

Original Research Paper

Stable Isotope Diet Reconstruction of Feral Horses on the Sheldon National Wildlife Refuge, Nevada, USA

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Abstract: Feral horse management has become a subject of significant controversy in the United States, resulting in part from opposing views of best management practices and limited empirical data. Feral horse densities increase in response to high reproductive rates and limited horse removal. With this increase, land managers are challenged with accurately quantifying horse diet selection and subsequent impacts on western rangelands. To accomplish this, we obtained tail hair isotopic values from samples collected from feral horses' tails in northwestern Nevada under the oversight of the Sheldon Refuge staff and animal use protocols. Plant samples were collected from two sites on the refuges (Little Sheldon and Badger Mountain). Plant biomass, abundance, and cover were determined at random locations within the two sites. The samples were dried and ground for analysis. Tail hair samples were taken from 10 mares and 10 stallions as they were being examined by Refuge staff. Tail hairs were cleaned and cut into 5 mm sections for analysis. The mean tail hair length was 807 mm. Horse tail hair has a growth rate of 0.72 mm/day which was used to set a timeline along the length of the hair strand, the bulb at time 0 to the end, approximately 1121 days. Carbon and nitrogen isotopic values were determined through mass spectroscopy on the tail hair section and the individual forage samples. IsoSource© software was used to compare isotopic values of tail hairs to those of plant tissues. The contribution of the various plant species to the tail hair mixture values was determined using. There were no plant isotope differences between the two sites. Chronology differences ($p < 0.05$) between mares and stallions were found. Chronology was divided into seasons and seasonal shifts were determined. Seasonal variations were noted between mares and stallions ($p < 0.05$) and between the three years ($p < 0.05$). The horse diet consisted of mainly grasses (~55%) and shrubs (~45%) with remainder forbs. Stallions consumed fewer forbs (5-6%) than mares (9-11%; $p < 0.05$). Forage availability and preference are most likely linked to seasonal consumption. The difference in mare and stallion foraging patterns was clearly shown using the isotope composition in the tail hair of these free-roaming horses. Combining plant species abundance in combination with tail hair is shown to be a method that can be used to monitor foraging patterns. Increasing the knowledge base of horse diets including plant species selection and forage consumption patterns allows land managers to make informed conservation decisions. This will then reduce negative habitat impacts and improve the prediction of appropriate horse population levels that sustain healthy feral horse populations.

Keywords: Free-Roaming Horses, Horse Diet, Forage Availability, Forage Selection, Stable Isotopes

Introduction

North American rangelands have seen an increase in the number of feral horses. This increase has been shown to have negative impacts on abiotic components of the ecosystem including hydrology and soil structure and biotic components of native plant and animal species (Bassett, 1980; Miller, 1983; Levin *et al.*, 2002; Ostermann-Kelm *et al.*, 2008; Beever and Aldridge, 2011; Davies *et al.*, 2014). There is limited data on feral horse impacts on habitat selection and forage consumption, resulting in land managers being challenged to establish management strategies for feral horse populations across diverse rangeland regions of the western United States.

Feral horse diets have been assumed to consist of grasses, forbs, and a limited intake of shrubs (Scasta *et al.*, 2016). Horses are hindgut fermenters where forage material consumed is mainly digested in the colon and large intestines (Stevens and Hume, 2004). Classified as grazers, horse dietary selection generally focuses on grasses. Hanley (1982) found that grass consumption made up the majority of the diet followed by forbs and shrubs. Variability in forage consumption was also found to be associated with the time of year. Scasta *et al.* (2016) presented a meta-analysis that compared feral horse diets with shifts throughout the seasons, but grass consumption remained the highest. They stated that in addition to winter stress, accessibility may be responsible for dietary shifts. Feral horse grass consumption includes annual grasses (i.e., *Bromus tectorum*, cheatgrass), short-rooted perennial grasses, and deeper-rooted perennial bunchgrasses (Beever and Aldridge, 2011; Davies *et al.*, 2014). Additionally, horse herbivory has been shown to extend from upland rangelands to lowland riparian and wet meadow environments (Davies *et al.*, 2014; Boyd *et al.*, 2017).

For managers to effectively assess rangeland's response to horse herbivory, particularly the plant community response, tools are needed that help identify specific plant species preferences. This includes the development of methods for detecting changes in plant species preference by feral horses throughout the season or year. Additionally, determining which plant species are most heavily selected can help improve the predictability of habitat use and the potential impacts of feral horse grazing on various wildlife and their habitats (Hall *et al.*, 2018). The conservation of these horses requires continual management to ensure the health and welfare of these animals and their interaction with other wildlife.

Garnick *et al.* (2018) conducted a literature survey of various methods of accessing diet selection of free-range herbivores including observations, near-infrared reflectance spectrometry, DNA barcoding, microbiology, cuticular wax alkanes, and stable isotopes. Advantages and disadvantages were pointed out for each technique.

Studies by Deniro and Epstein (1978); Tieszen *et al.* (1983); Ambrose and Deniro (1986); Burnik Šturm *et al.* (2017) have characterized animal diets using stable isotope analysis. The premise is the contribution of various food sources to an animal's diet can be determined by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (Michener and Schell, 1994). Schoeninger *et al.* (1998); Macko *et al.* (1999); O'Connell and Hedges (1999); Chambers and Doucett (2008) used hair to extract $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures from various mammalian species which are then used to characterize diet and track movements (Cerling *et al.* 2006). Hair is a good isotope chronology recorder because it grows quickly (Van Scott *et al.*, 1963) and once formed remains biologically inactive and resistant to degradation (Macko *et al.*, 1999). The isotopic chronologies are formed and limited only by the length of hair (West *et al.*, 2004; Dalerum and Angerbjörn, 2005). Short-term diet switches have been used in domestic horses (*Equus caballus*) to demonstrate changes in acute hair growth (West *et al.*, 2004).

Our objective for this study was to determine diet selection based on stable isotope analysis of feral horse tail hair and available forage species unique to the Sheldon National Wildlife Refuge (NWR) in northwestern Nevada, USA. We used stable isotopic signatures in the tail hair to provide chronological information on the dietary similarities or differences between stallions (males) and mares (females). We also wanted to determine the selection of forage types (grasses, forbs, and shrubs) based on plant availability.

