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Measuring Trace Element Concentrations in Artiodactyl Cannonbones using Portable X-Ray Fluorescence

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MEASURING TRACE ELEMENT CONCENTRATIONS IN ARTIODACTYL CANNONBONES USING PORTABLE X-RAY FLUORESCENCE

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by

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ABSTRACT

MEASURING TRACE ELEMENT CONCENTRATIONS IN ARTIODACTYL CANNONBONES USING PORTABLE X-RAY FLUORESCENCE

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Artiodactyl bones are the most common faunal remains found in Washington prehistoric archaeology sites, but they are often too fragmented to accurately identify a family, genus, or species. Traditional faunal analysis can only organize unidentifiable bone fragments into size class, and chemical methods often require the destruction of bone samples. In this thesis research, I tested a new, nondestructive faunal analysis technique using portable X-ray fluorescence (pXRF) to measure trace element concentrations in comparative collection and archaeological bone samples. Using cannonbones from five different artiodactyl species, I collected trace element data from 50 comparative collection specimens and 18 archaeological specimens previously identified to species. I used a Random Forest classification analysis to predict the family and species of modern comparative and archaeological specimens based on collected trace element data. Species identification accuracy was 70% for modern specimens and 22% for archaeological specimens, while family identification accuracy was 82% for modern specimens and 67% for archaeological specimens. These results suggest that identification pXRF method used in this thesis is promising, but would require further work to be definitive.

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CHAPTER I

INTRODUCTION

Broken bones compose the majority of faunal remains found in archaeology sites and have the potential to provide a wealth of knowledge regarding past subsistence practices, but identifications are limited using current methods (O'Connor 2008). Technological advancements in trace element research have the ability to expand our current identification techniques and strengthen our ability to taxonomically identify fragmented bones (Buddhachat et al. 2016a).

Due to the lack of diagnostic features, artiodactyl long bones, one of the most common sources of archaeological bone fragments in the Pacific Northwest (Lyman 1995:239-241) are often classified only by size class (Davis 1987:35). This problem stems from several compounding issues. Human and non-human taphonomic processes often leave bones fragmented and unidentifiable (Lyman 1994). In addition, the analysis of archaeological faunal material is often overlooked by project directors due to a lack of time and economic resources (Lipovitch 2013). Finally, the most common mammal groups found in archaeology sites in the Pacific Northwest are a number of similarly sized, and skeletally similar species including deer (*Odocoileus* sp.), bighorn sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus)*, and pronghorn antelope (*Antilocapra americana*) (Butler and Campbell 2004; Lyman 2007). The general similarity and lack of distinctive features of long bone fragments makes identifying artiodactyl species difficult (Todd and Rapson 1988:308; Zeder and Lapham 2010).

In an effort to identify artiodactyl bone fragments, past research has focused on finding osteological differences in skeletal features by way of morphology (e.g., Lawrence 1951; Olson 1964; Prummel and Frisch 1986). Although this method has proven to be effective in species identification (Driver 2011), it can only be used with diagnostic bone samples and does not work with most bone fragments. This method also takes a large quantity of time to record and compare each bone to reference samples. Further attempts to identify bone fragments have utilized chemical methods. Mass spectrometry, a common chemical analysis method, has been used for measuring bone chemistry in archaeological bone fragments (Buckley et al. 2009). A mass spectrometer is used to find identifiable bone collagen, but this requires a portion of the bone fragment to be ground into a powder, destroying the sample in the process (Buckley et al. 2009).

Although the use of skeletal morphology for species identification is nondestructive, it is typically a subjective analysis method for zooarchaeologists (Driver 2011; Prummel and Frisch 1986). Mass spectrometry on the other hand is replicable, but is a destructive analysis method (Buckley et al. 2009). Very little research has been done to develop techniques that are both nondestructive and replicable. Recent research has used portable X-ray fluorescence (pXRF) to identify species through trace element concentrations (Buddhachat et al. 2016a), but specific trace element research pertaining to artiodactyls and archaeological applications are absent.

The purpose of this thesis is to measure how bone trace elements differ in metapodial cannonbones bones from species in the Washington and Wyoming small artiodactyl group. I will use a Bruker Tracer 5i X-ray fluorescence analyzer to test

cortical bone samples found in cannonbones from two species of deer (*Odocoileus hemionus and Odocoileus virgininaus)*, bighorn sheep (*Ovis canadensis)*, mountain goat (*Oreamnos americanus*), and pronghorn antelope (*Antilocapra americana*). This study will omit caribou (*Rangifer tarandus*), as this species is located only in the far northeast corner of the state of Washington (Rominger et al. 1996:719). I will conduct my analysis at museum repositories (Central Washington University, the Burke Museum, and the Conner Museum) using identical settings and calibration of the pXRF unit. My principal research question is: are trace element concentrations consistent within an individual and species, and diagnostically different between the five species tested?

To answer this research question, the methodology of this thesis is split into three major objectives. Objective 1: Measure trace element concentrations with nondestructive pXRF on comparative collection cannonbone samples from the Burke Museum, Central Washington University, and the Conner Museum. Objective 2: Construct predictive identification models for each species by compiling measurements taken from comparative collection bone and applying them in statistical tests. Objective 3: Apply methods to a pilot study that will measure trace element concentrations from previously identified archaeological bone samples to compare results with comparative collection bone samples.

This thesis research will be significant to the field of archaeology because it will test a new nondestructive method for identifying bone fragments, which was previously limited to destructive and generalized techniques. Although trace element bone studies are currently limited due to the recent development of this method, it does show promise

for answering biochemical questions (Buddhachat et al. 2016a). New research needs to be conducted to continue the development of reliable nondestructive methods for measuring trace element concentrations.

Now that pXRF analyzers are becoming cost effective and readily used in some archaeological research, specifically for lithic sourcing, pigment and paint analysis, pottery provenance, and historic metals and glass analysis (Shackley 2011:12-14; Janssens et al. 2000), this research will explore a new use for this equipment. Portable X-ray fluorescence as a new method provides opportunities for instantaneous results which can save both time and resources.

This research is significant because of the possibilities for in-the-field analysis of bone fragments, and can enhance traditional bone fragment identification methods by providing more quantitative biochemical data. Nondestructive bone chemical analysis can improve our identification rate of bone fragments, something that has been a longstanding struggle for faunal analysis (Todd and Rapson 1988). Being able to identify larger quantities of bone fragments allows us to better reconstruct past animal populations, as well as human subsistence patterns throughout time (Wolverton 2014).

Organization of Thesis

This thesis continues in Chapter II where I discuss pertinent literature on such topics as species identification methods for archaeological bone, chemical analysis of bone, habitat and diet, and statistical tools for evaluating differences in bone. Chapter III discusses the methodology of the three objectives in this study that include measuring

trace element concentrations in modern comparative collection bone specimens, developing predicative identification models using statistical analysis, and a pilot study testing archaeological bones specimens. Chapter IV discusses the results from the statistical analyses on both the modern comparative collection and archaeological bone specimens. Chapter V, the final chapter, contains the discussion and conclusions which discuss results, key observations, and opportunities for future research.

CHAPTER II

BONE AND STATISTICAL ANALYSIS

In the analysis of bone, whether it be archaeological or modern, there are several standard methods that can be used. A discussion of well-studied species identification methods including skeletal morphology and DNA analysis is used in this section to support X-ray fluorescence and trace elements as a new method for identifying animal remains. Following the species identification section, there is discussion of chemical analyses in bone studies, and the effects of habitat and diet on bone chemistry of the selected species in this study. The final section of this chapter will review a series of statistical methods that are used to determine significant differences in modern and archaeological bones.

Species Identification Methods for Archaeological Bone

Faunal assemblages are considered to provide a wealth of knowledge for the purpose of understanding past human behavior. Since the inception of hunting by *Homo habilis* and *Homo erectus* (Lee-Thorp 2000:566), humans and human ancestors have shared an important relationship with animals for the purpose of survival. Bones are great indicators for various human behaviors. In the Pacific Northwest, the abundance of a particular species could provide new insights into environmental pressures or shifts in hunting and fishing practices (Butler and Campbell 2004).

The study of archaeological bones has been prevalent throughout the history of archaeology (Reitz and Wing 1999), but has only developed as a major sub-discipline starting in the 1960s with the emergence of processual archaeology (Binford 1962). With a newfound movement to study archaeological bone, it has been a primary focus to identify the species of animal remains found within a faunal assemblage. Identifying species from archaeological bones, what paleontologists sometimes call "species diagnosis" (e.g., Emery-Wetherell and Davis 2018), is invaluable for understanding cultural subsistence. There have been a few tried and true methods used to identify species from archaeological bone. The original method of species identification used by zooarchaeologists uses a classification system that groups bones by like features such as size or shape (Driver 2011:20). Comparative zooarchaeological collections and species-specific skeletal morphology are often used to assist in the identification of archaeological bones and bone fragments (Driver 2011:23). Studies that have developed these diagnostic osteological features have been invaluable for comparison to archaeological bones, especially for artiodactyls (e.g., Brown and Gustafson 1979; Lawrence 1951; Olson 1964).

Molecular methods have also been used within recent years to identify archaeological bone with more accuracy. DNA studies have come a long way in zooarchaeology. DNA can be identified in well-preserved bones that still maintain their organic structure. (Hagelberg and Clegg 1991:45-46), and can be used to differentiate bones by species (e.g., Grier et al. 2013). Grier et al. (2013) used DNA

from 70 salmonid vertebrae from the Dionisio Point site to discriminate three different salmonid species, which correlate to harvesting and occupation seasons.

Bone chemistry is also becoming a new method for species identification of archaeological bone fragments. Through the use of mass spectrometry, bone fragments are able to be identified to species from unique spectra of collagen peptides (peptide mass fingerprints or PMF) found inside the bone (Buckley and Collins 2011:1). Buckley et al. (2009: 3851-3853) tested this zooarchaeology by mass spectrometry (ZooMS) method on 32 known archaeological mammal bones. These tests concluded that peptides could identify 26 out of the 32 (81%) specimens using their modern equivalent as a comparative. Richter et al. (2011) also used the ZooMS method on 28 specimens from eight different species of modern and archaeological fish bone. Using peptide fingerprints within bone collagen, Richter et al. (2011) found that ZooMS could identify 25 out of the 28 (89%) fish specimens. Both DNA extraction and collagen peptide analysis are destructive sciences that requires bone samples to be ground to a fine powder (Buckley et al. 2009:3844; Hagelberg and Clegg 1991:46). Although destructive, these two techniques have demonstrated their validity.

Chemical Analysis of Bone

Early use of XRF on osseous tissue primarily focused on lead concentration in human bones as a way to determine how a harmful element like lead is absorbed into bone as a result of increased environmental exposure (Ahlgren et al. 1976; Hu et

al. 1991). Within recent years, trace elements and XRF have been primary analysis tools for bones. Bones tend to absorb elements from the surrounding environment, especially as an effect of diet. Bone trace element literature has primarily focused on how diet affects overall bone chemistry.

Reconstructing dietary behaviors is made possible by using mass spectrometry to look at a variety of isotopes and trace elements, including the carbon isotopes that are transferred to bone collagen with the consumption of certain variety of plants (Krueger and Sullivan 1984:206-213; Lee-Thorp et al. 1989:585). Isotope research has primarily focused on reconstructing paleodietary behavior of humans, including investigations of marine and terrestrial diets (Newsome et al. 2004; Richards and Hedges 1999; van der Merwe 1982). Diets based mostly on marine food sources can be determined by measuring the ^{15}N isotope value (Richards and Hedges 1999:717-719). Carbon isotopes are also used to construct the diets by various ungulate species (Richards and Hedges 1999: 717). Bones that show a higher concentration of ^{13}C compared to ^{12}C isotopes correlate to a diet based on C_4 photosynthetic pathway plants such as drought tolerant grasses, and often consumed by grazers like bighorn sheep, and intermediate feeders like pronghorn (Irwin et al. 1993: 415; Koerth 1984:561). Bones that show lower amounts of ^{13}C compared to ^{12}C isotopes correlate to a diet based on C_3 photosynthetic pathway plants such as trees, forbs, and shrubs, frequently chosen by browsers like deer (Cormie and Schwarcz 1996: 4161-4162).

Isotope analysis can be used to answer behavioral questions. New studies have focused on animal domestication practices (Barton et al. 2009; Britton et al. 2008; Thornton et al. 2016). During domestication, humans feed animals a diet of vegetation that differs from the normal diet of their wild counterparts, thus altering the stable isotope concentrations in their bones (Barton et al 2009: 5524). Due to the differences in diet between wild and domesticated species, stable isotopes of carbon and nitrogen are being used to analyze turkey remains to distinguish between wild and domesticated turkeys found in Maya lowland archaeology sites (Thornton et al. 2016: 583-586).

Mass spectrometry, the principal method used for intensive isotope analysis of animal and human osseous tissue, has been shown to be a valid method for archaeological remains. O'Connell and Hedges (1999) used mass spectrometry to test hair and bone samples from both archaeological and modern specimens in order to gain an understanding of how carbon and nitrogen isotopes differ between tissues, and if isotopes differ between archaeological and modern samples. Their findings demonstrated that modern and archaeological bone samples had similar isotopic values (O'Connell and Hedges 1999:663-664). This study did find a difference between hair and bone samples which indicates that isotope concentrations differ between different tissues of the body (O'Connell and Hedges 1999:664-645).

Other concerns regarding the use of mass spectrometry and the chemical analysis of bone stem from chemical alteration in post depositional environments, also known as diagenesis (Nelson et al. 1986: 1941-1942). Diagenesis in its most

basic of forms is the process by which the organic phase of bone collagen is lost, and the mineral phase of hydroxyapatite is altered (Hedges 2002: 319). Although the mineral phase of bone does change over time, there is evidence that isotope ratios and trace element concentrations can still be used for paleodietary analysis depending on the level of bone preservation (Katzenberg and Harrison 1997; Nelson et al. 1986:1947).

