

# Feeding the Children: A Paleodietary reconstruction of Juveniles from Kuelap, Peru

2018

Marley Denierio

Find similar works at: <https://stars.library.ucf.edu/honorsthesis>

University of Central Florida Libraries <http://library.ucf.edu>

 Part of the [Archaeological Anthropology Commons](#), [Biological and Physical Anthropology Commons](#), and the [Other Anthropology Commons](#)

FEEDING THE CHILDREN: A PALEODIETARY RECONSTRUCTION OF JUVENILES  
FROM KUELAP, PERU

by  
MARLEY S. DENIERIO

A thesis submitted in partial fulfillment of the requirements for the Honors in the Major Program  
in Anthropology in the College of Sciences and in the Burnett Honors College at the  
University of Central Florida  
Orlando, Florida

Fall Term, 2018

Thesis Chair: J. Marla Toyne, PhD

## ABSTRACT

Before reaching adulthood, every individual experiences a period of dependency, the juvenile period, during which they rely on the older, more experienced members of their society for their security, subsistence and care. This juvenile period is an important stage of life for human physical and physiological development. In bioarchaeology, there has been limited research conducted on juveniles, particularly, the development of their own social identity and influences. The research method of stable carbon (C) and nitrogen (N) isotope analysis is used to reconstruct the paleodiet of juveniles to determine their dietary composition. Specifically, this research is focused on Kuelap, located in the highlands of Peru, a large settlement inhabited from 900-1535 AD, including pre-Inca (900-1469 AD) and Inca periods (1470-1535 AD). The primary aim of this research is to determine if juveniles consumed different foods through different time periods. Another aim of this research is to determine if juveniles were treated differently than their adult counterparts. Bone collagen samples, primarily ribs, from 32 juveniles were analyzed. The average  $\delta^{13}\text{C}$  value for the pre-Inca juveniles was  $-13.1\text{‰}$ , and  $-13.4\text{‰}$  for the Inca period juveniles. There was no statistically significant difference in  $\delta^{13}\text{C}$  values between juvenile groups or between adult and juvenile subsamples. The average  $\delta^{15}\text{N}$  for the pre-Inca juveniles was  $+8.1\text{‰}$  and  $+7.8\text{‰}$  for the Inca period juveniles. The Mann-Whitney U test determined there was not a statistically significant difference in  $\delta^{15}\text{N}$  values between the juvenile burial groups; however, there was a statistically significant difference between the juvenile and adult subgroups. The findings suggest that there may have been preferential treatment toward or metabolic stressors on the juvenile. The results of this study offer insights to availability of dietary components, societal roles based on developmental age stages, and the potential role of parenting in Kuelap.

## **DEDICATION**

To my family,  
for inspiring my sense of adventure  
and instilling in me respect for diverse cultures and peoples.

## **ACKNOWLEDGEMENTS**

It is with profound appreciation that I extend my gratitude to my teachers and professors who challenged me to go above and beyond their expectations and who fostered my curiosity. I would especially like to thank Dr. Reyes-Foster who introduced me to the fascinating fields in anthropology and to Dr. Toyne for taking a chance on me as she inspired my lab work and dedicated countless hours to coaching and mentoring me. Our research in Peru was a life-changing experience.

I also thank my parents for demonstrating the courage to take me off the beaten path as we've traveled around the world. You developed in me an open mind, resiliency, cultural competence and appreciation for local diets for which I will be forever grateful.

# TABLE OF CONTENTS

|   |    |
|---|----|
| Chapter I. INTRODUCTION .....   | 1  |
| Basis of Research .....   | 1  |
| Juvenile Bioarchaeological Reconstruction .....                           | 1  |
| Research in the Andes .....   | 2  |
| Hypotheses .....  | 3  |
| Chapter II. BACKGROUND .....  | 6  |
| Archaeology of the Andean Region .....                                    | 6  |
| The Bioarchaeology of Juveniles .....                                     | 8  |
| Isotope Analysis .....  | 13 |
| Stable Carbon Isotopes .....  | 14 |
| Stable Nitrogen Isotopes and Presented Issues with Nitrogen Isotopes..... | 15 |
| Nitrogen Isotopes, Infants, and Weaning .....                             | 16 |
| Bioarchaeology and Stable Isotopes .....                                  | 16 |
| Chapter III. METHODS AND MATERIALS .....                                  | 17 |
| Sample Composition .....  | 17 |
| Methods .....   | 20 |
| Sample Preservation .....   | 21 |
| Analytical Methods .....  | 22 |
| Chapter IV. FINDINGS .....  | 23 |
| Calculated Sample Preservation .....                                      | 23 |
| $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Results .....             | 23 |
| Descriptive Statistics .....  | 24 |
| Statistical Analysis .....  | 25 |
| Chapter V. DISCUSSION .....   | 27 |
| Isotopic Change in Juveniles over Time .....                              | 27 |
| Juvenile and Adult Isotopic Variation.....                                | 30 |
| Dietary Composition of Juveniles and Adults.....                          | 31 |
| Preferential Treatment of Juveniles.....                                  | 32 |
| Chapter VI. CONCLUSIONS.....  | 34 |
| Limitations .....   | 34 |
| Sample Size and Time Limitations .....                                    | 35 |
| Sample Type Limitations .....   | 35 |
| Future Directions .....   | 36 |

|  |    |
|--|----|
| APPENDICES .....   | 38 |
| Appendix A: The modified Longin (1971) method used to extract collagen from bone.....  | 39 |
| Appendix B: Collagen yield, $\delta^{13}\text{C}$ values and $\delta^{15}\text{N}$ by juvenile subgroup (individuals are in order of ascending age, Table 3). .....          | 42 |
| Appendix C: $\delta^{13}\text{C}$ values and $\delta^{15}\text{N}$ values adjusted for diet by juvenile subgroup (individuals are in order of ascending age, Table 3). ..... | 43 |
| Appendix D: Cwt%, Nwt% and C/N ratios for juvenile samples (individuals are in order of ascending age, Table 3). .....   | 44 |

## LIST OF TABLES

|  |    |
|--|----|
| Table 1. Hypotheses and expectations.....  | 4  |
| Table 2. Age distribution of juveniles between the two burial contexts.....  | 17 |
| Table 3. Ages and bone samples analyzed by juvenile subgroup.....  | 19 |
| Table 4. Averages, standard deviation and ranges for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of juvenile<br>subgroups..... | 24 |
| Table 5. Averages and ranges for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of juvenile and adult<br>subgroups.....           | 24 |



## LIST OF FIGURES

|  |    |
|--|----|
| Figure 1. Map of Peru with focus on area of study, Kuelap.....   | 7  |
| Figure 2. Plan drawing of the archaeological site of Kuelap, Peru .....  | 8  |
| Figure 3. Example of juvenile remains utilized in bioarchaeological studies .....                                  | 11 |
| Figure 4. Bi-plot between juvenile burial and massacre $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values..... | 26 |
| Figure 5. Bi-plot between juvenile and adult subgroups $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values..... | 26 |
| Figure 6. $\delta^{15}\text{N}$ of juveniles by age, youngest to oldest, and subgroup.....                         | 29 |
| Figure 7. Andean food web comparing juvenile and adult subgroup isotope data adjusted for diet.....                | 32 |

## Chapter I. INTRODUCTION

### Basis of Research

The purpose of this research is to reconstruct paleodiet variation in the juveniles from Kuelap, Peru through the analysis of stable isotopes. The juvenile bone samples used in this study date from both the pre-Inca, Chachapoya, (900-1469 AD) and Inca periods (1470-1535 AD). The most useful stable isotopes in the reconstruction of paleodiet are carbon and nitrogen, as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (DeNiro & Epstein, 1978). The sample size of the juveniles for this research includes the bones of 32 individuals ranging in age from approximately 6 months to 19 years. Peru was selected as the country of interest for this research for its geographical diversity and its history of archaeological study and significance (Narváez, 1988; Ruiz Estrada, 2010). The 32 juveniles have been divided into two groups based on their chronological and burial context; a pre-Inca burial group and an Inca period massacre group.

Variation in the dietary composition between the two juvenile subgroups will be determined through the interpretation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. In addition, the research will include a comparison of the juvenile samples to adults from Kuelap to determine if there are any significant differences in dietary composition. The research aims to determine if juveniles consumed a different diet than adults. This can be used to elucidate customary practices in which juveniles were treated differently from adults. Reconstructing dietary trends and composition within these different populations can enhance the knowledge of the Chachapoya culture in Kuelap and identify how different members of society lived their daily lives.

## **Juvenile Bioarchaeological Reconstruction**

The sub-discipline of bioarchaeology aims to provide an understanding of human life and the environment in the past by analyzing bones or other archaeological materials. By completing bioarchaeological studies, both the social and biological factors of a population can be understood (Halcrow & Tayles, 2008). This research primarily focuses on the period of life before adulthood is reached, the juvenile period. Specifically, early life events and their consequences in later life, including disease incidences and nutritional patterns, can be better understood by studying juveniles (Lewis, 2007). Information about similarities and differences in how juveniles were treated throughout time or how they were treated compared with other populations enhances our understanding about past societies. Knowing more about early cultures, creates a greater appreciation for the past, enhances understanding of the treatment of different subgroups in a society, and helps to discover how stressors affect subgroups differently (Goodman & Armelagos, 1989). The research contributes to the current anthropological debate on whether juveniles are treated differently cross-culturally (Hill, 2013). Today, new research and media sources provide evidence that modern juvenile populations are nurtured and provided for differently based on the socioeconomic, religious, political or geographic influences in their community (Sofaer, 1994). The research also highlights the societal roles of subgroups and their impact on the greater community.

Stable isotopic analysis utilizes elements from bone, in this case carbon and nitrogen from extracted collagen, to reconstruct direct dietary components. In early stable isotopic analysis studies, there was not much emphasis on the reconstruction of diet in juveniles (DeNiro & Hastorf, 1984). Within the archaeological record, the value of juveniles was underestimated (Larsen, 2015). Additionally, juvenile remains were not as easily found nor analyzed, primarily

due to poor preservation or absence from archaeological sites, creating an archaeological bias (Halcrow & Tayles, 2012, Larsen, 2015). There was also a widely accepted belief that juvenile samples would not provide accurate or precise data upon which to base a bioarchaeological analysis. New bioarchaeological studies, however, offer evidence that juvenile remains provide good isotope recovery and potential in the reconstruction of past lives (Dupras & Tocheri, 2007; Tsutaya, 2017).

This thesis contributes an historical and cultural analysis of the change over time in the treatment of juveniles in the past. The data and conclusions of this research will contribute to future studies that compile dietary reconstructions from different locations and time periods to formulate an extensive cross-cultural analysis.

### **Research in the Andes**

In the last few decades, there has been a significant increase in bioarchaeological studies globally, including bioarchaeological studies conducted in the Andean region of South America (Andrushko et al., 2011; Knudson & Tung, 2010; Toyne 2015; Turner et al., 2013). This research contributes to bioarchaeological investigations, since there are so few studies directly conducted on juvenile remains, except in the case of human sacrifice (Andrushko et al., 2011; Toyne 2012; 2016; Turner et al., 2013). There have been stable isotopic studies conducted in the Andean region; however, there have not been studies that explicitly compare juveniles and their diets. The present research helps to create the framework for further bioarchaeological studies on stable isotopes from juvenile skeletal remains within this region and beyond.

The sample used for this research is comprised of 32 well-preserved and well-contextualized juvenile remains from Kuelap, Peru, located in the Andean highlands. These juveniles are from two different contexts; the burial subgroup, within the pre-Inca time period at Kuelap (900-1469 AD), and the massacre subgroup, within the time period after the Inca conquest of Chachapoya (1470-1535 AD). There are questions that arise when studying juveniles in any culture, many of which seek to understand if juveniles are treated the same way over different periods of time, or, if adults, the caretakers of the juveniles, treat their juveniles differently. This research attempts to address these aspects that have not been fully explored in past research conducted in the Andean region.

## **Hypotheses**

The research focus of this thesis aims to determine if juveniles were treated differently over time, or, treated differently than adults, to identify changes in social identities and parenting strategies. This research will test two major concerns: 1) the dietary differences between time periods of the juvenile burials; and 2) the dietary differences between the juveniles and an adult subgroup. Table 1 outlines the hypotheses and expectations for this research.

