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**Reconstructing Migration: Using Isotopic Analyses to Examine Ancient  
Maya Mobility in Northwestern Belize**

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**Reconstructing Migration: Using Isotopic Analyses to Examine Ancient  
Maya Mobility in Northwestern Belize**

**by**

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**Report**

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## **Dedication**

For Steve – who helped keep my sanity during the times I was sure I had lost it.

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## **Abstract**

### **Reconstructing Migration: Using Isotopic Analyses to Examine Ancient Maya Mobility in Northwestern Belize**

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Isotopic analysis has proven to be beneficial to the field of archaeology, aiding in the understanding of changing climatic conditions, diet, and mobility. This report proposes the use of Oxygen and Strontium isotope ratios to understand migration patterns of the Ancient Maya within the Program for Belize Archaeological Project (PfbAP) research area in northwestern Belize. Research seeks to first identify immigrants and then try to understand sociopolitical factors that may have influenced population movement as well as the consequences of that movement upon a region. Currently, our understanding of mobility and migration within this region is severely lacking. This report presents a general background on migration in archaeology as well as a general background on oxygen and strontium isotopes, their application to the field of archaeology, and how isotopic ratios can shed light on possible reasons for population movement. Additionally, this report outlines a protocol for each isotopic system and proposes future research for the PfbAP region.

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# CHAPTER 1

## Introduction

Mobility and migration have been part of human history since the dawn of the species (Little 2004). *Homo sapiens* have migrated both short and long distances for various reasons over time, and as civilizations developed, people continued to move regardless of what society or social class they belonged to (Freiwald 2011). Still, movement of people from the distant past was a research topic that was generally ignored by most archaeologists during the 1970s and 1980s (Anthony 1990; Burmeister 2000). However, within the past 20 years, migration studies in archaeology have found their way back into the research spotlight (Cabana and Clark 2011; Cameron 2013; Van Dommelen 2014; Price et al. 2012b) due to theoretical outlines and advances in methods.

Before we can begin discussing migration and mobility, it is important to first define them. Typically, migration has been thought of as a monumental population movement between long-distance regions while mobility has been thought of as short-movements, both permanent and non-permanent (Cameron 2013; Van Dommelen 2014; Cabana and Clark 2011; Kern 2012). For the purposes of this report, migration and mobility will be used interchangeably, as the focus here is on population movements within and into a region, regardless of the distance a person travelled.

Anthony (1990) defines migration as “a behavior that is typically performed by defined subgroups (often kin-recruited) with specific goals, targeted on known destinations

and likely to use familiar routes” (Anthony 1990: 895-6). Using this definition, and adding



*Figure 1 – Relation of the PfBAP area to the country of Belize. Courtesy of PfBAP.*

Cabana and Clark’s (2011) belief that migration need only include one person, this report will focus on research questions that encompass both long-distance and short-distance mobility in an attempt to understand the consequences of migration within a region. Additionally, this report will outline current geochemical methods employed to detect migration within a population. A research area like the Program for Belize Archaeological Project (PfBAP), nestled within the Rio Bravo Conservation and Management Area (RBCMA), allows for a large-scale regional study to be completed (Figure 1).

## **Report Organization and Objectives**

This report is organized into five chapters and provides an in-depth background on migration studies in archaeology and strontium and oxygen isotopes. It also proposes a method which incorporates using strontium and oxygen isotope ratios as geographic tracers of an individual’s place of origin within the Programme for Belize Archaeological Project research area in northwestern Belize. Chapter 2 introduces a literature review of how migration and isotope studies have been used in archaeology generally and in the Maya world specifically. In addition, chapter two provides information on the relevant research area, its geological and environmental setting, and culture history. Chapter 3 provides

background information about strontium and oxygen isotopic systems and how these can be applied to determine local and non-local populations. This chapter also discusses how strontium and oxygen are incorporated into the human skeleton and how they can be used as geographic tracers. Chapter 4 proposes a method for isotopic research to be used on human remains from the Programme for Belize Archaeological Project. Mass spectrometry is explained and a sample preparation protocol and analytical method are outlined for each isotope system. Finally, Chapter 5 outlines future research questions that will be addressed using strontium and oxygen analyses within the PfBAP region.

## **CHAPTER 2**

### **Literature Review**

While the fact that people frequently migrated in the past is generally acknowledged (Van Dommelen 2014; Smith 2014; Burmeister 2000; Osborne 1991), the theory behind migration studies is still widely debated (Anthony 1990; Burmeister 2000; Smith 2014). Still, there are common themes intertwined in each of the purposed theories and such themes can be seen in modern day migration studies by ethnographers, geographers, and sociologists. Most archaeologists admit that the present is not the perfect comparison for past events, yet, they agree that modernity does offer a starting point for the conversation of what an archaeologist can look for or identify when trying to study migration (Anthony 1990; Osborne 1992; Burmeister 2000; Cabana and Clark 2011; Smith 2014).

Observations from modern migration studies which may aid in viewing migration archaeologically include: 1) migration participant demographics – What are the ages and sex of the migrating individuals? If the majority of the immigrating population consists of adult females, we may be able to infer marriage alliances. (Anthony 1990; Burmeister 2000; Clark and Cabana 2011; Cameron 2013); 2) Spatial patterns – Do new sites form during population surpluses? How are sites grouped? Specific sites and/or house groups may represent ‘ethnic barrios,’ or groupings of immigrants from the same original location (Anthony 1990; Osborne 1991; Burmeister 2000; Smith 2014); 3) Return migration patterns – a migrant’s return to his/her original place of origin after an extended absence. Such occurrences have prompted new trade routes (Anthony 1990; Burmeister 2000;

Cabana and Clark 2011; Smith 2014); 4) Social networks – developed routes for migration from place A to place B through previous migrations, kinship ties, and/or trade and exchange networks. Are geographically similar people migrating along familiar routes due to information they obtained from previous migrants? (Anthony 1990: 903; Burmeister 2000; Smith 2014); and finally 5) “Negative push and positive pull factors at the place of origin and the destination” (Anthony 1990:898) – these can be anything from natural disasters, climate change, loss of resources, economic advantages, political upheaval, or warfare (Anthony 1990; Burmeister 2000; Cabana and Clark 2011; Cameron 2013).

Identifying these themes in the archaeological record through the sole analysis of cultural materials has proven to be difficult (Koch and Kupke 2012; Burmeister 2000). Typically, archaeologists use proxies such as architectural features, ceramics, and/or burial goods to identify local and non-local populations (Burmeister 2000; Koch and Kupke 2012); however, goods and ideas can cross boundaries through non-migratory means, such as trade. Additionally, assimilation may lead to a lack of ‘foreign’ goods for an originally non-local individual, while social status may lead to a collection of luxurious ‘foreign’ goods for a local, elite individual.

If we identify migration based solely on material goods uncovered from excavations and burials, we may misidentify individuals as local when they were actually foreign or vice versa (Koch and Kupke 2012; Evans et al. 2006b; Eckardt et al. 2009). In fact, a study conducted by Evans et al. (2006b) exemplifies how archaeologists originally misidentified both ‘local’ and ‘exotic’ burials “at the late Roman cemetery at Lankhills School, Winchester, southern England” (Evans et al. 2006b: 265).

G. Clark originally excavated this cemetery from 1967 to 1972 and assigned buried individuals as ‘exotic’ or ‘local’ based on their noted burial customs, associated burial goods, and the positioning of those goods relative to the body (Evans et al. 2006b). Using strontium and oxygen isotopic compositions, Evans et al. (2006b) tested the hypothesis G. Clark originally postulated – the ‘exotic’ population from the Lankhills cemetery were originally from Pannonia, located within the Danube region in central Europe.

Out of 18 re-analyzed burials, nine selected from the previously thought exotic population and nine from the previously thought local population, two of the nine supposed exotic burials were actually locals while four of the nine supposed locals were actually exotic (Evans et al. 2006b). Additionally, while the exotic population were all initially thought to come Pannonia, they actually varied geographically (Evans et al. 2006b).

