Elemental Analyses of Archaeological Bone Using PXRF, ICP-MS, and a Newly Developed Calibration to Assess Andean Paleodiets

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Elemental Analyses of Archaeological Bone Using PXRF, ICP-MS, and a Newly Developed Calibration to Assess Andean Paleodiet

by

Christine L. Bergmann

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Arts with a concentration in Archaeological and Forensic Sciences Department of Anthropology College of Arts and Sciences University of South Florida

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Keywords: human and animal bone specimens, archaeological science, paleodiet

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DEDICATION

I would like to dedicate this thesis to my family, close friends, and all the loved ones that I have lost while in the program. I could not have accomplished this without the love, support, and guidance of my family and close friends. I would like to especially thank my brother for providing me with this opportunity.
ACKNOWLEDGMENTS

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ABSTRACT

As a result of the quick rise of pXRF technology in archaeology, there are concerns regarding the reliability and validity of data output acquired from pXRF. In this study, I test the hypothesis that portable X-ray fluorescence (pXRF) spectrometry can provide reliable and valid results for the analysis of archaeological animal and human skeletal materials in prehistoric Peru to address hypotheses about ancient diet and trade, using newly developed calibration curves. While pXRF systems may come with calibration software, the few if any standards and reference materials provided with the instrument rarely correspond to the vast array of archaeological materials capable of being analyzed by pXRF, including archaeological bone specimens. Empirical calibration curves for Ba, Ca, Fe, and Sr were created using the linear regression analysis of 19 human and animal bone standards analyzed via pXRF and ICP-MS. The results suggest the calibrations for Ca and Sr are sound, but the calibrations for Ba and Fe need to be further improved.

In order to assess the reliability of pXRF (i.e. precision and accuracy), statistical analyses of 60 measurements on human bone specimens as well as on 19 human and animal bone specimens was performed in this study. The results indicate that the precision of pXRF is reliable, but additional work is needed with regard to accuracy. In contrast, the analysis of forty-four prehistoric human and animal bone specimens from varying regions in Peru were used to test the validity of pXRF. The pXRF data support the notion that pXRF is a valid technique to use in the analysis of
bone specimens to address archaeological questions regarding paleodiet and possible trade interactions among individuals that reside in the highland and coastal valley regions of Peru.
CHAPTER ONE:
RESEARCH OBJECTIVE

In this study, I test the hypothesis that portable X-ray fluorescence (pXRF) spectrometry can provide reliable and valid results for the analysis of archaeological animal and human skeletal materials in prehistoric Peru to address hypotheses about ancient diet and trade. In support of my hypothesis, I assess the reliability of the technique by developing a calibration, using matrix matched standards, for barium (Ba), calcium (Ca), iron (Fe), and strontium (Sr). Additionally, a methods comparison, as well as precision and accuracy testing, between elemental concentrations obtained via pXRF versus elemental concentrations gathered from Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) will be made to test the validity and reliability of pXRF data output. Subsequently, the elemental compositions of Ba, Ca, and Sr within bone are used to assess food resource consumption of individuals.

The concentration values of Ba and Sr distinguish between terrestrial versus marine resource consumption and the ratio of Ba/Ca versus Sr/Ca provide evidence for the trophic level of the organism consumed by individuals (Burton and Price 1990; Elias et al. 1982). The ability to extract information regarding trophic level of organisms consumed using Ba/Ca versus Sr/Ca allows for a distinction to be made among individuals that eat mainly agricultural, marine, or terrestrial animal organisms. The results provide archaeologists with information regarding paleodiet which
can be used to make interpretations about possible trade and exchange interactions in prehistoric times as they relate to food resource consumption and distribution.

In this paper, I use data from four archaeological sites (Cardal, Chokepukio, Pacopampa, and Tablada de Lurín) as case studies to demonstrate possible trade interactions between individuals that reside on the central coastal valley and individuals that reside in the highlands of Peru. Cardal and Tablada de Lurín are archaeological sites located on the central coast of Peru, while Chokepukio and Pacopampa are southern and northern highland sites in Peru respectively. Cardal and Pacopampa were occupied during the late Initial Period (200 B.C.E. – AD 600) while Chokepukio and Tablada de Lurín were occupied during the Early Intermediate Period (1800 – 900 B.C.E.). There is evidence from previous studies that trade among highland and coastal valley groups in Peru existed from the IP through the EIP through ethnohistoric evidence, the presence of artifacts, pathological conditions, and zooarchaeological evidence (Burger 1992; Kroeber and Strong 1924; Makowski 2002; Rostworowski 1970; Vradenberg et al. 2009). In support of trade inferences, the data must demonstrate that individuals from the highlands were consuming marine resources while individuals from the coastal valleys were consuming an abundant amount of terrestrial resources, such as camelids, and plant products that are only present in the highland regions, such as manioc.

Although pXRF’s applicability in addressing archaeological questions has seen a rise in the last decade, the technique is not without its limitations (Shackley 2010; Tykot 2016). For example, one main flaw in pXRF is the lack of appropriate matrix matched standard reference material for bone. While pXRF systems may come with calibration software, the few if any standards and reference materials provided with the instrument rarely correspond to the vast array of archaeological materials capable of being analyzed by pXRF (Speakman et al. 2011; Hunt and
According to Speakman and colleagues (2011) factory calibrations that come with pXRF instruments cannot adequately produce quantifications of elemental concentrations for nonmetallic archaeological materials and, for this reason, should be validated through the use of known reference materials similar in composition to the objects being analyzed.

There is therefore a pressing need to create the proper calibrations for the analysis of human bone using pXRF. According to Speakman and Shackley (2013:1436), there are at least 70 publications on the application of pXRF to obsidian analysis, but little has been published on the pXRF analysis of bone specimens (archaeological or modern). Previous studies applying pXRF to human bone include sorting commingled remains (Gonzalez-Rodriguez and Fowler 2013; Richards and Jones 2015; Perrone et al. 2014), assessing elements in bog bodies (Granite and Bauerochse 2013), measuring heavy metal content in archaeological bone (Little et al. 2014), and assessing the applicability of pXRF to the analysis of archaeological bone (Tonoike 2013). A possible reason for the lack of publications involving pXRF and bone is most likely the result of inadequate calibrations provided by the manufacturers to end users, in addition to issues associated with diagenesis of archaeological bones such as soil contamination and lack of preservation.

In this research, I create a calibration for pXRF using standard reference materials created from human and animal bones found at four archaeological sites in Peru (Cardal, Chokepukio, Pacopampa, and Tablada de Lurín), one archaeological site in Italy, and modern animal bones from Florida. The standard reference materials are created using elemental data obtained from pXRF spectra and ICP-MS concentration values. If successful, to the author’s knowledge, this study will be the first time that a calibration for the analysis of bone using pXRF will be created based solely on matrix matched standards; this calibration can therefore be used by future scholars.
researching dietary practices of prehistoric humans to permit archaeological interpretations based on reliable and valid data.

Research Goals - Summarized

1) Create an empirical calibration using matrix matched standard reference materials analyzed via ICP-MS and pXRF.

2) Test the reliability and validity of the calibration for the pXRF analysis of archaeological human and animal bone using paleodietary indicators to address an archaeological question.

3) Test the hypothesis that there were trade interactions between individuals that resided in the highlands of Peru and individuals that lived in the coastal valleys. This hypothesis will be tested through the use of paleodietary indicators.
CHAPTER TWO:

GEOGRAPHICAL BACKGROUND, CLIMATE, AND CHRONOLOGY OF PREHISTORIC PERU

The diverse geography of Peru allowed for the exploitation of many habitats by prehistoric individuals from the Preceramic Period (~9500 – 1800 B.C.E.) through the Early Intermediate Period (200 B.C.E. – AD 600). The eastern portion of Peru encompasses the Andes Mountains and the Amazon, while the western portion contains the arid desert coast and Pacific watershed river valleys (Haas and Creamer 2006:42-43) (Figure 2.1). The Andean geographical zones have their unique environments allowing for an array of microclimates to exist in Peru (Chauchat 1988). Consequently, populations along the coast and in the highlands had a varied diet comprising agricultural, riverine, marine, and terrestrial resources depending on exchange and trade networks as well as location of occupation (Burger 1992). For the purposes of this paper, only the coast and the highlands will be discussed.

The Peruvian coast and highlands comprise a multitude of different environmental zones within each geographical region. This allowed Peruvian populations to acquire a variety of different food resources. For example, the geography on the coastal region of Peru encompasses a desert environment with sandy and rocky shorelines as well as riverine coastal valleys surrounded by rich vegetation depending on seasonal water flow. These environments allowed for the exploitation of marine, riverine, and agricultural resources by prehistoric Peruvian populations.
The highland zones range from steep mountain slopes to rich inter-Andean valleys. Similar to the coast, a wide variety of agricultural and freshwater resources can be found in certain zones of the highlands (Pulgar Vidal 1981).

As a result of the geographical and environmental characteristics of each region there are marked differences in the available resources. For example, the arid climate of the coast limits the amount of vegetation available for the production of agricultural resources in the absence of irrigation agriculture (Bennett and Bird 1964) causing a heavy reliance on marine resources for sustenance. In contrast, the climate in the highlands is extremely varied among environmental zones leading to the availability of different plant resources. Additionally, in certain highland zones, such as the quechua and puna, there is plenty of land available for grazing animals (Pulgar Vidal 1981). In this chapter, I discuss the geography and climate of the highland and coastal regions as well as provide a brief description of the chronology used in this thesis.

Geographical Background

The Peruvian Coast

The desert coast of Peru is riddled with river valleys that stretch from the mountains to the Pacific Ocean. Most of these rivers are fed by water runoff from the Andes Mountains and flow into the ocean (Valdez 2007). Moseley (1972:26) defines five productive zones in the desert coast, or “chala” in which past humans exploited food: the river, the lomas, the sandy littoral, the rocky littoral, and the desert. The chala starts at the ocean and ends at an arbitrary line of 500 meters above sea level (m.a.s.l.); the chala is a productive agricultural zone. Additionally, individuals living close to the coast could have hunted sea lions, seabirds (such as the Huanay), mussels, clams, and anchovies. There is little terrestrial fauna available in the chala (Pulgar Vidal 1981).
The northern coast includes all of the valleys from Piura to Casma. The central coast includes all of the valleys from Huarmey to Lurín, (Bennett and Bird 1964) while the south coast extends from the Cañete Valley to Yauca (Quilter 2014). Geographically, each coastal valley is similar to their adjacent valleys, but the water flow and amount of arable land vary considerably (Haas and Creamer 2006:42-43). For example, the rivers in the northern valleys have permanent and abundant water during the highland rainy season while the rivers in the southern coastal valleys of Peru only carry water during the summer months and possess a more desert environment. Additionally, some of the northern valleys are bigger and have more arable land than the southern valleys. Moreover, the southern coastal valleys possess a more desert environment (Bennett and Bird 1964:68; Haas and Creamer 2006:42-43; Valdez 2007).

While the neighboring valleys may be geographically similar, there are general differences from the north to the south. The southern coast possesses the driest and narrowest river valleys in the Pacific watershed (Silverman 1996:98). As a general rule, agricultural productivity is lower in the southern valleys compared with the northern and central valleys (Reindel and Wagner 2009).
The Highlands

The highlands are comprised of several environmental zones and intermittent rivers that are fed by glacial runoff and precipitation. The zones are designated by their agricultural potential and have been classified by the geographer Javier Pulgar Vidal (Figure 2.2). Between the Pacific
coastal region and up into the highlands is the *yunga* zone which is located between 500 and 2,300 m.a.s.l. Products from the *yunga* zone include fruits such as pacay (*Inga feuillei*), guava, cherimoya (*Annona cherimola*), and coca, *aji* pepper (*C. baccatum*), and avocado (*Persea americana*). The *yunga* zone is broken into two geographical categories; the maritime *yunga* and the river *yunga*. The maritime *yunga* extends from 500 to 2,300 m.a.s.l on the bottom western slope of the Andes. It comprises narrow valleys capable of producing vegetation such as the willow, molle (*Schinus molle* or Peruvian pepper tree), and reed.

The *quechua* (2,300 to 3,500 m.a.s.l.) and the *suni* zones (3,500-4,000 m.a.s.l.) follow the *yunga* as one moves further upward into the Andes Mountains. The *quechua* is characterized by steep slopes created by the inter-Andean valleys and is a highly productive region. Crops such as, maize, beans, potatoes, olluco, squash, and arrachacha (although this crop is rarely grown in the south) flourish in this region (Burger 1992; Pulgar Vidal 1981; Rosas and Shady 1974). In contrast to the *quechua*, the *suni* has steeper slopes, rocky outcrops, and pampas. As a result of the geography of the *suni* there is limited agricultural land, but tubers (potato, oca, olluco, mashua), chenopodids (quinoa, achis, caniwa), barley, and wheat can grow in the region (the latter two being brought by the Spaniards).

