SEASONAL DIETS OF SABLE ANTELOPE (*HIPPOTRAGUS NIGER*): DETERMINED BY MICROHISTOLOGY AND TRNL SEQUENCE ANALYSIS

by

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ABSTRACT

I investigated the seasonal diets of sable antelope at Mason Mountain Wildlife Management Area from June 2013 to April 2014 using microhistological techniques and comparative sequence analysis of trnL gene fragments using next generation sequencing techniques on DNA obtained from fecal material. Forty samples were collected during summer 2013 with 20 samples collected in the fall, winter, and spring. Vegetational analyses were conducted simultaneously with the fecal collection to determine if sable antelope were selectively feeding. Herbaceous plants were sampled using the Daubenmire method. Woody plants were sampled using the line-intercept method. Annually, the bulk of the diet was comprised of little bluestem (*Schizachyrium scoparium*) and Texas wintergrass (*Stipa leucotricha*). During spring, summer, and fall little bluestem (*Schizachyrium scoparium*) was selected. During the summer, switchgrass (*Panicum virgatum*) was also selected. Sable antelope selected Texas wintergrass (*Stipa leucotricha*) during the winter. DNA analysis targeted a portion of the chloroplast trnL (UAA) intron. Thirteen samples were successfully amplified and sequenced and resulted in 24 unique plant sequences. The vast majority of plants consumed by sable antelope were grasses. While sable antelope may not compete for food resources with browsers such as white-tailed deer and greater kudu, careful consideration should be made when stocking with other grazers such as cattle, waterbuck, gemsbok, and scimitar-horned oryx.

1. INTRODUCTION

Exotic ungulates were first introduced to South Texas in the 1930s, when nilgai antelope were released on King Ranch. The number of individuals has grown from 13,000 in the 1960s to estimates that range from 275,000 to over 1 million in 2007 with the greatest concentration occurring in the Hill Country (Traweek 1995; Middleton 2007). The estimated number of exotic species has also grown from 13 species in the 1960s to 76 species last reported in 1995 (Traweek 1995). The large increase in exotic hoofstock populations and the popularity of exotic game ranching in Texas is largely due to high reproductive rates, ability to adapt to the Texas climate, and the hunting opportunity these species provide. With fluctuating profits in cattle ranching, many ranchers have turned to exotic game ranching for hunting (Middleton 2007). Reports by the Charlie Seale, executive director of the Exotic Wildlife Association of Ingram, Texas, (EWA, http://www.myewa.org) suggest that Texas has >5,000 landowners raising exotic ungulates, more than any other state (Middleton 2007).

Sable antelope (*Hippotragus niger*) is a species that has increased in popularity with hunters and ranchers. Hunters may spend from $12,500 up to $15,000 for a trophy sable antelope, depending on the length of its horns (Star S Ranch, http://www.star-s-ranch.com/HuntPrices.php). Sable antelope belong to the family Bovidae, subfamily Hippotraginae, and genus *Hippotragus* (IUCN 2008). There are four recognized subspecies of sable antelope: *H. n. niger*, *H. n. kirkii*, *H. n. roosevelti* and the critically endangered giant sable (*H. n. variani*) from Angola (Pitra et al. 2002; IUCN 2008). Sable antelope are native to flat and gently sloping grassy woodlands of southeastern Africa (parts of Botswana, Zambia,Angola, Zaire, Malawi, Kenya, Tanzania,

Mocambique, Zimbabwe, and South Africa, Wilson and Hirst 1977) where woody canopy cover is between 20 and 30% (Mungall and Sheffield 1994).

Although sable antelope have been stocked on several ranches in Texas, basic ecological information, such as food habits, is lacking for this exotic ungulate in Texas. Dietary information and other ecological data are necessary to maintain herd health, produce trophy bulls, assess dietary overlap with domestic livestock and other exotic hoofstock, and determine potential impact on habitats and native wildlife. These are major issues facing landowners that currently maintain or are contemplating stocking exotic species on their ranches (Middleton 2007).

The study of food habits has become an essential tool for understanding ecological relationships involving wildlife. In the absense of food habit studies, wildlife managers have turned to various forms of habitat manipulation to increase populations but failed to produce an increase in abundance. Gullion (1966) states that the health of game animals often depends on the availability of a single species of plant or very few species. Some food habit studies have attributed the failure of exotic game species becoming established, to a food supply that can not sustain them (Gullion 1966). Inadequate food resources have been regarded by wildlife managers as the most prevalent limiting factor of the size of big game herds and population growth (Gullion 1966). For these reasons food habit studies should be carried out for exotic species in both their native and non-native ranges. Food habit studies are also important for evaluating competition on rangelands between livestock and wildlife (Julander 1958). The overuse of palatable plants by livestock and wildlife results in forage competition which can cause range issues such as over- grazing (Julander 1958). For dietary data to have the

most meaning, emphasis must be placed on ecological relationships. Climate, seasonal and annual changes, food availability, animal competition, environmental manipulation, and population pressures should be recognized and interpreted (Medin 1970). A food habits study's amount of detail should be tied to the objectives of management (Bolen and Robinson 1995). To assess the success and possible competitive factors of sable antelope in Texas, a seasonal food habits study needs to illustrate the composition of food items consumed and the selectivity of those available plant species.

Limited research on sable diets within their native range suggests sable antelope are primarily grazers that minimally utilize browse (Mungall and Sheffield 1994). Wilson and Hirst (1977) used temporary feeding enclosures and examined rumen contents of sable in Africa to determine food preference. From the temporary feeding enclosures they found that sable were very selective foragers, eating only 15% of available grass species, and refusing to eat what was left of unpalatable grasses after only 10 days of feeding in enclosures. For the examination of rumen contents, a very small sample size was used because of a limit on how many sable could be harvested (Wilson and Hirst 1977). Owen-Smith et al. (2013) examined the selectivity of sable antelope in their native range by comparing their food habits with a generalist species. This study found sable antelope readily consumed grass species considered having low to moderate forage value. The ability to utilize lower quality forage enables them to occupy savannah woodlands associated with relatively infertile soils where the risk of predation is reduced (Owen-Smith et al. 2013).

The most common methods for quantifying food habits of large herbivores are: direct observation of a focal animal, utilization techniques, stomach analysis, and fecal

analysis (Holechek and Goss 1982). Direct observation, while widely used, can be problematic due to difficulties in plant identification and plant quantification (Holechek and Goss 1982). The oldest method, utilization or plant use, has a disadvantage because large portions of plants can be lost from weathering, trampling, and usage by other animal (Cook and Stoddart 1953). These issues can lead to an over estimation of use by the target animals (Cook and Stoddart 1953). While stomach content analysis is commonly used, it usually requires the sacrifice of the animal. This method would be difficult for research on such a prized and valuable game species such as sable antelope. Fecal analysis has become more common in last 30 years because it does not require interference with the habits or behaviors of animals and provides larger sample sizes.

Microhistological analysis compares the epidermal characteristics of plants found in the fecal or rumen contents to the epidermal characteristics in reference slides. The fecal or rumen material is often washed through a seive with water and then soaked in sodium hypochloride and alcohol to remove debri and clear plant material. Reference slides are made for comparison by collecting plants in the study area and scraping away the mesophyll until all that remains is the epidermal tissue. This method is time consuming and highly dependent on an extensive reference plant collection (Holechek 1982). Another common problem with this technique involves human error in the identification to species of epidermal plant material (Holechek 1982).

Recently, targeting the chloroplast trnL (UAA) intron for plant identification in fecal material, through DNA analysis, has been used to investigate large herbivore diets (Baamrane et al. 2012). Taberlet et al. 2007 recommends the trnL (UAA) intron for plant identification because it meets certain criteria. The region varies in composition between

taxon more than within taxon which aids in taxonomic assignment (Taberlet et al. 2007). The trnL intron also maintains highly conserved primer regions meaning that there is little variation between taxon in the primer region (Taberlet et al. 2007). The genomic region also has universal primers meaning that the primer regions are found in all plant chloroplast DNA. The region is also relatively short which is good when working with degraded DNA from fecal material (Taberlet et al. 2007). Research has also been conducted to compare the use of the trnL approach and microhistological techniques on rumen and fecal contents of herbivores to determine diet (Soininen et al. 2009; Murphree 2012). Soininen et al. 2009 found this type of DNA analysis to give a taxonomically more detailed picture of the diet for two species of small herbivores, *Microtus oeconomus* and *Myodes rufocanus*. However, Murphree (2012), concluded that DNA analysis did not enhance the ability to detect plant species in herbivore diets better than microhistological analysis. Analysis of plant DNA in fecal material might be a useful and fast technique for determining the food habits of large herbivores (Taberlet et al. 2007; Soininen et al. 2009). However this technique needs further investigation to determine its usefulness.

Objectives

To date, no research has been conducted to determine the food habits of sable antelope in Texas or elsewhere outside their native range. The goals of my research project are to a) quantify the seasonal food habits and food preferences of sable antelope in the Llano Uplift Natural Region of Texas using microhistological identification of plant material from sable fecal material, b) identify and quantify plant DNA from fecal material of sable antelope, and c) compare the results from both plant identification techniques. .

After determining the diet and assessing dietary selection by Sable Antelope in Central Texas, this basic ecological information can be used to: compare diets and address dietary overlap between sable antelope and native ungulates (as well as other exotics), compare sable antelope diet in Texas to the diet in their native range, assist those ranching exotic game in determining the feasibility of maintaining sable antelope on their property, assess the use of DNA analysis of fecal material as a technique for determing seasonal diets of large herbivores.

2. METHODS AND MATERIALS

Study Site

I studied the food habits of a herd of 20 sable antelope at Mason Mountain Wildlife Management Area (Mason Mountain WMA) in Mason County, Texas. Mason Mountain WMA is located in the western part of the Llano Uplift Natural Region of Texas. The Llano Uplift encompasses approximately 800,000 ha and the average rainfall is 60cm (NOAA 2013). The average annual temperature is 19.7° C and fluctuates from 8.9° C in January to 27.2°C in July (NOAA 2010). This region is characterized by gently rolling hills, monadnock features (granitic outcroppings), and predominately sandy soils (Singhurst et al. 2007). Vegetation ranges from oak woodlands in sandy, well watered areas, to mesquite savannahs on loamier soils with interspersed grasslands (LBJ School of Public Affairs 1978). The climate and vegetation of the region provide exotics with similar habitat requirements when compared to their native range of Africa during most months. However, major differences are recognized during the cold winter months when freezing can occur and affect survivorship in several exotic species (Mungall and Sheffield, 1994).

Mason Mountain WMA is approximately 2120 ha (5239 acres). Prior to public acquisition, the management area was a working exotic game ranch. The sable herd on Mason Mountain WMA is confined to a 1000 ha (2475 acres) pasture (Figure 1) surrounded by 2.4 m high fencing. Six species of exotic hoofstock, sable antelope, gemsbok (*Oryx gazella*), greater kudu (*Tragelaphus strepsiceros*), Thompson's gazelle (*Eudorcas thomsonii*), waterbuck (*Kobus ellipsiprymnus*), and axis deer (*Cervus axis*) are found in this pasture and provide unique opportunities to study the effects of African ungulates on habitats and native wildlife.