Eckardt et al. (2009) followed up on this study by analyzing an additional 40 burials (20 ‘local,’ 8 ‘Pannonian,’ and 12 ‘other’). They too found that the original burial interpretations as an indication of migration were incorrect. Only one out of eight supposed Pannonian burials was actually from the designated region previous archaeologists suspected based on the grave goods. Additionally, of the original 20 ‘local’ burials, nine were identified as ‘non-local’ (Eckardt et al. 2009)

These studies are important, because they shows us that relying solely on the archaeological record for migration identification may not be the best method. Instead, geochemistry provides us the evidence necessary to prove migration was occurring through the use of isotopic analysis (Van Dommelen 2014; for specific examples see also Bentley 2006; Freiwald 2011; Hodell et al. 2004; Kern 2012; Koch and Kupke 2012; Price et al.

2002; Price et al. 2012a; Price et al. 2012b; Rand 2012; Somerville 2010; Stuart-Williams et al. 1996; Sutinen 2014; Thornton 2011; Wright 2005; Wright et al. 2010).

## **MIGRATION AND ISOTOPES IN ARCHAEOLOGY**

In 1985, Jonathon Ericson proposed strontium isotopes be used as a means of identifying migration within archaeological populations. Until that point, proxies such as ceramics and architectural features (as briefly mentioned above) were the sole indicators archaeologists used to identify migrants. Ericson (1985) completed a small pilot study on three Chumash individuals from two different cemeteries in the Malibu area of Southern California and was able to show how strontium isotope ratios could be used to identify local and non-local people. Ericson outlines how strontium isotope ratios in human tooth enamel and bone can be used to identify geographic places of origin through variations within the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of bedrock. He cautions against low variability between locations, food sources, intraregional variability for a given population, and diagenesis. All of these concerns are taken into consideration when selecting a sample for analysis and will be discussed in chapter three.

Following Ericson's (1985) research, Schwarcz et al. (1991) identified oxygen as an additional indicator of geographical location. The assumption is that oxygen varies from one climatic region to another, with elevation, from one water reservoir to another, with humidity, and with precipitation patterns, all of which vary regionally. These early papers established key analytical techniques currently employed in bioarcheology and forensic anthropology. The use of these two isotopic systems to study migration has been

invaluable, and they have been employed to examine mobility within archaeological investigations across the globe (see for example Dupras and Schwarcz 2001; Price et al. 2002a; Evans et al. 2006a, 2006b; Price and Gestsdottir 2006; Eckardt et al. 2009; Slovak et al. 2009; Keenleyside et al. 2011; Gerling et al. 2012; Webb et al. 2012; Symonds et al. 2014).

Isotopic analyses as a means of identifying migration was introduced to archaeological research starting in the mid-1980s with strontium and the early 1990s with oxygen; however, it was not until the early 2000s when both systems became a staple in archaeological investigations. Perhaps the most intriguing aspect of the isotopic research conducted to date has been the amount of non-locals identified at various archaeological sites throughout the world. While the number of non-locals varies greatly, ranging anywhere from 3% non-local (Slovak et al. 2009) to 89% non-local (Symonds et al. 2014), the surprising fact is that every isotope study included in this report has been able to identify non-locals at a site, regardless of the sample size or the location.

#### **MIGRATION AND ISOTOPES IN MESOAMERICA AND THE MAYA WORLD**

In Mesoamerica, the findings are quite similar, with all isotope studies identifying non-local individuals at various archaeological sites during different times. Much of the isotopic analyses completed in broader Mesoamerica focus on the ethnic barrios and sacrificial victims at Teotihuacan (White et al. 1998, 2002, 2004a, 2004b, 2007; Price et al. 2000). Extensive studies indicate a large foreign population within the ethnic barrios – 29% at Tlajinga 33 (White et al. 2004), 80% at the Oaxaca Barrio (Price et al. 2000), 25%



at Ozttoyahualco (Price et al. 2000), 50% at Barrio de los Comerciantes (Price et al. 2000), 31% at Cueva de las Varillas (Price et al. 2000), and up to 80% at Tlailotlacan (White et al. 2004b). Additionally, the Moon Pyramid and the Feathered Serpent Pyramid also contained high quantities of foreign sacrificial victims – at least 57% at the Feathered Serpent Pyramid (White et al. 2002) and at least 86% at the Moon Pyramid (White et al. 2007).

Within the last 10 years, isotope studies have been applied to the Maya world to determine whether a link exists between Classic Maya cities and Teotihuacan. Many of these studies have focused on larger cities like Kaminaljuyu (White et al. 2000; Wright et al. 2010), Tikal (Wright 2005, 2012; Price et al. 2008), and Copan (Price et al. 2008, 2010). These sites represent the Maya during the peak of the Classic Period, and each site ranges in the number of identified immigrants -- 19% to 26% for Kaminaljuyu (Wright et al. 2010; White et al. 2000), 11% to 16% for Tikal (Wright 2005, 2012), and 26% for Copan (Price et al. 2010). While immigrants have been identified, connections to Teotihuacan have not. In fact, research has shown that immigrants are most likely coming from the Maya lowlands, such as the Petén in Guatemala or Belize. There have been few isotope studies completed in Belize; however, based on the results from Freiwald (2011), Belize holds promise for migration studies, even though it has much smaller communities.

Freiwald (2011) sampled a total of 148 individuals, encompassing 14 major and minor archaeological sites, to determine migration within the Belize River Valley. She was able to identify migration at almost every site sampled, regardless of time period or sample size. Of the 14 sites sampled, only three did not identify migrants, likely due to the small

sample sizes (n=3 per site). Still, throughout the region, immigrants make up, on average, 20.8% to 26.2% of all inhabitants, with many of the regions of origin unidentified (Freiwald 2011). This is most likely due to a lack of data.

I propose to add to the existing body of research by measuring strontium isotope and oxygen isotope ratios of human remains from the Rio Bravo Conservation and Management Area (RBCMA) in northwestern Belize. Using two different isotope systems will allow for a more complete understanding of where immigrants originally migrated from. The first advantage to using two isotope systems is this allows us to compare to a wider range of previously completed isotopic studies. Some researchers have completed strontium isotopic analysis; some oxygen; some have only analyzed bedrock and sediments. Analyzing both systems will allow the data from the PfBAP to be compared to a wider range of previously completed isotopic studies of various geographic regions.

The second advantage to using two isotope systems is their ability to complement one another and distinguish local from non-local when one system may be indistinguishable from another. When the data from Sr analysis are first given, Sr is reported with six significant figures; however, changes on the second, third and fourth decimal places are the most important. For this reason, Sr values reported in archaeological contexts are generally reported to the fourth decimal place (Price et al. 2010; Price et al. 2012b). Price et al. (2012b; Figure 2) provide a detailed map regarding the strontium



Figure 2. Map of varying Sr isotope ratios throughout Mesoamerica. Values represent the isotopic composition of modern local fauna and archaeological human dental enamel. Adapted from Price et al. 2012

isotope ratios in the Maya world; however, there is some overlap in Sr isotopic composition from sites geographically very distant. For instance, the Olmec major center of San Lorenzo, located on the Gulf Coast of Mexico, just south of the Isthmus of Tehuantepec,

