BREEDING ECOLOGY OF GREATER SAGE-GROUSE IN SOUTHWESTERN MONTANA

by

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ABSTRACT

The greater sage-grouse (*Centrocercus urophasianus*; hereafter: *sage-grouse*) is an umbrella species that needs large intact tracts of sagebrush habitat with habitat requirements that represent the needs of many other species found in the sagebrush biome of the American West. Much of the information collected to date on sage-grouse is from low-elevation, homogenous, landscapes, leaving information gaps for topographically complex, high elevation locations within the sage-grouse range. In this dissertation, I assess the following aspects of the breeding ecology of sage-grouse: 1) how females select nest and brood sites based on sagebrush type, along with livestock grazing features and other biotic and abiotic characteristics; 2) the influence of female nest-site selection on nest-survival outcomes; 3) experimentally derived isotopic discrimination values in domesticated gallinaceous birds as an estimation method for nutrient allocation strategies in wild sage-grouse; and 4) the degree to which females allocated nutrients from winter habitats for formation of offspring by comparing females nesting in southwest vs. central Montana. Based on the research, I found evidence that: 1) sage-grouse avoid a high-elevation sagebrush type that is the most common type in my study region and instead select for intermediate- or low-elevation sagebrush types for both nesting and brood rearing, 2) sage-grouse broods selected sites away from low-lying mesic areas and near ridgelines on upper slopes with south-facing aspects and sites further from cattle paths, 3) nest survival was (a) higher for nests placed away from fence lines, (b) lower in areas with more cow pies and taller dead grass, and (c) higher in areas with increased living grass cover, and 4) females from southwest Montana and in the high-elevation sagebrush type primarily allocated nutrients from winter habitats, whereas females from central Montana and in the low-elevation sagebrush type primarily allocated nutrient sources from spring habitats for offspring formation. My findings highlight a unique breeding strategy for sage-grouse residing in high-elevation sagebrush landscapes. Results described herein will allow managers in southwest Montana, and other regions in the northern Rocky Mountains, to better manage sage-grouse and sage-grouse habitats.

CHAPTER 1

INTRODUCTION TO THE DISSERTATION

Greater sage-grouse (*Centrocercus urophasianus*; hereafter: *sage-grouse*) have declined across their range from millions to only 200,000 individuals, and their current range is half of the historic extent (Schroeder et al. 2004). The sage-grouse is an umbrella species that needs large, intact tracts of sagebrush habitat and represents the needs of many other species found in the sagebrush biome of the American West (Rowland et al. 2006). Conservation efforts have rallied behind sage-grouse and their habitats, resulting in one of the largest coordinated conservation programs in the world. A ‘no listing’ decision was made for the sage-grouse under the Endangered Species Act (USFWS 2015) due to large-scale coordinated conservation efforts that addresses primary and secondary threats impacting the species and their sagebrush biome across the American West.

The sagebrush (*Artemisia* spp.) biome in the American West is one of the largest ecotypes in North America, yet it constitutes one of the most threatened major ecosystems in the United States (Noss et al. 1995). The biome has been reduced to half of its historical extent due to fragmentation and conversion for livestock forage, energy development, and urbanization (Knick et al. 2003). Across the biome, strong environmental gradients exist, driven largely by elevational-mediated soil temperature and moisture levels that are expressed by spatial changes in a multitude of sagebrush species and subspecies (hereafter: *sagebrush type*). Current conservation has, however, largely ignored the effect of sagebrush type, due to methodological limitations of identifying sagebrush type at large scales. In the Northern Rocky Mountains, distinct zones of sagebrush types exist. Given these differences, predator activity, abiotic and biotic characteristics, and grazing might differ by sagebrush type and influence the animal communities differently among sagebrush types. Understanding the contribution of sagebrush type to components of fitness in animals can help prioritize conservation in sagebrush types that contribute most to a species' population growth. Throughout my dissertation, I assess how the breeding ecology of sage-grouse is influenced by sagebrush type in various ways including on nest-site selection (Chapter 2) and brood-habitat selection (Chapter 5), nest survival (Chapter 2), and contribution of exogenous resources to sage-grouse chick formation (Chapter 3 and Chapter 4). Broad ecological differences occur among sagebrush types, including differences in plant species richness, vegetation structure and composition, and phenological differences in timing of green-up. The categorization of sagebrush type for conservation and management purposes also allows land managers to quickly understand variation in breeding strategy, habitat selection patterns, and nest survival.

Managers must make decisions regarding cattle grazing that involve stocking rates and spatial and temporal patterns of where and when to graze (Knick et al. 2003). Given how common livestock grazing is across the American West, I have integrated grazing effects into Chapters 2 and 5. I specifically focus on the effects of grazing infrastructure on nest selection, nest survival and habitat use during the brood-rearing period.

Grazing systems rely on intricate networks of linear and point features (hereafter: *LPF*; e.g., fences, water tanks), which have been shown to influence predator and prey populations (Dickie et al. 2016). Avian predators are more common in areas with increased LPFs (e.g., water tanks; Coates et al. 2016), and mortality from collisions with LPFs (e.g., fencelines) occurs frequently (Stevens et al. 2012, Van Lanen et al. 2017). Indirect effects of LPFs on animal behavior include avoidance and movement patterns through increased habitat edges, anthropogenic noises, and artificial light (Kociolek et al. 2011). Recent studies have revealed that LPFs also allow predators to travel farther over shorter times, which enhances their search efficiency and prey-handling time (Dickie et al. 2017, DeMars and Boutin 2017). However, impacts of LPFs related to grazing systems on behavioral cues and survival outcomes remains unknown, and are currently a research priority (Hovick et al. 2014). In arid landscapes such as the sagebrush biome, grazing infrastructure (e.g., fences and water tanks) strongly influences grazing pressure and utilization of plants as livestock are concentrated near water sources and are prohibited from freely ranging due to infrastructure (Bailey et al. 2015). In large grazing units that are limited by water, like those in my study region—some individual grazing units exceed 9,000 ha in the American West (Baily et al. 2015)—a low proportion of the area is utilized by cattle as cattle avoid traveling long distances from water (Bailey 2005). Thus, the distribution of water tanks and fences throughout large grazing units largely dictates the uniformity of grazing pressure, and subsequent utilization of plants, across the landscape. In the American West, agriculture has modified landscapes, yet little is known about whether agricultural changes alter the reliability of the cues animals use to identify habitat quality and selection habitats; which can ultimately form maladaptive breeding strategies where behavioral cues are mismatched with survival outcomes.

The following dissertation explores the variables that affect reproductive success for sage-grouse in the heterogeneous landscapes of the Northern Rocky Mountains, with particular focus on the effects of sage-brush type and grazing infrastructure. The dissertation was written as four standalone publications, presented as chapters here.

In Chapter 2, I evaluate sources of variation on nest-site-selection patterns that can influence survival of nests. Animals are expected to select habitats that maximize their fitness over evolutionary time scales. Yet in human-modified landscapes, habitat selection might not always lead to increased fitness because animals undervalue high-quality resources that appear less attractive than those of lower quality. Using the greater sage-grouse, a species highly dependent upon sagebrush landscapes, we (1) evaluated how females select nesting habitats based on sagebrush type, along with livestock grazing related linear and point features, and other biotic, abiotic characteristics, given hypothesized influences on hiding cover, microclimate and predator travel routes and perches; (2) compared habitat selection information with results for nest survival estimates to evaluate if selection appears to be adaptive or not; and (3) used our results to evaluate the most appropriate strategies for this species in a grazing-modified landscape.

In Chapter 3, I present a laboratory experiment on domesticated gallinaceous birds that I later use to apply to wild sage-grouse in Chapter 4. Before stable isotope techniques can be employed, researchers need reliable isotopic discrimination values. In this context, isotope discrimination compares the difference in the isotope ratio between the maternal-offspring tissue that occurs during nutrient transfer prior to egg laying. Currently, isotopic discrimination values are unknown between the maternal blood constituents—that reflect different temporal scales—and downy feathers of their offspring. Information on discrimination values can be used to assess dietary patterns of female birds during egg formation. In this study, I experimentally derive isotopic discrimination relationships between maternal diet-blood constituents for egg laying, and between maternal blood constituents-down feathers of offspring in an experiment with 3 types of domesticated Gallinaceous birds raised on known diets.

In Chapter 4, I evaluate sources of variation on nutrient allocation strategies for offspring production in female sage-grouse. I test whether nutrient allocation strategies for reproduction in sage-grouse differs with timing of breeding in different ecoregions: a high-elevation landscape, containing spatially complex vegetation (Rocky Mountains) versus a low-elevation, more homogenous landscape (Great Plains). I analyzed data collected from radio-telemetry and stable isotopes to assess the degree to which endogenous (body) reserves are used for reproduction and whether variation in allocation strategies was associated with time of year, ecoregion, habitat quality (including sagebrush type and plant greenness), or maternal characteristics.

In Chapter 5, I build a resource selection function to describe habitat selection patterns for sage-grouse females with broods. Studies from the Great Basin have consistently shown that sage-grouse with broods select mesic areas in sagebrush that contain green plants, but little information exists on whether selection is similar in mountainous landscapes in the Rocky Mountain ecoregion. Selection might be different in mountainous landscapes as upland rangelands provide mesic areas with more precipitation than what is available in the Great Basin. I used both broad- and fine-scale resource selection analyses to assess habitat selection patterns of sage-grouse broods in a high-elevation sagebrush landscape with strong gradients in abiotic and biotic conditions, along with a diverse network of grazing infrastructure.

In my concluding chapter (Chapter 6), I highlight key findings for each of my individual chapters. I then summarize broadly across all the chapters of the significance of this research. I end with suggestions of lines of future research that can build and expand upon my findings of the dissertation.

CHAPTER 2

MALADAPTIVE NEST-SITE SELECTION BY A SAGEBRUSH DEPENDENT SPECIES IN A GRAZING-MODIFIED LANDSCAPE

Contribution of Authors and Co-Authors

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Abstract

Animals are expected to select habitats that maximize their fitness over evolutionary time scales. Yet in human-modified landscapes, habitat selection might not always lead to increased fitness because animals undervalue high-quality resources that appear less attractive than those of lower quality. In the American West, agriculture has modified landscapes, yet little is known about whether agricultural changes alter the reliability of the cues animals use to identify habitat quality; ultimately forming maladaptive breeding strategies where behavioral cues are mismatched with survival outcomes.

Using the greater sage-grouse, a species highly dependent upon sagebrush landscapes, we (1) evaluated how females select nesting habitats based on sagebrush type, along with livestock grazing related linear and point features, and other biotic, abiotic characteristics, given hypothesized influences on hiding cover, microclimate and predator travel routes and perches, (2) compared habitat selection information with results for nest survival estimates to evaluate if selection appears to be adaptive or not, and (3) used our results to evaluate the most appropriate strategies for this species in a grazing-modified landscape. Nest-site selection for sagebrush type appears to be maladaptive: in the most-preferred sagebrush type, nest survival rate was one-fourth the rate realized by females nesting in the sagebrush type avoided. Nest survival was four times higher for nests placed away from (>100 m), rather than next to (1 m), the nearest fence, and survival was lower within sites with higher cow pie density (a proxy for previous grazing intensity). Live and dead grasses influenced selection and survival in opposing ways such that dead grass was selected for but resulted in reduced survival while live grass was avoided but resulted in increased survival. Results collectively provide the first empirical evidence that a

specific type of sagebrush acts as an ecological trap while another sagebrush type is undervalued. These results also suggest that adding more fences to control livestock grazing systems will likely reduce sage-grouse nest survival.

Introduction

Habitat selection evolved under natural selection, and its influence on subsequent fitness in avian taxa has been widely demonstrated (Clark and Shutler 1999). Many animals rely on environmental cues to assess a site's potential to maximize their fitness (Southwood 1977). These cues include tracking changes in food abundance, predators, and diverse environmental features. In dynamic landscapes, an animal's ability to track changes in resources can sometimes be inadequate to adapt to rapid environmental change (Hollander et al. 2017). In human-modified landscapes, numerous examples have been documented where animals select low-quality habitats even when high-quality ones exist in a scenario defined as an ecological trap (Robertson et al. 2013). Similarly, mechanisms that create ecological traps have corollaries that create undervalued resources; which in theory occur when high-quality resources appear less attractive than lower quality ones (Robertson and Chalfoun 2016). Yet, few empirical data exist that show how both forms of maladaptation govern adaptive selection across different habitat types (Robertson and Chalfoun 2016).

The sagebrush (*Artemisia* spp.) biome of the American West is one of the largest ecotypes in North America, yet it constitutes one of the twenty-two most imperiled major ecosystems in the United States (Noss et al. 1995). The biome has been reduced to half of its historical extent due to fragmentation and conversion for livestock forage, energy development, and urbanization (Knick et al. 2003). Across the biome, strong environmental gradients exist, driven largely by elevational-mediated soil temperature and moisture levels that are expressed by spatial changes in a multitude of sagebrush species and subspecies (hereafter: *sagebrush type*). Current conservation has largely ignored the effect of sagebrush type, largely due to methodological limitations of identifying sagebrush type at large scales. In the northern Rocky Mountains, distinct zonations of sagebrush type exist. Given these differences, predator activity, abiotic and biotic characteristics, and grazing might ultimately influence the animal communities differently across these sagebrush types. Understanding the contribution of different sagebrush types to components of fitness in animals can help prioritize conservation to those sagebrush types that contribute most to their population growth.

The greater sage-grouse (*Centrocercus urophasianus*; hereafter: *sage-grouse*) is an umbrella species that needs large tracts of habitat which is also dependent upon by many other species found in the sagebrush biome of the American West (Rowland et al. 2006). Sage-grouse have declined across their range from millions to now only 200,000 individuals, and their current range is half of the historic extent (Schroeder et al. 2004). Sage-grouse reproductive success is highly dependent on the structure and function of sagebrush habitats including relationships with shrub, grass, and forb thresholds (Connelly et al. 2000). Non-persistent stressors affecting sage-grouse populations include anthropogenic structures (Hovick et al. 2014).

Cattle grazing is the most common land-use practice in the American West (Knick et al. 2003). Grazing systems rely on intricate networks of linear and point features (hereafter: *LPF*; e.g., fences, water tanks), which have been shown to influence predator and prey populations (Dickie et al. 2016). Avian predators are more common in areas with increased LPFs (e.g., water tanks; Coates et al. 2016), and mortality from collisions with LPFs (e.g., fencelines) occurs frequently (Stevens et al. 2012, Van Lanen et al. 2017). Indirect effects of LPFs on animal behavior include avoidance and movement patterns through increased habitat edges, anthropogenic noises, and artificial light (Kociolek et al. 2011). Recent studies have revealed that LPFs also allow predators to travel farther over shorter times, which enhances their search efficiency and prey-handling time (Dickie et al. 2017, DeMars and Boutin 2017). However, impacts of LPFs related to grazing systems on behavioral cues and survival outcomes remains unknown, and are currently a research priority (Hovick et al. 2014).

Using the sage-grouse as our model species, we examined data on nesting ecology in a grazing-dominated landscape located in the northern Rocky Mountain region to (1) identify how they select nest sites based on sagebrush types that might result in maladaptive selection and (2) to evaluate whether a variety of suspected characteristics related to grazing LPFs and other biotic and abiotic characteristics might influence possible mismatches between selection cues and survival outcomes. We hypothesized that adaptive selection would be strongly influenced by sagebrush type driven by large differences in hiding cover, microclimate and/or predator travel routes and perches. We further hypothesized that reduced nest survival outcomes would be associated with grazing infrastructure given suspected predator use of infrastructure features for travel corridors and/or perches that increase their search efficiency and kill rates.

Materials and Methods

Study Area

We conducted the study on a 65,000-ha mountainous landscape in southwest Montana, USA, with the Greater Yellowstone ecosystem to the east and High Divide ecosystem to the west. The region is considered biologically diverse and supports a multitude of sagebrush types along with several breeding leks for sage-grouse as well as other charismatic megafauna including grizzly bears (*Ursus arctos horribilis*) and wolves (*Canis lupus*). Dominant stands of sagebrush types include: mountain big sagebrush (*Artemisia tridentata* ssp. *vaseyana*; hereafter: *MTSA*), basin big sagebrush (*Artemisia tridentata* ssp. *tridentata*; hereafter: *BBSA*), three-tip sagebrush (*Artemisia tripartita*; hereafter: *TTSA*) and low sagebrush (*Artemisia arbuscula*). Basin big sagebrush occurs in valley bottoms with deep soils, TTSA occurs at mid-elevation slopes, while MTSA occurs at the highest elevations. Low sagebrush was excluded from further consideration because birds did not use this sagebrush type for nesting. The dominant land-use practice includes livestock grazing with virtually all of the landscape receiving recent grazing activity. In most years, cattle begin grazing in low and basin big sagebrush during late-May and progress to higher elevation stands of TTSA and MTSA following the advancement of vegetation phenology. Most of the study area is on a rest-rotation grazing system, where a particular pasture is grazed for two-to-three years followed by a year of rest. Average stocking rate varies 1.2 to 2.0 ha per adult cow (weighting 454 kg; Ryan Martin, *personal communication*). An intricate network of grazing related LPFs that supports this agro-pastoral landscape includes fences, water tanks, paths, and roads.

Female Capture, Measurement, and Marking

We captured adult female sage-grouse across ten breeding leks using spotlights and dip nets from 2014 to 2016 (2014: $n = 51$, 2015: $n = 55$, and 2016: $n = 57$). We marked each female with either a 22-g (Advanced Telemetry System, Isanti, MN) or 12-g transmitter (American Wildlife Enterprises, Monticello, FL) necklace-style transmitter. Both transmitter types are <5% of the body weight; a threshold believed to have minimal impacts on flying animals (Cochran

1980). This research was conducted in compliance with state (2014-050, 2015-23, 2016-049), and animal care/use (2012-40, 2015-30) permits.

Bird Tracking and Nest Monitoring

We tracked prenesting females 2-3 times per week from mid-April until mid-July during the three years of study. After we discovered a nest, we monitored its survival 2 times per week until it successfully hatched ≥1 egg (as determined by the presence of a chick in the nest bowl, or evidence of an intact egg membrane that indicated hatching had occurred) or was destroyed by a predator. We assessed whether a nest was still active by using radio-telemetry from established points and bearings from an established location; usually 50-100 m away. We censored five nests from analysis of abandoned nests because the abandonments were believed to have been caused by observer disturbance. No failures were linked to cattle trampling. The community of nest predators includes coyotes (*Canis latrans*), ravens (*Corvus corax*), badgers (*Taxidea taxus jacksoni*), wolves and grizzly bears, with the first three species suspected of being responsible for most nest losses (*Kyle Cutting*, personal observation).

Sagebrush Type

We visually mapped four sagebrush types across the study area through detailed field mapping surveys of continuous patches >0.25 ha that were dominated (>50% cover) by a given sagebrush type. We assessed the accuracy of these visual surveys to the manual surveys described below and found high assignment accuracy (95.6% accuracy).

Linear and Point Feature Variables

We used Google Earth (image date: 19 July, 2014) to quantify distances from nest and random points to the nearest path. We could not differentiate between paths of cattle and those of wild ungulates, but based on our experience in the study area we think most paths were predominantly created by cattle given evidence of their tracks and feces, as well as most paths led to water tanks, fences, and roads used by livestock producers. Wild ungulates (elk [*Cervus elaphus*] and mule deer [*Odocoileus hemionus*]) also help maintain the network of paths. We compiled information on water tanks, roads and fences using existing data from our partners on grazing infrastructure for the study area. Roads are also utilized by recreationalists pursuing hunting and motorized use and are generally considered to have minimal to light traffic throughout the year. We confirmed, and modified when necessary, the relevant existing data layers based on results of intensive field surveys. We used ArcGIS10.5.1 (ESRI, Redlands, California, USA) to measure distances from nests and random points to LPFs.

Biotic Variables

We measured vegetation characteristics (i.e., live grass, dead grass, forb, and shrub) at nests at fate (either successful or predated) and at random locations throughout the nesting season. To assess selection patterns for nest sites, we stratified the number of random survey points based on the availability of a particular sagebrush type as determined by our visual mapping surveys using GIS. We conducted vegetation surveys at an average of 2.4 random points to every nest; with the number of random points varying based on the availability of a given sagebrush type (MTSA = 3.1, TTSA = 2.1, and BBSA = 1.3). We collected biotic plant data at 1-m intervals along three 30-m transects equally spaced at 120^{o} using a modified version of the line-point intercept method described by Herrick et al. (2017). We expected plant cover to vary according to sagebrush type with BBSA having the lowest, whereas MTSA having the highest. Therefore, we collected cover data on a vertical plane (height and number of times it crossed the plane) of up to four plant species at each sampling point, resulting in plant cover estimates exceeding 100%. We limited the number of species to four since this was generally the maximum number of different species that intersected the vertical plane across the various sagebrush types. If the number of layer 'hits' was still <4, we then recorded the type of ground surface present (e.g., litter, bare ground, rock, moss). At each stop along the vegetation transects, we dropped a pin flag and measured the height and cover of multiple vegetation layers along this vertical plane. We recorded different plant species that touched this plane beginning at the highest layer and proceeding to the lowest layer. The only time we recorded the same species was when it occurred as both dead and alive. This approach allowed us to characterize plant coverage estimates that exceed >100%. Accumulated cover estimates best characterize the known differences in complexity of the plant community across the various sagebrush types; given the lower layers provide additional concealment from predators. We centered each transect either over a nest bowl, or for the case of transects associated with random points, a randomly chosen nesting shrub >30 cm in height. Given the influence of vegetation cover on nest survival is dependent on the stage of the growing season, we controlled for the effect of growing season phenology on grass cover by following guidelines described in Gibson et al. (2016). As a proxy for previous grazing intensity, we recorded density of cow defecation piles within 1-m of either side of each transect.

Abiotic Variables

Terrain with a steep southwest facing aspect has the highest heat loading while terrain with steep northeast facing aspects has the lowest. Because snowpack is deeper and persists longer on north facing aspects, we predicted that heat loading would positively influence nest-site selection behavior given how early sage-grouse initiate nests in the spring, but would have marginal effects on nest survival (Gregg et al. 1994, Sveum et al. 1998, and Doherty el al. 2014). We calculated heat loading using the Geomorphic and Gradients toolbox (Evans et al. 2014) in ArcGIS. Weather patterns varied across years of study during the month of March, which is when lekking and prenesting behavior begins. Conditions in March of 2015 were, when compared to other years of study, warmer (average temperature: 2014 = -2.9 ^{o}C, 2015 = 0.4 ^{o}C, and 2016 = -2.1 ^{o}C) and drier (average precipitation: 2014 = 59.2 mm, 2015 = 8.3 mm, and 2016 = 58.2 mm; PRISM Climate Group 2018), and snowmelt occurred nearly 2 weeks earlier (2014 = 4 May, 2015 = 22 March, and 2016 = 23 April; Lakeview Ridge - 44° 35' 00" N, 111° 49' 00" W, 2350 m a.s.l., https://wcc.sc.egov.usda.gov/nwcc/site?sitenum=568).

Statistical Analyses

We conducted two separate analyses directed at assessing whether selection behavior was adaptive across three sagebrush types and by considering variables from biotic, abiotic, and grazing related model suites. Response variables for both nest-site selection (used vs. available sites) and nest survival (survived vs. predated) were binary and evaluated using appropriate methods for such response variables (see below) to evaluate competing models. To choose among competing models, we employed an automated model-selection procedure to evaluate the variables that most influence selection and survival within our study system from across suites.