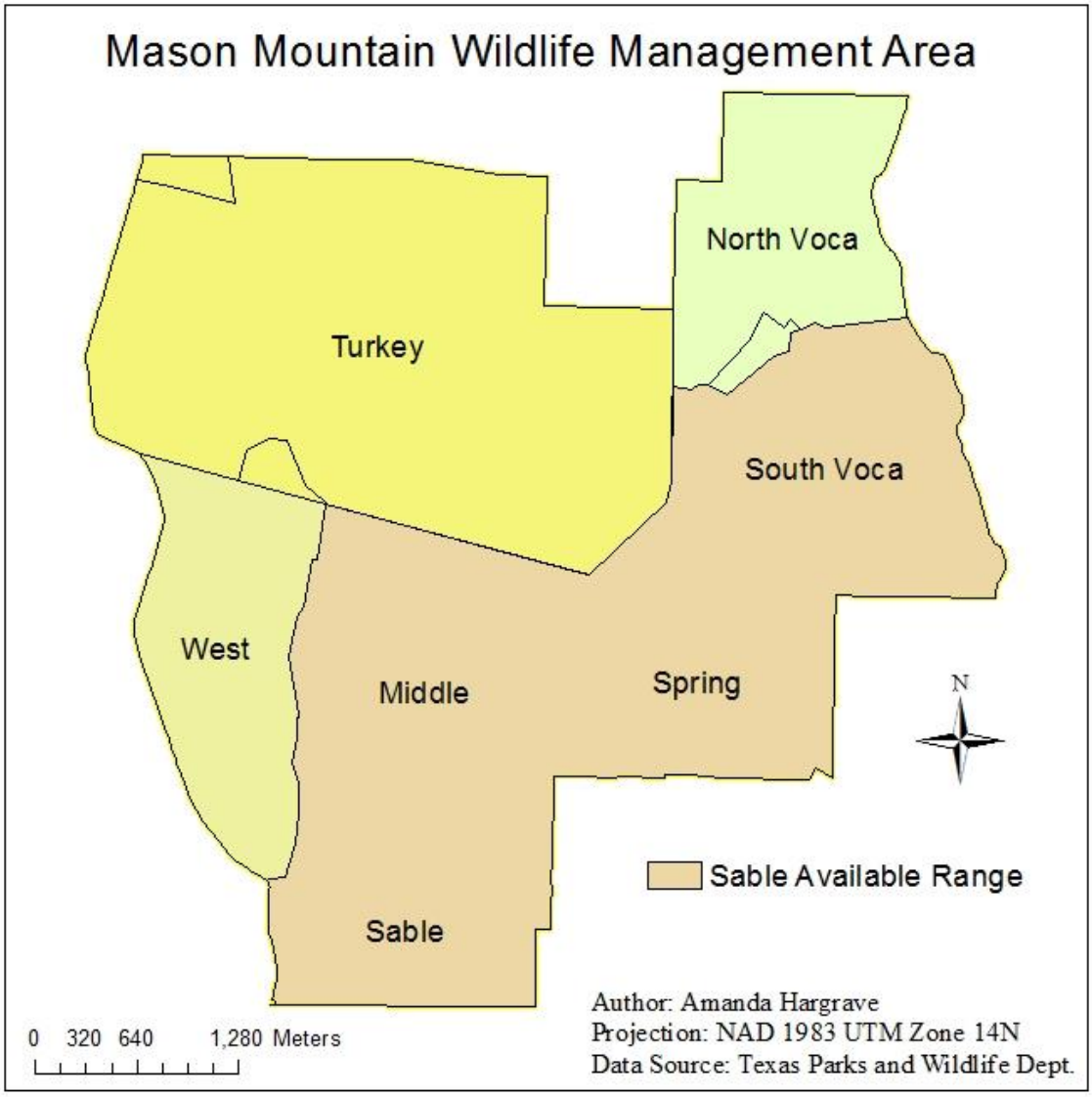


Figure 1. Pastures and sable antelope available range at Mason Mountain Wildlife Management Area, Mason County, Texas, 2013-2015.

Fecal Collection

I collected freshly deposited fecal samples seasonally beginning in June 2013. Once I located sable antelope, I observed them from a safe distance and recorded field notes on their behavior and feeding habits. Once they left, I searched the area for fresh fecal deposits and collect 6 pellets for microhistological analysis and 6 pellets for DNA

analysis from each deposit. To assure that I collected the freshest fecal material, only pellets that were soft, moist, and covered by mucus were selected (Green 1987). Sampling encompassed as many different fecal deposits as possible each season, with a minimum of 20 fecal samples per season collected. The collected fecal material was immediately frozen until processing and analysis.

Reference Slides

I made reference slides from the leaves and stems of the plants present in the vegetation surveys at Mason Mountain WMA to aid in the identification of epidermal fragments in the fecal samples. I also collected plants for the reference library in areas where sable antelope were observed grazing. I removed the mesophyl by scraping away the underlying material with a razor blade. The remaining piece of epidermis was cleared with sodium hypochloride, inverted on a slide and mounted in Permount™ mounting solution. I made reference slides of both the upper and lower epidermis of the plant. In cases where the epidermis was difficult to remove, I blended plant parts with sodium hypochloride and water in a household blender. Small samples of the resulting plant fragments were placed on a slide, cleared, and mounted. I took photomicrographs of the reference slides, using a Nikon Coolpix camera mounted on a Nikon microscope, for comparison of the sample slides.

Microhistological Analysis

Microhistological analysis is used commonly for dietary studies of herbivores. I thawed the collected fecal samples, then crushed and washed all 6 pellets through a 1-mm sieve to clear any debris. I also washed samples briefly (10 sec) with sodium hypochloride for clearing of plant material. I selected approximately 50 mg of washed

fecal material and deposited it onto a slide with a small paint brush. I used a wet mount for counting and observation of the material. I prepared 20 slides per sample by spreading the fecal material evenly on the slides so that approximately 3 large plant fragments were visible per each of five fields of view (Scott and Dahl 1980). The fields of view were determined by using premarked distances on the microscope stage. Fields of view were 10 mm apart. Each field of view was examined at a magnification of 100x and the epidermal fragment closest to the pointer was identified to species. I compared the epidermal characteristics of plants in the sample slides with the characteristics from the plant reference slides to identify species because the epidermis is resistent to digestion and contains diagnostic characteristics (Baumgartner and Martin 1939, Sparks and Malchek 1968, Litvaitis et al. 1996). I used characteristics such as cell size, cell shape, stomata, trichomes, and glands, as well as the arrangement of cells to determine plant species (Baumgartner and Martin 1939; Litvaitis et al. 1996).

Diet (Use)

Sable antelope diet was determined by comparing the number of plant fragments from each species to the total number of identified plant fragments to calculate percent composition. This was done annually and seasonally to investigate the seasonal changes in diet composition.

Vegetational Analyses

I conducted vegetational surveys as part of a team contributing to an ongoing vegetational database for MMWMA. The vegetational surveys were done seasonally simultaneously with the fecal collection to determine the availablity of plant species to sable. Random plant transect locations were selected using ArcGIS 9.3. Hawth's Tools

were used to select 4 random points per soil association. With 4 soil associations throughout the study area, there was a total of 16 random locations to place plant transects for vegetational analyses.

At each point, a 100 m plant transect was established in a random direction within the soil association. The line-intercept method (Gates 1949) was used to sample woody plants and estimate percent cover. Herbaceous plant material was sampled by using the Daubenmire method (Daubenmire 1959) and placing a 100 cm x 25 cm Daubenmire frame every 10 m along the transect line. I sampled herbaceous plant material each season and woody plants once.

Plant Selectivity

When presented with several food items, an animal will exhibit selectivity or preference for some and avoidance of others. Selectivity can be measured by comparing the usage of plant items (percent composition) to the availability in the environment (percent composition) (Krebs 1999). Because of the uncertainty of assigning individual fecal samples to specific animals I used a Design I general study for measuring selectivity (Manly et al. 1993). This method assumes that all measurements are made at the population level and individuals are not recognized. Krebs (1999) suggests that Manly's alpha is one of the best indices of selectivity for most situations. However, Manly's alpha unaccompanied by confidence intervals could be misleading. Therefore, I used a log-likelihood chi-square test with estimated proportions of available resources with Manly's alpha preference index to support the results.

The log-likelihood chi square test was used to test the null hypothesis that sable antelope used plants proportional to their estimated availability. The alternative

hypothesis was that sable antelope use plants more (show selectivity) or less (show avoidance) based on their availability. To show selectivity or avoidance, I constructed confidence intervals using observed frequencies (use) and compared with expected frequencies (availability). If the expected frequency fell within the observed confidence interval then sable antelope used the plant in proportion to its availability. If the expected frequency fell below the confidence interval then that plant was used more than its avaibility in the environment and indicates selectivity. If the expected frequency fell above the confidence interval, then that plant was used less than its availability and indicated avoidance. To maintain 95% confidence intervals, I corrected plant use confidence intervals using Bonferroni correction (α/n), which corrects the significance to maintain a stable error rate (Neu et al. 1974).

I calculated availability for each plant as described by Krebs (1999). The occurrence for each available herbaceous plant was calculated as the number of Daubenmire frames (n = 176) in which the plant made up 5% of the cover or more compared to the total number of Daubenmire frames. The occurrence for each available woody plant was calculated as the number of 10 m intervals (n = 160) where the plant made up more than 5% of the cover. Unknowns were left out of the calculations.

I used Manly's alpha selectivity index to support the log-likelihood chi-square test and confidence intervals (Manly et al. 1993). A Manly's alpha index number greater than 1/m (m = total number of plants available) indicated preference while an index number less than 1/m indicated avoidance (Krebs 1999, Manly et al. 1993).

DNA Analysis

Dietary research previously has been done comparing the trnL approach with microhistological techniques on stomach contents of small herbivores (Soininen et al. 2009). In this study, DNA analysis resulted in a taxonomically more detailed picture of the diet for two species of small herbivores. Baamrane et al. (2012) examined using the trnL approach on fecal material to determine the food habits of a large herbivore, the Moroccan dorcas gazelle (*Gazella dorcas*) . Both studies determined herbivore food habits using the techniques described below. However, studies comparing DNA and microhistological analyses used to analyze fecal matter of large herbivores are lacking. This is an important comparison to be made because of the degraded nature of the DNA and plant material found in feces compared to plant material in rumen contents.

For DNA analysis, I extracted DNA from 200 mg of fecal material using the E.Z.N.A.® Stool DNA kit following the manufacturer's (Omega Bio-tek) instructions. I carried out DNA amplification by using universal primers that target part of the trnL (UAA) intron and a PCR master solution providing the necessary materials for amplification. The master solution contained 0.1 µM primer trnL-c (^{5}'CGA AAT CGG TAG ACG CTA CG) and barcoded primers P6loop-h (^{5}'CCA TTG AGT CTC TGC ACC TAT C) (Taberlet et al. 2007), both with Illumina adapters, primer pads, and linkers as illustrated for 16 rRNA gene primers from the Earth Microbiome Project (www.earthmicrobiome.org/emp-standard-protocols/16s/), 1 x *Taq* buffer, BSA, 1U *Taq* polymerase, 2.5 mM $MgCl_2$, 0.2 µM dNTP, and 1 µl extracted DNA with a final volume of 100 µl. The solution was denatured for 10 minutes at 95°C, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 50°C, and 45 seconds at 72°C. Following the 40

cycles the solution ran for 7 minutes at 72°C for elongation. After PCR cleanup, I examined the PCR product using gel electrophoresis and a 2100 bioanalyzer from Agilent technologies to verify the presence and concentrations of DNA in the product. The clean PCR product was then sent to The University of Texas At Austin Genomic Sequencing and Analysis Facility (Austin, TX). The samples were run on the Illumina MiSeq v3 with paired end 2 x 250 bp reads using the respective sequencing and index primers from the Earth Microbiome Project (www.earthmicrobiome.org/emp-standard-protocols/16s/).

For analysis of the resulting sequences and taxonomic assessment, I created a DNA library in Geneious from trnL sequences collected from the NCBI database and the plant species at Mason Mountain WMA. For plant species that were not available in the NCBI database, I extracted their DNA and sequenced them. I then added these sequences to the Geneious library. I compared the sequences obtained from my samples to the sequences in the library to identify plant species within the sample. This was done using qiime, unix, python and R statistical software for bioinformatics.