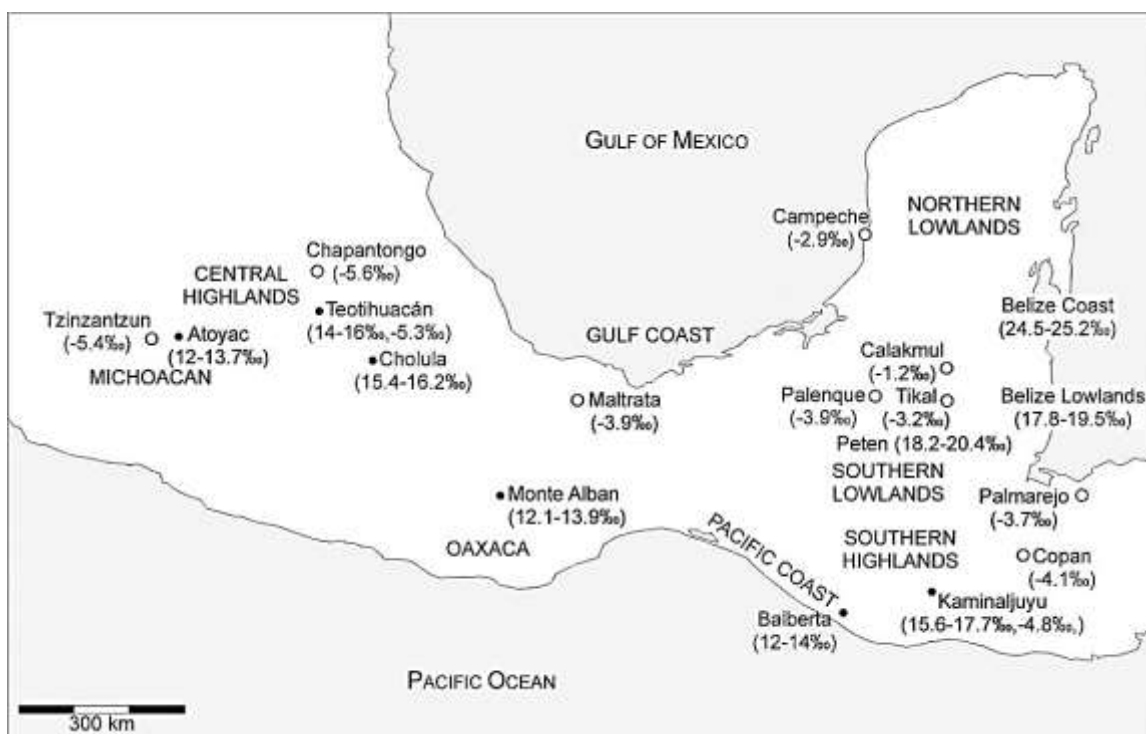


Figure 3. Map of Oxygen isotope variability in Mesoamerica. Values represent the isotopic ratios of archaeological human dental enamel and bone. This map includes both phosphate (dark circle or positive values) and carbonate (open circle or negative values) values. Adapted from Price et al. 2010.

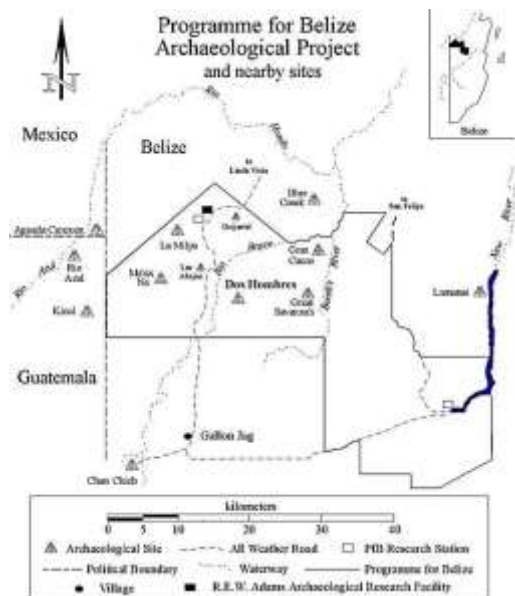
carries the same Sr isotope ratio as the Maya site of Coba, located near the Caribbean coast of the Yucatan Peninsula. Similarly, oxygen values may be similar in geographically distinct areas (Figure 3).

Again, we can see overlapping isotopic compositions for measured oxygen isotope ratios. The importance of using two isotope systems is to distinguish between individuals who may come from geographically distinct areas with similar values in one of the isotope systems. Generating a more comprehensive database of oxygen and strontium isotope ratios may help us distinguish where people were migrating from rather than simply acknowledging there are foreigners present.

## Program for Belize Archaeological Project (PfBAP)

## GEOLOGICAL AND ENVIRONMENTAL SETTING

The Rio Bravo Conservation and Management Area (RBCMA) is a private reserve in northwestern Belize that encompasses 260,000 acres of land, owned and operated by the non-governmental organization The Programme for Belize (PfB) (Figure 4) (Valdez, Jr. and Cortes-Rincon 2012). The RBCMA sits within a humid, tropical rain forest and is included in the Intertropical Convergence Zone (ITCZ) (Beach et al. 2011). The ITCZ



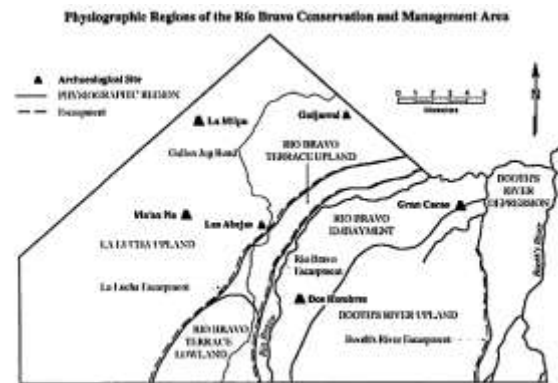
*Figure 4 – Location of the RBCMA and PfB. Courtesy of PfBAP.*

generates an average 1500mm of rain each year in this region, where the bulk of the rain (>200mm/month) comes during the “rainy season” (approximately June – January) while the region remains temperate (<10mm rain/month) during the “dry season” (approximately February – May) (Beach et al. 2002, 2008, 2009, 2011; Luzzadder-Beach and Beach 2008; Houk 1996; Sullivan 1997; Manning 1997; Lohse 2001; Bridgewater et al.

2002). Minor deviations occur within this pattern year to year.

Temperature variations are much less, with summer temperatures averaging around 26°C (80°F) and dropping to the lower 20s. Winter temperatures average around 24°C in

the daytime and can reach 10°C (50°F) during the night (Houk 1996; Sullivan 1997; Manning 1997; Lohse 2001; Bridgewater et al. 2002; Beach et al. 2008).



Cretaceous limestone (Lohse 2001; et al. 2002; Beach et al. 2002, 2008, 2009). These limestone outcrops were formed during the Eocene when Belize and most of the Yucatan Peninsula of Mexico were below sea level (Sullivan 1997). Belize gradually emerged from the water, creating terracing as the water slowly receded from the land. Three of these terraces resulted in fairly steep escarpments which now characterize the western portion of the RBCMA (Figure 5) (Houk 1996; Sullivan 1997; Manning 1997; Lohse 2001). The region of the RBCMA west of Booth's River is made up of wetlands and savannahs (Bridgewater et al. 2002; Beach et al. 2008).

The climate of the region will also help distinguish it from other regions in Mesoamerica. As noted above, the PfBAP is nestled within a humid, tropical rainforest located inside the ITCZ. It is also located in the Maya lowlands. The oxygen isotope ratios measured in the RBCMA will differ from oxygen isotope ratios measured in non-rainforest environments with less rainfall, in the mountainous Maya highlands, in coastal regions, in arid environments, in regions where the Pacific Ocean acts as the main source of air masses, and in regions which differ in latitude and consequently fall outside the ITCZ. Explanations of why oxygen isotope ratios vary in geographically distinct places are explained in further detail in Chapter 3.

## **CULTURAL HISTORY**

The Programme for Belize Archaeological Project (PfBAP), nestled within the Rio Bravo Conservation and Management Area (RBCMA), presents an ideal environment for mobility and migration studies. Occupation in the RBCMA spans from the Paleoindian Period (15,000 – 8,000 BCE) through the Historical Period (Valdez, Jr. and Cortes-Rincon 2012; Houk 1996; Lohse 2001; Manning 1997; Sullivan 1997). However, this report focuses on the migration within the region spanning the Late Preclassic (300 BCE – 250 CE) to the Terminal Classic (CE 800 – 900).

During this time interval, the RBCMA saw a rapid increase in population in the Late Preclassic, before experiencing a major decline in inhabitants at the end of the Early Classic (CE 250 – 550), commonly referred to as the Middle Classic Hiatus (approximately 530 CE). Then, in the Late Classic (CE 600 – 800), the region again saw a major influx of

residents on an even larger scale than it saw in the Late Preclassic. Population growth flourished during this period throughout the RBCMA before the region almost completely depopulated by the Terminal Classic. There is little evidence of occupation in the region during the Postclassic (CE 900 – 1600) (Houk 1996; Lohse 2001; Manning 1997; Sullivan 1997).