Materials and Methods

Study Site Description

The U.S. Fish and Wildlife Service manages the Sheldon National Wildlife Refuge, located in the northern Great Basin ecoregion in northwest Nevada, USA. Elevations range from 1,307-2,442, m across approximately 232,694, ha of the refuge. Summer temperatures range from 0-34°C and winter temperatures range between -29 and 14°C. Annual precipitation averages between 18 and 33, cm. The Refuge is a sagebrush-steppe ecosystem with associated habitats. Dominant vegetation consists of big sagebrush (*Artemisia tridentata* Beetle and Young) and little sagebrush (*A. arbuscula* Nutt.). Yellow rabbitbrush (*Chrysothamnus viscidiflorus* (hook.) Nutt.), rubber rabbitbrush (*Ericameria nauseosa* (Pall. Ex Pursh) G.L. Nesom and Baird), spineless horsebrush (*Tetradymia canescens* DC.) and antelope bitterbrush (*Purshia tridentata* (pursh) DC.) are other shrubs prominent in the ecosystem. Grasses consist predominantly of Idaho fescue (*Festuca idahoensis* Elmer), squirreltail (*Elymus elymoides* Raf. Swezey), Sandberg's bluegrass (*Poa secunda* J. Presl), and blue bunch wheatgrass (*Pseudoroegneria spicata*

(pursh) A. Love). Scattered open woodlands consist of western juniper (*Juniperus occidentalis* hook.) and curl-leaf mountain mahogany (*Cercocarpus ledifolius* nutt.) which occupy ridgelines and hillside slopes. Aspen (*Populus tremuloides* (michx)) and willows (*Salix* spp.) are found in scattered snow pockets and in areas of dependable water. Native wildlife also occupies the refuge including pronghorn (*Antilocapra americana*), mule deer (*Odocoileus hemionus*), bighorn sheep (*Ovis canadensis*), and Greater sage-grouse (*Centrocercus urophasianus*).

Tail Hair Collection and Analysis

We collected samples from horses in two separate areas within the refuge, Badger Mountain and Little Sheldon, representing horse-occupied and horse-removal areas, respectively. Between August and October of YR2, a small proportion of the Sheldon NWR horse population was gathered and corralled as part of ongoing management actions. As the horses passed through the handling chute, a staff member randomly pulled tail hair samples from 10 mares and 10 stallions. Hair samples were collected and placed in a paper bag for storage, keeping samples separate and to prevent mold or hair decay. The longest hair from each sample was chosen and analyzed for each horse (average length = 807±87 mm). The hair strand was cleaned with acetone, cut into 10 mm increments, and weighed (200-500 µg) on a microgram balance (Sartorius, data Weighing systems, Elk Grove, IL). A chronology of forage use by season was determined along each tail hair length starting from the root bulb (time 0, date of hair extraction) and working out to the end of the hair based on the continuous nature of tail hair growth 0.72 mm/day; (Ayliffe *et al.*, 2004; Dunnett, 2005; West *et al.*, 2004). Using the average hair length of 807 mm and the growth rate of 0.7 mm/day, the number of days from the bulb to the end of the hair was 1152±112. Dates were assigned to the isotope samples based on the hair length segment. Seasonal periods were divided into spring (March to May), summer (June to August), fall (September to November), and winter (December to February).

Forage Collection

Vegetation cover was mapped using 30m pixel Landsat imagery that identified major vegetation communities on Sheldon NWR (Tagestad, 2009). Using this map, a series of random points (n = 60) were generated for 5 major vegetation classes, separated by at least 10 m within each vegetation class to prevent overlap and minimize autocorrelation (Table 1). Since feral horses on the Sheldon NWR have access to adequate forage and water, they are able to maintain a smaller home range to meet their daily requirements. The home range was mapped and random sample locations were chosen for vegetation measurements.

Table 1: Major and minor vegetation classes within the two study sites

Major vegetation classes	Minor vegetation classes
Basin big sagebrush	Inter-mountain basin greasewood flat
Columbia plateau low sagebrush steppe	Inter-mountain basin mixed salt desert scrub
Inter-mountain basins big sagebrush shrubland	Inter-mountain basin playa
Inter-mountain basins montane sagebrush steppe	Inter-mountain juniper savannah
Inter-mountain basins semi-desert grassland	Inter-mountain mahogany woodland/shrubland
	Northern Rocky Mountains lower montane foothill deciduous shrubland
	Rocky mountain Aspen forest and woodland
	Rocky mountain subalpine-montane mesic meadow

Ten random points were generated for each of the eight minor classes. Within the two study areas, the Basin Big sagebrush habitat class was considerably more prevalent than either the inter-mountain basin mixed salt desert scrub or the inter-mountain basin greasewood flat. The basin big sagebrush class was a major class and the others were minor classes. The barren, inter-mountain basins cliff and canyon, open water and North American arid west emergent marsh vegetation types were not assigned sampling points due either to limited or no availability within the study site or inaccessibility for foraging within the 8 km radius area.

For each vegetation class two random points were selected along roadways in order of occurrence and vegetation was sampled at each selected site area. The random point served as the southwest corner for each 25 m². Within each plot (n = 60), we collected a representative sample of each plant species present (n = 1 sample collected per plot). Grass and forbs samples were clipped at ground level. Branch clippings were collected from the shrubs within the plot area. The plant samples were placed in a paper bag, labeled, and returned to the laboratory for analysis.

Plant samples were dried for 24 h at 60°C (Flinders and Hansen, 1972). Dried samples were ground using a 0.425 mm mill (Wiley Mini-mill, Thomas Scientific, Swedesboro, NJ). Ground samples of collected plants were weighed (600-700 µg) using a microgram balance (Sartorius, data Weighing systems, Elk Grove, IL). Ground subsamples of plants and tail hair sections were combusted using a Costech (ECS 4010, Cornusco MI Italy) elemental analyzer and then passed through a continuous-flow isotope-ratio mass spectrometry system (Delta-V, Thermo Fisher Scientific Inc., Waltham, MA) to determine δ¹⁵N and δ¹³C values.