While isotopes are not detectable by XRF, trace elements are, and plants contain distinct concentrations of specific trace elements (Brownell and Crossland 1972). Sodium (Na) is a necessary nutrient for C_4 plants, whereas C_3 plants do not require sodium (Brownell and Crossland 1972). Silicon (Si), a component of silicic acid in plants, is often more prevalent in C_4 plants. Manganese (Mn) acts as a catalyst for the metabolism of plants with short growing periods like legumes and forbs, whereas plants with longer periods of growth do not rely on manganese to support their metabolism (McHargue 1922:1597). Trace elements like sodium, silicon, and manganese are absorbed into the bones of the animals who consume these plants which enables the reconstruction of diet. Strontium (Sr) is another element that has concentrations of interest in paleodietary studies (Knudson et al. 2010). Strontium is absorbed into plants from soil and bedrock (Isermann 1981:66), which is then transferred to herbivores who consume these plants (Knudson et al. 2010:2). Strontium levels are higher in carnivores due to its concentrated accumulation at higher trophic levels (Knudson et al. 2010: 2-3). Other elements like arsenic (As) and cadmium (Cd) are also absorbed by plants, and up through the trophic chain through soil and water based on regional ecosystems (Sharma and Shupe 1977).

Trace elements that are absorbed into bone through the trophic chain can be measured with XRF (Snyder and Secord 1982).

Burton and Price (2000) discuss the concern that trace elements are often abused in paleodietary reconstruction due to the uncontrollable nature of contamination from surrounding sediment. Diagenesis is the process that changes the chemical and structural integrity of bone during burial (Hedges 2002:319). Bones tend to lose collagen and absorb mineral deposits from surrounding sediment, thus challenging trace element and XRF research (Hedges 2002: 320). One of the biggest challenges for trace element research is understanding what elements are susceptible to contamination by diagenesis (e.g., Millard and Hedges 1995). The current knowledge on the subject suggests that iron (Fe), manganese (Mn), and aluminum (Al) are common elements absorbed by buried bone (Karkanas et al. 2000; Sillen et al. 1989: 508).

Biochemical bone analysis of trace elements have also demonstrated their validity in the identification of species. For example, there have been several studies which have looked at trace element concentrations in elephant tusk with the objective of combating the black market ivory trade (Buddhachat 2016b; Kautenburger et al. 2004). Kautenburger et al. (2004) demonstrated positive results when they tested ivory samples from Asian and African elephant species using pXRF. They determined that nine selected trace elements including chlorine (Cl), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), nickel (Ni), silicon (Si), and zinc (Zn) could be used to identify the species of elephant based on elements that are absorbed through local soil, and unique to that species. Buddhachat et al. (2016b) completed a similar study using pXRF and a

much larger sample of ivory, finding a set of nine slightly different elements (silicon (Si), sulfur (S), chlorine (Cl), titanium (Ti), manganese (Mn), silver (Ag), antimony (Sb), tungsten (W,) and zirconium (Zr)) consistently differentiated between African and Asian elephants, with zirconium differing the most between the species (Buddhachat et al. 2016b).

New studies have also proposed that trace elements are diagnostic to a species level (e.g., Buddhachat et al. 2016a; Nganvongpanit et al. 2017). Buddhachat et al. (2016a) studied element differences in antler/horn, teeth and humerus bones of 14 different mammal species. They found that chlorine is only present in horn from bovids (identified at 75% accuracy) whereas V, Cr, Zr, Ag, Cd, Sn, and Sb are only present in antler from cervids (identified at 100%). Teeth resulted in eight element concentrations differing among the 14 species (identified at 78.4 % accuracy), and the humerus bones differed in concentrations of 13 elements (Al, Si, Ti, P, Ca, S, V, Cr, Mn, Fe, Zn, Sb, and Zr) with a prediction accuracy of 79.2% (Buddhachat et al. 2016a: 13-17). This study used a pXRF with instrument settings set to measure relative concentrations of elements magnesium (Mg) through bismuth (Bi). In another study using pXRF to discriminate species by differentiation of trace elements, Nganvongpanit et al. (2017) compared enamel and dentine of human teeth against 20 other mammal species. They concluded that human teeth can be discerned 83.2% of the time when compared with teeth from another mammal species.

Nondestructive XRF has recently been used for a variety of identification issues regarding bone. In an effort to organize scattered human remains from mass burials,

Gonzalez-Rodriguez and Fowler (2013) successfully used pXRF to separate five sets of mediaeval aged human skeletal remains that were within the same burial, and found that pXRF could separate individuals scattered in mass graves. An FBI-sponsored study conducted dispersive X-ray spectroscopy on multiple types of human bone and teeth to develop a database for identifying human elements from other animal bones and under a variety of taphonomic conditions (Ubelaker et al. 2002).

The method for using XRF contains its own contentious limitations. For pXRF units to measure at an accurate and optimal level, the analyzer requires samples to be a certain thickness and have a smooth surface. These conditions could be problematic for taking accurate measurements of irregular shaped objects such as bone. (Shugar 2013:180). Speakman and Shackley (2012) propose a set of international standards which can be used for archaeological pXRF research. They note that a common flaw in pXRF research is that without a set of standards, results cannot be validated due to the independent nature of each study and its methods. Recent studies that have utilized pXRF to analyze chemical composition of archaeological bones have all concluded that the method is effective, but requires further study to corroborate the results and refine the method (Buddhachat et al. 2016a; Nganvongpanit et al. 2017; Speakman and Shackley 2012).

Habitat and Diet

In order to distinguish between artiodactyl species using pXRF as proposed for this thesis, there would need to be systematic differences in bone chemistry between species. In this section, I discuss habitat and diet of the sample species to explore some potential differences in chemistry that might be caused by these factors. The species of interest are bighorn sheep, deer (mule and whitetail), mountain goat, and pronghorn.

As mentioned previously, C_3 photosynthetic pathway plants such as shrubs, woody trees, and some grasses grow and are predominant in areas associated with high precipitation. C_4 photosynthetic pathway plants such as sagebrush are considerably more drought-tolerant and can thrive in areas with minimal precipitation (Cormie and Schawrcz 1996). These plants tend to absorb different amounts of trace elements such as sodium, silicon, arsenic, and cadmium based on their photosynthetic pathway and ecosystem they are found in, thus being transferred to the animals who eat them (Brownell and Crossland 1972; Isermann 1981; Sharma and Shupe 1977). Animals considered *grazers* eat primarily grasses (C_4 plants), whereas animals considered *browsers* have a more mixed diet including a larger proportion of C_3 plants like forbs and shrubs (Janis 2008:30).

Deer are considered browsers in terms of their diet (Leslie et al. 1984:765). Deer tend to consume primarily shrubs, forbs, and trees (Campbell and Johnson 1983:489-890). Pronghorn are also primarily browsers. Modern studies from a variety of habitats show they eat mostly forbs and shrubs and little of grasses, and in

sagebrush steppe environments shrubs typically compose more than half of the diet (Yoakum 2004a). Among the species of interest, bighorn sheep and mountain goats are considered grazers (Hibbs 1967; Ruckstuhl 1998). Bighorn sheep move up and down slopes according to the prevalence of available grasses and forbs such as clovers and twinflowers (Festa-Bianchet 1988:580). Mountain goats are also primarily grazers and move around based on the availability of vegetation, but tend to be unselective and consume what is available (Côté and Marco Festa-Bianchet 2003).

Habitat preference differences between the species could also potentially have a role in variable bone chemistry if the substrate where they feed has different chemical makeup, like one might expect if there is different bedrock or sediment geology or vadose water. For Washington, the bedrock may be of limited concern, since the entire area is underlain by broadly similar Columbia River Basalts (Swanson et al. 1979). Bedrock in Wyoming tends to be more variable with different geological compositions (Downey 1986). Differences in surface sediments and ground water may be important. Thus, I will briefly discuss some habitat preference differences that may be relevant. Bighorn sheep prefer subalpine ecoregions, and are often found grazing along grassy slopes or cliffs (Festa-Bianchet 1988:581). Deer tend to prefer edge habitats (Kremstater and Bunnell 1999:121) near coniferous, riparian, and shrub-steppe environments that provide optimal cover and forage potential (Carson and Peek 1987:48). Pronghorn prefer semiarid sagebrush steppe, grassland, and desert environments with flat to low rolling slopes (Yoakum

2004b:409-411). Mountain goats have traditionally occupied steep cliffs along the Columbia River and in subalpine ecoregions east of the Cascades (Côté and Marco Festa-Bianchet 2003).

Statistical Tools for Evaluating Differences in Bone

For this thesis, I have identified several possible statistical methods to analyze the data collected from the five study species using the pXRF. The methods used may vary depending on the nature of the data. The most comparable prior study may be the bone pXRF study by Buddachat et al. (2016a). In that study, the authors calculated mean and standard deviations for each data set and used Student's *t* tests to compare those means. They also used stepwise discriminant function analysis to investigate the elemental content across species. Finally, they employed one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests to determine if there were statistically significant differences in elemental percentages between antler and frontal bone groups. Below, I will discuss these methods and other statistical methods commonly used in bone analysis. In my thesis, I expect to use the following methods in this order: principal component analysis, tests for normality, ANOVA, Tukey's post hoc test, and discriminant function analysis. I may also use *t* tests. They are discussed below.

Another topic which is not a specific statistical test per se is an approach sometimes called EDA, or exploratory data analysis. This approach suggests use of graphs and other devices to explore a data distribution and determine what methods of analysis might be appropriate. Traditional ideology of statistics suggests data

should fit rigid sets of rules for statistical analysis, when in fact archaeology as a social science should be implored to use flexible approaches to analyzing data (Drennan 1996). Exploratory data analysis allows for some of this freedom of subjectivity that social sciences require, and aids in identification of focus in large, multivariate data sets. Tukey, often considered the leader in the EDA movement (Drennan 1996), suggests that confirmatory statistical procedures can only be executed after a series of exploratory procedures (Tukey 1980:23).

The first topic is principal component analysis (PCA), a form of exploratory data analysis. Large data sets, like the trace element data sets in this thesis, often need to be scaled down for further work, and a good method for this is principal component analysis. Principal component analyses are used to transform large sets of correlated variables down to smaller sets of uncorrelated variables by combining variables into separate principal components (Baxter 1995:513). This is done using a matrix of rows (cases) and columns (factors) to organize the data from selected variables (Abdi and Williams 2010:433). Principal component scores are generated by multiplying the cases by their corresponding factors for each variable. Component loadings are the correlation coefficients between the original variables and the new principal component factors. When these factor loadings are squared, they describe what percent of variance of the original variables are explained by the factors (Abdi and Williams 2010:438). Due to the large number of variables, this method is commonly used to simplify morphometric and isotope data from studies that measure bones from many different species or breeds (Haruda 2017:554). Due to the complexity of scaling down large sets of data, a principal

components analysis is executed by using statistics software such as *SPSS* and *R.* Once the PCA is run, the user can identify the variables that account for most of the variation, possibly re-running the PCA with a smaller set of those variables.

Once the appropriate variables have been chosen based on the PCA, the next step is to test the data for multivariate normality, to determine whether methods of further analysis should be parametric or nonparametric. One test for normality is the Shapiro-Wilk test. Normality tests such as the Shapiro-Wilk are methods to assess if data fits a normal distribution (Shapiro and Wilk 1965:591). Normal distributions are symmetrical bell-shaped curves with the majority of the data falling within one standard deviation of the mean (Fletcher and Lock 2005: 60-61). The Shapiro-Wilk test is regarded as the most powerful normality test across all sample sizes when compared to similar normality tests (Shapiro et al. 1968:1371). A sample that passes the Shapiro-Wilk test with a p value of > 0.05 is normally distributed and can be further investigated with parametric tests, while a sample with a p value < 0.05 violates the normality assumption of most parametric methods and requires the use of non-parametric testing.

Distributions that fit the assumptions of normality can then be analyzed using an ANOVA, also known as an analysis of variance. ANOVA is a parametric method for statistically identifying significant differences between more than two distributions (Drennan 1996:171). The variance being measured in this test is equal to the square of the standard deviation for each distribution (Drennan 1996:172) An ANOVA, much like a Student's *t* test, has a dependent variable that is interval or ratio-scaled, and an independent variable with nominal values (Warne 2014:2). Because an ANOVA

measures the difference between two or more distributions, the independent variable will have two or more nominal values. For data that has two or more dependent variables, a MANOVA, or multivariate analysis of variance may be used (Warne 2014:2). An ANOVA can be used to analyze each dependent variable separately, but this procedure increases the likelihood of a Type I error in which the null hypothesis is falsely rejected (Warne 2014:3).

Within zooarchaeology, ANOVA has been used in several studies, three of which I mention here. Domínguez-Rodrigo and Yravedra (2009) use ANOVA to analyze cut mark patterns on a set of African archaeological bones to identify if some bones are more likely to contain cut marks than others. Another study used ANOVA statistics to identify regional plant variability in certain ecosystems using carbon isotope concentrations in deer bones (Emery and Thornton 2008). Stahl (2005) implemented principal component analysis, ANOVA, MANOVA, and other tests to question if there are osteological differences between wild and domesticated, male and female Muscovy ducks. Wild and domesticated duck specimens were examined using 64 standard osteometric measurements from comparative collections. Multivariate (MANOVA) and univariate (ANOVA) analyses of variance were used to test the null hypothesis that group mean measurements are equal between populations of male and female, domesticated and wild ducks (Stahl 2005: 917). Using means and 95% confidence intervals plotted around each sex mean, Stahl (2005:921) suggests that these same values can be used to distinguish unknown bones into three groups: (1) small domestic females, (2) large domestic males, and (3) a mixed group of small domestic males and wild ducks of both sexes. Three

archaeological specimens of Muscovy ducks were then classified using the means and 95% confidence intervals, with two determined to be domestic males and a third placed into the mixed group (Stahl 2005:925).

