Montana
Agencies: Fish, Wildlife & Parks and University of Montana
Grant: Pronghorn Movement and Population Ecology
Grant #: W-176-R
Time Period: 1 July, 2019 – 30 June, 2020

Executive Summary

The Montana Pronghorn Movement and Population Ecology Project was initiated to collect information on pronghorn movements, seasonal habitat use, and demographics in seven study areas across Montana that included the Big Hole, Paradise, Musselshell, Fergus-Petroleum, South Philips, Garfield-Rosebud, and Powder River-Carter study areas. In addition, an ongoing pronghorn study collecting identical information in the Madison is being included in this study and reporting.

For this reporting period, the primary objectives of the project were to: 1) capture, sample, and collar up to 60 pronghorn in each of the 8 study areas, 2) collect animal location data, and 3) initiate development of a pronghorn population model. Between January and early March 2020, we captured, sampled, and instrumented with GPS collars a total of 390 adult female pronghorn. We captured and collared a total of 60 pronghorn in the Musselshell, Fergus-Petroleum, South Philips, Garfield-Rosebud, and Powder River-Carter study areas, 46 in the Big Hole, 20 in the Madison, and 24 in the Paradise study area. We collared the 20 animals in the Madison to increase the sample size from 40 animals, which were captured and collared during January 2019, to a total of 60 animals. Including the 2019 Madison captures, we have captured, sampled, and collared a total of 430 animals as part of the project. Samples collected from each animal included blood serum for pregnancy and disease testing, blood gene cards and hair for genetic archiving, and fecal for diet analysis. Across all study areas, a total of 6 collars have malfunctioned, 63 animals have died, and 361 collars remain active and will continue to be monitored.

To date, we have collected 1,515,291 locations from 430 individuals. Movement patterns of individuals were diverse within and across study areas. Using these location data in combination with forthcoming spatial information we assemble on fences and fence types in the Garfield-Rosebud, Powder River-Carter, and South Philips study areas, we plan to develop a set of tools to identify and quantify pronghorn response to different types of fences during winter, summer, and migratory periods. To assist in identifying potential movement barriers within the study areas, we have developed a web interface that allows biologists to view pronghorn movement trajectories and begin identifying areas that may be barriers to pronghorn movements.

We began developing a pronghorn population model by assembling survey and harvest data for each hunting district containing the study populations. We have assembled the majority of survey and harvest data but are still receiving and aggregating data for a small number of hunting districts. When the data are aggregated, we will develop and use the population model to 1) identify important vital rates affecting population growth rate, 2) contrast important vital rates among populations, and 3) develop hypotheses to explain why some pronghorn populations experience limitations on population growth rate.

Project Background

Pronghorn (*Antilocapra americana*) provide important ecosystem functions and recreational opportunities in Montana, which hosts the 2nd largest population and harvest of pronghorn across their range. Ecologically, pronghorn may serve as an umbrella species for conserving sagebrush-grasslands and maintaining landscape connectivity of these systems (Rowland et al. 2006, Gates et al. 2012). Because of the important ecosystem functions and recreational opportunities pronghorn provide, conserving and managing pronghorn and their habitats is a priority for Montana Fish, Wildlife & Parks (FWP), land management agencies, private landowners, non-governmental organizations (NGOs), and numerous additional stakeholders.

Recently, there has been a focus in the western United States to identify and protect big game migration corridors and winter ranges, highlighted in the 2018 Secretarial Order (SO) 3362. The purpose of SO 3362 is to foster collaboration between the federal government, states, NGOs, and private landowners to identify, improve, and conserve winter range and migration corridors for mule deer, elk, and pronghorn. In response to SO 3362, FWP drafted a State Action Plan which identifies four priority conservation areas in Montana. Collaborations between state and federal wildlife, land management, and transportation agencies have since formed to delineate ungulate seasonal ranges and movement corridors, and design cooperative habitat or transportation projects to improve landscape connectivity and conserve big game populations.

In Montana, there are limited data available regarding pronghorn movements and population dynamics. Therefore, additional information is needed regarding pronghorn seasonal habitat use and migratory movements to direct and prioritize these important habitat and conservation efforts. In addition to collecting movement data, understanding population demography is needed to promote effective management strategies. Given widespread pronghorn population declines in portions of central and eastern Montana in recent decades, biologists need information regarding survival and demography to identify and understand potential issues limiting pronghorn population recovery.

Pronghorn populations were abundant and at or above regional population objectives/long-term averages (LTAs) throughout their range in Montana during the mid-2000s with harvest totaling 33,500 at the latest peak in 2007. Following widespread blue-tongue virus (BTV) outbreaks in the subsequent two years, then the record cold and snowy winter in 2010-2011, harvest fell to a low of 8,200 in 2013 (Montana Fish Wildlife and Parks 2020a). Pronghorn populations typically rebound quickly with favorable weather conditions, yet numbers of pronghorn in many of Montana's central and eastern populations are < 50% of population objective despite multiple years of favorable weather and minimal harvest. Meanwhile, mule deer and elk populations are exceeding objective levels over much of the region (Montana Fish Wildlife and Parks 2020b, 2020c)

The factors currently limiting pronghorn population recovery across central and eastern Montana are unknown. Stochastic events including severe winter weather may cause significant mortality events, leading to high variability in overwinter pronghorn survival rates (Martinka 1967, Pyrah 1987, O'Gara 2004a). Accordingly, survival of adult female pronghorn is lower or more variable than for other northern temperate ungulates, ranging from 0.29 to 0.87 in Montana (Boccadori 2002, Dunn and Byers 2008, Barnowe-Meyer et al. 2009, Jakes 2015). During winter 2010-2011, abnormally high snow depths in central and eastern Montana concentrated pronghorn on winter range, resulting in rapid exhaustion of browse, over exposure, and altered pronghorn distributions (Jakes et al. 2018a). In the Fort Peck Reservoir area of central Montana, flooding exacerbated the effects of the 2011 severe winters, as more than 2,000 pronghorn attempting to return north to fawning and summer ranges were stranded on the south side of the reservoir by unusually high floodwaters and were presumed to have died after exhausting nearby forage. Fences and roads may also act as barriers to movements within or between seasonal ranges, potentially affecting seasonal range selection and reducing habitat availability (Jakes et al. 2018b, Jones et al. 2019).

Pronghorn pregnancy and birth rates are generally high; however, these vital rates may also be affected by habitat or weather conditions and have the potential to limit pronghorn population recovery. Dunn and Byers (2008) recorded pronghorn reproductive failures on the National Bison Range (NBR), Montana, following severe drought in 2003 and none of the marked females that weaned fawns in 2003 gave birth in 2004. During this same period, annual counts of other ungulates on the NBR did not indicate exceptionally low survival or fecundity rates potentially because other ungulate species fall lower on the maternal energy-expenditure spectrum than pronghorn (Dunn and Byers 2008). Additionally, severe weather such as drought or harsh winters may have carryover effects on future reproductive success or survival (Webster et al. 2002). Although habitat or weather-related factors generally have a greater effect on pronghorn populations than predation, predation may limit recruitment and have important effects on population growth (O'Gara and Shaw 2004). Overall fawn mortality across 18 studies averaged 71%, with 76% of all mortalities being due to predation from coyotes (O'Gara and Shaw 2004). We expect that coyote predation is the main proximate cause of mortality of pronghorn fawns in central and eastern Montana, but its extent may vary due to habitat conditions (weather and land-use influences on vegetation), the abundance of alternate prey species (Hamlin and Mackie 1989, Berger et al. 2008, Berger and Conner 2008), or coyote control operations (Harrington and Conover 2007, Brown and Conover 2011).

In addition to the potential limiting effects of habitat, weather and predation on pronghorn survival and recruitment, disease events like BTV or epizootic hemorrhagic disease (EHD) can also impact pronghorn populations via direct mortality or negative effects on reproduction (Thorne et al. 1988, Dubay et al. 2006, Gray 2013). In July 2007, a BTV outbreak occurred across portions of central and eastern Montana and precipitated the decade-long decline in pronghorn populations (Montana Fish Wildlife and Parks 2012). Disentangling the effects of

BTV and other diseases on pronghorn reproductive rates will require serologic assays and pregnancy tests or other measures of productivity in years with and without disease outbreaks, and data from pronghorn in this study will begin to inform us on the influences of disease on pronghorn populations in Montana.

Several hypotheses exist concerning factors potentially limiting pronghorn population recovery. For example, 1) adult female survival and/or recruitment, as influenced by weather, habitat conditions, predation, and/or a combination of these factors, may be too low and therefore limiting the population, 2) carryover effects of past disease events or current infection may impact adult female survival and reproduction, and/or 3) potential barriers restricting seasonal movements of pronghorn may impact vital rates. We will develop an integrated population model using adult female survival data combined with population abundance and production estimates from survey and harvest data. This population model will 1) identify important vital rates affecting population growth rate, 2) contrast important vital rates between populations that are considered productive vs. limited in performance, and 3) develop hypotheses to explain why some pronghorn populations experience limitations on population growth rate. The population model will provide information towards developing more focused investigations into ecological and/or anthropogenic factors limiting pronghorn population recovery in central Montana and future population monitoring strategies.

The purpose of this project is to identify seasonal ranges and movement corridors and provide demographic data for pronghorn populations in 8 study areas across Montana (Figure 1). These areas have been selected based on local needs identified by FWP area biologists and where considerable community, conservation partner, and agency interest exists in mapping anthropogenic impediments or other habitat features that influence habitat/migratory pathway selection or fitness. Our objectives during this reporting period include:

1. Capture, sample, and collar up to 60 pronghorn in each of the 8 study areas.
2. Collect animal location data.
3. Initiate development of the pronghorn population model.

Location

The 8 study areas are located in the southwestern, central, and southeastern regions of Montana (Figure 1) and include the Big Hole, Madison, Paradise, Musselshell, Fergus-Petroleum, South Philips, Garfield-Rosebud, and Powder River-Carter.

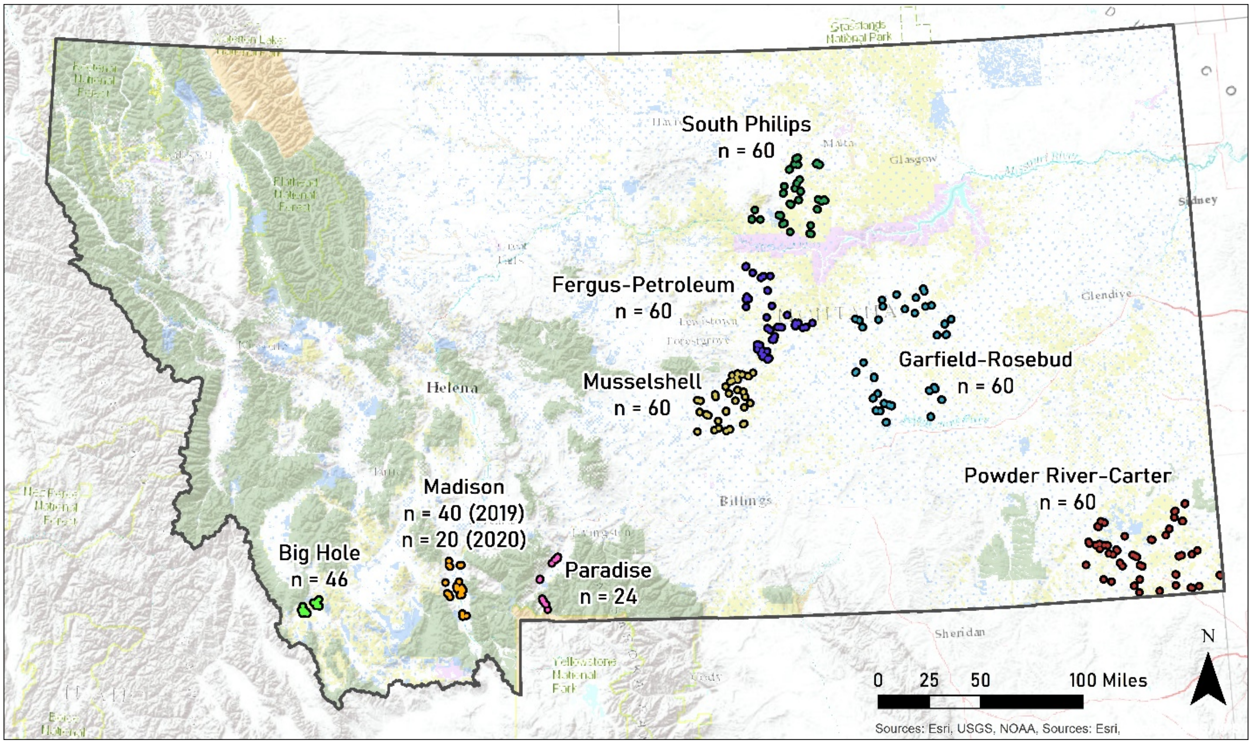


Figure 1. Capture locations and number of animals captured in the 8 study areas for the Montana Pronghorn Movement and Population Ecology Project during winter 2020.

