Title of Project:

Using Innovative Techniques for Assessing Herbivore Animal Diet

Name of Principal Investigator:

Jeff Cole

Timeframe covered by the report:

This report covers the timeframe from September 1st, 2014, when this project was approved for funding through June 30th, 2016, which marked the expiration of the agreement after all goals and objectives were completed.

Grant/award identifying number:

69-3A75-14-241

Date of submission:

September 10th, 2016

Deliverables identified on the grant agreement:

This project makes use of an innovative conservation technology and promotes the transfer of knowledge regarding data and procedures generated from the new technology. The following products are generated from the objectives of this project.

1.) Makes use of an innovative conservation technology by conducting next-generation sequencing on mule deer fecal matter collected from the Navajo Reservation.
    a. Provide baseline data regarding mule deer population diet for the NNDFW.
    b. Present a possibly more efficient, cost-effective, accurate method for assessing wildlife diet.

2.) Compare innovative conservation technology with proven historical technology by conducting microhistological diet analyses, and then comparing microhistological results to those from next-generation sequencing.
    a. Compare results from the new and innovative conservation technology to an older, proven conservation technology through the presence and absence of plant species found in each technique.
    b. Compare direct time, cost, and accuracy of results between the two techniques during the duration of the study. These comparisons will help determine the level of efficiency of next-generation sequencing over a proven diet assessment technology.

3.) Transfer baseline data and knowledge of technology use and its effectiveness to a Native American tribal program.
    a. Provide baseline data to aid Navajo Nation Department of Fish and Wildlife (NNDFW) in its management of mule deer on the Navajo Reservation.
    b. Provide knowledge of the use, benefits, and effectiveness of next-generation sequencing to the NNDFW for future use in their overall management practices.
    c. Present and demonstrate the tractability of next-generation sequencing to other Navajo tribal programs, and possibly non-tribal programs.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Summary of Activities:

We completed several **Natural Resources Conservation Service (NRCS) designated priorities** in regards to some of the overarching goals of the Conservation Innovation Grant (CIG) itself in that we successfully used a new diet assessment technology to study herbivorous diet of a wildlife species in collaboration with a Native tribe in the Navajo. We also made this technology available to the tribe to use for future studies examining the diet of both wild and non-wild herbivorous species, since this technology may have potential use for investigating the diet of livestock. Additionally, we demonstrated the efficacy and effectiveness of this new technology by comparing it with an long-standing diet assessment technology.

Our **goals** for this grant project were to: 1.) apply an innovative conservation tool in next-generation sequencing to assess the diet of an herbivorous species in mule deer, 2.) compare diet results identified through next-generation sequencing with that of a historical and long-standing herbivorous diet assessment method in microhistology, and 3.) collaborate with a Native American tribal entity in the Navajo Nation Department of Fish and Wildlife (NNDFW) by providing baseline biological data on mule deer as well as transfer knowledge of the innovative diet assessment technology, of which could be used in future herbivorous diet research.

Our **objectives** were to: 1.) make use of next-generation sequencing by using it on mule deer fecal samples collected from the Navajo Nation, 2.) compare results generated through next-generation sequencing to that of microhistology via the number of unique plant taxa (diet richness) and level of taxonomic resolution (e.g. species, genus, family) identified through each diet assessment method, as well as the monetary costs and time required to generate results from each method, and 3.) provide baseline mule deer diet data to the NNDFW, as well as demonstrate the effectiveness of next-generation sequencing for assessing herbivore diet to both tribal and non-tribal entities.

We **accomplished** all of our goals and objectives in that we successfully: 1.) used next-generation sequencing to assess mule deer diet on the Navajo Nation, 2.) compared results identified from next-generation sequencing to similar results generated from microhistology, and 3.) provided baseline data on mule deer diet to the NNDFW as well as disseminated the use of next-generation sequencing to tribal and non-tribal programs. In addition, we successfully carried out fieldwork to collect mule deer fecal samples and samples of plant species suspected to be in the diet in compliance with NNDFW rules and regulations, as well as aided in the creation of a genetic library database of plants identified in the mule deer diet.

All **goals** and **objectives** were met for this project. All fieldwork and lab work were completed **on time**, within the project timeline (see Appendix A: Figure 1). We requested, and were granted an **extension** through June 30th, 2016, to finalize data analyses of next-generation sequencing results, as well as for completing analytical comparisons between next-generation sequencing and microhistological results.

The **customers** that benefit from this grant specifically include the NNDFW, among other Navajo natural resource tribal entities, as well as the NRCS. The cost estimates for all work outlined in the grant proposal were accurate, and **project funds** would have been **spent** as anticipated, however we were unable to use any NRCS – CIG appropriated funds for this project. Therefore, the costs for this project were covered by the NNDFW. Our most significant obstacle in being able to use project funds was the convoluted system in gaining approval from multiple Navajo tribal departments to authorize use of funds appropriated by the NRCS.

The main method employed to demonstrate **alternative technology** within this project was the genetic sequencing of plant DNA, via next-generation sequencing, in mule deer fecal samples to assess mule deer diet. Our **quantifiable, physical results** of this project were the presence of unique plant taxa (diet richness) and the level of taxonomic resolution (e.g. species, genus, family) identified through each diet assessment method (i.e. next-generation sequencing and microhistology). Plant sequences were quantified as data in our genetic results and plant morphology was quantified in our microhistological results. Additionally, monetary costs and time until completion of each assessment were recorded as **economic results**.

There are **local programs** in the tribal government, such as the NNDFW, that may be able to implement this project and use this innovative technology in herbivore diet research. Next-generation sequencing may also be of interest to **state** game and fish, as well as **federal** natural resource departments, to use in future herbivore diet research.

Major **recommendations** from this project include a serious look at implementing next-generation in future herbivorous wildlife and non-wildlife diet studies. It is an up-and-coming technology that may produce more accurate results than other diet assessment methods for herbivores, and deliver the results in a more efficient manner. However, other **key recommendations** for interested parties who want to use this technology in herbivore diet research include becoming semi-proficient in genetic data analyses, creating a robust genetic reference library database of local plants expected to be in the diet, and being cautious as to the accuracy of any quantifiable amounts of plant taxa identified through next-generation sequencing and microhistology.

INTRODUCTION

Brief overview:

Much data are still unknown regarding herbivorous wildlife diet on Native American reservations. And there are numerous peer-reviewed studies that question the accuracy of long-standing herbivore diet assessment methods, including the microhistological method. The purpose of this study was to use an innovative diet assessment technique in next-generation sequencing to investigate the diet of mule deer populations on the Navajo Nation and compare those results with those generated through microhistology. Through these actions we would provide dietary data on a culturally and biologically important wildlife species to the Navajo tribe, as well as test the efficacy of an innovative diet assessment technology with that of a commonly used, long-standing method. We carried out diet assessments through fecal analyses of mule deer populations residing on the Navajo Nation, in collaboration with the Navajo Nation Department of Fish and Wildlife, the University of Arizona, and Washington State University, and Research and Testing Inc. This study took place during the years of 2015 and 2016, with fieldwork and lab work taking place during the first half of the time period and data analyses taking place during the second half of the study.

Key personnel:

Jeff Cole served as the project director and is the Wildlife Management Program Manager for the Navajo Nation Department of Fish and Wildlife, and has served in this capacity for several decades. He is the primary manager of the big-game management and conservation program on the Navajo Nation and has a wealth of knowledge regarding mule deer biology and local populations on the reservation. He served as the main point of contact for the NRCS and tribal offices for this project, and oversaw the progress of this study.

Chase Voirin served as the primary project collaborator between all entities involved with project. He was a graduate student at the University of Arizona pursuing an M.S. degree in Wildlife Management and Conservation and worked in the Conservation Genetics Lab at the university. He also conducted all fieldwork, initial lab work, and data analyses for the project. He also managed all required NRCS reports, and utilized his expertise in large-mammal biology and genetics to aid in the completion of the study.

Dr. Melanie Culver is an assistant professor at the University of Arizona of wildlife conservation genetics, and as Mr. Voirin's main graduate advisor, she helped oversee the validity of all genetic data generated from this project.

Dr. Dave Christianson is an assistant professor at the University of Arizona of wildlife management and conservation, and as Mr. Voirin's graduate committee member, he helped oversee the validity of the statistics used to compare next-generation sequencing and microhistological diet assessment results.

Bruce Davitt is a lab manager at Washington State University's Wildlife Nutrition Laboratory and he oversaw the main lab work and data analyses for the microhistological diet assessment.

Project goals and objectives:

The primary goal of this project was to make use of an innovative genetic conservation technology in next-generation sequencing to asses the diet of an herbivorous wildlife species in mule deer on the Navajo Nation. A secondary goal for this project was to compare results from next-generation sequencing to that of a long-standing diet assessment technology in microhistology in order to determine the effectiveness and accuracy of the new technology. A third goal of this project was to provide baseline data and transfer of innovative technology and knowledge to a Native American tribal program in the Navajo Nation Department of Fish and Wildlife (NNDFW).

Project objectives outlined:

1.) Make use of an innovative conservation technology.
    a) Conduct next-generation sequencing on mule deer fecal matter collected from the Navajo Nation.

2.) Compare innovative conservation technology with proven historical technology
    a) Compare results generated from next-generation sequencing with results generated from microhistology from mule deer fecal matter collected from the Navajo Nation.
    b) Compare cost and time required to obtain results between next-generation sequencing and microhistology.

3.) Transfer baseline data and knowledge of technology use and effectiveness to a Native American tribal program.
    a) Provide baseline data to aid the Navajo Nation Department of Fish and Wildlife (NNDFW) in its management of mule deer on the Navajo Nation.
    b) Provide knowledge of the use, benefits, and effectiveness of next-generation sequencing to the NNDFW for future use.
    c) Present and demonstrate the tractability of next-generation sequencing to other Navajo tribal programs, as well as possibly non-tribal programs.

Scope of Project Tasks:

Key tasks conducted to carry out project goals and objectives:

Task 1. Conduct fieldwork to collect mule deer fecal samples:

Mule deer fecal samples were collected from two distinct populations of mule deer on the Navajo Nation, with the purpose of examining summer and winter diet for both populations. Undecomposed mule deer fecal samples (Hubbard and Hansen, 1976), with mucus still present on the outer walls of the pellets, were collected opportunistically over approximately one-month periods (May and January) from both population's historical summer and winter ranges, with the assumption that collected fecal samples represented each population's respective summer and

winter diet. Fecal samples were collected over wide portions of each study area to gain as much representation for each habitat as possible, while decreasing the chances samples were collected from the same individual (Stewart et al. 2003). Fecal samples were collected in the same manner as in-field scat handling, collection, and storage methods by Naidu et al. (2011). Date, time, and location were recorded for each sample.

Task 2. Conduct laboratory work for next-generation sequencing and microhistological diet analyses:

A given subset of fecal samples were randomly selected for next-generation sequencing and microhistological diet analyses. Diet assessment results were then compared between the two techniques and final analyses regarding presence of unique plant taxa (diet richness) and taxonomic resolution (e.g. species, genus, family) were synthesized. Microhistological diet assessments took place at Washington State University's Wildlife Nutrition Lab (B. Davitt, Pullman, Washington, U.S.A), while diet assessments using next-generation sequencing took place at the University of Arizona's Wildlife Conservation Genetics Lab and Research and Testing Inc. (Lubbock, TX, U.S.A). Next-generation sequencing included identifying specific regions of chloroplast DNA shown to differentiate between plant species in the mule deer fecal samples (Valentini et al. 2009a).

Task 3. Transfer of information and results.

Results and procedures were shared through consistent reports to the NNDFW, as well as other tribal and non-tribal entities via conference and meeting presentations. Additionally, a permanent genetic reference library was established at Research and Testing Inc. (Lubbock, TX, U.S.A.) of plant species on Navajo suspected to be in the mule deer diet, and of which can be used in future herbivore diet research by the Navajo tribe.