Tukey's post hoc test is an additional test that can be applied to the results of a ANOVA test to determine which pairs of data are significantly different. Although an ANOVA may indicate if there are significant differences between sample populations, it will not specify where those differences are (Sheskin 2007a:891). Tukey's post hoc test is calculated by dividing the differences between a paired set of means by the square root of the mean squared within, divided by the number of observations (Sheskin 2007:892). If this calculation is larger than the associated value from the Tukey's critical value table, then there is a significant difference between the selected means (Sheskin 2007:893). In determining what trace element ratios significantly differed between mammalian osseous tissues of different species, Buddhachat et al. (2016a) used a Tukey's post hoc test following their analysis of variance test.

A final step in some studies similar to this thesis is a discriminant function analysis. Unlike the topics covered above, discriminant function analysis is a method to classify data into groups, not a specific statistical test (Sheskin 2007:1525). This method involves developing linear equations, also known as discriminant functions, to define new variables, and predict what categorical classification a certain variable will fit into based on its characteristics (Baxter 1994:185). A discriminant function analysis can be conducted using three different methods: standard, hierarchical, and stepwise (Sheskin 2007:1526). In developing the discriminant function equations for the standard method,

all variables are entered in simultaneously regardless of specific order (Sheskin 2007:1526). The hierarchical method uses an already established model or supporting theory to determine the order the variables are to be entered into the analysis (Sheskin 2007:1526). This thesis will most likely employ the stepwise method, which uses correlation to determine the order of variables to be entered, and selects the strongest correlated variables to develop discriminant function equations (Sheskin 2007:1527).

Trace element studies designed to discriminate bones into multiple categories often use ratios of two trace elements (Nganvongpanit et al. 2016:24). In archaeology, discriminant function analysis is often used to sex human remains by using morphometric data (Calcagno 1981; Šlaus and Tomiči 2005; Walker 2008). For example, Nganvongpanit et al. (2016) used stepwise discriminant function analysis to identify the best chemical element ratios that could be used to separate male and female modern *Homo sapiens* bones for crania, humeri, and os coxae.

Another common statistical test that may or may not be used in the thesis is the Student's *t* test. The Student's *t* test is a parametric test designed to determine the probability of significant differences between two independent sample populations. This test is calculated by dividing the difference between the means of the two samples by the standard error of the differences between the means (Fletcher and Lock 2005:96-97). Prominent limitations of *t* tests include the need for normally distributed data, requirement of large sample sizes, and the capacity to only compare two populations. When comparing more than two sample populations, and analysis of variance (ANOVA) must be used to avoid Type I errors.

In the analysis of faunal material, *t* tests are used to determine significance of intraspecies and interspecies differences. Ioannidou (2003) used a series of *t* tests on bone density data from humeri and tibiae to determine if density of domesticated cattle, sheep, pig, and wild boar bones were significantly different between species, breeds, age groups, and sexes (Ioannidou 2003: 356).

CHAPTER III

METHODS

This section will discuss the procedures for how this research met its objectives in understanding how trace element concentrations differ in bones between comparative collection and archaeological bone. The primary objectives for this study are to measure trace element concentrations in comparative collection bone, develop an identification model using unique trace element ranges for each species, and measure trace element concentrations of archaeological bone samples to be compared with the results of the previously measured comparative collection bones.

The first of these objectives was accomplished by using a Bruker Tracer 5i pXRF analyzer on 50 comparative collection specimens housed at Central Washington University, the Conner Museum, and the Burke Museum. To achieve the second goal, I used *R Studio* (RStudio Team 2016), an open source statistics processing software, to run a series of tests that identified species specific trace element ranges. For the last objective, using archaeological bone samples, I measured trace element concentrations with the pXRF and used the *R* software to compare the predictions generated from modern comparative samples with identified archaeological specimens. These three objectives are described in more detail below.

Objective 1: Methods for Measuring Trace Element Concentrations

This first objective for this thesis involved building a data set consisting of trace element concentrations from comparative collection (modern) metapodial bone samples for the target species, the five species of small native artiodactyls in Washington. I chose metapodials for their high bone shaft density (Lyman 1994:246-247) and identifiable shaft features (Dobney and Rielly 1988). I measured both the left and right metacarpal and metatarsal cannonbones from ten individual skeletons of each species from the Burke Museum, Central Washington University, and the Conner Museum, as shown in Table 3.01.

Table 3.01. Comparative Specimens Used in this Study

Species	Collected from	CWU	UW-Burke	WSU-Conner	Total
Antilocapra americana	Washington	0	0	0	0
	Wyoming	1	9	0	10
Odocoileus hemionus	Washington	3	1	4	8
	Wyoming	1	1	0	2
Odocoileus virginianus	Washington	1	0	6	7
	Wyoming	1	2	0	3
Oreamnos americanus	Washington	0	2	4	6
	Wyoming	0	4	0	4
Ovis canadensis	Washington	0	5	1	6
	Wyoming	0	3	1	4

The goal of this study was to use 10 specimens for each species with a provenience in Washington or Wyoming in the case of pronghorn. To control for regional variation, specimen samples were chosen from the Washington study area as possible

(Appendix A). However, this was not feasible for all specimens because (1) sometimes less than ten specimens from Washington were available at the three facilities, and specimens from Wyoming had to be substituted, and (2) pronghorn samples were only available from Wyoming, in part because there are no current modern populations in the state of Washington. Ideally, there would be the same number of Wyoming specimens for each species, but this was not feasible with the available skeletons.

Prior to all measurements taken for each bone sample, and to ensure the pXRF instrument was providing reliable measurements, two bone standards were made by setting 1 x 2 cm pieces of modern cow tibia shaft in resin. To determine if these two standards would yield replicable results, their chemical signatures were mapped to determine homogeneity using an FEI Quanta 250 Field Emission Scanning Electron Microscope (SEM) equipped with an Oxford Instruments X-MaxN Energy Dispersive Spectrometer (EDS) in the Department of Geological Sciences at CWU. The bone standards and corresponding SEM images are shown in Figure 3.01. Using the two chemically homogenous bone standards, the pXRF was monitored by measuring the same two standards day to day and before every specimen following protocol from obsidian XRF studies (Craig et al. 2007:2015; Shackley 2010:19; Sheppard et al. 2010:23).

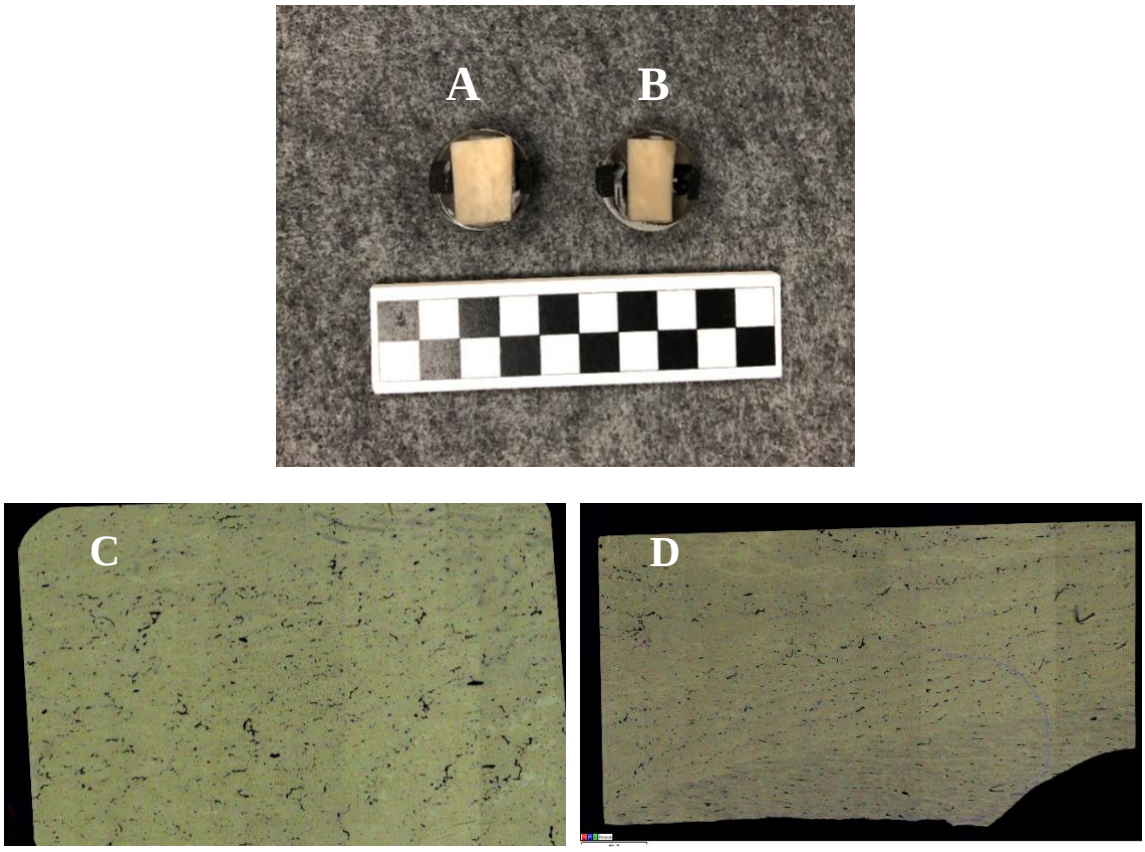

Figure 3.01. Images of bone standards. A) outside surface bone standard, B) inside surface bone standard, C) SEM calcium map of A, D) SEM calcium map of B. SEM calcium maps indicate calcium with red dots).

The process described above for creating calibration standards was inspired by the extensive methodologies of obsidian provenience research. The use of pXRF on obsidian is well studied, with many previous studies suggesting the need for calibration standards (Craig et al. 2007; Hampel 1984; Shackley 2011). The use of pXRF on bone is still being defined as a methodology and many of the early studies I have cited in this thesis such as Buddhachat et al. (2016a), Buddhachat et al. (2016b), Kautenburger et al. (2004), Nganvongpanit et al. (2016), and Nganvongpanit et al. (2017) have not created or used bone calibration standards in their studies. The two bone standards created for this

research will ensure that measurements will remain consistent and accurate throughout the data collection process.

Trace element data was captured with a Bruker Tracer 5i X-ray Fluorescence analyzer owned by the Central Washington University Department of Geological Sciences. This pXRF unit can detect 81 elements between Sodium, atomic number of 11, and Uranium, atomic number of 92 (Bruker Elemental 2017). Using elements identified from Nganvongpanit et al. (2016) and Buddhachat et al. (2016a), this study specifically aimed to detect 29 possible elements found to appear in the makeup of animal bones in trace levels (see Table 3.02). The 14 elements actually detected in this study are also indicated in that table.

Data was collected using a set-up consisting of the pXRF analyzer with a custom plastic pipe tabletop stand made by Dr. Bruce Kaiser, a laptop with associated XRF analysis software (*ARTAX*), and a tabletop ring stand with a three-fingered clamp to hold each bone sample above the pXRF analyzer for the designated shaft scan location. An example of this setup is shown in Figure 3.02. Before the measurement of every set of cannonbones (from one animal specimen), the two standards were scanned one time each and the resulting spectra were visually compared to the prior measurements of the standards. If the results were visually similar, scanning of the cannonbones proceeded. Each cannonbone was scanned three times at six locations on the bone consisting of the proximal shaft, middle shaft, and distal shaft on both the anterior and posterior sides (See Figure 3.03).

Table 3.02: List of Common Trace Elements Found in Bone[1].

Trace Element	Atomic Number	Abbreviation	Detected in this study[2]
Aluminum	13	Al	No
Silicon	14	Si	No
Phosphorus	15	P	No
Sulfur	16	S	No
Potassium	19	K	No
Calcium	20	Ca	Yes
Titanium	22	Ti	No
Vanadium	23	V	Yes
Chromium	24	Cr	Yes
Manganese	25	Mn	No
Iron	26	Fe	Yes
Cobalt	27	Co	No
Nickel	28	Ni	Yes
Copper	29	Cu	No
Zinc	30	Zn	Yes
Bromine	35	Br	No
Strontium	38	Sr	Yes
Zirconium	40	Zr	No
Molybdenum	42	Mo	No
Rhodium	45	Rh	Yes
Silver	47	Ag	No
Cadmium	48	Cd	No
Tin	50	Sn	Yes
Antimony	51	Sb	Yes
Hafnium	72	Hf	No
Mercury	80	Hg	No
Lead	82	Pb	No
Uranium	92	U	No

[1] After Nganvongpanit et al. (2016:64-65) and Buddhachat et al. (2016a:12).

[2] The elements detected in this study are the result of the 50 keV, 35μm energy level. The detection of lighter elements such as Phosphorus would have needed a lower energy level of 15 keV, 15 μm.