Isotopic Analysis

A mean carbon diet-hair fractionation (ϵ^*) for a variety of herbivores was demonstrated by Sponheimer *et al.* (2003a). Horses were not investigated in this study so an average value for all herbivores of 3.2% was subtracted from all tail hair $\delta^{13}\text{C}$ values. Sponheimer *et al.* (2003b) demonstrated nitrogen diet-hair fractionation values for a variety of species including horses so a value of 2.0% was subtracted from all tail hair $\delta^{15}\text{N}$ values used for analysis. Plant isotopic values were averaged for plant type (grass, forbs, and shrubs) across the sites to combine sources for the stable isotope mixing model (IsoSource 1.3, (Phillips and Gregg, 2003; Phillips *et al.*, 2005) SIAR, (Parnell *et al.*, 2010).

IsoSource and SIAR are software packages developed for multiple uses in stable isotope analysis using isotopic ratios to determine the proportional contribution of various sources to a mixture. This study uses plant sources that contribute to an isotope mixture associated with sections of tail hair. Various combinations of each source contribution are examined in a small increment (a value determined by the user) and a given mass balance tolerance value (also stated by the user). All source combinations that result in the predicted mixture signatures within the stated tolerance value were considered feasible solutions. In this study, both IsoSource and SIAR were used to calculate the ranges of source contributions of the plant species to the tail hair. Both models produced similar results so only the IsoSource results are presented.

Plant Productivity, Cover and Abundance

Vegetation availability for feral horse consumption on the Sheldon NWR was determined by measuring the following: Plant cover, plant productivity in the form of dry matter biomass, and a relative abundance score. These metrics were collected in July, August, and September of YR2 and YR3. Data were collected from both Badger Mountain and Little Sheldon areas where horses were captured and sampled. To quantify vegetation structure between both areas, we collected vegetation from both sites and compared these statistically.

Biomass Collection

Biomass samples were collected along the random locations described earlier and within 50 m transects. The direction was randomly determined from the central random point. Five 1 m² quadrants were placed at 10 m regular intervals along each transect. In each quadrat, total herbaceous vegetation was clipped at 3 cm above ground and separated by species. Clipped plants were placed in brown paper bags for proper weighing, preservation, and drying. Clipped samples were dried for 24 h at 60°C (Flinders and Hansen, 1972). It should be noted that shrub biomass samples were not collected in this study because of the size of the shrubs.

Table 2: Abundance scoring for plants within quadrants

Score	Abundance
1	Absent from the plot for specific plant species
2	Poor for specific plant species appeared to cover anywhere from 1-10% of the ground in the plot or had a number of plants ranging from 1-10 depending on the species
3	Fair for specific plant species covered from 5-25% of the plot had a number of plants between 2 and 20
4	Good for specific plant species covered between 20 and 40% of the plot had a number of plants between 5 and 30
5	Abundant for specific plant species by far the the most dominant type of cover in the plot, with cover >40% or numbers greater than 10-20

Plant Cover

Using the same transect and sample frequency as biomass, plant cover was determined for each habitat type. A step-point method described by Evans and Love (1957) was used to collect cover samples. Plants, bare ground, rock, and litter were used as surface features observed directly behind a notch in the tip of the boot and were recorded for a total of 100 points per plot. The total number of hits for a specific species divided by the total of all plant hits recorded gave the total percent plant canopy cover. Percent cover was recorded for each individual species, total grass, total forbs, total shrubs, and all "other" cover categories (bare ground, rock, pebble, cobblestone, dead shrub, litter, anthill, and manure).

Relative Abundance

The same transect method described previously using a 5×5 m² quadrat was placed in the southwest corner of that plot and each species present was observed and given an abundance score within the plot, (Table 2). The scores from 2-5 were dependent on the size of the plant species in question. Smaller species such as grasses were allowed to have more plants present while still falling in the poor abundance category while larger species such as shrubs were allowed fewer numbers while falling in this category. These values were given to each species in the plot relative to the abundance of that same species across the entire sampling area.

Statistical Analysis

Proc mixed (SAS, Inc, Cary, NC) was used to analyze hair and plant isotope data. Year, gender, collection site, and habitat type main effects, with interactions, were determined. Significance was determined at $p < 0.05$.

Systat 13 (Systat Software Inc, Chicago, IL; for ease of interpreting result format) was used to analyze biomass, abundance, and cover data, checking twice for normality. The data was transformed the first time using the square root transformation and then tested a second

time using skewness and kurtosis values for normality. Approximately half of the data was normal after transformation and the other half was still not normal. Normal data was analyzed with a parametric test (ANOVA) and non-normal data was analyzed with a non-parametric test (Kruskal-Wallis). Differences were determined significant at $p < 0.05$. To characterize vegetation structure and the differences between each, we also measured the significance of these metrics between both sites.

Results

$\delta^{13}C$ and $\delta^{15}N$ Isotope Results

Plant isotope values showed no difference between the time of year, years collected, collection sites, or habitat types ($p > 0.15$). The values for the plant isotopes were averaged by species for the two years and the various collection sites (Table 3).

The tail hair $\delta^{13}C$ and $\delta^{15}N$ values for each 5 mm section within gender were averaged for the seasonal periods of winter, spring, summer, and fall for years 1 (YR1), 2 (YR2), and 3 (YR3), respectively. The seasonal tail hair means were used in conjunction with plant $\delta^{13}C$ and $\delta^{15}N$ means for isotope analysis (Figs. 1-4).

Seasonal Variation

There was a seasonal variation of the horse diets for both $\delta^{13}C$ and $\delta^{15}N$ varying in similar chronological patterns between genders (Fig. 5). Though the isotope chronology pattern was similar, hair isotope signatures for $\delta^{13}C$ and $\delta^{15}N$ were significantly different between gender ($p < 0.001$) and for chronology ($p < 0.001$) but not for the interaction.