Table 1. Hypotheses and expectations

| <b>Hypothesis</b>   | <b>Expectation</b>  |
|---|---|
| There will not be a statistically significant difference between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of juvenile subgroups.    | Juveniles were eating the same dietary resources over time.<br><br>Inca conquest did not impact daily life. |
| There will be a statistically significant difference in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between the juvenile and adult subgroups. | Juveniles and adults did not eat the same dietary components due to parenting strategies.                   |

These hypotheses are tested using analytical statistics. I hypothesize that there will be no statistically significant variation in the dietary components,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , between the two juvenile subgroups. This will be tested by utilizing a non-parametric Mann-Whitney U test. If there is no statistically significant variation in the dietary composition between the time periods, it would signify that all juveniles had access to the same dietary components. If all juveniles were eating the same diet, it may indicate that juveniles were similarly provisioned for by the older members of the culture, regardless of the societal differences or subsistence practices in pre-Inca and post-Inca periods.

I also hypothesize that the juvenile  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  averages will be different than the adult averages. I expect that there will be a statistically significant difference in the stable isotope values between groups, also utilizing a non-parametric Mann-Whitney U test. I expect there to be differences in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. If there is a statistically significant variation between the subgroups, it would signify that juveniles were provided with different food resources. In order to make appropriate interpretations from the results of this research, literature will be

reviewed to understand the research already conducted in the Andes, juvenile bioarchaeology, and stable isotopic analysis.

## **Chapter II. BACKGROUND**

To situate this research, it is necessary to develop an understanding of Andean archaeology and the history of juvenile bioarchaeology. This chapter includes a review of the background literature for these topics to garner a better understanding and framework for this research.

### **Archaeology of the Andean Region**

Each region of the world provides unique insight to the past. The Andean region of northern Peru is rich in ecological diversity, ranging from alpine grasslands to thorn forests (Muscutt, 1998; Church & von Hagen, 2007). This variation in ecology influenced the customs and practices of Peru's different archaeological cultural groups.

Kuelap, an ancient major settlement, is located in the northeastern highlands of Peru and is an archaeologically significant site of interest (Figure 1). It sits at an elevation of 3000 meters. Inhabitants of Kuelap were members of the Chachapoya culture. The Chachapoya culture at Kuelap existed from 900-1535 AD, until the Inca conquered the region in 1470 AD (Narváez, 1988). The communities of Kuelap were large in scale, with long occupational time periods (Church & von Hagen, 2008). It is believed that Kuelap may have been a central location for the Chachapoya belief system (Toyne & Narváez, 2014).





setting. Most of the burials were located in the *Muralla Oeste*, the western wall. These burials occurred before the Inca conquest of Kuelap in 1470 AD.

The juvenile massacre subgroup from the *Platforma Circular* (Circular Platform) was the result of a violent event in which individuals were killed by blunt force trauma to the crania (Toyne & Narváez, 2014). These juvenile samples date to after the Inca occupation of Kuelap in 1470 AD. Figure 2 provides a drawing of Kuelap, with reference to the location of the two subgroups.

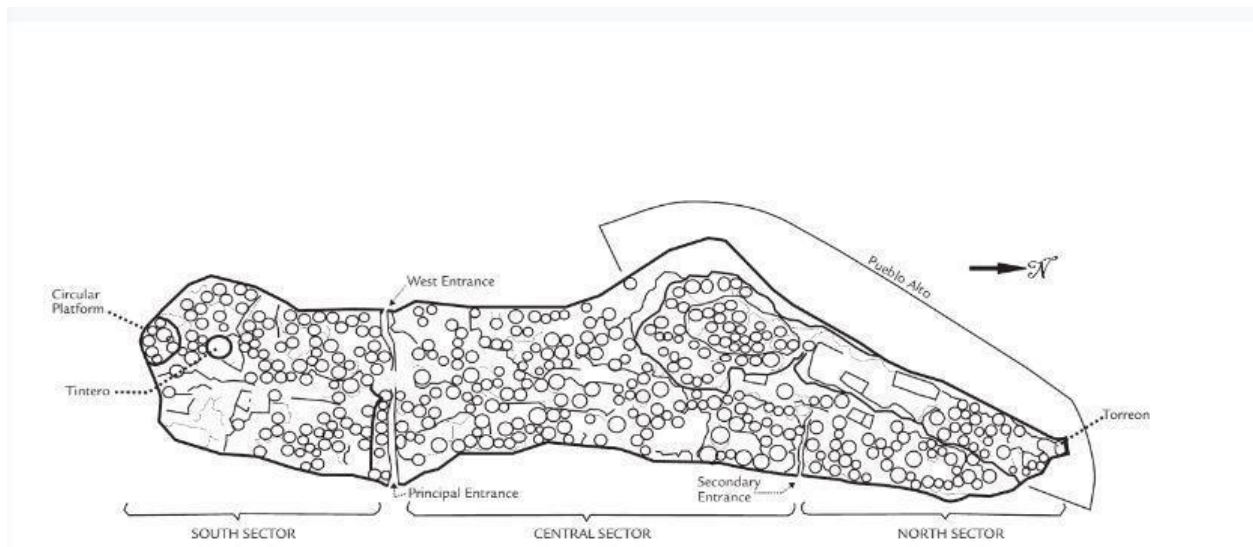


Figure 2. Plan drawing of the archaeological site of Kuelap, Peru (Courtesy of Dr. Toyne)

### The Bioarchaeology of Juveniles

The period of life that an individual is considered to be a juvenile, including the years of childhood, is unique to humans (Bird & Bird, 2000; Tsutaya, 2017). To facilitate the discussion of juvenile remains in the archaeological record, it is important to define who is considered a juvenile, biologically and socially. There are three different age types that have been defined by scholars, including, 1) age based on biological markers; 2) age based on time since birth; and 3) age based on social norms (Halcrow & Tayles, 2012). The actual juvenile age range varies by

scholar (Bogin, 1997). The juvenile age range can also vary cross-culturally, as societies may socially treat their younger populations differently and begin adulthood at different ages or stages. For the purposes of this research, juveniles are defined as individuals younger than 19 years. This definition is used to account for the various biological growth and development processes, from weaning to full maturity (Bogin, 1997).

Although a cross-cultural distinction does exist, the juvenile stage is generally identified by treatment or by certain expectations within a given culture (Kamp, 2001). There is a notion of a universal period of childhood based on a constructed social age in conjunction with biological and psychological indices (Sofaer, 1994). The timeframe in which an individual is considered to be a juvenile generally consists of a period of ‘dependency’; a concept developed in the early modern period (Sofaer, 1994). During this time, there are specific biological and osteological changes that occur in a juvenile, many of which are based on the environment; however, in all cultures, the juvenile years produce a mixture of learning and biological growth as well as social, political and economic learning (Sofaer, 1994). During this period of dependency, juveniles may be provided for and treated differently than their adult counterparts, within their cultural context.

Traditionally, across disciplines, juveniles have not been considered significant economic, social or political individuals in any given culture, or across time periods (Halcrow & Tayles, 2008). Generally, there is a lack of focus on juveniles that carries across disciplines, much of which comes from the belief that the behavior of juveniles is meaningless or simply projections of the imagination, such as building stone piles or digging holes (Lewis, 2007). Juvenile activities, however, serve as a training period, not only for physical development, but also to develop life skills as an adult and to cultivate personalities, belief systems and social identities (Sofaer, 1994). Research on health and mortality rates in juvenile populations has

focused on the disease itself, not necessarily the effect on the juvenile or their society. Many studies have simply recorded the number and cause of juvenile deaths within a particular region (Lewis, 2007). They have stopped short of assessing the societal effect of juvenile death. By studying the development of certain social identities, behaviors later in life can be understood.

Studying juveniles in the bioarchaeological record does not come without its challenges. Beyond the varying importance of juveniles within archaeological research, sample availability may be limited. There is often an under-representation and poor preservation of juveniles within mortuary contexts due to the porous nature of juvenile bones (Gordon & Buikstra, 1981; Chamberlain, 2000; Lewis, 2007). Soils high in alkalinity or acidity may degrade preservation (Gordon & Buikstra, 1981). There are situations, however, in which juvenile bones are deposited in the archaeological record that display excellent preservation. Figure 3 provides an example of juvenile remains that are preserved in excellent condition from Kuelap and that can be utilized for bioarchaeological studies.



Figure 3. Example of juvenile remains utilized in bioarchaeological studies (Courtesy of Dr. Toyne)

Bioarchaeological studies began to recognize the importance of juveniles at the same time that the importance of women in bioarchaeological studies was emphasized. The movement to incorporate female subgroups within bioarchaeological reconstructions began in the 1990s, with gender-focused and feminist studies (Baxter 2008; Halcrow & Tayles, 2011). The popularization of gender theory stimulated the discussion of juveniles in the archeological record (Lewis, 2007). Archaeological research developments, accompanied by methodological advancements, have improved juvenile recovery in the bioarchaeological record.

An initial case for incorporating children into archeological studies was made by Lillehammer (1989). Lillehammer (1989) argued that by studying the juvenile record, including burial context and osteology, connections and inferences may be made about adulthood. From the birth of an individual, everyday actions or treatments can influence how that individual lives their life up to, and through, adulthood, including, predilection, habits, and belief systems. Another case for the incorporation of juveniles into archeological studies was presented by Hirschfield (2002). Hirschfield (2002) noted that childhood should be of great interest to anthropologists since the primary purpose of anthropology is to discuss culture, and that culture is learned. To understand the ways in which a culture survived or existed in daily life, researchers should account for all demographic groups. This generates the largest scope possible to deduce the most information possible in reconstruction. It has been argued, that when a group, such as juveniles, are ignored in bioarchaeological studies, that the study can be rendered incomplete, as not all variables are considered (Ardren, 2006).

Recently, there has been ethnographic and ethnohistorical research documented in the Andean region (Abercrombie, 1998; Baitzel, 2017; Bolin, 2006; Sillar, 1994). Some of this research focuses on early parent-child interactions and child-rearing practices, but few studies exist that detail the juveniles themselves (Abercrombie, 1998; Baitzel, 2017). Past bioarchaeological studies in the Andean region have often focused on child sacrifices during Inca times, notably victim and trauma identification (Toyne, 2014). Conversely, this present research focuses primarily on juvenile reconstructions of burial and sacrificial contexts, by studying diet, a fundamental component of daily life.

## **Isotope Analysis**

An isotope is “a form of the same element that differ in the number of neutrons in the nucleus” (Fry, 2006, p.4). To be considered a stable isotope, the isotopes must not undergo radioactive decay. The stable isotopes used most often in bioarchaeological research include the elements hydrogen, carbon, nitrogen, oxygen, strontium, and sulfur (Meier-Augenstein, 2010). These are found in varying abundances and are classified into their different forms based on their mass. These classifications identify isotopes as ‘light’ or ‘heavy’. Light isotopes have fewer neutrons in the nucleus; whereas, heavy isotopes have more neutrons in their nucleus (Fry, 2006). The difference between light and heavy isotopes does not correlate with their chemical composition; but, is determined by chemical reactions, notably fractionation and mixing. Meier-Augenstein (2011) notes that heavy stable isotopes typically undergo chemical reactions at a slower rate than light stable isotopes.

Accounting for the processes of fractionation and mixing are vital in the reconstruction of stable isotopes in diet. Fractionation occurs when isotopes differentiate from one another; mixing occurs by creating a homogeneous substance (Fry, 2006). Fractionation is relatively more complex than mixing and can be considered the “power controlling isotope distributions on this planet” (Fry, 2006, p.12). There are several types of fractionation, such as equilibrium isotope fractionation and the fractionation of isotopes that occur within physiochemical or biochemical processes (Meier-Augenstein, 2010).

Stable isotope analysis has been selected as the methodology for this research as it is accepted as a valid method to reconstruct the paleodiet of individuals across cultures and time periods. It has been utilized in anthropological research to study migration, disease, and dietary patterns (Katzenberg, 2012). The stable isotopes most frequently analyzed in the reconstruction

of paleodiet are carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) (Schoeninger & Moore, 1992; Katzenberg, 2008). Carbon and nitrogen ratios can determine the food resources that comprised the diet of different individuals (DeNiro & Epstein, 1978). In dietary reconstruction, carbon isotopes reflect an individual's consumption of plants and nitrogen isotopes reflect an individual's consumption of protein (Ambrose & Norr, 1993).