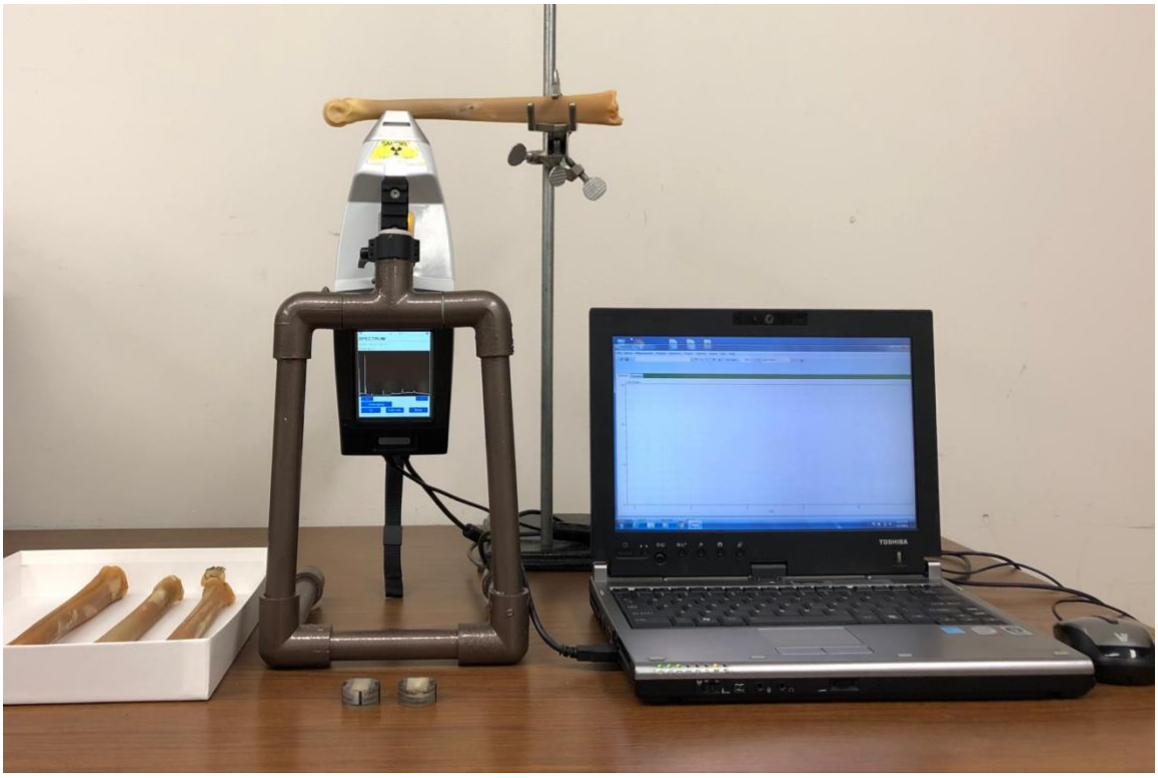

Figure 3.02: Example setup of pXRF, bone specimen, and stands. Photograph by author.

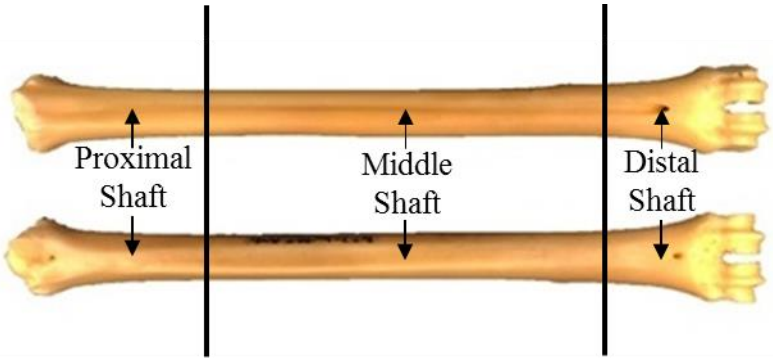

Figure 3.03: Scan locations on metapodial bones. From top to bottom: Anterior and posterior view of a left metatarsal cannon bone from pronghorn antelope PL057 with approximate shaft locations

The pXRF unit was used with a silicon drift detector and an 8 mm collimator. The measurement method was set to 50 keV, 35μm, and a filter of Cu 100μm Ti 25 μm Al μm to measure elements Potassium (19) through Barium (56). This specific energy level is known to get the most accurate results for the designated elements using the Bruker Tracer 5i pXRF analyzer (Bruker Elemental 2018). Assay times for the 50 keV scans were set to 60 seconds for each measurement location. I conducted all measurements at the institutions that housed each specimen. Each measurement was taken using the PVC pipe instrument stand to ensure the stability of the instrument and the consistency of shaft measurements. Data collected from each measurement location were then averaged, following prior researchers (Buddhachat et al. 2016a; Nganvongpanit et al. 2016). All four metapodials found in each comparative skeleton were measured. After each measurement, the measurement location and measurement number were manually recorded on metapodial data collection forms, and trace element data were downloaded from the pXRF unit via flash drive to an Excel file built by the pXRF unit.

Measurements taken with the pXRF are reported in photon counts over the course of the designated assay time, following Sánchez De La Torre et al. (2018). The principle is that higher photon counts equate to higher concentrations of associated trace elements. The majority of XRF studies in archaeology, mainly consisting of obsidian and other lithic material studies, report their results in parts per million or weight percentage (Shackley 2011:19). These quantitative results are made possible by using laboratory tested calibration standards. Because bone cannot as easily be calibrated for use in XRF studies (Sánchez de la Torre 2018), and the pXRF instrument owned by CWU lacked the

necessary software, the results reported for this thesis consist of photon counts for each trace element that were measured over the course 60 seconds.

Objective 2: Statistical Analysis Methods

The secondary objective involved a series of exploratory and statistical methods that were used to analyze the raw data collected from objective one with the goal of developing an identification model using unique trace element ranges for each species. The analysis described in this section is divided into eight steps and displayed in Figure 3.04. The first step in preparing the raw data for analysis was to group all measurements by specimen in the *ARTAX* spectra analysis software making sure to use a Bayesian deconvolution to correct the curve for each measurement (Rhode and Whittenburg 1993). I then converted the raw spectra to a spreadsheet format that reported the raw photon count for each trace element during the 60 second assay period (See Table 3.03). Some trace elements reported have multiple photon counts to account for the electron shell the photons originated from. Elements with lower atomic numbers will produce photons from the lower energy L to K transition whereas higher atomic number elements such as barium will produce photons from both the L to K and higher energy M to L shell transition (Shackley 2011:16-17). In this thesis, readings from the L to K transition are indicated as K12, and the readings from the M to L transition are indicated as L1. For the purpose of this study, I considered photon counts from all electron shells. All

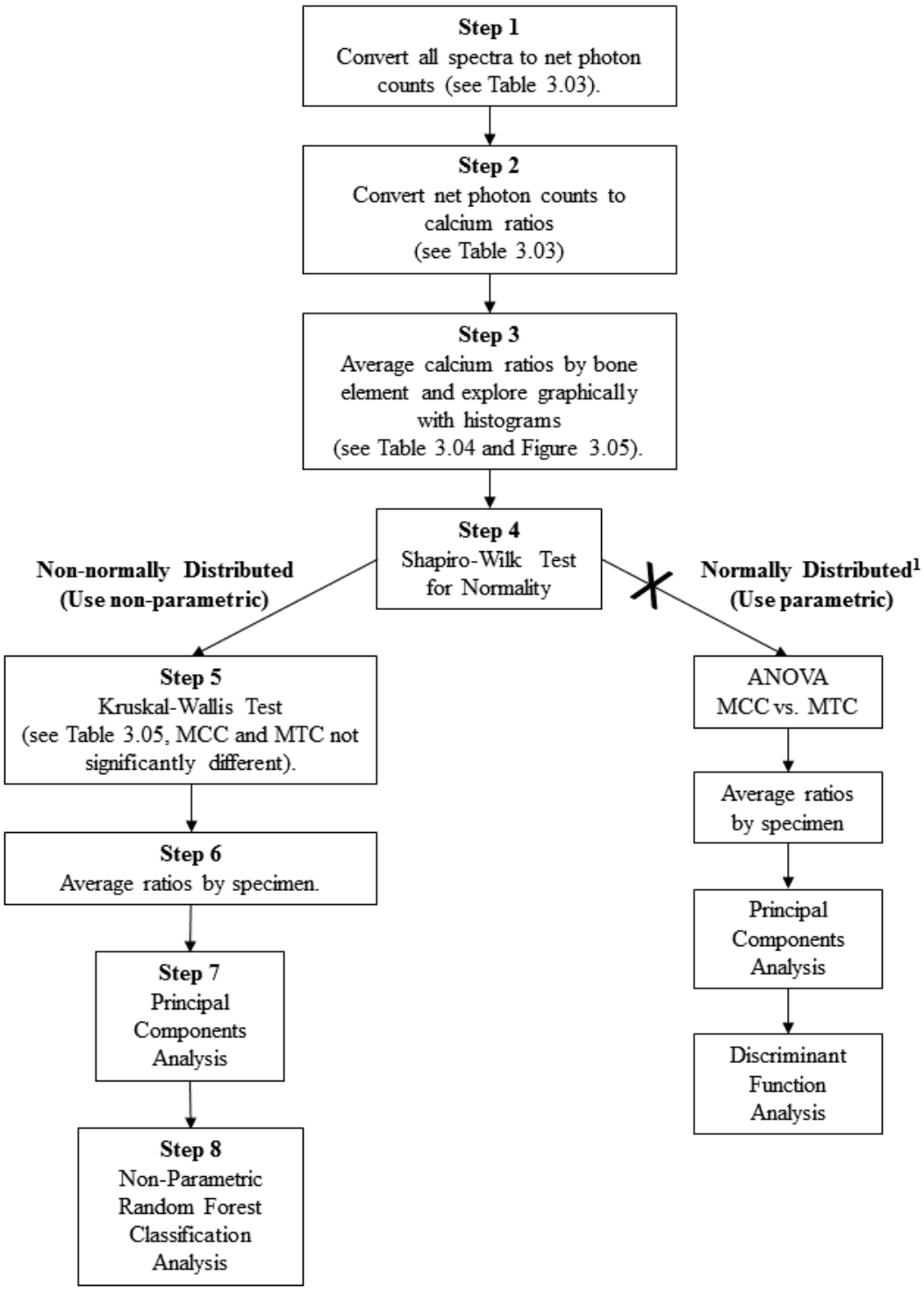

Figure 3.04: Data analysis process for Objective Two.
[1]This analysis did not pursue this avenue because the data was non-normally distributed.

Table 3.03: Example Data of Iron and Calcium Concentrations

Specimen #	Bone Element	Fe Net Photon Count	Ca Net Photon Count	Fe/Ca Ratio
PL554	Right MCC	2543	49977	0.0509
PL554	Right MCC	2694	81699	0.0330
PL554	Right MCC	3203	51662	0.0620
PL554	Right MCC	1901	77865	0.0244
PL554	Right MCC	2086	51677	0.0404
PL554	Right MCC	2045	32259	0.0634
PL554	Left MCC	1609	46906	0.0343
PL554	Left MCC	1907	54174	0.0352
PL554	Left MCC	1672	45871	0.0365
PL554	Left MCC	1605	62590	0.0256
PL554	Left MCC	1419	44742	0.0317
PL554	Left MCC	2106	45135	0.0467
PL554	Right MTC	2361	34500	0.0684
PL554	Right MTC	2585	56455	0.0458
PL554	Right MTC	2630	62519	0.0421
PL554	Right MTC	2510	54258	0.0463
PL554	Right MTC	2354	63214	0.0372
PL554	Right MTC	1760	47583	0.0370
PL554	Left MTC	2485	47548	0.0523
PL554	Left MTC	1886	52836	0.0357
PL554	Left MTC	2083	68455	0.0304
PL554	Left MTC	2274	59732	0.0381
PL554	Left MTC	2142	63636	0.0337
PL554	Left MTC	1326	50568	0.0262

spreadsheets for each specimen were merged into one central spreadsheet to prepare for initial exploratory data analysis in *RStudio*.

In *RStudio, a* statistical processing software which runs off the programing language *R* (RStudio Team 2016), I used the following *R* packages in the data analysis described in this chapter: *caret* (Kuhn 2018), *dplyr* (Wickham et al. 2018), *e1071* (Meyer et al. 2018), *ggplot2* (Wickham 2016), *mvShapiroTest* (Gonzalez-Estrada and Villasenor-Alva 2013), *npmv* (Burchett et al. 2017), *randomForest* (Liaw and Wiener 2002),

reshape2 (Wickham 2007), and *xlsx* (Dragulescu and Arendt 2018). These will be discussed as they are used in the analyses below.

In the second step, raw data for each of the 14 trace elements were divided by the calcium concentration to create a data set of calcium ratios (see Table 3.03) to follow methods used by Rasmussen et al. (2013) and Burton et al. (1999). These authors state that calcium is replaced by supplementary trace elements as an effect of environmental factors such as diet and biopurification through trophic levels. Using ratio data in this way was necessary due to the pXRF bone analysis method used in this thesis which lacked the capability of using a laboratory tested bone standard to produce externally replicable raw quantitative data. For Step 3, the newly produced calcium ratio data were averaged by bone element, side, and species (see Table 3.04) and explored graphically using histograms created in *ggplot2* (e.g., Figure 3.05).

Table 3.04: Example Data of Averaged Iron and Calcium Ratios by Bone Element.

Specimen #	Species	Bone Element	Fe/Ca Ratio
PL554	*Odocoileus hemionus*	Left MCC	0.0345
PL554	*Odocoileus hemionus*	Left MTC	0.0356
PL554	*Odocoileus hemionus*	Right MCC	0.0419
PL554	*Odocoileus hemionus*	Right MTC	0.0446

To determine which classification model and ANOVA to use for discriminating between species, I first tested the trace element calcium ratios for normality, using all specimen averages. In Step 4, I used the Shapiro-Wilk test for normality in the package mvShapiroTest in RStudio (Gonzalez-Estrada and Villasenor-Alva 2013), and found that most of the trace element ratios were statistically non-normally distributed ($p < 0.05$,

Table 3.05). This meant that for subsequent ANOVA tests I used non-parametric variants, and similarly for my classification analysis I used the non-parametric RandomForest test.

