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Understanding the Interaction Between Habitat Use of Feral Horses and the Abundance of

Greater Sage-Grouse in the Great Basin

Mikiah R. Carver

A thesis submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of

Master of Science

Steven L. Petersen, Chair Loreen Allphin Randy T. Larsen Brock R. McMillan

Department of Plant and Wildlife Sciences

Brigham Young University

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ABSTRACT

Understanding the Interaction Between Habitat Use of Feral Horses and the Abundance of Greater Sage-Grouse in the Great Basin

Mikiah R. Carver Department of Plant and Wildlife Sciences, BYU Master of Science

Environmental impacts of feral horses (Equus caballus) are a subject of conservation concern and controversial national policy. In North America, feral horses are considered an invasive species where they impact rangelands of the arid and semi-arid western United States. The greater sage-grouse (Centrocercus urophasianus) is a native sagebrush obligate bird species that relies on sagebrush habitats to sustain viable population levels. Recent literature suggests that feral horse presence can have a notable effect on the fitness of native and sagebrush obligate species throughout the arid and semi-arid western United States. The purpose of this thesis was to assess the potential impact of feral horses on population patterns and on late-brood rearing habitat of greater sage-grouse throughout the Great Basin. This was accomplished by pairing known sage-grouse use sites (leks and late brood-rearing habitat) to random sites for comparison. Within each pair, one site was located within Herd Management Area (HMA) boundaries (with assumed horse presence) while the other was located outside (with assumed horse absence). We then assessed lek attendance throughout the state of Nevada and compared attendance rates to known horse population estimates. Furthermore, paired late brood-rearing habitat sites were compared to one another to assess the effect of horse and cattle presence on habitat quality and characteristics. We determined that mean sage-grouse population size at leks is higher $(9.14 \pm$ 1.04 males) within HMA boundaries compared to areas outside of HMA boundaries (6.55 ± 0.74 males). Considering late brood-rearing habitat, we determined that statistical differences have occurred between horse and non-horse use sites in the following comparisons: annual grass frequency, percent annual grass cover, dung frequency, total plant height, vegetative height, and horse and cattle dung density. We suggest that feral horse presence can impact sage-grouse habitat, however, a more clear understanding of horse effects on rangeland wildlife habitat is needed to assess actual impacts on wildlife populations in consideration of multiple use management decisions.

Keywords: Artemisia, Centrocercus urophasianus, disturbance, Equus caballus, feral horses, grazing, late brood-rearing, lek, trampling

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CHAPTER 1

Comparison of Population Patterns of Greater Sage-Grouse (*Centrocercus urophasianus*) in Response to Feral Horses (*Equus caballus*) within the Great Basin

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ABSTRACT

Environmental impacts of feral horses (Equus caballus) are a subject of conservation concern and controversial national policy. In North America, feral horses are considered an invasive species where they impact rangelands of the arid and semi-arid western United States. The greater sage-grouse (Centrocercus urophasianus) is a bird species that relies on sagebrush habitats to sustain viable populations. Recent literature suggests that the presence of feral horses can have a notable effect on the fitness of sagebrush obligates throughout the arid and semi-arid western U.S. The purpose of this study was to assess the potential impact of federally managed feral horses on population patterns of native sage-grouse by assessing lek attendance. We used the Nevada Department of Wildlife's state-wide lek count, location, and site characteristic data to pair sites representing sage-grouse populations either inside or outside horse use areas. We separated horse use from non-use areas as those occurring within or outside Herd Management Area (HMA) boundaries, respectively. Similar habitat characteristics were identified for every location and lek sites were paired using non-metric multidimensional scaling (NMDS). To validate horse use designations and lek pairings, we conducted dung counts at 20 lek locations (n=100) throughout the study area. Using mixed model analysis of variance, we determined that

overall mean lek counts (i.e. sage-grouse male population size) were higher within HMA boundaries (\bar{x} =9.14 ± 1.04) than lek counts outside HMA boundaries (\bar{x} =6.55 ± 0.74). Contrary to our original hypothesis, mean population size of sage-grouse at leks were greater in areas where feral horses are managed (HMAs) compared to areas outside of HMA boundaries. This may be the result of several influences. First, leks are commonly found in disturbed/open areas and disturbed areas are often associated with recreation activities, fire, and domestic grazing, including feral horses. These disturbances are common within federally managed lands (HMAs) throughout the Great Basin. Second, horses likely do not utilize sage-grouse habitats evenly, suggesting that bird populations may not be impacted. Third, livestock, particularly cattle, range in both use and non-use areas and likely have a similar impact to sage-grouse as horses alone. Finally, horses are not explicitly bound to HMA areas and may impact leks outside HMA boundaries. These data can be used by rangeland managers as a broad-scale analysis of horse and sage-grouse population trends over the span of about 40 years. A better understanding of horse effects on rangeland wildlife habitat is needed to assess actual impacts on wildlife populations in consideration of multiple use management decisions.

INTRODUCTION

Invasive and feral species pose threats to native biodiversity and conservation efforts throughout the world (Andersen et al., 2004). Invasive species are known to frequently exploit resources in non-native environments as they often lack natural predators and regulation. When an invasive species successfully enters a new ecosystem, it can spread quickly and outcompete native plants and animals causing biodiversity loss and in some cases, monocultures (Goergen et al., 2011). In North America, feral horses (*Equus caballus*; hereafter, "horses") are considered an

invasive species that impact and semiarid ecosystems of western North America (Beever et al., 2018). Environmental impacts of horses are a subject of international conservation concern and controversial policy.

Following extirpation from North America at the end of the Pleistocene, horses were reintroduced by Spanish conquistadors in the mid 1500s and since then, numbers have increased by inadvertent or purposeful release and high birth rates. Today, federally managed horse populations on United States public lands exceed 95,114 horses across 10 western states (BLM Programs, 2020). Additionally, many horses also occur on tribal or reservation lands, with numbers exceeding 38,000 with an expectation to double within the next few years (Beever et al., 2018).

Populations of horses have experienced steady growth since the passage of the Wild Free-Roaming Horses and Burros Act in 1971. Expanding horse populations are often associated with degraded wildlife habitat, impaired ecosystem structure, and reduced rangeland health (Beever & Brussard, 2000; Beever, 2003; Beever & Aldridge, 2011; Garrott & Oli, 2013; Davies et al., 2014; Hall et al., 2016; Boyd et al., 2017; Beever et al., 2018; Davies & Boyd, 2019; Hennig et al., 2021). Additionally, horses differ from other domestic and wild ungulates found throughout the Great Basin both physiologically and in the way they are managed.

As hindgut fermenters, horses are potentially the least-selective ungulate grazer found in the Great Basin as opposed to their ruminant counterparts (cattle, sheep, pronghorn, elk and deer). Less selectivity and a wide home range may equate to fewer plant species remaining in horse-grazed areas compared to areas grazed by other ungulates. The use of a lower quality diet requires horses to consume 20-65% more forage than a ruminant of similar size (Beever, 2003).

Horses differ from other ungulates found in the Great Basin in the way they are managed as they are not rotationally grazed or herded as domestic animals are, nor are they hunted like big game species. Their federal protection and limitations in general population management faciliates habitat degradation in a largely unmanaged way (Beever et al., 2018). Subsequently, horses impact the structure of sagebrush communities, composition of vegetation, soils, and many native and sagebrush obligate species (Ostermann-Kelm et al., 2009; Beever & Aldridge, 2011).

Greater sage-grouse (*Centrocercus urophasianus*; hereafter sage-grouse) is a native species that relies on sagebrush habitats (i.e. sagebrush vegetation, forbs, invertebrates) to maintain healthy and viable populations. Populations of sage-grouse have generally been in decline for the past 4 decades, primarily in response to declining habitat, resource exploitation and overutilization of rangelands (Connelly & Braun, 1997; Connelly et al., 2000; Connelly et al., 2004; Crawford et al., 2004; Kaczor et al., 2011; Robinson & Messmer, 2013; Westover et al., 2016). On the other hand, predator management and bird translocation efforts with genetically compatible populations throughout the Great Basin have worked synonymously toward combating declining populations (Kohl et al., 2019).

Direct competition for forage plants and habitat disturbance associated with feral horse grazing and trampling may impact the fitness of sage-grouse through altered habitat as both horses and sage-grouse inhabit much of the same areas (Beever & Aldridge 2011). In addition to feral horse presence, improper grazing, fire, and recreation activities may impact local populations of sage-grouse. Large scale habitat continuity (i.e. percent of sagebrush dominated landscape), seasonal migrations, predator dynamics, adult hen survival, nest success, and chick survival are key factors relating to vital rates of sage-grouse and should be considered when

determining management decisions to prevent further population decline (Crawford et al., 2004; Dahlgren et al., 2010; Taylor et al., 2012; Guttery et al., 2013; Robinson & Messmer, 2013; Dahlgren et al., 2016).

During a sage-grouse's mating season, birds gather at lek sites. Leks are typically large and open sites that are sparsely vegetated to facilitate increased visibility of strutting males to females (Braun et al., 1977; Crawford et al., 2004). Leks are commonly located along ridgetops, swales, dry lake and riverbeds and other disturbed sites with little to no vegetation (Crawford et al., 2004). Anthropogenically disturbed sites (i.e. cleared roadsides, plowed fields, and burned areas) may also be used as leks by sage-grouse (Crawford et al., 2004).

Horses have been found to impact various components of the sagebrush ecosystem (Beever & Brussard, 2000; Beever, 2003; Beever & Aldridge, 2011; Davies et al., 2014; Hall et al., 2016; Boyd et al., 2017; Gooch et al., 2017). However, direct impacts of horses on the abundance of sage-grouse in regards to lek use and landscape-level population size trends is lacking in the literature (Beever & Aldridge, 2011; Hennig et al., 2021; Muñoz, 2021). Horses and livestock can degrade sage-grouse habitat and subsequently reduce population sizes as evidenced through reduced vegetation cover, declining plant height and density, soil compaction that can limit sage-grouse resources, and decreased sagebrush cover through trampling and foraging (Beck & Mitchell, 2000; Beever & Aldridge, 2011; Davies et al., 2014; Hall et al., 2016; Hennig et al., 2021).

The primary objective of our study was to assess the potential influence of federally managed feral horses on abundance of sage-grouse by assessing an index of abundance. Lek counts are used an index of abundance to assess populations of sage-grouse in a given area. We quantified the response of lek counts based on horse use throughout the Great Basin region of Nevada.

Specifically, we sought to evaluate how the presence and abundance of horses along with cattle grazing might influence the persistence of leks and the amount of sage-grouse attending lek sites. We hypothesized that horses, in conjunction with cattle grazing, may negatively influence habitat suitability for sage-grouse more than leks that are grazed by cattle alone. We suggested that lek sites with high density of ungulates (i.e. horses and cattle that exhibit high grazing and trampling) would have lower lek attendance than sites with reduced horse densities and cattle grazing only. This lower lek attendance might be a reflection of degraded sage-grouse habitat and lower grouse populations compared to similar sites without high horse densities.

MATERIALS AND METHODS

Study Area

Our study area occurs among sage-grouse occupied sites throughout the Great Basin region of Nevada (Figure 1.1). This area is characterized as a temperate desert with cold precipitous winters and hot, dry summers. With several mountain ranges and valleys, it is home to diverse ecosystems ranging from sagebrush dominated lowlands to alpine forests. Habitat within the study area are dominated by sagebrush and wet meadow plant communities. Dominant shrub species include Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*), mountain big sagebrush (*A. tridentata* ssp. *vaseyana*), low sagebrush (*A. arbuscula*), and black sagebrush (*A. nova*). The elevation of these areas range from approximately 1676 to 2014 m with a mean annual precipitation that ranges from 13.00 to 35.50 cm, mostly occurring as snowfall in the winter months. Temperatures during the summer months can reach as high as 42.20°C and as low as -12.80°C in the winter (U.S. Climate Data, 2020).

Nevada has the highest concentration and density of horse populations in the U.S., with an estimated total of 46,974 horses which is 59% of the total feral horse population within HMAs in the U.S. (BLM Programs, 2020). Additionally, this area also supports large, intact stands of suitable sage-grouse habitat, including areas with high overlap with horses inside of herd management areas. Horses are primarily managed by the Bureau of Land Management (BLM) with the aim of achieving a "thriving natural ecological balance" (The Wild Free Roaming Horses and Burros Act of 1971). To maintain this, efforts are made to regulate population sizes with fencing projects and feral horse removal programs. However, impacts from horses are still evidenced through degraded upland and riparian ecosystems, altered soil structure, and impaired wildlife habitat (Beever & Aldridge, 2011; Davies et al., 2014; Hall et al., 2016).

