David Little Livestock
Range Management Endowment
AT THE UNIVERSITY OF IDAHO

2020 Project Progress Report:

Plants in Pastures: Relationship between Cattle Grazing and Diet of Greater Sage-Grouse

By: Tyrell Styhl

PERSONNEL:

Dr. Dave Tank, Dept. of Biological Sciences, University of Idaho; **Dr. Lisette Waits**, Dept. of Fish and Wildlife Sciences, University of Idaho; **Dr. Courtney Conway**, USGS Cooperative Fish & Wildlife Research Unit; **Andrew Meyers**, Dept. of Fish and Wildlife Sciences; **Tyrell Styhl**, Ph.D. Student, Dept. of Biological Sciences and Dept. of Fish and Wildlife Sciences

PRELIMINARY RESULTS for 2020:

During the 2020 field season our project collected ~431 plants from across our study areas. Although these collections are not fully identified, initial identifications represent 44 families, 129 genera, and 280 species. During the 2020 field season 92 fecal pellets were collected from trapped adult greater sage-grouse. A total of 492 fecal surveys were conducted on 130 hens during brood rearing across the study areas, with 204 hen fecal samples and 157 brood fecal samples being collected. Fecal pellets collected from chicks ranged in age from 1 day after hatch to 76 days after hatch.

BACKGROUND:

Many species have substantial changes in dietary needs during development (i.e., juvenile to adult) and seasonally, which could affect our understanding of conservation needs. The link between food and habitat is an important area of inquiry because changes to wildlife habitat can affect the abundance, availability, quality, and diversity of food resources across the landscape. Furthermore, the feeding ecology of declining species is of upmost importance because understanding what dietary resources these species depend on can inform effective conservation policies (Duffy et al. 2007).

Greater sage-grouse rely on sagebrush (*Artemisia spp.*) for evading predators, nesting, and food. Following decades of gradual declines (Garton et al. 2011), greater sage-grouse have been petitioned for listing on the Endangered Species Act (ESA) numerous times. After the most recent petition, the U.S. Fish and Wildlife Service determined that protection under the ESA was not currently warranted because of a collaborative conservation initiative across greater sage-grouses' range. This initiative included management plans to ensure that ranchers, state legislatures, and federal and state agencies worked together so that greater sage-grouse could coexist on working landscapes with only the minimal necessary restrictions. However, the status of greater sage-grouse remains precarious, and in Idaho, male lek counts have declined significantly since 2016 (Idaho Fish and Game unpublished data). Thus, greater sage-grouse remain a topic of research priority for stakeholders that use or work within greater sage-grouse habitat, government agencies tasked with conserving the species, and the public.

Like other gallinaceous birds (i.e., "chicken-like"), greater sage-grouse hatch mobile young that have developmental shifts in diet during their progression from chick to adult. During the first weeks after hatching, juvenile greater sage-grouse are entirely dependent on invertebrates to sustain the rapid growth necessary for development (Peterson 1970). Following these crucial, protein-rich periods, juveniles begin to incorporate forbs, and small amounts of sagebrush, which allows them to maintain nutritional requirements and slowly build tolerances to the toxic terpenoids in sagebrush leaves. However, the exact timing of these dietary shifts in chicks is largely unknown and the precise species of forbs and insects that they prefer are not known. It is imperative that ranchers and other stakeholders better understand the proximate and distal factors that can deleteriously affect the diet of greater sage-grouse chicks, and ultimately, their recruitment and survivability.

Early studies of greater sage-grouse diet were restricted to direct observations of wild populations or within controlled captive populations, and little research has been conducted since the mid-20th century. Direct observations of crop contents require lethal take of greater sage-grouse and observation of foraging activities have limited success because of the difficulty associated with quantifying foraging events from a distance. These methods provide useful information about greater sage-grouse diet, but are low-resolution and lack details about the specific taxa that are consumed. The advent and subsequent development of genetic analysis methods, specifically non-invasive sampling and DNA metabarcoding of fecal samples, have allowed researchers to obtain accurate and detailed information on wildlife diets without the need for laborious observation or possible biases encountered in laboratory environments (Waits and Paetkau 2005, Sullins et al. 2018).