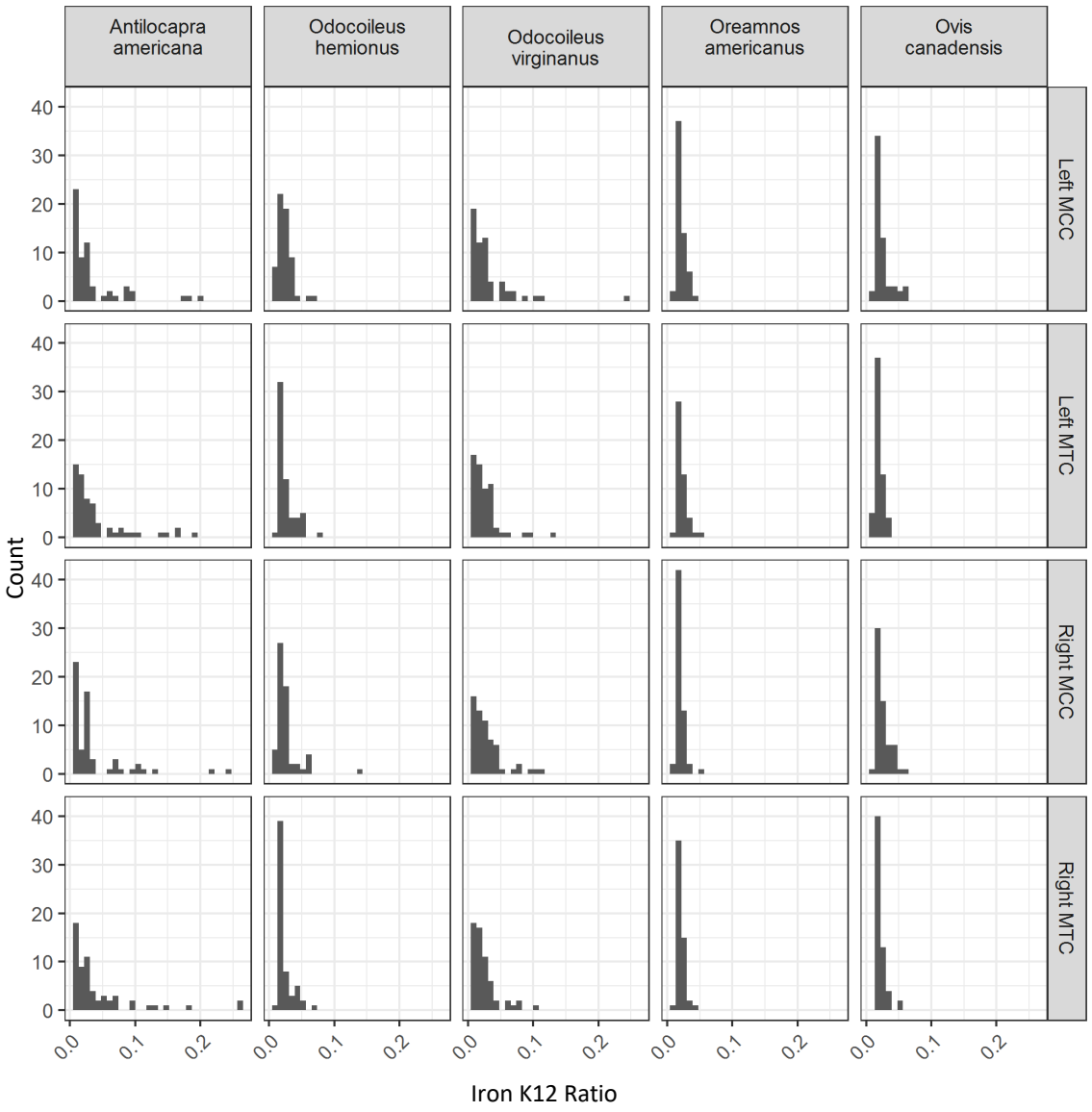

Figure 3.05: Histogram of example calcium ratio by bone element and species. This one is for iron using K12 only. MCC= metacarpal cannonbone, MTC= metatarsal cannonbone.

Table 3.05: Shapiro-Wilk *p* Values of Trace Element Ratios.[1]

Trace Element Ratios	*Antelocapra americana*	*Oreamnos americanus*	*Ovis canadensis*	*Odocoileus hemionus*	*Odocoileus virginanus*
Ba (K12)/Ca	0.036	0.002	0.637	0.081	0.021
Ba (L1)/Ca	0.001	0.006	0.319	0.001	0.002
Fe (K12)/Ca	0.005	0.350	0.297	0.155	0.003
Ni (K12)/Ca	0.006	0.368	0.535	0.313	0.611
Pd (K12)/Ca	0.073	0.810	0.552	0.113	0.002
Pd (L1)/Ca	0.007	0.252	0.760	0.805	0.001
Rb (K12)/Ca	0.197	0.470	0.065	0.229	0.008
Rh (K12)/Ca	0.073	0.631	0.534	0.121	0.004
Rh (L1)/Ca	0.003	0.107	0.159	0.009	0.007
Sb (K12)/Ca	0.174	0.662	0.980	0.283	0.017
Sb (L1)/Ca	0.001	0.089	0.364	0.196	0.006
Sn (K12)/Ca	0.109	0.494	0.975	0.066	0.006
Sn (L1)/Ca	0.181	0.001	0.007	0.145	0.001
Sr (K12)/Ca	0.014	0.007	0.321	0.312	0.029
Ta (L1)/Ca	0.125	0.610	0.477	0.079	0.105
Ta (M1)/Ca	0.444	0.381	0.160	0.130	0.231
V (K12)/Ca	0.332	0.529	0.001	0.085	0.261
Zn (K12)/Ca	0.315	0.543	0.007	0.109	0.432
Y (K12)/Ca	0.012	0.504	0.001	0.801	0.001

[1] Highlighted *p* values equal non-normal distributions $p < 0.05$

Before proceeding further, as Step 5, I tested if the trace element concentrations were consistent within an individual between the left and right metacarpal and metatarsal. To verify the homogeneity between cannon bones, as seen in Figure 3.05, I conducted a non-parametric version of ANOVA, the Kruskal-Wallis test, using the *stats* base package. The null hypothesis is that chemical element concentrations do not vary by bone element. The *p* value for all element ratios except for chromium showed no significant difference ($p > 0.05$) between the four cannonbones (see Table 3.06). Due to chromium having a *p* value of less than 0.05, chromium was not included in the subsequent analyses.

Table 3.06: Kruskal-Wallis *p* Values of Metapodial Bones.

Trace Element Ratio	H Statistic	n	*p* Value	Significant?
Ba (K12)/Ca	0.3328	197	0.9537	No
Ba (L1) /Ca	1.9076	197	0.5918	No
Cr (K12)/Ca	8.9972	197	**0.0293**	**Yes**
Fe (K12)/Ca	0.3228	197	0.9557	No
Ni (K12)/Ca	0.1499	197	0.9852	No
Pd (K12)/Ca	0.1798	197	0.9808	No
Pd (L1)/Ca	1.1663	197	0.7611	No
Rb (K12)/Ca	1.2381	197	0.7439	No
Rh (K12)/Ca	0.3017	197	0.9597	No
Rh (L1)/Ca	0.8019	197	0.8490	No
Sb (K12)/Ca	1.0912	197	0.7792	No
Sb (L1)/Ca	3.6747	197	0.2988	No
Sn (K12)/Ca	0.0852	197	0.9935	No
Sn (L1)/Ca	0.0371	197	0.8406	No
Sr (K12)/Ca	0.0074	197	0.9998	No
Ta (L1)/Ca	0.2075	197	0.9764	No
Ta (M1)/Ca	0.6685	197	0.8806	No
V (K12)/Ca	1.3805	197	0.7101	No
Y (K12)/Ca	2.7645	197	0.4294	No
Zn (K12)/Ca	1.0047	197	0.8001	No

The relative homogeneity of metapodial bones found in the previous step led to the sixth step of averaging all bone element ratios by specimen. For the example of iron for deer specimen PL-554 (Table 3.04), the 24 Fe/Ca ratio values average to 0.0392. These specimen ratios were used throughout the remaining analysis steps.

Step 7 included further exploratory data analysis where I completed a principal components analysis by specimen number using the *stats* base package to determine what trace elements ratios were the best at discerning differences between species. For this study, I used the first three principal components (Table 3.07) as a guide to choose the

best trace element ratios in the subsequent classification models. Larger PCA loading values indicate more importance in group separation on that axis.

Table 3.07: Principal Component Loading Values Based on Calcium Ratios.

Ratio	PC1	PC2	PC3
Sn (K12)/Ca	0.8044129	0.1272168	0.4412396
Sr (K12)/Ca	0.4465769	0.5947846	0.6612615
Pd (K12)/Ca	0.2266446	0.0384358	0.1390898
Ba (L1)/Ca	0.1993701	0.7568436	0.5647230
Rh (K12)/Ca	0.1913297	0.0427070	0.0865300
Ba (K12)/Ca	0.1207628	0.1273797	0.0597951
Zn (K12)/Ca	0.0626454	0.0103141	0.0030988
Fe (K12)/Ca	0.0522953	0.1136726	0.0354168
Ni (K12)/Ca	0.0462816	0.0693688	0.0313444
Ta (L1)/Ca	0.0329173	0.0062338	0.0257934
Sb (L1)/Ca	0.0237488	0.0093653	0.0181651
Sb (K12)/Ca	0.0206600	0.0052500	0.0078267
Pd (L1)/Ca	0.0130687	0.0021221	0.0090048
Rh (L1)/Ca	0.0071932	0.1403548	0.1233010
Sn (L1)/Ca	0.0060696	0.0002867	0.0076617
Rb (K12)/Ca	0.0050608	0.0002279	0.0084855
Ta (M1)/Ca	0.0045340	0.0029288	0.0001411
V (K12)/Ca	0.0009555	0.0003716	0.0000082
Y (K12)/Ca	0.0001810	0.0004227	0.0004559

Next, in Step 8, I used a classification model to determine diagnostic potential of the top elements suggested in the PCA analysis. Without normal distributions, my data violates the normally distributed assumptions of typically used classification analysis like Discriminant Function Analysis (Ghasemi and Zahediasl 2012:486-487). Note that other researchers (e.g., Buddhachat et al. 2016a, Nganvongpanit et al. 2016, Nganvongpanit et al. 2017) have used Discriminant Function Analysis without reporting normality tests. As a result, I instead reformatted my data using *dplyr* (Wickham et al. 2018) and *reshape2*

(Wickham 2007), then used the non-parametric Random Forest classification analysis using *randomForest* (Liaw and Wiener 2002.

The ratios with the highest factor loadings from the principal component analysis were the primary guide in the development of the Random Forest models, although other ratios with lower factor loadings were also considered. Before each model could be built, a training data set and multiple test data sets were constructed to guide the predicative modeling that each Random Forest model would be based from. Training sets and test sets of specimens are used in classification models to prevent over-fitting of the classification model (e.g., to ensure the model is identifying species trends, not individuals). The training set of specimens was initially built from a randomly selected sample of half the data, each containing five specimens from each species. With random data, I noticed that the accuracy of subsequent random forest models was highly erratic. There were several specimens which, if included in the training set, led to poor identification of the test set. To control for reliability and accuracy, I picked the five most accurately, and most consistently identified specimens of each species, and used them in the training set. This ensured the best specimens were included in the training set, restricting the number of outlier specimens that threw off the training set.

Using the training set, all models were built using combinations of element ratios that the principal component analysis showed were the best at separating species. I then ran each model in *RStudio* using the *randomForest* package (Liaw and Wiener 2002) against test data sets. The test data sets consisted of a) the remaining data, b) the full data set including the training, and c) the manufactured data that was built using a jitter

function from the base package in *R* on the entire real data set with a factor size of 20. This last test set was employed to simulate an entirely new data set that could test the accuracy of each random forest model, and to look for signs of model over-fitting. The results of predictions on these test sets were used to calculate model accuracy for each Random Forest model, provided in the results chapter below.

Objective 3: Testing Archaeological Bone Samples

The third objective is considered a pilot study, testing the validity of the predictive models developed in Objective 2 on archaeological bone samples. Ideally, I would test a sample of taxonomically identified metapodial bone fragments of the five study species from Washington archaeological sites. These would also be unburned specimens. However, an examination of identified faunal databases of local sites by and in the possession of Dr. Lubinski failed to yield very many specimens. For this reason, I expanded the possible samples to include burned specimens and fauna from Bernard Creek Rockshelter (Idaho) being examined by Dr. Lubinski as well as Wyoming sites being examined in our laboratory by Dr. Megan Partlow. The potential list is provided in Table 3.09, and the final sample of specimens tested is listed in the results chapter. Note that a search of these assemblages did not yield any identified metapodials: 45GR144 (Mesa 12), 45KT101 (Umtanum Creek), 45KT346 (Manastash Pines).

A total of 18 taxonomically identified archaeological specimens (Table 3.08) were chosen to measure with the pXRF. To avoid any predictive bias during the analysis, the analysis of the archaeological specimens was conducted blind. All bone specimens

were randomly assigned a number by Dr. Lubinski to keep the taxonomic identification unknown to me until after the completion of the analysis.

Table 3.08: Potential Archaeological Metapodial Bone Samples for Pilot Test.