Bioinformatics

With the help of an experienced bioinformatician, the sequence data obtained from the University of Texas's Genomic and Sequencing Analysis Facility was exposed to various computer programs and custom scripts to obtain unique plant sequences of high quality. The sequences obtained consisted of 3 files per sample; an index file, the forward read file, and the reverse read file. Since the entire region sequenced was located in the forward read file, the reverse read file was not used in further analysis. The index file and the forward read file were concatenated and then the split_libraries.py Qiime (Caporaso et al. 2010) script was used to filter out sequences with base pairs having

quality scores that fell below Q30. A grep search was used to filter out sequences that did not have a conserved 17 bp region corresponding to the universal trnL g primer sequence (5'-GGGCAATCCTGAGCCAA-3') within the trnL (UAA) intron (Taberlet et al. 2007). This ensured that only plant sequences were used for further analysis. To clean up the sequences the Qiime script "truncate_reverse_primer.py" (Caporaso et al. 2010) was used to remove the reverse primer and all base pairs that followed. The Qiime script "pick_de_novo_otus.py" (Edgar 2010) was used to identify unique sequences. These unique sequences were input into Geneious version R8 (Kearse et al. 2012) and using the custom blast search they were compared to sequences in the reference library. The sequences were also blasted to NCBI (NCBI 2015) to determine species.

3. RESULTS

Microhistological Analysis

Two thousand plant fragments were analyzed and 1939 plant fragments were identified to species. Five hundred plant fragments were analyzed per season. Twenty-two unique plant fragments were dectected through microhistological analysis (Table 1). Little bluestem (*Schizachyrium scoparium*) was detected in more samples (n = 69) than any other plant (Table 1). Other plants that were detected in a large number of samples included oak (*Quercus* spp.) (n = 68) and Texas wintergrass (*Stipa leucotricha*) (n = 35).

Table 1. Number of sable fecal samples in which plant species occurred (frequency of occurrence).

Species	# of Samples Where Detected	Frequency of Occurrence
Little Bluestem	69	0.211
Oak	68	0.208
Texas wintergrass	35	0.107
Plains lovegrass	20	0.061
Aristida	19	0.058
Unknown Grass	19	0.058
Gum Bumelia	16	0.049
Side Oats Grama	16	0.049
Unknown forb/browse	12	0.037
Witch Grass	12	0.037
Switchgrass	9	0.028
Green Sprangletop	7	0.021
Paspalum sp.	4	0.012
Barnyard grass	4	0.012
Hairy grama	4	0.012
Whorled dropseed	4	0.012
Tumble lovegrass	3	0.009
Spiderwort	2	0.006
Vine Mesquite	1	0.003
Canada wildrye	1	0.003
Persimmon	1	0.003
Knotroot bristlegrass	1	0.003

In summer, grasses made up 83.1% of the sable antelope diet (Figure 2). Within the grass family, little bluestem was present in the greatest amount (58.7%). Other grass species included sideoats grama (*Bouteloua curtipendula*,11.4%),three-awn spp. (*Aristida* spp., 5.6%), and switchgrass (*Panicum virgatum*, 2.7%). Browse made up approximately 16.9% of the sable antelop diet during the summer. Browse class in the summer diet was comprised of 10.1% oak and 6.8% gum bumelia (*Sideroxylon lanuginosum*). The category *other* consisted of 6 grass species, each contributing less than 1.5 % to the summer diet. No forbs were detected in the summer diet using microhistological analysis.

Fall grasses made up 96.3 % of the sable antelope diet. As illustrated in Figure 3, Little blustem, made up the majority (71.6%) of the fall diet. Other grasses included fall witchgrass (*Digitaria cognata*, 14.8%), plains lovegrass (*Eragrostis intermedia*, 4.3%), and green sprangletop (*Leptochloa dubia*, 2.4%). Browse contributed only 3.4% of the sable antelope's fall diet and was comprised of oaks (Figure 3). The category *other* consisted of 5 grasses and 1 forb species with each making up less than 1.5% of the diet.

The winter sable antelope diet was 80.4% grasses, primarily composed of Texas wintergrass (73.5%) (Figure 4). Other items in the winter diet were little bluestem (3.3%) and oak foliage (1.9%). The *other* category consisted of 1 browse and 3 grass species that each made up <1.9% of the winter diet. No forb species were identified in the winter samples.

In the spring diet, grasses made up 86.7% of the sable antelope's diet. As illustrated in Figure 5, Little bluestem was present in the greatest amount (72.1%). Other grasses included Texas wintergrass (5.4%), three-awn (3.8%), and plains lovegrass (2.7%). Oaks were the only browse species detected in the spring diet at 12.7% (Figure

5). The *other* category consisted of 5 grass and 1 forb species that each made up <1.5% of the spring diet.

In the the sable antelope's annual diet (Figure 6), percent composition of grasses was 87%, browse was 13%, and <1% of the diet consisted of forb species. Little Bluestem accounted for 51.5% of the annual diet and made up the majority of plants consumed (Figure 6). Other grass species in the annual diet included Texas wintergrass (19.8%), fall witchgrass (4.0%), sideoats grama (3.2%), three-awn (2.4%), plains lovegrass (2.0%), switchgrass (0.8%), green sprangletop (0.8%), and whorled dropseed (0.8%) (Figure 6). Browse species consumed by sable antelope annually primarily consisted of oaks (11.3%) and gum bumelia (1.7%).

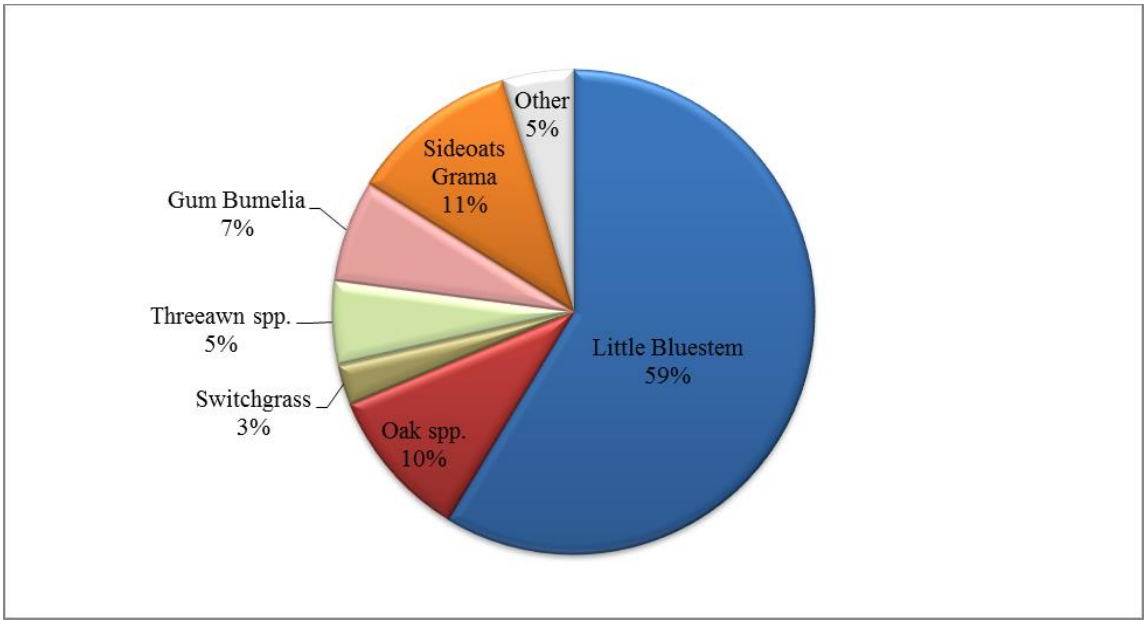


Figure 2. Percent occurrence of plants utilized by sable antelope during summer 2013 at Mason Mountain Wildlife Management Area. Data obtained from microhistological analysis.

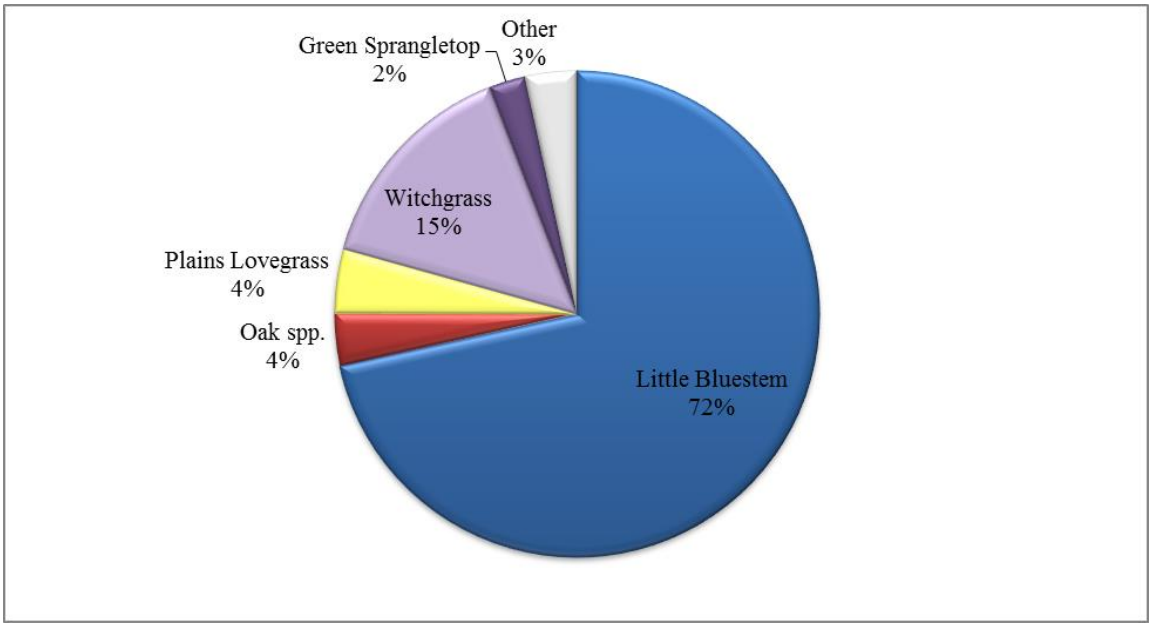


Figure 3. Percent occurrence of plants utilized by sable antelope during fall 2013 at Mason Mountain Wildlife Management Area. Data obtained from microhistological analysis.

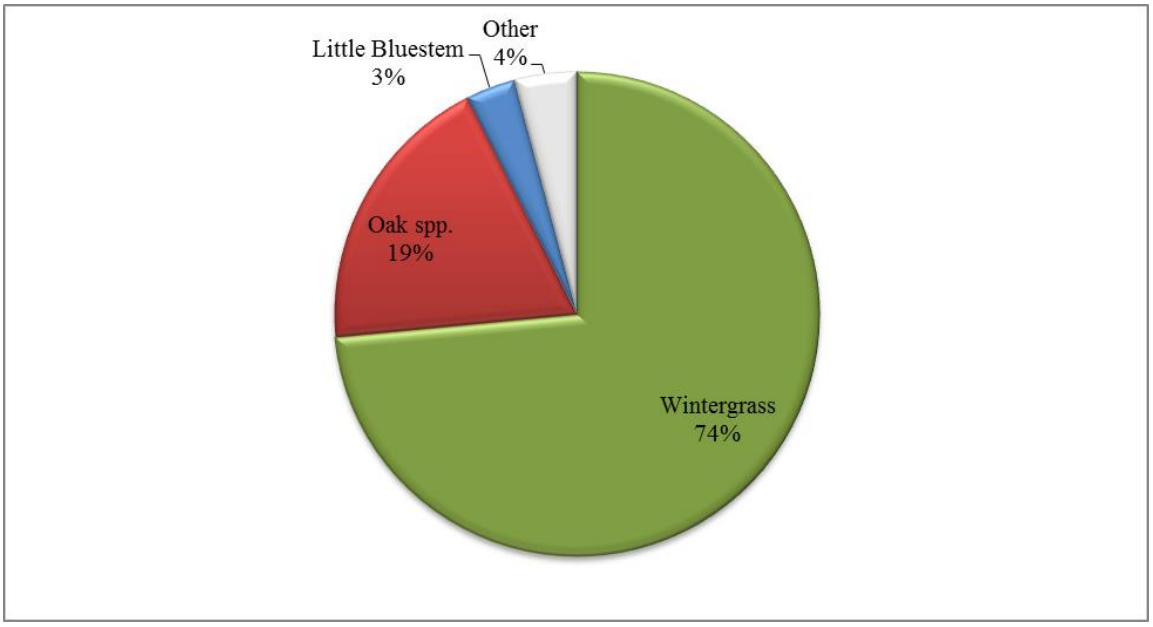


Figure 4. Percent occurrence of plants utilized by sable antelope during winter 2013-2014 at Mason Mountain Wildlife Management Area. Data obtained from microhistological analysis.

