8-2019

Nest-Site Selection of Golden Eagles and Ferruginous Hawks and Diet Composition of Sensitive Raptor Species Using Metabarcoding Analysis in the Uinta Basin and Ashley National Forest, UT, USA

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NEST-SITE SELECTION OF GOLDEN EAGLES AND FERRUGINOUS HAWKS AND DIET COMPOSITION OF SENSITIVE RAPTOR SPECIES USING METABARCODING ANALYSIS IN THE UINTA BASIN AND ASHLEY NATIONAL FOREST, UT, USA

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

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2019

ABSTRACT

Nest-site Selection of Golden Eagles and Ferruginous Hawks and Diet Composition of Sensitive Raptor Species using Metabarcoding Analysis

by

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Utah State University, 2019

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Habitat degradation and fragmentation of the North American sagebrush-steppe through development and climate change are causing population declines for numerous raptors species throughout their ranges. Because nest-site and prey availability are important factors in the population growth of raptors, it is important to understand the landscape characteristics of preferred nest sites and the diet of these species. Knowledge of preferred nest-sites and diet of Ferruginous Hawks (*Buteo regalis*) and Golden Eagles (*Aquila chrysaetos*) is lacking in portions of their range. In our study, we investigated the nest-site characteristics in the Uintah Basin, UT. We used maximum entropy modeling to characterize nest-site selection based on landscape variables and to predict nest-site likelihood in our study area. We found that slope, elevation, distance to nearest oil and gas well, geology, and northness were the most important variables in characterizing Golden Eagle nest-sites. Elevation, slope, vegetation type, and distance to nearest oil and gas well were the most important variables in characterizing Ferruginous Hawk nest-

sites. In addition, we used metabarcoding to analyze the diets of Golden Eagles, Ferruginous Hawks, and Northern Goshawk in the Uinta Basin, UT. Our results were validated by finding prey species consistent with previous diet studies as well as detecting novel prey items including the Western Whiptail *(Aspidocelis tigris*), Domestic Cow (*Bos Taurus*), Domestic Pig (*Sus scrofa*), and Rock Bass (*Amboplites rupestris*) within Ferruginous Hawk samples. Results from our study can provide managers with tools to better survey for nest-sites and to provide an alternative method of diet analysis to provide insight into prey species important to these raptors.

(56 pages)

PUBLIC ABSTRACT

Nest-site Selection of Golden Eagles and Ferruginous Hawks and Diet Composition of Sensitive Raptor Species through Genetic Analysis

Dylan J. Hopkins

Development and climate change in the sagebrush habitats are causing population declines of North American hawks and eagles. For these species, understanding the landscape features that are preferred for nesting and the prey they consume in sagebrush habitats are important in developing conservation plans. Specifically, we know little of the preferred nest-sites and diet of Ferruginous Hawks (*Buteo regalis*) and Golden Eagles (*Aquila chrysaetos*) many locales. In our study, we determined the landscape characteristics associated with nest sites for these two raptor species in the Uintah Basin, UT to predict where nests may occur in our study area. We found that slope, elevation, distance to nearest oil and gas wells, geology, and facing south were the most important variables in characterizing Golden Eagle nest-sites. Elevation, slope, vegetation type, and distance to nearest oil and gas wells were the most important variables in characterizing Ferruginous Hawk nest-sites. In addition, we looked at the diets of Golden Eagles, Ferruginous Hawks, and Northern Goshawks in the Uinta Basin, UT using a genetic analysis method novel to raptors. We found species consistent with previous diet studies and detected prey items not previously reported, including the Western Whiptail (*Aspidocelis tigris*), Domestic Cow (*Bos Taurus*), Domestic Pig (*Sus scrofa*), and Rock Bass (*Amboplites rupestris*) within Ferruginous Hawk samples. Results from our study

can provide managers with tools to better survey for nest-sites and to provide an alternative method of diet analysis to provide insight into prey species important to these raptors.

ACKNOWLEDGMENTS

I thank the Bureau of Land Management Vernal Field Office and Wildlife Biologist, Natasha Hadden, for providing financial and technical assistance for this project. I also wish to thank Utah State University Department of Biology and Ecology Center for providing financial assistance. I would also like to thank the members of the Utah Legacy Raptor Project for their collection of historical data. This project would not have been possible without technical aid of Dr. Matt Cross with the Toledo Zoo, the assistance of my lab partner, Marilyn Wright, and field technicians, Shala Brehm, Cal DeBerard, Ellis Juhlin, Daniel Kimball, Rachel Larson, Kathryn Luce, Jeff Merkley, Joanna Strange, Nolan Quinn, Meg Wolf, and Max Von Zastrow.

I also thank my committee members, Becky Williams and Julie Young, for their technical support, and my major advisor, Dr. Kim Sullivan, for her wisdom and patience guiding me through this project. My family, for being a strong foundation and supportive force throughout my life. Lastly, I thank my partner in life and science, Grace Carpenter, for her immense mental, emotional, and technical support on this project and throughout our endeavors.

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CHAPTER I

NEST-SITE SELECTION OF AND GOLDEN EAGLES (*AQUILA CHRYSAETOS*) AND FERRUGINOUS HAWKS (*BUTEO REGALIS*) USING MAXIMUM ENTROPY MODELING

Introduction

In recent decades, modifications to the sagebrush-steppe ecosystem from climate change and anthropogenic development have led to declines in avian populations (Knick et al. 2006). Among the declining avian populations are several species of concern that with low population numbers (Steenhof et al. 1997, Keough et al. 2015, Wallace et al. 2016). The Uintah Basin, a region largely composed of shrub-steppe habitat, is home to two such species, Ferruginous Hawks (*Buteo regalis*) and Golden Eagles (*Aquila chrysaetos*). These apex predators, found in low numbers and listed as species of concern by the Bureau of Land Management (Woffinden and Murphy 1989, Steenhof et al. 1999, Crandall et al. 2007, Keough et al. 2015, Wallace et al. 2016, BLM Sensitive Animals List, Bald and Golden Eagle Protection Act), rely on the shrub-steppe for foraging, breeding, and nesting. As some populations of these two species continue to decline, knowledge of the factors influencing their reproductive success are important in development of management strategies.

Understanding nest-site selection for bird species is important because the lack of suitable nest-sites often limits population growth and size in a region (Village 1983; Parra and Telleria 2004). Many studies across numerous species indicate that temperature, precipitation, surrounding habitat composition, food availability, disturbance, and

predation risk affect nest-site availability (Steenhof et al. 1999, Skagen and Adams 2012, Coates et al. 2014, Keough et al. 2015, Keeley et al. 2016, Wallace et al. 2016, McFarland et al. 2017). Nest sites can also be limited by the presence of specific features within an environment. For example, secondary cavity nesters rely on cavities excavated in snags (Gibbs and Melvin 1993, Newton 1994, Blewett and Marzluff 2019), seabird colonies require rocky islands isolated from mammalian predators (Strong et al. 2006), and sage-grouse need vegetation to provide shelter from extreme temperatures and to camouflage nests from predators (Watters et al. 2002). Thus, characterizing the factors determining nest-site selection for a species may help managers predict habitat suitability and identify locations of highest conservation need. Additionally, mapping areas of highest nest-site likelihood within management units can help managers design a survey plan which may lead to improved inventory and monitoring efforts.

Availability of nest sites is known to limit population size in raptors (Newton 1979, Village 1983, Parra and Tellería 2004,). Golden Eagles have historically used cliffs as their preferred nesting structure (McGahan 1968, Kochert et al. 2002). This is likely related to orographic lift providing upward drafts to aid flight (Crandall et al. 2007) and limited access by terrestrial nest predators. Certain characteristics of a cliff-face such as aspect or presence of overhanging structures can affect nest success through limiting exposure to abiotic factors such as radiant heat, wind, and precipitation (Charter et al. 2010, Salaberria et al. 2014). In contrast, Ferruginous Hawks select for a broader range of nest sites, choosing among nesting in juniper (*Juniperus* spp.), on the ground near a precipice, on rocky hoodoos, or on artificial nesting platforms (Wallace et al. 2016). The

availability of these nesting structures is fragmented throughout the population's range, especially in the intermountain west.