Currently, we do not know how people were moving around the region or how many non-locals were moving into the area as no migration studies have yet been completed in the RBCMA, or in northwestern Belize in general. However, with the ability to include human specimens from a variety of sites, we can understand migration and mobility at both a local level and the broader, regional scale. This will enhance our understanding of migrations, economic interactions, political and marital alliances, and commoner adaptations to changes in demographics.



## CHAPTER 3

### Isotope Background and Theory

A nuclide, in its simplest definition, is a nucleus, consisting of neutral neutrons and positively charged protons, surrounded by negatively charged electrons (Sharpe 2007; Meier-Augenstein 2010). Each element on the Periodic Table is defined by the number of protons within its nucleus. Thus the number of protons within the nucleus of a given element will always be the same; however, the number of neutrons within the nucleus of a particular element may vary (Katzenberg 2000; Banner 2004; Sharpe 2007; Meier-Augenstein 2010; Porcelli and Baskaran 2011). As such, an isotope can be defined as a nuclide of an element with varying amounts of neutrons.

The number of protons in the nucleus determines the number of electrons outside it, which essentially defines an element's chemical characteristics (Katzenberg 2000; Meier-Augenstein 2010). The mass of an electron does not impact a nuclide; however, neutrons, like protons, have an influential mass. As a consequence, the total mass of isotopes of the same element will vary from each other; however, the chemical characteristics that define the element, such as the valence or its electronegativity, will not be affected (Meier-Augenstein 2010; Katzenberg 2000). The physical characteristics of the element, such as kinetic properties and bonding energies, will vary with mass (Meier-Augenstein 2010; Porcelli and Baskaran 2011) which result in fractionation of isotopes from one another by chemical and physical processes.

A fractionation factor ( $\alpha$ ) can be determined by examining the ratios between two isotopes from the same element. In other words:

$$\alpha = \frac{R_A}{R_B}$$

where  $R_A$  is the isotope ratio in one phase (e.g. liquid water) and  $R_B$  is the isotope ratio in another phase (e.g. water vapor), and both are representative of the same element (e.g. Oxygen) (Sharp 2007; Porcelli and Baskaran 2011). There is a larger magnitude of fractionation in lighter elements due to the relative mass differences between isotopes. For example, hydrogen has the largest fractionation factor, while heavier elements, like strontium, have very small and usually considered insignificant fractionation. This can be explained by a simple mathematical equation:

$$\frac{M_{heavy} - M_{light}}{M_{atomic}}$$

where  $M_{heavy}$  is the mass of the heavy isotope,  $M_{light}$  is the mass of the light isotope, and  $M_{atomic}$  represents the atomic mass of the element.

Fractionation can occur in both equilibrium processes, where two reservoirs are engaged in equal isotopic exchange, and kinetic processes, where the distribution of isotopes is controlled by the rates of chemical and/or physical processes. One example of a kinetic process in situations related to diffusion or evaporation is when one phase is removed much more quickly than another. For example, evaporation is considered a kinetic effect whereby the rate of evaporation of  $H_2^{16}O$  is faster than the evaporation of  $H_2^{18}O$  (Sharp 2007; Porcelli and Baskaran 2011). A Rayleigh distillation equation can be used to

account for changes in the isotope compositions of water vapor in a cloud due to precipitation:

$$\frac{R}{R_i} = F^{\alpha-1}$$

where R is the measured isotope ratio (e.g.  $^{18}\text{O}/^{16}\text{O}$ ),  $R_i$  is the initial isotope ratio,  $\alpha$  is the fractionation factor as described above, and F is the fraction of water vapor remaining in the cloud (Sharp 2007; Porcelli and Baskaran 2011).

## **STABLE AND RADIOGENIC ISOTOPES**

Twenty-one elements on the Periodic Table are monoisotopic, including phosphorous, fluorine, sodium and aluminum; the remaining elements consist of stable and radiogenic isotopes (Sharp 2007; Meier-Augenstein 2010). Stable isotopes do not decay over time (Katzenberg 2000; Sharp 2007). Radiogenic isotopes are daughters generated from either alpha, beta, or gamma decay from a radioactive parent, due to the instability of a nucleus (Banner 2004; Porcelli and Baskaran 2011). Additionally, radiogenic isotopes can be either stable or radioactive (Sutinen 2014).

Stable and radiogenic isotopes have been used to investigate research interests in the earth sciences, chemistry, biology, and recently, archaeology (Price et al. 2002; Banner 2004; Sharp 2007; Meier-Augenstein 2010; Baskaran 2011; Porcelli and Baskaran 2011;). The use of both stable and radiogenic isotopes is complementary to one another and can help constrain places of origin where geographic variation may be low or where two

distinct geographic locations share the same isotopic compositions (Beard and Johnson 2000; Banner 2004; Hodell et al. 2004; Evans et al. 2006a; Eckardt et al. 2009; Keenleyside et al. 2011; Slovak and Paytan 2011; Price et al. 2012a).

The use of strontium and oxygen isotope ratios to identify migration in the distant past dates back to the early 1990s when researchers began to explore Ericson's (1985) in-depth proposal for their use (Katzenberg 2000). This technique works off the assumption that isotope ratios measured in human bone and dental enamel can be used as geographical tracers to determine points of origin or early childhood. In other words, an individual whose tooth enamel's isotopic composition matches the local isotopic ratios can be identified as a local, while an individual whose tooth enamel's isotopic ratios do not match the local isotopic composition can be identified as a non-local. Local values for strontium are determined by measuring isotope ratios of local sediments, plants, gastropods, and faunal remains; for oxygen, local values are determined through a statistical groupings of isotopic ratios measured in human bone. Two-sigma standard deviation outliers are generally considered non-local individuals. Increasingly, oxygen and strontium isotope ratios have been used to investigate research questions pertaining to human mobility in various regions of the world (see Wright and Schwarcz 1999; Price et al. 2000, 2008, 2010, 2012; Hodell et al. 2004; Bentley 2006; Slovak and Paytan 2011; Gerling et al. 2012; Koch and Kupke 2012; Frei and Price 2013; Fenner and Wright 2014).

## **Strontium**

There are four naturally occurring, stable isotopes of Strontium –  $^{84}\text{Sr}$  with a natural abundance of 0.56%;  $^{86}\text{Sr}$  at 9.87%;  $^{87}\text{Sr}$  at 7.04%; and  $^{88}\text{Sr}$  at 82.53%.  $^{87}\text{Sr}$  is a radiogenic

daughter product of  $^{87}\text{Rb}$  (Rubidium), due to beta decay processes (Beard and Johnson 2000; Price et al. 2000, 2008, 2012; Banner 2004; Hodell et al. 2004; Bentley 2006; Price and Gestsdottir 2006; White et al. 2007; Andrushko et al. 2009; Sommerville 2010; Thornton 2011; Freiwald 2011; Slovak and Paytan 2011; Sutinen 2014). While  $^{87}\text{Sr}$  is the product of radioactive decay, it is itself a stable isotope and will not decay further (Hodell et al. 2004; Bentley 2006; Andrushko et al. 2009; Sommerville 2010; Thornton 2011; Freiwald 2011; Sutinen 2014).  $^{87}\text{Sr}$  is normalized to a non-radiogenic isotope, such as  $^{86}\text{Sr}$ , to measure variations within isotopic ratios due to  $^{87}\text{Rb} \rightarrow ^{87}\text{Sr}$  decay over time and natural discrepancies within  $^{87}\text{Sr}$  geographically (Beard and Johnson 2000; Price et al. 2000, 2008).