We propose to use a molecular approach (i.e., DNA metabarcoding) to quantify, with high-resolution, the diet of greater sage-grouse from fecal pellets. By using non-invasively collected fecal pellets, an extensive local plant and insect reference collection, and DNA metabarcoding, our study will elucidate the seasonal nuances of greater sage-grouse diet. Sullins et al. (2018) demonstrated the utility of DNA metabarcoding for another omnivorous, gallinaceous bird: the lesser prairie-chicken. Invertebrate and plant diversity was highest in the diet of lesser prairie-chickens that inhabited grazed native grasslands (Sullins et al. 2018). Being under the umbrella of the Grouse & Grazing project, this research will also be able to test whether grazing affects the plant and invertebrate diversity in the diet of greater sage-grouse in southern Idaho. Hence, the results of this effort will provide much-needed context to the intensive vegetation plots and the intensive insect sampling that is being conducted on the Grouse & Grazing project (i.e., by showing the explicit species of forbs and species of insects that are most important for greater sage-grouse food). Moreover, the results from this project will inform management by providing stakeholders and land managers information about forbs, insects, and sagebrush spp. that are important for greater sage-grouse diets. Thus, providing rangeland stakeholders with the appropriate knowledge to manage rangeland for multiple uses, including greater sage-grouse conservation and grazing.

HYPOTHESIS or OBJECTIVES:

We will test the null hypothesis that cattle grazing has no effect on greater sage-grouse dietary composition. Our specific objectives include:

1) Conduct floristic surveys across 5 study areas in southern Idaho to compare dispersal and diversity patterns of plant taxa, and provide genetic references for greater sage-grouse diet analyses. This research will provide voucher specimens to the University of Idaho (UI) Stillinger Herbarium, giving researchers a temporal and spatial assemblage of sagebrush steppe taxa across ~400,000 hectares of southern Idaho. Moreover, collections will be publicly available online through the Consortium of the Pacific Northwest Herbaria data portal.

2) Employ innovative molecular and bioinformatic approaches to catalog greater sage-grouse diet across habitat patches and experimental pastures, to understand dietary preferences and the propensity of reliance on specific taxa. Using non-invasive sampling of greater sage-grouse fecal pellets, this research will document species composition and abundance of greater sage-grouse diet, through time, using high-throughput sequencing. To identify plant species, sequences will be compared to floristic collection sequences and identified to species using a phylogenetic bioinformatic pipeline.

PROCEDURES:

Our study will be conducted at 5 grazing allotments (hereafter, study areas) across southern Idaho. The study areas each support greater sage-grouse populations and have similar overstory composition (i.e., Wyoming big sagebrush;(*Artemisia tridentate ssp. wyomingensis*). Season temperatures at the study areas are highly variable (-10˚ C to 40˚ C). Most precipitation falls as snow, with the majority of rainfall occurring from April through June. Elevations at the study areas range from 1300 m to 2300 m. Predominantly sagebrush steppe rangeland, the study areas are dominated by sagebrush with understories composed of bluebunch wheatgrass (*Pseudoroegneria spicata*), other wheatgrass species, Sandberg bluegrass (*Poa secunda*), needle and thread grass (*Stipa comata*), Indian ricegrass (*Oryzopsis hymenoides*), and bottlebrush squirreltail (*Elymus elymoides*). Across southern Idaho moving from east to west, the study areas include Pahsimeroi/Challis, Big Desert, Browns Bench, Jim Sage/Malta, and Sheep Creek/Grasmere. At each study area, grazing is experimentally manipulated (i.e., via fencing, etc.) to alter the herbaceous offtake grazed by cattle. Experimental grazing treatments include: 1) areas where spring cattle grazing removes ~30% of the new grass biomass every other year but does not have any fall or winter grazing; 2) areas where spring and fall cattle grazing removes ~30% of the new grass

biomass, and 3) areas that are not grazed for at least four consecutive years. At each study area and within grazing treatments, we will conduct intensive floristics and fecal sampling to quantify greater sage-grouse diet using molecular techniques.