Increased fragmentation and disturbance caused by human development further limits nesting habitat (Coates et al. 2014, Carlisle et al. 2018). Golden Eagles in Montana do not avoid areas of human disturbance (Crandall et al. 2015), but it can be a primary cause of nest failure (Boekerl and Ray 1971). Ferruginous Hawks appear to be more sensitive to disturbance and will flush from a perch or nest when humans approach within 648 meters (Keeley and Bechard 2011). However, Keough et al. (2012) found that development of roads and increased habitat edges caused an increase in prey abundance which may have provided a reproductive advantage. Thus, the growing presence of disturbances such as oil and gas development near raptor breeding areas in the western U.S. may have varying effects on the populations of these two species. Tolerance thresholds may also exist for different disturbances associated with the operations including the construction of roads and buildings, and ongoing noise from roads and machinery (Keeley and Bechard 2011). Modeling the nest-site selection of these species in proximity to development activities may help managers determine appropriate buffer sizes needed to eliminate disturbance effects.

We used maximum entropy modeling, a presence-only distribution modeling, to predict the likelihood of Golden Eagle and Ferruginous Hawk nest-site occupancy in the Uintah Basin, UT, which contains important habitat for these species. Based on various species distribution modeling studies we hypothesized that elevation be an important landscape characteristic in nest site decisions (Loehle et al. 2015, Polasik et al. 2016).

Elevation is tied to many other factors, including the type of habitat available (Lenoir et al. 2008) and prey dependent on that habitat for either cover or forage (Hartová-Nentvichová et al. 2010). In addition, available nest substrates, such as juniper, are tied to specific bands of elevation due to temperature and precipitation gradients. Given that hydrological features create riparian areas and in dry, desert environments and are considered oases for prey species, we hypothesized that nests would be located near hydrological features. Although raptors can fly long distances on foraging bouts, shorter distances to an abundance of prey decreases time spent away from the nest. We predicted that nests would be located further away from potential anthropogenic disturbance, such as roads or oil and gas wells. As the preferred nesting substrate for Golden Eagles are rocky cliffs, we hypothesize that the type of rock or geology of the area would have an impact on nest-site selection. We hypothesized that Golden Eagles would prefer north facing aspects to avoid solar radiation. During hot summer months, direct exposure could cause desiccation in nestlings and adults roosting at the nest site and south facing slopes in the study area have greater exposure to solar radiation (Beecham and Kochert 1975). Also, we predicted slope to be important as Golden Eagles select cliffs as a preferred nesting substrate. Finally, we hypothesized that vegetation type would be important to Ferruginous Hawks due to juniper being a preferred nesting substrate (Keough and Conover 2012).

Methods

Study Area

We conducted the study in the Uintah Basin and the eastern part of the Uintah Mountains, which lie within the greater Colorado Plateau region, a 10,000 km^2 area encompassing Daggett, Duchesne, and Uintah counties in Utah. The centroid of the study area is located at 590437E 4459686N Zone 12N in the NAD83 datum. Elevation within the study area varies from 1300 to 4100 meters. Prominent habitat types as classified by LANDFIRE within the study area are Colorado Plateau Pinyon-Juniper Woodland, Inter-Mountain Basins Big Sagebrush Shrubland, and Inter-Mountain Basins Mixed Salt Desert Scrub. Around 6400 oil and gas wells were active at some point between 1999–present within the Uintah Basin. The area surveyed is managed by the Bureau of Land Management (BLM) Green River District Vernal Field Office.

Nest Surveys

The BLM provided a historic dataset of nest sites surveyed by the Utah Legacy Raptor Project, a multi-agency collaborative project, collected from 1999–2016. We used the Utah West Desert Raptor Nest Survey and Monitoring Protocol Manual provided by the Utah Legacy Raptor Project (2011) as our protocol to survey nest sites. We surveyed nest sites from May 15–August 11 in 2017 and May 14–August 10 in 2018 during the nesting season. We surveyed as many of the more than 2500 historical nest sites as we could, prioritizing sites that were active most recently and nearest to our duty station in Vernal, UT.   After arriving at a nest site, we recorded data regarding the nest-site

characteristics and nest structure, such as nest size, location, and quality (e.g., good, dilapidated, etc.). When visiting historical sites, we observed and recorded new sites *ad libitum* by seeking viewpoints that provided a broad view to scan potential nest substrate. If we determined a nest to be active, we revisited the nest site every 2 weeks until young fledged from the nest or the nest failed. Nest sites were typically visible from a vehicle due to the high density of roads in the area, though some nests were approached on foot, taking care to minimize disturbance of nesting raptors as per protocol. We attempted to maintain distance between our crew and nesting birds to avoid disturbance (Keeley and Bechard 2011). One technician approached nests in order to gather fecal material, record prey remains, or look for evidence of mortality. The technician spent less than five minutes at the nest site. We recorded nest-site locations using UTMs in the NAD83 Zone 12N datum. We conducted this survey method for all raptor species within the Uintah Basin as part of an ongoing BLM raptor monitoring effort, however we primarily focused site revisits on Ferruginous Hawks and Golden Eagles for the purpose of this study. We recorded nest site substrate, including if a nest site was located on an artificial nesting platform. Artificial nesting platforms were established as a mitigation method by power companies to provide an alternative nesting structure to utility poles.

Maxent Analysis

We obtained spatial data through online repositories including Utah Automated Geographic Reference Center (AGRC), the Department of Interior (DOI) LANDFIRE program, and the United States Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS). These data include landscape characteristics such as

elevation, hydrologic features, geology, roads, location of oil and gas wells, and vegetation layers (Table 1.1).

Table 1-1. Descriptions of variables used as input for the Maxent models after removal of highly correlated variables.

Variable	Description
elevation	Elevation of location (m).
slope	Slope (°) of hill/cliff nest is located on.
veg_type*	LANDFIRE description of vegetation type.
oilgas	Distance to nearest oil and gas wells that were not abandoned prior to 1999.
hydro2	Distance to nearest perennial water sources.
hydro1	Distance to nearest seasonal water source.
geology	Primary rock type.
roads	Distance to nearest road (paved and dirt).
eastness	Sine of aspect. -1 is West. 1 is East.
northness	Cosine of aspect. -1 is South. 1 is North.

*variable not included in Golden Eagle model due to high correlation with elevation

We downloaded all landscape characteristic layers in a 30 m resolution. We acquired a digital elevation map (DEM) from NRCS which represents the elevation of the nest-site in meters. We analyzed NRCS, hydrologic feature data in two categories based on persistence. “Hydro1” includes intermittent water sources, such as creeks and ephemeral ponds, that tend to be unavailable during summer months, while “Hydro2” includes perennial water sources which persist throughout the year. The geology layer is a geologic map database compiled by the USGS Mineral Resources Program which

represents the primary rock structure present in the area (e.g., limestone, sandstone, etc.) The NRCS roads layer is compiled from line data, which represents both paved and unpaved roads in the study area. The AGRC oil and gas well layer includes surface points that reference historically, or currently, active well sites compiled by the Utah Department of Natural Resources, Oil, Gas, and Mining Division. We only included oil and gas wells that were active after 1999 to coincide with our earliest nest-site records. The LANDFIRE vegetation layers used were vegetation type, vegetation cover, vegetation height, canopy height, and canopy cover. These layers are based on the Terrestrial Ecological Systems classification (NatureServe), US National Vegetation Classification, and the National Landcover Database (NLCD).

We used ArcMap to create other landscape characteristics such as aspect and slope (in degrees). We calculated the cosine and sine of aspect to generate the variables “eastness” and “northness,” which are measures of the aspect of a slope. A value of -1 represents west or south, while a value of 1 represents east or north-facing slopes. We used the Euclidean distance tool to generate distances to nearest hydrologic feature, roads, and wells, to see whether presence of water or anthropogenic disturbance had an impact on nest-site selection.

