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Stable Isotope Diet Reconstruction of Feral Horses (Equus caballus) on the Sheldon

National Wildlife Refuge, Nevada, USA

Megan K. Nordquist

A thesis submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of

Master of Science

Todd F. Robinson, Chair Steven L. Petersen Randy L. Larsen

Department of Plant and Wildlife Sciences

Brigham Young University

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ABSTRACT

Stable Isotope Diet Reconstruction of Feral Horses (*Equus caballus*) on the Sheldon National Wildlife Refuge, Nevada, USA

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Master of Science

Feral horse management has become a subject of significant controversy in the United States. This is because of differing opinions and minimal recent empirical data on feral horses. In recent years, numbers of feral horses have increased due to governmental horse removal restrictions (specifically the Wild Horse and Burro act of 1971). With increasing numbers of feral horses on rangelands, land managers are challenged with identifying the appropriate course of action for satisfying groups with differing opinions.

The purpose of this study is to characterize diet consumption through the use of stable isotope dietary analysis (δ^{15} N and δ^{13} C). We did this in order to measure the impact of feral horse forage consumption on rangelands and to propose strategies for improving habitat management and conservation. We obtained tail hair isotopic values from tail hair removed while horses that were held in squeeze chutes following a roundup. Resulting isotopic values were compared to plant isotopic values using plant samples obtained from the geographical areas as the horses in order to characterize diet. Contribution of the various plant species to the tail hair mixture values was determined using the EPA program IsoSource©. Initial analysis of tail hair isotopes demonstrated seasonal variation. During summer months, shrubs (mostly *Artemesia spp*, and *Purshia Tridentate*), *Elymus elymoides*, *Juncus balticus*, and *Festuca idahoensis* were the predominantly consumed vegetative species. During fall months, *Leymus cinereus* and *Juncus balticus* played a more significant role in feral horse diet. In the winter, shrubs were more heavily consumed along with *Poa secunda*. Springtime showed a shift towards forb consumption. Changes in seasonal consumption of forages are most likely linked to forage availability as well as equine preference.

We analyzed plant metrics (specifically biomass, abundance, and cover) to compare a site with horses present to a site where horses had been removed the previous year and found relatively few differences between the two sites. With nearly all differences we found higher plant production (forage availability) on the site where horses were still present. In riparian areas however, there was more vegetation (specifically *Carex rossii*, *Juncus balticus*, and *Poa secunda*) on the site where horses had been removed. Within riparian areas, only *Bromus tectorum* (a plant not typically found in riparian areas but characteristic of degraded areas) showed significantly greater amounts of biomass on the site with horses present. Knowledge of plant species consumption will allow land managers greater ability to make scientifically based decisions regarding feral horse population control which is important in determining appropriate management levels of populations.

Keywords: feral horses, stable isotopes, diet, forage selection, forage availability, IsoSource



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LITERATURE REVIEW INTRODUCTION

Feral horses (*Equus caballus*)¹ have drawn increasing interest and attention in recent years. With mounting numbers of feral horses on rangelands, negative impacts have been identified involving native plant and animal species as well as abiotic components of the ecosystem (i.e. hydrology, soil structure, etc.). The purpose of this chapter is to convey available information regarding feral horse population and habitat use characteristics (particularly regarding diet and forage selection) by presenting 1) a general description of feral horse habitat selection, time allocation, band structure, foaling rates, and population dynamics, 2) a description of grazing and feeding habits, with a particular emphasis on differences in diet between feral horses and other ungulates with which they compete, and 3) a review of available literature regarding potential and actual impacts horses have on rangelands. This review includes a discussion of the affects on wildlife, vegetation impacts, consequence to riparian areas, and changes to soils as well as a statement about how to mitigate such impacts. It will discuss some of the current management techniques employed by the Bureau of Land Management as well as the United States Fish and Wildlife Services.

This chapter serves as a springboard and introduction to our particular area of study involving stable isotope diet reconstruction of feral horses on the Sheldon National Wildlife Refuge (NWR) using stable isotopic analysis. It also provides a description of the physical

¹ The term feral refers to animals that were once domesticated. All free-ranging horses currently on North American rangelands (the principal area of study) came from domestic stock after native wild North American horses went extinct in the late Pleistocene (approximately 10,000 ybp) (Berger 1986). Despite the fact that feral horses are typically referred to as free-ranging, wild, or feral, for ease and convenience we have lumped them all into the term, "feral horses" throughout this paper.



characteristics of the Sheldon NWR (including its geographical location, the habitat, climate, and elevation).

The final section of this chapter introduces the reader to the use of carbon and nitrogen isotopes in dietary characterization. The focus of this introductory material will center on hair isotopic analysis in determining diet.

GENERAL FERAL HORSE CHARACTERISTICS

Habitat Selection

Feral horses spend the majority of their time in shrublands and meadows at relatively high elevations (Berger 1986). When horses are not in high-elevation sites, they preferentially select streamsides, bogs/meadows, and mountain sagebrush habitats (Linklater *et al.* 2000). Subsequently, feral horses allocate the majority of their time in sites of those habitat types and sites adjacent to or within close proximity to those habitat types (Linklater *et al.* 2000). Lowland sagebrush habitats, south-facing aspects, steep slopes, bare ground, and forest remnants are avoided (Crane *et al.* 1997). Habitat types are selected or avoided by feral horses due to three different factors: food, water, and shelter (Miller 1983).

Of these three factors, the most influential in habitat selection is food. Specifically, forage availability and succulence of available forages are factors that dominate habitat selection (Duncan 1983; Crane *et al.* 1997; Salter & Hudson 1979). Feral horses will typically select habitats with large quantities of high quality forage (defined as areas with >90g/m² of green plant matter) and prefer areas with a high concentration of preferred forage species (Duncan 1983).

Water

The availability of water is second to forage availability in importance for determining feral horse habitat selection (Miller 1983). During summer months, feral horses spend the



majority of their time within 4.8 km of a water source (Miller 1983). However, during late fall, winter, and early spring areas farther from water sources may be frequented. Shifts are due to changes in forage species availability within a plant community through the seasons (Miller 1983). Interestingly, the distance that feral horses remain from a water source is correlated with the average 7-day temperature. Colder weather causes horses to maintain winter patterns, meaning they reside farther away from water sources, and warmer weather causes horses to maintain summer patterns, remaining closer to water sources (Miller 1983).

Seasonal Variability in Habitat Selection

Fall/Winter

During the fall and winter, feral horses are more likely to be found in areas of higher elevations, ridges and areas with north-facing aspects. During fall and winter forage availability is proportionately greater in higher elevations and horses are able to find more protection from inclement weather in these areas (Miller 1983, Linklater *et al.* 2000). Within these higher elevations and ridges, habitat use typically focuses on sagebrush/shrubland and grasslands (Miller 1983, Berger 1986). The next most used habitat types are juniper stands followed by meadows (Berger 1986).

Spring/Summer

Early in the spring, feral horses are often found in habitats similar to fall/winter habitats (shrublands and grasslands). This is likely due to foaling and mating during the early spring (Linklater *et al.* 2000). During late spring and early summer, feral horses are more likely to spend time in short grassy vegetation/flush zones with lower elevations and gentler slopes, such as river bottoms and stream valley floors (Linklater *et al.* 2000). One study reports that horses in



Wyoming's Red Desert prefer habitat with saltbush-winterfat present during late spring and early summer (Miller 1983).

Allocation of Feral Horse Time

Feeding

Time budgeting for feral horses revolves around the central concept of feeding. Feral horses spend as much time as possible throughout their day obtaining food. Grazing patterns of feral ponies in Assateague Island show they spend 55% of nocturnal hours foraging (Keiper 1980). Time that horses spend grazing is limited by the need for other activities such as resting, moving, drinking, socializing, and reproduction (Berger 1986; Duncan 1985; Miller 1983). The quality of forage in a given home range of horses also affects grazing time (Berger 1986). In areas with higher quality forage (greater proportion of green matter), horses spend significantly less time foraging, whereas in areas of lower-quality forage (less green matter), horses spend a significantly longer time foraging (Berger 1986).

Experiments show that the onset of feeding is under endogenous control. This means that when blood glucose concentrations are high, the onset of feeding is delayed whereas when blood glucose concentrations are low, horses begin foraging (Mayes & Duncan 1986). It is also possible that feeding lengths, as well as sleep patterns, are determined by circadian rhythms. Therefore, when a horse is not resting or sleeping, it is induced to eat (Mayes & Duncan 1986). Along with glucose concentrations and activity of the horse, gut-fill plays a role in the induction of feeding. When gut-fill levels are low, horses begin to feed and when gut-fill levels are high, horses refrain from eating (Mayes & Duncan 1986).

Differences in Time Allocation between Reproductive Categories



Males spend significantly less time feeding than females (both lactating and non-lactating). Lactating females spend significantly more time feeding than non-lactating females (Berger 1986). This incongruity between lactating and non-lactating females may be explained through hormonal control or circadian rhythms (Berger 1986; Mayes & Duncan 1986). Breeding mares spend more time feeding (6-8% increase) during times of nutritional stress such as the peak of lactation in the end of winter (Duncan 1985).

Seasonal Differences in Time Allocation of Feral Horses

Due to the high protein content of forages in the springtime, horses spend less time foraging and more time resting than they do during any other season (Duncan 1985). This equates to approximately 75% of springtime daylight hours spent in foraging activities (Salter & Hudson 1979). In the summer, there is an increase in time spent walking and standing alert as compared to the other seasons. This increase is likely due to higher numbers of mosquitoes and other pests in the area (Duncan 1985). During the fall and winter, horses spend more time resting in sheltered areas than during the other seasons. This is particularly true during heavy rain and colder temperatures (Duncan 1985).

Band structure

Males are born in about the same numbers as females (Linklater *et al.* 2000; Berger 1986), but feral horses typically live in harem groups with a dominant stallion and between 1 and 11 mares (Feist & McCullough 1976; Linklater *et al.* 2000). In 88% of bands, only 1 stallion exists (Berger 1986) but up to four dominant stallions per band have been observed (Linklater *et al.* 2000). Approximately 12% of bands have more than one stallion protecting them (stallions in all the bands will fight on behalf of the band) although one stallion maintains both dominance over the other non-dominant stallions as well as breeding access to females (Berger 1986). Non-



dominant males fight with outside males more frequently than dominant stallions (Berger 1986). Older males form multi-male breeding bands more frequently than younger males (Berger 1986). Stallions without a harem, called bachelor stallions, either form small groups, with two to three individuals, or live solitarily (Berger 1986). Males only show aggression towards each other in 5% of all male-male interactions, thus only an average of 3% of all males die each year from combat-related injuries (Berger 1986).

The degree of stability exhibited by bands is determined by the degree of force exerted by the dominant stallion as well as the fidelity of group members (Feist & McCullough 1976). Young mares show less fidelity to a band than older mares (Berger 1986). In 55% of all cases of mares leaving a band, it is due to physical exclusion by a stallion, while 45% of the time, it is due to mares wandering away (Berger 1986).

