University of Nevada, Reno

Impacts of non-native ungulate grazing on reproduction and physiology in Greater Sage-grouse

A dissertation submitted in partial fulfillment of the requirements of the degree Doctor of Philosophy in Ecology, Evolution, and Conservation Biology.

by

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Abstract

Understanding the mechanisms underlying dynamics of animal populations is critical to planning appropriate management actions for species of concern. Greater Sage-grouse (*Centrocercus urophasianus;* hereafter "sage-grouse") face many threats across their range in North America. My dissertation examines the effects of two of these threats, livestock and feral horse grazing, on sage-grouse reproductive strategies and physiology.

In Chapter 1, I investigated influences on the start of the sage-grouse breeding process. Sage-grouse exhibit an intermediate pace on the life-history spectrum, which leads us to ask what kind of trade-offs they may experience and what costs might incur. I used data on the breeding status and survival of marked females to estimate breeding propensity and the potential cost of breeding under climatic variation and a range of non-native grazing pressure. I found both past and current weather influenced the decision to breed and increasing livestock numbers led to reduced breeding in drought years. Breeding rates increased slightly with feral horse presence, likely due to overlap in habitat use during drier conditions. Breeding birds had lower daily survival rates than birds what did not breed at all, revealing an immediate cost of reproduction.

In Chapter 2, I evaluated the physiological response of grouse to variation in climate and non-native grazers as a possible mechanism for population decline. I used an assay of feathers collected from wild sage-grouse to measure levels of the avian stress hormone, corticosterone. I also examined short-term demographic consequences of increased hormone levels. Both horses and livestock increased corticosterone, primarily

under dry climatic conditions. Nest survival was lower with increased hormone levels, but other demographic rates did not correlate with this measure.

Lastly, in Chapter 3 I addressed aspects of reproduction and the maintenance of genetic diversity in sage-grouse populations. I measured the occurrence of multiple paternity and conspecific nest parasitism using genetic samples from monitored nests. I found several nests that were the result of mating with multiple males, and one nest that had an egg laid by another female. These phenomena are likely important to female fitness, particularly for persisting through environmental variation.

The project goal was to better understand the complex interactions of sage-grouse, their habitat, and non-native grazers. I showed the importance of including precipitation in assessing the impacts of grazers, and that genetic analyses can reveal even more about these populations. This work adds to other recent evidence supporting the need for better management of livestock and feral horses across sage-grouse habitat.

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General Introduction

Greater sage-grouse (*Centrocercus urophasianus;* hereafter, "sage-grouse") are a symbol of the North American sagebrush ecosystem. The current range of sage-grouse has declined to half of the historic range, but still includes parts of 11 western U.S. states and 2 Canadian provinces (Schroeder et al., 2004). Land conversion, energy development and resource extraction, livestock grazing, wildfire, drought, and disease are among the many contributing factors to this decline (Knick & Connelly, 2011). Considerable effort has been made to understand the impact of some of these factors (Blickley et al., 2012; Chambers et al., 2014; Knick & Connelly, 2011; Naugle et al., 2004), but more research is necessary to quantify direct and indirect effects of grazing (Beck & Mitchell, 2000; Boyd et al., 2014; Dahlgren et al., 2015; Smith et al., 2018; Williamson et al., 2020). In the following chapters, I investigate factors likely to impact populations of sage-grouse through time and across much of their range. First, I look at the effects of non-native grazers and influence of precipitation on vital rates early in the annual cycle of sage-grouse. Then, I evaluate a bioindicator of population health and a potential mechanism of impact. Last, I explore breeding behavior and the genetic basis for population change. In this section, I review relevant literature on the Great Basin, feral horses, livestock, and sage-grouse.

Great Basin

Sage-grouse populations in the Great Basin experience unique circumstances compared to the rest of the species range. With only 15-30 cm of annual precipitation, wildlife rely on winter snowmelt, small natural springs, and scarce wet meadows (Donnelly et al., 2016; Suring et al., 2005) to support reproductive processes like the

growth of chicks. The region is increasingly under threat from prolonged drought, large-scale wildfires, and the spread of invasive annual grasses (Chambers et al., 2014). The limited supply and increasing demand for water have increased tensions between consumers including native wildlife, domestic livestock, and feral horses (Beschta et al., 2013; Runge et al., 2019).

Feral Horses

The Great Basin once had a native equid grazing across the northern landscape, but they went extinct over 10,000 years ago (Grayson, 2011; Luís et al., 2006). These animals co-existed with predator and vegetation communities that also differ from those that now occur in the region. The flora of the area changed considerably about 7,000 years ago, creating a new plant community unaccustomed to intensive grazing (Grayson, 2011). It wasn't until Spanish explorers introduced domestic stock more than 500 years ago that North America once again had horses. Through intentional release and accidental escapes, these horses became free-roaming. Through the years, "wild" horses became a symbol and the spirit of the American West, despite their relatively recent introduction.

The legal status of such horses changed with the Hunting Wild Horses and Burros on Public Lands Act of 1959, after a public awareness campaign spearheaded by "Wild Horse Annie" in Nevada (Public Law 86-234). Horse numbers had been severely reduced and there was public concern over the treatment of the remaining animals. In 1971, the Wild Free-Roaming Horses and Burros Act passed through the U.S. Congress, significantly altering how these animals could be managed (Public Law 92-195). The act includes a mandate that the BLM must "maintain thriving natural ecological balance in

combination with other uses." Appropriate Management Levels (AMLs) were established for different areas as target horse and burro population numbers. However, on many parts of the range, pushback from horse advocacy groups has impeded the removal of enough horses to meet those objectives (Scasta, 2019; Scasta et al., 2016).

As of March 2020, there are an estimated 95,000 free-roaming horses and burros on the range, which is more than three times the AML (Bureau of Land Management, 2020b). These animals inhabit parts of 10 western states, but over half of those horses and burros are in the state of Nevada. Two-thirds of the program's budget is required for maintenance of an additional 48,000+ animals in off-range holding facilities (Bureau of Land Management, 2020b). When unchecked by removals or other treatments, these herds can double in size every four years. Removals have been limited by public pushback, budget constraints, and a decrease in adoptions over time (Scasta et al., 2018). Current infertility treatment options are insufficient to reduce herds to AML, but could be useful for maintaining population levels once they have been reduced.

Numerous studies have demonstrated negative effects on wildlife and vegetation in areas of high horse use. Heavy horse use around water sources can lead to the disruption and even exclusion of native ungulates including mule deer (Hall et al., 2018), pronghorn (Gooch et al., 2017; Hall et al., 2018), and bighorn sheep (Ostermann-Kelm et al., 2008). The species richness of reptiles and the completeness of small mammal communities can also be affected (Beever & Brussard, 2004). Studies in the Great Basin have found decreased species richness of plants (Beever et al., 2008) and reduced aboveground herbaceous and grass biomass (Baur et al., 2018) with increasing grazing disturbance. A study on Sheldon National Wildlife Refuge, which has no domestic

livestock grazing, demonstrated the effects of horses by using grazing exclosures in riparian areas (Boyd et al., 2017). Herbaceous stubble heights were reduced up to 80%, visual obstruction was reduced up to 70%, and bare ground increased sevenfold outside compared to inside exclosures. Soil compaction can occur through direct effects of trampling by horses, but also through indirect effects on aboveground vegetation (Beever & Herrick, 2006). For sage-grouse, the effects of horses have not been well documented, other than the aforementioned habitat disturbance.

Horse grazing is likely to have unique effects on the ecosystem compared to grazing by livestock. Horses have cecal digestion, in contrast to the rumination of cows and native ungulates (Hanley & Hanley, 1982). Cecal digestion is faster but less efficient, thus horses consume 20-65% more plant volume than cows. Horses can also remove vegetation lower to the ground than cows because they have flexible lips and long incisors on both the lower and upper jaw. Horses also use the landscape less uniformly than cows, following trails, maintaining stallion territories, and preferring steeper slopes (Vavra & Ganskopp, 1987).

Livestock

Despite having a human presence for over 12,000 years, the Great Basin did not experience widespread anthropogenic change until European settlement around 150 years ago (Morris & Rowe, 2014). Domestic livestock, such as cattle and sheep, have been present in North America since the sixteenth century but reached substantial numbers by the early 1900s. The Taylor Grazing Act of 1934 came about after the recognition of the negative consequences of overgrazing on soil and vegetation (Morris & Rowe, 2014).

Currently, the BLM oversees livestock grazing on over 62 million hectares of public lands (Bureau of Land Management, 2020a).

Unlike feral horses, there are many different types and intensities of cattle grazing, both currently and historically. This spectrum of management likewise creates variation in the effect of livestock grazing on the habitat and native wildlife. Much of what we know about the impact of cows on the range comes from their removal. Riparian vegetation on Hart Mountain National Antelope Refuge in Oregon showed recovery 20+ years after cows were removed, including decreased bare soil and channel erosion, increases in grass/sedge/forb cover, and recovery of willow and aspen (Batchelor et al., 2015; Beschta et al., 2014; Dobkin et al., 1998). This coincided with an increase in wet meadow bird species returning to newly cow-free areas (Dobkin et al., 1998).

In a meta-analysis of grazing and grouse species globally, livestock grazing had negative effects on adult grouse and chick production (Dettenmaier et al., 2017). Quantifying the actual effects of livestock grazing on sage-grouse is complicated by the legacy of management actions taken for the benefit of livestock. Much of the reduction in sagebrush habitat in the mid-twentieth century can be attributed to chemical and mechanical treatments as well as seeding of non-native grasses to increase forage for livestock (Beck & Mitchell, 2000; Boyd et al., 2014). Indirect effects on sage-grouse can include decreased survival and increased nest predation near fences (Cutting et al., 2019; Stevens et al., 2012), increased incidence of West Nile from standing water sources (Naugle et al., 2004), and increased nest predation from ravens (Coates et al., 2016). Livestock grazing in the Great Basin also correlates with increased occurrence and prevalence of the invasive annual cheatgrass (*Bromus tectorum*) (Williamson et al.,

2020). Cheatgrass competes with native bunch grasses and decreases sagebrush cover through a positive feedback loop with fire. Comparison of sage-grouse trends with grazing records has suggested the importance of the timing of grazing within a year (Monroe et al., 2017). The Sage-Grouse Initiative has supported research on rotational grazing to better inform management strategies (Smith et al., 2018).

Study Species: Greater Sage-Grouse

Mating

Sage-grouse are a lekking species like many other birds, insects, fish, reptiles, and even some mammals (Höglund & Alatalo, 2014). The annual cycle begins with males congregating on leks, or strutting grounds, to display their elaborate plumage as well as vocalizations and mechanically produced sounds (Gibson, 1996; Gibson & Bradbury, 1985; Patricelli & Krakauer, 2009). Lek locations are relatively consistent through time, indicating strong site fidelity (Gibson et al., 2014) and may be associated with areas heavily used by females (Bradbury et al., 1989). These areas are characterized by large open areas with lower shrub cover allowing for male displays to be seen at a distance by females. Males begin to arrive as early as late February, and the lekking season can last as late as early June (Wiley, 1978). While daily attendance varies (Blomberg et al., 2013), the largest leks may have hundreds of males at the peak of breeding (Scott, 1942). Researchers have long observed that matings are highly skewed towards only a few males, which may lead to reduced genetic variation in populations (Gibson & Bradbury, 1985; Patterson, 1952; Scott, 1942; Stiver et al., 2008). Once a female decides on a male based on the complex signals, they copulate in mere seconds and the male's contribution

to reproductive success ends. The female is solely responsible for nest-site selection, incubation, and chick rearing.

Nesting

Females typically nest under sagebrush, however, nests have also been found under other shrubs, clumps of tall grass, and even rocks (Gibson et al., 2016; Kolada et al., 2009). The nest usually consists of a shallow bowl or depression under the shrub. Greater sage-grouse lay an average of 7-8 eggs per clutch, though this varies spatially (Blomberg et al., 2014; Knick & Connelly, 2011). Incubation requires approximately 27 days, after which, a successful nest will hatch and the precocial chicks will be ready to leave the nest with the hen immediately (Schroeder et al., 1999). Ravens and badgers are the main nest predators, but nest depredation by coyotes, snakes and bobcats have been documented as well (Coates et al., 2008). The presence of both horizontal and vertical cover protects from aerial and ground predators. Studies on nest-site selection and nest success show variable importance of sagebrush and other shrub cover, forb abundance, and residual grass height for nest success as well as chick survival immediately after hatch (Gibson et al., 2016; Gregg et al., 1994; Kolada et al., 2009).

Brood-rearing

Once hatched, mammalian, or reptilian predators may consume sage-grouse chicks (Gregg et al., 2007). Females brood their chicks at night for warmth, particularly through the turbulent weather of late spring and early summer. Chicks stay with the hen until late summer when they are nearly adult size and fully independent (Schroeder, 1997). Adoption of chicks by other brood-rearing females also occurs (Dahlgren et al., 2010). An abundance of insects, particularly Lepidoptera, and forbs are important for

chick growth and survival (Gregg & Crawford, 2009; Klebenow & Gray, 1968). The availability of suitable brood-rearing habitat is one of the factors limiting recruitment in much of sage-grouse range and particularly in the water-limited Great Basin (Atamian et al., 2010).

Conservation Issues

The widespread nature of this species has made the coordination of conservation efforts across states extremely difficult and prone to inconsistency. Much of the land still occupied by sage-grouse has value to humans for ranching and other agriculture, oil and gas development, and urbanization. Adding to the difficulty is the more recent understanding that not all populations of sage-grouse react to threats in the same way (Knick & Connelly, 2011). The habitat on which sage-grouse depend falls almost entirely on federal lands, which are then required to follow federal land management regulations. However, much of the management has been implemented by state agencies, local working groups, and conservation organizations (Duvall et al., 2017; Sage Grouse Initiative).

Study Area

Our project encompassed three main study regions in the northern Great Basin (Figure 1). Hart Mountain National Antelope Refuge (hereafter Hart Mountain) in southcentral Oregon was established in 1936 for the conservation of pronghorn antelope and other wildlife. The refuge encompasses over 112,500 hectares and ranges in elevation from about 1,360 to just over 2,445 m at the top of Warner Peak. Domestic livestock grazing ceased on Hart Mountain in 1991 (Boyd et al., 2017; Dobkin et al., 1998). Sheldon National Wildlife Refuge (hereafter Sheldon) in northern Nevada was originally

established in 1931 and then expanded in 1936. The current extent of the refuge includes over 231,840 hectares. The lowest elevation is approximately 1,300 m at the bottom of the Thousand Creek Gorge and the highest peak is Catnip Mountain, which reaches just over 2,220 m. Domestic livestock were removed from Sheldon in 1994, and nearly all feral horses were removed in a concerted effort in 2014-2015 (Boyd et al., 2017; Dobkin et al., 1998). The BLM-managed portion of our study is delineated by the Vya and Massacre Sage-grouse Population Management Units (hereafter Vya-Massacre) in northwestern Nevada and the border of northeastern California. Within these areas are many BLM allocated grazing allotments for domestic cattle, as well as Herd Management Areas (HMAs) for feral horses. Livestock are restricted to the allotments, whereas horses roam within and outside of the HMAs. Together, the Vya and Massacre units encompass approximately 711,200 hectares and range in elevation from 1200 m to 2700 m.

Data Collection

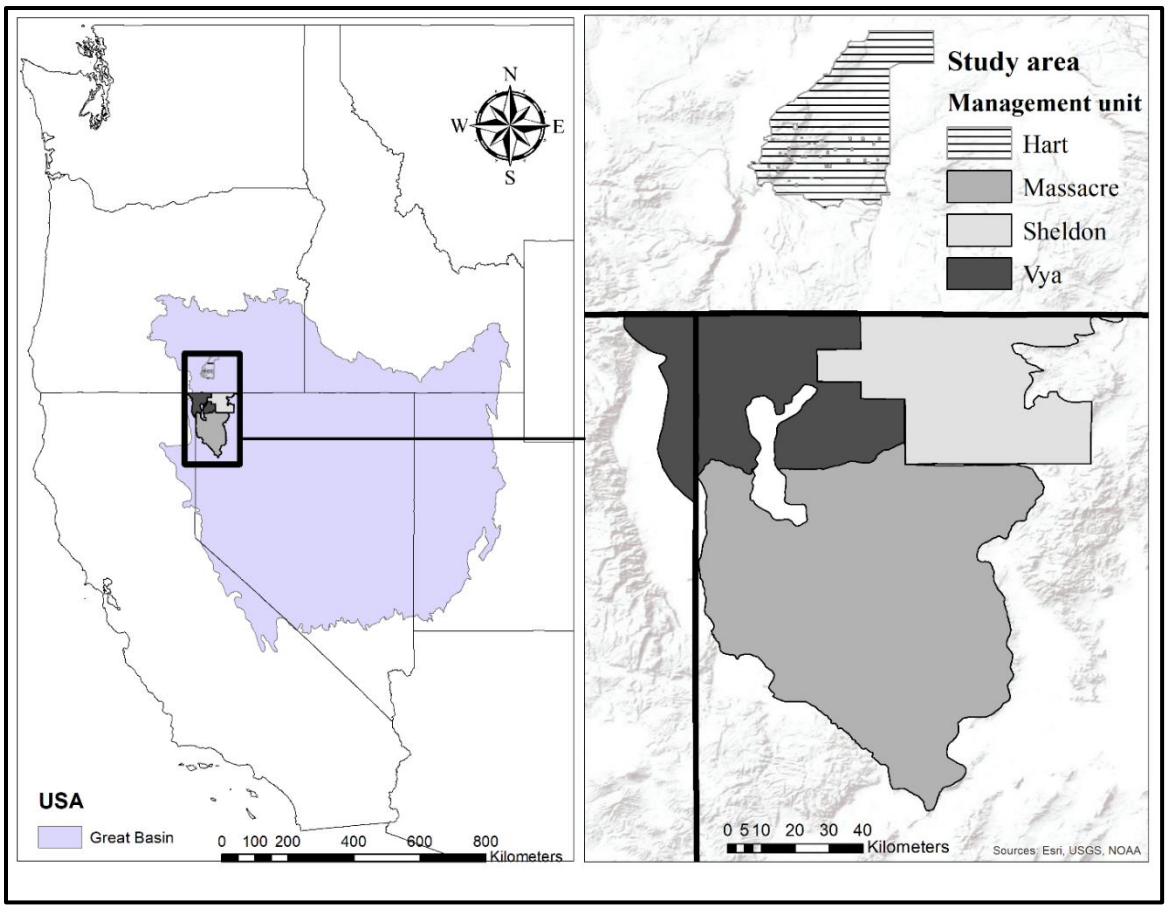
We trapped yearling and adult female sage-grouse during the spring every year, near active leks, and during the fall in some years. We captured birds at night using spotlights (Giesen et al., 1982) and handheld nets. Once captured, we banded females (n = 778) with unique metal bands (size 14) and fit them with 22g very high-frequency necklace-style radio-collars (VHF; Advanced Telemetry Systems, Isanti, MN, USA). These collars are equipped with a mortality signal that increased the beat frequency if a collar did not move for an extended period (8 hours for 2013 and 2014 collars, 2 hours for subsequent years). We measured culmen using dial calipers (± 1 mm), tarsus using dial calipers (± 1 mm), flat wing using a board (± 1 mm), and weighed each individual (±

1 g). We aged birds as juvenile or adult based on plumage characteristics (Crunden, 1963) and collected a breast feather sample.

We then monitored collared hens throughout the year. We accumulated over 7,000 telemetry locations during the course of the study. The highest level of effort took place during the breeding season, from March through July. We monitored females every 2-3 days during the early nesting period, to document nesting and success or failure of nests. We found and monitored over 800 nests. Our protocol for 2017-2019 included the collection of eggshell membranes from nests after hatch or failure. For hens that had successful nests, we began brood monitoring within the first 2-3 days after chicks hatched. We performed weekly video checks of these broods, to count the surviving chicks up to 42-49 days of age. We followed over 300 broods during the study. For the remaining months of the year, we employed aerial telemetry to assess live/dead status and location, approximately monthly.

Figures

Figure 0-1. Study areas map.