We used Maxent software (Phillips et al. 2019) to predict likelihood of nest occurrence of Ferruginous Hawks and Golden Eagles throughout the Uintah Basin. We checked for correlation among variables. Highly correlated variables (> 0.7) were removed from the analysis. Vegetation type was used as a representative variable and vegetation cover, vegetation height, canopy height, and canopy cover were removed, as

those vegetation layers were highly correlated. Although vegetation type and elevation were highly correlated, we included both because we hypothesized vegetation type to be biologically important due to the known nesting habits of Ferruginous Hawks. We removed vegetation type from the Golden Eagle model as Golden Eagles do not have a direct requirement of vegetation for a nesting structure in our study area. We used a subsample replicated run type to use 25 percent of nest sites for each species to test the model. We ran 15 replicates of the model for each species to measure the amount of variability. Bias files are used to reduce the likelihood that background points would be generated outside of the distribution range of each species and skew the model predictions, so we created a bias file using a buffered minimum convex polygon, which is used to encompass and visualize the nest occupancy area of each species. We ran a model using variables that were not highly correlated, except for vegetation type in the Ferruginous Hawk model, and then excluded variables that contributed less than five percent to the model output to avoid over-fitting (Warren et al. 2014). Our final model contained only variables with contributions to that model output that were greater than five percent in order to obtain the most parsimonious model. We did not include artificial nest platforms in the model because they do not represent natural nesting substrate and may bias the output if they were included.

We ran 15 replicates of the final model for each species. Each of the replicates generated an area-under-curve (AUC) value to measure the model's ability to map suitable nest-site locations. A model with an AUC value close to 0.50 suggests that the fit is no better than expected by random chance, while a model AUC of 1.0 suggests a

perfect fit (Baldwin 2009). Each model contained an AUC value which was then averaged over the 15 replicates and produced a standard deviation of the AUC for the averaged model.

Results

<u>General Survey Results</u>

We analyzed a combined dataset of 1046 nests collected from 1999 to 2018, which included 427 Ferruginous Hawk and Golden Eagle nests surveyed in 2017 and 2018. Of the 427 nests, 54 were new nests that were added to the combined database, 44 FEHA and 10 GOEA. Nests that were labeled as "did not locate" status in 2017 were not surveyed again in 2018.

<u>Model Results</u>

Golden Eagle

We created the Maxent model using 468 presence locations for training and 155 locations for testing. The average training AUC for the replicate runs was 0.874, while average test AUC was 0.875 with a standard deviation of 0.010, which suggests that the model was useful for predicting likelihood of nest-site selection (Baldwin 2009). Analysis of variable contributions showed that slope had the greatest percent contribution towards the Maxent model (52.6%; Table 1-2). Other important variables were elevation (24.3%), distance to nearest oil and gas well (oilgas; 10.5%), primary geological stratum (geology; 7.8%), and northness (4.8%). Slope was positively associated with the likelihood of occurrence, with the predictive output plateauing at greater than 50 degrees

Table 1-2. Maxent variable contributions and permutations of importance for the most parsimonious nest-site habitat model for Golden Eagle.

Variable	Percent contribution	Permutation importance
slope	52.6	58
elevation	24.3	35.1
oilgas	10.5	1.6
geology	7.8	3.6
northness	4.8	1.7

(Figure 1-1). Likelihood of occurrence plateaued between 1500 and 1750 meters in elevation then sharply declined. As distance to the nearest oil and gas well increases the likelihood of nets occurrence decreases. Northness was negatively associated with likelihood of nest occurrence. Spikes > 0.7 were observed at geology rocky type “47”, which represents claystone.

Ferruginous Hawk

The Maxent model was created using 291 presence locations for training and 96 locations for testing. The average training AUC for replicate

runs was 0.848, while average test AUC was 0.832 with a standard deviation of 0.009 suggesting the model is useful in predicting nest occurrence (Baldwin 2009). Analysis of variable contributions showed that elevation had the greatest percent contribution towards the Maxent model (43.1%; Table 1-3). Other important variables were slope (32.5%),

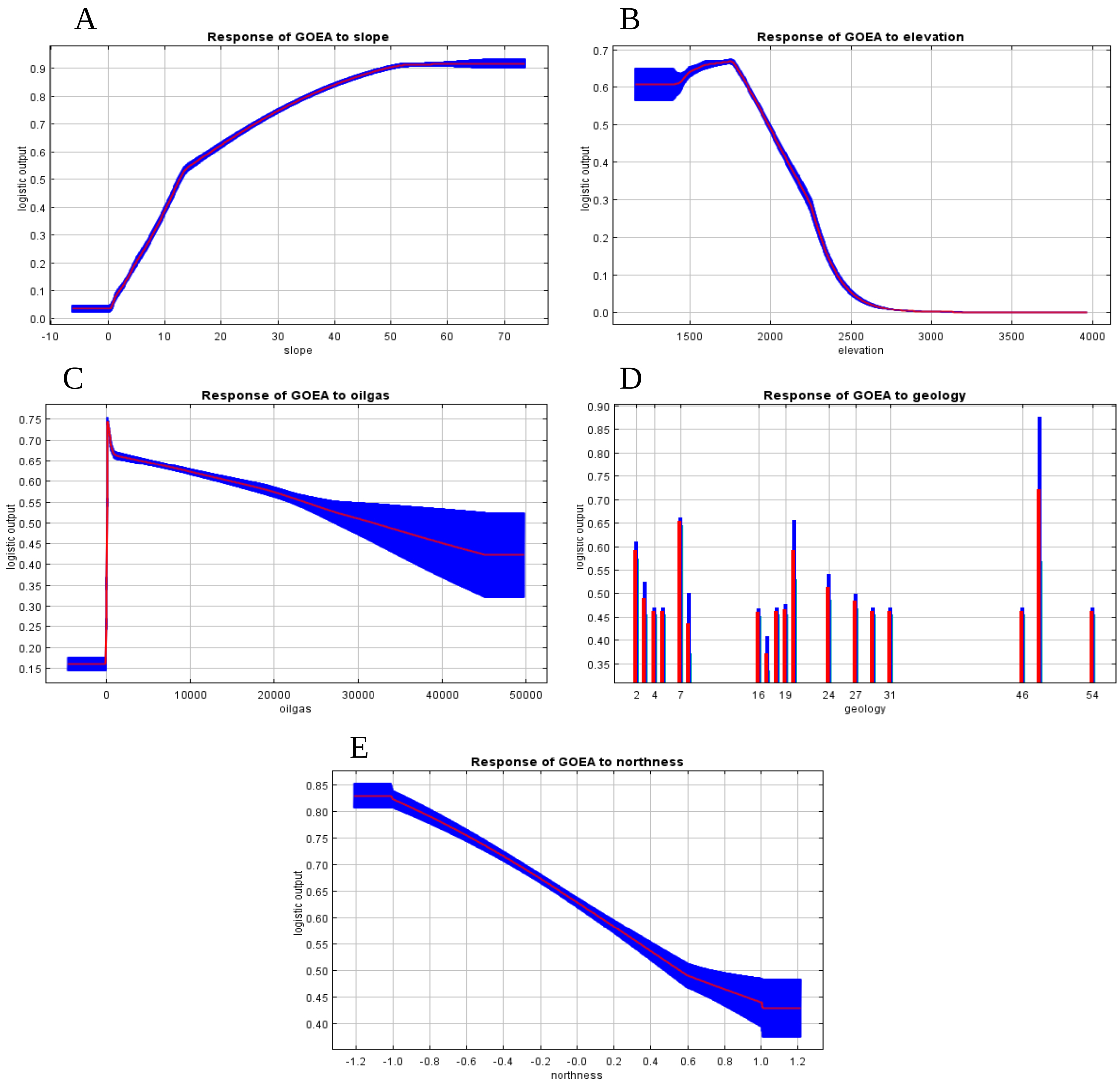

Figure 1-1. MaxEnt output graphs showing the response, logistic likelihood of nest-site occurrence, against explanatory variables. The red line represents the average logistic response value between the 15 replicate runs and the blue area is the standard deviation. The x-axis represents the variables A) slope, B) elevation, C) distance to nearest oil/gas well, D) primary rock type of area with spikes > 0.60 at 07 and 48, representing sandstone and claystone, respectively, and E) northness of the slope aspect. The y-axis represents the logarithmic predicted suitability.

Table 1-3. Maxent variable contributions and permutations of importance for the most parsimonious model for Ferruginous Hawk.

