EXPLORING HUMAN DIETARY VARIATION THROUGH STABLE ISOTOPE ANALYSIS OF HAIR

by

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES
(Anthropology)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

April 2017

Abstract

This thesis explores the use of carbon, nitrogen and sulphur stable isotope analysis of modern and archaeological human hair as an indicator of human diets. The thesis is focused around three distinct research projects, two on modern, living humans and one on an archaeological population.

The first project focuses on dietary variation among different populations in modern Ethiopia that share the same resource base but follow different economic and subsistence patterns. This research shows that economic and cultural patterns can cause very distinct and significant differences in diet among populations with access to the same resources. The second project uses data from modern Nicaraguan villagers to explore variability in isotopic signatures among demographic groups within one population. The data reveal significant differences among demographic groups, but the absolute differences are quite small, indicating that it is necessary to have a large sample size to determine isotopic differences within a population. The third project is an archaeological case study presenting the first serial isotopic analysis of human hair from the Basketmaker II (BMII) midden at the site of Turkey Pen Ruins on Cedar Mesa, in south-eastern Utah. These data show potential seasonality of diet at the site, with variations in the amount of C_4 protein being contributed to the diet.

Together these projects contribute to our understanding of how different scales of dietary variation can be interpreted and approached through isotopic analysis of human hair. The studies also show the applicability of both intra-individual and inter-individual isotopic analysis of human hair to our understanding of modern and ancient diets.

Preface

This thesis is composed of three parts: an introduction (Chapter 1), the main body of research that presents three distinct projects (Chapters 2 through 4), and the conclusion (Chapter 5). The three chapters comprising the main body of this thesis have been written as individually publishable articles, each of which have been published (Chapter 4) or are under review for publication (Chapters 2 and 3). The research presented in this thesis is the original work of the author, Catherine Cooper.

Chapter 2 is based on the manuscript titled: "Stable isotope analysis of hair from three groups of people in modern Ethiopia shows clear differences among isotopic signatures related to subsistence regimes", which has been submitted for publication. The paper is a collaboration with and co-authored by Karen Lupo, Ashenafi Zena, Dave Schmitt, and Michael Richards. Karen Lupo, and Dave Schmitt collected the hair samples during their fieldwork in Ethiopia. Ashenafi Zena was consulted on the ethnographic details of the region. All isotopic analyses were conducted at the Laboratory of Archaeology (LOA) Archaeological Isotope Laboratory at the University of British Columbia under the supervision of Michael Richards. I prepared and analysed all samples, did the statistical analyses, interpreted the data, and wrote the article.

Chapter 3 is titled "Dietary variability through isotopic analysis of modern human hair from Nicaragua: exploring significant differences in diet between and among demographic groups in a single population", which has been submitted for publication. I am principal author of this paper, co-authored with Angela Perri, Jessica Burns, Jeremy Koster, and Michael Richards. All hair samples were collected by Jeremy Koster during his ethnographic fieldwork in Nicaragua; Jeremy Koster also provided the map (Figure

3.1). Isotopic analyses were conducted at the LOA Archaeological Isotope Laboratory at the University of British Columbia under the supervision of Michael Richards. Angela Perri and Jessica Burns shared their expertise on the ethnography of the Nicaraguan research site. I prepared and analysed all of the hair samples for this study, analysed and interpreted the data, and wrote the manuscript.

Chapter 4 is titled "Short-term variability of human diet at Basketmaker II Turkey Pen Ruins, Utah: Insights from bulk and single amino acid isotope analysis of hair" published in 2016 in the *Journal of Archaeological Science: Reports* (vol. 5, pg. 10-18). The paper was a collaboration and was co-authored with Karen Lupo, R.G. Matson, William Lipe, Colin Smith, and Michael Richards; I was the primary author for the article. The samples were originally excavated by R.G. Matson, and William Lipe, both of whom shared their expertise and archaeological knowledge of Turkey Pen Ruins for the present study. The map and stratigraphic diagrams were drawn by S. Matson (Figures 4.1, 4.2) . The samples were provided by Karen Lupo. I prepared all samples for single amino acid stable isotope analysis at La Trobe University in Melbourne, Australia under the supervision of Colin Smith. I conducted all sample preparation and analysis for the bulk stable isotope data at the LOA Archaeological Isotope Laboratory at the University of British Columbia under the supervision of Michael Richards.

Assistance from non-co-authors is detailed in the Acknowledgements below.

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List of Symbols and Abbreviations

‰ – "per mil" refers to 1/1000.
$\delta^{13}C$ – difference between ^{13}C and ^{12}C, units in ‰
$\delta^{15}N$ – difference between ^{15}N and ^{14}N, units in ‰
$\delta^{34}S$ – difference between ^{34}S and ^{32}S, units in ‰

AIR – ambient inhalable reservoir; international scale for $\delta^{15}N$ data
BMII – Basketmaker II
EA-IRMS – elemental analyser-isotope ratio mass-spectrometer
MET – methionine standard
SUBC 1 – UBC LOA Archaeological Isotope Laboratory internal collagen standard
VCDT – Vienna Canyon Diablo Troilite; international scale for $\delta^{34}S$ data
VPDB – Vienna Pee Dee Belemnite; international scale for $\delta^{13}C$ data

UBC – University of British Columbia
LOA – Laboratory of Archaeology

Acknowledgements

This thesis would not have been possible without the generous assistance and support of my supervisors Professor Michael Richards and Professor David Pokotylo, and my committee member, Professor Michael Blake. My thanks, also, to the crew of the UBC LOA Archaeological Isotope Lab for their help and camaraderie throughout this process.

I would also like to thank the numerous brilliant and generous people who gave me the chance to collaborate on this wide range of projects: Karen Lupo, Colin Smith, R.G. Matson, William Lipe, Jeremy Koster, Angela Perri, Jess Burns, Ashenafi Zena, and Dave Schmitt.

The writing process would not have gone nearly as smoothly without my friends and family. To the wonderful people at PGIMF, the Rogue Folk Club, and WCS, thank you for keeping me balanced so I could complete this stretch of my educational journey. To my parents and the twins, thank you for reading many iterations of this thesis and telling me when I wasn't making sense. Thank you so much for the tactful and constructive critiques and calm assurances that I would finish.

To Hope and Anne

For continuing to write, and for helping me to continue writing as well. Thank you for your unfailing support as we all struggle with this thing called "scholarship".

1. Introduction

Traditionally, isotopic analyses of human remains for dietary reconstruction have focused on bone collagen and apatite as these materials are more commonly found in the archaeological record than hair, nails, or other soft tissues (Nehlich, 2015). Isotopic analysis of bone provides data on an individual's average diet for the last 10 to 30 years due to the constant process of bone remodelling during an individual's life (Britton et al., 2013; Lee-Thorp, 2008). Isotopic analyses of human bone have been used to address many archaeological questions, including changes in diet across archaeological periods (Coltrain, 2009; Vogel and van der Merwe, 1977), differences in diet between neighbouring, contemporaneous archaeological populations (Bollongino et al., 2013), migratory patterns due to variation between isotopic signatures of teeth and other bones (Quinn et al., 2008), and dietary differences between demographic groups within a population (Somerville et al., 2015). Hair keratin is another useful tissue for isotopic analysis, and can provide complementary information on human diet. Hair grows at a rate of approximately 1 cm per month with no remodelling of tissue once the hair has been formed (LeBeau et al., 2011). Thus, hair can be used to examine the last months of an individual's diet depending on the length of hair, and potentially diet variation over the course of months if hair is sampled serially (e.g., Britton et al., 2013; Webb et al., 2013). Another advantage of analysing the isotopic composition of hair is that it can be sampled non-invasively, allowing for relatively easy isotopic examination of living individuals (LeBeau et al., 2011).

Two main limitations in using hair as a substrate for isotopic analysis and dietary studies are: (1) understanding of the inherent sources of variability and (2) the limitations

of sample sizes. When interpreting isotopic variability, it is important to rule out growth patterns of hair, and normal fluctuations in isotopic signature that do not indicate changes in diet as sources of the variation. When using multiple hair strands for a larger sample size, it is critical to be aware of how growth patterns may differ between strands, and how this may affect the average isotopic signature and the resulting data interpretation (Huelsemann et al., 2009).

Isotopic studies, including those using human hair, frequently focus on dietary variability or dietary differences within and between groups. In archaeology, isotopic data are frequently used to identify different groups present in a site or region (e.g., Britton et al., 2013; Cheung et al., 2015; Knudson et al., 2007). However, one problem with such analysis is the lack of well-defined criteria for determining how large isotopic differences have to be in order to be considered significant, or how tightly the data need to cluster, in order to determine, with confidence, how many groupings might present.

In order to address these methodological problems, the papers in this thesis were designed to help improve our understanding and interpretation of hair isotope data though an examination of what constitutes significant differences in diet as distinct from natural isotopic variability within individuals or within a population. A better understanding of the difference between real isotopic - dietary variability and isotopic "noise" is critical to interpreting the data and drawing valid conclusions about the lives and diets of past individuals.

In order to address the questions of "noise" and significant difference, I examined patterns of dietary variation through three separate projects, each focused on a different scale of variation—among populations, within a single population, and within an

individual. The first two projects examine modern populations, which provide useful data for comparison and implications for archaeological interpretations. Each project is covered in a separate chapter, and is intended as a stand-alone paper. Detailed background and contextual information common to all three projects, including a more detailed discussion of hair chemistry, growth and variability, as well as human nutrition is presented in sections 1.2 and 1.3. The methods used in each project vary slightly, and are included in the project chapters 2, 3 and 4.

Chapter 2 uses carbon (C), nitrogen (N) and sulphur (S) stable isotopes to explore dietary variation among groups that have access to the same resource base but engage in different economic and subsistence strategies. This case study presents data from 51 individuals who belong to six different tribes in southern Ethiopia. Due to the small number of people involved in the study, individuals are grouped by their self-identification with one of three economic practices: pastoralism, farming, and fishing. The isotopic patterns that emerge from this data are that (1) isotopic differences among the economic groups are visually and statistically significant, and that (2) isotopic variation among groups is much larger than the isotopic variation within the different economic groups, despite the fact that some economic groups contain data from multiple tribes. Due to the fact that all of these individuals have access to the same resources through proximity and trade within southern Ethiopia, the dietary differences can be interpreted as economically and culturally mediated. These results are interesting and important because they show the importance of human agency and culture in selecting dietary components; data also provide an example of how distinct isotopic differences among diets can be, even when people have access to the same foodstuffs.

Chapter 3 presents isotopic data gathered from a single population in order to explore dietary variation at a narrower scale and examine how demographic and socioeconomic factors may affect diet within a single group of people. Nutritionists have shown that many socio-economic and demographic groupings have been found to influence human nutrition; wealth, for example, has been found as an important factor in both access to food and education on what to eat, which in turn influence an individual, or family's nutrition (Barros et al., 2010; Christiaensen and Alderman, 2004; Hadley et al., 2008; Sakisaka et al., 2006; Winking and Koster, 2015). In order to address demographic and socioeconomic relationships to diet, this case-study uses C and N isotopic analysis of hair from 352 individuals from two villages in the Bosawas Biosphere Reserve in Nicaragua; family affiliation, status, age, sex, and other factors for each individual in the Nicaraguan study were also recorded to compare with the isotopic data. A few of the demographic groupings examined in this study, such as sex, age, and wealth appear to be related to significant isotopic differences in this Nicaraguan population. These results show that dietary variability can be influenced by subgroups within a population and can increase the within-population spread of isotopic signatures. Due to the fact that the absolute differences in isotopic signatures are small, it is also important to note that these patterns may be detectable only through large sample sizes and statistical analyses.

Chapter 4 presents data that focuses on a yet-smaller scale of dietary variation—intra-individual dietary variation over short periods of time (on a scale of months). Examining dietary variation within an individual is important for understanding how much isotopic variation to expect when an individual is eating a "stable" diet, when that

diet changes with the seasons or regular migration, or how an individual's diet changes when he or she moves permanently between locations, or is ill. The data used in the Chapter 4 case study explores seasonality of diet in a few individuals by applying serial hair isotope analysis to a small number of archaeological hairs from Turkey Pen Ruins in the American Southwest. Serial C and N isotope analysis of 5 hairs was used to examine whether each individual's diet changed over the course of a few months to a year. These data were complemented by serial carbon isotope analysis of single amino acids a sixth hair from the site, which was used to further break down possible changes in diet over time. Though only six samples were analysed, the data show dietary variability at the site that may be connected to seasonality of food resources, which indicates that their horticultural crops were supplemented by seasonally accessible resources. The data also show that there was very little meat in the diet during the Basketmaker II period (~1000 BC—500 AD).

Together, these three case-studies show examples of how isotopic analysis of human hair can be used to explore different scales of dietary variation. They also show how the dietary variability at each scale affects the dietary variability of the next greater scale—individual dietary variability affects dietary variability within a population, and from there, also the dietary variability between populations. The implications for archaeology and anthropology are discussed further, and in more detail, in Chapter 5. I also identify future avenues of research to both expand on the research results presented here, and help fill in the gaps that remain in our understanding of how to apply isotopic analysis of human hair to the study of past diets.

1.1 Hair Chemistry and Growth

Hair provides a unique view into the short-term diet of an individual because of its quick growth rate (~1 cm/month) and unchanging chemistry after formation (LeBeau et al., 2011; Williams et al., 2011). This narrower temporal range makes it possible to relate hair isotopic data to more specific periods in an individual's life—for example, what an individual was eating in the months before he or she died, or whether an individual moved between regions and changed his or her diet (Bol et al., 2007; Wilson et al., 2007). This is in contrast with, but provides complementary information to, bone collagen, which has a turn-over rate of ~10-30 years and is being constantly remodelled such that the isotopic data from bone collagen represents an individual's average dietary signature from the past decade or more (Britton et al., 2013; Knudson et al., 2012).

Isotopic data from dental tissues, including enamel, dentine and calculus, are also complementary to hair isotopic analyses. Dental tissues are more similar to hair than bone in that once they have been formed and fully mineralized, they are not remodelled (Eerkens et al., 2014, 2016; Gregoricka, 2014). Isotopic analysis of different teeth allows for (1) comparison of dietary data from different periods in an individual's life through analysis of different teeth from the same individual that formed at different points in time (Gregoricka, 2014); and (2) serial analysis of dentine or enamel layers to examine changes in an individual's diet over the period of a few years, with each layer averaging 0.5 to 2 years of dietary input (Eerkens et al., 2016). These new developments in dental isotopic studies make it possible to look at dietary shifts over a period of years during childhood (1^{st} molars form between ages of 0—9) through young adulthood (3^{rd} molars form between ages 9—21) (Eerkens et al., 2016). Both of these approaches illustrate

changes in diet over time, but still focus on larger periods of time than hair isotopic analyses.

Hair analyses can focus on short-term diet in two ways: bulk analysis of hair to examine the average diet over the last few months or couple of years (represented by the total growth of hair used in the sample—the longer the hair, the longer the period of time represented by the sample), or serial analysis of segments of hair strands to look at sequential changes in diet over a period of months (Knudson et al., 2015; Schwertl et al., 2003; Webb et al., 2013).

There are a number of sources of potential error when relating hair isotope signatures to time, either in bulk hair analysis or serial hair analysis. First, human scalp hair grows, on average, 1.06 cm per month (LeBeau et al., 2011), but this average rate can vary due to individual factors such as health, age, nutrition or hormones. Also, hair from different body parts of the same individual can have varying growth rates (Fuller et al., 2006; LeBeau et al., 2011). In archaeological studies, the average growth rate of hair (1 cm/month) is commonly used to relate isotope values to time when the growth rate itself cannot be measured (Webb et al., 2013). The uncertainty around this average should still be acknowledged and addressed when relating isotopic signatures of hair to time when conducting serial analyses.

A second source of uncertainty in relating the isotopic signature measured in hair to time, is the growth cycle of hair. Hair growth follows three phases, only one of which (anagen) is active growth; the other two phases are catagen, the degeneration phase, and telogen, the rest phase (LeBeau et al., 2011; Schwertl et al., 2003; Williams et al., 2011). Healthy individuals have an average of about 85-90% of hair actively growing, with 10-

15% of hair in either the degeneration or rest phases (Williams et al., 2011). Knowing the growth phase of a hair strand is important when determining which few months are represented by that hair sample. Bundled batches of hair, if they are not all in the same stage of growth, can give an isotopic average that may represent a longer time than their segmented length may suggest (Knudson et al., 2015). The averaging effect caused by hair bundling is sometimes described as a "smearing" of the isotopic signature (Huelsemann et al., 2009). Another temporally confounding factor is sampling method, which can vary between live and deceased individuals. It takes about 2 weeks for newly formed hair to reach the surface of the scalp (LeBeau et al., 2011). This time lag can cause difficulty in relating isotopic signatures in hair to the time of growth, especially when hair samples are cut rather than pulled from the scalp, which is more common in live than deceased individuals (Huelsemann et al., 2009; Schwertl et al., 2003). When hair samples are cut from the scalp, it is important to know how far away from the root the sample was taken (LeBeau et al., 2011)

Further uncertainty in relating an individual's hair isotopic signatures to points of time in his or her life, is how long it takes for the body to equilibrate to a new diet (McCullagh et al., 2005). There is no standard value for the length of time it takes for the body reservoir to adjust to a new diet; rather, there are a few different values given in modern case studies, ranging from 2.3 to 4 months for complete turnover of the body's carbon reservoir (McCullagh et al., 2005; Petzke et al., 2010). It has also been noted that the rate of turnover can change, and increased physical activity level (PAL) was found to increase the rate of nitrogen turnover in the body (Huelsemann et al., 2009; Petzke et al., 2010).

One of the major questions in hair isotope analysis, partly due to this uncertainty, is what constitutes normal patterns of isotopic variation within an individual or population. For isotopic differences to be useful they must be greater than the spread of normal variation—but the needed difference between values is unknown. Interpreting isotopic variation in human hair is not consistent among published studies. One study examining intra-individual isotope signature variation in modern individuals eating "stable" diets determined that $\delta^{13}C$ and $\delta^{15}N$ variation within a range of 1.5‰ was stable (Fraser et al., 2006). In another study examining mobility within an ancient Peruvian population, a range of $\delta^{13}C$ values with a spread of less than 0.5‰ was considered stable; $\delta^{13}C$ variation between 0.5‰ and 2.7‰ was considered seasonal, and greater than 2.7‰ $\delta^{13}C$ variation was interpreted as multiple-zone resource exploitation (Webb et al., 2013). Williams and Katzenberg (2012) also used 0.5‰ variation as the limit for stable $\delta^{13}C$ isotopic spread (Williams and Katzenberg, 2012).

These measured isotopic differences must also be demonstrably larger than instrument error in order to be considered changes in diet, and when comparing hair isotopic data between studies, potential inter-laboratory variation needs to be acknowledged (Pestle et al., 2014). A recent examination of inter-laboratory variation of bone collagen and bone hydroxyapatite isotopic data proposed the use of the Minimum Meaningful Distance (MMD) in order to compare whether or not isotopic differences are real when comparing data between laboratories or previously published results (Pestle et al. 2014). The results of this study, while not directly applicable to hair isotopic data (which was not included), does note that small, repeatable differences in isotopic signatures collected from the same laboratory can be considered reliable—but the results

raise the point that the examination of MMD for hair isotopic signatures between laboratories may be advisable in the future (Knudson et al., 2015; Pestle et al., 2014).

Another concern is the relationship between hair and other tissues. Using hair for isotopic analysis in modern populations is of particular interest because it can be sampled non-invasively (Huelsemann et al., 2009; LeBeau et al., 2011; McCullagh et al., 2005). Hair (or nails) are therefore easier to sample from living individuals, but are rarely preserved in most archaeological sites (Nehlich, 2015; O'Connell and Hedges, 1999). The isotopic relationships between hair, nail and bone collagen isotopes are important in order to better compare modern and archaeological samples and studies (O'Connell et al., 2001; O'Connell and Hedges, 1999). Collagen is generally understood to be slightly enriched in both ^{13}C and ^{15}N, meaning that collagen has relatively more of the heavier isotopes when compared to hair and nail isotopic signatures (O'Connell et al., 2001). The exact value of this enrichment in collagen is not known; estimates are currently that bone collagen $\delta^{13}C$ is enriched over hair keratin by 1.41±0.45‰ while collagen $\delta^{15}N$ is enriched by 0.86±0.17‰ (O'Connell et al., 2001). The enrichment of collagen is attributed to differences in amino acid composition—collagen contains a greater percentage of glycine, an isotopically heavier amino acid, compared to keratin (O'Connell et al., 2001). There is no significant difference in hair and nail amino acid composition, so the observed $\delta^{15}N$ enrichment of nail over hair by 0.65±0.20‰ cannot be explained the same way; this indicates that there is more than one factor involved in which biological materials have higher (more enriched) isotopic signatures (O'Connell et al., 2001).

1.2 Human Nutrition

The intersection of diet and nutrition—what do our bodies require as opposed to what we actually eat—is a topic of considerable current academic and public interest. Although stable isotopic analysis cannot directly address the nutritive value of food, it can help objectively identify gross differences in diet between people. Biologically, human nutritive requirements are the same, irrespective of culture, but culture mediates what foods are eaten, in what quantities and when (James, 2004). Isotopic analysis is one way to determine (1) if there is a discrepancy between reported and actual diets, and (2) if there is a systematic difference in diet within or between cultural groups. This type of dietary information is useful to know when studying food security and health within a community, population, or country, and can complement nutritional data.

Biologically, humans need basic proportions of macronutrients (carbohydrates, fat, and protein) and micronutrients (vitamins and minerals) to be healthy (Leonard, 2000). The definition of food security given by the Food and Agricultural Organization of the United Nations (FAO)—that "food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life"—puts biological requirement alongside cultural preferences (Bacon et al., 2014). Different people can have different biological requirements; in particular, pregnant women and children have greater nutrient needs than other individuals due to greater biological demand (Black et al., 2008; Holliday, 1978). Children require consistent good nutrition to maintain normal growth patterns and mental development—chronic malnutrition can lead to stunting while acute malnutrition can cause wasting (Guerrant et al., 2008; Holliday, 1978;

Leonard, 2000). Acute malnutrition can also permanently affect a child's growth and future productivity (Guerrant et al., 2008). Malnutrition in adult women can lead to childbirth complications and death; a particular nutrient requirement of concern is iron—due to a woman's menstrual cycle, women need at least twice as much iron as men (Black et al., 2008).

A number of cultural and economic factors alongside biological ones can also impact an individual's nutrition. Education has been found to influence nutrition at a few different levels (Cochrane et al., 1982), as it not only influences an individual's job and income prospects, but can also affect an individual's approach to nutrition, such as how money is spent on food or what foods are prepared (Moore et al., 2009). Women's education has been found to be more influential to family nutrition than men's education: multiple studies have shown significant positive correlations between a woman's level of education and the food security of her family (Christiaensen and Alderman, 2004; Cochrane et al., 1982). Conversely, a mother's lack of education is significantly associated with risk of stunting in children under the age of two (Sakisaka et al., 2006). Gender can also be a factor depending on culturally driven sexual differentiation that might impact differential access to resources (Sakisaka et al., 2006). Two examples are, first, female children in Nicaragua are at higher risk of being underweight than male children and, second, adolescent girls report a higher level of food insecurity than boys in Ethiopia (Hadley et al., 2008; Sakisaka et al., 2006). Income is another important factor: it can buffer against food insecurity and malnutrition for all individuals of a household (Barros et al., 2010; Godoy et al., 2005; Hadley et al., 2011). Evidence of this includes the positive association of a father's financial support with his children's height for age Z

score (HAZ) (Lamontagne et al., 1998; Winking and Koster, 2015). Also, income is associated with greater diet variability, which is a common indicator of better nutrition (Belachew et al., 2013).

1.3 Scope and Implications

The scope of this thesis is limited by bounds of the three distinct, independent case-studies presented in chapters 2 – 4, but is tied into the greater literature on stable isotope analysis of hair through the implications of each study. Specifically, the research presented in this thesis shows the applicability of stable isotope data combined with rigorous statistical analysis to better understanding current and past human diets, and how those diets differ at different scales of social organization—economic, population, and individual. The following chapters that make up the body of this thesis provide the details of each individual study, and the final, concluding, chapter, expands on the implications these data have for understanding human dietary variation across different scales of dietary analysis.

2. Stable isotope analysis (C,N,S) of hair from Ethiopia shows clear differences in isotopic signatures related to subsistence regimes

2.1 Case Study Overview

This study characterizes and compares the stable isotope signatures of peoples with differing economic practices (farmers, pastoralists, fishers) in rural southern Ethiopia to determine if dietary differences were visible and measurable in their hair isotope values. Stable isotope ratios (C,N,S) of modern human hair were collected from 49 people. Overall, there were significant differences in all three elements that can distinguish these economic practices (carbon: $X^2 = 8.523$, $p = .014$; nitrogen: $X^2 = 35.372$, $p = .000$; and sulphur: $X^2 = 30.887$, $p = .000$). These data demonstrate the utility of isotopic methods as an indicator of diet, and show diverse dietary adaptations and economies occurring simultaneously in populations living side by side in this region of modern Ethiopia.