Mean dietary forage consumption percentage and standard error obtained from IsoSource are Figs. 1-4. The plant sources used for the isotope analysis were analyzed using intuition and observation. Plant species used in the isotope analysis included *Achnatherum* spp., *B. tectorum*, *Carex rossii*, *E. elymoides*, *F. idahoensis*, *Juncus balticus*, *Koeleria macrantha*, *Leymus cinereus*, *Poa* spp., *Artemisia* spp., *C. viscidiflorus* or *E. nauseosa*, *P. tremuloides*, *Salix* spp. and combined forb species. Grass and sedge species, shrub, tree, and forb isotope values were combined by plant type, with shrub and tree values combined, and the three plant type isotope values run through IsoSource for spring, summer, and fall.

IsoSource results between years showed similar patterns overall, but there were differences for some plant types. Forbs, *Salix*, and *P. tremuloides* were not used in the winter analysis because they were not available and *B. tectorum* was not included in the spring and summer analysis due to low palatability.

Table 3: Plant isotopic values (%) averaged^a between samples taken in YR2 and YR3. Data are expressed as value \pm SEM

Grasses/Sedges	$\delta^{13}C$	$\delta^{15}N$
<i>Achnatherum nelsoni</i>	-27.0 \pm 0.3	-0.7 \pm 0.5
<i>Achnatherum occidentale</i>	-26.6 \pm 0.2	-3.3 \pm 0.9
<i>Achnatherum thurberiana</i>	-26.9 \pm 0.4	-2.3 \pm 0.3
<i>Bromus tectorum</i>	-27.3 \pm 0.3	-2.3 \pm 0.3
<i>Carex rossii</i>	-28.4 \pm 0.4	-0.8 \pm 0.9
<i>Elymus elymoides</i>	-27.6 \pm 0.1	-1.4 \pm 0.2
<i>Festuca idahoensis</i>	-27.9 \pm 0.1	-2.7 \pm 0.2
<i>Hesperostipa comata</i>	-27.7 \pm 0.5	-0.6 \pm 0.6
<i>Juncus balticus</i>	-27.4 \pm 0.3	0.3 \pm 0.4
<i>Koeleria macrantha</i>	-26.9 \pm 0.6	-2.6 \pm 0.6
<i>Leymus cinereus</i>	-26.6 \pm 0.6	-1.7 \pm 0.6
<i>Poa pratensis</i>	-27.5 \pm 0.3	-2.8 \pm 0.5
<i>Poa secunda</i>	-27.3 \pm 0.3	-2.5 \pm 0.2
<i>Pseudoroegneria spicata</i>	-27.2 \pm 0.2	-2.5 \pm 0.3
<i>Stipa columbiana</i>	-26.2 \pm 0.6	-0.4 \pm 1.0
<i>Stipa hymenoides</i>	-26.9 \pm 0.4	-0.9 \pm 0.7
Forbs		
<i>Balsamorhiza sagittata</i>	-28.1 \pm 0.3	-0.9 \pm 0.6
<i>Crepis acuminata</i>	-28.1 \pm 0.2	-2.2 \pm 0.3
<i>Eriogonum</i> sp.	-29.1 \pm 0.2	-1.2 \pm 0.8
<i>Lupinus balticus</i>	-28.0 \pm 0.3	-1.4 \pm 0.2
<i>Phlox hoodii</i>	-26.3 \pm 0.4	-1.5 \pm 0.3
<i>Senecio canescens</i>	-29.7 \pm 0.7	-1.3 \pm 0.7
Shrubs/Trees		
<i>Artemisia arbuscula</i>	-27.8 \pm 0.2	-1.1 \pm 0.3
<i>Artemisia tridentata</i> ssp. <i>tridentata</i>	-27.7 \pm 0.4	-0.4 \pm 0.8
<i>Artemisia tridentata</i> ssp. <i>vaseyana</i>	-27.2 \pm 0.2	-0.2 \pm 0.3
<i>Artemisia tridentata</i> ssp. <i>wyomingensis</i>	-26.3 \pm 0.8	-2.0 \pm 0.7
<i>Cercocarpus ledifolius</i>	-25.8 \pm 0.5	-3.6 \pm 0.2
<i>Chrysothamnus nauseosus</i>	-28.0 \pm 0.2	-0.4 \pm 0.3
<i>Chrysothamnus viscidiflorus</i>	-28.2 \pm 0.1	-0.4 \pm 0.3
<i>Grayia spinosa</i>	-26.4 \pm 0.9	0.4 \pm 1.4
<i>Populus tremuloides</i>	-27.0 \pm 0.0	-0.1 \pm 0.2
<i>Purshia tridentata</i>	-26.2 \pm 0.2	-3.0 \pm 0.3
<i>Salix</i> sp.	-26.5 \pm 0.4	-1.4 \pm 0.6
<i>Symphoricarpos oreophilus</i>	-27.7 \pm 0.3	-2.7 \pm 0.3
<i>Tetradymia canescens</i>	-27.7 \pm 0.4	-1.4 \pm 0.3

statistical analysis was not significant for Year or Site, therefore the isotope values were averaged across Year and Site

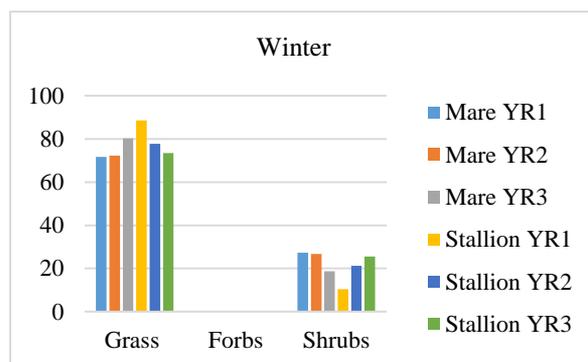


Fig. 1: Isotope analysis results of stallions and mares for winter YR1, YR2, and YR3 percentage of the forage consumed. SEM for the grass was 0.009 and for shrubs 0.010. Forbs were not included in the analysis. Forage means between forage types differed ($p < 0.05$). Between years forage means differed between genders ($p < 0.05$)

