DIETARY IDENTITY AT SALANGO: STABLE LIGHT ISOTOPE ANALYSIS OF GUANGALA PERIOD BURIALS (100 BCE – 800 CE)

by

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ABSTRACT

EMILIE MARIE COBB. Stable Light Isotopes of The Guangala Period: Dietary Identity at Salango. (Under the direction of DR. SARA JUENGST)

Salango is an archaeological site on the coast of Ecuador that has been inhabited for thousands of years by multiple different cultures, including the Guangala (100 BCE-400 CE). While research about mortuary treatment and socio-cultural characteristics has been conducted for the Guangala people, there is a lack of research about diet and/or using biogeochemical studies. This thesis utilizes stable isotope analysis of carbon and nitrogen in order to determine what dietary resources were consumed by the Very Early Guangala (VEG) and Early Guangala (EG) people buried at Salango. The sample includes individuals of various sexes and age groups. Results of this research show that these individuals were consuming mostly marine protein sources, which is to be expected for the area, and C_4 resources (maize). Comparisons between demographic groups (sex and age), time periods (VEG and EG), and other sites in Ecuador and the Andes region highlight some variation in diet. These comparisons show isotopes statistically differed between time periods, and between infants and older individuals in the sample. Comparisons between contemporaneous cultures in Ecuador show similar overall diets but differ in protein consumption, likely due to difference in geographic location. Overall, these data show that people living at Salango took advantage of abundant local resources and generally shared access to foodstuffs, although some restriction in dietary options occurred over time

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CHAPTER 1: INTRODUCTION

Salango is an archaeological site on the coast of Ecuador that has been inhabited since approximately 4000 BCE. Over thousands of years, various groups of people have lived there, including the Preceramic, Valdivia, Machalilla, Engoroy, Bahia II, Guangala, and Manteño peoples (Béarez and Lunniss 2003; Lunniss 2001, 2008, and 2017; Bythell 2019; Juengst et al. 2019). Evidence of the Guangala period (100 BCE to 800 CE) at Salango includes burials that contained the remains of high-status infants, juveniles, and adults, and grave goods. In 2019, Abigail Bythell and Dr. Sara Juengst examined these human skeletal remains and collected data regarding pathology, age, sex, and skeletal modification in order to address questions about health and status during the Very Early Guangala (VEG) (~100 BCE – ~50 CE) and Early Guangala (EG) (~50 CE – 300 CE) (Bythell 2019; Juengst et al. 2019; Lunniss 2017). However, questions remain regarding the identity and daily life of these individuals, in particular how food was shared across society.

Food and diet are important to investigate, as they are significant facets of cultural identity and essential to human life. Particularly in modern descendent and immigrant communities, food is a part of tradition that is passed down to other generations and can be indicative of how groups interact with their environment (Beoku-Betts 1995). Like ceramic work, body modifications, and attire, choices about diet can delineate individuals, and cultural and sub-cultural groups (Beoku-Betts 1995; D'Sylva and Beagan 2011; Mintz and Du Bois 2002; Parasecoli 2014). Diet can be impacted by age and sex due to expected social roles and access to resources associated with these categories cross-culturally, as demonstrated in other archaeological and sociocultural

investigations (Barrett and Richards 2004; Linderholm et al. 2008; Somerville et al. 2015; Turner et al. 2007). Stable isotope analyses, methods to investigate ancient diets, are thus beneficial for learning about the identities of the Guangala people of Salango.

For this project, I use stable isotope analysis to investigate diet and identity of Guangala individuals, likely of a high social status, at Salango. These samples came from VEG and EG period burials, and included individuals of various ages and sexes. During stable isotope analysis, bioarchaeologists isolate elements, usually carbon and nitrogen, and measure the amounts of elemental isotopes relative to a standard for resources in that area. For coastal Ecuador, these isotopic standards have been published by van der Merwe et al. (1993). The carbon and nitrogen isotope values differentiate between marine and terrestrial diets, and determine whether an individual's diet includes C_3, C_4, or CAM plants (Ambrose and Norr 1993; Bogaard and Outram 2013; Hastorf 1985; Katzenberg 2008; Schoeninger and Moore 1992). Hitherto, dietary isotopes have not been extensively studied in Guangala phase individuals; therefore, it is currently unknown if the Guangalan diet was the same for all individuals or varied throughout the population or within social groups.

CHAPTER 2: BACKGROUND INFORMATION

2.1 Regional Developmental Period of Ecuador

During the Regional Development Period (RDP) (500/200 BCE – 600/800 CE), distinct cultural groups formed in coastal Ecuador (Bushnell 1951; Bythell 2019; Masucci 2008). Dates marking the transition between the Late Formative Period and beginning of the RDP vary by location, but generally this period is known for its increase in complexity of sociopolitical structure, expanded trade and interregional relations, and creation of localized ceramic and art styles. Six distinct regional cultures on the southern coast of Ecuador emerged: the Guangala, Daule, Tejar, and Jambelí cultures (Bythell 2019; Masucci 1992, 2008). On the central and northern coast, the Bahía, Jama-Coaque, and Tiaone-Tolita cultures were formed. The majority of cultural groups in the RDP do not have indicators of sociopolitical hierarchy with the exception of cultures on the northern and central coast (Masucci 2008).

The Guangala Culture

The Guangala culture developed between 100 BCE and 800 CE and was present along the Ecuadorian coast in Guayas Province and southern Manabí (Bushnell 1951; Bythell 2019; Masucci 2008). This phase was initially identified by its pottery and is named after the modern town where this pottery was first located (Bushnell 1951). Guangala pottery is most distinct for the style change of bowls, cups, other ceramics (such as flutes, figures, and whistles), and thick-walled plates in multiple colors, including white-on-red painted pottery (Masucci 1997, 2000, 2008).

Guangala ceramics are divided up into six different classes based on specific characteristics such as differing sediments used in vessel creation. These different classes suggest that Guangala peoples traded ceramics and/or temper ingredients from different areas of the Ecuadorian coast and Northern Peru (Masucci 1997, 2000). A ceramic painting style diagnostic of this time period is "finger-painted" black designs on the body of the ceramic piece (Bushnell 1951; Masucci 2000, 2019). Decorated ceramic figurines are another frequent and notable Guangala feature. These figurines are often elaborately decorated with incised patterns that are possibly representative of ritual and daily clothing, body tattoos, paint, and/or adornment (i.e., earrings). The intricate detail of these pieces indicates specialized ceramic making (Masucci 2008).

Marine shell working and bead creation were also common during this period, and likely had a smaller, non-specialized role in the Guangala economy, compared with ceramic production. At the site of E1 Azúcar, marine shells were used primarily at a local level rather than as export items. Despite the non-specialized role of shell working in the Guangala economy, the *Spondylus* (*Spondylidae* spp.) shell was considered to be a heavily desired material throughout the Guangala period due to its cultural significance in pre-historic Ecuador (Masucci 1995). Specifically, chisels made from these shells dated to the Guangala phase have been located at the Salango site (Stahl and Norton 1987).

Guangala sociopolitical life is difficult to categorize in terms of hierarchy and interconnectivity between sites. While it is clear that the Guangala people had access to trade and non-local goods such as obsidian, gold, and copper (at least in the El Azúcar Valley), how the trade systems operated is currently unknown (Masucci 1995, 2008, 2019). Specialized ceramic creation can suggest that a social hierarchy and upper class

were present in the Guangala sociopolitical structure; however, there is no other evidence (e.g., grave goods indicating wealth or figurines depicting powerful or wealthy individuals) that this is the case (Masucci 2008; Stothert 1984). Furthermore, Guangala settlements were usually scattered agricultural sites throughout the area with no evidence of geographical hierarchy. It is likely that Guangala societies had chiefdom-based political structures, but were beginning to create more complex sociopolitical systems as suggested by types of ceramics being produced (Masucci 2008; Stothert 1984).

One way to investigate hierarchy and power relations is through access to resources and diet. Archaeological faunal remains indicate that Guangala diet included domesticated and non-domesticated animals, and marine life. At Guangala sites further inland (like El Azúcar), there is evidence of shellfish consumption that relied on trade from coastal sites, indicating that Ecuadorian trading systems included food resources not just craft materials (Masucci 1995). Zooarchaeological analysis at Salango has identified multiple non-marine sources of protein including the domesticated cavy (*Cavia porcellus*) and Muscovy duck (*Cairina moschata*). Non-domesticated animals that could have been used for food include deer, gulls, and marine fish (Stahl and Norton 1987; van der Merwe et al. 1993). People living at inland sites also may have been consuming pigeons, doves, and songbirds as evidenced by a high concentration of avian remains at El Azúcar (Tellkamp 2019).

Maize is likely to have been a feature in Guangala diets as well. Comparisons of dietary isotopes from previous time periods (Valdivia, Machalilla, and Chorrera) confirmed that once maize was introduced into the region, the crop slowly became an important feature in the diets of coastal Ecuadorians, and likely became a significant

dietary component by the Machalilla phase (1700 – 1100 BCE) (Tykot and Staller 2002; van der Merwe et al. 1993). However, it remains unclear if resources were accessible to everyone during the Guangala phases or if they were divided amongst the population; these divisions, if they are present, could correlate with specific identity groups (based on age or sex) or socioeconomic status.

2.2 Archaeology of Salango

Salango is an archaeological site on the coast of Ecuador (Manabí province) near a modern town also named Salango; the area has been occupied since approximately 4000 BCE (Fig. 1 and 2) (Bythell 2019; Lunniss 2001, 2008, 2017). Over time, Salango has been home to various groups of individuals from the Preceramic (~10450/10150 cal BCE – 4650 cal BCE) through the modern day. Archaeologists have worked at Salango since the 1980s in order to learn about these various groups of inhabitants and have concluded that the site was likely a sacred center for individuals living in this area (Béarez and Lunniss 2003; Lunniss 2001, 2008, 2017, 2020; Bythell 2019; Juengst et al. 2019).

Pre-Hispanic People of Salango

During the Formative Period of Ecuador (~4400 -300 cal BCE), Valdivia and Machalilla peoples occupied Salango (Zeidler 2008). Valdivia occupation occurred between 2700 and 1500 BCE (Lunniss 2001; Bythell 2019). Subsequently, the Machalilla established a fishing village at Salango (1500 to 900 BCE). Artifacts from Salango from this period include pottery fragments, shell remains, stone and bone tools (such as shellfish hooks and stone net sinkers), and faunal remains. Twenty-six human burials,

which have yet to be analyzed in a publication, also dated to this time at Salango (Béarez et al. 2012). The Machalilla people's main source of protein was marine animals, and zooarchaeological work concluded that 80% of the fish remains belonged to the *Scrombridae* family. Of the scrombrids, the most prevalent species were the yellowfin tuna, the black skipjack, and the skipjack tuna (Béarez et al. 2012). Excavation of these artifacts and burials confirms that Salango was established as a location where people subsisted on marine resources throughout different time periods.

Following the Machalilla, the Engoroy (a regional name for the Chorrera culture) people inhabited Salango from approximately 600 to 100 BCE (Béarez and Lunniss 2003; Zeilder 2008). Scrombrid fishing was also essential for the Engoroy, although zooarchaeological analysis showed that black skipjack was their main source of protein as opposed to yellowfin tuna (Béarez and Lunniss 2003). At this time, Salango was an important ceremonial center with large ceremonial spaces; the architecture was directly related to Engoroy spiritual practice (Lunniss 2001, 2008). Different structures at Salango had varied functions; for example, Salango inhabitants designated areas for recently deceased and ancestral individuals, everyday activities, and ritual activities. Religious leaders likely facilitated relationships with spirits (Lunniss 2008). The Engoroy people of Salango are also known for their *Spondylus* shell bead manufacturing as evidenced by white and red beads found in burials dated to this period (Bauer and Lunniss 2010).

During the Regional Development Period (100 BCE to 500 CE), Bahía II and Guangala people lived together at Salango (Béarez and Lunniss 2003; Lunniss 2001 and 2008; Bythell 2019). Based on pottery, artifacts related to ritualized coca consumption, and ceramic figurines, it appears that, compared to Bahía I, the Bahía II people of

Salango were more involved in structured religion and power (Lunniss 2017). In the Bahía II burials, evidence of coca consumption (lime paraphernalia), personal adornments made of various materials (including Spondylus shell), and figurines are present (Lunniss 2017). Burial offerings from the Guangala people included ceramic figurines of the Guangala style. These funerary objects indicated the importance of coca rituals for the Bahía II at Salango. It is possible that Guangala individuals joined them in rituals at Salango given that it is likely collaboration between the Guangala and Bahía II occurred at other sites in the area like Cerro Jaboncillo (Lunniss 2017).

Guangala burials at Salango were associated with two periods: the Very Early Guangala (~100 BCE – 50 CE) and Early Guangala (~50 CE – 300 CE) phases. These skeletons were incomplete, but researchers recorded pathologies such as caries and osteoarthritis (Bythell 2019; Juengst et al. 2019). The occurrence of stress related pathologies in juveniles and infants from VEG suggested that they experienced high levels of stress prior to death. Of particular interest were two VEG infants that were buried wearing “helmets” made from the skulls of previously deceased children, perhaps as part of ritual related to Guangala spirituality (Juengst et al. 2019). Guangala burials contained various grave goods such as spirit effigies and ceramics from both the Early and Middle Guangala periods (Lunniss 2018). After the Bahía and Guangala peoples, Salango was abandoned until later Manteño peoples settled there (500 CE to 1531 CE) (Béarez and Lunniss 2003; Lunniss 2001,2008; Bythell 2019).