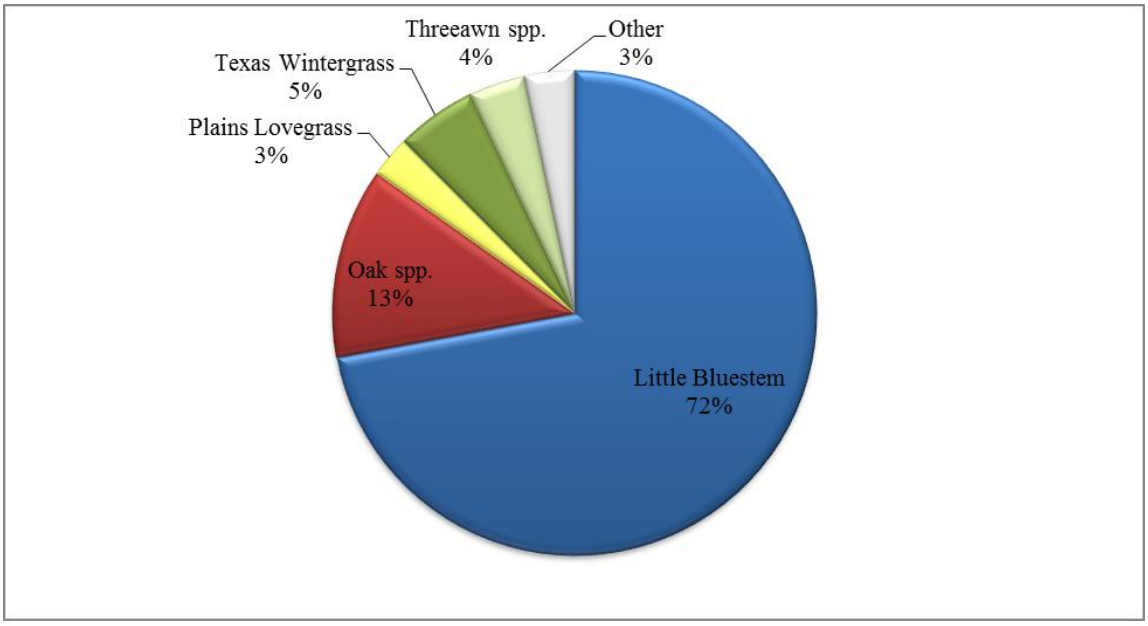


Figure 5. Percent occurrence of plants utilized by sable antelope during spring 2014 at Mason Mountain Wildlife Management Area. Data obtained from microhistological data.

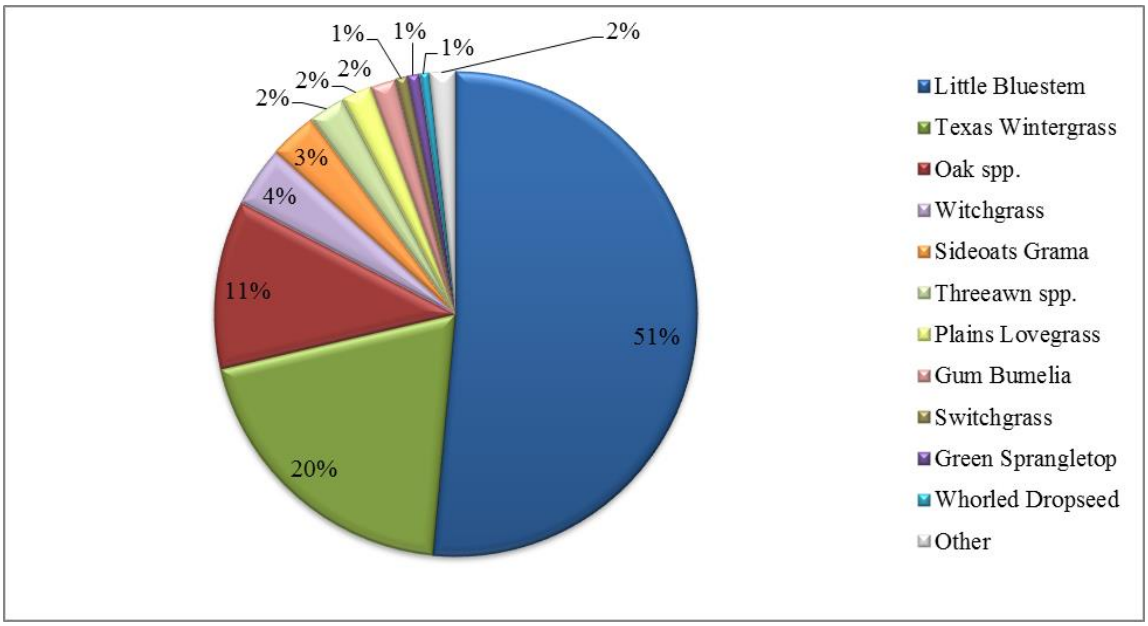


Figure 6. Percent occurrence of plants utilized by sable antelope annually at Mason Mountain Wildlife Management Area, 2013-2014. Data obtained from microhistological analysis.

Plant Selectivity

The null hypothesis that sable antelope use plants proportionally to their estimated availability was rejected for the summer season (χ_L^2 = 611.29, df = 6, P-value < 0.001). Sable antelope selected little bluestem and switchgrass during the summer (Table 2). The estimated availability (percent composition) of little bluestem in the habitat fell below the observed use (percent composition in the diet) confidence interval which indicates selection (Table 2). The Manly's alpha score was greater than 0.14 (m = 0.329) which also indicates selection (Table 3). The availability of switchgrass fell within the observed use confidence interval which suggests that sable antelope use switchgrass in proportion with its availability in the environment (Table 2). However, the Manly's alpha score (m = 0.391) was greater than 0.14 which suggests selectivity (Table 3). Oak and three-awn species had availability percentages that fell above observed use confidence intervals, suggesting these plants were avoided during the summer season (Table 2).

The null hypothesis was also rejected for the fall season (χ_L^2 = 877.58, df = 5, P-value < 0.001) with sable antelope selectively foraging on little bluestem and green sprangletop (Table 4). Little bluestem had an availability below the observed use confidence interval and a Manly's alpha score greater than 0.16 (m = 0.606) which indicates selectivity (Table 5). Green sprangletop, which had a low availability, had an available percentage that fell within the observed use frequency but a Manly's alpha score (m = 0.165) greater than 0.16 (Table 5). The confidence interval suggests that sable antelope use green sprangletop in proportion with its availability while the Manly's alpha score suggests selection (Table 5). Oak and plains lovegrass had availability percentages

(Table 4) that were greater than the observed use confidence intervals which suggests that these plants were avoided during the fall season.

During the winter sable selectively foraged on Texas wintergrass and the null hypothesis was rejected ($\chi_L^2 = 682.05$, df = 3, P-value < 0.001). Texas wintergrass had an availability that was less than the calculated observed use confidence interval (Table 6) and a Manly's alpha score (m = 0.810) greater than 0.25 (Table 7). Little bluestem had a low availability during the winter. Little bluestem and oak had available percentages that fell below the observed use confidence intervals and Manly's alpha scores less than 0.25 which suggests avoidance (Table 6).

Table 2. Comparison of the observed percent composition of plants in the diet and expected percent composition of plants in the environment, for the summer diet of sable antelope at Mason Mountain Wildlife Management Area, 2013. Hypothesis of proportional use was rejected ($\chi_L^2 = 611.29$, df = 6, P-value < 0.001).

Species	# of Plant Fragments	Use In Diet (Observed % Composition)	Availability (Expected % Composition)	Use 95% CI Lower Bound	Use 95% CI Upper Bound	Plants Utilized More (M) or Less Than Expected (L)
Little Bluestem	284	0.587	0.149	0.518	0.656	M
Oak	49	0.101	0.299	0.059	0.144	L
Switchgrass	13	0.027	0.006	0.004	0.050	-
Threeawn	27	0.056	0.213	0.024	0.088	L
Gum Bumelia	33	0.068	0.052	0.033	0.104	-
Sideoats Grama	55	0.114	0.098	0.069	0.158	-
Other	23	0.048	0.184	0.018	0.077	-

Table 3. Summary of results from Manly's alpha selectivity index for the summer diet of sable antelope at Mason Mountain Wildlife Management Area. α scores > 0.14 indicate preference.

Species	Manly's Alpha	Plants Utilized More (M) or Less Than Expected (L)
Little Bluestem	0.329	M
Oak	0.028	L
Switchgrass	0.391	M
Threeawn	0.022	L
Gum Bumelia	0.110	L
Sideoats Grama	0.097	L
Other	0.022	L

Table 4. Comparison of the observed percent composition of plants in the diet and expected percent composition of plants in the environment, for the fall diet of sable antelope at Mason Mountain Wildlife Management Area, 2013. Hypothesis of proportional use was rejected (χ_L^2 = 877.58, df = 5, P-value < 0.001).

Species	# of Plant Fragments	Use In Diet (Observed % Composition)	Availability (Expected % Composition)	Use 95% CI Lower Bound	Use 95% CI Upper Bound	Plants Utilized More (M) or Less Than Expected (L)
Little Bluestem	353	0.716	0.162	0.653	0.779	M
Oak	17	0.034	0.211	0.009	0.060	L
Plains Lovegrass	21	0.043	0.117	0.014	0.071	L
Witchgrass	73	0.148	0.142	0.099	0.198	-
Green Sprangletop	12	0.024	0.020	0.003	0.046	-
Other	17	0.034	0.348	0.009	0.060	M

Table 5. Summary of results from Manly's alpha selectivity index for the fall diet of sable antelope at Mason Mountain Wildlife Management Area. α scores > 0.16 indicate preference.

Species	Manly's Alpha	Plants Utilized More (M) or Less Than Expected (L)
Little Bluestem	0.606	M
Oak	0.022	L
Plains Lovegrass	0.050	L
Witchgrass	0.143	L
Green Sprangletop	0.165	M
Other	0.014	L

Table 6. Comparison of the observed percent composition of plants in the diet and expected percent composition of plants in the environment, for the winter diet of sable antelope at Mason Mountain Wildlife Management Area, 2013-2014. Hypothesis of proportional use was rejected ($\chi_L^2 = 682.05$, df = 3, P-value < 0.001).

Species	# of Plant Fragments	Use In Diet (Observed % Composition)	Availability (Expected % Composition)	Use 95% CI Lower Bound	Use 95% CI Upper Bound	Plants Utilized More (M) or Less Than Expected (L)
Wintergrass	355	0.735	0.195	0.673	0.797	M
Oak	92	0.190	0.415	0.135	0.246	L
Little Bluestem	16	0.033	0.122	0.008	0.058	L
Other	20	0.041	0.268	0.013	0.069	L

Table 7. Summary of results from Manly's alpha selectivity index for the winter diet of sable antelope at Mason Mountain Wildlife Management Area. α scores > 0.25 indicate preference.

Species	Manly's Alpha	Plants Utilized More (M) or Less Than Expected (L)
Wintergrass	0.810	M
Oak	0.099	L
Little Bluestem	0.058	L
Other	0.033	L

During the spring, sable antelope selectively foraged on little bluestem and null hypothesis was rejected (χ_L^2 = 617.67, df = 5, P-value < 0.001). Little bluestem had an available percentage of 0.199 which falls below the observed use confidence interval (Table 8) and a Manly's alpha score of 0.655 which is greater than 0.16 and indicates preference (Table 9). Plains lovegrass was used in proportion to its availability in the environment (Table 8). Oak, Texas wintergrass, and three-awn indicated avoidance with their available percentages being greater than the observed use confidence intervals (Table 8).