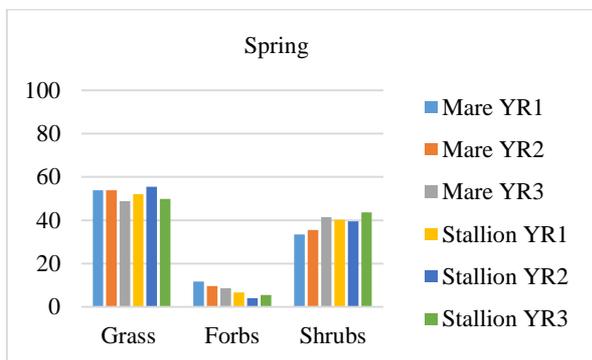


Fig. 2: Isotope analysis results of mares and stallions for spring YR1, YR2, and YR3 percentage of the forage consumed. SEM for the grass was 0.009, 0.010 for forbs, and for shrubs 0.010. Forage means between forage types differed ($p < 0.05$). Between years forage means differed between genders ($p < 0.05$)

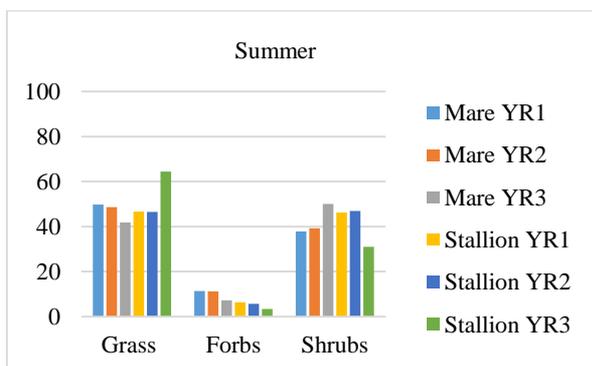


Fig. 3: Isotope analysis results of mares and stallions for summer YR1, YR2, and YR3 percentage of the forage consumed. SEM for the grass was 0.010, for 0.009 for forbs, and for shrubs 0.009. Forage means between forage types differed ($p < 0.05$). Between years forage means differed between genders ($p < 0.05$)

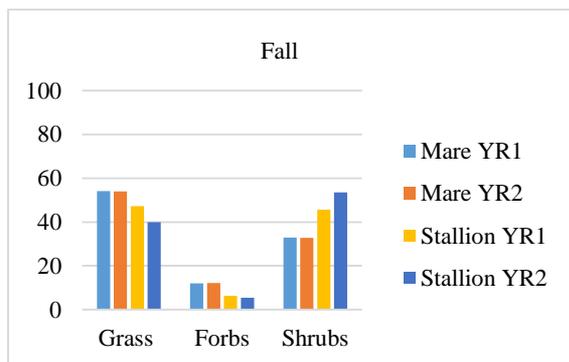


Fig. 4: Isotope analysis results of mares and stallions for fall YR1 and YR2 percentage of the forage consumed. SEM for grass was 0.009, for forbs 0.009, and for shrubs 0.010. Forage means between forage types differed ($p < 0.05$). Between years forage means differed between genders ($p < 0.05$)

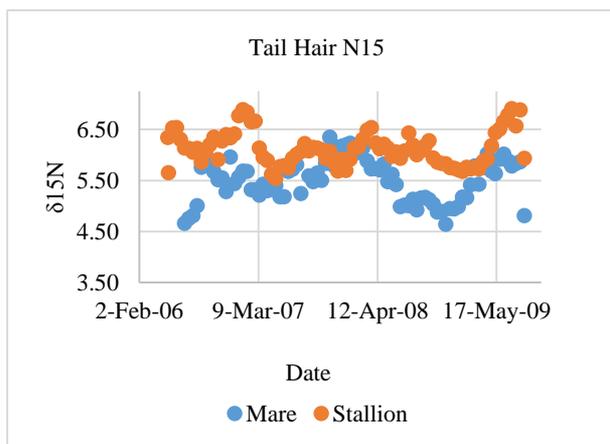
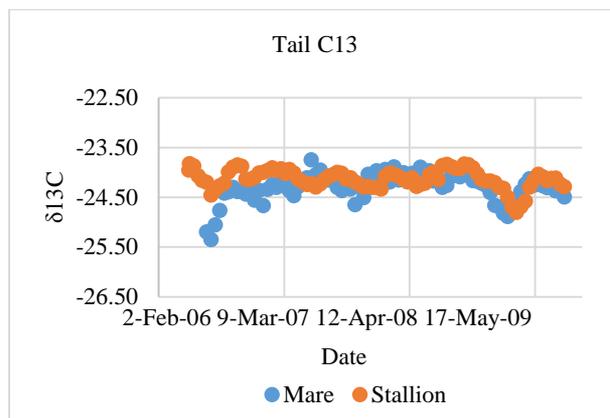


Fig. 5: Seasonal variation of carbon (13 C) and nitrogen (15 N) isotopic signatures of mares (o) and stallions (●). Differences ($p < 0.001$) were found between gender and chronology for carbon and nitrogen isotopes, but not for the interaction

Winter: Winter YR3 (Fig. 1) was significantly ($p < 0.05$) less than YR1 and YR2 for grass consumption by mares. Mare YR1 and YR2 are not different. The opposite pattern is noted in shrub consumption, with Mare YR3 significantly ($p < 0.05$) less than YR1 and YR2. Stallion grass consumption increased significantly ($p < 0.05$) between each of the years, while shrub consumption decreased.

Spring: Spring plant isotope values for (Fig. 2), *E. elymoides*, *F. idahoensis*, *J. balticus*, *C. Rossii*, and *Poa* spp. were the grass/sedges included in the IsoSource analysis. *P. tremuloides*, the shrubs *Artemisia* spp, *Chrysothamnus* spp, and *Salix* spp were included as well as forbs were also included in the analysis. Mare YR1 and YR2 were not different, while YR3 was less ($p < 0.05$). Mare YR1, YR2, and YR3 were different ($p < 0.05$) from each other, YR1 greater than YR2 greater than YR3. The opposite significant ($p < 0.05$) pattern was found for the shrub intake. Stallion results presented in Fig. 2 YR2 grass with a higher ($p < 0.05$) consumption followed by YR1

then YR3. Forb intake was the opposite with YR2 consumption less ($p<0.05$) the YR3 which was less than YR1. Stallion shrub intake was the same for YR1 and YR2, with YR3 consumption greater ($p<0.05$).