### **Stable Carbon Isotopes**

The reconstruction of plant dietary components in human diets can be determined with carbon stable isotopes.  $\delta^{13}\text{C}$  values can help identify the photosynthetic pathway of the plant consumed, including  $\text{C}_3$ ,  $\text{C}_4$ , and CAM photosynthetic pathways. There will be different  $\delta^{13}\text{C}$  values based on the photosynthetic pathway utilized by a plant. von Cremmerer (1992) states the range of  $\delta^{13}\text{C}$  ratio for  $\text{C}_3$  plants is from  $-21\text{‰}$  to  $-34\text{‰}$ ; the range of  $\delta^{13}\text{C}$  for  $\text{C}_4$  plants is from  $-8\text{‰}$  to  $-15\text{‰}$ ; CAM plants fall within a range most similar to  $\text{C}_4$  plants, but can overlap with the range of  $\text{C}_3$  plants, depending on the aridity of the climate (von Cremmerer, 1992). There are many examples of  $\text{C}_3$  plants, including rice, wheat, peanuts, beets, potatoes and spinach. Common  $\text{C}_4$  plants include sugarcane and millets. In the Andes, examples of  $\text{C}_3$  plants consumed are legumes; an example of highly consumed  $\text{C}_4$  is maize. The negative values correlate to the smaller ratio of  $^{13}\text{C}$  compared to a carbon standard (DeNiro & Hastorf, 1984; von Cremmerer, 1992). The standard that  $\delta^{13}\text{C}$  values are compared to is Vienna Pee Dee Belemnite (VPDB). The range of  $^{13}\text{C}/^{12}\text{C}$  is determined dependent on atmospheric  $\text{CO}_2$ , which reflects the conditions in which the plants grew. An enrichment factor of  $5\text{‰}$  for  $\delta^{13}\text{C}$  was applied to the raw data to reflect the original dietary source (DeNiro & Epstein, 1978).



## Stable Nitrogen Isotopes and Presented Issues with Nitrogen Isotopes

Nitrogen isotopes help to determine the type of protein an individual consumed; whether the protein is from a marine or terrestrial source (Ambrose, 1991). Like carbon, the  $\delta^{15}\text{N}$  value is presented as a ratio;  $^{15}\text{N}/^{14}\text{N}$  compared to AIR, as a standard. Nitrogen is incorporated into tissue, representing the protein resources consumed. Specifically, nitrogen isotopes help to determine the trophic level of the individual. The trophic levels correlate with the protein resource's placement in the hierarchy on a food chain, or food web. The higher the  $\delta^{15}\text{N}$  value, the higher the trophic level of the protein source consumed; indicating that it was higher up on the food chain (Hedges & Reynard, 2006). Different stressors in an individual can cause an alteration in nitrogen values. An individual in a diseased state or undergoing metabolic stress may have elevated  $\delta^{15}\text{N}$  levels (Reynard, 2006). Nitrogen can also be reflected in plants, based on their growing conditions, particularly soils. An enrichment factor of 3‰ for  $\delta^{15}\text{N}$  was applied to the raw data extracted to reflect the original dietary source (DeNiro & Epstein, 1978).

There are certain states of well-being that may also alter stable isotopic readings. Stressors, commonly disease stressors either throughout life or that cause mortality, can alter the chemical structure and readings of the bone (Fahy et al., 2017). These diseased states can cause an increase in  $\delta^{15}\text{N}$  values, creating a bias that may skew the data points when individuals may not actually be consuming foods from a higher trophic level. When in a disease state or under periods of extreme stress, bones may turn over more frequently (Olsen et al., 2013).

## **Nitrogen Isotopes, Infants, and Weaning**

In the early life of individuals, there is a period of life where an individual may directly rely on their mother for nutrient intake. Weaning can be defined as the period of time when a juvenile is slowing their consumption of breast milk and transitioning to solid foods more commonly found in an adult's diet (Williams et. al, 2005). Since these young children have often not yet fully weaned off breast milk, their  $\delta^{15}\text{N}$  values may be enriched compared to those individuals outside of the weaning period. Research has found that for weaning infants, the  $\delta^{15}\text{N}$  values are enriched by 2.4‰ (DeNiro & Epstein, 1978). For the purposes of this research, individuals were considered to be weaning if they were 1-3 years of age +/- 1 year.

## **Bioarchaeology and Stable Isotopes**

When performing stable isotopic analysis to reconstruct past diets, the type of tissues most frequently utilized are bones and teeth, since skeletons preserve more frequently due to the higher mineral content than other soft tissues (Ambrose, 1990). For the purposes of this research, bone samples were studied; specifically, the collagen of bone, which is part of the organic portion of bone. Bone is comprised of 70% inorganic components (hydroxyapatite) and 30% organic components (collagen) (Katzenberg, 2008). Collagen, a protein found in the bone, is typically utilized as a source for reconstruction of human tissue as it can survive for thousands of years (Tuross et al., 1980).

## Chapter III. METHODS AND MATERIALS

This chapter describes the sample and laboratory methodology utilized to complete this research. Since this research focuses on juveniles' dietary composition over time, this chapter outlines the different compositions and time periods of both the burial and massacre groups. This chapter also provides the methodology for the extraction of collagen and isotope analysis.

### Sample Composition

The sample size for this research is 32 bone samples from two distinct contexts at Kuelap, Peru. All samples were from juveniles with estimated ages ranging from 6 months to 19 years. These ages were estimated through biological and growth processes (Ubelaker & Buikstra, 1994). The earlier burial group includes 19 juveniles from a pre-Inca, Chachapoya, (900-1469 AD) occupation, most of which are from the *Muralla Oeste*, the western wall burials. The ages of these juveniles range from 6 months to 19 years old.

The massacre group includes 13 juveniles dating to the post-Inca period (1470-1535 AD), ranging from 9 months old to 17 years of age, many of which are from the *Platforma Circular*.

Table 2 summarizes the number of individuals in each age category found in both contexts.

Table 2. Age distribution of juveniles between the two burial contexts

| Age Categories<br>(in years) | Total      | Number of Individuals |                |
|------------------------------|------------|-----------------------|----------------|
|                              |            | Burial Group          | Massacre Group |
| Infant (0-2)                 | 10 (31.2%) | 7                     | 3              |
| Early Childhood (3-6)        | 7 (21.9%)  | 6                     | 1              |
| Late Childhood (7-11)        | 10 (31.3%) | 3                     | 7              |
| Adolescence (12-19)          | 5 (15.6%)  | 3                     | 2              |
| <b>Total Number</b>          | <b>32</b>  | <b>19</b>             | <b>13</b>      |

Juvenile rib bones were selected for analysis due to their assumed equal rates in bone turnover and bone remodeling; although, it should be noted that metabolic processes cause a higher bone turnover rate in individuals younger than one year of age (Tsutaya & Yoneda, 2013). Due to their location in the body, the ribs experience a faster turnover rate compared to some other bones in the body (Parfitt, 2002). The amount of pressure exerted on the ribs by the lungs and other internal organs cause the faster turnover rate in the rib bones. The faster turnover rate of the rib bones helps to identify the diet of the individual closer to their time of death. This is important in this dietary reconstruction as it can elucidate any dietary trends that may have occurred in the juvenile subgroups closer to death and can help aid in comparison with the adult sample, which were also rib samples. It should be noted that four of the juvenile samples utilized bones other than rib bones, including cranium, fibula, a long bone fragment and radius. These samples were included to augment sample size, contributing to a more viable and complete study. Table 3 provides a list of the individual samples, including the estimated age at death and skeletal element.

Table 3. Ages and bone samples analyzed by juvenile subgroup

|                    | <b>Sample Number</b>         | <b>Age</b>          | <b>Sample Fragment</b> |
|--------------------|------------------------------|---------------------|------------------------|
| Burial group       | KPANTorVa Ent3A              | 6 months            | Rib                    |
|                    | KSPlatI IIIÑ Ent3A           | 6 months            | Rib                    |
|                    | K-PAS MO -VIII T' ENT10      | 9 months            | Rib                    |
|                    | K-PAS MO -VIII T' ENT39      | 9 +/-3 months       | Rib                    |
|                    | K-PAS MO -VIII U' ENT69      | 2 years +/-8 months | Rib                    |
|                    | K-PAS MO -VIII U' ENT57      | 3 +/-1 years        | Rib                    |
|                    | K-PAS MO -VIII U' ENT52a     | 3-5 years           | Rib                    |
|                    | KSPlatI IIIÑ Ent1B           | 4-6 years           | Rib                    |
|                    | KC ME VK RH17                | 4-6 years           | Rib                    |
|                    | K-PAS MO -VIII T' ENT33      | 6-8 years           | Rib                    |
|                    | K-PAS MO -VIII T' ENT37      | 6-8 years           | Rib                    |
|                    | K-PAS MO -VIII T' ENT12      | 8-10 years          | Cranium                |
|                    | KSTIN-VU Ent3                | 8-10 years          | Rib                    |
|                    | K-PAC E1 -V Q' T2 ENT1       | 8-10 years          | Fibula                 |
|                    | K-PAS MO -VIII U' ENT46      | 9 +/-3 years        | Rib                    |
|                    | KSPlatI IIIÑ Ent4            | 9-11 years          | Rib                    |
|                    | KSPlatC E2-VIa' Ent22        | 10-12 years         | Rib                    |
|                    | KSTIN-IIIT Ent6              | 12-15 years         | Rib                    |
| KSTIN-IVV Ent11    | 15-19 years                  | Rib                 |                        |
| Massacre group     | KSPlatC E6-VIIz ENT105       | 3-9 months          | Rib                    |
|                    | KSPlatC E2-VIIa' Ent26       | 2-4 years           | Rib                    |
|                    | KSPlatC E6-VIIz,-VIIa' ENT75 | 2-4 years           | Rib                    |
|                    | K-PAS MO -VIII T' ENT22      | 4-6 years           | Rib                    |
|                    | KSPlatC E2-VIIa' Ent18c      | 5-7 years           | Rib                    |
|                    | KSPlatC E4-VIa' ENT61        | 6-9 years           | Rib                    |
|                    | KSPlatC-VIIz Ent2            | 7-9 years           | Long bone fragment     |
|                    | KSPlatC E2-VIIa' Ent16a      | 7-9 years           | Radius                 |
|                    | KSPlatC E3-VIa' Ent31        | 8-10 years          | Rib                    |
|                    | KSPlatC E3-VIIa' Ent37a      | 10-12 years         | Rib                    |
|                    | KSPlatC E4-VIa' ENT58        | 11-13 years         | Rib                    |
|                    | KSPlatC-VIA' ENT50           | 15-17 years         | Rib                    |
| KSPlatC-VIIz ENT91 | 15-17 years                  | Rib                 |                        |

The adult data utilized for comparison are from previous research conducted by Dr. Toyne in Kuelap. This subgroup is comprised of 27 individuals of both sexes, ranging from young adults to old adults. This research assumes that these adult individuals represent a uniform sample for comparison. They were assumed to have consumed similar dietary components and

that none of the adults were undergoing a diseased state or were under metabolic stress. The data regarding the adult sample can be found in Appendix C.

## **Methods**

There have been several methods developed to extract collagen for paleodiet reconstruction (Ambrose, 1990; Longin, 1971; Pestle, 2010). For the purposes of this research, the method utilized for dietary reconstruction is a modified Longin (1971) method. This method was selected as it thoroughly removes any humic acids or lipid substances that could skew the data.

The process of collagen extraction began by cleaning each sample manually and then with an ultrasonicator with distilled water to remove any dirt on the sample. Approximately ~1.0 gram samples were broken into uniform fragments for extraction. The lipids, and other organic materials, were removed utilizing a 2:1 chloroform methanol mixture. The samples were then slowly demineralized using 0.25 M hydrochloric acid (HCl), over a period of 2-3 weeks. Humic acids were then removed from the sample with 0.1 sodium hydroxide (NaOH) solution. Once humic acids were removed and samples reached a neutral pH of 7.0 by centrifuging multiple times with distilled water, the collagen was gelatinized by using 0.25 M HCl and placed into an oven to dry. The sample was heated in the oven at 90 degrees Celsius, and the leftover collagen substrate was pipetted out and dried. The resulting collagen was weighed to calculate the collagen yield. Appendix A outlines the step-by-step process used to extract the collagen.