Selection Analysis

To assess selection for particular sagebrush types, we used the Manly resource selection ratio (selection ratio = used/availability) as a measure for preference/avoidance patterns at the population level. A selection ratio >1 indicates preference of a certain sagebrush type relative to its availability on the landscape, and a ratio <1 indicates avoidance. We used the *adehabitat* package to assess nest-site selection (Calenge 2006) in the R programming language (R Core Development Team 2018).

Before conducting our analyses, we evaluated pairwise correlations and variance inflation factors (VIF) to assess possible collinearity issues in our covariates but did not find evidence of high multicollinearity (Pearson's $r \leq 0.7$, VIF <4). We hypothesized that the presence of grazing-related LPFs, cattle paths and road that provided predator travel corridors, and grazing infrastructure that provided perches for avian predator might be expected to negatively affect nests that were closer to such features and that nests placed in more topographically variable settings might be more obscured from predators. We therefore transformed all LPF variables to the natural logarithm to account for potential threshold effects. We used an exponential logistic resource selection function (RSF) to compare variables at nest sites with those at available sites using 99 bootstrap iterations. The RSF approach utilizes a used-available framework from a weighted distribution to generate a maximum likelihood estimate of the probability of use for each variable. We used the R package *Resource Selection* (Lele et al. 2017) to assess nest-site selection of variables spanning the three model suites (Table 2-1). We tested the fit of selection models to the data by applying the Hosmer and Lemeshow goodness-of-fit test using the *Resource Selection* package in R.

Nest Survival Analysis

We evaluated competing models of daily nest survival rates (hereafter: *DSR*) that allowed DSR to be a function of various combinations of variables from the LPF, biotic, and abiotic suites using generalized linear models with binomial distributed errors and a logit link (Dinsmore et al. 2002). We calculated nest survival rate (hereafter: *NSR*) as the probability that a nest with given covariate conditions would survive 38 days from the start of egg-laying through incubation. We used the delta method to calculate standard errors (Powell 2007) on estimated DSR and NSR. We also evaluated whether survival decreased across the nesting season, and whether possible temporal patterns in DSR across sagebrush types might have corresponded with differences in vegetation phenology and snowmelt between sagebrush types at low versus high elevations. We did so by using two models: the first contained an additive term between nest initiation date and sagebrush type, and the second contained an interaction term between the same variables. We used Program MARK (White and Burnham 1999) through the *RMark* package (Laake 2013) and its functions for estimating daily nest survival (Dinsmore et al. 2002).

We ran all possible additive combinations of covariates to describe selection and DSR using the *MuMIn* package (Bartoń 2017) in R. We assessed relative model support in two ways: (1) we considered the variables included in the best-supported model, i.e., the model with the lowest AIC_c score (Burnham and Anderson 2002) and evaluated the magnitude of each estimated coefficient and the extent to which each coefficients in the model overlapped zero, and (2) we implemented model averaging across all models that were within 4 AIC_c units of the top model and evaluated features of those coefficients as we did for the top model. Model averaging of uninformative variables across a set of models increases parameter uncertainty and is of questionable utility when variables across a set of models have low variation in the direction and effect size (Cade 2015). We found high agreement in both direction and magnitude of effect sizes from the two interpretation techniques for both the selection and survival analyses. Thus, for our specific case reported on here, we avoid redundancy by basing our inferences on just those coefficients found in the top model. To assess if the covariates in the most supported nest survival model varied across sagebrush type, we conducted an exploratory analysis by testing interaction effects between sagebrush type and each continuous variable and found no support for interactive effects.

Results

From 2014 to 2016, we monitored a total of 38 nests in BBSA, 69 nests in MTSA, and 47 nests in TTSA (Table 2-1). To assess selection patterns for nest sites, we collected data from a total of 365 random points across the three sagebrush types (BBSA: *n*=51, MTSA: *n*= 214, and TTSA: *n*=100). A total of 56 nests hatched at least one egg. All nest failures in the analyses were caused by nest predation.

Nest Site Selection

Female birds strongly selected stands of BBSA (7.5% of the study area), avoided stands of MTSA (63.5% of the study area) and exhibited no selection for TTSA (25.3% of the study area; Manly resource selection (mean ± 95% CI): BBSA = 3.29 ± 0.90, MTSA = 0.71 ± 0.12, TTSA = 1.21 ± 0.29). We did not find any females nesting in low sagebrush, which represents 3.7% of the study area.

A total of 35 models describing selection patterns were well-supported by the data (see subset of models on Table 2-2). We did not find evidence of lack of fit problems with our most general model (P = 0.10, Hosmer and Lemeshow goodness-of-fit test, Table 2-2). Estimates from the top model suggest females selected nest sites with less alive grass ($\hat{\beta} = -0.013$, SE = 0.005) and forb cover ($\hat{\beta}$ = -0.013, SE = 0.003), greater amounts of dead grass height ($\hat{\beta}$ = 0.001, SE = 0.0004), higher amounts of heat loading ($\hat{\beta}$ = 1.782, SE = 0.862; Figure 2-1), and greater selection in year 2016 ($\hat{\beta}$ = 0.570, SE = 0.240). Selection of nest sites was related to grazing LPFs in two divergent ways: birds tended to avoid cattle paths ($\hat{\beta}$ = 0.195, SE = 0.058) but tended to select nest sites that were closer to roads than expected based on the nature of available sites ($\hat{\beta}$ = -0.199, SE = 0.067; Figure 2-1).

Nest Survival

A total of 45 nest survival models were well-supported by the data (see subset of models on Table 2-2). Based on the average of the annual estimates for each sagebrush type from the top model, 38-day NSR is estimated as 0.083 (SE = 0.041), 0.311 (SE = 0.063), and 0.169 (SE = 0.058) in BBSA, MTSA, and TTSA, respectively.

Daily survival rate was higher for nests farther from fences ($\hat{\beta}$ = 0.156, SE = 0.069). For example, in 2014, in TTSA, DSR ± 1SE (NS ± 1SE) changed from 0.937± 0.022 (NS = 0.085 ± 0.077) to 0.974 ± 0.009 (NS = 0.365 ± 0.133), respectively, as covariate distance to fence went from its 2.5% quantile to its 97.5% quantile value in the observed dataset (Figure 2-3). In the top model, DSR was negatively associated with cow pie density ($\hat{\beta}$ = -0.198, SE = 0.099; Figure 2-3). As cow pie density ranged from the 2.5% quantile to its 97.5% quantile value in the observed dataset, DSR changed from 0.972 ± 0.009 (NS = 0.342 ± 0.132) to 0.942 ± 0.019 (NS = 0.104 ± 0.082), respectively. Nests with greater amounts of dead grass had lower DSR than those with lower amounts of dead grass ($\hat{\beta}$ = -0.001, SE = 0.0005; Figure 2-3). When ranged from 2.5% to 97.5% quantile in the observed dataset, influence of dead grass height on DSR decreased from 0.973 ± 0.008 (NS = 0.367 ± 0.118) to 0.935 ± 0.028 (NS = 0.079 ± 0.090), respectively. Daily survival rate was higher for nests with greater amounts of grass cover ($\hat{\beta}$ = 0.010, SE = 0.004; Figure 2-3). For example, in 2014, in TTSA, DSR changed from 0.942 ± 0.019 (NS = 0.104 ± 0.083) to 0.982 ± 0.007 (NS = 0.495 ± 0.150), respectively, as covariate grass cover went from its 2.5% quantile to its 97.5% quantile value in the observed dataset (Figure 2-3).

We found a common pattern that shows DSR increasing with increasing initiation date; regardless of what sagebrush type the nest was located in. For nests initiated on the same date, DSR was predicted to be highest in stands of MTSA intermediate in TTSA, and lowest in BBSA.

Discussion

Adaptive selection, where fitness is higher in preferred habitats, has been widely documented in animals (Chalfoun & Schmidt 2012, Clark & Shutler 1999). However, in human-modified landscapes, habitat selection can be maladaptive due to anthropogenic changes on the landscape. In our grazing-modified system, we observed a decoupling between selection behavior for nest sites and subsequent survival outcomes. Sage-grouse preferred stands of BBSA for nesting, yet overall nest survival in BBSA was one-fourth the rate for birds nesting in MTSA. We also found little support for congruence between factors associated with habitat selection and survival outcomes. Instead, we found support for indirect effects of grazing-related linear (fences) and point (cow pies) features that were associates with decreases nest survival in sage-grouse, a novel result for the species. Lastly, after controlling for grass phenology of live grasses, we found that live and dead grass had different relationships with nest survival. Collectively, these results have grazing implications, especially for public lands, where managers are trying to balance the need to provide high-quality wildlife habitat while simultaneously providing grazing opportunities to private livestock producers.

Factors Influencing Nest Site Selection

Our results indicate that sage-grouse prefer low-elevation sites for nesting. Such sites typically contain less snow and have advanced vegetation phenology relative to what is found in higher-elevation sagebrush types. Such differences are notable for sage-grouse on our study area because of how early in the year they start nesting relative to when snowmelt occurs. To our knowledge, this is the first study to document selection differences between sagebrush types. Our study site receives higher amounts of snowfall than other sagebrush regions in the American West. Thus, high-elevation sagebrush can hold snow later into the spring than sagebrush at lower elevations, resulting in delayed vegetation phenology. Females also tended to select nest sites in areas with greater amounts of heat, such as south facing aspects, which suggests that females might be attempting to reduce energy expenditure during times of low ambient temperatures. Interestingly, renesting rates were similar across elevations and birds did not switch sagebrush types between renesting attempts. These results collectively support our initial hypothesis that selection of nest sites would be favored in low rather than high elevations, and areas with greater amounts of heat.

The influence of herbaceous vegetation on sage-grouse nesting behavior and survival has received considerable attention, with most focusing on the height of dead grass (Connelly et al. 2004). In agreement with previous studies, we found that sage-grouse preferred nest sites with greater amounts of dead grass (Gregg et al. 1994, Sveum et al. 1998, Gibson et al. 2016). Grazing by domestic livestock during the growing season, in addition to compaction by deep snow during winter, can reduce the amount of standing vegetation the following spring, which leads to increased detection by predators. Although sage-grouse selected sites with higher amounts of dead grass for nesting, they also selected nest sites with lower grass cover from the current year's growth, supporting the hypothesis that females may be trying to balance tradeoffs between selecting sites that allow them to hide from predators while seeking sites that maximize localized movement and foraging efficiency for their precocial offspring (Gibson et al. 2017). Selection pressure was also greatest in 2016, a year with intermediate amounts of precipitation and temperature. Given temperature is expected to increase with climate warming, inter-annual variability in precipitation and other environmental factors may constrain to some degree the ability of sage-grouse to adapt to climate warming.

Sage-grouse avoid human structures (Hovick et al. 2014), yet no study has assessed LPF of grazing infrastructure on the birds' selection responses. Our selection analyses revealed that sage-grouse placed nests, on average, closer to roads and farther from cattle paths. Construction of primitive roads, which are largely found across our study site, oftentimes create edges that are angled towards the sun which increases the amount of heat at the ground surface during the nesting season. Given that cattle paths were the most ubiquitous LPF across the landscape, we suspect that predators might use cattle paths to navigate dense stands of sagebrush and thereby increase their kill rates and cause females to nest farther from paths. Dickie et al. (2017) found that wolves increased movement rates by selecting trails, and Atwood et al. (2004) reported that coyotes in rural landscapes selected linear habitat features. It would be useful if future research could assess path use by predators in sagebrush systems similar to ours.

Factors Influencing Nest Survival

Our modeling results reveal differences in the contribution of sagebrush type to nest survival. Nests in BBSA had low survival rates (0.08 across our study), which was one-fourth the rate of nests in MTSA, and one of the lowest values ever reported for sage-grouse (Taylor et al. 2012). Growth form of BBSA is columnar (tree-like), with elongated trunks and branches well-above the ground surface, which increases sightability of ground-dwelling predators (Gregg et al. 1994). Basin big sagebrush also grows in valley bottoms where both snowmelt and onset of vegetative growth occur earlier in the spring compared to what is found in other sagebrush types (Barker & McKell 1983), which likely causes predators to cue into these areas (Rose and Polis 1998, Koehler & Hornocker 1991). Nesting birds are forced to make the tradeoff between (a) nesting in snow-free areas with green vegetation but increased predation risk or (b) nesting in higher elevation areas that have delayed snowmelt and vegetation phenology but lower predation risk.

Nest survival across all the sagebrush types was lowest in 2015, a year when March temperatures were 2.5^{o}C warmer, and eight-times drier than in other years of our study. During 2015, the nesting season began 9 days earlier than in other years, which led to greater exposure of nests to predators because both nests and predators occur on a small portion of the overall landscape due to the distribution of snow, phenological stage of plants, and alternate foods, as demonstrated by the strong selection for low-elevation BBSA and avoidance of high-elevation MTSA. These results corroborate findings on sage-grouse from the Great Basin where reproductive success was reduced during years of warmer and drier conditions (Blomberg et al. 2013).

Assessments of indirect effects of LPFs related to grazing systems on both animal behavior and survival are rare (Hovick et al. 2014), even though they could be important for conservation planning if they exist. Indirect effects of LPFs on survival in animals might be even more severe than direct effects (e.g., fence collisions) because predators disproportionally use edge habitats, likely increasing interactions between predators and prey (Murcia 1995). Even though fences did not influence selection patterns of female sage-grouse, we still found that females that nested closer to fences had lower rates of nest survival than those that nested farther away. Similarly, we also found that nest survival was negatively related to density of cow defecation piles near nest sites. Even though our study site historically evolved with grazing by wild bison, spatial patterns of bison are highly variable across years due to their nomadic behavior. In contrast, grazing by domestic livestock occurs more frequently and usually under more consistent stocking rates with more consistent timing, resulting in different forms of grazing pressure between wild versus domestic grazers. Given fences are utilized as a critical component of livestock grazing operations, modifications could occur to reduce perches for avian predators and to reduce edge effects that would increase predator movements through fences by increasing the height of the bottom strand of wire.

Live and dead grass amounts are of great interest to land managers given its importance to grazing management and wildlife conservation. Surprisingly, we found opposing effects of dead versus live grass on nest survival. Our results suggest a decrease in nest survival with increasing amounts of dead grass. After controlling for plant phenology, we found that grass cover from the current year’s growth had a strong positive effect on nest survival. Variation in vegetation structure can greatly affect the distribution and detection probability of prey, which in turn can influence search and encounter efficiency by predators (Gorini et al. 2012). For instance, predators may be able to search most of the heterogeneous patches of dead grass within the sagebrush during the early season. In contrast, after the onset of the growing season when new grass is relatively more abundant, search and encounter efficiency by predators would be reduced. Future studies could assess the interactions between live versus dead grass on search efficiency and kill rates of predator communities across the growing season and sagebrush type.

Conclusions

We found that increasing live grass cover had the strongest, positive, association with nest survival as NSR varied by 39% across the range of grass cover measurements recorded. Other covariates had less but still measurable impacts on NSR which varied by 23-29% across the range of values recorded for variables including distance to fence, cow pie density, and dead grass, along with sagebrush type. It is important to note that the work reported here is focused only on the nesting aspect of the species' life history, when females appear to be making inappropriate decisions that could be leading to an ecological trap, especially in BBSA. Sage-grouse could compensate for the low observed nest survival in BBSA through increases in other vital rates such as chick survival. However, such compensation was not found in a previous study that found that chick survival was greater in high elevations (Gibson et al. 2017). Further, we found that adult survival during nesting was similar among sagebrush types, which suggests that females that nested in areas with lower nest survival rates were unable to compensate with increased adult survival. Basin big sagebrush could still be buffering against population stochasticity especially during winters of deep snowpack when other sagebrush types would be otherwise covered by snow forcing sage-grouse to migrate. Even though we did not find any birds nesting in low sagebrush, likely due to low stature and high amounts of bare ground, this sagebrush type is still important for lekking and for staging areas for bird’s en route to wintering quarters. Because agro-pastoral systems rely on grazing infrastructure to effectively implement targeted grazing practices, grazing systems that require the fewest amount of fences would be more desirable for use in sage-grouse nesting habitats. If this is not possible, we also speculate that (a) placing anti-perching devices on the top of fence posts and (b) increasing the height of the bottom strand of wire to reduce edge effects that are utilized by predators in search of nests in areas with fences. We are currently evaluating this idea by conducting an experiment in an area that supports high densities of nesting sage-grouse. Practitioners should seek to preserve and reduce threats in stands of MTSA, maintain cover of live grass in areas important for nesting, and modify fences to reduce search efficiency by predators.

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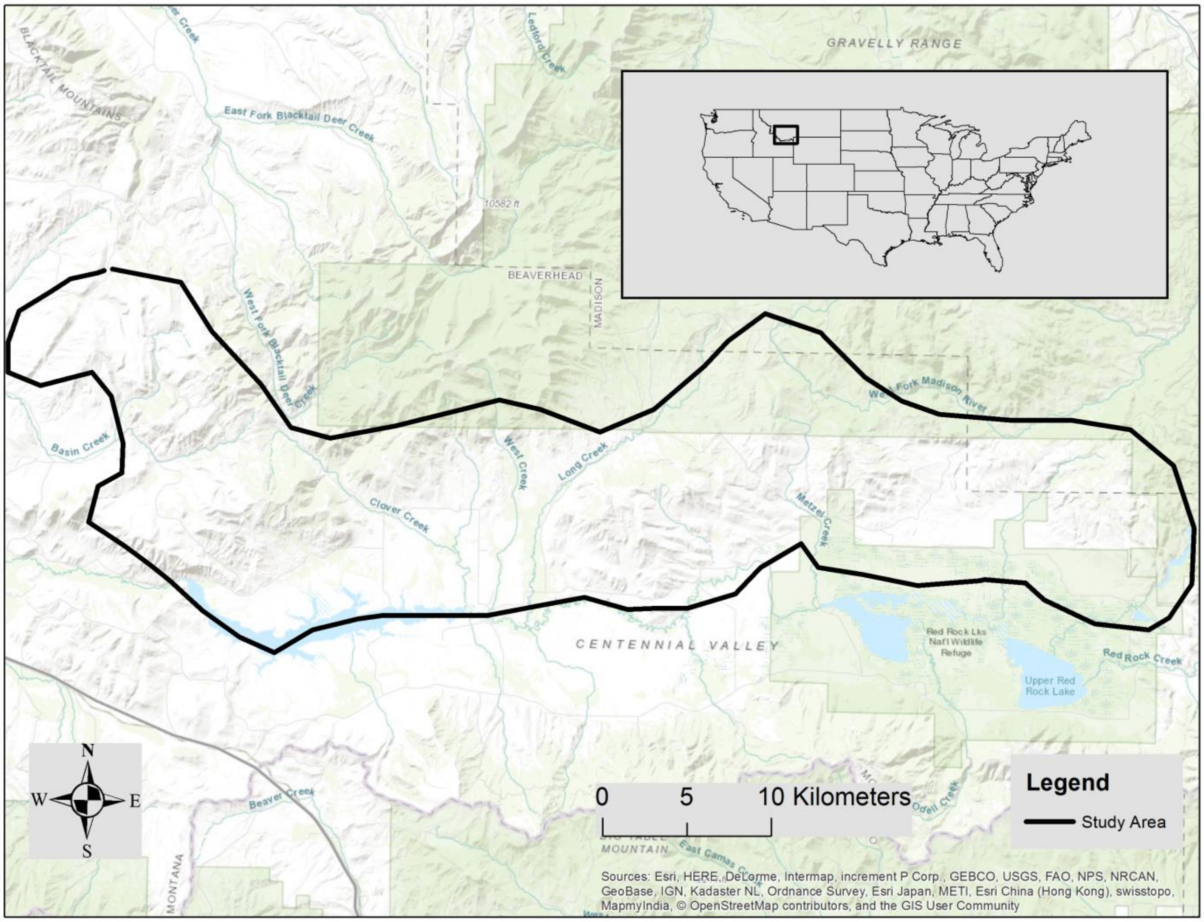
Figure 2-1. Map of the study area location in southwest Montana, USA.



Sources: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

Figure 2-2. Bean plots showing the distributions of parameters described in top model selection of nest sites and available sites. Shaded regions of the bean show density plots of the raw data. Mean values are indicated by white bolded lines.

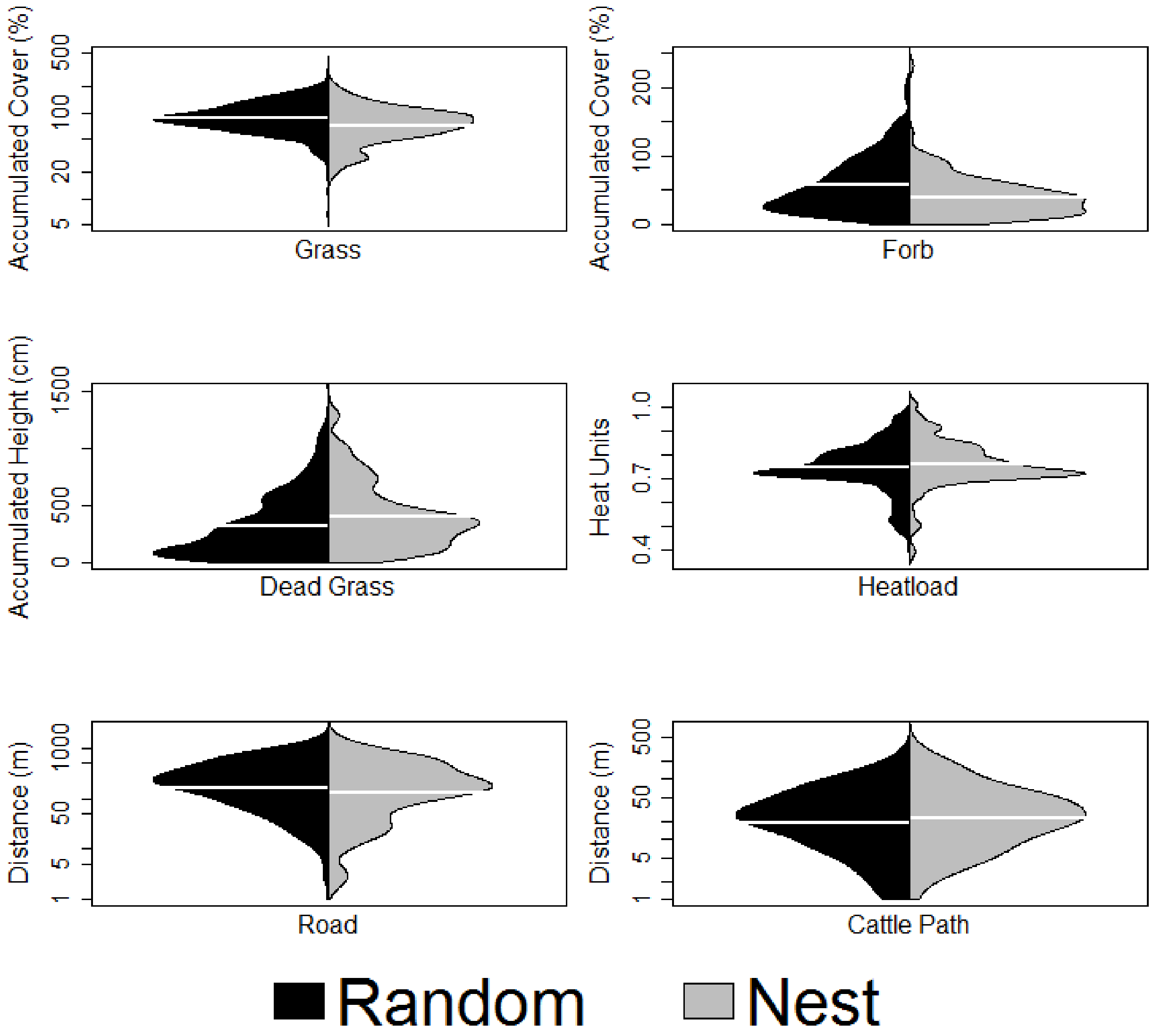


Figure 2-3. Predicted daily survival rates for sage-grouse nests. Continuous covariates (distance to fence, cow pie density, dead grass height, and grass cover) were held at their mean values. Shaded region represents 95% confidence interval, whereas solid line shows mean effect. Relationship between daily survival rate and sagebrush type were similar for all continuous variables. The range of x-values goes from the 2.5% to 97.5% quantiles of observed values in the data used in the analysis.