Business and Academic Relationships:

The project included collaborative efforts by the Navajo Nation Department of Fish and Wildlife (NNDFW) of which the majority of in-kind and monetary contributions were derived from. The in-kind contributions by the NNDFW included the time by Mr. Cole to oversee the progress of this project, as well as the time for an NNDFW botanist to aid in plant collection and identification of plant species suspected to be in the mule deer diet. Academic collaborators included University of Arizona assistant professors (Dr. Culver and Dr. Christianson) who put forth much time (in-kind) to the oversight of genetic and microhistological data analyses and synthesis of results. Another academic collaborator was Mr. Voirin (M.S. student), who conducted the majority of overall fieldwork, lab work, and data analyses. Key business collaborators included Washington State University's Wildlife Nutrition laboratory where microhisological lab work and initial analyses too place, and Research and Testing Inc. where genetic sequencing and initial analyses took place.

How project was funded:

The grant agreement was designed where half of the monetary support, mainly to cover indirect

lab costs and data analyses, would be provided through the NRCS. While 25% of total project costs would be in the form of monetary support to cover additional logistical and employment expenses of fieldwork by the NNDFW, and 25% of total project costs would be covered through in-kind contributions by the NNDFW. As it turns out, 100% of all in-kind and monetary costs were covered through by the NNDFW, without the use of any funding appropriated by the NRCS – CIG. Issues arose through the tribal offices not directly linked to this project that disallowed the use of federal funds for this study.

BACKGROUND

The Navajo Nation Department of Fish and Wildlife (NNDFW) is tasked with managing and conserving the natural flora and fauna on a reservation over 3.3 million acres in size. As with other tribal fish and wildlife programs, the NNDFW faces the challenges of managing natural resources over a vast landscape with limited funding, supplies, and personnel. In recent years, the NNDFW has sought out innovative techniques to help them achieve their goals of managing and conserving natural resources for the future, while keeping their cultural perspectives in mind. Mule deer are one of the most important species of interest for the Navajo people, both culturally and economically. Much of the revenue for the NNDFW stems from funding generated through the sale of hunting permits for mule deer. Despite the popularity of the species, little baseline data are known regarding its general biological niche on the Navajo Reservation, including its diet.

Microhistology is a long-standing diet assessment method involving the recognition of plant morphology in fecal matter under a microscope (Sparks and Malechek 1968), particularly in mule deer (Anthony and Smith 1974; Hansen and Reid 1975; Gill et al. 1983; Stewart et al. 2003). Historically, various wildlife agencies have used microhistology to conduct diet assessments for herbivorous wildlife they were managing. However, microhistology has several disadvantages including cost, precision, and the fact that it requires a considerable investment in time to become proficient at identifying plant species and genera (Holechek et al. 1982; Gill et al. 1983; Vavra and Holecheck 1980).

We sought to **mitigate both issues** by using an innovative genetic approach called next-generation sequencing, which allows for the identification of plant taxa through the recognition of plant DNA found in fecal samples. In this sense, we would be able to provide baseline biological data of mule deer diet to the NNDFW, examine the effectiveness of next-generation sequencing by comparing results generated from this method to those generated from a long-standing method in microhistology, and pass the education surrounding the use of the technology on to tribal and non-tribal entities.

There have been minimum **previous efforts** to examine mule deer diet on the Navajo Nation. And various case studies involving next-generation sequencing to assess herbivorous mammal diet through fecal material have recently occurred in foreign countries, such as diet analysis studies on moose, roe deer, and red deer in Poland (Czernik et al. 2013), chamois in France (Rayé et al. 2011), as well as tapirs in French Guiana (Hibert et al. 2013). Little research exists investigating the use of next-generation sequencing to assess diet through feces of any wild herbivorous mammal in North America. Next-generation sequencing is an emerging technology in terms of analyzing wild (Rayé et al. 2011; Czernik et al. 2013) and non-wild (Pegard et al. 2009) herbivore diets, so the uncertainty surrounding this technique is still great.

Diet assessment of wild herbivores via fecal analyses is a common practice today, especially among non-tribal wildlife agencies. We are hoping the NNDFW, as well as other tribal entities within the **environmental or agricultural sectors** interested in herbivore diet, will adopt these practices, including the use of next-generation sequencing, for routine examination of herbivore diet. Next-generation sequencing is appealing because it offers efficiency in terms of cost, time,

and accuracy compared to current diet assessment techniques, such as microhistology. Also, next-generation sequencing has the tractability for use among other entities outside of wildlife management, such as those that deal with livestock. **Agencies** dealing with habitat use by wildlife and livestock, such as the Bureau of Indian Affairs (BIA), NRCS, Bureau of Land Management (BLM), and U.S. Forest Service may want to consider using this technology to assess the diet of herbivorous animals. More specifically, studies examining overlap of diet between wildlife and livestock, or wild horses and burros, could be examined using this technology, and results could be generated in an efficient manner.

The **negative effects of the problem** to the environment would be a lack of important biological knowledge regarding a culturally and **economically** important wildlife species for the Navajo tribe. Understanding mule deer diet would help wildlife, and other natural resource agencies, better understand habitat use by this species, which may aid future land-use and outdoor recreation decisions. And with decreasing mule deer populations across the western half of the U.S., the NNDFW has great concern regarding the tribe's mule deer populations. Also, with an increase in livestock and feral horse and burro populations, coupled with negative effects from climate change, natural resource agencies would be wise to find creative ways and technology to mitigate those issues, such as techniques used in this study. Because negative effects regarding the aforementioned environmental issues stemming from the problem of a lack of knowledge and knowhow to mitigate those issues, will in turn create negative implications to local Navajo **communities** and their **economic welfare**.

REVIEW OF METHODS

What made this project **innovative** centered on the genetic technology used to carry out the mule deer diet assessment. Next-generation sequencing is a genetic technique that aids the use of DNA barcoding through its ability to sequence millions of bases of DNA, in the form of millions of short fragments of DNA of multiple organisms in a single sample, including plants (Deschamps et al. 2012). The combination of next-generation sequencing with targeting noncoding, conserved regions of chloroplast DNA (cpDNA) has allowed biologists to differentiate plant species of closely related taxa (Gielly and Taberlet 1994). In this study, we were able to use these techniques to amplify and sequence fragments of cpDNA from plant fragments in mule deer fecal samples, which essentially allowed us to investigate plants the mule deer were ingesting. More specifically, with the aid of Research and Testing Inc., we ran Polymerase Chain Reactions (PCR) for amplicons representing the whole *trnL* intron, as well as its associated P6 loop, using universal primers c,d,g, and h (Taberlet et al. 2007). The *trnL* gene, which is part of cpDNA, enabled us to differentiate between plant taxa allowing us to observe the breadth of mule deer diet.

The genetic methods we practiced could be defined as the recognition of plant taxa via the genetic makeup of the plants present in the samples. Our **innovative** methods in this study included comparing this new genetic method to investigate herbivore diet with a long-standing approach called microhistology, which is the recognition of plant morphology present in the samples. Few studies exist that analyzed direct comparisons of these two methods, possibly due to the fact that the genetic method we used (next-generation sequencing) is relatively new to the field of wildlife biology, especially in its use of investigating herbivore diet.

There are a few key differences when comparing next-generation sequencing methods to those of microhistology. In terms of **labor input**, microhistology requires whole mule deer fecal samples (i.e. pellets) to be diluted in an elution buffer so as to spread across a slide in order for final analyses to take place under a microscope, whereas the methods surrounding next-generation sequencing require small fragments of the mule deer pellets to eventually undergo DNA extraction and PCR before final sequencing and amplification takes place on a sequencing machine (i.e. Illumina MiSeq desktop sequencer). So, a key difference between each method may exist in the greater amount of lab work and time it takes to prepare samples for final sequencing using the next-generation sequencing method. There may be similarities in the relatively few amount of people required to complete each method, however, there are specialists within each field (i.e. next-generation sequencing and mircohistology) who may be more adept at certain aspects of specific methods over others, especially regarding the our genetic method where certain individuals specialized in lab work while others specialized in bioinformatics (i.e. genetic data analyses) at Research and Testing Inc. Each method took relatively the same amount of time to complete full lab work, examination, and analyses, which was approximately one month. However, it took about one full year from pellet preparation to final microhistological analyses to take place due to the backlog of other orders Washington State University's Wildlife Nutrion Lab had to complete before ours. Also, our next-generation sequencing method was exceptionally new so that our final bioinformatics and data analyses took much longer (approximately three months) than originally anticipated. Despite these delays in the project, estimated time to complete each method to generate final results upon collection of fecal samples

was approximately one month.

Differences in **material input** was significantly based on the multiple steps and materials required to complete genetic sequencing. For example, mule deer pellet preparation for microhistology included simply placing pellets in designated zip-lock bags to be sent to Washington State University's Wildlife Nutrition Lab, while our genetic method included extracting "plugs" of each mule deer pellet with a biopsy punch and placing them in small test tubes to send to Research and Testing Inc. (Lubbock, TX, U.S.A.) for sequencing and initial bioinformatics. Additional materials were needed for required PCR steps, such as lab equipment, chemicals, and reagents, not including the sequencing platform itself (i.e. Illumina MiSeq desktop sequencer), all of which was necessary to complete the next-generation sequencing method. Key materials needed for completing the microhistology method included lab equipment to implement elution and high-quality microscopes and computer processors to magnify plant fragments for morphological identification.

Differences in **economic input** were relatively similar in costs per sample analyses. For example, we conducted individual mule deer diet analyses of which microhistological costs were $100/sample and next-generation sequencing costs were $95/sample. Total costs of microhistological and next-generation sequencing analyses are outline in Table 1 (Appendix 2).

This project did not involve **marketing an alternative product**, nor was there a **producer** since this study was not part of an agricultural or livestock-related project.

Our schedule of events included sampling over over two 2-day intervals within each mule deer population's winter (January 2015) and summer (May 2015) ranges to investigate each population's respective summer and winter diet. Lab work was then conducted intermittently from June through September 2015. Data analyses to compare next-generation sequencing and microhistological results occurred September 2015 through May 2016, with the project closing on June 30th, 2016.

Our project map (Appendix A: Figure 2) shows the locations (i.e. within the black circles) of the two populations of mule deer where fecal collection took place, which are located at the Chuska and Carrizo Mountain ranges. Summer and winter field sites, where samples were collected to gain knowledge on summer and winter mule deer diet, exist within the circles highlighting the Chuska and Carrizo Mountain ranges. Summer habitat in the Chuska Mountains (2619–2773 m above sea level) consisted of subalpine grassland, Petran subalpine conifer forest, and Petran montane conifer forest (Brown and Lowe 1981 Vegetation Map, 2012) whereas winter habitat (2022–2140 m above sea level) consisted predominantly of Great Basin conifer woodland and Great Basin grassland (Brown and Lowe 1981 Vegetation Map, 2012). Summer habitat in the Carrizo Mountains (2681–2804 m above sea level) consisted of Petran montane conifer forest while winter habitat (1893 – 2036 m above sea level) consisted primarily of Great Basin conifer woodland (Brown and Lowe 1981 Vegetation Map, 2012).