The *puna*, or *jalca* zone, (4,000 to 4,800 m.a.s.l.) consists of grasslands, lagoons, lakes and streams. This high area supports large herds of camelids (e.g. vicuña, guanaco, alpaca and llama). A variety of tubers grow well in this region. Additionally, the highland lakes of the *puna* support waterfowl, amphibians, and aquatic plants, particularly totora reeds (Pulgar Vidal 1981; Rick
1988). There are geographical differences between the northern, central, and southern *punas*. For example, the *puna* in northern Peru has wetter grasslands than in the south, although limited, and is often referred to as *páramo* (the common native term is *jalka*) (Brush 1972; Burger 1992; Rick 1988:6). Llamas and alpacas grazed in this northern region, but according to Brush (1972:29) they do not anymore.

Finally, following the *puna* is the *cordillera* zone (4,800 to 6,768 m.a.s.l.). This high altitudinal zone has limited resources for human subsistence. However, there are some fauna, such as the male vicuña, vizcacha, and condor that can be found at this altitude (Pulgar Vidal 1981). Interestingly, in certain areas of Peru, the *yunga, suni, quechua*, and *puna* can be reached within one day (Burger 1992).

Climate

*The Peruvian Coast*

The climate of the Peruvian coast differs from other ecological zones of Peru as a result of interactions between trade winds and the Pacific Ocean. As winds blow west over the Pacific, they are chilled by the ocean waters. This prevents precipitation on the coast leading to an annual arid climate (Bennett and Bird 1964; Burger 1992:15; Chauchat 1988:43). The particular interactions between the trade winds and the Pacific Ocean affect the movement of the ocean’s currents. For example, the Humboldt Current flows north from Antarctica to the South American coast resulting in the replacement of warm water with cooler water. The Humboldt Current allows the proliferation of plankton which provide sustenance to fish, such as the anchovy (Royal Geographic Society 2015).
As a result of the effects of the Humboldt Current, seasonal fog oases, or “lomas”, form from a dense heavy fog (garua) which cannot exist far from the shore (Chauchat 1988:43). Lomas are seasonal cloud forests that provide natural vegetation in a desert environment. Lomas are dry between December and July, but coastal vegetation can arise from December to March (i.e. winter months) because coastal valleys receive a majority of water from run off from the highlands (Rick 1988). Individuals exploited the lomas as far back as 7000 B.C.E. for snails, wild potatoes, gourds, wild grasses, owls, lizards and wild seeds that could be ground into flour. Additionally, highland deer and guanaco that grazed in the lomas could be hunted during the winter months (Lanning 1965).

The Highlands

The climate in the yunga zone (the geographical interim of the coast and the highlands) is slightly humid and warm reaching 27°C (81°F) in the maritime yunga and 30 °C (86°F) in the river yunga with little rain during the summer months. The quechua zone is frost free and has a temperate-dry climate, with intermittent rains from December to March. In the suni zone, there is more rainfall than the quechua during the months of January to April. The average annual temperature in the suni is 11°C (52°F), with fluctuating low temperatures from May to June from -16°C (3°F) to -1°C (30°F) (Pulgar Vidal 1981).

The puna has an annual average temperature of around 7°C (46°F), varied precipitation, and the temperature can range from 0°C (32°F) to 20°C (68°F) within one day (Brush 1972; Pulgar Vidal 1981). The climate in the puna of the central portion of Peru has varied precipitation with wet seasons between November and April and dry seasons between May and October. In contrast, the puna in the southern highlands have more of a distinction between wet and dry seasons than
the north-central highland punas. (Rick 1988). Furthermore, the puna of north-central Peru (i.e. paramo) has an annual precipitation from 500 mm to 850 mm (Brush 1972). The snow and periglacial zone (i.e. the Cordillera region) has a cold climate with persistent snow and hail (Pulgar Vidal 1981).

Chronological Sequence for Peruvian Prehistory

There are two commonly used chronological systems for referencing the prehistory of Peru; one developed by Luis Lumbreras and one developed by John H. Rowe, Dorothy Menzel, and Lawrence Dawson. The chronology sequence suggested by Max Uhle, and finalized by the work of John H. Rowe, Dorothy Menzel, and Lawrence Dawson is the most commonly used chronology for the Central Andes in the United States (Quilter 2014) and will be used in this paper (Table 2.1). The chronological sequence is based on the analysis of ceramics from the Ica Valley. It has alternating “Horizons” and “Periods” with Horizons representing times in which there was a rapid spread of a consistent style throughout various regions of Peru (this would indicate that one cultural tradition spread and influenced other regions) while Periods represent times when cultures were more locally based and influenced (Menzel et al. 1964; Rowe 1962). Furthermore, I use the subdivisions of the Preceramic Period that were suggested by Lanning and Patterson (Lanning 1967:25), although the subdivisions are not fully supported by some scholars (Chauchat 1988; Quilter 1991; Rick 1988).
Table 2.1. Prehistoric chronology sequence for Peru.

<table>
<thead>
<tr>
<th>Cultural Sequence</th>
<th>Time Frame</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late Horizon</td>
<td>AD 1476-1532</td>
</tr>
<tr>
<td>Late Intermediate Period</td>
<td>AD 1000-1476</td>
</tr>
<tr>
<td>Middle Horizon</td>
<td>AD 600-1000</td>
</tr>
<tr>
<td>Early Intermediate Period</td>
<td>200 BCE-AD 600</td>
</tr>
<tr>
<td>Early Horizon</td>
<td>900-200 BCE</td>
</tr>
<tr>
<td>Initial Period</td>
<td>1800-900 BCE</td>
</tr>
<tr>
<td>Preceramic Period VI</td>
<td>2500-1800 BCE</td>
</tr>
<tr>
<td>Preceramic Period V</td>
<td>4200-2500 BCE</td>
</tr>
<tr>
<td>Preceramic Period IV</td>
<td>6000-42000 BCE</td>
</tr>
<tr>
<td>Preceramic Period III</td>
<td>8000-6000 BCE</td>
</tr>
<tr>
<td>Preceramic Period II</td>
<td>9500-8000 BCE</td>
</tr>
<tr>
<td>Preceramic Period I</td>
<td>?-9500 BCE</td>
</tr>
</tbody>
</table>
CHAPTER THREE:
BACKGROUND AND LITERATURE REVIEW

The Principles of Portable Energy Dispersive X-Ray Fluorescence Spectrometry

X-rays are a form of electromagnetic radiation with properties of waves and particles (i.e. photons) (Serway et al. 2009). X-rays are produced when high energy (a.k.a. high speed) electrons lose energy by slowing down, changing direction, or moving to a lower energy level in an atomic shell. Atomic shells are orbitals (also known as energy levels or energy shells), designated K, L₁, L₂, L₃, M₁…M₅, N₁…N₇ etc., that are occupied by a specific number of electrons and surround the nucleus of an atom. When an electron moves from an outer shell to an inner shell, these letters have further designations to include subshells of each energy level. The principle of the latter is the basis of X-ray fluorescence (Bruker 2016; Miller 1985).

X-ray fluorescence directs high energy X-rays (a.k.a. photons) at a sample to study the elemental composition of a substance. When an instrument, such as a pXRF spectrometer, has a flowing power source (e.g. electricity), X-ray radiation is generated from the X-ray tube when electrons accelerate from a cathode material to an anode material causing the electrons to decelerate and lose energy. Subsequently, primary (or incident) X-ray photons hit the sample, and therefore the atoms of an element within that sample, causing an electron to be ejected from the inner shell of an atom (now considered an ion). This is called the photoelectric effect in which part of the photon is used to overcome the binding energy of the electron and the rest is transferred as
kinetic energy, thus the photon is completely absorbed by the atom (Bruker 2016; Pollard et al. 2007; Serway et al. 2009; Shackley 2010).

When an electron is ejected from the inner shell of an atom, there is a vacancy in the inner shell. These vacancies produce instability in the atom and are thus filled by way of an electron moving from an outer shell of an atom into the vacated inner shell. When the inner shell vacancies are filled by the outer shell electrons, this transition gives off characteristic secondary X-rays (e.g. K\(_{\alpha 1}\), K\(_{\alpha 2}\), L\(_{\alpha 1}\), etc.) that are lower in energy than the incident X-rays and are picked up by the detector within the spectrometer. For example, when an electron is removed from the K shell of an atom and an electron from the L\(_2\) shell falls into the K shell this X-ray transition has a specific intensity that is designated K\(_{\alpha 2}\) and can be quantified. The secondary X-rays, called fluorescent radiation, are specific to a given element in which the total radiation intensity of these X-rays at a given wavelength is proportional to the concentration of that element (Bruker 2016; Miller 1985; Pollard et al. 2007; Serway et al. 2009; Shackley 2010).

Among other parameters, the specified amount of electricity in the form of voltage (energy) and current (the flow of energy) are responsible for enabling an X-ray photon to eject an electron from an inner energy shell (i.e. higher energy state) to an outer energy shell (i.e. lower energy state) in an atom. For this reason, it is important to be aware of the binding energies of electrons to atoms of an element. The binding energy, otherwise known as the absorption edge, is the minimum amount of energy it takes to move an electron from one shell to another. In order for an electron to move from a higher energy state to a lower one (and therefore emit secondary X-ray photons, or fluoresce), the voltage applied during analysis must be greater than the binding energy of the electrons in the inner shell of an atom for each element of interest. The resulting X-ray
photons are characteristic of specific elements because the amount of energy difference from one shell to another is known (Bruker 2016; Miller 1985).

Although the aforementioned appears straightforward, the X-ray spectrum produced from pXRF, is not limited to the appearance of solely elemental peaks of secondary X-ray photons. There are certain interactions between primary X-rays, the sample material, and the inner components of a pXRF spectrometer that can cause the data on the X-ray spectrum to be misleading. These “interactions” can lead to background noise, scattered radiation, spectral interferences, and sum and escape peaks on the X-ray spectrum. The aforementioned can cause incorrect spectral evaluation without knowledge of the physics behind pXRF. The section below describes this in detail.

*Inspecting the Spectrum*

There are several “phenomena” that can occur, and therefore be present on the resulting X-ray spectrum, when primary X-rays from the X-ray tube interact with a sample material: Bremsstrahlung (also referred to as continuum, white radiation, or background), Compton scatter (also referred to as inelastic or incoherent scattering), and Raleigh scatter (also referred to as elastic or coherent scatter). Bremsstrahlung means “braking radiation”. When electrons are accelerated then decelerated within the X-ray tube of a pXRF spectrometer, this causes continuous radiation (X-ray photons) to be emitted, which is evaluated on the X-ray spectrum as the regions that do not include the peaks of elements. Bremsstrahlung is important to consider when analyzing light elements because the background produced on the spectral interface is higher for the lighter elements. For this reason, when using ED XRF with a Rh anode in the X-ray tube, elements below Na cannot and are not analyzed in this research. Additionally, Bremsstrahlung radiation can be
minimized with the use of filters and high voltage tube settings. This research utilizes a filter and
a high voltage setting (Jenkins 1999:175; Pessanha et al. 2009; Pollard et al. 2007; Shackley 2011;

When using pXRF to analyze a sample, the analyte’s surface morphology has to be smooth
and flat to ensure the appropriate interaction between the sample and the incident X-ray beam
coming from the pXRF spectrometer (Shugar and Mass 2012:28). This involves being cognizant
of the morphological characteristics of the sample, including the shape and size of the material. If
the sample is not flat and close to the incident beam, this can cause more scattered radiation, such
as Rayleigh and Compton scattering from the X-ray tube to appear on the spectra which can affect
the qualitative and quantitative data. This was demonstrated by (Grave et al. 2012) in which
geological samples that were more morphologically diverse presented more noise on the X-ray
spectrum as a result of Rayleigh scattering. All samples in this research were placed as close to the
incident X-ray beam as possible and only the smoothest and flattest regions of the bones were
used.

The Compton scatter peak is the characteristic line on the X-ray spectrum coming from the
target material of the X-ray tube which is produced on the spectrum, at 18.5-19.5 keV, as the
element that is in the anode material of the X-ray tube (in the case of the Bruker III-SD it is the
Rhodium peak). It is beneficial to use when analyzing materials that have varying sizes and
surfaces because the area of the peak can demonstrate the density of a material (less dense material
will have a broader Compton peak). Finally, Raleigh scatter is produced when primary X-rays
from the X-ray tube hit a sample resulting in a lack of fluorescence, no loss of energy, and are
present on the X-ray spectrum as the element within the anode material (e.g. Rhodium in this case)
at ~20.2 keV (Ferguson 2012; Jenkins 1999; Pollard et al. 2007; Shackley 2010; Shugar and Mass
Raleigh scattered radiation is more broad than Compton scattered radiation when using lower photon energies, especially for high Z materials (Markowicz 2002:25).