Data Acquisition

We selected 100 study sites throughout Nevada's sagebrush steppe that exhibits horse and sage-grouse habitat, delineated from known lek sites and Herd Management Area (HMA) boundaries. Sites were selected through a vetting process to ensure each pair had one site within HMA boundaries and the other located outside HMA boundaries. This allowed for a more straightforward comparison between HMA sites and non-HMA sites. To manage the populations of these two species, both sage-grouse and horse densities are estimated from annual lek and HMA counts, respectively. State, federal, and private agencies typically have individuals assigned to conduct lek and horse counts each year. The BLM conducts the yearly HMA population estimates while state wildlife divisions typically update statewide lek count databases. A summarized Nevada HMA estimate table can be found in the Supplemental Material-Appendix 1 (Appendix Table 1). To determine population patterns of sage-grouse, we acquired

the state of Nevada's historical lek count dataset from the Nevada Department of Wildlife (NDOW). These data included lek center coordinate location and annual male lek counts for all known leks throughout Nevada ranging from years 1900 to 2019. In many cases, detailed notes were included by individuals who conducted the lek counts that indicated if horses were present in the area during individual lek counts. A summarized data table with all our study sites and their averaged lek counts can be found in Supplemental Material-Appendix 1 (Appendix Table 2).

To address our objective of determining the potential influence that federally managed feral horse presence has on sage-grouse attendance at lek sites in the Great Basin, we compared horse presence to non-horse presence on lek counts (which were used as an index of abundance of birds), using known lek sites that occur within HMA boundaries and pairing them with lek sites that were located outside of HMA boundaries. We identified lek and horse use sites that consisted of similar habitat characteristics (i.e. topography, dominant vegetation, and climate). We used these data to characterize population densities and trends for horses, cattle, and sagegrouse, and used survey results of dung counts to assess cattle and horse use at each site.

Site Pairing

We identified the potential influence of horses and cattle on populations of sage-grouse by pairing similar lek sites where horses were assumed to occur (within an HMA) and lek sites where horses were predicted to be absent (outside an HMA). To pair leks we used Non-metric Multidimensional Scaling (NMDS) in RStudio, Vegan Package (Oksanen et al., 2019; R Core Team, 2020). To minimize confounding factors due to abiotic variables, we paired sites with similar dominant vegetation, elevation, known horse presence and lek proximity to HMA

boundaries. In the process of selecting similar sites, we used a 30 m statewide digital elevation model (DEM), Landfire vegetation data, HMA boundaries (and subsequent lek counts) and lek GPS locations to generate site similarity for pairs. We created a 5 km buffer around each lek using ArcGIS Pro to incorporate crucial habitat and their respective attributes for analysis (ESRI, 2020). This buffer size was selected as most sage-grouse stay within approximately 3-5 km from a lek for most life stages (Braun et al., 1977; Wakkinen et al., 1992; Atamian et al., 2010; Robinson & Messmer, 2013).

To identify site similarity, we used 53 different Landfire cover types (Landfire, 2014) and their respective percentage of vegetation cover for the area within the 5 km buffer surrounding the lek. By taking into account the different cover types and their respective percentage of cover within the area surrounding a lek, we were able to account for heterogeneity and homogeneity of sites which enabled confident pairing. We paired 100 lek sites (50 pairs in total) for this analysis. To verify the viability of our site pairings (horse/non-horse) and to quantify the presence or absence of horses at the remotely paired sites, we conducted ungulate dung pile density counts at 20 random lek pairings found within the study area (10 total pairs).

To reduce error in study site pairing and selection, we omitted several lek sites with known horse presence from the original dataset that were located outside HMA boundaries due to the lack of population estimates associated with the areas. By cleaning out the non-HMA horse sites, we focused our comparisons solely on HMA related horse sites to non-horse sites (outside HMA boundaries).

Estimating Horse Density: Pellet Group and Dung Pile Analysis

In addition to feral horses, other domestic and wild herbivores inhabit the sagebrush ecosystems within our study area. The combined herbivory effect can include reduced plant cover and in cases a loss in habitat connectivity through habitat deterioration and fire (Connelly & Braun, 1997). Throughout this analysis, we addressed possible impacts from horses but did not explicitly deem them as the causal factor attributing to the overall decline in sage-grouse. We focused on horse impacts on sage-grouse habitat because of their high population densities and physiological ability to alter vegetation communities. However, we also included the potential influence of cattle on habitat condition.

To address this we measured cattle dung piles using the same methods that we used to assess horse use. It can be difficult to parse out horse impacts from other factors on a multiple use range, therefore, this study focused on horse presence as a potential contributing factor causing sage-grouse decline but we also considered other factors that could be contributing to decreased populations. These factors include domestic livestock grazing (primarily cattle), wildlife grazing and trampling, predator abundance, fire, translocated bird behavior, and anthropogenic influence through fragmentation, recreation and infrastructure (Connelly & Braun, 1997).

Dung pile counts and analysis have been found to be effective in determining ungulate use and density (Neff, 1968; Zabek et al., 2016). Thus, we used dung pile counts to quantify horse use in relation to other ungulates occurring at our paired study sites and to verify ungulate presence and absence at our remotely paired sites. Dung pile counts were conducted using methods similar to those used by David et al. (2007), Zabek et al. (2016) and Ahrestani et al. (2018). We randomly chose 10 of the 50 paired sites to visit and assess ungulate use.

At each of these locations, we sampled at the lek center (based on NDOW provided coordinates) and at 4 other randomly selected positions located within 1 km of each lek. At each random position, we implemented a wagon-wheel sampling design (3-100 m transects extending out from a given point 120° from each other) to count total dung piles and pellet groups by ungulate species. A predetermined bearing gave us one of 3 directions for the first transect (the other two were based on the first +/- 120°). We counted all ungulate dung within a 5 m radius (0.02 acre) plots at 10 m intervals along a 100 m transect. This resulted in 10 plots per transect, 30 plots per point and 150 plots per site. Due to the irregular amount of fecal matter in defecations, concentrations of fecal matter were determined to be dung piles after inspecting relative numbers, location, direction, and distance to other dung piles.

Dung freshness, as an indication of relative age, was determined by color and softness. Due to the special interest in horse and cattle influence on the landscape, horse and cattle dung were counted and given an age classification based on freshness: (1: fresh, 2: moderate- some weathering up to one year old, and 3: old- much weathering). Fresh piles (1) were categorized as dark or green, and moist with some insect presence. Moderate piles (2) were categorized as desiccated and dark in color. Old piles (3) were categorized as predominantly yellow or white in color, desiccated and breaking down. (Figure 1.2).

Dung piles were counted and identified to obtain an estimate of the horse use in these areas (Neff et al., 1968; Zabek et al., 2016). We also recorded all other ungulate dung (cattle, deer, pronghorn, sheep, and elk) to account for resource utilization. Due to the difficulty in determining dung pile numbers in horse stud piles, stud piles were treated as a single deposit (Zabek et al., 2016). Based on our data collection, cattle and horse use was represented both temporally and spatially throughout our study sites. Changes in habitat structure generally

require substantial time to occur, therefore, current or recent ungulate density may not be related to the cause of habitat structural change over time. The availability of historical data is valuable for a retrospective study, subsequently, we obtained records from the BLM of HMA horse counts over time and used this as a correlate with lek count values.

Statistical Analyses

Lek sites were paired using NMDS in RStudio, Vegan Package (Oksanen et al., 2019; R Core Team, 2020). The output produced a dissimilarity matrix which was used to pair the most similar sites within our dataset. Once lek sites were paired, we analyzed sage-grouse lek attendance estimates by comparing horse use to non-use areas. A mixed model analysis approach blocking on lek pairs was used to evaluate the difference between horse use and non-use areas. Our data were log transformed and analyzed due to skewness associated with original data. Tukey adjusted tests were used for all pairwise comparisons to obtain adjusted p-values and account for issues associated with multiple comparisons. We identified significance as α <0.05. This was a test of total effects as well as change over time in sage-grouse estimates within our study area. The data analysis for this paper was generated using SAS v.9.4 software. Copyright, SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS institute Inc., Cary, NC, USA.

RESULTS

Of the 10 paired sites that we sampled, the difference between horse and non-horse use sites were relatively consistent with a few exceptions. Five of the 10 non-horse sites that we visited had some horse presence. The mean horse dung piles at each of the five non-horse sites were \bar{x} =1.73 ± 1.98, \bar{x} =0.07 ± 0.26, \bar{x} =5.20 ± 5.13, \bar{x} =1.53 ± 2.20, and \bar{x} =14.67 ± 4.51. Three of the five sites had relatively low signs of horse presence (\bar{x} =1.73 ± 1.98, \bar{x} =0.07 ± 0.26, \bar{x} =1.53 ± 2.20), while the other two sites showed medium to high horse use (\bar{x} =5.20 ± 5.13 and \bar{x} =14.67 ± 4.5) (Table 1.3). Horse dung freshness at each of our sites were predominantly categorized as a 2 or 3 (2: moderate- some weathering up to one year old, and 3: old- much weathering). None of the non-horse sites had any sign of fresh horse dung (categorized as 1). Based on our field data, we noticed that cattle use was present at each of the 20 sites (Figure 1.3). Of all the lek sites we included in this study, counts ranged from 0-179 birds. The mean lek count across all the study sites was 7.24 ± 1.03 birds with a median of 9.97 birds.

We compared each decade with horse presence or absence (HMA or non-HMA) to test for significance on lek counts. The initial model we used tested decade and horse use and their interaction. The interaction between decade and horse use was not significant (F_{3, 152}=0.30, p=0.83). Additionally, we determined that the leks occurring within HMA boundaries had a higher mean lek count (\bar{x} =9.14 ± 1.04) (i.e. population estimate of male sage-grouse) than leks located outside of HMA boundaries (\bar{x} =6.55 ± 0.74) with assumed limited horse presence, but the comparison was not significant (F_{1,98}=3.62, p=0.06) (Table 1.1; Table 1.2).

To determine temporal patterns in lek use over time, we compared lek counts between the years 1980 and 2019. We parsed the historical lek count dataset up into four decades (1980's, 1990's, 2000's, and 2010's) due to missing annual lek count data for several lek sites. We determined a mean of 9.14 ± 1.04 males across all years at leks within HMAs compared to a mean lek population of 6.55 ± 0.74 males at sites outside of HMAs (Table 1.1). At most sites, lek counts were conducted more frequently during the 2000's and 2010's than they had been in

previous years (i.e. 80's and 90's). Subsequently, we did not see a more rapid decline in populations of sage-grouse in HMA areas compared to areas where horses are absent.

We observed a non-significant interaction between horse presence in HMAs and sage-grouse lek attendance. However, throughout the four decades, mean sage-grouse densities have fluctuated and these data indicate a negative overall statistical trend (Figure 1.4, Table 1.1). The test for differences in decades was suggestive (F_{3,155}=8.25, p<0.01). There were significant differences between the 1980s and 1990s (t-value=2.90, adjusted p=0.02), 1980s and 2000s (tvalue = 4.27, adjusted p<0.01), and 1980s and 2010s (t-value=4.89, adjusted p<0.01) where bird populations decreased over time. The other adjusted p-values ranged from 0.32 to 0.85 (Table 1.2). When dung counts were compared across sites, there were 3.22 times as much horse than cattle dung in horse sites than in the non-horse sites (F $_{1,13}$ =11.12, p<0.01) and 3.76 times as much cattle than horse dung in non-horse sites than in horse sites (F $_{1,15}$ =6.84, p=0.02).

DISCUSSION

Populations of sage-grouse throughout the range of the species have generally been in decline for the past 4 decades primarily in response to declining habitat, resource exploitation and over utilization of rangelands (Connelly & Braun, 1997; Connelly et al., 2000; Connelly et al., 2004; Crawford et al., 2004; Kaczor et al., 2011; Robinson & Messmer, 2013; Westover et al., 2016). Within our study area, we obtained support of a similar overall negative trend of sage-grouse populations in Nevada (Figure 1.3; Table 1.1). However, these trends could be influenced by the lek count data used for this analysis. We parsed the historical lek count dataset up into four decades (1980's, 1990's, 2000's, and 2010's) because there were lek sites with some annual lek count deficiencies within the dataset. Counts were not uniformly conducted throughout all

the years at all of the known lek sites. Furthermore, lek counts are simply an index for identifying population and do not directly measure population size and are often conducted quickly. The time restraint associated with conducting several lek counts in a given morning could produce slightly inaccurate count estimates. Additionally, the probability of finding a lek and routinely conducting lek counts improves if it is relatively easy to access. This could lead to roadside bias and suggest that unknown remotely located leks may show different trends. There are many factors that may have influenced the data obtained in this dataset, however, frequent lek counts have been suggested to be the best index of abundance when looking at populations of sage-grouse (Monroe et al., 2016).