The null hypothesis that sable antelope use plants proportionally to their estimated availability on an annual basis was rejected (χ_L^2 = 2129.16, df = 11, P-value < 0.001). Annually, sable antelope selectively foraged on little bluestem, Texas wintergrass, and switchgrass which had availability percentages below the lower confidence interval on observed use (Table 10). They also had Manly's alpha scores (Table 11) greater than 0.083 which indicates preference. Green sprangletop and gum bumelia had available percentages that fell within the observed use confidence intervals which indicates that sable antelope use them in proportion to their availability in the environment. Oak, fall witchgrass, sideoats grama, three-awn, plains lovegrass, and whorled dropseed had availability frequencies that were greater than the observed use confidence intervals which indicates avoidance (Table 10).

Table 8. Comparison of the observed percent composition of plants in the diet and expected percent composition of plants in the environment, for the spring diet of sable antelope at Mason Mountain Wildlife Management Area, 2013-2014. Hypothesis of proportional use was rejected ($\chi_L^2 = 617.67$, df = 5, P-value < 0.001).

Species	# of Plant Fragments	Use In Diet (Observed % Composition)	Availability (Expected % Composition)	Use 95% CI Lower Bound	Use 95% CI Upper Bound	Plants Utilized More (M) or Less Than Expected (L)
Little Bluestem	346	0.721	0.199	0.658	0.784	M
Oak	61	0.127	0.344	0.080	0.174	L
Plains Lovegrass	13	0.027	0.046	0.004	0.050	-
Texas Wintergrass	26	0.054	0.159	0.022	0.086	L
Threeawn	18	0.038	0.093	0.011	0.064	L
Other	16	0.033	0.159	0.008	0.059	L

Table 9. Summary of results from Manly's alpha selectivity index for the spring diet of sable antelope at Mason Mountain Wildlife Management Area. α scores > 0.16 indicate preference.

Species	Manly's Alpha	Plants Utilized More (M) or Less Than Expected (L)
Little Bluestem	0.655	M
Oak	0.067	L
Plains Lovegrass	0.106	L
Texas Wintergrass	0.062	L
Threeawn	0.073	L
Other	0.038	L

Table 10. Comparison of the observed percent composition of plants in the diet and expected percent composition of plants in the environment, for the annual diet of sable antelope at Mason Mountain Wildlife Management Area, 2013. Hypothesis of proportional use was rejected (χ_L^2 = 2129.16, df = 11, P-value < 0.001).

Species	# of Plant Fragments	Use In Diet (Observed % Composition)	Availability (Expected % Composition)	Use 95% CI Lower Bound	Use 95% CI Upper Bound	Plants Utilized More (M) or Less Than Expected (L)
Little Bluestem	999	0.515	0.159	0.480	0.550	M
Texas Wintergrass	385	0.198	0.078	0.170	0.226	M
Oak	219	0.113	0.285	0.091	0.135	L
Witchgrass	78	0.040	0.082	0.026	0.054	L
Sideoats Grama	62	0.032	0.088	0.020	0.044	L
Threeawn	47	0.024	0.127	0.013	0.035	L
Plains Lovegrass	39	0.020	0.064	0.010	0.030	L
Gum Bumelia	33	0.017	0.013	0.008	0.026	-
Switchgrass	16	0.008	0.001	0.002	0.015	M
Green Sprangletop	16	0.008	0.007	0.002	0.015	-
Whorled Dropseed	12	0.006	0.013	0.001	0.012	L
Other	34	0.018	0.079	0.008	0.027	L

Table 11. Summary of results from Manly's alpha selectivity index for the annual diet of sable antelope at Mason Mountain Wildlife Management Area. α scores > 0.083 indicate preference.

Species	Manly's Alpha	Plants Utilized More (M) or Less Than Expected (L)
Little Bluestem	0.202	M
Texas Wintergrass	0.158	M
Oak	0.025	L
Witchgrass	0.030	L
Sideoats Grama	0.022	L
Threeawn	0.012	L
Plains Lovegrass	0.019	L
Gum Bumelia	0.078	L
Switchgrass	0.342	M
Green Sprangletop	0.068	L
Whorled Dropseed	0.029	L
Other	0.014	L

DNA Analysis

Thirteen samples were successfully amplified and sent to the University of Texas at Austin's Genomic Sequencinging and Analysis Facility for illumina MiSeq sequencing. The 13 samples resulted in 24 unique plant sequences (Table 12). Three of the samples were from summer 2013 and resulted in 14 different plant sequences (Table 13). Only one fall sample was successfully amplified and sequenced and resulted in 11 different plant sequences (Table 14). Five winter samples were successfully amplified and sequenced. The winter samples yielded the most unique plant sequences with 21 detected (Table 15). Four spring samples were successfully amplified and sequenced resulting in 14 unique plant sequences (Table 16).

Four plants were detected in all of the 13 samples including little bluestem, oak , tall bush-clover (*Lespedeza stuevei*), and an unknown browse species. Other plants that were detected in each season but not in every sample included Texas wintergrass, switchgrass, unidentified mustard (*Arabidopsis* sp.), unidentified legume (Fabaceae sp.), gum rockrose (*Cistus ladanifer*), and Texas grama grass. Texas signalgrass (*Urochloa texana*) was detected in the summer, fall, and winter seasons. An unidentified *Euphorbia* sp. was detected in the fall and winter seasons. Poison ivy (*Toxicodendron radicans*) and an unidentified *Acacia* sp. were detected in the winter and spring seasons. Barnyard grass (*Echinochloa obtusiflora*) was detected in the summer and winter seasons. In the summer *Paspalum* sp.and an Asteraceae sp. where detected. Bur clover (*Medicago polymorpha*), white brush (*Aloysia gratissima*), an *Evovulus* sp., ashe juniper (*Juniperus ashei*), agarita (*Berberis* sp.), and rescuegrass (*Bromus catharticus*) were detected in the winter samples. Deer pea vetch (*Vicia ludoviciana*) was detected in 3 spring samples.

Table 12. Annual summary of plant species detected from DNA analysis. Table summarizes percent identity (percent of matching base pairs), number of sequences, number of samples the plant was detected, and whether or not the plant was detected in the microhistological analysis of the same samples.

Scientific Name	Common Name	ID %	# of Sequences	# of Samples	Detected in Microhis. Analysis
Quercus spp.	Oak spp.	100	1206426	13	Yes
Schizachyrium scoparium	Little bluestem	100	125451	13	Yes
Lespedeza stuevei	Tall bush-clover	99	150984	13	No
Elaeaocarpaceae	Unknown browse	93	433751	13	No
Arabidopsis sp.	Mustard sp.	100	118121	12	No
Cistus ladanifer	Gum rockrose	100	165797	11	No
Bouteloua rigidiseta	Texas grama	99	84842	11	No
Panicum virgatum	Switchgrass	99	45013	11	Yes
Fabaceae sp.	Legume sp.	96	59306	11	No
Stipa leucotricha	Texas wintergrass	100	251068	9	Yes
Echinochloa obtusiflora	Barnyard grass	100	5016	7	Yes
Acacia sp.	Thorn tree sp.	100	8354	6	No
Juniperus ashei	Ashe juniper	100	1741	5	No
Aloysia gratissima	White brush	100	1324	4	No
Urochloa texana	Texas signalgrass	99	2261	4	No
Vicia ludoviciana	Deer pea vetch	100	4072	3	No
Bromus catharticus	Rescuegrass	100	2182	3	No
Euphorbia sp.	Spurge sp.	98	975	3	No
Berberis sp.	Agarita	97	1096	3	No
Medicago polymorpha	Bur clover	100	1408	2	No
Toxicodendron radican	Poison ivy	100	942	2	No
Asteraceae sp.	Aster sp.	100	419	1	No
Evovulus sp.	Evovulus sp.	99	332	1	No
Paspalum sp.	Dallisgrass	99	233	1	No

Table 13. Summer 2013 summary of plant species detected from DNA analysis. Table summarizes percent identity (percent of matching base pairs), number of sequences, number of samples the plant was detected, and whether or not the plant was detected in the microhistological analysis of the same samples.

Scientific Name	Common Name	ID %	# of Sequences	# of Samples	Detected in Microhis Analysis
Quercus spp.	Oak spp.	100	226621	3	Yes
Cistus ladanifer	Gum rockrose	100	60569	3	No
Schizachyrium scoparium	Little bluestem	100	57333	3	Yes
Lespedeza stuevei	Tall bush-clover	100	56811	3	No
Arabidopsis sp.	Mustard	100	27970	3	No
Panicum virgatum	Switchgrass	99	29969	3	Yes
Bouteloua rigidiseta	Texas grama	99	3778	3	No
Fabaceae sp.	Legume sp.	96	45849	3	No
Elaeaocarpaceae	Unknown browse	93	34163	3	No
Echinochloa obtusiflora	Barnyard grass	100	1933	2	No
Stipa leucotricha	Texas wintergrass	100	1571	2	No
Asteraceae sp.	Aster sp.	100	419	1	No
Urochloa texana	Texas signalgrass	99	898	1	No
Paspalum sp.	Dallisgrass	99	233	1	No

Table 14. Fall 2013 summary of plant species detected from DNA analysis. Table summarizes percent identity (percent of matching base pairs), number of sequences, and whether or not the plant was detected in the microhistological analysis of the same samples. Only one sample was sequenced for fall.

Scientific Name	Common Name	ID %	# of Sequences	Detected in Microhistological Analysis
Quercus spp.	Oak spp.	100	204207	Yes
Schizachyrium scoparium	Little bluestem	100	14828	Yes
Arabidopsis sp.	Mustard sp.	100	9605	No
Stipa leucotricha	Texas wintergrass	100	1063	Yes
Cistus ladanifer	Gum rockrose	100	932	No
Lespedeza stuevei	Tall bush-clover	99	4346	No
Panicum virgatum	Switchgrass	99	376	No
Bouteloua rigidiseta	Texas grama	99	375	No
Euphorbia sp.	Spurge sp.	98	342	No
Fabaceae sp.	Legume sp.	96	1454	No
Elaeaocarpaceae	Unknown browse	93	943	No

Table 15. Winter 2013-2014 summary of plant species detected from DNA analysis. Table summarizes percent identity (percent of matching base pairs), number of sequences, number of samples the plant was detected, and whether or not the plant was detected in the microhistological analysis of the same samples.

Scientific Name	Common Name	ID %	# of Sequences	# of Samples	Detected in Microhis Analysis
Quercus spp.	Oak spp.	100	308767	5	Yes
Stipa leucotricha	Texas wintergrass	100	248045	5	Yes
Cistus ladanifer	Gum rockrose	100	97309	5	No
Arabidopsis sp.	Mustard sp.	100	67555	5	No
Schizachyrium scoparium	Little bluestem	100	20132	5	Yes
Lespedeza stuevei	Tall bush-clover	100	15909	5	No
Acacia sp.	Thorn tree	100	7262	5	No
Echinochloa obtusiflora	Barnyard grass	100	3083	5	Yes
Juniperus ashei	Ashe juniper	100	1741	5	No
Bouteloua rigidiseta	Texas grama	99	79964	5	No
Panicum virgatum	Switchgrass	99	13351	5	No
Elaeaocarpaceae	Unknown browse	93	11159	5	No
Aloysia gratissima	White brush	100	1324	4	No
Fabaceae sp.	Legume sp.	96	4471	4	No
Bromus catharticus	Rescuegrass	100	2182	3	No
Berberis sp.	Agarita	97	1096	3	No
Medicago polymorpha	Bur clover	100	1408	2	No
Euphorbia sp.	Spurge sp.	100	633	2	No
Urochloa texana	Texas signalgrass	99	957	2	No
Toxicodendron radican	Poison ivy	100	754	1	No
Evovulus sp.	Evovulus sp.	99	332	1	No

Table 16. Spring 2014 summary of plant species detected from DNA analysis. Table summarizes percent identity (percent of matching base pairs), number of sequences, number of samples the plant was detected, and whether or not the plant was detected in the microhistological analysis of the same samples.