Variable	Percent contribution	Permutation importance
elevation	43.1	48.8
slope	32.5	34.4
veg_type	18.9	11.1
oilgas	5.5	5.6

vegetation type (18.9%), and distance to nearest oil and gas well (oilgas) (5.5%). Likelihood of occurrence increased between 0 and 1650 meters in elevation then decreased after 1750 meters (Figure 1-2). There was a sharp increase in likelihood of occurrence up to around 5 degrees in slope which then gradually decreased beneath 0.5 near 50 degrees. Spikes in likelihood (> 0.7) were found at the vegetation types defined by LANDFIRE as: Inter-Mountain Basins Sparsely Vegetated Systems, Inter-Mountain Basins Mat Saltbush Shrubland, Rocky Mountain Wetland Herbaceous, and Introduced Upland Vegetation-Annual Grassland. Likelihood of occurrence near an oil and gas well spiked to 0.7 within 1000 meters of a well, then plateaued to 5000 meters and declined after.

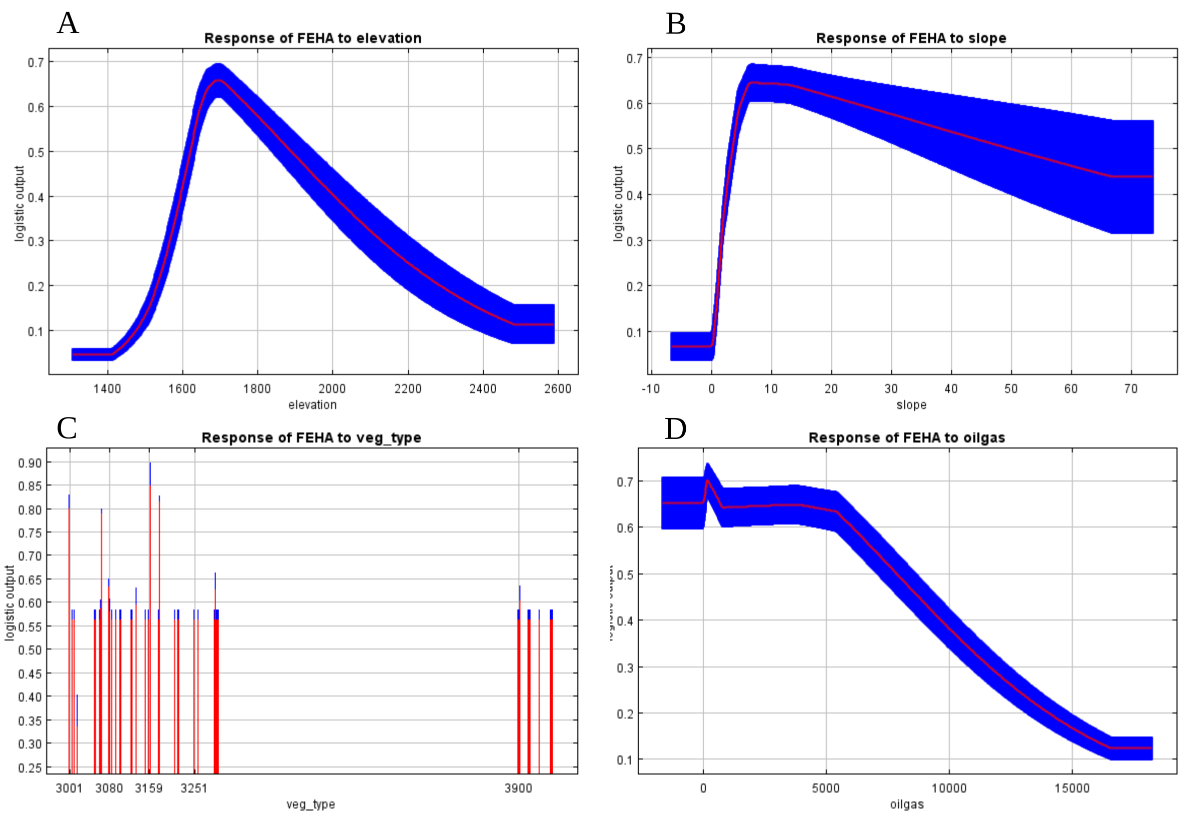

Figure 1-2. MaxEnt output graphs showing the response, logistic likelihood of nest-site occurrence, against explanatory variables. The red line represents the average logistic response value between the 15 replicate runs and the blue area is the standard deviation. The x-axis represents the variables A) elevation, B) slope measured in degrees, C) vegetation type as defined by LANDFIRE with spikes of > 0.70 likelihood at 3001 (Inter-Mountain Basins Sparsely Vegetated Systems), 3066 (Inter-Mountain Basins Mat Saltbush Shrubland), 3164 (Rocky Mountain Wetland Herbaceous), and 3181 (Introduced Upland Vegetation-Annual Grassland), and D) distance to nearest oil/gas well.. The y-axis represents the logarithmic predicted suitability.

There were 57 artificial nesting platforms that were used by FEHA. Thirty-two artificial nest platforms occurred in low probability areas (< 0.442) and 25 occurred in medium or high probability areas (> 0.442).

Discussion

A successful Maxent analysis predicts areas that are suitable for nesting based on characteristics at known nest sites. In our study the Maxent analyses performed well in identifying areas in the Uintah Basin that are likely suitable for nesting Golden Eagles (AUC = 0.874) and Ferruginous Hawks (AUC = 0.848). These areas will have similar site characteristics to the presence locations based on the landscape variables used as inputs in the model.

Golden Eagle

Slope and elevation emerged as the most important factors in nest-site selection of Golden Eagles, supporting our hypothesis. Other studies have shown Golden Eagles tend to select cliff faces as the preferred nesting structures to avoid terrestrial nest predators (McGahan 1968, Beecham and Kochert 1975, Kochert et al. 2002). Slope had a higher impact on the model than elevation for Golden Eagles. This may be due to lower elevation sites in the Uintah Basin containing many cliff-faces ideal for nesting. Previous studies have shown that Golden Eagles nest in mountainous areas (Kochert et al. 2002, López-López et al. 2007), therefore a sampling bias may have been present due to a lack of data available from higher elevation portions of the study area within the Ashley National Forest. The lack of nest surveys in the Ashley National Forest reduces the

predictive power of the model for high elevation areas.

Even though the distance to nearest oil and gas wells contributed to the model, the relationship was the opposite of what we predicted. Golden Eagles may nest near oil and gas wells because, while we were collecting data in the field, we noticed that some wells were located near the base of a cliff and our map does not take altitudinal distance into account. Also, the presence of cliffs may be more important than the presence of oil and gas wells or the eagles may have a behavioral tolerance to activity at the oil and gas well sites.

Spikes in the likelihood for primary rock type were observed for areas composed of claystone with sandstone being the second highest. Claystone and sandstone are commonly found throughout the Uintah Basin. The abundance of this rock type in the Uintah Basin and the selection of cliff substrate for Golden Eagle nest-sites may explain the spikes in likelihood of these primary rock types.

Northness was also an important variable in our model with Golden Eagles selecting for nest-sites with a south-facing aspect, which was contrary to our hypothesis. Golden Eagles initiate their egg-laying early in the year. In spring months, a south-facing slope will receive more solar radiation than a north-facing slope, which could provide heat to a brooding adult, eggs left unattended, or young chicks. This could outweigh the costs of the effects of intense solar radiation during the summer months when nestlings are older and better capable of thermo-regulation. Note the limited latitude in our present study; at more southerly locals, Golden Eagles may not choose north-facing slopes.

Distance to nearest road and distance to nearest hydrological feature did not

contribute to the model as much as we hypothesized. However, we did not look at intensity of road use in the area or the amount of road within a buffer around the nest site. It is possible that the level of activity associated with the nearby roads is important rather than just the presence of the road (Keeley and Bechard 2011). Likewise, the density of the roads in the area is high, so it may be hard to locate a site that is not near a road. Lastly, distance to nearest hydrological feature may not been as important relative to the other variables we analyzed. Golden Eagles have been known to cover large distances on regular foraging bouts within their home range (Marzluff et al. 1997) and may not need to select a site near water in order to benefit from its presence on the landscape.

Ferruginous Hawk

Our model showed that elevation, slope, vegetation type, and distance to nearest oil and gas wells were the most important factors in predicting occurrence of nest sites for Ferruginous Hawks. Elevation may have a strong contribution because it is correlated with vegetation type, prey availability, and nesting structures needed for Ferruginous Hawk occurrence. We did not predict that slope would have a significant impact, but it did contribute to the model. This may be due to Ferruginous Hawks selecting sites located within the desert “flats.” Most nesting locations were found in juniper habitat and rocky outcroppings that were both located in areas of rolling hills rather than steep canyon terrain, which is similar to other studies on Ferruginous Hawk nest-site selection (Gilmer and Stewart 1983, Bechard et al. 1990, Keough and Conover 2012) .