Ideal habitat for sage-grouse includes large sagebrush stands, tall and dense grasses, and high plant and insect species richness (Connelly et al., 2000; Crawford et al., 2004; Dobkin et al., 2008; Beever & Aldridge, 2011). Indirect effects of horses and other ungulates on sage-grouse may include reduction of insect availability, herbaceous understory and sagebrush cover by trampling mortality of sagebrush seedlings (Turner, 1987; Augustine & McNaughon, 1998; Connelly et al., 2000; Beever, 2003; Beever & Herrick, 2006; Boyd et al., 2017; Danvir, 2018; Davies & Boyd, 2019). Furthermore, direct competition for forage plants and habitat disturbance associated with excessive grazing (Dobkin et al., 2008) and trampling may impact the fitness of sage-grouse through altered habitat as they both inhabit much of the same areas (Beever & Aldridge 2011; Davies & Boyd, 2019). In addition to feral horse presence, improper grazing (Connelly et al., 2000; Crawford et al., 2004), fire (Connelly et al., 2000), anthropogenic recreation/development (Connelly et al., 2000) and bird translocations may impact local grouse populations.

Several studies have illustrated the impact of horses on their environments (Ganskopp & Vavra, 1986; Turner, 1987; Beever & Brussard, 2000; Beever, 2003; Beever & Herrick, 2006; Beever et al., 2008; Ostermann-Kelm et al., 2009; Beever & Aldridge, 2011; Davies et al., 2014; Hall et al., 2016; Boyd et al., 2017; Gooch et al., 2017; Danvir, 2018; Davies & Boyd, 2019; Hennig et al., 2021). Horses have been found to degrade sage-grouse habitat as a measure of lowered total vegetation cover, decreased sagebrush cover, reduced plant height and density, and higher soil compaction, all likely stemming from extensive foraging and trampling (Beever & Aldridge, 2011; Davies et al., 2014; Hall et al., 2016). Although there is ample research addressing impacts of horses on their environments, studies that characterize the direct impact of horse presence on sage-grouse lekking activity is minimal (Muñoz et al., 2021). We did not directly measure habitat characteristics for this study but rather population differences associated with paired sites by utilizing lek count estimates which is an index of abundance.

Contrary to our original hypothesis, our results suggested that mean sage-grouse population size at lek sites is greater in areas where feral horses are managed by the BLM compared to areas outside of HMA boundaries. The difference in mean bird counts between horse sites (9.14 ± 1.04) and non-horse sites (6.55 ± 0.74) is less than 3 birds and while close to being significant, it is arguably not biologically meaningful. A mean difference of 2-3 birds at each of these sites is not particularly helpful information. For instance, if we saw a mean difference of 9 birds or more at these sites, it might tell us that there truly is a significant difference between the sites. These results could be indicative that at this scale, horses do not show significant impacts on sage-grouse populations or the presence of cattle at the non-use sites confounds the true effect.

Muñoz et al. (2021) concluded that the probability of feral horses disrupting sage-grouse lekking activity was >75% and that non-native ungulate presence (horses and cattle) disrupted

lekking activity more than native ungulates. Their results are consistent with Beever (2003) who suggested native ungulates had a lesser effect on the sagebrush ecosystem than non-natives (horses and cattle). Muñoz et al. (2021) looked at 14 locations over the span of 6 years throughout the Great Basin while we looked at a 100 different lek sites with data dating back to the 1980's.

In comparison to these studies that assess the impact of feral horses and cattle on lek counts, our broad-scale approach may not identify these impacts, or suggests that impacts from horses observed at one particular area may not have the same influence on bird populations at different locations. Within our paired sites, cattle presence likely played a role in the impact that herbivory and trampling can have on sage-grouse lekking and even more importantly on sage-grouse habitat quality (Beever, 2003) (Figure 1.3). A possible explanation addressing why our results differed from Beever (2003) and Muñoz et al. (2021) may be because horse grazing alone was not as detectable as combined ungulate grazing.

We visited 20 random lek sites throughout our study area. Of the sites we visited, five of the 10 horse-absent sites showed some horse presence (Table 1.3). Based on their close proximity to several HMA boundaries, horses were able to more easily access these areas than those further away from HMA designated areas. Horses are not explicitly bound to HMA boundaries and we can expect a large population of horses to be present outside of HMA boundaries. Although horse dung counts were noticed, they were relatively low in comparison to most horse-use sites with one exception at site "MON-077" that had a mean horse dung count as high as \bar{x} =15.67 ±4.51 which was comparable to the other horse sites (Table 1.3). Furthermore, we did not come across any "fresh" horse dung at any of those five sites, suggesting they were not recently occupied by horses and thus were still relatively valid pairs to their horse counterparts. It is also

possible that some of the horse dung we came across came from domestic horses as domestic livestock occupy many of these areas as well.

Overall, our horse use sites all showed evidence of horse presence while most of our non-use sites were predominantly used by cattle. Cattle were consistently using all of our sites. Mean horse dung at the horse use and non-use sites were 11.97 ± 1.21 and 3.72 ± 1.34 , respectively. Mean cattle dung at the horse use and non-use sites were 2.28 ± 1.40 and 8.58 ± 1.47 , respectively (Figure 1.3). Based on the ungulate use from our study, more horses in an area often equate to fewer cattle and vice versa. Regardless of the site, horse or cattle use was notable. Ungulate usage throughout each of our sites seemed relatively consistent suggesting our results may be indicative of an overall ungulate issue and not explicitly a horse issue. With our results suggesting that lek counts are higher in HMA boundaries than outside, it may be a result of less cattle, and not more horses. Land managers adjust livestock numbers based on vegetation and soil characteristics of an area. Based on the presence of ungulates, if horses are not using an area, cattle or other ungulates will most likely utilize the area (i.e. adjustments to AUM allocations within grazing allotments). We know the horse population is well above the carrying capacity for the western United States. When we compound high livestock numbers in addition to horse use, the result can be an overstocked range and extensive competition for scarce resources by the combined use of all grazers. From these results, we recommend that both horse and livestock use be monitored carefully to prevent overstocking and reduce negative impacts on rangeland ecosystems. Additionally, our analysis raises a concern that the overall combined effect of ungulate grazing could potentially have a negative impact on native sagebrush obligate and facultative species. Populations of horses and cattle may all need to be reassessed when

determining stocking rates, particularly if our management goal is to improve habitats and the species that depend on these ecosystems such as sage-grouse.

Cattle use was higher in non-horse use areas and should be considered when making management decisions and designating land for grazing permits. If cattle and horse grazing occurs simultaneously at a given site, birds and other wildlife may have a more difficult time dealing with the combined grazing pressures. Furthermore, our results do not mirror those of other studies (Muñoz et al. 2021, Beever, 2003), as our data suggest that over 4 decades, mean sage-grouse densities at leks within HMA boundaries are higher (9.14 \pm 1.04 males) than leks outside HMA boundaries (6.55 \pm 0.74 males) (Table 1.1). However, disturbed areas are often characteristic of lek sites and may explain the higher lek counts in horse areas. Comparing our results to previous studies can be difficult due to differences in research questions and methodologies.

Several confounding factors could influence these numbers including natural and human related disturbance (i.e. fire) and livestock grazing. When interpreting population trends, it is also important to consider current and past conservation efforts (i.e. translocated sage-grouse populations, horse roundups, and potential fencing that may omit large ungulates from critical sage-grouse habitat). Furthermore, horses likely utilize HMA habitat inconsistently leading to heavily underutilized areas for sage-grouse to lek largely undisturbed. More investigation into other habitat (i.e. brood-rearing, nesting, etc.) may highlight different patterns than what we have found at lek sites. Further analysis should be done to assess the potential influence of horse and cattle presence on sage-grouse lek populations, to include habitat use patterns where these species coexist. This would assist in understanding if negative population trends of sage-grouse

are in fact related to non-native ungulate presence or something different entirely; since at a broad scale, conclusions tell a slightly different story compared to more fine-scale studies.

CONCLUSION

Overall, we determined that mean sage-grouse population size at leks is higher within HMA boundaries compared to areas outside of HMA boundaries. This is contrary to our original hypothesis where we predicted that sage-grouse lek populations would be greater in areas outside HMAs than areas inside HMAs. We predicted increased horse presence associated with HMA boundaries would decrease suitability of sage-grouse habitat. Lek sites may have a higher mean of birds per lek within HMAs because lek sites are commonly found in open and disturbed areas or because there may be less cattle presence. This study only assessed lek sites and not other sage-grouse habitat which commonly requires different characteristics than leks. Therefore, these results may not be directly applicable to other sage-grouse life history stages (i.e. nesting, brood-rearing, etc.). However, successful leks typically reside near appropriate nesting habitat as hens often stay within an approximate 5 km perimeter from the lek when nesting (Atamian et al., 2010). Therefore, more research should be conducted that focuses on brood-rearing habitat quality surrounding successful and historic lek sites.

At most of our lek sites, lek counts were conducted more frequently during the 2000's and 2010's than they were in the 1980's and 1990's. We also determined an overall negative population trend for sage-grouse throughout the decades included in our analysis. Our results could be indicative that at this scale horses do not show the suggested impacts on sage-grouse. This could be a reflection on how lands are managed at the landscape scale. Furthermore, other factors could be influencing these results including alternative grazing pressures, effects of fire,

plant community succession and predation pressures, all of which are outside the influence of horse and cattle grazing alone.

Management Implications

This study provides range managers with a large-scale analysis of horse and sage-grouse population trends over the span of about 40 years. Recent literature shows that horse presence has a notable effect on native and sagebrush obligate species fitness throughout the arid and semi-arid western US. Because horse management has been scrutinized for years, it is important to identify the true impact of horses on their arid environmental components. Further clarification would allow for making more specific management decisions and minimizing strategies that are based on a more emotional basis. Because horses are considered an invasive species and are impacting the arid western United States as such, it is important for researchers to identify the lasting impact they have on their habitat to make appropriate management decisions. If more research suggests horses are increasingly negatively impacting their surroundings, more roundups and subsequent adoptions may need to occur to reduce populations down to their appropriate management levels.

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FIGURES

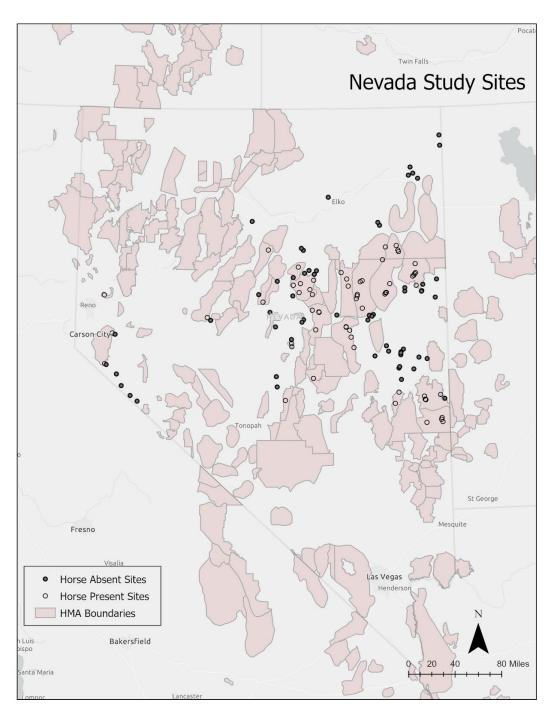


Figure 1.1: Paired study sites representing active greater sage-grouse leks in areas within and outside of herd management areas (HMAs). All study sites were located in Nevada, USA.

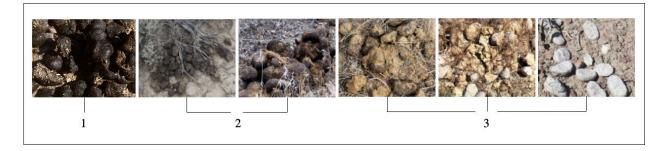


Figure 1.2: Age classification of horse dung piles based on freshness: (1: fresh, 2: moderatesome weathering up to one year old, and 3: old- much weathering). Fresh piles (1) were categorized as dark or green, and moist with some insect presence. Moderate piles (2) were categorized as desiccated and dark in color. Old piles (3) were categorized as predominantly yellow or white in color, desiccated and breaking down.