After extraction, the collagen was sent to the Light Stable Isotope Mass Spec Lab at the University of Florida in Gainesville Florida, to undergo the isotope-ratio mass spectrometry (IRMS) analysis. IRMS provides accurate and precise readings of stable isotope values (Meier-

Augenstein, 2011). Condensing Meir-Augenstien's work, the process begins with the combustion of the organic sample into a gas, such as CO<sub>2</sub>, that is equivalent in isotopic abundance to the original organic material. The gas flows into the IRMS and CO<sub>2</sub> and N<sub>2</sub> ions are created. A fixed voltage and magnetic fields dictate the movement of these ions into Faraday cup (FC) detectors. The type of detector depends on the atomic weight of isotope that is measured. The FC counts the ions that are drawn to the detectors and respond to an isotopologue. The ions can be differentiated and the abundance of the isotope can be determined. IRMS may vary in their consistency; therefore, known standards must be applied to ensure the validity. Any variation of variables in the process of stable isotopic analysis can affect the validity of a study. To maintain accurate, precise and comparable results, certain standards within methods or guidelines for variables should be set to provide justifiable and valid data and interpretations.

### **Sample Preservation**

The collagen yield for each sample was calculated to help determine if the sample was well-preserved. If not well-preserved, the samples may have undergone diagenetic processes that could have skewed the results. A sample with a collagen yield > 1% is considered to be well-preserved (Ambrose, 1990). The equation to calculate the collagen yield for samples is as follows:

$$\% \text{ Collagen Yield} = \frac{(\text{weight of vial with collagen} - \text{weight of vial without collagen})}{(\text{dry sample weight})} \times 100$$

Another indicator of good preservation of collagen is in the atomic C/N ratio; which is the ratio of the weight of collagen to the weight of nitrogen found in the collagen. The range indicating good collagen preservation is from 2.9-3.6 (Ambrose, 1990). A third indicator of well-

preserved materials is %C and %N. Well-preserved carbon value will be ~35%; well-preserved nitrogen value will be ~15%. The values of % weight carbon, % weight nitrogen and C/N ratio can be found in Appendix B.

### **Analytical Methods**

Statistical methods were used to analyze the data for each subgroup, including descriptive statistics of the average, range, and standard deviation for both carbon and nitrogen isotopic ratios. The standard deviation calculated the amount of variation within the data set for each juvenile subgroup. A non-parametric Mann-Whitney U test was performed to compare the means of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values within the sample set and determine if there is a statistically significant difference. The Mann-Whitney U test was chosen to be the most appropriate test for this sample set as the sample set was small and the samples were independent. Madrigal (2012) provides a formula for the calculation for the Mann-Whitney U-test:

$$U_1 = (n_1)(n_2) + - \sum R_1, \text{ and } U_2 = (n_1)(n_2) - U_1$$

Statistically significant different means would imply that the subgroups were consuming different dietary components on average in their lifetimes. This could determine if juveniles were eating differently based on their time period or differently from their adult counterparts. With accumulation of the raw data of the juvenile subgroups and adult subgroups, further analysis using the above statistics was completed. By utilizing these statistical methods, the difference between juvenile subgroups and between the juvenile and adult subgroups can be conducted. If there is a significant difference between subgroups, it would indicate a difference in dietary components.



## Chapter IV. FINDINGS

This chapter outlines the data produced and the results of the statistical analysis of the carbon and nitrogen values to determine the difference, or lack thereof, in dietary composition of juvenile subgroups and between adult and juvenile subgroups.

### Calculated Sample Preservation

If the sample has a collagen yield  $> 1\%$ , the sample is considered to be well-preserved (Ambrose, 1990). All of the 32 samples had a collagen yield above the 1% threshold (Table 4). These samples averaged 14.3% collagen yields; the lowest of these values was 6.3% yield; the highest was 23.8% yield; therefore, they were all considered to be well-preserved showing a high collagen content.

Another indicator of good preservation of collagen is in the atomic C/N ratio; which is the ratio of the weight of collagen to the weight of nitrogen found in the collagen. The range indicating good collagen preservation is from 2.9-3.6 (Ambrose, 1990). All of the values for the juvenile subsamples fell within this range (Appendix B).

### $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Results

The sample collagen yields and raw  $\delta^{13}\text{C}$  values and  $\delta^{15}\text{N}$  values for each sample separated by burial group are listed in Appendix B. The  $\delta^{13}\text{C}$  values and  $\delta^{15}\text{N}$  values adjusted for diet are listed in Appendix C. These adjustments were made, as fractionation processes that occur in the organic material of bone, collagen, do not directly represent dietary composition. The adjustment percentages for both the  $\delta^{13}\text{C}$  values and  $\delta^{15}\text{N}$  can be found in Chapter 2.

## Descriptive Statistics

Descriptive statistics were used to analyze the mean, range, and standard deviation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between the two juvenile subgroups, and between the juvenile subsamples and adult subsample. The summary of the descriptive statistics of both juvenile burial groups can be found in Table 4. The summary of the descriptive statistics for the juvenile subgroups and adult subgroup can be found in Table 5.

Table 4. Averages, standard deviation and ranges for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of juvenile subgroups

| Sample Type     | $\delta^{13}\text{C}$ Average | $\delta^{13}\text{C}$ Standard Deviation | $\delta^{13}\text{C}$ Range | $\delta^{15}\text{N}$ Average | $\delta^{15}\text{N}$ Range | $\delta^{15}\text{N}$ Standard Deviation |
|-----------------|-------------------------------|--|-----------------------------|-------------------------------|-----------------------------|--|
| Burial (n=19)   | -13.1‰                        | 1.6                                      | -16.1‰ to -10.2‰            | +8.1‰                         | +6.1‰ to +12.4‰             | 1.5                                      |
| Massacre (n=13) | -13.4‰                        | 0.9                                      | -15.0‰ to -12.1‰            | +7.8‰                         | +6.2‰ to +11.3‰             | 1.5                                      |

Table 5. Averages and ranges for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of juvenile and adult subgroups

| Sample Type     | $\delta^{13}\text{C}$ Average | $\delta^{13}\text{C}$ Range | $\delta^{15}\text{N}$ Average | $\delta^{15}\text{N}$ Range |
|-----------------|-------------------------------|-----------------------------|-------------------------------|-----------------------------|
| Juvenile (n=32) | -13.2‰                        | -16.1‰ to -10.2‰            | +8.0‰                         | +6.1‰ to +12.4‰             |
| Adult (n=27)    | -13.4‰                        | -15.5‰ to -11.6‰            | +7.4‰                         | +6.3‰ to +8.2‰              |

## Statistical Analysis

Figure 4 provides a bi-plot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the juvenile burial groups. Most of the juveniles'  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values overlap. The results of the Mann-Whitney U test between the two juvenile burial groups, the burial and the massacre groups, for  $\delta^{13}\text{C}$  produced a two-tailed significance of 0.57548, which is above the parameter for statistical significance at  $p \leq 0.05$ . The result of the non-parametric Mann-Whitney U test for  $\delta^{15}\text{N}$  between the juvenile burial groups is 0.11184, which is also above the parameter for statistical significance at  $p \leq 0.05$ . From this statistical analysis, it can be inferred that both juvenile subgroups were eating similar plant and protein resources.

Figure 5 provides a bi-plot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the juvenile and adult subgroups. The result of the non-parametric Mann Whitney U test for  $\delta^{13}\text{C}$  between the juvenile and adult subgroups is 0.36812, which is greater than the parameter for statistical significance at  $p \leq 0.05$ . The result of the non-parametric Mann-Whitney U test for  $\delta^{15}\text{N}$  between the juvenile and adult subgroups is 0.01178, which is within the parameter for statistical significance at  $p \leq 0.05$ . Therefore, there is a significant difference between the juvenile and the adult subgroups for  $\delta^{15}\text{N}$  values, but not  $\delta^{13}\text{C}$  values. From this statistical analysis, it can be inferred that the juvenile and adult subsamples were eating similar plant resources, but, different protein resources.

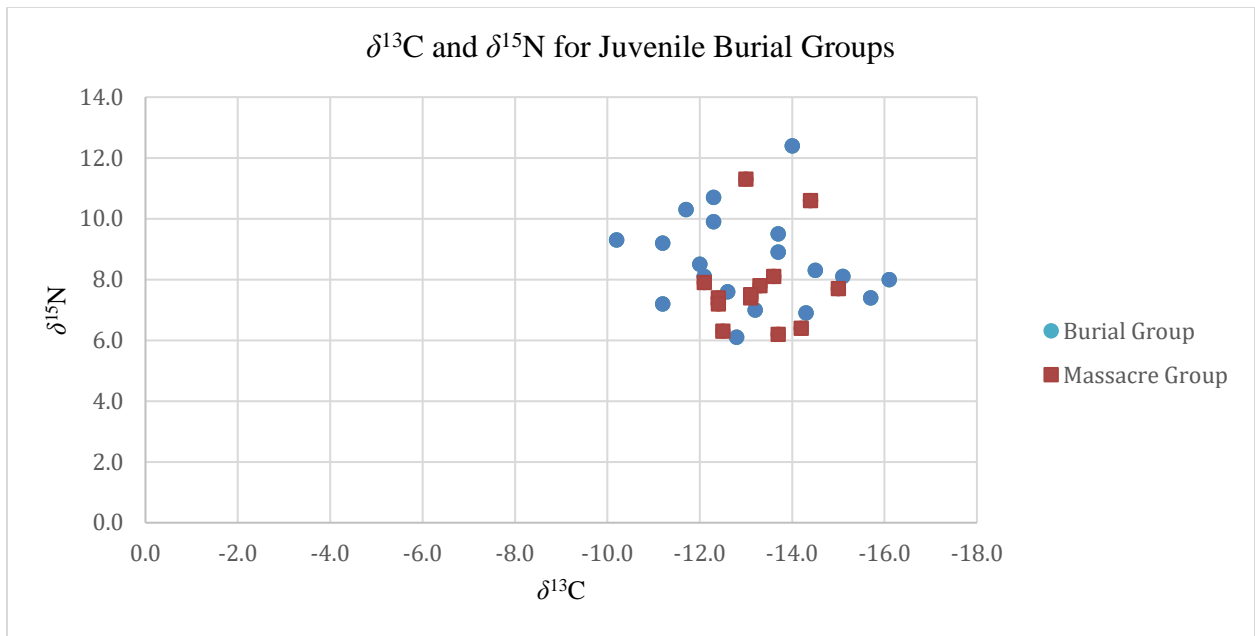


Figure 4. Bi-plot between juvenile burial and massacre δ<sup>13</sup>C and δ<sup>15</sup>N values.