The radiation that can come from the X-ray tube within a pXRF spectrometer can affect the elemental results by producing more background on the spectra as a result of the energy and intensity distribution of the scattered radiation. Pessanha and colleagues (2009) performed an experiment to evaluate the amount of scattered radiation produced as a result of Rayleigh and Compton effects on cultural heritage materials with different matrices using pXRF and ED-XRF. For the pXRF analysis, using 30 kV and 100 µA, the authors found that materials, such as certain plants with light matrices (composed of C, H, O) received more intensity from the Compton scattered radiation than the Rayleigh scattered radiation. As a result, elements such as Sr and Pb could not be accurately detected. For medium Z materials, such as bone ash and clay, composed of the elements Ca, Fe, and P, the background was less pronounced allowing for the detection of most of the elements in the materials except for elements under 30 ppm. The ability to obtain reliable Fe and Sr spectral data for materials with a mid-Z matrix, using a higher X-ray energy Kα excitation energy (40 keV) was further demonstrated by Grave and colleagues (2012) when analyzing archaeological hatchet blades. For materials with heavy matrices, composed of elements such as Cu and Ag, all of the elements were detected using pXRF (Pessanha et al. 2009). All of the samples in this research were analyzed using 40 KeV.

In addition to the phenomena that can be present on the X-ray spectrum as a result of interactions within the X-ray tube of a pXRF spectrometer, there are misleading peaks that can be present on the spectrum as a result of X-rays interactions with the detector. Sum peaks on the spectrum occur when two photons of the same characteristic fluorescent X-ray enter the detector at the same time. This results in one single X-ray event with double the original photon energy and
appears on the spectrum as more than one peak for a single element. This can be an issue when the amount of the calculated photon energy interferes with the photon energy of another element. To combat this issue, it is necessary to reduce the X-ray flux by lowering the flow of current (amperage) to the X-ray tube and/or using a filter (Shugar and Mass 2012:33). All of the samples in this research were analyzed using 11 µA and a filter.

Another issue that can arise as a result of the detector is the appearance of silicon escape peaks on the spectrum. These peaks come from the silicon in the detector (the Bruker III-SD uses a Silicon Drift Detector). When characteristic X-rays from the sample reach the detector, this can cause Si to fluoresce causing a reduction in the characteristic X-ray energy of certain elements by 1.74 KeV. For example, a silicon escape peak for copper can be misinterpreted as iron because it will have the same amount of energy as the energy it takes to cause Fe to fluoresce (Shugar and Mass 2012:33). In order for escape and sum peaks to not be misinterpreted on the spectrum, the analyst must be aware of the X-ray energies of elements.

Principles of ICP-MS

In recent decades, differing techniques within Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) have seen a rise in usage in archaeology for the trace and major elemental analyses of a variety of materials including ceramics, obsidian (Speakman et al. 2007; Tykot and Young 1996:121), metals (Tykot and Young 1996:120), marble (Ozdemir and Gucer 2016), and bone (Little et al. 2014; Shafer et al. 2008; Speakman et al. 2007:292). ICP-MS can provide accurate, precise, and fast results (after the samples have entered the instrument), is relatively inexpensive, and can output quantitative elemental data on a variety of elements with detection limits below parts per billion (ppb), even parts per trillion (ppt) depending on the instrument
(Pollard et al. 2007). The method has been used to demonstrate the accuracy of other analytical methods, such as pXRF, that appear to be less credible.

There are variants to the technique, such as ICP-OES, LA-ICP-MS, Q-ICP-MS, and MC-ICP-MS that differ in the manner of introduction into the plasma (i.e. whether the sample preparation involves creating a solution or solid from the sample), detection limits (ppb to ppt), and the type of magnetic separation applied to the ions. In this study, an ELAN DRC II ICP Mass Spectrometer, which is a quadrupole ICP-MS (Q-ICP-MS), is used for analysis of archaeological bones. The Q-ICP-MS uptakes the liquid analyte into a nebulizer via peristaltic pumps. While in the nebulizer, the aqueous analyte mixes with the argon gas to create an aerosol. After the aerosol has been created, it passes through a spray chamber for the removal of large droplets before being carried into a plasma torch. Subsequently, the atoms in a sample are ionized (i.e. charged) at a high temperature and injected into the quadrupole mass selector. The quadrupole mass selector, which consists of four metal rods, separates ions based on mass-to-charge ratio (m/z) using radio frequency (RF) and direct current (DC) voltages (Neff 2017; Pollard et al. 2007).

Previous Calibrations Used in pXRF Bone Analysis

pXRF is relatively inexpensive, non-destructive, and can provide elemental data rapidly (Tykot 2016). In light of the advantages to the technique, it has not been extensively applied to the analysis of bone as a result of the inadequate calibrations for bone analysis available to scholars. Calibration curves created using standards composed of geological materials, such as the “mudrock” calibration created by Dr. Harry Rowe (Rowe et al. 2012), have been utilized in the past for bone analysis using pXRF (Perrone et al. 2014; Tonoike 2013). Although the mudrock calibration is the preferred calibration to use for the analysis of bone using pXRF (Perrone et al. 2014; Tonoike 2013).
2014), the standards used to create the calibration are not matrix specific to bone; the calibration is made from geological materials, not human bone. The standards used for the calibration are composed of five “internationally-accepted” standards and 85 in-house reference materials that are made from mudstone and shale (Rowe et al. 2012).

The importance of using a matrix matched base calibration was demonstrated by Hunt and Speakman (2015) in their research involving the pXRF analysis of ceramics. Hunt and Speakman (2015) used the mudrock calibration to obtain elemental concentration values for ceramics. The results demonstrated that the mudrock calibration failed to produce suitable strontium values for the ceramic material. One possible reason for this was the lack of the use of matrix matching standards to calibrate the data.

Another calibration utilized in the pXRF analysis of bone is a “silicon-based” calibration, designed for the analysis of obsidian and other lithic material which was developed by Glascock and Ferguson (2012) (from now on referred to as the obsidian calibration). The obsidian calibration was developed using 40 obsidian reference standards. Similar to the mudrock calibration, the standards used for calibrating pXRF bone analysis data are composed of geological materials, not bone. Although the obsidian calibration provides a good range of values for Ba, with high and low standards ranging from 1 to 2287 ppm, it does not cover the range of values for Ca and Sr that can be found in bone. For example, the standards used for the “obsidian calibration” only have a maximum of 5% calcium. Additionally, the standards used only allow for the quantification of Sr up to 402 ppm (Glascock and Ferguson 2012); Sr values can be much higher than that as demonstrated from elemental analysis done on human bones from Peru (Farnum 1996).

Although the aforementioned calibrations have their own strengths and weaknesses, they clearly are not adequate for the analysis of bone. For this reason, the creation of an empirical
calibration for pXRF is necessary. Empirical calibrations are created by choosing standards that closely match the elemental compositions of the unknown samples. According to Drake (2013), five assumptions must be met for an empirical calibration to be successful: the sample must be fairly homogenous, the standards must be composed of the same material as the sample being analyzed, every element found in the sample must be present in the standard as well as encapsulate the minimum and maximum amount of elements found in the sample, and the data taken from the sample and the standard must use the same parameters (energy, filter, current, and atmosphere). In this research, an empirical calibration for Ba, Ca, Fe, and Sr is created by adhering to the five criteria suggested by Drake (2013).

Barium, Calcium, and Strontium as Paleodietary Indicators

Ba and Sr are alkaline-earth elements present in the local environment and then consumed by humans through the uptake of plants and animals (Burton et al. 2003). When analyzing the concentration values of Ba and Sr in human bone, it is possible to see a difference in the consumption of marine meat sources versus terrestrial meat sources due to the element's ability to be retained in bone at levels that reflect their dietary inputs (Burton and Price 1990, 1991). Sr concentrations in bone have been used in the past to study terrestrial versus marine resource consumption (Connor and Slaughter 1984; Nelson et al. 1986) but there can be an overlap in Sr values between terrestrial and marine organisms because of the high content of Sr in terrestrial sediments and seawater (Burton and Price 1991; Sealy and Sillen 1988). As a result, it is more advantageous to use the concentration values of Ba and Sr together to assess marine versus terrestrial resource consumption.
Ba, similar to Sr, is not engaged in any known metabolic processes and occurs as barium sulfate (BaSO₄). Barium sulfate is more physiologically discriminated against than Sr, has low solubility in water, and is more chemically stable. As a result, Ba is taken up by plants in lower quantities than Sr and is low in seawater. As a result, the concentration values of Ba and Sr will be higher for terrestrial organisms than marine organisms (Burton and Price 1990, 1991; Ezzo 1992; Pate 1994). It is also possible to examine the trophic level of an organism consumed by assessing the Ba/Ca and Sr/Ca ratios which can be very advantageous when analyzing the consumption of aquatic resources (Burton 2003).

Ba and Sr can substitute for calcium in hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂], the mineral portion of bone. This allows for a physiological process called biopurification to occur. During the biopurification process, following the intake of certain food sources, such as meat or plants, Ba and Sr are discriminated against in the mammalian gut while Ca is not (Burton et al. 2000; Meadors 1992). Ba and Sr can also be filtered in the mammalian gut from Ca such that the levels of Ba and Sr absorbed in bone are less than the levels in their diet, in proportion to the amount of Ca (Burton et al. 2003; Pollard et al. 2008). For example, according to Burton and colleagues (2000), because of this discrimination, the level of Sr decreases as one moves up the food chain resulting in an herbivore having 1/5 the Sr/Ca ratio of its diet. Furthermore, an organism feeding exclusively on an herbivore will have 1/5 the Sr/Ca ratio of the herbivore diet in its bones (Burton et al. 2000). The intestinal absorption ratio for Ca, Sr and Ba is, respectively, 10:5:1 (Gilbert et al. 1994).

Consequently, the principles of biopurification indicate soils have more Ba/Ca and Sr/Ca than the plants which grow in them. Plants have higher Ba/Ca and Sr/Ca ratios than herbivores, and herbivores have higher Ba/Ca and Sr/Ca ratios than the carnivores. As an organism moves up the trophic levels, the reduction of ratios relative to dietary ratios occur at fixed ratios and can be
used to estimate the average diet of a mammal (Burton et al. 1999; Burton 2003). Although not without its limitations, archaeologists have been able to exploit this process to estimate the average diet of prehistoric humans.
CHAPTER FOUR:
THE CREATION OF AN EMPIRICAL CALIBRATION: MATERIALS AND METHODS

In this chapter, I begin by outlining the five criteria for creating a successful empirical calibration. Second, I describe the pXRF analysis of human and animal bone specimens which are used as matrix matched standards to create calibration curves for barium (Ba), calcium (Ca), iron (Fe), and strontium (Sr). Third, I present the analytical procedure for the ICP-MS analysis of the same matrix matched standards used to create calibration curves for the same elements. Finally, I describe the process used to create the calibration curves, which include the linear regression analysis of data obtained from the pXRF and ICP-MS analyses.

Five Criteria for Creating an Empirical Calibration

First, it is necessary to ensure homogeneity of each standard when using a heterogeneous substance. Homogeneity mitigates the possibility of misrepresenting the elemental data in a standard and minimizes matrix effects. The traditional method used to accomplish homogeneity in the preparation of standards for XRF analysis is to create pressed pellets or fused glass beads (Aimers et al. 2012:435; Hein et al. 2002; Hunt and Speakman 2015; Reidinger et al. 2012; Rowe et al. 2012). Another method that can be used to ensure homogeneity of a heterogeneous material
is to crush the sample into a fine powder using a mortar and pestle (Shaffer, n.d.). This research used the latter method as a result of cost considerations.

Second, the standards must be composed of the same material as the analyte. This is referred to as creating a “matrix match” of the standards and the samples. When analyzing a sample, it is necessary to consider the size of the grains, the homogeneity or heterogeneity, and the mineralogical component of the material. All of these factors can interact to cause what is called a “matrix effect”. In order to minimize matrix effects, the standard should be composed of the same material being analyzed (Hunt and Speakman 2015; Shugar 2013:179). For this reason, archaeological and modern human and animal bone specimens were used as standards in this research because the analyte in bone specimens is Ca.

Third, the standards need to contain the full range of elemental concentration values that can be found in the analyte (Aimers et al. 2012:438; Drake 2012; Ferguson 2012:406; Shugar 2013:179). As demonstrated by Hunt and Speakman (2015), if the standards used to create a calibration do not cover the range of elemental concentration values that can be found in the analyte this can lead to the output of inaccurate concentration values. Archaeological bone can have a varying range of concentration values as a result of contamination and preservation issues (Allmae et al. 2012; Farnum et al. 1995). For this reason, archaeological human and animal bones were used as standards rather than internationally recognized standards. According to Shugar and Mass (2012:31), the range of elemental concentrations for unknown samples to be analyzed with the pXRF can be obtained through the use of another analytical technique, such as ICP-MS, and/or researching past data similar to the unknown samples. ICP-MS has been used in the past to obtain the elemental concentrations that can be present in archaeological bone specimens (Allmae et al.
2012). In this research, ICP-MS was used to get the quantitative elemental concentrations as well as the range of elements that can be found in archaeological bone.