2.2. Introduction

Isotopic analysis of human hair as a dietary and geographic indicator has a broad range of applications from forensic studies to archaeological research (e.g., Bol et al., 2007; Britton et al., 2013; Knudson et al., 2007; Mützel (Rauch) et al., 2009). Ideally, isotopic analysis of human remains can help us better understand past populations by providing quantitative data that may show dietary differences between or within groups of people. However, in archaeological contexts, using hair isotope analysis as a proxy for understanding past diets is problematic due to the number of different levels of approximation that must occur for interpretation. These include estimates of potential available food sources, differences in diet due to social status, and the number of populations present.

This study examines isotopic signatures among different neighbouring contemporary ethnic groups in Ethiopia. Although these populations regularly interact, each group practices distinct economic patterns. Due to their close physical proximity, access to markets, and use of trade networks, all of these ethnic groups likely have some overlapping dietary components (Amzaye, 2007; Förch, 2003). Thus, any differences in diet among the groups as reflected by isotopes can be attributed to economic practices or social differences in accessibility to resources. These samples provide the opportunity to explore what constitutes statistically significant differences between carbon, nitrogen, and sulphur isotopic signatures among neighbouring, interacting, and distinct ethnic groups.

2.3. Background

2.3.1 Ethiopia: Ethnography and Brief History

Ethiopia is a diverse country, both ecologically and ethnographically, with savannahs and mountain ranges that support more than 80 ethnic groups (Förch, 2003). This study focuses on individuals from six ethnic groups inhabiting areas south and north of the district of Konso, located in the Southern Nation Nationalities Peoples' Regional (SNNPR) State (Amzaye, 2007; Förch, 2003) (Figure 2.1). Though there is a range of ethnic groups included in this study, each individual self-identified with one of three main economic/subsistence groups—pastoralists, farmers, or farmers who supplement their crops with fish (fishing farmers).

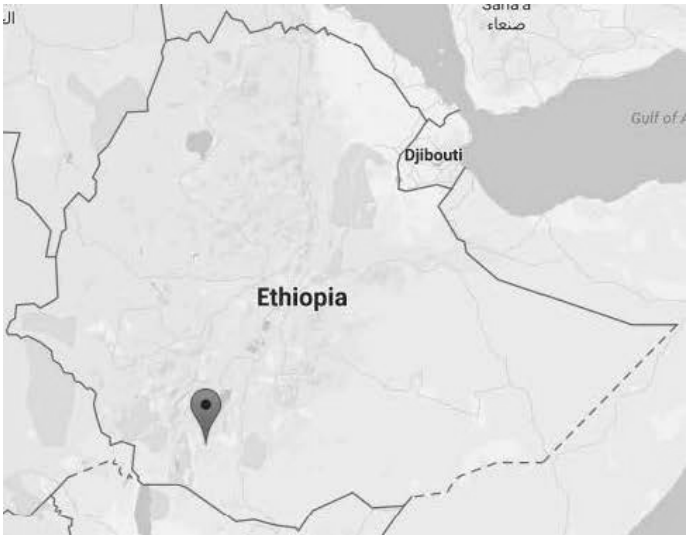

Figure 2.1: Map of Ethiopia; the city of Konso is marked by the bubble (Google Maps, 2017)

The history of this country is made more complex by the political upheaval and change over the last few decades, during which the country moved through periods of imperial rule, outsider occupation, a military regime, and recently, more democratic governance (Angassa and Oba, 2008; Holden and Yohannes, 2002; Tache and Oba, 2009). One of the largest changes occurred when the Haile Selassie I imperial regime was overthrown by the Derg military regime, replacing the old order with a socialist government in 1974 (Tache and Oba, 2009). One of the new governing actions was to institute the Land Reform Proclamations of 1975, resulting in the government's seizure and redistribution of almost all land in the country (Tache and Oba, 2009). The results of this policy had different impacts on pastoralists and farmers. Farm lands were redistributed according to family size, with no guarantees of land tenure (Holden and Yohannes, 2002). Pastoral grazing lands, traditionally organized according to water access, were redistributed with the aim of beginning ranch-like production (Tache and Oba, 2009). These new boundaries did not take into account traditional water-use rights and caused fragmentation of the pastoral communities (Tache and Oba, 2009). Both

farmers and pastoralists were also affected by a policy of resettlement and villagization aimed at "promoting the objectives of the state" by moving people where the government wanted them to be (Cohen and Isaksson, 1988). The military regime ended in 1991, but disputes over land and land use persist (Tache and Oba, 2009; Watson, 2006).

Pastoralists in southern Ethiopia traditionally focused on raising cattle but several aspects of pastoralists' lifeways have changed in recent times in response to the ecological and political climate (Boru et al., 2014). In more recent years, there is a trend towards supplementing cattle pastoralism with some crops (Angassa and Oba, 2008). There is also a trend towards livestock diversification, with some pastoralists also raising goats in addition to their traditional cattle herds, because goats are considered to be more drought tolerant than cattle (Angassa and Oba, 2008).

Farmers in Southern Ethiopia are known for their traditional methods of water and soil conservation. Along with staple crops such as ensete (false banana), maize, sorghum, coffee, barley and wheat, farmers also keep some livestock. Animals in these farming regions include cattle, goats, sheep and chickens, which are used mainly for their by-products—wool, milk, eggs, and dung (Förch, 2003).

2.3.2 Stable Carbon Isotope Analysis

Carbon and nitrogen isotope analyses create a useful bivariate data set for examining diets of past (and present) individuals (Lee-Thorp, 2008). Addition of other stable isotope analyses (sulphur, oxygen, or hydrogen), can improve our interpretation of the data and our understanding of past diets (Mützel (Rauch) et al., 2009). Different

types of dietary information can be gleaned from analysis isotopes of each element (Lee-Thorp, 2008).

Carbon isotope analysis compares the ratio of 1 ^{13}C to 1000 ^{12}C (expressed as $\delta^{13}C$ (‰)), and can differentiate between proteins garnered from C_3 and C_4 plants (van der Merwe and Vogel, 1978; Vogel and van der Merwe, 1977). The differentiation between these plant types is possible because C_3 and C_4 plants utilize different photosynthetic pathways to incorporate atmospheric CO_2 (Lee-Thorp, 2008). Compared to C_3 plants, C_4 plants are less likely to select for lighter atmospheric CO_2 containing ^{12}C, and so have a higher $\delta^{13}C$ ratio (Lee-Thorp, 2008). There is one other common photosynthetic pathway: crassulacean acid metabolism (CAM) which incorporates aspects of both C_3 and C_4 photosynthetic pathways such that CAM plants have differing $\delta^{13}C$ signatures depending on the climate (Ambrose and Norr, 1993).

2.3.3. Nitrogen Isotope Analysis

Nitrogen isotope analysis, comparing the ratio of 1 ^{15}N to 1000^{14}N (expressed $\delta^{15}N$ (‰)), can reveal an individual's trophic level given an approximate +3‰ enrichment in the heavier isotope, ^{15}N, at each increasing level in the food chain (Lee-Thorp, 2008; Schoeninger and DeNiro, 1984). Though the pattern of nitrogen isotopic enrichment is present in all food chains, the mechanism behind it is not well understood (Ambrose, 1991). Isotopic data from the bottom of the local, contemporaneous food chain is critical in these studies, however, as not all food chains start with the same nitrogen signature at the plant level (Ambrose, 1991; Lee-Thorp, 2008). Animals and

plants from arid climates, for example, have been found to have unusually enriched $\delta^{15}N$ values (Heaton et al., 1986).

2.3.4. Stable Sulphur Isotope Analysis

Sulphur isotopic analysis, comparing the ratio of 1 ^{34}S to 1000 ^{32}S (expressed as $\delta^{34}S$), can be applied to dietary and migration questions (Bollongino et al., 2013; Nehlich, 2015). There are three main reservoirs—the ocean, rocks, and gases—which can influence isotopic ratios. The ocean reservoir is relatively constant at about 20.3‰ $\delta^{34}S$; inland signatures can vary according to the underlying geology, and the majority of these signatures range from -20‰ to +30‰, though there are some extreme cases outside of this range (Nehlich, 2015). Freshwater environments are also variable in their $\delta^{34}S$ values—most falling between -5 and +15‰; these values reflect soluble portions of local rocks leaching into the freshwater system (Nehlich, 2015). When used in combination with carbon isotopes, it is possible to separate marine, freshwater, and C_3 terrestrial diets (Nehlich, 2015).

The Konso region in Southern Ethiopia is unlikely to be affected by sea-spray due to its distance from the coast, but $\delta^{34}S$ isotopes are still expected to vary across the region because of the geologic diversity of the landscape (Figure 2.1). The Southern Rift Valley in this region of Ethiopia is a volcanic feature that is highly geologically variable, and could influence the $\delta^{34}S$ signatures in this region (Tadesse et al., 2003; Woldegabriel et al., 1990).

2.3.5. Hair Growth and Chemistry

Hair provides a unique view into the short-term diet of an individual because of its quick growth rate and unchanging chemistry after formation (LeBeau et al., 2011; Williams et al., 2011). This contrasts with bone collagen, which has a turn-over rate of ~10-30 years and is constantly being remodelled (Britton et al., 2013). Hair analyses can focus on short-term diet in two ways: bulk analysis of hair to examine average diet over the last few months (represented by the total growth of hair used in the sample), or serial analysis of hair strands to look at sequential changes in diet over a period of months (Schwertl et al., 2003; Webb et al., 2013). In archaeological studies, an average hair growth rate of 1 cm/month is commonly used to relate isotope values to time (Webb et al., 2013). Even when using this average growth rate, it is important to be aware of the variability around the average when interpreting the isotopic signatures within a timeframe.

When considering bulk diets, it is important to know how long it takes for the body to equilibrate to a new diet (McCullagh et al., 2005). There is no standard value for the length of time for the body reservoir to adjust to a new diet; rather, there are a few different values given in modern case studies, ranging from 2.3 to 4 months for complete turnover of the body's carbon reservoir (McCullagh et al., 2005; Petzke et al., 2010). It has also been noted that the rate of turnover can change during a period of months or years; for example, increased physical activity levels (PAL) were found to increase the rate of nitrogen turnover in the body (Huelsemann et al., 2009; Petzke et al., 2010).

2.4. Ethics

The proposal for the ethnographic pilot study and collaborative isotopic analysis was submitted to the IRB ethics board at Southern Methodist University in December 2012 and accepted.

2.5. Materials and Methods

2.5.1 Hair Samples

The hair samples were collected on an opportunistic basis from people encountered walking on the highway between Konso and Arba Minch in southern Ethiopia (Figure 2.1). Local participants were told about the use of their hair, gave verbal consent, and were paid a small sum of money (~$2.50 USD) for a hair clipping. When the hair samples were collected, each individual was asked to self-identify with an economic strategy. Two blind-test samples—goat hair from a skin bag (expected herbivore), and a hair clipping from an American man (who ate a typical American omnivorous diet)—were also included to test the ability of multiple stable isotope analysis to differentiate between groups and act as controls.

In total, 49 Ethiopian individuals gave hair samples. These participants belong to six different ethnic groups that can be characterized by three different economic strategies.

Group 1: Pastoralists: The Tsemay (n=19) and Benna (n=1) are mainly cattle pastoralists who are seasonally mobile within a fixed territory and eat large quantities of butter and fermented milk. They trade cattle products (mostly butter) with neighbouring farmers for crops to supplement their diet.

Group 2: Farmers: The Konso (n=10), Gidole (n=10) and Gawada (n=1) are farmers whose major crops are sorghum, maize, coffee, cabbage trees and barley, with some wheat and banana grown commercially. Similar to most farmers in the region, these people raise small livestock such as goats and chickens and occasionally cattle for draft purposes, and they trade with pastoralists for cattle products.

Group 3: Fishing Farmers: The Gamo (n=8) are also a farming group, but have a narrower selection of crops. These individuals have focused on growing ensete and sorghum along with some maize and coffee but also supplement their diet with fish from a nearby inland lake. Due to their smaller selection of crops, and access to fish as an alternate source of meat protein, they are considered separately from the other farming communities for isotopic analysis and comparison.

2.5.2. Carbon and Nitrogen Isotope Analysis of Hair

All hair samples were cleaned before analysis using a modified version of the procedure laid out in O'Connell and Hedges (1999). Each hair sample was rinsed and sonicated twice in deionized water (DI H2O) for ten minutes. Next the hair samples were soaked in a mixture of 2:1 (v:v) chloroform:methanol twice, first for 10 minutes and second for two hours. Following the chloroform methanol rinses, the hair samples were rinsed with small amounts of DI H2O three times. The hair samples were then left to dry at 30°C overnight.

For each individual, the cleaned hair was cut into roughly 1 cm long segments, randomized and wrapped in tin weighing capsules for analysis. Samples weighed between 0.15 and 0.4 mg.

C and N isotopic values were measured using a MicroCube elemental analyser coupled to an Isoprime isotope ratio-mass spectrometer (EA-IRMS). All isotopic data were calibrated using USGS 40 (accepted $\delta^{13}C$ = -26.39, $\delta^{15}N$ = -4.52) and USGS 41 (accepted $\delta^{13}C$ = 37.63, $\delta^{15}N$ = 47.57). This calibration also scaled the data to international scales: Vienna Pee Dee Belemnite (VPDB) for carbon and the ambient inhalable reservoir (AIR) for nitrogen. Internal and international check standards were used, including USGS 42 (accepted $\delta^{13}C$ = -21.09, $\delta^{15}N$ = 8.05), USGS 43, (accepted $\delta^{13}C$ = -21.28, $\delta^{15}N$ = 8.44), Methionine (internal accepted $\delta^{13}C$ = -28.6, $\delta^{15}N$ = -5.0), and SUBC 1 (internal collagen standard with accepted $\delta^{13}C$ = -13.7, $\delta^{15}N$ = 17.4). Quality control and quality assurance measurements are included in Appendix 1.

2.5.3. Sulphur Isotope Analysis of Hair

All hair samples were cleaned in the same manner as for the carbon and nitrogen isotope analyses. The only differences in sample preparation were 1) vanadium pentoxide was included with all samples as an accelerant to assure the sample burned completely; and 2) ideal sample mass was 1 mg.

Sulphur isotopic measurements were made using a MicroCube elemental analyser coupled to an Isoprime 100 isotope ratio mass spectrometer (EA-IRMS). All isotopic data were calibrated to the international sulphur scale, Vienna Canyon Diablo Troilite, using S-1 (accepted $\delta^{34}S$ = -0.30) and NBS-127 (accepted $\delta^{34}S$ = 20.3). Internal and international check standards were used, including S-3 (accepted $\delta^{34}S$ = -32.3), S-2 (accepted $\delta^{34}S$ = 22.70), SO-5 (accepted $\delta^{34}S$ = 0.5), NIST 1577c (accepted $\delta^{34}S$ = 2.3), USGS 42 (accepted $\delta^{34}S$ = 7.84), and USGS 43 (accepted $\delta^{34}S$ = 10.46).

2.5.4. Statistical Tests and Modelling

Statistical tests and modelling were done using SPSS 22. Non-parametric (rank) tests were used due to small sample size and non-normal distribution of data within certain subsistence groups. Kruskal-Wallis tests for k-independent samples were used to examine whether there were significant differences between subsistence groups for each measured isotope. Significant Kruskal-Wallis tests were followed by post-hoc Mann-Whitney U tests. Examination and comparison of other variables (e.g., sex and age) were limited due to small sample size.

2.6. Results

Fifty one hair samples were analysed, including two samples that were included as controls—hair from a goat skin bag ($\delta^{13}C = -19.7 \pm 0.9$, $\delta^{15}N = 8.1 \pm 0.4$, $\delta^{34}S = 10.5$), and hair from one of the American ethnographers ($\delta^{13}C = -17.0 \pm 0.1$, $\delta^{15}N = 9.4 \pm 0.2$)—and will not be discussed further.

Isotopic averages for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ for each of the 49 individuals in the study groups are presented in Table 1. Although the C:N ratios of some analyses are outside the expected range (3.0-3.8) (O'Connell and Hedges, 1999), all samples were cleaned of all particulate and lipid contamination; also, all the samples are modern and cannot have undergone diagenetic alteration. The sulphur data was examined for poor %S and low peak height, which are indicative the sample not being burned completely. These runs are not included in the averages presented in Table 2.1.

Table 2.1: Isotopic averages and standard deviations for each individual examined.

Sample	FS	Subsistence	Group	Sex	Age	Age Category	Avg $\delta^{13}C_{VPDB}$	$\delta^{13}C$ stdev	Avg $\delta^{15}N_{AIR}$	$\delta^{15}N$ stdev	Avg $\delta^{34}S_{VCDT}$	%C	%N	% S	C:N
3815	FS 11	Pastoralist	Tsemay	Male	60-65	Elderly (50+)	-10.7	0.1	13.2	1.0	7.5	47.1	14.8	4.4	3.7
3817	FS 33	Pastoralist	Tsemay	Male	12 15	Child/Youth (Under 15)	-11.7	0.1	12.3	0.2	8.4	45.5	14.6	4.6	3.7
3819	FS 17	Pastoralist	Tsemay	Male	12 15	Child/Youth (Under 15)	-12.7	0.4	16.5	0.7	5.7	45.7	14.8	4.4	3.6
3823	FS 06	Pastoralist	Tsemay	Female	18-20	Adult (15-50)	-12.8	0.1	12.7	0.0	7.4	50.0	13.6	4.2	4.3
3824	FS 07	Pastoralist	Benna	Male	20-25	Adult (15-50)	-12.2	0.3	14.5	1.2	7.4	46.2	14.3	4.6	3.8
3825	FS 21	Pastoralist	Tsemay	Male	10 12	Child/Youth (Under 15)	-11.0	0.1	12.7	0.2	7.5	48.7	14.5	4.7	3.9
3826	FS 26	Pastoralist	Tsemay	Male	40-45	Adult (15-50)	-9.8	0.3	12.1	0.0	7.9	48.7	14.2	4.8	4.0
3827	FS 28	Pastoralist	Tsemay	Male	15-18	Adult (15-50)	-11.7	0.0	12.2	0.3	8.1	48.1	15.2	4.6	3.7
3832	FS 25	Pastoralist	Tsemay	Female	18-20	Adult (15-50)	-11.7	0.5	10.6	0.3	6.0	47.5	14.2	4.6	3.9
3836	FS 04	Pastoralist	Tsemay	Female	25-30	Adult (15-50)	-11.0	0.1	12.2	0.8	5.9	48.3	14.4	5.0	3.9
3837	FS 08	Pastoralist	Tsemay	Male	18-20	Adult (15-50)	-12.2	0.3	20.2	0.2	6.3	50.9	13.7	4.5	4.4
3842	FS 50	Pastoralist	Tsemay	Female	6 - 7	Child/Youth (Under 15)	-11.4	0.2	13.3	0.1	10.9	48.7	14.3	4.5	4.0
3843	FS 05	Pastoralist	Tsemay	Female	20-25	Adult (15-50)	-11.6	0.3	19.6	1.6	7.0	47.6	14.0	4.6	4.0
3844	FS 20	Pastoralist	Tsemay	Male	12 -14	Child/Youth (Under 15)	-12.6	0.3	14.1	0.4	6.9	49.3	14.1	5.2	4.1
3847	FS 32	Pastoralist	Tsemay	Male	60-65	Elderly (50+)	-11.4	0.4	13.1	0.0	6.4	49.7	13.8	4.7	4.2
3849	FS 49	Pastoralist	Tsemay	Male	40-45	Adult (15-50)	-10.9	0.1	12.7	0.1	6.8	49.6	13.7	5.0	4.2
3853	FS 35	Pastoralist	Tsemay	Male	20-25	Adult (15-50)	-11.1	0.5	11.8	0.3	7.3	48.4	14.1	4.6	4.0
3854	FS 38	Pastoralist	Tsemay	Male	18-20	Adult (15-50)	-12.2	0.2	14.3	0.2	7.3	47.3	13.6	4.4	4.1
3857	FS 45	Pastoralist	Tsemay	Female	20-25	Adult (15-50)	-12.1	0.3	13.3	1.0	6.8	46.7	13.9	5.4	3.9
4178	FS 34	Pastoralist	Tsemay	Female	20-25	Adult (15-50)	-11.9	0.3	12.8	0.5	5.0	48.0	13.6	4.2	4.1
3822	FS 03	Peasant Farmer	Gidolé	Female	25-28	Adult (15-50)	-12.1	0.1	8.4	1.3	10.2	49.0	14.7	4.4	3.9
3828	FS 46	Peasant Farmer	Konso	Female	40-45	Adult (15-50)	-10.6	0.0	8.2	0.1	10.8	47.8	14.3	4.6	3.9
3829	FS 13	Peasant Farmer	Konso	Male	70-75	Elderly (50+)	-10.5	0.1	8.3	0.4	10.6	47.9	14.5	4.5	3.9
3830	FS 16	Peasant Farmer	Gidolé	Male	50-55	Elderly (50+)	-9.2	0.1	9.2	0.9	12.1	49.3	16.3	5.4	3.5
3831	FS 18	Peasant Farmer	Konso	Female	20-25	Adult (15-50)	-13.7	0.1	6.2	1.1	9.1	47.8	14.7	4.3	3.8
3833	FS 36	Peasant Farmer	Gidolé	Male	15-18	Adult (15-50)	-11.5	0.1	8.8	0.1	11.7	48.0	14.2	4.5	3.9
3834	FS 37	Peasant Farmer	Gidolé	Male	30-35	Adult (15-50)	-10.5	0.3	8.4	0.2	11.4	49.7	14.1	4.7	4.2
3838	FS 19	Peasant Farmer	Gawada	Female	65+	Elderly (50+)	-13.1	0.6	9.3	0.1	6.8	52.7	14.1	5.1	4.4
3840	FS 47	Peasant Farmer	Gidolé	Male	65-70	Elderly (50+)	-9.7	0.2	9.1	0.0	11.2	48.0	13.8	5.1	4.1
3841	FS 48	Peasant Farmer	Konso	Male	65-70	Elderly (50+)	-12.7	0.2	9.1	1.2	10.4	48.0	14.3	5.1	3.9
3845	FS 22	Peasant Farmer	Konso	Female	18-20	Adult (15-50)	-14.5	0.2	6.5	1.4	11.3	47.4	13.9	5.3	4.0

Table 2.1 (cont.): Isotopic averages and standard deviations for each individual examined.

Sample	FS	Subsistence	Group	Sex	Age	Age Category	Avg $\delta^{13}C_{VPDB}$	$\delta^{13}C$ stdev	Avg $\delta^{15}N_{AIR}$	$\delta^{15}N$ stdev	Avg $\delta^{34}S_{VCDT}$	%C	%N	% S	C:N
3848	FS 39	Peasant Farmer	Konso	Female	18-20	Adult (15-50)	-14.5	0.4	6.1	0.5	10.5	48.7	14.3	5.1	4.0
3850	FS 02	Peasant Farmer	Konso	Male	20-25	Adult (15-50)	-13.8	0.0	7.2	0.2	9.7	50.2	14.3	4.7	4.1
3851	FS 09	Peasant Farmer	Konso	Female	30-35	Adult (15-50)	-11.0	0.1	7.9	0.8	11.7	47.2	14.5	5.0	3.8
3852	FS 24	Peasant Farmer	Gidolé	Female	17-19	Adult (15-50)	-12.7	0.3	7.7	1.3	11.9	48.2	14.1	4.4	4.0
3856	FS 42	Peasant Farmer	Konso	Male	18-20	Adult (15-50)	-14.3	1.3	5.7	0.3	11.7	50.9	14.4	5.4	4.2
3858	FS 10	Peasant Farmer	Gidolé	Female	8 - 10	Child/Youth (Under 15)	-11.8	0.2	9.2	1.7	11.1	49.0	14.2	4.6	4.0
3859	FS 12	Peasant Farmer	Konso	Male	45-50	Adult (15-50)	-12.1	0.2	7.4	1.4	12.5	47.4	13.9	4.8	4.0
3860	FS 14	Peasant Farmer	Gidolé	Male	15	Adult (15-50)	-12.4	0.1	7.9	1.4	12.7	48.0	14.4	5.4	3.9
3863	FS 29	Peasant Farmer	Gidolé	Male	16-18	Adult (15-50)	-9.8	0.2	10.0	1.0	12.2	47.1	14.5	4.4	3.8
3864	FS 30	Peasant Farmer	Gidolé	Female	20-25	Adult (15-50)	-12.6	0.0	6.7	0.0	11.5	49.2	13.5	4.3	4.3
3816	FS 44	Fishing Farmers	Gamo	Female	17	Adult (15-50)	-13.8	0.3	7.8	0.3	9.5	49.3	15.0	4.7	3.9
3820	FS 01	Fishing Farmers	Gamo	Female	14	Child/Youth (Under 15)	-14.5	1.2	7.3	1.3	9.0	45.7	14.9	5.3	3.6
3821	FS 31	Fishing Farmers	Gamo	Female	13-15	Child/Youth (Under 15)	-13.1	0.5	8.8	0.3	8.0	46.6	14.7	5.1	3.7
3835	FS 51	Fishing Farmers	Gamo	Female	15	Adult (15-50)	-13.3	0.1	10.3	0.2	8.4	46.6	14.8	4.6	3.7
3846	FS 27	Fishing Farmers	Gamo	Female	13	Child/Youth (Under 15)	-13.7	0.2	8.6	0.0	7.6	48.6	14.2	5.0	4.0
3855	FS 41	Fishing Farmers	Gamo	Female	18	Adult (15-50)	-13.1	0.3	9.3	1.0	8.7	46.1	14.2	4.8	3.8
3861	FS 15	Fishing Farmers	Gamo	Female	10-12	Child/Youth (Under 15)	-12.3	0.1	8.7	0.4	8.0	46.5	14.4	4.9	3.8
3862	FS 23	Fishing Farmers	Gamo	Female	11	Child/Youth (Under 15)	-11.4	0.3	8.5	0.7	8.1	48.2	14.5	4.6	3.9

Pastoralist hair isotopic signatures ranged from -12.8 to -9.8 with an average of -11.6±0.8 in $\delta^{13}C$, from 10.6 to 20.2 with an average of 13.7±2.4 in $\delta^{15}N$, and from 5.0 to 10.9 with an average of 7.1±1.2 in $\delta^{34}S$. Peasant farmer isotopic signatures ranged from -14.5 to -9.2 with an average of -12.1±1.6 in $\delta^{13}C$, from 5.7 to 10.0 with an average of 8.0±1.2 in $\delta^{15}N$, and from 6.8 to 12.7 with an average of 11.0±1.3 in $\delta^{34}S$. Fishing farmer isotopic signatures had the least variation overall, ranging from -14.5 to -11.4 with an average of -13.2±1.0 in $\delta^{13}C$, from 7.3 to 10.3 with an average of 8.7±0.9 in $\delta^{15}N$, and from 7.6 to 9.5 with an average of 8.4±0.6 in $\delta^{34}S$. Median values for each subsistence group are presented in Table 2.2.