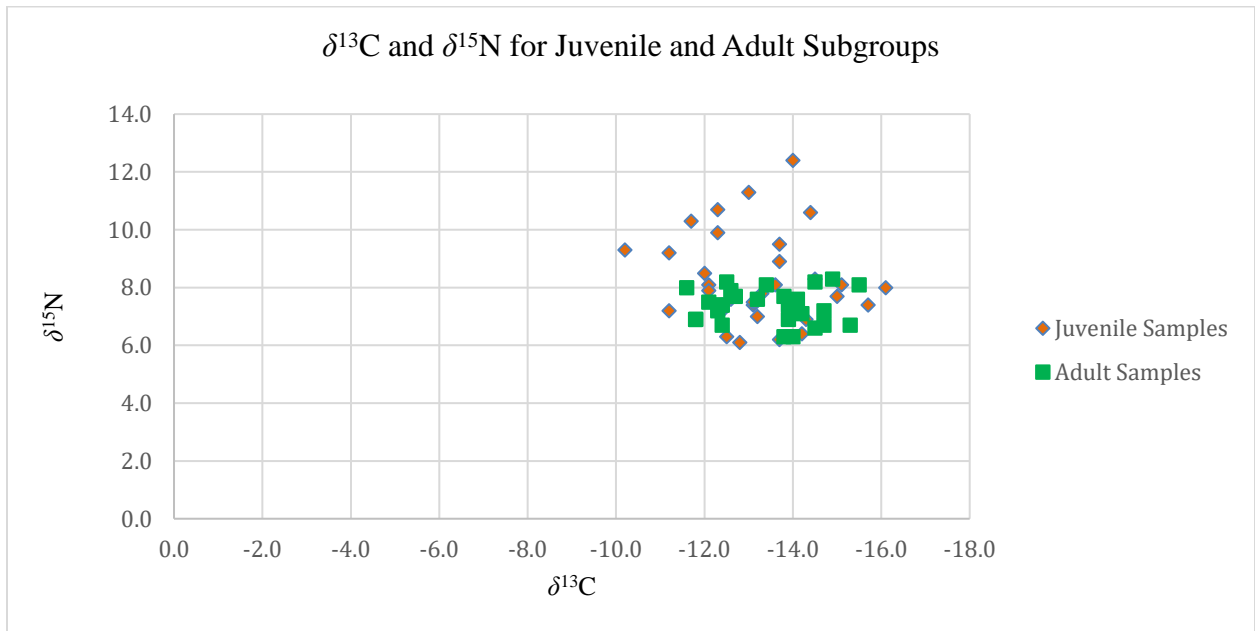


Figure 5. Bi-plot between juvenile and adult subgroups δ<sup>13</sup>C and δ<sup>15</sup>N values.

## Chapter V. DISCUSSION

There are several anthropological questions regarding paleodiet that can be answered based on the results of the stable isotope analysis. From the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the juveniles' diet, the results provide the context for better understanding of the daily lives of juveniles in the Kuelap community. This research tests the hypothesis that the juveniles from the burial and massacre groups would reflect the same dietary components; suggesting that the food resources in the community throughout time had not changed. The second hypothesis tested for differences in diet between the juveniles and the adult samples of the population. Although diet is not the only marker for different lifestyles, it is a necessity for all individuals and can reveal potential trends in other aspects of differential daily life. This chapter focuses on a discussion and interpretation of the results in relation to the stated hypotheses.

### Isotopic Change in Juveniles over Time

The first hypothesis stated that there would not be a significant difference in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  averages between the juvenile burial and massacre groups.

The non-parametric Mann-Whitney U test was used to determine whether there existed a statistically significant difference between the juvenile subgroups and their dietary preferences as determined by the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. There is not a statistically significant difference in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between the two burial groups. Thus, there was not a change in dietary resources over time, either in the consumption of plant materials or in the consumption of proteins by juveniles. The non-significant differences in the  $\delta^{13}\text{C}$  results between time periods in the juvenile subgroups could be attributed to a variety of factors, including, possible ecological

factors that may limit the growth of different food within Kuelap. In both time periods, there may be factors that do not allow for the farming of certain plant resources. Due to climate conditions and the availability of food resources, plant materials, such as maize or legumes, remained a staple of the diet throughout the region. The consistency in the plant materials that were consumed over time may also be explained by their ritual significance, their role in the subsistence strategy, and growing conditions.

The range of  $\delta^{15}\text{N}$  is 6‰ in the diets between the juvenile burial and massacre subgroups. This 6‰ difference suggests that there were protein dietary components consumed from two trophic levels. Figure 6 provides a graph of the  $\delta^{15}\text{N}$  values for each juvenile sample. The range in these values may indicate that different protein resources were consumed based on dietary preferences. Some individuals may have consumed a diet comprised of foods with a lower trophic level (i.e., vegetarians); other individuals may have consumed foods with a higher trophic level, potentially due to the status in the society of their guardians or based on ritual significance.

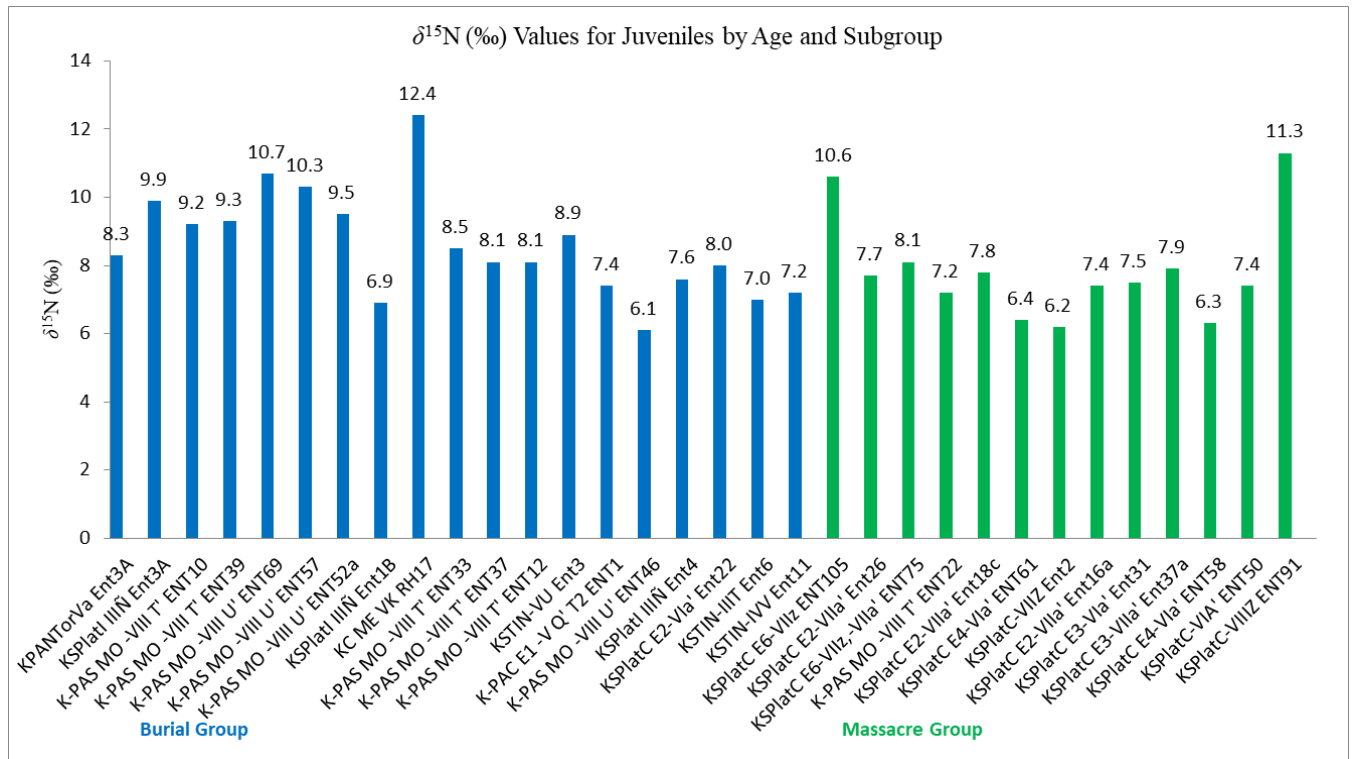


Figure 6. δ<sup>15</sup>N of juveniles by age, youngest to oldest, and subgroup

There was not a statistical significance in the protein resources consumed between juvenile subgroups. This suggests that even when the Inca administration took over in Kuelap in 1470 AD, the same protein sources remained common in the diet. This could be due to the availability of the protein resources in the region. If there was a primary source of protein that could easily be caught, cooked and consumed in Kuelap, regardless of the administration in charge, those same resources would be prevalent in the environment and best suited for as protein resources for consumption.

## Juvenile and Adult Isotopic Variation

The second hypothesis stated that there would be a statistically significant difference in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope values between the juvenile and the adult subgroups.

The Mann-Whitney U test determined that there is not a statistically significant difference between subgroups in  $\delta^{13}\text{C}$  values. This indicates that the diet of juveniles was patterned after those of their adult counterparts, or the resources that were available across the site. Similar to the comparison between the juvenile subgroups, the non-significant  $\delta^{13}\text{C}$  values may be due to the types of plant materials that could be grown in Kuelap, identification of certain plant materials in the culture based on ritualistic or systematic belief systems, or established subsistence strategies that would yield the best plant materials for ingestion.

Based on  $\delta^{15}\text{N}$  values, there is a statistically significant difference between the juvenile subgroups and the adult subgroups. The difference in these values may be explained by a variety of reasons. The  $\delta^{15}\text{N}$  values in the juvenile subgroups are almost always higher than the adult subgroup. This would indicate that juveniles were consuming proteins from a higher trophic level than the adults. Juveniles could have been fed differently than adults if they were considered to be special members of their population. Since some of the juvenile samples came from those in the weaning period of life (<3 years of age), it could be argued that weaning may have elevated the  $\delta^{15}\text{N}$  levels of those individuals. This could account for 8 out of the total 32 juvenile samples for which the weaning trophic elevation could be applied. The remaining juvenile samples are still elevated as compared to the adult samples, even though they exist outside of the weaning period. Although it could be assumed that the higher  $\delta^{15}\text{N}$  values in juveniles were due to eating proteins from a higher trophic level or weaning, these higher values may represent a stressor within the skeletons of the juvenile subgroups. Elevated  $\delta^{15}\text{N}$  values in



an individual may be indicative of a disease or condition that puts an individual under metabolic pressure, elevating their  $\delta^{15}\text{N}$  values. This explanation may be applied to these juveniles as individuals that do not reach adulthood are considered to be the non-survivors of the group; the juveniles may have died due to disease, marked isotopically in their bones.

### **Dietary Composition of Juveniles and Adults**

The primary goal of this research was to compare two juvenile subgroups and an adult subgroup to determine if there was any difference in the isotope values identified and thus, subsistence practices over time, or based on age. These data also shed light on what each individual at Kuelap may have been eating. Based on the adjusted for diet  $\delta^{13}\text{C}$  values for both juveniles and adults, there is an indication of a mixed diet of  $\text{C}_3$  and  $\text{C}_4$  plant resources. The presence of plants in the diet utilizing both photosynthetic pathways could be explained by the importance of  $\text{C}_3$  resources, and maize, a  $\text{C}_4$  plant resource, in the Andean diet (Narváez, 2013). Since there was a statistically significant difference in the  $\delta^{15}\text{N}$  values in the juvenile and adult subgroups, it can be assumed that the protein consumption for juveniles was different than the adults.

Figure 7 provides a graph of the values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  plotted to a previously constructed Andean food web. It should be noted that although most of the values overlap in their different dietary consumptions, there are some individuals, notably in the juvenile subgroups that do not overlap.

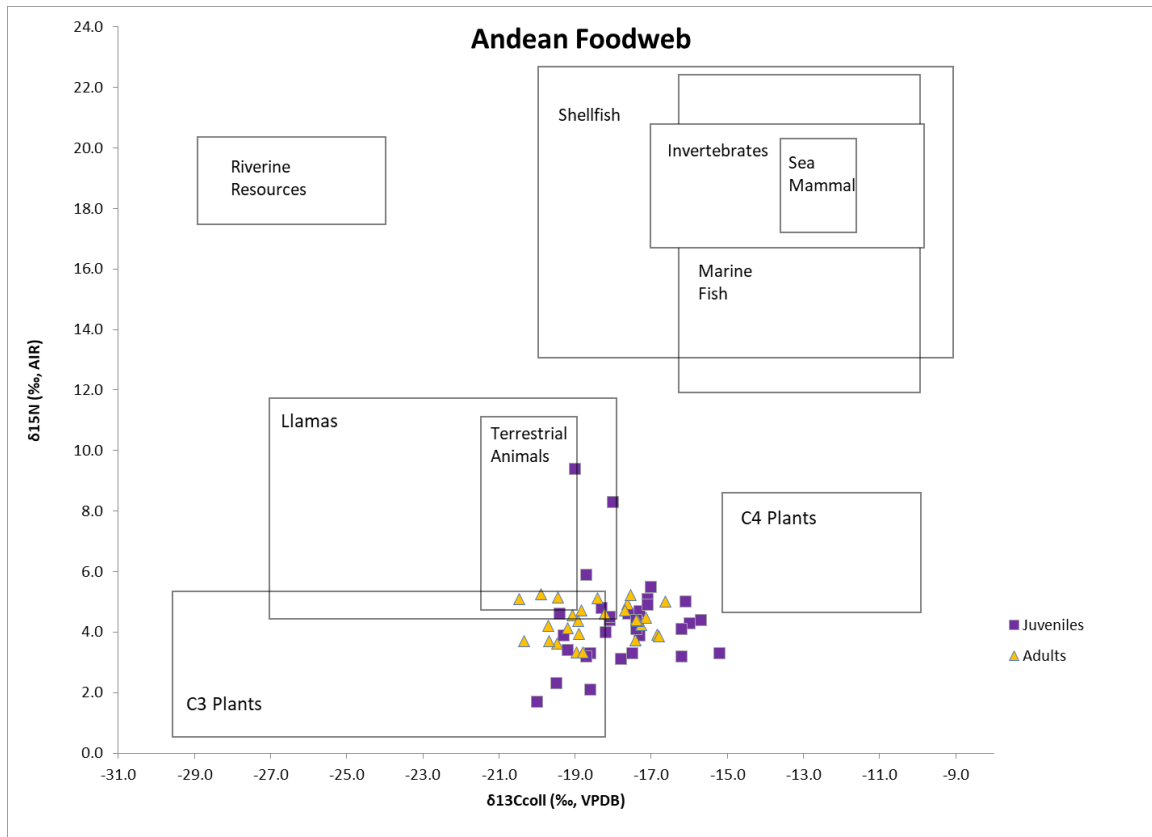


Figure 7. Andean food web (Courtesy of Dr. Toyne) comparing juvenile and adult subgroup isotope data adjusted for diet.