Scientific Name	Common Name	ID %	# of Sequences	# of Samples	Detected in Microhisto. Analysis
Quercus spp.	Oak spp.	100	466831	4	Yes
Lespedeza stuevei	Tall bush-clover	100	73918	4	No
Schizachyrium scoparium	Little bluestem	100	33158	4	Yes
Elaeaocarpaceae	Unknown browse	93	387486	4	No
Arabidopsis sp.	Mustard sp.	100	12991	3	No
Vicia ludoviciana	Deer pea vetch	100	4072	3	No
Fabaceae sp.	Legume sp.	96	7532	3	No
Cistus ladanifer	Gum rockrose	100	6987	2	No
Panicum virgatum	Switchgrass	99	1317	2	Yes
Bouteloua rigidiseta	Texas grama	99	725	2	No
Acacia sp.	Thorn tree sp.	100	1092	1	No
Stipa leucotricha	Texas wintergrass	100	389	1	Yes
Toxicodendron radican	Poison ivy	100	188	1	No
Urochloa texana	Texas signalgrass	99	406	1	No

4. DISCUSSION

Microhistological Analysis and Management Implications

At Mason Mountain Wildlife Management Area, sable antelope are primarily grazers that minimally utilize browse (Table 10). The use of browse and forbs may have been influenced by the seasonal availability of grass and forb species as dictated by local weather variation (UNL 2013). During summer 2013 Mason county experienced a record drought with an average of 5 cm of rainfall over the months of June, July, and August (NOAA 2010). During this time sable antelope were observed browsing when there was little green grass and forb forage available. This was also observed in the microhistological analysis with the detection of gum bumelia and oak spp. in the diet (Table 2). This is consistent with observations made by previous studies of sable antelope in their native range. Wilson and Hirst (1977) stated that sable antelope rarly forage on browse and on one reserve there was zero evidence of browse being consumed by sable antelope.

Winter diet was also likely affected by local weather. There was again little green forage in the Spring and Middle pastures. Most of the green forage was located on the southern part of the property. Several grazing exotics that previously occupied the South Voca, Spring, and Middle pastures, migrated to the southern part of the property to forage. Fecal collection was complicated due to the extreme cold weather that swept across the country January 5-8, 2014. As soon as sable left an area, the fecal material was frozen hard which made it difficult to identify fresh fecal samples from older fecal deposits. This issue was exacerbated by the increased herd size and the smaller area in which they foraged, creating numerous fecal deposits in the foraging areas. The herd was also highly aggressive during this time due to rivalry with other ungulates for forage, and

several young sable present in the herd, which further complicated the fecal collection. Most of the samples used for the winter analysis were collected during the first weekend of February when the samples could be confirmed as fresh.

This is the first study to investigate the diet of sable antelope in the United States. In their native range sable antelope have shown preference for grass species such as tanglehead (*Heteropogon contortus*), guinea grass (*Megathyrsus maximus*), red grass (*Themeda triandra*), and spiked crinkleawn (*Trachypogon spicatus*) (Owen-Smith et al. 2013; Wilson and Hirst 1977). While all of these grass species can be found in Texas, none of them were detected during vegetation surveys at Mason Mountain WMA. Tanglehead and spiked crinkleawn are considered native to parts of Texas. Guinea grass and red grass are considered introduced (USDA 2015). Little bluestem, tanglehead, red grass, and spiked crinkleawn all belong to the plant tribe Andropogoneae or the sorghum tribe (Skendzic et al. 2007). Switchgrass and guinea grass are similar in that they are both considered panicum grasses belonging to the tribe Paniceae and subtribe Setariinae (Gómez-Martínez and Culham 2000). The similarities between the grasses consumed in their native range and here in Texas supports Wilson and Hirst's conclusion that plant selection by sable antelope is an instinctive process, and probably modified by smell and texture.

Microhistological analysis indicates that the majority of the sable antelope diet is comprised of few grass species. This is consistent with findings from their native range by Wilson and Hurst (1977) where they found that over 75% of the sable antelope diet was comprised of two to four grass species, depending on the reserve. At Mason Mountain WMA, in the fall and spring, 72% of the diet was composed of 1 grass species,

little bluestem (Figure 3 and Figure 5). In the winter, 74% of the diet was 1 species, Texas wintergrass (Figure 4). During the summer 70% of the diet was composed of only 2 grass species. Annually, 82% of the sable antelope diet was composed of 3 grass species (Figure 6). Wilson and Hurst attribute the differences in number of species to palatability of available grasses and management practices such as prescribed burns.

During summer, fall, and spring seasons sable antelope primarily foraged on little bluestem. This was noted in field observations and verified using microhistological analysis. While little bluestem was an abundant grass with a high level of availability at Mason Mountain WMA, statistical analysis still confirmed selectivity. Little bluestem is a warm season perennial and did not provide palatable forage in the winter. Sable antelope then switched to Texas wintergrass. Texas wintergrass was abundant in the southern part of the property during the fall, winter, and spring seasons. Sable antelope were only observed selectively foraging on Texas wintergrass during the winter when little bluestem was unavailable. Texas wintergrass is a cool season perennialwith a higher nutrient content and palatability than other available forages during the winter (Mousel et al. 2006).

Sable antelope were observed foraging on little bluestem, switchgrass, gum bumelia, and blackjack oak (*Quercus marilandica*) during the summer months. These observations were verified in the microhistological (Table 2) and DNA analyses (Table 13) with the exception of gum bumelia being absent in the DNA analysis. During the fall, sable antelope were observed foraging mostly on little bluestem with an occasional bite of blackjack oak. This is consistent with the findings from the microhistological analysis with the majority of the diet consisting of little bluestem and the detection of oak

(Table 4). DNA analysis detected both species, but my field observations of oak use by sable were inconsistent with the high level of oak DNA sequences (Table 14) produced by the analysis. During the winter, sable antelope were only observed foraging on Texas wintergrass. This observation was verified by microhistological (Table 6) and DNA analysis (Table 15) with the detection of Texas wintergrass. Sable antelope were observed foraging on little bluestem, switchgrass, and blackjack oak during the spring season and this was also verified by microhistological (Table 8) and DNA analysis (Table 16). All plants detected by field observations were verfied by presence in the microhistological analysis. Most field observations, with the exception of gum bumelia, were verified to be present in the diet by DNA analysis.

Mason Mountain WMA has eight species of ungulates currently occupying the property. Sable antelope share pastures with approximately 20-30 waterbuck, 40-50 gemsbok, 2 female greater kudu, 3 Thompson's gazelles, axis deer, and white-tailed deer. Of these ungulates waterbuck (Kassa et al. 2008), gemsbok (Winters 2002), and Thompson's gazelles (Cerling et al. 2003) are known grazers. While a dietary study for waterbuck in Texas is lacking, Wilson and Hirst (1977) investigated competition between waterbuck and sable antelope. They found similarities between the rumen contents of waterbuck and sable antelope that were found foraging in the same area. However, waterbuck found in their typical riparian habitat did not show diet similarities with sable antelope. Waterbuck usually forage in more riparian and densly covered habitats which differs from sable antelope who prefer savannah woodlands on less fertile soils (Owen-Smith et al. 2013). At Mason Mountain WMA waterbuck were often found close to major water resources and areas where sable antelope rarely visited. If the riparian habitats were

not available to the waterbuck, competition between waterbuck and sable antelope could exist. While there is little dietary information available for waterbuck, it is important for landowners to consider available habitat types and stocking rates when stocking these species together.

Another common exotic species that has been stocked with sable antelope is gemsbok. Gemsbok are large grazers and are abundant on Mason Mountain WMA. One confrontation was observed during the summer of 2013 in which 3 gemsbok and 8 sable antelope were foraging in the same area. The male sable displayed aggression towards the gemsbok who eventually left the area. Annually, gemsbok diet primarily consisted of little bluestem, plains lovegrass, and sideoats grama (Winters 2002). They also foraged on Texas wintergrass during the winter season. Considering the overlap between the diet compositions of the two species, stocking rates should be considered to prevent over grazing and competition cannot be ruled out.

Although scimitar-horned oryx was not stocked with sable antelope at Mason Mountain WMA competition should be considered. At Mason Mountain WMA, scimitar-horned oryx are restricted to the turkey pasture which is part of the Edward's plateau ecological region. Scimitar-horned oryx are primarily grazers that selectively forage on Sporobolus spp. and Eragrostis spp (Robinson 2008). The scimitar-horned oryx were also observed foraging on little bluestem which made up approximately 18% of their diet at Mason Mountain WMA from June 2006- April 2007 (Robinson 2008). Texas wintergrass made up approximately 14% of the scimitar-horned oryx diet during that time (Robinson 2008). Seperation of the species and stocking rates are important factors when stocking both species.

While sable antelope might not compete with native browsers such as white-tailed deer, careful consideration should be made when stocking sable antelope with cattle, gemsbok, waterbuck, scimitar-horned oryx, and other grazers. Through public hunting programs Mason Mountain WMA has dramatically reduced the number of exotics on the property since acquisition. They continue to maintain appropriate stocking rates through the public hunting programs which has dramatically improved the condition of the property since it was acquired in 1997. This property continues to provide excellent opportunities to study the effects of African ungulates on local habitat, and interactions between exotic and native wildlife.

Future studies are need to assess competion between sable antelope and other ungulates and Mason Mountain WMA. Further studies are also needed to determine dietary differences between sex and age classes of sable antelope. Future food habit studies on sable antelope could also correct for differential digestion and compare rumen and fecal contents. Seasonal diet studies of sable antelope are also needed throughout the different ecological regions of Texas and United States.

DNA Analysis and Comparison to Microhistological Analysis

DNA analysis is an emerging technique for determining the food habits of large herbivores. With little published literature on the technique, I modified the technique for my research goals. Current published literature targeted the P6 loop region of the trnL intron (Taberlet et al. 2007; Baamrane et al. 2012; Murphree 2012; Soininen et al. 2009).However, I targeted a larger region of the trnL intron using the c and h primers to obtain sequenced between 270 – 300 bp. Taberlet et al. (2007) recommends using the trnL (UAA) p6 loop region for determining the diet of herbivores due to the degraded

nature of plant DNA in stomach and fecal matter. The larger region was used to obtain more precise species identification; however, this larger region could have contributed to the low concentration of PCR product obtained during amplification.

The microhistological analysis determined that sable antelope selectively foraged on little bluestem, Texas wintergrass, and switchgrass. While these plant species were also detected in the DNA analysis, the resulting DNA sequences cannot quantify the percent composition in the diet because of the process of DNA amplification and DNA sequencing. The results obtained from the DNA analysis are purely qualitative and thus selectivity cannot be determined by this method alone. This highlights a major drawback of using DNA analysis for dietary studies.

For the purpose of determining what plants are present or absent in the diet, DNA analysis can be a useful method. The DNA analysis using the trnL approach produced more identified plants than the traditional microhistological analysis. This is consistent with the findings from Soininen et al. (2009) who found DNA analysis to give a taxonomically more detailed picture of the diet than microhistological analysis. Murphee (2012), however, found a higher mean species composition was reported with microhistological analysis (79%) as
compared to DNA barcoding (50%). To compare the results of plants present in the diet between the two methods, only the microhistological results from the same samples used in the DNA analysis can be considered. Of the 24 plants detected using DNA analysis, 5 were also detected in the microhistological analysis of the same 13 samples (Table 12). These plants included little bluestem, Texas wintergrass, oak spp., switchgrass, and barnyard grass (Table 12). The microhistological analysis included an unidentified grass

and an unidentified forb species. The microhistological analysis detected 4 plant species that were not detected by DNA analysis; gum bumelia (*Sideroxylon lanuginosum*), plains lovegrass (*Eragrostis intermedia*), hairy grama (*Bouteloua hirsuta*), and sideoats grama (*Bouteloua curtipendula*). While this difference could be attributed to human error during the microhistological analysis, Texas grama (*Bouteloua rigidiseta*) was detected in the DNA analysis (Table 12) and is closely related to hairy grama and sideoats grama. Murphee (2012) investigated the differences between DNA and microhistological analysis on fecal material through a controlled feeding study. Pygmy goats were fed a controlled diet of 5 grass species, 5 shrub species, and 6 forb species. Murphee (2012) found that DNA analysis did not enhance the ability to detect plant species in herbivore diets with microhistological analysis having an average of 89% correct detection in control diets and DNA barcoding estimated 50% correct detection of species.