The vegetation types selected by the model as best the predictors of nest-site occurrence were Inter-Mountain Basins Sparsely Vegetated Systems (which includes

presence of Juniper), Inter-Mountain Basins Mat Saltbush Shrubland, Rocky Mountain Wetland Herbaceous, and Introduced Upland Vegetation-Annual Grassland (LANDFIRE). The vegetation types considered "sparsely vegetated" and "shrubland" fit with vegetation characterizations of areas occupied by Ferruginous Hawks in Idaho (Lehman et al. 1998), and Wyoming (Wallace et al. 2016), and fit with previous studies in the Uintah Basin, UT (Keough and Conover 2012). The vegetation type considered "annual grassland" fits with characterization of areas inhabited by Ferruginous Hawk in New Mexico (Keeley et al. 2016) and South Dakota (Gilmer and Stewart 1983). The "Rocky Mountain Wetland Herbaceous" vegetation type contributed to the model even though we did not find any nests in this vegetation type. Ferruginous Hawks may avoid nesting in wetland areas.

Ferruginous Hawks showed a higher likelihood of nest-site occurrence within 5000 meters of an oil and gas well. Similarly, to what we found with Golden Eagles, this is likely related to landscape characteristics that benefit both nest-site selection and anthropogenic development such as relatively flat, easily accessible, terrain. It is possible that nest sites were located near oil and gas wells because the Uinta Basin Ferruginous Hawk and Golden Eagle population is not affected by anthropogenic disturbance to the extent where the area is avoided or it could be that the positive characteristics of a nesting structure outweigh the negative effect of disturbance. We did not address nesting success in our study; it is possible that anthropogenic disturbance negatively affects nesting success, but not nest occurrence. Keeley and Bechard (2011) showed decreases in nesting success with human activities within 650 meters of a nest.

We hypothesized that distance to nearest hydrological feature would be important to nest-site selection, however it had little impact on the model. For similar reasons as the Golden Eagle, distance to nearest hydrological feature may be less important to nest-site selection than hypothesized because Ferruginous Hawks have a large home range size (Leary et al. 1998) and thus, are able to travel long distances on foraging bouts.

Nesting platforms were erected near oil and gas well sites in the Uinta Basin to discourage raptors from nesting on utility poles. In 2017 and 2018, five Ferruginous Hawk nests were located on artificial nesting platforms. Although these nest locations were not used in the model, we added the model output raster to a map then extracted the likelihood value associated with the artificial nesting platforms to examine how these platforms fit with the model. We found that 32 artificial nesting platforms from the historical dataset were in areas of low predicted likelihood, while 25 were in areas of medium to high predicted likelihood. These platforms, along with utility poles, are providing nesting structures in areas considered to be a low suitability by our model, thus allowing for expansion into previously unsuitable nesting areas. Prior studies have found that these artificial nesting platforms are not an ecological trap for the species (Wallace et al. 2016). We recommend future studies examine the impacts of nests located on artificial nesting platforms in close association with oil and gas development.

Our study did not take temporal selection into account as we lacked nest activity status prior to 2016. In order to properly understand the importance of site selection regarding human development, future studies may benefit from analyzing site selection data of active nests annually to look at how nest-site selection may change each nesting

season. A long-term study that continues to analyze nest-site selection of these two species will be beneficial in maintaining conservation of preferred nest site areas as well as informing future monitoring surveys and protocols within the Uinta Basin. Further research is needed to look at nest success to determine whether anthropogenic variables and climatic variables contribute to the success of young for these species. The nest-site selection model within the Uinta Basin can help inform management strategies for Golden Eagle and Ferruginous Hawk in this area by providing a foundation for a survey sampling method through generating random survey points within the modeled areas of nest-site occurrence.

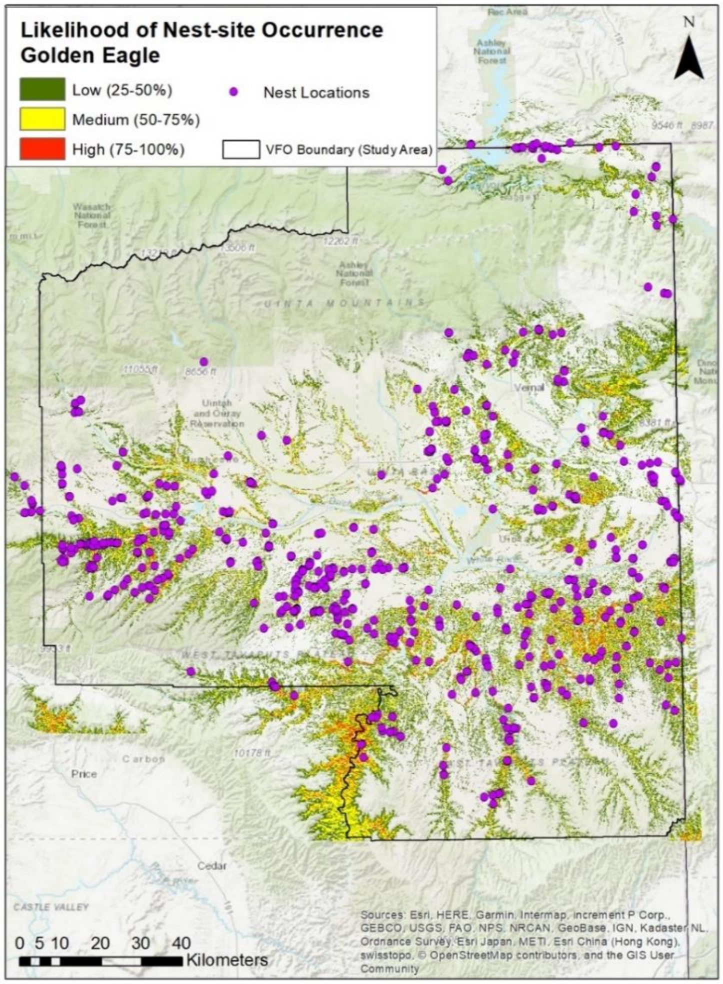

Figure 1-3. Map showing the Maxent output for likelihood of occurrence of Golden Eagle nest sites based on the variables selected in the final model. Values of likelihood < 0.0075 were removed from the output map based on the minimum training presence logistic threshold from the Maxent output table. Remaining values were then binned into 4 equal intervals and the lowest bin 0-25% removed; 25-50% represents (low), 50-75% (medium), and 75-100% (high) likelihood. 100% represents the likelihood value 0.875. Nest locations (purple circles) are presence locations used in the model. The Vernal Field Office management boundary is outlined in black. The Ashley National Forest was not involved in our study area.