Table 2.2 $\delta^{13}C$ and $\delta^{15}N$ medians by subsistence strategy

Subsistence Strategy	**n**	**$\delta^{13}C$ median**	**$\delta^{15}N$ median**	**$\delta^{34}S$ median**
Pastoralist	20	-11.7‰	13.0‰	7.1‰
Peasant Farmer	21	-12.1‰	8.2‰	11.3‰
Fishing Farmer	8	-13.2‰	8.1‰	8.5‰

Kruskal-Wallis tests were used to determine whether the isotopic signatures were significantly different between the three subsistence groups. The Kruskal-Wallis test of $\delta^{13}C$ values was statistically significant (X^2 (2, n=49) = 8.523, p = .014). The Fishing Farmers have a significantly lower mean rank (12.50) than the other subsistence groups. The Kruskal-Wallis test of $\delta^{15}N$ values was also statistically significant (X^2 (2, n=49) = 35.372, p < .001) (Table 2.2). The Peasant Farmers have the lowest mean rank (13.76) and Pastoralists have the highest mean rank (39.50) of the three subsistence groups. The Kruskal-Wallis test of $\delta^{34}S$ values was statistically significant (X^2 (2, n=49) = 30.887, p < .001) (Table 2.2). The medians between the three groups are spread out: Pastoralists (12.75) have the lowest mean rank, and Peasant Farmers (37.48) have the highest mean rank. All three isotopes (C, N, and S) are significantly different between the three groups, though the ranges do overlap (Figure 2.2).

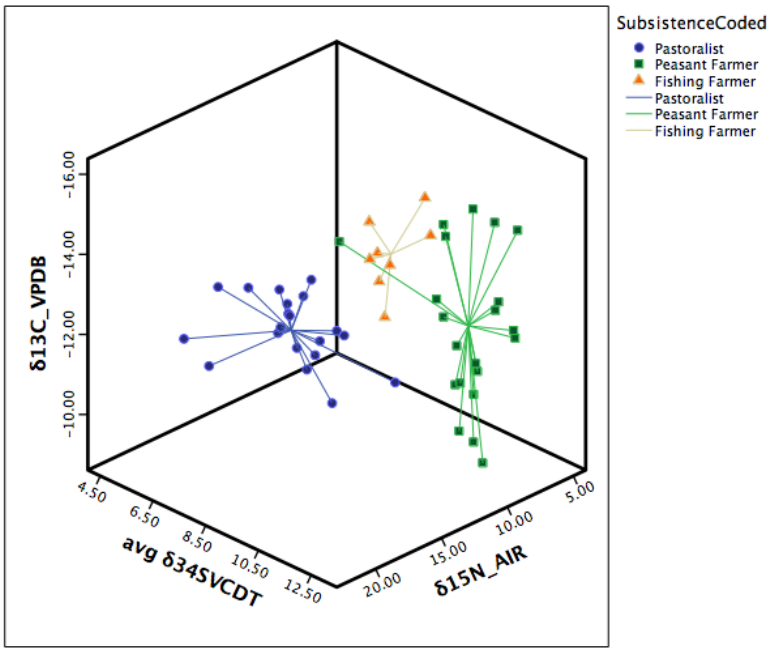

Figure 2.2: 3D scatterplot with centroids showing the separation of Peasant Farmers, Pastoralists and Fishing Farmers using $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values.

Posthoc pairwise comparisons were used to determine exactly where the significant differences in isotopic signatures exist between these three subsistence groups. As each of the three isotopes was compared between all three subsistence groups, Bonferroni adjustments were made to the significance level to avoid type one (false significance) errors, and α was adjusted to 0.017.

Three Mann-Whitney U tests were used to compare $\delta^{13}C$ values between Pastoralists (n=20, $\delta^{13}C$ median = -11.7‰), Peasant Farmers (n=21, $\delta^{13}C$ median = -12.1‰) and Fishing Farmers (n=8, $\delta^{13}C$ median = -13.2‰). The only significant difference in $\delta^{13}C$ values was between Pastoralists and Fishing Farmers (U=15.00; p=0.001) with the Fishing Farmers having significantly lower $\delta^{13}C$ values (Table 2.3; Figure 2.4)

Another series of three Mann-Whitney U tests was conducted to compare $\delta^{15}N$ values between Pastoralists ($\delta^{15}N$ median = 13.0‰), Peasant Farmers ($\delta^{15}N$ median = 8.2‰) and Fishing Farmers ($\delta^{15}N$ median = 8.7‰). Significant differences were found between the isotopic signatures of Pastoralists and Peasant Farmers (U=0.00; p=0.001, and between Pastoralists and Fishing Farmers (U=0.00; p=0.001) (Table 2.3).

The final series of Mann-Whitney U tests conducted to compare $\delta^{34}S$ values between Pastoralists ($\delta^{34}S$ median = 7.1‰), Peasant Farmers ($\delta^{34}S$ median = 11.3‰) and Fishing Farmers ($\delta^{34}S$ median = 8.2‰) revealed significant differences in $\delta^{34}S$ values between all three subsistence groups (Table 2.3).

Overall, this indicates that the only significant separation of all three isotopes between two subsistence groups is between Pastoralists and Fishing Farmers (Fig 2.2). $\delta^{13}C$ values are not distinctive between Pastoralists and Peasant Farmers or between Peasant Farmers and Fishing Farmers (Figures 2.3, 2.4). $\delta^{15}N$ values are not distinctive between Peasant Farmers and Fishing Farmers (Figures 2.3, 2.5). $\delta^{34}S$ is significant when comparing between all three groups (Figures 2.4. 2.5).

Table 2.3: Mann-Whitney Pairwise Comparisons between subsistence groups for carbon, nitrogen and sulphur isotopes. Mann-Whitney U values and p values presented for each comparison (row) by isotope (column). $\alpha = 0.017$ when significance level ($\alpha = 0.05$) is adjusted for 3 pairwise comparisons within each isotopic category.

Pairwise Comparison	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{34}S$**
Pastoralists and Farmers	U= 176.000 p = 0.375	U = 0.000 p < 0.001	U = 21.000 p < 0.001
Pastoralists and Fishers	U = 15.000 p = 0.001	U = 0.000 p < 0.001	U = 18.000 p = 0.002
Farmers and Fishers	U = 49.000 p = 0.088	U = 58.000 p = 0.205	U = 9.000 p < 0.001

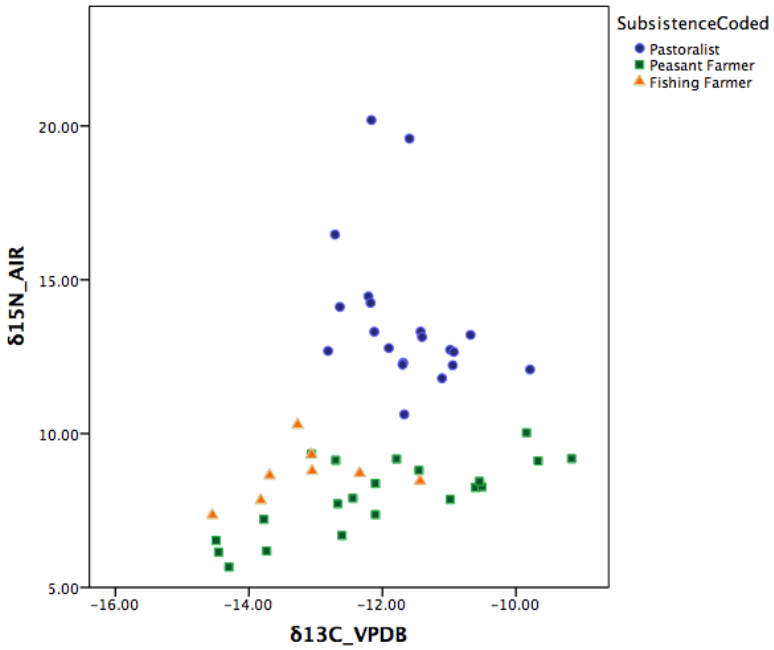

Figure 2.3: δ^{15}N by δ^{13}C values for Pastoralists, Peasant Farmers and Fishing Farmers shows the separation of δ^{15}N values between Pastoralists and Peasant Farmers and between Pastoralists and Fishing Farmers. The separation of δ^{13}C values between Pastoralists and Fishing Farmers is less visible but still statistically significant.

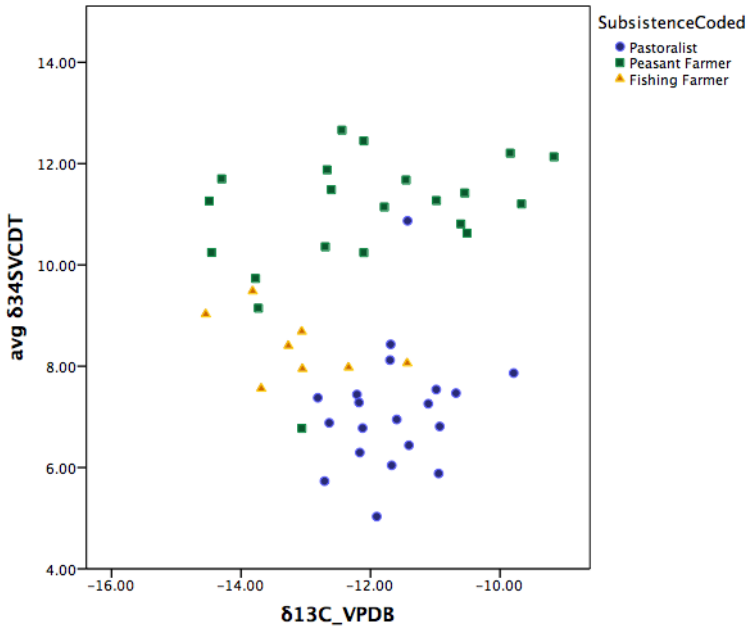

Figure 2.4: δ^{13}C by δ^{34}S values for Pastoralists, Peasant Farmers and Fishing Farmers shows the separation of δ^{34}S values between Pastoralists, Peasant Farmers and Fishing Farmers. The separation of δ^{13}C values between Pastoralists and Fishing Farmers is less visible but still statistically significant.

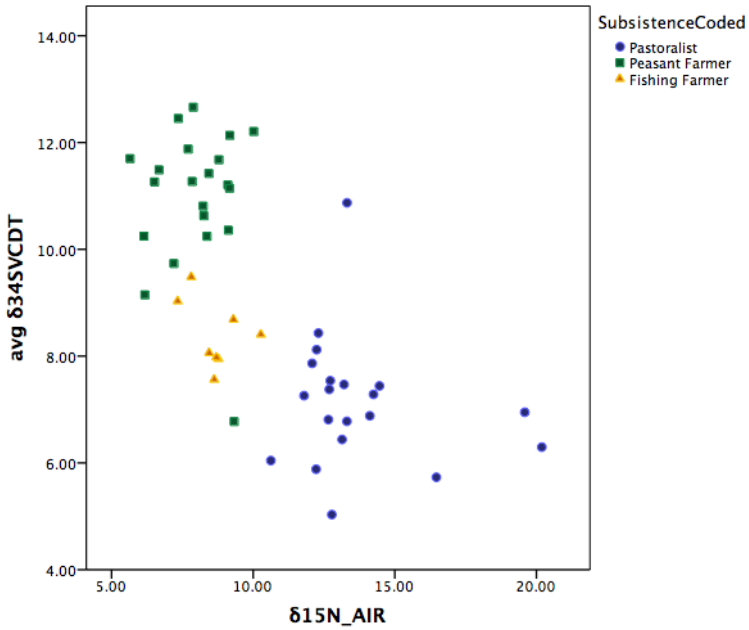

Figure 2.5: δ^{15}N by δ^{34}S values for Pastoralists, Peasant Farmers and Fishing Farmers shows the separation of δ^{34}S values between Pastoralists, Peasant Farmers and Fishing Farmers. The separation of δ^{15}N values between Pastoralists and the two Farming groups is also visible. Comparison of δ^{15}N and δ^{34}S creates the greatest visual separation in a bivariate scatterplot.

2.7. Discussion

The data show that interacting subsistence groups in southern Ethiopia have significantly different isotopic signatures. The dietary differences between the subsistence groups, however, are much clearer when examining all three isotopes together—carbon, nitrogen, and sulphur—because there is a higher degree of overlap in two of the three pair-wise isotopic ranges (Table 2.1).

The carbon isotopic values for all three subsistence groups fall within a relatively tight range from -14.5‰δ^{13}C (a Fishing Farmer individual) to -9.2‰δ^{13}C (a Peasant Farmer individual) (Table 2.1). These values are all consistent with a C_4 heavy diet, with variable amounts of C_3 sources contributing to the diet (Casey and Post, 2011; Vogel and van der Merwe, 1977). The lack of significant difference between Pastoralists and

Peasant Farmers (both of which are on the high end of the $\delta^{13}C$ range) indicates that these two groups are relying on similar plants at the bottom of the food chain (Figure 2.3). The similarity in carbon isotopic signatures between these two groups can be attributed to the plants being eaten either by the individuals themselves or their livestock, which are then consumed, because carbon does not fractionate much across trophic levels (Casey and Post, 2011). The recent adoption of plant cultivation by some pastoralists, and current governmental policies to encourage farming over pastoralism may also be contributing to the similar diets and, therefore, similar carbon isotopic signatures (Angassa and Oba, 2008; Fratkin, 2014).

The Fishing Farmers, however, have significantly lower $\delta^{13}C$ values than the Pastoralists, but do not show a significant difference when compared to the $\delta^{13}C$ values of the peasant farmers (Table 2.3). This indicates a greater difference in which plant resources are used between the Pastoralists and Fishing Farmers than between the two types of farmers in this region. The carbon isotopic signatures of the Fishing Farmers indicate that they may be eating foods with a higher proportion of C_3 based plants in the food chain. Another option is that the foods are the same, but the isotopic signatures of the plants may differ slightly due to regional climactic differences—access to water can change plant $\delta^{13}C$ values as well as species distributions, and access to the inland lake used by the Fishing Farmers may be indicative of a different microcline compared to the pastoral rangelands (Casey and Post, 2011; Farquhar et al., 1982). The difference in $\delta^{13}C$ values may also be due to more similarities in crop use between the farming communities than are shared between the fishing farmer community and the Pastoralist crop cultivars (Angassa and Oba, 2008)

Overall, $\delta^{13}C$ has the least amount of variability of the isotopes examined here (X^2= 8.523, p = 0.014) (Table 2.3). This means that examining plant protein sources in the diet between these three subsistence groups through $\delta^{13}C$ analysis alone is less diagnostic of dietary difference than either nitrogen isotopic analysis or sulphur isotopic analysis.

Nitrogen isotopic values have a greater overall range across the three subsistence groups, from 5.7‰$\delta^{15}N$ (a Peasant Farmer) to 20.2‰$\delta^{15}N$ (a Pastoralist) (Table 2.1). With an average enrichment of ~3‰ per trophic level, this spread indicates a range of meat intake across 4 to 5 trophic levels within the three subsistence groups (Lee-Thorp, 2008).

The Pastoralists have significantly higher $\delta^{15}N$ values (avg. 13.7±2.4‰) compared to both farming groups (Peasant Farmer $\delta^{15}N$ avg. 8.0±1.2‰; Fishing Farmer $\delta^{15}N$ avg. 8.7±0.9‰), indicating that the Pastoralists are regularly eating at a higher trophic level than the farmers (Figure 2.3). This is consistent with a greater reliance on cattle and cattle products. Pastoralists are known to consume a lot of butter and fermented milk; occasionally, blood will also be used (Galvin, 1992). The difference between the pastoralists and the farmers also indicates that Peasant Farmers and Fishing Farmers are not using their livestock as a major food source. This difference of livestock use is also consistent with ethnographic studies which show that livestock on farms are used mostly for their by-products, including dung (Förch, 2003).

The lack of a significant difference between Peasant Farmer $\delta^{15}N$ values and Fishing Farmer $\delta^{15}N$ values indicates that any fish supplementation by the Fishing Farmers does not change their trophic level by comparison (Table 2.3; Figure 2.3).

Though the Fishing Farmers have a slightly higher δ^{15}N signature compared to the Peasant Farmers, the lack of significant difference may indicate that (1) Fishing Farmers are not eating a significant quantity of fish; (2) the fish being eaten are low-trophic-level fish which are on the same trophic level as the terrestrial livestock; or (3) there were not enough individuals sampled, particularly from the Fishing Farmer group, to confirm the significance of this relatively small difference in δ^{15}N isotopic signatures between the groups.

Sulphur isotopic values are significantly different between all three groups. Pastoralists have the lowest δ^{34}S values (avg. 7.1±1.2‰), and Peasant Farmers have the highest δ^{34}S values (avg. 11.0±1.3‰); the Fishing Farmers δ^{34}S values (avg. 8.4±0.6‰) fall between the two other subsistence groups. The absolute differences between these groups is not large, but they are significant (Figures 2.4, 2.5).

Sulphur isotope values vary due to geology of the region as well as sea spray; they do not vary much across trophic levels (Nehlich, 2015) Sea spray is unlikely to be affecting these values due to Konso's distance from the coast. The sulphur values may instead indicate that the individuals in these different groups inhabit slightly different geological niches, such that the plants and food items that they rely on have different sulphur values. The geology of the Southern Rift Valley is volcanic based and highly variable which could also influence the variability of δ^{34}S in the diet (Tadesse et al., 2003; Woldegabriel et al., 1990).

2.8. Conclusions

The data in this study show that multi-isotope analysis is very useful for identifying differences in human diet among groups of people. Though pairwise comparisons of individual isotopic signatures between groups did not always test as significant (for example $\delta^{13}C$ values between peasant farmers and fishing farmers), each of the groups can be identified in three-dimensional space using the combination of carbon, nitrogen, and sulphur. These patterns of isotopic signature are strong enough that even though current governmental policies encourage convergence of subsistence systems, there are still significant differences in isotopic signatures between individuals who identify as pastoralists, peasant farmers or fishing farmers (Fratkin, 2014).

These results also show that, particularly when the isotopic data are combined, the isotopic variation among groups in the same region is greater than the isotopic variation within each of the groups. That these differences in isotopic signatures among groups occur even when the individuals have access to the same resource base indicates that differences in diet can be attributed to choice of consumables, potentially driven by culture and/or affiliation with a particular group of people or economic strategy.

3. Isotopic analysis of dietary variability between demographic groups of Nicaraguan villagers

3.1 Case Study Overview

Carbon and nitrogen stable isotope analysis of modern human hair from Nicaragua was used to explore what constitutes significant differences in gross diet between and among demographic groups within the same population. Our results show that, while the absolute differences between isotopic central tendencies of demographic groups are small, some are statistically significant. Socioeconomic categories that were found to have significantly different isotopic signatures between or among groups included age, location, and wealth.

3.2 Introduction

Isotopic analysis of human body tissues is a powerful tool for characterizing diets, especially protein sources in diets. In modern societies, nutritionists have identified differences in diet and nutrition between demographic groups, including between sexes (Christiaensen and Alderman, 2004; Hadley et al., 2008), among age groups (Lamontagne et al., 1998; Winking and Koster, 2015), among classes (Barros et al., 2010) or among families with differing levels of education (Christiaensen and Alderman, 2004; Sakisaka et al., 2006).

Here we present data from isotopic analysis of human hair from a modern population in Nicaragua to probe what constitutes a significant difference in $\delta^{13}C$ and $\delta^{15}N$ signatures among demographic groups. In particular, we focus on the demographic variables that nutritionists have noted as important factors in food security, including sex, age, parental education, wealth, and family size.

3.3 Background

3.3.1 Sampling Site

The modern hair samples were collected as part of a longer on-going study of the Mayangna and Miskito peoples who live in the villages of Arang Dak and Suma Pipi on the Bosawas Biosphere Reserve in Nicaragua (Figure 3.1). These villages are located 1 km apart on the Lakus River, and are relatively isolated, though some commercial goods are imported along the rivers (Koster, 2011). Although these two Indigenous groups are linguistically and ethnically distinct, they are considered part of the same population in this study due to 1) their close proximity, 2) close kin ties created by frequent intermarriage between the groups, and 3) similar subsistence strategies (Koster, 2011).

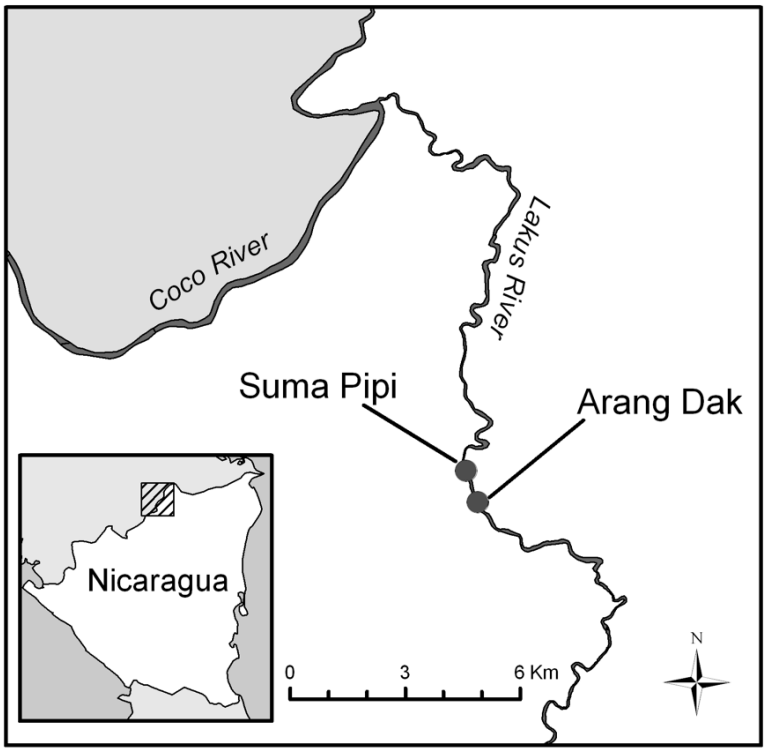

Figure 3.1: Map of Nicaragua showing the locations of Arang Dak and Suma Pipi.

The dietary components in these two villages include horticultural crops, domesticated animals and animal products, and wild game from hunting and fishing (Koster, 2011). Common crops grown in Arang Dak and Suma Pipi are plantains, manioc, maize, rice, beans and bananas (Koster, 2011; Koster and Leckie, 2014). Pigs, chickens, and turkeys are domesticated animals that are consumed on a regular basis, whereas cattle are valued primarily for their monetary value and so are slaughtered only rarely (Koster, 2011). Hunting and fishing also add to the available dietary protein. Hunted games species in the Bosawas Biosphere Reserve include pacas, armadillos, peccaries and tapirs, which are almost exclusively hunted by men. Fishing is an activity done by men, women and children (Koster et al., 2016).

The main social unit in these communities is the nuclear family (Koster, 2011; Winking and Koster, 2015). New households usually form after a couple has had their first child; until this point, most young couples will reside with the woman's family (Koster, 2011; Winking and Koster, 2015). Nuclear families can include some extended members as single mothers will reside with their parents; widowers and widows will also move in with one of their children if they are past reproductive age (Koster, 2011).

3.3.2 Nutrition Studies

Many nutritional studies focus on whether or not people are food secure. Food security is defined by the Food and Agricultural Organization of the United Nations (FAO)—as existing "when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (Bacon et al., 2014). To study food security, nutritionists take

anthropomorphic measurements such as height, age, weight, and mid-upper-arm-circumference (MUAC) to track growth, health and nutrition, but also pay attention to whether or not individuals worry about having enough food (Belachew et al., 2013; Reyes-García et al., 2008). Nutritionists have noted cross-cultural trends in food security associated with factors such as sex, age, education, and wealth.