The amount of time, effort, manpower, and equipment needed for fieldwork and lab work went about **as expected**, and these tasks were efficiently completed. The process that we had the most challenge with was the next-generation sequencing analyses to determine mule deer diet, and

more specifically creating a diligent bioinformatics method that would enable us to confidently determine what specific plant taxa were in the diet. We developed several iterations of bioinformatics and statistical analyses to determine mule deer diet via next-generation sequencing. One of the main challenges we encountered was that our first statistical iterations were unable to identify correct plant taxa from our study area based on the region of chloroplast DNA we were using. We mitigated this by collecting nearly 200 plant species from our study areas – both summer and winter areas in both the Chuska and Carrizo Mountain ranges – of which we suspected to be in the diet, and we genetically sequenced these plant species to create a genetic reference library. This genetic library added much greater clarification on what plant taxa were in the diet. Another challenge included the fact that it took several iterations on determining a conservative, yet thorough, statistical genetic analyses to determine what plant taxa were in the diet while reducing the chances of identifying false-positive taxa. Due to the lack of previous research in this area of study, specifically examining herbivore diet through chloroplast genes via feces, there was no concrete consensus on how to complete final genetic statistical analyses, so we had to develop our own. The creation of our genetic reference library and determining a suitable statistical analysis through our bioinformatics system added six months to our original projected timeline, and were clearly the tasks we **did not expect** to take so long to complete.

If the **project started today**, I would create my **genetic reference librar**y well ahead of time and make it robust by adding as many plant species as I would expect to be in the diet of the species of interest, as possible. Additionally, I would consult with various genetic sequencing labs to search for possibilities of expanding and strengthening my genetic sequencing results by sequencing **multiple regions of chloroplast DNA (cpDNA)** so as to add more clarity and possible accuracy to my final diet results. In this study, we only worked with one region of cpDNA (*trnL*), which proved to be a reliable region that has been used in previous research for similar diet studies, but accuracy may be improved with sequencing additional region(s) along with *trnL* to determine diet. Lastly, I would consult with a bioinformatics specialist to develop a thorough, concrete plan on the genetic statistical analyses that would take place before any work began. This would save much work and time in downstream analyses when its time to produce dietary results.

DISCUSSION OF QUALITY ASSURANCE

Our **project sites** were in the Chuska Mountains and Carrizo Mountains, and adjacent wintering habitat, of northeastern Arizona within the Navajo Nation (Appendix A: Figure 2). Summer habitat in the Chuska Mountains (2619–2773 m above sea level) consisted of subalpine grassland, Petran subalpine conifer forest, and Petran montane conifer forest (Brown and Lowe 1981 Vegetation Map, 2012) whereas winter habitat (2022–2140 m above sea level) consisted predominantly of Great Basin conifer woodland and Great Basin grassland (Brown and Lowe 1981 Vegetation Map, 2012). Summer habitat in the Carrizo Mountains (2681–2804 m above sea level) consisted of Petran montane conifer forest while winter habitat (1893 – 2036 m above sea level) consisted primarily of Great Basin conifer woodland (Brown and Lowe 1981 Vegetation Map, 2012).

Vegetative characteristics in summer habitats were similar at both **project sites** in that they consisted predominantly of mixed conifer, especially ponderosa pine (*Pinus ponderosa*). Vegetative characteristics in winter habitats were similar at both **project sites** in that they consisted predominantly of juniper (*Juniperus spp.*) and pinyon pine (*Pinus edulis*) with mixed sage (*Artemisia spp.*). We identified summer and winter habitats based on historical migration patterns of the mule deer populations, where they spend their summers in the mountains among lush forage and move to lower elevations at the base of the mountains during the winter when forage is unavailable and snow pack increases at higher elevations.

Sampling design and procedures:

We opportunistically collected non-decomposed mule deer fecal samples, with a shiny dark-brown outer surface on the pellets (Marshal et al. 2004; Marshal et al. 2012) from two distinct populations of mule deer on the Navajo Nation (hereafter referred to as Chuska and Carrizo populations). Sampling occurred over two 2-day intervals within each population's winter (January, 2015) and summer (May, 2015) ranges to investigate each population's respective winter and summer diets. We collected samples, consisting of approximately 40 pellets, from piles of ≥ 50 pellets to provide sufficient amounts for two separate diet analyses. We collected samples over wide portions (mean distance between transects = 3 km) of both populations' winter and summer areas primarily using game trails as transects with a minimum approximate distance of 2 km traveled, to gain as much representation for each habitat as possible with the assumption that each sample represented the diet of one individual deer (Stewart et al. 2003). We collected and stored samples following previous research by Naidu et al (2011), with the exception that our protocol involved the feces placed in paper sacks separate from the desiccant beads within the zip-lock bags. We used a hand-held GPS unit (eTrex® H, Garmin, Olathe, KS, U.S.A.) to record the collection, date, time, and location of eachsample. We stored samples at –20°C at the Culver Conservation Genetics Laboratory at the School of Natural Resources and the Environment, University of Arizona (Tucson, AZ, U.S.A.).

We collected 101 summer and 70 winter fecal samples from the Chuska population, and 101 summer and 82 winter fecal samples from the Carrizo population of mule deer. We randomly selected 20 individual fecal samples from each population, within seasons, resulting in 80 total individual samples that underwent both next-generation sequencing and microhistological

investigations and were included in final analyses. We collected and implemented sequencing of 198 distinct plant species from field sites and the Navajo Nation and Deaver Herbariums (Flagstaff, AZ, U.S.A.) to create our local genetic reference library (Appendix B: Table 3). We supplemented our genetic library with 55 additional *trnL* sequences downloaded from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/nucleotide), two of which were unique plant species (*Holodiscus discolor* and *Juniperus monosperma*) not included in our original reference database (Appendix B: Table 3). Following field sample collections, we randomly selected 20 samples (40 pellets/sample), within seasons, from both populations (80 total samples) for individual diet analyses (≈10 pellets/sample/method).

Microhistological diet analyses were conducted at Washington State University's Wildlife Habitat and Nutrition Laboratory (Pullman, WA, U.S.A.) following their standard protocol (Wildlife Habitat and Nutrition Lab, "Botanical Composition – Microhistology Methods", unpublished) which included the use of their local plant herbarium collection containing plant species represented in our study areas.

All DNA isolation and next-generation sequencing protocols were performed at Research and Testing Laboratory, Inc. (Lubbock, TX, U.S.A.). Whole DNA was extracted from fecal samples using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, U.S.A.). Plant chloroplast DNA (cpDNA) was isolated using the PowerPlant® Pro DNA Isolation Kit (Mo Bio Laboratories, Inc., CA, California, U.S.A.) per manufacturer instructions.

Research and Testing Inc. lab members prepared next-generation sequencing libraries for each sample from PCR amplicons representing the whole *trnL* intron, as well as its associated P6 loop, using universal primers c,d,g, and h (Taberlet et al. 2007). They performed PCR in 25 µl reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, CA, U.SA.), 1 µl of each 5 µM forward and reverse primer, and 1 µl of template under the following thermal profile: 95°C for 5 min, then 35 cycles of 94°C for 30 sec, 54°C for 40 sec, 72°C for 1 min, followed by one cycle of 72°C for 10 min and 4°C hold. They prepared samples using Illumina adapter sequences and they tagged individual samples with unique indices. They then pooled samples in equimolar concentrations for the final DNA library and ran all 80 fecal samples, and all plant library reference samples, on an Illumina MiSeq desktop sequencer (Illumina, model #: SY-410-1003, Hayward, CA, U.S.A.) using V2 chemistry and 2x250 flows.

Forward and reverse reads were merged in FASTQ format using the PEAR Illumina paired-end read merger (Zhang et al. 2014) after sequencing. Reads were run through an internally developed quality-trimming algorithm to assemble a query database. All denoising, chimera checking, Operational Taxonomic Unit (OUT) selection and subsequent quality control were conducted by Research and Testing Laboratory following their standard protocol (Research and Testing Inc., Data Analysis Methodology, http://www.researchandtesting.com/docs/Data_Analysis_Methodology.pdf). We conducted a FASTA alignment between the query database and genetic plant library reference database using Basic Local Alignment Search Tool (BLAST), only acquiring the top hit for every sequence that aligned with at least 98% identity between our query database and reference database, an e-value score of 1e-6 and 95% query coverage. Sequences that did not align with our reference library

database were aligned with the NCBI database (Nucleotide collection, blast/ncbi-2.2.28, 2016), using identical **statistical protocol**.

In order to make the results for each method comparable, diet analyses variables were measured in occurrences (hereafter termed "hits") found for each plant item, across all 80 samples, and converted into percentage values.

We measured and compared **diet richness and taxonomic resolution** through the presence and absence of plant items found in the diet, as well as the level of taxonomy (e.g. species, genus, family) each plant item was identified to. We compared percentages of hits distributed across major taxa (i.e. species, genus, family) only among individuals where hits of the aforementioned taxa were present.

While one could argue that this study was mainly designed to test the effectiveness of diet assessment methods, we did produce biological data that are **pertinent** to tribal management and conservation efforts for mule deer. Furthermore, previous studies claim that a composite of 15 individual fecal samples is minimally sufficient to estimate diet for mule deer for a given season (Anthony and Smith 1974; Anthoney and Smith 1977), and we examined the diet of 20 individuals per population, within season. Lastly, this study was not designed to give an in-depth view as to the full breadth of mule deer diet across multiple seasons, areas, and years, but instead it gives a snapshot of possible important forage within each population's respective summer and winter ranges. Despite these shortcomings, the sampling design, from the field to the lab to the analyses, was thorough and is recommended as a building block for future similar studies using next-generation sequencing as a method to examine herbivore diet via fecal analyses.

Custody procedures for this study started with obtaining a biological collection permit giving authority to the field worker to collect mule deer fecal samples and plant clippings/specimens from the aforementioned study areas. These procedures also included gaining tribal, and more specifically NNDFW, approval to make these data, as well as most work surrounding these data, public. We gained permission from the NNDFW to share the results and procedures of this study to tribal and non-tribal entities via various conferences and University of Arizona related functions. Further permission may be required to publish material in this study in a peer-reviewed journal.

Since we were not testing a new technology in the field, no **calibration procedures** were necessary other than the normal chemical and equipment calibrations that take place during PCR procedures for our genetic analyses. One method that improved the accuracy of our genetics results was to add plant species from our field sites that we suspected to be in the diet. This could possibly be included as some form of calibration in that the bioinformatics used to analyze gene sequences would have to target sequences in the feces that matched those in the reference library.

Our **data reduction** included Mr. Voirin synthesizing results into more understandable formats. These data have been presented at numerous conferences in front of the public and tribal and non-tribal natural resource entities. We presented our data in simple charts and diagrams that are interpretable to the public and various entities. Data were reviewed by both the microhistology and genetic laboratories, as well as multiple faculty and graduate students at the University of

Arizona who are keen on genetic research. Part of these data were incorporated and presented in Mr. Voirin's thesis research and defense presentation.

FINDINGS

Our main **findings** were that we identified more unique plant taxa through next-generation sequencing (i.e. greater diet richness) as well as more plant taxa at finer taxonomic scales (i.e. finer taxonomic resolution) in all mule deer diet samples, within seasons, than through microhistology. However, we did not find any statistical correlation of amounts of plants eaten between each method, therefore, we didn't know if either method would produce accurate projections of amounts of plant taxa eaten. Also, we found that next-generation sequencing was slightly cheaper than microshitology in producing final results, as well as producing results at a faster rate once all corrections to our genetics methods were taken into account. A list of costs and time associated with completing each method can be found on Tables 1 and 2 (Appendix B).

Our **findings supported our goals** in that: 1.) we made use of a **new and innovative conservation technology** in next-generation sequencing and successfully assessed wild herbivore diet (i.e. mule deer diet), as well as analyzed its **efficiency of use and the accuracy** of its results; 2.) we compared results from next-generation sequencing to that of a proven diet assessment technology in microhistology; and lastly 3.) we provided baseline data and **transfer of the innovative technology** to the Navajo Nation Department of Fish and Wildlife (NNDFW).

Overall, microhistological analyses revealed five species, 34 genera, 17 families across all 80 mule deer fecal samples. Comparatively, next-generation sequencing analyses identified 149 species, 97 genera, and 36 families (Figure 3: Appendix A). A comprehensive list of species, genera, and families identified between each method is shown in Table 4 (Appendix B). Every species that was identified through microhistology, with the exception of *Holodiscus dumosus*, was identified through next-generation sequencing (Appendix B: Table 4).