Objective #1: Capture, sample, and collar up to 60 pronghorn in each study area

1.1 Pronghorn capture, sampling, and collaring

From January to March 2020, we used helicopter net-gunning to capture and sample a total of 395 adult female pronghorn, of which 390 were outfitted with GPS collars (5 animals died during capture and sampling and were not collared). We captured and collared a total of 60 pronghorn in the Musselshell, Fergus-Petroleum, South Philips, Garfield-Rosebud, and Powder River-Carter study areas, 46 in the Big Hole, 20 in the Madison, and 24 in the Paradise study areas (Figure 1). The number of pronghorn captured in the Big Hole and Paradise was limited by the number of available animals and/or landowner permission to access properties. We collared the 20 animals in the Madison to increase the sample size from 40 animals, which were captured and collared during January 2019, to a total of 60 animals. Including the 2019 Madison captures, we collared a total of 430 pronghorn. From each animal, we collected biological samples that included blood serum for pregnancy and disease testing, blood gene cards and hair for genetic archiving, and fecal samples for diet analysis. From approximately half of the captured animals, we estimated a body condition score and/or obtained a measurement of rump fat thickness using ultrasound. We outfitted each animal with a Lotek LiteTrack Iridium 420 collar programmed to collect locations every hour for three years, transmit a VHF signal during daylight periods, and transmit a mortality alert and signal if the device is stationary for >5 hours. These collars upload locations via Iridium satellites to a web platform for viewing and downloading near-real-time data.

1.2 Sampling results

1.2.1 Serology

Blood serum samples were assayed for evidence of exposure to pathogens including *Anaplasma* bacteria, bovine herpesvirus, bovine respiratory syncytial virus, bluetongue virus, bovine viral diarrhea type 1, bovine viral diarrhea type 2, epizootic hemorrhagic disease, *Leptospira canicola*, *L. grippo*, *L. hardjo*, *L. ictero*, *L. pomona*, and parainfluenza-3. These pathogens were selected for screening because of either their known potential impact to individual or herd health (e.g., bluetongue virus and epizootic hemorrhagic disease) and/or because of their known association with livestock or wildlife health (e.g., *Leptospra* serovars, *Anaplasma*, bovine viral syncytial virus, and parainfluenza-3. All assays were conducted by the Montana Veterinary Diagnostic Laboratory (Bozeman, Montana), except for epizootic hemorrhagic disease which was conducted by the Washington Animal Disease Diagnostic Lab (Pullman, Washington). Evidence for exposure varied by pathogen and study area (Table 1). We found no serological evidence of exposure in any study area for bovine herpesvirus, *L. canicola*, *L. hardjo*, or *L. pomona*. We found evidence of exposure in all study areas for *Anaplasma* (ranging from 20 – 92% seroprevalence) and parainfluenza-3 (ranging from 69 – 100% seroprevalence). Below, we discuss each of the pathogens identified through serology in our study areas.

Anaplasmosis, or gall sickness, is a disease of blood cells primarily affecting domestic cattle that is caused by *Anaplasma* bacteria and transmitted by ectoparasites. Pronghorn are susceptible to infection of *Anaplasma*; however, serious clinical signs have not been recorded in pronghorn and little evidence exists that pronghorn act as important carriers (Kuttler 1984, O’Gara 2004b). We found serological evidence for exposure to *Anaplasma* in all study areas with seroprevalence averaging 53% (range: 20 – 92%) with Big Hole having the lowest seroprevalence and Paradise having the highest seroprevalence. Although we found evidence of exposure across all study areas, these results are not expected to impact individual or herd health.

Bovine respiratory syncytial virus is an infection associated with respiratory disease primarily affecting domestic cattle that can cause the formation of syncytial cells – the fusion of infected cells with neighboring cells. Pronghorn are susceptible to infection by the virus, which is most likely transmitted from cattle; however, serious clinical signs have not been recorded in pronghorn (O’Gara 2004b). We found serological evidence of low levels of exposure to bovine respiratory syncytial virus in only the Musselshell (5%) and Paradise (4%) study areas. Although evidence of exposure occurred in each of these study areas, these results are not expected to impact individual or herd health (O’Gara 2004b).

Bluetongue virus is transmitted by biting midges in the *Culicoides* genus and other arthropods and can cause acute and frequently fatal hemorrhagic disease in domestic and wild ungulates.

Pronghorn are susceptible to disease caused by the bluetongue virus which can result in large, all-sex and -age die-offs that occur primarily during late summer and early autumn (Thorne et al. 1988, O'Gara 2004b). There is evidence that pronghorn can, however, be exposed to this virus without suffering high rates of mortality or showing clinical signs (O'Gara 2004b). Exposure to bluetongue virus was only detected in Garfield-Rosebud (5%). These results were not atypical of exposure rates observed in pronghorn and do not necessarily indicate pathogenicity (O'Gara 2004b, Dubay et al. 2006).

Epizootic hemorrhagic disease virus is transmitted by biting midges in the *Culicoides* genus and other arthropods and can cause acute and frequently fatal hemorrhagic disease in domestic and wild ungulates. Pronghorn are susceptible to epizootic hemorrhagic disease which can result in large, all-sex and -age die-offs that occur primarily during late summer and early autumn. There is evidence that pronghorn can, however, be exposed to this virus without suffering high rates of mortality or showing clinical signs (O'Gara 2004b, Gray 2013). Epizootic hemorrhagic disease virus exposure was detected in all study areas except Big Hole, Madison, and Paradise, with seroprevalence averaging 22% (ranging 12 – 39%) in study areas where exposure was detected. These results were not atypical of exposure rates observed in pronghorn and do not necessarily indicate pathogenicity (Barrett and Chalmers 1975, O'Gara 2004b, Gray 2013).

Bovine viral diarrhea (types 1 & 2) is a disease caused by a virus that causes diarrhea and can induce immunosuppression, which allows for development of secondary bacterial pneumonia in domestic and wild ungulates. The different types (1 & 2) reflect differences in the antigens found on the viral surface protein and do not relate to the virulence of the virus. Pronghorn are susceptible to infection of bovine viral diarrhea, however, there is little evidence of serious clinical effects or that pronghorn act as important carriers. We found a low seroprevalence of both types of bovine viral diarrhea in the majority of study areas (0 – 7%) and seroprevalence ranging from 16 – 41% of both types of bovine viral diarrhea in Fergus-Petroleum. These seroprevalences were similar to those found in Alberta and Saskatchewan where no clinical signs were observed (Barrett and Chalmers 1975, Kingscote and Bohac 1986).

***Leptospira* spp.** are members of an infective serological group of bacteria that can infect nearly all mammals. Infection varies in severity from asymptomatic to fatal depending on the host and the serovar of *Leptospira*. Naturally occurring *Leptospira* infections in wildlife are usually asymptomatic, but may result in renal failure, lysis of red blood cells, fever, inappetence, hemorrhages on mucous membranes, jaundice, dehydration, infertility, abortion, stillbirths, or weakened neonates. Pronghorn are susceptible to *Leptospira* spp. infection which may cause some mortality; however, clinical disease in wildlife is rare and not likely a major limiting factor in pronghorn populations (O'Gara 2004b). We found low seroprevalence (2%) to *L. grippo* in only the Big Hole and low to moderate seroprevalence to *L. ictero* that averaged 10% (ranging 0.1 – 20%) in study areas where exposure was detected. We did not detect exposure to *L. ictero* in the Paradise. Although few previous studies have reported exposure to these *Leptospira* serovars in pronghorn and cross-reactivity of serovars makes interpretation of seroprevalence challenging, we do not suspect our results indicate pathogenicity and are within the range of normal exposure rates to other serovars of *Leptospira* in pronghorn (O'Gara 2004b).

Parainfluenza-3 is a virus capable of causing respiratory disease in domestic ungulates. The disease is usually associated with mild to subclinical infections, but may serve an important role as an initiator under severe stress that can lead to development of secondary bacterial pneumonia. Parainfluenza-3 exposure is highly variable among pronghorn from different areas and across years; however, there is no evidence of serious disease and the virulence is unknown in pronghorn (Barrett and Chalmers 1975, O'Gara 2004b, Dubay et al. 2006). We found an average seroprevalence of 90% (ranging from 69 – 100%) to parainfluenza-3 across all populations with Big Hole having the lowest seroprevalence and Madison and Powder River-Carter having the highest seroprevalence. Although evidence of exposure occurred in each study area, these results are not expected to impact individual or herd health (Barrett and Chalmers 1975, Stauber et al. 1980, O'Gara 2004b).

Table 1. Seroprevalence for anaplasmosis (ANPLSM), bovine herpesvirus (BHV), bovine respiratory syncytial virus (BRSV), bluetongue virus (BTV), bovine viral diarrhea type 1 (BVD1), bovine viral diarrhea type 2 (BVD2), epizootic hemorrhagic disease (EHD), *Leptospira canicola* (L. CAN), *L. grippo* (L. GRI), *L. hardjo* (L. HAR), *L. ictero* (L. ICT), *L. pomona* (L. POM), and parainfluenza-3 (PI3) based on serological screening of adult female pronghorn in the 8 study areas sampled during winter 2020 for the Montana Pronghorn Movement and Population Ecology Project.

Herd	**Statistic**	**ANPLSM**	**BHV**	**BRSV**	**BTV**	**BVD1**	**BVD2**	**EHD**	**L. CAN**	**L. GRI**	**L. HAR**	**L. ICT**	**L. POM**	**PI3**
Big Hole	# Sampled	45	45	45	45	45	45	45	45	45	45	45	45	45
	# Exposed	9	0	0	0	0	0	0	0	1	0	3	0	31
	% Exposed	20	0	0	0	0	0	0	0	2	0	7	0	69
Fergus-Petroleum	# Sampled	61	61	61	61	61	61	58	61	61	61	61	61	61
	# Exposed	33	0	0	0	1	25	14	0	0	0	8	0	59
	% Exposed	54	0	0	0	16	41	24	0	0	0	13	0	97
Garfield-Rosebud	# Sampled	61	61	61	61	61	61	49	61	61	61	61	61	61
	# Exposed	21	0	0	3	0	2	9	0	0	0	7	0	52
	% Exposed	34	0	0	5	0	3	18	0	0	0	12	0	85
Madison	# Sampled	21	21	21	21	21	21	21	21	21	21	21	21	21
	# Exposed	13	0	0	0	0	1	0	0	0	0	2	0	21
	% Exposed	62	0	0	0	0	< 1	0	0	0	0	< 1	0	100
Musselshell	# Sampled	59	58	58	59	57	57	22	59	59	59	59	59	57
	# Exposed	33	0	3	0	0	2	3	0	0	0	4	0	48
	% Exposed	56	0	5	0	0	4	14	0	0	0	7	0	84
Paradise	# Sampled	24	24	24	24	24	24	11	24	24	24	24	24	24
	# Exposed	22	0	1	0	0	0	0	0	0	0	0	0	22
	% Exposed	92	0	4	0	0	0	0	0	0	0	0	0	92
Powder River-Carter	# Sampled	61	57	57	61	61	61	33	61	61	61	61	61	57
	# Exposed	36	0	0	0	2	1	13	0	0	0	12	0	56
	% Exposed	59	0	0	0	3	2	39	0	0	0	20	0	98
South Philips	# Sampled	60	60	60	60	60	60	58	60	60	60	60	60	60
	# Exposed	32	0	0	0	1	4	7	0	0	0	7	0	58
	% Exposed	53	0	0	0	2	7	12	0	0	0	12	0	97

1.2.2 Pregnancy

Blood serum samples can be used to detect levels of progesterone and pregnancy-specific protein B (PSPB) in a variety of wild ungulates to determine pregnancy status (Wood et al. 1986, White et al. 1995, Noyes et al. 1997, Huang et al. 2000, Drew et al. 2001); however, to our knowledge serum assays for progesterone and PSPB have not been validated for accuracy in pronghorn (personal communications [A. Reinking, Colorado State University; G. Mastromonaco, Toronto Zoo]). To understand variation in and compare levels of progesterone and PSPB in the serum samples collected for this project, we sent a test batch of 30 samples from the same individuals in two study areas (15 samples from Big Hole and 15 samples from Garfield-Rosebud) to test for progesterone (Saint Louis Zoo Endocrinology Laboratory, Saint Louis, Missouri) and PSPB (Herd Health Diagnostics/BioTracking Testing Lab, Pullman, Washington). Progesterone levels averaged 3.71 ng/ml (standard deviation [SD] = 1.38 ng/ml, range 1.44 – 9.34 ng/ml) with an average of 3.85 ng/ml (SD = 1.58 ng/ml, range 1.84 – 9.34 ng/ml) in the Big Hole and an average of 3.58 ng/ml (SD = 1.15 ng/ml, range 1.44 – 5.43 ng/ml) in the Garfield-Rosebud (Figure 2). However, lack of evidence of a bimodal distribution or threshold values of progesterone levels indicate that it was not reasonable to define pregnancy based on progesterone levels alone.