Guangala Bioarchaeology

While we increasingly understand Guangala culture and the occupation of Salango in particular, there has been little bioarchaeology conducted at Guangala cultural sites (Bushnell 1951; Bythell 2019; Ubelaker 1983; Ubelaker and DeGaglia 2020). At La Libertad on the southern Santa Elena Peninsula, primary and secondary commingled Guangala burials were present and had associated artifacts (ceramic bowls and figurines, stone tools, shell tools, etc.). Analysis of these burials was focused on the associated artifacts and the description of Guangala culture (Bushnell 1951). Excavation of a Guangala cemetery at site OGSE-MA-172 on the Ecuadorian coast (in the town of Valdivia) revealed evidence of body modification, specifically cranial modification, and numerous side effects of transition to an agricultural society, such as higher rates of dental and infectious disease than previous societies (Ubelaker 1983). These studies supply important information about the mortuary treatment of Guangala individuals at sites other than Salango and give detailed descriptions of cultural objects specific to the Guangala phase.

Guangala remains from Torre Marina (located on the Ecuadorian coast) have been studied to examine impacts of community living on health (Ubelaker and DeGaglia 2020). These remains had low instances of periosteal lesions and porotic hyperostosis, but high levels of dental pathology (including caries, antemortem tooth loss, and alveolar abscesses). The high prevalence of dental pathologies observed here are consistent with maize consumption. Ubelaker and DeGaglia (2020) also conclude that severe antemortem tooth loss reflects a high life expectancy and adult longevity.

At Salango, a project in 2019 compared burials from the VEG and EG (Bythell 2019). Bythell found differences in the severity of stress pathologies between the two time periods; the VEG skeletons exhibited signs of high stress while the EG skeletons exhibited signs of moderate stress. This suggests a change in environmental or cultural stress load, difference in fragility, or sampling bias between the two samples. There were also differences in mortuary treatment between the burials. Mortuary treatment differences are possibly attributed to reestablishing social order after a stressful event or period of time (Bythell 2019).

Both of these recent bioarchaeological studies (Bythell 2019; Ubelaker and DeGaglia 2020) of the Guangala people provide essential information regarding the health of Guangala individuals. Although Ubelaker and DeGaglia (2020) suggest that consumption of maize contributed to the extensive dental pathology at Torre Marina, isotope analysis has not been conducted to confirm this, and neither of these studies explore dietary differences based on status or identity. Thus, this study uses a bioarchaeological framework to investigate Guangala identity at Salango, particularly focusing on connections between diet, age, sex, and gender to explore differences in lived experience of personhood.

2.3 Bioarchaeology of Social Identity

Biological anthropologists study both the cultural and biological aspects of an individual's life. In particular, bioarcheologists use the human skeleton to investigate how culture affects biology, and vice versa. An important aspect of studying an individual's life and culture involves the study of identity. Identity is a multifaceted

concept that encompasses gender, sex, status, age, and religion. It is also fluid and impacted by our communities (Agarwal and Glencross 2011; Knudson and Stojanowski 2008; Torres-Rouff and Knudson 2017). As stated by Knudson and Stojanowski (2008), "(i)dentities can be both personal and communal, ascribed and achieved, manipulated and feigned… Identities are about self-perception and self-promotion as well as constraints imposed by others" (2).

Age Estimation

Age in bioarchaeology is one of the first pieces of data collected when studying a human skeleton. This is often done by examining morphological changes such as tooth wear, joint surface destruction, tooth development, epiphyseal and suture fusions; however, pathologies (such as rickets and achondroplasia) can alter the estimated biological age of a skeleton (Bertrand et al. 2016; Buikstra and Ubelaker 1994; Gowland 2006; Knudson and Stojanowski 2008; Sofaer 2011). Age traditionally has been thought of as having three different types: biological or physiological age, chronological age, and social age. For this project, biological age and social age will be important, as biological age has previously been estimated for this sample and estimates about social age will be made based on diet reconstruction. While age is inherently tied to biology as it depends on the growth of the human body, it is important to remember that the life course of human beings involves cultural and physical/biological circumstances. The lifespan and physical body of a human is always impacted by the culture that they grow in (Gowland 2006; Knudson and Stojanowski 2008; Sofaer 2006, 2011; Torres-Rouff and Knudson 2017).

When estimating biological age, skeletons are often placed in categories such as infant/young child, juvenile, adolescent, and adult. The term "sub-adult" is often used to group together individuals who did not reach skeletal maturity before their time of death. There is debate about the use of this terminology as it can imply that younger individuals are inferior to older individuals, however; "juvenile" can represent individuals of specific ages in certain contexts (such as in European literature) (Halcrow and Tayles 2008; Lewis 2007; Torres-Rouff and Knudson 2017). Since the sample for this study involves the remains of children, it is important to recognize children as autonomous beings with their contribution and role within their society. The period of childhood and designation of children is dependent on culture. Cultures can also have evolving concepts of childhood and adulthood, such as in the case of Anglo-Saxon Britain where the age of legal adults changed from 10 years of age to 12 years of age in a few centuries (Gowland 2006; Halcrow and Tayles 2008; Mays et al. 2017; Perry 2008; Sofaer 2006).

Sex and Gender in Bioarchaeology

Sex estimation in bioarchaeology is conducted in order to learn more about the lives of people who died hundreds or thousands of years ago. It, along with age, is usually one of the first pieces of data gathered from a skeleton. Many assumptions and interpretations about these individuals are made based on sex in areas such as health and disease, possible manners of death, violence, and gender-based divisions of labor. This is in part due to the lack of distinction between sex, a biological and social spectrum, and gender, a spectrum of cultural performances (Claassen 1992; Fausto-Sterling 1993; Geller 2008, 2009; Hollimon 2017; Knudson and Stojanowski 2008; Stone 2012; Walker and Cook 1998). The advent of using aDNA analysis to determine sex could further

contribute to confusion on the difference between sex and gender as gender could become geneticized in studies that use this method (Geller 2017b). While the analysis of these topics in bioarchaeology is not new, considering how sex and gender intersect with them is a relatively fresh take (Hollimon 2011; Torres-Rouff and Knudson 2017). There are common assumptions that occur when considering sex and gender within these themes with some of them being problematic in nature. How sex and gender is researched and discussed in bioarchaeology (including bioarchaeology's affinity for binary systems) has been described in several studies of this topic (Agarwal 2021, 2017; Claassen 1992, 2001; Fausto-Sterling 1993; Geller 2008, 2009, 2017a, 2017b; Ghisleni et al. 2016; Hollimon 2011, 2017; Moral 2016; Novak 2017; Stone and Walrath 2006; Walrath 2017)

Isotopes and Identity

While reconstructing the diet of ancient peoples is valuable information by itself, dietary reconstruction can help us investigate other areas of an individual's identity or groups' culture. Anthropologists have used isotopes to study dietary differences based on sex or social differences (Ambrose et al. 2003; Barrett and Richards 2004; Linderholm et al. 2008; Somerville et al. 2015; White 2005), reconstruct the life history of an individual (Knudson et al. 2012), and study age-related dietary patterns (Tung et al. 2016; Tung and Knudson 2018; Turner et al. 2007). The methods and theoretical frameworks of these research projects can be applied to dietary isotopes of the Guangala people of Salango in order to learn more about how they lived.

2.4 Isotope Analysis to Investigate Diet

Isotopes are atoms of the same element that have the same number of protons but a different number of neutrons, and many different elements have been used in archaeological isotope analysis including strontium, carbon, nitrogen, strontium, and oxygen. Isotopes can be considered heavier or lighter based on their mass, which is determined by the sum of their protons and neutrons; heavier isotopes have a higher atomic mass and light isotopes have a lower atomic mass (Katzenberg 2008). Stable isotopes are isotopes that do not decay radioactively and are often used in archaeology to analyze human and faunal remains (Katzenberg 1989; Katzenberg and Harrison 1997; Katzenberg 2008; Schoeninger and Moore 1992). Beginning as a geochemical technique, isotope analysis can be used for multiple purposes including locating a person's place of origin, and breaking down the diets of past populations. For dietary studies, carbon (C) and nitrogen (N) are the two elements that are most commonly used. These elements enter the body through the consumption of macronutrients (protein, carbohydrates, and lipids). The isotopes are extracted from human bone samples that contain collagen or bone apatite which is usually the best-preserved organic material left after decomposition (Ambrose and Norr 1993; Hastorf 1985; Katzenberg and Harrison 1997; Katzenberg 2008; Schoeninger and Moore 1992; Yoder and Bartelink 2010). The values used for diet studies (expressed as $\delta^{13}C$ and $\delta^{15}N$) are determined using this notation:

$$\delta \; in \; \%_0 = \frac{R_{\,(sample)} - R_{(standard)}}{R_{(standard)}} \times 1000$$

In this notation, R is the ratio of heavier to lighter isotope ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). The lowercase delta notation, δ, is used to indicate the small amounts of change in the ratios of heavier to lighter isotopes (Katzenberg 2008). Aside from diet reconstruction, isotopic analysis has been explored as a method to estimate nutritional health as certain health conditions (such as pregnancy, diabetes, liver disease, and wasting diseases) have been found to impact element levels in the body (Fuller et al. 2005; Katzenberg and Lovell 1999; Reitsema 2013). Isotopes have also been used to study individual identity and community life (Barrett and Richards 2004; Knudson et al. 2012; Linderholm et al. 2008; Turner et al. 2007), diet of a geographical region over time (Turner et al. 2018), group mobility patterns (Knudson et al 2015), and manipulation of certain animal species for cultural activities (Somerville et al. 2010).

Carbon and Nitrogen Isotopic Analysis

Carbon has two stable isotopes: ^{13}C and ^{12}C. The values of ^{13}C relative to ^{12}C are compared to base levels of these isotopes in order to determine components of past diets, such as types of consumed plants and whether these were primarily terrestrial or marine (Ambrose and Norr 1993; Bogaard and Outram 2013; Hastorf 1985; Katzenberg 2008; Schoeninger and Moore 1992). Through photosynthesis, both marine and terrestrial plants gather carbon that is present in the atmosphere and in the ocean. Atmospheric carbon comes primarily from carbon dioxide (CO_2), and the value of $\delta^{13}C$ in terrestrial plants is different depending on which photosynthetic pathway a plant uses.

There are three different photosynthetic pathways for terrestrial plants that define three different plant groups: C_3, C_4, and CAM plants (Blake 2015; Ambrose and Norr

1993; Schoeninger and Moore 1992). C_3 plants (which include wheat, rice, all root crops, legumes, and vegetables) undergo a photosynthesis that produces three carbon atoms. $\delta^{13}C$ values for these types of plants usually range between -29 and -25‰ (Blake 2015). C_4 plants produce four carbon atoms with photosynthesis and are plants such as maize, sugarcane, and millets (Ambrose and Norr 1993; Schoeninger and Moore 1992). $\delta^{13}C$ signatures for C_4 resources typically fall within the range of -16 to -10‰ (Blake 2015). The final terrestrial photosynthetic pathway is "crassulacean acid metabolism" or CAM. CAM plants use either the C_3 or C_4 pathway depending on the conditions of their environment; therefore, the $\delta^{13}C$ value for CAM plants can fall between the values of C_4 and C_3 plants (Ambrose and Norr 1993; Schoeninger and Moore 1992). This group includes various succulents such as cacti, agaves, and bromeliads (Ambrose and Norr 1993; Schoeninger and Moore 1992).

Marine plants obtain carbon from a variety of sources such as terrestrial debris that enters the ocean, dissolved CO_2, and dissolved carbonic acid. Due to these multiple sources of carbon, marine plants can have $\delta^{13}C$ values that are similar to terrestrial plants. For instance, the $\delta^{13}C$ values of sea grasses can be similar to those of C_4 plants and cold-water plankton $\delta^{13}C$ values can be similar to C_3 plants (Katzenberg 2008; Schoeninger and Moore 1992). Carbon isotope values are taken from two sources in the body: bone collagen and bone apatite carbonate. Carbon isotopes from collagen are indicative of only the protein portion of an individual's diet, and carbon isotopes from apatite carbonate are representative of an individual's overall diet (Ambrose and Norr 1993; Katzenberg 2008).

Like carbon, nitrogen also has two stable isotopes (^{15}N and ^{14}N), and ^{15}N is relative to the ^{14}N atmospheric baseline. The majority of Earth's nitrogen is found in the

atmosphere as N_2 (nitrogen gas) or dissolved in bodies of water (Hastorf 1985; Katzenberg and Harrison 1997; Katzenberg 2008; Schoeninger and Moore 1992). Nitrogen is transferred through different organisms through two different methods. The first method involves N_2 fixing organisms in freshwater and saltwater (such as blue or green algae) and terrestrial plants with nodules of bacteria from the *Rhizobium* genus. Nitrogen fixing organisms are able to combine nitrogen with other elements so that it can be used; however, most terrestrial plants are unable to do this on their own (Katzenberg 2008; Schoeninger and Moore 1992). Legumes have *Rhizobium* bacteria living on their roots, and the bacteria is able to fix the nitrogen so that the legumes can access it for growth. Due to these bacteria, legumes often have $\delta^{15}N$ values close to that of atmospheric nitrogen which is 0%.