The size of home ranges for a band is positively correlated with band size (Linklater *et al.*2000). With both harem-structured and bachelor groups, bands frequently overlap in home range (this is true for both harem-structured groups as well as bachelor groups) (Feist & McCullough 1976; Berger 1986; Linklater *et al.* 2000). Due to this overlap in home ranges, stallions often leave their band (up to 1 km away) to investigate other horses and search for more potential females (Berger 1986).

Foaling

Foaling typically occurs in May or early June (Feist & McCullough 1976) though it occasionally will occur throughout the year (Berger 1986). In general, variability in ungulate birthing times of the year is a result of numerous factors such as predation (Estes 1976), nutrition (vegetation growth) (Anderson 1979; Rutberg 1984; Sadleir 1969), photoperiod (Spinage 1973;



Sadleir 1969), and past reproductive success/failure of individuals (Guinness *et al.* 1973). This is true of feral horses.

Foaling begins in females between the ages of 2-4 and continues annually until the female is approximately 22 years old. Often times, however, the female continues breeding past this date (Berger 1986; Wolfe 1986). In a population, 15-35% of the 2-year old females become pregnant and go on to reproduce each year. Once mares reach the age of 4, the frequency of pregnancy is 60-85% of the female population (Wolfe 1986).

Postpartum estrous cycles in the spring occur 11 days after parturition but are approximately 24 days later if parturition occurs in the winter instead of spring (Berger 1986). Due to physical stress during band take-over as well as harassment by new band stallions, pregnant mares in over-taken bands experience higher rates of prenatal loss than do stable bands (Berger 1986).

Gestation lengths are affected by social, physical, and ecological factors. Male foals induce a longer gestation length than female foals and larger/older mares have longer gestation rates than smaller/younger mares (Berger 1986). Foals are usually weaned between nine and 12 months but can be weaned up to 16 months in mares that are barren the year after a foal is born (Berger 1986; Feist & McCollough 1976).

Infanticide does not usually occur among feral horses; however stallions often show aggression towards male foals that are not their offspring (Berger 1986). Duncan (1982) reports the occurrence of infanticide when a male takes over a band and kills existing colt offspring.

Population Dynamics

The majority of feral horse deaths can be attributed to winter abrasion (extreme temperatures, inclement weather, lack of water and food). Most deaths (70% in the Great Basin)



occur at high altitudes (Berger 1986). Annual foal survival rates (in normal years) are between 90-95% which leads to an annual population increase between 15% and 23% (Berger 1986; USFWS 2009; Wolfe 1986). However, due to the high variability among factors affecting different feral horse populations in various regions, it is extremely difficult to make precise generalizations regarding population dynamics of feral horses (Wolfe 1986).

GRAZING AND FEEDING HABITS

General

Smith *et al.* (1998) in the *Southwestern Naturalist* report that feral horse diet consists of 91% grasses, 8% shrubs, and >1% forbs and unknowns and is consistent throughout the seasons. However, there were changes in habitat selection and a few of the consumed forages changed significantly throughout the year. Other studies report that diet does vary significantly throughout the year due to changes in forage availability. They also show that dietary quality as well as type varies seasonally (Salter & Hudson 1979).

Multiple studies reported gramineous plant species as the major constituent to feral horse diet in all seasons staying at or above 83% of the diet. Salter & Hudson (1979) report a value of 83% grasses. Smith *et al.* (1998) report 91% grasses. McInnis & Vavra (1987) report 88% grasses. Sedges (*Carex* spp.), found in bogs/meadows and streamsides, are also very important dietary components (Salter & Hudson *et al.* 1979; Crane *et al.* 1997). Shrubs and forbs play a significant though more limited role in feral horse diet (Krysl *et al.* 1984a).

The major grass components in feral horse diet most likely vary across sites depending on availability of forages. Several studies report the major constituents of feral horse diet. Hansen et al. (1977) in the *Journal of Range Management* claims the most important grasses are needlegrasses, wheatgrasses, and bromes. Krysl et al. (1984a) in the *Journal of Range*



Management report that needle and thread (Stipa comata), sandberg bluegrass (Poa secunda), thickspike wheatgrass (Elymus lanceolatus), Indian ricegrass (Achnatherum hymenoides), gray horsebrush (Tetradymia canescens), and winterfat (Krascheninnikovia lanata) are the most important dietary constituents. Salter & Hudson (1979) in the book The Ecology of Reproduction in Wild and Domestic Mammals report that hairy wild rye (Elymus innovates) and fescues (Festuca spp.) are the most important species. McInnis & Vavra (1987) in the Journal of Range Management report bottlebrush squirreltail (Sitanian hystrix), bearded bluebunch wheatgrass (Agropyron spicatum), and thurbers needlegrass (Stipa thurbariana) as heavily selected dietary components.

Feral horses consume forages that average 7.5% crude protein during the summer and are 6.1% in the winter. During the summer, average dry matter consumption is 52%, while during winter, dry matter consumption is 39% (Krysl *et al.* 1984b).

Feral horse diet compared to other ungulates

Throughout the course of a season, most large herbivores consume a wide variety of plant species; however, large herbivores generally have a diet of less than six major plant species per season (Olsen *et al.* 1977). Feral horse diets are quite similar to those of some of the other grazing large ungulates in the same geographical area (Olsen *et al.* 1977; Hubbard *et al.* 1976). This is particularly true with other non-selective grazing ungulates such as domestic cattle (*Bos taurus*) and elk (*Cervus canadensis*), whose diets consist more frequently of grasses and sedges (Olsen *et al.* 1977; Hubbard *et al.* 1976). Dietary overlap between horses and other ungulates such as pronghorn antelope (*Antelocapra americana*) and mule deer (*Odocoileus hemionus*) is typically much lower (between 7% in summer and 26% in winter). The wide range of dietary overlap between these browsers and grazers suggests that feral horse interactions with other



ungulates are a combination of noncompetitive coexistence and exploitative competition (McInnis & Vavra1987).

Cattle/elk vs. Feral Horses

Due to the fact that horses, cattle, and elk all compete heavily for similar food resources, they directly compete whenever resources are scarce (Hansen & Clark 1977). Due to similarities in feral horse habitat and diet, the potential for competition between horses and cattle is high (Menard *et al.* 2002). Various studies report differing numbers of similarity between feral horse and cattle diet ranging somewhere between 59% to 84% (Hubbard *et al.* 1976; Hansen *et al.* 1977; Krysl *et al.* 1984a). Not only are similar vegetative species chosen for consumption by cattle and horses but the food sources are also selected in a similar order (Krysl *et al.* 1984a).

During the summer months, horses frequent marshlands more and spend more time grazing on short grasses than cattle (Menard *et al.* 2002). Horses remove more vegetation per unit body weight and also use the most productive plant communities/species to a greater extent than cattle which leads to greater nutrient extraction in horses (Menard *et al.* 2002). They crop grasses (particularly more succulent grasses) shorter to the ground and leave a mosaic of short and tall patches of grass which contributes to structural diversity (Menard *et al.* 2002). Daily food intake per body mass is 63% greater in horses than in cattle which is in part due to the fact that cattle diets are more digestible than horse diets (Menard *et al.* 2002). This is because cattle use forbs, shrubs, and broadleaf plants more heavily than horses (Menard *et al.* 2002).

Mule deer/pronghorn antelope vs. Feral Horses

Dietary selection patterns differ between browsing (such as pronghorn and mule deer) and grazing ungulates (horses and cattle). Browsing animals that are often in direct habitat competition with feral horses include species such as pronghorn antelope and mule deer



(*Odocoileus hemionus*). These species select for browse, which in most cases is limited to sagebrush (*Artemesia* spp.), curl-leaf mountain mahogany (*Cerocarpus ledifolius*), antelope bitterbrush (*Purshia tridentata*), and western juniper (*Juniperus occidentalis*) (Hanley & Hanley 1982). Horses and pronghorn show a relatively low dietary overlap (7% to 26% depending on the season). This was determined to be not significant (McInnis & Vavra 1987).

Horses show a larger variation in diversity of plant consumption than do mule deer (Hansen *et al.* 1977). Diets of feral horses and mule deer are between 1% (Hansen *et al.* 1977) and 11% similar (Hubbard *et al.* 1976). Thus there is no evidence that large herbivores (specifically horses, cattle, or elk) compete directly with mule deer for food resources (Hansen & Clark 1977).

FERAL HORSE IMPACTS ON RANGELANDS

Wildlife

Competition with Other Large Ungulates

On the Sheldon National Wildlife Refuge, observations illustrate that several ungulate species, particularly pronghorn, are kept out of water sources through physical exclusion by feral horses. This is accomplished mostly by dominant stud horses (USFWS 2009).

Miller (1983) studied feral horse interactions with other ungulates at water sources and found that horses excluded pronghorn from water sources every time there was an interaction. Pronghorn sometimes had to wait longer than 5 hours for access to water; showing strong evidence for interspecific competition. While horses are surrounding water sources (up to 119 horses have been observed at one water source), pronghorn have been seen to circle, then leave only to return and repeat the behavior. Pronghorn typically won't drink unless there is enough space between individuals to stay at least 3 m away from horses and cattle (Miller 1983).



Habitat degradation is the destruction of natural communities which eventually decreases the productivity and the functionality of an area. Typically when habitat degradation occurs, biodiversity is reduced and organisms which previously used the habitat are destroyed or displaced. When habitat degradation is caused by feral horses, this may also leave pronghorn fawns particularly vulnerable to predation. This may affect other wildlife species that depend upon vegetative cover to hide (USFWS 2009).

There have been several studies that have shown the impacts feral horses have had on other ungulates (other than pronghorn) occupying the same ranges. Ostermann-Kelm *et al*. (2008) studied the effects of feral horses on bighorn sheep numbers. It was shown that at feral horse occupied watering sites, there was a 76% reduction in the number of groups of bighorn at these sites and a similar increase in numbers of bighorn groups visiting other non-horse occupied watering sites. Such a change in habits and movement in bighorn could potentially affect their survival as well as their impact on the environment.

Competition with Other Animals

The presence of feral horses not only impacts large mammals, but can also impact reptiles and small mammals. Beever and Brussard (2004) studied the differences between horse-occupied and horse-absent sites on squamate reptiles and granivorous small mammals. It was determined that species richness was not different between occupied and unoccupied sites but the sites with horses showed less biotic integrity, meaning horse-occupied sites showed signs of habitat degradation as compared to unoccupied sites. There were 1.1-7.4 times more deer mice in horse-occupied sites, whereas there were more reptiles in horse-free sites. In areas where horses were excluded from springs, there was an increased number of small mammal burrow entrances (Beever *et al.* 2000). Species expansion combined with species reduction can lead to



changes in ecological roles as well as the impact on their immediate environment (Beever & Brussard 2004).