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Chapter 1: Climate and non-native herbivores influence reproductive investment in Greater Sage-grouse

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Abstract

Highly seasonal environments can increase competition among herbivores for nutrients, leading to consequences affecting rates of reproduction and survival. We estimated breeding propensity, the annual proportion of females attempting a nest, for Greater Sage-grouse using 7 years of data from the northwestern Great Basin, USA. We investigated the effects of weather and sympatric non-native herbivores, feral horses and domestic cattle, on reproductive rates and female survival. Adults bred at a higher rate than yearlings. If the first nest failed, renesting rates were similar between adults and yearlings. Birds in better body condition at the start of the season bred more, and moderately snowy winters had the highest breeding propensity. Drier conditions led to poor breeding, particularly in areas with dense cattle grazing. Female survival was lower for breeders, indicating a survival cost of reproduction. Areas with abundant feral horses had slightly higher breeding propensity, though other research suggests negative impacts later in the breeding cycle. Sage-grouse face life-history trade-offs that may be shifting due to changing climatic conditions. Our work provides a framework to evaluate these conditions, and suggests that the effects of adverse weather on sage-grouse life-history may be exacerbated by competition with non-native ungulates.

Introduction

Competition between herbivores is well established (Arsenault & Owen-Smith, 2002), frequently acting through depletion of nutritious foods (Augustine & Springer, 2013) with secondary effects on nutritional status reducing fitness among competitors (DeGabriel et al., 2009). Herbivory can also activate plant chemical defenses (Janssen et

al., 2002), making them less palatable (Frye et al., 2013), increasing toxicity, and reducing nutritional quality of foods (DeGabriel et al., 2009). Reduction of resources through seasonal or persistent drought increases the fitness consequences of this competition (Donnelly et al., 2018b). Such effects can reduce recruitment, intrinsic rate of population increase and equilibrium population size (Coates, O'neil, et al., 2021; Dettenmaier et al., 2017; Eldridge et al., 2020; Monroe et al., 2017).

While nutritional status primarily influences investment in individual offspring in herbivorous mammals (Keech et al., 2000; Monteith et al., 2013; Veiberg et al., 2017), it can also influence the decision of whether to breed at all in herbivorous birds. Females in long-lived avian species that fail to attain a minimum threshold of nutrition forgo breeding (Reynolds, 1972), likely because such females ultimately have a low probability of producing a clutch of eggs, successfully incubating them and rearing the resulting offspring (Alisauskas & Ankney, 1992, 1994; Guillemain et al., 2008). Life-history theory predicts that longer-lived species may forgo breeding if the cost of breeding is too great in a given year and if there are opportunities to breed in future years (Souchay et al., 2014; Stearns, 1992).

The decision to breed has been overlooked, relative to other demographic rates (Sedinger et al., 2001) but can be as important to both individual fitness (Sedinger et al., 2008) and population dynamics as other components of reproduction. For example, Sedinger et al. (2011) showed that wintering location was strongly predictive of the probability that female Black Brant (*Branta bernicla nigricans*) nested and that warmer sea surface temperatures on wintering areas reduced breeding probability during the next summer (Sedinger et al., 2006). Blomberg et al. (2017) showed that the probability that a

female Greater sage-grouse (*Centrocercus urophasianus*; hereafter sage-grouse) initiated a nest was strongly negatively density dependent, ranging from ca. 0.6 to 0.95, even though their lek breeding system means that breeding partners are not a limiting factor (Gibson & Bradbury, 1985).

Greater sage-grouse, a species of conservation concern (Coates, Prochazka, et al., 2021; Knick & Connelly, 2011), are herbivorous as adults and forage nearly exclusively on various species of sagebrush (*Artemesia* spp.) during fall and winter (Remington & Braun, 1985). Palatability and nutritional quality of sagebrush responds to browsing by herbivores (Forbey et al., 2013; Frye et al., 2013) and influences the availability of foods for sage-grouse during winter. Thus, presence of other herbivores has the potential to affect nutrients available to sage-grouse, which could affect nutritional status of female sage-grouse before breeding and their investment in reproduction (Barnett & Crawford, 1994; Gregg et al., 2008; Gregg et al., 2006).

Feral horses (*Equus ferus caballus*) and livestock, mostly in the form of cattle (*Bos bos*), are the competing herbivores comprising the greatest biomass in North America's Great Basin (Zeigenfuss et al., 2014), a landscape with highly seasonal fluxes in resource availability (Donnelly et al., 2018a). Feral horses are socially dominant to all other herbivores (Gooch et al., 2017; Hall et al., 2018; Ostermann-Kelm et al., 2008) and are currently at nearly four times the numbers considered to be consistent with ecosystem sustainability by the U. S. Bureau of Land Management (BLM; Bureau of Land Management, 2020b). Livestock numbers, in contrast, have been relatively stable over the past several decades (Bureau of Land Management, 2020a). Abundance of feral

horses (Coates, O'neil, et al., 2021) and livestock (Monroe et al., 2017) was recently negatively associated with the finite rate of population increase (λ) in sage-grouse.

Our objective was to assess the hypothesis that non-native ungulates influence breeding effort of female sage-grouse and that such effects could be exacerbated by drought conditions. We estimated breeding propensity of female sage-grouse in the Great Basin in response to use of the landscape by feral horses and livestock over 7 years using Bayesian multi-state models to estimate daily transition probabilities of females to nesting and renesting. We used numbers of ungulates on the landscape, based on BLM's surveys of horse numbers and billed number of cows in each BLM allotment for livestock as measures of the abundance of these two herbivores. We also assessed body condition, as a measure of nutritional status, of female sage-grouse as a response to presence of ungulates and as a predictor of breeding propensity by sage-grouse. We estimated female survival based on breeding status to address the possible trade-offs between reproduction and survival.

Methods

We collected sage-grouse telemetry data from 2013 to 2019 in southeastern Oregon and northwestern Nevada (Figure 1). Hart Mountain National Antelope Refuge in southern Oregon covers 112,500 hectares of habitat that has been free of grazing by non-native ungulates since 1991 (Boyd et al., 2017; Dobkin et al., 1998). Sheldon National Wildlife Refuge in Nevada is 231,840 hectares, and has been livestock-free since 1994, but feral horses were only more recently removed in 2014-2015 during this study (Boyd et al., 2017; Dobkin et al., 1998). Our remaining study lands encompass approximately

711,200 hectares managed by the BLM in northern Nevada. This area has both livestock grazing allotments and Herd Management Areas (HMAs) for feral horses (Bureau of Land Management, 2020a, 2020b).

Field Methods

We trapped yearling and adult female sage-grouse near active leks during the spring every year and during the fall in some years. We captured female sage-grouse at night using spotlights (Giesen et al., 1982) and handheld nets. Once captured, we banded females (n = 778) with unique metal bands (size 14) and fit them with 22g very high-frequency necklace-style radio-collars (VHF; Advanced Telemetry Systems, Isanti, MN, USA). Radio collars are equipped with a mortality signal that increased the beats per minute if a collar did not move for an extended period (8 hours for collars applied in 2013 and 2014, and 2 hours for collars applied in subsequent years). We measured culmen length using dial calipers (± 1 mm), tarsus length using dial calipers (± 1 mm), flat wing using a board (± 1 mm), and weighed each individual (± 1 g).

We monitored females every 2-3 days during the early nesting period, to document nesting and success or failure of nests. We flushed females from nests approximately 10 days after the nest was found in order to count and float two eggs to estimate nest age (Gibson et al., 2015). If a female's first nesting attempt failed, we continued to track her every 2-3 days to document renesting attempts. Females were monitored until their first nest hatched, their second nest failed, or until July 1st (Blomberg et al., 2017; Gregg et al., 2006).

Individual Covariates

We assigned the age as assessed at capture as a yearling or an adult female based on plumage characteristics (Crunden, 1963). If yearling females survived the first breeding year, we advanced their age to "adult." We used body measurements and mass at capture to create a metric of body condition, the residuals of body mass regressed on the first principal component of female structural size (Blomberg et al., 2014; Freeman & Jackson, 1990). We assigned zero values for any individuals that were not measured.

Environmental Covariates

We used cumulative precipitation from December 1 through February 28 ('WPRCP') from PRISM (PRISM Climate Group, 2011) precipitation data downloaded from Climate Engine (Huntington et al., 2017) at a 4-km resolution as a measure of current conditions. For a longer assessment of drought conditions, we used the prior year precipitation ('PYP'; cumulative from October 1 to September 30) corresponding to the next breeding year, also from PRISM (PRISM Climate Group, 2011; Huntington et al., 2017). This measure did not overlap with the winter precipitation, thus we included both in the model.

We used annual population estimates for the Herd Areas and Herd Management Areas based primarily on alternate-year aerial surveys (Bureau of Land Management, 2020b). Estimates between survey years were based on interpolation by BLM between survey estimates. We estimated domestic cattle usage by billed number of cows in each grazing allotment times the number of days they were present (BLM Applegate Field Office unpublished data) in the year prior to our monitoring for each female. So that estimates of each of the ungulate species were comparable, we multiplied the number of

horses by 365 days (because horses are present throughout the year), and we divided cow- and horse-days by the area of the HMA or allotment. Our final covariates were horse or cow use days per km^2 for the year prior to breeding.

We calculated averages of all four environmental variables for a 5-km buffer around the leks where females were marked (Coates et al., 2016). These buffers encompassed the majority of locations occupied by females before breeding and the nesting period (Bradbury et al., 1989; Gibson et al., 2014).

Breeding Propensity Model

We implemented a Bayesian multistate model to estimate daily transition probabilities between partially observable latent states of biological interest. We considered seven states (Figure 2), and estimate daily transition probabilities between these states over a 122 days, from March 1st to July 1st. We assumed that technicians could accurately determine the female's state each time she was located. We also assumed that females did not transition to another state and back between consecutive locations of the female in the same state. For example, if a female was located on a nest on days 50 and 55, we assumed she remained in the nesting state during days 51-54. If females transitioned from one state to another between locations, we considered her state to be unknown and let the model determine when this transition occurred. Under these assumptions, we stored our data in an array $y_{i,t,s}$, where each individual *i* at time *t* was assigned to state *s*. Using these data we estimate daily transition probabilities ($\psi_{i,\,t,\,s}$) as:

$$y_{i,t,s} \sim Multinomial(\psi_{i,\,t,\,s}) \quad (1)$$

Because transitions of females between some states were impossible, we constrained $\psi_{i,\,t,\,s}$ with the transition matrix

$$\begin{bmatrix} (1-\psi_{gn1,i,t})sg & \psi_{gn1,i,t}sg & 0 & 0 & 0 & 0 & 1-sg \\ 0 & \psi_{n1n1,i,t}sn & \psi_{n1f1,i,t}\,sn & 0 & 0 & \psi_{n1h,i,t}sn & 1-sn \\ 0 & 0 & 1-(\psi_{n1f2,i,t})sf & \psi_{f1n2,i,t}sf & 0 & 0 & 1-sf \\ 0 & 0 & 0 & \psi_{n2n2,i,t}sn2 & \psi_{n2f2,i,t}sn2 & \psi_{n2h,i,t}sn2 & 1-sn2 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \quad (2)$$

where ψ_{gn1} was the probability of a female transitioning from a non-breeding state (g = general, non-breeding) to a first nest (n), ψ_{n1n1} was the probability of a female remaining on her first nest, ψ_{n1f1} was the probability the first nest failed, $\psi_{f1,n2}$was the probability of a female attempting a second nest conditioned on the first nest having failed, ψ_{n2n2} was the probability of a female remaining on a second nest, ψ_{n2f2} was the probability a female's second nest failed, and lastly, ψ_{n1h} and ψ_{n2h} were the probabilities that either the first or second nest hatched. All of these transition probabilities were conditioned on each female's survival. To assess whether females were surviving at different rates depending on their breeding state, we evaluated four survival probabilities where *sg*, *sn*, *sf*, and *sn2* were the survival probabilities of females estimated to be non-breeding, attending a first nest, having a failed nest, and attending second nest respectably. The last three rows in the transition matrix represent three terminal states indicating a failed second nest, hatched first or second nest, or female mortality. Once a female transitioned to any of these three states, we truncated the capture history data as we were not interested in these probabilities.

Model selection and reporting of parameters

Before we fit any models, we evaluated the data for correlations among the 6 covariates of primary interest. Our full Bayesian multi-state model to evaluate breeding propensity, adult survival, and nest survival had long run times (approximately 5 days to run 1,000 MCMC iterations on 8 cores at a speed of 3.4GHz with 16 GB RAM) causing model selection analysis on the full model to be computationally expensive. In an effort to increase efficiency in the model selection process we used a 3 step approach.

First, because breeding propensity was the primary parameter of interest, we created a model to evaluate that life history stage individually. For this model, the data ($y_{i,t}$) was 0 for each day (t) female (i) was known to be alive but not breeding, and a 1 for the first time the female was detected on the nest (1^{nest_i}). We right-censored the data for each female after she was detected on the nest. This data structure allowed us to fit a Bernoulli trial (Equation 3) analogous to ψ_{gn} from the full model, where logit ($\psi_{gn,i,t}$) was a function of the design matrix $\beta_{gn}X$. This model would run in minutes rather than days and allow us to test hypotheses associated with breeding propensity.

$$y_{i,t} \sim \text{Bernoulli}(\psi_{gn,i,t}), \quad t = 2, ...,1^{nest_i} \tag{3}$$

Second, we had several hypotheses containing interactions and quadratic relationships. To assess support for these hypotheses, we specified $\beta_{gn,}X$ to contain all six covariates of interest as additive effects and considered 6 different structures for the interactions and quadratic effects. These 6 models represented a balanced model set that contained all combination of either an interaction between the individual covariates and the weather covariates, quadratic effects for the weather covariates, or a combination of the interactions and quadratic effects. Abbreviated model structures can be found in

Table 1, and full model structures can be found at the end of the manuscript indexed as Models 1-6. Because females were not observed breeding early or late during the season, we considered all models to contain a quadratic term fit to the number of days since March 1st in any given year, which allowed the probability of transitioning to nesting to approach zero early and late in the breeding season. Because females were not observed breeding early in the season we used a prior for ($\beta_{\text{gn},0} \sim normal(-10,\text{SE}=5)$) rather than a prior centered on 0; this reflected transitions approaching zero when transformed from the logit scale. For the priors $\beta_{\text{gn,c}}$ for each covariate c, we fit a prior $\sim$ normal(0,SE=1.5) (Northrup & Gerber, 2018), and we used deviance information criterion (DIC) to rank the models in terms of their expected predictive error.

Once the best model structure for Equation 3 was found with respect to our interactive and quadratic coefficients, we fit the full model conditioned on survival. When there were more than two transition probabilities from a given state, we used a multinomial logit link (mlogit) to constrain the transition probabilities from each state to sum to 1. Blomberg et al. (2017) highlight the importance of nest age in the probabilities of nest survival. To control for nest age, we incorporated age structure into the linear models associated with each transition probability. Age for each individual at time *t* in each state was calculated as the $\sum y_{i,1:t,s}$, or the amount of time a female had been in each state. From the onset of a first nest, a female could remain on that nest (ψ_{n1n1}), modeled as:

$$mlogit(\psi_{n1n1,i,t}) = \beta_{n1n1,0} + \beta_{n1n1,age} \times age_{n1,t} \quad (4)$$

where $age_{n1,t}$ was the age of the nest in days at time *t*. The nest could hatch (ψ_{n1h}), modeled as:

$$mlogit(\psi_{n1h,i,t}) = \beta_{n1h,0} + \beta_{n1h,age} \times age_{n1,t} \quad (5)$$

At any point after initiation, the nest could fail (ψ_{n1f}), calculated as:

$$\psi_{n1f1,i,t} = 1 - \psi_{n1h,i,t} - \psi_{n1n1,i,t} \quad (6)$$

where failure was 1 minus the probability of either hatching or remaining on the nest. For females whose first nest failed, they could renest (ψ_{f1n2}), modeled as:

$$\begin{aligned} logit(\psi_{f1n2,i,t}) = {} & \beta_{f1n2,0} + \beta_{f1n2,age} \times age_{f,t} + \beta_{f1n2,age} \times age_{f,t}{}^{2} + \\ & \beta_{f1n2,wprcp} \times WPRCP + \beta_{f1n,cond} \times Cond + \\ & \beta_{f1n2,adult} \times Adult \end{aligned} \quad (7)$$

where $age_{f,t}$ is the number of days since the first nest failed, applied as a quadratic term. We included winter precipitation (*WPRCP*), body condition (*Cond*), and female age (*Adult*) as covariates for renesting. To evaluate seasonal variability we derived daily transition probabilities with mean covariate values for each day ($\psi_{gn1,t}$). We then derived a population level probability that a female would attempt breeding at least once as $1 - \prod_{t=1}^{122}(1 - \psi_{gn1,t})$. Similarly, we calculated the probability of renesting, conditioned on failure of a first nest as $1 - \prod_{t=1}^{122}(1 - \psi_{f1n2,t})$. We report means, 95% credible intervals, and ν, the proportion of the posterior distribution on the same side as the mean.

Results

We captured and followed 778 unique females (842 female-year combinations) throughout the breeding season (March 1st – July 1st) to evaluate breeding and survival probabilities. The strongest correlations between the covariates were a negative correlation between horses and prior year precipitation (-0.431) and a negative correlation between horses and winter precipitation (-0.257). The negative correlations indicate that areas where we had high horse numbers and marked sage-grouse had drier weather patterns during our study. The correlations between the other covariates were all less than 0.2 (Figure 3).

Transition to nesting

The overall probability of transitioning to a nest was essentially zero on March 1st ($\beta_{gn,0} = -15.28$, 95% CI, -16.38 – -14.279) and increased daily ($\beta_{gn,day} = 0.453$, 95% CI, 0.413 – 0.495, $\nu > 0.99$), peaking the last 10 days of April (Figure 4), before starting to decline ($\beta_{gn,day2} = -0.004$, 95% CI, -0.004 – -0.004, $\nu > 0.99$). Adult females initiated nests at higher rates than yearling females (Figure 5, $\beta_{gn,adult} = 0.286$, 95% CI, 0.058 – 0.51, $\nu = 0.993$). We found evidence that females with a higher body condition index nested at higher rates (Figure 6, $\beta_{gn,adult} = 0.064$, 95% CI, -0.019 – 0.150, $\nu = 0.935$).

We determined the best model structure (Table 1) to include a quadratic term when modeling winter precipitation, and allowed prior year precipitation to interact with livestock and feral horse grazing. Females that attended leks in years receiving moderate amounts of winter precipitation bred at higher rates than those with lower values (Figure 7, $\beta_{gn,WPRCP} = 0.146$, 95% CI, -0.005 – 0.296, $\nu = 0.971$), and high values ($\beta_{gn,WPRCP2} = -0.137$, 95% CI, -0.225 – -0.052, $\nu > 0.99$). At mean values of horse and livestock grazing, females that attended leks with high values of prior year precipitation breed at

rates higher than those with low values ($\beta_{gn,PYP}$ = 0.235, 95% CI, 0.129 – 0.344, ν > 0.99). We found little evidence for an effect of livestock ($\beta_{gn,livestock}$ = −0.017, 95% CI, -0.101 – 0.064, ν = 0.649) at mean values of prior year precipitation, but there was evidence that livestock had a negative effect on breeding propensity after females had experienced a low water year value (Figure 8, $\beta_{gn,PYP,livestock}$ = 0.075, 95% CI, -0.021 – 0.178, ν = 0.928). Unlike livestock and opposite of our hypothesis, at mean values of prior year precipitation we found evidence that females were breeding at higher rates in areas with more feral horses (Figure 8, $\beta_{gn,horse}$ = 0.229, 95% CI, 0.109 – 0.348, ν > 0.99). In years with low values of prior year precipitation this effect increased ($\beta_{gn,PYP,horse}$ = 0.066, 95% CI, -0.028 – 0.161, ν = 0.912).

Overall breeding propensity, for mean covariate values, was 0.931 (95% CI, 0.904 – 0.953) for adults, and 0.867 (95% CI, 0.802 – 0.922) for yearling females (Figure 5).