PSPB levels (measured as an optical density) averaged 0.35 (SD = 0.08, range 0.08 – 0.47) with an average of 0.33 (SD = 0.08, range 0.08 – 0.47) in the Big Hole and an average of 0.37 (SD = 0.06, range 0.12 – 0.43) in the Garfield-Rosebud (Figure 2). PSPB values were classified into categories that are typical for wildlife species: open (values ≤ 0.210) and pregnant (values > 0.210). Based on these preliminary classifications, 90.0% were pregnant and 10.0% were open in the Big Hole and 96.7% were pregnant and 3.3% were open in the Garfield-Rosebud.

Comparisons between the serum assays indicated that progesterone and PSPB levels were not correlated, and animals classified as open based on PSPB were not those with the lowest progesterone values (Figure 3). Progesterone levels in fecal samples have been found to vary, with values for pregnant and nonpregnant animals overlapping (O'Gara 2004c). Given the potential unreliability of progesterone samples and indication that our PSPB assays were providing meaningful results based on previous tests of other ungulates (A. Merk, Herd Health Diagnostics, personal communication), we tested a remaining sample of 35 animals per population for PSPB. We validated the results of these tests with reproduction observations collected during necropsies of dead or relocations of alive collared animals (e.g., single fetus, twin fetuses, single fawn at heel, etc.). A total of 14 reproduction observations were used for validation, of which 4 (28.6%) were incorrectly classified as pregnant based on PSPB classifications. Based on these results and the substantial variation of PSPB values across study areas (Figure 4), we determined that PSPB were inaccurate and unreliable. To our knowledge, an accurate test for pregnancy from blood serum samples does not exist for wild pronghorn.

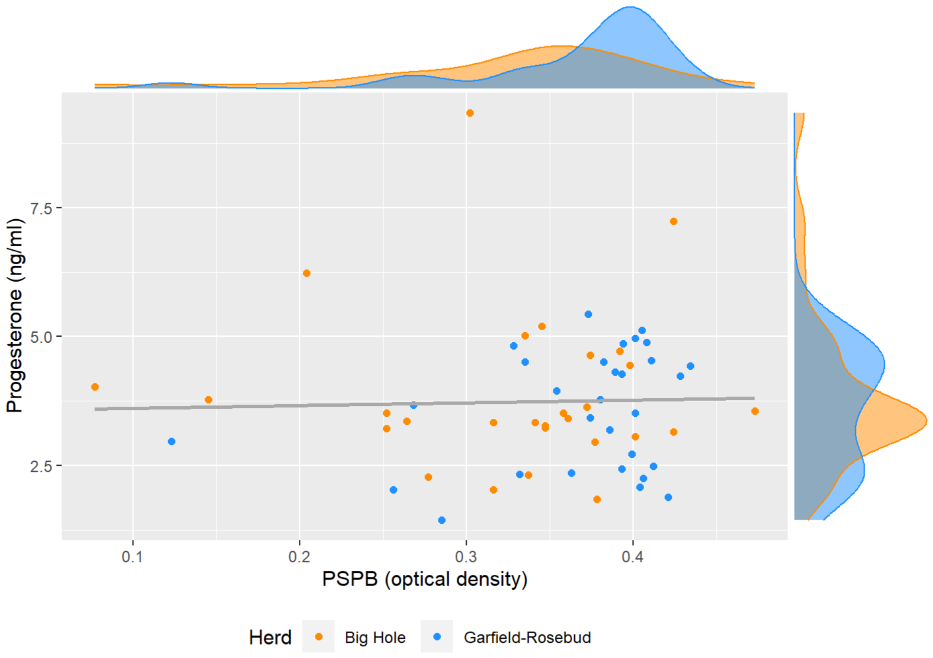


Figure 2. Serum progesterone and PSPB levels in test batches from the same individuals in a western (Big Hole) and eastern (Garfield-Rosebud) population of the Montana Pronghorn Movement and Population Ecology Project collected during winter 2020.

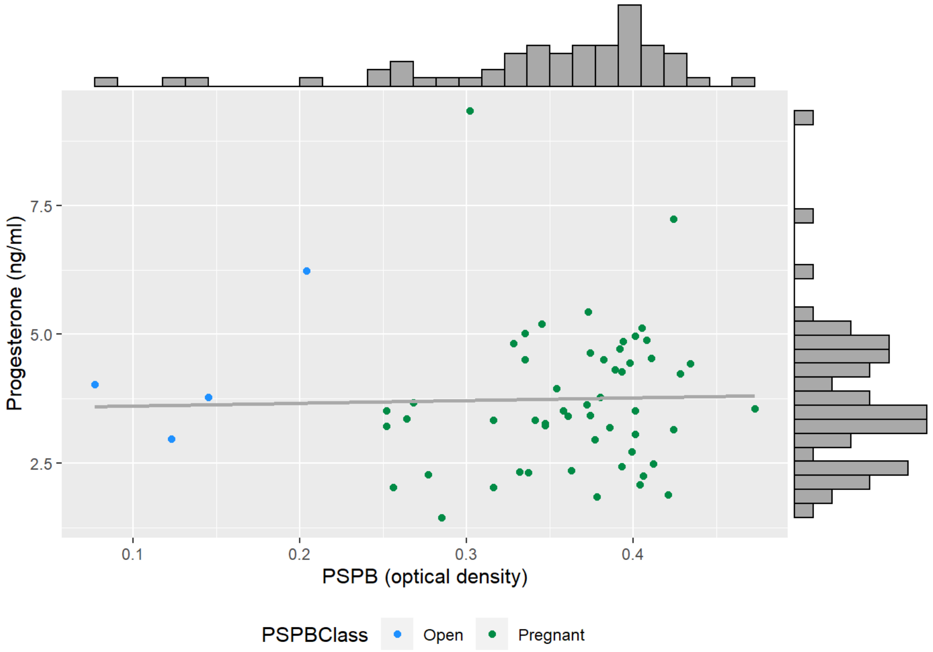


Figure 3. Serum progesterone and PSPB levels highlighted by PSPB pregnancy classification (Open = not pregnant) in test batches from the same individuals in a western (Big Hole) and eastern (Garfield-Rosebud) study area of the Montana Pronghorn Movement and Population Ecology Project collected during winter 2020.

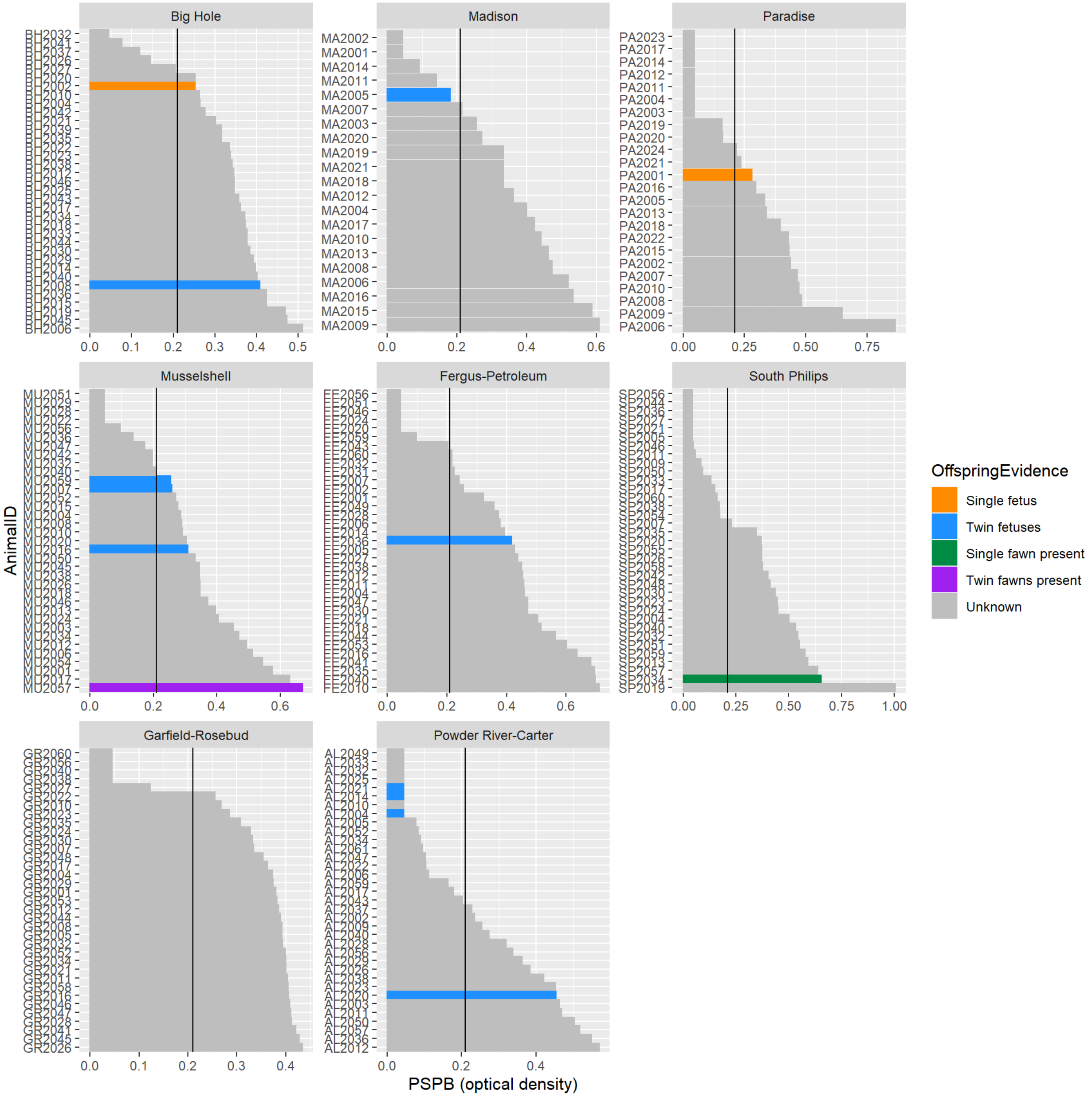


Figure 4. Serum PSPB levels validated by observations of offspring from necropsies of dead or relocations of alive collared animals in the 8 study areas of the Montana Pronghorn Movement and Population Ecology Project collected during winter 2020. The vertical line demarcates non-pregnant (left of line) and pregnant (right of line) animals based on the PSPB value 0.210 that is traditionally used to define pregnancy for wild ungulates. Animals with the lowest PSPB levels (0.045) are the lowest levels measurable by the assay. Evidence of offspring was collected for 14 individuals and used to validate PSPB classifications of pregnancy.

1.2.3 Winter diet

Fecal samples can be used to understand ungulate diets by analyzing fecal pellets to identify plant species. Two possible methods exist that include microhistology (i.e., plant fragment analysis) and DNA metabarcoding. Microhistology reflects relative biomass intake. This technique identifies plant species based on cell wall structures. The number of cell walls observed is used to estimate relative plant abundance in the diet. More digestible plants may be underrepresented because their cell walls are digested and broken down faster than less digestible plants. DNA metabarcoding, on the other hand, reflects relative protein intake. This technique identifies plant species based on the DNA of chloroplasts, the number of which scales with protein and not the number of cell walls. More digestible plants may be overrepresented because high quality plants have more chloroplasts then lower quality plants, and chloroplasts do not decompose during the digestive process.

Our goal was to compare the two methods of determining pronghorn diet by sending test samples collected during captures to be analyzed for plant fragments (Micro Composition Laboratory, Broomfield, Colorado) and DNA metabarcoding (Jonah Ventures, Boulder, Colorado). To each laboratory, we sent a test batch of 3 composite samples (1 pellet from each of 5 animals) from each of 4 study areas that included Big Hole and Madison in western Montana and Powder River-Carter and Fergus-Petroleum in eastern Montana. Currently, we have received results only for the DNA metabarcoding.