Feral horse populations have been shown to affect avian populations in Argentina. Zalba and Cozzani (2004) looked at the effects of grazing horses on abundance of birds. Some birds (such as the southern lapwing) are more common in horse grazed areas whereas other birds (such as pipits) are common in less grazed areas. Areas that have mostly tall grass show the highest species richness as well as the greatest total abundance of birds. Also, the presence of feral horses may be linked to increased egg predation by opportunistic carnivores, such as the culpeo fox (*Lycalopex culpaeus*), due to increased nest exposure (Zalba & Cozzani 2004).

Estuarine Systems

With regards to estuarine sites, where freshwater from streams mixes with saltwater from the sea's tide, the presence of horses correlates with less vegetation diversity, higher diversity of grazing birds, higher densities of crabs, and lower densities/species richness of fishes compared to sites without horses present (Levin *et al.* 2002). Predation upon various fish species increases in grazed areas (less vegetation means less cover for fishes) compared to ungrazed areas. The shift in vegetation in marsh areas may account for a shift in fish behavior which would lead to increased predation (Levin *et al.* 2002).

Vegetation

Feral ungulates (specifically feral horses, feral cattle, feral sheep and feral goats) influence vegetation community dynamics by lowering the above ground annual growth in saltmarsh and grass-shrub areas. The rate of succession and the spread of maritime forest are slowed in these areas due to grazing pressure. In a study by Beever *et al.* (2007), differences between high elevations and lower elevations showed that at higher elevation, vegetation reached



heights 2.8 times greater than vegetation grazed by horses only and that vegetation reached maximum heights 4.5 times greater than vegetation grazed by horses and cattle (Beever *et al.* 2000). Sites where feral horses had been removed for 10-14 years versus sites where feral horses continue to graze, show greater shrub cover, greater total plant cover, greater species richness, and greater frequency of native grasses, compared to exotic plants and grazing-resistant forbs, than did horse-occupied sites (Beever *et al.* 2007).

Horse grazing can significantly reduce the main grass species found in particular areas, especially marshland sites (Bassett 1980). Within these marshland sites, areas without horse grazing show significantly greater amounts of overall vegetative biomass than do sites with horse grazing (Levin *et al.* 2002). Feral horses may have indirect impacts on salt marsh communities by altering a habitat, potentially making it more or less suitable for certain species to exist in a particular ecosystem (Levin *et al.* 2002; Bassett 1980; Wood *et al.* 1987).

The removal of a key herbivore such as a feral horse has no significant impact on perennial plant cover in the desert. However, areas with feral horses removed show a significant increase in annual forbs and plant litter. Also species composition and dominance are altered and many species specific changes occur (Ryerson & Parmenter 2001).

Horse and cattle grazing can either perpetuate or decrease the rate of expansion of particular plant species. For example, in the Netherlands, *Sambucus nigra*, or Elder Bush, expansion is slowed by heavy cattle grazing. This is due to the fact that only ruminants are capable of detoxifying the cyanogenic glycosides found within this particular species. Whereas the rate of expansion of *S. nigra* is perpetuated when heavy horse grazing occurs. This is because horses, as hindgut fermenters, are incapable of detoxifying this species and as such it is



avoided. Thus grazing by different species may alter vegetation in different ways just as cattle and horse grazing affect vegetation (specifically Elder Bush) differently (Vulink *et al.* 1984).

Dung piles from feral horse populations may be viewed as a window into impact on vegetation. The vegetation on top of dung piles is significantly different than the vegetation in control plots. Once horse visitation has ceased in an area, the vegetation near abandoned dung piles shows increased total vegetation cover, species richness, diversity and evenness compared to areas with currently used dung piles. Non-native plant species are also frequently associated with dung piles as compared to control plots (Loydi & Zalba 2009).

Riparian Areas

Studies have shown that feral horses visit watering locations daily, typically during crepuscular hours. They typically inhabit areas that are within 4.8 km of water (Pellegrini 1971, Meeker 1979; Ganskopp & Vavra 1986; Miller 1983). Due to the daily visitation of feral horses to watering areas, riparian areas are negatively impacted from trampling, herbivory, and fecal/urine contamination (USFWS 2009).

Riparian Areas on the Sheldon NWR

Areas with springs present that have exclosures to prevent horses from entering the spring-area exhibit significantly greater plant species richness, percent cover, and abundance of grasses and shrubs. Figure 1 shows a photograph that was taken at the Sheldon National Wildlife Refuge at Big Spring Creek in September 2002. An exclosure was placed over the square with the tall vegetation (cage is on the left) to protect against herbivory. Stubble height outside of the cage was found to be 4 cm and inside the cage, it was found to be 35 cm.



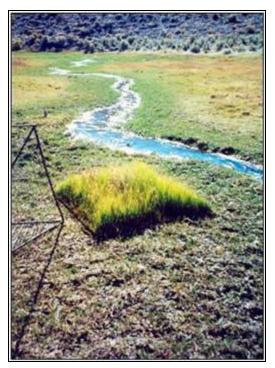


Figure 1. Shows the differences between an area where grazing was allowed and where grazing was excluded. Areas where grazing occurred showed stubble height of approximately 4 cm. The excluded area showed heights of approximately 35 cm. Figure taken from http://www.fws.gov/sheldonhartmtn/sheldon/horseburro.html, 2010.

Figure 2 contains two photographs taken from the Sheldon NWR in 2009. An exclosure study was set up in order to determine effects of grazing horses on riparian areas. The exclosures were set up at a height that allowed ungulates (such as mule deer and pronghorn antelope) to enter and graze but did not allow horses to enter (USFWS 2011).



Figure 2. Two photographs from the Sheldon NWR showing the difference in vegetation in two different riparian areas in horse excluded areas as well as horse occupied areas. Fences represent exclosures where horses are excluded but other ungulates are allowed.

Figure 3 shows the difference in a riparian area from before horses were removed from an area (horses present) to after a horse gather in the same area (horse numbers significantly reduced). The two photographs were taken one year apart at the same location (Big Spring Creek), at the same angle showing the same site. The upper was taken in August 2004 before the horse gather and the lower was taken in August 2005 after 293 horses had been removed from Big Spring Butte. The vegetative response from reduced grazing pressure is apparent but the restoration of woody vegetation will take many years to occur (USFWS 2011).





Figure 3. These two photographs were taken from the USFWS website for the Sheldon NWR. They show the same area one year apart when horses were present (2004) and when horses were reduced (2005). Taken from http://www.fws.gov/sheldonhartmtn/sheldon/horseburro.html, 2010

Soils

Soils are compacted after repeated use by multiple horses (USFWS 2008). With soil compaction and degradation, infiltration decreases, grasses and other vegetation are unable to grow, and soil is degraded and/or lost. Recovery from degradative impacts requires thousands of years in order to build the layers within soils. Even more time is needed within a desert climate such as is often found in the western United States where the majority of the United State's feral horses reside (USFWS 2008).

Feral Horse Management

Population growth rates on the Sheldon NWR have been estimated to be between 17 and 23% (USFWS 2009). Relatively little natural predation occurs on feral horses (occasionally a mountain lion will predate upon a foal but occurrences are rare according to USFWS managers)



and reproduction is high. The Bureau of Land Management (BLM) has estimated appropriate management levels (AMLs) for the numbers of feral horses in the western United States (Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, and Wyoming). All states (excluding Idaho and Oregon) have horse counts above the established AML (see Table 1, BLM 2010). Due to high population counts, feral horses in the Western United States (including the Sheldon NWR) are periodically "rounded-up" in an attempt to obtain desired appropriate management levels (AMLs). Grazing effects by large ungulates (particularly feral horses) may not be significant if populations are managed at appropriate levels (Detling 1998).

Table 1. These values represent each respective state's current population estimate information as of October 28, 2010. Most of the estimates represent direct counts and are not adjusted for animals not observed. AMLs are established as a randge with upper and lower levels. The numbers displayed here represent the upper limit (BLM 2010).

	Populations			Total
	Horses	Burros	Total	AML
AZ	400	2,248	2,648	1,676
CA	4,079	1,069	5,148	2,190
CO	888	0	888	812
ID	596	0	596	617
MT	150	0	150	120
NV	17,711	1,177	18,888	12,688
NM	119	0	119	83
OR	2,461	15	2,476	2,715
UT	2,724	164	2,888	1,956
WY	4,564	0	4,564	3,725
TOTAL	33,692	4,673	38,365	26,582

With increasing horse numbers, management budgets for horse conservation have increased as well. Table 2 shows the BLM budget breakdown for the BLM Horse and Burro Management Program since 2005. The majority of the budget is spent in removing horses that exceed AMLs from rangelands and caring for them in long-term holding facilities. Long-term holding facilities are employed in conjunction with the "no kill" policy adopted in the Wild and Free-Roaming Horses and Burros Act of 1971. After adoptions, horses and burros that are not adopted are placed in long-term holding facilities where they are fed and cared for until they are either adopted or they die. Increases in budgeting are allocated to offset increasing expenses for housing horses that have been removed from rangelands in long-term holding facilities. The increase in budgeting from 2009 to 2010 is due mostly to increased expenditures in land purchases in the mid-west. The purpose of these purchases is to increase the numbers of long-term holding facilities with hopes of decreasing feed expenses due to more productive grasslands which can hold larger numbers of grazing species.

Table 2. BLM Horse and Burro Management Program budget from 2005-2010. BLM officials reported that budgets are primarily used in helicopter gathers (and on costs associated with these) and caring for horses in long-term holding facilities (BLM 2010).

Budget Breakdown
2005 - \$29,524,000
2006 - \$29,051,000
2007 - \$36,045,000
2008 - \$36,362,000
2009 - \$36,354,000
2010 - \$63,986,000



SHELDON NATIONAL WILDLIFE REFUGE

Location

The Sheldon National Wildlife Refuge (NWR) in northwestern Nevada is located on Highway 140, 14 miles west of Denio, NV and 68 miles east of Lakeview, OR. It is home to many wildlife species including pronghorn antelope, bighorn sheep, mule deer, rabbits (including pygmy rabbits), greater sage grouse, several species of water birds, many species of migratory birds, and feral horses and burros (USFWS 2011).

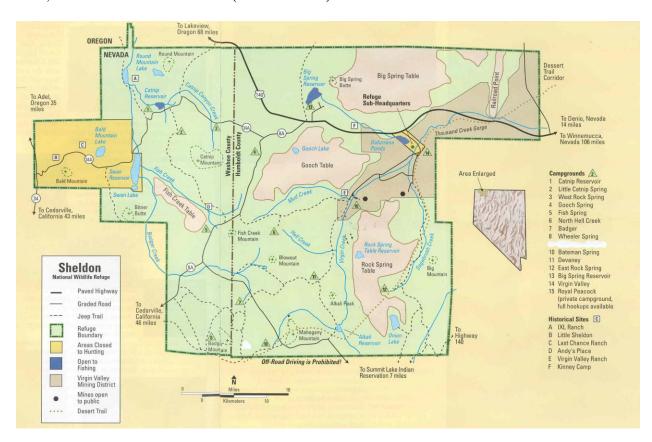


Figure 4. Map of the Sheldon NWR.