Fourth, the standards need to contain all of the elements that can be present in the analyte (Shugar 2013:179). Although ICP-MS can provide the majority of elements present in archaeological bone specimens, with the use of an appropriate calibration, only calibration curves for Ba, Ca, Fe, and Sr were created in this research because these elements have been demonstrated to be adequate paleodietary and diagenetic indicators (Buikstra et al. 1989; Burton and Price 1990; Ezzo 1995; Price et al. 1992). Depending on the instrument, the portable X-ray fluorescence spectrometer may be optimized for elements ranging from Na to U (Bruker 2016). As a result, the elements of interest in this research can be analyzed via pXRF with the use of an appropriate calibration. Finally, in order for a calibration to be effective, the standards and samples must be taken using the same parameters (energy, filter, current, and atmosphere). All of the standards and samples used in this research adhere to the final criterion.

pXRF Analysis

Samples and Instrumentation

Nineteen animal and human bone powder samples (Table 4.1) from the USF Archaeological Science Laboratory and the USF Osteology Laboratory were analyzed using a Bruker Tracer III-SD obtained from Dr. Robert H. Tykot at the University of South Florida. The Bruker Tracer III-SD used in this research possesses a Silicon Drift Detector with a typical resolution of 145 eV at 100,000 counts per second (cps) that is cooled by an internal Peltier device and utilizes an X-ray tube with a rhodium target as its excitation source.
Table 4.1. Standards analyzed via ICP-MS and pXRF, specimen, site, region, and time period.

<table>
<thead>
<tr>
<th>Site</th>
<th>Bone Specimen</th>
<th>Number of Samples used as Standards</th>
<th>Region</th>
<th>Time Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardal</td>
<td>Human</td>
<td>2</td>
<td>Central Coast, Peru</td>
<td>Initial Period</td>
</tr>
<tr>
<td>Pacopampa</td>
<td>Human</td>
<td>1</td>
<td>Northern Highlands, Peru</td>
<td>Initial Period – Early Horizon</td>
</tr>
<tr>
<td>Tablada de Lurín</td>
<td>Human</td>
<td>4</td>
<td>Central Coast, Peru</td>
<td>Early Intermediate Period</td>
</tr>
<tr>
<td>Chokepukio</td>
<td>Human and llama</td>
<td>4</td>
<td>Southern Highlands, Peru</td>
<td>Early Intermediate Period – Late Intermediate Period</td>
</tr>
<tr>
<td>Sovizzo</td>
<td>Human</td>
<td>4</td>
<td>Italy</td>
<td>7th Century AD</td>
</tr>
<tr>
<td>N/A</td>
<td>Pig</td>
<td>2</td>
<td>Tampa, FL United States</td>
<td>Modern</td>
</tr>
<tr>
<td>N/A</td>
<td>Cow</td>
<td>1</td>
<td>Tampa, FL United States</td>
<td>Modern</td>
</tr>
<tr>
<td>Tierra del Fuego</td>
<td>Sea lion</td>
<td>1</td>
<td>Bolivia</td>
<td>Early Horizon</td>
</tr>
</tbody>
</table>

*pXRF Analysis of Samples*

Prior to analysis, each whole bone sample was ultrasonically cleaned in deionized water (DI) using the heat setting for 10 minutes. This was repeated twice per sample. The bones were then dried in an oven overnight at a low temperature. When the bones were completely dry, each bone was drilled using a diamond bit drill to obtain powder. When possible, the inside of the bone was drilled to minimize surface contamination that can occur on the outer surface of bone as a result of being buried in the ground. The powder was then homogenized with a mortar and pestle.

Subsequently, each powder sample was placed in a SPEX SamplePrep X-Cell 31 mm sample holder and put on the spectrometer. The spectrometer was mounted on a secure tripod to ensure stability of the instrument. Each sample holder contained at least 100 mg of powder to meet the 3 mm infinite thickness requirement set by Bruker AXS (Nazaroff et al. 2010). Each sample inside of the holder covered the X-ray beam path completely to optimize photon counts and reduce potential backscatter. pXRF spectrometers typically measure a 5x7 mm area, although they can
measure smaller areas depending on the size of the beam hole on the instrument (McGlinchy 2012). All analyses were measured for 120 live seconds using tube settings turned to 40 keV and 11 µA, with a 12 mil Al, 1 mil Ti, 6 mil Cu filter placed in the X-ray path, and no vacuum. Each sample was measured twice, mixing the powder in between measurements.

ICP-MS Analysis

Samples and Instrumentation

The same archaeological and modern human and animal bone specimens (n=19) used in the previous pXRF analysis from the University of South Florida Archaeological Science Laboratory and the University of South Florida Osteology Laboratory were analyzed via ICP-MS. A Perkin-Elmer Elan DRC II Quadrupole Inductively Coupled Plasma Mass Spectrometer from the University of South Florida, Department of Geology, Center for Geochemical Analysis Plasma Instrument Laboratory was used to analyze five human and animal bone specimens. The additional 14 specimens were analyzed using an Agilent 7500cx ICP-MS with a ASX-500 autosampler from the College of Marine Science at the University of South Florida – Saint Petersburg.

Preparation for ICP-MS Analysis

Prior to analysis, each whole bone sample was ultrasonically cleaned in deionized water (DI) using the heat setting for 10 minutes. This was repeated twice per sample. The bones were then dried in an oven overnight at a low temperature. When the bones were completely dry, each bone was drilled using a diamond bit drill to obtain powder. When possible, the inside of the bone was drilled to minimize surface contamination that can occur on the outer surface of bone as a
result of being buried in the ground. The powder was then homogenized with a mortar and pestle. Subsequently, the bone powder samples were weighed (2 mg) using an analytical balance and placed into 5 mL Teflon containers. The Teflon containers were washed prior to placing the samples in them using hydrochloric acid and DI water (1:1) then were dried in the oven.

1. Hydrofluoric Acid (HF): Nitric Acid (HNO3) Digestion

   It is necessary with Q-ICP-MS to completely dissolve the powder in order for the sample to go into the nebulizer as a liquid. This was done via acid dissolution. Each sample was digested in a 1 mL solution of 1:4 HF: HNO₃ as follows: 2 mL of HF was placed into each 5 mL Teflon container housing the 2 mg sample of bone powder, including the blank (14 human and animal bone powder samples and a blank). Subsequently, 0.8 mL of HNO₃ was placed into each sample. The caps were put back on each sample and each container was gently swirled ensuring all powder would be coated with the liquid. The samples were then placed on a hot plate at 80 - 90° C for 24 hours to dry to hardness. When the samples were hard they were diluted 5000 times by adding 10 mL of solution according to the following procedure:

   - Add 2 mL of 2% HNO₃ to the Teflon containers to pick up the dry sample
   - Swirl the solution and pour into pre-weighed 15 mL centrifuge tubes
   - Rinse the containers twice with 2 mL of 2% HNO₃ with caps on to dissolve the residue
   - Rinse once more with 1 mL of 2% HNO₃
   - Add 2 mL of 10 ppb Indium and Ge in 2% HNO₃ spike
   - Bring samples up to 10 mL, by weight, with 2% HNO₃

2. Making the Quality Control Standards

   In order to test the quality of the ICP-MS analysis, quality control standards, comprised of certified reference standards (NCS-DC77306, GSJ-JLS-1, NIST 120c, and NIST 1486), were
digested and analyzed in conjunction with the samples. The quality control standards are triple the recipe used for the samples.

3. Making the Matrix-Matching Stock Solution (MMSS)

200 mg CaCO$_3$ and 100 mg P$_2$O$_5$ were weighed and combined into a Teflon container and digested in 1mL HF and 4 mL HNO$_3$ (concentrated) for two hours on a hot plate. Then the cap was open and the solution dried to hardness. When hard, it was picked up in 5 mL concentrated HNO$_3$ and put back on the hot plate for 30 minutes, uncapped. The solution was then diluted to 100 mL and an internal spike was added as follows:

- Pour solution from the Teflon into a 100 mL glass beaker
- Rinse the Teflon three times with 2% HNO$_3$ within 10 mL and pour into the beaker
- Rinse the Teflon cap three times with 2% HNO$_3$ within 5 mL and pour into the beaker
- Add 1 mL of 10 ppm Indium
- Fill the glass beaker to 100 mL with 2% HNO$_3$ by weight (assume 1 g = 1 mL)

4. Making the Trace Element Stock Solution

A trace element stock solution (TESS) was created by pipetting individual element, high purity liquid standards of Ba, Cu, Fe, K, Mg, Mn, Na, Sr, and Zn into a 50 mL centrifuge tube. The standards were taken down from 1000 ppm ± 3 µg/mL stock solutions in 2% HNO$_3$ to 2 ppm by adding 0.1 mL of each element of interest into 50 mL of solution with deionized water ($(1,000$ ppm * 0.1 mL)/50 mL = 2 ppm).
5. Making the Trace Element Calibration Standards

Four standards were created from the TESS and matrix-matching standard solution to calibrate for trace elements according to the following procedure:

Add 25 mL of DI water, 1 mL HNO₃, and 5 mL MMSS to each 50 mL centrifuge tube. Subsequently, add 0.1 mL TESS to the first centrifuge tube to create the first standard, 1 mL TESS to the second centrifuge tube to create the second standard, 2.5 mL TESS to the third centrifuge for the third standard, and 5 mL TESS to the fourth centrifuge tube to create the fourth standard. Fill each of the four tubes with DI water to 50 mL. The resulting concentrations can be found in Table 4.2.

Table 4.2. Concentrations from trace element calibration standards.

<table>
<thead>
<tr>
<th>2 ppm TESS</th>
<th>Concentration from 2 ppm TESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mL</td>
<td>8 ppb</td>
</tr>
<tr>
<td>1 mL</td>
<td>40 ppb</td>
</tr>
<tr>
<td>2.5 mL</td>
<td>100 ppb</td>
</tr>
<tr>
<td>5 mL</td>
<td>200 ppb</td>
</tr>
</tbody>
</table>

Combining the ICP-MS and pXRF Data to Create an Empirical Calibration

The process for calibrating the pXRF spectrometer began with an evaluation of the intensity of photon counts versus energy spectra in S1PXRF. For each element of interest, the photon counts were calculated by looking at the net area under the peak of each element. This was done by obtaining the region of interest (ROI) per element, using the K-lines for Ca, Fe, and Sr and the L-lines for Ba. Although correction for background can be eliminated when there is minimal variation in elemental concentration ranges between the composition of the standards and the composition of the unknowns, the baseline was locally corrected by normalizing to the
Compton as an additional measure to combat interferences that can result from background scatter. Gathering data in such a way accounts for background scatter so that it is not included in the final quantitative analysis, which can lead to erroneous concentration values being reported later (McGlinchy 2012; Vries and Vrebos 2002). The photon counts were then used with the concentration data from ICP-MS to create calibration curves for each element of interest in Microsoft Excel.

The empirical calibration curves for Ba, Ca, Fe, and Sr were created using linear regression analysis in Excel which include the line of best fit \( y = mx + b \) and the coefficient of determination \( (R^2) \). The equation of a straight line \( y = mx + b \) is translated as \( I_p = M_iC_i + I_b \) in which \( I_p \) is the peak intensity of an element, \( M_i \) is the slope of the line which allows for the conversion of peak intensities into concentrations, \( C_i \) is the concentration of an element within the standard, and \( I_b \) is background intensity (Rousseau 2001). The coefficient of determination \( (R^2) \) that results from the linear regression equation on a calibration curve is an indicator of how well the model will be at predicting elemental concentration values from unknown samples; the model is most optimal as the \( R^2 \) value approaches one.
CHAPTER FIVE:
RESULTS: EMPIRICAL CALIBRATION

In this chapter, I discuss the results of the empirical calibration. First, I present the concentration values obtained via ICP-MS and the photon counts acquired via pXRF. Second, I present the calibration curves that were generated to get the linear regression equation. The linear regression equation for each element is used to convert photon counts from pXRF into concentration values for barium (Ba), calcium (Ca), iron (Fe), and strontium (Sr) which will be demonstrated in Chapter 6.

ICP-MS and pXRF

Nineteen bone specimens were analyzed via ICP-MS to get concentration values for Ba, Ca, Fe, and Sr (Table 5.1). The standard deviation, minimum and maximum were calculated for the ICP-MS data for each element with the exception of the three specimens that yielded negative results for Ba and the five specimens that were not analyzed for Fe. Ba, Fe, and Sr are reported as ppm and Ca is reported as weight percent. The standard deviations for Ba, Ca, Fe, and Sr are 112, 3, 407, and 319 respectively. The minimum concentration values for Ba, Ca, Fe, and Sr are 0.6, 15.9, 86, and 35 respectively. The maximum concentration values for Ba, Ca, Fe, and Sr are 418, 32.5, 1530, and 1164.
The same specimens were analyzed by pXRF to get photon counts for Ba, Ca, Fe, and Sr (Table 5.1). All of the specimens yielded results. Overall, Ba has the lowest average photon counts while Ca has the highest average photon counts out of all of the elements. The standard deviation of the photon counts for Ba, Ca, Fe, and Sr are 15, 504, 60, and 2097 respectively. Sr has the highest amount of variation in the data set while Ba has the lowest.