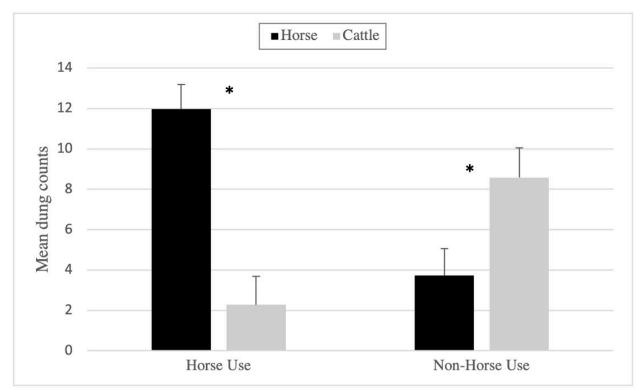


Figure 1.3: Comparison of mean horse and cattle dung data that was collected at the horse use and non-use sites in Nevada, USA in 2020. Horse use sites were located within HMA boundaries, non-horse use sites were located outside HMA boundaries with an assumed reduction in horse presence. Mean horse dung at the horse use and non-use sites were 11.97 ± 1.21 and 3.72 ± 1.34 , respectively. Mean cattle dung at the horse use and non-use sites were 2.28 ± 1.40 and 8.58 ± 1.47 , respectively. We identified significance as p<0.05 and p-values for the horse and cattle dung at the horse use and non-horse use sites were p<0.01 and p=0.02, respectively.

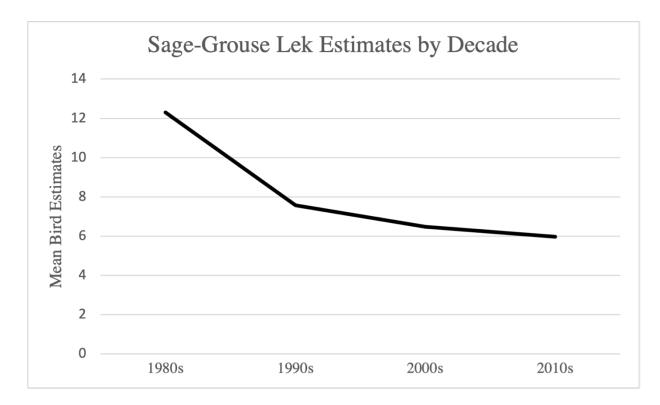


Figure 1.4: Mean greater sage-grouse lek estimates by decade. Leks were located throughout Nevada, USA and male lek counts estimates were conducted over a series of decades. Lek counts were grouped into decades (1980s, 1990s, 2000s and 2010s) and averaged over the decade to obtain a mean bird estimate for that decade. Mean bird estimates for the lek sites included in this analysis show a negative overall trend for the past 40 years with mean estimates set at 12.30 ± 1.61 , 7.57 ± 0.94 , 6.47 ± 0.63 , and 5.96 ± 0.54 birds for the 1980s, 1990s, 2000s and 2010s, respectively.

TABLES

Table 1.1: Mixed model analysis least squares means output table with comparisons of each decade and treatment (horse/non-horse). Data were collected throughout Nevada, USA in 2020 and analyzed in SAS v. 9.4. The data were log transformed. The result estimates and standard errors in the table are shown as back transformed to the original scale. Estimates are abundance means of male sage-grouse per lek based on the association of decade and treatment in the table. T-values and p-values are all shown on the log scale.

	Least Squares Means												
Effect	Decade	Treatment	Estimate	Standard Error	DF	t Value	Pr > t						
Decade	1980s		12.30	0.15	155	16.57	<.0001						
Decade	1990s		7.57	0.14	155	14.18	<.0001						
Decade	2000s		6.47	0.11	155	17.19	<.0001						
Decade	2010s		5.96	0.10	155	17.90	<.0001						
Treatment		Horse	9.14	0.13	98	17.25	<.0001						
Treatment		Non-Horse	6.55	0.13	98	14.80	<.0001						

Table 1.2: Differences of least squares means table with comparisons of each decade and treatment (horse/non-horse). Data were collected throughout Nevada, USA in 2020 and analyzed in SAS v. 9.4. Estimates are population means of male sage-grouse per lek based on the associate of decade and treatment in the table. Data were analyzed using a log transformation and estimates and standard errors shown here are back transformed to the original scale. P-values are represented in the table on the log scale. The Tukey-Kramer test was used to determine which pairwise comparisons were significant by accounting for issues of multiple comparisons.

	Differences of Least Squares Means												
Effect	Decade	Treatment	Decade	Treatment	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P		
Decade	1980s		1990s		1.63	0.17	155	2.90	0.0042	Tukey-Kramer	0.0219		
Decade	1980s		2000s		1.90	0.15	155	4.27	<.0001	Tukey-Kramer	0.0002		
Decade	1980s		2010s		2.06	0.15	155	4.89	<.0001	Tukey-Kramer	<.0001		
Decade	1990s		2000s		1.17	0.14	155	1.11	0.2672	Tukey-Kramer	0.6817		
Decade	1990s		2010s		1.27	0.14	155	1.72	0.0873	Tukey-Kramer	0.3164		
Decade	2000s		2010s		1.09	0.10	155	0.80	0.4226	Tukey-Kramer	0.8525		
Treatment		Horse		Non-Horse	1.40	0.18	98	1.90	0.0599	Tukey-Kramer	0.0599		

Table 1.3: Mean dung counts for each study site that was visited (10 pairs). Horse use sites are shown in the top table while non-horse use sites are illustrated in the bottom table. Each site was located in Nevada, USA and randomly selected from a larger list of study sites that were used for the analysis of this project. Dung pile counts were conducted at each site to verify if horse/non-horse pairs were valid and to assess ungulate use. Fifteen-100 m transects were extended and walked at each study site to conduct dung pile counts. Horse and cattle dung were of special concern but other ungulate dung piles were counted as well. Horse dung was present at five of the 10 non-horse sites with means of each of those sites equating to 1.73, 0.07, 5.20, 1.53, and 15.67. These means were averaged along 5 transects at each site. It is possible some horse dung came from domestic herding horses. The two leks with notable horse dung (DIA-029 and MON-077) were located relatively close to HMA boundaries and could be a result of feral horses escaping HMA boundaries from lack of fencing.

Horse Sites										
LEK	Horse Dung	±SE	Cattle Dung	±SE	Other Dung	±SE				
3BAR-008	15.60	2.20	0.73	0.37	1.60	1.00				
BUBUWP-051	9.20	1.62	0.73	0.33	1.87	0.65				
BUBUWP-141	15.40	1.46	1.60	0.54	1.60	0.74				
BUBUWP-142	10.20	0.81	0.87	0.39	0.53	0.17				
BUBUWP-008	14.47	2.57	8.07	1.96	0.47	0.13				
LIN-006	20.20	2.36	0.53	0.22	16.47	15.04				
BUBUWP-159	24.27	2.99	0.53	0.17	1.13	0.35				
DIA-015	17.93	2.73	6.67	2.90	0.67	0.21				
DIA-030	5.87	1.32	0.07	0.07	2.67	0.73				
RUVA-062	17.00	1.31	4.07	0.79	1.60	0.41				
		N	on-Horse Sites							
LEK	Horse Dung	±SE	Cattle Dung	±SE	Other Dung	±SE				
BUBUWP-039	1.73	1.98	19.73	9.65	0.73	0.33				
MON-001	0.07	0.26	0.00	0.00	0.40	0.21				
DIA-029	5.20	5.13	0.27	0.46	0.33	0.16				
LIN-001	0.00	0.00	4.60	7.40	0.73	0.30				
SCAN-037	1.53	2.20	0.00	0.00	34.13	8.44				
STCA-024	0.00	0.00	41.47	10.58	1.73	1.32				
BUBUWP-043	0.00	0.00	4.33	4.98	0.47	0.24				
BUBUWP-041	0.00	0.00	14.13	15.30	0.93	0.36				
STCA-019	0.00	0.00	40.13	27.08	2.93	1.10				
MON-077	15.67	4.51	0.00	0.00	0.93	0.40				

SUPPLEMENTAL MATERIAL

Appendix 1

Appendix Table 1: Nevada Herd management area (HMA) annual estimates for the HMAs that leks resided in that were focused on for this analysis. All lek sites that were deemed "horse use" were located within these 15 HMAs. Each column includes each HMA's appropriate management level (AML) that identifies the carrying capacities deemed for each HMA and their respective annual population estimates conducted by the Bureau of Land Management. Some HMAs do not have population estimates dating back to 2005 (Triple B, Pancake, Eagle and Silver King). Further information on these data can be found at:

https://www.blm.gov/programs/wild-horse-and-burro/about-the-program/program-data#:.

Herd	ROBERTS	MAVERICK-			CLAN					SAND	PINE NUT		SOUTH		
Management	MOUNTAIN	MEDICINE	TRIPLE B	PANCAKE	ALPINE	FISH CREEK	EAGLE	SEVEN MILE	STONE	SPRINGS	MOUNTAIN	ANTELOPE	SHOSHONE	SILVER	CALLAGHAN
Areas	(AML 150	(AML 276	(AML 518	(AML 493	(AML 979	(AML 180	(AML 210	(AML 50	CABIN (AML	WEST (AML	S (AML 179	(AML 324	(AML 100	KING (AML	(AML 237
Aleas	Horses)	Horses)	Horses)	Horses)	Horses)	Horses)	Horses)	Horses)	364 Horses)	49 Horses)	Horses)	Horses)	Horses)	128 Horses)	Horses)
2005	270	335	0*	0*	441	337	0*	196	325	239	132	160	239	0*	454
2006	317	875	0*	0*	541	57	0*	33	330	48	132	190	267	0*	569
2007	372	190	0*	0*	534	67	0*	40	154	56	146	230	299	0*	669
2008	123	228	0*	0*	612	100	0*	100	171	48	190	310	63	0*	847
2009	158	675	555	897	685	118	505	118	198	246	177	372	88	365	134
2010	186	863	667	1076	657	139	606	138	397	246	215	365	187	505	238
2011	248	553	1217	1291	503	163	200	162	476	285	182	239	224	200	221
2012	229	489	415	1005	560	215	522	129	246	200	218	349	250	262	249
2013	307	586	498	1,081	600	256	626	154	316	120	293	344	282	314	322
2014	369	763	1,107	1,111	724	461	751	185	379	149	351	413	336	377	361
2015	551	910	1460	1400	700	351	1142	420	332	187	279	669	476	789	512
2016	560	1155	1600	1800	685	421	1322	172	560	230	336	861	560	912	387
2017	596	1309	1702	1800	882	476	1350	257	452	213	646	1271	606	850	425
2018	715	781	1262	2160	1005	538	1859	308	652	287	775	1525	850	1020	543
2019	887	1652	1514	2503	1206	645	1929	369	782	333	360	1830	1021	228	602
2020	1020	1640	1348	3004	1447	202	964	391	938	386	232	1830	1217	387	684

0* indicate no estimates for that specific HMA for that given year.

Appendix Table 2: All 100 paired study sites throughout Nevada, USA that were included in the analysis for this project. The left half of the table includes all horse use sites and the right half includes all non-horse sites. Mean sage-grouse lek counts for the 4 decades (80s, 90s, 00s and 10s) that were included in the analysis.