Function

The Sheldon NWR was formed in 1931 for three different purposes: 1. To provide habitat specifically for pronghorn antelope but also for secondary species such as mule deer, sage



grouse, and song-birds. 2. To protect threatened and endangered species (fish, wildlife, and plants). 3. To be used as a sanctuary for migratory bird species (USFWS 2011).

Habitat/Climate/Elevation

Habitat in the Sheldon NWR is high desert dominated by stands of sagebrush (basin big, mountain big, and arbuscula), rabbittbrush, juniper, mountain mahogany, aspen, meadows and playa. Climate is hot in summer and cold in winter; however, temperatures can vary greatly and can drop below freezing at any time of the year. Precipitation is rarely greater than 12 inches per year. Elevations range from 2,223 m to 1,280 m. As a general trend, elevation drops from west to east throughout the refuge (USFWS 2011).

CARBON AND NITROGEN ISOTOPES

How they are used

Stable isotope technology does not directly measure the nutritive value of various food sources. It measures the difference between atoms by their mass-to-change-ration. Isotopes are atoms of a common element with the same number of protons and electrons but differing numbers of neutrons than the common form (Sulzman 2007). There are approximately 300 stable isotopes (Hoefs 1997). As an example of an isotope, carbon normally has an atomic mass of 12 with the most common isotope of carbon being C¹³. Thus C¹³ has an extra neutron with an atomic mass of 13 (Sulzman 2007).

Due to the fact that isotopes occur naturally, organisms will sequester them in their tissues through biochemical pathways as dietary nutrients are metabolized. For example, in plants, carbon enters the plant in the form of atmospheric CO₂ through various photosynthetic processes (Marshall *et al.* 2007). Nitrogen enters plants through detris sources. As these isotopes, as well at the element in its natural state, are brought into the plant they are stored in



species-specific ratios. Animals then take in the ratios of various isotopes through the consumption of plant material. When the isotopic signature is consumed, it remains in the animal's tissues specific to the plant species and growing conditions. Using isotope ratio mass spectrometry (IRMS), the isotope ratio can be determined by separating the charged atoms on the basis of their mass-to-charge-ratio (Sulzman, 2007). The result of the mass-to-charge ratio is a number that can be positive or negative. A positive "delta" (δ) indicates that the sample has more of the heavy isotope (ie. ¹³C or ¹⁵N) than does the standard and that a more negative value of "delta" (δ) indicates the sample being measured has less of the heavy isotope than the standard (Sulzman, 2007). In other words a more negative value is said to be depleted for the heavy isotope.

Stable isotope dietary information may be used in three different ways to reveal patterns of dietary change. First, one can compare isotope ratios from the same tissue type repeatedly over time. This will reveal both long and short term dietary variation (depending on length of time that the tissue is sampled). Second, one can compare the differences between assimilation of isotopes in different tissues. Due to the fact that various tissues assimilate isotopes at different rates, one can see short term dietary changes simply by comparing different tissues. Third, tissues with progressive growth (such as hair, feathers, teeth, and claws) will show an isotopic chronology of dietary changes (Dalerum *et al.* 2005).

Both carbon and nitrogen occur in two natural isotopic forms, 12 C, 13 C, 14 N, and 15 N. These isotopes are compared to a standard and reported as a ratio of the two isotopic forms in the written form of δ^{13} C and δ^{15} N. The actual values are reported as parts per thousand (‰) (Ehleringer & Rundel 1988). All food sources should be corrected prior to further analysis. This is necessary in order to account for fractionation and assimilation during digestion (Phillips &



Gregg 2003). Tissues are typically more enriched in δ^{13} C and δ^{15} N than their food sources. But the degree of fractionation values differ between species, diets, and tissue types (Hobson & Clark 1992).

δ^{13} C and δ^{15} N

Carbon isotopes (δ^{13} C) will typically reveal some of the major biochemical fractions of the diet, specifically the lipid, carbohydrate, and protein fractions (DeNiro & Epstien 1978). Δ^{13} C values of tissue samples are closely related to the δ^{13} C values of plant samples (Teeri & Schoeller 1979; Ramsay & Hobson 1991). Whereas Nitrogen isotopes (δ^{15} N) are only useful if the potential food sources had varying δ^{15} N values (many food sources can be confounded). Values of Nitrogen isotopes may be incorrect due to the presence of chemical fertilizers which have changed the distribution of the nitrogen isotopes in food sources (Deniro & Epstein 1981). Δ^{15} N values are often elevated (giving a falsely enriched appearance to tissue samples) after fasting or stress (Hobson *et al.* 1993). Δ^{15} N values increase going up trophic levels. As such they not only indicate diet but also trophic level (Ben-David *et al.* 1997).

Hair

Hair samples are generally more enriched in δ^{13} C values than other tissue samples (Tieszen *et al.* 1983). However, hair samples also have a much longer turn-over rate than other more metabolically active tissues (Tieszen *et al.* 1983). Hair has been used in many studies of various mammal species to extract δ^{13} C and δ^{15} N signatures which are then used to characterize diet (Chambers & Doucett 2008; Macko *et al.* 1999; O'Connell & Hedges 1999; Schoeninger *et al.* 1998) and track movements (Cerling *et al.* 2006). Hair is well-suited for isotope analysis because it grows quickly (van Scott *et al.* 1963) and once formed is biologically inactive and resistant to degradation (Macko *et al.* 1999). As such, isotopic chronologies, meaning a record



of isotopic values through time, can be formed, limited only by the length of hair (West *et al.* 2004; Dalerum *et al.* 2005).

IsoSource

Stable isotopes can be used to determine the relative contribution of various food sources to an animal's diet (Michener & Schell 1994). Determining the contribution of various sources to a mixture of isotopic signatures, as is the case with tail hair, can be accomplished through the use of a computer program called IsoSource which can be obtained and used from the website http://www.epa.gov/wed/pages/models.htm. It functions through the testing of all possible mixture ratios in increments of 1 to 100 (this number is set by the user). This is called the source increment and is typically 1% or 2%. Next, all possible combinations from the source increments are created and the predicted isotope signatures for the mixture are computed. Third, the observed mixture values, the tail hair for example, and the predicted mixture combinations (from IsoSource) are compared. If the predicted and actual values are the same or similar, within a certain value called the mass balance tolerance, generally 0.1%, it is a possible combination and is stored in the data set. All the possible combinations are reported. These are given a minimum and a maximum value which represents the range of possible contributions to the mixture (Phillips & Gregg 2003).

After output, it might be necessary to consider other factors that would rule out certain sources. This may include known dietary requirements (Minagawa 1992), observed food sources that are known not to be consumed, microhistological fecal analysis, etc (Phillips & Gregg 2003). After decreasing the amount of potential sources, it will be easier to narrow down the actual contributing sources as well as the percentage of contribution. Another option for decreasing the amount of potential sources is to combine sources that are statistically similar as



well as related enough to warrant source aggregation to decrease numbers of sources. This is called source aggregation (Phillips *et al.* 2005).

CONCLUSION

Many studies have reported the habits of feral horses. Despite previous research, there are still numerous gaps in data sets that are required in order to make informed and scientific decisions regarding feral horse management. By looking at the current knowledge we possess, it makes policy making easier and more beneficial to all parties involved. This chapter has attempted to inform the reader of such habits as well as impacts upon rangelands.

The concluding portion of the chapter has informed the reader of background specific to the research project entitled "Stable isotope diet reconstruction of feral horses on the Sheldon National Wildlife Refuge." A background of the particular study site was given informing the reader of what sort of conditions exist on the Sheldon NWR. After the study site characteristics, a brief introduction to the use of stable isotopes was presented. This introduction specifically introduced nitrogen and carbon isotopes with relation to tail hair isotopes, in the use of diet reconstruction.

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Stable Isotope Diet Reconstruction of Feral Horses (*Equus caballus*) on the Sheldon National Wildlife Refuge, Nevada, USA

ABSTRACT

Feral horse management has become a subject of significant controversy in the United States. This is because of differing opinions and minimal recent empirical data on feral horses. In recent years, numbers of feral horses have increased due to governmental horse removal restrictions (specifically the Wild Horse and Burro act of 1971). With increasing numbers of feral horses on rangelands, land managers are challenged with identifying the appropriate course of action for satisfying groups with differing opinions.

The purpose of this study is to characterize diet consumption through the use of stable isotope dietary analysis (δ^{15} N and δ^{13} C). We did this in order to measure the impact of feral horse forage consumption on rangelands and to propose strategies for improving habitat management and conservation. We obtained tail hair isotopic values from tail hair removed while horses that were held in squeeze chutes following a roundup. Resulting isotopic values were compared to plant isotopic values using plant samples obtained from the geographical areas as the horses in order to characterize diet. Contribution of the various plant species to the tail hair mixture values was determined using the EPA program IsoSource©. Initial analysis of tail hair isotopes demonstrated seasonal variation. During summer months, shrubs (mostly *Artemesia spp*, and *Purshia Tridentate*), *Elymus elymoides*, *Juncus balticus*, and *Festuca idahoensis* were the predominantly consumed vegetative species. During fall months, *Leymus cinereus* and *Juncus balticus* played a more significant role in feral horse diet. In the winter, shrubs were more heavily consumed along with *Poa secunda*. Springtime showed a shift



towards forb consumption. Changes in seasonal consumption of forages are most likely linked to forage availability as well as equine preference.

We analyzed plant metrics (specifically biomass, abundance, and cover) to compare a site with horses present to a site where horses had been removed the previous year and found relatively few differences between the two sites. With nearly all differences we found higher plant production (forage availability) on the site where horses were still present. In riparian areas however, there was more vegetation (specifically *Carex rossii*, *Juncus balticus*, and *Poa secunda*) on the site where horses had been removed. Within riparian areas, only *Bromus tectorum* (a plant not typically found in riparian areas but characteristic of degraded areas) showed significantly greater amounts of biomass on the site with horses present. Knowledge of plant species consumption will allow land managers greater ability to make scientifically based decisions regarding feral horse population control which is important in determining appropriate management levels of populations.

INTRODUCTION

Feral horses (*Equus caballus*) have drawn increasing interest and attention in recent years. With mounting numbers of feral horses on rangelands, negative impacts have been identified involving native plant and animals species as well as abiotic components of the ecosystem (i.e. hydrology, soil structure, etc.) (Miller 1983; Ostermann-Kelm *et al.* 2008; Bassett 1980; Levin *et al.* 2002). With a lack of empirical data regarding feral horse habitat selection, ecological impacts, and forage consumption, it has been nearly impossible and highly debatable for land managers to agree on appropriate management levels for feral equine populations within various areas across the western United States.