Sex is a commonly examined factor when comparing nutritional data and exploring which demographic groups are most at risk for food insecurity (Cronk, 2000). One study of food security of adolescents in Ethiopia reported that 40% of girls reported feeling food insecure when their brothers did not (Hadley et al., 2008). A nutritional study focused on children under the age of two in Granada, Nicaragua noted that being female was a risk factor for a child being underweight (Sakisaka et al., 2006). The opposite trend was found in another study based in Ethiopia, where data showed that boys were more likely to be malnourished than girls (Christiaensen and Alderman, 2004).

Nutrition and food security has also been noted to vary due to an individual's age. One factor in nutritional differences between age groups is that parents may create a buffer to protect their children from nutritional stress (Hadley et al., 2008; Leonard, 1988). The other factor is whether older or younger children are buffered from food-stress. A previous study focused on the people of Arang Dak and Suma Pipi found a negative correlation of height-for-age scores (HAZ), which indicates the children were falling further below height standards as they grew up (Winking and Koster, 2015).

Many nutritional studies point to education as being crucial for good nutrition. In both Nicaragua and Ethiopia, the data show a positive correlation between the mother's education and her children's nutrition as measured by HAZ scores (Christiaensen and

Alderman, 2004; Cochrane et al., 1982; Sakisaka et al., 2006). The father's education did not have as strong a correlation with children's health (Christiaensen and Alderman, 2004).

Wealth is also considered a very important factor. In a meta-analysis of data from over 200 published articles and from data collected in an additional 100 surveys, Barros et al. (2010) found that not only is wealth correlated with better living conditions, but also that children from poorer households are more likely to be undernourished compared to children from more affluent families. One study focused on a population in the Bolivian Amazon and found that wealth, particularly wealth invested in livestock, was positively correlated with the nutritional status of adults, showing that wealth can affect the nutrition of all individuals in a family (Godoy et al., 2005). The importance of wealth has also been noted at our study site: a measure of men's paternal investment was positively correlated with household wealth and children's growth (Winking and Koster, 2015).

3.3.3. Hair Growth and Chemistry

Hair is a useful substrate for isotopic analysis for two main reasons. First, hair provides a unique view into an individual's short-term diet due to its fast rate of growth and stable chemistry after formation (LeBeau et al., 2011; Williams et al., 2011). Second, hair can be sampled from living individuals non-invasively, which makes it an ideal substrate when studying modern populations (LeBeau et al., 2011).

As with all tissues in the human body, the chemical composition of a human's hair is directly related to diet as compounds that are consumed are routed or broken down

and used in the construction of all tissues (O'Connell et al., 2001). Human hair grows at a rate of approximately 1cm per month, and is unaltered after formation, making it possible to use isotopic analysis of hair to probe a variety of different questions about an individual's diet (Webb et al., 2013). Bulk analysis of hair can be used to probe an individual's average diet over the past few months (Mützel (Rauch) et al., 2009). Serial isotopic analysis of hair, either through regular resampling from an individual, or from analysing sequential segments of hair, can show change in diet over time (Huelsemann et al., 2009; Knudson et al., 2007). Methodologies for sampling hair become more complex when relating the isotopic information from hair samples to specific periods of time (Huelsemann et al., 2009; LeBeau et al., 2011; Schwertl et al., 2003).

3.3.4 Carbon and Nitrogen Isotopes in Dietary Studies

Carbon and nitrogen isotope analyses create a useful bivariate data set for examining diet (Lee-Thorp, 2008).

Carbon isotope analysis compares the ratio of 1 ^{13}C to 1000 ^{12}C (expressed as $\delta^{13}C$ (‰)), and can differentiate between proteins ingested from C_3 and C_4 plants (van der Merwe and Vogel, 1978; Vogel and van der Merwe, 1977). C_3 and C_4 plants use different photosynthetic pathways to transform atmospheric CO_2 into sugar and biomass, which results in differences in the ratio of ^{13}C to ^{12}C (Lee-Thorp, 2008). C_4 plants are less likely than C_3 plants to discriminate against "heavy" CO_2 containing ^{13}C, and so have a higher $\delta^{13}C$ ratio (more ^{13}C to relative to ^{12}C) (Lee-Thorp, 2008). There is one other common photosynthetic pathway: crassulacean acid metabolism (CAM) which uses

aspects of both C_3 and C_4 photosynthetic pathways depending on the climate (Ambrose and Norr, 1993).

Nitrogen isotope analysis, comparing the ratio of 1 ^{15}N to 1000^{14}N (expressed $\delta^{15}N$ (‰)), can reveal an individual's trophic level because of an approximate +3‰ enrichment in ^{15}N at each increasing level in the food chain (Lee-Thorp, 2008; Schoeninger and DeNiro, 1984). Though the pattern of nitrogen isotopic enrichment is present in all food chains, the mechanism behind it is not well understood (Ambrose, 1991). Isotopic data from the bottom of the local, contemporaneous food chain is critical in these studies, however, because not all food chains start with the same nitrogen signature at the plant level (Ambrose, 1991; Lee-Thorp, 2008). Animals and plants from arid climates, for example, have been found to have unusually enriched $\delta^{15}N$ values (Heaton et al., 1986).

3.4 Ethics

The Institutional Review Board at the University of Cincinnati reviewed and approved the field study.

3.5 Materials and Methods

3.5.1 Hair Samples

In February 2013, hair samples were collected from each individual living in Arang Dak (n = 262) and Suma Pipi (n = 82). Hair samples were also collected from a family of individuals whose permanent residence was in a nearby community, Tawan Raya (n = 8), but had relocated to Arang Dak on a semi-permanent basis during the study

period. Hairs of approximately 1 to 2 inches (2.54 to 5.08 cm) in length were collected, taking care to retain only the lengths that are proximal to the scalp.

3.5.2 Carbon and Nitrogen Isotopic Analysis

Each hair sample was weighed and put into an individual, labelled test tube. The samples were then rinsed twice in deionized water (DI H_2O) and sonicated for 10 minutes during each rinse. The samples were then soaked in 2:1 v:v mixture of chloroform:methanol, first for 10 minutes, and then for 2 hours. Following the chloroform:methanol soaks, the samples were quickly rinsed with DI H_2O four times. The samples were then dried overnight at 30°C.

Once the samples were dried, each hair sample was cut into cm-long segments, randomized, and packaged into tin weigh boats for analysis. C and N isotopic analyses were carried out using a MicroCube elemental analyser coupled to an Isoprime isotope ratio-mass spectrometer (EA-IRMS).

All isotopic data were calibrated using USGS 40 ($\delta^{13}C$ = -26.39, $\delta^{15}N$ = -4.52) and USGS 41 ($\delta^{13}C$ = 37.63, $\delta^{15}N$ = 47.57). This calibration also scaled the data to international scales: Vienna Pee Dee Belemnite (VPDB) for carbon and the ambient inhalable reservoir (AIR) for nitrogen. Internal and international check standards were used, including USGS 42 ($\delta^{13}C$ = -21.09, $\delta^{15}N$ = 8.05), USGS 43, ($\delta^{13}C$ = -21.28, $\delta^{15}N$ = 8.44), Methionine (internal accepted $\delta^{13}C$ = -28.6, $\delta^{15}N$ = -5.0), and SUBC 1 (internal collagen standard with accepted $\delta^{13}C$ = -13.7, $\delta^{15}N$ = 17.4). Quality control and quality assurance measurements are included in Appendix 1.

3.5.3 Statistical Analyses

Data was collected at two different scales for different variables in this study: some data was collected at the individual level (e.g. isotopic measurements, age, sex), other variables reflect family-level data (e.g. wealth, family size) (Tables 3.1, 3.2). Along with traditional bivariate scatterplots to visually determine isotopic differences between groups, Kruskal-Wallis and Mann-Whitney tests were used to determine whether there were statistically significant differences in isotopic signatures between demographic groups. Non-parametric tests were used due to sample size and normality concerns.

δ^{13}C and δ^{15}N isotopic measurements for all individuals were used when comparing sex and age demographic groups. Age groupings were created according to ethnographically determined age categories (Koster, 2007). The infant group was defined as ages 0-2 due to the fact that 90% of women reported that their infants were weaned between the ages of 1 and 2. As it is known that breastfeeding infants have higher δ^{15}N signatures than their mothers (Fuller et al., 2006), it was important to keep this group separated from other children.

When analysing differences using family-level variables, individual isotopic measurements were combined to into measurements of isotopic central tendency and variability for the family level. For each family, the mean, median, range and IQR were calculated for δ^{13}C and δ^{15}N. Due to the known isotopic difference of breastfeeding infants, all infants were excluded from the family isotopic calculations.

Table 3.1: Individual variables collected, with descriptions of how the data were gathered and notes on how the data were categorized (if applicable).

Variable	**Measured/synthetic**	**How measured/synthesized**	**Notes**
$\delta^{13}C$	Measured	Hair sample analysed on EA-IRMS	
$\delta^{15}N$	Measured	Hair sample analysed on EA-IRMS	
Sex	Recorded	As reported	M/F binary
Age Group	Synthetic	Individuals grouped by ethnographically determined age categories	Infant: 0-2 (as defined by mode weaning age) Child: 3-12 Adolescent: 13-17 Adult: 18-64 Elderly: 65+ (age when adults have stopped working)
Village	Recorded	Where individuals were living when the hair samples were collected	Villages included Arang Dak, Suma Pipi, and Tawan Raya

Family level variables such as education, family size, and wealth, were each recoded into two groups—families with measurements at the median or below, and families with measurements above the median for the given variable. This created groupings between which it was possible to compare whether or not there was a significant difference between isotopic central tendencies or isotopic variation of high and low scoring families.

Table 3.2: Family variables collected, with descriptions of synthetic variables (calculated from the measured/collected variables, Table 3.1) and notes on synthesis/treatment.

Variable	Measured/synthetic	How measured/synthesized	Notes
δ^{13}C mean	Synthetic	Mean of all δ^{13}C signatures within the family	δ^{13}C signatures of infants were excluded from calculated value
δ^{13}C median	Synthetic	Median of all δ^{13}C signatures within the family	δ^{13}C signatures of infants were excluded from calculated value
δ^{13}C range	Synthetic	Range of all δ^{13}C signatures within the family	δ^{13}C signatures of infants were excluded from calculated value
δ^{13}C IQR	Synthetic	IQR of all δ^{13}C signatures within the family	δ^{13}C signatures of infants were excluded from calculated value
δ^{15}N mean	Synthetic	Mean of all δ^{15}N signatures within the family	δ^{15}N signatures of infants were excluded from calculated value
δ^{15}N median	Synthetic	Median of all δ^{15}N signatures within the family	δ^{15}N signatures of infants were excluded from calculated value
δ^{15}N range	Synthetic	Range of all δ^{15}N signatures within the family	δ^{15}N signatures of infants were excluded from calculated value
δ^{15}N IQR	Synthetic	IQR of all δ^{15}N signatures within the family	δ^{15}N signatures of infants were excluded from calculated value
Family Size	Measured	Reported number of family members	Infants included in this count Grouped scores as ≤ median (median = 8) and greater than median
Good mask fisher in family	Measured	Binary: 0 = no, 1 = yes	Refers to a man in the family
Good bow fisher in family	Measured	Binary: 0 = no, 1 = yes	Refers to a man in the family
Good female fisher in family	Measured	Binary: 0 = no, 1 = yes	

Table 3.2 (cont.): Family variables collected, with descriptions of synthetic variables (calculated from the measured/collected variables, Table 3.1) and notes on synthesis.

Variable	Measured/synthetic	How measured/synthesized	Notes
Good hunter in family	Measured	Binary: 0 = no, 1 = yes	
Good male fisher in family	Synthetic	Combined score of good mask fisher in family + good bow fisher in family	Resulted in scores ranging from 0 to 2
Good fisher in family	Synthetic	Combined score of good mask fisher in family + good bow fisher in family + good female fisher in family	Resulted in scores ranging from 0 to 3
Good provider in family	Synthetic	Combined score of good mask fisher in family + good bow fisher in family + good female fisher in family + good hunter in family	Resulted in scores ranging from 0 to 4
Male-head Spanish knowledge	Measured	Value is a percentage of the number of other villagers who said yes, the subject spoke fluent Spanish	This score reflects *perceived* knowledge of Spanish. Grouped scores into binary ≤ median (median = 44) and > median
Female-head Spanish knowledge	Measured	Value is a percentage of the number of other villagers who said yes, the subject spoke fluent Spanish	This score reflects *perceived* knowledge of Spanish. Grouped scores into binary ≤ median (median = 15) and > median
Male-head knowledge	Measured	Value is a percentage of the number of ethnobiological knowledge questions the subject answered correctly	Grouped scores into binary ≤ median (median = 86) and > median
Female-head knowledge	Measured	Value is a percentage of the number of ethnobiological knowledge questions the subject answered correctly	Grouped scores into binary ≤ median (median = 81) and > median
Wealth	Measured	Total monetary value of all key household goods in Nicaraguan cordobas	Grouped scores into binary ≤ median (median = 31446) and > median

3.6 Results

All hair samples were modern and clean, so all isotopic values were included in the analyses, even those whose C:N ratios fall outside of the "expected" range of 3.0-3.8 (O'Connell and Hedges 1999). To determine if there were significant differences in isotopic signatures between demographic groups, each demographic was considered separately.

3.6.1 Sex

Visually, no distinct isotopic groupings are apparent in a bivariate plot of $\delta^{13}C$ and $\delta^{15}N$ (Figure 3.2). The overlap of isotopic values on each axis result in non-significant differences in both carbon and nitrogen isotopic signatures of male and female villagers (Figure 3.3). There was no statistically significant difference (U = 13920, p = 0.102) in $\delta^{13}C$ values between males (n=172, median = -22.6 ‰) and females (n=180, median = -22.6‰). There was also no statistically significant difference (U=14679, p=0.401) in $\delta^{15}N$ values between males (n=172, median = 9.6‰) and females (n= 180, median = 9.6‰).

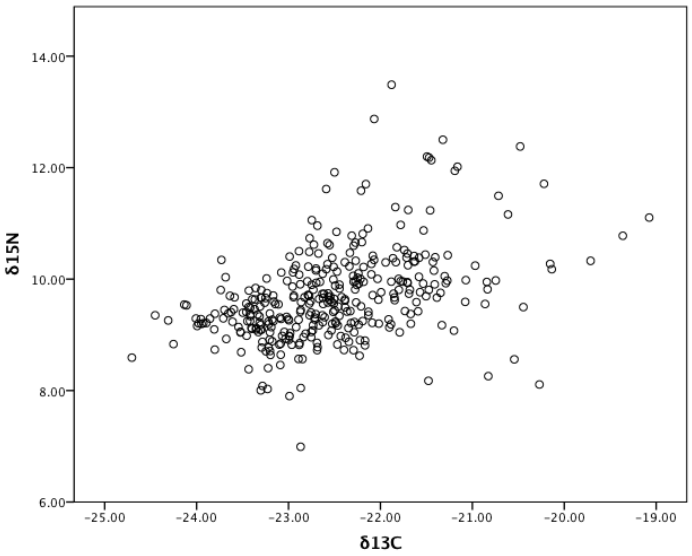

Figure 3.2: δ^{13}C and δ^{15}N scatterplot of all individuals

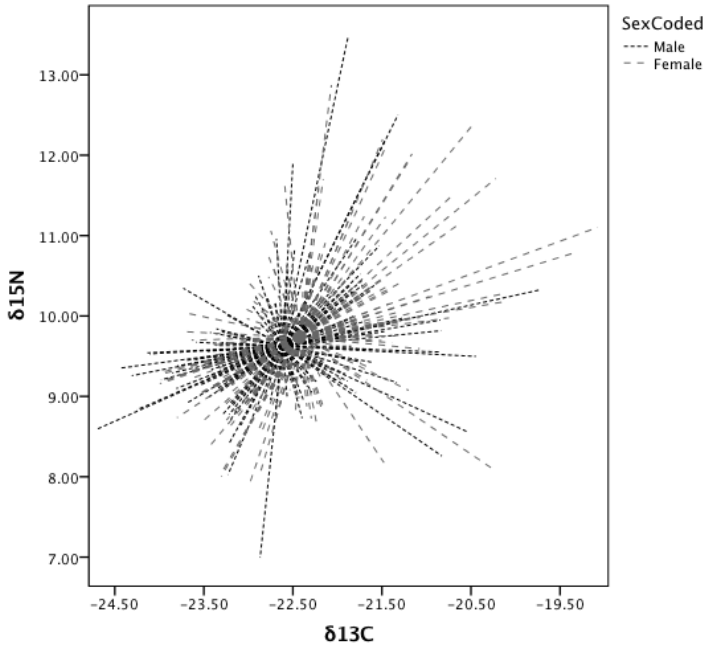

Figure 3.3: δ^{13}C and δ^{15}N scatterplot with centroids of all individuals coded by sex. Coordinates for each individual are found at the ends of the lines.

When controlling for which village each individual lives in, there was no statistically significant difference in δ^{13}C values or δ^{15}N values between males and females. There was, however, a statistically significant difference (U = 1709.000, p =

0.015) in δ^{13}C values between male (n=61, median = -23.2‰) and female (n=74, median = -23.0‰) children (Figure 3.4).

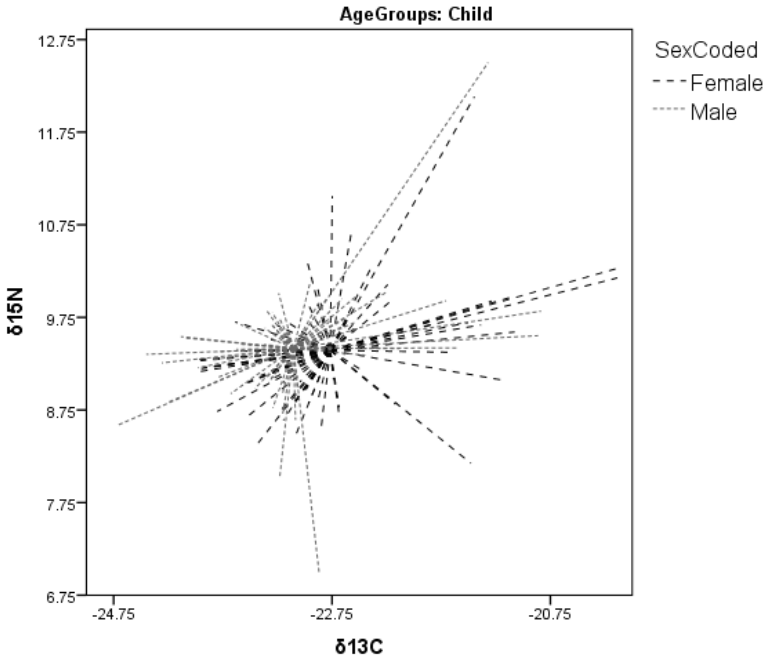

Figure 3.4: δ^{13}C and δ^{15}N scatterplot with centroids of all children, coded by sex

3.6.2 Age Groups

Kruskal-Wallis tests comparing isotopic values between age groups showed significant differences in δ^{13}C ($X^2 = 76.99$, df = 4, $p < 0.001$) and δ^{15}N ($X^2 = 86.85$, df = 4, $p < 0.001$). These results were followed up by post-hoc Mann-Whitney tests to determine which age groups have significantly different isotopic signatures. Due to the relatively small sample size of the Elderly category, this age group was removed from post-hoc testing. A total of 6 post-hoc tests for significant difference in δ^{13}C were used, requiring a Bonferroni adjustment, making $\alpha = 0.008$. The same Bonferroni adjustment ($\alpha = 0.008$) was used for the 6 post-hoc tests examining significant differences in δ^{15}N.

Visually, these differences among age groups cannot be identified using a traditional scatterplot, but become clearer when using a scatterplot with centroids (Figures 3.5, 3.6).

There were a number of significant differences in $\delta^{13}C$ between age groups (Tables 3.3, 3.4; Figure 3.5). Infant $\delta^{13}C$ signatures (median = -22.1‰) were significantly higher than child $\delta^{13}C$ signatures (median = -23.1‰). Infant $\delta^{13}C$ signatures were also significantly higher than adolescent $\delta^{13}C$ signatures (median = -22.7‰). Adolescents have significantly higher $\delta^{13}C$ signatures than children. Adults (median = -22.3‰) have significantly higher $\delta^{13}C$ signatures than children and adolescents.

There were also significant differences in $\delta^{15}N$ between age groups (Tables 3.3, 3.4; Figure 3.6). Infants had the highest $\delta^{15}N$ values (median = 10.9‰). Infant $\delta^{15}N$ values were significantly higher than children $\delta^{15}N$ values (median = 9.4‰). Infant $\delta^{15}N$ isotope values were also significantly higher than adolescent $\delta^{15}N$ values (median = 9.3‰), and significantly higher than adult $\delta^{15}N$ values (median = 9.8‰). Child and adolescent $\delta^{15}N$ values were not significantly different from each other, but both were significantly lower than adult $\delta^{15}N$ values.

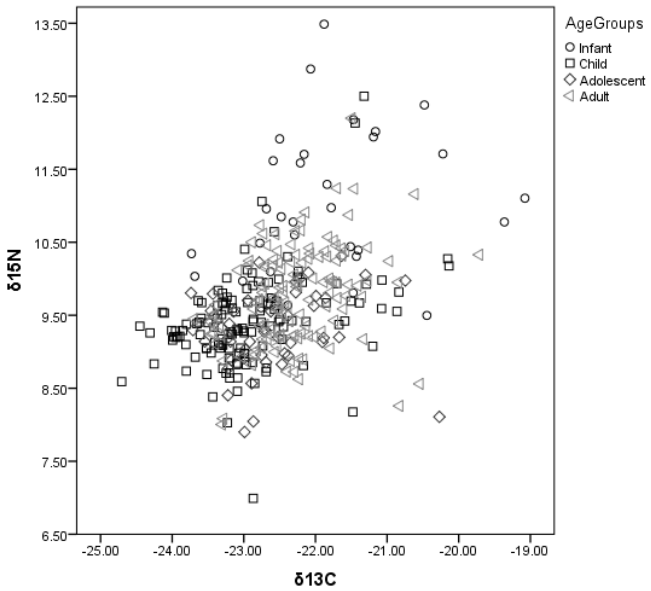

Figure 3.5: Scatterplot of δ^{13}C and δ^{15}N data for all individuals by age group

Table 3.3: Central tendencies of isotopic measurements by age group

Age Groups	**N**	**δ^{13}C mean**	**δ^{13}C median**	**δ^{15}N mean**	**δ^{15}N median**
Infants	31	-21.8 ± 1.1	-22.1	11.0 ± 1.0	10.9
Children	135	-22.9 ± 0.8	-23.1	9.4 ± 0.6	9.4
Adolescent	47	-22.6 ± 0.7	-22.7	9.3 ± 0.6	9.3
Adults	132	-22.3 ± 0.7	-22.3	9.8 ± 0.7	9.8
Elders	7	-21.7 ± 0.5	-21.7	10.1 ± 0.8	10.0

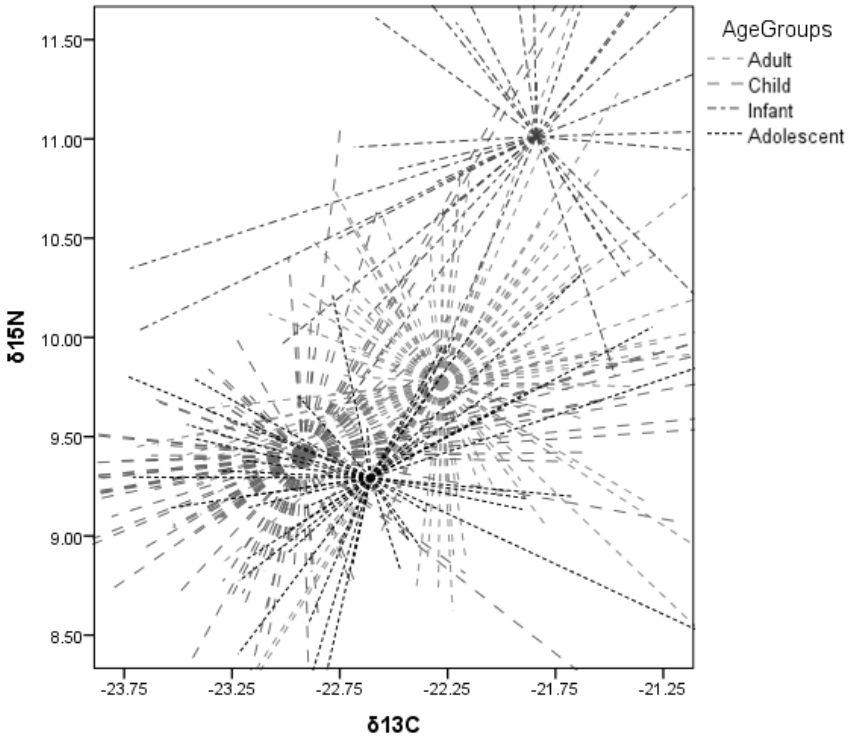

Figure 3.6: Scatterplot with centroids for age group data from all individuals except elders. Infants and adults are the most distinct groups; the centroids for children and adolescents are much closer together. The graph is focused on the centroids for visual clarity; some individuals fall outside the ranges of $\delta^{13}C$ and $\delta^{15}N$ values shown.