LEK	1980sSG Mean	1990sSG Mean	2000s SG Mean	2010sSG Mean	Pair	Treatment	HMA	LEK	1980sSG Mean	1990s SG Mean	2000sSG Mean	2010sSG Mean	Pair	Treatment	HMA
3BAR-008	0.00	0.00	25.14	7.25	1	Harse	Roberts Mountain	BUBUWP-039	3.67	0.00	0.00	0.00	1	Non-Horse	N/A
3BAR-009	27.40	18.70	15.30	11.00	2	Harse	Roberts Mountain	EAVA-006	0.00	0.00	13.17	16.62	2	Non-Horse	N/A
3BAR-014	49.00	9.80	25.70	25.08	3	Harse	Roberts Mountain	3BAR-030	41_00	36.40	36.90	28.06	3	Non-Horse	N/A
38AR-058	0.00	0.00	12.00	8.00	4	Harse	Roberts Mountain	BAMO-005	0.00	0.00	950	13, 10	4	Non-Horse	N/A
3BAR-062	0.00	0.00	0.00	7.75	5	Harse	Roberts Mountain	COR-014	7.00	0.00	0.00	0.25	5	Non-Horse	N/A
BUBUWP-006	12.50	0.00	28.90	16.87	6	Horse	Marerick Medicine	BUBUWP-034	14.00	0.00	0.00	0.00	6	Non-Horse	N/A
BUBU WP-008	17.00	0.00	23.00	1.20	7	Harse	Marerick Medicine	SCAN-037	0.00	0.00	11.30	6.00	7	Non-Horse	N/A
BUBUWP-016	16.00	0.00	8.00	17.47	8	Harse	Marerick Medicine	BUBUWP-115	10.33	14.10	9.30	10.50	8	Non-Horse	N/A
BUBUWP-017	57.75	73.00	40.14	29.12	9	Horse	Triple B	SNA-049	0.00	23.00	19.56	11.76	9	Non-Horse	N/A
BUBUWP-049	0.00	0.00	15.00	16.17	10	Hase	Triple B	STCA-012	8.67	13.00	3.75	6.00	10	Non-Horse	N/A
BUBUWP-051	7.50	4.67	4_50	0.36	11	Harse	Triple B	MON-001	23.00	0.00	0.00	5.10	11	Non-Horse	N/A
BUBUWP-056	9.67	2.00	0.84	1152	12	Harse	Triple B	SCAN-045	0.00	0.00	0.00	5.00	12	Non-Horse	N/A
BUBUWP-060	0.00	0.00	30.00	28.03	13	Horse	Triple B	LIN-024	0.00	0.00	0.00	5.00	13	Non-Horse	N/A
BUBUWP-061	7.20	9.88	0.00	0.00	14	Horse	Triple B	MON-051	0.00	0.00	6.00	7.67	14	Non-Horse	N/A
BUBUWP-064	19.00	14.67	9.33	3.00	15	Harse	Triple B	GOL-131	0.00	0.00	6.00	3.13	15	Non-Horse	N/A
BUBUWP-068	7.00	5.50	4_91	11.13	16	Harse	Triple B	STCA-011	7.60	6.88	11.00	16.91	16	Non-Horse	N/A
BUBUWP-084	12.00	18.00	8.67	1.92	17	Horse	Pancake	RUVA-018	0.00	30.00	0.00	0.00	17	Non-Horse	N/A
BUBUWP-085	450	0.00	0.00	0.00	18	Harse	Pancake	SCAN-029	0.00	0.00	10.50	650	18	Non-Horse	N/A
BUBUWP-087	7.00	3.00	0.00	0.00	19	Harse	Pancake	DCFA-010	0.00	0.00	12.00	12.67	19	Non-Horse	N/A
BUBUWP-133	0.00	0.00	31.00	32.29	20	Harse	Pancake	SCAN-044	0.00	0.00	0.00	8.33	20	Non-Horse	N/A
BUBUWP-137	0.00	0.00	5.00	7.80	21	Harse	Triple B	SPSV-019	0.00	10.00	7.10	4.08	21	Non-Horse	N/A
BUBUWP-141	0.00	0.00	0.00	7.29	22	Horse	Triple B	DIA-029	8.71	4.29	0.00	0.33	22	Non-Horse	N/A
BUBUWP-142	0.00	0.00	0.00	8.00	23	Harse	Triple B	LIN-001	0.00	0.00	3.67	050	23	Non-Horse	N/A
BUBUWP-159	0.00	0.00	0.00	567	24	Horse	Pancake	BUBUWP-043	5.40	2.00	0.00	0.00	24	Non-Horse	N/A
CLAL-001	0.00	0.00	25.00	6.68	Б	Horse	Clan Alpine	DESA-009	0.00	0.00	26.38	11.52	Б	Non-Horse	N/A
DIA-015	23.00	1.60	0.00	0.00	26	Hase	Fish Greek	BUBUWP-041	22.70	26.50	18.60	15.03	26	Non-Horse	N/A
DIA-016	5.00	4.00	0.55	1.00	27	Horse	Triple B	SNA-043	0.00	0.00	16.33	250	27	Non-Horse	N/A
DIA-030	0.00	19.00	0.00	4.00	28	Horse	Fish Greek	STCA-019	12.33	4.89	0.00	0.00	28	Non-Horse	N/A
DIA-040	0.00	0.00	0.00	9.88	29	Horse	Fish Greek	STCA-014	0.00	8.00	15.00	8.00	29	Non-Horse	N/A
LIN-002	0.00	0.00	34.14	25.74	30	Horse	Eagle	3BAR-002	5.00	0.00	5.00	0.00	30	Non-Horse	N/A
LIN-003	0.00	0.00	10.50	7.10	31	Harse	Eagle	TOI-068	16.70	17.90	20.40	9.78	31	Non-Horse	N/A
LIN-006	0.00	0.00	4.25	025	32	Horse	Eagle	STCA-024	0.00	18.00	17.30	8.09	32	Non-Horse	N/A
LIN-007	0.00	0.00	7.75	1.00	33	Horse	Eagle	MOGR-006	6.00	2.00	029	058	33	Non-Horse	N/A
LIN-017	0.00	0.00	10.64	875	34	Horse	Eagle	BUBUWP-036	9.50	0.00	0.00	0.00	34	Non-Horse	N/A
LIN-022	0.00	0.00	9.11	1155	35	Harse	Eagle	MOGR-034	0.00	0.00	0.00	175	35	Non-Horse	N/A
MON-015	0.00	0.00	3.67	3.70	36	Horse	Seven Mile	MON-027	35.00	0.00	0.00	0.00	36	Non-Horse	N/A
MON-046	0.00	0.00	4.50	0.14	37	Harse	Stone Cabin	3BAR-005	0.00	6.88	18.22	5.86	37	Non-Horse	N/A
MON-047	0.00	0.00	6.13	7.58	38	Horse	Fish Creek	SCAN-028	0.00	0.00	6.00	3.29	38	Non-Horse	N/A
MON-079	0.00	0.00	0.00	575	39	Harse	Sand Springs West	SCAN-024	2.00	0.00	0.00	19.33	39	Non-Horse	N/A
PINU-012	0.00	0.00	0.00	10.00	40	Horse	Pine Nut Mountains	PINU-005	0.00	0.00	425	5.67	40	Non-Horse	N/A
RUVA-060	0.00	0.00	5.00	2.56	41	Horse	Triple B	RUVA-026	8.00	6.00	2.00	8.26	41	Non-Horse	N/A
RUVA-062	0.00	7.00	10.50	857	42	Harse	Triple B	MON-077	0.00	0.00	14.00	5.00	42	Non-Horse	N/A
SCAN-002	11.50	8.00	26.20	12.69	43	Horse	Antelope	GOL-085	0.00	0.00	13.00	8.00	43 44	Non-Horse	N/A
SCAN-COG	6.00	6.00	18.89 16.67	4.68	44	Harse	Antekape	STCA-010 SCAN-014	13.00 0.00	10.00 0.00	2.60	0.00	44 45	Non-Horse Non-Horse	N/A
SCAN-025 SCAN-040	0.00	0.00		13.13	-6 	Horse	Anteiope				2.80		46		N/A
SCAN-040	0.00	0.00	0.00	13.68	46	Horse	Antekape	STCA-008	0.00	5.00	13.56	14.49		Non-Horse	N/A
SHO-012	0.00	0.00	10.00	3.07	47	Harse	South Shashane	NOFO-146	0.00	0.00	8.00	6.00	47	Non-Horse	N/A
STCA-027	0.00	0.00	9.11	12.71	48	Harse	Silver King	SPSV-002	0.00	0.00	1.83	2.40	48	Non-Horse	N/A
STCA-081	0.00	0.00	0.00	22.00	49	Horse	Silver King	TOI-012	0.00	2.50	1.00	0.00	49	Non-Horse	N/A
T01-004	0.00	0.00	0.00	2150	50	Harse	Callaghan	00R-063	0.00	0.00	0.00	4.00	50	Non-Horse	N/A

CHAPTER 2

Comparison of Greater Sage-Grouse (*Centrocercus urophasianus*) Late Brood-Rearing Habitat in Areas With and Without Feral Horses (*Equus caballus*)

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ABSTRACT

Environmental impacts of feral horses (Equus caballus) are a subject of international conservation concern and controversial national policy. In North America, feral horses are considered an invasive species where they impact arid and semi-arid rangelands. The greater sage-grouse (Centrocercus urophasianus) is a sagebrush obligate bird species that relies on sagebrush habitats to maintain viable population levels. Sage-grouse late brood-rearing habitat consists of abundant forbs, insects, succulent mesic vegetation and adequate sagebrush cover to fledge broods. Overabundant local populations of feral horses may impact sagebrush community structure, vegetation composition, soils, and many native and sagebrush obligate species. In areas where sage-grouse and horses co-exist, resource managers are concerned overabundant horses may decrease suitability of late brood-rearing habitat, reduce chick survival, and species recruitment. We assessed the potential impact of horse presence on sage-grouse late broodrearing habitat within the Great Basin in western Utah and eastern Nevada. We used coordinate locations acquired from global positioning system (GPS) and very-high frequency (VHF) collared birds to delineate late brood-rearing habitat in Utah and Nevada and compared eight paired sites within horse use and non-use areas to assess the impact of horse presence on sagegrouse late brood-rearing habitat. For each pairing, one site was located within and the other

outside of a BLM Herd Management Area (HMA) boundary. Each pairing shared similar habitat characteristics (i.e. topography, dominant vegetation, and climate). We selected reference paired sites outside HMA boundaries that were 3-31 km from their respective paired site to mitigate any bias related to horse seasonal migrations. We collected vegetation and dung count data at each site to assess site quality for sage-grouse brood-rearing, based on ungulate presence and impact. We used a mixed model analysis of variance (ANOVA) to detect differences between each paired site comparison (α <0.01). No horse presence was identified at our non-horse sites allowing for a clean comparison between paired sites. We determined statistical differences in the following comparisons: annual grass frequency, percent annual grass cover, dung frequency, total height, vegetative height, and horse and cattle dung density. These data can be useful for rangeland management as key habitat differences are notable in areas that experience horse and cattle vs. just cattle grazing pressure. If crucial habitat is protected from grazing pressure, sagebrush ecosystems and associated obligate species populations could improve.

INTRODUCTION

Invasive species, including feral animals, pose threats to native biodiversity and conservation efforts throughout the world (Beever et al., 2019). Environmental impacts of feral horses (*Equus caballus*; hereafter, "horses") are a subject of international conservation concern and controversial policy. In North America, horses are considered an invasive species where they impact rangeland health and ecological resilience within arid and semi-arid areas, including the Great Basin Region, USA. Following extirpation from North America at the end of the Pleistocene, horses were reintroduced by Spanish conquistadors in the mid 1500s and since then, horse densities have greatly increased as a result of high reproductive success, limited predation,

and inadvertent or purposeful release. Furthermore, populations of horses have experienced continued steady growth since the passage of the Wild Free-Roaming Horses and Burros Act in 1971 further restricting limited resources in the arid Great Basin. Today, federally managed horse populations on United States public lands exceed 95,114 horses across 10 western states (BLM Programs, 2020). Additionally, many horses also occur on tribal or reservation lands that are not accounted for in this value, with numbers exceeding 38,000 with an expectation to double within the next few years (Beever et al., 2018).

Expanding horse populations are often associated with degraded wildlife habitat, impaired ecosystem structure, and reduced rangeland health (Beever & Brussard, 2000; Beever, 2003; Beever & Aldridge, 2011; Garrott & Oli, 2013; Davies et al., 2014; Hall et al., 2016; Boyd et al., 2017; Beever et al., 2018; Davies & Boyd, 2019). Horses differ from many of the domestic and wild ungulates found throughout the Great Basin both in how their populations are managed and in their anatomy and physiology. As a protected species, horses are not hunted (like most wild ungulates) and are not managed through rotational grazing strategies (as most domestic ungulates).

As large hindgut fermenters, horses are potentially the least-selective ungulate grazer found in the Great Basin as opposed to their ruminant counterparts (cattle, pronghorn, sheep, elk and deer) (Beever, 2003). Less selectivity and an expansive home range may equate to reduced plant species diversity, impaired vegetation structure, and lower ecosystem resilience in horse-grazed areas compared to areas grazed by other native ungulates (Beever, 2003, Boyd et al., 2017). Additionally, a lower quality diet requires horses to consume 20-65% more forage than a ruminant of similar size (Menard et al., 2002). Subsequently, horses can effectively impair sagebrush community structure, diminish vegetation composition, reduce soil stability, and

displace native and sagebrush obligate species (Ostermann-Kelm et al., 2009; Beever & Aldridge, 2011, Hall et al., 2016, Gooch et al., 2017).

Greater sage-grouse (*Centrocercus urophasianus;* hereafter sage-grouse) is a species that relies on sagebrush habitats (i.e. sagebrush vegetation, forbs, invertebrates) to maintain viable population levels. Populations of sage-grouse have generally been in decline for the past four decades, primarily in response to declining habitat, resource exploitation and over-utilization of rangelands (Connelly & Braun, 1997; Connelly et al., 2000; Connelly et al., 2004; Crawford et al., 2004; Atamian et al., 2010; Kaczor et al., 2011; Robinson & Messmer, 2013; Westover et al., 2016). Direct competition for forage plants and habitat disturbance associated with grazing and trampling may impact the fitness of sage-grouse through altered habitat as both horses and sagegrouse typically inhabit similar areas (Beever & Aldridge 2011).

In addition to improper livestock grazing, fire, anthropogenic recreation, and sage-grouse translocations, horses may also directly and indirectly impact local sage-grouse populations. Large-scale habitat continuity (percent of sagebrush dominated landscape), seasonal migrations, predator dynamics, adult hen survival, nest success, and chick survival have been directly related to sage-grouse vital rates and are factors that should be considered when determining management decisions and actions to prevent further population decline (Crawford et al., 2004; Dahlgren et al., 2010; Taylor et al., 2012; Guttery et al., 2013; Robinson & Messmer, 2013; Dahlgren et al., 2019).

