Carbon and Nitrogen Stable Isotope Analysis of Human and Faunal Skeletal Remains from the Formative Period of the Northern Highlands of Ecuador

by

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Abstract

In Ecuador, the diet of prehispanic populations has been interpreted mostly based on the evidence recovered by archaeobotanical and zooarchaeological studies, if available. In contrast, stable isotope analysis for reconstructing diet is still a novel method that has been employed on only a few occasions. This study presents the results from the first stable isotope analysis that has been conducted on Formative sites (3500 – 500 BC) from the northern highlands. Values are reported for a total of 61 samples (n= 47 human and 14 faunal) collected from the sites Rancho Bajo (n= 13), Cotocollao (n= 27), and Las Orquídeas (n= 21).

Besides identifying intra-site dietary patterns, the main purpose of this study was to examine any shifts in diet that may have occurred between the Early and the Late Formative Periods. Given that isotopic data was available for the sites La Florida, Tajamar, and NAIQ from the Regional Development and Integration periods, the results of these studies were compared to examine the diet over the three subsequent periods, giving special emphasis to observing the role played by maize in their subsistence.

The results indicate that Formative groups had a predominantly C_3 diet that also included small quantities of maize, and that there was no apparent age or sex-based differential consumption of this C_4 resource. In the following periods the increase in the consumption of the maize crop is evident until it became a staple, supporting the connection between the intensification of maize consumption and the increasing sociopolitical complexity that characterizes the subsequent periods.

Preface

This thesis is an original work by Paula Nathaly Torres Peña. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name "Carbon and Nitrogen Stable Isotope Analysis of Human and Faunal Skeletal Remains from the Formative Period of the Northern Highlands of Ecuador", No. Pro00071391, March 22, 2017.

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Chapter 1: Introduction

Stable isotopes were discovered in 1913 by the Nobel Laureate Joseph John Thomson; however, it was not until the late 1970s that these were employed in archaeology (Bethard, 2013). The first publication was made by scholars Vogel and van der Merwe (1977), who found evidence of maize agriculture in North America through the application of chemical analysis of bone, specifically through carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis (Bethard, 2013; Katzenberg, 2008). Since the introduction of this method to the field, major advances have occurred in terms of equipment and the number of stable isotopes now employed in research. Many researchers have used carbon, nitrogen, oxygen, strontium, sulphur, and lead stable isotope analysis in their search for answers to innumerable questions that had been thought to be unanswerable (Bethard, 2013). Some of these questions are related to the subsistence, place of residence, and/or mobility of an individual, group or society (Katzenberg, 2008).

The application of stable isotope analysis is certainly not new in South America or the Andes. The latter region has been the center of interest for many scholars. To cite only a few examples, for Peru, Finucane et al. (2006) used stable isotope analysis to examine the role of maize in the subsistence of a Middle Horizon (ca. 600-100 AD) Wari polity; this analysis also revealed valuable information about the use of different animal husbandry practices. Turner and Armelagos (2012) studied pathological conditions and early-life diet as an approach to examine residential mobility in the Inca site of Machu Picchu. Another example is the work of Somerville et al. (2015), who focused on determining whether the differential consumption of chicha (a fermented beverage based on maize) in Tiwanaku colonies was based on sex, as in the Inca Period. As examples in Chile, Knudson et al. (2012) conducted the analysis of the remains of an isolated individual found in the middle of the Atacama Desert, an analysis which led to the discovery of evidence that the individual was a traveller who made frequent trips between the coast and the highlands. In Argentina, Fernández et al. (1999) examined mummified remains found on Mount Aconcagua. Tessone et al. (2015) studied weaning and breastfeeding practices of hunter-gatherers from Patagonia. In Ecuador, researchers have also used stable isotope analysis to answer questions about diet or weaning and breastfeeding practices; however, this work is limited to only a few studies.

This study aims to contribute to a better understanding of the diet of human groups that lived during the Formative Period (3500 – 500 BC) in the northern highlands of Ecuador. This study is done through the analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes of bone collagen, which have proven to give insights into the subsistence of different populations across the globe.

The specific objectives of this research are: 1) to establish the main food resources consumed at each site; 2) to identify particular intra-site dietary patterns, and whether they are age or sex-based; 3) to distinguish any dietary trends in the northern highlands during the Formative Period; and 4) to compare the results from this study to the isotopic data presented by other researchers in order to understand the diet in the northern highlands over three consecutive periods: the Formative, the Regional Development, and the Integration periods. By using stable isotope analysis as an approach for studying Formative diets, this study also aims to add to the literature for the area, as stable isotope analysis has been previously used only a few times in Ecuador.