Table 3.4: Post-Hoc Mann-Whitney tests for $\delta^{13}C$ and $\delta^{15}N$ comparisons between Age Groups

	Infant	**Child**	**Adolescent**	**Adult**
Infant		$\delta^{15}N$ U = 262.000 $p < 0.001$	$\delta^{15}N$ U = 65.000 $p < 0.001$	$\delta^{15}N$ U = 604.000 $p < 0.001$
Child	$\delta^{13}C$ U = 794.000 $p < 0.001$		$\delta^{15}N$ U = 2941.000 $p = 0.457$	$\delta^{15}N$ U = 5776.000 $p < 0.001$
Adolescent	$\delta^{13}C$ U = 388.000 $p = 0.001$	$\delta^{13}C$ U = 2315.000 $p = 0.006$		$\delta^{15}N$ U = 1811.000 $p < 0.001$
Adult	$\delta^{13}C$ U = 1542.000 $p = 0.033$	$\delta^{13}C$ U = 4260.000 $p < 0.001$	$\delta^{13}C$ U = 2154.000 $p = 0.002$	

Significant differences in isotopic values between age groups were also tested for each village separately. Due to its small sample size, Tawan Raya was not included in this set of tests.

At Arang Dak, significant differences in isotopic signatures between age groups were strong for both $\delta^{13}C$ values ($X^2 = 55.965$, df = 4, $p < 0.001$) and $\delta^{15}N$ values ($X^2 = 66.025$, df = 4, $p < 0.001$). Six post-hoc Mann-Whitney tests for each $\delta^{13}C$ and $\delta^{15}N$ required a Bonferroni adjustment of $\alpha = 0.008$.

Table 3.5: Central tendencies of isotopic measurements by age group at Arang Dak

Age Groups	**N**	**$\delta^{13}C$ mean**	**$\delta^{13}C$ median**	**$\delta^{15}N$ mean**	**$\delta^{15}N$ median**
Infants	23	-21.2 ± 1.1	-21.9	11.2 ± 1.0	11.0
Children	91	-22.7 ± 0.8	-23.0	9.5 ± 0.7	9.4
Adolescent	37	-22.5 ± 0.7	-22.6	9.3 ± 0.6	9.2
Adults	105	-22.2 ± 0.6	-22.3	9.8 ± 0.7	9.9
Elders	6	-21.6 ± 0.5	-21.6	10.2 ± 0.8	10.1

Post-hoc tests show that Arang Dak infant $\delta^{13}C$ isotopic values (median = -21.88‰) are significantly higher than child (median = -23.0‰) and adolescent (median = -22.6‰) $\delta^{13}C$ values, but are not significantly different than adult $\delta^{13}C$ values (median = -22.3‰) (Tables 3.5, 3.6). Children and adolescents at Arang Dak do not have significantly different $\delta^{13}C$ isotopic signatures, but both are significantly lower than adult $\delta^{13}C$ values (Table 3.6; Figure 3.7).

Post-hoc tests exploring $\delta^{15}N$ values at Arang Dak show that infants (median = 11.0‰) again have significantly higher $\delta^{15}N$ signatures than children (median = 9.4‰), adolescents (median = 9.2‰), and adults (median = 9.9‰) (Tables 3.5, 3.6). Adults have the next highest $\delta^{15}N$ signatures, which are significantly higher than children and

adolescent δ^{15}N values. Children and adolescents do not have significantly different δ^{15}N values (Table 3.6; Figure 3.7).

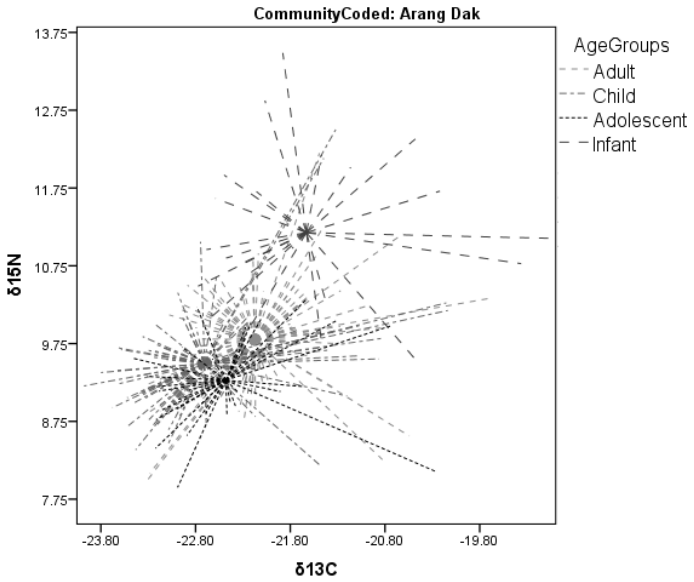

Figure 3.7: Scatterplot with centroids for age group data from all individuals living at Arang Dak. Infants and adults are the most distinct groups; the centroids for children and adolescents are much closer together

Table 3.6: Post-Hoc Mann-Whitney tests for δ^{13}C and δ^{15}N comparisons at Arang Dak

	Infant	**Child**	**Adolescent**	**Adult**
Infant		δ^{15}N U = 151.000 p < 0.001	δ^{15}N U = 34.000 p < 0.001	δ^{15}N U = 309.000 p < 0.001
Child	δ^{13}C U = 350.000 p < 0.001		δ^{15}N U = 1404.000 p = 0.142	δ^{15}N U = 3205.000 p < 0.001
Adolescent	δ^{13}C U = 197.000 p = 0.001	δ^{13}C U = 1251.000 p = 0.023		δ^{15}N U = 1065.000 p < 0.001
Adult	δ^{13}C U = 859.000 p = 0.031	δ^{13}C U = 2412.000 p < 0.001	δ^{13}C U = 1333.000 p = 0.005	

The significant differences in isotopic signatures between age groups were also strong for both δ^{13}C values (X^2 = 13.774, df = 4, p = 0.008) and δ^{15}N values (X^2 = 20.117, df = 4, p

< 0.001) at Suma Pipi. Six post-hoc Mann-Whitney tests for each δ^{13}C and δ^{15}N required a Bonferroni adjustment of $\alpha = 0.008$.

Table 3.7: Central tendencies of isotopic measurements by age group at Suma Pipi

Age Group	**N**	**δ^{13}C mean**	**δ^{13}C median**	**δ^{15}N mean**	**δ^{15}N median**
Infant	7	-22.5 ±1.1	-22.8	10.39 ± 0.8	10.3
Children	40	-23.3 ± 0.7	-23.3	9.22 ± 0.6	9.3
Adolescents	9	-23.0 ± 0.7	-23.2	9.35 ± 0.6	9.5
Adults	25	-22.7 ± 0.6	-22.6	9.69 ± 0.6	9.5
Elders	1	N/A	N/A	N/A	N/A

Post-hoc tests exploring δ^{13}C values at Suma Pipi show that the only significant difference is between adult (median = -22.6‰) and child (median = -23.3‰) δ^{13}C signatures (Tables 3.7, 3.8; Figure 3.8).

Post-hoc tests on δ^{15}N values between age groups revealed two significant differences. Infant δ^{15}N (median = 10.3‰) are significantly higher than child δ^{15}N values (median = 9.3‰) and adolescent δ^{15}N values (median = 9.5‰) (Tables 3.7, 3.8; Figure 3.8).

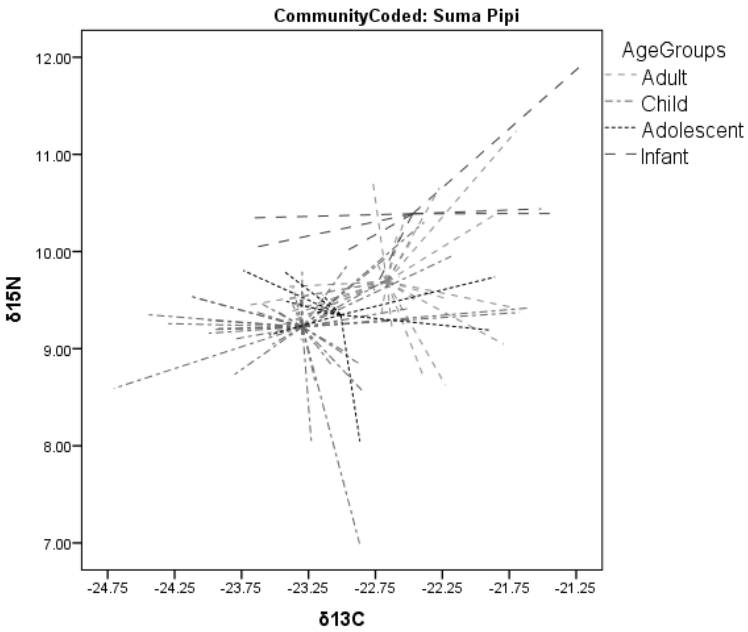

Figure 3.8: Scatterplot with centroids for age group data from all individuals living at Suma Pipi. Infants and adults are the most distinct groups

Table 3.8: Post-Hoc Mann-Whitney tests for $\delta^{13}C$ and $\delta^{15}N$ comparisons at Suma Pipi

	Infant	**Child**	**Adolescent**	**Adult**
Infant		$\delta^{15}N$ U = 10.000 p < 0.001	$\delta^{15}N$ U = 3.000 p = 0.003	$\delta^{15}N$ U = 36.000 p = 0.019
Child	$\delta^{13}C$ U = 78.000 p = 0.064		$\delta^{15}N$ U = 139.000 p = 0.290	$\delta^{15}N$ U = 302.000 p = 0.008
Adolescent	$\delta^{13}C$ U = 21.000 p = 0.266	$\delta^{13}C$ U = 148.000 p = 0.409		$\delta^{15}N$ U = 90.000 p = 0.380
Adult	$\delta^{13}C$ U = 83.000 p = 0.837	$\delta^{13}C$ U = 254.000 p = 0.001	$\delta^{13}C$ U = 72.000 p = 0.114	

3.6.3 Village

Mann-Whitney tests were used to compare the $\delta^{13}C$ and $\delta^{15}N$ values of people living in Arang Dak (n = 262, $\delta^{13}C$ median = -22.5‰; $\delta^{15}N$ median = 9.7‰) and Suma Pipi (n = 82, $\delta^{13}C$ median = -23.1‰; $\delta^{15}N$ median = 9.4‰) (Figure 3.9). The village of

Tawan Raya was left out of this comparison because of its small sample size (n = 8). There were significant differences in δ^{13}C values (U = 5992.000, p < 0.001) and δ^{15}N values (U = 8926.000, p = 0.021) between the two villages.

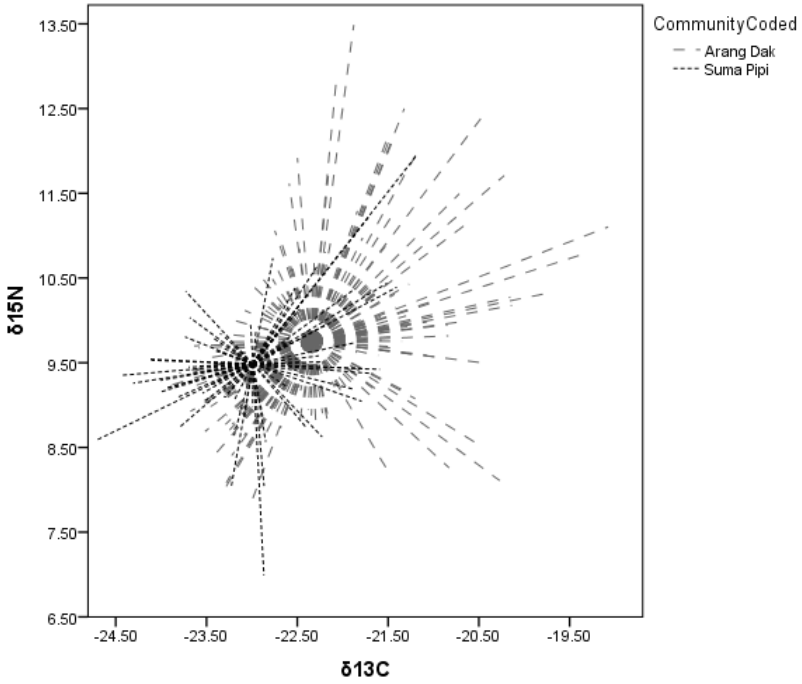

Figure 3.9: Scatterplot with centroids for δ^{13}C and δ^{15}N data for each individual by village

3.6.4 Education

Education variables were recorded for male and female heads of household. Because men had significantly higher scores than women in both perceived knowledge of Spanish (U = 456.000, p < 0.001), and general knowledge (U = 486.000, p < 0.001), significant differences in isotope signatures were explored separately for each measure of male and female education.

There were no significant differences in δ^{13}C mean, δ^{13}C median, δ^{13}C range, δ^{13}C IQR, δ^{15}N mean, δ^{15}N median, δ^{15}N range, or δ^{15}N IQR when comparing families

with more educated male or female heads of household to families with less-educated male or female heads of household.

3.6.5 Family Size

Family size, the total number of individuals living in the household, was divided into two groups of above median family size and median-and-below family size; the median family size in this population was 8 members. Mann-Whitney tests showed no significant differences in isotopic central tendency (mean, median), but significant differences in isotopic variability between larger and smaller family sizes. Larger families had significantly greater ranges of $\delta^{13}C$ (U = 155.000; p = 0.030) and $\delta^{15}N$ (U = 115.000; p = 0.002) isotopic values than smaller families (Table 3.9).

Table 3.9: Mean isotopic ranges by family size

Family Size	**Mean $\delta^{13}C$ range**	**Mean $\delta^{15}N$ range**
Median or below	1.4 ± 0.7	1.2 ± 0.6
Above median	1.8 ± 0.7	1.9 ± 0.8

3.6.6 Wealth

Household wealth, measured as the total value (in Nicaraguan córdobas) of all key possessions owned within the household. The households were then grouped into those with above median wealth (31,446 córdobas), and those with below or at median wealth, for statistical comparison.

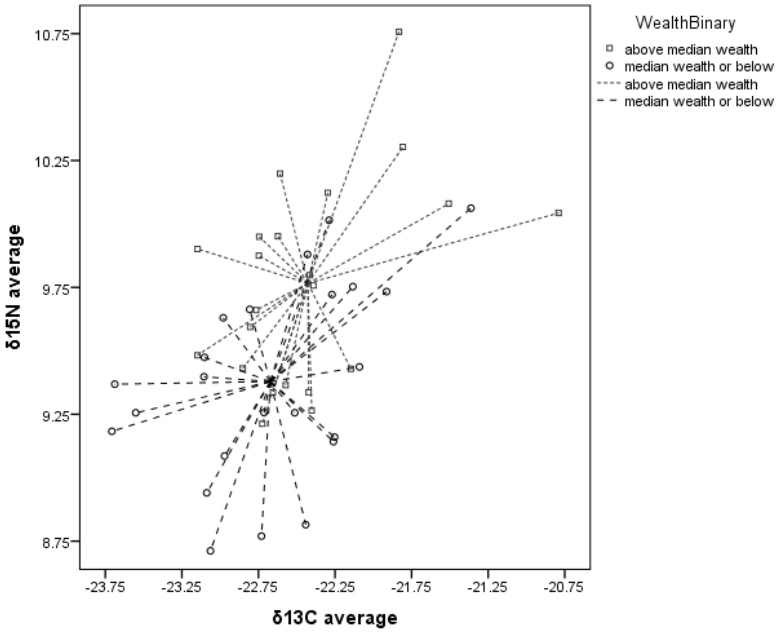

Figure 3.10: Scatterplot with centroids of δ^{13}C median and δ^{15}N median for each family, markers defined by wealth grouping.

Mann-Whitney tests to examine isotopic measures of central tendency and variation between wealth groups showed a significant difference in the central tendency of δ^{15}N values. The wealthier families had significantly higher mean δ^{15}N (U = 122.000, p = 0.003), and median δ^{15}N (U = 148.000, p = 0.017) compared to the poorer families (Table 3.10; Figure 3.10).

Table 3.10: Comparison of δ^{15}N central tendencies by wealth grouping

Wealth Grouping	**Mean of family δ^{15}N mean values**	**Median of family δ^{15}N mean values**	**Mean of family δ^{15}N median values**	**Median of family δ^{15}N median values**
Median wealth or below	9.4 ± 0.4‰	9.4‰	9.4 ± 0.3‰	9.4‰
Above median wealth	9.8 ± 0.4‰	9.8‰	9.7 ± 0.4‰	9.7‰

3.7 Discussion

Overall, there is a relatively large range of isotopic signatures within this population—$\delta^{13}C$ values range from -24.7‰ to -19.1‰, and $\delta^{15}N$ values range from 7.0‰ to 13.5‰ (Figure 3.2). The $\delta^{13}C$ values are consistent with a mainly C_3 based diet with varying contributions of C_4. The $\delta^{15}N$ values have a range of 6.5‰, which is consistent with people eating protein from different trophic levels (Lee-Thorp, 2008). Although no distinct isotopic groupings are readily apparent in a bivariate plot of $\delta^{13}C$ and $\delta^{15}N$ (Figure 3.2), probing the data further shows some statistically significant differences among demographic groups.

Though all individuals are considered to be from the same population, there are some isotopic differences between people from Arang Dak and those living in Suma Pipi (Figure 3.9). $\delta^{13}C$ and $\delta^{15}N$ values of people living in Arang Dak ($\delta^{13}C$ median = -22.49‰; $\delta^{15}N$ median = 9.67‰) are slightly, but significantly higher than the isotopic signatures of people living in Suma Pipi ($\delta^{13}C$ median = -23.11‰; $\delta^{15}N$ median = 9.44‰). This indicates a difference in diet that may be due to a slight difference in food types or difference in access to food between the two villages; individuals in Arang Dak, may be eating higher trophic level foods, or have easier access to maize or other C_4 crops.

Differences in diet between males and females were plausible based on previously published research. Differences in diets and food security between males and females have been noted in nutritional studies (Christiaensen and Alderman, 2004; Hadley et al., 2011; Sakisaka et al., 2006). In this current study among the Mayangna and Miskito in Nicaragua, there is also evidence for sexual differentiation: women scored significantly lower than men in both measures of education—perceived knowledge of Spanish and an

objective test of ethnobiological knowledge. There was, however, no overall difference in isotopic signatures between men and women to indicate significant differences in diet between males and females (Figure 3.3). Examining isotopic signatures between sexes within each age group showed only a significant difference between $\delta^{13}C$ values of male and female children (Figure 3.4). Male children had a median $\delta^{13}C$ signature of -23.22‰, which is only slightly lower than the median signature for female children, -22.95‰, and would not appear readily distinct without the large sample size (n = 135).

There were a number of significant differences in isotopic signature between age groups. Infants (ages 0 to 2) had the highest $\delta^{15}N$ signatures (median = 10.85‰), which is consistent with babies being breastfed by their mothers. Babies who are breastfeeding exclusively without formula supplements typically have isotopic signatures that are one trophic level above their mothers until they begin the weaning process, at which time their $\delta^{15}N$ signatures begin to drop (Fuller et al., 2006). Infant $\delta^{15}N$ signatures were significantly higher than the $\delta^{15}N$ values of all other age groups, including adults (Tables 3.3, 3.4; Figure 3.6). Infants also had $\delta^{13}C$ values that were significantly higher than child and adolescent $\delta^{13}C$ signatures. Infant $\delta^{13}C$ values were not significantly higher than adult $\delta^{13}C$ values due to the more conservative significance level of $\alpha = 0.008$ required to rule out false-positives during the series of post-hoc tests.

Adult $\delta^{15}N$ isotopic signatures (median = 9.76‰) were also significantly different from all other age groups, being higher than child and adolescent $\delta^{15}N$ values and lower than infant $\delta^{15}N$ values (Tables 3.3, 3.4; Figure 3.6). This indicates that adults were eating more higher-trophic level foods than children or adolescents. Adults also had

significantly higher $\delta^{13}C$ values than children and adolescents, indicating a greater contribution of C_4 foods in their diets.

Children and adolescent isotopic signatures were the most similar to each other. Adolescents had a significantly higher $\delta^{13}C$ values, than children, but the $\delta^{15}N$ values were indistinguishable between the two age groups (Tables 3.3, 3.4; Figure 3.6). There were two outliers in the child age group, with $\delta^{15}N$ values consistent with breastfeeding infants; these individuals were 3 years old and likely were still being breastfed when their hair was sampled. These two infants show the potential variation around the population's mode weaning age of 2 years old.

These patterns also appear when the inhabitants of Arang Dak are explored separately from those living in Suma Pipi or traveling to Tawan Raya. The only exception is that at Arang Dak, there is no significant difference between the $\delta^{13}C$ or $\delta^{15}N$ values of children and adolescents (Tables 3.5, 3.6; Figure 3.7).

There are fewer significant differences between age groups at Suma Pipi. Infant $\delta^{13}C$ values at Suma Pipi are indistinguishable from all other age groups; infant $\delta^{15}N$ values are still significantly higher than child and adolescent signatures, but are not significantly different from adult $\delta^{15}N$ values. Adults at Suma Pipi have significantly higher $\delta^{13}C$ values than children, but otherwise, adults, adolescents and children in the village are isotopically indistinguishable (Tables 3.7, 3.8; Figure 3.8).

Differences in isotopic signatures among age groups indicate that different members of the household have different diets. One hypothesis regarding differences in diet between adults and their dependents is buffering—parents may try to protect their children from food stress (Hadley et al., 2008; Leonard, 1988). Buffering of this nature

does not appear to be happening in this population. Previous studies of this population have shown that children fall further behind standard growth patterns as they get older (Winking and Koster, 2015). This pattern indicates that younger children have better access to the nutrients they need to grow and are less able to meet their nutrient needs over time. Protein is one of the critical macronutrients in growth and maintenance of the body (Leonard et al., 2000). Though protein can be ingested from many types of food, meat is still an important source. It is interesting to note that adults (median = 9.76‰) have a greater proportion of meat in their diet, compared to children (median = 9.38‰) and adolescents (median = 9.28‰). These isotopic data provide another line of evidence showing differential access to food between age groups.

Of the other social and demographic categories explored at the family level, only one could be related to significant differences in isotopic values and therefore differences in diet. Parental education level, which has been shown to be positively correlated with food security and nutrition in children (Christiaensen and Alderman, 2004; Cochrane et al., 1982; Sakisaka et al., 2006), was not found to be related to significant differences in diet in the Mayangna and Miskito. The lack of a pattern may be due to the lack of relationship between education and isotopic signatures in this population, or lack of relationship between the types of knowledge that were measured and family diet. The measures of education used here were not tailored to measure an individual's knowledge of food, diet or nutrition. Rather, these measurements focused on general ecological knowledge and fluency in Spanish, which may have no bearing on diet.

However, there were significant differences in diet between poorer and wealthier families. Families with above-median wealth for the population had significantly higher

δ^{15}N values (median of family medians = 9.72‰) than families with wealth at the median or below (median of family medians = 9.35‰) (Table 3.10). This is consistent with other nutritional studies which show that families of greater affluence have better nutrition, partially due to a greater diversity of food (Barros et al., 2010; Belachew et al., 2013). In this population, for example, cattle are considered an investment of wealth, and while not eaten for food, families with cattle will have access to milk, which can contribute to higher δ^{15}N values. This dietary difference between wealthier and poorer families may also be a contributing factor in the isotopic difference in signatures between individuals living in Arang Dak and Suma Pipi because there is a higher concentration of wealth in Arang Dak compared to the smaller village of Suma Pipi.

3.8 Conclusion

The isotopic data show that these individuals, from a dietary perspective, are part of the same population, with no distinctly different outlying groups. Further exploration of the data using statistical analyses reveals some significant differences between or among certain demographic groups. These significant differences are consistent with nutritional literature, such as the difference in diet between wealthier and poorer individuals. Conversely, some of the differences noted in nutritional studies did not correlate to significant differences in isotopic signatures.

The absolute differences between demographic groups that tested as statistically significant were generally quite small—usually within 1 – 2‰. These slight differences, though showing slight distinctions in dietary composition, may not relate to differences in nutrition. It is also possible that this small but significant patterning is being teased out

due to the large sample size used in this study, which may be beyond the scope of some archaeological studies with limited access to well-preserved remains. These data show the importance of sample size for determining statistically significant differences in diet as well as determining the full range of isotopic signatures. Second, these data show that it is possible for some groups to have large ranges of isotopic signatures without the tight clustering that is frequently used to define populations in archaeological studies.

As an archaeological analogue, these data show that isotopic analysis is one way in which archaeologists may try to determine if individuals are from the same or different populations. However, this definition of population—that everyone is eating similar diets—may or may not coincide with other archaeological data. In this case study, for example, the Mayangna and Miskito are linguistically and ethnographically distinct groups, but from a dietary isotopic perspective, they cannot be differentiated. In archaeology, isotopic analysis is only one tool—the isotopic data may or may not coincide with distinct differences in material remains. Within each context, there is the challenge of interpreting multiple types of data, and how isotopic and more traditional archaeological analyses may or may not coincide.