Significant research has been conducted on the life history of horses and their impacts on various components of the sagebrush ecosystem (Beever & Brussard, 2000; Beever, 2003; Beever & Aldridge, 2011; Davies et al., 2014; Hall et al., 2016; Boyd et al., 2017; Gooch et al., 2017; Hennig et al., 2021). However, research assessing the direct impacts of feral horses on late

brood-rearing habitat of sage-grouse is limited. Horses may degrade sage-grouse habitat by lowering vegetation cover, in particular sagebrush (*Artemisia*) cover, reducing plant height and density, and compacting soils that stem from excessive trampling and foraging (Beever & Aldridge, 2011; Davies et al., 2014; Hall et al., 2016). Adult female sage-grouse do not nest every year, potentially due to deterioration of range conditions and decreased nutrition for prelaying hens (Connelly et al., 2000).

Sage-grouse require varying habitat conditions throughout the year to meet their life-history requirements (Connelly et al., 2000; Crawford et al., 2004; Dahlgren et al., 2016). Sage-grouse habitats can be generally defined into three categories: breeding, summer, and winter habitat (Connelly et al., 2000; Dahlgren et al., 2016). Breeding habitat consists of areas used for prelaying, lekking, nesting, and early brood-rearing (Connelly et al., 2000; Dahlgren et al., 2016). Sage-grouse chicks typically hatch between late May to early June (Dahlgren et al., 2016). Sage-grouse females utilize specific habitats for rearing their brood which maximizes safety and nutrient requirements for their chicks. Females with broods often select sites with increased vegetation cover and height (Hagen et al., 2007; Kaczor et al., 2011). Early brood-rearing areas are typically similar in land cover as areas surrounding lek sites. The first 2-3 weeks of brood-rearing occur close to nests which are typically located within 5 km of the lek where breeding took place (Atamian et al., 2010).

Early brood-rearing occurs in upland sagebrush areas where forbs, grasses, sagebrush and insects are all present (Connelly et al., 2011). Broods typically stay in sagebrush dominated environments until desiccation (typically around June or July) at which time they move to habitats that is often characterized by wetter conditions that support greener and more succulent vegetation (Braun et al., 1977; Connelly et al., 2000). Late brood-rearing areas are often

dominated by montane sagebrush, riparian shrubland, desert grassland and big sagebrush (Atamian et al., 2010). Sage-grouse typically choose sites higher in elevation with more moisture and riparian shrubs or montane sagebrush as these areas provide an abundance of succulent forbs and insects that last later into the summer and fall (Braun et al., 1977; Crawford et al., 2004; Atamian et al., 2010; Kaczor et al., 2011). Brood habitat that has high invertebrate abundance and protective cover has been shown to increase productivity as both are crucial for growth, development and survival of chicks (Crawford et al., 2004; Kaczor et al., 2011). However, sage-grouse have been found to prefer lower vegetation and succulent forb growth stimulated by moderate grazing in riparian brood-rearing habitat (Crawford et al., 2004). Chicks that are fed in forb rich/high invertebrate areas typically gain more weight than those fed in forb-poor environments and consequently aid in better recruitment rates (Kaczor et al., 2011).

The purpose of this study was to determine the potential influence of large ungulate grazing, specifically horses and cattle, on late brood-rearing habitat structure in western Utah and eastern Nevada. We focused our research on the effects that ungulate herbivory can have on big sagebrush (*A. tridentata*) and black sagebrush (*A. nova*) plant communities where late sage-grouse brood-rearing is known to occur. We were primarily interested in assessing plant community structure that is necessary for sage-grouse hiding and nesting cover and for forage availability within areas commonly used by horses.

Horse utilization and cattle grazing are influences that can be difficult to differentiate in their influence on sagebrush ecosystems. Understanding this relationship, we considered the combined habitat use of horses and cattle, but maintained a focus on horse utilization impacts on late brood-rearing habitat. We hypothesized that combined horse/cattle utilization in these habitats would decrease the structure and suitability of habitat for sage-grouse during late brood-rearing

and therefore impact local population stability. Specifically, we expected horse sites to have lower overall vegetation height, higher invasive species presence, and lower frequency and percent cover of grasses and forbs. We predicted that areas with heavier grazing and trampling potential would have less suitable habitat conditions, potentially leading to lower population densities of sage-grouse, likely due to unusable habitat and subsequently lower chick survival and recruitment rates. Our goal in this study was not to focus on adult fitness, chick survival, or recruitment levels directly, but instead to quantify the quality of late brood-rearing habitat and its probable relationship to fitness and population trends of sage-grouse.

MATERIALS AND METHODS

Study Area

We established 8 study sites in 4 pairs (Benmore Pastures/Government Creek (North UT Pair), Butcher Troughs/Hamblin Wash (South UT Pair), High Schell/Spring Gulch (East NV Pair), Pony Express/Egan Canyon (West NV Pair)) located within the Great Basin region (Figure 2.1). Each of these sites were located in high elevation, cool desert ecosystems characterized by hot, dry summers and cold winters. Dominant vegetation common for all study sites included Wyoming big sagebrush (*A. tridentata spp. wyomingensis*), black sagebrush (*Artemisia nova*), broom snakeweed (*Gutierrezia sarothrae*), yellow rabbitbrush (*Chrysothamnus viscidiflorus*), crested wheatgrass (*Agropyron cristatum*), Sandberg bluegrass (*Poa secunda*), bottlebrush squirreltail (*Elymus elymoides*), cheatgrass (*Bromus tectorum*), and bur buttercup (*Ceratocephala testiculata*). Sites were located between approximately 1676 and 2014 m elevation and paired distances ranged from 3 to 31 km from each other. Annual temperatures in these sites range from -10°C to 31°C. The mean annual precipitation in each of these areas ranges from 0.76 cm to 3.30 cm (U.S. Climate Data).

Data Acquisition

Within the delineated late brood-rearing habitat area, we established 5 randomly located plot center points. At each plot center we extended a 100 m transect from which variables representing ecological structure important for sage-grouse late brood-rearing were sampled. These metrics included 1) vegetation height (max vegetative and max reproductive), 2) vegetation biomass (total and by species), 3) species frequency, 4) percent foliar cover, and 5) dung density (horse and cattle). All plots were measured along the left side of each transect to minimize plot disturbance (i.e. trampling). Transect bearings were randomly oriented ranging in bearing between 0-359 degrees. Dung counts of large ungulates were conducted at each site to develop an index for quantifying ungulate use at each location.

The 8 study sites were used to compare horse use with non-use areas by pairing late-broodrearing habitat within and outside of herd management areas (HMA), respectively. Sites within HMAs exhibited both horse and cattle grazing while sites outside HMAs experienced only cattle grazing. To delineate late brood-rearing habitat ranges, we utilized coordinate locations obtained from collared sage-grouse that were monitored year-long within each area.

The late-brood rearing areas that we selected for the Northern pair were determined from data collected from monitored (translocated and resident) sage-grouse between 2016-2020 by technicians associated with Utah State University. To monitor sage-grouse vital rates and habitat-use, all individuals were monitored weekly throughout capture to August and intermittently throughout the fall and winter for very-high frequency (VHF) marked individuals

and all year for global positioning system (GPS) marked individuals. For the GPS transmitters, four to six locations were programmed each day with four seasons, coinciding with sage-grouse lekking, nesting brooding, and late fall seasons. For each location, the date, time, elevation and speed were recorded. Late-brood rearing locations were delineated as four weeks post-hatch to the designated 50-day brood age. Included in these data were 22 translocated females' broods and 8 resident females' broods (see Supplemental Material-Appendix 1). The Southern Utah pair was determined using similar methods. The Nevada late-brood rearing location data was obtained from the Nevada Department of Wildlife (NDOW) historic sage-grouse brood survey database. Once use sites were identified, a minimum convex polygon (MCP) was used to identify study area boundaries using ArcGIS Pro (ESRI, 2020). We then paired 4 selected late brood-rearing sites within HMAs to the nearest points outside of HMA boundaries with similar habitat characteristics.

Along each transect, we systematically measured plant species frequency, percent vegetative cover by species, and percent bare-ground cover. Percent shrub canopy cover was sampled using the line-intercept method. Herbaceous vegetation (i.e. perennial/annual grasses, perennial/annual forbs) and soil surface properties (i.e. bare-ground, rock, litter) were measured using a 0.25 m² nested frequency quadrat (Greig-Smith, 1983). We systematically placed the nested frequency quadrat frame along the transect at 10 m intervals for a total of 10 quadrats per transect.

Using a nested frequency sampling method, we assessed the frequency of functional groups of shrubs, perennial grasses, annual grasses, forbs and dung. Functional groups were given a classification of 1-4 based on where they were located within the quadrat frame. If a functional group was located within the smallest frame it was given a value of 4 suggesting it was more common. If a plant was found only in the largest frame it was given a value of 1 and was

suggested to be more rare. Because of this method, we did not explicitly measure frequency as a percentage. Instead frequency is expressed using a mean value between 1-4 with lower values (closer to 1) suggesting higher frequency and higher values (closer to 4) suggesting lower frequency.

We estimated maximum plant height by averaging the highest plant growth (including inflorescence) from all sampled plants per plot per transect. Similarly, we measured maximum vegetative height but only included non-reproductive (vegetative) material in this measure. We measured shrubs and/or herbaceous plant growth within each plot. We used a meter measuring stick to determine plant height along the transect at each point where the nested frequency quadrat was placed. All respective heights along each transect were averaged.

Plant biomass was sampled by clipping all above-ground plant tissue by species within quadrats equal in size to the nested frequency quadrat (0.25 m²). We grouped species into functional groups which included shrubs, perennial grasses, annual grasses, and forbs. Plots were clipped at two locations along each transect (25 m and 75 m). Clipped vegetation was placed in paper bags in the field, and then later dried in the lab at approximately 70° Fahrenheit for 48 hours. Dried samples were then immediately weighed to determine the weight of dried plant biomass. Species biomass was determined by percentage of dried biomass.

Dung piles and pellet groups were counted to identify ungulate presence. At each random point we implemented a wagon-wheel sampling design (3-100 m transects extending out from the predetermined given point 120° from each other) to count total dung by ungulate species. A predetermined bearing gave us one of 3 directions for the first transect (the other two were based on the first +/- 120°). Observers walked along the transect and stopped every 10 m along the 100 m transect to count all ungulate and sage-grouse dung piles within a 5 m radius (approx. 0.02

acre) circular plot from the center point (10 per transect/30 per point). Due to the irregular amount of fecal matter in defecations, concentrations of fecal matter were determined to be dung piles after inspecting relative numbers, location, direction, and distance to other dung piles. Stud piles were counted as a single deposit due to the difficulty in parsing out individual dung piles within a stud pile.

Cattle use was consistent throughout all the paired sites, and horse use was only present in the HMA locations. This allowed for a clean separation between horse use in a study site compared to the control (non-horse), while maintaining similar plant composition and close proximity. This pairing approach reduced potential confounding factors resulting from ecological differences. If a pair was selected that did not have the same dominant plant species or environmental conditions as the other pair or pairs, it was removed from the analysis and a new site was selected.

Statistical Analysis

We used a mixed model analysis of variance (ANOVA) blocking on pairs with horse use vs. non-use as the independent variable to detect differences (or lack of) between the comparison of vegetation and dung variables. To account for issues of multiple comparisons often associated with ANOVA, we conducted a pseudo-Bonferroni test by identifying significance as α <0.01. The data analysis for this paper was generated using SAS v.9.4 software. Copyright, SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS institute Inc., Cary, NC, USA.

RESULTS

Our results were structured to identify differences between horse use and non-use sites (Figure 2.2; Table 2.2). We determined statistical differences from the following comparisons: annual grass frequency, percent annual grass cover, dung frequency, total height, vegetative height, and horse and cattle dung density (Figure 2.2; Table 2.1). When comparing horse to non-horse use sites, the frequency of annual grass (\bar{x} =0.56, p<0.01) and the percent of annual grass cover (\bar{x} =3.75, p<0.01) were both higher in horse sites than in non-horse sites. Overall dung frequency was higher in horse sites than non-horse sites (\bar{x} =0.17, p<0.01). Total (\bar{x} =-9.7, p<0.01) and vegetative (\bar{x} =-5.1, p=0.014) plant height were significantly lower at horse sites than at non-horse sites. Horse dung frequency (\bar{x} =23.45, p<0.01) was greater at horse sites and cattle dung presence was overall, greater at non-horse sites (\bar{x} =-19.97, p<0.01) (Table 2.1). All measurement means, standard deviations and standard errors for each of the 8 study sites can be found in Supplemental Material-Appendix 2 (Table 1).