Based on the metabarcoding results, plant species in the diet varied by study area (Figure 5). *Artemisia* species comprised the majority (39.3 – 66.7%) of the diet in each study area except the Madison, which was comprised primarily of plants in the Asteraceae family (23.7%). In the Big Hole, common diet species included plants in the Poaeceae (22.2%) and Asteraceae (9.2%) families and *Eriogonum effusum* (13.9%). In the Madison, common diet species included *Populus angustifolia* (14.3%) and *Comandra umbellata* (13.7%). In Fergus-Petroleum, other diet species included *Eriogonum effusum* (9.8%), plants in the Poaceae family (9.7%), and *Bromus tectorum* (5.6%). In the Powder River-Carter, other diet species found to be important included *Eriogonum effusum* (6.7%) and plants in the Poaeceae (4.1%) and Asteraceae (3.3%) families and the *Eriogonum* genus (4.3%). The metabarcoding results also demonstrate a substantial limitation of DNA metabarcoding: often multiple species match a given DNA sequence resulting in a general plant classification to genus or family rather than to species (e.g., Asteraceae or Poaeceae family).

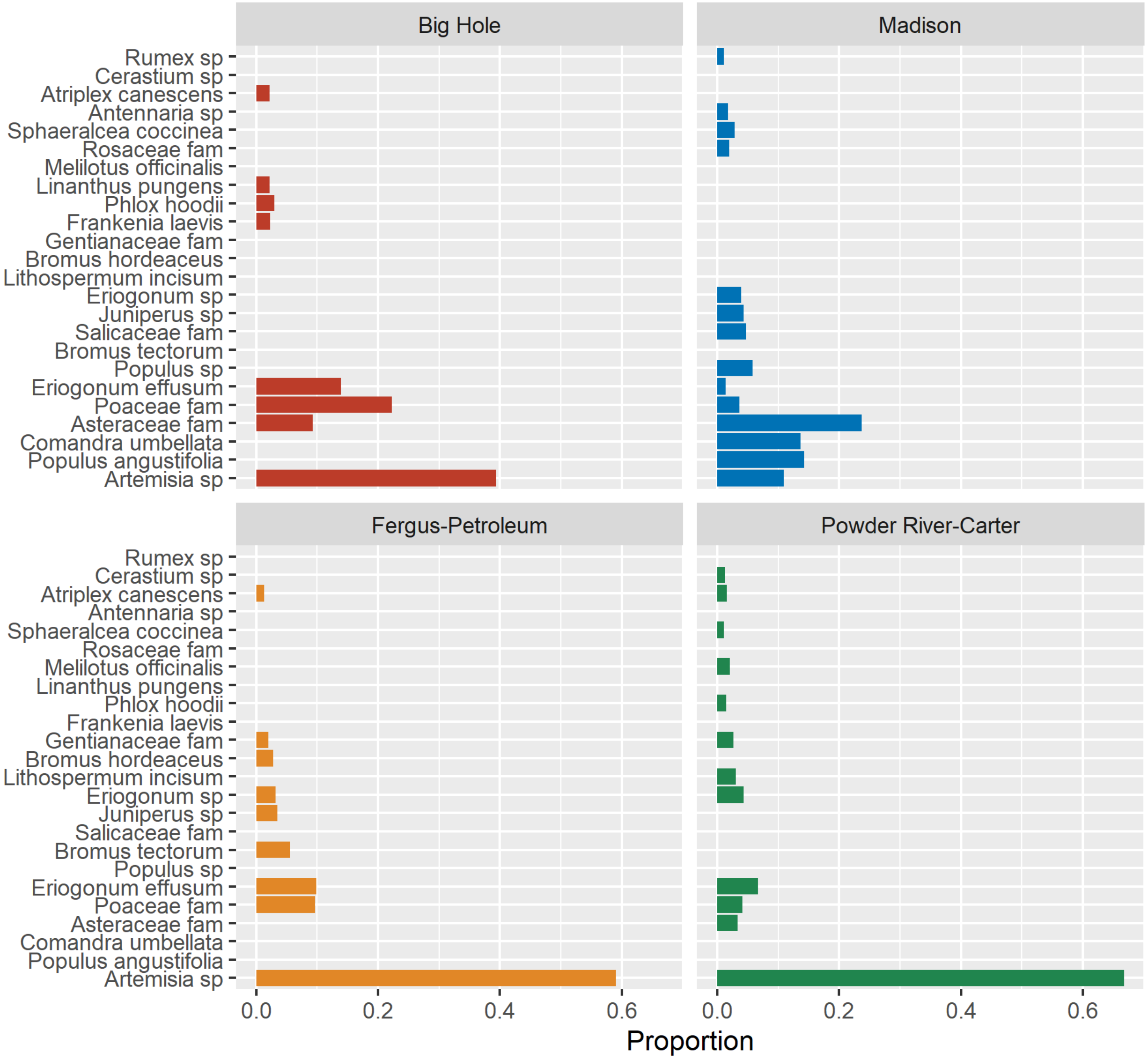


Figure 5. Proportion of plant species in the pronghorn winter diet based on DNA metabarcoding of fecal samples collected during winter 2020 for the Montana Pronghorn Movement and Population Ecology Project. Proportions of all species in the diet of each herd are averaged across 3 composite samples that were comprised of 1 pellet from each of 5 animals and are based on the number of times a DNA sequence associated with each plant classification was read by the sequencer. Only plant species with proportions ≥ 0.01 of the total diet are displayed.

1.2.4 Genetics

Blood gene cards and hair samples were collected and stored for future genetic testing.

1.2.5 Animal body condition

We estimated a body condition score for 183 (42.5%) captured animals and measured the maximum rump fat thickness (Maxfat) using ultrasonography for 181 (42.1%) captured animals (Cook et al. 2010). Body condition scores are based on manual palpation of the spine and hips to estimate fat deposits and can range 1 (very poor) to 5 (very high). Average body condition scores within study areas ranged 2.7 – 4.1 and were lowest in Paradise and Powder River-Carter and highest in Garfield-Rosebud and South Philips (Figure 6). Body condition scores and Maxfat measurements were not evaluated for the Madison and Big Hole, as this sample of 66 animals were used as test cases to develop scoring and measuring methods. Average Maxfat measurements within study areas ranged 0.18 – 0.50 cm and were lowest in Paradise and Powder River-Carter and highest in Garfield-Rosebud and South Philips (Figure 7).

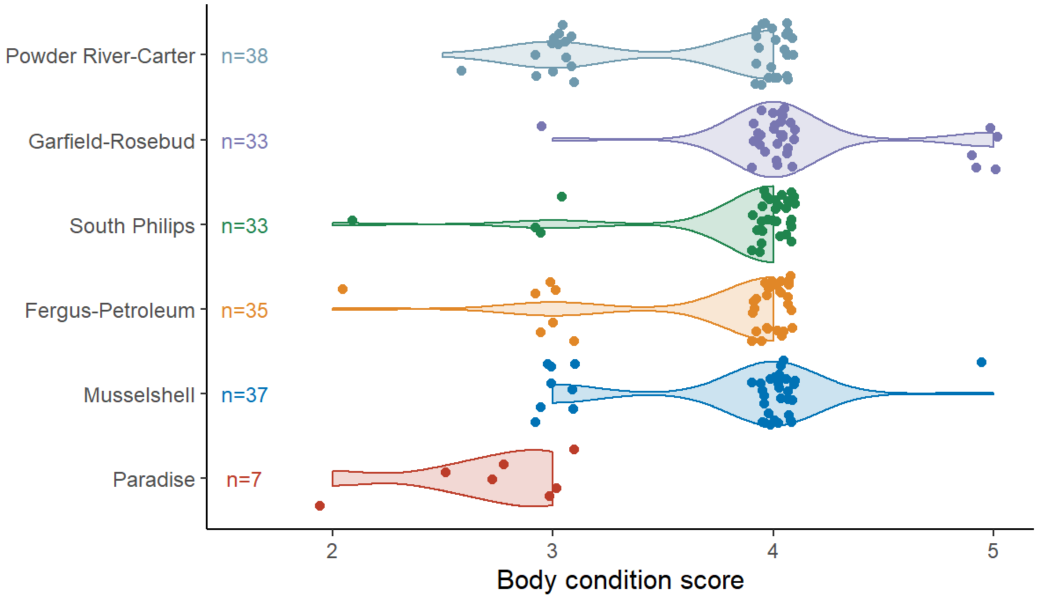


Figure 6. Body condition scores of adult female pronghorn captured in each study area during winter 2020 for the Montana Pronghorn Movement and Population Ecology Project. Body scores were not evaluated in Madison and Big Hole study areas.

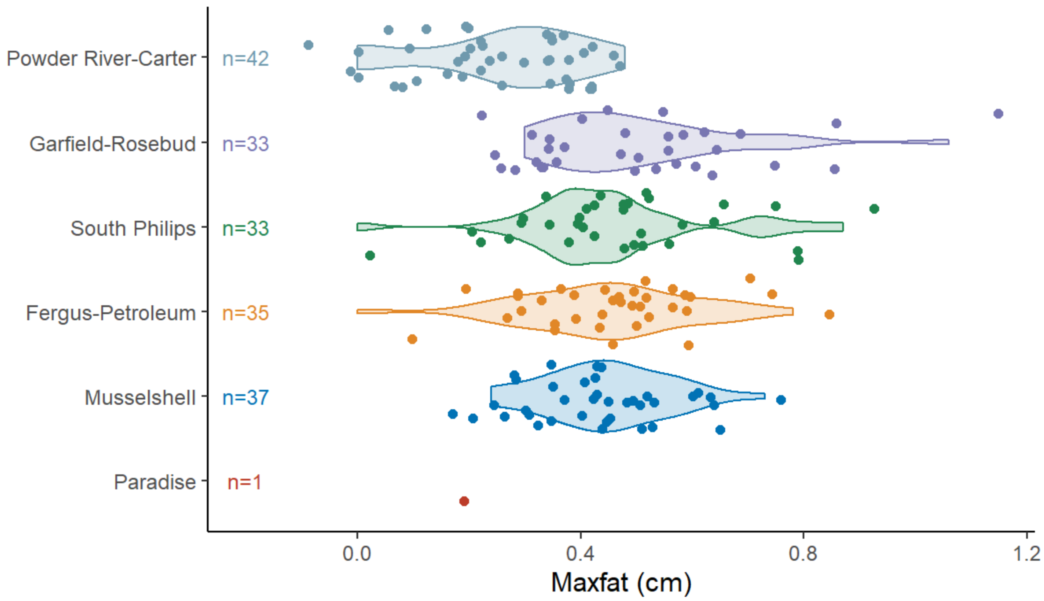


Figure 7. Maximum rump fat thickness (Maxfat) measured by ultrasonography of adult female pronghorn captured in each study area during winter 2020 for the Montana Pronghorn Movement and Population Ecology Project.

1.3 Survival monitoring

Of the 430 animals captured and collared, a total of 63 (14.7%) animals have died, ranging 5 – 14 (7.9 – 22.2%) animals in each study area, and 6 (1.4%) collars have malfunctioned (Figure 8). Mortality investigations were completed as soon as possible after receiving the morality alerts. Mortalities associated with capture operations (capture myopathy or injury) predominated and totaled 17 mortalities, ranging 0 – 5 (0.0 – 8.3%) mortalities in each study area (Figure 9). The remaining mortalities were classified as unknown (n = 19), predation (n = 13), natural (n = 8), disease (n = 2), legal harvest (n = 2), human-related (n = 1), and injury (n = 1). A total of 361 collared pronghorn are being monitored.