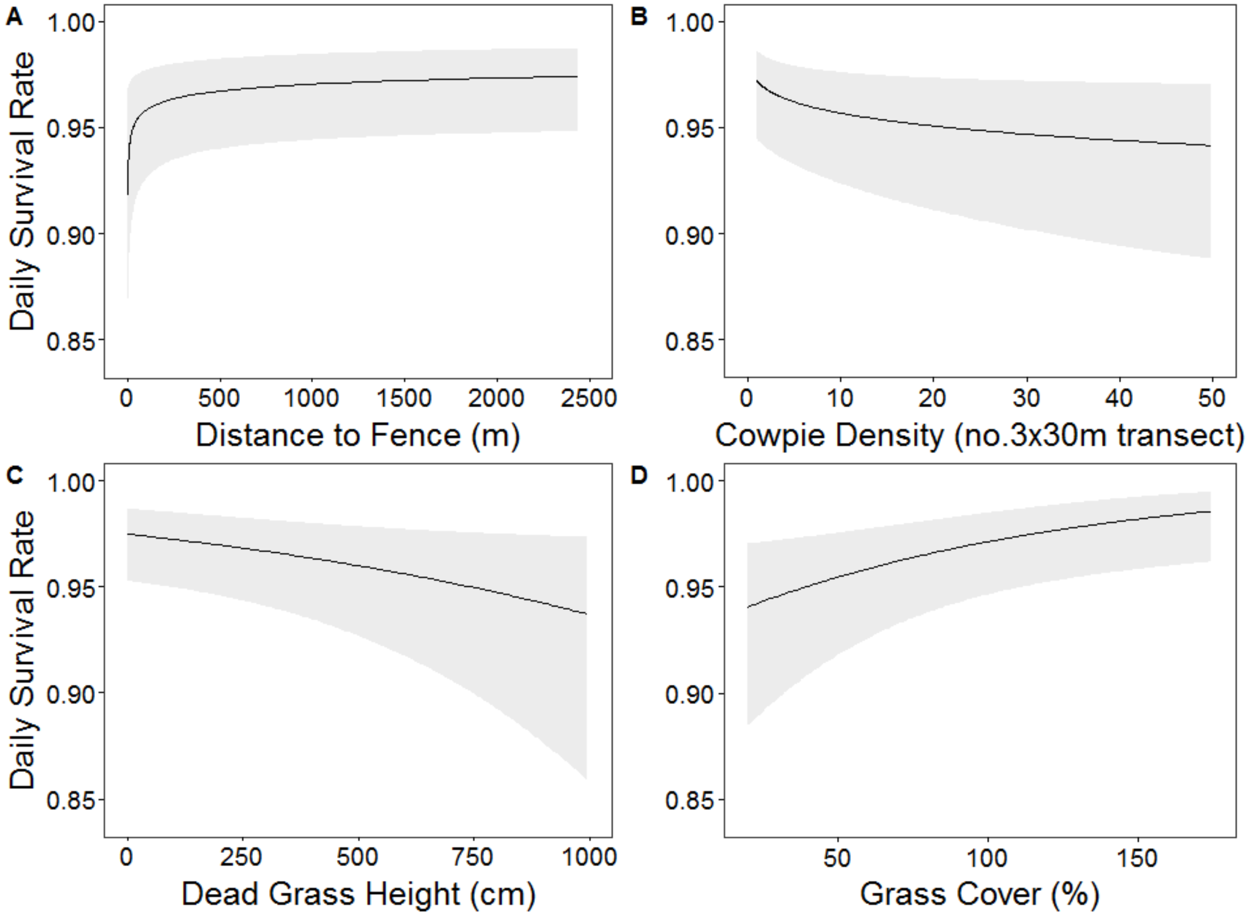


Table 2-1. Covariates used in the nest site selection and survival analyses. Each variable is categorized according to our main hypotheses.

Category	**Variable**	**Description**	**Type**
Biotic	Grass Cover	Accumulated cover of grasses	3x30m transect
Biotic	Dead Grass Height	Accumulated height of dead grass	3x30m transect
Biotic	Forb Cover	Accumulated cover of forbs	3x30m transect
Biotic	Shrub Cover	Accumulated percent cover of shrubs	3x30m transect
Biotic	Year	Annual effects	Factor
Biotic	Sagebrush Type	Dominant shrub type	Factor
Abiotic	Heat Load*	Combines aspect and slope	GIS 30m^2
Linear and Point	Road	Natural logarithm of distance to road	GIS
Linear and Point	Path	Natural logarithm of distance to path	Google Earth
Linear and Point	Fence	Natural logarithm of distance to fence	GIS
Linear and Point	Water tank	Natural logarithm of distance to water tank	GIS
Linear and Point	Cow pie	Natural logarithm of cow pie density	3x30m transect

* Heat Load (Evans et al. 2014)

Table 2-2. Candidate models, AICc, ΔAICc, Akaike weight, log-likelihood (LL) and number of parameters (K) for nest site selection (A) and survival (B) of female sage-grouse in southwest Montana, 2014-2016. All models within 2 AICc units from the model with lowest AICc value are shown.

(A) Selection

Model	**AIC$_c$**	**ΔAIC$_c$**	**AIC$_c$ weights**	**Model Likelihood**	**K**
Year + forbs + grass cover + dead grass + road + path + heat load	1750.01	0.00	0.19	-866.51	8
Year + forbs + grass cover + dead grass + road + path + heat load + shrubs	1750.14	0.13	0.18	-865.45	9
Year + forbs + grass cover + dead grass + road + path + heat load + water tank	1750.91	0.90	0.12	-865.84	9
Year + forbs + grass cover + dead grass + road + path + heat load + shrubs + water tank	1751.06	1.04	0.11	-864.77	10
Year + forbs + grass cover + dead grass + road + path + shrubs	1751.38	1.37	0.09	-867.20	8
Year + forbs + grass cover + dead grass + road + path + heat load + cow pie	1751.45	1.43	0.09	-866.10	9
Year + forbs + grass cover + dead grass + road + path + heat load + shrubs + cow pie	1751.83	1.82	0.08	-865.15	10
Year + forbs + grass cover + dead grass + road + path + heat load + fence	1751.87	1.86	0.07	-866.32	9
Year + forbs + grass cover + dead grass + road + path	1751.96	1.95	0.07	-868.60	7

(B) Survival

Model	**AIC$_c$**	**ΔAIC$_c$**	**AIC$_c$ weights**	**Model Likelihood**	**K**
Sagebrush type + year + fence + grass cover + dead grass + cow pie	672.92	0.00	0.24	-327.42	9
Sagebrush type + year + fence + grass cover + dead grass + cow pie + heat load	673.64	0.72	0.17	-326.77	10
Sagebrush type + year + fence + grass cover + dead grass + cow pie + water tank	674.38	1.47	0.12	-327.15	10
Sagebrush type + year + fence + grass cover + dead grass + cow pie + shrub	674.52	1.61	0.11	-327.22	10
Sagebrush type + year + fence + grass cover + dead grass + cow pie + forb	674.78	1.86	0.10	-327.34	10
Sagebrush type + year + fence + grass cover + dead grass + cow pie + road	674.85	1.93	0.09	-327.38	10
Sagebrush type + year + fence + grass cover + dead grass + cow pie + path	674.88	1.96	0.09	-327.39	10
Sagebrush type + year + fence + grass cover + dead grass	674.90	1.99	0.09	-329.42	8

CHAPTER 3

NUTRIENT SOURCES FOR OFFSPRING FORMATION: DIET-MOTHER AND MOTHER-OFFSPRING ISOTOPIC DISCRIMINATION IN DOMESTICATED GALLINACEOUS BIRDS

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

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Contributions: Conceived idea. Analyzed the data and wrote the paper.

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Contributions: Outlined and wrote the paper.

Co-Author: James A. Waxe

Contributions: Conceived idea, performed the experiment, and provided critical inputs on the paper.

Co-Author: Erika Nunlist

Contributions: Conceived idea, performed the experiment, and provided critical inputs on the paper.

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Contributions: Conceived idea. Provided critical inputs on the paper.

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Isotopes in Environmental and Health Studies

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Abstract

Stable isotope techniques can be used to assess nutrient acquisition and allocation strategies that females use to produce offspring. Before stable isotope techniques can be employed, researchers need reliable isotopic discrimination values. In this context, isotope discrimination compares the difference in the isotope ratio between the maternal-offspring tissue that occurs during nutrient transfer prior to egg laying. Currently, isotopic discrimination values are unknown between the maternal blood constituents—that reflect different temporal scales—and downy feathers of their offspring. Information on discrimination values can be used to assess dietary patterns of female birds during egg formation. In this study, we experimentally derive isotopic discrimination relationships between maternal diet and blood constituents for egg laying, and between maternal blood constituents and down feathers of offspring in an experiment with 3 types of domesticated Gallinaceous birds raised on known diets. Relative to diet, $\Delta^{13}C$ values for maternal blood constituents were negative, on average (plasma: -1.0 ‰, red blood cells [RBC]: -1.1 ‰), while $\Delta^{15}N$ values were positive (plasma: 3.8 ‰, RBC: 2.5 ‰). Relative to blood constituents of the adult female, $\Delta^{13}C$ of offspring down were positive for both plasma ($\Delta^{13}C$: 0.7 ‰) and RBC ($\Delta^{13}C$: 0.8 ‰). Similarly, relative to maternal blood constituents, $\Delta^{15}N$ of offspring down was positive for RBC (1.6 ‰), but did not change strongly for plasma. Our experiment is the first to report isotopic discrimination values for maternal blood constituents and down of offspring in avian taxa that does not make assumptions of diet that are routine when estimating source contributions to offspring formation. Our study also provides a sampling technique that is less invasive than previously available as collecting down does not require sampling viable eggs or individuals. With the isotopic discrimination values provided here, future researchers can

identify nutrient sources—along with the timing of nutrient acquisition—used by adult female birds to form the down feathers of offspring.

Introduction

Stable isotope analysis (SIA) is a powerful tool in ecology that is used to gain insights into characteristics that are difficult to observe in nature such as migration, niche partitioning, and nutrient-allocation (Klaassen et al. 2006, Young et al. 2010, Hobson 2011). SIA depends on knowing the isotopic discrimination values for the tissues and isotopes involved. Isotopic discrimination is the measured difference in the isotopic value between the nutrient source and the consumer tissue, or between maternal tissue and offspring tissue (Ciancio et al. 2016, Jenkins et al. 2020). Isotopic values in the two tissue types will differ because of the metabolic processes and isotopic routing involved in tissue synthesis (Phillips and Koch 2002, Carter et al. 2019, Whiteman et al. 2020). Reliable information on isotopic discrimination values and associated variation in values is critical for making accurate inferences using SIA techniques. Unfortunately, discrimination values have only been described for tissues for a few species. Thus, researchers often use values from other taxa even though discrimination values can vary by species, diet, body size, and physiology (Bearhop et al. 2006, Caut et al. 2009). However, experimentally deriving discrimination values for species-specific, or closely related proxies will lead to the most robust inference using SIA techniques (Healy et al. 2018).

Income breeding animals, those who rely on local nutrient sources consumed during time of offspring formation, route nutrients directly from their diet to the formation of their offspring. In contrast, capital breeders, who accumulate and store nutrients for future reproduction, route nutrients from endogenous (i.e., body) reserves (e.g., muscle or lipid sources) to the formation of their offspring. Most studies to date have found that birds exhibit a mixed breeding strategy (Williams et al. 2017). The amount of income vs. capital resources used during offspring formation appears to be related to body size with larger individuals capable of storing more nutrients in endogenous reserves for reproduction as a way to deal with environmental variability or uncertain access to food (Hupp et al. 2018, Whiteman et al. 2020). Using experimentally derived isotopic discrimination values between maternal tissues and downy feathers from young will allow researchers to assess the degree to which dietary sources are used by females to produce offspring, and can help identify the timing of when nutrients for offspring formation were acquired (Frankel et al. 2012). Chick down can be sampled noninvasively from young birds following hatch, which is preferable to destructive sampling of eggs or lethal sampling of juveniles. In addition, the collection of multiple tissues types with differing turnover rates can be used to directly assess the timing of nutrient acquisition by breeding adults for offspring production. For instance, isotopic turnover in blood plasma reflects nutrients acquired immediately prior to egg laying (e.g., spring) whereas turnover in red blood cells (hereafter, *RBC*) reflects distant diets acquired during a previous season (e.g., winter; Carter et al. 2019). In terms of assessing which seasonal habitats were used for nutrient allocation for offspring formation, the collection of multiple tissues types is logistically easier and allows greater certainty when defining contributions of different sources during statistical estimation through the use of an isotope mixing model when compared to working with assumed dietary sources. The collection of tissues with different turnover rates is especially beneficial for research on migratory species and/or those occurring over vast geographic areas for which it would be logistically difficult to collect dietary sources prior to offspring formation. Using discrimination values directly from the mother's tissues to infer the sources of nutrient acquisition can circumvent assumptions of food preferences of breeding individuals during embryonic development, especially in migratory species that move long distances during the seasons leading up to breeding. However, researchers currently lack information on mother-offspring stable isotope discrimination values in avian taxa.

In this study, we experimentally derived isotopic discrimination values between: (1) diet-blood constituents (i.e., plasma and RBC) of mothers and (2) maternal blood constituents-down feathers of offspring using 3 types of domesticated Gallinaceous birds (2 chickens and 1 quail). Researchers could potentially use these values to assess dietary sources in wild gallinaceous birds to elucidate income foraging strategies used for egg production along with the timing of nutrient acquisition. The isotopic discrimination values established in the current study for carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotopes. The goal of the study is to estimate isotopic discrimination values in three types of Gallinaceous birds for: (1) diet-blood constituents, and (2) blood constituents-down feathers of their offspring. All female captive birds were raised on a consistent, known diet from hatching until they laid eggs and were fed *ad libitum,* which represents an income breeding strategy. We sampled plasma and red blood cells from the adult females when they reached breeding age, and down feathers from their offspring upon hatch, to estimate isotopic discrimination values for diet-maternal blood constituents, and maternal blood constituents-down feather of offspring.

Methods

Experimental Design

We raised Australorp and Barred Rock (*Gallus gallus domesticus* spp.) chickens from chick stage and incubated Japanese quail (*Coturnix japonica*) eggs at 37.5°C until hatch to comprise our experimental flock. We housed the captive flock in 3 holding stalls (1/bird type) and provided ample floor, roosting, and nesting space (floor space: 0.25 m^2/chicken, 0.01 m^2/quail; roost space: 20.3 cm/chicken [ground-dwelling quail did not require roosting space]; nesting space: 1 nest box/4 females for each spp. - nest box = 35.6 cm wide x 35.6 cm long x 30.5 cm tall). We fed chickens with Purina® Medicated Start and Grow Feed®, and we fed quail with Purina® Non-Medicated Start and Grow Feed®, which was formulated with prebiotics, probiotics, and yeast to support digestive health and immune function. Both chicken and quail feed contained 3% crude fat and 18% crude protein. We tested the isotope values of the feed throughout the duration of the experiment by collecting 3 random samples per bag of feed for both chicken (12 bags) and quail (3 bags). The isotopic composition of the chicken feed was -19.7±1.5 ‰ for $\delta^{13}C$ (mean±SD) and 1.4±0.5 ‰ for $\delta^{15}N$, while the isotopic composition of the quail feed was -17.2±1.2 ‰ for $\delta^{13}C$ and 1.2±0.3 ‰ for $\delta^{15}N$. The C/N ratio of the feed by atomic mass was 18.3/1. We allowed birds to feed *ad libitum* throughout the duration of the experiment.

Birds were fed a constant diet source from chick stage to breeding age for both chicken types (5 months) and quail (2 months). When females reached breeding age, we placed a male of the breed with the females (1 male/8 female chickens; 1 male/3 female quail) for mating and egg fertilization. After fertilization, we collected all eggs that were laid by the females and placed them in a forced air incubator with an automatic egg turner. We set the incubator temperature at 37.5°C and kept the humidity between 55 – 65% until 3 days before hatching when we raised the humidity to 70 – 80%. Three days before hatch, we removed the eggs from the automatic egg turner and placed them directly on the bottom of the incubator until hatch. Once the chicks hatched, we allowed them to dry (~5 hours) before collecting their down feathers.

Sample Collection

We raised 27 female chicks to breeding age (9 black Australorp, 9 Barred Rock, and 9 Japanese Quail), and collected and incubated their eggs, which produced 40 chicks (12 Barred Rock, 13 Australorp, and 15 Japanese quail). We obtained isotope tissue from all females and their chicks. Once adult females reached breeding age, we collected blood samples. We collected a 2-3 ml sample of whole blood from each female by brachial venipuncture. We placed blood samples in plastic vacutainer containing spray coated sodium heparin to avoid clotting. We stored the whole blood samples on ice in a cooler until being centrifuged to separate plasma and RBC within 3 hours of collection. For chick tissue samples, we collected ~5 mg of down feathers from the breast region of individual birds.

Blood samples were freeze dried and then crushed into small pieces. Surface oils were removed from offspring downy feathers using a 2:1 chloroform to methanol rinse, solution then decanted, and samples allowed to dry. Samples were encapsulated in tin cups (3*x*5 mm in dimension) to a weight of 0.6 mg (±0.1 mg) prior to SIA. Isotope samples were analyzed for stable-carbon (δ^{13}C) isotopes using continuous-flow isotope-ratio mass spectrometry at the University of New Mexico Stable Isotope Laboratory. Stable isotope values were reported in parts per thousand (‰), relative to various in-lab organic protein standards. Estimated analytical error was 0.1 ‰ and 0.3 ‰ for δ^{13}C and δ^{15}N, respectively, based on *n*=200 replicate within-run measurements of various standards.

SIA and Statistical Analysis

We conducted analyses directed at assessing the differences in δ^{13}C and δ^{15}N between diet-blood constituent, and maternal blood-down feathers of their offspring for 3 types of Galliformes. Isotope ratios for both analyses were continuous, and both covariates, bird variety and tissue type, were categorical. We implemented the analyses using an ANOVA model within the *rstanarm* package in R (R Core Team 2021). Each model was run using 4 chains with 1,000 warm-up iterations and 1,000 subsequent iterations per chain. We assessed model convergence using the Gelman–Rubin ($\hat{R}$) statistic (Gelman and Rubin 1992). The model was validated using posterior predictive checks obtained using the R package *shinystan* (Gabry 2018) to compare predicted values based on the model to observed values. We built one *a priori* model that included an interaction with species and tissue type for both δ^{13}C and δ^{15}N as a means of quantifying isotopic discrimination values.

We derived isotopic discrimination values (hereafter; Δ) in diet-blood constituents and blood constituents-chick down to create differences across bird type that approximates isotopic discrimination values. To do so, we summarized (mean ± 90% credible intervals) estimates based on all stored values in the posterior distribution of coefficients for each bird type *x* tissue type combination. We evaluated the magnitude of each estimated discrimination value by assessing whether the 90% credible interval for the estimated discrimination value overlapped zero. We then subtracted these estimates from the summarized estimates for the diet and the chick down for a given bird type. Finally, we derived average discrimination values across the 3 bird types by combining all rows stored in the posterior distribution of coefficients for a given tissue type and using the same method as described above.

Results

Model Validation

Inspection of model diagnostics indicated that models converged for all estimated parameters ($\hat{R}$ <1.01). Posterior predictive checks indicated that the two models could produce predicted values with characteristics similar to those for the observed data.

Diet-Blood Constituent Discrimination

Isotope discrimination for both δ^{13}C and δ^{15}N between diet-blood constituents differed depending on tissue type (i.e., RBC or plasma) but showed a similar pattern across bird types (Table 3-1). Relative to diet, the δ^{13}C values of plasma and RBC were negative (Figure 3-1), which resulted in negative discrimination values between diet-blood constituent (Table 3-1). Overall difference of discrimination values for δ^{13}C between diet-plasma and diet-RBC were similar (Table 3-1). For δ^{15}N, we found that both plasma and RBC had higher isotope values than their diet (Figure 3-1), which resulted in positive discrimination values between diet-blood for both constituents (Table 3-1). However, discrimination for δ^{15}N in diet-blood constituent was greater in plasma than in RBC (Figure 3-1). Variation in discrimination between diet-blood constituents was less in δ^{15}N than δ^{13}C (Table 3-1).

Blood-Down Feather Discrimination

Isotope discrimination for both δ^{13}C and δ^{15}N between maternal blood constituents-down feathers of chicks differed depending on blood constituent type but showed a similar pattern across bird types (Table 3-1). Relative to chick feathers, the δ^{13}C values of plasma and RBC were negative for the adult females across the 3 bird types (Figure 3-2), resulting in positive discrimination values between maternal blood-down feathers of offspring (Table 3-1). For δ^{15}N, offspring down feathers had consistently higher isotope values than maternal RBC regardless of bird type (Table 3-1, Figure 3-2), resulting in positive discrimination values. Discrimination value for δ^{15}N overlapped zero between plasma-down feathers. Variation in discrimination between blood constituent-feather was less in δ^{15}N than δ^{13}C (Table 3-1).

Discussion

In our study, we experimentally derived discrimination values for diet-blood constituents and blood constituents-down feather for 3 types of domesticated gallinaceous birds. We found evidence that isotopic discrimination is largely similar between diet-blood constituents, and between blood constituents-down feather across different types of Gallinaceous birds. By establishing diet-blood and blood-down discrimination values, the current study allows researchers working on gallinaceous birds the opportunity to potentially assess nutrient allocation strategies of females during offspring formation, along with the opportunity to estimate the timing of nutrient acquisition for offspring formation given the differences in turnover rates of blood plasma and RBC. The current study also provides a sampling technique that is less invasive than previously available methods that compares the blood isotope values of the mother with the down feathers of her offspring, which avoids sampling viable whole eggs (but see Morrison and Hobson 2004 for alternative technique to sample egg tissue while avoiding whole egg collection) or lethal sampling of juveniles. Previous studies established discrimination values between diet to egg constituents (i.e., albumen, yolk protein, and membrane; Hobson

1995) and egg constituents to chick feather (Federer et al. 2012, Hahn et al. 2012). The current study avoids the use of multiple discrimination values for a single egg by providing only one discrimination value per blood constituent to assess nutrient strategies used by the female to produce down feathers in her chick.