Strontium isotope ratios differ geographically due to the original amount of Rb within a rock and the rock's age. (Ericson 1985; Price et al. 2000, 2002, 2008; Hodell et al. 2004; Price and Gestsdottir 2006; White et al. 2007; Slovak and Paytan 2011; Wright 2012; Frei and Price 2014). Rb is incompatible with the mantle and incorporated into partial melts of the mantle; thus, it is high in certain crustal materials, such as shales, sandstones, and granites while low in others, such as limestones, basalts, and marbles (Beard and Johnson 2000). The age of the rock also contributes greatly to the variances in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. Because they started with higher concentrations of Rb when they formed, old metamorphic rocks (>100Ma) will have some of the highest  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios (Hodell et al. 2004; Evans et al. 2006a, 2006b; Price and Gestsdottir 2006; White et al. 2007; Slovak and Paytan 2011; Frei and Price 2013). This is because Rb was plentiful in the rock when it formed and has had ample time to decay into  $^{87}\text{Sr}$ . The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of younger rocks (1-10Ma) will have much lower  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios because Rb has not had as much time to

decay. (Price et al. 2000, 2002, 2008; Hodell et al. 2004; Evans et al. 2006a, 2006b; Price and Gestsdottir 2006; White et al. 2007; Slovak and Paytan 2011; Wright 2012; Frei and Price 2013). Furthermore, the value of a given marine sedimentary rock, such as a calcium carbonate, will not vary much over time due to an initial lack of Rb. Strontium and calcium are chemically similar, so Sr often substitutes for Ca, excluding Rb. For these reasons, calcium carbonates like limestone represent the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the ocean during the time of their formation (Hodell et al. 2004).

We know that the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio varies over time and location due to radioactive decay from  $^{87}\text{Rb}$ . While the differences in ratios can seem quite small (0.0001 to 0.001), such variations between two regions can have immense impacts on data interpretations. To try to make small changes in ratios easier to understand, results may be presented in epsilon notation ( $\epsilon^{87}\text{Sr}$ ):

$$\epsilon^{87}\text{Sr} = \left( \frac{\frac{^{87}\text{Sr}}{^{86}\text{Sr}}_{\text{measured}}}{\frac{^{87}\text{Sr}}{^{86}\text{Sr}}_{\text{bulk earth}}} - 1 \right) * 10,000$$

where  $^{87}\text{Sr}/^{86}\text{Sr}_{\text{measured}}$  is the amount of  $^{87}\text{Sr}/^{86}\text{Sr}$  measured in your sample and  $^{87}\text{Sr}/^{86}\text{Sr}_{\text{bulk earth}}$  is equal to 0.7045, an internationally accepted value.

This variation between locations is what makes Sr appealing for migration studies within an archaeological context. The Sr ratios from an individual's tooth enamel can be used to determine a geographic place of origin when compared to local samples of bedrock, sediments, and/or modern or archaeological faunal remains (Beard and Johnson 2000;

Price et al. 2002, 2008; Wright 2005; Evans 2006a, 2006b; Eckardt et al. 2009; Gerling et al. 2012). Furthermore, because the half-life of  $^{87}\text{Rb}$  is  $4.88 \times 10^{10}$  years, there is no concern that archaeological investigations will be affected by the decay of  $^{87}\text{Rb}$  into  $^{87}\text{Sr}$ , since the time scale archaeologists work on is so short in comparison (Price et al. 2000, 2002, 2008; Hodell et al. 2004; Bentley 2006; Andrushko et al. 2009; Sommerville 1010; Thornton 2011; Freiwald 2011; Sutinen 2014).

### ***Strontium in the Human Body***

Strontium has the ability to act as a geographic tracer of an individual's place of origin through tropic cycles (Ericson 1985; Hodell et al. 2004; Wright 2005; Bentley 2006; Andrushko et al. 2009; Sommerville 1010; Thornton 2011; Freiwald 2011; Sutinen 2014). The underlying bedrock, limestone in the case of PfbAP, carries an  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio. After traveling through the bedrock, water containing Sr with this isotopic ratio percolates out into the soils and is absorbed by local plants. Animals consume the plants and drink the water; followed by humans eating the animals and plants and drinking the same water. Isotopic fractionation between  $^{87}\text{Sr}$  and  $^{86}\text{Sr}$  is relatively small; it has been noted that mass fractionation is almost completely absent between strontium isotopes (Ericson 1985; Beard and Johnson 2000; Price et al. 2000, 2002; Hodell et al. 2004; Wright 2005 Evans et al. 2006a, 2006b; Eckardt 2009; Slovak and Paytan 2011; Frei and Price 2013). In the rare event that any fractionation does occur during analysis, it can be corrected for by measuring known standards alongside the unknowns. The computer software used to operate the mass spectrometer then calculates what the true  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the unknowns are. In other words, the local person who eats local foods and drinks local waters, should be

representative of the local Sr isotopic values (Beard and Johnson 2000; Hodell et al. 2004; Bentley 2006; Evans et al. 2006a, 2006b; Andrushko et al. 2009; Eckardt et al. 2009; Slovak and Paytan 2011; Frei and Price 2013). After ingestion, Sr is incorporated into human tissues like fingernails, hair, bone, and dental enamel (Slovak and Paytan 2011; Frei and Price 2013).

Bones and teeth are the only remaining human tissues available to us at the PfBAP and make up the samples to be used for analysis. Human bone, commonly referred to as bioapatite, is a hard mineral matrix composed of insoluble calcium phosphate hydroxyapatite with a chemical formula of  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$  (Price et al. 2000, 2012a, 2012b; White et al. 2007; Grimes and Pellegrini 2013). Strontium and calcium (Ca) are chemically similar, and as such, a portion of Sr substitutes for Ca in bone and tooth enamel during mineralization phases (Budd et al. 2000; Price et al. 2000, 2008, 2012a, 2012b; Wright 2005; Bentley 2006; White et al. 2007; Slovak and Paytan 2011). Since no fractionation occurs within the tropic cycle or during these mineralization phases, the ratio of  $^{87}\text{Sr}/^{86}\text{Sr}$  in an individual's bone or tooth enamel can be measured and used to identify whether or not the individual consumed local foods during tooth mineralization periods.

For this research, tooth enamel is preferred over bone samples for multiple reasons. As mentioned in the Geological and Environmental Setting section of Chapter 2, the PfBAP is located in a very humid, tropical rainforest. Acidic soils and excessive rainfall are not ideal conditions for the preservation of bones. As such, teeth represent the bulk of the available specimens. Additionally, studies have shown that tooth enamel, unlike bone, is highly resistant to diagenetic contamination which often occurs during postmortem



deposition (Beard and Johnson 2000; Bentley 2006; Price and Gestsdottir 2006; Slovak et al. 2009; Price et al. 2012a, 2012b).

Further, tooth enamel does not remodel after formation. The mineralization phases for tooth enamel are in the early stages of a person's life and vary in time depending upon the tooth. While the tooth is forming, it incorporates the local  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio through the substitution of strontium for calcium. Once the tooth has finished forming, the chemical composition does not change, thus reflecting the Sr isotope ratio of the bedrock of where the tooth formed (Beard and Johnson 2000; Budd et al. 2000; Schweissing and Grupe 2003; Hodell et al. 2004; Wright, 2005, 2012; Price and Gestsdottir 2006; White et al. 2007; Slovak et al. 2009; Slovak and Paytan 2011; Gerling et al. 2012; Price et al. 2000, 2002, 2008, 2010, 2012a, 2012b; Boric and Price 2013).

Incisors develop first, followed by canines, and finally molars (Wright and Schwarcz 1998, 1999; Beard and Johnson 2000; White et al. 1998, 2000, 2002, 2004a, 2004b, 2007; Keenleyside et al. 2011; Wright 2012; Webb et al. 2014), with Sr isotopic compositions decreasing with later tooth formation phases due to a change from weaning to eating local food sources (Beard and Johnson 2000). Choosing the same tooth (e.g. the first molar or pre-molar) for every individual to be analyzed ensures a similar time of reference across an entire population regardless of differences in the age the individuals were at their time of death or a difference between burial time scales (e.g. Late Preclassic vs. Late Classic).