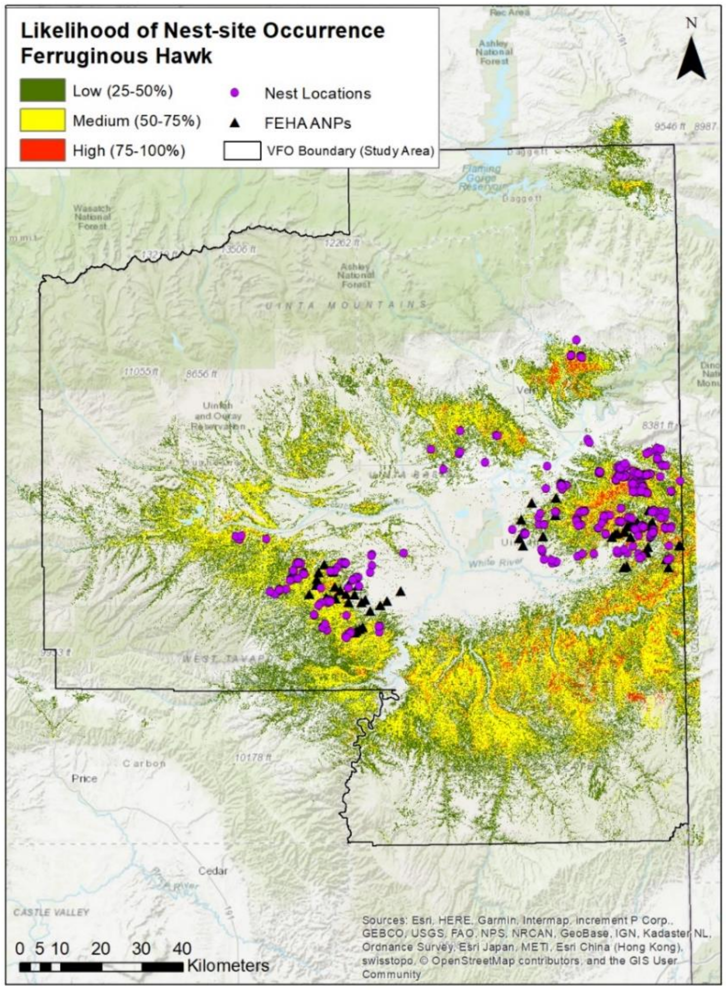

Figure 1-4. Map showing the Maxent output for likelihood of occurrence of Ferruginous Hawk nest sites based on the variables selected in the final model. Values of likelihood < 0.0471 were removed from the output map based on the minimum training presence logistic threshold from the Maxent output table. Remaining values were then binned into 4 equal intervals and the lowest bin 0-25% removed; 25-50% represents (low), 50-75% (medium), and 75-100% (high) likelihood. 100% represents the likelihood value 0.832. Nest locations (purple circles) are presence locations used in the model. The Vernal Field Office management boundary is outlined in black. The Ashley National Forest was not included in our study area.

CHAPTER II

EXAMINING DIETS OF RAPTORS IN NORTHEASTERN UTAH THROUGH DNA METABARCODE ANALYSIS

Introduction

Raptors, acting as apex predators, play an important role in sagebrush-steppe ecosystems (Sergio et al. 2006). Apex predators influence community diversity and, consequently, energy and nutrient cycling by preying upon herbivores and carnivores. They impact the surrounding plant and animal communities via a trophic cascade (Schmitz et al. 2000). As the sagebrush-steppe ecosystem undergoes dramatic changes caused by climate change and anthropogenic development, the landscape has shifted in habitat composition and populations reliant on the shrub-steppe habitat have declined (Steenhof et al. 1997, Keough et al. 2015, Wallace et al. 2016). To properly manage raptor populations, it is important to determine which sagebrush-steppe prey species currently compose raptor diets. Raptor diet composition can inform managers about prey species and their corresponding habitats that are necessary to maintain a healthy predator population in a changing ecosystem (Holt 1977).

Previous studies have determined diet through direct observation of raptor foraging, passive observation using cameras at nest sites, or by collecting pellets and prey remains near nest or resting sites (Collopy 1983, Gatto et al. 2005, Zarybnicka et al. 2011, Preston et al. 2017, Harrison et al. 2019). The traditional methods work well and can be used to answer different questions related to diet, but each method has its own

limitations. Direct observation is a method that requires a great amount of time by the observer and the predator may consume additional prey items away from the observer (Marti et al. 2007). Collecting pellets or recording prey remains at a nest site may not provide a full representation of the diet as prey items may be eaten away from the nest during foraging bouts (Lindsay and Meathrel 2008). Invertebrate prey remains may be difficult to locate or identify and soft-bodied prey items may not leave behind remains (Marti et al. 2007). This may bias results toward larger prey items (Marti et al. 2007). Passive observation through cameras requires setting up trail cameras at an occupied nest site and repeated visits to change out storage or batteries in the camera, which can cause disturbance (Harrison et al. 2019). Also, while there is greater success in identify prey compared to analysis of pellets and prey remains, only whole prey items can be identified (Harrison et al. 2019).

Metabarcoding has the potential to contribute to prey use studies and help fill in the gaps caused by limitations of the existing methods. Recent improvements through next-generation sequencing has allowed simultaneous sequencing of millions of amplified DNA templates from fecal samples (Crisol-Martinez et al. 2016). Researchers have had success in using metabarcoding to determine diet from fecal samples in several bird species, for example Yang et al. (2016) found ten unique food items that could be identified to species in herbivorous waterbirds. Jedlicka et al. (2017) found 66 arthropod species within the diets of Western Bluebirds (*Sialia mexicana*) including novel prey items and evidence of ingestion of ectoparasites. This technique has been primarily been used in studies of herbivorous and insectivorous birds, raptor diet studies have typically

used traditional methods of diet analysis. While there is a study using gastrointestinal tract contents of airport mortalities (Coghlan et al. 2013), currently there are no published studies using fecal samples to analyze raptor diets through metabarcoding. Thus, we wanted to look at whether this method provides useful raptor diet information and could be an informative tool for managers in addition to prior diet analysis methods.

Our objectives were to 1) determine if we could use fecal metabarcoding to determine diet in raptors, 2) to characterize the diets of raptors in the Uintah Basin, and 3) detect whether Greater Sage-grouse was present in the diets of Golden Eagles (*Aquila chrysaetos*) and Ferruginous Hawks (*Buteo regalis*) in the Uintah Basin.

Methods

Study Area

Our study area consisted of the Bureau of Land Management (BLM) managed lands of the Uinta Basin and United States Forest Service (USFS) managed lands of Ashley National Forest, which lies at the eastern end of the Uinta Mountains. The basin is primarily sagebrush-steppe with areas of pinyon-juniper woodland. Forests are dominated by lodgepole pine (*Pinus contorta*) and Douglas Fir (*Pseudotsuga menziesii*).

Field Collection

In 2017 and 2018, we collected fecal samples during the nesting season for Ferruginous Hawks, Northern Goshawk (*Accipiter gentilis*), and Golden Eagles. Nests were not approached during the laying or incubating phase as this is when raptors are most likely to abandon their nest (Keeley and Bechard 2011). We sampled nests when the

chicks were greater than two weeks old. After approaching a nest site, we used nitrile gloves and clean popsicle sticks to scrape fecal matter from tree branches and rocks beneath nest sites into sterile 2 ml microtubes. In 2018, we added plastic sheeting underneath nest sites and left it overnight to collect fresh fecal samples and compared these to samples of unknown age collected on nearby nesting strata. The plastic sheeting was placed the evening before and left overnight. Samples were collected the next morning if they were found on the sheeting. All samples were stored on dry ice in the field and then transferred to -80 °C freezers at Utah State University Uinta Basin (USUUB) at the end of the day. For each site, ~3–10 samples were collected from different piles as replicates.

Molecular Methods

We used a commercial vendor, Jonah Ventures Lab, for DNA extraction, sequencing, and bioinformatics (e.g., Craine et al. 2015). For each sample, an approximately 100–400 bp segment of 12S mtDNA was sequenced with a general vertebrate primer for the region. The samples were processed according to Protocol ID 104.02, using the Ac12S vertebrate primers (Evans et al. 2016), 2-step PCR, and open reference database. Genomic DNA from samples was extracted using the MoBio PowerSoil htp-96. Both primers also contained a 5' adaptor sequence to allow for subsequent indexing and sequencing on the MySeq Illumina Platform. Each 40 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI), which included 12.5ul Mastermix, 0.5 µl of each primer, 3.0 µl of gDNA, and 7.5 ul DNase/RNase-free H_2O. DNA was PCR amplified

using the following conditions: initial denaturation at 94 °C for 3 minutes, followed by 40 cycles of 30 s at 94 °C, 30 s at 55 °C, 60 s at 72 °C, and a final elongation at 72 °C for 10 minutes.

Amplicons were then cleaned by incubating amplicons with Exo1/SAP for 30 minutes at 37 °C following by inactivation at 95 °C for 5 minutes. A second round of PCR was performed to give each sample a unique 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µl of template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95 °C for 3 minutes followed by 8 cycles of 95 °C for 30 sec, 55 °C for 30 seconds, and 72 °C for 30 seconds. Five µl of PCR products of each sample were visualized on a 2% agarose gel. Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). Twenty-five µl of PCR amplicon was purified and normalized using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol. Samples were then pooled together.