Comparisons of plant taxa identified, at the genus level of taxonomy, between both diet assessment techniques for summer and winter seasons are shown in Figures 4 and 5 (Appendix A). Due to a high discrepancy in the amounts and types of plant species identified through each method, we decided to show comparisons in genera idenified instead. These data will be useful to the NNDFW for future management and conservation decisions for mule deer. Also, we were able to successfully sequence the *trnL* chloroplast gene for 198 distinct plant species from the Navajo Nation, which will be available for future use in any herbivore diet or plant-genome related study for the tribe, including the NNDFW. Also, we presented at least two presentations to the NNDFW, as well as other tribal personnel, regarding the technology used in this study, which further demonstrates a transfer of biological and technological information to the tribe.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, our expectations were met in that we identified more unique plant taxa from overall diet richness with next-generation sequencing and finer taxonomic resolution in the form of a greater number of plant items identified to species and genus levels of taxonomy for each population, within seasons. To our knowledge, no similar study has been conducted for mule deer via fecal analyses, but these results are consistent with the few similar studies that compared next-generation sequencing and microhistological/visual diet assessment methods; including herbivorous fish (Budarf et al. 2011), microalgal prey of copepods (Nejstgaard et al. 2008), Moroccan dorcas gazelle (Baamrane et al. 2012), woodland caribou (Newmaster et al. 2013), and rodents (Soininen et al. 2009). For example, a similar study investigating differences between next-generation sequencing and microhistological results in vole diets via gut content found next-generation sequencing showed greater richness and finer resolution of plants, as well as a greater proportion of hits at the species level of taxonomy (Soininen et al. 2009). Additionally, Baamrane et al (2012) found far greater diet richness and finer resolution using next-generation sequencing techniques in relation to microhistological results in the diets of Moroccan gazelles via fecal analyses. More specifically, our next-generation sequencing results were consistent to those of previous studies using cpDNA, namely the *trnL* gene, to investigate diet variables of next-generation sequencing for wild ungulates that found exceptional diet richness and taxonomic diet resolution among their respective species of focus (Rayé et al. 2010; Kowalczyk et al. 2011; Hibert et al. 2013; Czernik et al. 2013).

Our expectations were met in regards to the little significance in agreement of percent of families found with both methods, among all samples. However, skepticism regarding the reliability in proportional estimates of specific plant taxa, and even plant forage groups, in herbivore animal diets have been addressed in previous research for both microhistological (Vavra and Holecheck 1980; Gill et al. 1983) and next-generation sequencing (Soininen et al. 2009; Valentini et al. 2009b; Rayé et al. 2010; Kowalczyk et al. 2011; Hibert et al. 2013; Czernik et al. 2013) techniques. More specifically, differences in digestibility may affect microhistological analyses assuming the epidermis of each plant species survives digestion sufficiently to be identifiable in the feces (Stewart 1970; Anthony and Smith 1974; Vavra and Holecheck 1980), and this may not be present in an equitable ratio to when the plant taxon entered the animal. This same bias can occur in genetic analyses, as well as in the differences in sequence amplification through PCR and next-generation sequencing among several different plant taxa at the *trnL* region (Valentini et al. 2009b). These factors may lead to an over or underestimation of those taxa in final analyses, therefore, it is understandable we found little significant similarity or dissimilarity in proportions of plant families among all samples.

Through this study we found several analyses and results that are applicable to wildlife biologists considering the use of next-generation sequencing to assess wildlife diet, and we **recommend** the following: 1.) It is imperative to assemble a robust reference library of DNA sequences from plants from the given study area. Since the intergenic region of the *trnL* gene is relatively short, a species of plant from one study area may vary in its *trnL* region from the same species of plant in another study area. Creating a sequence library from local plant species may improve resolution and reduce identification errors between plant taxa, 2.) A wildlife biologist with a basic

understanding of the bioinformatics that goes into parsing and analyzing sequencing data, will help to understand the outcome of those data. There is currently no universal method to analyze next-generation sequencing data for herbivore diet, thus, one can choose a technique that best fits their needs in assessing wildlife diet. Understanding these methods will aid in understanding why certain methods provide better resolution of one's dataset than other methods, and 3.) As with previous wildlife diet studies that used next-generation sequencing techniques, we caution against putting excess importance on the proportional information of a taxon within a sample due to the aforementioned biases that occur when such estimates are made. It should also be noted that similar studies comparing the application of these methods may yield different results based on diet content and the organism of focus.

The development of next-generation sequencing methods to efficiently and accurately assess diet richness along with strong discrepancy in taxonomic resolution make it an effective method for non-invasive, fecal diet analyses. Studies have proven that plant diet richness can be calculated even among highly degraded cpDNA that has passed through the digestive systems of herbivores using next-generation sequencing methods (Taberlet et al. 2007). Additionally, the decreasing costs of such applications may make this method more appealing to wildlife biologists.

With continued decreases in sequencing costs and increases in sequencing accuracy, next-generation sequencing will offer a wealth of possibilities towards wildlife management. In fact, technological improvements have been so rapid with genomic sequencing platforms, that currently the software and algorithm development is falling behind reductions in sequencing costs and improved quality of data generated through sequencing (Hamilton and Buell 2012). It has been anticipated that continued improvements to genomics, and subsequently next-generation sequencing, will include length and quality of output and quality of algorithms and bioinformatics software necessary to handle large genome datasets (Hamilton and Buell 2012).

Understanding wildlife diet, and more specifically mule deer diet, is important from a management perspective by allowing managers to better understand how a species of interest functions in a given habitat. The use of genetic sequencing to understand wildlife diet may provide better understanding of nutritional quality and correlate quality of habitat with items present in the diet. Understanding the correlation of nutritional value of plants with diet selection of herbivores may shed light on habitat use by mule deer across seasons. This, in turn, could aid wildlife managers with decisions involving habitat improvement and in limiting human-related disturbances. Additionally, managers could identify the diet and nutritional quality of "healthy" mule deer populations, and try to apply changes to the habitat and forage quality of mule deer populations to increase population size in other areas.

Lastly, this technology could be applied to other herbivorous species, other than mule deer, in determining their diets within various ecosystems. However, due to the relative lack of a universal data analyses method, as well as the lack of peer-reviewed literature and studies surrounding this method, it is not recommended for common usage as the sole method to understand herbivore diet. We recommend further research and trial tests of this method for different herbivorous species in various environments before this is brought to common usage or supersedes commonly used, reliable diet assessment methods. However, we encourage further research and application of this method in trial tests, as well as comparing it to results of other

diet assessment techniques, which we accomplished through this study.

APPENDIX A: FIGURES

Figure 1. Proposed Project Timeline.

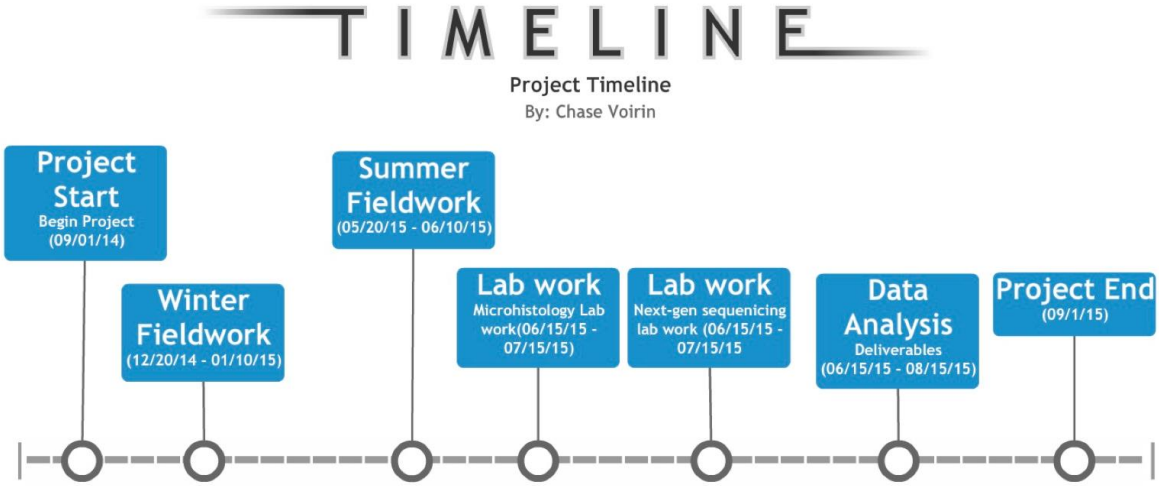


Figure 2. Locations of Carrizo Mountain (furthest north) and Chuska Mountain study areas. Both summer and winter habitats are within black circles.

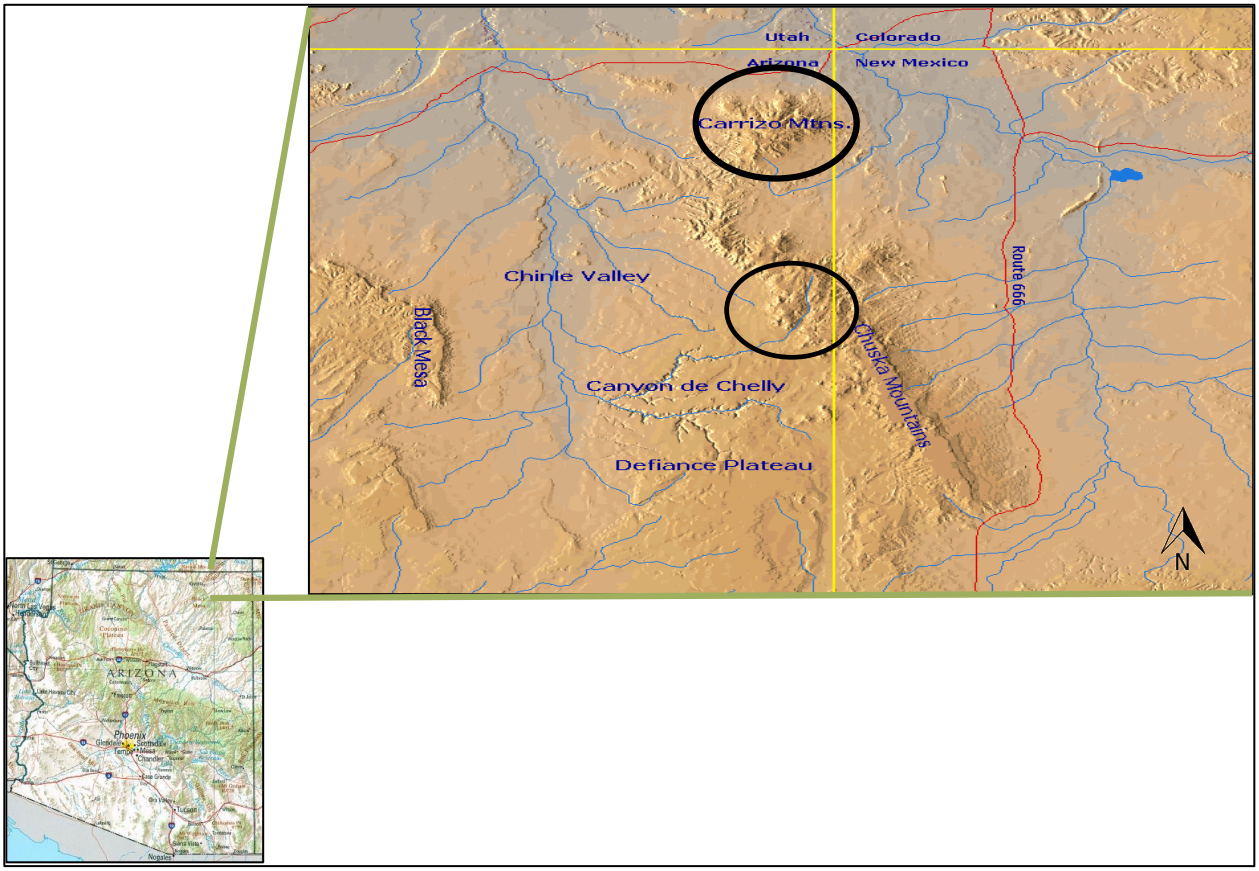


Figure 3. Number of unique plant taxa identified between each method across all 80 samples.