The second method, for plants without the *Rhizobium* bacteria, involves absorbing nitrogen that is available during the decomposition of dead organisms (Katzenberg 2008; Schoeninger and Moore 1992). Vascular plants are able to absorb nitrogen from compounds such as ammonia (NH_3) and nitrate (NO_3) that are produced when bacteria breakdown dead organisms. Because these plants are getting nitrogen from another source, they usually have $\delta^{15}N$ values higher than atmospheric nitrogen. In marine environments, most of the nitrogen available comes from bacterial denitrification and the organisms absorbing this nitrogen usually have $\delta^{15}N$ values more positive than N_2 (Katzenberg 2008; Schoeninger and Moore 1992). A baseline graph of stable carbon and nitrogen values for various organisms in different environments can be seen in Figure 3 (Blake 2015:142).

The $\delta^{15}N$ values of different species are dependent on their trophic level, or their level within the food chain. While $\delta^{13}C$ also varies on trophic level, the values of $\delta^{15}N$ vary much more than the carbon isotopes (Bogaard and Outram 2013; Katzenberg and Harrison 1997; Katzenberg 2008; Schoeninger and Moore 1992). An example of how $\delta^{15}N$ values are affected by the trophic level of a species is clearly seen with herbivores. Herbivores consume plants, which causes a concentration of ^{15}N relative to ^{14}N. These herbivores then have a higher $\delta^{15}N$ value than the plants that they have eaten (Schoeninger and Moore 1992). Due to this reflection of trophic levels, herbivores and carnivores have approximately 3% higher $\delta^{15}N$ values than the species that they consume (Katzenberg 2008; Schoeninger and Moore 1992).

It is important to note that stable isotope nitrogen values are significantly impacted by periods of nutritional stress, pathologies (such as osteomyelitis and diabetes), health conditions like pregnancy, and breastfeeding (Bogaard and Outram 2013; Fuller et al. 2006; Katzenberg and Harrison 1997; Katzenberg and Lovell 1999; Reitsema 2013). For instance, the $\delta^{15}N$ range in skeletal material from infants consuming breast or herbivore milk is usually 3-5% higher than the range in juvenile or adult skeletal material (Turner et al. 2007).

Oxygen Isotope Analysis

While this study focuses primarily on carbon and nitrogen isotopes in order to elucidate dietary patterns, oxygen isotopes are also reported for this sample and they are briefly analyzed. There are three stable oxygen isotopes that occur in nature: ^{16}O, ^{17}O, and ^{18}O (Pederzani and Britton 2019). Isotope analysis of oxygen usually only includes

analysis of the ratio between ^{16}O and ^{18}O ($^{18}O/^{16}O$) which is expressed as $\delta^{18}O$. These values are normalized using the VSMOW (Vienna Standard Mean of Water) and SLAP (Standard Light Antarctic Precipitation) scale; although, oxygen values are normally reported on the VPDB (Vienna Pee Dee Belemnite) scale (Juengst et al. 2021; Katzenberg 2008; Pederzani and Britton 2019). Oxygen isotopes enter the phosphate and carbonate components of human bone and enamel from body water and water that is consumed through diet. Because past populations generally consumed locally sourced water, the oxygen isotopes extracted from human bone can be indicative of an individual's migratory status or give details about an individual's place of origin (Lightfoot and O'Connell 2016; Pederzani and Britton 2019). Typically, $\delta^{18}O$ values decrease with elevation, increase in latitude, increasing distance from the coast, and lower temperatures (Juengst et al. 2021; Pederzani and Britton 2019).

Oxygen isotope analysis can be used for a variety of different studies in bioarchaeology. $\delta^{18}O$ values are most commonly utilized in paleoclimate reconstruction, to study human mobility (often in conjunction with strontium isotopes), and in the analysis of human life histories (Juengst et al. 2021; Katzenberg 2008; Pederzani and Britton 2019). Like carbon and nitrogen isotopes, oxygen isotopes can be impacted by a variety of factors including breastfeeding, culinary processes, and sociocultural practices. Like nitrogen isotopes, oxygen isotopes can be higher in breastfeeding infants than in non-breastfeeding individuals (Pederzani and Britton 2019). Culinary processes such as boiling, stewing, fermentation, and distillation can also result in $\delta^{18}O$ values that are not consistent with the research population. As diet is impacted by the sociocultural practices of culture, for instance individuals of various classes or ages may be consuming different

resources, they can modify oxygen isotope values as well (Pederzani and Britton 2019). Notably, the $\delta^{18}O$ value spread for individuals drinking water from the same source can be approximately 2‰, and research that included behavioral and physiological variability in the study of oxygen spreads concluded that the range of $\delta^{18}O$ values in an archaeological population is likely greater than 3‰ (Lightfoot and O'Connell 2016; Pederzani and Britton 2019).

2.5 Stable Isotope Analyses in South America

In South America, isotopes have been used to answer a variety of questions about identity and diet. For example, isotopic study and grave good analysis have been beneficial for research on the relationship between diet and social structure in Middle Horizon Period colonies of Tiwanaku in the Moquegua Valley, Southern Peru (Somerville et al. 2015). Individuals living at these settlements ate a combination of C_3 and C_4 food, and occasionally marine foods; males had higher $\delta^{13}C_{collagen}$ values suggesting they consumed more C_4 but otherwise diet between the sexes was mostly similar. (Somerville et al. 2015). Somerville et al. concludes that this difference may occur due to increased consumption of chicha by estimated males (2015). Chicha is a drink created by fermenting maize; the fermentation process of maize can make protein and certain amino acids more bioavailable in chicha (Gagnon and Juengst 2018; Somerville et al. 2010). Therefore, while likely all members of the Tiwanaku colony consumed chicha, it is clear that males consumed more than the other sex groups (Somerville et al. 2015).

Life history and dietary differences based on age are other areas of study that can utilize stable isotopes. For example, isotopic analysis of carbon, nitrogen, oxygen, and strontium provide information regarding the life of an Andean traveler who was moving inland from the coast of modern-day Chile. Bioarchaeologists estimated the individual to be a male between the ages of 28 and 35 years after excavation of his tomb (dated to the Late Formative Period), which is located in the Chilean portion of the Atacama Desert (Knudson et al. 2012). Based on the biogeochemical data, the researchers conclude that it is likely this individual traveled from the Chilean coast, and consumed both marine and terrestrial resources as he moved. After death, he was buried by people who were possibly travelling with him (Knudson et al. 2012; Knudson et al. 2015).

Age, an important factor in one's identity and social status, can have an impact on diet and lifestyle as well, as exemplified by the isotopic analysis of sites in Peru before, during, and after Wari Empire control and influence (Tung and Knudson 2018; Tung et al. 2016). The site of Beringa was not directly under the control of the Wari, but fell under the empire's sphere of influence. Isotopes of carbon and nitrogen were isolated from pre-Wari and during Wari archaeological collagen and keratin (Tung and Knudson 2018). These isotopes show that diet did not change when Wari influence began at Beringa and original food sources remained in place; however, there was a difference based on age. Juveniles in Beringa had lower $\delta^{13}C$ values than adults, which suggests that younger individuals were not consuming large amounts of maize. This may indicate that it was a cultural practice for Beringa individuals to make maize a part of their diets as they reach puberty or adulthood, as it was a culturally important food (Tung and Knudson (2018).

In a different study by Tung et al., isotopic and trauma analysis were used to investigate diet, inequality, and status based on age. Samples for isotope values were taken from the sites of Monqachayoq, Vegachayoq Moqo, and rural caves in the Peruvian Andes in this research. Tung et al. found that Wari $\delta^{13}C$ values were significantly higher than post-Wari values, suggesting a shift in juvenile diet from the Middle Horizon period to the Late Intermediate Period (Tung et al. 2016). Dietary differences were also observed between rural and urban sites for breastfeeding adults, and juveniles in the post-Wari period; this differs from the Wari-era when rural and urban sites had similar dietary isotope values (Tung et al. 2016). These isotope values in tandem with lethal skeletal trauma paint a picture of social inequality that emerged after the collapse of the Wari empire (Tung et al. 2016). This analysis of dietary isotopes and violence answers archaeological questions about how the lives of Andeans changed after Wari control.

Stable isotope studies in Ecuador

In Ecuador, stable isotope studies are limited, but a few notable studies exist. Important for this study are faunal baselines of carbon and nitrogen isotopes for Ecuador published by van der Merwe et al. (1993). These faunal signatures come from both modern and archaeological samples; however only one archaeological sample (belonging to the remains of a deer) contained enough collagen for analysis. Thus, the food web reconstructed here is primarily from modern animals (Fig. 4). Also included in this research are isotopic measurements of human remains from various cultural phases. Although the landscape of Ecuador has changed significantly over time, van der Merwe et al. (1993) state that it is highly likely that the marine environment has been mostly preserved for the past 6000 years (with the exception of population decline) (98). Marine

specimens included different types of seaweed, octopus, shellfish, crabs, shrimp, and various fish species. Riverine signatures were collected from different species of fish, crab, and shrimp; these $\delta^{13}C$ and $\delta^{15}N$ signatures proved to be significantly different than marine signatures. Terrestrial mammals were also sampled from remains provided by local hunters, and these signatures established the terrestrial environment as being primarily C_3. No signatures from mangrove swamps were able to be collected due to the endangerment of the swamp environment (van der Merwe et al. 1993).

Human isotopic signatures published in this study came from the Preceramic, Valdivia, Machalilla, Chorrera, Guangala, Bahía, and Manteño cultures. Preceramic samples came from the site of Las Vegas, where it was determined that the people ate some marine foods and C_4 foods (van der Merwe et al. 1993). Valdivia isotopes were collected from skeletons at Loma Alta and Real Alta; the diets of these individuals were primarily C_3 terrestrial and similar but with added marine sources. Likely mangrove oysters were a significant part of the Valdivia diet based on archaeological evidence, but this could not be confirmed isotopically (van der Merwe et al. 1993). Machalilla period isotopes were extracted from burials at Salango, where marine diets were common and maize was possibly being introduced at this time. Chorrera phase individuals had a similar diet with slight changes in $\delta^{13}C$ values. Guangala individuals at the site of Valdivia had signatures indicating a marine diet similar to the Chorrera phase with an increased consumption of maize. Finally, a similar dietary pattern as the Chorrera and Guangala people was indicated through analysis of Bahía and Manteño people at Salango (van der Merwe et al. 1993).

Stable isotope analysis has been conducted at a few sites other than those described by van der Merwe et al. in 1993. Stable isotope values from La Florida (a Chaupicruz Phase, Regional Development Period site) in the highlands of Ecuador reveal dietary differences based on status. Here, $\delta^{13}C$ values between high-status and low-status individuals were statistically significant, but $\delta^{15}N$ were not (Ubelaker et al. 1995). These findings contradict accounts of high-status individuals having more access to meat than lower-status individuals. Based on these signatures and a lack of dental pathology among elite Chaupicruz individuals, it is suggested that the differences in $\delta^{13}C$ values occur due to a higher consumption of maize beer (*chicha*) among the elite at La Florida (Ubelaker 2000; Ubelaker et al. 1995).

Information on paleodiet and mobility, and environmental baselines of the Quito Basin during the Integration Period were published by Pennycook (2013). The environment of the Quito Basin (located in the Ecuadorian highlands) was determined to contain mostly C_3 species, with the exception of some deer that consumed maize from human agricultural areas (Pennycook 2013). Quito Basin sites Tajamar and Nuevo Aeropuerto Internacional de Quito had different $\delta^{13}C$ and $\delta^{15}N$ values. At Tajamar, individuals ate mostly C_4 resources, likely maize, with males consuming slightly more protein than females. In contrast, the Nuevo Aeropuerto Internacional de Quito diet consisted of C_3 and C_4 plants, but relied heavily on C_3 resources (Pennycook 2013). There were no differences in values between sex, age, or burial type. Evidence of breastfeeding for individuals under the age of two years was present at both sites; however, there was no evidence of a singular, universal weaning method at either site. Therefore, it is possible that weaning methods varied throughout this region. Slight

differences in isotope values indicate that juveniles may have eaten marginally different protein sources than adults, diet was the same for adults and children by late childhood at both Tajamar and Nuevo Aeropuerto Internacional de Quito (Pennycook 2013).