Table 5.1. ICP concentration values and pXRF photon counts.

<table>
<thead>
<tr>
<th>Samples</th>
<th>ICP-MS</th>
<th></th>
<th>pXRF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ba (ppm)</td>
<td>Ca (wt. %)</td>
</tr>
<tr>
<td>CH 16</td>
<td>144</td>
<td>28.8</td>
<td>No*</td>
</tr>
<tr>
<td>CH 72</td>
<td>46</td>
<td>24.3</td>
<td>723</td>
</tr>
<tr>
<td>CH 82</td>
<td>141</td>
<td>25.1</td>
<td>No*</td>
</tr>
<tr>
<td>CH 10039</td>
<td>320</td>
<td>30.8</td>
<td>1530</td>
</tr>
<tr>
<td>Modern Cow</td>
<td>77</td>
<td>21.9</td>
<td>283</td>
</tr>
<tr>
<td>Modern Pig 1</td>
<td>-8*</td>
<td>15.9</td>
<td>204</td>
</tr>
<tr>
<td>Modern Pig 2</td>
<td>-6*</td>
<td>24.5</td>
<td>277</td>
</tr>
<tr>
<td>Sea Lion 1152</td>
<td>79</td>
<td>28.1</td>
<td>321</td>
</tr>
<tr>
<td>1153</td>
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*the values were not included in the statistical analyses
Calibration Curves

Calibration curves for Ba, Ca, Fe, and Sr were created using linear regression analysis of human and animal bones to assess the strength of the linear relationship between the concentration values from the ICP-MS analysis versus the photon counts from the S1PXRF region of interest data (Figures 5.1, 5.2, 5.3, 5.4). Certain standards were used to create each calibration curve in order to acquire the most optimal coefficient of determination ($R^2$), and therefore best linear relationship between the ICP-MS and pXRF data. The $R^2$ value for Ba, Ca, Fe, and Sr is .9994, .945, .9996, and .9918 respectively. Aimers et al. (2012:440) suggest a relatively good linear fit on a calibration curve can be indicated by an $R^2$ value of .8575 and .8744. However, a perfect linear fit between the ICP-MS and pXRF data is indicated by an $R^2$ value of one; it is favorable to have an $R^2$ value as close to one as possible.

As a result of the standards chosen to create the calibration curves, there are certain limits of quantification for each calibration. Ba has a minimum and maximum quantification limit at 57 ppm and 180 ppm respectively. Ca has a minimum and maximum quantification limit of 24.3 wt. % and 30.8 wt. % while Fe has a minimum and maximum quantification limit at 283 ppm and 1530 ppm. Sr has a minimum and maximum quantification limit at 35 ppm and 814 ppm.
Figure 5.1. Calibration curve for barium created using four animal and human bone standards. The X-axis displays the concentration values obtained via ICP-MS while the Y-axis displays the photon counts obtained through pXRF. The coefficient of determination is .9994.

Figure 5.2. Calibration curve for calcium created using four animal and human bone standards. The X-axis displays the concentration values obtained via ICP-MS while the Y-axis displays the photon counts obtained through pXRF. The coefficient of determination is .945.
Figure 2.3. Calibration curve for iron created using three animal and human bone standards. The X-axis displays the concentration values obtained via ICP-MS while the Y-axis displays the photon counts obtained through pXRF. The coefficient of determination is .9996.

Figure 5.4. Calibration curve for strontium created using 12 animal and human bone standards. The X-axis displays the concentration values obtained via ICP-MS while the Y-axis displays the photon counts obtained through pXRF. The coefficient of determination is .9918.
CHAPTER SIX: FURTHER TESTING

In this chapter, I attempt to assess the reliability and validity of using portable X-ray fluorescence spectrometry (pXRF), with a newly developed calibration, to analyze archaeological human and animal bone specimens as well as the ability of the technique to address an archaeological question. I demonstrate the reliability of data acquired via pXRF through precision and accuracy testing of human and animal bones using pXRF and ICP-MS. Following reliability testing, I highlight one of the biggest limitations associated with the pXRF of heterogeneous archaeological materials – diagenesis. Diagenesis of standards used to create the calibration curves for Ba, Ca, Fe, and Sr is assessed by statistically viewing the correlation between Fe and Sr as well as the Ca:P ratio. Additionally, I assess the validity of pXRF by testing the ability of certain elements (Ba, Ca, Sr) to be used as paleodietary indicators. I conclude by presenting a case study in which archaeological bones from four prehistoric sites in Peru are used to demonstrate the validity of pXRF to answer questions regarding paleodiet and trade interactions.

Reliability

Precision

To ensure the reliability of pXRF to the elemental analysis of human bone, precision testing is necessary. Precision (i.e. repeatability) is defined here as the ability to obtain the same results
using the same parameters for each sequential measurement per sample (Hughes 1998:6). Typically, an assessment of precision involves repeatedly testing international standards or in-house standards and reporting the data using “internationally recognized” units, such as ppm or weight % (Hughes 1998:108). However, precision testing can be done by taking repeated measurements on an unknown material that is not an international standard (Craig et al. 2007; Shaffer n.d.; Tykot 2016) and can be reported as elemental intensities (Shaffer n.d.), such was done in this study. It can be said that this method of precision testing is more about repeatability and works only to assess the precision of one particular instrument under specific conditions (Frahm 2013), but if the goal is to check repeatability of the data (i.e. precision) to assess a deviation in the data over time, and thereby instrumental drift, than internally consistent data (although frowned upon) should be sufficient enough to demonstrate, at the very least, precision of a particular instrument which can still provide valuable information for the end user that operates this particular instrument.

**Precision Methods**

Precision testing was performed using pXRF on 3 archaeological human femur bone samples collected from the surface of two looted tombs located in the Chincha Valley of southern Peru. Prior to analysis, each whole bone sample was cut into 4 cm pieces and ultrasonically cleaned for 10 minutes in deionized water. Ultrasonic cleaning was repeated once for each sample. The samples were then dried in air overnight. Each whole bone sample was analyzed using a Bruker Tracer III-SD. The spectrometer used in this research possesses a Silicon Drift Detector with a typical resolution of 145 eV at 100,000 counts per second (cps) which is cooled by an internal
Peltier device and utilizes an X-ray tube with a rhodium target as its excitation source. The spectrometer was mounted on a secure tripod before placing each sample on the instrument.

Analysis was performed on two different regions of each bone (the freshly cut interior and the exterior). Each bone was placed at a 45° angle to the beam hole, covering the X-ray beam path completely, to optimize photon counts and reduce potential backscatter and analyzed in each spot 10 times consecutively using identical parameters (40 KeV, 11 µA, 12 mil Al, 1 mil Ti, 6 mil Cu filter, 120 seconds) for a total of 60 analyses. For two samples (Individual 1 and 2), the same parameters were set after each run and the spectrometer was physically turned on at the beginning of each analysis. For the third sample (Individual 3), a different setting was used in which I allowed the S1PXRF software program to analyze the sample 10 times consecutively on its own using the parameters that I initially set. This was done with the thought that I would get the same results in a timelier manner.

**Precision Results**

Precision can be quantified by calculating the relative standard deviation of the values (Frahm 2012). Saiki et al. (1999) suggest RSD values below 14% to indicate good precision. RSD values for all of the elements indicate good precision with the exception of Ba for Individual 3 (Table 6.1) which has a RSD of 17.9%. In fact, although the individuals have RSD values that fall within 14%, Individual 3 has higher RSD values for most of the elements when compared with the rest of the individuals. This can be a consequence of random error associated with the random fluctuations within the instrument that can occur while the sample is being run on the pXRF spectrometer.
Table 6.1. Precision test of 3 human femur bones using pXRF.

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<tr>
<td>INDIVIDUAL 3 EXTERIOR</td>
<td>107.3</td>
<td>5723.4</td>
<td>296.6</td>
<td>265.4</td>
<td>528.4</td>
</tr>
<tr>
<td>INDIVIDUAL 3 EXTERIOR</td>
<td>113.6</td>
<td>5611.3</td>
<td>319.5</td>
<td>322.3</td>
<td>510.5</td>
</tr>
<tr>
<td>INDIVIDUAL 3 EXTERIOR</td>
<td>118.8</td>
<td>5525.6</td>
<td>321.1</td>
<td>275.2</td>
<td>524.3</td>
</tr>
<tr>
<td>INDIVIDUAL 3 EXTERIOR</td>
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<td>5685.7</td>
<td>316.0</td>
<td>291.5</td>
<td>537.4</td>
</tr>
<tr>
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<td>6929.1</td>
<td>395.3</td>
<td>385.2</td>
<td>657.3</td>
</tr>
<tr>
<td>SD</td>
<td>23.6</td>
<td>428.5</td>
<td>33.0</td>
<td>38.9</td>
<td>45.3</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>131.9</td>
<td>5733.3</td>
<td>314.5</td>
<td>295.3</td>
<td>538.5</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>17.9</td>
<td>7.5</td>
<td>10.5</td>
<td>13.2</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Standard deviation, average, and relative standard deviation highlighted for each element.
Accuracy of the Newly Developed Calibration

In addition to precision, accuracy is considered a measure of the reliability of data (see Frahm 2013 for a different perspective). Accuracy is defined as the closeness of measured values to actual values. Accuracy can be measured by statistically comparing results from the same sample set using a different analytical method (Hughes 1998:108). Concentration values from ICP-MS were compared with concentration values from pXRF on the same set of bone specimens to test the accuracy of the empirical calibration created for the pXRF analysis of bone specimens.

Accuracy Methods

*Inter-instrument Elemental Data Comparison*

A total of 19 archaeological and modern human and animal bone powder samples were tested via ICP-MS and pXRF (Table 6.2). The concentration values from ICP-MS and the photon counts from pXRF underwent linear regression analysis to obtain calibration curves for Ba, Ca, Fe, and Sr (see Chapter 4 for methodology and Chapter 5 for results). The resulting linear regression equation for each element was used to input photon counts obtained via S1PXRF to provide concentration values using the newly developed calibration curves.

Accuracy Results

Statistical analysis of the concentration values obtained from ICP-MS and pXRF for Ba, Ca, Fe, and Sr was performed to demonstrate significant or insignificant correlations between the two data sets. Spearman’s rank order correlation was performed on the standards to assess the strength and direction of the association between the concentration values obtained via ICP-MS
and the concentration values acquired from the calibrated pXRF data. There is a statistically
significant positive correlation between the ICP-MS and pXRF data for Ca (Spearman’s $r = .511$;
$n = 19$; $p = .025$) as well as Sr (Spearman’s $r = .977$; $n = 19$; $p < .001$). In contrast, there is no
significant correlation for the Ba data (Spearman’s $r = .118$; $n = 19$; $p = .664$) and Fe data
(Spearman’s $r = .530$; $n = 19$; $p = .051$).

Table 2.2. ICP-MS and pXRF concentration values (using the author’s calibration) for Ba, Ca,
Fe, and Sr.