The difference in isotopic discrimination for δ^{13}C between mother-to-offspring down depended on blood type; however, isotopic patterns and subsequent discrimination values followed a similar pattern across the considered bird types. Specifically, blood-to-offspring down for Δ^{13}C in RBC and plasma were consistently positive across the bird types. This pattern reflects biochemical and metabolic routing involved in tissue formation, which indicates that relatively uniform patterns exist for isotopic discrimination between maternal tissues to chick down formation (Hobson 1995). The change in Δ for δ^{13}C between material blood constituents-down feather formation of chicks ranged from 0.3 to 1.0 ‰, which is similar to values reported in other studies (Δ for δ^{13}C from -1.3 to 1.5 ‰; Hobson 1995, Hahn et al. 2012). However, previous studies assessed isotopic discrimination between female diet-to-eggs, or eggs-to-chick down, so previous findings are not directly comparable to our current study. For comparison, variation in blood-down Δ for δ^{15}N were more similar across bird types, with plasma having no measurable discrimination than RBC across all bird types. Values for blood-down Δ for δ^{15}N in our study ranged from 0.1 to 1.7 ‰, which is lower, on average, than values from previous studies, which reported Δ for δ^{15}N for diet-egg to range from 2.8 to 3.6 ‰ and egg-chick down to range from 1.7 to 3.1 ‰. Results for Δ in δ^{15}N are, however, more comparable to the isotopic discrimination of turtles for mother epidermis-blood of offspring (epidermis: -0.2 to 1.4 ‰; whole blood: 2.1 to 2.3 ‰; Carpentier et al. 2015). Our results suggest that researchers wanting to apply SIA to wild gallinaceous species for which discrimination values are lacking and lethal sampling is not possible (e.g. due to critically low population size) could use plasma and RBC to assess foraging patterns along with timing of nutrient acquisition for offspring formation as the patterns of isotopic discrimination were largely similar for a given tissue type across three different gallinaceous bird types.

Application to Avian Conservation

It is important to note that individuals in our study had unlimited access to food, which might make the inferences less applicable to wild birds residing in seasonal environments. Air temperature (Carleton and Del Rio 2005), diet quality and quantity (Williams et al. 2007), and nutritional stress (e.g., fasting; Hobson et al. 1993) can influence isotopic discrimination values. One potential way to lessen the impacts of these known sources of variation is to use δ^{13}C, which appears less sensitive than δ^{15}N to known sources of variation (e.g., stress; Hobson et al. 1993) and has lower discrimination values than δ^{15}N. Future studies that include a greater diversity of gallinaceous species with different breeding strategies (e.g., income vs. capital breeders) fed a variety of diet types would be useful. Future studies can also assess compound specific analyses of essential and non-essential amino acids to determine how isotopes routing between diet-blood and blood-feather and corresponding changes to isotopic discrimination (Herbert et al. 2016, Whiteman et al. 2020). For now, the discrimination values developed for bulk tissues in the current study can help avian researchers address foraging patterns along with timing of nutrients acquisition and later used for offspring formation (Williams et al. 2017).

Acknowledgments

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Figure 3-1. Box plots summarizing observed carbon (δ13C; A) and nitrogen (δ15N; B) values (points), empirical means for each tissue x bird combination, and modeled estimates (error bars: mean ± 90% credible interval) for diet and adult female plasma and red blood cell (RBC) tissues for different domesticated gallinaceous bird types. Black dot shows the observed mean value for each cluster

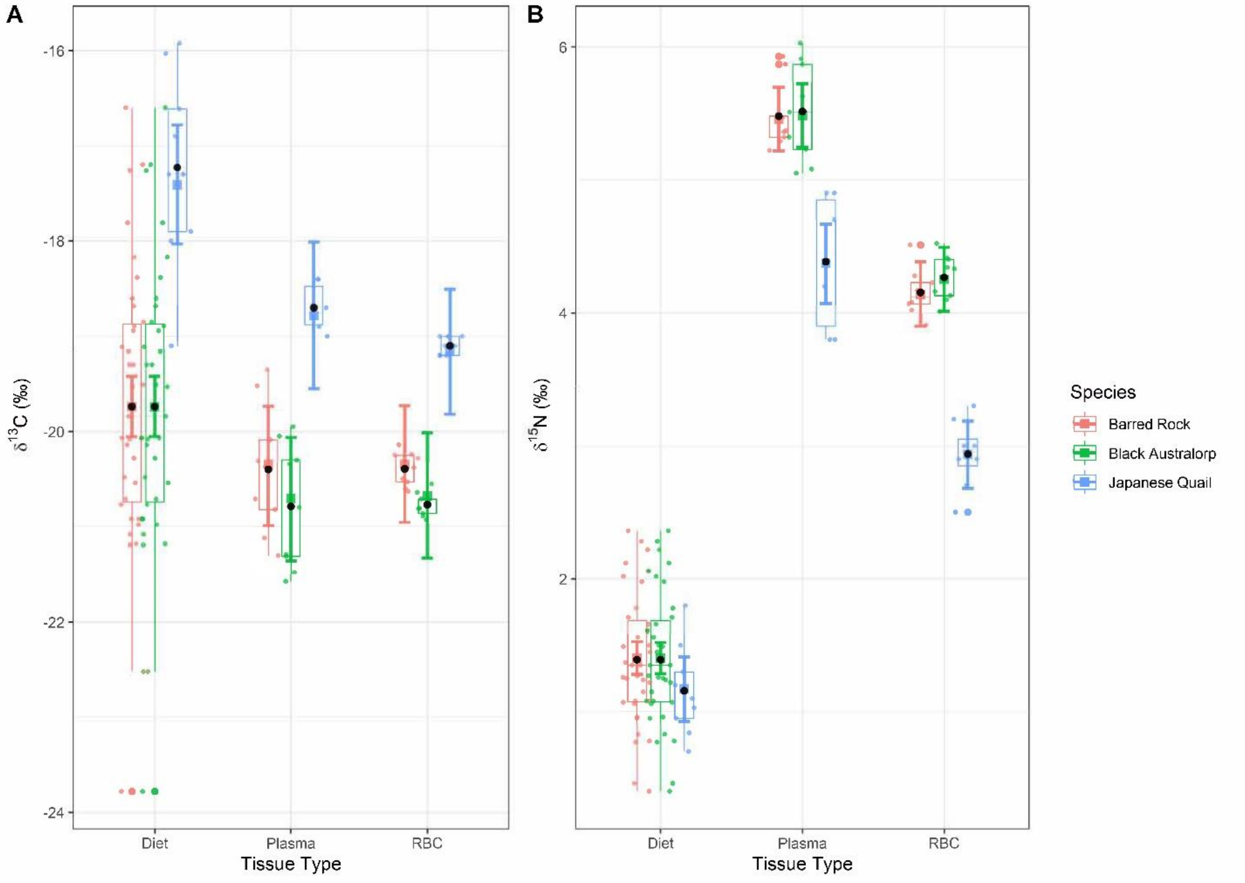

Figure 3-2. Box plots summarizing observed carbon (δ13C; A) and nitrogen (δ15N; B) values (points), empirical means for each tissue x bird combination, and modeled estimates (error bars: mean ± 90% credible interval) for adult female plasma and red blood cells (RBC) and down feathers of offspring for different domesticated gallinaceous bird types. Black dot shows the observed mean value for each cluster.

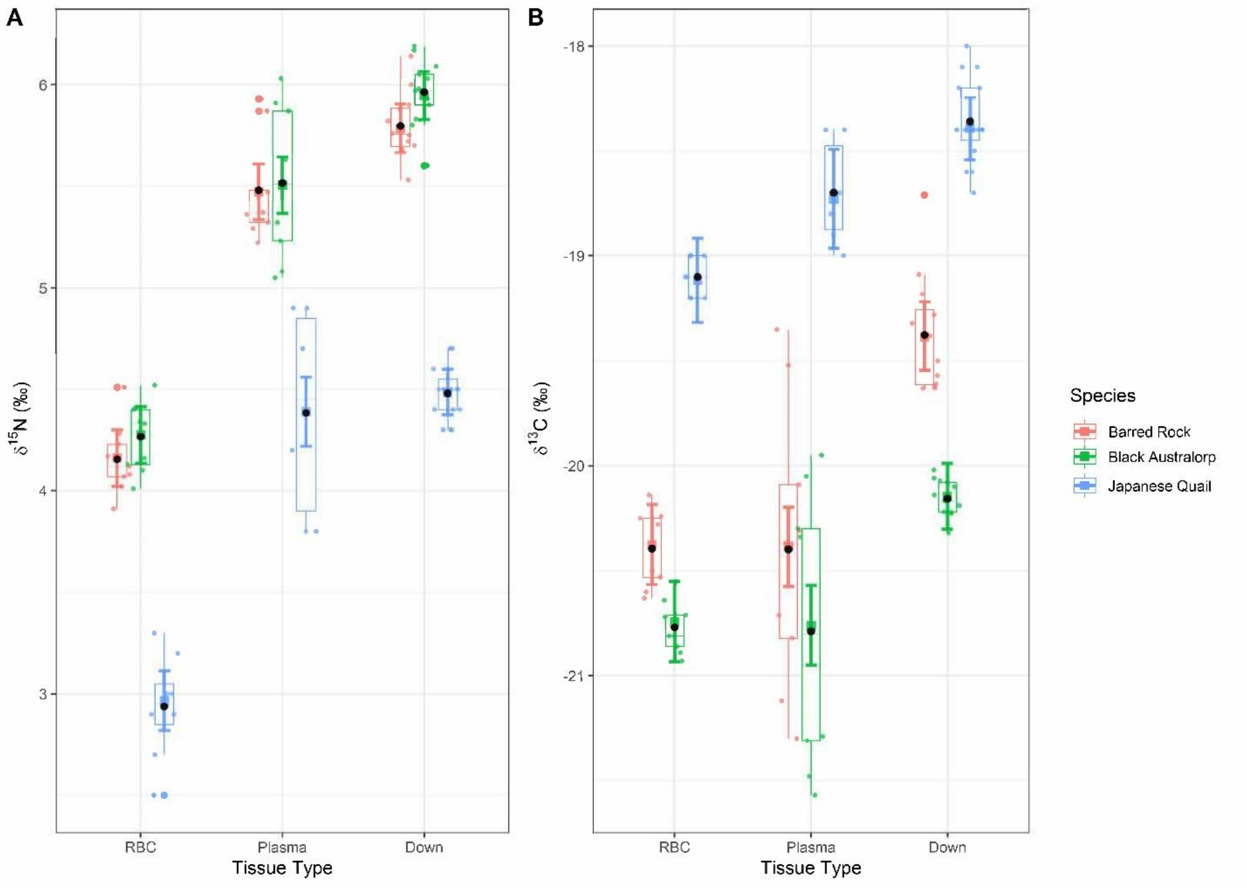

Table 3-1. Average (± 1SD) discrimination values (denoted Δ13C and Δ15N) for diet-blood and blood constituent-down feather of chickens (Barred Rock and Black Australorp) and Japanese Quail. Birds were fed ad libitum a consistent source of grain diet. Asterisks indicate coefficients that had 90% credible intervals that did not include 0.

Source	**Tissue**	**Species (n)**	**Δ^{13}C**	**Δ^{15}N**
Diet to:	Plasma	Barred Rock (9)	-0.6 (0.4)	4.0 (0.2)*
	RBC		-0.6 (0.4)	2.7 (0.2)*
	Plasma	Black Australorp (9)	-1.0 (0.4)*	4.1 (0.2)*
	RBC		-0.9 (0.4)*	2.9 (0.2)*
	Plasma	Japanese Quail (9)	-1.4 (0.6)*	3.2 (0.2)*
	RBC		-1.7 (0.6)*	1.8 (0.2)*
	Plasma	Overall (27)	-1.0 (0.6)*	3.8 (0.5)*
	RBC		-1.1 (0.7)*	2.5 (0.5)*
Plasma to:	Down feather	Barred Rock (9)	1.0 (0.2)*	0.3 (0.1)*
	Down feather	Black Australorp (9)	0.6 (0.1)*	0.4 (0.1)*
	Down feather	Japanese Quail (9)	0.3 (0.2)*	0.1 (0.1)
	Down feather	Overall (27)	0.7 (0.3)*	0.3(0.2)
Red blood cells to:	Down feather	Barred Rock (9)	1.0 (0.2)*	1.6 (0.1)*
	Down feather	Black Australorp (9)	0.6 (0.2)*	1.7(0.1)*
	Down feather	Japanese Quail (9)	0.7 (0.2)*	1.5 (0.1)*
	Down feather	Overall (27)	0.8 (0.2)*	1.6 (0.1)*

CHAPTER 4

RESOURCE ALLOCATION EFFECTS ON THE TIMING OF REPRODUCTION IN AN AVIAN HABITAT SPECIALIST

Contribution of Authors and Co-Authors

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Contributions: Conceived idea, study design, and data collection, and provided critical inputs on the paper.

Manuscript Information

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Abstract

Variation in nutrient allocation can influence the timing of breeding and ultimately reproductive output. Time and space constraints might exist, however, if fewer food resources are available to meet the costs of reproduction early during the reproductive season. Here, for the first time, we test whether nutrient allocation strategies for reproduction in a shrub-dependent avian species differs with timing of breeding in different ecoregions: a high-elevation landscape, containing spatially complex vegetation (Rocky Mountains) versus a low-elevation, more homogenous landscape (Great Plains). We analyzed data collected from radio-telemetry and stable isotopes to assess the degree to which endogenous (body) reserves are used for reproduction and whether variation in allocation strategies was associated with time of year, ecoregion, habitat quality (including sagebrush type and plant greenness), or maternal characteristics. Using a Bayesian statistical framework, we found that females relied on a similar amount of endogenous reserves for reproduction in first nesting and renesting attempts. Additionally, endogenous contributions declined more rapidly throughout the nesting season in the Rocky Mountains than in the Great Plains. Individuals in high- and intermediate-elevation sagebrush types in the Rocky Mountains used similar amounts of endogenous reserves, whereas females nesting in low-elevation sagebrush used less. Females nesting at intermediate elevations, which experience the greatest flush of new green vegetation during the nesting season, switched their reliance from endogenous to exogenous sources for reproduction as green vegetation became available during spring. Our study highlights adaptations of a nutrient-allocation strategy across areas with varying levels of resources in time and space in a habitat specialist bird. Nutrient allocation by individuals residing in high-elevation areas favors a strategy that mainly

uses nutrients gained from wintering habitats, whereas individuals residing in low-elevation areas mainly use exogenous sources for reproduction.

Introduction

Variation in allocating nutrients towards reproduction within a season is common in many species (Daan et al. 1988) and can provide resilience to environmental change (Jönsson 1997), influencing the timing of breeding and ultimately, reproductive success (Drent and Daan 1980). Individuals in a relatively good condition early in the breeding season, breed early and produce larger clutches, while individuals in an initially poor condition, delay breeding to improve their state, but do not achieve a high condition due to the negative effects of late breeding on offspring growth and survival (Drent and Daan 1980). Thus, the timing of reproduction can be critical to maximize reproductive output, as early attempts commonly produce larger numbers of young for many animal taxa (Lindström 1999). However, challenges might exist to meet the costs of offspring development for earlier attempts, especially for avian herbivores, as the timing of their breeding season is often initiated ahead of vegetation green-up due to the time required for egg formation, egg laying, and incubation (Sedinger and Raveling 1986).

Energy demands used to initiate breeding can be met using body reserves acquired prior to the breeding season and stored endogenously, exogenous sources obtained during the breeding season; or a combination of these strategies (Stephens et al. 2009). Individuals using endogenous reserves can start the nesting season early and renest more quickly and more frequently if earlier nest attempts fail (Ankney and MacInnes 1978, Alisauskas and Ankney 1992). Females in better condition can rely more on endogenous reserves for reproduction than can females in poorer condition (Bêty et al. 2003). However, carrying extra reserves can be costly due to increased risk of being killed by a predator because heavier individuals are slower to escape (Klaassen et al. 2006). The contribution of endogenous reserves for breeding can be influenced by proximal factors (e.g., vegetation greenup) encountered by the female during offspring development (Drent, Fox, and Stahl 2006, Meijer and Drent 1999), along with individual age and ability to acquire and store nutrients during the nonbreeding season (Hupp et al. 2018). To date, much research on the use of endogenous reserves for breeding has focused on where different species fall along the endogenous-to-exogenous continuum (Stephens et al., 2009), along with temporal changes in nutrient reserves of females (Alisauskas and Ankney 1992).

Few studies have evaluated the effect of habitat quality and ecoregional differences on the use of endogenous reserves for breeding. High-elevation sites are characterized by colder temperatures, later snowmelt, and delayed green-up during spring, resulting in fewer or less predictable food resources compared to what is available at lower elevations. Individuals nesting at higher elevations have adapted to more hostile environmental conditions by increasing reliance on endogenous reserves for breeding (Jönsson 1997). As a result, the reliance on endogenous reserves would decline with increasing laying date within a population as environmental conditions improved (Drent and Daan 1980).

We assessed variation in the use of endogenous reserves on the timing of reproduction in the greater sage-grouse (*Centrocercus urophasianus*; hereafter: *sage-grouse*). Females raise a single brood per year at most, and clutch size strongly declines throughout the nesting season (Schroeder 1997). Sagebrush leaves are the winter food for sage grouse, but the leaves have a lower nutritional profile (e.g., crude protein, calcium, and phosphorus) than green forbs (Barnett and Crawford 1994). Although previous research indicates that female sage-grouse rely heavily on exogenous sources from the local environmental during spring (Gregg et al. 2006), we do not understand the potential for temporal and spatial variation in nutrient-allocation strategies. Grouse species are limited by protein given their herbaceous diet (Sedinger 1997, Meijer and Drent 1999, Gregg et al. 2008). The species occupies a broad geographic range that encompasses gradients of habitat quality. Some individuals begin nesting early; while others delay, especially those individuals who cannot store enough endogenous reserves in areas with hostile environmental conditions. They also renest if their first nest is unsuccessful; these later breeding attempts are initiated after green-up has occurred (Gregg et al. 2006, Schroeder 1997). Thus, sage-grouse appear to maximize their reproductive output by adaptively selecting for early breeding.

In this study, we use data collected from females to determine the (1) timing of egg laying and (2) stable isotopes collected from blood constituents (i.e., red blood cells and plasma) and the feathers of their offspring to test whether the use of endogenous reserves may be an adaptively selected trait and whether this response relates to timing of reproduction. Using a Bayesian statistical framework, we first measure the extent to which individual females used endogenous reserves for offspring formation and evaluated several possible sources of variation in resource allocation using data from two distinctly unique ecoregions with contrasting climates. Second, we analyze a six-year dataset from the Rocky Mountains in southwest Montana to evaluate if variation in resource allocation was related to sagebrush type, plant greenness, and maternal body condition. We used the two datasets to test the following hypotheses: 1) the contribution of endogenous reserves for offspring formation is greater early as compared to late in the nesting season, 2) endogenous reserve use is greater in first versus second nesting attempts, 3) endogenous reserves are greater in high- versus low-elevation ecoregions and sagebrush types and in higher-quality habitats (based on plant greenness), and 4) endogenous reserves are positively related to maternal body condition.

Methods

Study Site and System

Data were collected across two sagebrush-dominated ecoregions: The Great Plains of eastern Montana, and the Rocky Mountains of southwest Montana, USA (Figure 4-1). The Great Plains site is located near the town of Roundup, which contains rolling topography ranging in elevation from 975m to 1250m. The dominant sagebrush species are Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) and silver sagebrush (*A. cana* ssp. *cana*), with an understory of native perennial bunch grasses and forbs. Average annual precipitation and temperature is 359 mm and 9.2°C, respectively (National Climatic Data Center 2019). The Rocky Mountain site is located near the town of Lakeview in southwest Montana. Sagebrush in this ecoregion occurs across an elevation gradient of 2,000 m to 2,900 m. Mountain big sagebrush (*A. tridentata* ssp. *vaseyana*; hereafter; *MTSA*) occurs at the highest elevations where snowmelt and vegetation green-up occur latest during the spring. Three-tip sagebrush (*A. tripartita*; hereafter; *TTSA*) occurs on south-facing mid-elevation slopes where snowmelt and vegetation green-up occur earliest. Basin big sagebrush (*A. tridentata* ssp. *tridentata*; hereafter; *BBSA*) occurs in valley bottoms with deep soils where drifting of snow results in a later snowmelt date, but the earliest vegetation senescence. Average annual precipitation and temperature at Lakeview is 500mm and 1.6°C, respectively.

Data Collection

We captured female sage-grouse using dipnets and spotlights. Upon capture, we collected blood samples from individual female sage-grouse by brachial venipuncture using a 23-gauge needle with 3 mL syringe. We collected blood samples from a subsample of individual female sage-grouse from the Great Plains site in both 2016 and 2017 (*n*=10 females each year), and from every captured individual at the Rocky Mountain site from 2014 to 2019 (*n*=91). We centrifuged blood samples within 12 hours of capture and separated the plasma and red blood cells constituents into separate vials. Samples were stored frozen until we prepped samples for stable isotope analysis.

On each study site, we tracked individual female grouse 2–3 times per week during the pre-nesting season (April to June) until we discovered a nest. Once we discovered a nest, we monitored nest status two times per week until the nest either hatched or failed. We considered a nest to be successful if ≥1 egg hatched as determined by the presence of any of the following in the nest bowl: a chick, an intact egg membrane, and/or eggshell cap. We counted membranes after a nest hatched and estimated when the first egg was laid (hereafter; *initiation date*) by assuming an egg laying interval of 1.5 days and a 28-day incubation period to determine the date (Moynahan et al. 2007). For failed nests, we continued to track the female until mid-June to determine whether a second nest was initiated. We included only individuals who successfully hatched young, since our primary focus was on explaining variation in nutrient allocation to offspring production.

If a nest was successful, we captured chicks when they were 2 to 8 days old (average = 3 d) and collected down feathers for stable isotope analysis. Downy feathers from the breast are present at the time of hatch and are predominantly keratin that reflect nutrient (protein) pools used by adult females during egg development (Williams 2012). We captured chicks by hand in the early morning by flushing the brooding female off her dependent young. We stored feather samples from individual chicks in separate paper envelopes prior to stable isotope analysis.

Laboratory Experiment

Stable isotope techniques can be used to determine the timing of nutrient acquisition and then how they are allocated for reproduction (Hobson 2006). To quantify a female's reliance on endogenous versus exogenous sources, we generated isotopic discrimination values from a controlled laboratory study for red blood cells and plasma of the adult females to her offspring's chick feathers from three varieties of domesticated *Galliformes* (i.e., Barred-rock, Australorp, and Japanese Quail; Cutting, *unpublished data*). Discrimination value for $\delta^{13}C$ from red blood cells of adult females to their offspring downy feathers was 0.80‰ (± 1SD: 0.22‰), and discrimination values of plasma to offspring downy feathers was 0.67 (0.35‰; Cutting, *unpublished data*).

Stable Isotope Analysis

The stable isotope technique can quantitatively assess the degree to which an individual relies on endogenous versus exogenous sources for reproduction (Hobson 2006, Cutting et al. 2011). For example, isotope values of red blood cells in avian taxa represent habitat use during the previous 1-2 months, whereas isotope values of blood plasma reflect diet use in the previous 2 weeks (Hobson and Clark 1992, Odgen et al. 2004).