In order to achieve this goal, a total of 56 human samples and 19 faunal samples were collected from four settlements discovered in three archaeological sites. The first site is

Rancho Bajo, dated to ca. 1610 – 1450 cal. BC (Early Formative). The second site is Cotocollao, which has two settlement phases, the first one dated to ca. 2405 – 1255 cal. BC (Early Formative), and the second one dated to ca. 1206 – 361 cal. BC (Late Formative). The third site is Las Orquídeas, dated to ca. 800 – 400 cal. BC (Late Formative). The first two sites were discovered in Pichincha Province and are located in the Quito Basin, relatively close to each other; Las Orquídeas was discovered in Imbabura Province, north of Pichincha Province. The reason behind the selection of these sites was the opportunity of examining cemeteries that represented both the Early and Late Formative periods, in order to observe changes in diet that may have occurred throughout the Formative period in the same region. It is important to mention that none of these sites have been examined isotopically before, so all the previous inferences about diet have relied solely on the analysis of archaeobotanical or zooarchaeological remains if available.

The data generated by this study will also be compared to the records of carbonized seeds, phytoliths, and pollen from these sites when possible, as well as to the interpretations of diet that were previously developed for each site. This comparison is done to evaluate whether the new data change our present understanding of the subsistence of these groups, or confirm it. In addition, the results from this study will provide a new line of dietary evidence for those sites that is not based on phytolith, pollen, and/or seed analysis.

Finally, the new data will be compared to the results presented by Ubelaker et al. (1995) and Pennycook (2013), who worked with human remains from the northern highlands that date to periods subsequent to the Formative. Ubelaker et al. (1995) examined the diet of high and low-status individuals buried in the site La Florida (Quito Basin) dated to around 1610 BP (uncalibrated), in the Regional Development Period. Pennycook (2013) provided isotopic information from the human skeletal remains recovered from the sites Tajamar and

NAIQ (Quito Basin) dated to the Integration Period (500/600-1532 AD). Thus, this research will give insight into dietary changes that occurred during the transition from the Formative to the Regional Development Period and to the Integration Period.

This thesis is organized into eight chapters. Chapter 1 introduces the research question and details the general and specific objectives of this study. It also summarizes the content of each chapter.

Chapter 2 is divided into two parts. The first part presents an overview of the development of the concept of the Formative Period in America, and describes the historical context of this period in Ecuador. The second part presents information that can contribute to a better understanding of the diet of Formative groups in the Northern Highlands. Therefore, in this chapter there will be a description of the environment and topography of Ecuador; and a brief summary of the archaeobotanical, zooarchaeological, and stable isotope evidence available for this period.

Chapter 3 gives detailed information about the archaeological finds at Rancho Bajo, Cotocollao, and Las Orquídeas. Specific information related to the sex, age, stature, pathologies, and grave goods that accompanied the individuals buried in each site is also presented.

Chapter 4 provides a brief summary of the theoretical and methodological backgrounds of stable isotope analysis, with an emphasis on carbon and nitrogen stable isotope analysis. An explanation of how the data recovered from this analysis are interpreted is also given. In addition, this chapter presents some key examples of stable isotope research conducted in the Andes.

Chapter 5 offers a general discussion of the human and faunal skeletal samples that were collected for this study. A description of the most commonly used methods for the preparation of bone collagen is included, as well as the protocols that were followed in the laboratory to extract the collagen and to submit the samples for carbon and nitrogen stable isotope analysis. This chapter also describes the criteria used to determine sample quality.

Chapter 6 presents the values obtained for each of the collagen preservation indicators – collagen yield, atomic C:N, %C, and %N – that are considered in this study. It also includes the results of the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis of both human and faunal samples. The relationships between these various measures are also examined.

Chapter 7 contains an interpretation of the diet of the Formative groups settled in the northern highlands, based on the $\delta^{13}C$ and $\delta^{15}N$ values. It also presents further discussion in terms of what the isotopic evidence tells us about the diet at the beginning and end of the Formative Period. The results from this study are also compared to the stable isotope study conducted by Ubelaker et al. (1995) for the Regional Development Period and the data presented by Pennycook (2013) for the Integration Period. After comparing the results of these studies, this chapter explains the dietary trends observed over time.