The data from the Mayangna and Miskito, however, is one illustration of how isotopic data can be used to identify different groups in archaeological populations. The individuals in this population do not have isotopic signatures that are tightly clustered together, rather, this population encompasses a range of values. The key to this data set is the number of samples—without a large sample size, it is possible that different groups can not be statistically separated. These data and results do show, however, that the use of isotopic data can reveal differences in diet between demographic groups and can be

used to explore gross differences in diet in an objective way that complements nutritional studies. Though not all socioeconomic data can be related to dietary differences, combining the datasets shows the potential for further complementary studies of diet through isotopic analysis with ethnographic studies of nutrition.

4. Short-term variability of human diet at Basketmaker II Turkey Pen Ruins, Utah: Insights from bulk and single amino acid isotope analysis of hair

4.1 Case Study Overview

Strands of human hair excavated from Basketmaker II Turkey Pen Ruins in Utah were examined using stable carbon and nitrogen isotope analysis and show that these individuals were heavily reliant on maize but their diet fluctuated over a period of months. Through serial bulk carbon and nitrogen analysis and serial single amino acid carbon isotope analysis of individual strands of hair, this study addresses the questions of how human diets at the site varied over a period of months and if domesticated turkeys (*Meleagris gallopavo*) were part of the diet during any point of the year. Hair carbon isotope values range from -9.2‰ to -12.4‰ with an average of -10.8 ± 1.2, and nitrogen isotope values range from 6.0‰ to 7.3‰ with an average of 6.6‰ ± 0.5. There was also variability in the single amino acid $\delta^{13}C$ values, with an overall pattern of terrestrial C_4 signatures of individual amino acids ($\delta^{13}C$ for leucine averaged -17.8‰ ± 1.7). $\Delta^{13}C_{valine\text{-}phenylalanine}$ values ranging from -1.6‰ to 2.6‰ also point strongly to a terrestrial diet. From the stable isotope data presented here, we suggest that BMII diet, while being heavily maize based, did vary during the year, and that turkeys were never a diet staple.

4.2 Introduction

There have been a number of archaeological studies designed to better understand the introduction and use of domesticates such as maize, squash, beans and turkeys in the American Southwest (Coltrain and Janetski, 2013; Decker and Tieszen, 1989; Matson, 1991; Munro, 1994, 2006). Archaeological evidence has shown that maize was present in the northern Southwest by 2200 BC (Huber, 2005; Huckell, 1996). By the

Basketmaker II (BMII) period (~1000 BC—500 AD), there is also evidence of domesticated squash and turkeys, although beans do not appear in the archaeological record until the Basketmaker III period (BMIII) (Decker and Tieszen, 1989; Kidder, 1924; Lipe, 1978; Matson, 1991; Spangler et al., 2010).

This study focuses on two questions using serial stable carbon, nitrogen, and serial single amino acid carbon isotope analyses of human hair from the BMII midden at Turkey Pen Ruins: (1) Was the relative proportion of maize to foraged material constant throughout the year, or were there periods of time (seasons) when these BMII individuals relied more heavily on foraged foods in their diet? (2) Multiple studies have shown that domesticated turkeys were fed maize during the BMII period (Aasen, 1984; Munro, 1994; Nott, 2010), and that humans from this period have carbon isotopic signatures that would be consistent with eating mainly maize, or eating meat from maize-fed turkey (Matson and Chisholm, 1991); within this established context, were the individuals from BMII Turkey Pen Ruins eating enough turkey on a regular or seasonal basis to contribute to their high maize-consumption isotopic signatures?

4.3 Background

4.3.1 Archaeology of Turkey Pen Ruins on Cedar Mesa

In 1972, as part of the Cedar Mesa Project in southeastern Utah, R.G. Matson and William Lipe excavated a test pit in the BMII midden at the Turkey Pen site located in the neighbouring Grand Gulch, in an alcove named for the "turkey pen" structure dating to a later Puebloan occupation (Figure 4.1) (Matson, 1991, 2015a). Three columns were taken from the midden—two columns were excavated and sifted in situ to expose the

central column between them (Matson, 1991). This central column was removed intact and examined stratum by stratum (Figure 4.2) (Matson, 1991). Organic materials such as coprolites, hair, and feathers were well preserved by arid conditions at the site (Matson, 1991, 2015a). Radiocarbon analysis of numerous organic inclusions in the columns dated the BMII occupation of Turkey Pen Ruins to approximately 100 BC to AD 200 (Lipe et al., 2011; Matson, 2015a; Matson and Chisholm, 1991).

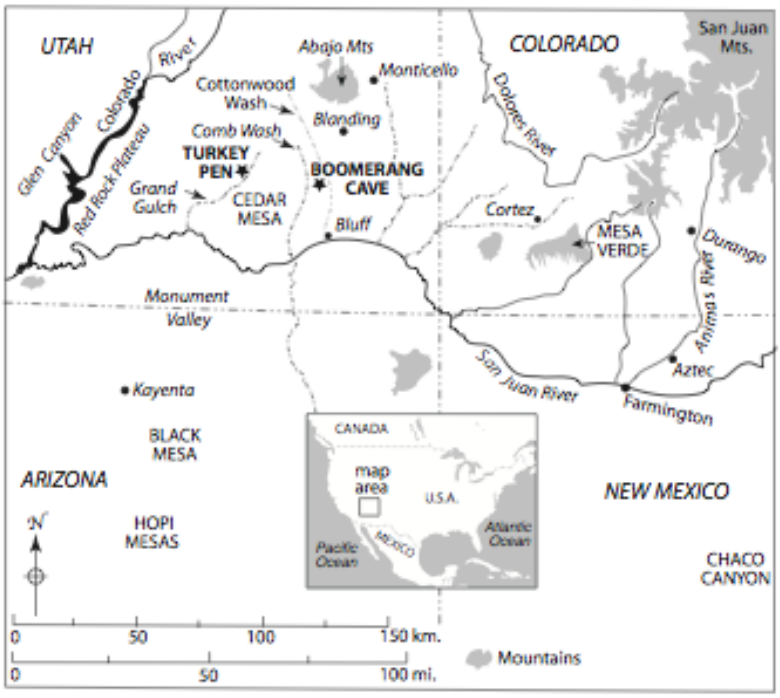

Figure 4.1: Map of Four Corners Region with Turkey Pen Ruins

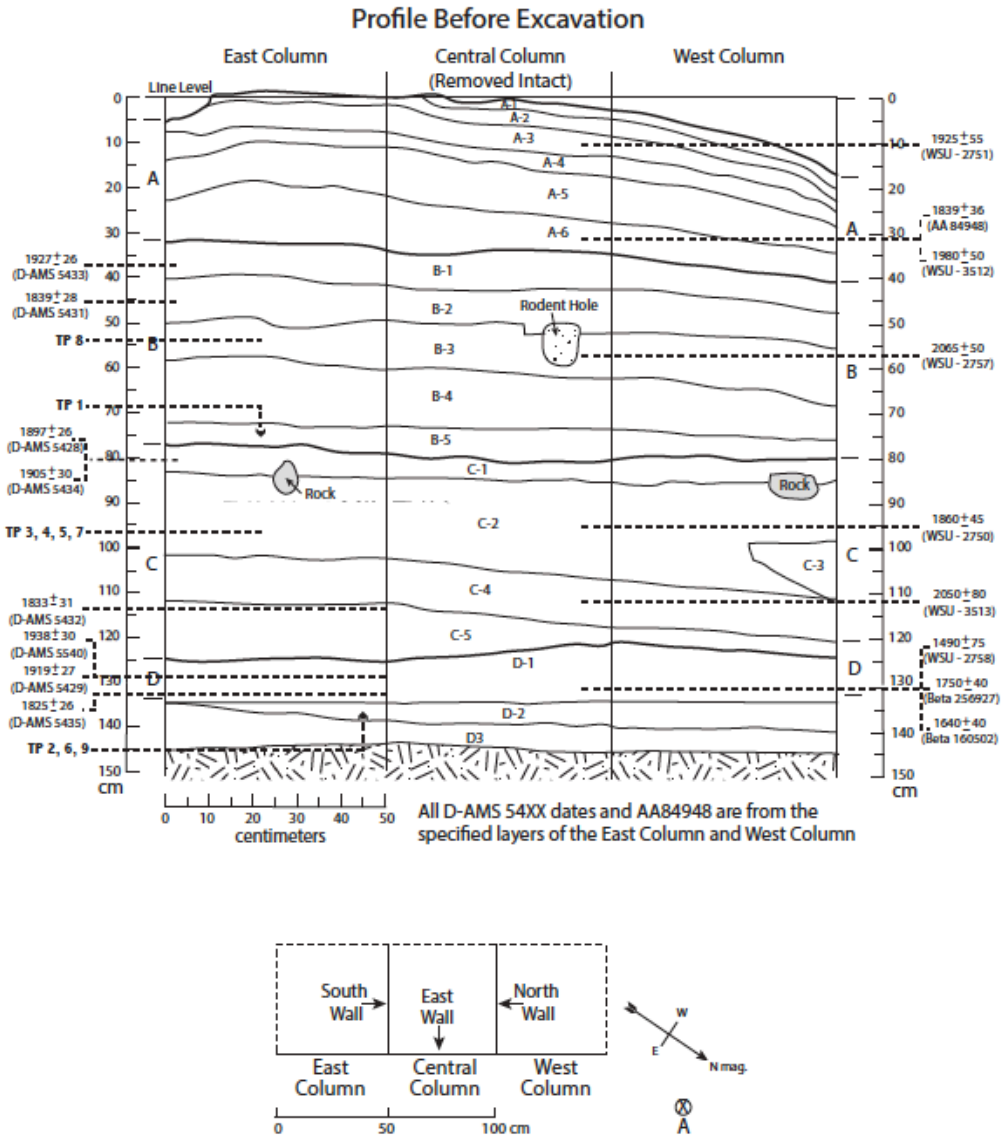

Figure 4.2: Stratigraphy of the BMII midden with radiocarbon dates shows orientation and relationship of the excavated Central Column to the West and East Columns

Maize (*Zea mays*) was a dietary staple at BMII Turkey Pen Ruin (Aasen, 1984; Matson, 1991, 2006; Matson and Chisholm, 1991). The BMII settlement pattern on Cedar Mesa is similar to those of later occupations and there is no evidence of a transition phase between a hunting and gathering tradition to a horticultural one (Matson, 1991). This evidence agrees with the human coprolite analysis, which found maize in a majority

of the Turkey Pen coprolites (Aasen, 1984; Matson et al., 1988; Matson, 1991). Maize was also the greatest contributor to the macrofossil weight in most of the coprolites (Aasen, 1984; Matson et al., 1988). Coprolite and palynological analyses show that lesser contributors to the BMII diet include domesticated squash (*Cucurbita* sp), pinion pine nuts (*Pinus*), ricegrass (*Stipa hymenoides*) chenopods and amaranth (Aasen, 1984; Matson and Chisholm, 1991). Isotopic analysis of human bone collagen from individuals excavated from the surrounding mesa resulted in similar conclusions being drawn, concurring with an overall high-maize consumption by Cedar Mesa BM II individuals (Chisholm and Matson, 1994; Matson and Chisholm, 1986, 1991).

Turkeys found in archaeological contexts in the American Southwest have been identified as belonging to two mitochondrial haplogroups: one representing the local Merriam type (*Meleagris gallopavo merriami*) and the other one interpreted as a domesticate (Speller et al., 2010). This domesticate is more closely related to subspecies that are today found to the east of the Pueblo Southwest , *M. g. silvestris* or *M. g. intermedia* (Speller et al., 2010). Analysis of turkey coprolites from BMII Turkey Pen Ruins shows that the turkeys were fed maize (Aasen, 1984; Nott, 2010). The purpose of keeping domesticated turkeys is hypothesized to be for their feathers, which had ritual and economic value as ceremonial objects such as trimming on costumes or feather bundles and their use in feather blankets (Lipe et al., 2016; Munro, 1994, 2006). During the BMII period there is a lack of large assemblages of turkey bones with cut-marks or evidence of burning; this has been interpreted as indicating that turkeys were not a major source of food (Munro, 1994, 2006). Isotopic data from human bone collagen similarly suggests that the individuals from BMII mainly relied on plants and not meat for food

(Chisholm and Matson, 1994; Coltrain and Janetski, 2013). Only one BMII study examining BMII human coprolites suggests that meat may have been a significant part of the diet in this region (Androy, 2003).

Seasonality of diet at Turkey Pen Ruins has only been briefly addressed. Aasen suggested that the use of the site was from the spring to the fall on the basis of pollen and macrofossil content of the coprolites, but the data was not conclusive (Aasen, 1984). This interpretation of seasonal use was supported by Androy's analysis of BMII coprolites from Boomerang Shelter (Figure 4.1), which included a suggestion that meat might have been eaten in greater proportions during winter (Androy, 2003).

4.3.2 Stable Carbon and Nitrogen Isotope Analysis

Carbon and nitrogen isotope analyses can be used to create a useful bivariate data set for examining diets of past (and present) individuals (Lee-Thorp, 2008). Carbon isotope analysis compares the ratio of 1 ^{13}C to 1000 ^{12}C (expressed as $\delta^{13}C$ (‰) relative to an international standard), and can differentiate between proteins garnered from C_3 and C_4 plants (van der Merwe and Vogel, 1978). The differentiation between these plant types is possible because C_3 and C_4 plants utilize different photosynthetic pathways to incorporate atmospheric CO_2 (Lee-Thorp, 2008). C_4 plants are less likely to select for lighter atmospheric CO_2 containing ^{12}C, and so often have a higher $\delta^{13}C$ ratio compared to C_3 plants (Lee-Thorp, 2008). There is one other common photosynthetic pathway: crassulacean acid metabolism (CAM) which incorporates aspects of both C_3 and C_4 photosynthetic pathways such that CAM plants have differing $\delta^{13}C$ signatures depending on the climate (Ambrose and Norr, 1993). Of the plants used at BMII Turkey Pen Ruins,

maize is C_4, squash, piñion pine, and Indian rice grass are C_3, and chenopods, yucca and amaranths are CAM plants (Coltrain and Janetski, 2013).

Nitrogen isotope analysis, comparing the ratio of 1 ^{15}N to 1000^{14}N (expressed $\delta^{15}N$ (‰) relative to an international standard), can reveal an individual's relative trophic level because of an approximate +3‰ enrichment in the nitrogen signature at each increasing level in the food chain (Lee-Thorp, 2008; Schoeninger and DeNiro, 1984). Potential prey at BMII Cedar Mesa included cottontail rabbits, jackrabbit, mule deer, mountain sheep, and wild-type and domestic turkeys, all of which are considered herbivorous (Chisholm and Matson, 1994; Coltrain and Janetski, 2013).

Serial stable isotope analysis of human hair has been successfully applied to examine short-term diet variation in Nubian and Egyptian mummies (White, 1993; White et al., 1999), and prehistoric individuals from Peru and Western Alaska (Britton et al., 2013; Webb et al., 2013; Williams and Katzenberg, 2012). Preliminary results from the American Southwest have also been presented (LeBlanc and Morgan, 2010).

4.3.3 Single Amino Acid Stable Carbon Isotope Analysis

Single amino acid isotope analysis provides a more detailed look at the components of diet by determining the carbon isotope ratio of each amino acid fraction present in the protein being analysed (Smith et al., 2009). Classifying amino acids can be problematic (Reeds, 2000) however they are generally classified into three categories depending on whether the body can synthesize them under normal conditions (non-essential amino acids) or whether they must be ingested from the diet (as the body cannot synthesize them - essential amino acids). A third category is defined as amino acids that

the body can manufacture but under normal conditions it is energetically preferable to ingest (conditionally essential amino acids) (Fromm and Hargrove, 2011).

Ideally, $\delta^{13}C$ values for individual amino acids show different components of the diet from protein directly incorporated from food (essential amino acids) to average total diet including protein, carbohydrates and lipids being more represented by the non-essential amino acid isotope values (Choy et al., 2010).

Previous studies comparing the $\delta^{13}C$ values of individual amino acids have demonstrated that it is possible to distinguish between marine, terrestrial and omnivorous diets when nitrogen isotope data is inconclusive (Corr et al., 2005). For example, the difference between $\delta^{13}C_{valine}$ and $\delta^{13}C_{phenylalanine}$, and the difference between $\delta^{13}C_{glycine}$ and $\delta^{13}C_{phenylalanine}$ increase with increasing marine protein in the diet (Honch et al., 2012 and Corr et al., 2005 respectively).

4.3.4 Hair Growth

Hair provides a unique view into the short-term diet of an individual because of its growth rate of 1.06 ± 0.06 cm per month (LeBeau et al., 2011). This estimate is a general average calculated from multiple hair growth studies published in the literature and discussed in LeBeau et al. 2011. The high degree of variation in hair growth rate can be caused by factors such as health, age, nutrition, and hormones (LeBeau et al., 2011). Another source of uncertainty in hair growth rates is the growth cycle followed by each follicle such that some hairs are actively growing, and some are dormant (LeBeau et al., 2011; Schwertl et al., 2003). Despite the uncertainty around the average, 1 cm/month is

the value used in archaeological studies and represents the shortest time-frame for isotopic analysis of diet in archaeology (Webb et al., 2013).

4.4 Materials and Methods

4.4.1 Hair Samples

The seven individual human hairs here examined were found as loose inclusions in the Turkey Pen Ruins BMII midden excavated by Matson, and as such, are not directly associated with any particular individual or individuals. Hairs in this midden were identified as human due to similarity with human hair cordage also found in the midden, and a distinct lack of other sources. Other animal remains within the midden context included turkey feathers and coprolites, scraps of deer or sheep hide and rabbit skins with some fur intact (Matson, 1991). Human hair was frequently collected for use in cordage by Basketmaker II individuals from SE Utah and NE Arizona, similar to remnants found in the Turkey Pen midden (Guernsey and Kidder, 1921).

TP hairs 1 through 9 represent a subsample of what was found, and were chosen to represent a range of stratigraphic contexts. Individual hairs from the different contexts were chosen for optimum length and preservation; two hairs (TP 5 and 6) were held back for future destructive analysis. Though not directly dated, the stratigraphic location of each hair is known (Figure 4.2) and all can be assumed to be from the Basketmaker II period. Unfortunately, the root end of these hairs were not present, so knowing which end is oldest could not be determined with the tools available.

4.4.2 Bulk Stable Carbon and Nitrogen Isotope Analysis of Hair

For bulk C and N stable isotope analysis, each hair was cleaned using a modified version of the O'Connell and Hedges (1999) method. Briefly, the hair was rinsed twice in deionized (DI) water, sonicating for 10 minutes each time. This was followed by soaking the hair twice in 2:1 v:v chloroform:methanol; the first soak lasted five minutes and the second soak was 18 hours. The hairs were given a final set of four rinses with DI water, and then left to dry overnight.

Each hair was cut into 2 to 2.5 cm long sequential segments with masses of approximately 0.08 mg. Each segment was bundled into a tin capsule after it was cut off from the hair strand.

The bulk C and N stable isotope analyses were carried out simultaneously using a MicroCube elemental analyser coupled to an Isoprime isotope ratio-mass spectrometer (EA-IRMS) at the Archaeological Chemistry Lab at the University of British Columbia. All samples were compared to two standards, the Vienna Pee Dee Belemnite (VPDB) standard for carbon and the ambient inhalable reservoir (AIR) for nitrogen.

4.4.3 Single Amino Acid Stable Carbon Isotope Analysis of Hair

Hair sample TP 4 was selected for single amino acid carbon isotope analysis because it came from the same stratigraphic context as TP 3 and TP 7. TP 4 was cut into 5 mm long segments with a scalpel and placed in hydrolysis tubes. Samples were hydrolyzed using 250 μL of amino acid free 6M HCl in vacuum at 110°C. Hydrolysis was continued for 2 days, if the hair had not dissolved an additional 250 μL of 6M HCl was added and the hydrolysis continued for a maximum total of 4 days. After hydrolysis

samples were transferred to clean glass vials and dried using a rotary vacuum desiccator and frozen until analysis. In preparation for analysis the samples were resolved in milliQ water to a concentration of approx. 6 μg sample/15 μL. 10 μL of internal standard (2-amino butyric acid) was added to the vial, and the rest of the calculated volume made with milliQ H_2O. The samples were transferred to small volume injection vials with glass inserts. The samples were analysed on a ThermoScientific LC-IRMS using the three phase chromatographic method developed by Smith et al. (2009).

4.5 Results

4.5.1 Bulk Carbon and Nitrogen

The carbon and nitrogen stable isotope values of these hairs show a range of variation from an extreme shift along the length of one hair (TP 9) to very little change in another (TP 8) (Table 4.1; Figure 4.3). Though included in the table, TP 2 has C/N ratios outside the accepted range of 3.0-3.8 determined for well preserved hair and will not be discussed further (O'Connell and Hedges, 1999).

Table 4.1: Segmental carbon and nitrogen stable isotope data for Turkey Pen Ruins human hairs shows isotope ratio data for each hair, broken down into segments and listed sequentially from the thickest end to the thinnest end of the hair in lieu of a root end to the hair.

Hair	Segment	Length (cm)	mass (mg)	C/N	$\delta^{13}C$	$\delta^{15}N$
TP 1	1	2.5	0.090	3.7	-10.1	6.9
	2	2.5	0.095	3.7	-13.1	6.8
	3	2.5	0.108	3.7	-14.1	7.1
TP 2	1	2	0.077	3.9	-11.3	6.9
	2	2	0.092	4.3	-12.9	7.9
TP 3	1	2	0.107	3.6	-10.6	7.2
	2	2	0.115	3.5	-12.1	7.5
	3	2	0.120	3.5	-10.2	7.5
	4	≈ 2	0.115	3.6	-11.2	7.0
TP 7	1	2	0.137	3.4	-11.4	7.3
	2	2	0.153	3.5	-8.6	6.8
	3	2	0.153	3.5	-8.7	6.2
	4	≈ 2	0.133	3.6	-8.6	6.4
	5	2	0.146	3.5	-8.8	6.1
TP 8	1	2.25	0.089	3.6	-10.8	6.0
	2	2.25	0.086	3.6	-9.9	6.2
	3	2.5	0.105	3.6	-9.6	6.9
TP 9	1	2.5	0.104	3.7	-9.9	7.2
	2	2.5	0.123	3.6	-9.9	6.1
	3	2.5	0.127	3.5	-14.3	6.9
	4	2.5	0.130	3.6	-11.5	4.8
	5	2.5	0.132	3.4	-10.0	5.1
	6	2.5	0.134	3.4	-10.4	5.9

The average change along the length of a hair is 2.9‰ $\delta^{13}C$ and 1.1‰ $\delta^{15}N$. The largest shifts in isotope value along a strand of hair are from sample TP9: the carbon isotope shift is 4.4‰ and the nitrogen shift 2.4‰. The smallest shifts occur in different hairs: the smallest variation in carbon along a single strand is 1.2‰ in hair TP 8 and the smallest variation in nitrogen is 0.3‰ in hair TP 1 (Figure 4.3), both of which are within the range of variation seen in constant diet (<1.5‰) (Fraser et al. 2006).

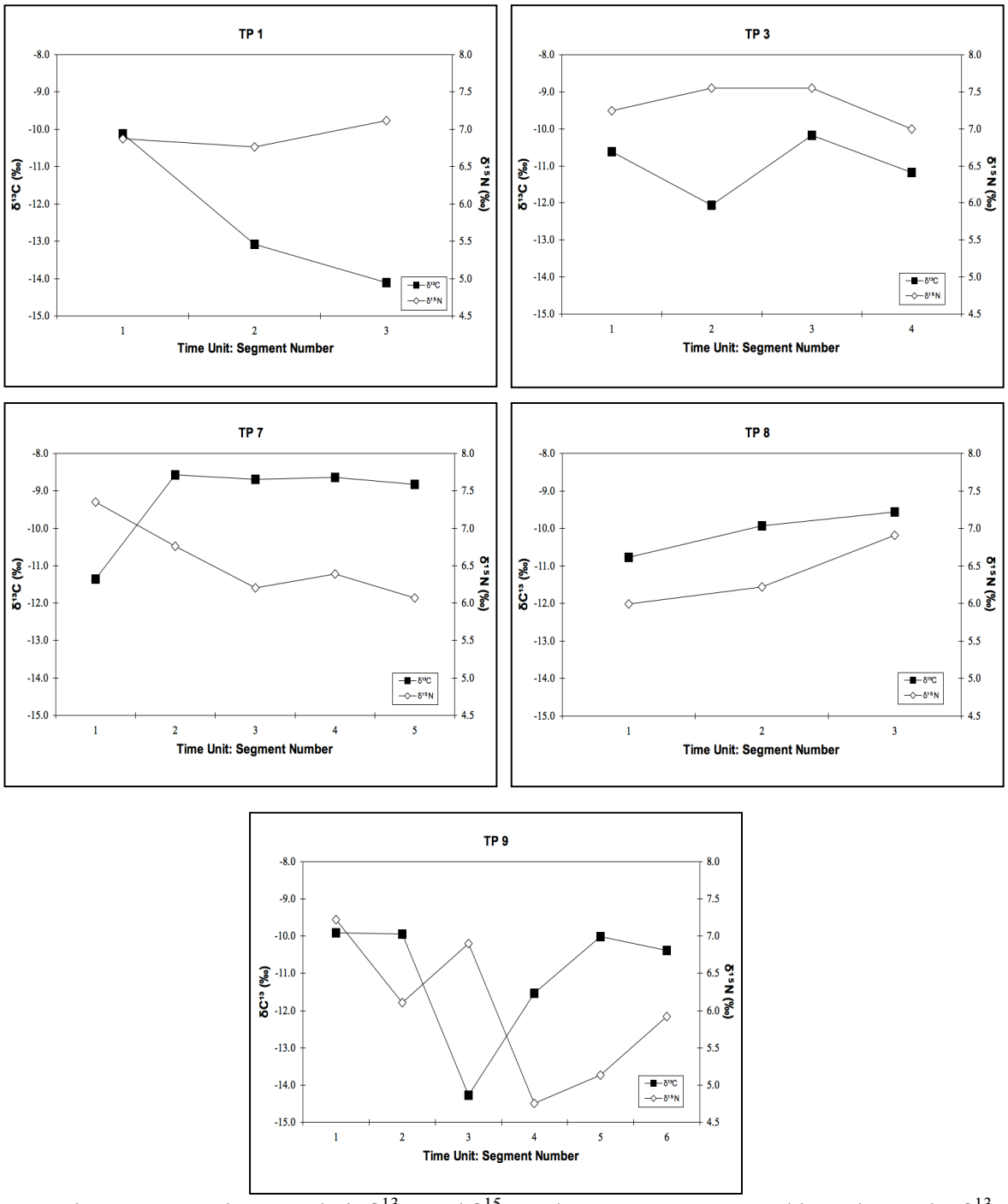

Figure 4.3: Turkey Pen hair $\delta^{13}C$ and $\delta^{15}N$ values across segments/time shows the $\delta^{13}C$ and $\delta^{15}N$ values for each segment of hair samples TP 1, TP 3, TP 7, TP 8 and TP 9. Each segment represents approximately 2 to 2.5 months of the individual's life.