Nest survival

Once a female had transitioned to a nest, she could remain on the nest, the nest could fail, or the nest could hatch. The probability that a female would remain on a nest was high at day 1 (*nn*=1, 95% CI, 0.999–1), but decreased with each day she remained on the nest ($\beta_{nn,age}$ = −0.238, 95% CI, -0.263 – -0.217, ν > 0.99). With an intercept of $\beta_{nf,0}$ = 4.174 (95% CI, 3.658 – 4.817), and an age effect of $\beta_{nf,age}$ = −0.2 (95% CI, -0.229 – -0.176, ν > 0.99) and its dependency on the probability a female remained on the nest or hatched, the probability of a nest failing was low at day 1 (Figure 4, 0.021, 95% CI, 0.017 – 0.025), highest at day 28 (0.045, 95% CI, 0.037 – 0.055), before dropping to its lowest point at day 41 (0.015 , 95% CI, 0.009 – 0.024). The probability of a nest hatching was 0 at day 1 and remained low until day 24, after which the daily transition probability increased to 0.828 (95% CI, 0.762 – 0.889) at 41 days. Based on these estimates, the median time it took for a female to hatch a nest was 27 days of incubation.

Transition to Second Nest (Renesting)

We monitored 411 females that attempted a first nest and failed, of which 103 attempted a second nest. We found that the daily probability of attempting a second nest was low immediately after the first nest failed ($\beta_{fn2,0}$ = -6.141, 95% CI, -7.151 – -5.176), but increased daily ($\beta_{fn2,age}$ = 0.322, 95% CI, 0.201 – 0.452, $\nu > 0.99$) until 15 days after the first nest failed, before declining ($\beta_{fn2,age2}$ = -0.011, 95% CI, -0.015 – -0.007, $\nu > 0.99$) to nearly 0 at 30 days after the first nest failed (Figure 4). Unlike first nests, there was no apparent effect of female age ($\beta_{fn2,adult}$ = 0, 95% CI, -0.544 – 0.583, $\nu = 0.514$); however, body condition positively ($\beta_{fn2,cond}$ = 0.265, 95% CI, 0.062 – 0.487, $\nu = 0.996$) affected the probability a female would attempt a second nest. Once a female transitioned to a second nest, we assumed the probability of remaining on the nest, the nest failing, and hatching were the same as the first nest. Renesting probability (conditioned on failure of the first nest), for mean covariate values, was 0.349 (95% CI, 0.292 – 0.410) for adults, and 0.353 (95% CI, 0.217 – 0.507) for yearling females (Figure 5).

Survival

Out of the 842 females, 94 died before they either hatched a nest, had a failed second nest, or July 1st if they never attempted a nest. When modeling these probabilities, we allowed survival to vary by state. Daily female survival (Figure 9) was highest for nonbreeding females at 0.9996 (95% CI, 0.9993 – 0.9997). Survival was lower for females attending a first nest (0.9976 95% CI, 0.9966 – 0.9985) and females that attempted a first nest that failed (0.9979 95% CI, 0.9968 – 0.9989). Daily survival was lowest for females who were attending a second nest (0.9956 95% CI, 0.9919 – 0.9982).

Discussion

The decision to breed is the first step in the annual cycle for female sage-grouse. Our results support that this decision is influenced by both individual attributes and the

environment. Increasing female body condition and age led to higher breeding rates. Precipitation immediately preceding and in the prior year influenced breeding. The density of non-native herbivores, in association with prior year precipitation, had opposing effects on reproduction. The outcome of the breeding decision also impacted female survival.

We found that female sage-grouse initiated nests at lower rates when livestock were abundant and precipitation was low during the previous year. This finding is consistent with the known impacts of livestock on understory vegetation in rangelands (Beck & Mitchell, 2000; Dettenmaier et al., 2017; Dobkin et al., 1998; Hockett, 2002), which we have also documented in our current work (Street, 2020). We report elsewhere that a proportion of the study population expressed elevated levels of corticosterone in their feathers when livestock use was high and precipitation low (Behnke et al. in prep). While there is a several month lag between the growth of new feathers and the next breeding season it is possible that carry-over effects from the state of females during feather growth influenced their decision or ability to breed the next spring (Blomberg, Sedinger, et al., 2013; Fairhurst et al., 2017; Hansen et al., 2016). Finally, while cattle do not typically consume large quantities of sagebrush, unless other foods are lacking (Hanley & Hanley, 1982; Scasta et al., 2016), only minimal browsing is required to elicit a chemical response sufficient which to reduces the palatability and nutritional quality of sagebrush for sage-grouse (Forbey et al., 2013; Frye et al., 2013).

Recent findings show that nutrients stored before breeding govern the investment by female sage-grouse in reproduction (Cutting et al., 2021) so reductions in nutritional quality of sagebrush, the principal food of sage-grouse during winter (Remington &

Braun, 1985) could reduce breeding propensity the next spring. Our finding that females in better body condition initiated both first and second nests at higher rates is also consistent with this hypothesis. Irrespective of the precise mechanism, these results are consistent with the general concern about the impact that livestock grazing can have on sage-grouse population dynamics, particularly expressed over the past two decades (Beck & Mitchell, 2000; Boyd et al., 2014; Dettenmaier et al., 2017; Monroe et al., 2017).

We detected a significant survival cost of nesting for female sage-grouse. Based on our estimates of daily survival, females had an 8% chance of dying during a typical 36 day egg laying and incubation period (Blomberg et al., 2015), in contrast to a 1.4% chance of dying during the same length period before initiating a nest. Females attending a second nest experienced the greatest mortality risk (15%) during a typical egg laying and incubation period. Mortality risk for females attending second nest represents about one third of the expected annual mortality (0.42, Taylor et al., 2012) for the average female. Substantial increase in mortality risk associated with breeding has been identified in at least one other population of sage-grouse (Blomberg, Sedinger, et al., 2013) in which the increased mortality was attributed primarily to predation (Blomberg, Gibson, et al., 2013). Irrespective of the cause of mortality, female sage-grouse clearly experience a substantial mortality cost of breeding, which has important implications for understanding population dynamics and the evolution of life-histories (Blomberg et al., 2012; Connelly et al., 2011; Stearns, 1992; Sæther & Bakke, 2000).

Our finding that breeding propensity is positively associated with precipitation during the previous winter and calendar year is also consistent with one other study that estimated breeding propensity in sage-grouse (Blomberg et al., 2017). Winter

precipitation is typically associated with the production of forbs that sage-grouse chicks require during growth and development (Gibson et al., 2016, 2017; Smith et al., 2019) but our findings indicate that such precipitation also influences the decision about whether to breed or not and provide additional support for the critical role of precipitation before the breeding system in the recruitment of sage-grouse into the breeding population (Blomberg et al., 2012). We caution that the apparently strong quadratic pattern in the influence of winter precipitation could reflect an artifact of fitting a relationship where the response has an upper threshold combined with very sparse data for both the explanatory and response variables over most of the region of the decline in the response (Figure 7).

In contrast to our hypothesis, we found that breeding propensity increased with increasing numbers of horses, although the interaction between horse use and prior year precipitation indicated lower breeding propensity following years of low precipitation similar to the situation with livestock grazing. Given the well documented impact of free-roaming horses on Great Basin ecosystems (Beever & Brussard, 2000, 2004; Beever et al., 2008; Boyd et al., 2017; Davies & Boyd, 2019) this result is surprising but could reflect occupation of similar habitats by breeding sage-grouse and horses (Coates, O'neil, et al., 2021; Muñoz et al., 2021; Street, 2020).

The probability of initiating a first nest (adult = 0.93; yearling = 0.86) was higher than the reported average for sage-grouse in the western portion of their range (0.784; Knick & Connelly, 2011). Renesting was also higher (0.35 for both yearlings and adults) than the western average of 0.299, but well within the range of variation (SD=0.264; Knick & Connelly, 2011). In sage-grouse, breeding propensity has received little

attention when compared to demographic rates associated with other life-history traits. Our model provides the foundation for not only estimating this little-studied demographic rate, but also considering tradeoffs among multiple life-history traits (Stearns, 1989).

Increasing risk of nest failure as incubation increased (Figure 4) is consistent with findings in other studies (Davis et al., 2015; Kolada et al., 2009), and would be consistent with increased awareness of the nest location by predators such as common ravens (*Corvus corax*) and coyotes (*Canis latrans*) through time (Coates et al., 2008; Dinkins et al., 2016; Taylor et al., 2017). The median 15-day interval between loss of a nest and initiation of a new nest (Figure 4) is consistent with time required for females to restore some endogenous nutrients (Blomberg et al., 2014; Gregg et al., 2006) and the time required for the development of follicles until they are ready for ovulation (Bacon & Cherms, 1968; Birrenkott et al., 1988; Donham et al., 1976; Grau, 1976).

In conclusion, we found that dry weather patterns result in lower breeding rates for female sage-grouse. These effects were exacerbated by livestock grazing. Climate models predict that the frequency and intensity of drought events are likely to increase in the future (Snyder et al., 2019). If this is the case, current livestock management strategies are unlikely to result in robust sage-grouse populations. While our results regarding feral horses are less clear, when combined with recent literature (Coates, O'neil, et al., 2021; Street, 2020) it is unlikely that feral horses are resulting in increases in sage-grouse populations. It is important to note that within our study area, livestock are present in much higher rates than feral horses, and we suggest management of both of these non-native species.

Tables

Table 1-1. Results from step 1 of our model selection strategy to find the best fit for interaction terms and quadratic terms for our simplified breeding propensity model (Equation 1) based on DIC. ‘PYP’ represents prior year precipitation and ‘WPRCP’ is winter precipitation.

Name	Interaction	Quadratic	DIC
mod:4	Cow * PYP + Horse * PYP	$WPRCP^2$	5430.60
mod:1		$WPRCP^2 + PYP^2$	5432.71
mod:6	Cow * WPRCP + Horse * WPRCP	PYP^2	5438.09
mod:3	Cow * PYP + Horse * PYP		5442.24
mod:2	Cow * WPRCP + Horse * WPRCP		5442.47
mod:5	PYP * WPRCP		5447.86

Figures

Figure 1-1. Map of study areas (right) in context of the Great Basin of the Western United States (left).

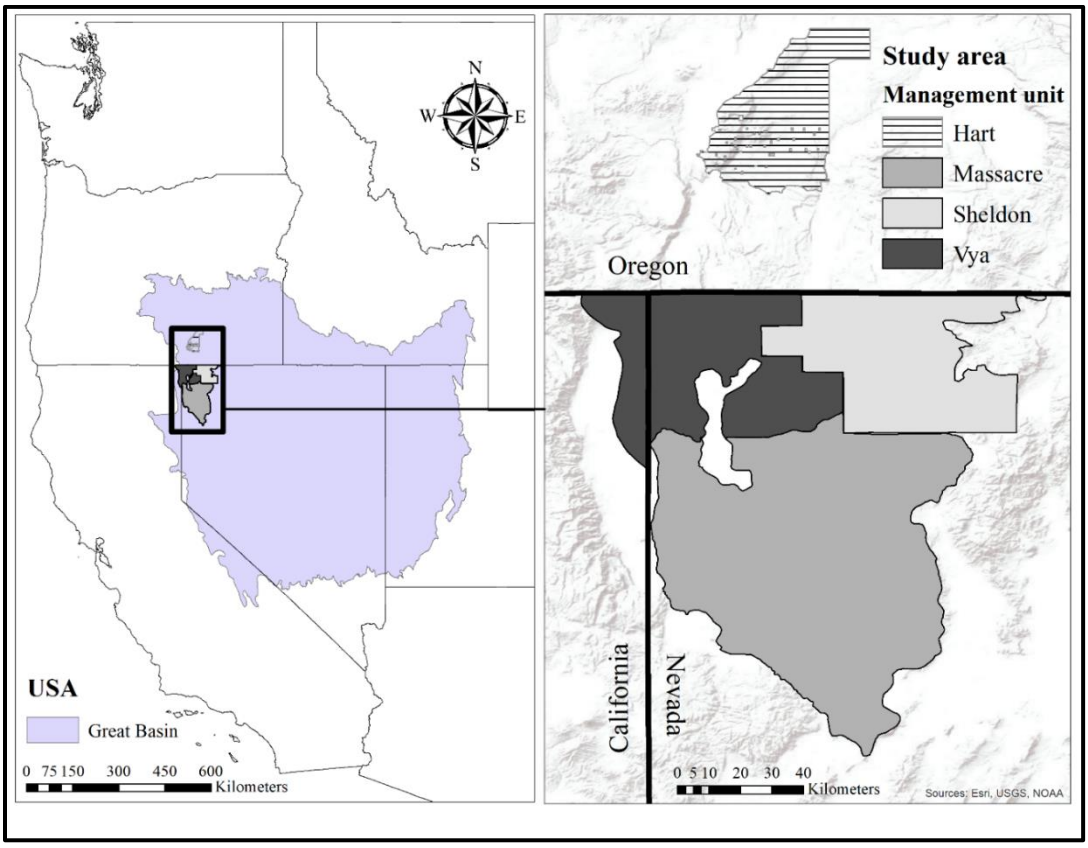


Figure 1-2. All females started in a general non-breeding state (g). Females could survive with the probability ϕ and transition to a primary nest (ψ_{gn1}), they could survive and remain in a non- breeding state (1- ψ_{gn1})ϕ, or they could die (1- ϕ). Once females have transitioned to a primary nesting state, the nest can survive with the probability ψ_{n1n1} or fail (ψ_{n1f1}) and transition to a failed state (f1). Females have to transition through this state before they can attempt a secondary nest (n2). We had three terminal states; hatched (h), failed secondary nest (f2), or dead (d).

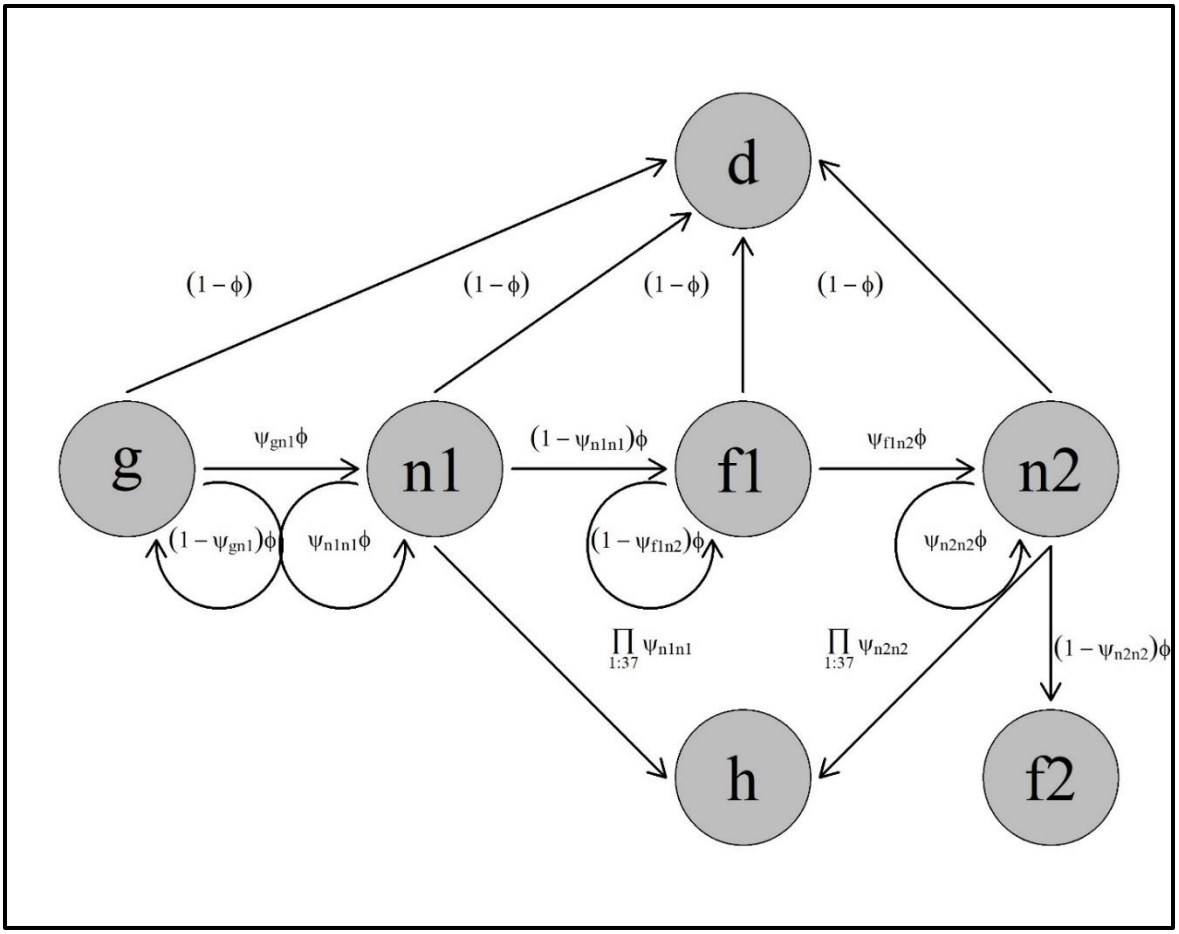


Figure 1-3. Correlation plot for the 6 covariates of primary interest – livestock, horses, prior year precipitation, winter precipitation, female age, and body condition.

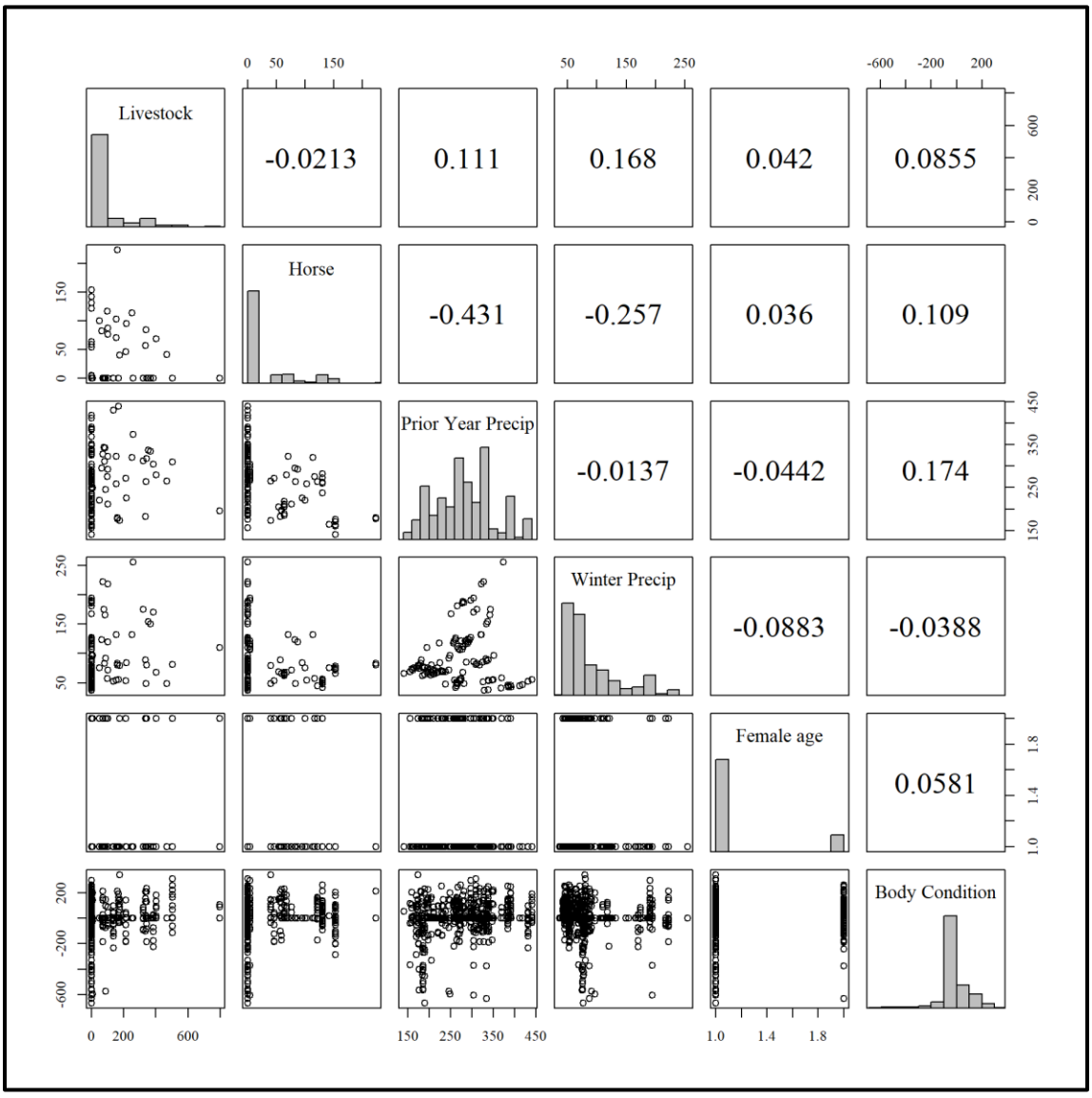


Figure 1-4. Daily transition probabilities based on either the day of the year, or the time spent within a state (age for a nest).