### Preferential Treatment of Juveniles

Based on the data analyzed and discussed, it can be deduced that juveniles were treated differently from their adult counterparts at Kuelap, in regards to protein consumption. There was no statistically significant difference in  $\delta^{13}\text{C}$ , as determined by the non-parametric Mann-Whitney U test; however, there was a statistically significant difference found in the  $\delta^{15}\text{N}$  values. The plant foods consumed by adults may have been fed to juveniles in order to help them survive. There appears to be the same plant dietary components consumed by all the juveniles in Kuelap, whether due to environmental stress, belief system or subsistence patterns. Regardless of the reason, the juveniles were given some form of special dietary treatment. Juveniles are

considered to be dependent on the older members of the community; therefore, they may provide for juveniles differently, in order to extend their life expectancy (Lewis, 2007).

## Chapter VI. CONCLUSIONS

The aim of this research is to provide an insight into the diet of juveniles and feeding practices, in ancient Kuelap, Peru using stable isotopic analysis in order to conclude if juveniles were treated differently than adults. There are several bioarchaeological applications for the outcomes of this research.

Notably, in our modern North American culture, we do notice a differential treatment for our juveniles; we emphasize special treatment for juveniles (Hill, 2013). Even postnatally, children are treated differently than their adult counterparts. They are given special supplements and foods with specific fortifications to aid the growth and development process. These same supplements and fortifications may not present in the more mature members of a society. Based on the differential societal roles, there is a practice of providing for children, patterned after their parent's preferences. There are instances, however, in which juveniles are treated differently than their adult counterparts. More than likely, preferential treatment may be indicative of parents or guardians concerned for the health and fitness of younger individuals in a culture.

### **Limitations**

The research process and theory of stable isotopic analysis were beneficial in testing the hypotheses. There is, however, as with all scientific investigations, room for improvement. Paleodietary reconstruction is an important research methodology that analyzes stable isotopic values extracted from the organic material in bone. Although stable isotopes are considered to be the prime methodology to reconstruct diet, stable isotopic analysis provides a direct evidence of an individual's diet, but, bulk sampling reflects patterns in overall long term dietary composition.

### **Sample Size and Time Limitations**

Sample size is often a limitation in research studies. The 32 juveniles are a good sample size for a base study; however, with a longer time period to conduct research, more data could have been extracted for analysis. The semesters during which the research was conducted provided enough time for the process of stable isotope analysis of the samples provided. A larger sample size would possibly be able to account for more variation or to elucidate other dietary trends in the juvenile populations. Possible dietary trends based on age could be determined if there were more individual samples within each age group. A larger number of individuals included in the adult subsamples may have also been helpful in the same respect. If the study is revisited in the future, it may also be beneficial to include adult samples from the same time period as each juvenile subgroup to further account for differences in dietary patterns.

### **Sample Type Limitations**

Although the ribs are useful to reconstruct the diet of juveniles, it would be interesting to conduct studies on different skeletal elements. By conducting stable isotopic analysis on other skeletal elements, different portions of the juvenile's life could be considered, not just the period of time closer to death. The analysis of teeth would be useful to incorporate, as they reflect the diet of individuals when the teeth were first formed (Ambrose, 1990). Utilizing different portions of the tooth as well, such as the tooth or crown, can produce insight about the ages at which individuals consumed different dietary components throughout their lifetime (DeNiro & Epstein, 1978). Any differences between dietary composition while teeth were forming and after may further determine if juveniles had been provided for differently from other subgroups, based on their time within the juvenile period.

## **Future Directions**

The research focused on juveniles from Kuelap, Peru, and not any other cultures or archaeological sites. Cultures throughout different time periods have been examined for their juveniles' dietary composition and treatment. A cross-cultural comparison of regions during the same time period, in locations relative to Kuelap, to determine dietary differences would be beneficial to determine a pattern or trend of differential treatment for juveniles.

Although the research conducted focused on one cultural group and primarily aimed to determine whether juveniles were treated differently than adults in respect to dietary compositions, the findings can be applied to the broader anthropological field. Familial and cultural patterns may be identified based on the differential treatment and food resources provided to juveniles. These results can help reconstruct everyday life in a culture, the structure of a culture, and the subsistence strategies of a culture.

Researchers may be able to provide insight into possible nutritional deficiencies or disease patterns expressed in ill individuals at the time of death. This could be done by comparing the isotopic readings of the juveniles to any known evidence of skeletal or dental pathologies found within these skeletal remains. This may also be done by utilizing any pathological data found on the skeletal remains of the juveniles to help narrow down the possibility of a juvenile having a chronic illness that may have elevated these nitrogen levels.

By reconstructing the diet of juveniles, a more complete record of what individuals consumed can be better developed for the past. The stable isotopic values may reveal what juveniles were eating, but, the implications of what they were eating go beyond diet. Food is vital to existence. By understanding the food resources juveniles consumed, we also develop an understanding about survival strategies, ritual practices, and physical aspects of individuals

within any given society. Overall, looking at the change over time in the diet of individuals, between age groupings and within age groupings, can allow for a better understanding of how human societies have developed overtime.

Determining differences and similarities in the diet between juveniles and adults can help reveal other trends in daily life for the ancient Chachapoya people. A statistically similar diet in both the juvenile and adult subgroups highlights similarities in dietary patterns. In future research, or a cross-cultural comparison with research already completed, the differential or similar treatment of juveniles may be better understood.

## **APPENDICES**



## **Appendix A: The modified Longin (1971) method used to extract collagen from bone**

1. Each sample was dry cleaned manually with a toothbrush, dental pick or Dremel to remove any visible dirt or soils. Once the primary cleaning was completed, the sample was placed into an ultrasonicator, with distilled water, to remove any remaining dirt found within the sample.
2. The sample was dried in an oven at 60 degrees Celsius for ~24 hours.
3. The rib bone samples were broken down using a mortar and pestle to sizes from 2mm to 5mm until the sample weighed (~1.0 gram). During the process of breaking down the rib sample, any further dirt found and the trabecular bone were removed, to avoid data contamination by those two factors.
4. The weighed samples were placed into individual, labeled, centrifuge tubes.
5. Lipids were removed from each sample to mitigate any errors from reading the lipid signature over the carbon and nitrogen within the bone. Each sample was brought to a fume hood with ~10 ml of 2:1 chloroform and methanol, agitated and left for ~20 minutes.
6. The samples were removed from the fume hood and placed into a centrifuge for 10 minutes at 2.4 rpm.
7. The chloroform and methanol solution was pipetted off and steps 5-6 were repeated two more times.
8. After steps 5-6 were completed three times in total, the samples were left to dry overnight in the lab fume hood.

9. The bone samples were demineralized. This process began by adding 0.25 M HCl to the centrifuge tubes.
10. After 24 hours, the 0.25 M HCl solution was pipetted off and 10 mL of distilled water was added, before placing in the centrifuge for 10 minutes at 2.4 rpm.
11. Step 10 was repeated until reaching the pH of 2.5-3.0 and the samples were fully demineralized. This process took 2-3 weeks, depending on the samples and their degree of preservation. The bone samples that took the longest period of time to finish the process were those that were the best preserved.
12. Humic acids were removed by adding ~10 mL of 0.1 M NaOH to each centrifuge tube. The tubes were capped and slightly agitated every 5 minutes for 20 minutes.
13. After 20 minutes, the samples were centrifuged for 10 minutes at 2.4 rpm.
14. The samples were observed for potential color change. A golden color or no color change indicated that the NaOH in the sample remained relatively clear. If the sample exhibited a color change, Step 13 would be repeated until there was no color change.
15. Once all of the samples exhibited no color change, the NaOH was removed and ~10 mL of distilled water was added to each sample and centrifuged for 10 minutes at 2.4 rpm.
16. Step 15 was repeated until the pH of each sample reached  $7 \pm 1.0$  pH. Although variation existed between the samples, it generally took ~12 rinses to neutralize each sample.
17. The process of gelatinizing and drying collagen began by adding 10 mL of 0.25 M HCl into each centrifuge tube and centrifuging for 10 minutes at 2.4 rpm.
18. The HCl was pipetted off, 5 mL of distilled water was added, and the pH was checked to ensure that the pH was in the 2.5-3.0 range.

19. The centrifuge tubes were capped and placed into an oven at 90 degrees Celsius for ~16 hours.
20. Once removed from the oven, the samples were centrifuged for 10 minutes at 2.4 rpm.
21. The collagen solution, minus the precipitate formed on the bottom of the tube, was pipetted out and placed into pre-weighed glass test tubes.
22. The glass test tubes, uncapped, were placed into the oven at 90 degrees Celsius for ~24-36 hours.
23. The samples were weighed to calculate collagen yield.
24. The samples were sent to the Light Stable Isotope Mass Spec Lab in the Department of Geological Sciences at the University of Florida to undergo the IRMS process. The resulting data was returned to the University of Central Florida's laboratory.

**Appendix B: Collagen yield,  $\delta^{13}\text{C}$  values and  $\delta^{15}\text{N}$  by juvenile subgroup (individuals are in order of ascending age, Table 3).**