Archaeology Site	Element	Cat#/ Specimen #	Identified Species[1]	Fragment Length (cm)	Reference
10IH83	Metatarsal	4/7.08	*Odocoileus* sp.	7	Day 2014
10IH83	Metacarpal	11/12.13	*Ovis canadensis*	15	Day 2014
10IH83	Metacarpal	11/12.19	*Ovis canadensis*	7	Day 2014
10IH83	Metacarpal	24/29.08	*Ovis canadensis*	16	Lubinski in prep
10IH83	Metatarsal	24/29.14	*Odocoileus* sp.	7	Lubinski in prep.
10IH83	Metatarsal	26/35.06	*Ovis canadensis*	6	Lubinski in prep
10IH83	Metatarsal	36/49.20	*Odocoileus* sp.	8	Lubinski in prep
10IH83	Metatarsal	36/49.22	*Ovis canadensis*	9	Lubinski in prep
10IH83	Metatarsal	40/52.25	*Ovis canadensis*	8	Lubinski in prep
10IH83	Metatarsal	40/53.02	*Odocoileus* sp.	10	Lubinski in prep
45GR76 (Sam Israel HP)	Metatarsal	831	*Ovis* sp.	5	Olsen 1997:59
45GR76	Metatarsal	1158	*Ovis* sp.	4	Olsen 1997:75
45KT12 (HITW Canyon)		450/41.22	*Odocoileus* sp.		Johnson 2018
45KT12		472/43.04	*Ovis canadensis*	10	Johnson 2018
45KT13 (French Rapids)		45/5.09	*Odocoileus* sp.	10	Johnson 2018
45KT301 (Grissom)		10110/12.09	*Odocoileus* sp.	9	Spencer 2018
48SU7579 (Pedestal)	Metatarsal	854/30.03	*Antilocapra americana*	18	Partlow 2018
48SW19464 (Sourdough II)	Metatarsal	263/1.01	*Antilocapra americana*	9	Partlow 2019

[1] species identification verified by Lubinski

Before taking each measurement, following methods used by Sánchez De La Torre et al. (2018), each metapodial fragment was cleaned with distilled water and let dry

so as to remove the exterior sediment contamination. After a drying period of 24 hours, each archaeological sample was measured three times at the center-most point on the cortical shaft surface using the same XRF settings as described for the modern specimens in Objective 1. Using the methodology described in Objective 2, data were averaged and converted to calcium ratios for each trace element. These calcium ratios were then entered into the most accurate Random Forest model developed from the modern comparative collection data set in Objective 2. To maximize the predictive accuracy, the Random Forest model used the complete modern data set as the training set for archaeological specimens instead of only half which was used in the initial comparative collection analysis.

CHAPTER IV

RESULTS

Modern Comparative Collection

Using the non-parametric Random Forest classification described in the previous chapter, 10 Random Forest models were developed using a combination of trace element/calcium ratios. The models were developed as follows: RF 1, 2, 4, and 5 were exploratory ratio combinations selected by the author, RF 3 was inspired by Burton et al. (1999) which employed Ba/Ca and Sr/Ca ratios for dietary analysis, RF 6 includes all 20 measured element ratios, RF 7 consisted of all transition metals, and RF 8-10 include the top six elements from principal components 1-3. These 10 models (RF 1 through RF 10) were tested against three data sets for predictive accuracy: a jittered data set, the full unaltered data set, and a half data set consisting of specimens not used in the training data set. Table 4.01 displays all 10 models and their predictive accuracy for all three test data sets.

The top three models with the highest prediction accuracy rates for the three data sets included models RF 3, RF 7, and RF 10. RF 10 had the highest accuracy rates of about 70% for the jittered and full data sets, and about 41% for the half data set. The half data set is the most conservative test set for prediction as the full and jittered data sets contain data used in the training set, so may include overfitting. Using a variable importance plot for model RF 10 as seen in Figure 4.01, strontium and barium are considered the most important variables with the highest mean decrease in accuracy

values. Barium and strontium are also included in the model RF 3 which had an accuracy rate of 67% for the full and jittered data sets and 35 % for the half data set.

Table 4.01: Random Forest Models and Associated Accuracy for Predicting Species.

Model Name	Trace Element Ratios Used in Model[1]	Jittered Data (n=50)	Full Data (n=50)	Half Data (n=25)
RF 1	Ba(L Shell), Ba(K Shell), Sn, Sr	0.60	0.60	0.22
RF 2	Ba(L Shell), Sn, Sr	0.49	0.49	0.36
RF 3	Ba(L Shell), Sr	0.67	0.67	0.35
RF 4	Rh(K Shell), Rb, Ni	0.65	0.65	0.31
RF 5	V, Y	0.52	0.52	0.22
RF 6	Ba(K Shell), Ba (L Shell), Cr, Fe, Ni, Pd (K Shell), Pd (L Shell), Rb, Rh(K Shell), Rh(L Shell), Sb(K Shell), Sb(L Shell), Sn(K Shell), Sn(L Shell), Sr, Ta(L Shell), Ta(M Shell), V, Y, Zn	0.63	0.63	0.27
RF 7	Fe, Ni, Pd(K Shell, Pd(L Shell), Rh(K Shell), Rh(L Shell), Sn(K Shell), Sn(L Shell), Ta(L Shell), Ta(M Shell), V, Y, Zn	0.65	0.65	0.30
RF 8	Sn(K Shell), Ba(L Shell), Ba(K Shell), Sr, Pd(K Shell), Rh(K Shell)	0.63	0.63	0.27
RF 9	Sn(K Shell), Ba(L Shell), Ba(K Shell), Sr, Rh(K Shell), Fe	0.61	0.61	0.22
RF 10	Sn(K Shell), Ba(L Shell), Sr, Pd(K Shell), Rh(K Shell), Rh(L Shell)	0.70	0.70	0.41

[1] All trace element values were divided by calcium values.

After determining that model RF 10 produced the highest accuracy rate for identifying specimens to species, I examined the predicted species in more detail to see if there were patterns in misidentification. Commonly, but not in all cases, the two species of deer were often predicted interchangeably. I observed this same prediction error between mountain goat and bighorn sheep as well. Even when species was misidentified,

taxonomic family seemed to be correctly identified by the model. These observations prompted the Random Forest models to be run again, but instead of predicting by species, the models were set to predict by family. Table 4.02 provides species and family predicted by the RF 10 model compared with known identification for all 50 specimens.

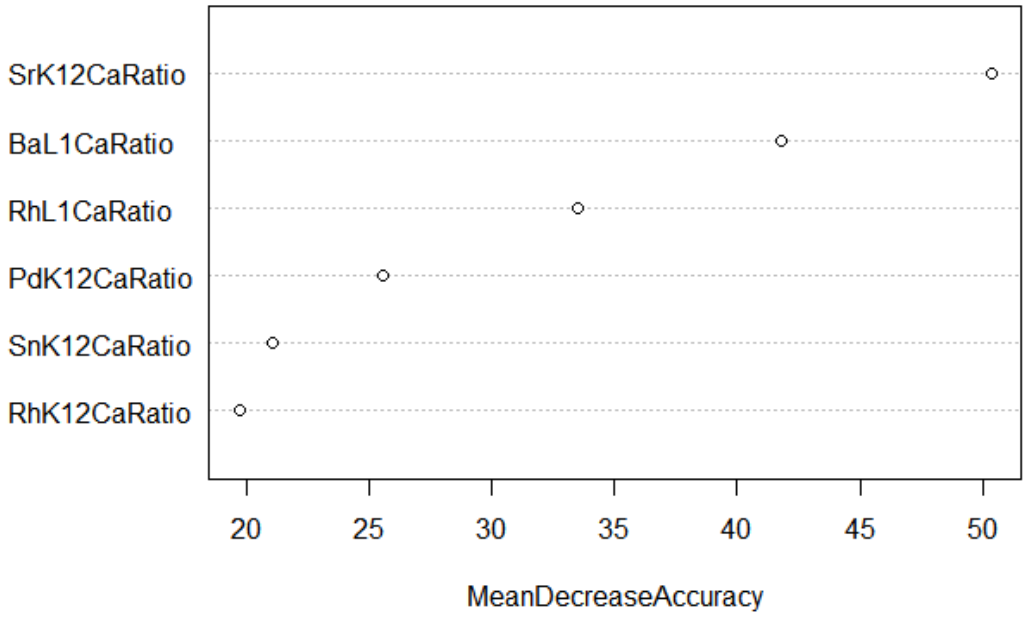

Figure 4.01: Variable importance plot for Random Forest Model RF 10. Higher mean decrease in accuracy equates to more important classification variables. In other words, the plotted values are the resulting loss in relative accuracy of the model if this element was removed.

In this study, there are three families present which include Cervidae (*Odocoileus virginanus* and *Odocoileus hemionus*), Bovidae (*Ovis canadensis* and *Oremanos americanus*), and Antilocapridae (*Antilocapra americana*). As shown in Table 4.02, the accuracy of the RF10 model for predicting family is 82% overall. But the accuracy is variable by family, with 14/20 (70%) for Cervidae, 20/20 (100%) for Bovidae, and 7/10 (70%) for Antilocapridae.

Table 4.02: Random Forest Model 10 Predictions by Species and Family.[1]

Specimen Number	Known Species	Predicted Species	Known Family	Predicted Family
32519	*Antilocapra americana*	*Ovis canadensis*	Antilocapridae	Bovidae
33496	*Antilocapra americana*	*Antilocapra americana*	Antilocapridae	Antilocapridae
33498	*Antilocapra americana*	*Antilocapra americana*	Antilocapridae	Antilocapridae
33500	*Antilocapra americana*	*Antilocapra americana*	Antilocapridae	Antilocapridae
34314	*Antilocapra americana*	*Antilocapra americana*	Antilocapridae	Antilocapridae
38617	*Antilocapra americana*	*Antilocapra americana*	Antilocapridae	Antilocapridae
38619	*Antilocapra americana*	*Antilocapra americana*	Antilocapridae	Cervidae
38620	*Antilocapra americana*	*Antilocapra americana*	Antilocapridae	Antilocapridae
38622	*Antilocapra americana*	*Antilocapra americana*	Antilocapridae	Antilocapridae
PL057	*Antilocapra americana*	*Ovis canadensis*	Antilocapridae	Bovidae
34272	*Odocoileus hemionus*	*Odocoileus hemionus*	Cervidae	Cervidae
41-364	*Odocoileus hemionus*	*Odocoileus hemionus*	Cervidae	Cervidae
46-270	*Odocoileus hemionus*	*Odocoileus hemionus*	Cervidae	Cervidae
47-172	*Odocoileus hemionus*	*Odocoileus hemionus*	Cervidae	Cervidae
49-482	*Odocoileus hemionus*	*Odocoileus hemionus*	Cervidae	Cervidae
59671	*Odocoileus hemionus*	*Ovis canadensis*	Cervidae	Bovidae
PL059	*Odocoileus hemionus*	*Oreamnos americanus*	Cervidae	Bovidae
PL554	*Odocoileus hemionus*	*Odocoileus hemionus*	Cervidae	Cervidae
PL613	*Odocoileus hemionus*	*Ovis canadensis*	Cervidae	Bovidae
PL627	*Odocoileus hemionus*	*Odocoileus virginanus*	Cervidae	Cervidae
32122	*Odocoileus virginianus*	*Odocoileus virginanus*	Cervidae	Cervidae
32131	*Odocoileus virginianus*	*Antilocapra americana*	Cervidae	Antilocapridae
32135	*Odocoileus virginianus*	*Odocoileus virginanus*	Cervidae	Cervidae
41-42	*Odocoileus virginianus*	*Odocoileus hemionus*	Cervidae	Cervidae
41-44	*Odocoileus virginianus*	*Odocoileus virginanus*	Cervidae	Cervidae
49-43	*Odocoileus virginianus*	*Oreamnos americanus*	Cervidae	Antilocapridae
76-563	*Odocoileus virginianus*	*Ovis canadensis*	Cervidae	Bovidae
86-271	*Odocoileus virginianus*	*Odocoileus virginanus*	Cervidae	Cervidae
90-133	*Odocoileus virginianus*	*Odocoileus virginanus*	Cervidae	Cervidae
PL286	*Odocoileus virginianus*	*Odocoileus virginanus*	Cervidae	Cervidae

Table 4.02: Random Forest Model 10 Predictions by Species and Family [1] (continued).

Specimen Number	Known Species	Predicted Species	Known Family	Predicted Family
32103	*Oreamnos americanus*	*Ovis canadensis*	Bovidae	Bovidae
34310	*Oreamnos americanus*	*Odocoileus hemionus*	Bovidae	Bovidae
34311	*Oreamnos americanus*	*Ovis canadensis*	Bovidae	Bovidae
35995	*Oreamnos americanus*	*Ovis canadensis*	Bovidae	Bovidae
42-27	*Oreamnos americanus*	*Ovis canadensis*	Bovidae	Bovidae
47-184	*Oreamnos americanus*	*Oreamnos americanus*	Bovidae	Bovidae
48-449	*Oreamnos americanus*	*Oreamnos americanus*	Bovidae	Bovidae
49-22	*Oreamnos americanus*	*Oreamnos americanus*	Bovidae	Bovidae
59674	*Oreamnos americanus*	*Oreamnos americanus*	Bovidae	Bovidae
59675	*Oreamnos americanus*	*Oreamnos americanus*	Bovidae	Bovidae
39467	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
39468	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
39469	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
39480	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
65-60	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
80-250	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
81686	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
81687	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
81822	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
82330	*Ovis canadensis*	*Ovis canadensis*	Bovidae	Bovidae
Prediction Accuracy		70%		82%

[1] Highlighted predictions equal incorrect classifications.

Table 4.03 displays the same 10 Random Forest models used to predict species in Table 4.01, but were set to instead predict family. The results of the newly run Random Forest models suggest an overall improvement in identification accuracy for all 10 models, compared to species predictions. Model RF 10 had the highest prediction accuracy rate of 82% for both the jittered and full data sets, along with a 64 % accuracy rate for the half data set.