The average $\delta^{13}C$ values for each hair range from -9.2‰ to -12.4‰ while the nitrogen averages range from 6.0‰ to 7.3‰ (Table 4.2). The average carbon isotope value for all of the hairs is -10.8‰ ± 1.2 (Figure 4.4). The average nitrogen isotope value for all the hairs is 6.6‰ ± 0.5 (Figure 4.4).

Table 4.2: Carbon and nitrogen isotope averages for each hair sample with ranges around each average.

Sample	**$\delta^{13}C$ ‰ Segment Average**	**$\delta^{13}C$ ‰ Segment Range**	**$\delta^{15}N$ ‰ Segment Average**	**$\delta^{15}N$ ‰ Segment Range**
TP 1	-12.4	-10.1 to -14.1	6.9	6.8 to 7.1
TP 3	-11.0	-10.2 to -12.1	7.3	7.0 to 7.5
TP 7	-9.2	-8.6 to -11.4	6.5	6.1 to 7.3
TP 8	-10.1	-9.6 to -10.8	6.4	6.0 to 6.9

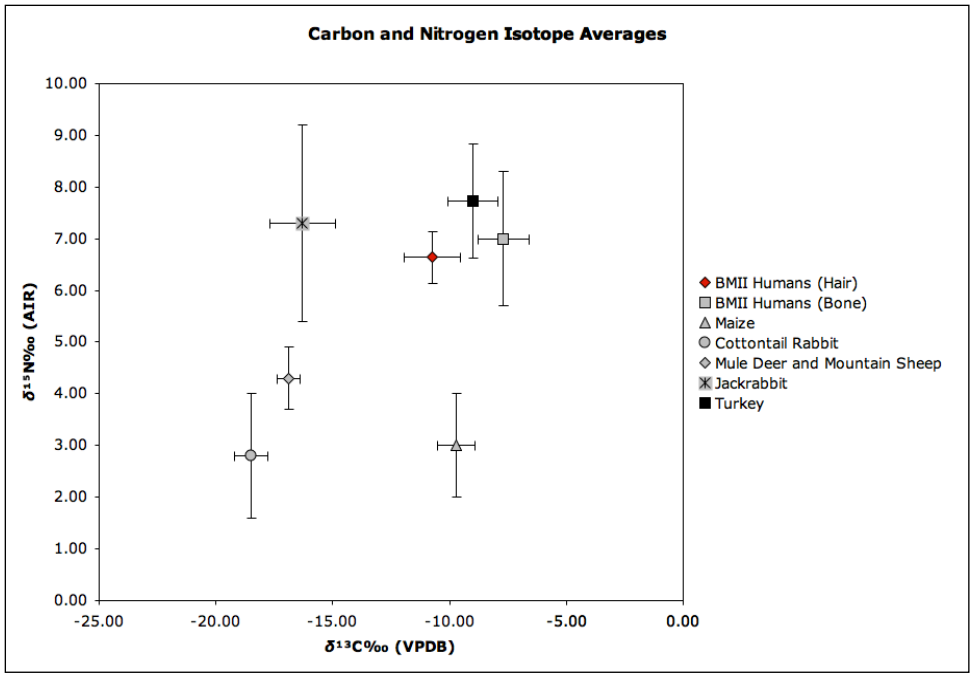

Figure 4.4: Average carbon and nitrogen isotope values for Turkey Pen hair with baseline and BMII collagen isotope values. The red point is the average of bulk CN hair isotope analysis (Table 4.2). Grey data points are from Coltrain and Janetski 2013. The black data point is from Rawlings and Driver 2010.

4.5.2 Single Amino Acid Analysis

The essential and non-essential amino acids show different patterns of variation along the length of hair TP 4 (Figures 4.5, 4.6). There is similar variation in $\delta^{13}C$ values along the length of the hair in each essential amino acid; the average variation of the essential amino acids is 6.2‰ (Figure 4.5). The smallest overall shift in $\delta^{13}C$ values is in valine at 4.8‰ and the greatest shift is in threonine at 11.5‰ (Table 4.3).

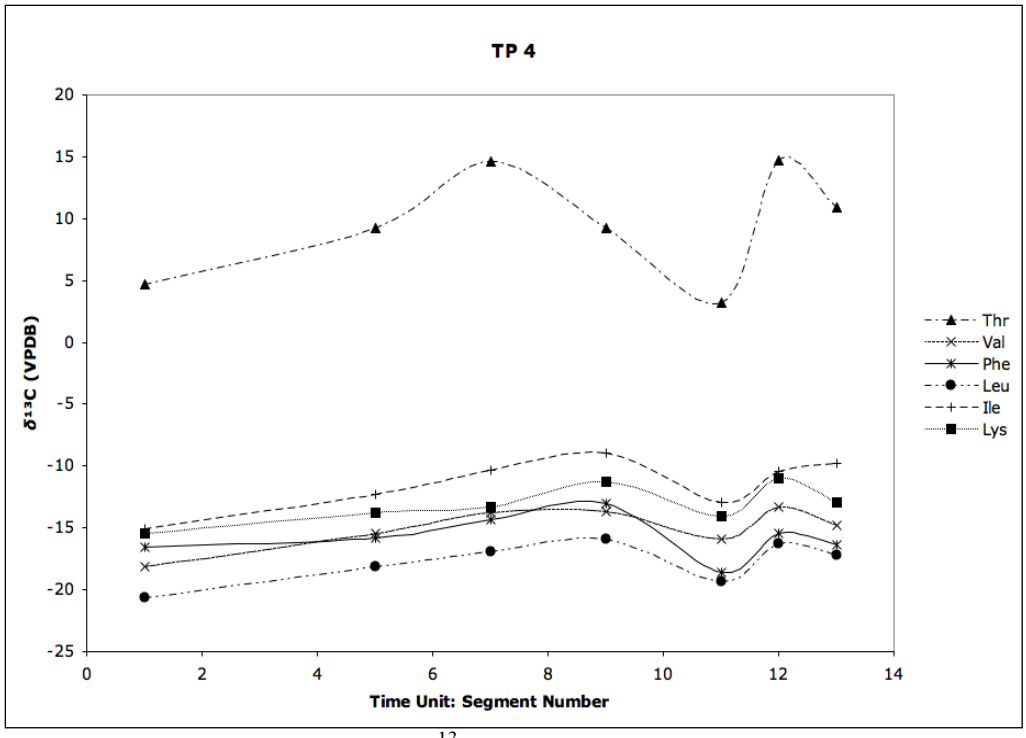

Figure 4.5: Essential amino acid $\delta^{13}C$ variation along TP 4 with data for segments 1, 5, 7, 9, 11, 12 and 13. Each segment represents about half a month

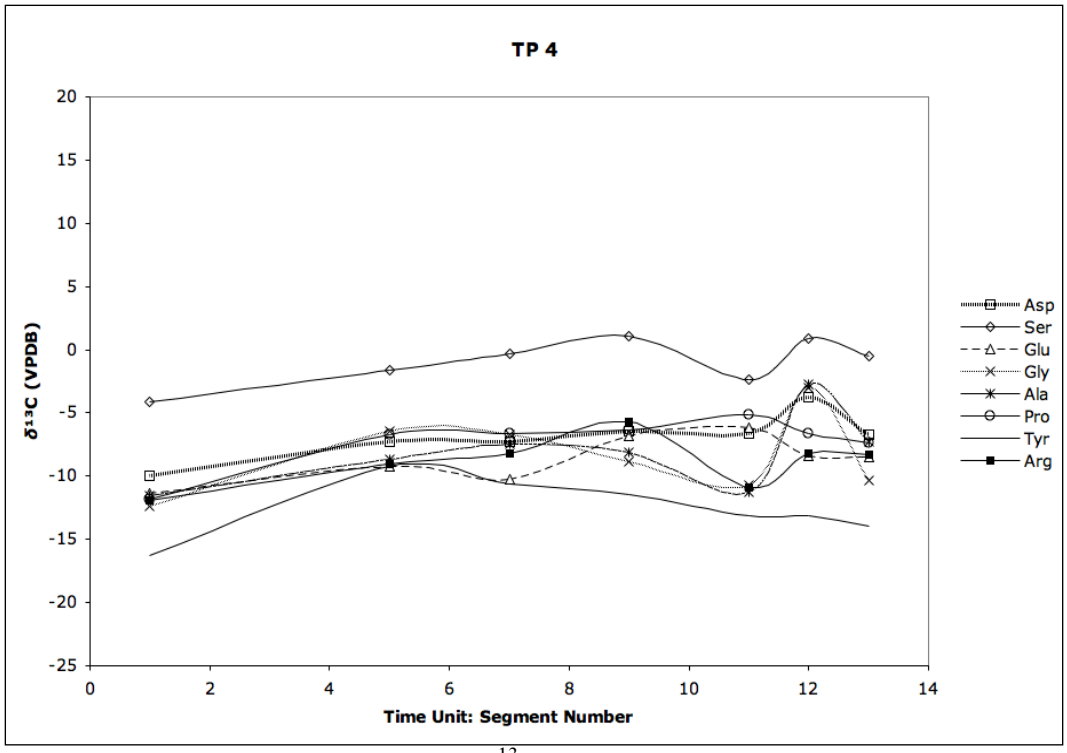

Figure 4.6: Non-essential amino acid $\delta^{13}C$ variation along TP 4 with data for segments 1, 5, 7, 9, 11, 12 and 13. Each segment represents approximately half a month.

The non-essential amino acids follow the same pattern as the essential amino acids except for tyrosine, proline and glutamine/glutamic acid (Figure 4.6). The average shift in the non-essential amino acids is 6.7‰, with serine shifting the least at 3.8‰ and glycine shifting the most at 9.4‰ (Table 4.3).

Table 4.3: Single amino acid carbon isotope values for hair TP 4. Values are all in ‰

Segment	Asp	Ser	Glu	Thr	Gly	Ala	Pro	IS	Val	Cys	Met	Ile	Leu	Lys	His	Tyr	Arg	Phe	$\Delta^{13}C_{Lys-Phe}$
1	-9.9	-4.1	-11.3	4.7	-12.4	-11.5	-11.9	-31.8	-18.1	-8.2	12.5	-15.1	-20.7	-15.5	-8.6	-16.3	-12.0	-16.5	1.0
5	-7.3	-1.6	-9.2	9.3	-6.5	-8.7	-6.7	-30.2	-15.4	N/A	N/A	-12.3	-18.2	-13.8	-5.9	-9.2	-9.0	-15.8	2.0
7	-7.3	-0.3	-10.2	14.6	-6.7	-7.5	-6.6	-29.8	-13.8	N/A	N/A	-10.4	-16.9	-13.3	-2.3	-10.6	-8.2	-14.4	1.1
9	-6.4	1.1	-6.8	9.3	-8.9	-8.1	-6.3	-31.3	-13.7	-6.1	9.3	-9.0	-15.9	-11.2	-1.5	-11.5	-5.7	-13.0	1.8
11	-6.6	-2.4	-6.2	3.2	-10.8	-11.3	-5.2	-31.4	-15.9	N/A	N/A	-12.9	-19.4	-14.0	-9.0	-13.1	-10.9	-18.6	4.6
12	-3.8	0.9	-8.4	14.8	-3.0	-2.7	-6.6	N/A	-13.3	-15.6	11.0	-10.4	-16.3	-11.0	-1.3	-13.1	-8.2	-15.4	4.4
13	-6.7	-0.5	-8.5	10.9	-10.3	-7.3	-7.4	-29.9	-14.8	-12.5	5.3	-9.8	-17.2	-12.9	-9.0	-14.0	-8.3	-16.4	3.5
Average	**-6.9**	**-1.0**	**-8.7**	**9.5**	**-8.4**	**-8.2**	**-7.2**	**-30.7**	**-15.0**	**-10.6**	**9.5**	**-11.4**	**-17.8**	**-13.1**	**-5.4**	**-12.5**	**-8.9**	**-15.7**	**2.6**
StDev	**1.8**	**1.9**	**1.8**	**4.4**	**3.2**	**2.9**	**2.1**	**0.9**	**1.7**	**4.3**	**3.1**	**2.1**	**1.7**	**1.6**	**3.6**	**2.3**	**2.0**	**1.8**	**1.5**

4.6 Discussion

The human hair from BMII Turkey Pen Ruins had average isotopic values of -10.8‰ ± 1.2 δ^{13}C and 6.6‰ ± 0.5 δ^{15}N, which we interpret as signs of these people eating a mainly C_4, maize based diet that was low in animal protein (Figure 4.3). When compared to baseline isotope values from the American Southwest, it is possible to see that the human hairs have a similar δ^{13}C value (-10.8‰ ± 1.2) to ancient North and Central American maize (-9.7‰ ± 0.8) as opposed to foraging animals which ate the local C_3 wild flora (see Figure 4.3) (Coltrain and Janetski, 2013). The range of δ^{13}C values in the bulk hair from -8.6‰ to -14.3‰ all fall within expected ranges of a C_4, maize based diet (Tables 4.2, 4.3; Figure 4.2) (van der Merwe and Vogel, 1978). These values are slightly lower, however, than the average human bone collagen δ^{13}C value of -7.7‰ ± 1.1 from this region (Coltrain and Janetski, 2013).

The human hair δ^{15}N values indicate that these individuals were eating at a relatively low trophic level when compared to a local baseline (Coltrain and Janetski, 2013; Rawlings and Driver, 2010). The δ^{15}N average from Turkey Pen Ruins (6.6‰ ± 0.5) is within the same δ^{15}N range as local wild turkeys (7.7‰ ± 1.1) and jackrabbits (δ^{15}N 7.3‰ ± 1.9) (Figure 4.3) (Coltrain and Janetski, 2013; Rawlings and Driver, 2010). These δ^{15}N values are approximately +3‰ above local maize (δ^{15}N 3.0‰ ± 1.0), suggesting that the humans and turkeys were eating at the same trophic level (Coltrain and Janetski, 2013; Rawlings and Driver, 2010). Also, increases in δ^{15}N do not coincide with large increases in δ^{13}C, indicating that in increase in meat protein does not relate to increases in C_4 protein.

The isotopic comparison between individuals from Turkey Pen Ruins and turkeys in this case, however, is not ideal. The turkeys from the Rawlings and Driver study were from Baskemaker III through Pueblo III periods, with most samples coming from Pueblo II and Pueblo III contexts (2010). Very few turkey bones were found in the BMII midden at Turkey Pen Ruins and were unavailable for isotopic analysis. Other coprolite analyses, however, suggest that both domesticated turkeys and their contemporaneous human keepers were consuming a high proportion of maize in their diets in the BMII period (Aasen, 1984; Androy, 2003; Munro, 1994; Nott, 2010). These results suggest that isotope data from later period domesticated turkeys such as presented by Rawlings and Driver 2010 may be substituted for the trophic level comparison because they were eating similar diets.

The single amino acid carbon isotope data support the bulk isotope findings that Turkey Pen Ruins BMII individuals experienced short-term variation in diet. Essential amino acids are a direct reflection of dietary protein because they cannot be made by the body (Choy et al., 2010; Corr et al., 2005). The shift of the essential amino acid $\delta^{13}C$ values show that the diet of this individual did change over a period of approximately 6.5 months (Figure 4.4). It is also interesting to note that though the $\delta^{13}C$ values of each essential amino acid differ, they follow a similar shifting pattern along the length of the hair.

The interpretation that TP 4 reflects a C_4 diet is supported by comparing the amino acid $\delta^{13}C$ signatures to bone collagen and hair data from previous studies (for example (Choy et al., 2010; Honch et al., 2012; Raghavan et al., 2010). The values of the

amino acids are high when compared to C_3 and freshwater fish consumers indicating a C_4 diet as the likeliest interpretation.

When focusing on maize consumption (or maize-fed turkeys) as a particular C_4 diet it is important to consider the essential amino acids leucine (Leu), the most abundant amino acid in maize, and lysine (Lys) which in maize is deficient from a dietary perspective (Katz et al., 1974). Lysine is not only present in low quantities in maize but what is present is not readily bioavailable (Katz et al., 1974). Food preparation strategies used at Cedar Mesa during the BMII period have been examined experimentally and the results suggest that some lysine can be made available from maize by nixtamalization, the process of stone boiling maize kernels with limestone (Ellwood et al., 2013; Katz et al., 1974). The amount of lysine made available through stone boiling maize, however, is not enough to support human dietary needs (Ellwood et al., 2013; Matson, 2015b).

Considering these factors we suggest that in a maize (or maize-fed turkey) rich diet, lysine is likely to come from other sources (C_3) in the diet as maize alone will not supply enough lysine. Consequently the $\delta^{13}C$ values of lysine will be more negative than expected on a C_4 diet with sufficient lysine, an observation that Honch et al. identified in their data (Honch et al., 2012). For TP 4, the average Leu $\delta^{13}C$ value is -17.8‰ ± 1.7, which is similar to values reported for C_4 consumers by Corr et al (2005) and more positive than the values presented for humans eating a high marine protein diet (Choy et al., 2010; Corr et al., 2005), confirming that hair TP 4 came from an individual eating a C_4 based terrestrial diet and not (albeit unlikely) a high marine protein diet. Moreover, the $\Delta^{13}C_{valine\text{-}phenylalanine}$ values for TP 4 range from -1.6‰ to 2.6‰ and are similar to those for the terrestrial food consumers analysed by Honch et al. 2012. Typically lysine is

relatively light compared to other essential amino acids, and in C_3, high freshwater fish and high marine protein diets is typically 5‰ or more lighter than phenylalanine (Choy et al., 2010; Honch et al., 2012). In C_4 consuming individuals this difference is typically around 3‰ or less and attributed to the contribution of C_3 lysine lowering the overall $\delta^{13}C$ value of the lysine in bone collagen analysed (Honch et al., 2012). It is anticipated that in a high lysine C_4 diet, $\Delta^{13}C_{Lys\text{-}Phe}$ would be greater, but this has not been explicitly tested. In hair TP 4 the $\delta^{13}C$ value for lysine (-13.1‰ ± 1.6) and values for $\Delta^{13}C_{Lys\text{-}Phe}$ (which range from 1.0- 4.6), are consistent with a largely low lysine C_4 diet (of maize or maize-fed turkey) with some periods of mixed C_4/C_3 consumption (Table 4.3).

Based on the similarities between the six hairs analysed using bulk carbon and nitrogen isotopes and the single hair analysed using single amino acid carbon isotopes, it appears that all of the individuals examined ate a C_4 based diet with some seasonal variation including months when C_3 plants (most likely piñon pine nuts or ricegrass) were consumed in greater quantities relative to the total diet. Moreover, the single amino acid $\delta^{13}C$ data backs the notion of a high maize (or maize-fed turkey) diet. The low $\delta^{15}N$ values from the bulk hair analysis (average 6.6‰ ± 0.5) indicate that these individuals were eating on the same trophic level as the local herbivores throughout the year, and the single amino acid $\delta^{13}C$ values likely tied to maize rather than maize-fed turkey. For the bulk hair $\delta^{13}C$ values, TP 9 shows the strongest pattern of semi-sinusoidal curvature, suggesting a regular, potentially seasonal variation in diet supplementation while still remaining based on maize. The other hairs show variability, but not as strongly. This may be due to the short lengths of the hair where only a few months are captured rather than over a year as is seen in TP 4. Variability of hair isotope values could also be

affected by differences in diet between demographic groups, but as the samples in this study are not connected to specific individuals, such a relationship cannot be addressed.

Overall, the low nitrogen isotope values indicate that these individuals did not consume a large proportion of dietary protein from animal sources and were therefore unlikely to be consuming large quantities of domesticated turkeys on a regular basis; had the humans been ingesting turkey meat, there would be a trophic level shift between humans and turkeys, which is not shown in the data. Other research on BMII individuals from this region came to similar conclusions; it is thought that in this period the turkeys were intentionally raised for other reasons, possibly to use their feathers in creating feather blankets and ceremonial objects for ritualistic use (Lipe et al., 2016; Munro, 1994; Rawlings and Driver, 2010). If part of a ritualistic use incorporated eating the turkeys on a very occasional basis, this would not result in a significant change in isotopic signature, as consistent eating patterns are required to affect the protein isotope signature (Lee-Thorp, 2008).

4.7 Conclusions

This study is one of the first serial isotope analyses of human hair from the American Southwest. It also provides the first serial amino acid isotope data for this region and provides another point of comparison for amino acid signatures of C_4 diets.

The findings of our study complement the existing body of data from this site and region, showing quantitatively that the human diet at BMII Turkey Pen Ruins, while reliant on maize horticulture, was variable over a period of months and was never high in animal protein. Analysing individual strands of human hair allow us to examine the short-

term variation within the overall dietary pattern of these BMII individuals. The single amino acid carbon isotope analysis similarly confirms these conclusions, while also allowing a detailed comparison between lysine and leucine $\delta^{13}C$ signatures to suggest that they likely come from different food sources. The variability in $\delta^{13}C$ signatures along the individual hairs in both bulk keratin and single amino acid isotope analyses indicates probable seasonal supplementation of the diet with wild C_3 plants.

5. Conclusions

The three different hair isotope projects presented in this thesis were conducted to improve our understanding and use of isotopic data to better interpret variations in human diets. Each project focused on a different scale of dietary variation in order to see the range of challenges inherent in interpreting isotopic data among populations, within populations, and within individuals. The examination of these three scales of dietary variation also highlight research questions that can be asked and how the different scales of dietary variation influence each other. Overall, these three studies show that the identification of significant dietary differences requires either a wide spread of isotopic signatures (as seen in the Ethiopian data set in Chapter 2), or large sample sizes (e.g. the Nicaraguan data set in Chapter 3). Determining statistically significant shifts in diets when sample sizes are small, as seen in the intra-individual study of hair from Turkey Pen Ruins, was not possible. These data are still useful for illuminating patterns of dietary shift over short periods of time.

This chapter presents the major findings and implications of each study, followed by a broad analysis of the three scales here presented. The chapter finishes with a discussion of future directions for study—highlighting both possible avenues for expanding the three case-studies and future avenues for expanding applications of hair isotope analysis in collaborative and archaeological work.

5.1 Major Findings

The first project (Chapter 2) explores dietary variation among multiple groups in Ethiopia with distinctly different subsistence strategies through carbon, nitrogen and

sulphur isotopic analyses. These data provide the first application of stable isotope analysis of hair from modern individuals in this region and are a component of an ethnographic pilot study in the region. This study also shows isotopic and dietary variation among groups that had access to the same subsistence resource base through trade and proximity in Southern Ethiopia

The data from this study provide important implications for archaeologists, showing that populations or distinct groups can have visually and statistically different isotopic ranges (and thus have been eating distinctly different diets) even when they have access to the same foodstuffs. These differences in diet when populations have access to the same resource base are interpretable as variations in economic strategy or cultural preference. Previous studies have examined isotopic and dietary differences between groups to show the temporal succession of different cultural groups within the same region—usually interpreted as one group of people replacing another (Britton et al., 2013; Coltrain, 2009)—or the differential use of resources geographically (Boyd et al., 2008). The data presented in the modern Ethiopian study are a reminder to also examine the possibility of multiple groups co-habiting a region yet remaining isotopically and dietarily distinct, and that this can be a question we address with our data.