DISCUSSION

We sampled vegetation and surface (bare ground, rock, litter) characteristics at each paired site as these characteristics delineate probable selection of an area for brood-rearing. Specific sampling techniques included percent vegetation and surface feature cover, vegetation production (biomass), species frequency, and vegetation height. Kaczor et al. (2011) determined their best model of resource selection of broods, in the Great Basin, included total herbaceous cover, maximum grass height and sagebrush height. Total cover and grass height were positively associated with brood-rearing site selection.

We measured percent cover of shrubs, perennial and annual grasses, forbs, litter and bare ground at each of our sites. Annual grass cover was the only functional group that exhibited a significant differential response to horse use. Annual grass in this study consisted solely of cheatgrass (Bromus tectorum) which had higher cover in horse sites than in the non-horse sites. Consistent horse grazing pressures can result in changes in vegetation community structure, including invasive species (Beever & Aldridge, 2011; Boyd et al., 2017). The establishment and dominance of cheatgrass may be an indicator of an ecological disturbance and lower habitat quality. Cheatgrass invasion is considered a significant ecological issue throughout the Great Basin as it has invaded and established monoculture plant communities in many areas often leading to not only reduced perennial plant diversity but also decreased insect diversity and densities. This annual grass has also led to exacerbated fires in the Great Basin as it serves as a prolific fuel source. The compounded effect of increased fire and decreased biodiversity associated with increased cheatgrass presence may negatively impact sage-grouse chicks. It is common for overall vegetation cover to be lower at horse grazed sites throughout the Great Basin (Beever & Brussard, 2000; Ostermann-Kelm et al., 2009; Beever & Aldridge, 2011). Davies et al. (2014) noted reduced perennial grass cover in heavily grazed sites. Although it did not meet our significance level of p<0.01, perennial grass cover approached significance p=0.02 with percent cover lower at the horse use sites. This could indicate that with more samples, combined cattle and horse grazing might reduce hiding cover for sage-grouse chicks and forage availability for wildlife, including invertebrate availability through reduced perennial grass cover. Shrub, forbs, litter and non-living cover groups were not significantly different between the horse and non-horse sites.

Frequency of annual grasses (*Bromus tectorum*) and dung piles were significantly higher in horse sites than in non-horse sites. Shrubs, and forbs were more frequent in horse sites than in non-horse sites but differences were not significant. Frequency of perennial grass was slightly higher in non-horse sites than in horse sites. Perennial grasses are useful for cover and as sources of food for sage-grouse chicks through insect abundance (Atamain et al., 2010). Although differences between sites were not significant, it may suggest that the combined effect of horses and cattle in horse use sites are degrading late-brood rearing habitat faster than cattle alone.

Total plant height was measured as the overall height of the plant including reproductive inflorescences while the vegetative plant height included overall plant height without the reproductive material. Total plant height and vegetative plant height were both higher in nonhorse locations than in horse locations which could also impair nesting and brood-rearing success. In their studies, Boyd et al. (2017) and Beever & Brussard (2000) noticed vertical vegetation structure was negatively influenced by horse presence. Although we didn't measure invertebrate populations directly, we did hypothesize that this food source for sage-grouse chicks could also be reduced through excessive vegetation reduction in late brood-rearing habitat potentially impairing brood success. Furthermore, higher plant height aids in concealment cover for chicks. If plant height and subsequent hiding cover is reduced, recruitment may not be very successful if predators can more easily hunt chicks.

We measured biomass of shrubs, perennial grasses, annual grasses and forbs at each of our sites. Boyd et al. (2017) suggested a negative correlation between plant biomass and horse presence, however, we did not detect significant differences in above ground plant biomass at our sites. Biomass of shrubs and perennial grasses were lower at horse sites than non-horse sites but annual grasses and forbs were higher at horse sites than non-horse sites. Although these

differences were not significant, they follow the results we got for frequency and percent cover at our study sites.

Ostermann-Kelm et al. (2009) suggested reduced vegetation cover and compacted soils were often associated with horse trailing. Furthermore, Davies et al. (2014) and Beever & Aldridge (2011) noted greater soil compaction and lower shrub density in areas with horses. We did not notice significant differences in soil compaction or shrub density between sites. However, soil was slightly more compacted at the non-horse sites than at the horse sites. These differences may be due in part to cattle presence in our non-horse sites as well as differences in research questions and methodologies.

Dung frequency was higher at horse-use locations due to the combined effect that occurs with both horse and cattle grazing. Ostermann-Kelm et al. (2009) determined an increase in native plant diversity was often associated with dung presence. Interestingly, there were more cattle dung at non-horse sites than at horse sites suggesting that managers adjust grazing levels to minimize the potential of overgrazing in areas where both horses and cattle occur.

Late brood-rearing areas are commonly dominated by montane sagebrush, riparian shrubland, desert grassland and big sagebrush (Atamian et al., 2010). These are common characteristics of the study sites that we included in this study. Brood-rearing habitat that has high invertebrate abundance and protective cover has been shown to increase productivity (Crawford et al., 2004; Kaczor et al., 2011). Additionally, taller grass is helpful as a protective cover in providing concealment from predators (Gregg et al., 1994; Kaczor et al., 2011). Overall, we found lower grass and plant height associated with our horse sites, suggesting that horse and cattle presence combined could largely impact the fitness of sage-grouse chicks through reduced plant height. However, Crawford et al. (2004) found that sage-grouse prefer lower vegetation and

succulent forb growth stimulated by moderate grazing in riparian brood-rearing habitat. This could suggest that although vegetation height was lower, it may not impact sage-grouse as much as we might expect. However, this may only be true in riparian areas and most of our sampling was done in very dry locations. Chicks that are fed in forb rich/high invertebrate areas typically gain more weight than those fed in forb-poor environments (Kaczor et al., 2011). In all of our metrics, forbs were more common in horse areas than in non-horse areas. This could indicate that horse presence did not seem to impact forb abundance as much as cattle presence in our non-horse sites. Overall, we assessed differences between horse sites and non-horse sites with cattle use consistent between the sites. The differences we see may not be resulting from horse presence but a combined ungulate pressure on these areas.

Nesting and late brood-rearing habitat of sage-grouse often occur in very high risk areas prone to overutilization and development (Aldridge and Boyce, 2007). Atamian et al. (2010) found that within their study site, the high quality late brood-rearing habitat, on which sage-grouse chicks were successfully reared, represented less than 3% of the area and was extremely restricted in distribution. Consequently, late brood-rearing habitat can be limiting populations of sage-grouse throughout their range as it becomes more restricted through development and grazing pressures. It is important that we conserve these habitats for sage-grouse and all sagebrush obligates.

CONCLUSION

Our objective of this study was to determine the potential influence of herbivory by ungulates (horses and cattle) on late brood-rearing habitat. We hypothesized that feral horse utilization in conjunction with cattle grazing in these habitats would decrease the structure and suitability of

habitat for sage-grouse during late brood-rearing and therefore impact local population stability. Based on our hypothesis, we predicted areas with heavier grazing and trampling to have less suitable habitat conditions that could potentially lead to lower sage-grouse densities, likely due to lower chick survival and recruitment rates. We determined that statistical differences did occur between horse and non-horse use sites in the following comparisons: annual grass frequency, percent annual grass cover, dung frequency, total height, vegetative height, and horse and cattle dung density. Based on these results, intense ungulate grazing and the combined utilization of late-brood rearing habitat by feral horses and livestock may decrease habitat suitability for hens rearing their young.

Management Implications

Overall, our findings were similar to current published research with only a few exceptions. We determined statistical differences between sites from the following comparisons: annual grass frequency, percent annual grass cover, dung frequency, total height, vegetative height, and horse and cattle dung density. This suggests and supports the conclusion that unmanaged grazing in arid and semi-arid habitats can disrupt habitat condition and suitability for native and sagebrush obligate species. Whether through direct competition for resources (Gooch et al., 2017) or indirectly through habitat degradation (Beever & Brussard, 2000; Beever, 2003; Beever & Aldridge, 2011; Davies et al., 2014; Hall et al., 2016; Boyd et al., 2017; Beever et al., 2018; Davies & Boyd, 2019) horses have been shown to impair habitat structure and ecological resilience in Great Basin ecosystems. Understanding this relationship can aid rangeland managers in identifying key habitat differences that are notable in areas that experience horse and cattle vs. just cattle grazing pressure. Understanding factors that directly impact sage-grouse juvenile survival can be critical in effectively managing the fluctuating population dynamics of sage-grouse. If crucial habitat is protected from grazing pressure, sagebrush obligate species populations could improve.

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doi:10.1371/journal.pone.0156290

FIGURES

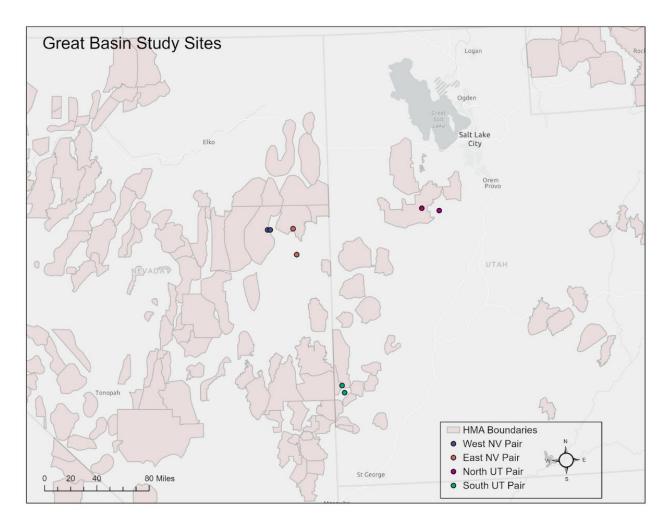


Figure 2.1: Map of eight paired study sites throughout the Great Basin. Each pairing had one horse use site and one non-horse site. Sites were compared to determine differences in habitat characteristics.

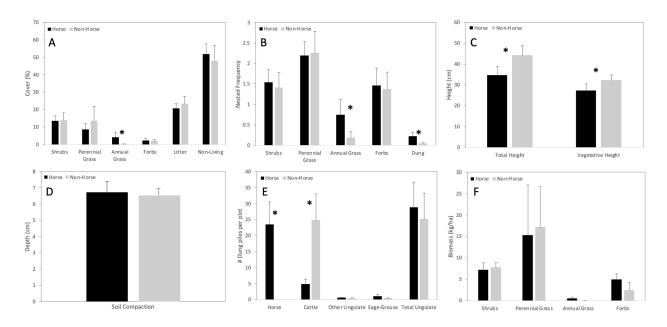


Figure 2.2: Graphs A-F: Comparison of all measurements between horse and non-horse use sites. A: percent cover by functional groups; B: nested frequency value averages that range from 1-4, the higher the value, the higher the frequency, the lower the value, the lower the frequency; C: plant height (total height is the overall height that includes reproductive inflorescences, vegetative height includes overall plant height without reproductive material); D: soil compaction depth measured in centimeters using a soil penetrometer; E: number of dung piles per 5 meter plot; F: biomass. *Significant differences (p<0.01) between horse and non-horse use sites.

TABLES

Table 2.1: Statistical comparison of habitat variables associated with greater sage-grouse habitat comparing the treatment effect (horse use vs. non-use) at each paired site. The estimate is the result from the values associated with horse use sites subtracted by non-use sites. Positive estimates indicate higher values at the horse sites while negative estimates indicate higher values at the non-horse sites.