Chapter 8 presents the conclusions of this study, and suggests possible directions for future research.

Chapter 2: Diet in the Ecuadorian Formative Period

This chapter provides a general background of the region under study, starting with a description of its environment and topography. Then, it presents a brief summary of the development of the concept of the Formative Period in the Americas, as well as a brief description of the different archaeological sites discovered in the highlands, and in the western and eastern lowlands of Ecuador, that have been identified as part of the Formative Period. It also introduces the known evidence for Formative diets that has been obtained through palaeobotanical analysis, from the zooarchaeological record, and through stable isotope studies.

2.1. Environment and Topography of Ecuador and the Northern Highlands

Taking into consideration the geography of a country or region is of great importance when examining any cultural aspect of its human groups; this aspect acquires even more relevance when diet is under study, since the acquisition of food resources will depend on the local area and its environment. Understanding both factors, environment and topography, will contribute to a better comprehension of the dynamics between people and their surroundings, and how the latter could have affected human settlements in the past.

2.1.1. Ecuador

Ecuador shares its borders with Colombia in the north, Peru in the east and south, and the Pacific Ocean in the west. Its territory is divided into four regions: the Coast, the Sierra (Highlands), the Amazon, and an insular region (the Galapagos Islands) (see Fig. 2.1).

Fig. 2.1 - Map of Ecuador, displaying its four regions and provinces[1]

Ecuador's mainland is crossed in its longitudinal axis by two mountain ranges: the Western Cordillera, which has an elevation of between 3000 and 3600 masl (metres above sea level); and the Eastern Cordillera Real, with an elevation that starts at 3600 masl and exceeds 4000 masl. These longitudinal cordilleras (Eastern and Western) form the Interandean valleys whose catchment basins ("*hoyas*") are separated by transverse ranges

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[1] Map modified after d-maps.com (https://d-maps.com/carte.php?num_car=3401&lang=es) and wikipedia.org (https://en.wikipedia.org/wiki/File:Ecuador_relief_location_map.svg#file)

(“*nudos*”) bathed by hydrographic systems (Villalba 1988; Sarmiento and Frolich, 2002; Torres, 2014).

The climate is influenced by Ecuador’s position on the equator and the varied topography that characterizes the country. Similar to other equatorial climates, there is no seasonal cycle; instead, the tropical climate of Ecuador is characterized by a wet season and a dry season. These vary regionally by geographical and altitudinal location. The coastal region has a humid tropical climate with a rainy season affected by the El Niño current and the El Niño phenomenon, which cause an increase of sea surface temperatures in the Pacific Ocean and, consequently, heavy rainfall and faster wind currents. In the highlands and mountain valleys, the temperatures are mild year-round; and the climate is temperate and relatively dry. In the Amazon basin (eastern lowlands), the climate is characteristic of rainforests (Burke et al., 2010).

Ecuador is also characterized by the presence of more than 80 volcanoes, of which a total of 30 showed significant volcanic activity during the Holocene and Pleistocene. Most of the materials produced by these volcanic events were deposited in the Interandean Valleys of the Sierra, although occasionally they have also been deposited in the western and eastern lowlands (Hall and Mothes, 1998).

The features described above combine to create varied ecosystems and ecological niches that have made Ecuador one of the most biodiverse countries in the world. At the same time, these characteristics have influenced human settlements in aspects including food availability and the development of strategies for the acquisition of food resources, the development of specific agricultural techniques, commerce, and mobility, as well as the abandonment and relocation of settlements due to volcanic events.

2.1.2. The Northern Highlands

As previously mentioned, two cordilleras cross the country from north to south in the highlands. These along with transverse mountain ranges form the Interandean valley, which has been affected by different volcanic events. The most important volcanic eruptions in the Western Cordillera are those associated with the Cuicocha, Pululahua, Guagua Pichincha, Ninahuilca, and Quilotoa volcanoes. In the Eastern Cordillera the most relevant events are linked to the Imbabura, Cayambe, Cotopaxi, and Tungurahua volcanoes (Villalba and Alvarado, 1998; Hall and Mothes, 1998). The majority of these volcanic eruptions have in fact taken place in the northern highlands.