The importance of maize in the highlands of Ecuador during the Formative Period has been studied at sites of La Chimba, Socapamba, La Emerenciana, Rancho Bajo, Cotocollao, and Las Orquídeas. At La Chimba and Socapamba, $\delta^{13}C$ values suggested that maize was a significant portion of the inhabitants' diet, but not so much so as to be considered a staple (Tykot et al. 1996 as cited in Torres Peña 2018). Isotopes at La Emerenciana confirmed this and determined that freshwater and terrestrial resources contributed the most to Formative diets (Tykot et al. 2002). More recently, the sites of Rancho Bajo, Cotocollao, and Las Orquídeas have had human and faunal samples taken for isotopic analysis in order to learn more about food in the Formative Period (Torres Peña 2018). At these sites, the $\delta^{13}C$ and $\delta^{15}N$ values indicate that the diet of these individuals was composed of primarily C_3 organisms and terrestrial animals. Maize was confirmed to have been consumed by the individuals of Rancho Bajo, Cotocollao, and Las Orquídeas; however, the amount of maize consumption varied from insignificant to highly significant between the sites (Torres Peña 2018).

CHAPTER 3: SAMPLE AND METHODS

3.1 Study Sample

I investigated the daily lives and identities of people who lived at Salango by assessing isotopic signatures of carbon and nitrogen from skeletal and dental remains. Guangala skeletons excavated from Salango by Dr. Richard Lunniss between 2014 and 2016 were sampled by Abigail Bythell and Dr. Sara Juengst in 2018. This included 24 individuals from the VEG, EG, and Integration Periods. These remains were stored at the Universidad Tecnica de Manabi, and the 2018 excavation and research was also funded

For this project, individuals between birth and 2 years of age at the time of death were considered infants, juveniles were those between the ages of 4 and 5 years, those estimated to be 12-18 years were categorized as adolescents, young adults were classified as individuals between 19-29 years, and middle adults were 30-50 years. VEG individuals included two middle adults, one young adult, one young adolescent, and seven infants based on the Todd and Suchey-Brooks methods (Bythell 2019). Following standard sex estimation methods (Buikstra and Ubelaker 1994), Bythell estimated there to be one male, one female, and one probable female. Of the EG individuals, there were four indeterminate aged adults, two middle adults, three young adults, one older adolescent 16 to 18 years old, one juvenile 6 to 11 years old, and one juvenile 4 to 5 years old. The EG adults were estimated to include one female, three probable males, and six intermediates. There was one individual estimated to be from Integration Period (IP) who was a young adult of indeterminate sex (Bythell 2019).

Of these 24 individuals, 16 were sampled for isotopic analyses. The isotope sample includes: four infants (0-2 years), one juvenile (4-5 years), two adolescents (12-18), four young adults (19-29 years), and five middle adults (30-49 years). Sex was not estimated on juveniles under the age of 16 years; therefore, there were six samples without sex estimates. The sample of individuals over 16 years of age included two females, one probable female, one male, two probable males, and four adults of indeterminate sex. Three of the samples were teeth, and the remaining samples were bones or bone fragments from the postcranial skeleton. Samples were selected from individuals that had duplicate elements (i.e. multiple ribs, or corresponding teeth from the other side of the mouth) in order to limit destruction and allow for future studies of these individuals.

3.2 Methods: Stable Isotope Analyses

Samples for this project were prepared using established methodologies (Turner et al. 2013; Turner et al. 2018) at Georgia State University's Bioarchaeology Laboratory under the supervision of Dr. Bethany Turner-Livermore. All samples were abraded and then cleaned twice ultra-sonically in distilled, deionized water (ddH_2O). Samples being prepared for collagen were crushed using an agate mortar and pestle, and then demineralized in 0.5M hydrochloric acid (HCl) at 4°C until translucent. The HCl was changed every 48-72 hours during demineralization (Turner et al. 2013; Turner et al. 2018). Once translucent, the samples were soaked in a 0.2% solution of potassium hydroxide (KOH) for 48-72 hours to remove humic residue (contaminants from humus, organic matter belonging to soil) (Garvie-Lok et al., 2004; Turner et al. 2013, 2018). Then the samples were gelatinized in a 0.05M HCl solution for approximately 8 hours at

95°C. The gelatinized samples were filtered through 0.045μm millipore syringe tips into 5ml borosilicate tubes and dried in an evaporation oven to prevent spillage during depressurization. Finally, the collagen samples were freeze dried for 36 hours (Turner et al. 2013; Turner et al. 2018). Modifications to previously established methods include the exclusion of soaking the samples in a methanol, chloroform, and double-distilled water as the samples did not have a significant content of lipids, the exclusion of a second soak in HCl following the KOH soak as extra demineralization was not required, and the addition of drying the samples in an evaporation oven (Turner 2020). After preparation, the samples were analyzed in a Thermo Electron DeltaV Advantage IRMS with a ConFlo II interface connected to a Carlo Erba NA 1500 CNHS Elemental Analyzer at the Department of Geological Sciences at the University of Gainesville, Florida. The analytical precision for this IRMS was ±0.22‰ for $\delta^{15}N$ and ±0.14‰ for $\delta^{13}C_{collagen}$ (Turner et al. 2018).

Bone and enamel samples prepared for carbonate were powdered with an agate mortar and pestle, and then soaked in a solution of 2% bleach (NaOCl) and ddH_20 for 24 to 72 hours until the cessation of degassing (which indicates that the majority of organic material has been removed). After degassing, the samples were centrifuged, rinsed with ddH_20, and submerged a 0.2% acetic acid solution at 4°C for 2-4 hours to ensure the removal of contaminants (Turner et al. 2018). Following this, the carbonate samples were centrifuged, rinsed with ddH_20, and freeze dried. These samples were analyzed in a Finnigan-MAT 252 IRMS with a Keil III carbonate preparation device at the Department of Geological Sciences at the University of Gainesville, Florida. The analytical precision for this IRMS was ±0.07‰ for $\delta^{18}O$ and ±0.02‰ for $\delta^{13}C_{carbonate}$ (Turner et al. 2018).

The isotopic values from this project were compared to modern and archaeological faunal baselines published by van der Merwe et al. (1993), and studied in the context of Salango during the VEG and EG Phases. The modern baselines were gathered from marine, riverine, and terrestrial sources including algae, mollusks, salt and freshwater fish, and land mammals. Only one archaeological sample (deer) had enough collagen for isotope extraction; therefore, mainly modern baselines were used (van der Merwe et al. 1993). Statistical analyses were performed in Microsoft Excel and Statistical Package for the Social Sciences (SPSS) 26. These analyses included comparison to regression formulae by Kellner and Schoeninger (2007) and centroids developed by Froehle et al. (2011). The model developed by Kellner and Schoeninger (2007) plots $\delta^{13}C_{co}$ values against $\delta^{13}C_{ca}$ values to provide regression lines of C_3, C_4, and marine protein; position on the line indicates the energy source (1). Froehle et al.'s (2011) model includes $\delta^{13}C_{collagen}$, $\delta^{13}C_{carbonate}$, and $\delta^{15}N$ values in cluster and discriminant function analysis; this model aims to eliminate limitations of a bivariate carbon model with the addition of $\delta^{15}N$ values (1).

CHAPTER 4: RESULTS

The results of this project are displayed in Figures 4-7 and Table 1. Table 1 shows the isotopic and demographic information for all samples. The C:N ratio was between 3.2 and 3.4 indicating that the collagen was well preserved without contamination; therefore, the isotopic values can be considered a good representation of diet (Ambrose 1990; DeNiro 1985; Juengst et al. 2021).

Collagen $\delta^{13}C$ ($\delta^{13}C_{co}$) values for the whole sample ranged from -7.04 and -12.74 with an average of -8.94 and standard deviation of ±1.49. This indicates significant contribution from C_4 resources in the protein portion of the diet. Carbonate $\delta^{13}C$ ($\delta^{13}C_{ca}$) values ranged from -13.01 to -17.70 with an average of -14.74 and standard deviation of ±1.29, overall, more negative than collagen values and indicating less C_4 contributions from the overall diet. Oxygen ($\delta^{18}O_{VPDB}$) values ranged from -1.34 to -3.06 with an average of -2.29 and standard deviation of ±0.42, consistent with environmental water sources for the coast (Wright 2017). Carbon spacing (the difference between $\delta^{13}C_{ca}$ and $\delta^{13}C_{co}$, represented as $\delta^{13}C_{ca\text{-}co}$) values for the samples are displayed on Figure 8 with dietary interpretations from Ambrose and Norr (1993). Using the Ambrose and Norr (1993) dietary interpretations, it can be estimated that the majority of individuals consumed a mixed diet of marine and C_4 resources with the exception of an outlier (burial 339). Nitrogen ($\delta^{15}N$) values for the entire sample ranged from 13.07 and 18.42 with an average of 14.68 and standard deviation of ±1.43, indicating a range of experience in access to protein.

ANOVA tests were used to compare all values between periods and demographic groups (age and sex). Averages by sex estimate, time period, and age category are displayed in Tables 2-4. When comparing time periods, the $\delta^{13}C_{ca}$ values (indicative of whole diet) significantly varied ($f= 4.048$, $p= 0.041$). This difference appears to be caused by the lone sample from the IP; however, differences between the VEG and EG are qualitatively apparent on the scatter plots (Figs. 4-7). Other isotopic values did not significantly vary by time period with $p>0.05$. Values were not significantly different between adults and juveniles; however, the $\delta^{15}N$ values did significantly differ between more specific age groups ($f= 8.492$, $p=0.003$). Values were not significantly different between sex estimates.

When compared with dietary models established by Froehle et al. (2011) (Fig. 9), results from this study cluster closest to Centroid 3, the centroid associated with a 50:50 C_3:C_4 diet that consists of marine protein. Notably, the values appear to be pulled more negatively by the $\delta^{13}C_{ca}$ component of the diet. When plotted with the Kellner and Schoeninger (2007) regression formulae (Fig. 10), this pull is more seen more clearly. This likely indicates these individuals consuming marine proteins and a significant amount of C_4 resources (such as maize).

Collagen $\delta^{13}C$ and $\delta^{15}N$ values clustered closely with modern baselines from marine sources provided by van der Merwe et al (1993) with the exception of two outliers (Fig. 4). Specifically, these values are clustered near marine fish, crustaceans, and mollusks. Values do not correlate with those from terrestrial and riverine sources, indicating that very little or none of these individuals' protein intake came from such sources. The aforementioned outliers are from the VEG period and are infants.

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1 Discussion

General Trends

Generally, the isotopic values of this sample were consistent with expectations for individuals living in coastal Ecuador during the Guangala phase (100 BCE to 800 CE). Individuals from this period likely had a diet that consisted mainly of marine protein and C_4 resources, with a low intake of C_3 resources as evidenced by the comparison of these values with the Froehle centroids (2011), the regression formulae by Kellner and Schoeninger (2007), dietary assumptions by Ambrose and Norr (1993), and dietary baselines from van der Merwe et al. (1993). The collagen values presented here cluster most closely with marine fish, mollusks, and crustaceans. Notably, they are distributed the furthest away from riverine and terrestrial sources indicating that these sources were rarely consumed.

Temporally, there was not a statistically significant difference between collagen carbon values; however, there was a significant difference between $\delta^{13}C_{ca}$ value. Therefore, there is no significant difference between dietary protein resources but there is a difference between overall dietary resources. While this difference is likely caused by the IP sample's high $\delta 13C_{ca}$ value, scatter plot analysis reveals there may be slight differences between the VEG and the EG. When displayed on a scatter plot of $\delta^{13}C_{ca}$ and $\delta^{18}O$ values, the VEG individuals do not cluster as tightly as the EG individuals. These observations suggest that while the protein sources do not change over time, the overall

diet consumed by the Guangala individuals does change between the VEG and EG. It is likely that VEG individuals had more variety in their diets than EG individuals.

This may be explained by a variety of scenarios. First, it is possible that EG individuals had a narrower diet due to stricter control of resources at this time. Although groups do not have statistically different isotope values in the EG, systems of power could be emerging that limited access to resources. This would be consistent with hypotheses that Guangala sites were organized in chiefdoms but did not have a highly stratified social hierarchy. (Masucci 2008; Stothert 1984).

Alternatively, food scarcity during the VEG could have resulted in the residents of Salango consuming a wider variety of foods than they would normally. Bythell (2019) showed that individuals of this time experienced high levels of physical stress; skeletal pathology identified by this research includes: cribra orbitalia, porotic hyperostosis, periosteal reactions, osteomyelitis, and endocranial lesions (32). Bythell hypothesized that these pathological reactions were a result of a health crisis related to agricultural failure, possibly caused by a volcanic eruption that occurred in the Ecuadorian highlands around this time (Bythell 2019:41). If this agricultural failure did occur, it is possible that VEG individuals ate more varied foods in order to survive, compared to their preferred or traditional diets.

While there was no significant difference between the broader age groups of adult and juvenile, there was a significant difference in the $\delta^{15}N$ values of more specific age groups of infants (0-2yrs), juvenile (4-5yrs), adolescents (12-18yrs), young adults (19-29yrs), and middle adults (30+yrs). As seen in the means plot (Fig.11), infants had

significantly higher $\delta^{15}N$ than the other age groups. These high $\delta^{15}N$ values are likely the result of breastfeeding, as previous studies have shown $\delta^{15}N$ values to be higher in infants or young children who are breastfeeding. When an individual is breastfeeding, both the $\delta^{13}C$ and $\delta^{15}N$ values increase by 1% and 2-3% respectively. $\delta^{13}C$ values generally decrease more quickly than $\delta^{15}N$ due to the smaller increase of these values during the breastfeeding period (Fuller et al. 2006, Turner et al. 2007). It should be noted that collagen values for the four- to five-year-old juvenile were not available; thus, I could not determine if breastfeeding continued past the age of two in this study. The sample size used here was also not large enough to make general statements about Guangala weaning practices outside of Salango but seems to indicate that these infants were still breastfed until at least two years old.