Blood samples were dried in a freeze drier, and then crushed into small pieces. Surface oils were removed from offspring downy feathers using a 2:1 chloroform to methanol rinse, solution then decanted, and samples allowed to dry. Samples were encapsulated in tin cups (3x5mm in dimension) to a weight of 0.6mg (±0.1mg) prior to stable isotope analysis.

Stable-carbon isotopes are ideal for tracing nutrient allocation for reproduction by assessing the bulk carbon pathways of plants consumed by females (Hobson 2006). Isotope samples were analyzed for stable-carbon ($\delta^{13}C$) isotopes using continuous-flow isotope-ratio mass spectrometry at the University of New Mexico Stable Isotope Laboratory. Stable isotope values were reported in parts per thousand (‰), relative to various in-lab organic protein standards. Estimated analytical error was 0.1‰ for $\delta^{13}C$, based on n=200 replicate within-run measurements of various standards.

Statistical Analysis

We implemented all statistical analyses in a Bayesian framework. We first estimated the contribution of endogenous reserves used for offspring formation by using stable isotope-mixing models. We then modeled variation using a beta regression model with a logit link with weakly informative priors to model associations between percent contribution of endogenous reserves and covariate conditions including nesting attempt, ecoregion, and within and across breeding season. Using data from the Rocky Mountains, we finally evaluated if variation in resource allocation was related to sagebrush type, plant greenness, and maternal body condition of individual females. Results describe where sage-grouse fall on the endogenous/exogenous breeding spectrum and identify potential mechanisms that influence nutrient allocation for reproduction. All data were analyzed using R version 4.0.2 (R Development Core Team 2021).

Isotope Mixing Model

We deployed a two-endpoint, one-isotope (δ^{13}C), Bayesian stable isotope mixing model to estimate the contribution of endogenous reserves for offspring protein formation using the R package *MixSIAR* (Stock et al. 2018), which constructs and fits models in JAGS (Plummer 2003). We implemented the mixing model using 300,000 iterations from 3 Markov chain Monte Carlo (MCMC) chains after a burn-in of 200,000 steps. We assessed model convergence by ensuring the Gelman-Rubin diagnostic of $\hat{R}$ was <1.1 (Gelman and Rubin, 1992). We included the blood constituents (plasma and red blood cells) of females as a fixed effect, and brood ID as a random effect to derive mean reliance of endogenous versus exogenous contributions for individual broods. We then carried forward the mean value of the dependent variable for modeling against covariates of interest. We did not propagate error around the mean values as they were estimated quite precisely in the isotope mixing model (90% credible interval for mean estimate at Great Plains = 25.2%, and Rocky Mountains = 12.7%). The effect of the error measurement around the mean value of the dependent variable is to only increase the unexplained part of the regression model (Sokal and Rohlf 1995).

Modeling Sources of Variation in Endogenous Reserves

Before modeling, we evaluated pairwise correlations among covariates and did not find evidence of high multicollinearity (Pearson's $r \leq 0.7$). For Normalized Difference Vegetation Index (hereafter; *NDVI*) values across different sagebrush type, we assessed differences using a regression model, and found that NDVI values were higher across the growing season in TTSA than in either MTSA and BBSA, while NDVI values were similar in both MTSA and BBSA (Figure 4-5). We then used a generalized linear model with beta distributed errors and a logit link to model the associations between endogenous sources and covariate conditions. We fit all beta regression models in Stan via the R package *rstanarm* using weakly informative priors with a mean of 0 and a standard deviation of 2.5 for both the intercept and coefficients with four chains, each with 1000 iterations of burn-in, and 1000 iterations of sampling (Carpenter et al. 2017, Stan Development Team 2017). The goal of the regression modeling was to evaluate how the estimates of the proportion of endogenous reserves for offspring formation was associated with nesting attempt, time of year, ecoregion, habitat quality, and/or maternal characteristics.

Model Covariates

Maternal Characteristics We calculated avian body condition for individual females during the pre-breeding season using a principal component analysis. Body condition index was calculated using the *prcomp* function in R using body mass, head length, and wing length measurements. We extracted values for the first principal component for each individual and then regressed individual bird weight and PC1 values. We finally added the residuals from the simple linear regression to the mean bird weight to get the final BCI value (Warren et al. 2013). Body condition metrics better characterize structural differences in birds than body mass alone (Freeman and Jackson 1990).

Habitat Quality NDVI is derived from satellite imagery and provides a measure of vegetative cover, productivity, and phenology (Johnston et al. 2018). Given that sage-grouse consume new green-plant growth during the pre-egg laying stage (Barnett and Crawford, 1994), and tend to have higher nest survival in areas with greater amounts of new grass growth (Cutting et al., 2019, but see Gibson et al., 2016), we consider NDVI as a proxy of both forage quality and hiding cover from predators (Johnston et al., 2018). We describe the phenological stage of herbaceous vegetation surrounding a nest site using NDVI values (250-m spatial resolution) obtained from the Moderate Resolution Imaging Spectroradiometer sensors using the R package MODIStsp (Busetto and Ranghetti 2016). Satellite-derived NDVI data are collected on 2-week intervals. We therefore used the NDVI image closest to initiation date for a given nest. If a nest initiation date was in the middle of two NDVI images, we then averaged the image values for the given pixel where the nest occurred. Johnston et al. (2018) defined NDVI of ~0.10 as being the transitional period between winter and spring, ~0.15 as being the start of the growing season in early-spring, and 0.40 as being the maximum instantaneous rate of green-up. We used a site-specific field map of different sagebrush types to determine the dominant sagebrush type at a given nest site using ArcGIS 10.5.1 (ESRI, Redlands, California, USA).

Model Selection

We built *a priori* models for both analyses that included various additive and interactive combinations (Table 4-2). We compared Bayesian model performance across our set of candidate models based on the expected log pointwise predictive density (ELPD) obtained via 10-fold cross-validation in *rstanarm*. We assessed convergence by visual inspection of trace plots and by ensuring that $\hat{R}$ was <1.1 (Gelman and Rubin 1992). For each covariate in a well-supported model, we evaluated the magnitude of the estimated coefficient, the degree to which the 90% credible intervals (hereafter; *CrI*) for the covariate overlapped 0, and the degree to which values of the proportion of endogenous reserves were predicted to change as the covariate value was altered. We treated the covariate *year* as a factor with sum to zero contrasts (Chambers and Hasite 1992), which allowed us to assess the contribution of endogenous reserves for an ‘average year’. The model was validated using posterior predictive checks obtained using the R package *shinystan* (Gabry 2018) by comparing predicted values based on the model to observed values. We plotted the estimated relationships between the proportion of endogenous reserves to various covariates by making predictions across a relevant range of covariate values while holding other covariates constant at specified values. We then plotted relationships of covariates that were strongly associated with variation in endogenous contributions by using values in the posterior to create 90% CrI.

Results

Results of Isotope Mixing Model

We collected down feathers for stable isotope analysis from 459 chicks from 91 broods at the Rocky Mountain site, and 158 chicks from 95 broods at the Great Plains site, from 2014 to 2019. Seventy-four and 17 individuals raised young on their first and second nesting attempt, respectively, at the Rocky Mountain site, and 75 and 20, respectively, at the Great Plains site. Summarized isotope data across years are presented in Figure 4-4.

Summary of Covariate Conditions

For the Great Plains, average initiation dates for first nesting versus renesting attempts were 22 April (range: 2 April to 20 May) and 12 May (range: 25 April to 28 May), respectively. In the Rocky Mountains, average initiation date for first nesting versus renesting attempts were 4 May (range: 9 April to 5 June) and 16 May (range: 28 April to 30 May), respectively.

Endogenous Contributions to Reproduction Across Breeding Season, Year, and Ecoregion

All models yielded substantial improvements over the null model based on ELPD score (Table 4-2). MCMC chains for all estimated parameters in models converged ($\hat{R}$ <1.1) and posterior predictive checks indicated that predicted values from the model were similar to the observed response values. The model that performed best included nest attempt, ecoregion, year, and an interaction between ecoregion and initiation date (Table 4-3, Figure 4-2). Contrary to our prediction, the contribution of endogenous reserves was similar between first nesting attempts and renesting attempts. For instance, for a nest that was initiated on the average date for either a first nesting or renesting attempt during an average year, the predicted value of endogenous contributions remained similar for first nesting and renesting attempts in both ecoregions (Figure 4-2). In support of our prediction, endogenous contributions also varied by year, and were greater in the Rocky Mountains than the Great Plains across all years (Table 4-3; Figure 4-2). Finally, also in support of our prediction, the contribution of endogenous reserves decreased across the nesting season, but at a faster rate in the Rocky Mountain than in the Great Plains (Figure 4-2).

Influence of Habitat Quality and Maternal Characteristics on the Contribution of Endogenous Reserves for Reproduction

Over the six field seasons in the Rocky Mountains, we monitored 296 nests, of which 121 hatched young. All offspring from broods that died before chicks were 3 days old (*n*=30); were excluded from further analysis. Average initiation dates were similar among the sagebrush types considered (BBSA: 12 May ± 14 days SD; TTSA: 6 May ± 14 days SD; and MTSA: 6 May ± 11 days SD). NDVI values were collected over a range of time that spanned from winter (-0.02) to the maximum instantaneous rate of vegetation green-up (0.48). Most broods (58%) in our sample were in high-elevation MTSA, 30% were found in mid-elevation TTSA, and 12% were found in low-elevation BBSA. Female body condition index (BCI) ranged widely from poor (BCI=1,344) to high (BCI = 1,640) quality individuals (Table 4-5).

All *a priori* models yielded substantial improvements over the null model with one exception: the model containing nesting attempt and body condition based on ELPD score was worse than the null model (Table 4-2). The model that performed best included nest attempt, sagebrush type, NDVI, and the interaction between sagebrush type and NDVI (Table 4-4, Figure 4-3). In support of our prediction, for a first nesting attempt from the start of the growing season, the contribution of endogenous reserves was predicted to be similar in high-elevation MTSA and mid-elevation TTSA, but lower in low-elevation BBSA (Table 4-4, Figure 4-3). Contrary to our prediction, a female on her first nesting attempt and at the average date of initiation, the predicted contribution of endogenous reserves declined dramatically as plant greenness (NDVI) increased in TTSA, but not in MTSA or BBSA (Table 4-4, Figure 4-3). The estimated coefficient associated with the body condition covariate in the model containing body condition and nesting attempt overlapped zero (mean: -0.001; 90% CrI = -0.003 to 0.001).

Discussion

Variation in the allocation of nutrients towards reproduction can influence the timing of breeding, and ultimately reproductive output, as animals who breed earliest often have the greatest reproductive success (Drent and Daan 1980). Here, we carried out the first large-scale study on patterns of avian endogenous reserve dynamics by using modern stable isotope and statistical modeling techniques to test whether endogenous reserve storage may be an adaptively selected trait, and whether it is associated with breeding attempt and timing of reproduction. We found that the contribution of endogenous reserves for reproduction tended to be similar for first nesting and renesting attempts, which supports the hypothesis that females restore reserves during incubation and between clutches for future reproductive attempts. The contribution of endogenous reserves for reproduction was nearly twice as high in the Rocky Mountains as compared to values for the Great Plains ecoregion. This is consistent with the prediction that the contribution of endogenous reserves for breeding is greater where new plant growth is less available to females during egg production, especially at higher elevation sites in the Rocky Mountains. However, within-season declines in the use of endogenous reserves occurred more rapidly in the Rocky Mountain than Great Plains ecoregion, suggesting females in the Rocky Mountains begin nesting before vegetation green-up and are more dependent on endogenous reserves than females in the Great Plains. Females that nested later in the season in TTSA were more reliant on exogenous food sources to produce their clutches because they could not store sufficient reserves. Finally, we found little evidence of an association between maternal body condition and variation in endogenous reserves for offspring formation. For instance, Gregg et al. (2006) found that adult female sage-grouse initiated nests earlier than yearlings, regardless of female body condition, which could indicate that older, more experienced individuals may be utilizing more endogenous reserves than younger birds and be able to do so because of their larger size, storage capacity, experience fueling up, and knowledge of where to forage. Future studies could improve our understanding of how age and/or experience interact with body condition to influence reproductive output.

In support of the hypothesis that individuals use body reserves for renesting (Milonoff 1991), we found a similar contribution of endogenous reserves for renesting individuals. Except for extreme cases of certain species of geese, swans and emperor penguins (Klassen et al. 2006, Whiteman et al. 2020), the nutrients deposited into a clutch are a balance between endogenous nutrients acquired both distantly and locally, along with dietary nutrients (Stephens et al. 2009). Even though we found similar contributions of endogenous sources between first and subsequent renesting attempts, clutch size in sage-grouse declines precipitously throughout the nesting season, which indicates declines in the absolute amount of endogenous sources used for egg formation. From an evolutionary fitness perspective, females likely invest their endogenous nutrients in their first clutches for two reasons. First, the nutrients invested in the first clutch are more likely to affect fecundity, whereas nutrients that remain for a second clutch only come into play if the first clutch fails. Second, offspring from the first clutch may be more “valuable” because of their earlier hatch dates (Rowe et al. 1994). Our study supports the hypothesis that sage-grouse have a mixed breeding strategy in terms of the contribution of endogenous and dietary food sources for reproduction. We found that maternal sage-grouse used endogenous reserves for offspring production acquired both before and after initiation of the first nest as they used similar amounts of endogenous reserves across different breeding attempts (Klassen et al. 2006). Both maternal nutrition and endogenous stores can help buffer the costs of egg production and incubation, and both sources can allow females to quickly renest if their first nest fails, improving the chance of breeding success, increasing the condition of their offspring, and maximizing survival of their young (Moss et al. 1975, Sedinger and Raveling 1986). Future studies on how the timing of endogenous reserve acquisition (i.e., prior to or after the start of nesting) for breeding influences both the timing of renesting following the loss of the first nest, along with the quality and survival of their offspring will further elucidate the physiological mechanisms influencing reproductive success.

We found that this habitat specialist used greater amounts of endogenous reserves during the early season than in the later season, which was especially pronounced for the Rocky Mountain ecoregion. The principal advantage of stored reserves for breeding is that the reserves allow individuals to nest earlier than they otherwise would if they relied entirely on dietary nutrients. Early breeding is beneficial to maximize reproductive output in many temperate zone birds, including sage-grouse that raise a single brood per year at most. Using endogenous reserves for reproduction allows females to have greater flexibility as to when and where they initiate nesting, allowing them to quickly renest if their first attempt fails (Martin 1987), and to ensure that the timing of hatch is matched with optimal conditions for raising young (Dann et al. 1988). Results from our study suggest that focusing solely on conserving habitat used during the spring may not adequately meet the nutritional demands for breeding, especially for sage-grouse breeding in high-elevation landscapes.

Biologically important elevational gradients that occur over small spatial scales create heterogeneity in vegetation phenology (Crimmins et al. 2010), which promotes an evolved response to environmental variation in life-history strategies of animals (Bears et al., 2009, Stoner et al. 2020). Sage-grouse females tend to be philopatric to their nest sites, so females that first nest at high elevations continue to do so through their life. We found evidence that sage-grouse deal with low resource periods by deploying two nutrient-allocation strategies across a strong elevational gradient. The first major finding is that sage-grouse nesting in the high- and mid-elevation MTSA and TTSA had a greater contribution of endogenous reserves at the start of the nesting season compared to values for individuals nesting in low elevation BBSA. Blomberg et al. (2014) found that sage-grouse that nested at higher elevations had larger clutch sizes, and consequently greater fecundity (Cutting et al. 2019), than did birds that nested at low elevations. Another major finding is that sage-grouse relied more heavily on local exogenous food sources as NDVI values increased in TTSA. TTSA is found predominately at intermediate elevations and on south-facing slopes that receive higher solar radiation (Cutting et al., 2019) and where the green flush of new plant growth is generally most pronounced during the spring nesting season (Figure 4-5). Ungulates and other bird species alter the timing of their seasonal movements to the emergence of green plants (Thorpe et al., 2017; Geremia et al., 2019). Middleton et al. (2018) demonstrated that elk (*Cervus canadensis*) in the Greater Yellowstone Ecosystem moved to areas with green plants as a foraging tactic, resulting in gains of fat reserves that are positively correlated with reproduction and survival outcomes. For the first time, we show direct benefits on resource allocation strategies for offspring production by nesting in areas with more green food plants in this habitat specialist. Future work conducted in landscapes that have a diversity of plant phenologies should consider if sage-grouse and other species are preferentially feeding, producing larger clutch sizes and raising higher quality offspring in sites as they green up (e.g., Gibson et al. 2017), and, if so, whether individuals that do so have higher demographic rates than individuals that do not.

Conservation Implications

Our results show a striking physiological adaptation for early breeding using endogenous nutrient reserves in a habitat specialist. Sage-grouse conservation is primarily focused on improving habitat quality near leks (e.g., < 6.5 km from leks; Montana Sage-Grouse Habitat Conservation Program). Conserving habitats only near lekking areas may not protect all resources birds use to meet their nutritional requirements for reproduction, especially for migratory populations. The importance of habitat used during winter, where individuals build endogenous reserves are highlighted especially for individuals from the Rocky Mountains. Many birds in the Rocky Mountain landscape migrate long distances (>120 km) to their winter grounds (Cutting, *unpublished data*), so prioritizing these wintering areas for conservation is warranted. Frye et al. (2013) showed that wintering sage-grouse in southcentral Idaho selected areas with plants containing greater nutrient concentrations (i.e., crude protein) and lower concentrations of defensive chemistry profiles, relative to unused sites. Hupp and Braun (1989) demonstrated that sage-grouse in Colorado select areas with low snow depths (i.e., south and west aspects) and where mountain big sagebrush is above the snow for feeding during winter. We suspect that the two sage-grouse populations in this study from ecosystems in Montana are following a similar pattern, especially in the Rocky Mountain region given the high diversity of sagebrush types and topographic variability with varying snow depths. Importantly, areas that green-up earliest during spring, especially TTSA sagebrush located in intermediate elevations with south facing aspects, also appear to contribute greatly towards meeting the nutrient requirements for reproduction in this habitat specialist. This habitat also benefits many other species, such as elk, that use it during spring, which supports prioritization of this habitat for conservation. Future studies should couple stable isotope data with locational data to assess both *when* and *where* nutrients were acquired for breeding attempts in both successful and unsuccessful individuals, and to tie this to population dynamics models that incorporate individual variation in demographic performance (e.g., Ellner and Rees, 2006, Grimm et al., 2006).

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Figure 4-1. Map of the sagebrush ecosystem and greater sage-grouse distribution in the western United States. The Great Plains study site is located in eastern Montana (dashed oval), while the Rocky Mountain study site (solid oval) is located in southwest Montana, USA. Map layer for sage-grouse distribution is from Schroeder et al. (2004): layer for sagebrush ecosystem is from U.S. Geological Survey (2002).

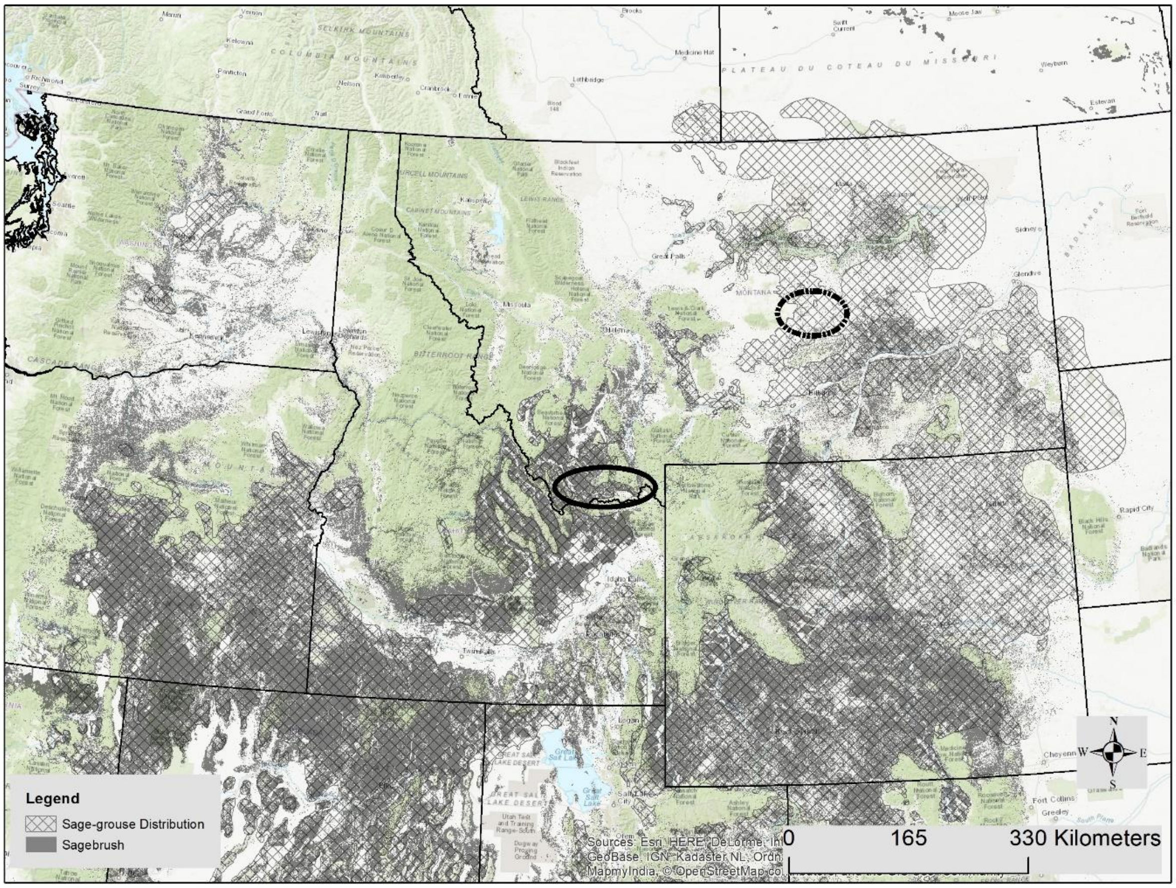

Figure 4-2. The predicted relationship between contribution of endogenous reserves for offspring formation in sage-grouse across two ecoregions, nesting attempt, and year. Lines connect predicted mean value while error bars depict 90% credible intervals.