Amplicons were sequenced using an Illumina MiSeq housed in the CU Boulder BioFrontiers Sequencing Center using the v2 500-cycle kit (cat# MS-102-2002). Each run yields approximately 10 million reads; each sample is represented by 1–10 thousand reads and each read yielded approximately 100–400 bp segments of 12S mtDNA.

Jonah Ventures also performed a bioinformatics analysis. The Ac12S amplicons were processed via a joint QIIME (Caporaso et al. 2010) and UPARSE (Edgar 2013) pipeline similar to that of Andrei et al. (2015), with modification. Sequences were

demultiplexed by taking advantage of Golay barcodes (Caporaso et al. 2012) via QIIME v1.9.1 (Caporaso et al. 2010). The following options were used to output raw unfiltered fastq files for both forward and reverse reads: split_libraries_fastq.py -q 0 --max_bad_run_length 250 --min_per_read_length_fraction 0.0001 --sequence_max_n 250 --store_demultiplexed_fastq… Primer sequences were trimmed using cutadapt v1.8.1 (Martin 2011) in 'paired-end mode' to remove the primers. Trimmed paired-ends where then merged by the –fastq_mergepairs option of usearch v8 (Edgar 2010). From here, the general quality filtering and OTU construction was completed as per the UPARSE pipeline (Edgar 2013), with the following modifications: operational taxonomic units (OTUs) were generated by clustering the reads at 99% sequence similarity, and the OTU table was generated by mapping quality filtered reads back to the OTU seeds by performing an exhaustive search via the search_global command of usearch. This ensures that individual reads are correctly mapped to their respective OTUs.

Taxonomy was assigned by recording the top BLAST (Camacho et al. 2009, Altschul 2001) hit for any sequence in which the query coverage and identity exceeded 95% and 80% respectively. GenBank was used as the reference database. Any OTUs with taxonomy assignments not meeting these criteria were removed from the OTU table. Each OTU was identified to the level of genus or species where possible and the frequency of each OTU recovered in each sample was included in the table. We used a Shannon-Weaver diversity index to compare diet diversity between Ferruginous Hawk samples found on the plastic sheeting and those found on the ground or other natural substrate.

Results

During the 2017 and 2018 field seasons, we collected 247 fecal samples: 90 Ferruginous Hawk, 120 Northern Goshawk, 37 Golden Eagle, and 8 Red-tailed Hawk. We sent in 82 samples for sequencing and received successful diet results from the focal species in 75 samples; 42 Ferruginous Hawk, 27 Northern Goshawk, and 6 Golden Eagle. We recovered 17 diet items identifiable to species, not including the host, from these samples (Table 2-1). Three samples contained results that were not identifiable to species, genus, family, class, or order; raw data is available in supplementary materials for these samples identified to metazoan and chordata (Table A-1). Human contamination was found in ten of the samples and likely domestic canid contamination in two of the samples (Table A-1).

We compared the Ferruginous Hawk samples collected from plastic sheeting to samples collected off the sheeting. No significant difference in number of reads from prey items was observed ($t = -0.28$, $n= 29$, $P > 0.05$).

<u>Ferruginous Hawk</u>

The prey item found to be most prevalent among samples was the white-tailed prairie dog (n=25; *Cynomys leucurus*) with desert cottontail (n=12; *Sylvilagus audubonii*) and thirteen-lined ground squirrel (n=8; *Ictydomys tridecemlineatus*) also commonly present in the samples. Prey items not previously reported in prior diet studies include: Rock bass (n=1, *Ambloplites rupestris*), white-tailed antelope squirrel (n=1, *Ammospermophilus leucurus*), western whiptail (n=5, *Aspidoscelis tigris*), domestic cow (n=1, *Bos taurus*), and domestic pig (n=2, *Sus scrofa*, Table 2-1).

Northern Goshawk

Common prey items were Northern Flicker (n=3; *Colaptes auratus*), red squirrel (n=3; *Tamiasciurus hudsonicus*), and house mouse (n=4; *Mus musculus*, Table 2-1).

Golden Eagle

Common prey items were cottontail (n=3; *Sylvilagus sp*), white-tailed prairie dog (n=2), and house mouse (n=2, Table 2-1).

Discussion

We were able to successfully recover species-specific dietary results from raptor feces using metabarcoding. Samples collect in 2017 that contained mostly uric acid or “hawk chalk” returned results of host DNA and degraded prey DNA which could only be identified to kingdom, phylum, or class. Collection of the fecal material is recommended as it is more likely to provide species-specific dietary results. We found common prey items that were previously reported in prior diet studies using different methods of analysis (Blair and Schitoskey 1982, Miller et al. 2014, Keeley et al. 2016; Figure 2-1). We also discovered novel prey items such as western whiptail, domestic cow, domestic pig, and rock bass within the Ferruginous Hawk diet that have not been previously reported in other diet studies (Table 2-1).

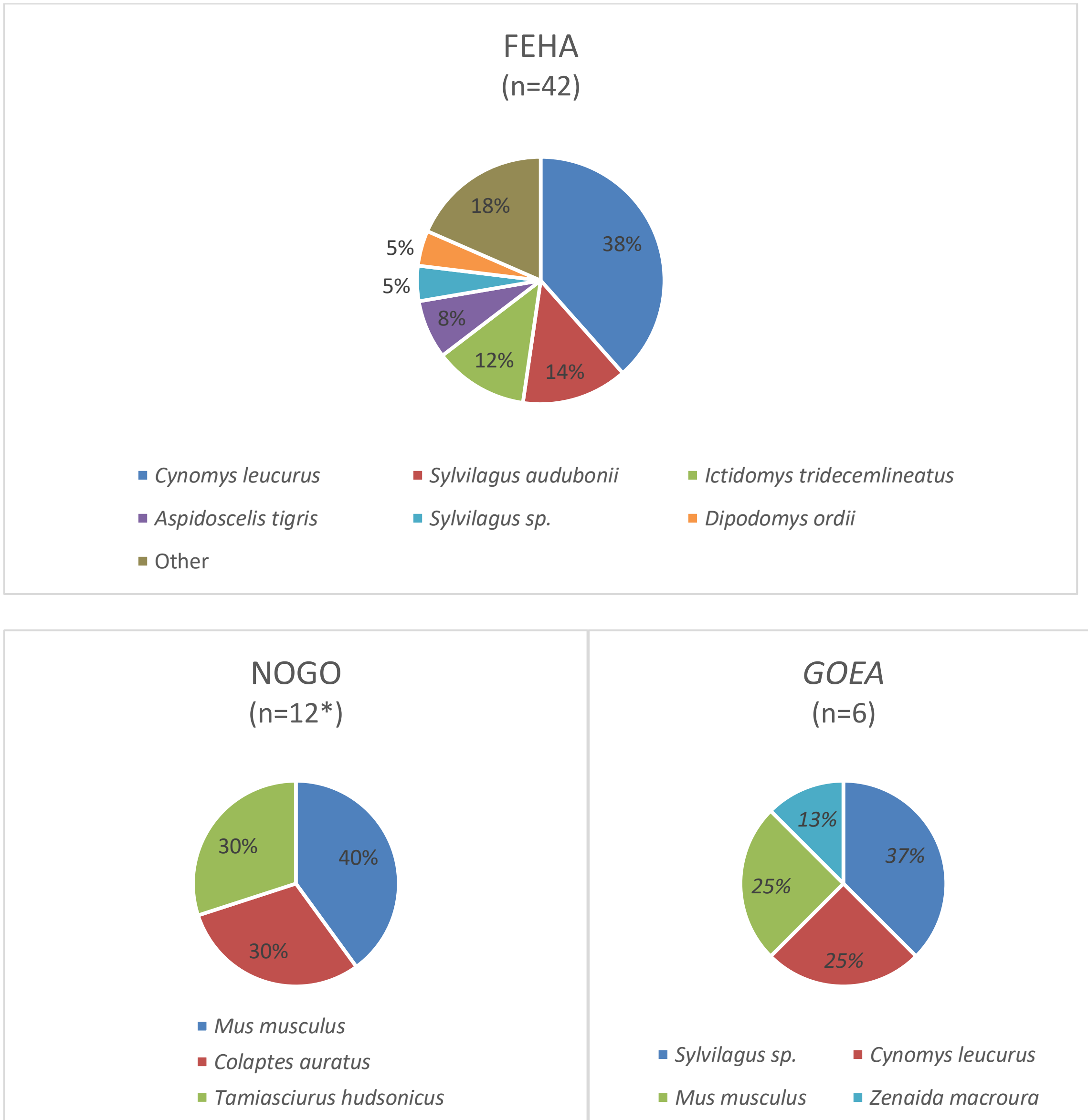

Figure 2-1. Diet items identified for Ferruginous Hawks (FEHA) and Golden Eagles (GOEA) of the Uinta Basin and Northern Goshawks (NOGO) of Ashley National Forest, UT, USA. Included are prey items identified from family to species and the number of fecal samples in which prey items were present. Numbers represent the percentage of samples the prey item was found in of the total collected.