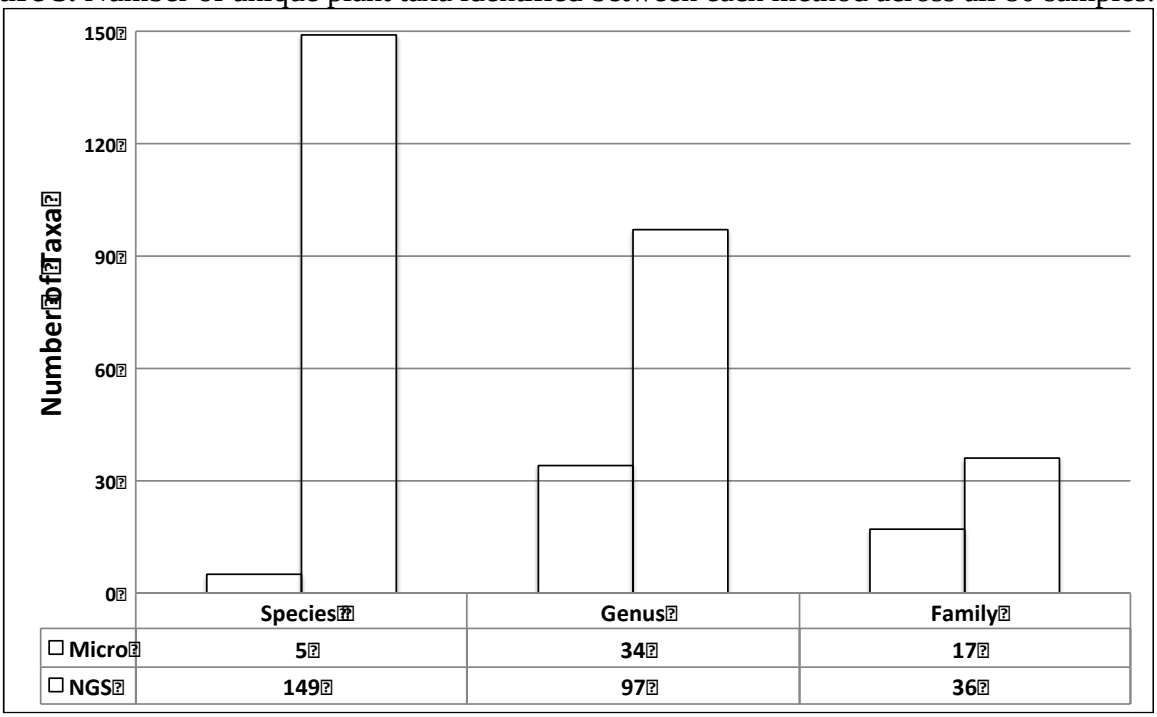


Figure 4. Plant genera identified between each method for each population's winter diet.

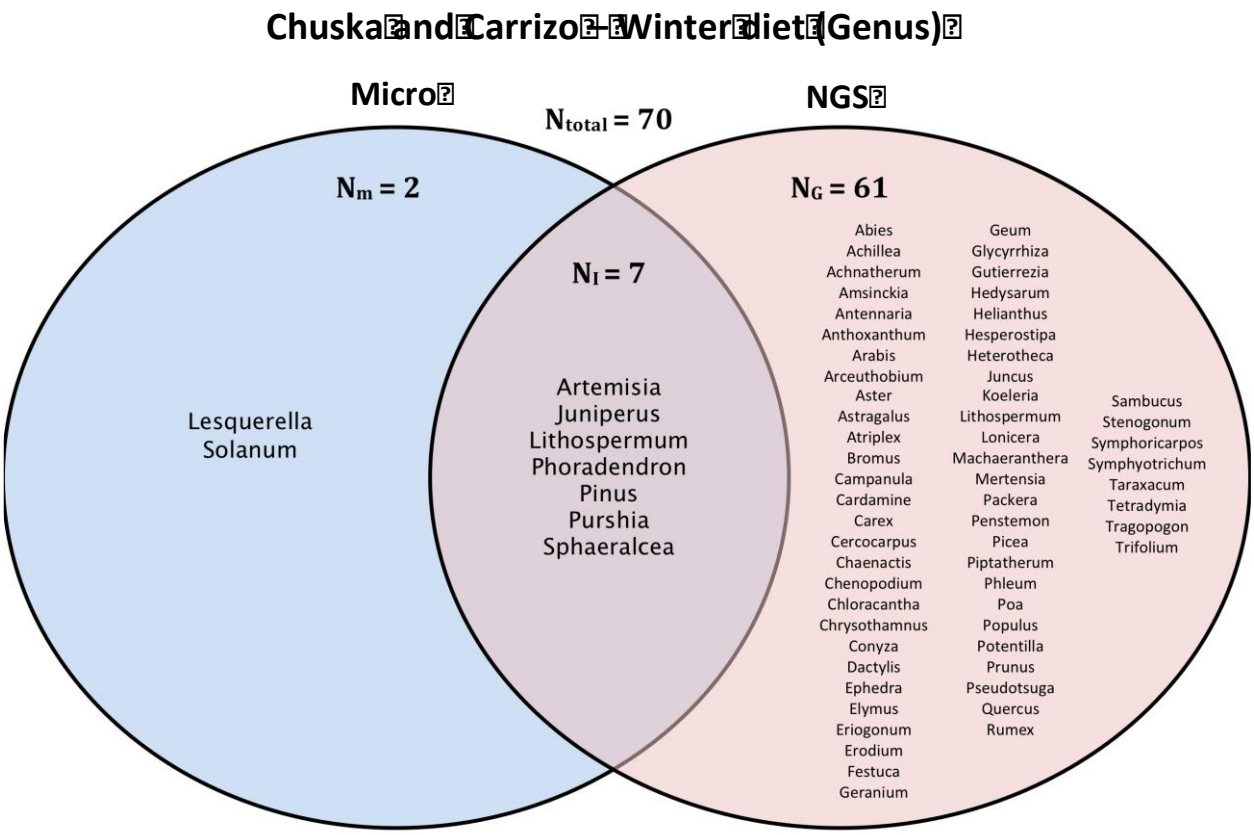
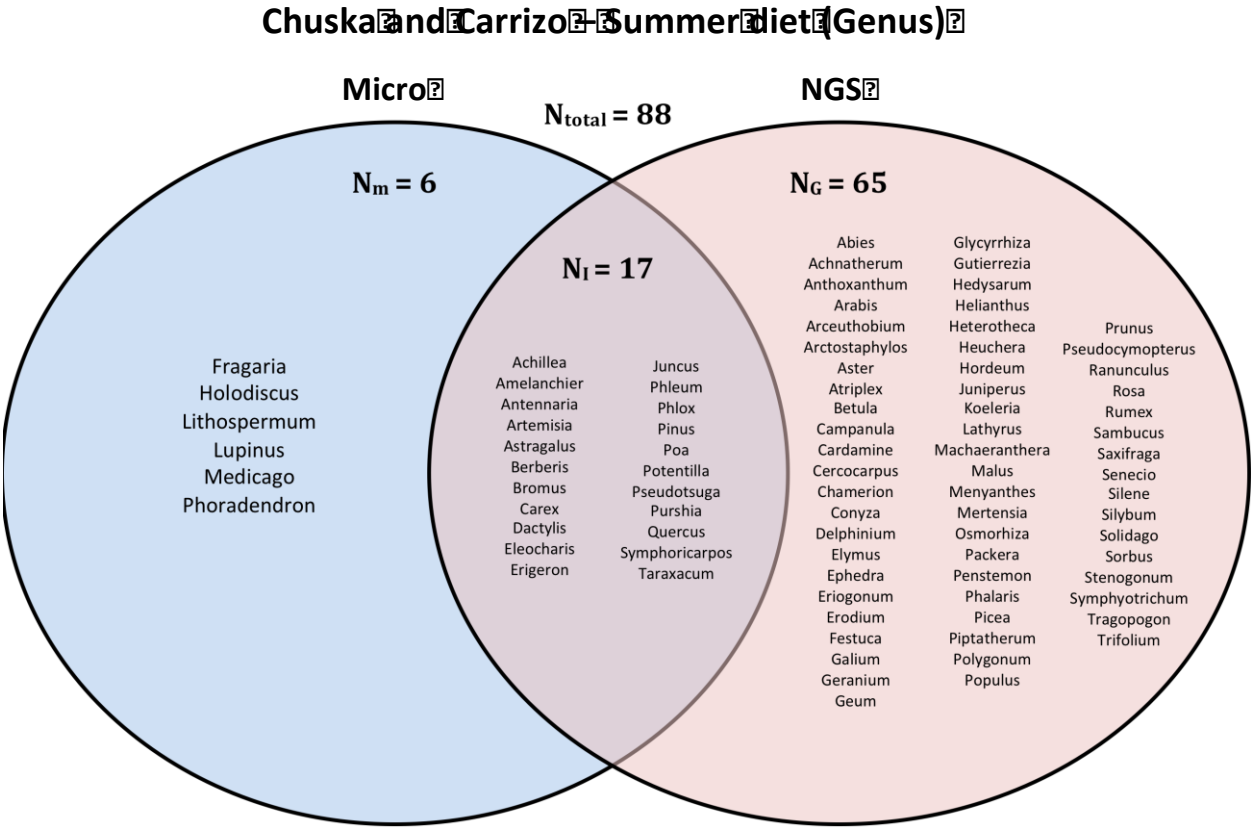


Figure 5. Plant genera identified between each method for each population's summer diet.



APPENDIX B: TABLES

Table 1. Cost comparison of next-generation sequencing and microhistology.

	Microhistology	Next-generation sequencing
Cost per sample	$100	$95
Number of samples	80	80
Total Costs	$8,000	$7,600

Table 2. Amount of time to complete diet assessments.

	Microhistology	Next-generation sequencing
Fieldwork	4 days	4 days
Lab work	14 weeks	7 days
Data analyses	7 days	7 days
Total days	25	18

Table 3. Genetic plant reference library.