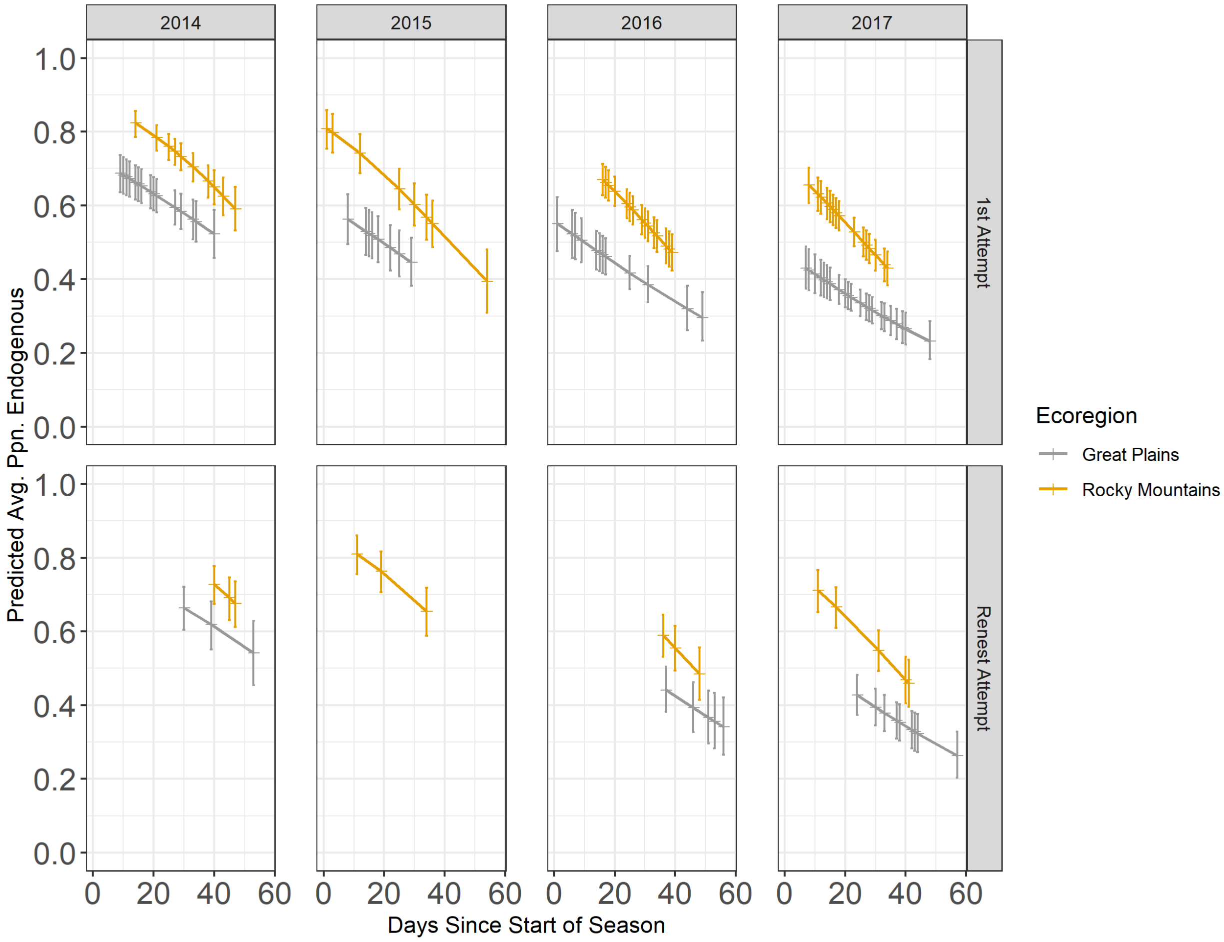

Figure 4-3. The predicted relationship between endogenous contributions for sage-grouse offspring formation and sagebrush types and plant greenness based on NDVI. Lines connect predicted mean value while gray shaded areas depict 90% credible intervals. Predictions were made for birds on their first nesting attempt, along with the average date of nest initiation for the three sagebrush types; initiation varied by only 6 days across the various sagebrush types.

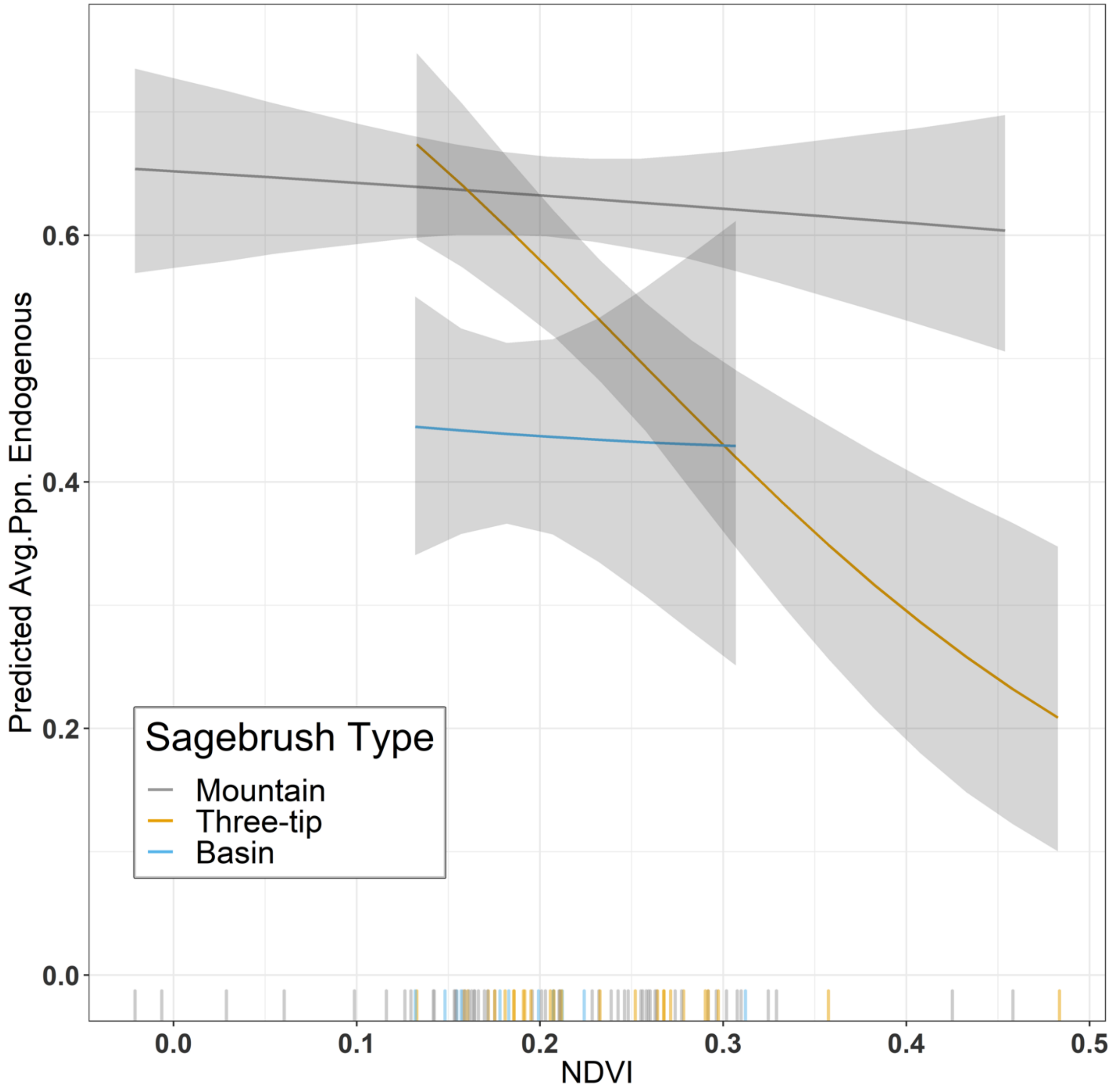

Figure 4-4. Stable carbon isotope results for adult female red blood cells (left of vertical white line), blood plasma (right of vertical white line), and offspring feather samples of sage-grouse collected across multiple years from Great Plains (top panel) and Rocky Mountains (bottom panel) ecoregions in Montana. Red blood cells represent nutrients acquired on winter habitats, while blood plasma represents nutrient acquired on spring habitats, based on isotope turnover in these different body tissues.

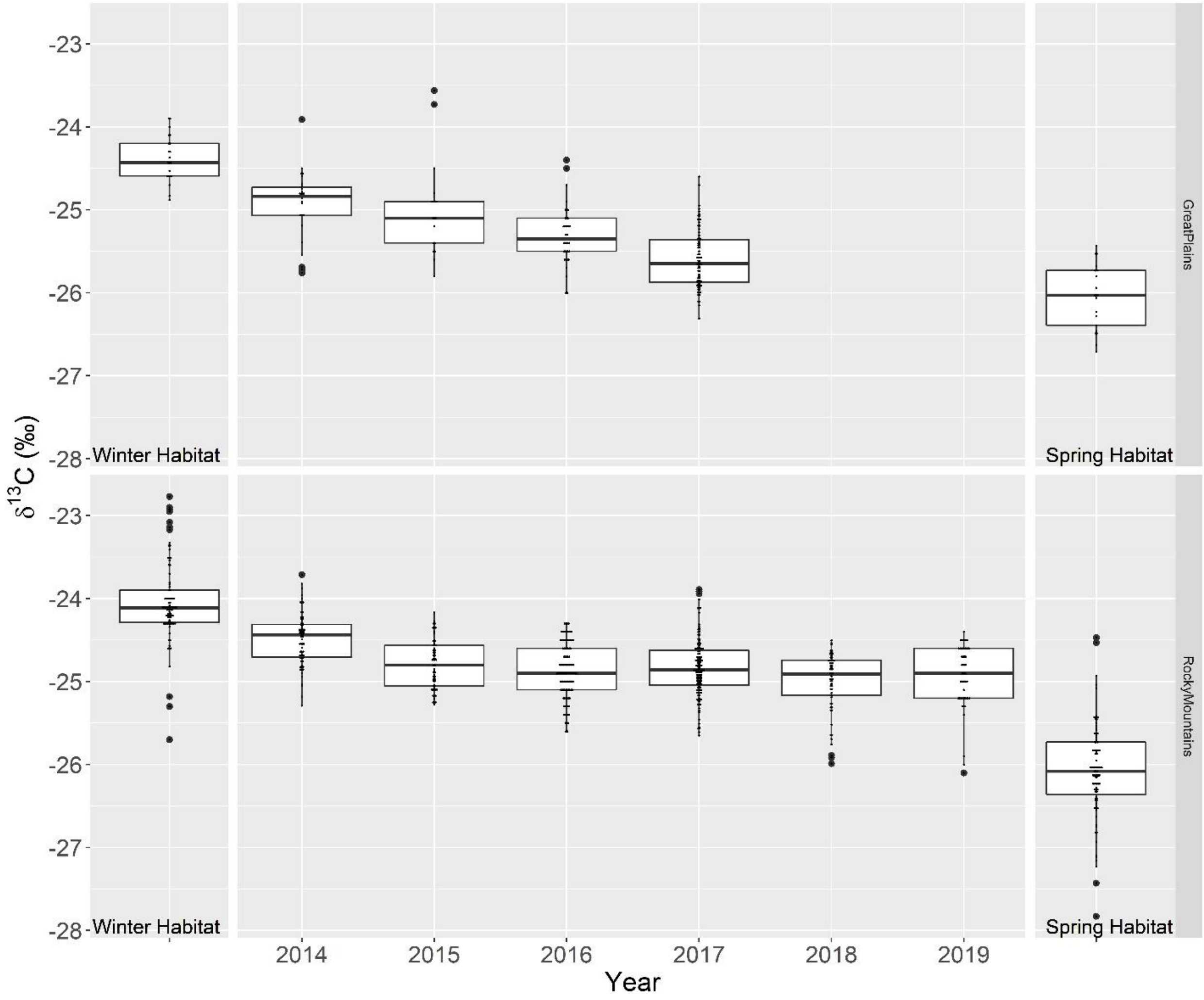

Figure 4-5. NDVI plant greenness values at sage-grouse nest sites across different sagebrush types from early (left panel), middle (middle panel), and late (right panel) in the nesting season, Rocky Mountains of southwest Montana. Barplots represent mean values while the error bars depict 95% confidence intervals from a regression model containing sagebrush type and initiation date as additive terms and NDVI as the response variable.

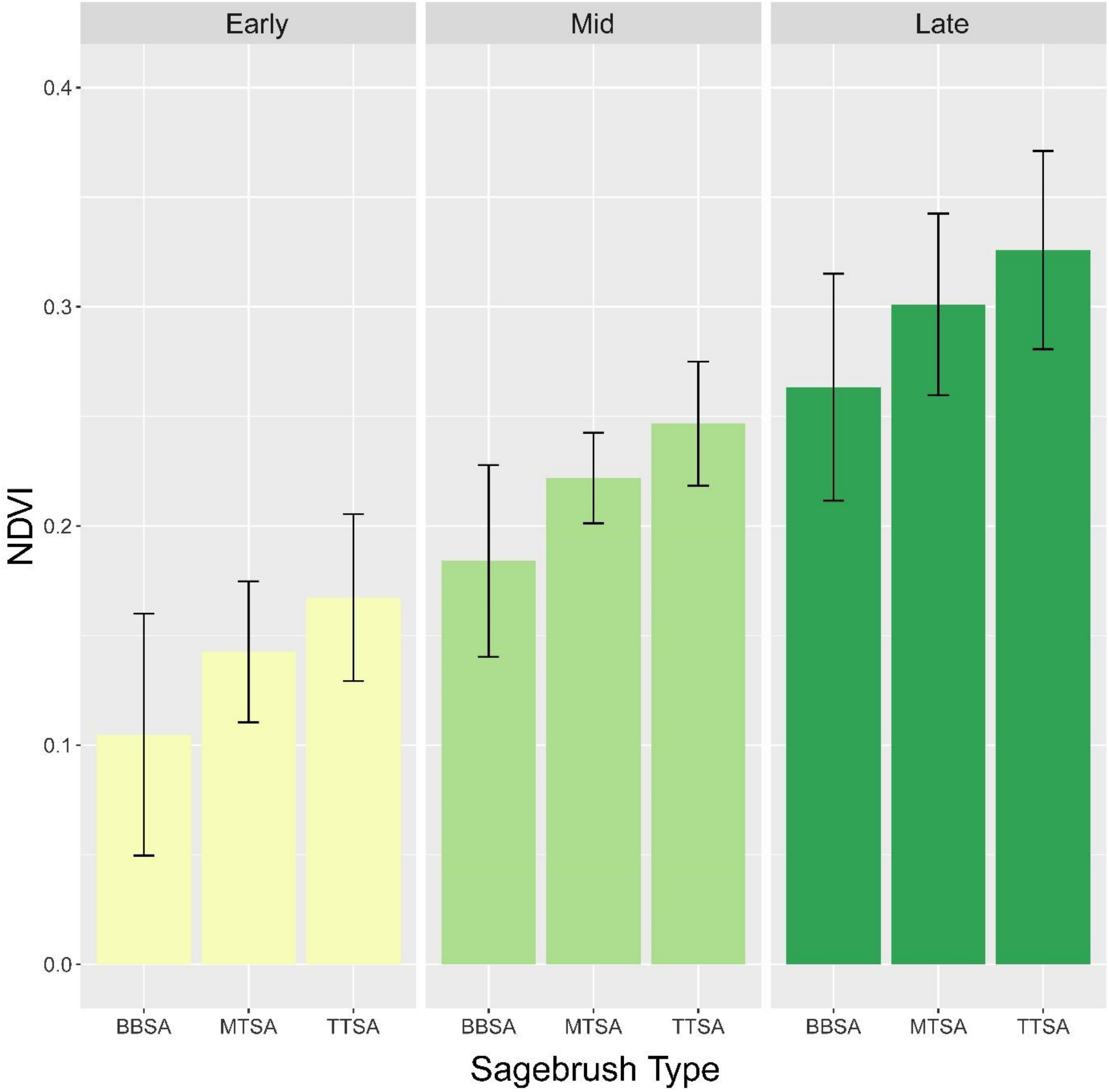

Table 4-1. Predictor variables used to explore variation in endogenous contributions to offspring formation in sage-grouse. Each variable class is categorized according to our main objectives (A and B).

A) Objective 1) *Endogenous Contributions to Offspring Across Ecoregions*

Variable	**Description**	**Type**
Nesting Attempt	Hatch offspring on first nesting vs. renesting attempt	Binary
Ecoregion	Great Plains vs. Rocky Mountains	Binary
Initiation Date	Date of initiation of each nest standardized within each ecoregion	Continuous
Year	Year of breeding seasons	Factor

B) Objective 2) *Endogenous Contributions to Offspring Within the Rocky Mountain Ecoregion*

Variable	**Description**	**Type**
Nesting Attempt	Hatch offspring on first nesting vs. renesting attempt	Binary
Initiation Date	Date of initiation of each nest standardized	Continuous
Body Condition	Size-adjusted body-condition for prebreeding female	Continuous
Sagebrush Type	Sagebrush type at nest site found across different elevations	Factor
Primary Productivity	Satellite-derived vegetative index (NDVI) at nest site	Continuous

Table 4-2. Comparisons between model suites describing the contribution of endogenous reserves for offspring production in sage-grouse. ELPD difference obtained through 10-fold cross-validation for Bayesian models, and SE difference is the estimated standard error of the difference.

a) *Objective 1: Endogenous Contributions to Offspring Across Ecoregions*

Model	ELPD difference	SE difference
Nest Attempt + Ecoregion * Initiation Date + Year	0	0
Nest Attempt + Ecoregion + Initiation Date + Year	-0.5	1.9
Nest Attempt + Initiation Date + Year	-24.3	6.4
Null	-52.12	9.8

b) *Objective 2: Endogenous Contributions to Offspring Within the Rocky Mountain Ecoregion*

Model	ELPD difference	SE difference
Nesting Attempt + MTSA*NDVI + TTSA*NDVI + Initiation Date	0.0	0.0
Nesting Attempt + MTSA^2*NDVI + TTSA*NDVI + Initiation Date	-0.2	1.0
Nesting Attempt + MTSA + TTSA + NDVI + Initiation Date	-2.9	2.5
Nesting Attempt + MTSA + TTSA + NDVI*Initiation Date	-4.3	2.7
Nesting Attempt + NDVI*Initiation Date	-11.1	3.8
Null	-13.7	5.1
Nesting Attempt + Body Condition	-15.6	5.2

Table 4-3. Parameter estimates from the top models describing variation of endogenous contributions to offspring formation in sage-grouse across ecoregion, nesting attempt, initiation date, and year, Great Plains and Rocky Mountains, Montana, USA.

Covariate	***Mean***	***sd***	***5% CrI***	***95% CrI***	$\hat{R}$
Intercept	0.746	0.227	0.458	1.038	1.001
Nest Attempt – First	-0.369	0.129	-0.531	-0.202	1.000
Ecoregion - Rocky Mountain	1.062	0.236	0.761	1.362	1.001
Initiation Date	-0.022	0.006	-0.03	-0.015	1.000
Initiation Date:Ecoregion	-0.013	0.008	-0.024	-0.003	1.001
Year contrasts	—	—	—	—	—
2014	0.613	0.088	0.499	0.729	1.000
2015	0.049	0.114	-0.095	0.196	1.001
2016	-0.157	0.082	-0.259	-0.052	1.000

Note: Sum-to-zero contrasts were used on the covariate *year* such that this parameter estimate represents the log-odds of endogenous contributions during an average year. The intercept is for a nest during the ‘average year’ from the second attempt (coded as 0 for renest) in Great Plains ecoregion (coded as 0 for Great Plains) with an initiation date value of 0.

Table 4-4. Parameter estimates from the top model describing variation of endogenous contributions to offspring formation in sage-grouse across nesting attempt, initiation date, plant greenness (NDVI), and sagebrush type, Rocky Mountains, Montana, USA.

Covariate	***mean***	***sd***	***5% CrI***	***95% CrI***	$\hat{R}$
Intercept	1.242	0.934	-0.252	2.812	1.001
Nest Attempt – Second	0.181	0.176	-0.105	0.470	1.000
Initiation Date	-0.011	0.006	-0.021	-0.001	1.000
MTSA	0.804	0.700	-0.346	1.921	1.003
TTSA	1.709	0.780	0.430	2.980	1.004
NDVI	-0.430	3.568	-6.403	5.425	1.004
MTSA:NDVI	-0.025	3.558	-5.704	5.888	1.003
TTSA:NDVI	-5.653	3.839	-11.888	-0.752	1.004

Note: Calculation of endogenous contributions for specific NDVI in different sagebrush types includes: 1) BBSA = -0.430, 2) MTSA = -0.430 + -0.025, and 3) TTSA = -0.430 + -5.653. The intercept is for a nest during the first attempt (coded as 0 for renest =1) in BBSA such that the NDVI covariate value is for BBSA.

Table 4-5. Variables describing endogenous reserve contributions for offspring production in Great Plains and Rocky Mountains of Montana, USA. Mean of metrics are listed with either ±1 SE or range of values.

Ecoregion:	**Great Plains** (mean ± SE)		**Rocky Mountain**	
Variable	First Attempt (*n* =75)	Renest Attempt (*n* =20)	First Attempt (*n* =74)	Renest Attempt (*n* =17)
Egg Lay Date	112.0 (± 1.3)	132.9 (± 2.0)	124.4 (± 1.4)	135.5 (± 2.4)
# Nests in MTSA	-	-	45	8
# Nests in TTSA	-	-	22	5
# Nests in BBSA	-	-	7	4
NDVI	-	-	0.20 (SE ± 0.01)	0.25 (SE ± 0.02)
Female Condition	-	-	1474.6 (range: 1344 to 1640)	1467.8 (range: 1380 to 1547)

CHAPTER 5

NONCONFORMITY IN SAGE-GROUSE BROOD SELECTION OF MESIC AREAS IN A HIGH-ELEVATION SAGEBRUSH ECOSYSTEM: IMPLICATIONS TO GRAZING MANAGEMENT

Abstract

Studies from the Great Basin have consistently shown that sage-grouse with broods select for sagebrush mesic areas containing green plants, but little information exists on whether selection is similar in mountainous landscapes in the Rocky Mountain ecoregion. Selection might be different in mountainous landscapes because upland rangelands in the Rocky Mountains have more mesic areas, with more precipitation, than what is available in the uplands of the Great Basin. I used both broad- and fine-scale resource selection analyses to assess habitat selection patterns of sage-grouse broods in a high-elevation sagebrush landscape with strong gradients in abiotic and biotic conditions along with a diverse network of grazing infrastructure. I found evidence that sage-grouse broods selected for more xeric sagebrush types, and for fine-scale topographic features. Grazing effects were also evident as sage-grouse broods avoided cattle paths. My findings differ from what would be predicted based on results from studies in the Great Basin. My results indicate that broods selected areas away from low-lying mesic and hydric areas, and near ridgelines on upper slopes with south-facing aspects that were further from cattle paths. Given differences in the selection patterns between the Great Basin and northern Rocky Mountains, my results suggest that management plans should account for the selection of upper slope areas located on south facing hillsides in low and intermediate

elevations. To avoid impacts to sage-grouse brood rearing habitat in mountainous landscapes, grazing managers should place grazing infrastructure, which can concentrate cattle and create paths, on the lower and/or flatter portions of a hillslope on north-facing aspects. My findings highlight the value of fine-scale studies that match the scale of most management and habitat management plans that are occurring in the sagebrush landscapes of the American West.