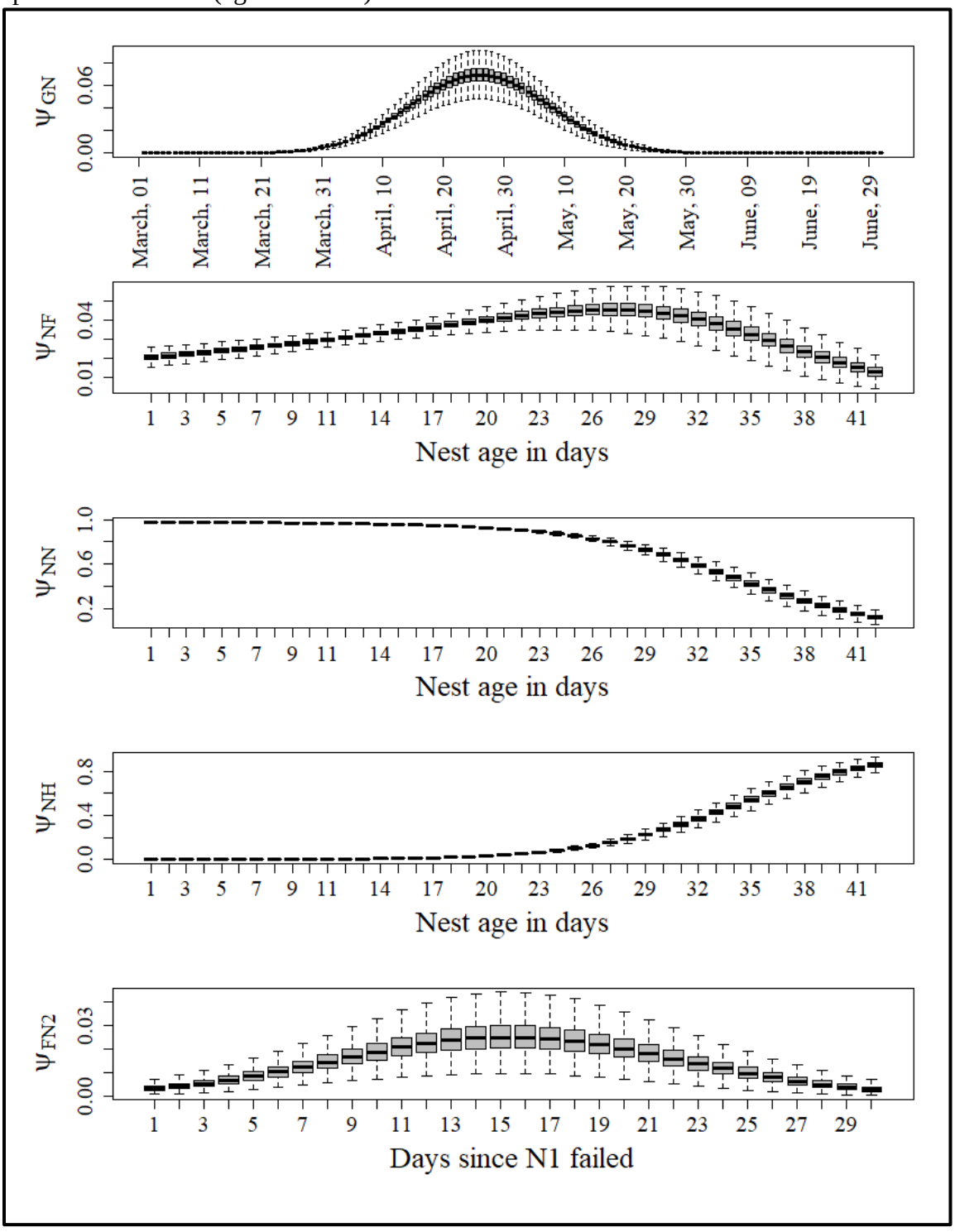


Figure 1-5. Probability of initiating a first nest based on female age with mean values of all the other covariates. The probability of initiating a second nest is conditioned on a female attempting to build a first nest and that nest failing. Sample sizes (n) are the number of females of each age class in the data used to train the model.

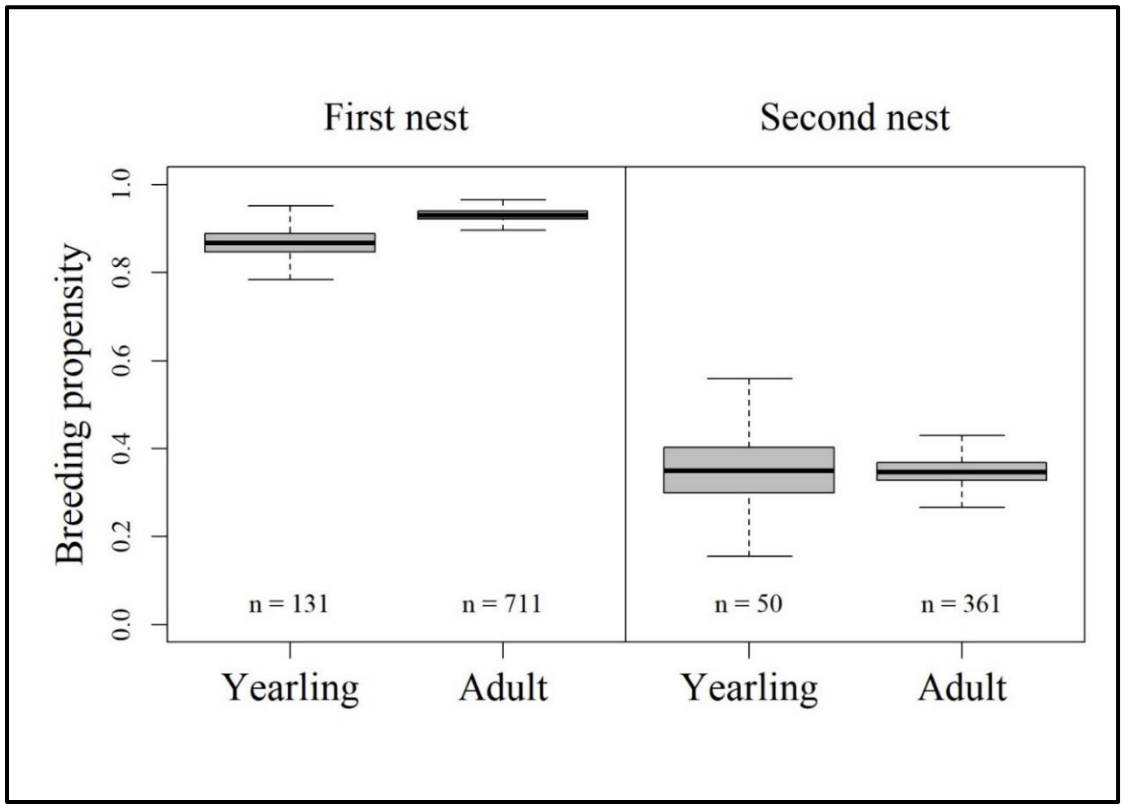


Figure 1-6. Probability of initiating a first and second nest when body condition is varied and all other covariates are held constant at the mean.

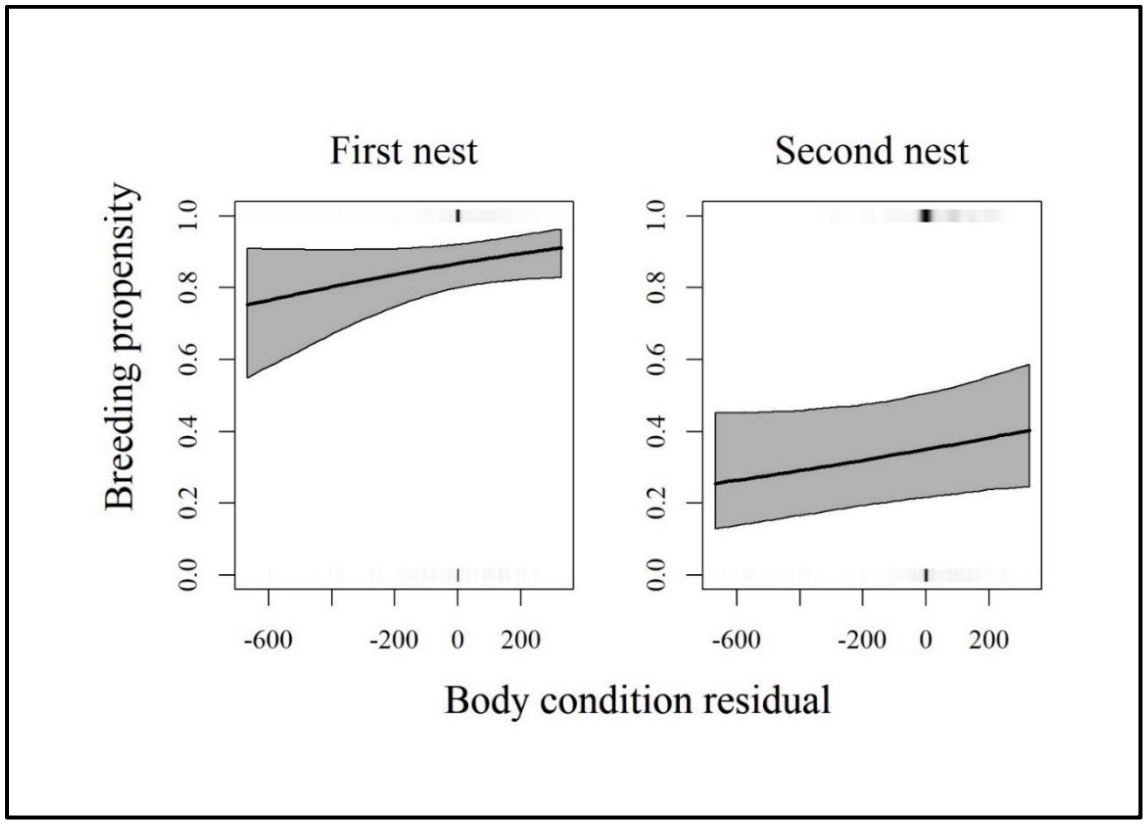


Figure 1-7. Effect of prior year precipitation and winter precipitation on breeding propensity at mean levels of grazing.

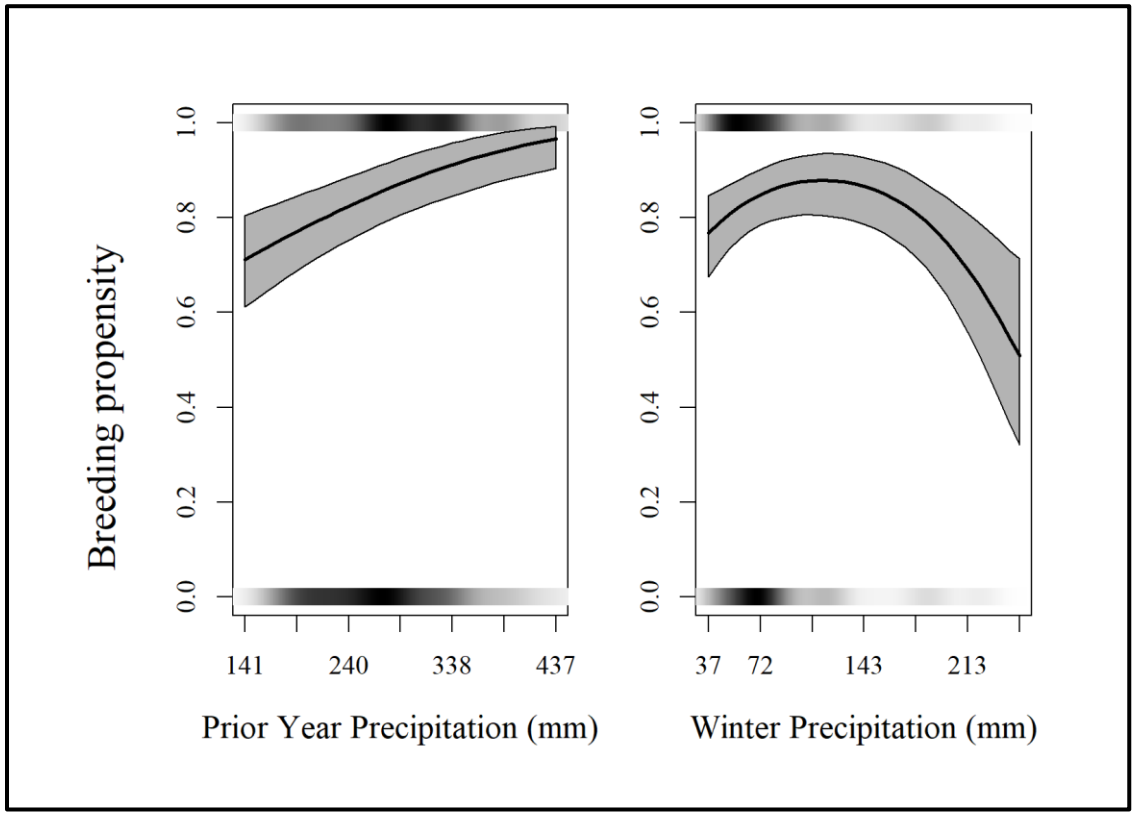


Figure 1-8. Probability of breeding as a function of grazing pressure and prior year precipitation, at the mean of other covariates. Blue surface represents breeding propensity estimates, with grey surfaces for 95% credible intervals.

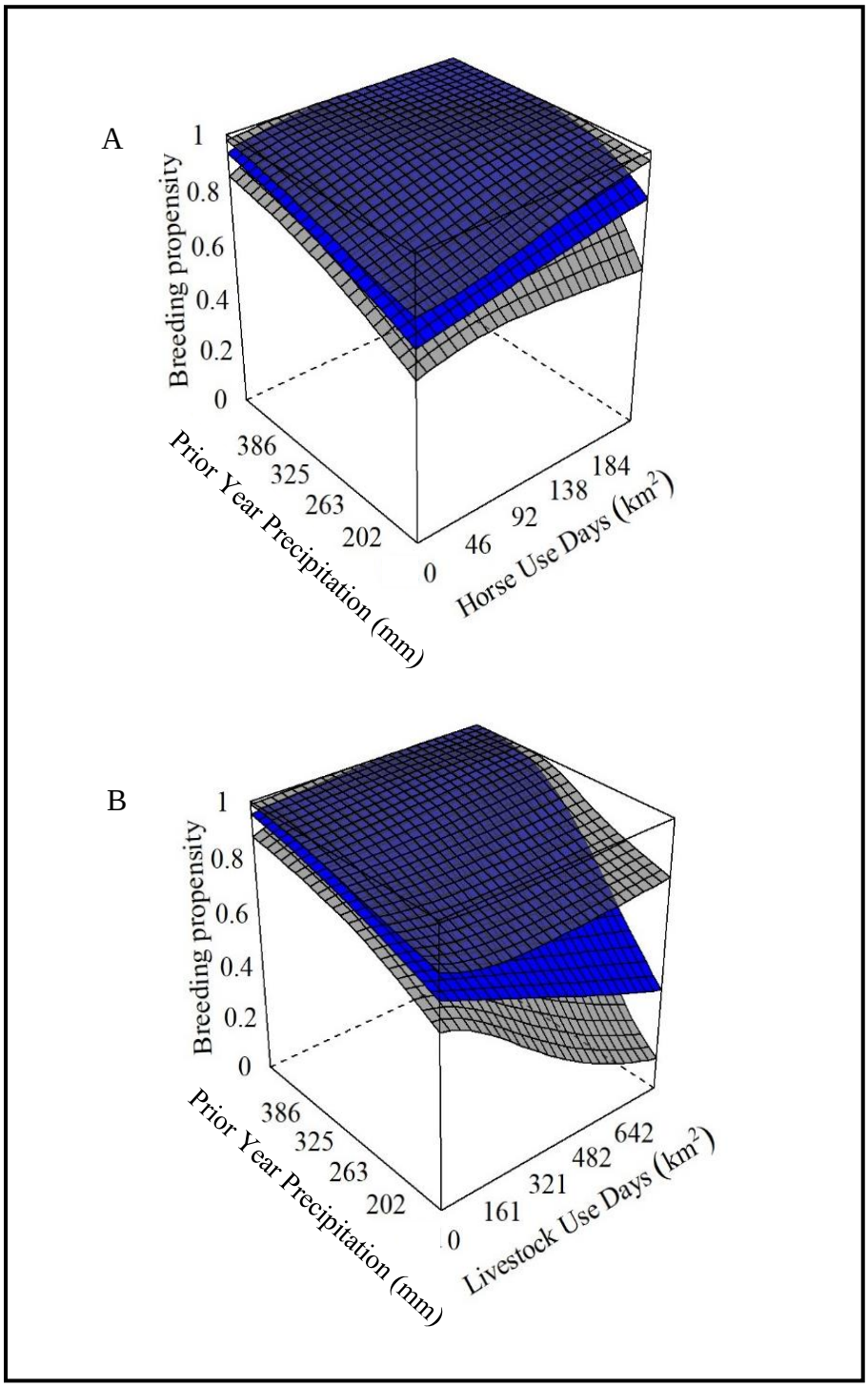
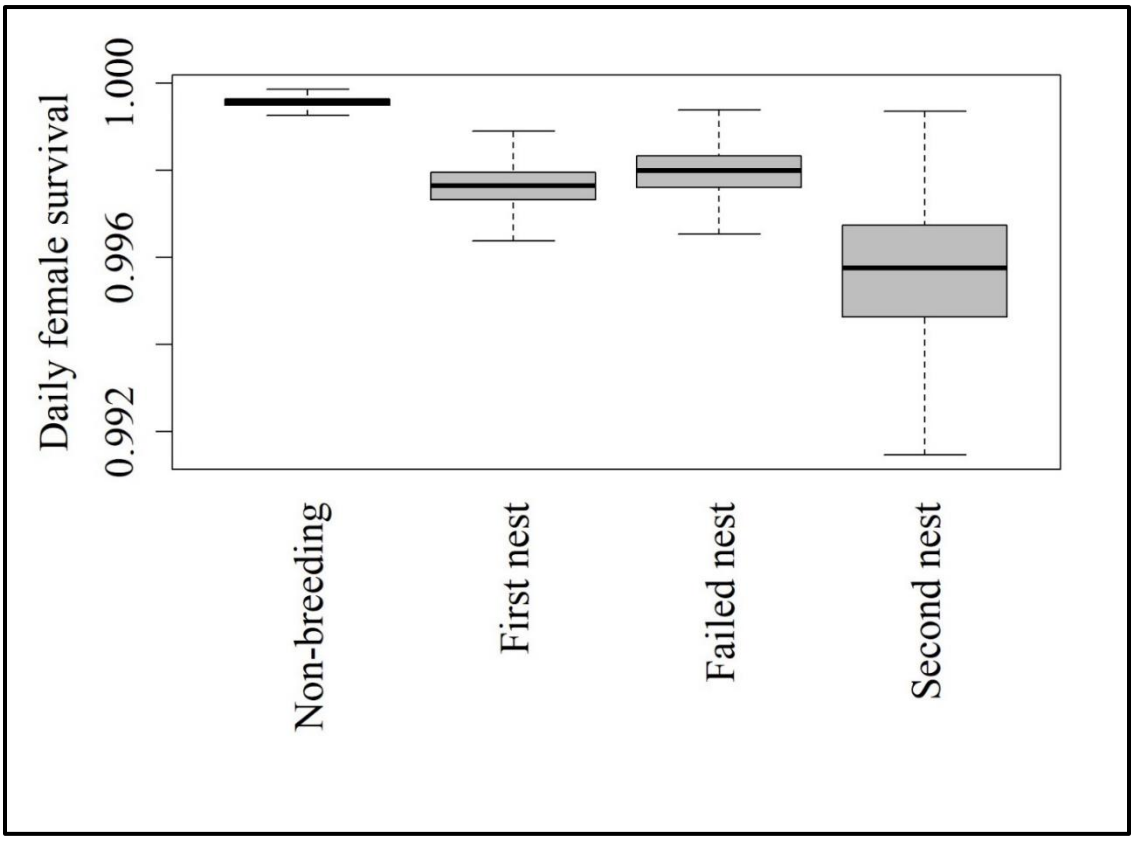


Figure 1-9. Daily survival probability for each non-terminal state.