Origin	Catalog #	Species	Origin	Catalog #	Species
NAVA	5695	Abies concolor	NAVA	9641	Baccharis wrightii
ASC	88878	Abies lasiocarpa var. lasiocarpa	NAVA	10832	Berberis fremontii
NAVA	6453	Achillea millefolium	NAVA	8500	Berberis repens
ASC	70380	Achnatherum hymenoides	ASC	95706	Boechera perennans/Arabis perennans
ASC	59246	Amelanchier alnifolia	NAVA	6606	Bromus carinatus
NAVA	7250	Amelanchier utahensis var. utahensis	NAVA	9140	Bromus ciliatus
NAVA	6533	Antennaria marginata	NAVA	6924	Bromus inermis
NAVA	11404	Antennaria parvifolia	NAVA	5468	Bromus tectorum
NAVA	10006	Antennaria rosea	NAVA	6937	Campanula parryi
NAVA	5933	Antennaria rosulata	ASC	32810	Campanula rotundifolia
NAVA	10007	Arabis fendleri var. fendleri	NAVA	7436	Cardamine cordifolia
NAVA	2963	Arceuthobium divaricatum	NAVA	11550	Carex athrostachya
NAVA	5934	Arceuthobium douglasii	NAVA	6503	Carex geophila
NAVA	10079	Arctostaphylos uva-ursi	NAVA	8899	Carex microptera
NAVA	7454	Artemisia arbuscula	NAVA	10566	Carex occidentalis
NAVA	7131	Artemisia bigelovii	NAVA	4796	Carex pellita
NAVA	9199	Artemisia campestris subsp. borealis	NAVA	1153	Carex praegracilis
NAVA	6838	Artemisia carruthii	NAVA	11553	Carex rossii
NAVA	6680	Artemisia dracunculus	NAVA	4384	Carex utriculata
NAVA	7320	Artemisia filifolia	NAVA	6527	Cercocarpus montanus
NAVA	6793	Artemisia frigida	NAVA	6173	Chrysothamnus nauseosus subsp. Bigelovii/Ericameria nauseosa ssp. Subaridum
NAVA	8355	Artemisia ludoviciana var. latiloba	ASC	103494	Collomia linearis
NAVA	9028	Artemisia pygmaea	NAVA	4486	Conioselinum scopulorum
NAVA	4979	Artemisia spinescens	NAVA	6995	Conyza canadensis
NAVA	8544	Artemisia tridentata	NAVA	2142	Dactylis glomerata
NAVA	1250	Astragalus albulus	NAVA	5560	Descurainia sophia

NAVA	10537	Astragalus amphioxys	NAVA	850	Draba aurea
NAVA	11463	Astragalus chuskanus	ASC	100826	Ephedra torreyana
NAVA	10358	Astragalus flavus var. candicans	NAVA	5864	Ephedra viridis var. viscida/Ephedra cutleri
NAVA	4206	Astragalus humistratus	NAVA	8567	Erigeron flagellaris
NAVA	6765	Astragalus lentiginosus	NAVA	6972	Eriogonum alatum
NAVA	10768	Astragalus lonchocarpus	NAVA	9190	Eriogonum cernuum
NAVA	10285	Astragalus wingatanus	NAVA	9690	Eriogonum jamesii
NAVA	5075	Astragalus zionis	NAVA	10917	Eriogonum racemosum
NAVA	8739	Atriplex canescens var. canescens	NAVA	6899	Eriogonum umbellatum var. subaridum
NAVA	6186	Atriplex confertifolia	NAVA	5444	Erodium cicutarium
NAVA	10839	Atriplex obovata	NAVA	9008	Erodium texanum
NAVA	10362	Atriplex powellii	NAVA	10008	Erysimum capitatum
NAVA	6266	Atriplex saccaria	NAVA	4848	Fragaria virginiana

Table 3. Genetic plant reference library continued…

Origin	Catalog #	Species	Origin	Catalog #	Species
ASC	92813	Geranium caespitosum	NAVA	10573	Lupinus caudatus subsp. cutleri
ASC	62774	Geranium richardsonii	NAVA	6836	Lupinus kingii
NAVA	4866	Geranium viscosissimum	NAVA	6449	Machaeranthera canescens var. canescens
NAVA	2645	Geum macrophyllum	NAVA	524	Machaeranthera grindelioides
NAVA	4871	Geum triflorum	NAVA	6414	Malus baccata
NAVA	6056	Gilia haydenii	ASC	72327	Malus pumila
NAVA	4519	Gilia longiflora	NAVA	9170	Malus sylvestris
NAVA	10515	Gilia multiflora/Ipomopsis multiflora	NAVA	1520	Medicago lupulina
NAVA	10003	Glycyrrhiza lepidota	NAVA	5928	Medicago sativa
NAVA	9704	Hedysarum boreale	NAVA	6944	Melilotus alba
NAVA	469	Helianthus annuus	NAVA	800	Mertensia franciscana
NAVA	470	Helianthus ciliaris	NAVA	11402	Mertensia fusiformis
NAVA	9055	Helianthus petiolaris	ASC	76433	Microsteris gracilis/Phlox gracilis
ASC	64818	Hesperostipa comata/Stipa comata	NAVA	4940	Osmorhiza depauperata
NAVA	7998	Heterotheca villosa var. villosa	NAVA	81	Oxypolis fendleri
NAVA	9712	Heuchera parviflora	NAVA	5149	Packera multilobata/Senecio multilobatus
NAVA	10561	Holodiscus dumosus	NAVA	11591	Phemeranthus parviflorus
NAVA	11474	Ipomopsis aggregata	NAVA	4104	Phleum pratense
NAVA	1714	Juncus articulatus subsp. articulatus	NAVA	6703	Phlox austromontana
NAVA	6800	Juncus balticus subsp. ater/Juncus arcticus	Field site	*	Phlox cluteana
ASC	78393	Juncus drummondii	NAVA	2397	Phlox hoodii
NAVA	9995	Juncus longistylis	NAVA	6222	Phoradendron juniperinum
NAVA	9351	Juncus saximontanus	NAVA	7972	Picea engelmannii
NAVA	11041	Juncus torreyi	NAVA	7971	Picea pungens
NAVA	8473	Juniperus communis	NAVA	3189	Pinus edulis

NAVA	8177	Juniperus osteosperma	ASC	87921	Pinus ponderosa
NAVA	8790	Juniperus scopulorum	NAVA	7036	Piptatherum micranthum
NAVA	6752	Koeleria macrantha	NAVA	6771	Poa annua
NAVA	7293	Lathyrus eucosmus	NAVA	6584	Poa bulbosa
NAVA	1483	Lathyrus lanszwertii var. leucanthus/Lathyrus arizonicus	NAVA	6620	Poa compressa
NAVA	11348	Lesquerella rectipes/Physaria rectipe	NAVA	7306	Poa fendleriana
NAVA	6914	Ligusticum porteri	Field site	*	Poa pratense
NAVA	10666	Lithospermum multiflorum	NAVA	10912	Polygala acanthoclada
NAVA	11009	Lobelia cardinalis	ASC	102061	Polygala alba
NAVA	4327	Lonicera involucrata	NAVA	7661	Polygonum amphibium
NAVA	8171	Lonicera korolkowii	NAVA	5525	Polygonum aviculare
NAVA	8134	Lotus wrightii	NAVA	8052	Polygonum douglasii subsp. johnstonii
NAVA	8773	Lupinus argenteus	NAVA	7102	Populus angustifolia

Table 3. Genetic plant reference library continued…

Origin	Catalog #	Species	Origin	Catalog #	Species
NAVA	7310	Populus deltoides subsp. wislizenii	NAVA	7421	Senecio spartioides
NAVA	7271	Populus tremuloides	NAVA	6118	Solanum elaeagnifolium
NAVA	9166	Populus x acuminata hybrid	NAVA	9191	Solanum jamesii
NAVA	9315	Potentilla anserina	NAVA	6852	Solanum rostratum
NAVA	2659	Potentilla fruticosa	NAVA	6435	Solanum triflorum
NAVA	4112	Potentilla hippiana	NAVA	7835	Sorbus dumosa
ASC	104942	Prunus emarginata	NAVA	3707	Sorbus scopulina
NAVA	6531	Prunus virginiana	NAVA	6602	Sphaeralcea coccinea
ASC	77857	Pseudocymopterus montanus	NAVA	6247	Sphaeralcea fendleri
NAVA	6513	Psoralidium lanceolatum	NAVA	6544	Sphaeralcea leptophylla
NAVA	8214	Purshia stansburiana	NAVA	10361	Stenogonum salsuginosum
NAVA	9576	Purshia tridentata	Field site	*	Symphoricarpos rotundifolius/Symphoricarpos oreophilus
ASC	88959	Quercus gambelii	NAVA	11423	Symphyotrichum falcatum
NAVA	7267	Rosa woodsii subsp. arizonica	NAVA	7481	Symphyotrichum lanceolatum subsp. hesperium
NAVA	4893	Rubus idaeus	ASC	88856	Taraxacum laevigatum
NAVA	3224	Rumex acetosella	ASC	106118	Taraxacum officinale
NAVA	6708	Rumex aquaticus var. fenestratus	NAVA	9517	Thelypodiopsis purpusii
NAVA	8145	Rumex crispus	NAVA	929	Thelypodium wrightii
NAVA	4117	Sambucus racemosa	NAVA	3484	Thlaspi montanum
ASC	94394	Saxifraga rhomboidea/Micranthes rhomboidea	NAVA	6423	Tragopogon dubius
NAVA	6190	Senecio eremophilus	NAVA	5796	Trifolium longipes
NAVA	10009	Senecio neomexicanus/Packera neomexicana	NAVA	6646	Vicia americana subsp. americana

Table 3. Genetic plant reference library continued…

Origin	Accession #	Species	Origin	Accession	Species
NCBI	gi|365775303|gb|JN935708.1|	Abies concolor	NCBI	gi|312182251|gb|HM590316.1|	Medicago sativa
NCBI	gi|49823400|gb|AY603268.1|	Achillea millefolium	NCBI	gi|4927011|gb|AF124232.1|	Melilotus alba
NCBI	gi|379322775|gb|JQ392190.1|	Amelanchier utahensis	NCBI	gi|590107589|gb|KF416842.1|	Mertensia franciscana
NCBI	gi|312182174|gb|HM590239.1|	Antennaria parvifolia	NCBI	gi|590107592|gb|KF416845.1|	Mertensia fusiformis
NCBI	gi|260079485|gb|GQ244576.1|	Antennaria rosea	NCBI	gi|86169369|gb|DQ353964.1|	Phleum pratense
NCBI	gi|30721770|gb|AY288216.1|	Arceuthobium douglasii	NCBI	gi|260080157|gb|GQ245248.1|	Phlox hoodii
NCBI	gi|444745861|gb|JX073753.1|	Artemisia arbuscula	NCBI	gi|920668080|gb|KT264132.1|	Picea engelmannii
NCBI	gi|444745897|gb|JX073789.1|	Artemisia bigelovii	NCBI	gi|150416834|gb|EF440560.1|	Picea pungens
NCBI	gi|568218873|gb|KF736812.1|	Artemisia campestris	NCBI	gi|22779316|dbj|AB081127.1|	Pinus ponderosa
NCBI	gi|444745865|gb|JX073757.1|	Artemisia filifolia	NCBI	gi|86169388|gb|DQ353983.1|	Poa annua
NCBI	gi|444745910|gb|JX073802.1|	Artemisia pygmaea	NCBI	gi|86169447|gb|AH015559.1|SEG_DQ354038S	Poa bulbosa
NCBI	gi|4099033|gb|U82016.1|ATU82016	Artemisia tridentata	NCBI	gi|675583636|gb|KF940773.1|	Populus tremuloides
NCBI	gi|575501503|dbj|AB732921.1|	Bromus carinatus	NCBI	gi|19032448|gb|AF348556.1|	Potentilla anserina
NCBI	gi|38892946|gb|AY367959.1|	Bromus ciliatus	NCBI	gi|19032449|gb|AF348557.1|	Potentilla fruticosa
NCBI	gi|312182183|gb|HM590248.1|	Bromus inermis	NCBI	gi|19032454|gb|AF348562.1|	Purshia tridentata
NCBI	gi|560068161|gb|KF600709.1|	Bromus tectorum	NCBI	gi|312182275|gb|HM590340.1|	Rosa woodsii
NCBI	gi|12006496|gb|AF284870.1|	Carex rossii	NCBI	gi|62468163|gb|AY818240.1|	Rubus idaeus
NCBI	gi|312182206|gb|HM590271.1|	Dactylis glomerata	NCBI	gi|291173286|gb|GU591014.1|	Solanum elaeagnifolium
NCBI	gi|312182208|gb|HM590273.1|	Descurainia sophia	NCBI	gi|241897597|gb|GQ149755.1|	Solanum rostratum
NCBI	gi|58978779|gb|AY900368.1|	Draba aurea	NCBI	gi|78099907|gb|DQ180457.1|	Solanum triflorum
NCBI	gi|10281062|gb|AF163523.1|	Fragaria virginiana	NCBI	gi|805307942|gb|KP208387.1|	Sphaeralcea coccinea
NCBI	gi|34765646|gb|AY216058.1|	Helianthus annuus	NCBI	gi|62003415|gb|AY958583.1|	Thelypodium wrightii
NCBI	i|19032438|gb|AF348546.1|	Holodiscus discolor**	NCBI	gi|37195455|gb|AY154800.1|	Thlaspi montanum
NCBI	gi|38565348|gb|AY437961.1|_	Juncus articulatus			
NCBI	gi|7620581|gb|AF211519.1|	Juniperus communis			
NCBI	gi|307602535|gb|HM024577.1|	Juniperus monosperma**			
NCBI	gi|7620588|gb|AF211526.1|	Juniperus osteosperma			
NCBI	gi|307602557|gb|HM024599.1|	Juniperus scopulorum			
NCBI	gi|312182239|gb|HM590304.1|	Koeleria macrantha			
NCBI	gi|225200401|gb|FJ789856.1|	Lithospermum multiflorum			
NCBI	gi|53627278|gb|AY618502.1|	Lupinus argenteus			
NCBI	gi|298570122|gb|GQ488612.1|	Medicago lupulina			

NAVA = Navajo Nation Herbarium
ASC = Deaver Herbarium
*not yet accessioned
**species not included from herbariums or field sites

Table 4. Total number of taxa identified in diets (diet richness).