Table 4.03: Random Forest models and associated accuracy for predicting family

Model Name	Trace Element Ratios Used in Model[1]	Jittered Data (n=50)	Full Data (n=50)	Half Data (n=25)
RF 1	Ba(L Shell), Ba(K Shell), Sn, Sr	0.76	0.76	0.52
RF 2	Ba(L Shell), Sn/Ca, Sr	0.76	0.80	0.60
RF 3	Ba(L Shell), Sr	0.74	0.80	0.60
RF 4	Rh(K Shell), Rb, Ni	0.76	0.78	0.56
RF 5	V, Y	0.68	0.72	0.44
RF 6	Ba(K Shell), Ba (L Shell), Fe, Ni, Pd(K Shell), Pd(L Shell, Rb, Rh(K Shell), Rh(L Shell), Sb(K Shell), Sb(L Shell), Sn(K Shell), Sn(L Shell), Sr, Ta(L Shell), Ta(M Shell), V, Y, Zn	0.76	0.76	0.52
RF 7	Fe, Ni, Pd(K Shell, Pd(L Shell), Rh(K Shell), Rh(L Shell), Sn(K Shell), Sn(L Shell), Ta(L Shell), Ta(M Shell), V, Y, Zn	0.80	0.80	0.6
RF 8	Sn(K Shell), Ba(L Shell), Ba(K Shell), Sr, Pd(K Shell), Rh(K Shell)	0.70	0.70	0.4
RF 9	Sn(K Shell), Ba(L Shell), Ba(K Shell), Sr, Rh(K Shell), Fe	0.78	0.78	0.56
RF 10	Sn(K Shell), Ba(L Shell), Sr, Pd(K Shell), Rh(K Shell), Rh(L Shell)	0.82	0.82	0.64

[1] All trace element values were divided by calcium values.

Archaeological Specimen Results

Eighteen archaeological specimens were measured with the pXRF in this pilot study. Using the RF10 model which yielded the best prediction results for modern specimens, archaeological specimens were predicted on both a species and family level. As seen in Table 4.04, identifying the 18 archaeological specimens by species was fairly inaccurate at 22%. These results mirror the modern comparative specimen results as the bovid species were often misidentified with each other as well as the cervid, and

antilocaprid species. Using the more accurate method of identifying by family yielded more successful results with an identification accuracy of 67%. Because there are three families possible, RF 10 has a one in three probability ($p = 0.3333$) of correctly identifying each archaeological bone fragment to the correct family purely by random chance for each trial. Following methods used by Emery-Wetherell and Davis (2018), an exact binomial probability test was performed in *R* to verify that the 12 out of 18 correctly identified bones (12 successes out of 18 trials) were not the result of random chance. The exact binomial probability function calculates the probability of a certain number of successes (12 here) in a certain number of trials (18 here) by chance given a known probability of an individual successful outcome (0.33 here). The calculated probability ($p = 0.0045$) indicates far less than a 1% chance that 12/18 successes could occur by chance.

The archaeological specimens used in this study have all undergone some level of chemical alteration due to processes regularly seen amongst archaeological material. Taphonomic processes such as burning can be seen in bone fragment (Cat # 1158) from 45GR76. This fragment, despite its burned nature was correctly identified. The lower accuracy rate of archaeological specimens as a whole seems to suggest that a natural chemical alteration process such as diagenesis may affect the bone chemistry of all archaeological bones (Nielsen-Marsh and Hedges 2000). In this experimental study, the degree of chemical alteration from taphonomy and diagenesis is uncertain and will be discussed further in the discussion and conclusions chapter.

Table 4.04: Prediction Accuracy of Model RF10 on Archaeological Metapodials [1]

Archaeology Site	Cat#/ Specimen #	Identified Species	Predicted Species	Predicted Family
10IH83 (Bernard Creek)	4/7.08	*Odocoileus* sp.	*Oreamnos americanus*	*Cervidae*
10IH83	11/12.13	*Ovis canadensis*	*Oreamnos americanus*	*Bovidae*
10IH83	11/12.19	*Ovis canadensis*	*Oreamnos americanus*	*Cervidae*
10IH83	24/29/08	*Ovis canadensis*	*Ovis canadensis*	*Bovidae*
10IH83	24/29.14	*Odocoileus* sp.	*Ovis canadensis*	*Cervidae*
10IH83	26/35.06	*Ovis canadensis*	*Oreamnos americanus*	*Bovidae*
10IH83	36/49.20	*Odocoileus* sp.	*Odocoileus hemionus*	*Cervidae*
10IH83	36/49.22	*Ovis canadensis*	*Oreamnos americanus*	*Cervidae*
10IH83	40/52.25	*Ovis canadensis*	*Oreamnos americanus*	*Cervidae*
10IH83	40/53.02	*Odocoileus* sp.	*Ovis canadensis*	*Cervidae*
45GR76 (Sam Israel HP)	831	*Ovis* sp.	*Ovis canadensis*	*Bovidae*
45GR76	1158	*Ovis* sp.	*Ovis canadensis*	*Bovidae*
45KT12 (HITW Canyon)	450/41.22	*Odocoileus* sp.	*Ovis canadensis*	*Cervidae*
45KT12	472/43.04	*Ovis canadensis*	*Oreamnos americanus*	*Bovidae*
45KT13 (French Rapids)	45/5.09	*Odocoileus* sp.	*Ovis canadensis*	*Cervidae*
45KT301 (Grissom)	10110/12.09	*Odocoileus* sp.	*Ovis canadensis*	*Bovidae*
48SU7579 (Pedestal)	854/30.03	*Antilocapra americana*	*Ovis canadensis*	*Cervidae*
48SW19464 (Sourdough II)	263/1.01	*Antilocapra americana*	*Oreamnos americanus*	*Cervidae*
Prediction Accuracy			**22%**	**67%**

[1] Highlighted predictions equal incorrect classifications.

CHAPTER V

DISCUSSION AND CONCLUSIONS

The experimental pXRF and Random Forest classification methods used in this thesis highlighted several innate issues that affected the results of my study. This final chapter concludes my experimental thesis research by discussing these issues such as diagenesis and taphonomy, two processes that alter the chemical makeup of bone. This is followed by a discussion of taxonomic prediction success and other observations that were made during the Random Forest classification analysis. Finally, this chapter closes with my conclusions regarding the method used in this work as well as avenues for future research and plans to complete a journal manuscript for submission to a peer-review journal.

Taphonomy and Diagenesis

While promising, the prediction accuracy of 67% for taxonomic family of archaeological bone fragments, as compared to 82% for modern bones, suggests that there could be confounding factors introduced in the archaeological bones, such as caused by taphonomy and/or chemical diagenesis. One taphonomic factor is thermal alteration which changes the chemical state of bone. When bone is exposed to extreme levels of heat, the mineral/crystalline phase of bone restructures (Shipman et al. 1984; Stiner et al. 1995). When exposed to heat, bone hydroxyapatite crystals (where many trace elements are stored) do not degrade like bone collagen, but rather gradually change and increase in size as temperatures increase (McCutcheon 1992; Pemmer et al. 2013;

Shipman et al. 1984). The chemical composition of bone hydroxyapatite remains relatively unaffected after the burning process (Zimmerman et al. 2015:386), presumably including trace elements in the hydroxyapatite matrix. In the archaeological pilot study included in this thesis, there was one bone fragment (Cat # 1158) from 45GR76 that exhibited evidence of burning that included a blackened exterior surface. It is worth noting that despite the observed thermal alteration of this specimen, it was correctly identified using the RF 10 Random Forest model. The correct identification of this one specimen does seem to suggest that the chemical makeup of burned bone is robust enough to be identified using this method.

Following Hedges (2002), I will use the term diagenesis to refer to chemical changes to bone after burial. The primary diagenetic process to be discussed occurs as a result of being exposed for long periods of time to local geology and hydrology of surrounding sediment post deposition. Diagenetic change results in the loss of collagen or the organic phase of bone, and the restructure of the mineral phase as hydroxyapatite crystals are dissolved and re-precipitated throughout the bone, and as surrounding mineral content is added to the inorganic matrix (Hedges 2002). This chemical alteration of bone also brings into question whether archaeological bones can be identified at all using the method described in this thesis.

To explore potential diagenetic change in the sample bones, I compare the pXRF results for modern and archaeological bone of one species. I use bighorn sheep, which have similar sample sizes for both archaeological (n = 9) and modern (n = 10) cannonbones in this study. Figure 5.01 illustrates the difference in raw calcium

concentration between archaeological and modern bighorn sheep bones. The archaeological specimens clearly show a higher concentration of calcium, likely the result of exposure to surrounding calcium rich sediment and re-precipitation of calcium within the bone fragment, as typically expected in diagenesis (Hedges 2002, Nielsen-Marsh and Hedges 2000). This difference in calcium concentration with diagenesis is one reason this study used trace element/calcium ratios, on the assumption that the concentration of all trace elements is likely to shift in a similar way as calcium concentrations (after Gonzalez and Fowler 2013:410).

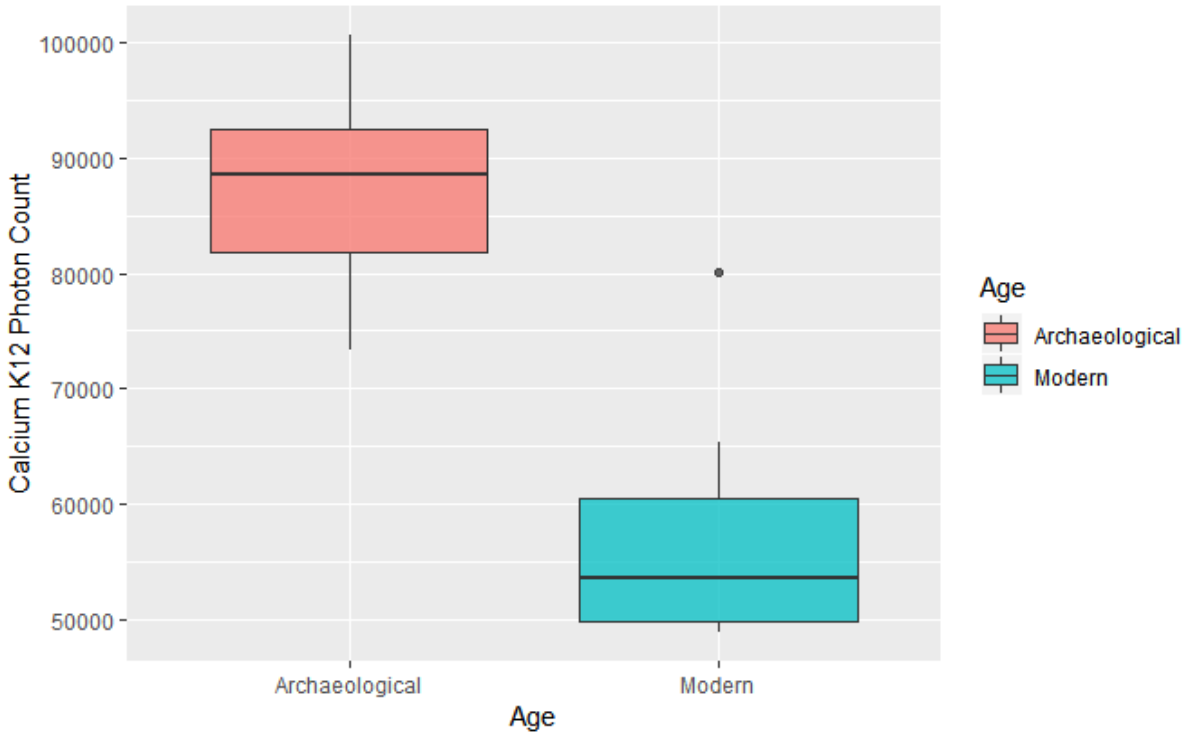

Figure 5.01: Box plot of raw calcium concentrations for modern and archaeological bighorn sheep. Both are plots of average values, with the archaeological data based on three scans per specimen and the comparative on 24 scans per animal

A second major difference was observed in the trace element/calcium ratios. Here we examine the six ratios employed in the RF 10 model that had the highest accuracy. All ratios but the Ba L1/Ca showed significant differences between archaeological and modern bone as demonstrated by a Welch's two sample *t* test (see Figure 5.02 and Table 5.01). Out of the six ratios in RF 10, the Sr/Ca ratio was found to be the only one where values were found to be much higher in archaeological specimens than modern; in the others, it is lower. Strontium is well studied as an element that easily transfers into bone through diagenetic processes because of its relative geologic abundance and its unique characteristic to naturally replace calcium in bone hydroxyapatite (Byrnes and Bush 2016:1042). Barium, being the only ratio found to not significantly differ between groups is also well studied as a robust element, less susceptible to diagenesis (Pearsall 2016:409). This may suggest that certain chemical elements are better suited for the identification of archaeological bone. Despite there being notable chemical differences between archaeological and modern bone, the RF 10 model still maintained a fairly high prediction accuracy.

The differences in trace element/calcium ratios and prediction accuracies due to the effects of diagenesis may explain in part why the RF 10 model is not 100% effective on archaeological specimens. Despite the noted chemical changes, the identification accuracy of archaeological bones in this thesis does suggest that a moderate 67% accuracy can be achieved despite the effects of diagenesis. The results of this thesis clearly demonstrate that there are chemical differences between modern and

archaeological bone. That being said, these differences due to diagenesis are not substantial enough as to make this identification by bone chemistry method unusable.