Furthermore, two VEG infants (burials 339 and 375) were outliers for $\delta^{13}C_{co}$ and $\delta^{15}N$ values (Fig. 7). These differences in collagen isotope values could be attributed solely to breastfeeding; however, pathological conditions are also known to increase nitrogen values (Katzenberg and Lovell 1999; Reitsema 2013). Bythell (2019) states that both of these individuals were clearly afflicted with prolonged stress and/or infection (35). Burial 339 has evidence of healing periosteal reactions, active porotic hyperostosis, and endocranial lesions and Burial 375 has evidence of healing porotic hyperostosis and cribra orbitalia (Bythell 2019; Juengst et al. 2019). The VEG overall had many individuals exhibiting similar symptoms of stress and/or infection. Thus, the elevated collagen isotopic values of the outliers here were likely the result of breastfeeding in addition to stress or infectious disease (Bythell 2019).

This study contained two pairs of bone and tooth enamel samples stemming from the same individual, which allows us to compare carbonate isotope samples throughout an individual's life. Enamel typically develops while an individual is *in utero* and early childhood while bone constantly remodels over an individual's lifetime (White et al. 2011). Therefore, isotopic values from enamel samples are indicative of what resources their gestational parent was consuming or their diet in childhood, while isotopic values from bone reflect the resources consumed in the years prior to death. Samples SLG 17 (tooth) and SLG 18 (bone) both came from Burial 359, an adult female between the ages of 30 and 34 years, and samples SLG 25 (tooth) and SLG 26 (bone) both came from Burial 363, an adolescent between the ages of 12 and 14 years. Burial 359's values were markedly consistent across their lifetime, indicating that their dietary resources did not greatly vary throughout their life. However, the carbonate values for Burial 363 clearly vary (Fig.12), suggesting that their diet and water sources may have changed over their life course.

It is important to note that previous research has indicated that $\delta^{16}O$ isotopes vary within an archaeological population by ~2‰ to over 3‰ (Lightfoot and O'Connell 2016; Pederzani and Britton 2019). Thus, it is likely that Burial 363 did not migrate outside of the same oxygen value range as Burial 359. A possible explanation for this variation could be that while they consumed similar resources as Burial 359 at one point in their life, their social or economic status may have changed resulting in their dietary resources altering slightly from Burial 359 (Juengst et al. 2021; Pederzani and Britton 2019). Burial 363 is recorded to have healing porotic hyperostosis and osteomyelitis, and active endocranial lesions by Abigail Bythell (2019). Burial 359 experienced similar health

concerns; however, it is possible that their diet may have shifted in response to pathology (Bythell 2019). While it cannot be certain that Burial 363 experienced any significant dietary changes before death, this individual was experiencing some health-related stress that could have caused their dietary resources to change during life.

In the Context of Salango

The consumption of marine protein at this site is not unusual given the history of Salango. As an Ecuadorian sacred center on the coast, Salango has been inhabited by many different groups of people of which the Guangala are only one. Before the Guangala, individuals of the Machalilla (1500-900 BCE) established a fishing village at the site (Béarez and Lunniss 2003; Béarez et al. 2012; Lunniss 2001, 2017). Evidence of this includes shellfish hooks, stone net sinkers, a bone spear-thrower hook, and marine fish remains (Béarez et al. 2012, 197). Fishing and marine life was also important to the Middle and Late Engoroy (600-100) people who lived at Salango following the Machalilla (Béarez and Lunniss 2003; Lunniss 2001, 2008, 2020). Marine fish remains and fishing hooks dating to this cultural phase show that fishing for subsistence continued after Machalilla occupation; although, ceremonies related to fishing or shellfish harvesting were likely not a regular occurrence (Béarez and Lunniss 2003; Lunniss 2008, 2017, 2020). Adding to this rich history of fishing and marine resource consumption at Salango is the data presented in this project. While I did not include artifact analysis in my research, the carbon and nitrogen values make it clear that the VEG and EG people were consuming significant portions of marine protein like the cultures before them.

Comparison to other Ecuadorian RDP Isotope Studies

When compared to van der Merwe et al. (1993), the values presented here are strongly consistent with the contemporaneous individuals buried at Valdivia (Fig. 13). This indicates that throughout the Guangala period, individuals on the coast at various sites consumed diets high in marine protein and with significant amounts of C_4 resources. Therefore, proximity to the coast was an important factor of diet selection and that resources did not vary by site. Thus, there is no evidence of differential access to food sources, as might be indicative of site hierarchy, amongst Guangala costal sites. Of course, a sample size of two archaeological sites is not extremely significant; therefore, conducting isotopic research at other Guangala period sites would be an interesting topic of future investigation.

Individuals from La Florida (a contemporaneous Chaupicruz Phase site), in the Ecuadorian highlands have similar $\delta^{13}C_{co}$ values to the Salango sample, but have lower $\delta^{15}N$ values. (Fig. 13) (Ubelaker et al. 1995). Although they stemmed from a different culture, this indicates that RDP individuals in lowland and highland environments consumed similar sources of carbon (C_4 resources, likely maize and perhaps consumed in liquid form as *chicha*) (Ubelaker et al. 1995). However, sources of protein differed between regions. This result is perhaps unsurprising given that La Florida is in a geographically different area of Ecuador (within the upper elevations of the Andes Mountain Range) (Fig. 16) and likely would not have access to marine resources without extensive trading. However, these results are further evidence that the consumption of maize and maize products (such as *chicha*) was not restricted to a certain location or groups of people in Ecuador, even when protein sources varied.

Comparison to the Northern Peruvian Coast

Contemporaneous to the Guangala were several cultures along the Peruvian coast including the end of the Salinar phase (400 BCE to 1 CE), the Gallinazo phase (1 CE to 200 CE), the Early Moche Phase (200 CE to 300 CE), and the Moche Phase (300 CE to 800 CE) (Lambert et al. 2012; Gagnon 2004, 2006). Stable isotope analysis has been conducted at the site of Cerro Oreja, a large urban center in Perú's Moche Valley (Gagnon 2006). Evidence of dietary shift toward increased maize consumption at this site is present during the Gallinazo phase at Cerro Oreja. Lambert et al. (2012) theorize that marine resource consumption decreased during the preceding Salinar phase, and that maize consumption increased in the Gallinazo phase, an argument further bolstered by high rates of carious lesions in the Gallinazo phase individuals (Lambert et al. 2012). They suggest that this dietary shift occurred due to changes in domestic and political economies; likely farmers were responding to an increased demand for maize from the Cerro Oreja polity (Lambert et al. 2012:162).

Comparatively, the Guangala individuals presented here consumed more marine protein than those at Cerro Oreja (where marine protein consumption declined during the Salinar phase). This may have been related to geographic location (Cerro Oreja is near the Río Moche, but inland from the Pacific Ocean). However, both locations were consuming significant amounts of C_4 resources (maize). This increase of maize consumption places both locations within the general trend of maize becoming a larger portion of Andean diets during this time period (Lamber et al. 2012). Maize consumption was likely increasing during this time due to its growing sociopolitical importance in the Andes. Maize has been documented as an important crop in this area (Gagnon and

Juengst 2018; Somerville et al. 2010, 2015; Tykot and Staller 2002; Ubelaker et al. 1995), especially for its use in making corn beer or *chicha*. These results show that between 100 BCE and 800 CE maize was a socially significant and widely eaten food source.

Comparisons between other Ecuadorian sites and sites in Perú help position Salango in an isotopic documentation of diet in the Andes. At Salango, these results show that social hierarchy or identity was not connected to diet; although, access to food sources did become narrower in the EG. At Cerro Oreja and La Florida, consumption of maize either as a solid food or as *chicha* was also not exclusively consumed by one group but did increase over time. This suggests that at this time, most individuals of the same socioeconomic status in the Andes had equal access to maize. This is true at Salango and also in the highlands of Ecuador and coast of Perú. Therefore, it appears that the lack of social divisions based on age, sex, or gender in diet is consistent throughout the Andes at this time. It appears that maize agriculture in increasing at this time across the Andes as well.

5.2 Conclusion

Food and diet are essential components of an individual's life and culture. Stable isotope analysis has been used in numerous instances to investigate identity, culture, life history, and social hierarchy (i.e., Ambrose et al. 2003; Barrett and Richards 2004; Knudson et al. 2012; Linderholm et al. 2008; Somerville et al. 2015; Tung et al. 2016; Tung and Knudson 2018; Turner et al. 2007; White 2005). Heretofore, it was unknown whether or not diet was a factor of identity or dictated by status for the Guangala peoples

at Salango. Using dietary isotope analysis of carbon and nitrogen, this research was able to determine that the Guangala people at this site consumed a diet primarily composed of marine protein and C_4 resources, most likely maize, consistent with isotope values of other Guangala individuals in coastal Ecuador (van der Merwe et al. 1993). Diet and access to resources was likely not stratified for Guangala peoples at Salango among high status individuals, as there were no dietary differences between demographic groups. The only outliers were infants with elevated nitrogen values, likely attributable to illness, breastfeeding, or a combination of the two, rather than differences in social identity or emerging hierarchy (Fuller et al. 2006; Katzenberg and Lovell 1999; Reitsema 2013).

The lack of dietary differences between social groups is an indicator that if a social hierarchy based on sex, gender, or age was established within Guangala culture, it was not expressed through diet. This is consistent with previous studies of the culture which show that intra-status variation was also not expressed through grave goods (Masucci 2008; Stothert 1984). The emergent dietary differences between VEG and EG individuals of a higher status could have been the result of a regional health crisis related to agricultural struggle (as evidenced by isotopic and pathological data); however, it is also possible that a sociopolitical hierarchy within status groups that limited resources was beginning to be established between the two periods.

This research contributes to the larger literature on Ecuadorian archaeology and to that of the Regional Development Period. While stable isotope analysis has been conducted in this region (Pennycook 2013; Torres Peña 2018; Tykot et al. 2002; Ubelaker et al. 1995; van der Merwe et al. 1993), dietary isotope research is lacking in general for the Regional Development Period. This research has contributed to a growing

body of literature on regional dietary baselines. More specifically, I demonstrate that Guangala individuals at Salango had similar diets to those from the same cultural phase at the site of Valdivia, and were consuming similar C_4 resources to individuals of the Chaupicruz phase at La Florida and Peruvian contemporaries. This indicates that even in different geographic locations, maize consumption was increasing at this time. Future stable isotope studies in conjunction with excavation of securely dated burials from Salango and other contemporaneous sites can help us elucidate these patterns more fully.

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APPENDIX A: TABLES

Sample #	Period	Burial #	Age	Age Cat	Sex	Sample Description	wt %N	wt %C	%coll yield	C:N	$\delta^{15}N$ (vAIR)	$\delta^{13}C_{ca-co}$	$\delta^{13}C_{ca}$ (vPDB)	$\delta^{13}C_{co}$	$\delta^{18}O$ (vPDB)	$\delta^{18}O$ (vSMOW)
SLG 07	VEG	460	30-46yrs	A	M	rib	2.61	7.56	5.35	3.4	13.9	5.13	-14.9	-9.7	-2.6	27.7
SLG 54	VEG	522/511	26-44yrs	A	PF	metatarsal 2	12.44	34.02	12.40	3.2	13.4	6.86	-14.4	-7.6	-2.4	27.9
SLG 17	VEG	359	30-34yrs	A	F	maxillary molar 1						13.35	-13.4		-2.2	28.2
SLG 18	VEG	359	30-34 yrs	A	F	rib	10.73	31.12	0.49	3.4	14.0	3.97	-13.9	-9.9	-2.0	28.3
SLG 11	VEG	339	6-9mnths	J	NA	metacarpal	12.36	34.3	12.94	3.2	16.1	12.74		-12.7		
SLG 23	VEG	375	1-2 yrs	J	NA	rib	11.65	32.24	10.84	3.2	18.4	6.8	-17.7	-10.9	-2.0	28.3
SLG 25	VEG	363	12-14 yrs	J	NA	mandibular incisor						15.17	-15.2		-1.9	28.5
SLG 26	VEG	363	12-14 yrs	J	NA	foot phalanx	10.04	28.49	3.17	3.3	13.3	6.99	-16.5	-9.5	-3.1	27.2
SLG 36	VEG	403	1-2yrs	J	NA	rib	11.45	31.75	7.39	3.2	16.4	5.98	-14.0	-8.0	-1.3	29.0
VEG AVG							10.18	28.50	7.51	3.28	15.07	8.55	-14.98	-9.76	-2.18	28.12
VEG StD							3.45	9.43	4.78	0.08	1.95	4.06	1.46	1.74	0.52	0.54
SLG 32	EG	424	39-57yrs	A	PM	rib	11.5	32.16	7.72	3.3	14.0	6.19	-13.8	-7.7	-2.5	27.8
SLG 52	EG	P14 E5	35-46yrs	A	PM	metacarpal	12.52	34.56	7.48	3.2	14.1	5.68	-14.1	-8.5	-2.3	28.0
SLG 39	EG	430	25+yrs	A	Ind	rib	12.09	34.44	1.18	3.3	14.2	5.63	-14.2	-8.6	-2.9	27.4
SLG 57	EG	P14 E6/7	25+ yrs	A	Ind	rib	11.83	33.17	11.63	3.3	14.7	6.09	-14.4	-8.3	-2.3	28.0
SLG 59	EG	P14 E4	25+yrs	A	Ind	femoral fragment	11.79	32.73	9.02	3.2	15.5	7.58	-14.6	-7.0	-2.6	27.7
SLG 42	EG	174/176	16-18yrs	J	F	cuneiform	12.08	33.71	15.86	3.3	13.1	5.08	-14.1	-9.0	-2.0	28.3
SLG 34	EG	P12	0-1yr	J	NA	rib	11.97	33.19	11.17	3.2	15.0	7.19	-14.7	-7.5	-2.4	27.9
SLG 49	EG	P12 1005	4-5yrs	J	NA	maxillary molar 1						13.01	-13.0		-1.9	28.4
EG AVG							11.97	33.42	9.15	3.26	14.38	7.06	-14.13	-8.08	-2.36	27.94
EG StD							0.32	0.88	4.54	0.03	0.78	2.54	0.53	0.70	0.31	0.32
SLG 29	IP	P7	25+ yrs	A	Ind	hand phalanx	12.67	34.93	14.54	3.2	14.1	8.13	-17.3	-9.1	-2.6	27.7
Total AVG							11.18	31.22	8.75	3.27	14.68	7.87	-14.71	-8.94	-2.29	28.01
Total StD							2.47	6.75	4.68	0.06	1.43	3.32	1.29	1.49	0.42	0.43