<table>
<thead>
<tr>
<th>Samples</th>
<th>ICP-MS</th>
<th></th>
<th></th>
<th>pXRF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ba (ppm)</td>
<td>Ca (wt. %)</td>
<td>Fe (ppm)</td>
<td>Sr (ppm)</td>
<td>Ba (ppm)</td>
<td>Ca (wt. %)</td>
</tr>
<tr>
<td>CH 16</td>
<td>144</td>
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<td>*</td>
<td>1164</td>
<td>129</td>
<td>27.5</td>
</tr>
<tr>
<td>CH 72</td>
<td>46</td>
<td>24.3</td>
<td>723</td>
<td>814</td>
<td>106</td>
<td>24.7</td>
</tr>
<tr>
<td>CH 82</td>
<td>141</td>
<td>25.1</td>
<td>No*</td>
<td>837</td>
<td>105</td>
<td>27.0</td>
</tr>
<tr>
<td>CH 10039</td>
<td>320</td>
<td>30.8</td>
<td>1530</td>
<td>927</td>
<td>66</td>
<td>31.7</td>
</tr>
<tr>
<td>Modern Cow</td>
<td>77</td>
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<td>283</td>
<td>220</td>
<td>75</td>
<td>25.4</td>
</tr>
<tr>
<td>Modern Pig 1</td>
<td>-8*</td>
<td>15.9</td>
<td>204</td>
<td>35</td>
<td>87</td>
<td>25.3</td>
</tr>
<tr>
<td>Modern Pig 2</td>
<td>-6*</td>
<td>24.5</td>
<td>277</td>
<td>66</td>
<td>103</td>
<td>26.9</td>
</tr>
<tr>
<td>Sea Lion</td>
<td>79</td>
<td>28.1</td>
<td>321</td>
<td>523</td>
<td>73</td>
<td>28.9</td>
</tr>
<tr>
<td>1152</td>
<td>-0.5*</td>
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<td>338</td>
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</tr>
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<td>1153</td>
<td>4</td>
<td>29.9</td>
<td>140</td>
<td>339</td>
<td>37</td>
<td>25.4</td>
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<tr>
<td>410</td>
<td>180</td>
<td>28.1</td>
<td>*</td>
<td>372</td>
<td>180</td>
<td>27.7</td>
</tr>
<tr>
<td>425</td>
<td>0.6</td>
<td>29.9</td>
<td>642</td>
<td>244</td>
<td>130</td>
<td>29.3</td>
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<tr>
<td>427</td>
<td>178</td>
<td>27.6</td>
<td>No</td>
<td>379</td>
<td>102</td>
<td>30.4</td>
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<td>428</td>
<td>418</td>
<td>24.2</td>
<td>*</td>
<td>415</td>
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<tr>
<td>429</td>
<td>38</td>
<td>32.5</td>
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<td>503</td>
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<td>29771</td>
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<td>183</td>
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<td>29781</td>
<td>89</td>
<td>29.9</td>
<td>310</td>
<td>177</td>
<td>55</td>
<td>29.4</td>
</tr>
<tr>
<td>29782</td>
<td>99</td>
<td>29.9</td>
<td>1091</td>
<td>158</td>
<td>54</td>
<td>26.3</td>
</tr>
<tr>
<td>Mean</td>
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<td>27.2</td>
<td>497</td>
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<td>85</td>
<td>27.7</td>
</tr>
<tr>
<td>Standard Deviation</td>
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<td>3.9</td>
<td>407</td>
<td>319</td>
<td>37</td>
<td>1.9</td>
</tr>
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<td>15.9</td>
<td>86</td>
<td>35</td>
<td>37</td>
<td>24.7</td>
</tr>
<tr>
<td>Max</td>
<td>418</td>
<td>32.5</td>
<td>1530</td>
<td>1164</td>
<td>180</td>
<td>31.7</td>
</tr>
</tbody>
</table>

*the values were not included in the statistical analyses either because they could not be quantified using ICP-MS
(e.g. barium) or they were not analyzed for that particular element (e.g. iron).
Assessment of Preservation and Contamination of Standards

The accuracy of the concentration values obtained from an empirical calibration can be compromised as a result of using contaminated standards which can jeopardize the reliability of pXRF analysis. Following deposition into the soil, the inorganic portion of bone (mainly composed of hydroxyapatite) can undergo postmortem chemical alterations as a result of ion exchange, ion substitution and other biological processes between the ions in bone and the ions in the surrounding environment. This is due in part to the breakdown of the organic portion of the bone (i.e. collagen) which causes a greater susceptibility to diagenesis as the preservation of the bone declines and allows for ion exchange and substitution in the crystal lattice of hydroxyapatite (Pate et al. 1989). Preservation of the 19 bone standards was assessed by calculating the ratio of Ca to P (Table 3).

The Ca:P ratio was used because secondary Ca carbonates can be accumulated in bone more so than phosphates which can result in a higher Ca:P ratio in archaeological bone than modern bone. The Ca:P ratio can increase the longer bone is buried in the soil and subject to elemental alterations (Pate et al. 1989; Sillen 1981). All of the standards, with the exception of three, fell within the acceptable range for modern bones which is 1.6 to 2.3 (Fabig and Herrmann 2002; Zwanziger 1989).

As an additional test for possible contamination of the standards statistical analysis, using Spearman rank correlation of Fe and Sr, was performed on the same 19 bone standards (Table 6.3). If Fe and Sr correlate this is an indication that ions from the soil have penetrated the surface of the bone and could not, or were not, eliminated during ultrasonic cleaning and abrasion (Allmae 2012). The results (Spearman’s r =.323; n = 19; p = .26) indicate that there is no significant correlation between Fe and Sr.
Table 6.3. Assessment of contamination of standards.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ca (%)</th>
<th>P (%)</th>
<th>Fe (ppm)</th>
<th>Sr (ppm)</th>
<th>Ca/P %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>21.9</td>
<td>11.3</td>
<td>284</td>
<td>220</td>
<td>1.9</td>
</tr>
<tr>
<td>Pig 1</td>
<td>15.9</td>
<td>8.8</td>
<td>204</td>
<td>35</td>
<td>1.8</td>
</tr>
<tr>
<td>Pig 2</td>
<td>24.6</td>
<td>12.8</td>
<td>278</td>
<td>67</td>
<td>1.9</td>
</tr>
<tr>
<td>Sea Lion</td>
<td>28.1</td>
<td>13.6</td>
<td>321</td>
<td>524</td>
<td>2.1</td>
</tr>
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<td>USF 1152</td>
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<td>86</td>
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<td>928</td>
<td>2.6</td>
</tr>
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<td>11.6</td>
<td>504</td>
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<td>2.1</td>
</tr>
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<td>14.7</td>
<td>30.6</td>
<td>183</td>
<td>137</td>
<td>0.5</td>
</tr>
<tr>
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<td>30.0</td>
<td>14.4</td>
<td>310</td>
<td>177</td>
<td>2.1</td>
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<td>CB 8987</td>
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<td>CB 428</td>
<td>24.2</td>
<td>10.9</td>
<td>*</td>
<td>*</td>
<td>2.2</td>
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</table>

Validity

Applying Hughes (1998) levels of validity for obsidian provenance research to this study, the first level of validity addresses the question of whether or not the newly developed calibration can be used to distinguish different food resource consumption in prehistoric humans. Tykot (2016) was successful in demonstrating a clear distinction between individuals that consumed mainly marine organisms versus individuals that consumed terrestrial organisms using Ba and Sr values. The author analyzed 67 clean human bones via pXRF and calibrated the data using a silicon-based calibration typically used for geological materials. The results suggested that there was minimal contamination of the samples and pXRF is a valid technique to use in assessing the consumption of marine versus terrestrial food resources. Although this study is successful in
producing valid results, the elemental data from the calibration is not reliable. If a successful calibration, using matrix matched standards, is created than the results would be reliable and valid for differentiating marine versus terrestrial resource consumption.

**Methods**

Forty-four prehistoric human and animal bone specimens from varying regions in Peru were used to test the ability of the calibrations for Ca and Sr to distinguish between marine versus terrestrial resource consumption and trophic level of organism consumed (Table 6.4). Two of the main limitations of the pXRF analysis of bone specimens is contamination (Little et al. 2014) and lack of preservation (Tykot 2016). To mitigate the effects of these limitations, it is typically necessary to abrade the surface of the bone. Previous research suggests that abrading the surface of bone can reduce, and in some cases eliminate, elemental contaminants caused by soil staining, such as Pb (Little et al. 2014), Fe (Ezzo 1995) and Sr (Byrnes et al. 2016). Previous precision testing in this chapter indirectly demonstrated the need to abrade the surface of the bone specimens before analysis using pXRF; ultrasonic cleaning did not remove all of the possible contaminants present on bone that can come from the environment. There are higher levels of Fe and Sr on the outside of the bone than the interior. For this reason, each bone was ultrasonically cleaned and the surface was abraded before analysis.

The samples were analyzed with a Bruker Tracer III-SD pXRF spectrometer (40 KeV, 11 μA, 12 mil Al, 1 mil Ti, 6 mil Cu filter, 120 seconds). The spectrometer was placed in an upright fixed stationary position to permit analysis of the bone samples in a laboratory setting. Each whole bone sample was placed as close to the X-ray path as possible by completely covering the beam
path. This procedure was done to maximize the amount of X-rays bombarding the sample and to maximize the amount of photons gathered from the sample by the detector (Nazaroff et al. 2010). The photon counts for each sample were obtained from the region of interest data from S1PXRF and put into the calibration equations calculated from the linear regression analysis of Ca and Sr. The concentration values for Ba were obtained from the use of a calibration called the “obsidian” calibration which was created by Glascock and Ferguson (2012). This calibration was used because the concentration values from my calibration did not have a statistical relationship with the ICP-MS concentration values which resulted in a nonlinear relationship between the Ba and Sr. The graphs were created in IBM SPSS Statistics.

Table 6.4. Samples used for dietary reconstruction.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of Bone Specimen</th>
<th>Region</th>
<th>Time Period</th>
<th>Number of Samples used for Dietary Reconstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardal</td>
<td>Human</td>
<td>Central Coast, Peru</td>
<td>Initial Period</td>
<td>8</td>
</tr>
<tr>
<td>Chokepukio</td>
<td>Human and llama</td>
<td>Southern Highlands, Peru</td>
<td>Early Intermediate Period – Late Intermediate Period</td>
<td>11</td>
</tr>
<tr>
<td>Pacopampa</td>
<td>Human and Deer</td>
<td>Northern Highlands, Peru</td>
<td>Initial Period – Early Horizon</td>
<td>10</td>
</tr>
<tr>
<td>Tablada de Lurín</td>
<td>Human</td>
<td>Central Coast, Peru</td>
<td>Early Intermediate Period</td>
<td>12</td>
</tr>
<tr>
<td>University of Florida</td>
<td>Animal</td>
<td>Ayacucho, Peru and Pacific Ocean</td>
<td>Comparative Collection</td>
<td>3</td>
</tr>
</tbody>
</table>
Results

Case Studies Used for Paleodietary Reconstruction

*Cardal and Pacopampa during the Initial Period*

The Initial Period (1800-900 B.C.) in the coastal valleys of Peru is characterized by the appearance of ceramics (although there are some archaeological sites from the Initial Period that do not possess ceramics, such as El Paraíso (Quilter 1985), Salinas de Chao, and Alto Salaverry (Pozorski and Pozorski 1990), an increase in monumental architecture, specifically U-shaped civic ceremonial centers, especially along the central coast, and the intensification of agricultural production through the use of irrigation networks. During the Initial Period, coastal villages on the northern and central coast began to move more inland, most likely, to utilize arable land, with access to canal uptakes, for agricultural production in the valleys (Burger 2014: 224; Moseley 1992: 57; Pozorski and Pozorski 2008:614; Kembel and Rick 2001: 52).

The increasing use of irrigation canals is evidenced by the presence of a wider variety of cultivated plants found in comparison with the Preceramic Period (Pineda 1988:82; Moseley 1992:153). These cultivated plants included cotton, squash, gourds, lima and kidney beans, peanuts, peppers, maize, pacay, lucuma, avocado, and guava (Burger et al. 2012:402; Pineda 1988), although some of these crops were already present during the Preceramic Period (Bennett and Bird 1964; Bird 1951). Additionally, the people of the Initial Period were also consuming herbivores that could have been organisms hunted on the lomas or in the riverine environment such as deer, camelids, birds, and vizcachas (Burger 2003).

During the Initial Period, with a new emphasis on agricultural foodstuffs, there is also evidence for technological innovations, especially on the northern and central coast. Perhaps the most
obvious innovation is the creation of pottery which, according to Burger (2014:1084), came about as a result for the need to store and cook food. For example, at Cardal, analysis of grains in cooking pots revealed evidence of foods such as manioc, sweet potatoes, potatoes, *achira*, and maize (Burger et al. 2012:403). Given the increase in agricultural goods, it would be expected that the populations on the northern and central coast during the Initial Period relied minimally on seafood, but according to isotopic studies done on the coast, this is not the case (Tykot et al. 2006; Burger and van der Merwe 1990).

During the latter part of the Initial Period, there were two additional important innovations that occurred. One innovation was the use of domesticated llama (Pozorski 1979) for local and long distance transport of goods and the other was the production of freeze-dried potatoes (*chuño*), documented from residue analysis of cooking pots from the Lurín Valley (Burger 2014:1088). These innovations provide clear evidence for the beginning stages of possible trade between coastal valley populations and the people of the highlands.

In contrast to the coast, during this time, individuals residing in the northern highlands had a subsistence base that involved rainfall farming and hunting. During the Initial Period, the northern highlands of Peru were characterized by non-urban monumental civic ceremonial centers, small residential populations, and trade interactions with populations from the coast and tropical forest. Centers with public architecture, such as Pacopampa and Kuntur Wasi, were situated in the *quechua* and *yunga* zones in which agricultural resources, such as maize, cotton, cherimoya, beans, cassava, pumpkin, squash, and manioc could be grown on the steep valley slopes. In addition to consuming the aforementioned plants, individuals subsisted mainly on deer and wild game with little reliance on camelids. Llamas at some Initial Period northern centers, such as Pacopampa, were occasionally acquired possibly from the central and southern highlands and used as beasts of
burden, but were not regularly consumed during the late Initial Period (Burger 1992; Flores 1975; Miller and Burger 1995; Morales 1998).