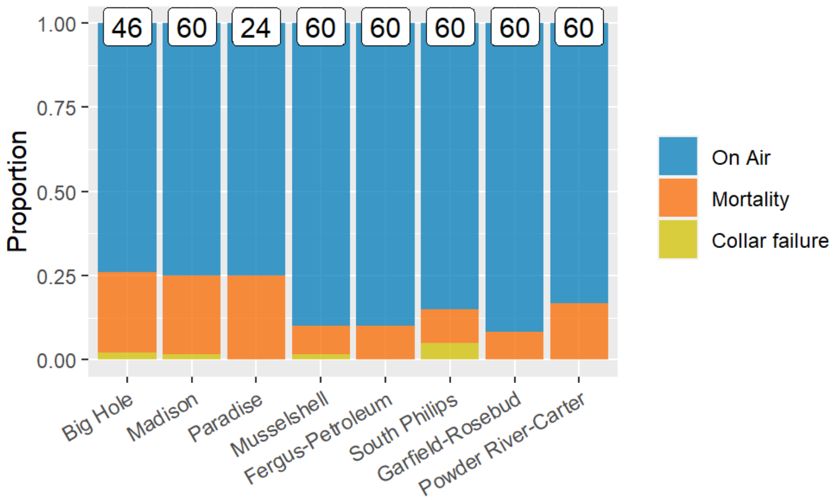


Figure 8. Proportion of collared animals remaining on air, dead, or with a malfunctioned collar in each study area in the Montana Pronghorn Movement and Population Ecology Project as of August 1, 2020. The total number of collared animals in each study area is labeled at the top of each bar.

Figure 9. Proportion of collared animals remaining alive, dead, or with a malfunctioned collar in each study area through June 30, 2020. Cause of death was determined by field investigations.

Objective #2: Collect animal location data.

2.1 Pronghorn location and movement data

To date, we have collected 1,515,291 locations from 430 individuals, averaging 3,582 (range: 22 – 12,896) locations per individual. Movements patterns of individuals were diverse within and across study areas (Figure 10 – 17). We have generated monthly reports of the most recent animal distributions and movements. Preliminary estimates of seasonal ranges and movement corridors will be delineated after a full year of data collection, and finalized at the end of location data collection.

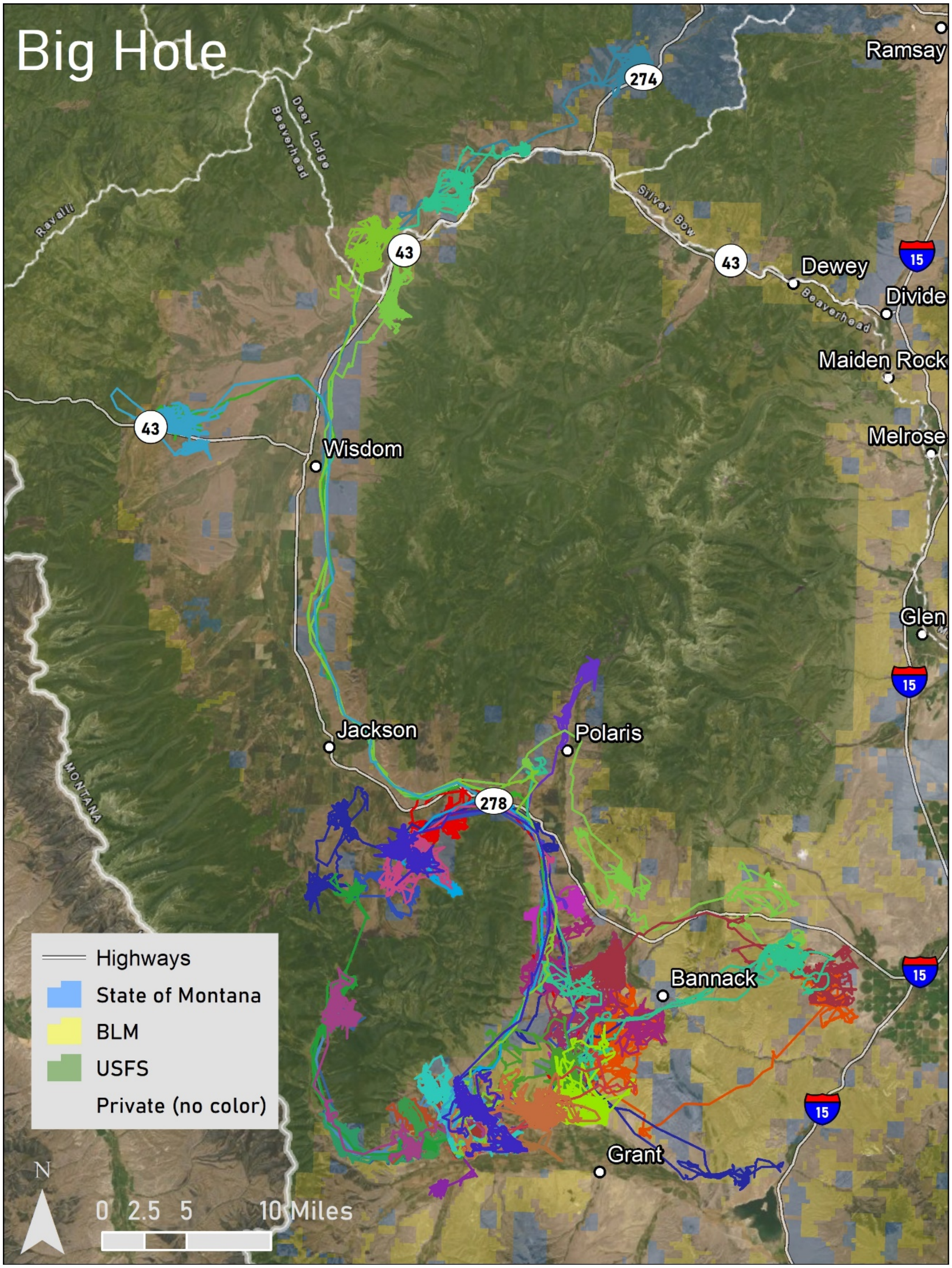


Figure 10. Movements of collared adult female pronghorn (colored by individual) in the Big Hole study area for the Montana Pronghorn Movement and Population Ecology Project, as of August 1, 2020.

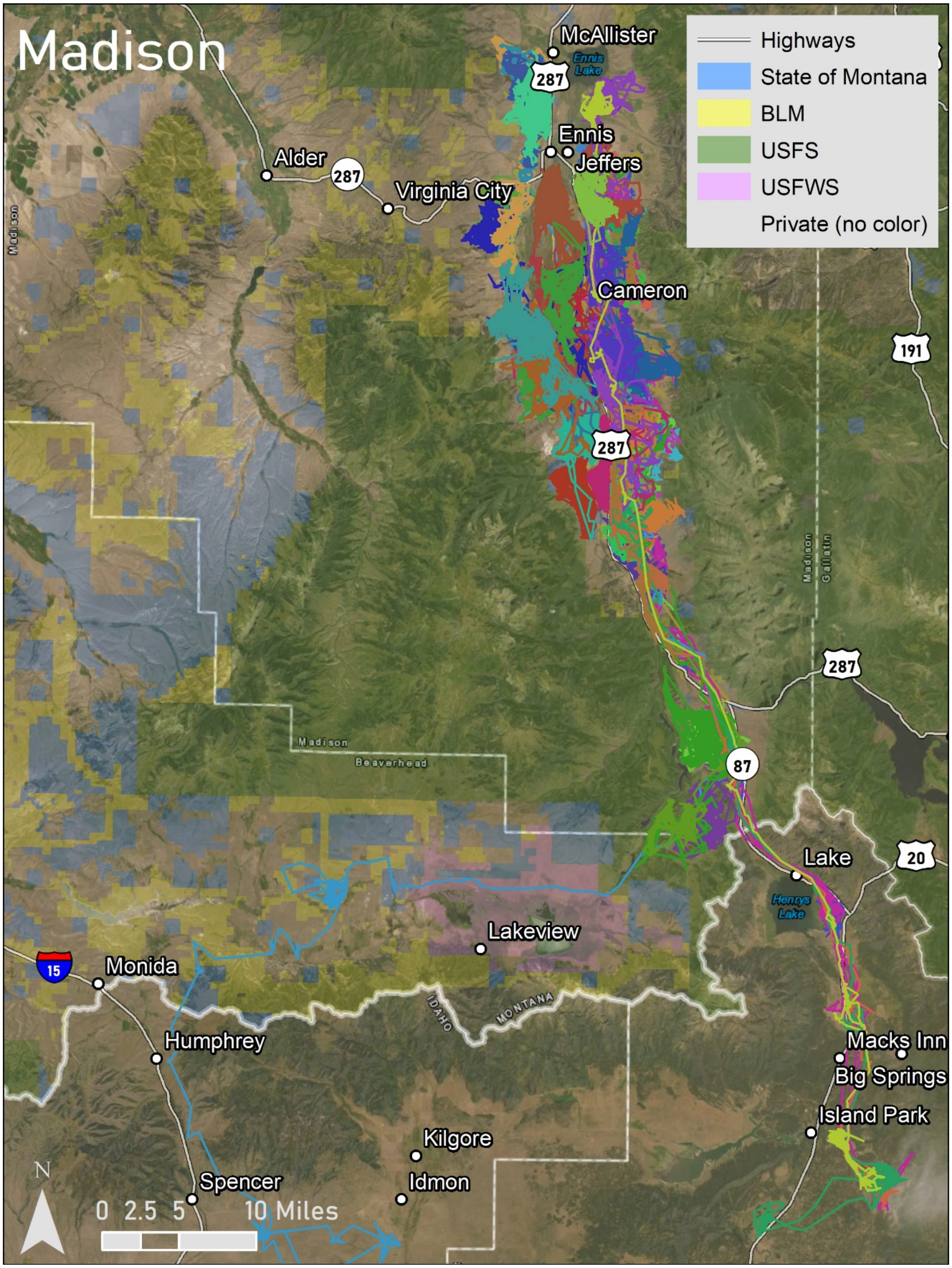


Figure 11. Movements of collared adult female pronghorn (colored by individual) in the Madison study area for the Montana Pronghorn Movement and Population Ecology Project, as of August 1, 2020.

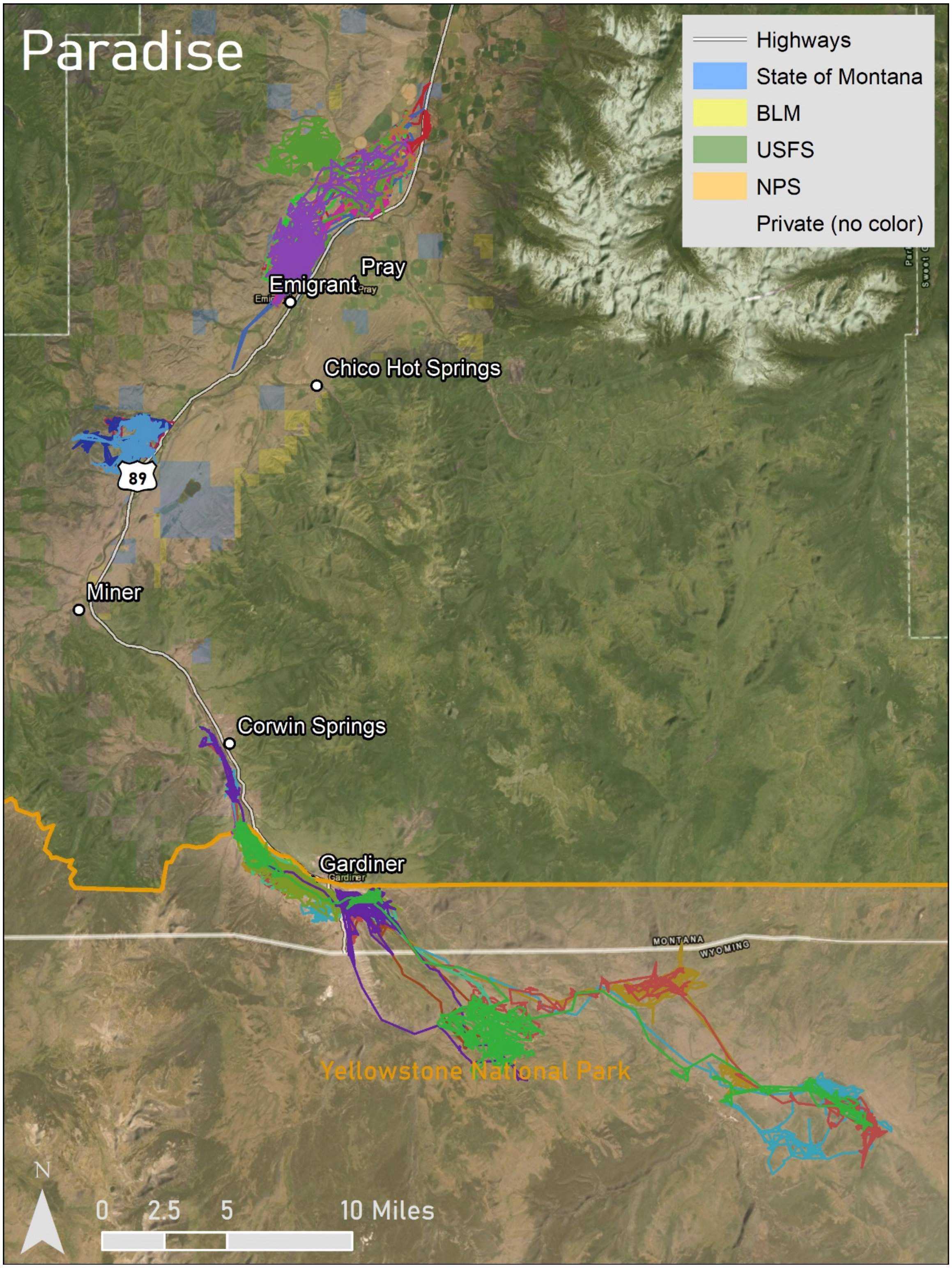


Figure 12. Movements of collared adult female pronghorn (colored by individual) in the Paradise study area for the Montana Pronghorn Movement and Population Ecology Project, as of August 1, 2020.