Table 1. Results and demographic information. Lines highlighted in green are the averages for the VEG and EG, orange lines are the standard deviations for the VEG and EG, and lines highlighted in yellow are the averages and standard deviations for the entire sample. A= adult, J= juvenile, M= male, PM= probable male, Ind= indeterminate, PF= probable female, F= female, and NA= not available.

Sex Estimate	$\delta^{15}N$	$\delta^{13}C_{ca\text{-}co}$	$\delta^{13}C_{ca}$	$\delta^{13}C_{co}$	$\delta^{18}O$
M	13.9	5.13	-14.9	-9.7	-2.6
PM	14.1	5.94	-12.3	-8.1	-1.9
Ind	12.7	7.29	-12.3	-4.8	-1.9
PF	13.4	6.86	-14.4	-7.6	-2.4
F	13.9	7.29	-13.5	-8.3	-2
NA	14	7.29	-12.4	-8.1	-1.9

Table 2. Average by sex estimate.

Time Period	$\delta^{15}N$	$\delta^{13}C_{ca\text{-}co}$	$\delta^{13}C_{ca}$	$\delta^{13}C_{co}$	$\delta^{18}O$
VEG	15.07	8.55	-14.98	-9.76	-2.18
EG	14.38	7.06	-14.13	-8.08	-2.36
IP	14.1	8.13	-17.3	-9.1	-2.6

Table 3. Average by time period. Statistically significant values are highlighted in yellow (N=9, F=4.048, p=0.041).

Age Category	$\delta^{15}N$	$\delta^{13}C_{ca\text{-}co}$	$\delta^{13}C_{ca}$	$\delta^{13}C_{co}$	$\delta^{18}O$
Infants (0-2yrs)	15.16	8.65	-15.15	-9.2	-2.21
Juvenile (4-5yrs)	--	13.01	-13.01	--	-1.94
Adolescent (12-18yrs)	14.29	7.55	-14.97	-8.49	-2.33
Young Adults (19-29)	14.45	7.04	-14.43	-8.129	-2.29
Middle Adults (30+)	14.61	7.99	-14.74	-9.13	-2.27

Table 4. Average by age category. Statistically significant values are highlighted in yellow (N=4, F=8.492, p=0.003). Data was not available for cells with dashes.

APPENDIX B: FIGURES

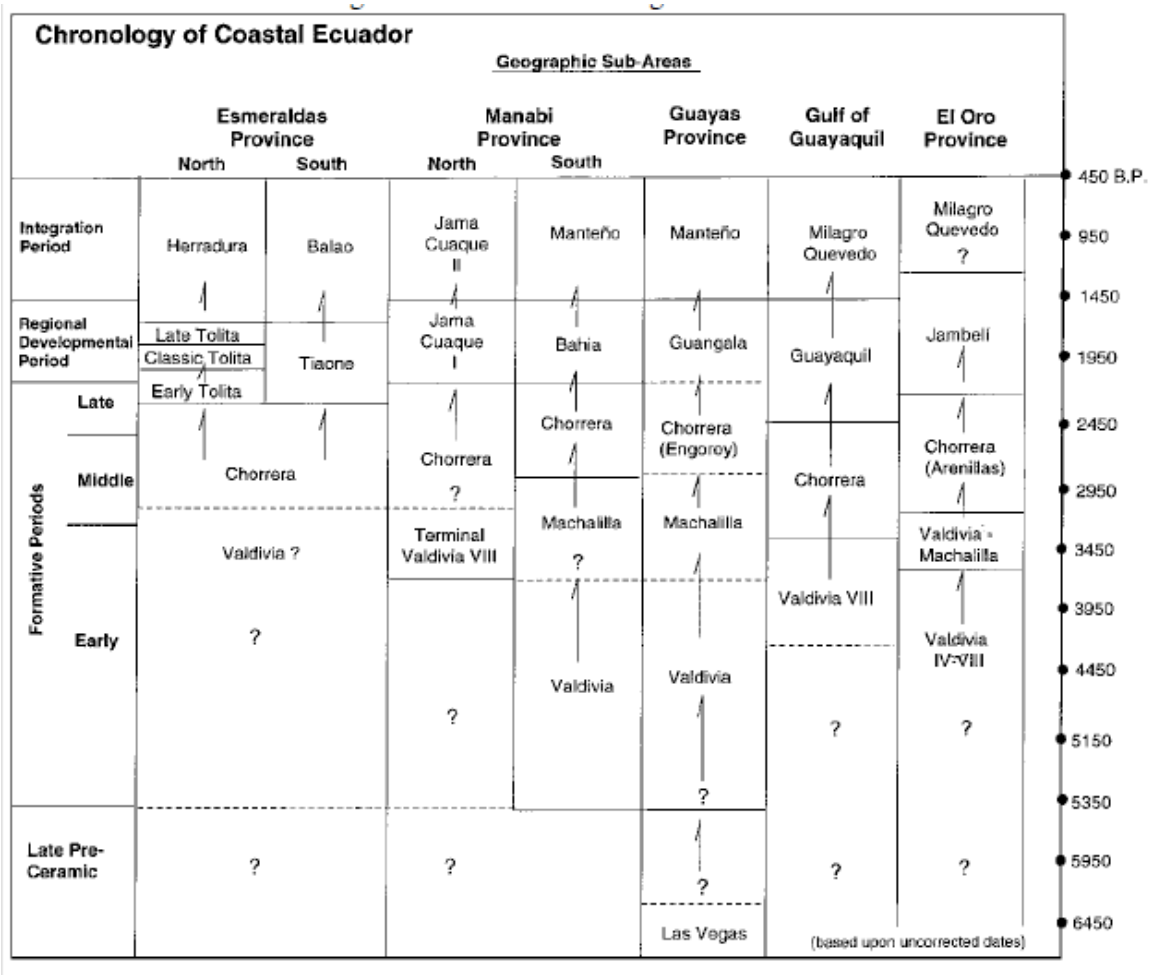

Figure 1. Archaeological chronology of Ecuador from Staller 2001

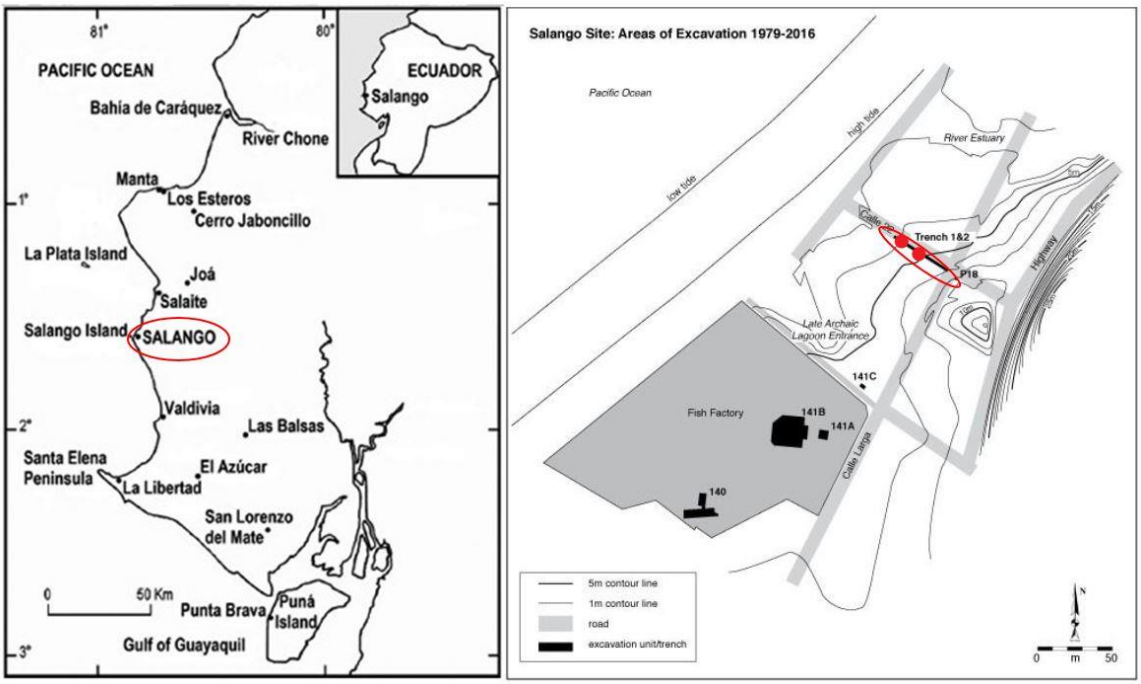

Figure 2. Map of Coastal Ecuador indicating location of Salango and approximate location of burials included in this study (from Juengst et al. 2019).

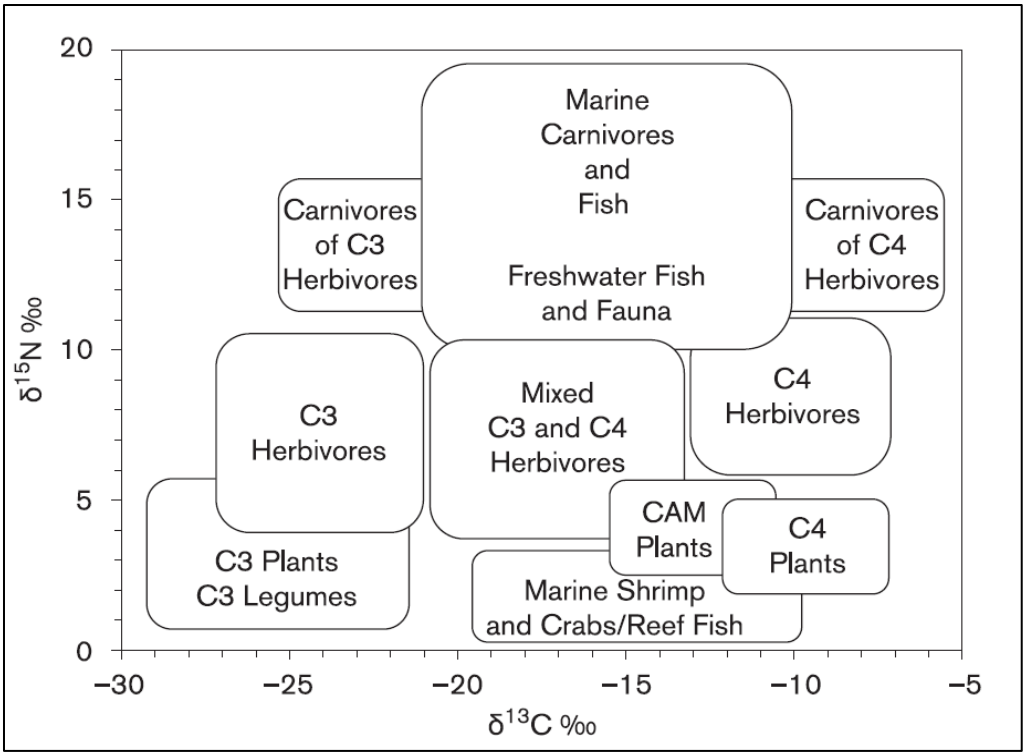

Figure 3. Distribution of collagen carbon and nitrogen values for various organisms from different environments (Blake 2015)