Introduction

The Sage-Grouse Initiative (SGI) is one of the world's largest conservation efforts that is occurring in the sagebrush biome across the American West (NRCS 2015). The program is centered on collaborative conservation between governmental agencies and private landowners. This has resulted in one of the largest coordinated conservation programs in the world, which undoubtably contributed to a 'no listing' decision for the greater sage-grouse (*Centrocercus urophasianus*) under the Endangered Species Act (USFWS 2015). The initiative has protected nearly 2.5-million hectares of sagebrush, providing habitat for sage-grouse, over the last 10 years by addressing primary conservation threats through the investment of nearly a billion-dollars. Sagebrush mesic areas is one of six key priorities for SGI (NRCS 2015); resulting in a multitude of conservation partners focused on restoring degraded mesic areas for water conservation (Silverman et al. 2016), soil moisture enhancement (Blankenship et al. 2014), and ecological benefits particularly to sage-grouse (Donnelly et al. 2016). Mesic habitat restoration has focused on low-lying meadows where low-tech restoration structures are installed to slow water flow and spread water to adjacent meadow surface to increase moisture levels or more area to improve plant growth (Zeedyk and Clothier 2014) and sage-grouse habitat (Nash et al. 2018, Silverman et al. 2020). Yet, for the Rocky Mountains, it is unknown if sage-grouse broods rely on these low-lying mesic areas, as sage-grouse have been shown to do in the arid landscapes of the Great Basin Ecoregion (Donnelly et al. 2016).

Models used to prioritize conservation at the landscape level have shown the importance of sagebrush mesic habitats for the survival of young sage-grouse during summer across the western portion of the Great Basin in the American West (Donnelly et al. 2016). Sagebrush mesic habitats provide succulent plants and abundant insect resources for young sage-grouse during summer months when water and moisture are limiting (Donnelly et al. 2016). Donnelly et al. (2016) demonstrates that populations of sage-grouse in the Great Basin increase, and are buffered against drought, when leks are near mesic, low-lying habitats, as sage-grouse move their broods to these habitats, especially during summer (Connelly et al. 2000). Fine-scale studies have corroborated this finding (Fischer et al. 1996, Atamian et al. 2010, Connelly et al. 2011, Casazza et al. 2011) as areas with moderate or hydric moisture regimes are often located next to flowing streams in low-lying mesic areas (Silverman et al. 2020). The sagebrush ecosystem in the Great Basin Ecoregion is largely considered xeric where moisture is the most limiting factor influencing plant growth during summer (Comstock and Ehleringer 1992). However, in mountainous landscapes located in the Rocky Mountains, mesic areas can also be found in high elevations and on north-facing aspects where moisture lasts longer into the summer (Miller et al. 2012). These sites also receive a greater amount of annual precipitation during the summer months than does the Great Basin Ecoregion (Chambers et al. 2017). It could be possible, however, that mesic areas are more limiting in arid places than in high-elevation sagebrush. High-elevation sagebrush rangelands of the Rocky Mountains contain most of the mesic habitat resources (Donnelly et al. 2018), as this region receives high precipitation and contains the coolest temperature regimes across the sagebrush biome (Maestas et al. 2016, Chambers et al. 2017). Thus, it is currently unknown if the resource selection models developed from the Great Basin Ecoregion conform to habitat selection patterns of sage-grouse broods in the Rocky Mountains.

Mack and Thompson (1982) argue compellingly that areas with strong coevolutionary history of large mammalian grazers do better with grazing pressure than areas without. For instance, bison once roamed the Rocky Mountains and Great Plains ecoregions where the plant communities have coevolved with herbivory as compared to the Great Basin ecoregion where large mammalian grazers were historically absent and is considered detrimental to the plant communities (Mack and Thompson 1982). In arid landscapes, grazing infrastructure (e.g., fences and water tanks) strongly influences grazing pressure and utilization of plants as livestock are concentrated near water sources and are prohibited to freely range due to infrastructure (Bailey et al. 2015). In large grazing units that are limited by water—some individual grazing units exceed 9,000 ha in the American West (Baily et al. 2015)—a low proportion of the area is utilized as cattle avoid traveling long distances from water (Bailey 2005). Thus, the distribution of water tanks and fences throughout large grazing units largely dictates the uniformity of grazing pressure and subsequent utilization of plants across the landscape (Cutting et al. 2019). Therefore, a common practice by rangeland managers is to relocate grazing infrastructure away from low-lying mesic areas as a mean to protect sensitive low-lying mesic areas from congregating livestock (Raynor et al. 2021). Relocating water tanks also provides greater distribution of cattle and utilization of vegetation, which influences weight gains in livestock (Augustine et al. 2013). Relocating water tanks is occurring across the mountainous landscape of southwest Montana, with a primary justification that sage-grouse broods are selecting low-lying mesic areas, as sage-grouse have been shown to do in the arid landscapes of the Great Basin Ecoregion (Donnelly et al. 2016). Cattle tend to concentrate their use on flat and low-lying mesic areas, which suggests a potential conflict between livestock grazing and sage-grouse broods. Relocating grazing infrastructure needs to be based on accurate data for a given site as sage-grouse are a surrogate species to conserve biodiversity (Rowland et al. 2006), and are used to generate financial support for grazing management projects focused at redistributing livestock to upland habitats, thereby facilitating mesic habitat improvements.

The most critical habitats selected by sage-grouse with young occur during the first few weeks of life as chick survival is lowest during this period (Dahlgren et al. 2010). Given the importance of chick survival to population growth in the species (Taylor et al. 2012), understanding habitat selection patterns immediately following hatch can help inform conservation plans and rangeland improvement projects. Sage-grouse young depend on their mothers for their survival up until 50 days of age (Atamian et al. 2010); after which time they become more independent and begin to flock with other birds. In mountainous landscapes of the Rocky Mountains, sage-grouse can move their broods to high-elevation sites and/or to northerly aspects to find mesic habitats and thereby have a lower reliance on low-lying meadow habitats as compared to birds in the Great Basin Ecoregion. Rangeland managers from sagebrush mountainous landscapes need information to confirm whether sage-grouse broods are using mesic habitats, either the low-lying mesic sites or the upper elevation mesic sites, so they can better manage grazing across the landscape. Thus, in the research reported here, I evaluate if habitat selection by sage-grouse females raising broods in high-elevation ecoregion of the northern Rocky Mountains show similar patterns for selection of mesic meadow habitats, or related habitat features that increase moisture later into the summer, similar to previous studies from the Great Basin.

Methods

Study Area

We conducted the study on a 65,000-ha mountainous landscape in southwest Montana, USA. The region is considered biologically diverse, supporting a multitude of sagebrush types. Sagebrush in my study area occurs across an elevation gradient from 2,000 m to 2,900 m. Dominant stands of sagebrush include: mountain big sagebrush (*Artemisia tridentata* ssp. *vaseyana*; hereafter: MTSA), basin big sagebrush (*Artemisia tridentata* ssp. *tridentata*; hereafter: BBSA), and three-tip sagebrush (*Artemisia tripartita*; hereafter: TTSA). Basin big sagebrush occurs in valley bottoms with deep soils, TTSA occurs at mid-elevation south-facing aspects, while MTSA occurs at the highest elevations and/or north facing aspects. The dominant land-use practice across all of the American West, including my study site, is livestock grazing. Virtually all of the study area has experienced recent grazing activity as grazing units in the study area have a maximum of 1-3 growing seasons worth of rest in-between grazing. Average annual precipitation and temperature at my study site is 50 cm and 1.6^{o}C, respectively.

Female Capture, Measurement, and Tracking

We captured 238 female sage-grouse across ten breeding leks using spotlights and dip nets from 2014 to 2019 (2014: n = 51, 2015: n = 55, 2016: n = 57, 2017: n = 45, 2019: n = 30). Capture and radio-telemetry techniques can be found in Cutting et al. (2019). The research was conducted in compliance with state (2014-40, 2015-023, 2016-049, 2017-043-W, 2018-003-W, 2019-078-W), and animal care/use (2012-40, 2015-30, 2019-88) permits.

We tracked individual radio-marked female grouse to discover their nests from early-April to mid-June. Once a nest was found, we monitored it until it either hatched or failed. If a female's nest failed, we continued to track the female until mid-June to determine whether she initiated a second nest. If a female's nest hatched, we obtained daytime locations between the hours of 7:00 to 20:00 for a total of 2-3 locations per week for 30 days after hatching. We randomized the order in which we obtained locations for each brood, each day

Habitat Selection Design

We assessed fine-scale selection defined as Type IV following Johnson (1980). To assess fine-scale habitat-selection patterns for sage-grouse broods, we paired use and random locations by brood and date. Given an average home range size of about 100 hectares for sage-grouse broods in Montana (Wallestad 1971), We paired each use location with one randomly selected available point that was 100-500 m away given the home range size (Wallestad 1971) of sage-grouse broods from Montana based on a randomly chosen distance and bearing. The number of use/available points ranged between 1-12 pairs of points per brood.

Given most mortality of sage-grouse young occur in the first 2 weeks of life (Dahlgren et al. 2010), we included age group as a covariate for younger (1-15 d old) and older (16-30 d old) broods. We collected at least 1 pair of use-random locations for a given individual per age class. We decided if a female still had young by flushing the brooding female roughly once per week during the twilight hours prior to sunrise when air temperatures were above freezing and when the preceding night did not receive precipitation. We ceased tracking and data collection for females that lost young at the last location they were observed.

Mesic Variables

We compiled information on covariates that characterize mesic areas in mountainous landscapes including elevation, slope position, aspect, and distance to low-lying area that contains high amounts of moisture (Table 5.1). In mountainous landscapes, slope and aspect vary greatly by location and affect site-specific productivity and reproductive patterns in sage-grouse (Stoner et al. 2020). We predicted that sage-grouse broods would select higher elevation sites as these areas contain more mesic areas than intermediate and low elevation sites. We predicted sage-grouse broods in the mountainous landscape would select locations as compared to available that were 1) higher elevations, 2) lower on hill slopes that tend to contain more moisture, 2) more northerly aspects, and/or 3) closer to low-lying mesic areas (Donnelly et al. 2016, Donnelly et al 2018). We calculated elevation, slope and aspect, of each use and random location using a digital-elevation model (30-m resolution) in the Geomorphic and Gradients Toolbox (Evans et al. 2014) of ArcGIS. Aspect position ranges from -1 to 1 with the former being located on south facing while the latter is located on north facing. Slope position reflects the portion of a hillslope on which a point is located such that negative values are nearest the bottom, and positive values are located on upper portions of a slope. We calculated the distance from each location to the nearest low-lying mesic water source using the National Hydrography Dataset.

Grazing Variables

Cattle grazing is the most common land use practice in the sagebrush biome of the

American West (Knick et al. 2003). It seems plausible that the placement of grazing infrastructure can influence the selection patterns of sage-grouse broods, through direct or indirect effects, if the infrastructure is placed in critical habitats for sage-grouse, given the species has been shown to avoid infrastructure (Hovick et al. 2014). We compiled information on covariates related to livestock grazing including: 1) distances to the nearest fence, 2) water tank, and 3) cattle path for each paired use and random site (Table 5.1). We used Google Earth (image date: 19 July 2014) to measure distances from brood use and random sites to the nearest path. We assumed that most paths in my study area are created and used by cattle because most paths had at least one end at a water tank, fence, or road. We used ArcMap 10.7.1 (ESRI, Redlands, California, USA) to measure distances from the brood use and random points to the nearest tank or fence. We calculated distances to the nearest fence and water tank from a brood use or random point using existing data on grazing infrastructure for the study area. We confirmed, and when necessary modified, the locations of water tanks and fences based on results of intensive field validation surveys. We predicted sage-grouse broods would avoid locations as compared to available related to grazing infrastructure or cattle paths.

Cover Variables

We measured vegetation characteristics that might be indicators of mesic areas at small spatial scales (i.e., grass, and shrub cover) at use and random locations using a modified line-point intercept method (Herrick et al. 2017). Detailed descriptions of the plant survey protocol can be found in Cutting et al. (2019). To minimize investigator disturbance, we recorded field measurements for vegetation and insects when broods moved away from previous locations (average number of days between brood location and field derived measurement was 23 days [±

14 SD]). We predicted sage-grouse broods would prefer locations with high amounts of vegetation cover as compared to available locations as these sites contain more vegetation that provides better hiding cover from predators and weather and with increased food.

Food Variables

We compiled information on forb cover (described previously) and insect biomass of preferred food items and used them as covariates in the 'food' model suite. We placed pitfall trap grids at each use and random location. Each pitfall grid consisted of 5 plastic cups (11 cm in diameter, 14 cm deep) placed at the axis and endpoints of two perpendicular transects with 30 m spacing between cups. We added water to each cup (~3 cm deep) to prevent insects from escaping. Invertebrate samples were emptied from cups every 2 days and preserved in 95% ethanol solution. We defined catch per unit effort (mg per trap day) by adjusting invertebrate biomass by the number of trap nights an individual trap (cup) was deployed. We sorted invertebrate samples to taxonomic order that are commonly consumed by sage-grouse chicks, including grasshoppers (*Orthoptera* spp.), beetles (*Coleoptera* spp.), ants (*Hymenopterans* spp.), and caterpillars (*Lepidoptera* spp.; Peterson 1970). We dried samples of each invertebrate order at 60^{o}C for 48 hours and weighed them to the nearest 0.1g. We summed insect orders to derive total insect biomass of preferred sage-grouse chick food items. For 11 of 302 trap grids at use sites that were destroyed by ungulates, we estimated insect biomass using a linear regression model based on data from other traps to predict insect biomass for the given sagebrush type, date, and year for the trap loss occurrence. We predicted that sage-grouse broods would prefer locations with high amounts of insect biomass as compared to available given their importance to the development of young sage-grouse during the first few weeks of life.

Statistical Analyses

We assessed the importance of covariates from different covariate suites (i.e., mesic, grazing, cover, food) on habitat selection at fine scales using the following steps. We tested for collinearity among covariate values using variance inflation factors and pairwise correlations. As we found all values to have low VIF and correlation (VIF < 1.5, and correlation <0.50 for all variables), all covariates were used in further analysis. To assess potential threshold effects in covariate conditions on brood selection, we took the natural logarithm of all variables. We then compared support from the data for pseudo-threshold and linear functional forms (Franklin et al. 2000) of each covariate using AIC_c and used whichever form was better supported in further analyses. We used conditional logistic regression ('clogit') models in the *survival* package to evaluate competing models of habitat selection at fine scales. Given the nested structure of the data between the use and available points, all competing models included individual brood as the 'cluster' term and the date when data for a given use-random pair of points were collected as the 'strata' term. We then built a total of 4 separate models one for each covariate suite that contained additive terms for all covariates within a given suite (Table 5-1). In addition, we also built a global model containing all variables across suites, and a null model. We then evaluated the influence between *brood age* and other covariates from the best supported model to assess if selection changed as broods aged. For each model that was within 4 AIC_c units of the best-supported model (lowest AIC_c score; Burnham and Anderson 2002), we evaluated the magnitude of each estimated coefficient and the extent to which the 90% confidence interval for the estimate overlapped one. We used odds ratio for all interpretations to describe habitat selection as values <1 suggests selection against a given covariate, whereas >1 indicates selection for.

We projected brood site selection across the study area for coefficients that had 90% confidence intervals that did not include 1, and where we had continuous GIS raster layers. As an approximation of average brood selection patterns, we included only the mean effect of a given covariate on brood selection. Given distance to cattle paths was a field-derived variable, we could not include this variable in the map's creation. We projected these results by creating a map representing brood site selection based on the relative strength of selection by presenting odds ratios: 0.4-1 (i.e. no selection), 1-2 (i.e. weak selection), 2-3 (i.e. moderate selection), and 4-7 (i.e. strong selection). The map represents the mean effect of the well-supported covariates on brood selection. We masked out habitats not inhabited by sage-grouse broods, including lakes and forests.

Results

From 2014 to 2019, we monitored 96 different sage-grouse broods and collected data at 304 pairs of use and random locations including 198 pairs of locations from 96 younger broods (1-15 days old) and 106 pairs from 47 older broods in age (16-30 days old).

Local-Scale Selection

To assess fine-scale selection patterns, we present results as odds ratio, which express the difference in odds between the selection of a site where the covariate level increases, versus a baseline for a given covariate. Among the 4 individual covariate suites considered, the grazing suite was clearly best as it had the lowest AIC_c score (Table 5-2), and the covariate distance to cattle path had an estimated coefficient that had a 90% confidence interval that did not overlap 1. The global model containing covariates from all model suites performed best, including the variables distance to nearest cattle path, and several covariates from the mesic suite, including elevation, slope position, aspect position, and distance to nearest low-lying mesic water source, while the effect of other variables were estimated imprecisely. All of these coefficients had 90% confidence intervals that did not include 1 (Table 5-3; **Error! Reference source not found.**). While holding other variables at their mean constants, sage-grouse broods were more likely to select sites that were further from cattle paths ($\hat{\beta}_{\text{cattle path}} = 1.241$, SE = 0.070), as broods were 1.41 times (90% CI: 0.96-2.09) more likely to select sites 1,119 m (i.e., natural logarithm of distance = 7.02) than sites <1m (i.e., natural logarithm of distance = -6.90) from cattle paths. Sage-grouse broods tended to select lower elevation sites ($\hat{\beta}_{\text{elevation}} = 0.99$, SE = 0.0004), as broods were 0.90 (90% CI: 0.69-1.18) times more likely to select a site in the lowest (i.e., 2,032 m) than a site in the highest (i.e., 2,603 m) elevation. Sage-grouse broods were more likely to select sites that were on the upper portions of a slope, near ridgelines ($\hat{\beta}_{\text{slope}} = 1.008$, SE = 0.003), as broods were 1.71 times (90% CI: 1.22-2.39) times more likely to select sites on the uppermost slope position than a site on the bottom. Sage-grouse broods selected for more south facing aspects ($\hat{\beta}_{\text{aspect}} = 0.585$, SE = 0.169); broods were 1.25 times (90% CI: 0.85-1.83) more likely to select a directly south facing aspect than a directly north facing aspect. Finally, sage-grouse broods selected for areas further from the nearest low-lying mesic habitat ($\hat{\beta}_{\text{distance to water source}} = 1.141$, SE = 0.070); broods were 4.27 times (90% CI: 2.08-8.73) more likely to select sites 1,775 m (i.e., natural logarithm of distance = 7.48) than a site 6m (i.e., natural logarithm of distance = 1.79) from a mesic source. A model containing brood age as an interaction term with all covariates within the global model performed worse than the top model ($\Delta AIC_c = 22.19$); the estimated coefficients plus the 90% confidence interval between early and late ages overlapped each other for all variables in the global model.

Spatial variation in selection for preferred brood sites (i.e., odds ratio >1) indicates that selected brood sites are a minor component of the total sagebrush landscape at the study site (**Error! Reference source not found.**).

Discussion

Sagebrush mesic areas are critical for water conservation, forage production for livestock, and habitat for diverse wildlife populations in arid regions of the American West (Silverman et al. 2019, Donnelly et al. 2016). Results from previous studies conducted in the Great Basin indicate that female sage-grouse with broods tend to move to low-lying mesic habitats with less-variable topography (e.g., wet meadows, riparian areas, wetland margins, and agriculture fields) during summer as a direct response to environmental drying and desiccation of plants (Fischer et al. 1996, Atamian et al. 2010, Connelly et al. 2011, Casazza et al. 2011, Donnelly et al. 2016, Baxter et al. 2017). However, in high-elevation landscapes that receive greater amounts of precipitation and have diverse topographic features (e.g., slope, aspect, elevation), moisture can be present later in the growing season than it is in more arid landscapes such as the Great Basin allowing sage-grouse to use these additional areas. We found sage-grouse broods prefer low and intermediate elevations, respectively, more than high elevations. We also found that sage-grouse broods selected habitats on upper slopes located on southerly aspects further from low-lying mesic areas. These upper-slope, south-facing habitats provide a diversity of environmental and topographic characteristics that positions sage-grouse broods to deal with the highly stochastic conditions common to the Rocky Mountain Ecoregion (e.g., variable weather, heterogeneous food conditions, and predation risk; (Cutting et al. 2019).

In the northern Rocky Mountains, sage-grouse broods appear to favor sagebrush in low and intermediate elevations over high-elevations. The northern Rocky Mountain Ecoregion receives nearly twice the precipitation as the Great Basin Ecoregion, which allows sage-grouse broods to use upland rangelands for brood-rearing habitat (Chambers et al. 2017). Donnelly et al. (2018) reported that the greatest proportion of mesic habitat in the Rocky Mountains occurs in the upland rangelands, whereas in the Great Basin it tends to be found in low-lying riparian and wet meadow areas. Vegetation at our study site stays mostly green throughout much of the brood-rearing season, suggesting mesic habitat is not limiting during the time when most chick mortality occurs (Dahlgren et al. 2010). For instance, satellite derived NDVI plant greenness values at both brood use and random location were >0.5 (K. Cutting, unpublished data), which is greater than the threshold of 0.3 indicating mesic areas (Donnely et al. 2016). Locations with higher moisture, similar to sites commonly found in the northern Rocky Mountains, allow sage-grouse broods to have smaller home ranges (Wallestad 1971, Drut et al. 1994), which could increase survival as movement rates have been found to negatively impact brood survival (Gibson et al. 2017). During summer, low-lying mesic habitats in arid landscapes are often the greenest locations, which concentrates both predators and prey (Coates et al. 2016) and could create an ecological trap (Robertson et al. 2013), a possibility that merits further study. Future studies should evaluate if survival differs for chicks that spend more time in different sagebrush types, and across different types of mesic resources.

At my study site, sage-grouse broods were associated with upper-slope positions that are primarily found on south-facing aspects and away from low-lying mesic areas (e.g., meadow, riparian, agricultural areas). Use of south-facing and upper hillsides near ridgelines in mountainous sagebrush landscapes provides several benefits to sage-grouse broods. For instance, on or near upper slope ridgelines, vegetation cover is lower and rock cover is greater, which allow young sage-grouse to increase their micro-scale movements and foraging efficiency. South-facing ridgelines are also warmer, with enhanced solar radiation producing warmer soil temperatures, earlier green-up of forbs and availability of insects consumed by adults and young (Blomberg et al. 2013, Cutting et al. 2021), than north-facing aspects. Birds using ridgelines have better vantage points for seeing predators, and young birds learning to fly can move more easily downhill than they can when attempting to fly out of low-lying meadows. I also found evidence that broods tended to select areas that were further from cattle paths than what was typically available, which has grazing implications; cattle tanks are commonly moved to ridgelines which likely impacts critical brood rearing habitats through the creation of cattle paths that radiates outward from the water tank. My results contrast strongly with findings from arid regions (Donnely et al. 2016, Fischer et al. 1996, Gibson et al. 2017, Dahlgren et al. 2010), suggesting a one-size-fits-all approach to conservation of sage-grouse brood habitat could be detrimental to the species who reside in biologically diverse, high elevation ecosystems located in the northern Rocky Mountains region.