By comparing forage availability to forage consumption, it is possible to determine which plant species horses are selecting. A knowledge of heavily selected plant species will lead to a better understanding of horse habitat selection which in turn leads to an understanding of horse impacts on various habitats.

Studies have reported the use of isotopic analysis to characterize animal diet for several decades (Ambrose & Deniro 1986; Deniro & Epstein 1978; Tiezen et~al. 1983). The premise is that δ^{13} C and δ^{15} N signatures can be used to determine the contribution of various food sources to an animal's diet (Michener & Schell 1994). Hair has been used in many studies of various mammal species to extract δ^{13} C and δ^{15} N signatures which are then used to characterize diet (Chambers & Doucett 2008; Macko et~al. 1999; O'Connell & Hedges 1999; Schoeninger et~al. 1998) and track movements (Cerling et~al. 2006). Hair is well-suited for isotope analysis because it grows quickly (van Scott et~al. 1963) and once formed is biologically inactive and resistant to degradation (Macko et~al. 1999). As such, isotopic chronologies (meaning a record of isotopic values through time) can be formed, limited only by the length of hair (West et~al. 2004; Dalerum et~al. 2005). Isotopes in domestic horse (Equus~caballus) tail hair have been studied and short-term diet switches can be noted within isotope signatures (West et~al. 2004).

The objective of this study is to reconstruct the diet of wild horses on the Sheldon National Wildlife Refuge (NWR) in Nevada using stable isotope analysis of feral horse tail hair and available forage species. We determined the dietary differences between various reproductive categories of wild horses (originally we tested studs, wet mares, dry mares but ultimately we combined wet mares and dry mares). We then compared dietary selection available forage found on the Sheldon NWR in order to determine whether horses were selecting forages based on availability or to determine whether they were targeting specific plant species



for consumption. We then compared plant biomass, abundance, and cover across a site with horses present to a site where horses had been removed the year before in order to determine whether significant differences exited between the two sites.

METHODS

Tail Hair Collection

Feral horses annually are removed from the Sheldon National Wildlife Refuge in Nevada, USA (see figure 5). In August 2009, 353 feral horses were captured near the Little Sheldon region (UTM coordinate: 11T 294052.83 E, 4639510.97 N) and removed from the refuge. During the processing (in preparation for adoption or spaying/vasectomizing), the longest available sections of hair were pulled at the root of the hair, placed in paper bags, and labeled. We sampled 59 dry mares, 57 wet mares, 63 foals, and 174 studs.

Habitat in the Sheldon NWR was high desert dominated by stands of sagebrush (basin big, mountain big, and low sagebrush), rabbittbrush, juniper, mountain mahogany, aspen, meadows and playa. Climate was hot in summer and cold in winter; however, temperatures can vary greatly and can drop below freezing at any time of the year. Precipitation was rarely greater than 12 inches per year. Elevations ranged from 2,223 m to 1,280 m. As a general trend, elevation dropped from west to east throughout the refuge (USFWS 2011).

Ten random samples were selected from each four reproductive categories (i.e. 10 mare samples and 10 stud samples for mass spectrometer analysis). The longest hair was analyzed from each horse. Hairs were cleaned by repeated rubbings with acetone, divided into 5-20 mm increments (typically 10 mm used), and weighed on a microgram balance (Sartorius, Data Weighing Systems, Elk Grove, IL) with a target weight of 200-500 µg (lengths varied according to weights needed for analysis). Each hair sample was then placed into tin capsules (Costech,



Valencia, CA) and combusted using a Costech (ECS 4010, Cornusco MI, Italy) elemental analyzer. The sample was passed through a continuous-flow isotope-ratio mass spectrometer (Delta-V, Thermo Fisher Scientific Inc., Waltham MA) to obtain δ^{15} N and δ^{13} C values.

Variance between reproductive categories was analyzed using ANOVA (R project, Institute for Statistics and Mathematics, Vienna University of Economics and Business). Differences between the groups, where present, were then determined through the Tukey-Kramer method. Due to the continuous nature of tail hair growth, a time frame was obtained to represent each tail hair (West *et al.*, 2004; mm/day). This allowed us to determine the seasonality patterns within the tail hair. Once compared to forage samples, we were able to determine the differences in diet sources not only through different seasons but throughout specific months of 2006 (this was the oldest date, most tail hairs only supplied data as far back as the end of 2007) to the sample time in 2009.

Forage Collection

Cover Map

United States Fish and Wildlife Service (USFWS) personnel developed a cover type map that classified vegetation communities on the Sheldon (NWR). This map was produced in 2009 by J.D. Tagestad and associates of the Department of Energy's Pacific Northwest National Laboratory (PNNL) in Richland, Washington. They used three Landsat 7 images from 2002, each at 30-m spatial resolution. The three images were converted to nine images using coefficients for brightness (a measure of overall reflectance), greenness (contrast between near-infrared and visible reflectance), and wetness (contrast between shortwave-infrared and visible/near-infrared reflectance)—also known as a TM Tasseled Cap transformation (Crist and Cicone 1984). An additional two images were incorporated—elevation from the USGS National



Elevation Dataset for the greater Sheldon area, and shaded relief using ERDAS Imagine—providing an 11-band image stack (Tagestad 2009). The elevation and shaded relief datasets were used to develop a range of topographic moisture conditions and a topographic moisture index (Tagestad 2009). Data from 130 ground truth polygons were collected from representative areas of fifteen cover types (Tagestad 2009).

Because traditional supervised classification techniques rely on normally distributed data, and because some spectral variables such as shaded relief and greenness often have non-normal distributions, standard classifications such as maximum likelihood can produce results that do not accurately represent the population (Tagestad 2009). To address this, PNNL researchers developed a customized nonparametric classification routine which compares every known pixel in the image to every unknown pixel, removing the dependency on the distribution of the data, and thereby representing a cover type with many different signatures rather than by integrating all signatures (Tagestad 2009). The output of this process was one of the fifteen vegetation cover classes that the pixel most closely matched (Tagestad 2009). Post-classification edit and analysis of the topographic moisture index produced two additional vegetation cover classes (basin big sagebrush was produced from big sagebrush areas with a high index value, and rocky mountain subalpine-montane mesic meadow was produced from wet meadow areas with a low index value) (Tagestad 2009). Manual edits were also applied to two locations of mountain mahogany (Tagestad 2009). Figure 6 shows the produced map.

Random Point Generation

Collecting a sample that would be representative of all vegetation available to the horse is paramount for accurate diet reconstruction. In order to represent not only species diversity, but also potential variation within species across the landscape, a series of random sampling



locations were selected within each vegetation class in both the Little Sheldon and Badger Mountain areas. Based on data from monitoring collars, it has been shown that the horses generally remain within a five miles of the respective catch site. Therefore, a 5-mile radius circle was created around each catch site (Little Sheldon catch site: 11T 294052.83 E, 4639510.97 N; Badger Mountain catch site: 11T 308412 E, 4616894 N; provided by USFWS personnel), and the vegetation raster map was limited to show only areas within this 5-mile radius of each catch site. These clipped areas then needed to be narrowed to distances accessible by road to sampling personnel in the field. A 0.5-mile buffer was set around all mapped roadways, and the circular areas were again clipped to exclude all locations outside this buffer (Figure 7).

Figure 8 shows the relative proportions of cover type for each 5-mile radius circle, the 0.5-mile road buffer areas, and the overall proportion across the Sheldon NWR. The graphs demonstrate that the area proportions of the buffer zones are very similar to those of the 5-mile radius areas.

Maps were converted from raster data to polygon features to facilitate random point generation using Hawth's Analysis Tools (www.spatialecology.com). In both areas thirty random points were generated for each of five major classes (basin big sagebrush, columbia plateau low sagebrush steppe, inter-mountain basins big sagebrush shrubland, inter-mountain basins montane sagebrush steppe, and inter-mountain basins semi-desert grassland), and ten points were generated for each of eight minor classes. Across the entirety of the Sheldon NWR, both the mixed salt desert scrub and greasewood flat classes were more prominent than the basin big sage class. However, basin big sage was considerably more prominent in the Little Sheldon and Badger Mountain areas than either the mixed salt desert scrub or the greasewood flat. As such, basin big sage was considered a major class and the others minor classes. The barren,



inter-mountain basins cliff and canyon, open water, north american arid west emergent marsh, and unclassified classes were not assigned sampling points due either to absence of the vegetation type within either study site, inaccessibility for foraging within the 5-mile radius areas, or inappropriateness for horse foraging. Random points were generated with a minimum distance of ten meters between points. Finally, UTM coordinates were determined for all points for later field sampling (Figure 9).

Field Plant Collection

Prior to entering the field, two random points from each vegetation class were selected in order of occurrence along various roads and vegetation was sampled at each selected site in both the Little Sheldon and Badger Mountain areas. Some random points were discarded for use due to road closures, inaccessibility on foot or within a vehicle, or deemed as an inaccurate vegetation classification. The random point coordinate location served as the southwest corner of each plot. A 5-meter tape was used to measure all four sides of the plot and markers were placed in each corner in order to create a 25 m² plot for use in plant collection for isotopic analysis. One example of each plant species present within the plot was clipped, placed in a paper bag, labeled, and returned to the laboratory.

Laboratory Plant Analysis

In the laboratory, plant samples were dried for 24 hours at 60°C (Flinders & Hansen 1972). Samples were then ground using a 0.425 mm mill (Wiley Minimill, Thomas Scientific, Swedesboro, NJ) and shaken to produce a fine uniformly distributed sample.

Subsamples of collected plants were weighed using a microgram balance (Sartorius, Data Weighing Systems, Elk Grove, IL) with a target weight range of 600-700µg. Subsamples were combusted using a Costech (ECS 4010, Cornusco MI Italy) elemental analyzer then passed



through a continuous-flow isotope-ration mass spectrometry system (Delta-V, Thermo Fisher Scientific Inc., Waltham, MA) to determine carbon and nitrogen isotope levels.

IsoSource

Sponheimer *et al.* (2003a) demonstrated a mean carbon diet-hair fractionation (ϵ^*) for a variety of herbivores. Horses were not specifically studied so an average value for all herbivores was taken and 3.2% was subtracted from all tail hair δ^{13} C values used for analysis. Sponheimer *et al.* (2003b) demonstrated nitrogen diet-hair fractionation values for a variety of species. Horses demonstrated a value of 6.5% which was subtracted from all tail hair δ^{15} N values used for analysis. The plant isotopic values were averaged according to species across the sites in order to combine sources for a stable isotope mixing model (Phillips *et al.* 2005). For all tail hair data points that occurred during 2009, plant data was collected for that same year as a direct comparison. For all other tail hair data points, plant data points that had been averaged across sites and years were used.