Model Structures

$$\begin{aligned}\text{logit}(\psi_{gn,i,t}) = {} & \beta_{gn,0} + \\ & \beta_{gn,\,day} \times \text{Day}_t + \beta_{gn,\,day^2} \times \text{Day}^2_t + \\ & \beta_{gn,\,cond} \times \text{Cond} + \\ & \beta_{gn,\,adult} \times \text{Adult} + \\ & \beta_{gn,\,WPRCP} \times \text{WPRCP} + \beta_{gn,\,WPRCP^2} \times \text{WPRCP}^2 + \\ & \beta_{gn,\,PYP} \times \text{PYP} + \beta_{gn,\,PYP^2} \times \text{PYP}^2 + \\ & \beta_{gn,\,horse} \times \text{Horse} + \\ & \beta_{gn,cow} \times \text{Cow}\end{aligned} \qquad \text{(Model 1)}$$

$$\begin{aligned}\text{logit}(\psi_{gn,i,t}) = {} & \beta_{gn,\,0} + \\ & \beta_{gn,\,day} \times \text{Day}_t + \beta_{gn,\,day} \times \text{Day}^2_t + \\ & \beta_{gn,\,cond} \times \text{Cond} + \\ & \beta_{gn,\,adult} \times \text{Adult} + \\ & \beta_{gn,\,WPRCP} \times \text{WPRCP} + \\ & \beta_{gn,\,PYP} \times \text{PYP} + \\ & \beta_{gn,\,horse} \times \text{Horse} + \\ & \beta_{gn,\,cow} \times \text{Cow} + \\ & \beta_{gn,\,horse,\,WPRCP} \times \text{Horse} \times \text{WPRCP} + \\ & \beta_{gn,\,cow,\,WPRCP} \times \text{Cow} \times \text{WPRCP}\end{aligned} \qquad \text{(Model 2)}$$

$$\text{logit}(\psi_{gn,i,t}) = \beta_{gn,\,0} + \beta_{gn,\,day} \times \text{Day}_t + \beta_{gn,\,day} \times \text{Day}^2_t + \beta_{gn,cond} \times \text{Cond} + \beta_{gn,\,adult} \times \text{Adult} + \beta_{gn,\,WPRCP} \times \text{WPRCP} + \beta_{gn,\,PYP} \times \text{PYP} + \beta_{gn,\,horse} \times \text{Horse} + \beta_{gn,\,cow} \times \text{Cow} + \beta_{gn,\,horse,\,PYP} \times \text{Horse} \times \text{PYP} + \beta_{gn,\,cow,\,PYP} \times \text{Cow} \times \text{PYP} \quad \text{(Model 3)}$$

$$\text{logit}(\psi_{gn,i,t}) = \beta_{gn,\,0} + \beta\text{gn, day} \times \text{Dayt} + \beta\text{gn, day} \times \text{Day}^2\text{t} + \beta_{gn,\,cond} \times \text{Cond} + \beta_{gn,\,adult} \times \text{Adult} + \beta\text{gn, WPRCP} \times \text{WPRCP} + \beta\text{gn, WPRCP}^2 \times \text{PRCP}^2 + \beta_{gn,\,PYP} \times \text{PYP} + \beta_{gn,\,horse} \times \text{Horse} + \beta_{gn,\,cow} \times \text{Cow} + \beta_{gn,\,horse,\,PYP} \times \text{Horse} \times \text{PYP} + \beta_{gn,\,cow,\,PYP} \times \text{Cow} \times \text{PYP} \quad \text{(Model 4)}$$

$$\begin{aligned}\text{logit}(\psi_{gn,i,t}) = {} & \beta_{gn,\,0} + \\ & \beta_{gn,\,day} \times \text{Day}_t + \beta_{gn,\,day} \times \text{Day}^2_t + \\ & \beta_{gn,\,cond} \times \text{Cond} + \\ & \beta_{gn,\,adult} \times \text{Adult} + \\ & \beta_{gn,\,WPRCP} \times \text{WPRCP} + \\ & \beta_{gn,\,PYP} \times \text{PYP} + \\ & \beta_{gn,\,horse} \times \text{Horse} + \\ & \beta_{gn,\,cow} \times \text{Cow} + \\ & \beta_{gn,\,WPRCP,\,PYP} \times \text{WPRCP} \times \text{PYP}\end{aligned} \qquad \text{(Model 5)}$$

$$\begin{aligned}\text{logit}(\psi_{gn,i,t}) = {} & \beta_{gn,\,0} + \\ & \beta_{gn,\,day} \times \text{Day}_t + \beta_{gn,\,day} \times \text{Day}^2_t + \\ & \beta_{gn,\,cond} \times \text{Cond} + \\ & \beta_{gn,\,adult} \times \text{Adult} + \\ & \beta_{gn,\,WPRCP} \times \text{WPRCP} + \\ & \beta_{gn,\,PYP} \times \text{PYP} + \beta_{gn,\,PYP} \times \text{PYP}^2 + \\ & \beta_{gn,\,horse} \times \text{Horse} + \\ & \beta_{gn,\,cow} \times \text{Cow} + \\ & \beta_{gn,\,horse,\,WPRCP} \times \text{Horse} \times \text{PRCP} + \\ & \beta_{gn,\,cow,\,WPRCP} \times \text{Cow} \times \text{WPRCP}\end{aligned} \qquad \text{(Model 6)}$$

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Chapter 2: Physiological and demographic responses by Greater Sage-grouse to non-native grazers

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Abstract

Non-native ungulate grazing has negatively impacted native species across the globe, leading to massive loss of biodiversity and ecosystem services. Despite their pervasiveness, interactions between grazers and native species are not fully understood. We often observe declines in demography or survival of these native species, but lack understanding about the mechanisms underlying these declines. Physiological stress represents one mechanism of (mal)adaptation but data are sparse. We investigated glucocorticoid levels in a native avian herbivore exposed to different intensities of non-native grazing in the cold desert Great Basin ecosystem, USA. We measured corticosterone, a glucocorticoid in birds, in feathers for a large sample (n = 280) of female Greater sage-grouse (*Centrocercus urophasianus*) from three study areas in Northern Nevada and Southern Oregon with different grazing regimes of livestock and feral horses. We found greater feral horse density increased corticosterone levels, and this effect was exacerbated by drought conditions. Livestock grazing produced similar results; however there was more model uncertainty about the livestock effect. Subsequent nesting success was lower with increased feather corticosterone, but corticosterone levels were not predictive of other vital rates. Our results indicate a physiological response by sage-grouse to grazing pressure from non-native grazers. We found substantial among-individual variation in the strength of the response. These adverse effects were intensified during unfavorable weather events, highlighting the need to reevaluate management strategies in the face of climate change.

Introduction

Glucocorticoid hormones (GCs) link a vertebrate's internal condition and its external environment (Sapolsky et al., 2000). Baseline levels of GCs have been related to components of fitness in several species (Bonier et al., 2009; Cyr & Romero, 2007; Hau et al., 2010; Henderson et al., 2017; Madliger & Love, 2016; Sorenson et al., 2017; Wingfield & Sapolsky, 2003). The Cort-Fitness Hypothesis, proposed by Bonier et al. (2009), suggests that elevated baseline levels of GCs reflect a physiological response to environmental or social resource limitation that in turn results in lower reproductive investment or survival, thereby lowering fitness. However, existing data are conflicting as to the nature of this relationship (Madliger & Love, 2016; Sorenson et al., 2017), leaving us with no consensus on the interplay between GCs and fitness. Understanding is poor in part due to lack of context (Romero, 2004; Vera et al., 2017) about the specific environment as experienced by wild individuals.

Introduced ungulates, both domestic and wild, are known to negatively impact habitats for native species across numerous ecosystems (Dettenmaier et al., 2017; Eldridge et al., 2020; Spear & Chown, 2009; Volery et al., 2021), thereby having the potential to chronically elevate GC levels. Grazing by domestic cattle (*Bos bos*) has been a prominent land use in the western U.S for more than a century (Bureau of Land Management, 2020b). Feral horses (*Equus ferus caballus*) have increased dramatically over the past two decades, largely associated with sociopolitical resistance to removing horses from rangelands (Garrott, 2018). Both livestock and horses graze plants that are preferred by native herbivores because of their higher nutritional content (Hanley &

Hanley, 1982; Scasta et al., 2016; Veblen et al., 2015). Livestock and horses also cause physical damage to riparian areas (Batchelor et al., 2015; Beever & Herrick, 2006; Beschta et al., 2014; Boyd et al., 2017; Dobkin et al., 1998), that provide essential foods and water during dry periods in the cold deserts of western North America (Batzer & Baldwin, 2012; Donnelly et al., 2018b). Such impacts are likely to be exacerbated when seasonal moisture is reduced because primary productivity is lower under such conditions in already dry environments (Chambers et al., 1999; Donnelly et al., 2018b; Zeigenfuss et al., 2014).

Greater sage-grouse (*Centrocercus urophasianus*; hereafter, "sage-grouse") are herbivorous birds that are sympatric with feral horses and/or livestock throughout their range in the western United States (Schroeder et al., 2020). They rely on sagebrush as their main food source outside the breeding season but expand their diet during breeding to include insects and other plants (Rowland et al., 2006). Sage-grouse are highly selective of specific sagebrush plants because individual shrubs can vary in nutrient content and anti-herbivore secondary compounds (Frye et al., 2013; Remington & Braun, 1985).

Following nesting in early spring, adult female sage-grouse and their offspring feed on forbs and associated invertebrates (Casazza et al., 2011; Gregg et al., 2008; Gregg & Crawford, 2009). As the offspring age the days get longer, the temperature increases, the precipitation decreases, and by early summer (June) these foods are available primarily in higher elevation sites or riparian areas. These areas represent as little as 2% of the landscape (Atamian et al., 2010; Donnelly et al., 2016). Growth rate of young, which influences both prefledging and postfledging survival (Blomberg, Sedinger,

et al., 2014; Gibson et al., 2016) is directly dependent on the quality and quantity of food (Blomberg, Poulson, et al., 2013; Smith et al., 2019). It seems likely that adult females also restore some depleted nutrient reserves during this period because forbs represent the highest protein content foods females encounter (Gregg et al., 2008) and protein is a limiting nutrient for herbivorous birds (Sedinger, 1997). These high-quality foods potentially regulate individual fitness and population dynamics of sage-grouse. Consequently, non-native ungulates have the potential to negatively influence sage-grouse through their influence on key food plants used by sage-grouse. In fact, recent analyses by Coates et al. (2021) demonstrates that abundance of feral horses is negatively correlated with the rate of growth of local sage-grouse populations.

Assessment of the potential effects of domestic cattle and feral horses on sage-grouse has primarily been through the lens of habitat modification or behavioral disturbance (Beck & Mitchell, 2000; Boyd et al., 2014; Dahlgren et al., 2015; Dettenmaier et al., 2017; Williamson et al., 2020). Direct assessment of the effects of livestock or horses on fitness, demography, and population dynamics of sage-grouse is lacking. Possible physiological mechanisms for demographic effects resulting from coexistence with these non-native ungulates are also largely unaddressed (Monroe et al., 2017).

We measured variation in corticosterone levels, the predominant GC in birds, in feathers of female sage-grouse across a gradient of livestock and feral horse densities in multiple years that varied in precipitation to assess the elevation of GC levels in relation to the abundance of livestock and feral horses under variable climatic conditions. Feather corticosterone (fCort) is an indicator of chronic stress, which may alter an individual's

ability to allocate resources to reproduction or survival over time. In house sparrows (*Passer domesticus*) and Eurasian Sparrowhawks (*Accipiter nisus)* higher fCort levels in feathers were associated with lower survival in the following season (Koren et al., 2012; Monclus et al., 2017). High GC concentrations in feathers led to a higher likelihood of skipping breeding in the following breeding season for harlequin ducks (*Histrionicus histrionicus*) (Hansen et al., 2016) and successfully breeding giant petrels (*Macronectes* spp.) had 1.5 times higher fCort than non-breeders (Crossin et al., 2013), indicating some cost to reproduction. Other studies have found no support for links between fCort and fitness (Henderson et al., 2017; Madliger & Love, 2016), which suggests that these relationships may depend on circumstances. We tested the relationships between fCort and important demographic rates, including adult survival, probability of initiating breeding, nest success, and probability of producing fledged young. We assessed potential sources of chronic stress and the demographic consequences for female sage-grouse.

Methods

Study Areas

We studied sage-grouse in three study regions in the northern Great Basin, USA (Figure 1). Hart Mountain National Antelope Refuge (hereafter Hart Mountain) in southcentral Oregon encompasses over 112,500 hectares and ranges in elevation from 1,360 to just over 2,445 m at the top of Warner Peak (U.S. Fish and Wildlife Service). Domestic livestock grazing ceased on Hart Mountain in 1991 and feral horses occur only in very low numbers outside areas where we studied sage-grouse. Sheldon National Wildlife Refuge (hereafter Sheldon) in northern Nevada includes over 231,840 hectares

(U.S. Fish and Wildlife Service). The lowest elevation is approximately 1,300 m at the bottom of the Thousand Creek Gorge and the highest peak is Catnip Mountain, which reaches just over 2,220 m. Domestic livestock were removed from Sheldon in 1994. Feral horses were abundant but nearly all feral horses were removed in 2014-2015. The Bureau of Land Management (BLM)-managed portion of our study is delineated by the Vya and Massacre Sage-grouse Population Management Units (hereafter Vya-Massacre) which encompass approximately 711,200 hectares in northwestern Nevada and northeastern California (Nevada Department of Wildlife). There are 22 grazing allotments for domestic cattle, as well as 7 Herd Management Areas (HMAs) for feral horses in the BLM portion of our study area.

Field methods

We trapped yearling (ca. 10 months old at the time of capture) and adult female sage-grouse near active breeding leks (Gibson & Bradbury, 1987) during March of 2013 to 2017. We captured birds at night using spotlights (Giesen et al., 1982) and handheld nets. Once captured, we banded females (n = 778) with unique metal bands (size 14) and fit them with 22g very high-frequency necklace-style radio-collars (VHF; Advanced Telemetry Systems, Isanti, MN, USA). We measured culmen using dial calipers (±1 mm), tarsus using dial calipers (±1 mm), flat wing using a board (±1 mm), and weighed each individual (±1 g). We aged birds as yearlings or adults based on plumage characteristics (Crunden, 1963). During capture, we plucked 2 breast feathers, which are thought to be grown in fall (Clait Braun, personal comm.) from each individual. We labeled feathers by individual and stored them frozen in small plastic storage bags (2013-2016) or dry in small paper coin envelopes (2017-2019). For this analysis, we used

feathers collected from Hart Mountain (2013-2015), Sheldon (2013-2017), and Vya-Massacre (2013-2017).

We monitored females every 2-3 days during the early nesting period, to document nesting and success or failure of nests. For females that had successful nests, we began monitoring their broods within the first 2-3 days after chicks hatched (Street et al. in review). We performed weekly checks of these broods, to count the surviving chicks up to 42-49 days of age. For the remaining months of the year, we employed aerial telemetry, approximately monthly, to assess live/dead status and location.

Additional Data

We used reported data to assess horse and cow usage across our study region (Figure 1). For cows, we used BLM data for each allotment, which included the number of cows present and the amount of time spent in the allotment (BLM Applegate Field Office unpublished data). To account for variation in the number of days cows were actually on each allotment, we multiplied the number of cows by number of days they were on an allotment, then divided by the size of the area to produce a variable reflecting cow use days per unit area. For horses, we used annual population estimates reported for each Herd Management Area (HMA; (Bureau of Land Management, 2020a). Because horses are on the landscape year-round, we multiplied number of horses by 365 days and divided by the size of the HMA. Thus, our variables for analysis were horse and cow use days per square kilometer.

Our study region experienced significant variation in precipitation among years (150 – 410 mm). We used cumulative precipitation from October to September, referred to as the ‘northern water year’, as a measure of annual drought conditions (Huntington et

al., 2017; PRISM Climate Group, 2011). Because introduced annual grasses displace native vegetation and can be detrimental to sage-grouse fitness and population dynamics (Blomberg et al., 2012), we included annual grass cover as an explanatory variable in some candidate models of fCort levels. We used year-specific layers of estimated annual grass percent cover, which in this area is primarily cheatgrass (*Bromus tectorum*) (Boyte et al., 2019), for this purpose.

Because feathers were collected at the time of capture, in late winter or early spring, the exact geographic locations of the birds during feather growth was unknown. Site fidelity of sage-grouse for specific seasonal habitats is high (Fischer et al., 1993), thus we used locations collected after capture as an index of where the female was the prior season. We extracted values for horse and cow density, drought, and annual grass cover for each individual sage-grouse at each location and averaged the covariate values across all locations. We used the locations for the year corresponding to feather growth, rather than the year the bird was captured for these estimates.

We calculated body condition as residuals of body mass regressed on female structural size (Blomberg, Gibson, et al., 2014; Freeman & Jackson, 1990). When measurements were missing (n = 16), we assigned the individual the mean condition value. As noted above, measurements were conducted at the time of capture so our analyses of condition assessed whether fCort was predictive of body condition a few months after feather growth.

Hormone Assays

We extracted corticosterone from feathers following Bortolotti et al. (2008), with some modifications. We first removed the calamus before measuring the length of each

feather. We cut each feather into pieces < 5 mm^2, mixed the pieces with 7 mL of HPLC grade methanol, and incubated the samples in a sonicating water bath at room temperature for 30 min. After sonication, we incubated the samples overnight in a shaking water bath at 50 °C. To separate the feather pieces and methanol, we used vacuum filtration with grade 4 filter paper (Whatman, Little Chalfont, United Kingdom). We also washed the sample tube, feather pieces, and filter apparatus twice with 2 mL of methanol, with washes added to the methanol extract. We dried the methanol extracts under air in a 50 °C heat block, reconstituted the extracts in assay buffer provided with the assay kit (see below), and froze them until assay.

We quantified corticosterone using enzyme-linked immunoassay according to the manufacturer's instructions (Enzo Life Sciences, Farmingdale, NY, USA). To validate this assay, we verified that a serially diluted sample was parallel with the corticosterone standard curve. The average intra- and inter-assay coefficients of variation were 4.7% and 16.9%, respectively. The average assay sensitivity was 8.3 pg/mL. Because of the way feathers grow (Bortolotti et al., 2009; Bortolotti et al., 2008), we normalized all final corticosterone values by feather length.