Fecal samples will be collected opportunistically from male and female adult greater sage-grouse while capturing and radio collaring for the Grouse and Grazing project. Chick and hen fecal pellets, of known individuals, will be collected at evening roost sites after clutches have hatched and the hen and chicks leave their nest sites. To determine an evening roost site, radio collared hens will be tracked before sunrise and the approximate location of the roost will be recorded with hand-held GPS units. Fecal collections from a hen with chicks will occur every 3-5 days to minimize disturbance, yet still provide the highest resolution of their individual diets. Chick fecal pellets at evening roost sites will be determined based on size. Hen fecal pellets will be collected separately. When fecal pellets are found, a portion of the bowl movement (~10 mg) will be placed in sterile tubes with Zymo Research DNA/RNA shield solution and the remainder of the bowl movement will be placed in coin envelopes, placed in zipper bags with silica gel to dry, and stored at room temperature until being returned to the University of Idaho genetics lab, where they will be stored at -20 ˚C until DNA extraction.

Diet composition will be analyzed by molecularly attaching unique barcodes to DNA fragments to differentiate unique DNA sequences. Complete fecal pellet samples will be mechanically mixed prior to DNA extraction. Following mixing, ~1 ml of fecal pellet sample will have DNA extracted using a DNA extraction kit (i.e., Zymo Research *Quick*-DNA Plant/Seed mini-kit) following the manufactures recommendations and optimizations for breaking apart cell walls. Because fecal pellets contain high volumes of tiny insect and vegetative fragments, sequences from multiple species will be generated from a single fecal sample. Reference sequences of plants and insects will be generated using universal PCR primers (i.e., conserved regions of DNA across many species that allow for comparison). Following the molecular methods developed in Dr. David Tank's lab at UI, sequences will be grouped by species for each individual, and identified using an evolutionary placement algorithm. To test differences in fecal collection and storage methods, samples stored in Zymo Research DNA/RNA shield solution will be compared to dried samples stored in silica gel for the same individuals. Developmental shifts of chick diet will be compared among and within study areas. Moreover, greater sage-grouse diet will be analyzed among study areas and within grazing treatments to assess differences among diet across southern Idaho and to infer if grazing affects the dietary composition of greater sage-grouse.

The David Little Livestock Range Management Endowment funds that our project received has been/will be spent on technician support for plant identifications, and fecal DNA extraction. The technician has been focused on accurately identifying plant collections. In the coming weeks the technician will transition to extracting DNA from fecal samples so DNA's can be sequenced and analyzed. The funding received from the David Little Livestock Range Management Endowment has/will greatly increase our ability to process fecal and plant collections, which ultimately will enable us to accurately identify the diet of greater sage-grouse broods as they develop.

ACCOMPLISHMENTS or RESULTS:

Thus far, this project has accumulated ~1,084 collections from across our study areas. Although these collections are not fully identified, initial identifications represent 56 families, 194 genera, and 441 species. A total of 208 fecal pellets were collected from trapped male and females. During 2019 and 2020 brood rearing across the study areas, 621 fecal surveys were conducted on 164 hens. Brood rearing fecal surveys yielded 262 hen fecal samples and 220 brood fecal samples. Pellets collected from chicks ranged in age from 1 day after hatch to 76 days after hatch. DNA extractions began in the Spring of 2020 but have stopped due to COVID-19 – we are hoping to resume soon. To date, we have extracted DNA from 40 fecal samples that were collected during the 2019 field season.

PUBLICATIONS or OUTPUTS:

The 2020 field season was the second for this study and we are in the process of conducting analyses, thus we have not yet produced products to disseminate our results. However, all of 2019 floristic collections have been databased onto the Consortium of the Pacific Northwest Herbaria data portal and are available for the public or other researchers to use – with 2020 plant collections not far behind. We plan to continue collecting data in the coming years to best estimate the diet of greater sage-grouse. As the study progresses, we plan to share our results through Tyrell Styhl's Ph.D. dissertation, peer-reviewed journal articles, conference presentations, public presentations, a white paper/brochure, and field tours with ranchers and BLM field staff. We are confident that this study will inform management and policy decisions by providing detailed knowledge about the diet of greater sage-grouse and help document the effects of grazing on explicit species of forbs and insects important in sage-grouse diet.

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University of Idaho
Rangeland Center