IsoSource version 1.3 is a Microsoft Visual Basic™ software package developed for multiple uses in stable isotope analysis including using isotopic ratios to determine the proportional contribution of several different sources to a mixture (as in using various plant sources that contribute to an isotope mixture which in this case we used a section of tail hair). All possible combinations of each source contribution are examined in a small increment which is a value determined by the user and a given mass balance tolerance value is stated by the user. All source combinations that result in the predicted mixture signatures within the stated tolerance value are considered feasible solutions. The results of IsoSource show a mean value for each source along with a range of possible source contributions (maximum and minimum values)



(Phillips & Gregg 2002). In this study, IsoSource was used to calculate ranges of potential source contributions of various plant species to the tail hair (the mixture).

Vegetation Collection

In order to classify vegetation availability for feral horses on the Sheldon NWR, the following plant metrics were obtained: plant productivity (in the form of dry biomass), plant cover, and a relative abundance score.

Biomass collection

Biomass samples were collected along 52 randomly located 50m transects. The direction of the tape from the central random was randomly determined. Along each transect, five 1-m² quadrates were placed at 10 m regular intervals. In each quadrat, total herbaceous vegetation was clipped and separated by species. Clipped plants were placed in brown paper bags for properly weighing, preserving, and drying. Samples were initially weighed in the field, however, inclement conditions influenced total plant water content, therefore, wet weight was excluded from data analysis. In the laboratory, samples were dried for 24 hours at 60°C (Flinders & Hansen 1972) and weighed to the tenths.

Plant cover

Plant cover was determined for each habitat type using the same transect and sample frequency as biomass. Cover samples were collected using a step-point method described by Evans and Love (1957). The surface feature (plants, bare ground, rock, litter) observed directly behind a notch in the tip of the boot was recorded for a total of approximately 100 points per plot. Total percent plant canopy cover was determined by dividing the total number of hits for that species by the total of all plant hits recorded. 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

Using the same transect description previously, a 5x5m² quadrat was placed in the southwest corner of that plot. Within the 5x5m² plot, each species present was observed and given a score according to the following scale: 1-absent (the specific plant species was absent from the plot), 2-poor (the specific plant species appeared to cover anywhere from 1 to 10% of the ground in the plot or had numbers of plants ranging from 1 to 10 depending on the species - smaller species were allowed to have more plants present while still falling in the poor abundance category while larger species such as shrubs were allowed few numbers while falling in this category), 3-fair (the specific plant species covered from 5 to 25% of the plot or had numbers of plants between 2 and 20, again depending on the size of the plant), 4-good (the specific plant species covered between 20 and 40% of the plot or had numbers of plants between 5 and 30), and 5-abundant (the specific plant species was by far the most dominant type of cover in the plot, with cover greater than 40% or numbers greater than 10 to 20, again depending on the size of the plant species in question). 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

Biomass, abundance, and cover data were analyzed through the use of Systat 13 (Systat Software Inc, Chicago, IL). Data was checked twice for normality. The first time it was transformed using the square root transformation, then it was tested a second time for normality using skewness and kurtosis values. Approximately half of the data was normal after transformation and the other half were 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).

Horse Density

Horse density was estimated by USFWS personnel in July of 2010. Numbers of feral horses were recorded from flight sightings of horse bands in various areas across the Sheldon NWR.

RESULTS

Plant Results

Differences between horse present and horse absent sites

It was determined that between the two sites studied (Little Sheldon and Badger Mountain), there were relatively few differences between plant productivity and availability. Only 26 out of 218 parameters were found to differ (p < 0.05) between the two sites (Table 3). Of those that did differ significantly 22 of the 26 had higher plant biomass on the Badger site (where horses had not been removed). Only *Artemisia arbuscula* (both abundance and cover), *Artemesia cana* (cover), and rock (cover) showed significantly higher amounts on the Little Sheldon site compared to the Badger site.

Further analysis comparing the differences between horse present and horse absent sites identified an interaction between site and habitat type. Thus, after looking specifically at various habitats between the two sites, there were more differences between sites.

Previous research has demonstrated that feral horses can have degradative impacts to riparian areas. After looking at the various habitat differences between the two sites studied, one habitat type, the mesic meadow, was of particular interest due to previous research which has demonstrated that feral horses can have degradative impacts to riparian areas (Levin *et al.* 2002;



Wood *et al.* 1987; Ganskopp & Vavra 1986). From all comparisons tested, only biomass showed significant differences and within the biomass sampled species, four plant species out of all plant species sampled showed significant differences (see table 5).

Plant species availability

Plant species available for feral horse consumption across the Badger Mountain and the Little Sheldon site were similar for biomass, abundance, and percent cover. Table 6 shows the top 10 results in biomass, abundance, and cover for our study plots. Shrubs and forbs were each included in the table as a combined entity. It should be noted that shrub biomass samples were not collected in this study.

Horse Density Results

Total density was found to be much higher in the Badger site than the Little Sheldon site.

This was expected, as there was a roundup and subsequent removal of horses from the Little

Sheldon site in August of 2009, just one year prior to the USFWS count (see figure 10).

δ^{13} C and δ^{15} N Results

Initial observation of isotopic results shows seasonal variation of feral horse diet with both carbon and nitrogen isotopes varying in similar patterns annually (see figure 11). There was no significant difference between animals within the same reproductive categories, the only difference being lengths of hair. The ten examples of each reproductive category were averaged and one value was represented per date.

Plant Isotopic Results

Plant isotope values showed little variation between sites and habitat types. This led to plant isotopes being averaged between the Little Sheldon site as well as the Badger Mountain



site. This was done for 2009 and for 2010. Plant isotopes were averaged between the two sample years and are shown in table 7.

Statistical Isotopic Results

Initially, analysis of variance of tail hair isotope results demonstrated that 75 out of 160 tests showed significance (p < 0.05) for differences between date and reproductive category. A Tukey-Kramer test was used for all significant results in order to determine where the significance between the various groups exists. Due to the significant results of the Tukey-Kramer tests, and the results from tail hair isotopic results, we selected 11 data points to run in IsoSource; studs and mares in Aug 2009, studs and mares in May 2009, studs and mares in Sept 2008, mares in Jan 2008, studs and mares in Aug 2007, combined values (studs and mares) in Nov 2006, and combined values (studs and mares) in July 2006.

IsoSource Results

Mean isotopic and range values (with minimum and maximum values) obtained from IsoSource are shown in figure 12.

In the summer of 2006, the major dietary constituents consisted mostly of FEID, BRTE, and shrubs. By winter of that year, ELEL, FEID, and POSE were the major dietary components. In the summer of 2007, studs consumed mostly JUBA, followed by shrubs and, then STTH while mares showed a preference for shrubs, JUBA, and ELEL. In the winter of 2008, mares consumed STTH, forbs, ELEL. During fall 2008, studs consumed LECI, JUBA, and ELEL in order of preference; mares consumed JUBA, shrubs, then ELEL. In the spring of 2009, studs ate PSSP, FEID, and ELEL and mares ate PSSP, BRTE, and STTH. Finally, in 2009 both mares and studs consumed shrubs in the greatest abundance followed by FIED and ELEL for mares JUBA and ELEL for studs.



DISCUSSION

Feral horse diet is largely determined by what food sources are available for consumption. Thus it logically follows that feral horse diet will change with changing seasons. Initial isotopic results demonstrated seasonal patterns with depleted and enriched carbon and nitrogen isotopic values (see figure 6). This finding is consistent with findings in previous literature (Salter & Hudson 1979). Dietary shifts throughout the seasons to a limited degree are dependent on the reproductive categories to which the horse belongs. Though no studies specify the reasons why horses choose the food sources that they do, previous literature has found that ungulates in general select forages on the basis of four morphological parameters: body size, type of digestive system, rumino-reticular volume to body weight ratio, and mouth size (Hanley 1982). Potential areas for future study could include why seasonality exists in forage selection of feral horse diet and why differences exist between reproductive categories (different dietary needs, dietary preferences, or spatial patterning differences between various bands and herds of horses).

Dietary selection among feral horses is also likely to be dependent on forage availability. With shrubs, forbs, *Elymus elymoides*, and *Poa secunda* commonly found in abundance across the Sheldon NWR (and more specifically within the Little Sheldon and the Badger Mountain areas where the horses of this study reside), it is likely that their presence and their ease of access significantly influence equine dietary selection. Interestingly, *Elymus elymoides* was a frequent prominent player in IsoSource results and *Poa secunda* was for the most part not an important component. *Festuca idahoensis* was less commonly found (in abundance, cover, and biomass) but it was still much more heavily selected for according to IsoSource results. It is interesting to note that *Festuca idahoensis* is an important native species that was nearly eliminated during the



turn of the twentieth century due to excessive overgrazing by sheep. The effects are still seen today. It is also interesting that feral horses still seek it out even though it is not as prevalent as other vegetative species. It is likely that some other factor is involved in feral horse dietary preference such as palatability. Table 8 shows various scores of palatability for horses of a few important gramineous species. In previous research, *Festuca idahoensis* has been reported to be the main grass selected in cattle and horse diets in Oregon (Vavra & Sneva, 1979).

Moderate levels of grazing of *Festuca idahoensis* actually promote plant vitality and can increase community stability. The amount of grazing that Idaho fescue can sustain is directly determined by the conditions of the site. It is difficult to determine the health of the vegetation and typically it is best to look at the long-term response of the vegetation (USDA Forest Service, 2011).

The differences between two sites – one with horses still present and one with horses removed the previous year – showed surprising results. With statistically higher amounts of vegetation on the Badger site (the site with horses present) compared to the Little Sheldon site, the likely explanation is that there is simply more vegetation on the Badger site than the Little Sheldon site. These differences are likely due to elevation, precipitation, soil types, or any number of other factors. Due to differing amounts of vegetation across the two sites, one can see that even within a few miles, certain areas have higher or lower carrying capacities. With differing carrying capacities, it is recommended that appropriate management levels of horse herd population sizes be adjusted according to individual sites (not just according to a large area such as an entire state).

After looking at the differences available between the vegetation across the habitats, it was determined that *Carex rossii*, *Juncus balticus*, and *Poa secunda* were significantly higher on



the Little Sheldon site than the Badger site. These are species frequently and abundantly found within riparian areas. The one species that was significantly higher in riparian areas on the Badger site than the Little Sheldon site was *Bromus tectorum* which is often found disturbed areas (Knapp 1996, Hunter 1991, Rice & Mack 1991). This disturbance was possibly caused by feral horse impacts (though other factors almost certainly have an impact).

MANAGEMENT IMPLICATIONS

The issues involved with the management of feral/wild horses are highly debatable and controversial. Issues include but are not limited to whether feral horses need to be removed from public lands, values at which appropriate management levels (AMLs) should be set, the most humane way to take care of excess horses, and how to deal with public relations and threats of litigation. Many have varying ideas as to how feral/wild horses should be managed and how rangeland integrity should be maintained for the benefit of all wildlife and for the well being of the horses. Options range from leaving current population as they are; eliminating excess horses (counts above AML) via euthanization and/or meat production, adoption programs, or transfer to long-term holding facilities; and completely removing horses from public rangelands.