Corticosterone Model

We analyzed fCort levels in a Bayesian framework using JAGS UI (Kellner, 2015; Plummer, 2003) in Program R (R Core Team, 2019). We modeled fCort rounded to the nearest pg/mm for each bird *i*, with negative binomial variation approximated by a mean fCort level (μ), and shape term (r) to account for the highly skewed distribution. For all models we considered r to be constant. We modeled individual variation among μ with covariates of age (yearling or adult) and body condition. We included horse and cow

densities, precipitation, and annual grass cover to assess the potential for impacts of grazing by non-native ungulates on fCort. We included interaction terms for horses x precipitation, cows x precipitation, and horses x annual grass to allow for spatial-temporal variation in the effects of non-native grazers in response to variation in climate or ecological conditions. Continuous covariates were z-standardized so that the estimates of each beta value are comparable to each other. Thus, our full model, for each individual female, i, was,

$$log(\mu_i) = \beta_0 + \beta_{age} * age_i + \beta_{body\ condition} * body\ condition_i + \beta_{cow} * cow_i + \beta_{horse} * horse_i + \beta_{precipitation} * precipitation_i + \beta_{annual\ grass} * annual\ grass_i + \beta_{horse,precipitation} * horse_i * precipitation_i + \beta_{cow,precipitation} * cow_i * precipitation_i + \beta_{horse,annual\ grass} * horse_i * annual\ grass_i .$$

We calculated the mean absolute prediction error (MAPE) from an 8-fold cross-validation as a measure of the predictive ability of our model (Hooten & Hobbs, 2015). We divided the dataset into 8 clusters. We then withheld one data cluster from each model run and compared the predicted values from the model to the recorded data values of the withheld data. We used normal priors centered on 0 for all of our beta coefficients. The prior for β_0 had a variance of 10. For all other beta coefficients, we used Bayesian regularization to optimize the variance on the priors to minimize the MAPE (Hooten & Hobbs, 2015). We performed an iterative search, fitting the model with variances of 0.001, 0.01, 0.05, 0.1, 0.15, 0.2, 0.5, and 1. This allowed for selection of the variance with the minimum MAPE across model runs (Supplemental Figure 1). We then ran the model with the whole dataset, using the best optimized variance for the priors. For the

shape parameter of the negative binomial, we used a uniform prior from 0 to 500. The model was run with a burn-in of 5,000 iterations followed by additional 10,000 iterations.

fCort and Demographic Rates

We included additional data collected from radio-marked birds to estimate the effects of fCort on adult survival, breeding propensity, nest success, and overall breeding success using a Bayesian framework. For adult female survival, we used a known fate binomial survival model with a weekly interval (Royle & Dorazio, 2008). We also included seasonal offsets, a 1 or 0 assigned for that occasion, on survival for the spring (March 1 – May 31), summer (June 1 – July 31), fall (August 1 – October 31), and winter (November 1 – February 28/29). We only modeled survival during the first year following collection of a feather sample. We modeled probability of survival for each individual female, i, at time, t, as:

$$logit(s_{i,t}) = \beta_0 + \beta_{cort} * cort_i + \beta_{age} * age_i + \beta_{spring} * spring_t + \beta_{summer} * summer_t + \beta_{fall} * fall_t.$$

The winter season served as the intercept. Birds were assigned a 1 for age if first sampled as adults, or a 0 if sampled as yearlings, thus β_{age} reflects the difference between adults and yearlings.

For each female, i, we investigated whether fCort affected different components of the recruitment process. Breeding propensity ($y_{bp,i}$) was recorded as a 1 if we observed a female on a nest, and a 0 if we never observed a nesting attempt. Similarly, nest success ($y_{ns,i}$) was recorded as a 1 if birds successfully hatched a nest, and a 0 otherwise. We

modeled the probability of success for each component of the recruitment process (d) as $y_{d,i} \sim Bernoulli(p_{d,i})$, where,

$$logit(p_{d,i}) = \beta_{d,0} + \beta_{d,cort} * cort_i + \beta_{d,age} * age_i.$$

Overall breeding success ($y_{bs,i}$) was considered to be a 1 if the bird fledged at least one chick. To estimate the number of chicks that each female successfully fledged ($y_{f,i}$), we modeled $y_{f,i} \sim Zero - inflated\ Poisson\ (bs_i * \mu)$ where μ was the mean and variance of the number of chicks fledged, based on,

$$log(y_{f,i}) = \beta_{f,0} + \beta_{f,cort} * cort_i + \beta_{f,age} * age_i.$$

Results

Corticosterone Model

We estimated fCort for 280 female sage-grouse. We detected substantial among-individual variation in fCort levels, with 65% of the sample having fCort levels < 10 pg/mm, while 20% of the sample had fCort levels > 100 pg/mm feather. There was no effect of age, and body condition only had a small negative effect on mean fCort (Table 1). In contrast, horse and cow densities interacted with precipitation to influence fCort levels. Increasing numbers of horses increased fCort in drought years (Figure 2; Table 1). This interaction was similarly positive but the effect was not as large for cows (Figure 3; Table 1). FCort levels were also positively associated with the interaction between horse density and percent cover of annual grasses (Figure 4; Table 1). Despite substantial among-individual variation, fCort levels tended to be highest in grazing allotments or horse management areas containing the largest numbers of cows and horses.

Demographic Model

Mean fCort was not strongly predictive of future survival or reproductive success (Table 2). The posterior distribution for the beta estimate for nest success suggested the potential for a negative relationship between fCort and nest success (Figure 5; Table 2).

Discussion

Mean fCort levels in female sage-grouse were higher when density of non-native ungulates was higher under drought conditions. Ungulates in arid environments can remove a high percentage of primary productivity (Baur et al., 2018; Veblen et al., 2015), an effect that is exacerbated under drought conditions when primary production is reduced (Donnelly et al., 2018a; Zeigenfuss et al., 2014). Our findings are, therefore, consistent with the hypothesis that grazing by ungulates, likely through a reduction in late summer food availability for sage-grouse (Street, 2020), acts as a chronic stressor for a significant number of female sage-grouse. Horses in particular remove vegetation closer to the ground than other ungulates and consume upwards of 20% more plant matter than cows (Hanley & Hanley, 1982). This may explain the stronger effect found with horses than with cows. The increase in fCort due to the combined effect of horses and annual grass cover is not surprising, given that horses can help spread cheatgrass (King et al., 2019) and sage-grouse recruitment suffers in areas with higher cheatgrass cover (Blomberg et al., 2012). We cannot completely rule out the potential that social interactions between ungulates and sage-grouse are responsible for elevated GCs; we believe this hypothesis is unlikely for two reasons. First, fCort was deposited during fall when livestock are only present in a few allotments and in reduced numbers in sage-grouse habitat, because they have been removed under permitted grazing practices (BLM

Applegate Field Office, unpublished data). Second, while numerous species avoid riparian areas when horses are present, observations of aggressive behaviors by horses tend to be restricted to other ungulates (Gooch et al., 2017; Hall et al., 2018; Ostermann-Kelm et al., 2008).

Our findings are consistent with other research demonstrating that environmental or social restrictions on energy or nutrient intake can produce chronic physiological stress (Fairhurst et al., 2017; Johns et al., 2018; Will et al., 2015; Wingfield et al., 1997; Wingfield et al., 2017; Wingfield & Sapolsky, 2003). Drought conditions are known to reduce reproductive investment by female sage-grouse (Blomberg et al., 2017; Blomberg et al., 2012; Blomberg, Sedinger, et al., 2013b) and male sage-grouse were less likely to attend leks during drought, especially at higher population densities (Blomberg, Sedinger, et al., 2013a), indicating that factors reducing food availability between breeding seasons constrain reproductive activities in sage-grouse. The fact that grazing by ungulates under drought conditions increased fCort levels is consistent with the general linkage between nutrient intake and elevated GCs, and elevated levels and reduced breeding investment (Hansen et al., 2016; Harms et al., 2015; Williams et al., 2008). We caution that our results, while consistent with the hypothesis that habitat degradation and food restriction elevate GCs in some females, cannot be used to establish a direct linkage between a chronic physiological stress and reproductive investment because we assessed the GC levels several months before breeding began. Delayed impacts on current breeding and survival (carry-over effects) have been demonstrated for other variables in various avian species, such as previous breeding experience (Souchay et al., 2014; Warren et al., 2014) and population density (Blomberg et al., 2017).

Our data suggest that not all individuals responded the same way to grazing impacts (Figures 2 & 3). This could result from underlying intrinsic differences in reactivity to stressors (Cockrem, 2007, 2013) or other aspects of individual quality. Unknown circumstantial influences during the period of feather growth such as a predator attack, prior breeding effort, or disease status could also contribute to variation in response (Wingfield et al., 2017). Females that attended a brood during the summer delay molt of their primary feathers (Braun et al., 2020). If replacement of body feathers is also delayed, the timing of fCort deposition could differ by previous reproductive state. However, we found no effect of age on fCort, thus adults showed similar levels to yearlings who had no previous reproductive experience.

We found weak support that fCort was related to decreased nesting success, but otherwise was not predictive of vital rates. The time interval between the elevated GCs and nesting, however, suggests that both reflect nutritional condition, rather than the GC elevation causing lower nest success in some females. Because we captured females in spring of the year following feather growth, our sample only contains those females who survived from fall to spring. If high fCort levels had more immediate survival consequences, we would have been unable to detect them in this analysis because mortality would have occurred before females entered our demographic samples.

Using Bayesian methods to address the uncertainty in our estimates, we were able to identify the variation in female responses to stressors. We likely would have missed this important pattern with traditional methods used for comparing hormone levels. We observed a significant amount of variation in fCort among females. We modeled variation in the mean of the negative binomial distribution with fCort levels rounded to

the nearest pg, and allowed the rate (variance term) to be constant. We also attempted to model variation in the rate term of the negative binomial and with a Poisson distribution, but observed poorer fit. Jankowski et al. (2014) standardized fecal corticosterone so that they could fit a linear model with a normal distribution. The highly skewed distribution of fCort levels we found could have influenced conclusions in studies that assumed a normal distribution for the residual variance. We emphasize that this variation is important to our understanding of the physiological response of chronic stressors. Our results suggest that there will likely be females with elevated fCort levels even when chronic stress is low, and there will likely be females with low fCort levels when chronic stress is high. Yet, the proportion of birds with high GC levels is higher when chronic stress is higher, and that adverse weather conditions combined with higher levels of either livestock of feral horses represent one source of chronic stress in our system.

Climate projections for the Great Basin indicate more frequent and longer lasting drought (Bradford et al., 2020; Snyder et al., 2019). Our results suggest that these conditions, along with unchecked increases in feral horse populations, will lead to extreme chronic stress in a substantial proportion of sage-grouse. Birds may acclimate to grazing pressure during wet or average years, but such responses are insufficient under drought conditions when resources become scarce. While short-term spikes in GCs facilitate survival and onset of breeding, chronic stress can lead to suppression of immune response, reproduction, and growth. Downregulation of the stress response system due to chronic stress can impede an individual's ability to respond to acute stressors when needed (Rich & Romero, 2005). Our results suggest that under increased drought, female

sage-grouse could experience an increased frequency of physiological stress under current grazing regimes.

Our study used a large dataset of feather corticosterone across a key sage-grouse habitat region of over 1 million hectares. This allowed us to assess both individual variation and effects of a gradient of biotic and abiotic environmental variables. Declines of sage-grouse populations have continued (Coates et al., 2021) despite intensive conservation efforts over the last few decades. Investigating the physiological links between the birds, their habitat, and the threats to both is important for informing future conservation actions.

Tables

Table 2-1. Model estimates for the full fCort model with covariates. LCI and UCI represent lower and upper 95% credible intervals, respectively. Pr < 0 and Pr >0 were the proportions of the posterior distribution below and above zero, respectively.

Term	*Estimate*	*SD*	*LCI*	*UCI*	*Pr <0*	*Pr >0*
Intercept	3.779	0.107	3.574	3.99	0	1
Cows	0.016	0.064	-0.107	0.143	0.405	0.595
Horses	-0.056	0.077	-0.206	0.096	0.764	0.236
Body Condition	-0.037	0.061	-0.159	0.081	0.726	0.274
Age	-0.004	0.087	-0.174	0.168	0.517	0.483
Annual Grass	-0.078	0.073	-0.219	0.065	0.86	0.14
Annual Precipitation	-0.125	0.068	-0.258	0.008	**0.967**	0.033
*Horses * Precipitation*	-0.125	0.069	-0.261	0.01	**0.965**	0.035
*Horses * Annual Grass*	0.075	0.086	-0.095	0.243	0.19	**0.81**
Cows Precipitation*	-0.043	0.06	-0.161	0.073	**0.759**	0.241

Table 2-2. Estimated effects of fCort on each demographic rate. LCI and UCI represent lower and upper 95% credible intervals, respectively. Pr < 0 and Pr >0 were the proportions of the posterior distribution below and above zero, respectively.

Demographic rate	*Estimate*	*SD*	*LCI*	*UCI*	*Pr < 0*	*Pr > 0*
Adult Survival	0.001	0.001	-0.002	0.004	0.252	0.748
Breeding Probability	0	0.002	-0.003	0.003	0.435	0.565
Nest Success	-0.002	0.002	-0.006	0.001	**0.873**	0.127
Breeding Success	-0.001	0.001	-0.004	0.002	0.691	0.309

Figures

Figure 2-1. Study area map showing areas with horses, cows, or both during the course of the study. Bird locations include all known locations of the birds with fCort measurements. Note- Hart Mountain did have a few horses (<31) in 2012-2013, but these were not in the same areas as monitored sage-grouse.

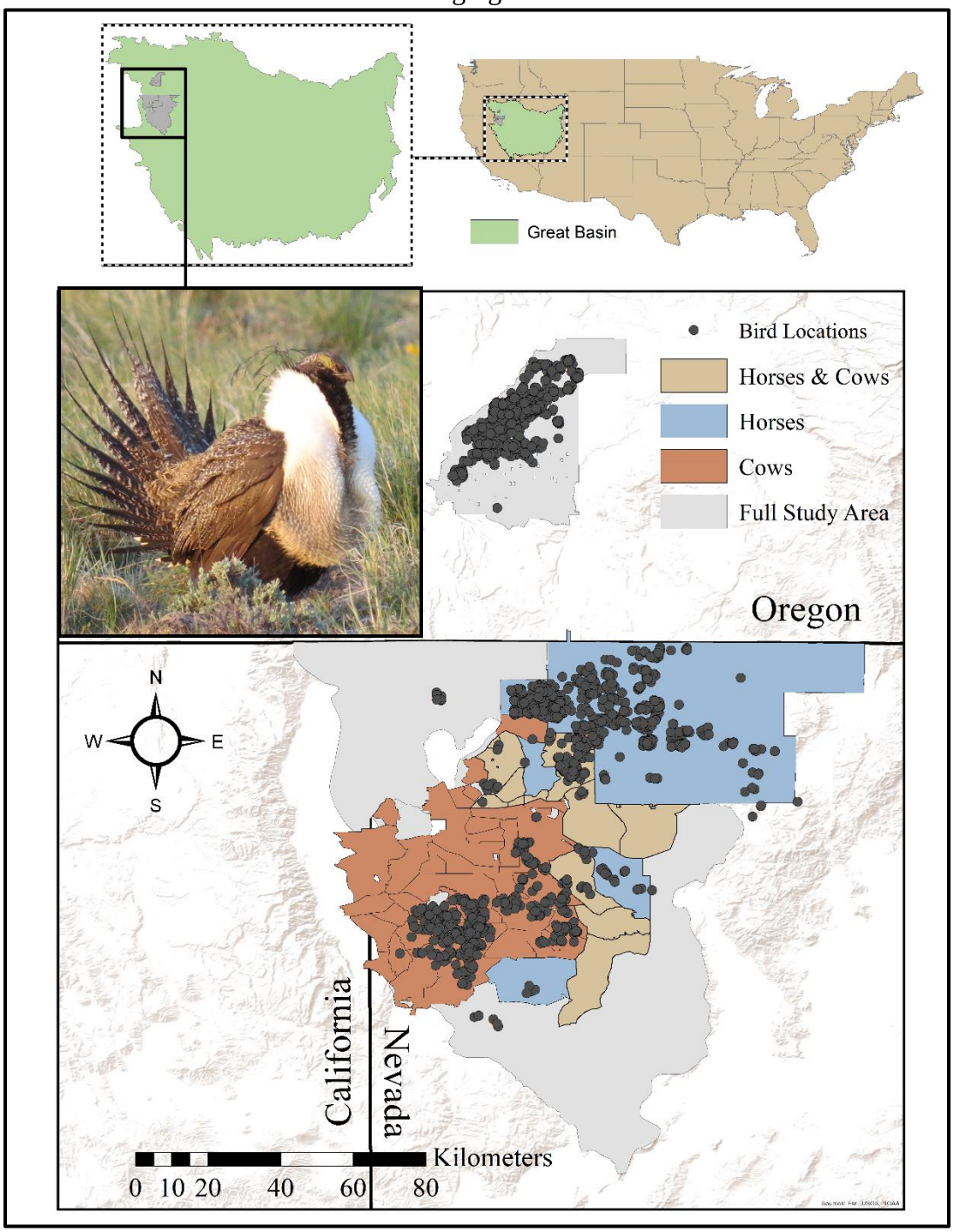


Figure 2-2. Interaction effects of horses (horse use days per km^2) and annual precipitation (mm) on fCort (pg/mm feather), at the mean of all other covariates. Grid surface represents the estimated interaction of effects from the full model. Colored points are observed values of fCort.

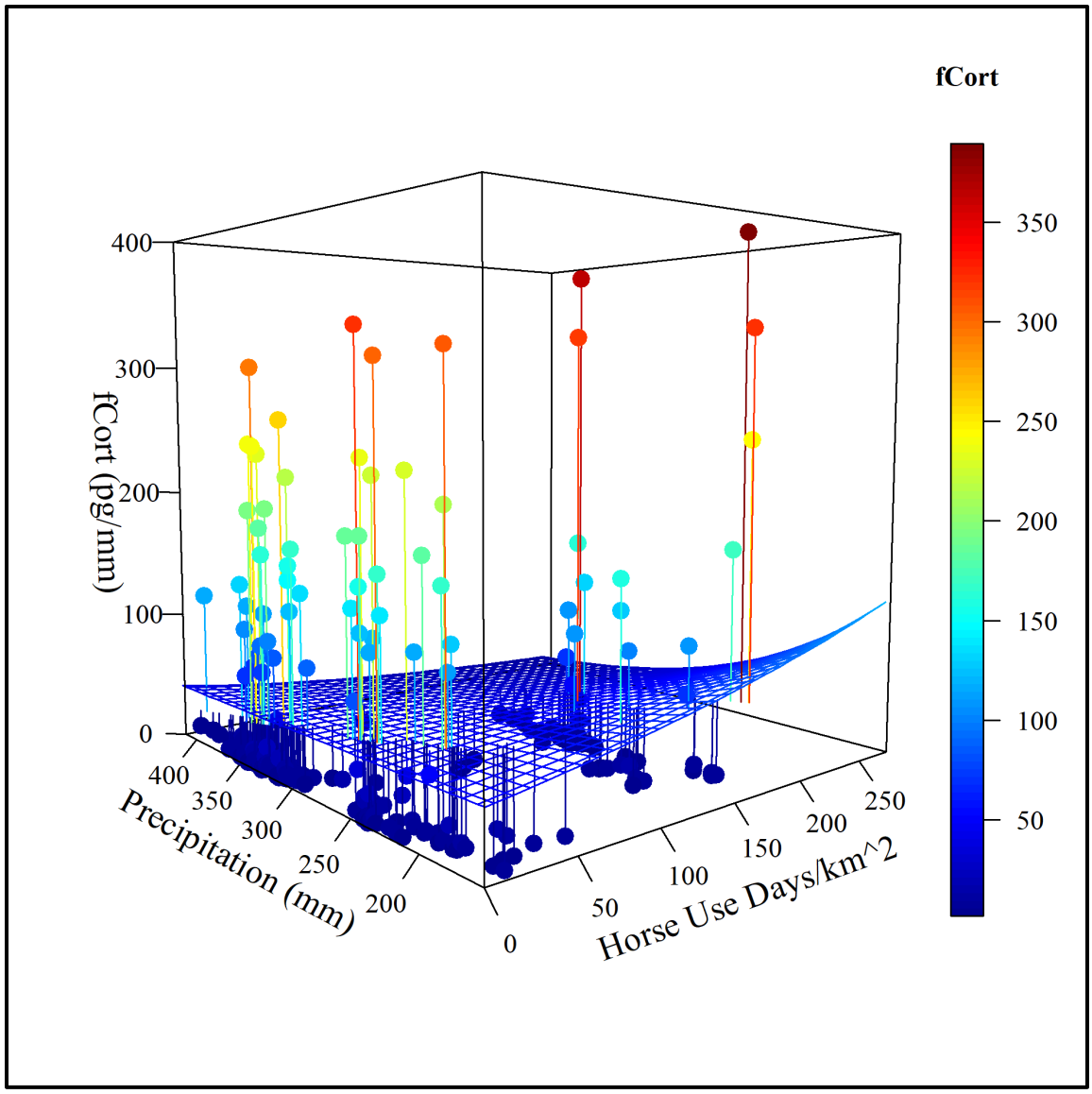


Figure 2-3. Interaction effects of cows (cow use days per km^2) and annual precipitation (mm) on fCort (pg/mm feather), at the mean of all other covariates. Grid surface represents the estimated interaction of effects from the full model. Colored points are observed values of fCort.