The number of DNA sequences detected per species was not consistent with the dietary percent composition produced by microhistological analysis nor with detailed field notes taken during the course of the study. The plant with the highest number of matching DNA sequences (1,206,426) was oak (Table 12) rather than grasses as indicated by both microhistological analysis and field observations. The differential digestion of chloroplast in a grazer such as sable antelope should be considered. Tannins are present in most forb, shrub and woody species but generally absent from grass species (Haslam 1979). Tannins have shown to be digestibility reducers (Mole and Waterman 1987) depending on characteristics of both the tannin and consumer (Hagerman et al. 1992). McArthur and Sanson (1993) demonstrated how the presence of tannins in browse and shrub species affects the digestibility of nutrients in both browsers and grazers. They

attributed the low digestibility of browse nutrients in grazing herbivores to a lack of physiological adaptations by grazers. Tannins present in oak species might affect the digestibility of oak chloroplast and result in more oak chloroplast present in the fecal matter which would result in more oak DNA being extracted. Since the trnL (UAA) intron is a chloroplast gene, our analysis may have detected oak and forb species in greater amounts than actually consumed by sable antelope.

Difficulties encountered when using DNA analysis were lack of successful amplification, variation in concentrations between samples, and obtaining high enough concentrations (500ng/µl) for illumina sequencing. The master mix used in other studies was unsuccessful when I attempted, so I altered it to obtain PCR product using the master mix described in this study. I also found that using the stock extracted DNA yielded higher PCR product concentrations than using a water dilution mix. After using a bioanalyzer to determine concentrations, I found that concentrations varied greatly between samples. Some samples required amplification multiple times in order to obtain appropriate concentrations. I began with 24 samples (6 per season) and only sequenced 13. One sample per season was thrown out due to a lack of PCR product. This issue may be attributed to the degraded nature of the DNA. An additional 7 samples were not sent for sequencing due to a human error made during PCR cleanup that resulted in low concentrations.

In all, both the process of DNA analysis and microhistological analysis are tedious and very time consuming, requiring several hours in the lab each day to improve the technique. For both methods, reference material must be obtained and organized. Both methods depend on quality reference material for correct identification of plant

material. The DNA analysis resulted in 2 ambiguous plant identifications. One plant sequence that was found in every sample only exhibited a 93% match to a plant species found in the family Elaeaocarpaceae (Table 12). Another plant sequence that exhibited ambiguity was a Fabaceae sp. that had a 96% match. These limitations demonstrate the need for more quality reference material for the trnL region.

One major difference that should be considered when choosing the technique to be used is costs. There were very little associated costs using the microhistological technique. The cost for slides and mounting solutions was minimal in comparison with cost of the DNA analysis. Expenses for the DNA analysis included Extraction kits, PCR clean-up kits, next-generation primers, barcoded primers, PCR reagents, bioanalyzer usage, and Illumina sequencing. The Illimuna sequencing was by far the most expensive aspect of the DNA analysis. Twenty-four samples can be analyzed for approximately $1,800. This does not include the bioinformatics or analysis of resulting sequences. Due to the purely qualitative data obtained and the high costs associated with using DNA analysis for determining diets, I would suggest the traditional microhistological analysis. At its current stage of development, DNA analysis should be considered an incomplete method for investigating food habits.

The methods outlined in this study can be used and altered for future food habit studies. Further studies are needed to investigate the use of DNA analysis on fecal material for determining the food habits of large herbivores. Most studies concentrated on the P6 loop region of the trnL intron and future studies should investigate the use of other regions. The taxonomic library that was created for this study can be used for other food habit studies wishing to use DNA analysis on rumen or fecal samples in Texas. This

study has contributed not only in helping ecologist understand the seasonal food habits of sable antelope but also in understanding the feasibilty of DNA analysis for dietary studies.

APPENDIX SECTION

Appendix 1. Line intercept data for woody plants during spring 2001 at Mason Mountain Wildlife Management Area. (Percent cover = length of species/total length of intercept lines (1600 m) * 100).

Scientific Name	Common Name	Total Length (m)	Percent Cover
Quercus stellata	Post Oak	239.09	14.94
Opuntia engelmannii	Prickly Pear	224.818	14.05
Quercus fusiformis	Live Oak	132.78	8.30
Prosopis glandulosa	Mesquite	84.8	5.30
Quercus marilandica	Blackjack Oak	36.93	2.31
Acacia sp.	Mimosa sp.	30.26	1.89
Aloysia gratissima	White Brush	13.27	0.83
Diospyros texana	Persimmon	13.05	0.82
Opuntia leptocaulis	Pencil Cactus	10.02	0.63
Mahonia trifoliolata	Agarita	9.29	0.58
Yucca constricta	Buckley's Yucca	8.47	0.53
Celtis laevigata	Sugarberry Elm	7.03	0.44
Smilax bona-nox	Greenbriar	6.49	0.41
Ulmus americana	American Elm	4.42	0.28
Rhus lanceolata	Flameleaf Sumac	2.91	0.18
Forestiera pubescens	Elbowbush	2.44	0.15
Sideroxylon lanuginosum	Gum Bumelia	2.42	0.15
Sapindus saponaria	Soapberry	1.8	0.11
Celtis reticulata	Netleaf Hackberry	1.3	0.08
Zanthoxylum sp.	Prickly Ash	0.82	0.05
Parthenocissus quinquefolia	Virginia Creeper	0.77	0.05
Rhus trilobata	Skunkbush Sumac	0.61	0.04
Toxicodendron radicans	Poison Ivy	0.07	0.00

Appendix 2. Daubenmire percent coverages of herbaceous plants during summer 2013 at Mason Mountain Wildlife Management Area.

Scientific Name	Common Name	Sum of Midpoints	Percent Cover
Litter	Litter	8065.5	45.83
Bare ground	Bare ground	3205	18.21
Rock	Rock	1195.5	6.79
Aristida spp.	Threeawn	749.5	4.26
Schizachyrium scoparium	Little bluestem	547	3.11
Lechea san-sabeana	San-saba pinweed	381.5	2.17
Sida sp.	Sida sp.	360.5	2.05
Bouteloua curtipendula	Sideoats grama	318	1.81
Eragrostis spp.	lovegrass	270.5	1.54
Digitaria cognata	Fall witchgrass	208.5	1.18
Fabaceae sp.	Unknown legume	167.5	0.95
Poaceae sp.	Unknown grass	151	0.86
Tragia ramosa	Branched noseburn	140	0.80
Paspalum spp.	Paspalum spp.	138.5	0.79
Seteria spp.	Bristle grass spp.	127.5	0.72
Bothriochloa ischaemum	KR bluestem	114.5	0.65
Evolvulus sericeus	White evovulus	107.5	0.61
Spikemoss	Spikemoss	98	0.56
Ambrosia sp.	Western ragweed	85	0.48
Sedge	Sedge	76.5	0.43
Bouteloua hirsuta	Hairy grama	67	0.38
Croton sp.	Croton sp.	66.5	0.38
Froelichia gracilis	Snake cotton	61.5	0.35
Phyllanthus sp.	Knotweed leaf flower	56	0.32
Wedelia texana	Orange zexmenia	51.5	0.29
Digitaria patens	Texas cottontop	46.5	0.26
Dichanthelium acuminatum	Lindheimer rosette grass	38.5	0.22
Liatris mucronata	Gay-feather	38	0.22
Dichanthelium oligosanthes	Scribner's dicanthelium	36	0.20
Convolvulus equitans	Texas bindweed	31	0.18
Digitaria californica	Arizona cottontop	31	0.18
Cynanchum barbigerum	Cynanchum	31	0.18
Sporobolus sp.	Dropseed sp.	31	0.18
Hedeoma sp.	Mock pennyroyal	20.5	0.12
Panicum sp.	Panicum sp.	20.5	0.12
Talinum parviflorum	Talinum	20.5	0.12
Unknown forb 2	Unknown forb 2	20.5	0.12
Physalis sp.	Groundcherry	18	0.10
Panicum hallii	Hall's panicgrass	18	0.10

Solanum elaeagnifolium	Silverleaf nightshade	18	0.10
Rhynchosia sp.	Snout bean	18	0.10
Chloris cucullata	Hooded windmill	18	0.10
Bothriochloa saccharoides	Silver bluestem	15.5	0.09
Hilaria belangeri	Curly mesquite	15.5	0.09
Phyla incisa	Frog-fruit	15.5	0.09
Lythrum salicaria	Purple loosestrife	15.5	0.09
Salvia leucantha	Mexican sage	15.5	0.09
Euphorbia sp.	Unknown spurge	15.5	0.09
Solanum dimidiatum	Western horsenettle	15.5	0.09
Ratibida columnifera	Mexican hat	12.5	0.07
Unknown forb	Unknown forb	12.5	0.07
Eriogonum sp.	Tall buckwheat	7.5	0.04
Oxalis sp.	Yellow wood sorrel	7.5	0.04
Tradescantia sp.	Spiderwort	5	0.03
Callirhoe involucrata	Winecup	5	0.03

Appendix 3. Daubenmire percent coverages of herbaceous plants during fall 2013 at Mason Mountain Wildlife Management Area.

Scientific Name	Common Name	Sum of Midpoints	Percent Cover
Litter	Litter	5063	28.77
Bareground	Bareground	2892.5	16.43
Aristida spp.	Threeawn	1071.5	6.09
Sida sp.	Sida sp.	900.5	5.12
Digitaria cognata	Fall witchgrass	879	4.99
Schizachyrium scoparium	Little Bluestem	862	4.90
Rock	Rock	845	4.80
Bouteloua curtipendula	Sideoats grama	803	4.56
Eragrostis spp.	Lovegrass	683	3.88
Lechea san-sabeana	San-saba pinweed	545.5	3.10
Paspalum spp.	Paspalum spp.	431.5	2.45
Unknown forb 2	Unknown forb 2	380	2.16
Stipa leucotricha	Texas wintergrass	378.5	2.15
Bouteloua hirsuta	Hairy grama	375	2.13
Froelichia gracilis	Snake cotton	331	1.88
Tragia ramosa	Branched noseburn	309.5	1.76
Sedge	Sedge	251	1.43
Phyllanthus sp.	Knotweed leaf flower	244.5	1.39
Evolvulus sericeus	White evovulus	233.5	1.33
Digitaria patens	Texas cottontop	230.5	1.31
Bothriochloa ischaemum	KR Bluestem	223.5	1.27
Fabaceae sp.	Unknown legume	182	1.03
Croton sp.	Croton sp.	178	1.01
Digitaria californica	Arizona cottontop	170	0.97
Solanum elaeagnifolium	Silverleaf nightshade	163.5	0.93
Seteria spp.	Bristle grass spp.	132	0.75
Ambrosia sp.	Western ragweed	130.5	0.74
Wedelia texana	Orange zexmenia	116.5	0.66
Bothriochloa saccharoides	Silver bluestem	105	0.60
Convolvulus equitans	Texas bindweed	96.5	0.55
Callirhoe involucrata	Winecup	90	0.51
Solanum dimidiatum	Western horsenettle	89.5	0.51
Leptochloa dubia	Green sprangletop	84.5	0.48
Commelina erecta	Whitemouth dayflower	77.5	0.44
Sporobolus sp.	Dropseed sp.	69	0.39
Dichanthelium acuminatum	Lindheimer rosette grass	53.5	0.30
Verbena halei	Texas vervain	49	0.28
Rhynchosia sp.	Snout bean	46.5	0.26
Dichanthelium oligosanthes	Scribner's dicanthelium	46	0.26
Salvia leucantha	Mexican sage	40	0.23
Chloris sp.	Windmill grass sp.	36	0.20
Spikemoss	Spikemoss	33.5	0.19
Unknown grass	Unknown grass	33.5	0.19
Talinum auranticum	Flame-flower	31	0.18
Hedeoma sp.	Mock pennyroyal	31	0.18
Dichondra recurvata	Ponyfoot	31	0.18

Physalis sp.	Groundcherry	23	0.13
Unknown forb	Unknown forb	20.5	0.12
Oxalis sp.	Yellow wood sorrel	20.5	0.12
Phyla incisa	Frog-fruit	15.5	0.09
Muhlenbergia sp.	Muhly grass	15.5	0.09
Stillingia sylvatica	Queen's delight	15.5	0.09
Eriogonum sp.	Tall buckwheat	15.5	0.09
Paronychia virginica	Whitlow wort	15.5	0.09
Verbena canescens	Gray vervain	7.5	0.04
Euphorbia sp.	Unknown spurge	5	0.03

Appendix 4. Daubenmire percent coverages of herbaceous plants during winter 2013 at Mason Mountain Wildlife Management Area.