Unfortunately, relatively little experimental research has been conducted with feral/wild horses in recent years and as such relatively little is known about their behavior and their impact on their surroundings. Without knowledge of these facts, feral horse management will continue to be a controversial issue. Both managers and the general public will continue to be perplexed about the best way to handle this tenuous situation.

With the knowledge of what feral/wild horses are consuming on rangelands, policy makers and land managers can know better how to handle tenuous situations involving feral horses. Management strategies can become less emotionally based and more strategic. Tax



money (of which a considerable amount is spent on horse management annually) can be spent in more beneficial ways.

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Table 3. Significant differences between horse removed sites and horse present sites. This table shows mean values (with SEM values) for the various parameters of both the Little Sheldon and the Badger Mountain sites. Biomass is shown in kg/ha, abundance in a relative score, and cover in percentage of hits.

Parameter	Little Sheldon	Badger Mountain	Level of significance (p<0.05)
Biomass (kg/ha)			<u> </u>
Achnatherum thurbarianum	2.35±0.45	6.58±0.81	0.000
Annual Forbs	0.00 ± 0.00	1.06±0.36	0.001
Elymus elymoides	5.62±0.50	8.86 ± 0.77	0.000
Festuca idahoensis	1.52±0.73	7.09 ± 1.15	0.000
Forbs	10.35±1.01	13.72 ± 1.02	0.019
Grasses	44.61±8.39	43.70±2.06	0.916
Hesperostipa 53olumb	0.00 ± 0.00	0.94 ± 0.41	0.052
Oryzopsis hymenoides	0.00 ± 0.00	0.09 ± 0.09	0.013
Perennial Forbs	10.35±1.01	12.66±1.01	0.008
Stipa 53olumbiana	0.00 ± 0.00	0.78 ± 0.33	0.013
Abundance (relative score)			
Acnatherum nelsonii	0.00 ± 0.00	0.98 ± 0.30	0.002
Artemesia arbuscula	1.58±0.39	0.38 ± 0.22	0.012
Cryptantha sp.	0.00 ± 0.00	0.42 ± 0.18	0.020
Elymus elymoides	2.23±0.27	3.10 ± 0.23	0.007
Forbs	10.46±1.10	13.38±1.29	0.092
Grasses	10.19±0.82	13.02 ± 0.81	0.018
Lupinus argenteus	0.54 ± 0.26	1.27 ± 0.29	0.013
Phlox hoodii	0.35±0.17	1.15 ± 0.30	0.031
Shrubs	5.96±0.79	7.27 ± 0.78	0.245
Tetradymia canescens	0.00 ± 0.00	0.50 ± 0.22	0.020
Cover (%)			
Artemesia cana	0.15±0.09	0.00 ± 0.00	0.039
Elymus elymoides	2.62 ± 0.49	6.00 ± 0.81	0.001
Forbs	9.00±1.24	9.50 ± 2.75	0.869
Grasses	27.46±3.41	24.04±3.64	0.496
Hesperostipa columbiana	0.00 ± 0.00	0.81 ± 0.31	0.005
Litter	6.31±1.38	12.04±1.33	0.000
Phlox hoodii	0.23 ± 0.23	0.62 ± 0.25	0.030
Rock	8.38±2.56	1.62 ± 0.56	0.031
Shrubs	22.38±0.42	19.58 ± 2.65	0.415
Stipa columbiana	0.00 ± 0.00	1.08 ± 0.40	0.001
Stipa nelsonii	0.00 ± 0.00	0.23 ± 0.13	0.039

Table 4. Sites analyzed during ANOVA showing habitat interactions, site interactions, or habitat and site interactions. Significant site differenced show differences between the Little Sheldon and the Badger Mountain sites. Significant habitat differences show differences between the 13 different habitat types found on the Sheldon NWR. Significant differences between both show differences between various habitats on the two sites.

Parameter	Site(p-value)	Habitat(p-value)	Both(<i>p</i> -value)
Biomass (kg/ha)			
Achnatherum thurbarianum	0.000	0.026	0.001
Carex rossii	0.724	0.005	0.000
Elymus elymoides	0.000	0.002	0.004
Forbs	0.019	0.000	0.000
Grasses	0.916	0.029	0.004
Poa Secunda	0.152	0.000	0.000
Abundance (relative score)			
Achnatherum thurbarianum	0.482	0.410	0.018
Elymus elymoides	0.006	0.156	0.355
Festuca idahoensis	0.494	0.002	0.820
Forbs	0.092	0.032	0.032
Grasses	0.018	0.608	0.063
Shrubs	0.245	0.001	0.041
Cover (%)			
Forbs	0.234	0.470	0.114
Grasses	0.171	0.005	0.290
Shrubs	0.076	0.001	0.242

Table 5. Significant differences between biomass of species in riparian areas. Mean values (with SEM values) are shown for both the Little Sheldon and the Badger Mountain sites in grams.

Parameter	Little Sheldon B	adger Mountain	Level of significance ^a
Biomass (kg/ha)			
Bromus tectorum	0.00 ± 0.00	3.12±1.29	0.030
Carex rossii	6.74 ± 1.70	1.62 ± 1.08	0.035
Forbs	6.00±1.03	8.80 ± 2.58	0.334
Grasses	72.86 ± 15.00	56.62 ± 7.52	0.350
Juncus balticus	14.10±1.67	6.27 ± 1.89	0.012
Poa secunda	10.07±1.41	3.64±1.49	0.011

^ap-values adjusted with Tukey's Honest significant difference.



Table 6. Biomass, abundance, and cover summed across the Badger Mountain and the Little Sheldon sites. Biomass values are in kg/ha, abundance are a summed abundance score value, and cover values are total number of hits per cover type out of 5100 total hits.

Biomass (kg/ha)		Abundance (relative score)		Cover (%)	
Forbs	3129	Forbs	620	Shrubs	1091
Poa Secunda	1884	Shrubs	344	Forbs	481
Elymus elymoides	1882	Elymus elymoides	139	Poa secunda	287
Bromus tectorum	1598	Poa secunda	124	Elymus elymoides	224
Stipa thurberiana	1160	Stipa thurberiana	68	Festuca idahoensis	152
Leymus cinereus	1142	Festuca idahoensis	62	Stipa thurberiana	124
Festuca idahoensis	1118	Pseudoroegneria spicata	44	Juncus balticus	123
Pseudoroegneria spicata	641	Bromus tectorum	39	Leymus cinereus	81
Carex rossii	618	Achnatherum nelsonii	26	Agropyron cristatum	78
Poa pratensis	396	Juncus balticus	23	Lepidium perfoliatum	60

Table 7. Plant isotopic values (‰) as an average between the Little Sheldon and the Badger Mountain sites. Values shown are an average between samples taken in 2009 and 2010. An average between all forb values is given as well as the most common forbs found in the area. Data are expressed as value ±SEM

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



Table 8. Ratings of gramineous plant palatability of key species found on the Sheldon NWR. Data taken from http://www.fs.fed.us/database/feis/plants/graminoid/index.html, 2011.

Plant species	Rating
Achnatherum nelsoni	Good in all seasons
Achnatehrum thurberiana	Good
Bromus tectorum	Good in spring and mild winters
Carex rossii	Fair
Elymus elymoides	Fair (palatability decreases as awns lengthen)
Festuca idahoensis	Good
Juncus balticus	Fair to moderately low
Poa secunda	Good
Pseudoroegneria spicata	Good



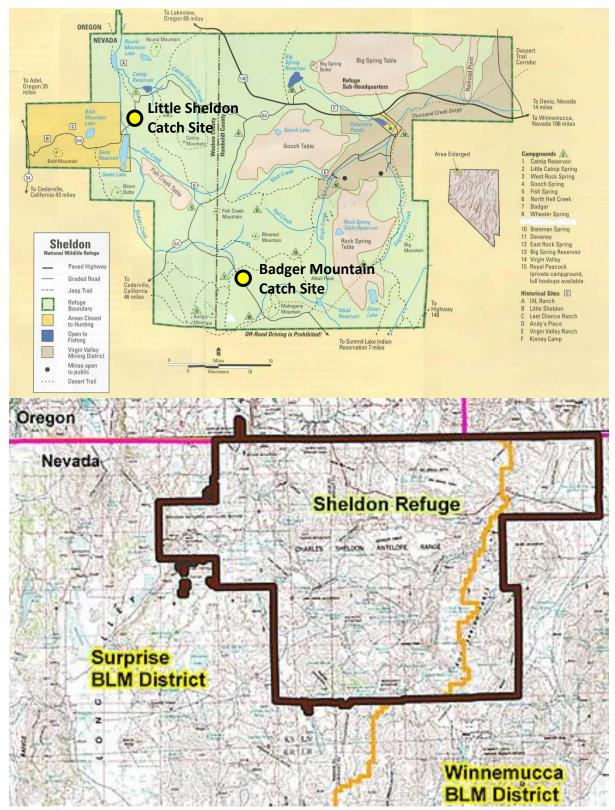


Figure 5. Map of the Sheldon NWR with two tail hair and plant sampling areas pointed out.



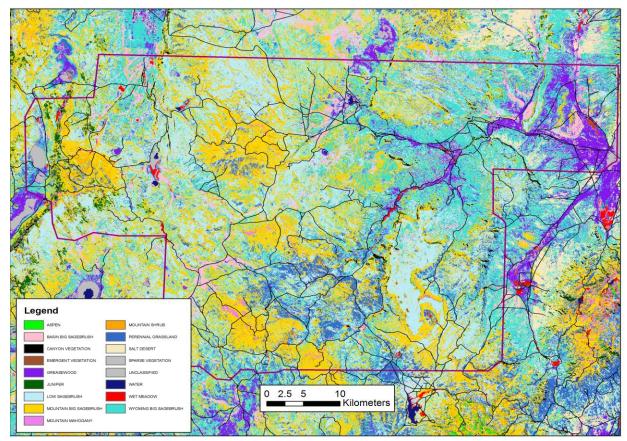


Figure 6. Map produced representing vegetation classes on the Sheldon NWR.



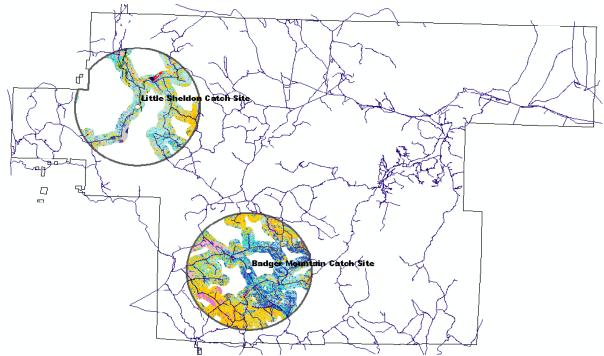


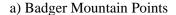
Figure 7. Potential areas for sampling based on 0.5-mile distance from roadways within 5-mile radius of each catch site.