Conservation Implications

Influential conservation-based programs are currently focused on restoring degraded mesic habitats across the sagebrush biome for sage-grouse brood-rearing habitat (NRCS 2015). I found that the Sage-Grouse Initiative could improve their conservation delivery by using information for specific sites and/or localized regions. I found that habitat selection of sage-grouse broods from the northern Rocky Mountains, a location important for sage-grouse conservation and also one of the most biological diverse sagebrush ecosystems in the entire American West (Maestas et al. 2016), does not conform to selection patterns that have been reported for the arid Great Basin Ecoregion. Though we do not question the value of mesic habitats for water storage, conservation, soil moisture enhancement, and forage production to other wildlife and livestock, we do question the importance of mesic habitats to sage-grouse brood rearing habitats during a time when most mortality occurs, at least for the northern Rocky Mountains. Movement of grazing infrastructure to upper slopes, and south-facing ridgelines, at intermediate elevations, will likely negatively impact critical brood rearing habitat. Additionally, south-facing upper slope areas are also the most suitable areas for cheatgrass invasion (West et al. 2017), which could be further promoted through the establishment of grazing infrastructure along with the creation of cattle paths. At least from a sage-grouse conservation perspective, we encourage land managers to consider moving grazing infrastructure to north aspects and the flatter benches at the bottom part of a hillslope to reduce impacts to sage-grouse brood rearing habitat. Additionally, these same sites are the least suitable for cheatgrass growth (West et al. 2017). It is important to note that sage-grouse in our study area may simply be selecting non-mesic areas simply because the low-lying mesic sites are impaired. It is also important to note that we assessed habitat selection during the early and mid-summer (May-August), a time when moisture is still high, and the environment has yet to dry. In making these management recommendations based on brood-use data we are assuming that the brood habitat selection that we have observed is ‘adaptive’, meaning that sage-grouse broods in my sample are making wise choices that maximize their survival. We will test this assumption in the future using chick survival and fitness data that extends beyond just habitat selection patterns for broods.

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Figure 5-1. Predicted habitat selection patterns (odds ratios) for sage-grouse broods. Values for one continuous covariate were altered while all others variables were held at their mean values for the following covariates: elevation, aspect position, slope position, stream distance [natural logarithm of, meters], path distance [natural logarithm of, meters], fence distance [natural logarithm of, meters], water tank distance [natural logarithm of, meters], insect biomass [natural logarithm of, meters], forb cover, grass cover, shrub cover [natural logarithm of]. Shaded region represents 90% confidence interval, whereas solid line shows mean effect.

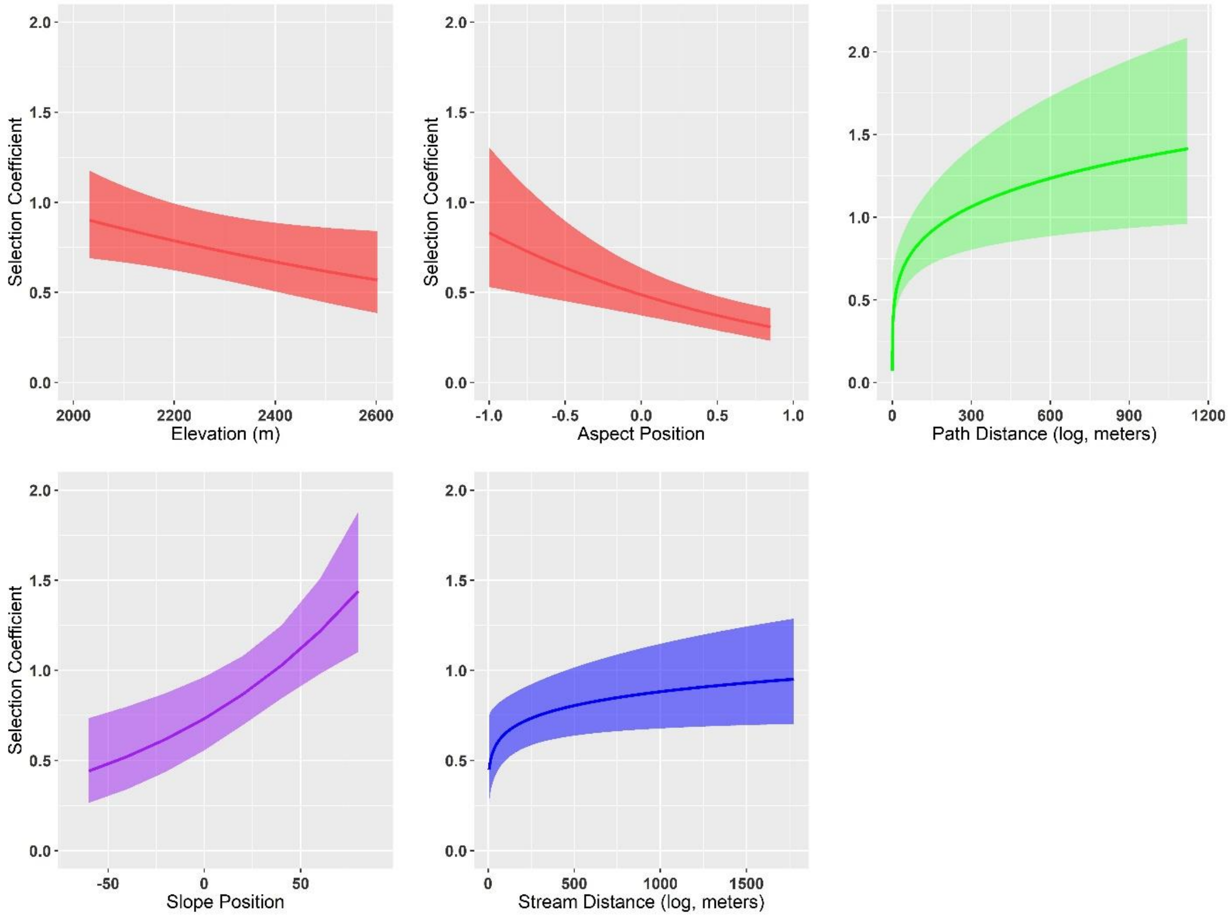

Figure 5-2. Map of brood habitat selection scores predicted from a resource selection function developed from sage-grouse brood locations. Brood site selection was modeled using a conditional logistic regression model of used and random locations paired for a given bird and date. The average estimated coefficient was used to create the map from a model containing slope, aspect, and distance to water source. Continuous values were reclassified based on the odds ratio from the top model. Locations of sage-grouse broods were collected using radio-telemetry in southwest Montana, United States, 2014-2019.

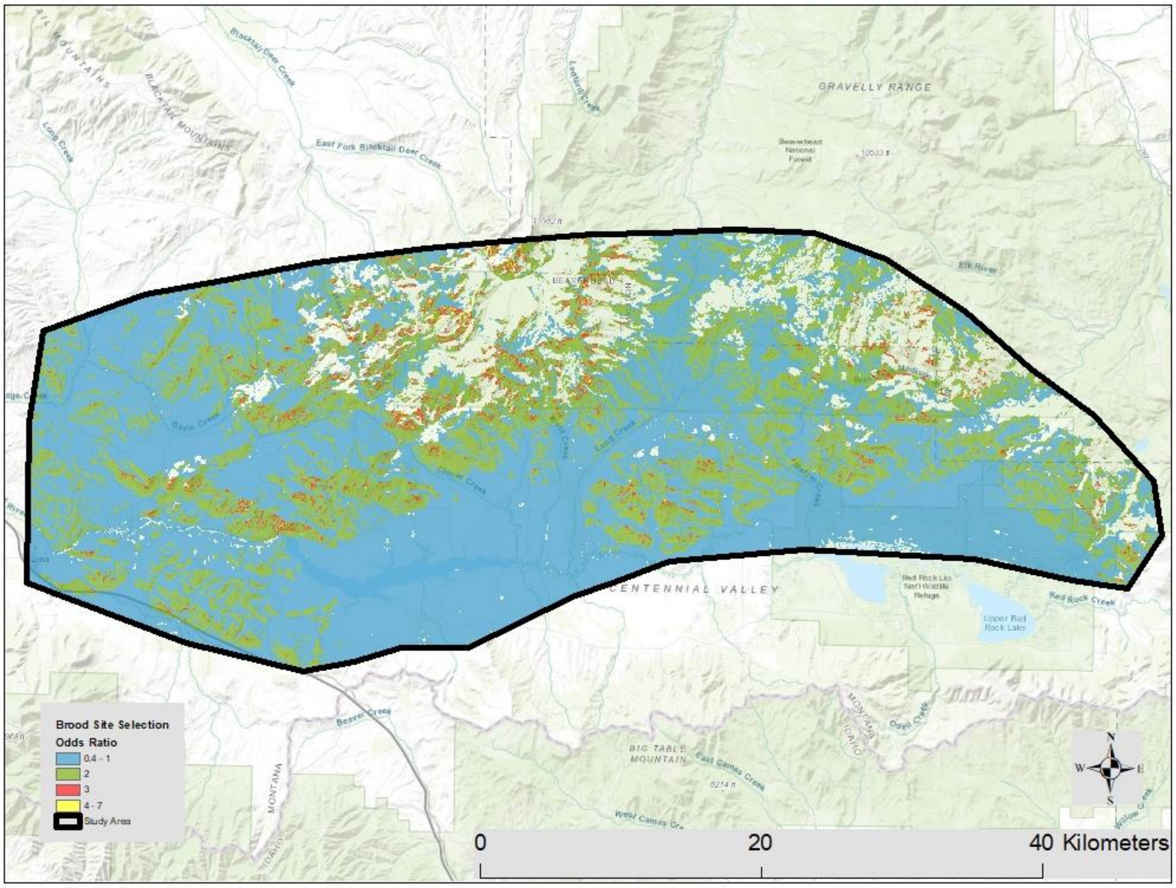

Table 5-1. Covariates used in the brood selection analyses. Each variable is categorized according to the covariate model suite.

Category	**Variable**	**Description**	**Type**
Mesic	Elevation	Elevation (m)	GIS 30 m^2
Mesic	Slope Position	Relative position from bottom (negative value) to top (positive value) of hill slope	GIS 30 m^2
Mesic	Aspect Position	Relative orientation to north (1) /south (-1) aspects	GIS 30 m^2
Mesic	Water Source	Natural logarithm distance	GIS
Grazing	Path	Natural logarithm distance	Google Earth
Grazing	Fence	Natural logarithm distance	GIS
Grazing	Water tank	Natural logarithm distance	GIS
Cover	Grass Cover	Accumulated percent cover	3x30 m transect
Cover	Shrub Cover	Accumulated percent cover	3x30m transect
Food	Forb Cover	Accumulated percent cover	3x30 m transect
Food	Insect	Biomass	Pitfall Trap

Table 5-2. Model-selection results for competing models of habitat selection by sage-grouse broods. Model suite names indicate the covariates considered within each suite. The global model contains all variables across all suites. Minimum AIC_c score for top model is 864.5.

Model Suite	**ΔAIC_c**	**AIC_c weights**	**Number Parameters**
Global	0	0.83	11
Grazing	3.23	0.17	3
Mesic	28.21	0	4
Food	36.73	0	2
Cover	37.38	0	2
Null	39.09	0	1

Table 5-3. Coefficient estimates from top model describing sage-grouse brood selection. Models assessed influence of mesic, grazing, cover, and food variables on sage-grouse brood selection. Asterisks next to a given covariate indicates that the estimated coefficient had a 90% confidence interval that did not overlap 1 suggesting selection for (>1) or against (<1) the given covariate.

Category	Covariate	Estimate	SE	90% LCI	90% UCI
Mesic	Elevation*	0.99	0.0004	0.998	1.000
Mesic	Slope Position*	1.01	0.003	1.00	1.01
Mesic	Aspect Position*	0.59	0.17	0.31	0.87
Mesic	Water Source*	1.14	0.07	1.03	1.26
Grazing	Path*	1.24	0.07	1.13	1.35
Grazing	Fence	1.06	0.05	0.98	1.13
Grazing	Water tank	1.06	0.04	0.99	1.13
Cover	Grass Cover	1.00	0.002	1.00	1.00
Cover	Shrub Cover	1.02	0.06	0.92	1.11
Food	Forb Cover	1.00	0.003	0.99	1.01
Food	Insect Biomass	1.05	0.06	0.95	1.15

CHAPTER 6

CONCLUSIONS

In this dissertation, I assess various components of the breeding ecology of sage-grouse in the high-elevation landscape of southwest Montana. One of my chapters (Chapter 3) covers another ecoregion that compares nutrient allocation strategies for breeding birds in southwest versus central Montana. I assessed the following aspects throughout the dissertation: 1) how females select nest and brood sites based on sagebrush type, along with livestock grazing features, and other biotic and abiotic characteristics; 2) the influence of female nest-site selection on nest-survival outcomes; 3) experimentally derived isotopic discrimination values for a new technique intended to assess nutrient allocation strategies for breeding in wild sage-grouse; and 4) the degree to which females allocate nutrients from winter habitats for breeding. Below, I summarize key findings across all the chapters and end by recommending (a) management actions to conserve and improve sage-grouse habitat, (b) future research that would be useful, and (c) overall status of the sage-grouse population and grazing in the Centennial Valley.

Sage-grouse nest survival is an important vital rate that influences population size of the species (Taylor et al. 2012). In Chapter 2, I found that, based on nest survival outcomes, sage-grouse were making poor decisions (i.e. maladaptive) when selecting sagebrush type for nesting: in the most-preferred (i.e. low-elevation) sagebrush type, nest survival rate was one-fourth the rate realized by females nesting in the sagebrush type avoided (i.e. high-elevation). Next, I found evidence of a grazing effect linked to fences that is relevant for future grazing management. Nest survival was four times higher for nests placed away from (>100 m), rather than next to (1 m), the nearest fence. I also found that nest survival was lower within sites with higher cow pie density (a proxy for previous grazing intensity). Live and dead grasses influenced selection and survival in opposing ways such that dead grass was selected for but resulted in reduced survival while live grass was avoided but resulted in increased survival. Other variables that were meaningfully related to sage-grouse nest site selection included: forb cover, distance to road, heat loading and cattle path. Sage-grouse selected sites with more heat loading index, such as those that are south facing which receive more solar radiation in the spring. Sage-grouse also avoided cattle paths, which indicates that land managers should avoid placing infrastructure in key nesting areas located on south facing hillsides especially in three-tip and mountain big sagebrush. Results in aggregate provide the first empirical evidence that a specific type of sagebrush acts as an ecological trap while another sagebrush type is undervalued. These results also suggest that adding more fences to control livestock grazing systems could be reasonably expected to reduce sage-grouse nest survival. Finally, these results demonstrate how land managers could strategically place grazing infrastructure across the landscape that provides greater sustainability to grazing programs by considering wildlife and their habitat.

In Chapter 3, I presented the first ever experiment to report isotopic discrimination values for maternal blood constituents-down feathers of offspring in avian taxa. This new technique can be used in grouse conservation as it’s a new way of prioritizing when nutrients were acquired that are later used for breeding. Stable isotope techniques can directly trace nutrients across time and space as nutrient pools are stored in body tissues (e.g., muscle) of female birds and later deposited for offspring formation. The power of using blood constituents to assess timing of nutrient allocation is that it circumvents the assumptions of assumed foraging patterns in birds, as is the traditional use of the isotopic technique. For instance, red blood cells represent diet during a period of 1-2 months prior to sampling while plasma represents a period of 1-2 weeks prior to sampling. Dietary sampling to assess nutrient dynamic strategies using stable isotope techniques carries the assumption that the correct dietary items were sampled during the previous periods of interest which is problematic for species that migrate long distances from wintering areas to breeding areas and where a diversity of habitat types are used for foraging during migration. My experiment also provides a sampling technique that is less invasive than those previously available; collecting down does not require destructive sampling of viable eggs or individual birds. With the isotopic discrimination values provided here, future researchers working on gallinaceous birds can use these isotopic discrimination values to identify nutrient sources—along with the timing of nutrient acquisition—used by adult female birds for offspring development.

Using the experimental results from Chapter 3, I assessed in Chapter 4 how the timing of breeding in sage-grouse across different ecoregions influences the amount of endogenous reserves acquired from winter habitats for offspring formation. It is well-established that in most avian species including sage-grouse who breed early have the greatest reproductive success as compared to individuals who breeding late in the season. There are consequences to individuals that attempt to nest early, especially in high elevations like the Centennial Valley, given harsh conditions early in the spring that increase in severity with elevation. I found that females: 1) relied on a similar amount of endogenous reserves for reproduction in first nesting and renesting attempts, 2) declined more rapidly throughout the nesting season in the Rocky Mountains than in the Great Plains, 3) individuals at high- and intermediate-elevation sagebrush types in the Rocky

Mountains used similar amounts of endogenous reserves while females nesting in low-elevation sagebrush used less and 4) females nesting at intermediate elevations switched their reliance from endogenous to exogenous sources for reproduction as green vegetation became available during spring. My study highlights adaptations of a nutrient-allocation strategy across areas with varying levels of resources in time and space in sage-grouse. Results prioritize winter habitats as most nutrients are acquired during this period at least for sage-grouse in southwest Montana.

In my last data chapter of the dissertation (Chapter 5), I report evidence that sage-grouse broods selected sagebrush in low and intermediate elevations, along with fine-scale topographic features and grazing related variables. These results however contrast with those that are expected from the Great Basin. My results indicate that sage-grouse broods selected areas away from low-lying mesic areas and near ridgelines on upper slopes with south-facing aspects that were further from cattle paths. Given differences in the selection patterns between the Great Basin and northern Rocky Mountains, my results suggest management plans should account for the unique ecological and topographical characteristics for a given region that defines critical brood rearing habitat. It is important to note that sage-grouse avoid cattle paths for not only the brood-rearing season, but also the nesting season, which I discuss in the following paragraph.

Results described in my dissertation will hopefully allow land managers to better manage the sagebrush landscape across the Centennial Valley and neighboring watersheds spanning an area of 125,000 ha (300,000 acres) for breeding and 450,000 ha (1.1 million acres) during the winter season. Broadly speaking, mountainous landscapes are characterized by strong environmental gradients driven primarily through topography and elevation, which creates a mosaic of various sagebrush types that exist across topographic and elevational gradients. Land managers can use sagebrush type as a starting point to understand its influence on sage-grouse habitat selection and nest survival outcomes. Mountain big sagebrush is clearly the most important sagebrush type that contributes to nest survival of sage-grouse, yet it is undervalued for both nesting and brood rearing sage-grouse. Though not assessed in my dissertation, it is important to note that mountain big sagebrush fledged 62% of all broods assessed, as compared to 30% in three-tip sagebrush and only 8% in basin big sagebrush (K. Cutting, unpublished data). Mountain big sagebrush also supports the greatest diversity of plant species, and also contains the most diverse topographic variation. This provides sage-grouse with the most options for habitat choices depending on the needs of an individual bird along with the associated environmental conditions that exist during that time.

I also found grazing effects on sage-grouse nests and broods that pertains to: 1) fences, 2) cattle paths, and 3) grazing intensity. Sage-grouse consistently avoided areas with cattle paths during both the nesting and brood-rearing periods. To avoid creating more cattle paths in important nesting and brood-rearing areas, managers could strategically place water tanks and/or fences away from important nesting and brood rearing areas. Additionally, land managers should consider moving grazing infrastructure away from upper slope ridgelines and south facing aspects, to lower slope and flatter bench areas on north facing aspects. North-facing aspects contain greater amounts of moisture along with cooler temperatures and these sites can resist against cheatgrass invasion than is possible on more arid south facing aspects. Waiting to begin grazing until after the nesting season (late-June/early-July) appears to be compatible with the nesting needs of sage-grouse. Fortunately, most livestock producers begin to graze sagebrush later in the summer during the later stages of vegetation phenology, and after the flowering of larkspur (*Delphinium* spp.), a poisonous plant that commonly results in numerous cattle dying each year from poisoning. I also show that sage-grouse rely on distant winter habitats for the site of nutrient investment that is later used for offspring formation. Conservation action should address large-scale habitat loss in the Upper Snake River in southeast Idaho associated with center pivots and habitat degradation associated with cheatgrass. Finally, management guidelines for diverse sagebrush regions such as those found in the northern Rocky Mountains should be based on data from similar sites or watershed than research from lower and drier regions. For instance, using data from the Great Basin will likely not reflect the requirement for sage-grouse in the northern Rocky Mountains such as southwest Montana. Southwest Montana is higher in elevation and receives more precipitation that results in greater ecological resistance to change and greater reliance to return to its original state should a disturbance occur.

One caveat to my study includes the fact that it was conducted in one geographic region (except for Chapter 4) over a short time period. Given we captured sage-grouse from most every lek on the northside of the Centennial Valley, results should be good representation of sage-grouse biology in this largely intact watershed. Furthermore, my study site contains a diversity of sagebrush species and subspecies that occur on the wet-cool half of the sagebrush spectrum as sagebrush is found from dry-hot to wet-cool regions across the American West (Chamber et al. 2017). So, results are assumed to be transferrable to similar sites that exhibit similar wet-cool climatic patterns. However, this is an assumption, and it is not yet known how well these results from the Centennial Valley region apply to other such high-elevation regions.

The sagebrush ecosystem of southwest Montana is one of the most biological diverse sagebrush ecosystem across the entire American West (Chambers et al. 2017). It is largely remote where one can travel all day without seeing another person or seeing evidence of strong human impacts especially in the sagebrush rangelands where native plant communities still flourish. The citizens of southwest Montana have the unique opportunity to continue to live sustainably off the land, while still providing high quality wildlife habitat along with thriving populations of wildlife. The Centennial Valley of southwest Montana is a wonderful example of collaborative conservation across private and public boundaries at the intersection of land management, ranching, and applied science through the board coalition of people. Very few places exist in the American West like those founds in the remote corner of southwest Montana. My dissertation is one small piece to the conservation story at the intersection of collaborative conservation at the landscape scale. Results described herein are intended to improve land management decisions for ranchers and land practitioners. Results from my dissertation are largely observational and correlational, which are not intended to suggest causation. Findings described herein can hopefully be a tool to maximize sustainability of ranching, along with providing practitioners information to conserve sage-grouse and their habitats.

Finally, the following future research needs are intended to build off the findings from the current study and further understanding in general of sage-grouse and its habitat needs across the American West. Future research should address the following: 1) assess how winter habitats influence adult female quality during the prebreeding season along with female condition impacts to offspring productivity, 2) experimentally verify the observed association between nest survival and distance to fence (i.e. Chapter 2) through assessment of a large scale fence modification project on both sage-grouse nest survival and changes in predator behavior, 3) assess source-sink dynamics related to sagebrush type on sage-grouse fitness, 4) add to Chapter

5, or individually assess in a separate manuscript, how factors including grazing variables influence chick survival, and 5) identify migration corridors and critical staging areas for female sage-grouse during the seasons leading up to breeding including fall, winter, and spring seasonal habitats.

The Centennial Valley and neighboring watersheds of southwest Montana serve as an important landscape for sage-grouse conservation. A strong alliance of people is currently working together to conserve this iconic landscape. From my dissertation, I found that mid- and especially high-elevation sagebrush communities appear to be carrying the population of sage-grouse. My opinion is that low-elevation sagebrush will likely not support long-term conservation of sage-grouse populations assuming similar low survival and productivity trends that I observed continue into the future. Future management should focus on maintaining high-elevation sagebrush communities from various forms of human and natural disturbance. Restoration work could also occur in low elevation sites that are impaired that could better sage-grouse populations. With respect to cattle grazing, I found both positives and negatives of grazing on the breeding ecology of sage-grouse. In my opinion, the positives of grazing appear to largely outweigh the negatives. The most obvious positive benefit of grazing in the Centennial Valley is the alliance formed between diverse groups of people working together to ensure ranching heritage is continued. Ranching will also help maintain landscape scale conservation as these people oftentimes own and manage tens of thousands of acres of land that are critical to wildlife including sage-grouse. Additionally, grazing in the Centennial Valley largely occurs after the nesting season, so little conflict will occur between nesting sage-grouse and livestock. Based on these findings, I suspect the population of sage-grouse in the Centennial Valley will remain viable into the future as there are currently no significant threats (e.g. oil, gas, agricultural conversion, home development) to this population.

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