Cardal is a civic ceremonial site (1150-800 B.C.) located in the central coastal valley of Peru, one kilometer from the Lurín River and 14 kilometers from the Pacific Ocean (Meadors 1992). Cardal exist at an elevation of 150 m.a.s.l. on the desert coast and is set in the lower Lurín Valley situated by seasonal fog vegetation called lomas (Burger and Salazar-Burger 1991; Burger 1992). Given the increase in agricultural goods, it would be expected that the populations on the central coast during the Initial Period relied minimally on seafood, but according to previous zooarchaeological, isotopic and elemental studies done at Cardal, this is not the case; there is evidence for a mixed diet of marine, terrestrial, agricultural, and riverine foods consumed at Cardal (Bergmann 2013; Burger 2012; Tykot et al. 2006).

In contrast to the central coast during the Initial Period, in which marine resources appeared to be the dominant food source for individuals, individuals in the Peruvian highlands had access to more terrestrial plant and animal resources, such as domesticated camelids, potatoes, manioc, and quinoa (Pearsall 2008). For example, the land by the El Mirador hill of the Pacopampa center was suitable for cultivation of maize, arracacha, pumpkin, potato, and squash. In excavations by Morales, cervid, camelid bones, and land snails were found in refuse at the site (Morales 1998). Pacopampa is a highland valley center with associated public architecture located in the Chotano Valley at an altitude of 2140 m.a.s.l. in the northern highlands of Peru and is among the most northerly of early Peruvian ceremonial centers (Burger 1989, 1992). Morales interprets El Mirador as a residence for individuals in charge of the center’s activities (Burger 1992:108).

Pacopampa was established in the late Initial Period and occupied into the Early Horizon. Pacopampa is located in the rich agricultural lands of the quechua zone (1800-3200 m.a.s.l.) and
exist 70 km from the Pacific Ocean and near the Marañon River (Burger 1992). Previous stable isotope analysis of bone collagen samples from Initial Period Pacopampa ($\delta^{13}$C = -19.3 ± 0.4‰, n = 11) indicate a pure C$_3$ diet, with minimal contribution of seafood, and the bone apatite results ($\delta^{13}$C = -10.3 ± 1.2‰, n = 10) suggest individuals were consuming animals such as llama, guinea pigs, and wild deer (Tykot et al. 2006). Excavations by Flores (1975) support this interpretation in which deer, llama, and guinea pig remains were found at the site. Although Miller and Burger (1995:450) state that the people of Pacopampa did not heavily rely on wild camelids for the meat portion of their diet, but rather subsisted on deer and wild game.

**Tabla de Lurín and Chokepukio during the Early Intermediate Period**

During the Early Intermediate Period (200 B.C. – A.D. 400) there was a thriving culture on the central coast that existed during the Early Intermediate Period specifically in the valleys of Lurín, Rimac, Chillón, and Chancay, but the structure of settlements and style of monumental architecture were markedly different from what was seen during the Initial Period and the amount of monumental architecture constructed was negligible compared to the Initial Period. Also, elites in the Lurín Valley were thought to reside behind the monumental civic ceremonial centers during the Initial Period but, during the EIP elites started to move their residences further inland to hilltops in seemingly defensive locations (Conklin and Moseley 1988; Earle 1972) and extensive cemeteries with a lack of residential and public architecture, such as Tablada de Lurín, appear on the central coast.

Tabla de Lurín is a mortuary site located 12 km from the Pacific Ocean on the central coast of Peru in the lower Lurín Valley at 100 m.a.s.l. There is evidence for use of the cemetery during the late Early Horizon and into the EIP (200 B.C. – AD 200) (Makowski 2002; Tykot et al.)
Previous bioarchaeological investigations of teeth suggest individuals at Tablada de Lurín subsisted on agricultural goods, with minimal consumption of animal protein (Pechenkina et al. 2007). However, isotopic analysis of human bone collagen and apatite indicate high seafood consumption by individuals at Tablada de Lurín followed by a reliance on C₄ plants, such as maize, over time (Tykot et al. 2006).

Similar to the central coast during the EIP, fortresses, warfare, and population increase were evident in the southern highlands (Lanning 1967). Cities, such as Tiahuanaco, Pucara, and Huari formed. Additionally, food items that could be exploited in the highlands during the Initial Period were similar to the foods that could be present during the EIP. For example, potatoes and quinoa were still commonly grown in the southern highlands, especially the antiplano (Brush 1967) and previous excavations at Chokepukio revealed terrestrial fauna in the refuse. Chokepukio is an archaeological site located 3138 m above sea level at the southern end of the Cuzco Valley (McEwan et al. 1995). The site is situated in the Lucre Basin, 30 km southeast of Cuzco. Chokepukio was occupied by various ethnic groups from the EIP (400 B.C.) through the Late Horizon (AD 1476 - 1534). In this study, only bones from the EIP were used for analysis. During the EIP, Chokepukio was occupied by a small farming community.

The results indicate a good linear relationship between the Ba and Sr values and the ratios of Ba and Sr to Ca (Figures 6.1, 6.2). The individuals from the coastal valley region of Tablada de Lurín have the lowest values of Ba and Sr while the samples from the coastal valley site of Cardal are mixed with the samples from Tablada de Lurín and Pacopampa. The samples from the northern highland site of Pacopampa have higher Ba and Sr values than the Tablada de Lurín samples. Above the samples for Pacopampa are the individuals from the southern highland site of Chokepukio along with the guinea pigs from the southern highland site of Ayacucho and the llama.
from Chokepukio. Interestingly, three of the individuals from Chokepukio are above the llama and guinea pig suggesting mainly plant resource consumption.

Figure 6.1. Paleodietary reconstruction of human and animal bones from Peru. Ba values calculated using a silicon based calibration and Sr concentrations calculated using the author’s calibration.
Figure 6.2. Paleodietary reconstruction of human and animal bones from Peru. Ba values calculated using a silicon based calibration and Ca and Sr concentrations calculated using the author’s calibration.
CHAPTER SEVEN:

DISCUSSION

A fundamental aspect of all archaeological research that utilize analytical methods to address archaeological questions is the need to ensure the most valid and reliable data output from instruments. This mitigates misinterpretations of archaeological data. As a result of the quick rise of pXRF technology in archaeology, there are concerns regarding the validity and reliability of data output acquired from pXRF (Shackley 2010; Speakman and Shackley 2013). In the past, scholars have claimed that non-scientists and researchers collecting qualitative and quantitative data from pXRF have erroneously interpreted pXRF data and misused pXRF spectrometers as a result of a lack of knowledge regarding X-ray physics, as well as not being aware of the limitations and inner workings of the instruments (Aimers et al. 2012:423; Shackley 2010; Shugar and Mass 2012:19). This is essentially stated by Shugar and Mass (2012:18) regarding the use of pXRF spectrometers in the cultural heritage sector:

“Too often these instruments are sold and used as rapid “point and shoot” solutions for materials analysis problems in cultural heritage with little training on X-ray physics and spectrometry, and the identification of instrument artifacts. In addition, there is insufficient discussion on XRF being a surface analysis technique only (the penetration depth of the X-rays varies depending on the object’s density and the tube voltage), and that cultural heritage objects almost invariably have complex, layered surfaces with heterogeneous microstructures” (Shugar and Mass 2012:18).

Some researchers allege that most archaeologists using pXRF do not care about reliability and validity and that they deem “internally consistent” results as adequate for “compositional
analysis” (Shackley 2010; Speakman and Shackley 2013). Furthermore, the same scholars have asserted that we have reached a time of “silo science” in which researchers believe “it is perfectly fine to provide results that are that are only internally consistent, and do not conform to international standards and data…where each researchers’ data is self-contained, independent, and cannot be verified externally” (Speakman and Shackley 2013:1435).

In an attempt to rectify these accusations, some researchers have tried to establish the validity and reliability to the use of pXRF in the analysis of archaeological materials by shining a light on the limitations associated with pXRF, such as low precision and accuracy of pXRF data output, photon optimization with regard to the sample’s surface morphology and size, and calibrations used to convert photon counts to concentration values (Forster et al. 2011; Frahm 2013; Grave et al. 2012; Nazaroff et al. 2010; Newlander et al. 2015; Ogburn et al. 2013; Speakman and Shackley 2013 also see Speakman and Shackley 2013 for a different view on Frahm 2013 results). Most of the results demonstrated a lack of reliability, with regard to accuracy (Craig et al. 2007; Nazaroff et al. 2010), but did suggest a high level of validity in addressing archaeological questions involving obsidian and basalt provenance studies (Craig et al. 2007; Frahm 2013; Grave et al. 2012; Nazaroff et al. 2010).

Evaluation of Contamination of Standards

As Shugar (2013:180) stated, although empirical calibrations can be very accurate “The accuracy…is limited by experimental issues including the quality of the standards used, sample preparation, similarity between the matrices of the standards and samples, and the repeatability and reproducibility of the measurements.” For this reason, an assessment of possible diagenetic alteration of the standards is necessary. pXRF is an analytical technique that measures the surface
of a material (Tykot 2016). This can present an issue when analyzing archaeological materials, such as bone, because they are susceptible to surface contamination as a result of being buried in soil. This is one of the key limitations to pXRF and can be understood better by having the appropriate knowledge of what it means for a material to be “infinitely thick” and the depth at which primary X-rays can penetrate a material and the depth at which atoms of certain elements within a specific matrix can fluoresce and reach the detector. This is important because the concentration of an element in a sample is determined by how many photons reach the detector.

A sample being analyzed via pXRF need to be infinitely thick in order to optimize the amount of primary X-ray photons that are absorbed by the sample as well as allow for secondary X-rays to reach the detector (Shugar 2013). The depth at which an incident X-ray can penetrate a sample depend on the amount of energy used to bombard the sample with X-ray photons and the density of the sample while the depth at which secondary X-rays are emitted depend on the atomic number of the element and the energy of the X-ray line (Bezur and Casadio 2013:277). The equation below demonstrates this concept:

\[ I = I_0 \exp \left( -\frac{\mu}{r}x \right) \]

where \( I \) is the beam intensity with attenuation, \( I_0 \) is the beam intensity without attenuation, \( r \) is the density of the material, \( \mu / r \) is the mass attenuation coefficient for a given material at a given X-ray energy, and \( x \) is the mass thickness which is obtained by multiplying the density \( r \) by the thickness \( t \) (\( x = rt \)) (Kaiser and Shugar 2013). Shugar (2013) provides a nice example of this concept in which the author analyzed a ceramic sherd with a paste and slip in four different ways using pXRF. It was clear that when the slip had been removed the elements that were present when the slip was there were either not detected or were minimized. Also, after the slip had been removed, this led to an enhancement of the peaks and concentrations of elements that make up the material under
the slip. The author recommended removing the slip and/or homogenizing the paste. Another way to combat this issue non-destructively is to average several elemental readings of the same sample (Grave et al. 2012).

The example previously presented illustrates the importance of the size as well as the characteristics of the surface of the material measured by pXRF. The bones and bone powder involved in this study present a non-issue as far as meeting the infinite thickness requirement. However, such as demonstrated with the effect of the slip on the ceramic samples, the elemental data can be affected by what is present on the surface of archaeological bone, such as soil, which can provide elemental signatures, such as increased Fe (Buikstra et al. 1989) and Sr (Price et al. 1992) in bone that do not represent paleodiet.

Preservation and contamination of the standards used to create the calibration curves were assessed by evaluating the Ca:P ratio and the correlation between Fe and Sr. In fresh bones, the ratio of Ca to P in hydroxyapatite can range from 1.6 to 2.3 (Fabig and Herrmann 2002; Zwanziger 1989). Additionally, the results (Spearman’s \( r = .323; \ n = 19; \ p = .26 \)) indicate that Fe and Sr from soil contamination did not penetrate the bone. Overall, this suggests that most of the standards are adequately preserved and not contaminated.

Calibration Curves for Barium, Calcium, Iron, and Strontium

There is not a high standard deviation in photon counts for Ba resulting in a non-linear relationship between the ICP-MS and pXRF data when all of the standards were combined. Additionally, the photon counts for Ba are much lower than the photon counts for Ca, Fe and Sr. The results suggest that the Ba concentration values in the bone specimens are below the limit of detection of pXRF. If Ba is below the limit of detection than the instrument’s software cannot
differentiate between signal and noise. This was demonstrated by Pessanha and colleagues (2009) in which Ba was not detected for bone ash and clay materials that possess a mainly Ca, P, and Fe matrix because the Ba concentration was lower than 30 ppm. Barium can be as low as 3 ppm in archaeological bone (Ezzo 1995). Hunt and Speakman (2015) performed an experiment using pXRF which demonstrated that the Ba L lines in archaeological ceramics could not be measured accurately using pXRF. Archaeological ceramics can have trace amounts of Ba, similar to the trace amount that can be present in bone, that are too small to be detected by pXRF spectrometers that have a maximum voltage of 40 KeV or below.

Similar to Ba, Fe had low photon counts when compared to Ca and Sr, but this was most likely the result of simply having less Fe in the bone than Ca or Sr. When all of the standards were combined to obtain the calibration curve for Fe, there was a linear relationship between photon counts from pXRF and concentration values from ICP-MS, but there were outliers in the data causing a low coefficient of determination ($R^2 = .5936$). This problem can be rectified by adding more standards to the curve (Aimers et al. 2012). High concentration values of Fe need to be added to support the curve.