|                     | Sample                       | Collagen Yield<br>% | $\delta^{13}\text{C}$<br>(‰, VPDB) | $\delta^{15}\text{N}$<br>(‰, AIR) |
|---------------------|------------------------------|---------------------|------------------------------------|-----------------------------------|
| Burial<br>group     | KPANTorVa Ent3A              | 22.3                | -14.5                              | +8.3                              |
|                     | KSPlatI IIIÑ Ent3A           | 17.4                | -12.3                              | +9.9                              |
|                     | K-PAS MO -VIII T' ENT10      | 13.9                | -6.2                               | +9.2                              |
|                     | K-PAS MO -VIII T' ENT39      | 7.7                 | -10.2                              | +9.3                              |
|                     | K-PAS MO -VIII U' ENT69      | 10.7                | -12.3                              | +10.7                             |
|                     | K-PAS MO -VIII U' ENT57      | 16.3                | -11.7                              | +10.3                             |
|                     | K-PAS MO -VIII U' ENT52a     | 7.2                 | -13.7                              | +9.5                              |
|                     | KSPlatI IIIÑ Ent1B           | 19.7                | -14.3                              | +6.9                              |
|                     | KC ME VK RH17                | 17.0                | -14.0                              | +12.4                             |
|                     | K-PAS MO -VIII T' ENT33      | 16.6                | -12.0                              | +8.5                              |
|                     | K-PAS MO -VIII T' ENT37      | 12.8                | -12.1                              | +8.1                              |
|                     | K-PAS MO -VIII T' ENT12      | 19.9                | -11.1                              | +8.1                              |
|                     | KSTIN-VU Ent3                | 18.9                | -13.7                              | +8.9                              |
|                     | K-PAC E1 -V Q' T2 ENT1       | 12.6                | -15.7                              | +7.4                              |
|                     | K-PAS MO -VIII U' ENT46      | 9.8                 | -12.8                              | +6.1                              |
|                     | KSPlatI IIIÑ Ent4            | 23.8                | -12.6                              | +7.6                              |
|                     | K-PAS MO -VIII T' ENT22      | 18.8                | -10.1                              | +8.0                              |
|                     | KSTIN-III T Ent6             | 19.9                | -13.2                              | +7.0                              |
| KSTIN-IVV Ent11     | 19.7                         | -11.2               | +7.2                               |                                   |
| Massacre<br>group   | KSPlatC E6-VIIz ENT105       | 13.                 | -14.4                              | +10.6                             |
|                     | KSPlatC E2-VIIa' Ent26       | 12.4                | -15.0                              | +7.7                              |
|                     | KSPlatC E6-VIIz,-VIIa' ENT75 | 6.2                 | -13.6                              | +8.1                              |
|                     | KSPlatC E2-VIa' Ent22        | 12.1                | -12.4                              | +7.2                              |
|                     | KSPlatC E2-VIIa' Ent18c      | 12.2                | -13.3                              | +7.8                              |
|                     | KSPlatC E4-VIa' ENT61        | 9.6                 | -14.2                              | +6.4                              |
|                     | KSPlatC-VIIZ Ent2            | 6.8                 | -13.7                              | +6.2                              |
|                     | KSPlatC E2-VIIa' Ent16a      | 10.6                | -13.1                              | +7.4                              |
|                     | KSPlatC E3-VIa' Ent31        | 9.3                 | -13.1                              | +7.5                              |
|                     | KSPlatC E3-VIIa' Ent37a      | 6.3                 | -12.1                              | +7.9                              |
|                     | KSPlatC E4-VIa' ENT58        | 12.7                | -12.5                              | +6.3                              |
|                     | KSPlatC-VIA' ENT50           | 19.3                | -12.4                              | +7.4                              |
| KSPlatC-VIIIZ ENT91 | 20.1                         | -13.0               | +11.3                              |                                   |

**Appendix C:  $\delta^{13}\text{C}$  values and  $\delta^{15}\text{N}$  values adjusted for diet by juvenile subgroup (individuals are in order of ascending age, Table 3).**

|                 | Sample                       | $\delta^{13}\text{C}$ (‰ to Diet) | $\delta^{15}\text{N}$ (‰ to Diet) |
|-----------------|------------------------------|-----------------------------------|-----------------------------------|
| Burial Group    | KPANTorVa Ent3A              | -19.5                             | +5.3                              |
|                 | KSPlatI IIIÑ Ent3A           | -17.3                             | +3.9                              |
|                 | K-PAS MO -VIII T' ENT10      | -11.2                             | +6.2                              |
|                 | K-PAS MO -VIII T' ENT39      | -15.2                             | +6.3                              |
|                 | K-PAS MO -VIII U' ENT69      | -17.3                             | +7.7                              |
|                 | K-PAS MO -VIII U' ENT57      | -16.0                             | +7.3                              |
|                 | K-PAS MO -VIII U' ENT52a     | -18.6                             | +3.3                              |
|                 | KSPlatI IIIÑ Ent1B           | -19.3                             | +3.9                              |
|                 | KC ME VK RH17                | -19.0                             | +9.4                              |
|                 | K-PAS MO -VIII T' ENT33      | -17.0                             | +5.5                              |
|                 | K-PAS MO -VIII T' ENT37      | -17.1                             | +5.1                              |
|                 | K-PAS MO -VIII T' ENT12      | -17.1                             | +5.1                              |
|                 | KSTIN-VU Ent3                | -18.7                             | +5.9                              |
|                 | K-PAC E1 -V Q' T2 ENT1       | -15.7                             | +4.4                              |
|                 | K-PAS MO -VIII U' ENT46      | -17.8                             | +3.1                              |
|                 | KSPlatI IIIÑ Ent4            | -17.6                             | +4.6                              |
|                 | K-PAS MO -VIII T' ENT22      | -16.1                             | +5.0                              |
|                 | KSTIN-III T Ent6             | -18.2                             | +4.0                              |
| KSTIN-IVV Ent11 | -16.2                        | +4.1                              |                                   |
| Massacre group  | KSPlatC E6-VIIz ENT105       | -19.4                             | +7.3                              |
|                 | KSPlatC E2-VIIa' Ent26       | -20.0                             | +4.7                              |
|                 | KSPlatC E6-VIIz,-VIIa' ENT75 | -18.6                             | +5.1                              |
|                 | KSPlatC E2-VIa' Ent22        | -17.4                             | +4.1                              |
|                 | KSPlatC E2-VIIa' Ent18c      | -18.3                             | +4.8                              |
|                 | KSPlatC E4-VIa' ENT61        | -19.2                             | +3.4                              |
|                 | KSPlatC-VII Z Ent2           | -18.7                             | +3.2                              |
|                 | KSPlatC E2-VIIa' Ent16a      | -18.1                             | +4.4                              |
|                 | KSPlatC E3-VIa' Ent31        | -18.1                             | +4.5                              |
|                 | KSPlatC E3-VIIa' Ent37a      | -17.1                             | +4.9                              |
|                 | KSPlatC E4-VIa' ENT58        | -17.5                             | +3.3                              |
|                 | KSPlatC-VIA' ENT50           | -17.4                             | +4.4                              |
|                 | KSPlatC-VIIIZ ENT91          | -18.0                             | +8.3                              |

**Appendix D: Cwt%, Nwt% and C/N ratios for juvenile samples (individuals are in order of ascending age, Table 3).**

|                     | <b>Sample</b>                | <b>Cwt%</b> | <b>Nwt%</b> | <b>C/N Ratio</b> |
|---------------------|------------------------------|-------------|-------------|------------------|
| Burial group        | KPANTorVa Ent3A              | 47.1        | 17.3        | 3.2              |
|                     | KSPlatI IIIÑ Ent3A           | 42.8        | 15.7        | 3.2              |
|                     | K-PAS MO -VIII T' ENT10      | 47.6        | 17.3        | 3.2              |
|                     | K-PAS MO -VIII T' ENT39      | 46.6        | 17.3        | 3.1              |
|                     | K-PAS MO -VIII U' ENT69      | 42.9        | 15.9        | 3.1              |
|                     | K-PAS MO -VIII U' ENT57      | 45.7        | 16.9        | 3.1              |
|                     | K-PAS MO -VIII U' ENT52a     | 46.9        | 17.2        | 3.2              |
|                     | KSPlatI IIIÑ Ent1B           | 45.4        | 16.7        | 3.2              |
|                     | KC ME VK RH17                | 43.5        | 16.0        | 3/2              |
|                     | K-PAS MO -VIII T' ENT33      | 45.2        | 16.7        | 3.2              |
|                     | K-PAS MO -VIII T' ENT37      | 45.3        | 16.7        | 3.2              |
|                     | K-PAS MO -VIII T' ENT12      | 45.7        | 16.5        | 3.2              |
|                     | KSTIN-VU Ent3                | 46.7        | 16.9        | 3.2              |
|                     | K-PAC E1 -V Q' T2 ENT1       | 47.0        | 16.9        | 3.2              |
|                     | K-PAS MO -VIII U' ENT46      | 44.0        | 16.0        | 3.2              |
|                     | KSPlatI IIIÑ Ent4            | 42.6        | 15.5        | 3.2              |
|                     | K-PAS MO -VIII T' ENT22      | 47.3        | 17.1        | 3.1              |
|                     | KSTIN-IIIT Ent6              | 42.2        | 15.3        | 3.2              |
| KSTIN-IVV Ent11     | 39.1                         | 14.2        | 3.2         |                  |
| Massacre group      | KSPlatC E6-VIIz ENT105       | 44.2        | 16.2        | 3.2              |
|                     | KSPlatC E2-VIIa' Ent26       | 45.0        | 16.5        | 3.2              |
|                     | KSPlatC E6-VIIz,-VIIa' ENT75 | 47.0        | 17.4        | 3.1              |
|                     | KSPlatC E2-VIa' Ent22        | 46.8        | 17.1        | 3.2              |
|                     | KSPlatC E2-VIIa' Ent18c      | 47.2        | 17.3        | 3.2              |
|                     | KSPlatC E4-VIa' ENT61        | 43.0        | 15.7        | 3.2              |
|                     | KSPlatC-VIIZ Ent2            | 38.2        | 14.4        | 3.2              |
|                     | KSPlatC E2-VIIa' Ent16a      | 40.2        | 14.6        | 3.2              |
|                     | KSPlatC E3-VIa' Ent31        | 46.7        | 17.2        | 3.2              |
|                     | KSPlatC E3-VIIa' Ent37a      | 41.2        | 15.0        | 3.2              |
|                     | KSPlatC E4-VIa' ENT58        | 45.7        | 16.7        | 3.2              |
|                     | KSPlatC-VIA' ENT50           | 40.0        | 14.5        | 3.2              |
| KSPlatC-VIIIZ ENT91 | 47.3                         | 17.6        | 3.1         |                  |

**Appendix E:  $\delta^{13}\text{C}$  values and  $\delta^{15}\text{N}$  for adult subgroup.**

| Sample                 | Subsection         | Sample Fragment | $\delta^{13}\text{C}$ (‰ VPBD) | $\delta^{13}\text{C}$ to Diet | $\delta^{15}\text{N}$ (‰ AIR) | $\delta^{15}\text{N}$ to Diet |
|------------------------|--------------------|-----------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|
| KCAC1 IIN Ent1         | Acceso 1 - N       | Rib             | -12.3                          | -17.3                         | +7.2                          | +4.2                          |
| KCAC1 IIN Ent2         | Acceso 1 - N       | Rib             | -12.1                          | -17.1                         | +7.5                          | +4.5                          |
| KPANTorVIIA Ent7       | Torreón            | Rib             | -11.8                          | -16.8                         | +6.9                          | +3.9                          |
| KPANTorVIIA Ent8       | Torreón            | Rib             | -11.6                          | -16.6                         | +8.0                          | +5.0                          |
| KSPlatII -IIIÑ Ent3A   | Plataf 2           | Rib             | -13.9                          | -18.9                         | +6.9                          | +3.9                          |
| KSPlatII -IIIÑ Ent4    | Plataf 2           | Rib             | -12.4                          | -17.4                         | +7.4                          | +4.4                          |
| KSSbP1III O Ent1A      | Sub Plataf 1       | Rib             | -15.3                          | -20.3                         | +6.7                          | +3.7                          |
| KSSbP1III O Ent1B      | Sub Plataf 1       | Rib             | -14.9                          | -19.9                         | +8.3                          | +5.3                          |
| KSSbPlt1 -IIP Ent1     | Sub Plataf 1       | Rib             | -13.8                          | -18.8                         | +7.7                          | +4.7                          |
| KSSbPlt1 -IIP Ent2     | Sub Plataf 1       | Rib             | -15.5                          | -20.5                         | +8.1                          | +5.1                          |
| KSSbPlt1 -IP Ent2      | Sub Plataf 1       | Rib             | -11.8                          | -16.8                         | +6.9                          | +3.9                          |
| KSTIN-III T Ent4       | Tintero            | Rib             | -14.0                          | -19.0                         | +6.3                          | +3.3                          |
| KSTIN-III T Ent5       | Tintero            | Rib             | -14.5                          | -19.5                         | +6.6                          | +3.6                          |
| KSTIN-III V Ent7       | Tintero            | Rib             | -14.7                          | -19.7                         | +7.2                          | +4.2                          |
| KSTIN-III U-IIV Ent10A | Tintero            | Rib             | -12.4                          | -17.4                         | +7.4                          | +4.4                          |
| KSTIN-VU Ent1          | Tintero: E:U Circu | Rib             | -14.1                          | -19.1                         | +7.6                          | +4.6                          |
| KSPlatC-VII Z Ent4     | PlatCir            | Rib             | -14.2                          | -19.2                         | +7.1                          | +4.1                          |
| KSPlatC-VIA' Ent12A    | PlatCir            | Rib             | -13.2                          | -18.2                         | +7.6                          | +4.6                          |
| KSPlatC-VIIA' Ent15    | PlatCir            | Rib             | -12.6                          | -17.6                         | +7.9                          | +4.9                          |
| KSPlatC-VIIA' Ent32    | PlatCir            | Rib             | -12.5                          | -17.5                         | +8.2                          | +5.2                          |
| KSPlatC-VIIA' Ent39A   | PlatCir            | Rib             | -14.7                          | -19.7                         | +6.7                          | +3.7                          |
| KSPlatC-VIIA' Ent40    | PlatCir            | Rib             | -13.9                          | -18.9                         | +7.4                          | +4.4                          |
| KSPlatC-VIA' ENT65     | PlatCir            | Rib             | -13.8                          | -18.8                         | +6.3                          | +3.3                          |
| KSPlatC-VIII Z ENT83   | PlatCir            | Rib             | -12.7                          | -17.7                         | +7.7                          | +4.7                          |
| KSPlatI IIIÑ Ent1c     | Plataf 1           | Rib             | -14.5                          | -19.5                         | +8.2                          | +5.2                          |
| KSPlatI IIIÑ Ent2c     | Plataf 1           | Rib             | -13.4                          | -18.4                         | +8.1                          | +5.1                          |
| KSPlatII -IIIÑ Ent1    | Plataf 2           | Rib             | -12.4                          | -17.4                         | +6.7                          | +3.7                          |