Identifying a representative local signature can be difficult. We know that geologic sources can be used to determine the  $^{87}\text{Sr}/^{86}\text{Sr}$  values for a location. The first step may be

to sample the bedrock in the region to try to assess the local strontium isotopic composition and any variations within it (Beard and Johnson 2000). While this will give you ratios of the underlying bedrock, it may not provide an accurate signature for local inhabitants. There are additional contributors to an individual's strontium intake – soils, rainfall, ground waters, and the atmosphere. Soils will have the largest impact on Sr values, especially in the PfBAP region where clays are abundant, but precipitation and atmospheric components have also been shown to contribute (Beard and Johnson 2000; Price et al. 2002; Hodell et al. 2004). Moreover, Sr ratios can vary within a single piece of rock and between rocks, and people are not completely sedentary. Local populations may not live on the same formation of bedrock (assuming the two rocks have different  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios). Thus, it is more accurate to compare the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of human specimens to the ratios of other biologically available samples, such as faunal remains, gastropods, and, if available, other human bone samples from the site being studied (Price et al. 2002; Hodell et al. 2004; Wright 2005; Bentley 2006; Price and Gestsdottir 2006; Andrushko et al. 2009; Slovak et al. 2009; Frei and Price 2013).

## Oxygen

Oxygen has three naturally occurring, stable, non-radiogenic isotopes:  $^{16}\text{O}$  with an abundance of 99.762%;  $^{17}\text{O}$  at 0.038%; and  $^{18}\text{O}$  at 0.20%. Oxygen isotope ratios are compared to a standard and are notated in permil as  $\delta$  values, such that

$$\delta = \left( \frac{R_{sam} - R_{std}}{R_{std}} \right) * 1000$$

where  $R_{\text{sam}}$  is equal to the heavy/light isotope ratio ( $^{18}\text{O}/^{16}\text{O}$ ) in the sample and  $R_{\text{std}}$  is equal to the heavy/light isotope ratio in the standard (White et al. 2000; 2002; 2004a; 2004b; 2007; Sharp 2007; Price et al. 2012; Wright 2012; Sutinen 2014; Symonds et al. 2014). Values are multiplied by 1000 so the rather small  $\delta$  values are converted to a more reasonable quantity in parts per mil (‰) (Sharp 2007; Porcelli and Baskaran 2011; Sutinen 2014; Symonds et al. 2014). If the  $\delta$  values are positive, the ratio of heavy to light isotopes of the sample is greater than the standard. If the  $\delta$  values are negative, the ratio of heavy to light isotopes of the sample is less than the standard (Price et al. 2012).

Two standards are used for oxygen analysis, one for low temperature carbonates (Pee Dee Belemnite [PDB]) and one for water, silicates, and quartz (Standard Marine Ocean Water [SMOW]) (Price et al. 2012). Oxygen isotope ratios can be measured in the carbonate or phosphate component of human dental enamel and bone. The carbonate component utilizes the PDB standard while the phosphate component utilizes the SMOW standard. The two can be related using the following:

$$\delta^{18}\text{O}_{\text{SMOW}} = 1.03091 (\delta^{18}\text{O}_{\text{PDB}}) + 30.91$$

This equation seeks to relate the SMOW and PDB scales. Once the scales have been related, the calculated  $\delta^{18}\text{O}_{\text{SMOW}}$  is then substituted into the below equation to account for the mineralization of human dental enamel at body temperature:

$$\delta^{18}\text{O}_{\text{phosphate}} = 0.98 (\delta^{18}\text{O}_{\text{SMOW}}) - 8.5$$

Oxygen isotopes are representative of the local meteoric rainwater, and vary depending upon the distance travelled from the ocean, cooling history of the air, humidity, elevation, latitude, and precipitation patterns (Stuart-Williams et al. 1996; White et al. 1998, 2000, 2002, 2004a, 2007; Wright and Schwarcz 1998; Dupras and Schwarcz 2001; Evans et al. 2006a ; Eckardt et al. 2009; Price et al. 2010, 2012a; Wright et al. 2010; Keenleyside et al. 2011; Gil et al. 2014). Essentially, as clouds form over oceans, they take in evaporating water molecules. As they travel from the ocean over the land, the heavy oxygen isotope ( $^{18}\text{O}$ ) is preferentially incorporated into the liquid water during condensation and removed from the air mass as precipitation occurs, as a result the isotopic signatures closest to the ocean will be heaviest (more positive values). As the clouds continue to move over land and up in elevation, the falling rain from the clouds has much lower  $\delta^{18}\text{O}$  values, resulting in isotopically lighter water (more negative values) (Price et al. 2010; Wright et al. 2010). As such, rain that occurs on the coast is typically isotopically heavier (meaning more  $^{18}\text{O}$ ) than rain falling further inland (Price et al. 2010)

Unlike Sr, the mass fractionation between oxygen isotopes is much greater due to the larger relative mass difference between  $^{18}\text{O}$  and  $^{16}\text{O}$ . As such, oxygen isotopes are susceptible to biological and environmental processes (Price et al. 2012a; Webb et al. 2013; Symonds et al. 2014). It is possible that changing environmental factors, such as hurricanes and heavy flooding, and evaporation during the dry season affected the  $\delta^{18}\text{O}$  values of the Maya aguadas, where the Maya would have gotten their water during the dry seasons.

Evaporation during the dry seasons has the ability to increase  $\delta^{18}\text{O}$  values in standing water (like the aguadas) and waxy leaves. It is because of these changing environmental factors that oxygen isotopes are relevant to migration studies (Dupras and Schwarcz 2001; Evans et al. 2006a; Keenleyside et al. 2011) as sites within regions will carry their own independent oxygen isotopic values.

### ***Oxygen in the Human Body***

Oxygen isotope analysis for human provenance focuses on subtle differences in meteoric water sources between geographic regions (White et al. 1998; Dupras and Schwarcz 2001; Gerling et al. 2012; Webb et al. 2013; Gil et al. 2014), and relies on two expectations: 1) human teeth will represent the region in which they formed; and 2) geographic regions are isotopically distinct from one another (White et al. 1998, 2000, 2004a, 2007; LaPorte et al. 2009; Price et al. 2010, 2012a; Wright 2012; Webb et al. 2013, 2014; Gil et al. 2014). Though consumption of local waters, oxygen is integrated into the phosphate  $[\text{PO}_4]$ , carbonate, and hydroxyl  $[\text{OH}]$  components of human tooth enamel, formed during early childhood (typically before the age of 12 in all teeth), and of bone (Stuart-Williams 1996; Wright and Schwarcz 1998; Stephan 2000; Dupras and Schwarcz 2001; Evans et al. 2006a, 2006b; Eckardt et al. 2009; Wright et al. 2010; Freiwald 2011; Keenleyside et al. 2011; Slovak and Paytan 2011; Gerling et al. 2012; Price et al. 2012a; Grimes and Pellegrini 2013; Webb et al. 2013; Gil et al. 2014; Sutinen 2014; Symonds et al. 2014).

There are additional factors that contribute to the oxygen values in teeth and bone, resulting in multiple difficulties when measuring the local oxygen values of the past. The first struggle is that it is hard to find a proxy of what the local signature was since oxygen isotope values can seasonally vary (Price et al. 2010; Wright 2012; Symonds et al. 2014). Modern faunal remains cannot be used due to the variability of oxygen isotopes in an annual cycle (Dupras and Schwarcz 2001). Additionally, animals often obtain their water much differently than humans. Humans often rely on meteoric water sources as their main intake of water while animals often rely on leaf water as their main source (Dupras and Schwarcz 2001). Due to the oxygen fractionation which occurs during species-specific bone mineralization and the evaporation effects on oxygen from the plants consumed as water resources by the animal, values are not representative across species (Wright and Schwarcz 1998; Dupras and Schwarcz 2001; Wright et al. 2010).

The second obstacle is that meteoric water is not the only contributor to oxygen values in humans. Breastfeeding has proven to have an influence on the  $\delta^{18}\text{O}$  in infants and consequently teeth that form during infancy, like incisors and canines, since breastmilk has a heavier isotopic value than water due to its formation from body water rather than meteoric waters (Wright and Schwarcz 1998, 1999; White et al. 2002, 2004a, 2007; Eckardt et al. 2009). Still, the enrichment from breastfeeding can be accounted for isotopically as researchers have shown through the measurement of lighter  $\delta^{18}\text{O}$  values as tooth formation ages increase (Wright and Schwarcz 1998, 1999). In other words, the third molar, which mineralizes between ages 9 to 13, has lighter oxygen values than the second molar which has lighter values than the first molar, etc. In the Maya region, children began

the weaning process between two and four years old (Wright and Schwarcz 1998, 1999; White et al. 2002, 2004a, 2007), so samples can be selected to avoid influence of breastfeeding. In the event that no molars are available for sampling, 1‰ can be subtracted from tooth samples that formed before weaning (White et al. 2002, 2004a, 2007; Eckardt et al. 2009). Choosing a tooth that formed after the weaning period has ended (e.g. the first molar) ensures the measured oxygen ratios reflect the local values and not that of the mother's breastmilk which is more enriched in oxygen (Wright et al. 2010); however, research has shown that if molars are not available for selection and if canines or pre-molars are used, oxygen values can be subtracted to remove any influence the mother's breastmilk had on the forming tooth (Wright 2005; Wright et al. 2010).