In contrast to Fe and Ba, the concentration values for Sr are abundant in the bone standards. As are result of the abundance of Sr in the standards there is no issue with regard to the limit of detection of pXRF for Sr. Additionally, most of the standards analyzed via ICP-MS and pXRF were able to be incorporated into the calibration curve for Sr. However, although there is tight point clustering about the calibration line and a good coefficient of determination ($R^2 = .9918$), there are outliers. This can be corrected by adding more standards to the curve (Aimers et al. 2012).
The calibration curve for Ca had similar photon counts and similar ICP-MS values which resulted in a cluster about the calibration line when all of the standards were plotted. This is expected given the low range of Ca values that can be found in bone (Juyal et al. 2005). One would not expect really low or really high Ca concentrations in bone unless some type of diagenetic alteration has occurred. As a consequence of the results, only certain standards were used to create the calibration line.

The calibration curves for Ba, Ca, Fe, and Sr result in certain limits of quantification (Table 7.1) which can affect the concentration values that are calculated from the unknown samples using the calibration. If a concentration value in bone is below or above the limit of quantification than this will produce inaccurate concentration values. This was demonstrated after a statistical analysis of the standards revealed no significant correlation in concentration values between ICP-MS and pXRF using the newly developed calibration for Ba (Spearman’s r = .118; n = 19; p = .664) and Fe (Spearman’s r = .530; n = 19; p = .051). In contrast, there is a significant correlation between the ICP-MS and pXRF concentration values for Ca and Sr. For this reason, the calibration curves for Ba and Fe were not used to reconstruct paleodiet.

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba (ppm)</td>
<td>57</td>
<td>180</td>
</tr>
<tr>
<td>Ca (wt. %)</td>
<td>24.3</td>
<td>30.8</td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>283</td>
<td>1530</td>
</tr>
<tr>
<td>Sr (ppm)</td>
<td>35</td>
<td>814</td>
</tr>
</tbody>
</table>
Reliability

Precision

Precision is measured by analyzing the same bone sample in the same spot multiple times over a period of time with no change to the current, voltage, run time, and atmospheric conditions. According to Frahm (2013) to test the precision of an instrument, analysis should be done on a regular basis, using the same material, over the course of months or even years because values reported for the same material analyzed can change over time as a result of instrumental drift. In contrast, Shackley (2010) tested the reliability and stability of a pXRF instrument in less time than recommended by Frahm by analyzing a pure copper pellet for 24 hours and a standard of similar composition to the analyte every 20 runs. Although the sample size is small and the samples were not measured anywhere near the amount of time suggested by Frahm (2013) and Shackley (2010) to indicate precision, this is sufficient to illustrate repeatability of the data.

Although the relative standard deviation values fall within an acceptable range to indicate good precision for the samples tested using pXRF, there was a slight discrepancy in the data with regard to individual 3. The elements for Individual 3 had higher relative standard deviation values than most of the other individuals. This was most likely the result of instrument error which could have come from voltage generator variation or a faulty X-ray tube.

Accuracy

The notion of unreliable, yet valid data, is an interesting one in which a closer look at internally consistent data and the definitions of what it means for data to be reliable and valid is necessary. Elemental data from pXRF can be deemed internally consistent, and thus according to
some, unreliable (Speakman and Shackley 2013) when results cannot be accurately verified using another analytical technique that involves elemental analysis, such as laboratory XRF, on the same samples (e.g. Craig et al. 2007). In other words, spectral data coming from pXRF need to be empirically calibrated, using matrix matched material, to create concentration values and then evaluated using another analytical method that involves empirical calibrations to produce elemental concentrations for a material (Shackley 2011). This allows for a comparison to be made between data sets as well as gives an indication of the level of accuracy of the data coming from pXRF.

The results from the inter-instrument test suggest there is a statistically significant positive correlation between the ICP-MS and pXRF data for Ca (Spearman’s r = .511; n = 19; p = .025) as well as Sr (Spearman’s r = .977; n = 19; p < .001). In contrast, there is no significant correlation for the Ba data (Spearman’s r = .118; n = 19; p = .664) and Fe data (Spearman’s r = .530; n = 19; p = .051). Consequently, the concentration data obtained from the Ca and Sr calibration curves can be deemed as accurate while data from the Ba and Fe calibrations cannot. For this reason, the Ba calibration that was created by the author is not used to assess paleodiet in the next section.

Validity

There is a dueling consensus among researchers regarding the validity of applying pXRF spectrometry to archaeological materials as a means to answer archaeological questions, but previous researchers have been successful at demonstrating the validity of pXRF analysis to address archaeological questions regarding provenance studies of obsidian (Craig et al. 2007; Nazaroff et al. 2010; Frahm 2013; Tykot et al. 2013) and basalt (Grave et al. 2012), and ceramic production and trade (Tykot et al. 2013). Elemental analysis of Sr, Ba, and Ca obtained from
archaeological human bones have been used in the past to study paleodiet using various analytical methods, such as Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), and more recently pXRF (Ezzo et al. 1995; Gilbert et al. 1994; Burton and Price 1990, 1991; Price 1989; Price et al. 1992; Tykot 2016). I demonstrate the ability of pXRF to be a valid technique to use to distinguish between different food resource consumption as well as address an archaeological question by presenting a case study involving prehistoric sites in Peru.

Discussion of Case Studies

The results from Chapter 6 suggest that the individuals from Cardal had a varied diet of marine, terrestrial, and plant resources while the majority of individuals from Pacopampa had a diet consistent with terrestrial and plant resource consumption. The results are consistent with previous isotopic analysis done on individuals from Cardal and Pacopampa (Tykot et al. 2006). The results suggest there could have been trade of food resources, on a minimal scale, between the northern highlands and central coast during the Initial Period as evidenced by the presence of certain agricultural resources, such as manioc and freeze-dried potatoes (chuño) documented at Cardal from residue analysis of cooking pots; manioc is not a common food resource on the central coastal valley, but manioc is abundant at Pacopampa (Burger et al. 2012; Burger 2014:1088; Morales 1998). However, it appears as though the individuals at Cardal consumed mainly marine resources. Additionally, individuals at Pacopampa had an absence of marine resources in their diet. As a result, it is unclear what individuals at Cardal could have provided to individuals at Pacopampa in order to obtain the highland resources. Rather, this supports a hypothesis for a possible exchange network between the coast and the inland valley. In sum, the results suggest
there were more interactions among coastal and inland individuals through the presence of certain food resource consumption than among inland central coastal valley individuals and northern highland individuals.

In contrast to the results from Cardal and Pacopampa, there is negligible overlap in Ba and Sr values among the Tablada de Lurín and Chokepukio individuals. The results suggest the individuals from Tablada de Lurín were mainly consuming high trophic level marine organisms while the individuals at Chokepukio were consuming low trophic level terrestrial organisms and/or agricultural resources. The results appear to indicate that there was little interaction, in the form of food resource trade, among the southern highland and central coastal individuals during the Early Intermediate Period. However, though there is no evidence in the form of food resource trade between the two sites during this time, there is evidence for interactions among individuals at Tablada de Lurín and individuals from the highlands in the form of pottery; the burials at Tablada de Lurín have coastal and highland pottery associated with them (Makowski 2002).

In conclusion, it appears that there were interactions between the coast and the highlands during the Initial Period and Early Intermediate Period, but the interactions were not heavily based on the trading of food resources. Rather, the pXRF data demonstrate more of a local highland-highland and coastal-coastal valley exchange system during the Initial Period and Early Intermediate Period. Additionally, the pXRF data support the notion that pXRF is a valid technique to use in the analysis of bone specimens to address archaeological questions regarding paleodiet and possible trade interactions among individuals that reside in the highland and coastal valley regions of Peru. However, additional calibration efforts need to be employed to improve the accuracy of the elemental data and allow comparison with results from other studies.
CHAPTER EIGHT:  
CONCLUSION

In recent decades archaeology has evolved into a field in which transdisciplinary endeavors have become more and more common and necessary to support archaeological theories and interpretations. Prior to the revolution of transcending beyond the world of traditional archaeological methods and techniques to address archaeological questions - archaeology existed in a silo. This placed archaeologists at a disadvantage because the lines of evidence used to support theories and interpretations were not substantiated with analytical data obtained from the hard sciences. It has become imperative to incorporate analytical sciences into archaeological research.

Even though this is a very exciting time in the realm of archaeology in which the inclusion of hard sciences has worked to enhance the reliability and validity of data to address archaeological questions, there are certain responsibilities that archaeologists need to be aware of to adequately accomplish this. For example, if archaeologists wish to use pXRF on archaeological materials than they need to be cognizant of the inner workings and limitations of the instrument as well as the principles behind the technique. As stated in previous chapters, it is not enough to “point and shoot” an archaeological material. This can lead to erroneous results that are deemed as reliable and valid. As archaeological scientists, we must be held to a higher standard than that which is believed by “real” scientists. In this paper, I have attempted to go beyond the “point and shoot”
mentality of pXRF by creating a valid and reliable calibration for the analysis of human and animal bone specimens to address an archaeological question.

Research Goals

The first research goal involved the creation an empirical calibration using matrix matched standard reference materials analyzed via ICP-MS and pXRF. Nineteen animal and human bone specimens were used as standards to create calibration curves for Ba, Ca, Fe, and Sr using linear regression analysis of elemental concentrations obtained via ICP-MS and photon counts from pXRF. Certain standards were used to create each calibration curve in order to acquire the most optimal coefficient of determination ($R^2$), and therefore best linear relationship between the ICP-MS and pXRF data. As a result, a relatively small number of samples were used for each curve (with the exception of Sr) which resulted in certain limits of quantification for each calibration. This was to be expected given the small number of standards used in this research, but each of the elements of interest had an adequate coefficient of determination indicating a good linear relationship between the ICP-MS and the pXRF data.

The second research goal tested the reliability and validity of the calibration for the pXRF analysis of archaeological human and animal bone using paleodietary indicators to address an archaeological question. Precision testing of Ba, Ca, Cu, Fe, and Sr was performed on 3 archaeological samples to assess the repeatability of the data using a Tracer Bruker III-SD. This yielded good relative standard deviation values of each element for the three samples; however, there are higher RSD values for the third individual in which the instrument was permitted to run on its own consecutively. This can be a consequence of random error associated with the random
fluctuations within the instrument that can occur while the sample is being run on the pXRF spectrometer. This should be addressed further to assess the exact reason for the deviation.

In addition to the test for precision, accuracy of pXRF data and the contamination of standards, using the newly developed calibration curves, were tested to assess the reliability of the calibration. Statistical analysis of ICP-MS concentration values versus pXRF concentration values indicated a significant positive correlation between the ICP-MS and pXRF elemental data for Ca and Sr while Ba and Fe had no significant correlation. The accuracy of the data for Ba was comprised based on the limits of detection of the pXRF spectrometer used in this research while the Fe data did not have a significant correlation because of the lack of standards used in this research. Additionally, the Ca:P for all of the standards, with the exception of three, fell within the acceptable range for modern bones indicating a lack of contamination. Furthermore, there was no significant correlation between Fe and Sr for all of the standards further indicating an absence of contamination for a majority of the standards.

The third research goal tested the hypothesis that there were trade interactions between individuals that resided in the highlands of Peru and individuals that lived in the coastal valleys through the use of paleodietary indicators. The results provided a clear distinction between individuals that consumed marine resources versus individuals that consumed terrestrial plant and animal resources.

Future Research

This paper has demonstrated the need for further research to be performed to enhance the reliability and validity of pXRF in the analysis of human and animal bone specimens. To enhance the reliability of the data, additional precision tests should be performed, preferably using an
international standard for a long period of time to assess precision of a particular instrument. Additionally, to test the precision of the calibration, the calibration should be employed using photon data from a different pXRF spectrometer and in a different location. Also, if there is a need to quantify Ba via pXRF, than the use of a pXRF spectrometer with higher detection limits is necessary. To rectify the limits of quantification for the elements of interest, more standards need be added to the calibration curves for Ca, Fe, and Sr.

As well as the future research needed to improve the reliability of the data, there is a need to strengthen the validity of the data to address archaeological questions regarding paleodietary studies. This can be accomplished by adding more animal bones to the analysis which will provide a baseline for the food resource consumption of humans. Although this research provided adequate results using the newly developed calibrations, there is a big assumption in this research in which it is assumed that ICP-MS is a more reliable method to use in the analysis of human and animal bone specimens than pXRF, but further tests should be employed using different analytical methods, such as Neutron Activation Analysis and Laser Ablation Inductively Coupled Mass Spectrometry to ensure the best method is used to compare results with pXRF.
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Tykot, R. H., R. L. Burger, and N. J. van der Merwe

Tykot, R. H., K. P. Freund, A. Vianello

Tykot, R.H., and S.M. Young

Valdez, Lidio M.

Zwanziger, H.
APPENDIX A: PERMISSIONS

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