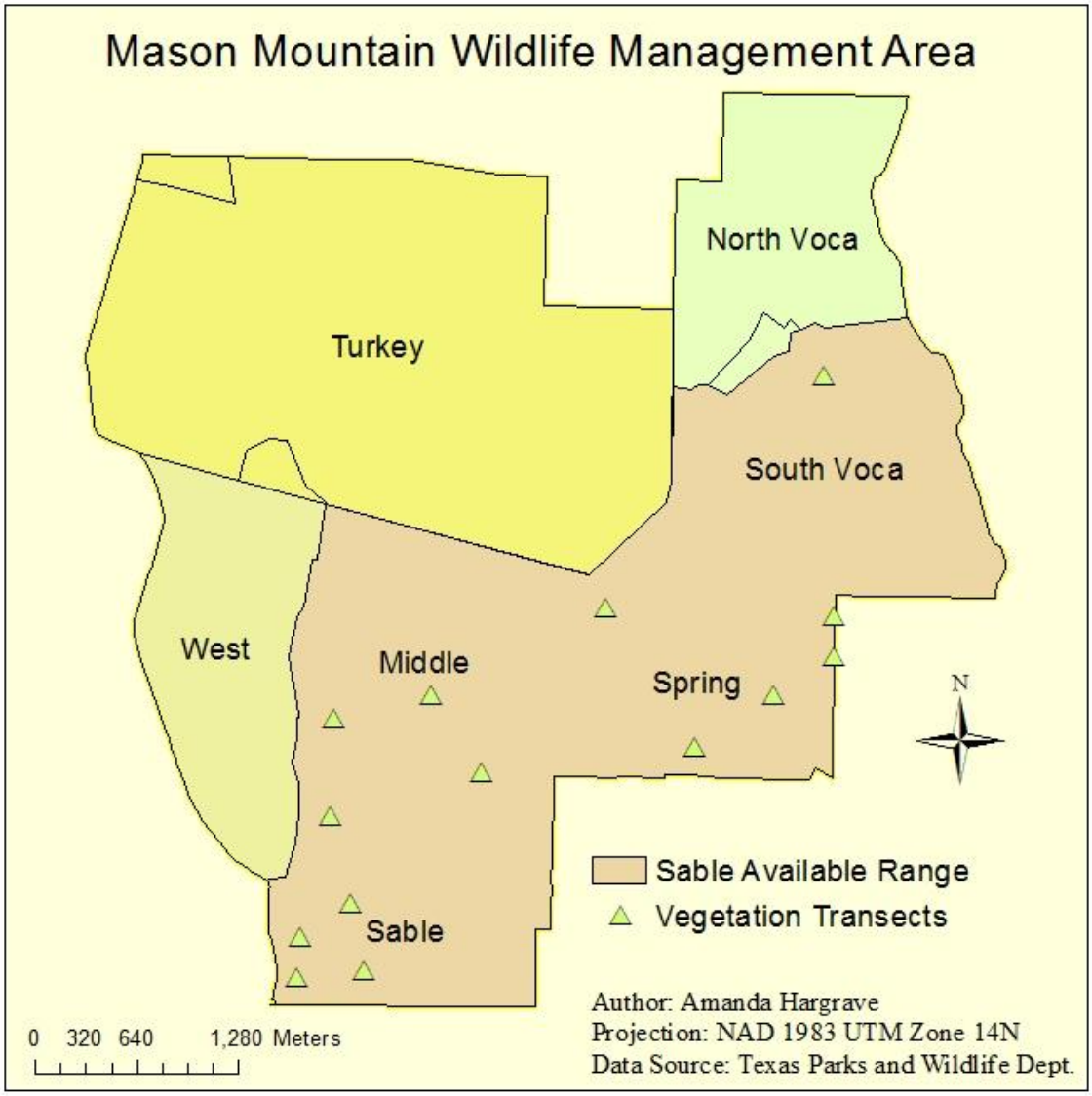
Scientific Name	Common Name	Sum of Midpoints	Percent Cover
Litter	Litter	7481.5	42.51
Bare ground	Bare ground	2343.5	13.32
Rock	Rock	873.5	4.96
Lechea san-sabeana	San-saba pinweed	838.5	4.76
Stipa leucotricha	Texas wintergrass	713.5	4.05
Unknown forb	Unknown forb	463.5	2.63
Schizachyrium scoparium	Little bluestem	225	1.28
Spikemoss	Spikemoss	218.5	1.24
Eragrostis spp.	lovegrass	208.5	1.18
Sida sp.	Sida sp.	201.5	1.14
Lupinus texensis	Bluebonnet	191.5	1.09
Callirhoe involucrata	Winecup	178	1.01
Tragia ramosa	Branched noseburn	160	0.91
Bothriochloa saccharoides	Silver bluestem	159.5	0.91
Aristida spp.	Threeawn	145	0.82
Achillea millefolium	Yarrow	132	0.75
Evolvulus sericeus	White evovulus	129	0.73
Oxalis sp.	Yellow wood sorrel	115.5	0.66
Dichondra recurvata	Ponyfoot	111	0.63
Bouteloua curtipendula	Sideoats grama	93	0.53
Paspalum spp.	Paspalum spp.	87	0.49
Physalis sp.	Groundcherry	51.5	0.29
*Phyllanthus*sp.	Knotweed leaf flower	51.5	0.29
Dichanthelium oligosanthes	Scribner's dicanthelium	49	0.28
Unknown grass	Unknown grass	49	0.28
Ambrosia sp.	Western ragweed	40.5	0.23
Panicum sp.	Panicum sp.	38	0.22
Verbena halei	Texas vervain	18	0.10
Solanum dimidiatum	Western horsenettle	18	0.10
Digitaria cognata	Fall witchgrass	18	0.10
Talinum auranticum	Flame-flower	15.5	0.09
Verbena canescens	Gray vervain	15.5	0.09
Hedeoma sp.	Mock pennyroyal	15.5	0.09
Muhlenbergia sp.	Muhly grass	15.5	0.09
Solanum elaeagnifolium	Silverleaf nightshade	15.5	0.09
Croton sp.	Croton sp.	2.5	0.01
Geranium sp.	Geranium sp.	2.5	0.01

Appendix 5. Daubenmire percent coverages of herbaceous plants during spring 2013 at Mason Mountain Wildlife Management Area.

Scientific Name	Common Name	Sum of Midpoints	Percent Cover
Litter	Litter	5911	33.59
Bare ground	Bare ground	3261	18.53
Stipa leucotricha	Texas wintergrass	767	4.36
Lechea san-sabeana	San-Saba pinweed	739	4.20
Schizachyrium scoparium	Little bluestem	702.5	3.99
Rock	Rock	649.5	3.69
Coreopsis sp.	Tickseed sp.	473.5	2.69
Tragia ramosa	Branched noseburn	420.5	2.39
Lesquerella argyraea	Silver bladderpod	410.5	2.33
Evolvulus sericeus	White evolvulus	385.5	2.19
Aristida spp.	Threeawn	343	1.95
Ratibida columnifera	Mexican hat	298.5	1.70
Sida sp.	Sida sp.	292.5	1.66
Lupinus texensis	Bluebonnet	267	1.52
Plantago spp.	Plantain spp.	263	1.49
Astragalus sp.	Vetch sp.	260.5	1.48
Gaillardia pulchella	Indian blanket	188	1.07
Digitaria cognata	Fall witchgrass	186	1.06
Ambrosia sp.	Western ragweed	185.5	1.05
Hedeoma sp.	Mock pennyroyal	175.5	1.00
Lepidium virginicum	Virginia pepperweed	165.5	0.94
Callirhoe involucrata	Winecup	160	0.91
Achillea millefolium	Yarrow	131	0.74
Lespedeza stuevei	Tall bush-clover	118	0.67
Fabaceae sp.	Unknown legume	115.5	0.66
Bouteloua hirsuta	Hairy grama	111	0.63
Oxalis sp.	Yellow wood sorrel	111	0.63
Eragrostis spp.	Lovegrass spp.	108.5	0.62
Senecio ampullaceus	Yellow Texas groundsel	95.5	0.54
Bouteloua curtipendula	Sideoats grama	93	0.53
*Phyllanthus*sp.	Knotweed leaf flower	90	0.51
Paspalum spp.	Paspalum spp.	82.5	0.47
Dichondra recurvata	Ponyfoot	77.5	0.44
Seteria spp.	Bristle grass spp.	71.5	0.41
Aster sp.	Yellow aster	67	0.38
Unknown forb	Unknown forb	64.5	0.37
Gaura sp.	Beeblossom	62	0.35
Spikemoss	Spikemoss	62	0.35
Apiaceae sp.	Parsley sp.	54	0.31

Hymenoxys scaposa	Bitterweed	49	0.28
Sedge	Sedge	46.5	0.26
Dichanthelium oligosanthes	Scribner's dicanthelium	46.5	0.26
Rhynchosia sp.	Snout bean	46.5	0.26
Dichanthelium acuminatum	Lindheimer rosette grass	36	0.20
Erodium texanum	Texas stork's bill	36	0.20
Unknown forb 3	Unknown forb 3	36	0.20
Wedelia texana	Orange zexmenia	33.5	0.19
Unknown forb 2	Unknown forb 2	33.5	0.19
Elymus virginicus	Virginia wild rye	33.5	0.19
Convolvulus equitans	Texas bindweed	31	0.18
Allium sp.	Wild onion	31	0.18
Oenothera sp.	Primrose	31	0.18
Solanum elaeagnifolium	Silverleaf nightshade	31	0.18
Froelichia gracilis	Snake cotton	31	0.18
Physalis sp.	Groundcherry	20.5	0.12
Bromus catharticus	Rescuegrass	18	0.10
Verbena halei	Texas vervain	18	0.10
Croton sp.	Croton sp.	15.5	0.09
Engelmannia peristenia	Engelmann's daisy	15.5	0.09
Phyla incisa	Frog-fruit	15.5	0.09
Geranium sp.	Geranium sp.	15.5	0.09
Argemone sp.	Prickly poppy	15.5	0.09
Stillingia sylvatica	Queen's delight	15.5	0.09
Lygodesmia texana	Texas skeleton plant	15.5	0.09
Tradescantia sp.	Spiderwort	15.5	0.09
Sporobolus sp.	Dropseed sp.	15.5	0.09
Talinum parviflorum	Talinum	15.5	0.09
Krameria lanceolata	Trailing ratany	15.5	0.09
Malvaviscus arboreus	Turk's cap	15.5	0.09
Tridens albescens	White tridens	15.5	0.09

Appendix 6. A map illustrating the location points of the 16 vegetation transects.



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