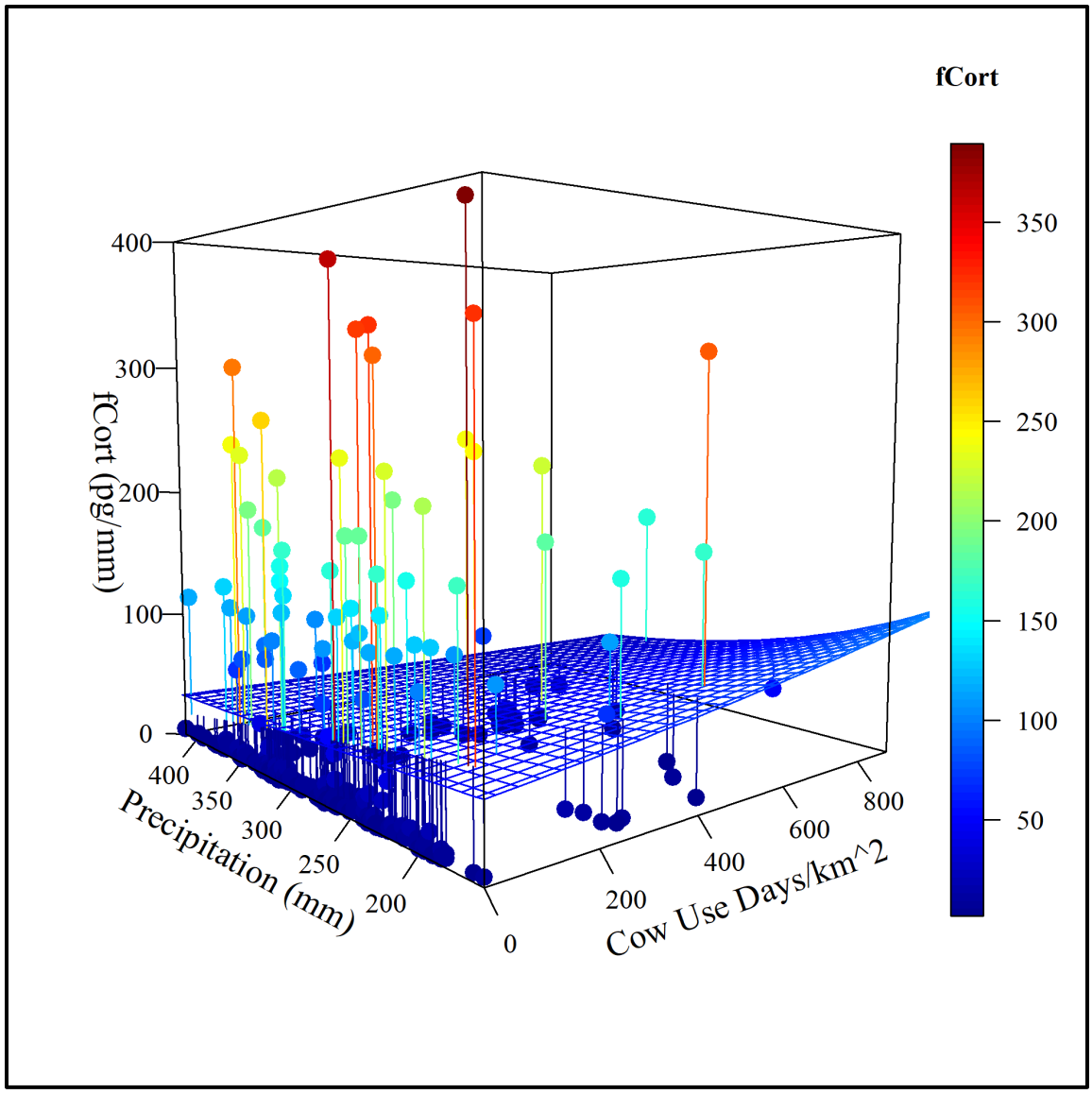


Figure 2-4. Interaction effects of horses (horse use days per km^2) and annual grass cover (% cover) on fCort (pg/mm feather), at the mean of all other covariates. Grid surface represents the estimated interaction of effects from the full model. Colored points are observed values of fCort.

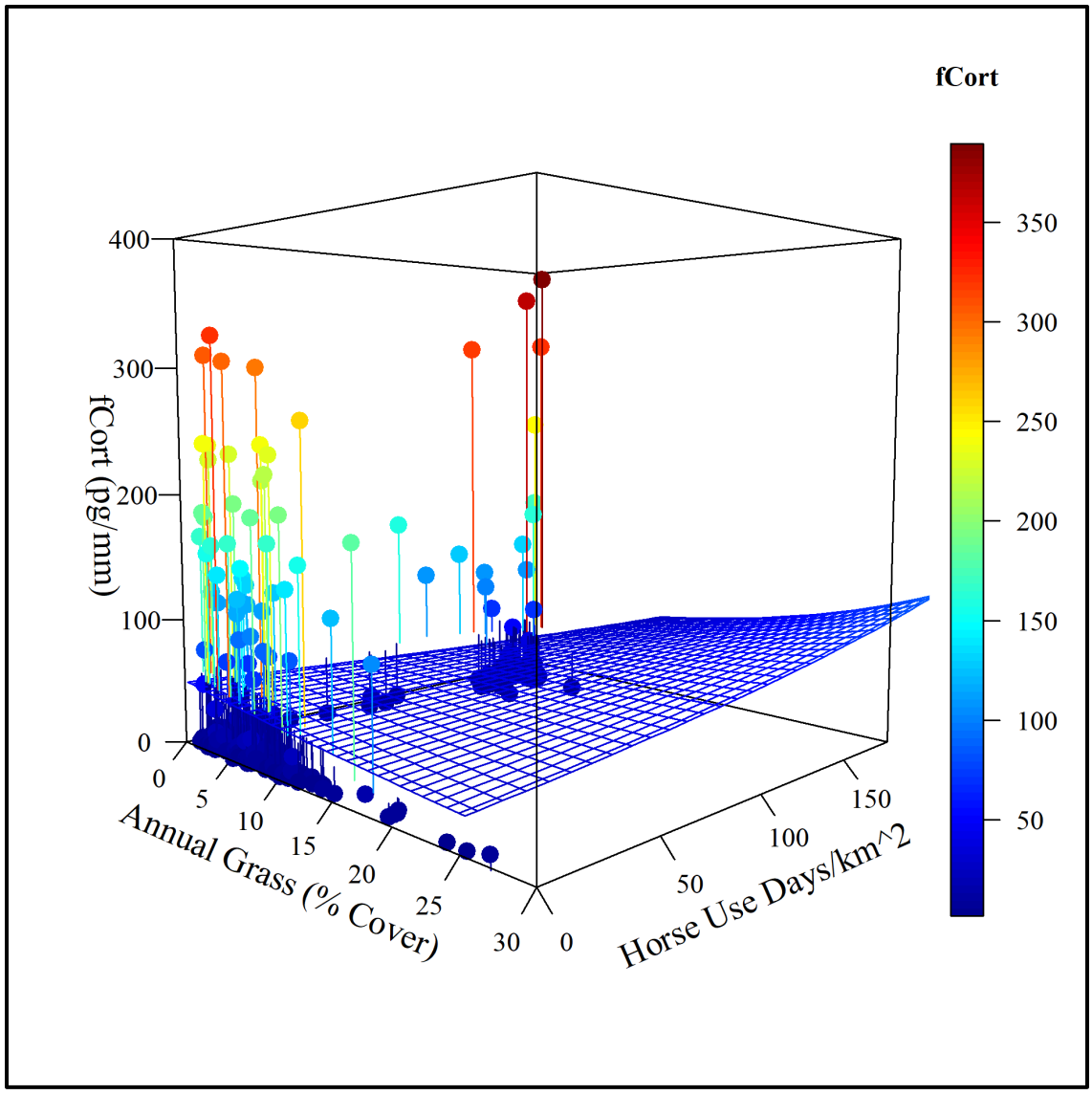
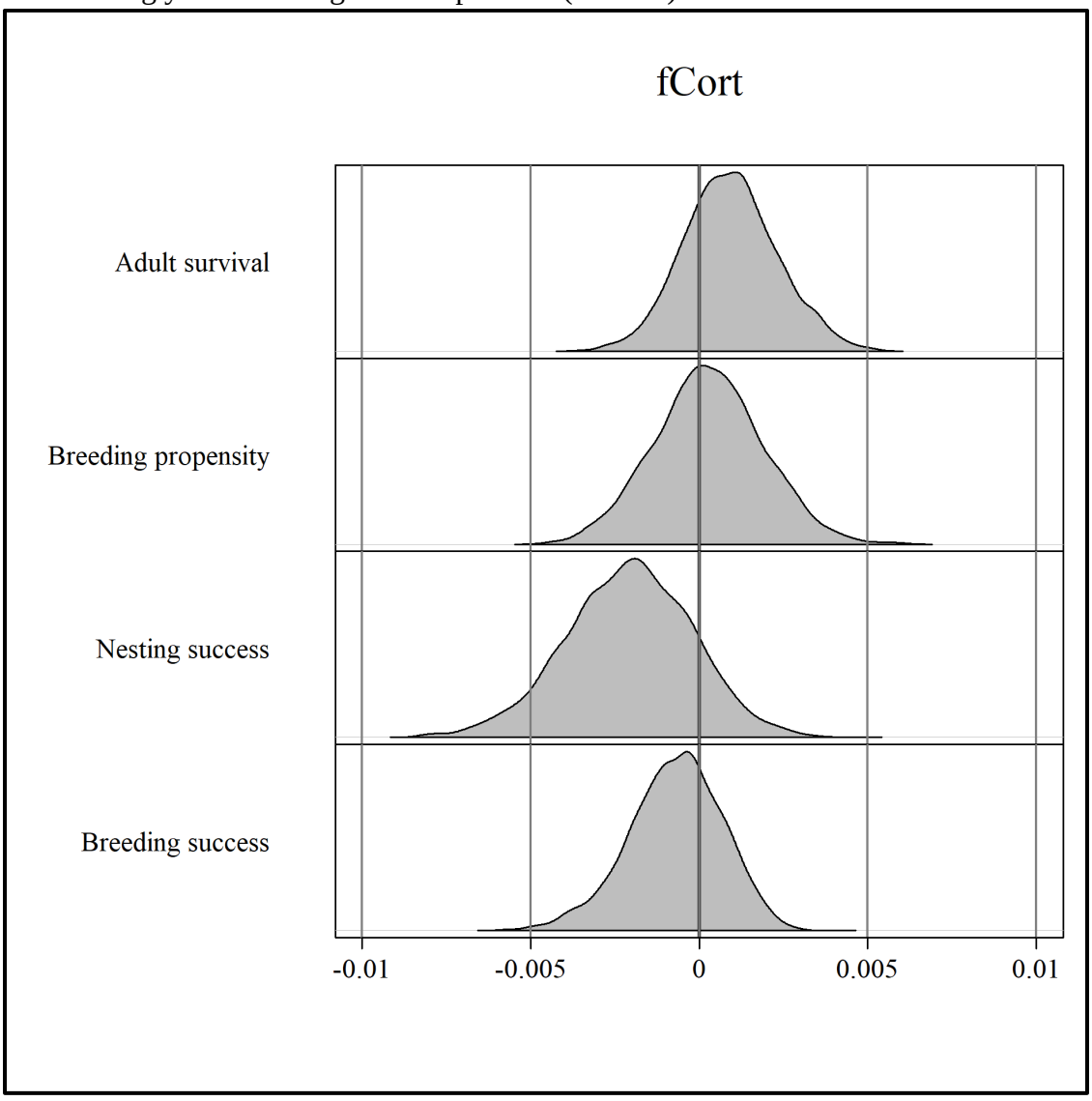
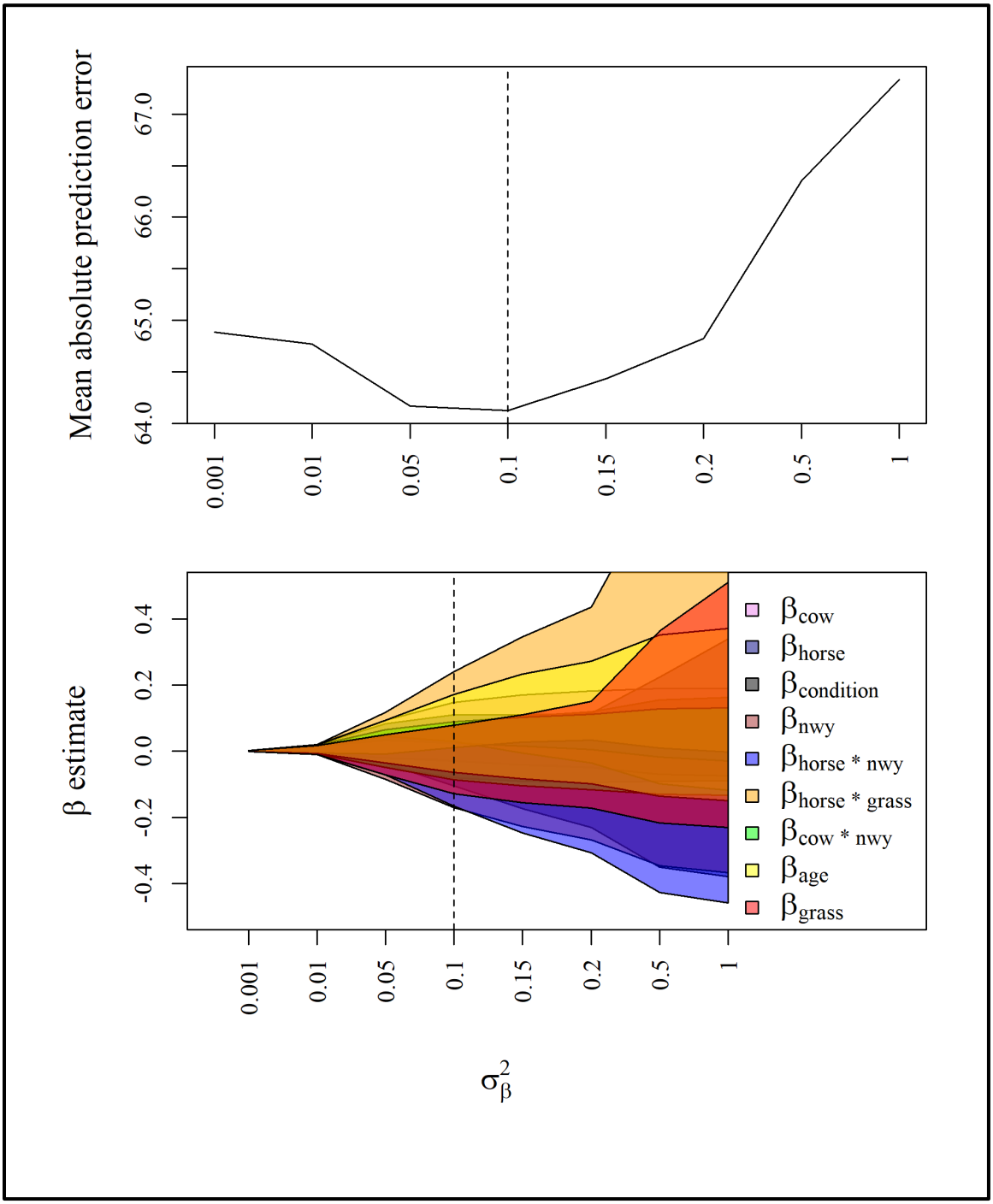


Figure 2-5. Beta distributions from the models using fCort as a predictor of demographic rates – adult survival (known-fate binomial), breeding propensity (Bernoulli), nest success (Bernoulli), and overall breeding success (zero-inflated Poisson and Bernoulli) in the breeding year following fCort deposition (Table 2).



Supplemental Figure 2-1. Sigma optimization. Upper plot shows the mean absolute prediction error assessed for sigma values from 0.001 to 1. Lower plot shows the range of beta estimates associated with each sigma. Sigma = 0.1 minimized the absolute prediction error of the beta estimates for the covariates and was used for the main model.



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Chapter 3: Parentage within and among clutches in Greater Sage-grouse

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Abstract

The reproductive choices of females are important to understanding population dynamics genetics over time. Polyandry and nest parasitism, two female-based strategies, are becoming better documented, but information for one prominent species, Greater Sage-grouse (*Centrocercus urophasianus*), has so far been limited to populations on the periphery of the species range. Though males are often quite visible on leks during the breeding season, female mating behavior is more secretive leading to the need for genetic analysis to reveal true parentage of clutches. We extracted DNA from 268 eggs in 39 clutches and feathers of putative mothers from our study site in Northwestern Nevada. Using the female feather samples, we verified maternity to examine nest parasitism, and then reconstructed possible male genotypes and looked for evidence of multiple paternity. We used 15 microsatellite loci in 39 clutches from northwestern Nevada. We found multiple paternity in 4 clutches (10.25%) and one possible parasitic egg in an additional clutch (2.56%). Multiple parentages in clutches may help maintain genetic diversity for the population or allow females to hedge their bets on male quality, and therefore the quality of offspring. These results provide important insights about sage-grouse breeding behavior that observational studies alone cannot.

Introduction

Females may employ different reproductive strategies to enhance their fitness (Andersson, 2017; Garcia-Gonzalez et al., 2015; Sinervo & Zamudio, 2001; Whittingham & Dunn, 2010; Wittenberger, 1978), but less common tactics can be difficult to observe. Genetic approaches to parentage of offspring can help detect such strategies to determine

how frequently they occur and under what circumstances (Lank et al., 2002; Sorenson & Payne, 2002; Trimbos et al., 2009). Two such alternative reproductive strategies of particular interest in avian species are multiple paternity and nest parasitism

Polyandry, a female mating with more than one male, is common in nature (Taylor et al., 2014). In a system without direct benefits related to paternal care of young or resource guarding, the benefits of polyandry are less obvious. Yet, females may reap indirect benefits through good genes from the male or genetic diversity among their offspring (Yasui, 1998; Yasui & Garcia-Gonzalez, 2016). The magnitude of such benefits may depend on variability in the environment in which those genes will be expressed and the female's ability to discern the fit of a particular male's genes to the environment (Garcia-Gonzalez et al., 2015; Yasui, 1998). Mating with multiple males may also be a bet hedging strategy by females (Yasui & Garcia-Gonzalez, 2016), which allows for imperfect assessment of male quality and the uncertainty about which genes will be beneficial under future conditions. Polyandry is predicted to be uncommon in lek mating systems, which are found in many taxa including birds, insects, fish, reptiles, and mammals, but often corresponds with characteristics of lekking species such as strong sexual selection on males and skewed sex-ratios (Höglund & Alatalo, 2014). Nevertheless, many examples of polyandry have now been documented in various species of lekking birds (Bird et al., 2013; Hess et al., 2012; Lank et al., 2002; Lesobre et al., 2010; Rivers & DuVal, 2020). In one population of lekking Greater Prairie-Chicken, 44% of clutches sampled had more than one and up to four different sires (Hess et al., 2012).

Conspecific nest parasitism has also been documented in many egg-laying species and ranges from opportunistic to obligate parasitism (Andersson, 2018; Lyon & Eadie, 2008; Sorenson & Payne, 2002). Laying eggs in another female's nest can directly increase the parasitic female's fitness through several pathways, while potentially increasing that of the host through kin selection if they are closely related (Andersson, 2017). If a female only lays parasitic eggs, and does not attempt a nest of her own, she eliminates the potential costs of incubation and brood rearing (Blomberg et al., 2013b). If clutch size is limited by either incubation ability or costs of large broods (Nur, 1988), parasitism may increase the number of genetic offspring fledged for the parasitizing female. Finally, this strategy spreads risk among nests, which may be especially beneficial in species with low or highly variable nest success (Knick & Connelly, 2011).

Greater Sage-grouse (*Centrocercus urophasianus*) are a classic example of a species with a lek mating system. Males gather seasonally on designated lek areas to strut and produce a variety of noises to attract the females (Gibson & Bradbury, 1985; Wiley, 1978). Mate choice is based on complex signaling – vocalizations, mechanically produced sounds, plumage, display behaviors, responsiveness to females, and territory centrality (Gibson & Bradbury, 1985, 1987; Patricelli & Krakauer, 2009). Female mating decisions may also be influenced by the choices of other females, particularly when direct assessment of mates may be costly due to predation or other risks (Gibson et al., 1991). Dominant males on a particular lek typically get most of the observable copulations (Gibson & Bradbury, 1985; Gibson et al., 1991; Wiley, 1973), however, additional matings may occur off the lek as well. Males exhibit high lek site fidelity among years (Fremgen et al., 2017; Gibson et al., 2014), which could lead to lower genetic diversity

within the local population. Female grouse may partially counteract this pattern by mating with multiple males. Females could mate with non-dominant males from the same lek, or visit other leks nearby (Semple et al., 2001). If females cannot accurately assess kinship between themselves and the dominant male on a lek, some cases of multiple paternity could buffer inbreeding and genetic incompatibility (Zeh & Zeh, 1996).