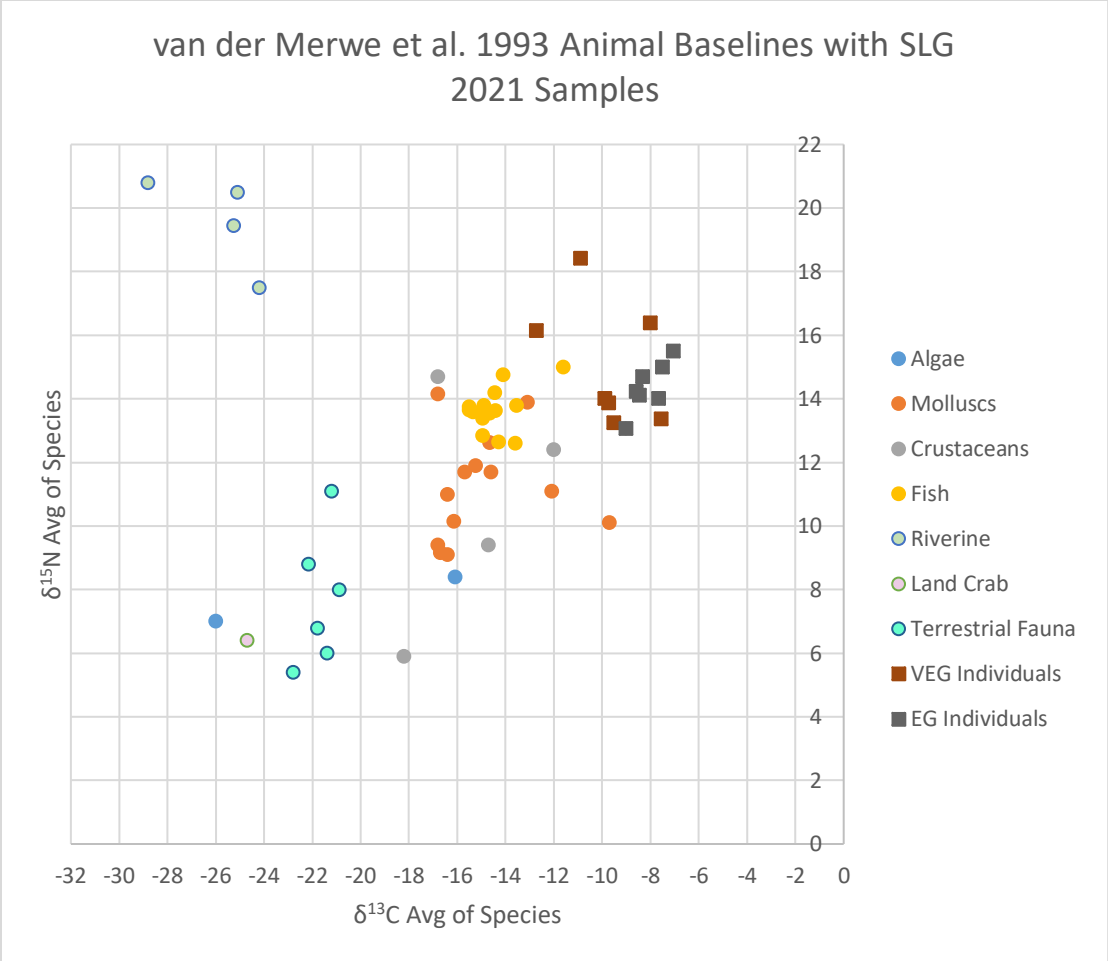

Figure 4. van der Merwe et al. 1993 Animal Baselines compared with SLG 2021 samples. Human samples are emphasized by a square marker.

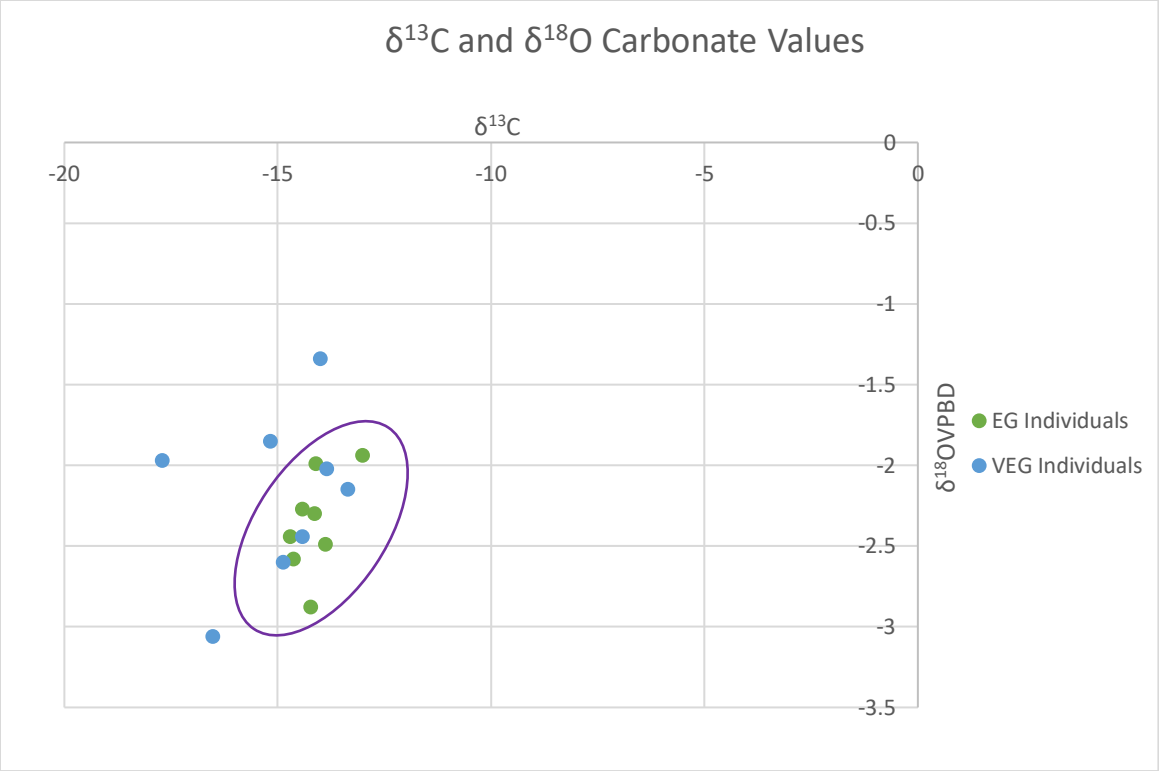

Figure 5. Scatterplot of SLG 2021 carbonate values. Clustered points with similar values are circled.

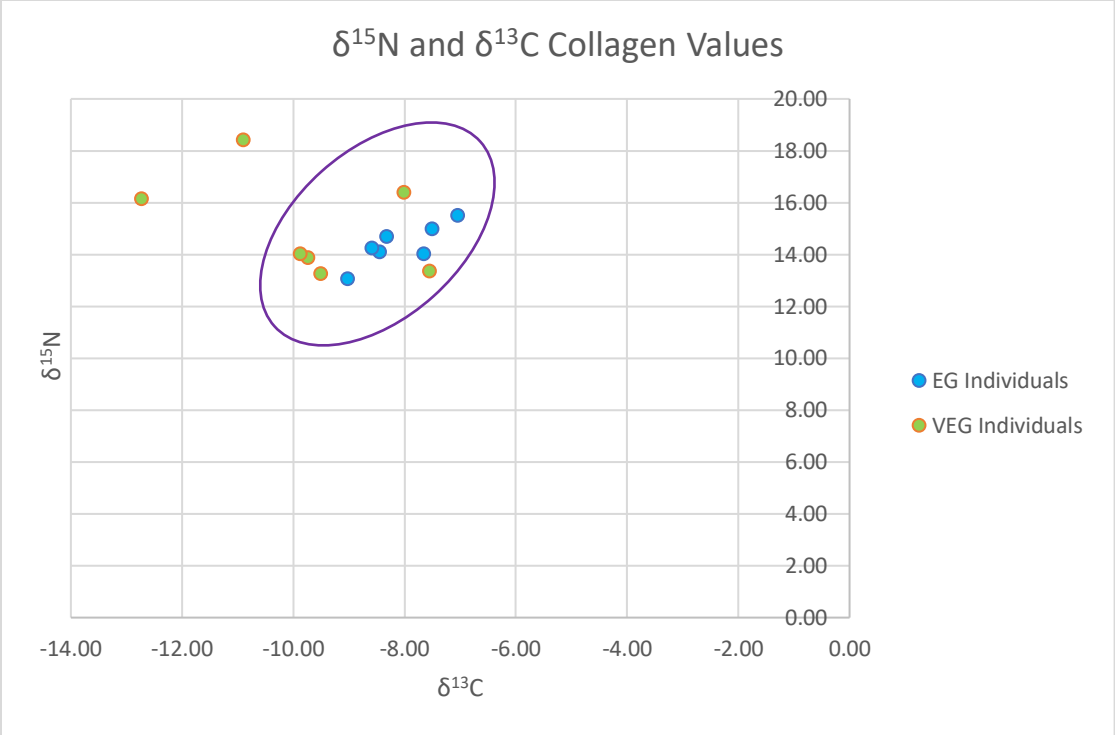

Figure 6. Scatterplot of SLG 2021 collagen values. Clustered points with similar values are circled.

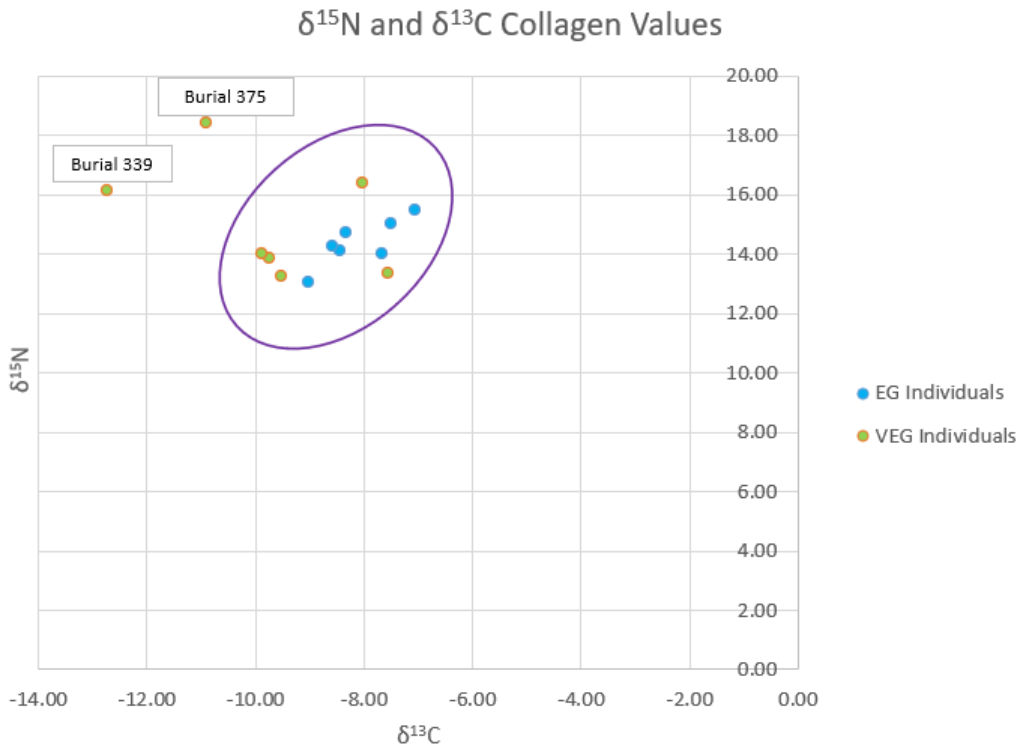

Figure 7. Collagen scatterplot with labelled outliers. Clustered points with similar values are circled.

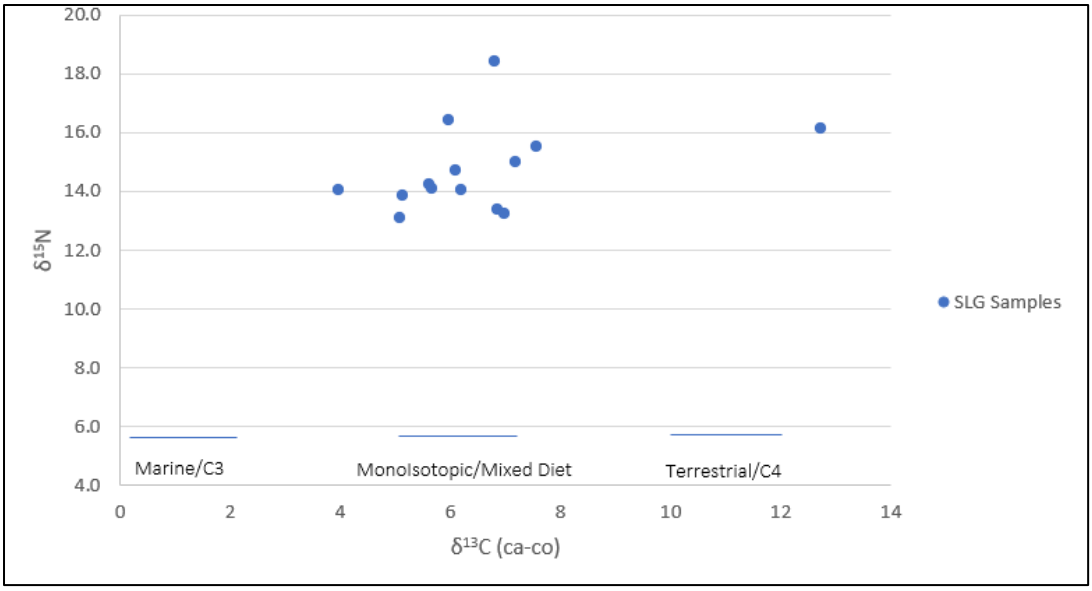

Figure 8. Data plotted with dietary interpretations from Ambrose and Norr 1993.