Summer: Summer plant isotope sources included *E. elymoides*, *F. idahoensis*, *J. balticus*, *C. Rossii* and *Poa* grass/sedges, *Artemisia* spp, *Chrysothamnus* spp, *P. tremuloides* and *Salix* spp shrubs and Forbs. Mare YR1 and YR2 for grass/sedge, forbs, and shrubs are not different between the two years. Grass/sedge, forb, and shrub YR3 were different ($p<0.05$) from YR1 and YR2. Stallion YR1 and YR2 were not different from each other, while YR3 grass/sedge was greater ($p<0.05$) than YR1 and YR2. For YR3 and shrubs, values were less ($p<0.05$) than YR1 and YR2.

Fall: Fall data is only presented for the YR1 and YR2 years because the tail hair collection occurred in October of YR3 (Fig. 4). Mare YR1 was not different from YR2 for grass. Forbs or shrubs, while stallion YR1 was greater ($p<0.05$) than YR2 for grass and forbs. Stallion YR2 shrub consumption was greater ($p<0.05$) than YR1. Mare grass consumption for grass and forbs was greater ($p<0.05$) than for stallions. Shrub intake by the stallions was greater ($p<0.05$) than mares.

Year Variation

Comparing season to season across the 3 years mare grass intake was higher ($p<0.05$ during the winter (72%) than the spring and fall at 53% for YR1 and YR2 but was different ($p<0.05$) for summer, which was less. Grass intake for YR3 winter was greater ($p<0.05$) at 80% than the spring and summer YR3 intake at 49 and 42% respectively. Forb intake by the mares was similar across spring, summer, and fall for the three years, while shrub intake was greatest ($p<0.01$) for summer followed by spring, fall then winter.

Across the four seasons, stallion grass intake was greatest ($p<0.05$) during winter. The stallions consumed very little forbs each of the three years.

Vegetation Biomass, Cover and Availability

Comparison of plant biomass, abundance, and cover between the two sampling sites (Badger Mountain and Little Sheldon), between the various habitat types studied, and both site and habitat differences are found in Table 4. A comparison of biomass, abundance, and cover for the different horse habitat areas is found in Table 5. The differences in plant biomass observed in riparian areas are found in Table 6. Table 7 presents the top 10 plant species that exhibit the highest biomass, abundance, and cover within our study plots. Forbs and shrubs were each included in the table as a combined entity.

Table 4: Comparison of plant biomass, abundance, and cover between Little Sheldon and Badger Mountain sites

Parameter	Site	Habitat	Site X habitat
Biomass (kg/ha)	NS	0.030	0.004
Grasses			
<i>Achnatherum thurbarianum</i>	0.001	0.030	0.001
<i>Carex rossii</i>	NS	0.005	0.001
<i>Elymus elymoides</i>	0.001	0.002	0.004
<i>Poa Secunda</i>	NS	0.001	0.001
Forbs	0.020	0.001	0.001
Abundance (relative score)	0.020	NS	NS
Grasses			
<i>Achnatherum thurbarianum</i>	NS	NS	0.020
<i>Elymus elymoides</i>	0.006	NS	NS
<i>Festuca idahoensis</i>	NS	0.002	NS
Forbes	NS	0.030	0.030
Shrubs	NS	0.001	0.040
Cover (%)	NS	NS	NS
Grasses			
Forbes	NS	0.005	NS
Shrubs	NS	0.001	NS

Table 5: Biomass (kg/ha), abundance (relative score), and cover (percent of hits) for feral horse Little Sheldon (LS) and Badger Mountain (BM) habitat areas within the Sheldon National Wildlife Refuge

Parameter	LS	BM	P-value
Biomass (kg/ha)	44.61±8.39	43.70±2.06	NS
Grasses			
<i>Achnatherum thurbarianum</i>	2.35±0.45	6.58±0.81	0.001
<i>Elymus elymoides</i>	5.62±0.50	8.86±0.77	0.001
<i>Festuca idahoensis</i>	1.52±0.73	7.09±1.15	0.001
<i>Hesperostipa</i>	0.00±0.00	0.94±0.41	0.050
<i>Oryzopsis hymenoides</i>	0.00±0.00	0.09±0.09	0.010
<i>Stipa columbiana</i>	0.00±0.00	0.78±0.33	0.010
Forbs	10.35±1.01	13.72±1.02	0.020
Annual Forbs	0.00±0.00	1.06±0.36	0.001
Perennial Forbs	10.35±1.01	12.66±1.01	0.008
Abundance (relative score)	10.19±0.82	13.02±0.81	0.020
Grasses			
<i>Achnatherum nelsonii</i>	0.00±0.00	0.98±0.30	0.002
<i>Elymus elymoides</i>	2.23±0.27	3.10±0.23	0.007
Forbs	10.46±1.10	13.38±1.29	NS
<i>Cryptantha</i> sp.	0.00±0.00	0.42±0.18	0.020
<i>Lupinus argenteus</i>	0.54±0.26	1.27±0.29	0.010
<i>Phlox hoodii</i>	0.35±0.17	1.15±0.30	0.030
Shrubs	5.96±0.79	7.27±0.78	NS
<i>Artemisia arbuscula</i>	1.58±0.39	0.38±0.22	0.010
<i>Tetradymia canescens</i>	0.00±0.00	0.50±0.22	0.020
Cover (%)	27.46±3.41	24.04±3.64	NS
Grasses			
<i>Elymus elymoides</i>	2.62±0.49	6.00±0.81	0.001
<i>Hesperostipa columbiana</i>	0.00±0.00	0.81±0.31	0.005
<i>Stipa columbiana</i>	0.00±0.00	1.08±0.40	0.001
<i>Stipa nelsonii</i>	0.00±0.00	0.23±0.13	0.040
Forbs	9.00±1.24	9.50±2.75	NS
<i>Phlox hoodii</i>	0.23±0.23	0.62±0.25	0.040
Shrubs	22.38±0.42	19.58±2.65	NS
<i>Artemesia cana</i>	0.15±0.09	0.00±0.00	0.040
Litter	6.31±1.38	12.04±1.33	0.001
Rock	8.38±2.56	1.62±0.56	0.030