Low levels of multiple paternity have been observed in Canadian populations of Greater Sage-grouse (Bird et al., 2013), as well as in the Bi-state population in California and Nevada (Semple et al., 2001). In one study, nest parasitism by conspecifics has been documented in sage-grouse (Bird et al., 2013). We used molecular methods to assess the parentage of individuals within clutches from northern Nevada populations of sage-grouse to assess multiple paternity and nest parasitism as possible alternative female breeding strategies and contributors to the maintenance of local genetic diversity.

Methods

Field Methods

We trapped yearling and adult female sage-grouse near active leks in northwestern Nevada during March 2017 – 2018. We focused on leks in the western section of the Sheldon National Wildlife Refuge (hereafter Sheldon) managed by the United States Fish and Wildlife Service and near Boulder Mountain within the Massacre sage-grouse Population Management Unit (hereafter Massacre), managed by the Bureau of Land Management (Figure 1). The two focal leks on Sheldon were just over 4 km apart, with average peak male lek counts of 36 and 43 over the previous 5 years. The two leks on Massacre were approximately 4.8 km apart and had average peak counts of 67

and 100 males over the previous 5 years. The two lek clusters were 65-70 km from each other.

We captured birds at night using spotlights (Giesen et al., 1982) and handheld nets. Once captured, we banded females (n = 778) with unique metal bands (size 14) and fit them with 22g very high-frequency necklace-style radio-collars (VHF; Advanced Telemetry Systems, Isanti, MN, USA). We aged birds as juvenile or adult based on plumage characteristics (Crunden, 1963). We then monitored collared females throughout the year. We monitored females every 2-3 days during the early nesting period, to document nesting and success or failure of nests. We collected samples of eggshell membranes upon hatch or nest failure. When possible, we collected samples from the entire clutch (27/39 full clutches after necessary exclusions).

Laboratory Methods

We stored membranes at room temperature in individual paper envelopes within a plastic bag containing silica gel. When whole unhatched eggs were present, we collected them and stored them frozen. At capture, we collected breast feathers from hens, which we stored in individual envelopes or bags at room temperature.

Samples were analyzed at the U.S. Geological Survey Molecular Ecology Lab at the Fort Collins Science Center in Fort Collins, Colorado. DNA was extracted from eggshell membranes and feathers using a Qiagen DNeasy Blood & Tissue kit following the user-developed protocol for the purification of total DNA from hair, nails, and feathers (Qiagen).

Samples were amplified across 15 grouse-specific microsatellite loci (MSP11, MSP18, SGMS06.4, SGMS06.6, SGMS06.8; (Oyler-McCance & St. John, 2010),

SGCA5, SGCA11, SGCTAT1; (Taylor et al., 2003), TUT3, TUT4; (Segelbacher et al., 2000), BG6; (Piertney & Höglund, 2001), SG28, SG36, SG29, SG39; (Fike et al., 2015). The primers for 2 loci were redesigned for better performance (SGCA5F: CGGACAGGTACATCCTGGAA, SGCA5R: GGGAAAAGATGTCAGAATCTACAAA, SGCA11F: GCAGTAAAGAAAATTTGGAAGCA, SGCA11R: TCTTGAACTGATGTTGGATTTG). The sex of each sample was determined by amplifying a region of the CDH gene using the primers 1237L and 1272H (Kahn et al., 1998).

Amplifications were performed in four 10 μL multiplexed PCRs using the 2x Qiagen Multiplex PCR Master Mix (Qiagen) following the manufacture's protocol. The loci were grouped in multiplexes based on their annealing temperature, size and primer label. Multiplexes were comprised of the following loci: Set 1; MSP18, SGCA5, Sexing, SGMS06.4, and BG6, Set 2; TUT3, SGCA11, SGMS06.6, MSP11, SGMS06.8, and SGCTAT1, Set 3; SG28, SG36 and SG39, Set 4; SG21, SG29 and TUT4. All PCRs consisted of 2 μL of template DNA, 1 μL primer mix, 5 μL 2x Master Mix, and 2 μL water. Amplification conditions for multiplex sets were as follows: 95ºC for 15 min, then 94ºC for 30 sec, annealing temperature (55 ºC: sets 1 and 2, 60 ºC: sets 3 and 4) for 1.5 min, 72ºC for 1 min for 40 cycles, then 60ºC for 45 min. PCR products were combined with GeneScan LIZ 600 internal lane size standard (Applied Biosystems), and electrophoresed through a capillary gel matrix using an AB3500 Genetic Analyzer (Applied Biosystems). Each multiplex PCR was run separately on the AB3500. Allele sizes were determined for each locus using GeneMapper v5 software (Applied

Biosystems). All feather samples and 20% of the eggshell membrane samples were amplified twice at each locus to verify genotypes. If genotypes differed between these two runs, the sample was run a third time. If two out of three runs matched, the genotype was called as the match, otherwise the locus was treated as missing data.

Parentage Methods

From the duplicate runs of a portion of our samples, we estimated an error rate of less than 1% for each locus. We excluded samples that failed to amplify at 7 or more loci (n=13). We checked for repeat sampling of eggs within clutches and discarded duplicates (n=20). We analyzed the microsatellite data using the programs Cervus (Kalinowski et al., 2007) and Colony (Jones & Wang, 2010) as well as by-hand assessments.

Of the 39 clutches sampled, 7 were from depredated nests and the rest were from hatched nests. Twelve of the samples were intact eggs from otherwise successful nests. Of these whole eggs, 3 had fully developed chicks that failed to hatch properly and the remaining 9 only had yolks. We sampled nests from 3 of the females in two different years.

We used Cervus to conduct an allele frequency analysis, a simulation, and a maternity check. First, we analyzed allele frequencies to establish the suitability of the loci we used for parentage analysis. The loci chosen are known to be highly polymorphic in sage-grouse, but we checked alleles for Hardy-Weinberg equilibrium to confirm their utility for this population of birds. Then we ran the maternity simulation to get estimates of exclusion probabilities. Using these probabilities, we then ran the maternity assignment analysis to determine whether putative mothers matched their clutches.

To check for nest parasitism, we compared genotypes of eggs against the putative mother. After matching the mother to her clutch, we subtracted the maternal alleles from the offspring genotypes to reconstruct the possible male genotypes. We used the Colony program to assess likelihood of full and half siblingships within and among clutches. This also helped us identify if a male sired multiple clutches in the sample. Colony also estimates the probability of maternity for each egg in the clutch, which we compared to the findings from the Cervus analysis.

We considered eggs with 2 or more loci that did not match the maternal alleles and sibling genotypes to have been laid by another female (Bird et al., 2013). We considered eggs with 2 or more loci that did not match the sire reconstruction for the majority of the clutch, after accounting for the maternal genotype, to be the result of mating with multiple males (Bird et al., 2013).

Results

After exclusion of duplicates and samples with 7 or more missing loci, we retained 39 clutches (10 from Massacre, 29 from Sheldon) containing 268 unique eggs as well as feather samples from 36 putative mothers for parentage analyses.

The mean number of alleles per locus for our 15 loci was 9.667. Two loci (WY BG6 & TUT3) were out of Hardy-Weinberg equilibrium, but we retained them for parentage assessment (Supplementary Material Table S1). Only 1 locus (reSGCA11) had a somewhat high proportion of null alleles (0.06), but exclusion of this locus did not change results. The combined non-exclusion probability for both the first and second parent was less than 0.0001.

Maternity

We found one egg from Sheldon that is most likely the result of intraspecific nest parasitism (0.37% of eggs; 2.56% of clutches). The egg was the only unhatched egg in an otherwise successfully hatched nest. There were mismatches at 4 loci and the Colony probability of a different mother than the rest of the clutch was 0.97 (Supplementary Material Table S2). There were also 3 single locus mismatches across all clutches, which is within the estimated error and mutation rates. All remaining clutches completely matched their putative mothers.

Paternity

We identified 7 eggs (out of 268; 2.61%), across 4 clutches (out of 39; 10.25%), as having a different sire than the majority of the clutch based on reconstructions of male genotypes (Supplementary Material Table S2). All clutches with multiple paternity were nests from the Sheldon site in 2018.

Based on the paternal genotype reconstructions, we were able to identify several dominant males. On the Massacre site, 2 males sired 2 clutches each (together, 40% of Massacre clutches). On the Sheldon site, there were 5 males that sired multiple and partial clutches. The most dominant male in 2018 sired 4.5 clutches (15.5% of Sheldon clutches). One male sired 3 clutches in 2017 (10% of Sheldon clutches) and two males sired 2 clutches each in 2018 (6.9% of clutches, each). One male in 2018 sired 1.5 clutches (5% of clutches). None of the clutches of the 3 females sampled in two years were sired by the same male in both. In 2 of the clutches with multiple fathers, one of the fathers was a dominant male.

Discussion

We found evidence for both multiple paternity and nest parasitism in this population segment of Greater Sage-grouse. Our estimate of 10.25% of clutches with multiple sires was slightly higher than Bird et al. (2013) found in the Canadian population (7.9% of clutches), but lower than the 20% found by Semple et al. (2001). The one egg likely be the result of nest parasitism gave us a lower rate of 2.56% to the estimate from Canada of 9.6% of clutches parasitized (Bird et al., 2013). We also documented several dominant males that sired multiple full clutches and partial clutches.

We cannot determine if these females are visiting multiple leks, as documented in California (Semple et al., 2001), or mating at the same lek with multiple males as in Canada (Bird, 2013). The clear presence of dominant males in these areas creates several motivations for females to mate multiple times. Females could be mating with the most likely dominant male, based on his attributes or social cues from other females (Gibson, 1996; Patricelli & Krakauer, 2009), and then securing an additional mating to bet hedge against incompatibility or copulation failure (Yasui, 1998; Yasui & Yoshimura, 2018). Alternatively, females could mate with one male, then come across a more desirable male and mate again (Lank et al., 2002). Females have been observed making several visits to leks before nesting (Gibson, 1996). Fluctuations in lek attendance by males within (Wann et al., 2019) and between seasons (Blomberg et al., 2013a) could also influence the options available to females as they prepare to nest. Lek size might influence female choices, such as whether there is a trade-off between time to select a mate and predation risk or of nest initiation.

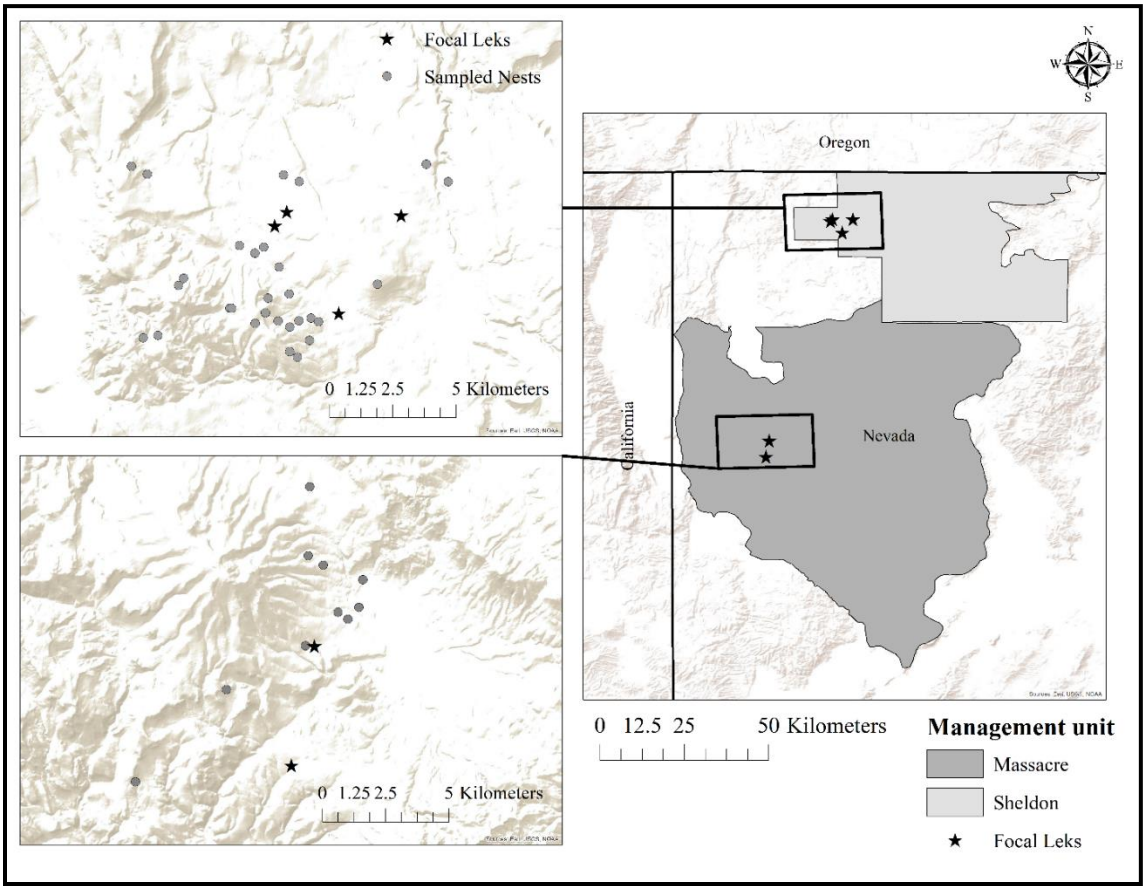
Closely related mothers or fathers could have caused us to underestimate the number of parents for a clutch through shared alleles, particularly without candidate male

genotypes for comparison (Wang & Santure, 2009). The proximity of leks in both study areas (Figure 1) coupled with sage-grouse site fidelity (Fremgen et al., 2017; Gibson et al., 2014) make it possible that breeding individuals (males or females) within our samples were related to each other. Consequently, our estimates of both nest parasitism and multiple paternity should be considered minimum estimates.

Lek-based systems stimulate complex questions in the study of female reproductive decisions. We have provided evidence that multiple parentage occurs at low rates in Greater Sage-grouse. We were unable, however, to relate multiple parentage to specific attributes of female Greater Sage-grouse or the ecological conditions in which they bred. Even with diligent lek observation, such as by Gibson & Bradbury (1985, 1987), it would be difficult to get enough nest samples from females with known mating histories to fully document the circumstances of female choices. Nest samples from additional populations, with larger and smaller leks, coupled with adult genetic samples could help us determine more about the conditions for this phenomenon. Understanding the genetic dynamics of these populations is important information for ongoing and future conservation efforts.

Figures

Figure 3-1. Map of the study region in northwestern Nevada with insets of sampled nests and focal leks.



Supplemental Materials

Supplementary Material Table S1. Characteristics of 15 loci evaluated to test nest parentage.

Locus	Alleles	N	H_O	H_E	NE-1P	NE-2P	HW	F(Null)
MSP18	9	304	0.763	0.753	0.633	0.451	NS	-0.0047
SGMS06.4	7	304	0.707	0.72	0.695	0.518	NS	0.0072
WY BG6	13	300	0.837	0.855	0.451	0.289	*	0.0095
reSGCA5	6	300	0.72	0.75	0.658	0.48	NS	0.0201
MSP11	13	304	0.852	0.832	0.499	0.329	NS	-0.0124
SGCTAT1	9	304	0.727	0.717	0.676	0.494	NS	-0.011
SGMS06.6	15	301	0.884	0.877	0.398	0.248	NS	-0.0047
SGMS06.8	11	300	0.86	0.852	0.461	0.296	NS	-0.0077
TUT3	5	303	0.706	0.716	0.696	0.519	***	0.0037
reSGCA11	7	301	0.638	0.722	0.674	0.491	NS	**0.0637**
SG28	14	304	0.816	0.84	0.482	0.315	NS	0.015
SG36	7	302	0.642	0.706	0.71	0.535	NS	0.0461
SG39	10	300	0.84	0.826	0.51	0.338	NS	-0.009
SG29	10	304	0.753	0.748	0.637	0.458	NS	-0.005
TUT4	9	300	0.71	0.753	0.634	0.453	NS	0.0251

Supplementary Material Table S2. Loci listed had 3 or more paternal alleles, after exclusion of maternal genotype.

	Locus						
Individual	MSP18	WYBG6	SGC TAT1	SGMS 06.8	TUT3	reSGCA 11	SG29
Female 100579	102/108	289/289	94/94	119/119	152/152	185/187	120/120
Egg 1	108/110	281/289	94/94	119/135	148/152	185/191	120/120
Egg 2	102/104	281/289	86/94	119/147	148/152	185/189	120/124
Egg 3	108/108	273/289	94/106	119/123	152/156	177/187	120/134
Egg 4	104/108	289/289	94/94	119/135	148/152	185/189	120/124
Egg 5	102/110	281/289	86/94	119/135	152/152	185/191	120/120
Egg 6	108/110	281/289	94/94	119/147	152/152	185/191	120/120
Paternal alleles	**104,108, 110**	**273,281, 289**	**86,94, 106**	**123,135, 147**	**148,152, 156**	**177,189, 191**	**120,124, 134**
	MSP18	SGMS 06.4	WYBG6	MSP11	SGMS 06.6	reSGCA 11	SG36
Female 14456	108/108	134/158	281/293	206/208	161/164	187/187	227/235
Egg 1	102/108	134/158	281/293	208/228	155/164	187/191	227/243
Egg 2	108/114	134/134	253/281	208/228	134/164	187/187	235/243
Egg 3	108/114	134/158	281/293	206/228	155/161	187/187	227/231
Egg 4	108/108	158/158	261/281	206/208	140/161	187/187	235/235
Egg 5	108/110	158/158	253/281	206/210	161/164	185/187	227/235
Egg 6	108/110	134/150	253/293	208/210	161/164	185/187	227/231
Paternal alleles	**102,108, 110,114**	**134,150, 158**	**253,261, (281/293)**	**(206/208), 210,228**	**134,140,155, (161/164)**	**185,187, 191**	**231,235, 243**
	SGMS 06.4	MSP11	SGC TAT1	SGMS 06.8	SG28	SG36	SG39
Female 14410	130/134	206/210	94/94	119/135	117/137	239/239	175/175
Egg 1	134/150	206/210	94/94	119/143	135/137	239/239	154/175
Egg 2	130/150	208/210	94/94	119/143	135/137	239/239	172/175
Egg 3	130/150	208/210	94/102	119/143	117/135	239/239	172/175
Egg 4	130/150	206/210	94/102	119/135	135/137	239/239	154/175
Egg 5	130/150	208/210	94/94	119/119	117/135	235/239	172/178
Egg 6	130/158	210/228	94/106	119/123	117/121	231/239	169/175
Egg7	134/158	210/228	94/102	119/147	117/117	231/239	169/175
Paternal alleles	**134,150, 158**	**206,208, 228**	**94,102, 106**	**119,123, 143,147**	**117,121, 135,145**	**231,235, 239**	**154,169, 172**

Supplementary Material Table S2 (continued). Loci listed had 3 or more paternal alleles, after exclusion of maternal genotype. The nest of female 100518 had one egg with additional alleles suggesting nest parasitism by another female.

	Locus	
Individual	MSP11	SG29
Female 100508	210/228	120/134
Egg 1	210/228	120/120
Egg 2	210/228	120/134
Egg 3	210/220	120/122
Egg 4	210/220	120/122
Egg 5	210/210	120/138
Egg 6	210/220	120/134
Paternal alleles	**210,220, 228**	**120,122, 138**

	MSP11	SGMS06.8	SG36	SG29
Female 100518	228/238	123/147	231/235	120/124
Egg 1	222/238	147/147	231/235	120/134
Egg 2	222/238	123/147	231/231	124/134
Egg 3	222/228	147/147	235/235	120/134
Egg 4	222/228	123/147	231/231	124/134
Egg 5	228/234	123/147	231/235	124/134
Egg 6	222/238	147/147	235/235	120/134
Egg 7	206/222	135*/147	231/239	120/136*
Maternal alleles	**228,238,206**	**123,147,135***	**231,235,239**	**120,124,136***

*could be paternal allele, but only occurred in this offspring

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