Of the events listed above, the most devastating were caused by the eruption of the Pululahua, Guagua Pichincha, and Quilotoa volcanoes. In the late Holocene the Pululahua volcano produced powerful pyroclastic flows as well as blocks and ash that covered the plains from San Antonio de Pichincha to Pomasqui (Hall and Mothes, 1998). Evidence of this eruption was discovered in the Quito basin at the Cotocollao site in the form of an ash layer of about 10 cm in thickness dated to 2,300 BP (Hall and Mothes, 1998). The layer was found overlying a cemetery dated to the Late Formative Period (Villalba, 1988). Excavations conducted by Ugalde (2013a) in Rancho Bajo revealed a similar ash layer from the Pululahua event, although it was situated in an upper stratigraphic deposit not directly associated with the cemetery discovered at the site, which dated to the Early Formative Period. According to Hall and Mothes (1988), these ash falls surely destroyed the agriculture and lacustrine life in the Quito basin, where the Cotocollao and Rancho Bajo sites, considered in this study, were located. Ash falls from this volcano also reached the west, affecting the area of Tulipe, associated with the Cotocollao culture, as well as the sites of Nueva Era, El Mocorral, Tamayo, and Agua Blanca, on the coast (Hall and Mothes, 1998; Isaacson and Zeidler, 1998).

On the other hand, the volcanic events associated with the Guagua Pichincha volcano had a recurrence of approximately 500 years. Layers of ash and pyroclastic material from this volcano spread over the Quito basin up to Tumbaco and Pifo. As for the Quilotoa volcano, ash falls and pyroclastic flows dated to 810 BP filled the Toachi River basin, transforming it into an arid and inhospitable landscape (Hall and Mothes, 1998).

The above are only some examples of volcanic events that altered the northern highlands; and although certain time periods experienced more volcanic activity than others, the devastation and transformation of the landscapes by pyroclastic flows and ash falls may have lasted at least hundreds of years. Therefore, these events may have played a major role in the stability of human groups, and also in the abandonment and relocation of sites as they could have impeded the use of the land where the settlements were located (Hall and Mothes, 1998).

Other factors that characterize the northern highlands and that must be considered are the presence of a large number of ravines that have given this region an irregular topography, as well as the presence of lagoons. The aforementioned appear to have been relevant for human groups deciding where to establish their settlements, since many human groups settled in these areas successively throughout different periods (Villalba and Alvarado, 1998). In the case of the Quito Basin, two lagoons, Turubamba and Iñaquito, played a significant role in settlement patterns in the past until they dried up and eventually disappeared (Villalba and Alvarado, 1998).

In terms of climate, it has previously been stated that local temperatures vary according to altitude; however, in general the northern highlands are described as a temperate and relatively dry region. The areas of Quito and Ibarra, which are of interest to this study, have a mild climate, constant temperatures, moderate rainfalls and fertile soils, resulting in several ecological niches that provided abundant and varied food resources that were exploited by groups in the past.

The last factor to consider here has to do with the strategic location of both Quito and Ibarra, in the sense that they constituted an obligatory point for the transit of human groups and exchange of products with the Coast and the Amazon region, through mountain passages and depressions (Villalba and Alvarado, 1998; Dyrdahl, 2017). This observation has support in ethnohistoric sources, which documented *mindalá* (merchant) routes, one of them being through the Chota-Mira Valley in the proximity of Ibarra. This route may have been involved in the procurement of raw materials such as cotton, coca and spondylus (Dyrdahl, 2017). Therefore, the strategic location of both areas needs to be considered, as it may have also played an important role in terms of the acquisition of raw materials and goods, exchange, and mobility during the Formative Period.

2.2. The Formative Period

A thorough review of how the notion of the Formative Period was conceived, and later adapted to the Ecuadorian case, can be found in the work of Marcos (2003) and Zeidler (2008). According to these authors, the Formative Period was originally defined in the 1950s by Gordon Willey and Philip Phillips on the basis of "the presence of maize and/or manioc agriculture and by the successful socioeconomic integration of such agriculture into a well-established sedentary village life" (Willey and Phillips, 1958: 144). This definition resembles that created by Gordon Childe for the Neolithic in the Old World (Marcos, 2003; Zeidler, 2008); however, the term "Formative" was apparently used by Americanists as an effort to avoid committing to any terminology that could imply Old World relationships (Ford, 1969).