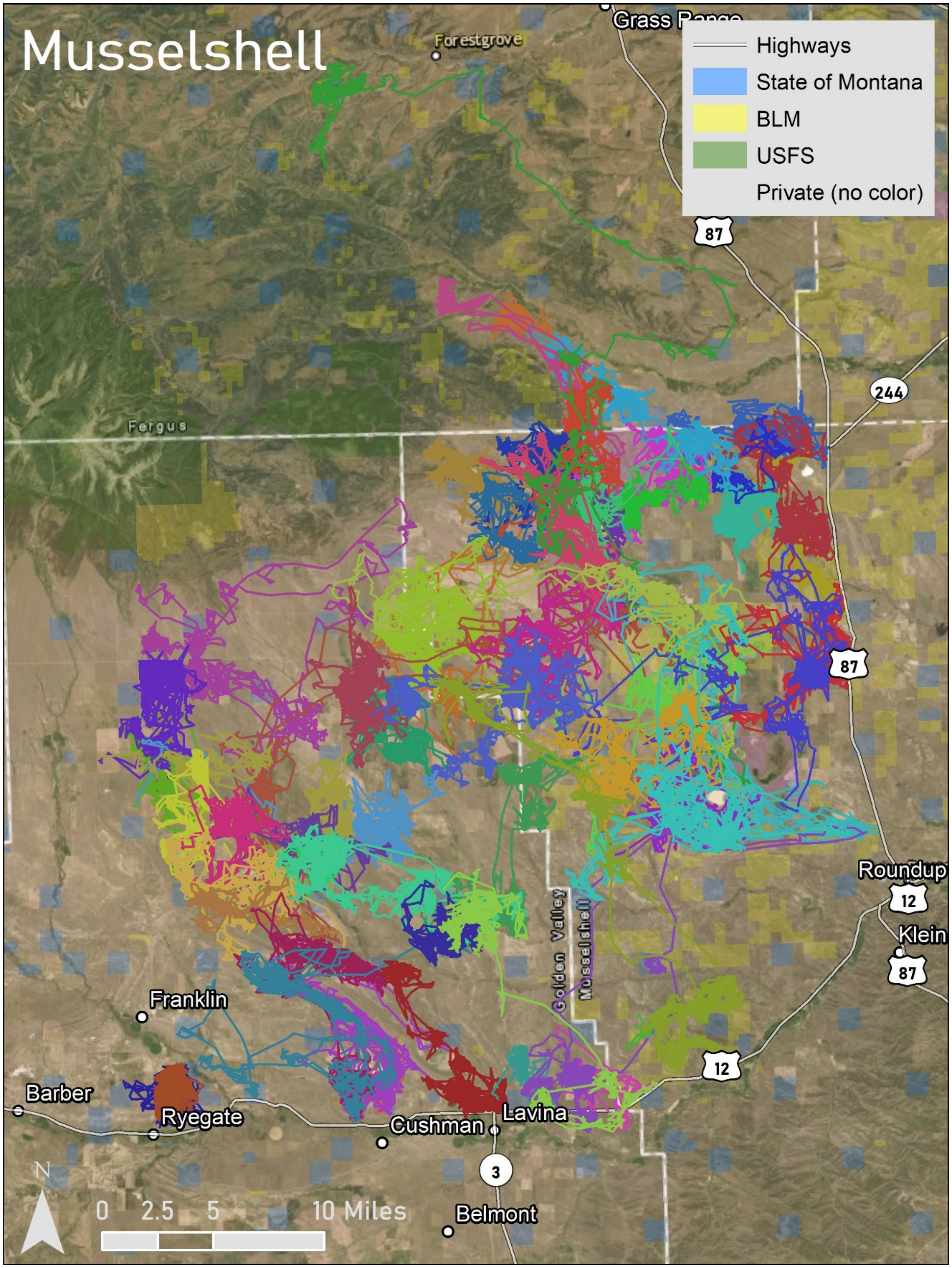


Figure 13. Movements of collared adult female pronghorn (colored by individual) in the Musselshell study area for the Montana Pronghorn Movement and Population Ecology Project, as of August 1, 2020.

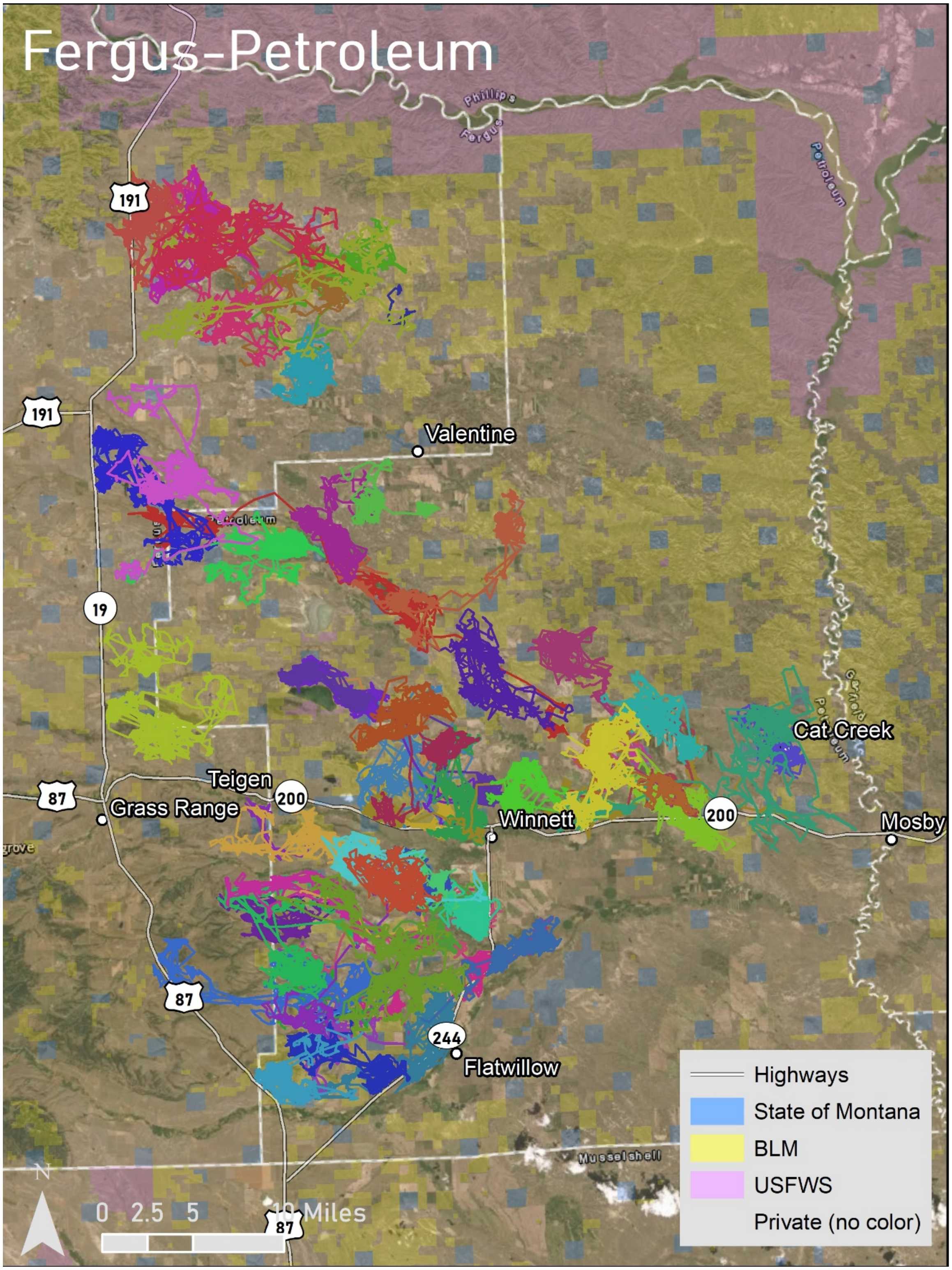


Figure 14. Movements of collared adult female pronghorn (colored by individual) in the Fergus-Petroleum study area for the Montana Pronghorn Movement and Population Ecology Project, as of August 1, 2020.

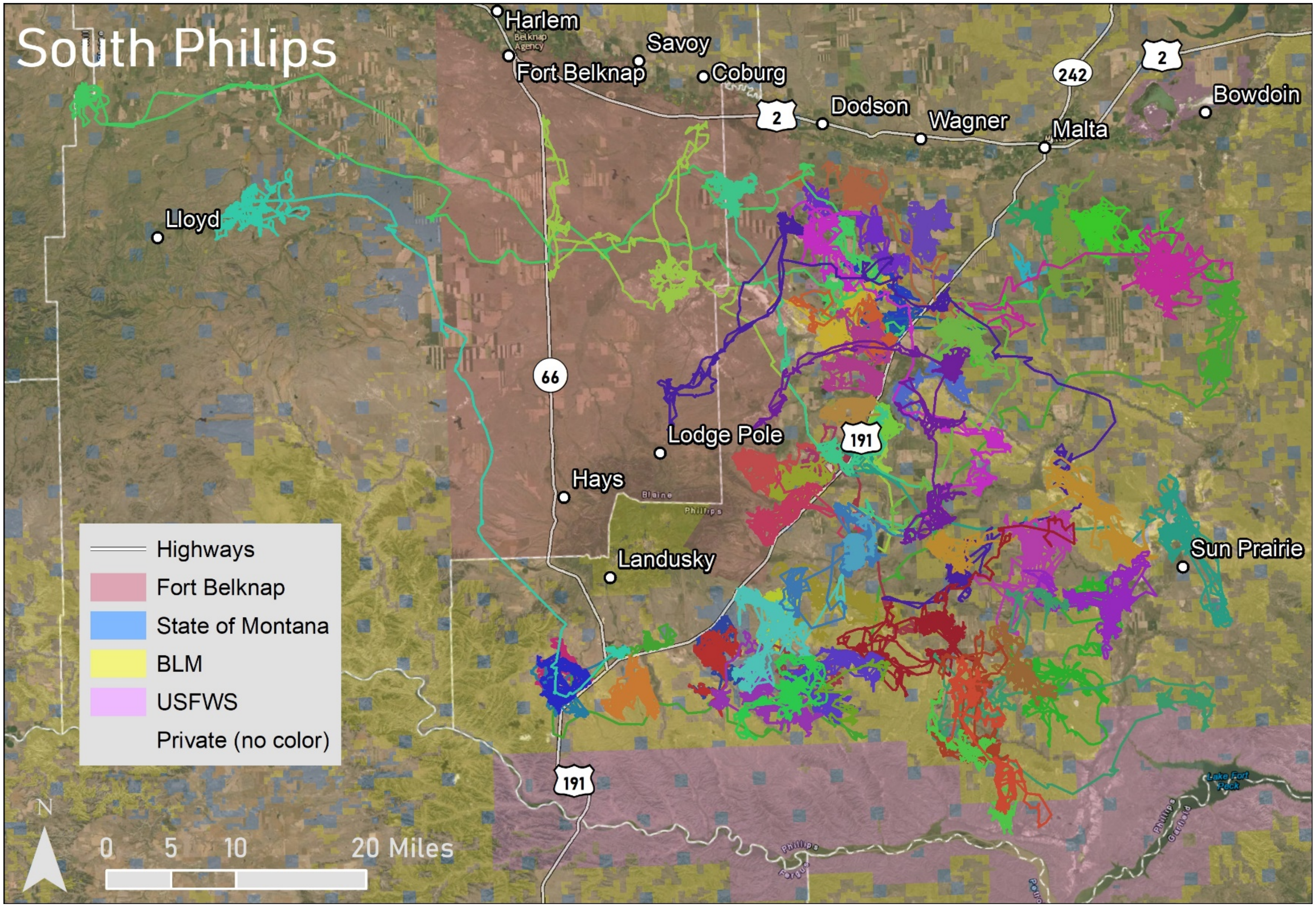


Figure 15. Movements of collared adult female pronghorn (colored by individual) in the South Philips study area for the Montana Pronghorn Movement and Population Ecology Project, as of August 1, 2020.