Taxa	Chuska				Carrizo			
	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)
Species								
Abies concolor		✓				✓		✓
Abies lasiocarpa var lasiocarpa		✓						
Achillea millefolium		✓		✓		✓		✓
Achnatherum hymenoides		✓		✓		✓		
Amelanchier utahensis		✓				✓		
Amsinckia tessellata				✓				✓
Antennaria rosea		✓		✓		✓		✓
Antennaria rosulata		✓		✓		✓		✓
Anthoxanthum odoratum		✓						✓
Arabis fendleri var fendleri		✓		✓		✓		✓
Arceuthobium divaricatum		✓				✓		
Arceuthobium douglasii		✓		✓		✓		✓
Arctostaphylos uva ursi		✓						
Artemisia bigelovii				✓		✓		✓
Artemisia filifolia				✓		✓		✓
Artemisia ludoviciana var latiloba		✓		✓		✓		✓
Artemisia pygmaea				✓		✓		✓
Artemisia scopulorum				✓		✓		
Artemisia tridentata		✓		✓		✓		✓
Astragalus chuskanus		✓						✓
Atriplex canescens var canescens		✓		✓				✓
Atriplex saccaria				✓				✓
Berberis repens		✓				✓		
Bromus ciliatus		✓		✓		✓		
Bromus inermis		✓						
Bromus tectorum		✓		✓		✓		✓
Campanula parryi		✓						
Campanula rotundifolia		✓				✓		✓
Cardamine cordifolia		✓		✓		✓		✓
Carex athrostachya		✓				✓		
Carex geophila		✓		✓		✓		✓
Carex occidentalis		✓		✓				✓
Carex praegracilis		✓						
Carex rossii		✓						
Carex utriculata						✓		
Cercocarpus montanus		✓		✓				✓
Chaenactis macrantha								✓
Chamerion latifolium		✓						
Chenopodium leptophyllum				✓		✓		✓
Chloracantha spinosa								✓
Chrysothamnus nauseosus subsp bigelovii				✓				
Conyza canadensis		✓				✓		✓
Dactylis glomerata	✓	✓		✓		✓		✓
Delphinium barbeyi		✓						
Descurainia sophia						✓		
Eleocharis acicularis		✓						
Ephedra torreyana		✓				✓		✓
Ephedra viridis								✓
Eriogonum alatum		✓						✓
Eriogonum cernuum		✓		✓				✓
Eriogonum jamesii		✓				✓		
Eriogonum umbellatum var subaridum		✓		✓		✓		✓
Erodium cicutarium		✓		✓		✓		✓

Table 4. Total number of taxa identified in diets (diet richness) continued…

Taxa	Chuska				Carrizo			
	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)
Species								
Erodium texanum								✓
Festuca arundinacea		✓						✓
Galium boreale		✓						
Geranium caespitosum		✓		✓		✓		✓
Geranium richardsonii		✓				✓		
Geranium sylvaticum		✓						
Geranium viscosissimum		✓		✓		✓		✓
Geum macrophyllum		✓		✓		✓		✓
Geum triflorum		✓		✓		✓		✓
Glycyrrhiza lepidota		✓		✓				✓
Gutierrezia sarothrae		✓		✓				✓
Hedysarum boreale		✓		✓				✓
Helianthus annuus		✓		✓		✓		
Helianthus petiolaris		✓		✓		✓		✓
Hesperostipa comata		✓						✓
Heterotheca villosa var villosa		✓		✓		✓		
Heuchera parviflora		✓				✓		
Holodiscus dumosus					✓			
Hordeum vulgare		✓						
Juncus articulatus subsp articulatus				✓				✓
Juncus balticus subsp ater		✓						
Juncus dichotomus		✓						
Juncus drummondii		✓		✓		✓		
Juncus longistylis		✓				✓		
Juncus saximontanus		✓						
Juncus triglumis		✓						
Juniperus monosperma		✓		✓		✓		✓
Juniperus osteosperma				✓				✓
Juniperus scopulorum				✓		✓		✓
Koeleria macrantha		✓		✓		✓		✓
Lathyrus eucosmus		✓						
Lithospermum multiflorum				✓				
Lonicera korolkowii						✓		✓
Machaeranthera canescens var canescens				✓				✓
Machaeranthera grindelioides		✓		✓		✓		✓
Malus pumila						✓		
Malus sylvestris		✓				✓		
Menyanthes trifoliata		✓						
Mertensia fusiformis		✓				✓		✓
Osmorhiza depauperata		✓						
Packera multilobata		✓				✓		
Packera neomexicana		✓		✓		✓		✓
Penstemon strictiformis		✓						✓
Phalaris arundinacea		✓						
Phleum pratense		✓				✓		✓
Phlox hoodii		✓				✓		
Phoradendron juniperinum				✓				✓
Picea pungens		✓		✓		✓		
Pinus aristata		✓				✓		
Pinus edulis		✓	✓	✓		✓	✓	
Pinus ponderosa	✓	✓	✓	✓	✓	✓		
Pinus ponderosa var. scopulorum		✓						
Piptatherum micranthum		✓		✓				

Table 4. Total number of taxa identified in diets (diet richness) continued…

Taxa	Chuska				Carrizo			
	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)
Species								
Poa annua		✓		✓		✓		
Poa bulbosa		✓		✓		✓		✓
Poa compressa		✓						
Poa palustris		✓				✓		
Poa pratense		✓		✓		✓		✓
Polygonum amphibium		✓						
Polygonum aviculare		✓						
Polygonum douglasii subsp johnstonii		✓				✓		
Populus tremuloides		✓						✓
Populus x acuminata		✓						
Potentilla fruticosa		✓		✓		✓		✓
Potentilla hippiana		✓		✓		✓		✓
Prunus virginiana		✓				✓		✓
Pseudocymopterus montanus		✓				✓		
Pseudotsuga menziesii	✓	✓		✓		✓		
Puccinellia nuttalliana						✓		
Purshia tridentata		✓		✓		✓		✓
Quercus gambelii		✓		✓		✓		✓
Rosa woodsii		✓				✓		
Rumex acetosella		✓		✓		✓		✓
Rumex aquaticus var fenestratus		✓						
Rumex crispus		✓						
Sambucus racemosa		✓		✓		✓		✓
Saxifraga rhomboidea		✓						
Senecio spartioides		✓				✓		
Silene drummondii		✓						
Silene latifolia		✓						
Silene noctiflora		✓						
Silybum marianum		✓						
Solanum triflorum						✓		
Solidago multiradiata		✓						
Sorbus scopulina		✓				✓		
Sphaeralcea leptophylla				✓				✓
Stenogonum salsuginosum		✓		✓		✓		✓
Symphoricarpos rotundifolius		✓		✓		✓		✓
Symphyotrichum falcatum		✓		✓		✓		✓
Symphyotrichum lanceolatum subsp hesperium		✓		✓				✓
Taraxacum laevigatum		✓						
Taraxacum officinale		✓		✓		✓		✓
Tetradymia canescens				✓				✓
Tragopogon dubius		✓		✓		✓		✓
Trifolium longipes		✓		✓		✓		✓
Vicia americana subsp americana						✓		

Table 4. Total number of taxa identified in diets (diet richness) continued…

Taxa	Chuska				Carrizo			
	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)
Genus								
Abies		✓				✓		✓
Achillea	✓	✓		✓		✓		✓
Achnatherum		✓		✓		✓		
Amelanchier	✓	✓			✓	✓		
Amsinckia				✓				✓
Antennaria	✓	✓		✓	✓	✓		✓
Anthoxanthum		✓						✓
Arabis		✓		✓		✓		✓
Arceuthobium		✓		✓		✓		✓
Arctostaphylos		✓						
Artemisia		✓	✓	✓	✓	✓	✓	✓
Aster		✓		✓				✓
Astragalus	✓	✓			✓			✓
Atriplex		✓		✓				✓
Berberis	✓	✓				✓		
Betula		✓						
Bromus	✓	✓		✓	✓	✓		✓
Campanula		✓				✓		✓
Cardamine		✓		✓		✓		✓
Carex	✓	✓		✓	✓	✓		✓
Cercocarpus		✓		✓				✓
Chaenactis								✓
Chamerion		✓						
Chenopodium				✓		✓		✓
Chloracantha								✓
Chrysothamnus				✓				
Conyza		✓				✓		✓
Dactylis		✓		✓		✓		✓
Delphinium		✓						
Descurainia						✓		
Eleocharis	✓	✓						
Elymus		✓		✓				
Ephedra		✓				✓		✓
Erigeron	✓				✓	✓		
Eriogonum		✓		✓		✓		✓
Erodium		✓		✓		✓		✓
Festuca		✓						✓
Fragaria					✓			
Galium		✓						
Geranium		✓		✓		✓		✓
Geum		✓		✓		✓		✓
Glycyrrhiza		✓		✓				✓
Gutierrezia		✓		✓				✓

Table 4. Total number of taxa identified in diets (diet richness) continued…

Taxa	Chuska				Carrizo			
	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)
Genus								
Hedysarum		✓		✓				✓
Helianthus		✓		✓		✓		✓
Hesperostipa								✓
Heterotheca		✓		✓		✓		
Heuchera		✓				✓		
Holodiscus								
Hordeum		✓						
Juncus		✓		✓	✓	✓		✓
Juniperus		✓	✓	✓		✓	✓	✓
Koeleria		✓		✓	✓	✓		✓
Lathyrus		✓						
Lesquerella							✓	
Lithospermum	✓			✓	✓		✓	
Lonicera						✓		✓
Lupinus					✓			
Machaeranthera		✓		✓		✓		✓
Malus		✓				✓		
Medicago	✓							
Menyanthes		✓						
Mertensia		✓				✓		✓
Osmorhiza		✓						
Packera		✓		✓		✓		✓
Penstemon		✓				✓		✓
Phalaris		✓						
Phleum	✓	✓				✓		✓
Phlox		✓			✓	✓		
Phoradendron	✓		✓	✓			✓	✓
Picea		✓		✓		✓		
Pinus	✓	✓		✓		✓		✓
Piptatherum		✓		✓				
Poa	✓	✓		✓	✓	✓		✓
Polygonum		✓				✓		
Populus		✓						✓
Potentilla		✓		✓	✓	✓		✓
Prunus		✓				✓		✓
Pseudocymopterus		✓				✓		
Pseudotsuga		✓		✓		✓		
Puccinellia						✓		
Purshia	✓	✓	✓	✓		✓	✓	✓
Quercus	✓	✓		✓	✓	✓		✓
Ranunculus		✓						
Rosa		✓				✓		
Rumex		✓		✓		✓		✓
Sambucus		✓		✓		✓		✓
Saxifraga		✓						
Senecio		✓				✓		
Silene		✓						
Silybum		✓						
Solanum			✓			✓	✓	