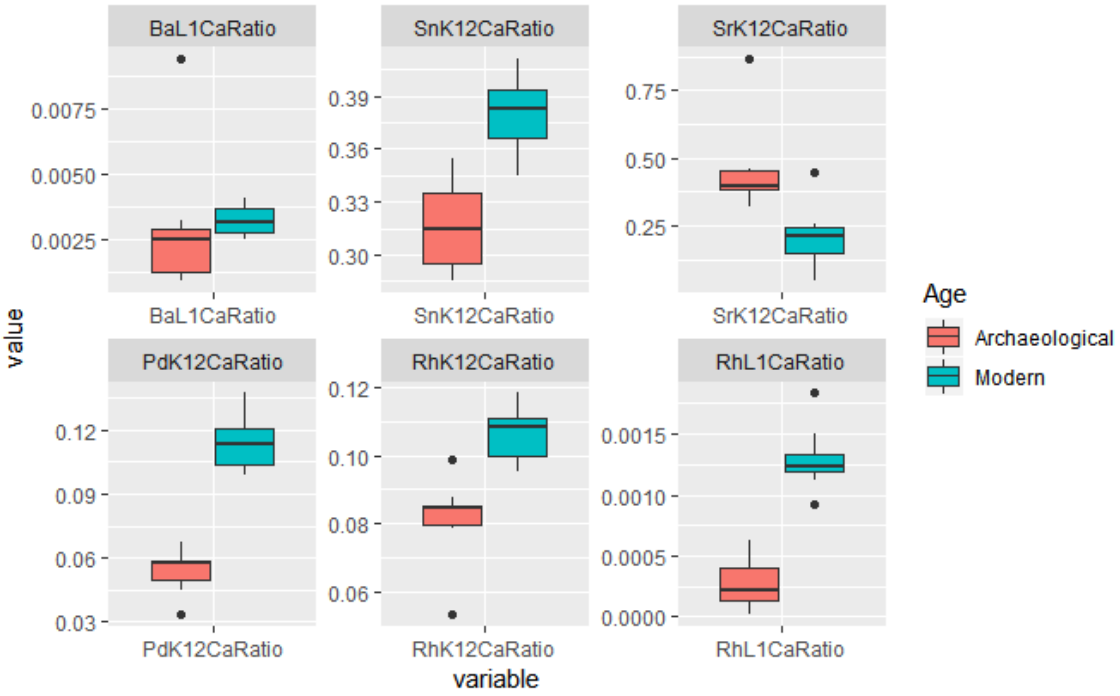

Figure 5.02: Box Plots of trace element/calcium ratios in RF 10 for modern and archaeological bighorn sheep. Both are plots of average values, with the archaeological data based on three scans per specimen and the comparative on 24 scans per animal.

Table 5.01: Welch's *t* test of RF 10 Trace Element/Calcium Ratios for Bighorn Sheep.

Trace Element Ratio	*t* Statistic	n (Archaeological)	n (Modern)	*p* Value	Significant?
Ba (L1) /Ca	-0.3237	9	10	0.7539	No
Pd (K12)/Ca	-11.417	9	10	< **0.0001**	**Yes**
Rh (K12)/Ca	-5.2685	9	10	**0.0001**	**Yes**
Rh (L1)/Ca	-9.705	9	10	< **0.0001**	**Yes**
Sn (K12)/Ca	-6.1986	9	10	< **0.0001**	**Yes**
Sr (K12)/Ca	3.8475	9	10	**0.0019**	**Yes**

Identification Accuracy

Several key observations were made regarding the identification of the study species using the Random Forest models. The first of these occurred during the switch from identifying by species to identifying by family in the modern specimens. Because species in the same family were often identified interchangeably, identifying by family was the logical choice. The three families present in this study have relatively different dietary behaviors which could account for the improvement in accuracy. Both cervids and bovids had relatively high prediction rates in both the modern and archaeological tests respectively (see Table 5.02). These two families are representative of grazing (bovids) and browsing (cervids) dietary behaviors. Antelope on the other hand did not fare as well in the archaeological tests with the two samples being identified as one of the other two families. Although there was a substantially small sample size for archaeological antelope metapodial fragments, the results could reflect the dietary behavior of antelope which exhibit both grazing and browsing behaviors.

Table 5.02: Identification Accuracy of RF 10 by Family.

Sample Size	Cervidae	Bovidae	Antilocapridae	Total
Modern known specimens	20	20	10	50
Correctly Identified [1]	14	20	7	41
Correct %	**70%**	**100%**	**70%**	**82%**
Archaeological known specimens	7	9	2	18
Correctly Identified [2]	6	6	0	12
Correct %	**86%**	**67%**	**0%**	**67%**

[1] RF 10 predictions for modern specimens were based on a training set of half the modern specimens
[2] RF 10 predictions for archaeological specimens were based on a training set of all modern specimens

Identification by family, although not the original goal for this analysis, is still a valid identification method when geographical context is considered. For example,

pronghorn antelope are the only member of their family that live in North America. Thus, if this method was applied and confirmed with traditional morphological analysis, the only identification option would be pronghorn. The same can be said for the two species in the Bovidae family. Bighorn sheep and mountain goats tend to live in different geographical locations in the Pacific Northwest, bighorn sheep preferring dryer subalpine regions east of the Cascades, and mountain goats preferring subalpine environments with modern populations living in the Cascade and Olympic ranges. Thus, if this method was applied and confirmed with morphological analysis, one can use geographical context of where the archaeological remains were found to draw final identification conclusions.

The overall identification accuracies of modern and archaeological bone in this thesis fall within ranges of identification accuracies of similar chemical studies, some of which I have discussed previously in Chapter II. Modern specimen identification, with an identification accuracy by species and by family at 70% and 82% respectively are comparable to the prediction accuracy of Buddhachat et al. (2016a) which had a 78% accuracy rate for identifying modern humeri to species. A 67% family identification accuracy of archaeological fragments in this thesis was found to be lower than comparable ZooMS studies by Buckley et al. 2009 (81% accuracy) and Richter et al. 2011 (89% accuracy). Although having a lower accuracy rate for archaeological specimens, the results of this experimental thesis are not far behind the results of a well-established and destructive identification method such as ZooMS.

Conclusions

It is important to note that the identification model used in this study was based on a relatively small sample size of 10 modern specimens of each species. To account for more intraspecies variation and variation within individual specimens, future studies should seek to increase the sample size of modern specimens. This thesis demonstrated the relative homogeneity of trace element concentrations in all four cannonbones of individual specimens using a Kruskal-Wallis test that was reported in Chapter III. Measuring trace element concentrations in skeletal elements from the same specimen is not reported in similar bone chemistry studies (e.g., Buddhachat et al. 2016a; Nganvongpanit et al. 2016; Richter et al. 2011). Regarding modern specimen research and their use to develop predictive identification models for archaeological bone, future studies should be sure to measure trace element concentrations of skeletal elements to account for variation within individual specimens. Based on the local availability, this study was only able to acquire 18 taxonomically identified archaeological fragments of the selected study species, only one of which was burned. Using the method described in this thesis, larger sample sizes of burned bone should also be considered for future experimental studies. Future studies should also seek to expand the modern and archaeological sample size to continue testing this identification method.

Bone chemistry and the application of XRF has in recent years garnered more of a following in archaeological communities. However, using this method to identify archaeological fauna is a fairly new concept, especially with Pacific Northwest artiodactyls. In this exploratory work, a new nondestructive analysis method was tested

on similarly sized artiodactyls, resulting in a family identification accuracy of 67% for archaeological specimens. Although this result may be a lower accuracy rate than ideal, it does suggest that there are some discernable differences in bone chemistry between similarly sized artiodactyls, specifically between bovids and cervids of the Pacific Northwest.

The application of pXRF in the development of species identification models is promising, but requires further investigation. As demonstrated in this thesis, pXRF is an exemplary tool to use as a relatively quick, nondestructive, and objective analysis method. In combination with more traditional morphological analysis, pXRF and the identification by bone chemistry method suggested here, have the potential to increase the number of identified specimens and is worth expanding on with larger modern and archaeological sample sizes which are likely to improve the identification model.

Peer Reviewed Journal Manuscript

This project will continue after the completion of this thesis as Dr. Wetherell and myself will pursue writing a peer reviewed journal manuscript for submission in the *Journal of Archaeological Sciences: Reports.* With the methods and results of this thesis research at its base, the planned journal manuscript will seek to take a more in depth look at the Random Forest classification models used in this thesis to discuss the validity and applicability of this method for classifying data from archaeological materials.

One adjustment I plan to make to the journal manuscript with my coauthor(s) is to examine and employ the probabilities used by Random Forest to make its family

predictions. In other words, we will refine the Random Forest classification analysis used in this thesis by examining the posterior probabilities. In the context of the Random Forest method described in this thesis, posterior probabilities will inform us the probability of each specimen prediction. We will use posterior probabilities to tune the model and set a selection criteria where the random forest model will only predict a certain family if the probability meets our predetermined criteria (e.g., a family is predicted only if $p > 80\%$). By removing low probability predictions, this new approach will increase the prediction accuracy and confidence in identification of archaeological specimens.

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APPENDIX A:

MODERN SPECIMENS SAMPLED

Species	Specimen No.	Institution	Sex	Collection Location
Odocoileus hemionus	47-172	Conner Museum	M	Okanogan County, WA
Odocoileus hemionus	46-270	Conner Museum	M	Pierce County, WA
Odocoileus hemionus	41-364	Conner Museum	F	Asotin County, WA
Odocoileus hemionus	49-482	Conner Museum	M	Jefferson County, WA
Odocoileus hemionus	PL554	CWU	F	Kittitas County, WA
Odocoileus hemionus	PL059	CWU	M	Sweetwater County, WY
Odocoileus hemionus	PL613	CWU	Unk.	Kittitas County, WA
Odocoileus hemionus	PL627	CWU	Unk.	Kittitas County, WA
Odocoileus hemionus	34272	Burke Museum	F	King County, WA
Odocoileus hemionus	59671	Burke Museum	M	Chelan County, WA
Odocoileus virgianus	49-43	Conner Museum	F	Columbia County, WA
Odocoileus virgianus	86-271	Conner Museum	F	Whitman County, WA
Odocoileus virgianus	41-42	Conner Museum	M	Wahkiakum County, WA
Odocoileus virgianus	41-44	Conner Museum	M	Wahkiakum County, WA
Odocoileus virgianus	76-563	Conner Museum	M	Whitman County, WA
Odocoileus virgianus	90-133	Conner Museum	M	Whitman County, WA
Odocoileus virgianus	PL286	CWU	M	Stevens County, WA
Odocoileus virgianus	32122	Burke Museum	M	Wahkiakum County, WA
Odocoileus virgianus	32131	Burke Museum	F	Wahkiakum County, WA
Odocoileus virgianus	32135	Burke Museum	M	Wahkiakum County, WA
Ovis canadensis	82330	Burke Museum	F	Chelan County, WA
Ovis canadensis	81822	Burke Museum	M	Okanogan County, WA
Ovis canadensis	81686	Burke Museum	Unk.	Chelan County, WA
Ovis canadensis	81687	Burke Museum	Unk.	Chelan County, WA
Ovis canadensis	39480	Burke Museum	F	Carson County, WY
Ovis canadensis	39469	Burke Museum	F	Bighorn County, WY
Ovis canadensis	39467	Burke Museum	M	Albany County, WY
Ovis canadensis	39468	Burke Museum	M	Teton County, WY
Ovis canadensis	80-250	Conner Museum	F	Okanogan County, WA
Ovis canadensis	65-60	Conner Museum	M	Blue Mountain Herd, WA
Oreamnos americanus	48-449	Conner Museum	F	Chelan County, WA
Oreamnos americanus	49-22	Conner Museum	F	Okanogan County, WA
Oreamnos americanus	42-27	Conner Museum	F	Okanogan County, WA
Oreamnos americanus	47-184	Conner Museum	M	Okanogan County, WA
Oreamnos americanus	59674	Burke Museum	Unk.	Pierce County, WA

MODERN SPECIMENS SAMPLED (CONTINUED)

Species	Specimen No.	Institution	Sex	Collection Location
Oreamnos americanus	59675	Burke Museum	Unk.	Pierce County, WA
Oreamnos americanus	32103	Burke Museum	F	Clallam County, WA
Oreamnos americanus	34310	Burke Museum	F	Clallam County, WA
Oreamnos americanus	34311	Burke Museum	F	Clallam County, WA
Oreamnos americanus	35995	Burke Museum	M	Pierce County, WA
Antilocapra americana	32519	Burke Museum	F	Carbon County, WY
Antilocapra americana	33496	Burke Museum	F	Converse County, WY
Antilocapra americana	38622	Burke Museum	F	Fremont County, WY
Antilocapra americana	38617	Burke Museum	M	Carbon County, WY
Antilocapra americana	38619	Burke Museum	M	Natrona County, WY
Antilocapra americana	33498	Burke Museum	M	Converse County, WY
Antilocapra americana	33500	Burke Museum	F	Converse County, WY
Antilocapra americana	34314	Burke Museum	M	Natrona County, WY
Antilocapra americana	38620	Burke Museum	M	Carbon County, WY
Antilocapra americana	PL057	CWU	M	Sweetwater County, WY

APPENDIX B

ADDITIONAL DATA

The *R* script used for the statistical analysis along with the digital data including raw spectra, raw photon counts, and averaged trace element/calcium ratios for all modern and archaeological specimens are available through contacting either Dr. Patrick Lubinski of Central Washington University, or Joshua Henderson. Dr. Lubinski can be contacted via email at Pat.Lubinski@cwu.edu. Josh Henderson can be contacted via email at Joshua.L.Hend@gmail.com. Raw spectra are recorded in spx. file format. Raw photon count and ratio data are recorded in Excel spreadsheets.