Table 6: Differences between biomass (kg/ha) of predominant species in riparian areas. Mean values (with SEM values) are shown for both the Little Sheldon (LS) and the Badger Mountain (BM) sites in kilograms

Plants	LS	BM	P-value
Grasses	72.86±15.00	56.62±7.52	NS
<i>Bromus tectorum</i>	0.00±0.000	3.12±1.29	0.03
<i>Carex rossii</i>	6.74±1.700	1.62±1.08	0.04
<i>Juncus balticus</i>	14.10±1.670	6.27±1.89	0.01
<i>Poa secunda</i>	10.07±1.410	3.64±1.49	0.01
Forbs	6.00±1.030	8.80±2.58	NS

Table 7: Biomass, abundance, and cover of feral horse habitat areas within the Sheldon National Wildlife Refuge measured during spring and summer. Biomass values are in kg/ha, abundance is a summed abundance score value and cover values are the total number of hits per cover type out of 5100 total hits

Biomass (kg/ha)	Abundance (relative score)		Cover (%)		
Forbs	3129	Forbs	62	Shrubs	21
<i>Poa Secunda</i>	1884	Shrubs	344	Forbs	9.4
<i>Elymus elymoides</i>	1882	<i>Elymus elymoides</i>	139	<i>Poa secunda</i>	5.6
<i>Bromus tectorum</i>	1598	<i>Poa secunda</i>	124	<i>Elymus elymoides</i>	4.4
<i>Stipa thurberiana</i>	1160	<i>Stipa thurberiana</i>	68	<i>Festuca idahoensis</i>	3.0
<i>Leymus cinereus</i>	1142	<i>Festuca idahoensis</i>	62	<i>Stipa thurberiana</i>	2.4
<i>Festuca idahoensis</i>	1118	<i>Pseudoroegneria spicata</i>	44	<i>Juncus balticus</i>	2.4
<i>Pseudoroegneria spicata</i>	641	<i>Bromus tectorum</i>	39	<i>Leymus cinereus</i>	1.6
<i>Carex rossii</i>	618	<i>Achnatherum nelsonii</i>	26	<i>Agropyron cristatum</i>	1.5
<i>Poa pratensis</i>	396	<i>Juncus balticus</i>	23	<i>Lepidium perfoliatum</i>	1.2

Discussion

Forages collected within the Badger Mountain and Little Sheldon sites showed no significant isotopic species variation ($p>0.26$) within or between the two sites, time of year sampled, or between years ($p>0.15$). Because of this, averaged plant species isotopic values were used. Because there was no difference between the late spring and late fall forage isotope values, we felt confident that the average species values could be used for all four seasons presented.

Tail Hair

A tail hair growth value of 0.72 mm/d was used to determine chronology. Dunnett (2005) in a study of 29 horses, found tail hair growth was linear for a year and determined climate had no effect. They also found no gender differences. Nutrition has been shown to decrease hair growth (Lewis, 1995). Burnik Šturm *et al.* (2015) showed tail hair growth rate was between 0.72 and 1.28 mm/d. The 1.28 was determined on one horse. Based on the growth range, we chose 0.72 mm/d due to the estimated lower nutrient-quality diet of the horses. The isotopic signatures revealed there were differences between mares, and stallions, and a temporal relationship for C13 and N15. These findings were different from a similar study where the feral horse temporal values stayed relatively constant through time (Burnik Šturm *et al.* 2017). The differences between the two studies could be due to different vegetation species and the other study focused on

comparing the feral horses with Przewalski's horses and khulans (Burnik Šturm *et al.* 2017).

Diet-hair Fractionation

Diet-hair $\delta^{13}C$ fractionation (ϵ^*) has not been determined on horses, so an average of published herbivore values outlined by Sponheimer *et al.* (2003a) of 3.2% was used. We acknowledge that this is an approximation and is an area that needs to be researched further. The value used for $\delta^{15}N$ for diet-hair fractionation was 2.0%. Sponheimer *et al.* (2003b) looked at the dietary protein response between low-protein coastal Bermuda grass and alfalfa fed to horses and showed a decrease from 4.5-2.0%. For our study, we used 2.0% for the different seasons assuming the protein intake of the feral horses would more closely approximate the Bermuda grass.

Site Forages

The biomass, abundance, and cover data together are good indicators of the forage available to horses and other herbivores. In combination with the isotope data, the grasses, forbs, and shrubs consumption can be estimated by species of each forage type.

The forage differences between both sites demonstrated that *C. Rossii*, *J. balticus*, and *Poa* spp. were higher ($p<0.05$) on the Little Sheldon site, 6.7, 14.1, and 10.1 kg/ha respectively. Badger Mountain site *B. tectorum* was more prevalent ($p<0.05$) in the Badger Mountain riparian area than the Little Sheldon, 3.1-0.0 kg/ha respectively. This is attributed to the Badger Mountain riparian areas being more disturbed by horses (Hunter, 1991; Rice and Mack, 1991; Knapp, 1996). Grasses, in general, made up most of the biomass with *J. balticus*, and *Poa* as the predominant grasses for both the little Sheldon and the Badger Mountain (Table 5). Though grasses were the prominent biomass, *P. tremuloides* and *Salix* spp. were present.

Dietary Shifts

The feral horse diet is largely determined by what food sources are available for consumption, meaning they are opportunistic to a degree, so it logically follows that the diet would change with changing seasons and plant species abundance. Dietary shifts throughout the seasons, in Fig. 5, indicate the mares and stallions are foraging differently ($p<0.05$), like seasonal pattern shifts from previous studies (Salter and Hudson, 1979). The changes in seasonal forage consumption (Figs. 1-4) are further evidence of this. We show Fall shrub intake is less ($p<0.05$) for mares than summer, 33-38-50% respectively. Whereas the winter shift was more towards the grass and fewer shrubs (18-27%). It is logical that the horses would shift more to grass in the winter due to the dormancy of the shrubs (aspen and willows). Our assumption is that the horses were eating leaves during the winter.