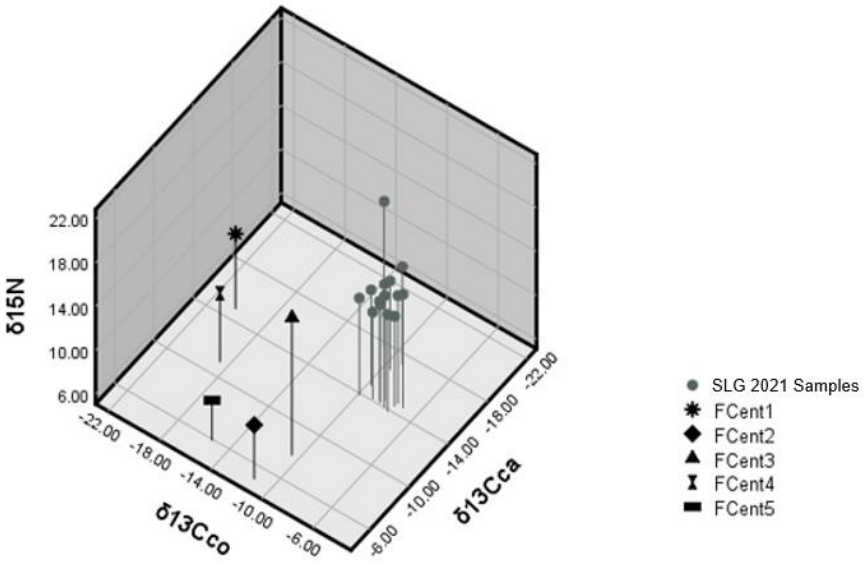

Figure 9. Data with Froehle et al. centroids (2011).

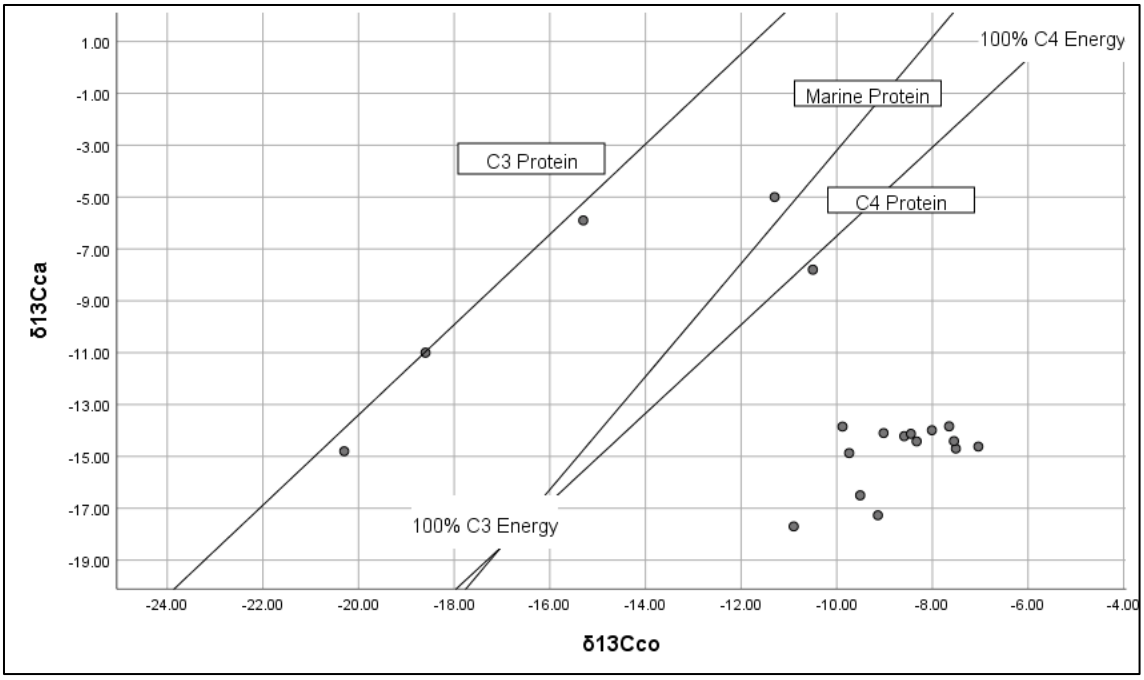

Figure 10. Data plotted with the regression formulae by Kellner and Schoeninger (2007).

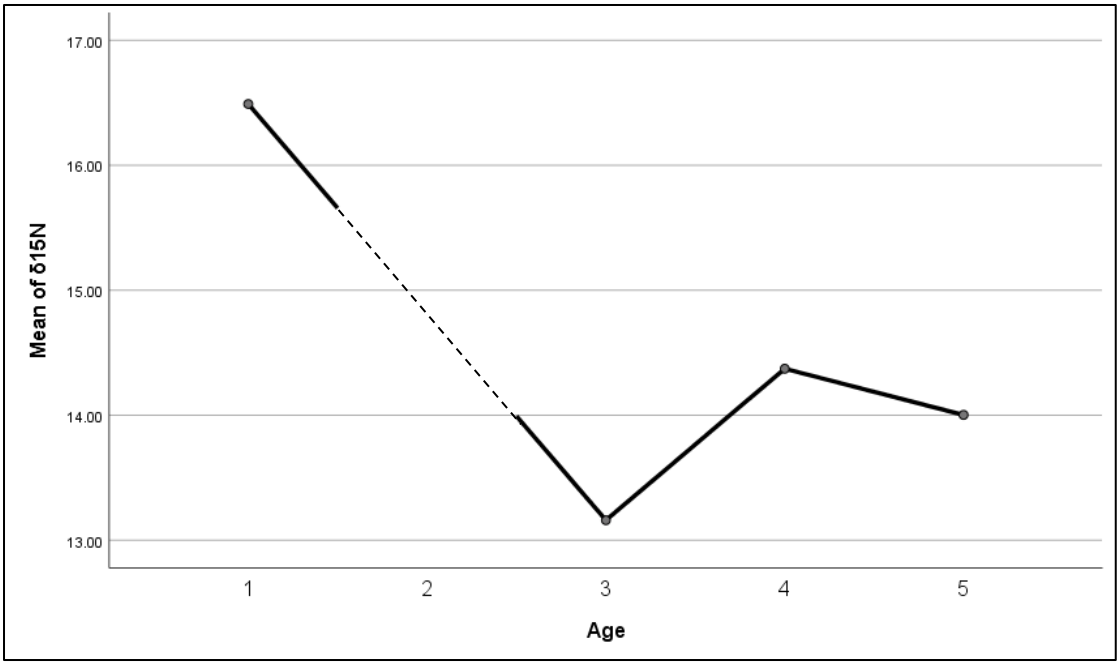

Figure 11. Means plot from ANOVA test by age category. 1= infants, 2= juveniles, 3=adolescents, 4= young adults, and 5= middle adults. Note that there is no collagen data available for the one juvenile in this sample; this is indicated by the dashed line.

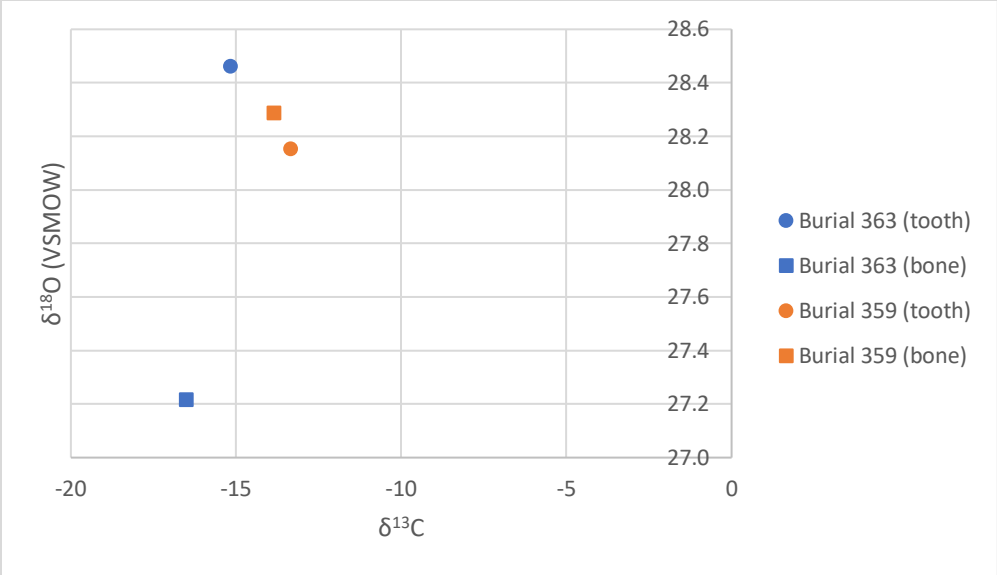

Figure 12. Carbonate values for burials 363 and 359.

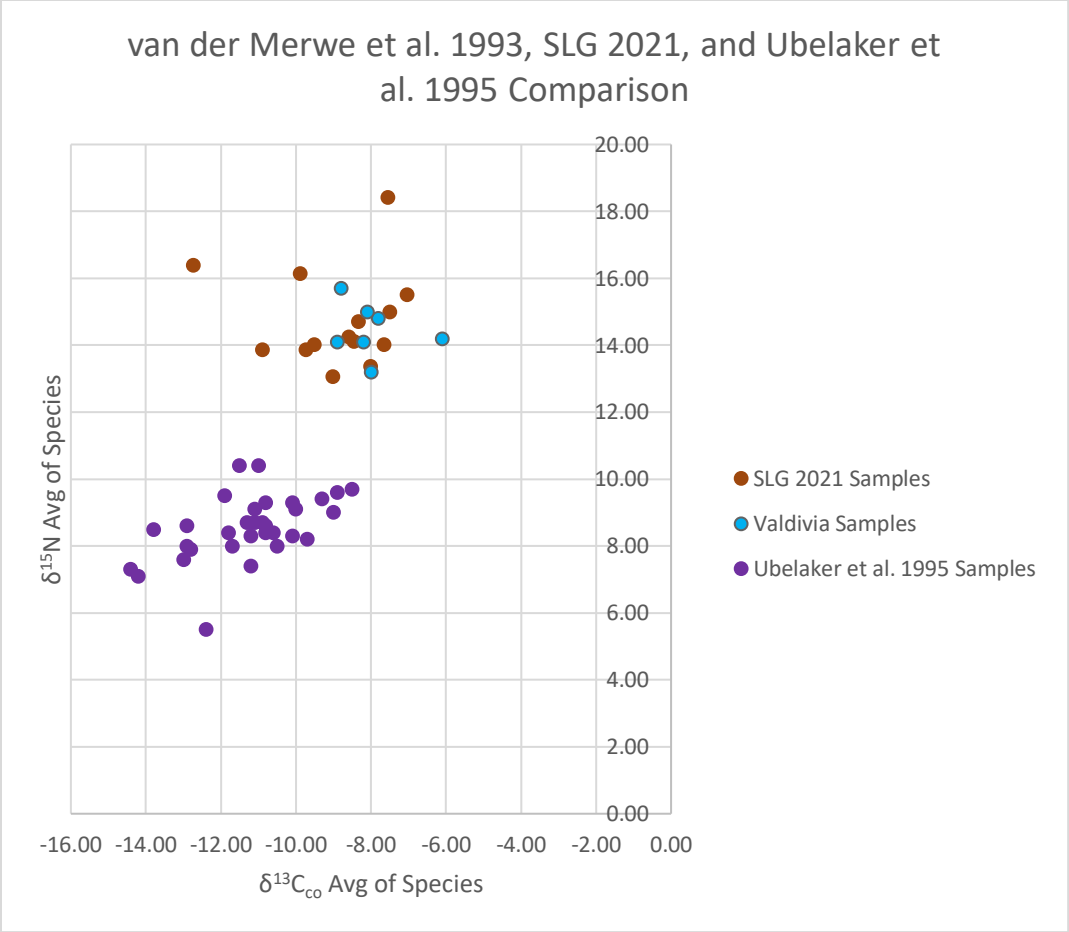

Figure 13. Data plotted with Valdivia samples, Salango 2021 samples, and Ubelaker et al. 1995 samples. (Ubelaker et al. 1995; van der Merwe 1993).