Table 2-1. The number of fecal samples in which prey items were present within the diets of Ferruginous Hawk, (FEHA), Northern Goshawk (NOGO), and Golden Eagle (GOEA), in the Uinta Basin and Ashley National Forest, UT, USA. Number in parentheses is the proportion of samples the prey item was found in of the total collected as a percentage.

CLASS	ORDER	FAMILY	GENUS	SPECIES	FEHA (N=42)	NOGO (N=27)	GOEA (N=6)
ACTINOP-TERYGII	Perciformes	Centrarchidae	*Ambloplites*	*rupestris*	1 (2.4)		
AVES	Columbiformes	Columbidae	*Zenaida*	*macroura*			1 (16.7)
	Passeriformes	Alaudidae	*Eremophila*	*alpestris*	1 (2.4)		
	Piciformes	Picidae	*Colaptes*	*auratus*		4 (14.8)	
MAMMALIA	Artiodactyla	Bovidae	*Bos*	*taurus*	1 (2.4)		
		Suidae	*Sus*	*scrofa*	2 (4.8)		
	Carnivora	Canidae				1 (3.7)	
			Canis	spp.		2 (7.4)	
	Lagomorpha	Leporidae	*Lepus*	*californicus*	1 (2.4)		
			Sylvilagus	*audubonii*	10 (23.8)		
			Sylvilagus	spp.	2 (4.8)		3 (50.0)
	Rodentia	Geomyidae	*Thomomys*	*talpoides*	1 (2.4)		
		Heteromyidae	*Dipodomys*	*ordii*	2 (4.8)		
		Muridae	*Mus*	*musculus*		4 (14.8)	2 (33.3)
		Sciuridae	*Ammospermophil-us*	*leucurus*	1 (2.4)		
			Callospermophilus	*lateralis*	2 (4.8)		
			Cynomys	*leucurus*	23 (54.8)		2 (33.3)
			Ictidomys	*Tridecemlineat-us*	6 (14.3)		
			Tamiasciurus	*hudsonicus*		3 (11.1)	
REPTILIA	Squamata	Teiidae	*Aspidocelis*	*tigris*	2 (4.8)		

Through metabarcode analysis, we were also able to identify four species previously unknown in the diet of Ferruginous Hawks: domestic cow, domestic pig, rock bass, and western whiptail. Although western whiptail was a novel diet item, it is expected as prior Ferruginous Hawk diet studies have shown small reptiles to be a part of the diet. The larger prey items along with the rock bass could be evidence of scavenging behavior. Previous studies have reported that Ferruginous Hawks will scavenge on smaller prey items such as Richardson's ground squirrel that have been killed using lead bullets or poison (Knopper et al. 2006), but have not shown scavenging on these larger prey items. Ingestion of prey items containing lead bullets or rodenticide can lead to mortality. Through analysis of the diet and detecting prey species that are being targeted through poisoning or shooting, we can determine if there is a potential risk for Ferruginous Hawks scavenging for prey items in the Uinta Basin.

Human and canid contamination were found in 15 samples collected in 2017. The canid results returned in the Northern Goshawk samples are likely due to contamination as this seems uncharacteristic for the species. Training technicians on proper use of gloves and field collection of genetic material to reduce possible contamination events is imperative. We placed plastic sheeting under some nest sites to reduce potential contamination of fecal samples with genetic material on the ground. Additionally, the plastic sheeting made it easier to locate fecal samples on the ground, in areas with vegetative litter, specifically in forested Northern Goshawk nesting areas or Ferruginous Hawks nesting in juniper.

The automated bioinformatics pipeline sometimes produces unexpected results

and researchers should be ready to conduct further analysis on certain samples. For example, Douglas squirrel was reported in a Northern Goshawk sample, which is a species native to California, Oregon, and Washington (IUCN Red List). We input the Douglas squirrel sequence from our results into BLAST. The first result was Douglas squirrel (96.98%), but the second result was red squirrel (96.48%). These two species of the genus *Tamiasciurus* are closely related, however the red squirrel is commonly found throughout the Uinta Mountains (IUCN Red List) and thus, more likely to be the actual prey item. This sample was collected during the middle of the nesting season, so it is unlikely that it was a prey item was picked up during migration or dispersal. Other results that required further interpretation were black-tailed prairie dog and rock bass. We found that white-tailed prairie dog was the second result (98.49% identity) when inputting black-tailed prairie dog sequence (100% identity). The rock bass result did return a percent identity value of 98.97. Rock bass were introduced to reservoirs around the Wasatch range of Utah but have not been reported in the Uintah Basin. We recommend that further verification of results such as these may be needed if abnormal diet items are found.

There are limitations to DNA metabarcoding diet identification methods. It is a genetic method which relies on physical collection of fecal matter in the field, making it prone to contamination. This technique does not produce true prey abundance data. The results are reported as number of reads, which does not suggest a unique prey item per sample but rather the number of times the DNA sequence for a species is identified. The same unique individual prey item could result in multiple readings. However, it is

possible to receive quantitative information if the study uses a relative read abundance (RRA) method (Deagle et al. 2019).

If enough samples are preserved and sent in bulk, this technique could be a cost-effective alternative in comparison to the cost of analyzing game camera footage or field observation as these methods require many observer hours. We were able to identify novel prey items which is important to conservation of a predator species. Managers can use this information to promote conservation of prey items previously unknown in a raptor's diet or find novel behaviors (e.g. such as scavenging on large prey items) to gain a better understanding of the natural history of raptors and better conserve sensitive raptor species.

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APPENDICES

Table A-1. Number of total OTU reads for each prey item present within the fecal samples of Ferruginous Hawk, (FEHA), Northern Goshawk (NOGO), and Golden Eagle (GOEA), in the Uinta Basin and Ashley National Forest, UT, USA. Host reads, contamination events, and unrefined results included.

KINGDOM	PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES	FEHA (N=42)	NOGO (N=27)	GOEA (N=6)
Metazoa							25	1394	
	Chordata						263	3212	
		Actinop-terygii	Percifo-rmes	Centrarc-hidae	*Ambloplites*	*rupestris*	63		
		Aves							45
			Accipitr-iformes	Accipitri-dae	**Accipiter*	*gentilis*		108130	
					**Aquila*	*chrysaetos*			13490
					**Buteo*	*regalis*	101529		
			Columb-iformes	Columbi-dae	*Zenaida*	*macroura*			136
			Passerif-ormes	Alaudid-ae	*Eremophila*	*alpestris*	446		
			Picifor-mes	Picidae	*Colaptes*	*auratus*		641	
		Mamma-lia							19
			Artioda-ctyla	Bovidae	*Bos*	*taurus*	70		
				Suidae	*Sus*	*scrofa*	231		
			Carnivo-ra	Canidae				60	

				Canis	spp.		466	
		Lagomorpha	Leporidae	*Lepus*	*californicus*	39		
				Sylvilagus	*audubonii*	5926		
					spp.	263		10808
		Primates	Hominidae	*Homo*	*sapiens*	155	11811	
		Rodentia	Geomyidae	*Thomomys*	*talpoides*	213		
			Heteromyidae	*Dipodomys*	*ordii*	671		
			Muridae	*Mus*	*musculus*		4250	5513
			Sciuridae	*Ammospermophilus*	*leucurus*	162		
				Callospermophilus	*lateralis*	122		
				Cynomys	*leucurus*	27312		916
					ludovicianus	27		
				Ictidomys	*tridecemlineatus*	5135		
				Tamiasciurus	*douglasii*		86	
					hudsonicus		5208	
	Reptilia	Squamata	Teiidae	*Aspidocelis*	*tigris*	75		

* Denotes host species