In addition to enrichment from breastfeeding, humans'  $\delta^{18}\text{O}$  values are depleted through sweat, urine, feces, and carbon dioxide (White et al. 2000, 2002, 2004a, 2004b, 2007; Dupras and Schwarcz 2001; Price et al. 2012a). As a result, statistical analyses must be completed on burials from each site to try to obtain a representative local signature. Outliers larger than a 2-sigma standard deviation from the averaged values of the population are considered to be non-local. Research has shown variation within a given population is typically less than 2‰ (Price et al. 2012a).

As mentioned previously, tooth enamel is the preferred choice for identifying local and non-local individuals because it does not remodel over time, it represents a formation period during childhood across an entire population, and is highly resistant to diagenetic alteration while buried (Lécuyer et al. 1993; Rink and Schwarcz 1995; White et al. 1998, 2000, 2004a, 2004b; Wright and Schwarcz 1998, 1999; Evans et al. 2006a; Eckardt et al.

2009; Price et al. 2010; Wright et al. 2010; Keenleyside et al. 2011; Wright 2012; Webb et al. 2014). An individual whose tooth enamel falls within the local values will be considered local, while an individual whose tooth enamel falls outside the local values will be considered non-local (Keenleyside et al. 2011). This is different for oxygen isotopes in human bones, where O is constantly replenished during bone growth and remodeling. As such, O is also a great indicator of mobility throughout an individual's lifetime (Evans et al. 2006a; Price et al. 2012; Stuart-Williams et al. 1996).

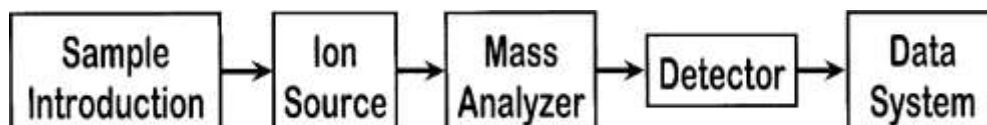
While the sample preparation is much more intensive, I propose to analyze the oxygen isotope ratios from the phosphate component from human tooth enamel and bone rather than the carbonate component. The bond between phosphorous and oxygen is very strong and as such is highly resistant to diagenetic alteration (White et al. 1998; Wright and Schwarcz 1998, Vennemann et al. 2002; Wright et al. 2010; Grimes and Pellegrini 2013). While researchers have studied the removal of diagenetic contamination from the calcite portion of enamel and bone, there is a loss of the original oxygen isotope ratios, resulting in altered numbers (White et al. 1998; Dupras et al. 2001; Grimes and Pellegrini 2013). Phosphates allow for the analysis of original  $\delta^{18}\text{O}$  values in both bone and tooth specimens, which will help constrain individuals geographically.



## CHAPTER 4

### Mass Spectrometry

As discussed in Chapter 3, oxygen and strontium values are represented as ratios, and these ratios are measured by a mass spectrometer. What type of mass spectrometer you use will depend upon what you are trying to analyze; however, the process of analysis for all spectrometers is relatively similar (see Figure 5; reference Sharp 2007; Smith and Thakur 2010).



*Figure 6 – Basic components of a Mass Spectrometer. Borrowed from Smith and Thakur 2010.*

Samples are first introduced into the instrument through a sample inlet. In the case of strontium, where, a Thermal Ionisation Mass Spectrometer (TIMS) is used, samples are simply loaded on filaments and placed in the instrument. For oxygen, where an Isotope Ratio Mass Spectrometer is used, samples are converted from either a solid or a liquid to a gaseous mixture. Once this has occurred, the gas mixture travels to the ion source where the molecules are ionized by blasting them with electrons. The TIMS ionizes by heating the samples (Smith and Thakur 2010). This ionization results in the loss of a single electron from each molecule, creating cations, or positively-charged ions.

The cations are then focused as they travel, under vacuum, through a series of cones and plates. These cones and plates ensure that the kinetic energy of each ion is the same as the sample travels through the flight tube. The beam is accelerated as it reaches the Mass Analyzer.

In the mass analyzer, the beam encounters a magnet and bends. Ions travel at different trajectories depending upon their mass to charge ( $m/z$ ) ratio. Heavier masses will not bend as much as lighter masses, and as such, heavier masses will travel along outer routes, while lighter masses travel along inner paths. As the ions leave the mass analyzer, they enter the detector where they are counted either sequentially (e.g. ICP-MS) or simultaneously (e.g. TIMS and IRMS) by faraday cups. The collection of ions is measured as a voltage and can be displayed graphically as a spectrum or given as ratios.

The type of precision achieved by a mass spectrometer depends upon its detector and the number of faraday cups. An instrument like an IRMS measures the mass/charge ratio of a select number of voltages represented by various molecules (e.g. mass 44, 45, and 46 to monitor CO<sub>2</sub> gas) and measures them simultaneously, resulting in precision to the second decimal place in per mil notation (Sharp 2007) . An instrument like a TIMS measures the mass/charge ratio of a select number of currents represented by a single element of interest, and also measures them simultaneously, resulting in high precision to the sixth significant figure of a ratio (Price et al. 2012a, 2012b). Before either of these instruments can be utilized for measuring strontium and oxygen isotope ratios within dental enamel and bone specimens, samples must be cleaned, dissolved, and re-precipitated as solids that have isolated the constituent of interest.

## **Proposed Method for Future Research**

### **OXYGEN ISOTOPES**

Following protocols outlined by Stephan (2000), Meier-Augenstein (2010), and Grimes and Pellegrini (2013), samples will be prepared in the Stable Isotope Mass Spectrometry laboratory located at the Jackson School of Geosciences (JSG) at the University of Texas at Austin (UT). The ultimate end goal of sample prep is to remove any organic material and diagenetic contamination from the teeth and bones, dissolve the bioapatite sample, and reprecipitate as silver orthophosphate ( $\text{Ag}_3\text{PO}_4$ ). This process is designed to isolate the phosphate component for oxygen isotope analysis using a Temperature Conversion Elemental Analyzer (TC/EA) coupled to an Isotope Ratio Mass Spectrometer (IRMS). Samples may be analyzed at UT or they may be sent to an established laboratory for analysis.

Sample prep occurs in four major stages. The first stage concerns exterior cleaning and sample drilling. To remove the exterior surface and any contaminants, samples are first abraded 100 $\mu\text{m}$  using a rotary tool with a tungsten carbide brush attachment. Samples are then placed into an ultrasonic cleaner filled with double distilled water ( $\text{ddH}_2\text{O}$ ) for approximately one hour. This ensures any remaining surface contaminants (e.g. dust) are removed prior to sample homogenization. Once the samples have been ultrasonically cleaned, they are placed into a drying oven set at 50°C for two hours. Ultrasonic cleansing and drying are repeated as many times as needed until all surface contaminants are removed.

Once the samples have been cleaned, a known quantity is sectioned out. Using a rotary tool with a diamond drill bit, samples are drilled longitudinally. This ensures homogenization of the sample where isotope values may vary. Approximately 40mg of powdered enamel or bone is removed from each sample and placed into a 15mL centrifuge tube. To remove apatite powder and ensure no cross contamination occurs, drill bits and the workspace are wiped down with a diluted bleach solution and latex gloves are changed between each sample.