The first project also shows that using isotopic data to identify distinct dietary groups becomes a more powerful tool as isotopes of more elements are examined. Carbon and nitrogen, the most commonly used pairing of isotopic analyses when studying dietary variation, were only able to differentiate between the Pastoralists and the Peasant Farmers, but not between the Peasant Farmers and the Fishing Farmers (Figure 2.3). The proportion of C_3 to C_4 plants in the diets of Peasant Farmers and Fishing

Farmers was too similar to distinguish, and there was no significant difference in the trophic level of protein being contributed to the diet. Adding sulphur isotopic data made it possible to differentiate between all three economic strategies (Figures 2.4, 2.5). Though expensive, sulphur is a very useful addition to isotopic analyses for identifying whether fish protein is contributing to the diet, as well as if there is a distinct difference in the geology of different locations (Bollongino et al., 2013; Nehlich, 2015).

The findings presented in Chapter 2 reinforce the importance of cultural background, economic strategy, and food preferences on what individuals are eating. By focusing on differences in isotopic signatures between groups of individuals who follow distinct economic strategies within the same resource catchment, it is possible to see that though there is isotopic spread within each of the economic groupings, the overall differences within each group are smaller than the isotopic differences between economic strategies. At this scale of isotopic analysis, the isotopic differences between economic groups are both visually and statistically identifiable (Table 2.3; Figure 2.2). Within this region, these data illustrate the fact that despite government pressure to homogenize peoples in Ethiopia, people following different economic strategies remain dietarily distinct (Cohen and Isaksson, 1988; Tache and Oba, 2009). Beyond examining modern Ethiopians, this pattern of between-group isotopic variation being larger than within-group isotopic variation is an example of one way for archaeologists to statistically identify populations by their dietary preferences or strategies.

The second project (Chapter 3) examines dietary differences through carbon and nitrogen isotopic analysis among demographic groups in Nicaragua considered as a single population. First, the data presented in the second project addresses the question

of what constitutes a distinct population when we attempt to interpret large isotope datasets from an archaeological study. Data were collected from Nicaraguans who live in two different villages—but they were determined to be part of the same population due to intermarriage, similarity of economic strategy, and the proximity of the two villages (Koster, 2011, 2007; Winking and Koster, 2015). The isotopic data supports this interpretation of both villages being part of the same population: there are no large separations in the isotopic distributions, and the slight but significant difference in isotopic signature between the two villages may be due to differences in diet between wealthier and poorer families (Figure 3.10). There were significant isotopic and nutritional differences between the wealthier and poorer families in this population, and a disproportionate number of the wealthy families were found in Arang Dak (Winking and Koster, 2015).

These data show that there can be dietary variability within a single population, resulting in a large spread in both carbon and nitrogen isotopic signatures, and that there can be some significant differences in isotopic signatures among demographic groups that contributes to isotopic variability within the population. The complementary ethnographic data gathered from the Mayangna and Miskito in Nicaragua made it possible to explore dietary variation among multiple different demographic (age, sex) and socio-economic (wealth, village) groups, some of which were significantly different. There are archaeological studies that apply isotopic analyses of bone collagen to similar questions of demographic differences in diet—for example, one study used $\delta^{13}C$ and $\delta^{15}N$ data to show dietary differences between men and women in an archaeological

population from Peru (Somerville et al., 2015), and another used isotopes to explore the relationship between status and diet at Cahokia (Ambrose et al., 2003).

Further, the Nicaraguan hair isotope data show that it is possible to find patterns of distinct isotopic differences through statistical analysis that are not clear as visual patterns, as illustrated by slight—but significant—differences in diet among age groups (Figures 3.5, 3.6) The archaeological importance of this study is that it shows the value of probing isotopic data beyond visual separation of groups. There is potential for dietary variation within one population, as well as subtle, but significant, demographic differences in diet that can be identified if enough samples are present and analysed (Ambrose et al., 2003; Somerville et al., 2015).

These data, though they show statistical differences in diet, may not actually indicate differences in nutrition. The patterns found in the Nicaraguan isotopic data do agree with some of the nutritional studies—for example, the correlation between increased wealth and better nutrition is mirrored in the isotopic data (Figure 3.8) (Barros et al., 2010; Godoy et al., 2005; Winking and Koster, 2015). Further examination of isotopic dietary data alongside nutritional data, such as anthropometric measurements, would better illuminate the relationship between differences in isotopic data (as an indicator of diet) and differences in nutritional markers (as indicators of individual's heath and food security) (Bacon et al., 2014; Belachew et al., 2013). Combining isotopic and nutritional data would be useful when expanding studies on questions such as whether nutritional education influences feeding habits and health (Moore et al., 2009) or if perceived differences in social status are correlated with differences in diet and/or nutrition (Reyes-García et al., 2008)

The third project (Chapter 4) examines intra-individual dietary variation through serial isotopic analysis of hair. This project used serial carbon and nitrogen isotopic analysis of five hair strands to examine the overall variability of diet within individuals, as well as serial single amino acid carbon isotopic analysis of one hair strand as complementary strategies to examine how diets at Basketmaker II Turkey Pen Ruins may have varied. These data show that, first, it is possible to see variations in diet when serially analysing human hair in this region, and second, that intra-individual variability may not be the same across all members of a population. Furthermore, these data show that isotopic variation can be interpreted as shifts in diet, while also remaining cautious about interpreting these variations as significant—without increased numbers of samples, statistical analysis is not viable (Fraser et al., 2006; Knudson et al., 2007; Williams and Katzenberg, 2012).

This third study also shows that the detailed data collected using stable carbon isotope analysis of single amino acids is complementary to the more common carbon and nitrogen isotopic analyses. In the Turkey Pen Ruins study, the single amino acid carbon isotope data confirmed the results found through carbon and nitrogen stable isotope analysis. The data also show shifts in diet over even shorter periods of time, allowing analysis of an individual's diet over two week increments (Figures 4.5, 4.6). This technique would be most powerfully applied to exploring diets at archaeological sites where carbon and nitrogen isotopic analyses alone cannot reveal dietary patterning, or in instances where isotopic data from very narrow time-windows are needed (Choy et al., 2010; Corr et al., 2005; Honch et al., 2012).

5.2 Isotopic Sectioning Points and Scales of Dietary Variation

Multiple studies in the literature have discussed the topic of how large a shift in isotopic signatures needs to be in order to be considered a significant change in diet (Fraser et al., 2006; Fuller et al., 2006; Knudson et al., 2015; Webb et al., 2013). A few different "sectioning points" have been used specifically in intra-individual dietary studies to decide whether or not differences in isotopic signatures indicate significant differences in diet (Fraser et al., 2006; Williams and Katzenberg, 2012). The acceptable amount of variability (measured as difference between the highest and lowest isotopic signatures for an individual) within a "stable" diet ranges from 1.5‰ (Fraser et al., 2006) for δ^{13}C and δ^{15}N to 0.5% for δ^{13}C (Webb et al., 2013 and William and Katzenberg, 2012); there has been no published data on the amount of isotopic variation expected in δ^{34}S for an individual with a stable diet. At another scale of dietary variation, the amount of isotopic variability expected within a population has also not been discussed.

These sectioning point values and techniques are most directly comparable to the data from Turkey Pen Ruins, which examines intra-individual dietary variation. Using either the 1.5‰ or 0.5‰ break points, hair strands TP 1, TP 3, and TP 7 would be considered to show dietary variation beyond a stable diet. Hair strand TP 8 has a 1.2‰ spread of δ^{13}C values, which is indicative of dietary change by one metric, but would be considered stable by the other. Only one hair strand, TP 9, has a δ^{15}N range that is above the sectioning point of 1.5‰ published by Fraser et al. (2006).

Most of the isotopic differences between demographic group medians in the Nicaraguan data set are smaller than 1.5‰, and some are even below 0.5‰, indicating that these could be considered generally stable diets (Table 5.1). The small, but

statistically significant, differences among demographic groups may be partly due to individuals eating stable diets, resulting in tighter clustering of the isotopic signatures and the ability to identify dietary differences among subgroups of a population. Combining a large demographic isotope study with intra-individual isotopic analysis will help illuminate how these two scales of dietary variation relate.

Table 5.1: Absolute differences between Nicaraguan demographic group isotopic medians that tested as significantly different (see Chapter 3)

Comparison between	**Difference between δ^{13}C medians**	**Difference between δ^{15}N medians**
Male and female children	0.2‰	
Infants and children	1.0‰	1.5‰
Infants and adolescents	0.6‰	1.6‰
Infants and adults		1.1‰
Children and adolescents	0.4‰	
Adults and children	0.8‰	
Adults and adolescents	0.4‰	
Families with above median wealth and families with below median wealth		0.3‰
Residents of Arang Dak and residents of Suma Pipi	0.6‰	0.3‰

Examining the significant differences among economic groups in Ethiopia, however, shows that all of the significant isotopic differences between δ^{13}C and δ^{15}N values are greater than the more stringent sectioning point for a variable diet. These differences show that despite variation within these different economic groups, overall differences between the groups is quite large.

5.3 Future Directions

This thesis has used the data from the three case studies in order to discuss how we handle and interpret isotopic data collected from human hair and what constitutes significant differences in diet. The three projects have each examined a different scale of dietary variation—among populations, within a population, and within individuals—and in so doing have affirmed the utility of isotopic analysis for determining dietary differences (Ambrose et al., 2003; Nakamura et al., 1982; Vogel and van der Merwe, 1977), highlighted the necessity for statistical rigor and illustrated some new directions that isotopic analysis of hair can take.

There are three main avenues through which these research projects could be expanded: (1) addition of other isotopic analyses (e.g., S, O, H) to complement the data in the three case studies presented here; (2) increasing the number of archaeological and ethnographic samples analysed; and, (3) conducting ethnographic isotopic analyses of individuals in other regions to compare isotopic variation either within or among populations.

First, expanding the data sets through further isotopic analyses such as sulphur (for the second and third projects) oxygen or hydrogen, would provide another variable for comparison through which dietary differences may become apparent (Bol et al., 2007). Depending on the archaeological questions being asked—whether they are about mobility, diet, or climate—using multiple, complementary isotopic analyses is an important strategy for showing differences among or within populations or individuals as long as the process of isotopic fractionation reflects the human process in question. Sulphur would be particularly useful for expanding the dataset of the Nicaraguan case

study because it would potentially help identify fish-based protein in individuals' diets (Nehlich, 2015). Oxygen and hydrogen would be useful particularly in the Ethiopian case study to determine whether the geographical landscapes used by the different populations are isotopically different, and how large the trading area is (Knudson et al., 2012; Thompson et al., 2009; Wilson et al., 2007).

Second, increasing the number of samples, particularly for the Ethiopian and Turkey Pen Ruins case studies, would make it possible to do more robust statistical analyses. Ideally, expanding the Ethiopian data set to collect more samples from the same populations and include people from all demographic groups, would allow for analysis of isotopic difference both among the economic groups and within each economic group to strengthen these studies of dietary variation. Particularly, it would be useful to determine if there are differences in diet between sexes in the Fishing Farmer group, which would change the isotopic spread for that economic strategy (Christiaensen and Alderman, 2004; Hadley et al., 2008). Expanding the number of hair samples serially analysed from Turkey Pen Ruins would enable further comparison of intra-individual variability to see if seasonality of diet was a widespread or unusual dietary pattern among individuals from this population (LeBlanc and Morgan, 2010).

Third, it would be useful to expand on the research in this thesis by analysing hair samples from more modern and archaeological populations. Ideally, the patterns found at each scale of dietary variation could be compared to data collected from populations in other parts of the world to see how the patterning of isotopic variability varies. Do other interacting populations with different subsistence and economic strategies have identifying isotopic signatures that separate the populations? Are the same demographic

variables of age and wealth factors in the isotopic spreads of other semi-isolated populations? Which economic and subsistence patterns result in significant seasonal variability of diet? Isotopic analysis of human hair from more modern and archaeological populations would begin to answer these questions.

Though it is not found in all archaeological contexts, hair remains an important substrate for isotopic analysis in archaeology. Hair can be found in multiple areas across the globe, including (but not limited to) sites in Peru (Knudson et al., 2015; Webb et al., 2015), Chile (Macko et al., 1999), Egypt (White et al., 1999), Iran (Pollard et al., 2008), Alaska (Britton et al., 2013), the southwest United States (LeBlanc and Morgan, 2010), England (O'Connell and Hedges, 1999) and the Alps (Macko et al., 1999). Due to the difference in tissue growth of hair and bone, it is possible to ask questions about seasonal diet (Williams and Katzenberg, 2012), seasonal mobility (Knudson et al., 2012), or whether an individual's diet changed soon before death (Wilson et al., 2007). It is also possible to use hair isotopic signatures for examining dietary trends that do not need to be directly related to changes over a period of months or a couple of years. As these studies continue, we will have more data across which we can compare ways to interpret intra-individual dietary variation, and how that impacts dietary variation within and between populations. These data can also be used to examine how or if dietary seasonality has changed for modern populations in a rapidly globalizing world (Bacon et al., 2014).

This dietary information that can be gleaned from hair isotopic analyses is complementary to the data gathered from isotopic analyses of bone collage, tooth enamel, dentine or calculus, which are found more commonly in the archaeological record. Isotopic analysis of each of these tissues can be used to explore past diet, but each is

suited to different temporal foci. These different tissues makes it possible to examine diet over different scales of time in an individual's life—bone collagen averaging an individual's diet from the years or decades preceding death (Britton et al., 2013; Knudson et al., 2012) , teeth reflecting an individual's average diet from the time of formation (Gregoricka, 2014), and hair reflecting an individual's average diet over a period of weeks or months (LeBeau et al., 2011). It is possible to use a few of these tissues to look at dietary changes over the course of an individual's life, either through a combination of different bones (Cheung, 2015), teeth (Gregoricka, 2014), or serial analyses (Eerkens et al., 2016; Knudson et al., 2007; Webb et al., 2013). Teeth, hair and nails can be used for serial isotopic analysis, but focus on changes in an individual's diet at different points in their lives due to when the tissues were formed (Eerkens et al., 2016; Knudson et al., 2007). Archaeologists can use hair isotopic data as a way to compare isotopic signatures of past individuals to modern ones, as well as in helping refine serial isotopic analyses, which are growing more common in using both hair and dental samples (Eerkens et al., 2016; Webb et al., 2013).

Currently, very few ethnographic studies supplement their data with isotopic data, despite its proven utility and the availability of fingernails and hair as potential substrates for isotopic analysis (Nardoto et al., 2006, 2011). I would encourage further collaborative work in this direction for two reasons. First, understanding how dietary differences in modern individuals appear isotopically, when we can supplement our data and refine our interpretation with ethnographic data, will help us be able to better identify patterns in archaeological populations. Second, collaborations among stable isotope chemists, ethnographers, and nutritionists, and the resulting complexes of data, could

begin to help us understand how differences in diet may or may not relate to differences in nutrition and food security (Petzke et al., 2010).

5.4 Concluding Remarks

The focal point of this thesis has been examining the applications of stable isotope analysis of human hair, and evaluating the use and interpretation of these data. It is evident from these data that there is a great deal of information that can be gleaned from isotopic data of hair. Hair is the ideal substrate for studying short-term intra-individual dietary variation as well as dietary variation among modern and archaeological individuals. In archaeological settings, this data is complementary to isotopic analysis of bone collagen when hair samples are available because hair is useful for mapping dietary variation among individuals without needing to account for the diet-over-time averaging effects inherent in analysis of bone collagen. In modern populations, the analysis of hair or fingernails makes it possible to conduct isotopic analysis non-invasively, and provide complementary data in ethnographic studies. As such, these case studies can be used as a point of reference for examining dietary variation at multiple scales, and how distinct (or indistinct) the patterns may be, as well as a suggestion of how isotopic analysis can be used in future collaborative and interdisciplinary work.

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Appendix 1: Supplemental Information

Table S1: CN Instrument QA/QC data for Chapter 2 including isotopic averages and standard deviations for each standard in each analytical run

Run ID	Standard	n	Average δ13CVPDB			Average %C			n	Average δ15NAIR			Average %N		
12092014	MET	9	-28.58	±	0.08	43.88	±	2.43	9	-4.91	±	0.27	9.24	±	0.27
12092014	SUBC-1	7	-13.74	±	0.24	44.32	±	2.21	7	17.34	±	0.13	15.25	±	0.40
12092014	NIST 1577c	3	-17.31	±	0.01	44.45	±	0.61	3	8.20	±	0.03	10.17	±	0.10
12092014	USGS42	4	-21.09	±	0.02	45.10	±	0.87	4	7.98	±	0.03	14.78	±	0.26
12092014	USGS43	3	-21.28	±	0.02	44.52	±	0.65	3	8.41	±	0.06	14.77	±	0.64
18082014	MET	6	-28.62	±	0.05	39.62	±	1.44	6	-4.97	±	0.22	9.55	±	0.17
18082014	SUBC	4	-13.68	±	0.03	42.70	±	2.23	4	17.26	±	0.11	16.51	±	0.99
18082014	USGS-42	4	-21.13	±	0.09	45.77	±	1.69	4	8.20	±	0.06	15.15	±	0.24
18082014	USGS-43	5	-21.26	±	0.04	42.96	±	1.05	5	8.54	±	0.10	15.11	±	0.44
19082014	MET	8	-28.61	±	0.02	39.89	±	1.71	6	-4.87	±	0.24	9.16	±	0.14
19082014	SUBC	4	-13.73	±	0.06	43.92	±	1.58	4	17.37	±	0.13	16.01	±	0.85
19082014	USGS-42	4	-21.14	±	0.04	45.48	±	1.59	3	7.94	±	0.12	14.79	±	0.36
19082014	USGS-43	5	-21.26	±	0.02	45.14	±	1.22	5	8.40	±	0.09	14.90	±	0.72

Table S2: S Instrument QA/QC data for Chapter 2 including isotopic averages and standard deviations for each standard in each analytical run

Run ID	Standard	n	average $\delta^{34}S$			Average %S		
20141028	IAEA S-1	3		±	0.47		±	1.32
20141028	IAEA S-3	2	-31.27	±	1.79	13.77	±	0.46
20141028	MET	3	8.21	±	0.58	23.81	±	1.10
20141028	NBS-127	3		±	0.29		±	0.72
20141028	USGS-42	3	6.71	±	0.37	4.66	±	0.33
20141028	USGS-43	3	9.52	±	0.66	5.18	±	0.07
20141030	IAEA S-1	3		±	0.40		±	0.22
20141030	IAEA S-3	3	-31.62	±	0.63	13.32	±	0.92
20141030	MET	4	8.21	±	0.43	22.74	±	0.89
20141030	NBS-127	3		±	1.89		±	2.83
20141030	USGS-42	3	6.70	±	0.77	4.34	±	0.26
20141030	USGS-43	3	9.49	±	0.61	4.66	±	0.17
20151125	Casein	3	6.18	±	0.08	0.66	±	0.03
20151125	IAEA S-1	3		±	0.77		±	0.90
20151125	MET	2	9.65	±	0.17	23.06	±	1.32
20151125	NBS-127	3		±	0.48		±	0.24
20151125	NIST 1577b	3	1.97	±	0.56	0.78	±	0.05
20151125	USGS43	3	9.68	±	0.28	4.77	±	0.14
20151126	Casein	3	7.49	±	0.10	0.76	±	0.12
20151126	IAEA S-1	3		±	0.19		±	0.11
20151126	MET	2	9.18	±	0.12	23.24	±	1.72
20151126	NBS-127	4		±	0.40		±	0.34
20151126	NIST 1577b1	4	1.66	±	0.38	0.78	±	0.05
20151126	USGS43	3	9.71	±	0.81	5.09	±	0.14
20151130	Casein	3	7.39	±	0.60	0.68	±	0.21
20151130	IAEA S-1	3		±	0.38		±	0.29
20151130	MET	2	9.28	±	0.15	22.61	±	0.06
20151130	NBS-127	3		±	0.16		±	0.68
20151130	NIST 1577b	3	1.91	±	0.32	0.79	±	0.02
20151130	USGS43	3	9.13	±	0.41	4.65	±	0.13
20151201	Casein	3	7.44	±	0.64	0.80	±	0.04
20151201	IAEA S-1	3		±	0.54		±	0.49
20151201	MET	3	9.06	±	0.76	23.69	±	1.47
20151201	NBS-127	3		±	0.93		±	0.68
20151201	NIST 1577b	3	1.85	±	0.37	0.80	±	0.04
20151201	USGS43	3	9.96	±	0.52	4.74	±	0.11

Table S3: CN Instrument QA/QC data for Chapter 3 including isotopic averages and standard deviations for each standard in each analytical run

Run ID	Standard	n	Average δ^{13}C VPDB			Average δ^{15}N AIR			Average %C			Average %N			Average C/N		
20150925	MET	6	-28.64	±	0.05	-5.04	±	0.05	40.49	±	2.43	9.36	±	0.41	5.04	±	0.10
20150925	USGS-40	7		±	0.04		±	0.07		±	0.88		±	0.17		±	0.04
20150925	USGS-41	6		±	0.14		±	0.09		±	0.46		±	0.08		±	0.04
20150925	USGS-43	8	-21.32	±	0.06	8.38	±	0.12	43.70	±	0.35	14.60	±	0.31	3.49	±	0.07
20150928	MET	6	-28.61	±	0.04	-5.00	±	0.07	40.05	±	1.76	9.27	±	0.36	5.04	±	0.06
20150928	USGS-40	7		±	0.04		±	0.05		±	0.83		±	0.19		±	0.02
20150928	USGS-41	6		±	0.04		±	0.07		±	0.79		±	0.20		±	0.02
20150928	USGS-43	6	-21.42	±	0.32	8.50	±	0.34	50.31	±	14.69	16.76	±	4.89	3.50	±	0.03
20150929	MET	6	-28.55	±	0.06	-5.05	±	0.04	40.27	±	1.75	9.29	±	0.31	5.05	±	0.08
20150929	USGS-40	7		±	0.03		±	0.25		±	1.28		±	0.28		±	0.03
20150929	USGS-41	6		±	0.05		±	0.06		±	0.32		±	0.10		±	0.03
20150929	USGS-43	8	-21.31	±	0.03	8.37	±	0.06	44.11	±	0.65	14.63	±	0.31	3.52	±	0.07
20150930	MET	6	-28.59	±	0.05	-5.05	±	0.10	40.23	±	1.61	9.28	±	0.39	5.06	±	0.06
20150930	USGS-40	7		±	0.05		±	0.05		±	0.66		±	0.23		±	0.05
20150930	USGS-41	6		±	0.10		±	0.18		±	0.28		±	0.13		±	0.04
20150930	USGS-43	8	-21.25	±	0.06	8.35	±	0.14	44.13	±	0.59	14.55	±	0.42	3.54	±	0.07
20151001	MET	6	-28.61	±	0.04	-5.01	±	0.09	40.24	±	1.85	9.24	±	0.31	5.07	±	0.09
20151001	USGS-40	7		±	0.03		±	0.08		±	0.81		±	0.21		±	0.03
20151001	USGS-41	6		±	0.03		±	0.20		±	0.37		±	0.12		±	0.02
20151001	USGS-43	6	-21.29	±	0.04	8.38	±	0.06	43.79	±	0.90	14.64	±	0.41	3.49	±	0.03
20151002	MET	6	-28.62	±	0.07	-5.03	±	0.05	40.07	±	1.71	9.29	±	0.25	5.03	±	0.09
20151002	USGS-40	7		±	0.07		±	0.06		±	0.74		±	0.17		±	0.02
20151002	USGS-41	6		±	0.09		±	0.07		±	0.25		±	0.08		±	0.04
20151002	USGS-43	6	-21.29	±	0.07	8.39	±	0.06	43.91	±	1.06	14.69	±	0.44	3.49	±	0.05
20151005	MET	7	-28.63	±	0.03	-5.05	±	0.06	39.83	±	1.65	9.28	±	0.25	5.01	±	0.08
20151005	USGS-40	7		±	0.02		±	0.04		±	0.78		±	0.11		±	0.04
20151005	USGS-41	6		±	0.06		±	0.18		±	2.04		±	0.44		±	0.05
20151005	USGS-43	7	-21.29	±	0.05	8.37	±	0.06	43.83	±	1.01	14.80	±	0.25	3.45	±	0.05
20151102	MET	6	-28.88	±	0.13	-5.05	±	0.10	38.08	±	3.29	8.82	±	0.83	5.04	±	0.05
20151102	NIST 1577c	2	-17.70	±	0.05	8.11	±	0.01	47.87	±	0.38	9.88	±	0.06	5.65	±	0.01
20151102	SRM-1	2	-16.99	±	3.16	8.73	±	9.69	41.23	±	1.69	14.63	±	0.37	3.29	±	0.05
20151102	SRM-2	1	-14.70	±		15.54	±		40.45	±		14.41	±		3.27	±	
20151102	SUBC-1	3	-13.61	±	0.07	17.39	±	0.03	43.46	±	2.38	16.09	±	1.09	3.15	±	0.04
20151102	USGS40	6		±	0.35		±	0.25		±	0.79		±	0.22		±	0.04
20151102	USGS41	6		±	0.22		±	0.25		±	0.36		±	0.10		±	0.04
20151102	USGS43	1	-20.80	±		7.90	±		44.84	±		14.29	±		3.66	±	