Relative Abundance of Vegetation Classes 50.00% 45.00% 40.00% 35.00% 30.00% 25.00% 20.00% 15.00% 10.00% 5.00% 0.00% ■ Vegetation Map ■ Little Sheldon (0.5-mile buffer from roadways) Little Sheldon (5-mile radius area) ■ Badger (0.5-mile buffer from roadways) ■ Badger (5-mile radius area)

Figure 8. Proportion of vegetation classes across the Sheldon NWR.





b) Little Sheldon Points

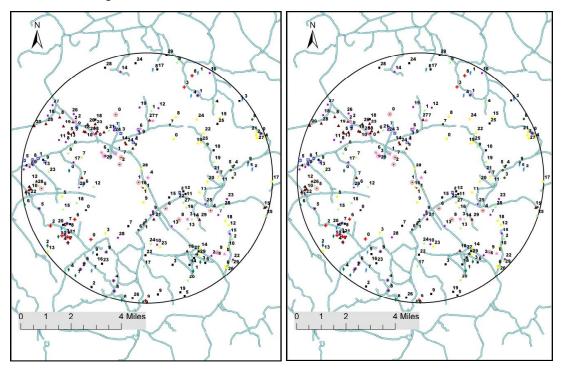


Figure 9. Maps generated through the use of ArcGIS November 2009 showing random points for a) the Badger area of the Sheldon NWR and b) the Little Sheldon area of the Sheldon NWR. 30 random points within 0.5 miles of a road were generated for the 5 main vegetation and 10 random points were generated for the remaining 8 vegetation types that are pertinent to the study. These maps were later used in field data collection.



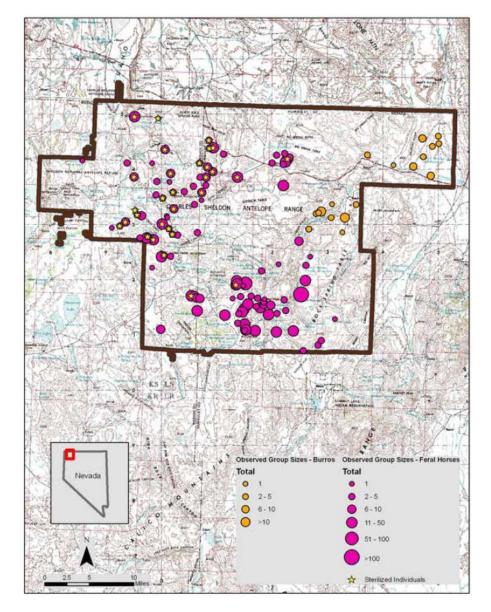
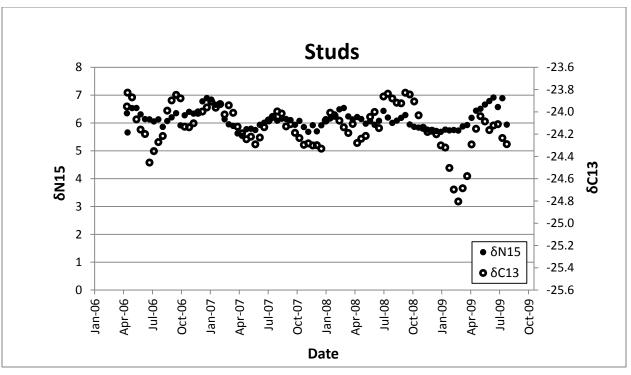


Figure 10. Feral horse group sizes and location across the Sheldon NWR as of July 2010. Stars indicate at least one sterilized individual present.





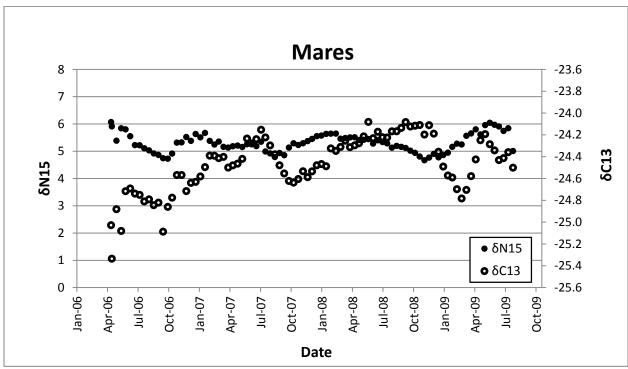
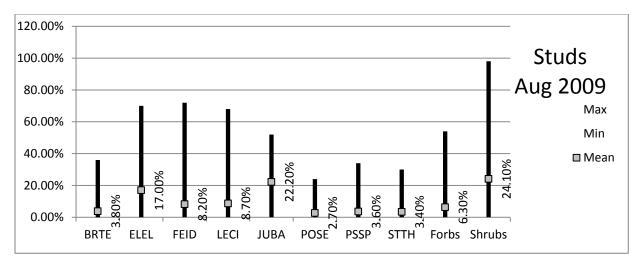
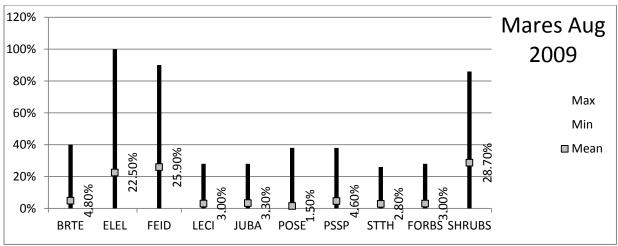
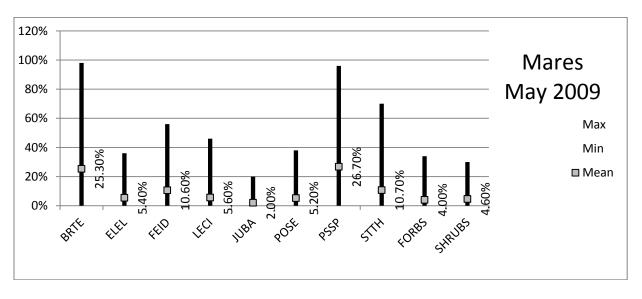


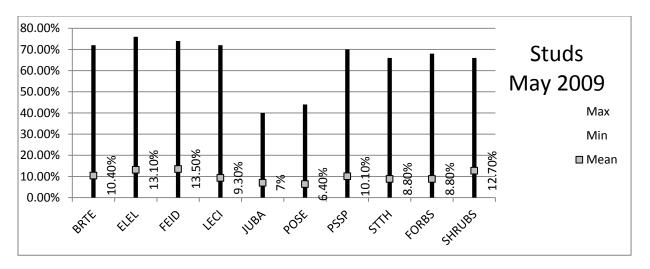
Figure 11. Seasonal variation of isotopic signatures within each reproductive category. Graphs shown are an average of 10 randomly selected individuals within each reproductive category.

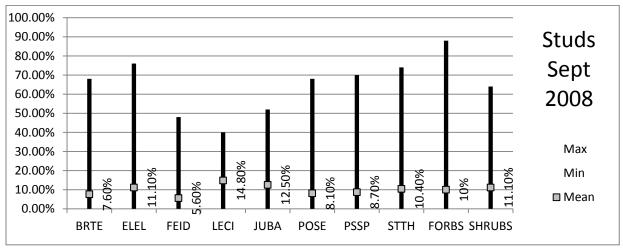


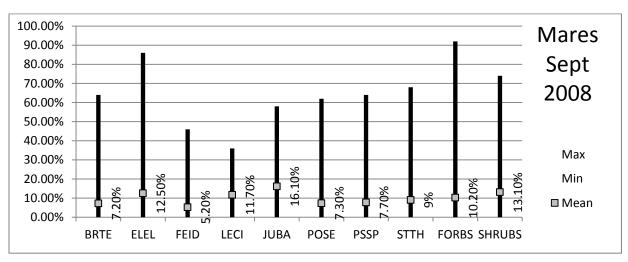




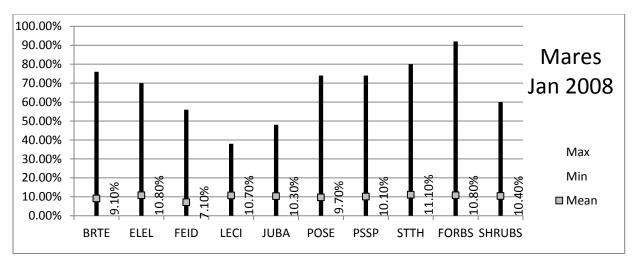


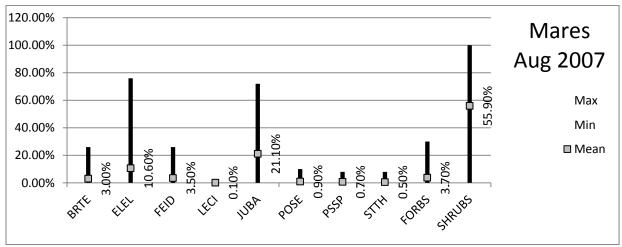


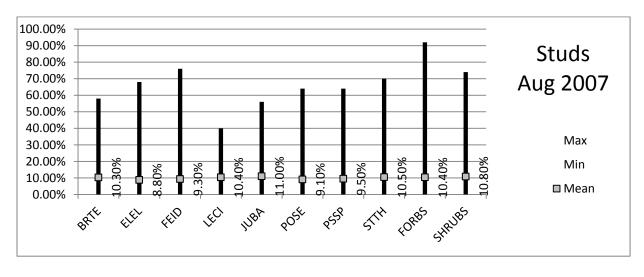




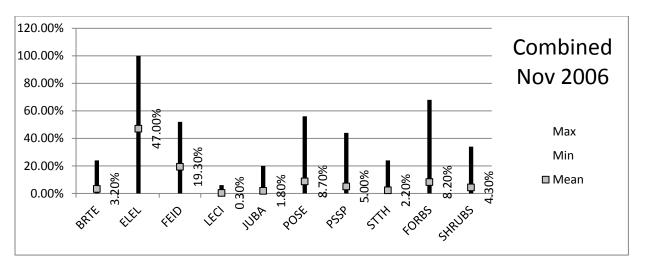












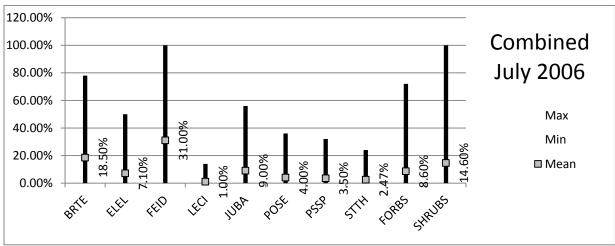


Figure 12. IsoSource results showing a maximum, minimum, and a mean value (as recommended by Phillips & Gregg 2003). BRTE: *Bromux tectorum*, ELEL: *Elymus elymoides*, FEID: *Festuca idahoensis*, LECI: *Leymus cinereus*, JUBA: *Juncus balticus*, POSE: *Poa secunda*, PSSP: *Pseudoroegneria spicata*, STTH: *Stipa thurberia*.