The second stage involves removing any organic material and/or diagenetic contaminants that may affect the analysis. 2mL of 2.5% sodium hypochlorite (NaOCl) is added to each sample tube before the samples are gently agitated for 24hours. Samples are then centrifuged for five minutes at 10,000rpm before the supernatant is discarded. The sample pellet is thoroughly washed with ddH<sub>2</sub>O before it is centrifuged for another five minutes at 10,000 rpm. This process is repeated until the supernatant pH reaches neutral. When neutrality is reached, a few drops of the final supernatant is added to 0.5mL of 1M silver nitrate (AgNO<sub>3</sub>) to check for chloride, which is indicated by a formation of milky precipitation. If one occurs, the rinse procedure is repeated until the final supernatant is chloride free.

Once the supernatant is chloride free, 2mL of 0.125M sodium hydroxide (NaOH) is added to the sample pellet and gently agitated for 48h. This ensures any remaining organic material is dissolved. After 48h, the samples are centrifuged for five minutes at 10,000rpm. Sample pellets are repeatedly washed with ddH<sub>2</sub>O and centrifuged until a neutral pH is reached.

The third phase involves dissolving the phosphate component from the sample pellets and into an aqueous solution. 2mL of 2M hydrofluoric (HF) is added to the sample pellet for a 24h digestion period. The samples are then centrifuged for five minutes before the supernatant is pipetted into a 250mL beaker (filled with 3mL of 2M potassium hydroxide (KOH) to neutralize the HF) and kept for the final stage. The centrifuge tubes containing the sample pellet are then filled with ddH<sub>2</sub>O, centrifuged again, and the supernatant added to the neutralized HF solution. Double distilled water is added to fill each beaker to 200mL.

In the final phase, the samples are re-precipitate as Ag<sub>3</sub>PO<sub>4</sub>. 15mL of buffered silver ammine solution is added to each beaker before they are placed on a hotplate and slowly warmed to 70°C. Samples are held at this temperature for 3h. After 3h, samples are removed from the hotplate and slowly cooled to room temperature before the supernatant pH is rechecked for neutrality. The greenish crystals that have formed are filtered using pre-weighed 0.2µm filters, washed three to four times with distilled water, and dried at 50°C for approximately 12 hours. Once dried, 0.2mg of each sample is placed into tightly folded (4 x 3.2 mm), pre-cleaned silver capsule prior to analysis.

## **STRONTIUM ISOTOPES**

Following the protocol outlined by Price et al. (2000), samples will be prepared in the Isotope Clean Laboratory also located in the JSG at UT. The goal of the sample prep is to remove organic material and diagenetic contamination for the teeth, dissolve the bioapatite sample, isolate the strontium, and precipitate the strontium component as a salt

for analysis. This process allows for the isolation of strontium for analysis using a Thermal Ionization Mass Spectrometer (TIMS). Samples will be analyzed with the Thermo Scientific Triton TIMS located in the TIMS Laboratory within the JSG at UT.

Ultrasonic cleaning and sample sectioning is identical to oxygen sample preparation. Once the samples have been cleaned and sectioned out, diagenetic contaminants are removed by adding 2mL of 5% ultrapure acetic acid to each sample vial before being sonicated for 15 minutes. After 15 minutes of ultrasonic cleansing, the samples are left to soak in acetic acid for 12 hours. This has been shown to remove most contaminants (Price et al. 2000). After 12 hours of soaking, samples are centrifuged at 10,000 rpm for five minutes before being rinsed with deionized water. The rinse is repeated three times, removing the supernatant after each centrifuge before samples are placed on a hot plate set to 50°C, and left to dry for 24 hours.

The samples will then be dried down and sent through a second phase of dissolution to remove any remaining minerals or precipitates. This includes first transferring the samples to sterile silica glass tubes that have previously been cleaned in nitric acid (HNO<sub>3</sub>). This ensures no organic contaminants are on the glass tubes. Samples are ashed in a muffle furnace set to 825°C for 8 hours.

Samples are redissolved in 2mL of ultrapure 0.5M HNO<sub>3</sub> before strontium isolation begins. Elemental separation for Sr will be completed using Eichrom Sr-specific resin. Once the Sr is isolated, 10 µl of phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) will be added to the sample before being dried down. A drop of concentrated 15M HNO<sub>3</sub> will then be added to remove any remaining organics. Samples will be dried down before being loaded onto Rhenium

filaments with Tantalum Fluoride slurry and analyzed on the Thermo Triton TI thermal-ionization mass spectrometer (TIMS), also located in the JSG at UT.

NIST NBS987 will be used as a calibration standard to ensure precision of the instrument, data will be normalized to the accepted value of NBS987 of 0.710248 and the six-month lab average, and BHVO will be used as a secondary standard to measure accuracy. The TIMS software automatically corrects for any mass fractionation, normalizing to  $^{88}\text{Sr}/^{86}\text{Sr} = 8.375209$  using an exponential law and also uses the accepted  $^{87}\text{Sr}/^{85}\text{Sr} = 0.3856$  to correct for any Rb interferences. Laboratory blanks will be calculated using an Isotopic Dilution Equation and will determine any lab contamination, which may affect the results.

## **CHAPTER 5**

### **Future Research**

Immigration and human mobility are constant features in contemporary national discourses, bringing up complex questions pertaining to social sustainability. It is not unreasonable to suggest that the articulation between migration and sustainability was also of concern to past societies. To better understand the consequences of ancient Maya mobility through time, I will combine archaeological research with oxygen and strontium isotopic analyses.

As mentioned in Chapter 2, this research is associated with the Programme for Belize Archaeological Project (PfBAP), which includes archaeological sites dating from the Late Preclassic (300 BCE–250 CE) to the Terminal Classic (800–900 CE). I want to understand the consequences of mobility and determine if major population changes during this time interval were related to intraregional mobility, long distance immigration, or both. In other words, what are the demographic shifts throughout the PfBAP area over time? Is mobility restricted to local people moving around the region or can we also identify foreigners? Do new communities develop because of population overflow? Are these sites representative of specific people? Can we determine marriage alliances/kinship ties through migration patterns?

We do not yet know how people were moving throughout the PfBAP region; however, a robust dataset of human remains exists which will help clarify our understanding of demographic changes. I have currently obtained permission to analyze tooth and bone samples from 130 human specimens excavated from the PfBAP area and



43 individuals from external sites. Additional samples will be added over the next two to three years. I will compare the isotopic signatures of individuals from the PfBAP area with external sites and previously published data from the Maya region and outside it to determine the percentage of non-local individuals who were buried within the region and to try to pinpoint their place of origin.

Using the methods outlined in Chapter 4, I will analyze the oxygen and strontium components of human teeth and bone. Upon identifying non-locals, I will examine spatial patterns and demographics of the non-local population to test my hypothesis, which proposes population movements within the PfBAP area encompassed people from a wide range of places and distances, establishing marriage and kinship alliances between sites and resulting in the development of new sites in the Late Classic. This research seeks to measure isotopic values to reflect the origin of and length of time individuals spent in the PfBAP area and the consequences for population changes.

## **Conclusion**

Migration affects both the migrating population and the native population, the place of emigration and the place of origin, and society at large (Kern 2012). Migrants help facilitate the movement of new cultural practices and beliefs, dietary habits and foods, and diseases (Little 2004). Trying to fully understand a group of people cannot be completed without understanding the consequences of demographic changes. These fluctuations may impact the environmental conditions of a site, the social complexities of a civilization,

and/or the biological changes in a population and can occur over an extended period of time rather than the inert snapshot shown to the archaeologist (Little 2004; Osborne 1991).

Strontium and oxygen isotopic analyses have proven to be beneficial in identifying non-locals from various archaeological projects around the world (for specific examples see Bentley 2006; Eckhart et al. 2009; Evans et al. 2006; Freiwald 2011; Hodell et al. 2004; Kern 2012; Koch and Kupke 2012; Price et al. 2002; Price et al. 2012a; Price et al. 2012b; Rand 2012; Somerville 2010; Stuart-Williams et al. 1996; Sutinen 2014; Thornton 2011; Wright 2005; Wright et al. 2010), and these analyses provide the foundation needed to address consequences of migration. Once migrants have been identified in the PfBAP region, I will employ methodologies to test the effects of mobility and immigration through qualitative and quantitative analyses of settlement patterns and distributions (e.g. the emergence of new communities) and changes in material goods.

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