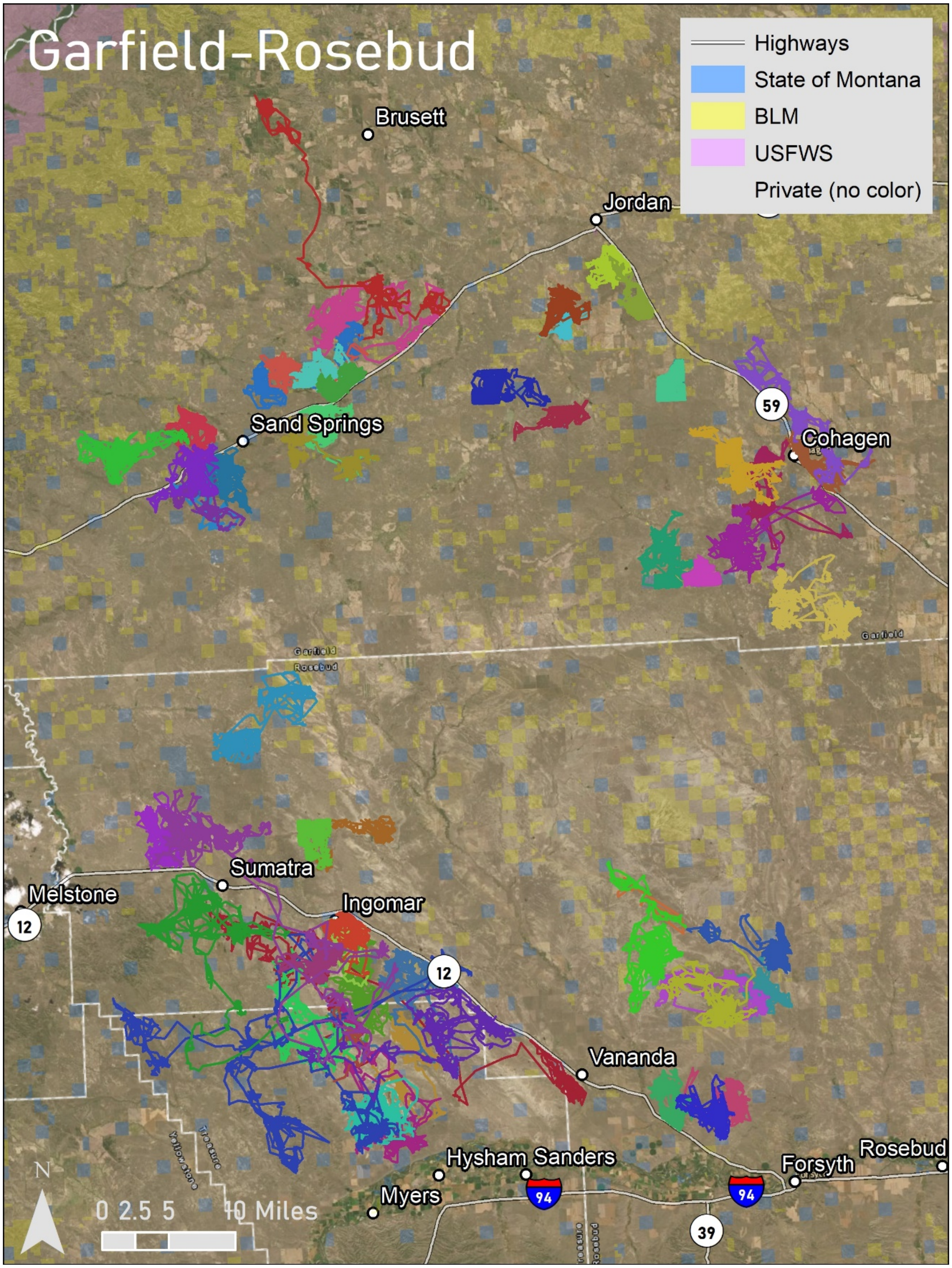


Figure 16. Movements of collared adult female pronghorn (colored by individual) in the Garfield-Rosebud study area for the Montana Pronghorn Movement and Population Ecology Project, as of August 1, 2020.

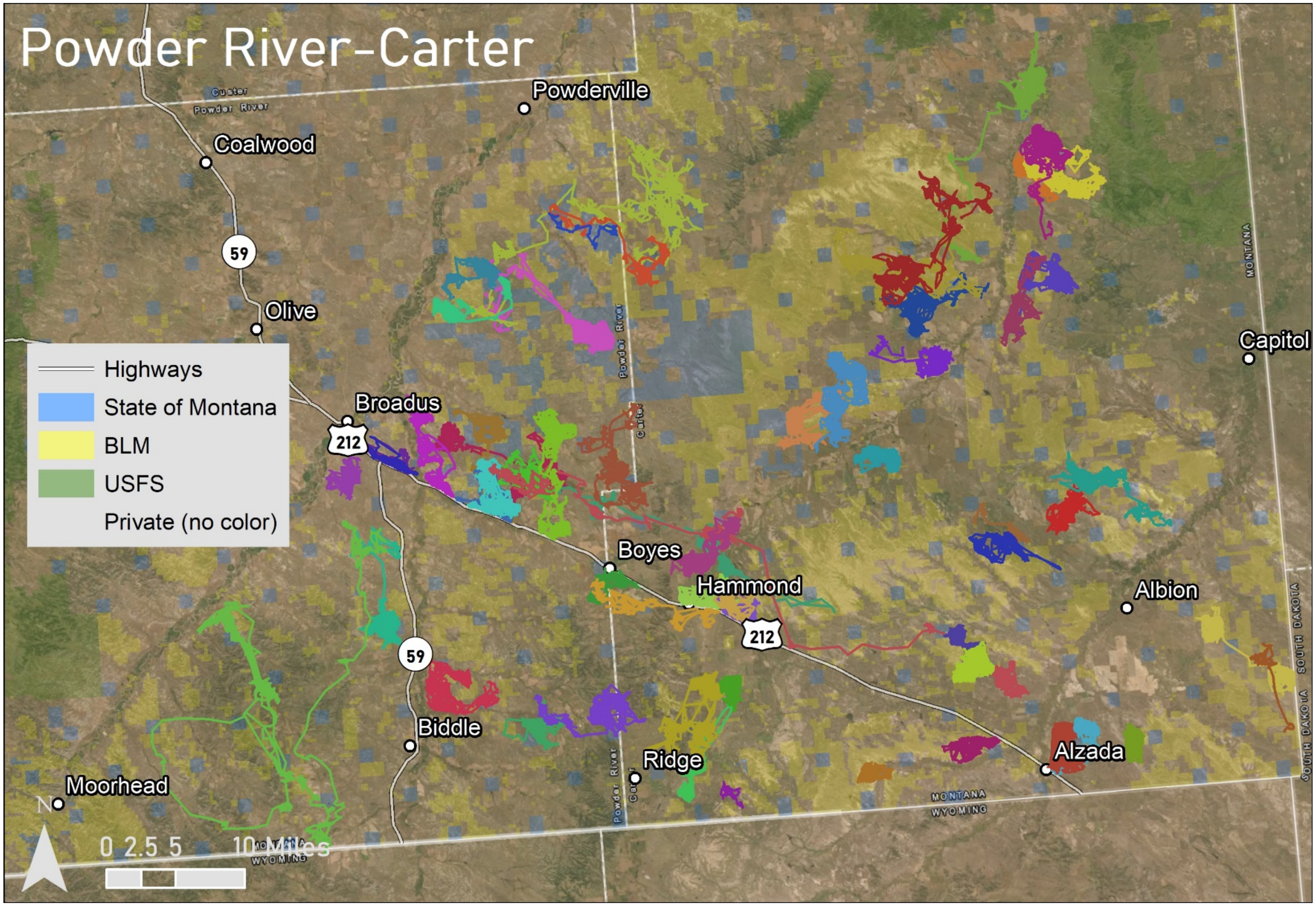


Figure 17. Movements of collared adult female pronghorn (colored by individual) in the Powder River-Carter study area for the Montana Pronghorn Movement and Population Ecology Project, as of August 1, 2020.

2.2 Identification and quantification of pronghorn responses to fences

Using the collar location data in combination with spatial information we plan to assemble on fences and fence types in the study areas, we will develop a set of tools to identify and quantify pronghorn behavioral responses to fences during winter, summer, and migratory periods. Management biologists in the Garfield-Rosebud, Power River-Carter, and South Philips study areas have initiated fence mapping projects. Information on fence location, style, and height are being collected and digitized into a GIS layer. We will use this fence mapping data together with tools that are currently being developed at University of California, Berkley (Xu, W., personal communication) to categorize pronghorn responses to fences, and then estimate how different types of fencing influence pronghorn responses to fencing. Additional methodologies may be used to quantify pronghorn responses to fences, including estimating step lengths before and after the crossing, estimating movement rates in proximity to different fences, etc. We may also consider a model-based analysis that incorporates fence style and location attributes as well as snow depth and other weather variables as covariates predicting responses to fences. After evaluating pronghorn responses to difference styles of fences, our goal will be to rank fences by occurrence/frequency of behavior types in order to identify the most "problematic" styles of fences that may impeded animal movements. This will allow for prioritization of remediation efforts across the study areas. Additionally, we may compare "modified" fences that meet current wildlife friendly standards to other types of fences to determine how modifications influence pronghorn responses to fences. Better understanding of how fence style and configurations effect pronghorn movements will inform wildlife-friendly fencing strategies and conservation efforts aimed at improving landscape permeability.

To assist in assembling fence and movement barrier data, we have developed an online platform through ArcGIS Online that allows biologists to visualize pronghorn movements and record information on potential barriers. We are currently in the beginning stages of identifying barriers to pronghorn movement and will continue to work on this as additional movement data become available.

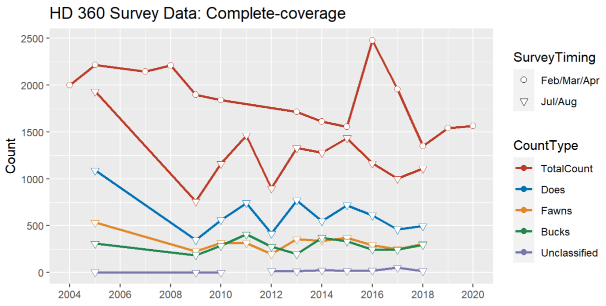
Objective #3: Initiate development of the pronghorn population model.

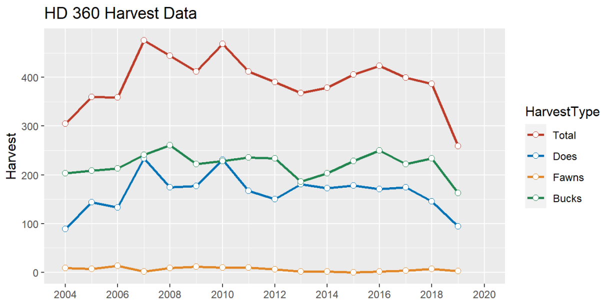
3.1 Survey protocols and data assembly

Integrated population models (IPMs) can integrate known-fate survival from marked adults, recruitment and abundance data from count and classification surveys, and harvest data to provide estimates of vital rates and improve inferences into the underlying driers of variation of these vital rates (Kéry and Schaub 2011, Schaub and Abadi 2011). Management decisions can be improved by the use of IPMs in several ways that include: sensitivity and elasticity analyses for determining the vital rate most important in driving population abundance and targeting management actions specific to that vital rate (Johnson et al. 2010a, Eacker et al. 2017); retrospective analyses for estimating vital rates (Proffitt et al. *in review*) and population abundances and assessing the factors influencing annual variability in vital rates (Paterson et al. *in press*); and prospective analyses for projecting population abundances under different management scenarios under consideration (e.g., what harvest rates increase or decrease populations by how much and over what amount of time; Johnson et al. 2010b, Mitchell et al. 2018). Integrated population models, therefore, can be a powerful learning tool that may help resource managers to understand the mechanisms driving population performance and to adapt management strategies accordingly.