James Ford (1969) was the first to question the concept for the Formative in the Americas (Marcos, 2003; Zeidler, 2008). In his opinion, the definition of Willey and Phillips did not apply to the Formative in all regions of the Americas. He emphasized the fact that plant cultivation developed many centuries before the appearance of ceramics and polished stone tools, which have been commonly accepted as Formative traits. Examples that support this observation can be seen in Mexico and Peru, where sedentism, agriculture, polished stone tools, and ceramics did not appear simultaneously. In addition, Ford stated that although small New World Formative settlements seem to have been sedentary, that does not mean they represented a "well-established village life" as specified in Willey and Phillips' definition (Ford, 1969: 4-5; Marcos, 2003: 7).

Ford also pointed out the fact that the earliest ceramics were not made by agricultural peoples, but instead may have been manufactured and spread by coastal groups whose subsistence relied on the consumption of shellfish. "The marriage of agriculture and ceramics seems to have taken place halfway through the 3000-year long Formative in Andean South America" (Ford, 1969: 5). It was Ford who originally suggested using a bipartite division for the Formative period in South America and not a tripartite division as in Mesoamerica. Although the names coined by Ford for this bipartite division did not last, the division itself is still used; and has become known as the Early and Late Formative Periods (Marcos, 2003).

2.3. Historical Background of the Formative Period in Ecuador

The Formative Period in Ecuador arguably starts around 3500 BC and ends approximately 500 BC, following the chronology most commonly used by the Museo Nacional del Banco Central of Ecuador (Torres, 2014). A revised and slightly more recent age range was given by Dyrdahl (2017), who suggested that the Formative runs from 3000 to 300 cal BC.

Fig. 2.2 - Major Formative sites in Ecuador. Redrawn after Dyrdahl (2017)[2]

The definition of this period in Ecuador was first developed based on the work of Meggers, Evans, and Estrada in the coastal region in the 1950s, particularly at the site of

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[2] Map modified after d-maps.com (https://d-maps.com/carte.php?num_car=3401&lang=es).

Valdivia (Meggers et al., 1965; Marcos, 2003). This site was originally identified in 1956 by Emilio Estrada, and subsequently investigated by Smithsonian archaeologists Betty Meggers and Clifford Evans in the late 1950s (Zeidler, 2008: 461). It is located in the Valdivia river valley (Santa Elena province), near seashores and estuaries. Cultural materials recovered at G-31 (Valdivia occupation site) included "Venus" figurines, chipped stone artifacts, faunal material (terrestrial, fish, and shellfish species), and elaborately incised ceramics. The ceramics were used by said researchers to establish a four-phase sequence of ceramic styles (Zeidler, 2008), and to characterize the Valdivia culture (4400 - 1450 BC). The culture was described to have had a semi-sedentary lifestyle restricted to the margin of the sea, which provided the people with more food resources than those they could have obtained inland before the development of agriculture. Later, incipient farmers appeared in the area and probably visited the inner regions seasonally or periodically for the purpose of collecting fruits and other wild plants (Meggers, 1966). In other words, from early excavations at the type site, Meggers and colleagues described Valdivia as a "semi-sedentary maritime adaptation of egalitarian fishermen and shellfish gatherers having only a marginal reliance on horticulture" (Zeidler, 2008: 462). Furthermore, they stressed that tools and utensils of shell, stone, and bone were quickly made only to fulfill their function, while pottery was decorated with a wide range of motifs produced using various techniques (Meggers et al., 1965; Meggers, 1966). Based on the above, Meggers stated that "the Formative Period sees the introduction of ceramics and agriculture" (Meggers 1966: 26).

Years later, however, the discovery of nearly 100 other Valdivia sites (Marcos, 2003) and further research at the most important sites of this culture such as Real Alto, Salango, and Loma Alta have suggested that the Valdivia culture differed from what Meggers et al. (1965) stated (Zeidler, 2008). Instead, Valdivia represented a:

> “Fully sedentary society of village horticulturalists characterized by progressive demographic growth and an increasing degree of social ranking and status inequality through time. Beginning as early as Middle Valdivia times, long-distance maritime as well as terrestrial trade may have provided an impetus for social change leading to greater complexity in the later Valdivia phases. […] Evidence for obsidian trade with the Quito basin exists. […] Agricultural production throughout the sequence is diversified, with both maize and a variety of root crops being staples. Maize as well as a series of root crops (manioc, achira, arrowroot, and llerén) are ubiquitous in both domestic and ceremonial contexts” (Zeidler, 2008: 464).