Generally, the mares consumed a wider variety of forages, while the stallions focused more on grasses and shrubs. Salter and Hudson (1979) found that seasonal forage consumption variability was due to palatability and abundance. Studies indicate various factors that influence forages and forage types chosen by feral horses. These factors include forage quality, water source, and availability (Salter and Hudson, 1979). Throughout the seasons and years, there was a shift between grass consumption (58-89%) to shrubs and forbs. Crane *et al.* (1997) also found shrubs and forb consumption ranged from 1-23% combined. Scasta *et al.* (2016) showed wild horses diet composition was between 77-89% grass, 4-15% forbs and 3-10% browse (shrubs). King *et al.* (2019) using micro-histology and DNA barcoding showed horses consumed 79 and 69% of graminoids (grasses), respectively. Horses in our study consumed a larger amount of shrubs ranging from 13-37%, while forb intake ranged from 4-17% across the three years.

Fleurance *et al.* (2009) concluded that feral horses prefer areas with abundant biomass to increase the efficiency of foraging due to their digestive physiology. Girard *et al.* (2013) found that feral horses in Alberta, Canada foothills preferred open grassland and shrubland areas. *Artemisia* spp., *Chrysothamnus* spp., species along with *P. tremuloides* and *Salix* spp. were the predominant shrub species found on the range and in the riparian corridors. The foraging of these shrub species was affected by gender and season.

Forbs and grass species commonly found in abundance (Table 4) within our study sites likely influenced dietary selection due to presence and abundance. Vavra and Sneva, (1978) showed that *F. idahoensis* was a grass highly selected by cattle and horses in Oregon. Additionally, Crane *et al.* (1997) found that grazing impacts to streamside and meadow areas were highest in mountain big sagebrush communities during the growing season. Springs, riparian areas, and wet meadows provide important habitat to other large and small mammalian herbivores (Hall *et al.*, 2016; Gooch *et al.*, 2017; Hall *et al.*, 2018).

Wildlife Impact

The interaction of the feral horse and other herbivorous species is of great importance, especially with the sage-grouse. Plant community composition is affected by grazing in the wildlife habitat of open shrubland and open ground change (Holechek *et al.*, 1989) where sage-grouse may be found. Zalba and Cozzani (2004) found that the grazing of feral horses on the grasslands of Argentina resulted in an increase in egg predation due to increased nest visibility. Beever and Aldridge (2011) described the effects of feral horses on sage steppe ecosystems showing that not only did they consume shrubs and grasses, but trampling was also a factor in the degradation of the

ecosystem. These factors were responsible for decreasing grass height and shrub canopy cover in horse-grazed ecosystems. Reduced shrub cover has been shown to negatively impact sage-grouse nesting and brood-rearing metrics (Beever and Aldridge, 2011).

Gregg *et al.* (2008) assessed the diet selection and nutrition of preincubating sage-grouse and found that because of the higher forage quality of forbs, sage grouse selected forbs prior to incubation to prepare for reproductive activity. Grouse hens shifted forage selection from sagebrush to forbs during the spring higher crude protein, calcium, and phosphorus; Barnett and Crawford, (1994). Forb selection by feral horses in our study was not a major component of the mare's diet and less in the stallions during the spring of each year. Forb biomass was two times as high in sagebrush steppe communities where mares were observed grazing as opposed to riparian areas that were frequented more often by stallions.

The influence of horse grazing is shown to influence plant cover, which may potentially influence sage-grouse nesting and brood rearing (Beever and Aldridge, 2011). Forb-rich areas also provide important summer forage during early and late brood-rearing periods for sage-grouse and potentially impact insects for dietary consumption, habitat, and population characteristics of the sage-grouse (Beever and Aldridge, 2011; Johnson and Boyce, 1990).

Girard *et al.* (2013) showed a 44% biomass removal by horses and other herbivores, including cattle, by mid-July and concluded that carrying capacity and rangeland health were important in the management of feral horse populations.

Studies have shown overlap in dietary preferences between feral horses and wild ungulates including deer, elk, and pronghorn and that season was a factor (Hubbard and Hanson, 1976; Hansen *et al.*, 1977). This implies that not only does the rangeland condition need to be monitored for the benefit of the feral horses, but also the other ungulates they compete with.

Conclusion

Measuring tail hair carbon and nitrogen isotope concentrations provides information on the chronological foraging differences between stallions and mares. Using the isotopic values of plant species abundant within the home range of feral horses in conjunction with the isotope values of horse tail hair is a tool to determine the plant type composition selected for by mares and stallions. Grass species made up the largest percentage (55%) of the feral horse diets, while shrubs (including immature willow and aspen; 45%) and forbs made up the remainder in our study. These percentages of intake combined with the measured plant biomass, cover, and abundance data are helpful in determining which plant species are most heavily selected for. This information will help improve

the predictability of habitat use and the potential competition effects of feral horse grazing on various wildlife and their habitats. The consumption of immature aspen and willow trees in this study can inform managers how to conserve areas through exclusions, moving horses to a different area within the refuge, or other methods to improve forest regions within the refuge. Understanding the grazing habits of feral horse populations and the forages they consume will benefit conservation group members and land and horse managers to better understand, oversee, and ensure the ecosystem's success. Data, like these presented, can be used to make management discussions on the carrying capacity of the range for the various wildlife, specifically the feral horse. Feral horse population size can be reduced to a level that the range can handle and horses can be moved to other sites like contracted facilities.

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Author's Contributions

Megan K. Nordquist and Gail H. Collins: Research data collected, data analysis, manuscript preparation.

Todd F. Robinson and Randy L. Larsen: Data analysis, manuscript preparation.

Melissa A. Myrick: Manuscript preparation.

Steven L. Petersen: Research data collection and analysis.

Ethics

The authors declare there are no ethical issues that may arise after the publication of this manuscript. The

handling of the horses and sample collection was under the approval of the Sheldon National Wildlife Refuge staff and their animal use protocols.

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