Our objective is to develop a pronghorn IPM based on adult female survival from monitoring collared animals, abundance and production estimates from surveys, and harvest data collected for each study population. We will use this model to 1) identify important vital rates affecting population growth rate, 2) contrast important vital rates between populations that are considered productive vs. limited in performance, and 3) develop hypotheses to explain why some pronghorn populations experience limitations on population growth rate. The population model will provide information towards developing more focused investigations into ecological and/or anthropogenic factors limiting pronghorn population recovery in central Montana and future population monitoring strategies.

For this reporting period, our goal was to begin developing the IPM by assembling survey and harvest data for each population. We are defining populations based on hunting districts (HDs) in which captures occurred and collared pronghorn used during winter. We have assembled count and classification data from aerial and ground surveys and harvest data for each population (e.g., Figure 18) collected as part of FWP's wildlife survey and inventory program. Methods of count and classification data varied by HD. The Big Hole, Madison, and Paradise study areas are surveyed with complete-coverage surveys every 1 – 2 years. The South Philips, Garfield-Rosebud, and Powder River-Carter study areas have trend area surveys conducted every year. The Musselshell and Fergus-Petroleum study areas have complete-coverage surveys every 3 – 5 years and trend area surveys every year. We are still in the process of assembling count and classification data for these HDs.





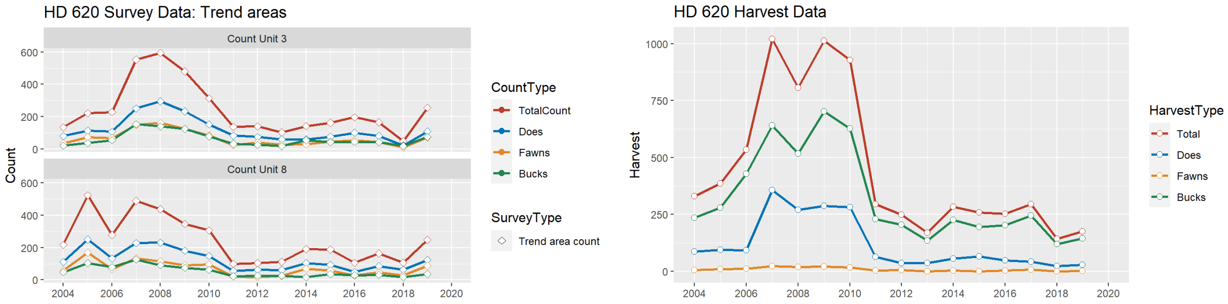


Figure 18. Examples of survey (left column) and harvest (right column) data for 2 hunting districts (HDs) encompassing study areas (HD 360 = Madison, HD 620 = South Philips) assembled for the Montana Pronghorn Movement and Population Ecology Project. Complete-coverage surveys, where the entire HD is surveyed, is demonstrated by HD 360, and trend area surveys, where areas representative of the entire HD are surveyed, is demonstrated by HD 620. Note that the data shown here may not be complete.

Literature Cited

Barnowe-Meyer, K. K., P. J. White, T. L. Davis, and J. A. Byers. 2009. Predator-specific mortality of pronghorn on Yellowstone’s northern range. Western North American Naturalist 69:186–194.

Barrett, M. W., and G. A. Chalmers. 1975. A serologic survey of pronghorns in Alberta and Saskatchewan, 1970-1972. Journal of Wildlife Diseases 11:157–163.

Berger, K. M., and M. M. Conner. 2008. Recolonizing wolves and mesopredator suppression of coyotes: impacts on pronghorn population dynamics. Ecological Applications 18:599–612.

Berger, K. M., E. M. Gese, and J. Berger. 2008. Indirect effects and traditional trophic cascades: a test involving wolves, coyotes, and pronghorn. Ecology 89:818–828.

Boccadori, S. J. 2002. Effects of winter range on a pronghorn population in Yellowstone National Park. Thesis. Montana State University, Bozeman, Montana, USA.

Brown, D. E., and M. R. Conover. 2011. Effects of large-scale removal of coyotes on pronghorn and mule deer productivity and abundance. Journal of Wildlife Management 75:876–882.

Cook, R. C., J. G. Cook, T. R. Stephenson, W. L. Myers, S. M. Mccorquodale, D. J. Vales, L. L. Irwin, P. B. Hall, R. D. Spencer, S. L. Murphie, K. A. Schoenecker, and P. J. Miller. 2010. Revisions of rump fat and body scoring indices for deer, elk, and moose. Journal of Wildlife Management 74:880–896.

Drew, M. L., V. C. Bleich, S. G. Torres, and R. G. Sasser. 2001. Early pregnancy detection in mountain sheep using a pregnancy-specific protein B assay. Wildlife Society Bulletin 29:1182–1185.

Dubay, S. A., T. H. Noon, J. C. deVos, and R. A. Ockenfels. 2006. Serologic survey for pathogens potentially affecting pronghorn (Antilocapra americana) fawn recruitment in

Arizona, USA. Journal of Wildlife Diseases 42:844–848.

Dunn, S. J., and J. A. Byers. 2008. Determinants of survival and fecundity through a population bottleneck in pronghorn (Antilocapra americana). Journal of Mammalogy 89:1124–1129.

Eacker, D. R., P. M. Lukacs, K. M. Proffitt, and M. Hebblewhite. 2017. Assessing the importance of demographic parameters for population dynamics using Bayesian integrated population modeling. Ecological Applications 27:1280–1293.

Gates, C. C., P. Jones, M. Suitor, A. Jakes, M. S. Boyce, K. Kunkel, and K. Wilson. 2012. The influence of land use and fences on habitat effectiveness, movements and distribution of pronghorn in the grasslands of North America. Pages 277–294 Fencing for Conservation. Springer New York, New York, NY.

Gray, S. S. 2013. Investigation of diseases occurring in pronghorn. Federal Aid Project W-127-R Segment 20. Big Game Research and Surveys, Texas Parks and Wildlife.

Hamlin, K. L., and R. J. Mackie. 1989. Mule deer in the Missouri Breaks, Montana: A study of population dynamics in a fluctuating environment. Montana Department of Fish, Wildlife & Parks.

Harrington, J. L., and M. R. Conover. 2007. Does removing coyotes for livestock protection benefit free-ranging ungulates? Journal of Wildlife Management 71:1555–1560.

Huang, F., D. C. Cockrell, T. R. Stephenson, J. H. Noyes, and R. G. Sasser. 2000. A serum pregnancy test with a specific radioimmunoassay for moose and elk pregnancy-specific protein B. Journal of Wildlife Management 64:492–499.

Jakes, A. F. 2015. Factors influencing seasonal migrations of pronghorn across the Northern Sagebrush Steppe. Dissertation, University of Calgary, Calgary, Alberta, CA.

Jakes, A. F., C. C. Gates, N. J. DeCesare, P. F. Jones, J. F. Goldberg, K. E. Kunkel, and M. Hebblewhite. 2018a. Classifying the migration behaviors of pronghorn on their northern range. Journal of Wildlife Management 82:1229–1242.

Jakes, A. F., P. F. Jones, L. C. Paige, R. G. Seidler, and M. P. Huijser. 2018b. A fence runs through it: A call for greater attention to the influence of fences on wildlife and ecosystems. Biological Conservation 227:310–318.

Johnson, H. E., L. S. Mills, T. R. Stephenson, and J. D. Wehausen. 2010a. Population-specific vital rate contributions influence management of an endangered ungulate. Ecological Applications 20:1753–1765.

Johnson, H. E., L. Scott Mills, J. D. Wehausen, and T. R. Stephenson. 2010b. Combining ground count, telemetry, and mark-resight data to infer population dynamics in an endangered species. Journal of Applied Ecology 47:1083–1093.

Jones, P. F., A. F. Jakes, A. C. Telander, H. Sawyer, B. H. Martin, and M. Hebblewhite. 2019. Fences reduce habitat for a partially migratory ungulate in the Northern Sagebrush Steppe. Ecosphere 10:e02782.

Kéry, M., and M. Schaub. 2011. Bayesian population analysis using WINBUGS: a hierarchical perspective. Academic Press, Inc., New York, USA.

Kingscote, B. F., and J. G. Bohac. 1986. Antibodies to bovine bacterial and viral pathogens in pronghorns in Alberta, 1983. Journal of Wildlife Diseases 22:511–514.

Kuttler, K. L. 1984. Anaplasma infections in wild and domestic ruminants: a review. Journal of Wildlife Diseases 20:12–20.

Martinka, C. J. 1967. Mortality of northern Montana pronghorns in a severe winter. Journal of Wildlife Management 31:159.

Mitchell, M. S., H. Cooley, J. A. Gude, J. Kolbe, J. J. Nowak, K. M. Proffitt, S. N. Sells, and M. Thompson. 2018. Distinguishing values from science in decision making: Setting harvest quotas for mountain lions in Montana. Wildlife Society Bulletin 42:13–21.

Montana Fish Wildlife and Parks. 2012. Big game surveys and inventory, antelope, Region 5. Statewide Wildlife Inventory, Federal Aid Project W-130-R-26. Montana Fish, Wildlife, and Parks, Billings.

Montana Fish Wildlife and Parks. 2020a. Montana harvest reports. Accessed 30 June 2020. https://myfwp.mt.gov/fwpPub/harvestReports.

Montana Fish Wildlife and Parks. 2020b. Montana statewide elk management: population and distribution. Accessed 30 June 2020. http://fwp.mt.gov/fishAndWildlife/management/elk/.

Montana Fish Wildlife and Parks. 2020c. Deer management: population and distribution. Accessed 30 June 2020. http://fwp.mt.gov/fishAndWildlife/management/deer/.

Noyes, J. H., R. G. Sasser, B. K. Johnson, L. D. Bryant, and B. Alexander. 1997. Accuracy of pregnancy detection by serum protein (PSPB) in elk. Wildlife Society Bulletin 25:695–698.

O'Gara, B. W. 2004a. Mortality factors. Pages 379–408 *in* B. W. O'Gara and J. D. Yoakum, editors. Pronghorn ecology and management. Wildlife Management Institute, Washington D.C. and University Press of Colorado, Boulder, Colorado, Boulder Colorado.

O'Gara, B. W. 2004b. Diseases and parasites. Pages 299–336 *in* B. W. O'Gara and J. D. Yoakum, editors. Pronghorn ecology and management. Wildlife Management Institute, Washington D.C. and University Press of Colorado, Boulder, Colorado.

O'Gara, B. W. 2004c. Reproduction. Pages 275–298 *in* B. W. O'Gara and J. D. Yoakum, editors. Pronghorn ecology and management. Wildlife Management Institute, Washington D.C. and University Press of Colorado, Boulder, Colorado.

O'Gara, B. W., and H. G. Shaw. 2004. Predation. Pages 337–377 *in* B. W. O'Gara and J. D. Yoakum, editors. Pronghorn ecology and management. Wildlife Management Institute, Washington D.C. and University Press of Colorado, Boulder, Colorado.

Pyrah, D. B. 1987. American pronghorn antelope in the Yellow Water Triangle, Montana: a study of social distribution, population dynamics, and habitat use. Montana Department of

Fish, Wildlife and Parks, Helena, Montana.

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

Schaub, M., and F. Abadi. 2011. Integrated population models: A novel analysis framework for deeper insights into population dynamics. Journal of Ornithology 152:S227–S237.

Stauber, E. H., R. Autenrieth, O. D. Markham, and V. Whitbeck. 1980. A seroepidemiologic survey of three pronghorn (Antilocapra americana) populations in southeastern Idaho, 1975-1977. Journal of Wildlife Diseases 16:109–115.

Thorne, E. T., E. S. Williams, T. R. Spraker, W. Helms, and T. Segerstrom. 1988. Bluetongue in free-ranging pronghorn antelope (Antilocapra americana) in Wyoming 1976 and 1984. Journal of Wildlife Diseases 24:113–119.

Webster, M. S., P. P. Marra, S. M. Haig, S. Bensch, and R. T. Holmes. 2002. Links between worlds: unraveling migratory connectivity. Trends in Ecology & Evolution 17:76–83.

White, P. J., R. A. Garrott, J. F. Kirkpatrick, and E. V. Berkeley. 1995. Diagnosing pregnancy in free-ranging elk using fecal steroid metabolites. Journal of Wildlife Diseases 31:514–522.

Wood, A. K., R. E. Short, A.-E. Darling, G. L. Dusek, R. G. Sasser, and C. A. Ruder. 1986. Serum assays for detecting pregnancy in mule and white-tailed deer. Journal of Wildlife Management 50:684.