## REFERENCES CITED

- Abercrombie, T.A. (1998). *Pathways of memory and power: ethnography and history among an Andean people*. Madison: University of Wisconsin Press.
- Ambrose, S.H. (1990). Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Sciences*, 17, 431-451.
- Ambrose, H.S., & Norr, L. (1993). Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In J.B. Lambert & G. Grupe (Eds.), *Prehistoric Human Bone* (pp. 1-29). Berlin, Germany: Springer.
- Andrushko, V.A., Buzon, M.R., Gibaja, A.M., McEwan, G.F., Simonetti, A., & Creaser, R.A. (2011). Investigating a child sacrifice event from the Inca heartland. *Journal of Archaeological Science*, 38(2), 323-333.
- Ardren, T. (2006). Setting the table: Why children and childhood are important in an understanding of ancient Mesoamerica. In T. Ardren & S. Huston (Eds.). *The Social Experience of Childhood in Ancient Mesoamerica* (pp. 3-24). Boulder; University Press of Colorado.
- Baitzel, S. (2017). Parental grief and mourning in the ancient Andes. *Journal of Archaeological Method and Theory*, 25, 178-201.
- Baxter, J.E. (2008). Introduction: The archaeology of childhood in context. *Archaeological Papers of the American Anthropological Association*, 15, 1-9.
- Bird, D.W., & Bird, B.R. (2000). The ethnoarchaeology of juvenile foragers: Shellfishing strategies among Meriam children. *Journal of Anthropology and Archaeology*, 19, 461-476.
- Bolin, I. (2006). *Growing up in a culture of respect: child rearing in highland Peru*. Austin: University of Texas Press.
- Bogin, B. (1997). Evolutionary hypotheses for human childhood. *Yearbook of Physical Anthropology*, 40, 63-89.
- Buikstra, J. E. & Ubelaker, D.H (1994). *Standards: For data collection from human skeletal remains*. Fayetteville, Arkansas: 3rd Printing.
- von Cremmerer, S. (1992). Carbon isotope discrimination in C3-C4 intermediates. *Plant, Cell and Environment*, 15, 1063-1072.
- Chamberlain, A.T. (2000). Minor concerns: A demographic perspective on children in past societies. In: Derevenski Sofaer J., (Ed.). *Children and Material Culture*. New York: Routledge. P206-212.
- Church, W.B., & von Hagen, A. (2007). Chachapoyas: Cultural development at an Andean cloud forest crossroads. In Silverman H., Isbell W.H. (Eds.). *The Handbook of South American Archaeology*. Springer, New York, NY.



- Dean, C. (2002). Andean androgyny and the making of men. In C.F. Klein (Ed.), *Gender in pre-Hispanic America: A symposium at Dumbarton Oak* (pp. 143-182). Washington, D.C.: Dumbarton Oaks Research Library and Collection.
- DeNiro, M.J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, 42, 495-506.
- DeNiro, M.J., & Hastorf, C.A. (1984). Alteration of  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios of plant matter during the initial stages of diagenesis: Studies utilizing archaeological specimens from Peru. *Geochimica et Cosmochimica Acta*, 49, 97-115.
- Dupras, D.L., & Tocheri, M.W. (2007). Reconstructing infant weaning histories at Roman Period Kellis, Egypt using stable isotope analysis of dentition. *American Journal of Physical Anthropology*, 134, 63-74.
- Fahy, G.E., Deter, C., Pitfield, R., Miskiewicz, J.J., Mahoney, P. (2017). Bone deep: Variation in stable isotope ratios and histomorphometric measurements of bone remodelling within adult humans. *Journal of Archaeological Science*, 87, 10-16.
- Finucane, B.C. (2007). Mummies, maize and, manure: Multi-tissue stable isotope analysis of late prehistoric human remains from the Ayacucho Valley, Peru. *Journal of Archaeological Sciences*, 34-2115-2124.
- Fry, B. (2006). *Stable Isotope Ecology*. New York: Springer.
- Garvie-Lok, S.J., Varney, T.L., Katzenberg, M.A. (2004). Preparation of bone carbonate for stable isotope analysis: the effects of treatment time and acid concentration. *Journal of Archaeological Science*, 31, 763-776.
- Gordon, C.C. & Buikstra, J. (1975). Nutritional individuality. In M. Katz, G. T. Keusch & L. J. Mata (Eds.) *Interactions of Nutrition and Infection*. Guatemala City, Panama: *American Journal of Diseases in Childhood*, pp. 422–424.
- Halcrow, S. E., & Tayles, N. (2008). The bioarchaeological investigation of childhood and social age: Problems and prospects. *Journal of Archaeological Method and Theory*, 15, 190–215.
- Halcrow, S. E., & Tayles, N. (2011). The bioarchaeological investigation of children and childhood. In: S.C. Agarwal & B. Glencross (Eds). *Social Bioarchaeology*, 333-360.
- Hill, M.D. (2013). Growing up Quechua: Ethnic identity, narrative, and the cultural politics of childhood migration in Cusco, Peru. *Childhood*, 20, 383-397.
- Hedges, R.E.M., & Reynard, L.M. (2007). Nitrogen isotopes and the trophic level of humans in archaeology. *Journal of Archaeological Science*, 34, 1240-1251.
- Katzenberg, M.A. (2008). Chapter 13: Stable isotope analysis: A tool for studying past diet, demography and life history. In: M.A. Katzenberg & S.R. Saunders (Eds.), *Biological Anthropology of the Human Skeleton*. (2<sup>nd</sup> Ed. pp. 413-441). New York: John Wiley and Sons, Inc.

- Koch, P.L. (2007). Isotopic study of the biology of modern and fossil vertebrates. In: R. Michener & K. Lajtha (Eds.), *Stable Isotopes in Ecology and Environmental Science* (2<sup>nd</sup> Ed. Pp. 99-154). Oxford, UK: Blackwell.
- Larsen, C.S. (2015). *Bioarchaeology: Interpreting behavior from the human skeleton*. (2<sup>nd</sup> Ed.) Cambridge: Cambridge University Press.
- Lewis, M.E. (2007). *The bioarchaeology of children*. New York: Cambridge University Press.
- Longin, R. (1971). New method of collagen extraction for radiocarbon dating. *Nature*, 230, 241-242.
- Meier-Augenstein, W. (2011). *Stable isotope forensics and introduction to the forensic application of stable isotope analysis*. Hoboken, NJ: Wiley-Blackwell.
- Michell, S.M. (2018). *Animals of the cloud forest: Isotopic variation of archaeological faunal remains from Kuelap, Peru*. Orlando, FL: University of Central Florida.
- Muscutt, K. (1998). *Warriors of the cloud: Lost civilization in the upper Amazon of Peru*. Albuquerque: University of New Mexico Press.
- Narváez, L.A. (1988). Kuelap: Una ciudad fortificada en los Andes nororientales de amazonas, Perú. *Arqueol Arquit*, 115-142.
- Nystrom, K.C., Buikstra, J.E., Muscutt, K. (2010). Chachapoya mortuary behavior: A consideration of method and meaning. *Revista de Anthropologia Chilena*, 42, 477-495.
- Olsen, K.C., White, C.D, Longstaffe, F.J., von Heykin, K., McGlynn, G., Grupe, G., Rühli, F.J. (2013). Intraskelatal isotopic compositions ( $\delta^{13}\text{C}$   $\delta^{15}\text{N}$ ) in bone collagen: nonpathological and pathological variation. *American Journal of Physical Anthropology*, 1-7.
- Parfitt, A.M. (2002) *Misconceptions (2): turnover is always higher in cancellous than in cortical bone*. *Bone* 30: 807–809.
- Pestle, W.J. (2010). Chemical, elemental and isotopic effects of acid concentration and treatment duration on ancient bone collagen: an exploratory study. *Journal of Archaeological Science*, 37, 3124-3128.
- Ruiz Estrada, A. (2010). Huaytapallana, arte rupestre de chachapoyas. *Investigaciones sociales*, 14, 53-65.
- Schoeninger, M.J., Moore, K. (1992). Bone stable isotope studies in archaeology. *Journal of World Prehistory*, 6, 247-296.
- Sillar, B. (1994). Playing with god: cultural perceptions of children, play and miniatures in the Andes. *Archaeological review from Cambridge*, 13(2), 47–63.
- Sofaer Derevenski, J. (1994). Where are the children? Accessing children in the past. *Archaeological Review Cambridge*, 13, 7-20.
- Sofaer Derevenski, J. (2000). *Children and material culture*. New York: Routledge.

Szpak, P., White, CD., Longstaffe, FJ., Millaire, JF., & Vásquez Sánchez, VF. (2013). Carbon and nitrogen isotopic survey of northern Peruvian plants: baselines for paleodietary and paleoecological studies. *PLOS ONE*, 8, e53763.

Tieszen, L.L., & Fagre, T. (1993). Carbon isotopic variability in modern and archeological maize. *Journal of Archaeological Sciences*, 20, 25-40.

Toyne, J.M. (2015). Tibial surgery in ancient Peru. *International Journal of Paleopathology*, 8, 29-35.

Toyne, J.M., Church, W.B., Tello, J.L.C., & Gamarra, R.M. (2017). Exploring imperial expansion using an isotopic analysis of paleodietary and paleomobility indicators in Chachapoyas, Peru. *American Journal of Physical Anthropology*, 1-22.

Toyne, J.M. & Narváez, L.A. (2014) Chapter 13: The fall of Kuelap. Bioarchaeological analysis of death and destruction on the Eastern slopes of the Andes. In: A.K. Scherer & J.W. Verano (Eds.) *Embattled Bodies, Embattled Places: War in Pre-Columbian Mesoamerica and the Andes*. Washington, D.C.: Dumbarton Oaks Research Library and Collection.

Tsutaya, T. (2017). Post-weaning diet in archaeological human populations: A meta-analysis of carbon and nitrogen stable isotope ratios of child skeletons. *American Journal of Physical Anthropology*, 164, 546-557.

Tsutaya, T. & Yoneda, M. (2013). Quantitative reconstruction of weaning ages in archaeological human populations using bone collagen nitrogen isotope ratios and approximate Bayesian computation. *PLOS ONE*, 10(3), e0119778.

Turner, B.L., Klaus, H.D., Livengood, S.V., Brown, L.E., Saldaña, F., & Wester, C. (2013). The variable roads to sacrifice: Isotopic investigations of human remains from Chotuna-Huaca de Los Sacrificios, Lambayeque, Peru. *American Journal of Physical Anthropology*, 151(1), 22-3.

Williams JS, White CD, and Longstaffe FJ. 2005. Trophic level and macronutrient shift effects associated with the weaning process in the Postclassic Maya. *American Journal of Physical Anthropology* 128(4):781-790.

Wood, J.W., Milner, G.R, Harpending, H.C., & Weiss, K.M. (1992). The osteological paradox: Problems of inferring prehistoric health from skeletal samples. *Current Anthropology*, 33, 343-363.

Zanden, M.J.V, & Rasmussen, J.B. (2001). Variation in trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, 46, 2061-2066.