Table 4. Total number of taxa identified in diets (diet richness) continued…

Taxa	Chuska				Carrizo			
	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)	Summer (Micro)	Summer (NGS)	Winter (Micro)	Winter (NGS)
Genus								
Solidago		✓						
Sorbus		✓				✓		
Sphaeralcea				✓			✓	✓
Stenogonum		✓		✓		✓		✓
Symphoricarpos	✓	✓		✓	✓	✓		✓
Symphyotrichum		✓		✓		✓		✓
Taraxacum	✓	✓		✓	✓	✓		✓
Tetradymia				✓				✓
Tragopogon		✓		✓		✓		✓
Trifolium		✓		✓		✓		✓
Vicia						✓		
Family								
Adoxaceae		✓		✓		✓		✓
Amaranthaceae		✓		✓		✓		✓
Apiaceae		✓				✓		
Asteraceae	✓	✓	✓	✓	✓	✓	✓	✓
Berberidaceae	✓	✓				✓		
Betulaceae		✓						
Boraginaceae	✓	✓		✓	✓	✓	✓	✓
Brassicaceae		✓	✓	✓		✓	✓	✓
Campanulaceae		✓				✓		✓
Caprifoliaceae	✓	✓		✓	✓	✓		✓
Caryophyllaceae		✓						
Cupressaceae		✓	✓	✓		✓	✓	✓
Cyperaceae	✓	✓		✓	✓	✓		✓
Ephedraceae		✓				✓		✓
Ericaceae		✓						
Fabaceae	✓	✓		✓	✓	✓		✓
Fagaceae	✓	✓		✓	✓	✓		✓
Geraniaceae		✓		✓		✓		✓
Juncaceae		✓		✓	✓	✓		✓
Malvaceae				✓			✓	✓
Menyanthaceae		✓						
Onagraceae		✓						
Pinaceae	✓	✓	✓	✓	✓	✓	✓	✓
Plantaginaceae		✓				✓		✓
Poaceae	✓	✓		✓	✓	✓		✓
Polemoniaceae		✓			✓	✓		
Polygonaceae		✓		✓		✓		✓
Ranunculaceae		✓						
Rhamnaceae		✓						
Rosaceae	✓	✓	✓	✓	✓	✓	✓	✓
Rubiaceae		✓						
Salicaceae		✓						✓
Santalaceae/Viscaceae	✓	✓	✓	✓		✓	✓	✓
Saxifragaceae		✓				✓		
Solanaceae			✓			✓	✓	
Ulmaceae		✓						

REFERENCES

Anthony, R. and N. Smith. 1974. Comparison of Rumen and Fecal Analysis to Describe Deer Diets. Journal of Wildlife Management 38:535–540.

Anthony, R. and N. Smith. 1977. Ecological Relationships between Mule Deer and White-Tailed Deer in Southeastern Arizona. Ecological Monographs 47:255-277.

Baamrane, M. A. A., W. Shehzad, A. Ouhammou, A. Abbad, M. Naimi, E. Coissac, P. Taberlet and M. Znari. 2012. Assessment of the food habits of the Moroccan dorcas gazelle in M'Sabih Talaa, west central Morocco, using the trnL approach. PloS One 7:e35643.

Brown, D.E. and C.H. Lowe. Digital version of David E. Brown and Charles H. Lowe's 1981 mapv[map]. 1:1,000,000. "Brown and Lowe's Biotic Communities of the Southwest". June 2012. http://www.arcgis.com/home/item.html?id=42b40ca784294e13a3360883339cb0ff. (August 10, 2015).

Budarf, A. C., D. D. Burfeind, W. K. W. Loh and I. R. Tibbetts. 2011. Identification of seagrasses in the gut of a marine herbivorous fish using DNA barcoding and visual inspection techniques. Journal of Fish Biology 79:112–21.

Czernik, M., P. Taberlet, M. Swisłocka, M. Czajkowska, N. Duda and M. Ratkiewicz. 2013. Fast and efficient DNA-based method for winter diet analysis from stools of three cervids: moose, red deer, and roe deer. Acta theriologica 58:379–386.

Deschamps, S., V. Llaca and G. D. May. 2012. Genotyping-by-Sequencing in Plants. Biology 1:460–83.

Gielly, L. and P. Taberlet. 1994. The Use of Chloroplast DNA to Resolve Plant Phylogenies: Noncoding versus rbcL Sequences. Molecular Biology and Evolution 11:769–777.

Gill, R., L. Carpenter, R. Bartmann, D. Baker and G. Schoonveld. 1983. Fecal analysis to estimate mule deer diets. The Journal of Wildlife Management. 47:902–915.

Hamilton, J. P. and C. R. Buell. 2012. Advances in plant genome sequencing. The Plant Journal 70:177–190.

Hansen, R. and L. Reid 1975. Diet overlap of deer, elk, and cattle in southern Colorado. Journal of Range Management 28:43–47.

Hibert, F., P. Taberlet, J. Chave, C. Scotti-Saintagne, D. Sabatier and C. Richard-Hansen. 2013. Unveiling the Diet of Elusive Rainforest Herbivores in Next Generation Sequencing Era? The Tapir as a Case Study. PloS One 8:e60799.

Holechek, J., M. Vavra and R. Pieper. 1982. Botanical Composition Determination of Range Herbivore Diets: A Review. Journal of Range Management 35:309–315.

Hubbard, R. and R. Hansen. 1976. Diets of wild horses, cattle, and mule deer in the Piceance Basin, Colorado. Journal of Range Management 29:389–392.

Kowalczyk, R., P. Taberlet, E. Coissac, A. Valentini, C. Miquel, T. Kamiński and J. M. Wójcik. 2011. Influence of management practices on large herbivore diet—Case of European bison in Białowieża Primeval Forest (Poland). Forest Ecology and Management 261:821–828.

Marshal, J. P., V. C. Bleich, N. G. Andrew and P. R. Krausman. 2004. Seasonal forage use by desert mule deer in southeastern California. The Southwestern Naturalist 49:501–505.

Marshal, J. P., V. C. Bleich, P. R. Krausman, M-L. Reed and A. Neibergs. 2012. Overlap in diet and habitat between the mule deer (Odocoileus homionus) and feral ass (Equus asinus) in the Sonoran Desert. The Southwestern Naturalist 57:16–25.

Naidu, A., L. A. Smythe, R. W. Thompson and M. Culver. 2011. Genetic Analysis of Scats Reveals Minimum Number and Sex of Recently Documented Mountain Lions. Journal of Fish and Wildlife Management 2:106–111.

National Center for Biotechnology Information (NCBI). Nucleotide search. http://www.ncbi.nlm.nih.gov/nucleotide. Accessed January, 2015.

Nejstgaard, J. C., M. E. Frischer, P. Simonelli, C. Troedsson, M. Brakel, F. Adiyaman, A. F. Sazhin and L. F. Artigas. 2007. Quantitative PCR to estimate copepod feeding. Marine Biology 153:565–577.

Newmaster, S. G., I. D. Thompson, R. A. D. Steeves, A. R. Rodgers, J. Fazekas, Aron, J. R. Maloles, R. T. McMullin and J. M. Fryxell. 2013. Examination of two new technologies to assess the diet of woodland caribou: video recorders attached to collars and DNA barcoding 900:897–900.

Pegard, A., C. Miquel, A. Valentini, E. Coissac, F. Bouvier, D. François, P. Taberlet, E. Engel and F. Pompanon. 2009. Universal DNA-Based Methods for Assessing the Diet of Grazing Livestock and Wildlife from feces. Journal of agricultural and food chemistry 57:5700–6.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rayé, G., C. Miquel, E. Coissac, C. Redjadj, A. Loison and P. Taberlet. 2010. New insights on diet variability revealed by DNA barcoding and high-throughput pyrosequencing: chamois diet in autumn as a case study. Ecological Research 26:371–374.

Research and Testing Laboratory. Data Analysis Methodology. Nov. 11, 2014. http://www.researchandtesting.com/docs/Data_Analysis_Methodology.pdf

Soininen, E. M., A. Valentini, E. Coissac, C. Miquel, L. Gielly, C. Brochmann, A. K. Brysting, J. H. Sønstebø, R. A. Ims, N. J. Yoccoz and P. Taberlet. 2009. Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. Frontiers in Zoology 6:16.

Sparks, D. and J. Malechek. 1968. Estimating percentage dry weight in diets using a microscopic technique. Journal of Range Management Archives 21:264–265.

Stewart, D. 1970. Survival during digestion of epidermis from plants eaten by ungulates. Revue de Zoologie et de Botanique Africaines:343–348.

Stewart, K. M., R. T. Bowyer, J. G. Kie, B. L. Dick and M. Ben-David. 2003. Niche partitioning among mule deer, elk, and cattle: Do stable isotopes reflect dietary niche? Ecoscience 10:297–302.

Taberlet, P., L. Gielly, G. Pautou and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17:1105–9.

Taberlet, P., E. Coissac, F. Pompanon, L. Gielly, C. Miquel, A. Valentini, T. Vermat, G. Corthier, C. Brochmann and E. Willerslev. 2007. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic Acids Research 35:e14.

a.)Valentini, A., F. Pompanon and P. Taberlet. 2009. DNA barcoding for ecologists. Trends in Ecology & Evolution 24:110–7.

b.)Valentini, A., C. Miquel, M. A. Nawaz, E. Bellemain, E. Coissac, F. Pompanon, L. Gielly, C. Cruaud, G. Nascetti, P. Wincker, J. E. Swenson and P. Taberlet. 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. Molecular ecology resources 9:51–60.

Vavra, M. and J. Holechek. 1980. Factors influencing microhistological analysis of herbivore diets. Journal of Range Management 33:371–374.

Wildlife Habitat and Nutrition Laboratory. “Botanical Composition – Microhistology Methods”. Lab protocol. Washington State University. Pullman, Washington. n.d. Print.

Zhang, J., K. Kobert, T. Flouri and A. Stamatakis. 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics (Oxford, England) 30:614–20.

APPENDIX D: PRESENTATIONS

Society for the Advancement of Chicanos and Native Americans in Science (SACNAS) National Conference (October, 2016) – Long Beach, CA – Conservation Biology Panel – oral presentation and Q&A Session – Title: "Expanding Wildlife Assessment Strategies: A Novel Genetics Approach to Investigate Mule Deer Diet on the Navajo Nation"

Native American Fish and Wildlife Society (NAFWS) Southwest Region Conference (August, 2016) – Twin Arrows, AZ – oral presentation – Title: "Expanding techniques to understand herbivore diet on tribal lands: Investigating mule deer diet on the Navajo Nation as a model"

Joint Annual Meeting (JAM) of the New Mexico and Arizona Chapters of The Wildlife Society (TWS) and American Fisheries Society (AFS) (February, 2016) – Flagstaff, AZ – oral presentation – Title: "Exploring past and present techniques to investigate composition variables for mule deer diet on the Navajo Nation"

The Wildlife Society (TWS) National Conference (October, 2015) – Winnipeg, Manitoba, Canada – poster presentation – Title: "Using past and present techniques to estimate diet richness and dietary taxonomic resolution for mule deer on the Navajo Nation"

University of Arizona School of Natural Resources and the Environment (SNRE) Plenary Earth Week Poster Session (April, 2015) – Tucson, AZ – poster presentation – Title: "Mule deer diet analyses: Comparing past with present techniques"

Joint Annual Meeting (JAM) of the New Mexico and Arizona Chapters of The Wildlife Society(TWS) and American Fisheries Society (AFS) (February, 2015) – Las Cruces, NM – oral presentation – Title: "Mule deer diet analyses: Comparing past with present techniques"