Variables	Estimate	SE	DF	tValue	Probt
Nested Frequency Shrubs	0.1355	0.1236	35	-1.10	0.2803
Nested Frequency Perennial Grass	-0.05750	0.1836	35	0.31	0.7561
Nested Frequency Annual Grass	0.5600	0.1563	35	-3.58	0.0010*
Nested Frequency Forbs	0.08250	0.1988	35	-0.41	0.6807
Nested Frequency Dung	0.1725	0.04939	35	-3.49	0.0013*
Cover Shrubs (%)	-0.5000	1.4143	35	0.35	0.7258
Cover Perennial Grass (%)	-4.9000	2.1207	35	2.31	0.0269
Cover Annual Grass (%)	3.7500	1.3434	35	-2.79	0.0084*
Cover Forbs (%)	0.1500	0.6124	35	-0.24	0.8079
Cover Litter (%)	-2.6000	2.0277	35	1.28	0.2082
Cover Non-Living (%)	3.9500	3.1346	35	-1.26	0.2160
Biomass Shrubs (kg/ha)	-0.6180	2.2667	35	0.27	0.7867
Biomass Perennial Grass (kg/ha)	-1.8665	9.4516	35	0.20	0.8446
Biomass Annual Grass (kg/ha)	0.4115	0.3619	35	-1.14	0.2633
Biomass Forbs (kg/ha)	2.5055	1.3419	35	-1.87	0.0703
Soil Compaction (cm)	0.2100	0.3780	35	-0.56	0.5820
Total Height (cm)	-9.7000	2.8727	35	3.38	0.0018*
Vegetative Height (cm)	-5.1000	1.9834	35	2.57	0.0145*
Horse Dung (per plot)	23.4500	2.5202	35	-9.30	<.0001*
Cattle Dung (per plot)	-19.9667	3.4981	35	5.71	<.0001*
Other Ungulate Dung (per plot)	0.2167	0.2249	35	-0.96	0.3419
Sage-Grouse Dung (per plot)	0.5833	0.3176	35	-1.84	0.0747
Total Ungulate Dung (per plot)	3.7000	3.1790	35	-1.16	0.2523

sites combined.	Horse $\overline{\mathbf{x}}$	Sites SE	Non-Horse Sites $\overline{\mathbf{x}}$ SE				
Nested Frequency Shrubs	1.53	0.33	1.40	0.38			
Nested Frequency Perennial Grass	2.19	0.35	2.25	0.54			
Nested Frequency Annual Grass	0.74	0.38	0.18	0.15			
Nested Frequency Forbs	1.45	0.44	1.37	0.42			
Nested Frequency Dung	0.21	0.11	0.04	0.04			
Cover Shrubs (%)	13.25	3.20	13.75	4.50			
Cover Perennial Grass (%)	8.45	3.62	13.35	8.44			
Cover Annual Grass (%)	3.95	3.17	0.20	0.20			
Cover Forbs (%)	2.00	1.31	1.85	0.91			
Cover Litter (%)	20.60	2.95	23.20	4.43			
Cover Non-Living (%)	51.75	5.93	47.80	9.17			
Biomass Shrubs (kg/ha)	7.12	1.70	7.74	1.11			
Biomass Perennial Grass (kg/ha)	15.25	11.76	17.11	9.52			
Biomass Annual Grass (kg/ha)	0.45	0.37	0.04	0.03			
Biomass Forbs (kg/ha)	4.91	1.36	2.41	1.85			
Soil Compaction (cm)	6.74	0.77	6.53	0.49			
Total Height (cm)	34.49	4.46	44.19	4.72			
Vegetative Height (cm)	27.08	3.42	32.18	2.53			
Horse Dung (per plot)	23.45	7.10	0.00	0.00			
Cattle Dung (per plot)	4.73	1.63	24.70	8.34			
Other Ungulate Dung (per plot)	0.55	0.11	0.33	0.25			
Sage-Grouse Dung (per plot)	1.00	0.64	0.42	0.19			
Total Ungulate Dung (per plot)	28.73	8.04	25.03	8.26			

Table 2.2: Comparison of all mean habitat measurements for Horse (4) and Non-Horse (4) sites combined.

SUPPLEMENTAL MATERIAL

Appendix 1

Capture and Translocations

In efforts to prevent extirpation of sage-grouse in the Sheeprock Mountains, researchers at Utah State University partnered with Utah Department of Wildlife Resources to implement a translocation program. Translocations followed guidelines outlined by Reese and Connelly (1997) and Baxter et al. (2008). During the breeding seasons of 2016-2019, 146 sage-grouse (40 males and 106 females) were translocated from genetically compatible populations of sagegrouse located in Box Elder County and in Parker Mountain (Reese & Connelly, 1997; Oyler-McCance et al., 2005). These populations are greater than 50 km away from the Sheeprock Sage-Grouse Management Area, where the birds were released (Reese & Connelly 1997; Oyler-McCance et al., 2005). The source populations were approved by the Utah Regional Advisory Councils, the Wildlife Board, the Resource Development Coordination Council (RDCC), and the West Desert, Parker Mountain, and West Box Elder local working groups.

In the Sheeprock population, we captured 35 resident sage-grouse (23 females and 12 males) between the breeding seasons of 2016 to 2020. Rump-mounted, solar-powered Global positioning system (GPS) transmitters (Microwave Telemetry, Inc., Columbia, MD, USA, and GeoTrak, Inc., Apex, NC, USA) were deployed on 6 male and 15 females of the resident population, with the remaining individuals being fitted with 18-gram necklace-style very-high frequency (VHF) radio-collars (Advanced Telemetry systems, Insanti, MN, USA). They were immediately released following processing after capture. With the population being so low in the Sheeprock SGMA, capturing up to 10 grouse per year represented a realistic goal (Robinson & Messmer, 2013).

We captured individuals at night using all-terrain vehicles, spotlights, and long handled nets near active leks (2100hr to 200hr; Connelly et al., 2003). The translocated sage-grouse were either processed immediately upon capture or brought to a central area and processed before departing the source site. Individuals were either fitted with an 18-gram necklace-style the VHF radio transmitter or with the solar-powered GPS transmitters following capture protocols mentioned previously (Connelly et al., 2003). Five males and 38 females were marked with GPS transmitters with the remaining individuals marked with VHF radio collars. Processing included mounting the transmitter, ageing, sexing, weighing, marking with a 14-16 leg band for females and males, respectively, taking a feather sample for genetic analyses, and recording the capture location (UTM, 12N, NAD 83).

Field Monitoring

All marked sage-grouse were monitored from 2016-2020. To monitor sage-grouse vital rates and habitat-use, all translocated and resident sage-grouse were monitored weekly throughout capture to August and intermittently (monthly after August and an aerial telemetry flight performed once between December and February) throughout the fall and winter for VHF marked individuals. The GPS transmitted birds had a duty cycle of 5 days, where data were uploaded to the Argos System and accessed through Movebank at the end of each duty cycle. Four to six locations were programmed each day with four seasons, coinciding with sage-grouse lekking, nesting, brooding, and late fall seasons, that collect data at different times during the year: March 1 (0100hr, 0700-0800, 1300, 1700-1800), May 1 (0100-0700-0800, 1300, 1800-1900), June 16 (0000, 0200, 0700, 1300, 1600, 2000), and October 1 (0000, 0800, 1600, 2000). For each location, the date, time, elevation and speed were recorded. Mortality for the GPS

transmitters was determined using the data, which indicate a potential mortality after several fixes at the same location. After a mortality was detected, the observer located the transmitter and determined the cause of death, if possible.

During the nesting season, beginning around late March through early June, all radio-marked females were located 2 to 3 times per week to determine the date of nest initiation. Once a nest was confirmed and either hatched after 26-28 days of incubation or failed, the clutch size was estimated by counting the number of egg shells after the female leaves the nest. If a nest failed, the observer attempted to identify the cause for failure and that female was monitored 2 to 3 times a week to document re-nesting attempts. Broods were visually radio-tracked 3 times a week until the brood reached 50 days old post-hatch, at which point they were determined to be successful if there was at least one chick present with the female. Females that did have broods were monitored 1 to 2 times per week.

Late-brood rearing locations were delineated as four weeks post-hatch to the designated 50day brood age. Included in these data were 22 translocated females' broods and 8 resident females' broods. Appendix 1: Supplemental Material References:

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Appendix 2

Appendix 2 Table 1: Means of overall data collected at each of the 8 study sites. Sites are paired up by horse and non-horse sites (i.e. Benmore Pasture/Government Creek). Horse sites are designated with an asterisk (*).

	Benmore Pasture	Government Creek*	Butcher Troughs	Hamblin Wash*	High Schell	Spring Gulch*	Pony Express	Egan Canyon*
Nested Frequency Shrubs	0.25	0.64	1.90	1.88	1.63		1.81	1.74
SD	0.25	0_39	0_52	0.51	0.19	0.31	0.42	0_44
SE	0.11	0.17	0.23	0.23	0.08	0.14	0.19	0.20
Nested Frequency Perennial Grass	3.51	2.40	2.96	2.48	1.16		1.36	2.72
SD	0_11	0.43	0.17	0.43	0.25		0.56	0.17
SE	0.05	0.19	0.07	0.19	0.11		0.25	0.08
Nested Frequency Annual Grass SD	0.12	1.66 0.52	0.00	0.45	0.12		0.46	0.78 0.47
SE	0.03	0.32	0.00	0.30	0.05		0.32	0.47
Nested Frequency Forbs	0.86	0.78	1.77	2.69	0.51		2.34	1.14
SD	0.49	0.46	0.54	0.43	0.34			0.42
SE	0.22	0.21	0.24	0.19	0.15		0.08	0.19
Nested Frequency Dung	0.00	0.38	0.12	0.24	0.00	0.14	0.04	0.09
SD	0.00	0.26	0.13	0.23	0.00		0.07	0.09
SE	0_00	0.11	0_06	0.10	0.00		0.03	0_04
Cover Shrubs (%)	1.40	4.80	14.20	11.80	16.60		22.80	17.60
SD SE	1_14 0_51	1_30 0_58	6.02 2.69	3.83 1.71	1.82 0.81		7_29	4.72 2.11
Cover Perennial Grass (%)	37.20	18.20	13.40	5.00	1.40		1.40	9.20
SD	3_56	4.02	10.01	1.58	0.55		1.40	2.95
SE	1.59	1.80	4.48	0.71	0.24		0.68	1.32
Cover Annual Grass (%)	0.00	13.40	0.00	0.60	0.00			1.80
SD	0.00	8.65	0.00	1.34	0.00		0.84	L64
SE	0_00	3.87	0.00	0.60	0.00		0.37	0.73
Cover Forbs (%)	3.20	0.00	3.60	5.80	0.00			1.60
SD	2_49	0.00	3_21	2.05	0.00		0.55	0.89
SE Cover Litter (%)	1.11 33.20	0.00 28.20	1_44 23.80	0.92 13.80	0.00		0.24 24.20	0.40 20.00
SD	6.72	4_27	4.60	5.22	4.83		5.67	7.35
SE	3.01	1.91	2.06	2.33	2.16		2.54	3.29
Cover Non-Living (%)	25.80	35.80	44.80	62.60	70.40		50.20	50.00
SD	7_82	13.42	9.78	5_59	4.51		9.42	5.61
SE	3_50	6.00	4_37	2.50	2.01	2.91	4.21	2.51
Biomass Shrubs (kg/ha)	4.81	9.35	8.62	10.23	7.49		10.06	6.16
SD	6.60	12.61	6.38	10.30	4.67		6.22	3.73
SE Diama Danamial Carao (ha fa a)	2.95	5.64	2.85	4.61	2.09			L67
Biomass Perennial Grass (kg/ha) SD	42.06 13_75	50.22 84_31	21.67 16.97	7.85 7.13	4.40 3.85		0.32	1.88 1.05
SE	6.15	37.71	7.59	3.19	1.72		0.17	0.47
Biomass Annual Grass (kg/ha)	0.02	1.57	0.00	0.05	0.02		0.13	0.15
SD	0.04	3.20	0.00	0.08	0.04		0.29	0.21
SE	0.02	L43	0.00	0.03	0.02	0.05	0.13	0.10
Biomass Forbs (kg/ha)	0.91	0.88	7.93	6.64	0.00	6.41	0.78	5.72
SD	0_86	1.18	6_82	6.48	0.00		0.41	3_97
SE	0_38	0.53	3.05	2.90	0.00		0.18	1.77
Soil Compaction (cm)	6.80	5.71	5.28	6.36	6.40		7.62	5.85
SD SE	0_97 0_43	0.72 0.32	0.58	0.61 0.27	0.28		0_30 0_14	0.82 0.36
Total Height (cm)	49.61	45.21	52.73	24.18	31.38		43.02	37.23
SD	5.13	16.53	5.76	3.14	3.11		7.17	2.27
SE	2.29	7.39	2.57	1.41	1.39		3.20	1.02
Vegetative Height (cm)	33.73	32.70	33.31	17.51	24.95	26.94	36.74	31.18
SD	3.81	10_50	6.09	235	3.49	2.58	6.20	2.46
SE	1.70	4.70	2.72	1.05	1.56		2.77	L10
Horse Dung (per plot)	0.00	41.67	0.00	27.73	0.00			11.47
SD SE	0_00 0_00	8.34 3.73	0.00 0.00	4.23 1.89	0.00		0.00	6.08 2.72
Cattle Dung (per plot)	44.67	5.07	32.07	9.20	13.07		9.00	2.60
SD	20_06	1.80	13.31	1.95	7.77			1.42
SE	8_97	0.81	5.95	0.87	3.48			0.64
Other Ungulate Dung (per plot)	0.20	0.27	0.07	0.53	1.07		0.00	0.60
SD	0_30	0.37	0.15	0_38	1.74			0.64
SE	0_13	0.16	0_07	0.17	0.78			0.29
Sage-Grouse Dung (per plot)	0.67	2.73	0.80	0.00	0.20			0.07
SD	0.67	1.48	1.30	0.00	0.45			0.15
SE Total Ungulate Dung (per plot)	0_30 44.87	0.66 47.00	0.58 32.13	0.00 37.47	0.20			0.07 14.67
SD	20.00	47.00 9.73	13.26	5.11	8.00			4.89