Valdivia, arguably one of the most studied Formative cultures, demonstrates that through this period some sites experimented with a sedentary village life, maize or manioc agriculture, and the production of polished stone tools and ceramics, as well as a higher population density and complexity at a fairly early stage (Zeidler, 2008). Nevertheless, it also shows, once compared to other coastal sites and sites in the highlands and eastern lowlands, that different subsistence systems and forms of organizational structure took place at the same time. What is more, it shows that agriculture and ceramics did not necessarily appear simultaneously.

2.3.1. The Formative Period in the Western Lowlands

The cultures known from this period are Valdivia (4400-1450 cal BC), associated with the Early Formative Period; Machalilla (1430-830 cal BC), representing the Middle Formative; and Chorrera (1300-300 cal BC), seen in the Late Formative Period (Zeidler, 2008:460). The Valdivia culture was briefly explained in the previous section, so this section will focus on describing Machalilla and Chorrera.

The Machalilla culture was initially identified by Bushnell (1951) in excavations at sites on the Santa Elena Peninsula (Zeidler, 2008: 466). Several authors are convinced, based on radiocarbon dates, stratigraphy, and stylistic analysis of ceramics, that Machalilla evolved from terminal Valdivia (Zarrillo, 2012: 50). The Machalilla peoples, like their Valdivia predecessors, practiced a mixed economy that relied heavily on maritime resources and on farming based on maize, achira, root crops, and tree fruits (Pearsall 2003; Zeidler, 2008). These staples were also accompanied by terrestrial sources of protein, especially deer (Zeidler, 2008). In terms of settlement patterns, Machalilla was characterized by small hamlets or homesteads dispersed along higher grounds close to alluvial floodplains in riverine areas, as well as by the absence of large civic-ceremonial centers and mound building. These characteristics suggest an apparent shift in site distribution and social hierarchy from the Valdivia settlement pattern (Zeidler, 2008). The ceramic style also seems to have evolved from Terminal Valdivia. As evidence of this conclusion, a Valdivia-Machalilla transition was found in San Lorenzo del Mate (Guayas Province) and La Emerenciana (El Oro Province). Several stylistic similarities have been found between Machalilla ceramics and ceramics from Formative sites in the highlands that were probably a result of long-distance trade. The sites in the highlands are Cotocollao (Pichincha Province), Alausí (Chimborazo Province), Cerro Narrío (Cañar Province), and Catamayo (Loja Province) (Zeidler, 2008:466-467).

Chorrera is a culture of the Late Formative Period that has been considered the most geographically widespread of Ecuador's prehispanic cultures, as it covered the entire coastal lowlands and extended its influence into the highlands (Zeidler, 2008: 468). Chorrera had a different ceramic style from Machalilla and Valdivia that included zoomorphic and phytomorphic effigy bottles with whistling spout-and-strap handles, mold-made anthropomorphic figurines, and well-made utilitarian wares with elaborate decoration. As to the settlement pattern, an archaeological survey covering an area of 785 km^2 located 239 sites or site components. The majority of these were found in alluvial areas of a valley, with a few located in upland zones or along higher elevations; none were adjacent to the coastal strip (Zeidler, 2008).

2.3.2. The Formative Period in the Highlands

Unlike the coast, the highlands do not have uniform and geographically expansive cultural traditions such as Valdivia, Machalilla, and Chorrera, except for the ties to Machallila seen at Cotocollao. On the contrary, even sites that are relatively close to each other show significant differences. Although two attempts to define a generalized ceramic style are worth noting (the “Challuabamba tradition” and the “Cerro Narrío style”), these are not understood in the same way as the cultural traditions of the coast (Zeidler, 2008: 471).

The first major site in the highlands is Cotocollao (Pichincha province), excavated by Villalba (1988). It is characterized by the presence of early and late components, and includes both habitation contexts and cemeteries. It is also probably the best known site of the Formative in this region, one of the reasons why it was chosen for this study. Detailed information regarding this site can be found in Chapter 3.

Another site in the northern highlands is La Chimba (Imbabura province), which has been studied by Athens (1995). Radiocarbon dates have dated this site between around 900 BC and 200 cal AD. This site shows several similarities with Cotocollao, one of them being ceramic decorative techniques and vessel forms. The second similarity is that both groups were fully agricultural, as indicated by the presence of cultigens such as maize, potatoes, oca, quinoa, and beans. Cotton was also present (Pearsall 2003; Zeidler, 2008). In La Chimba, however, there is also evidence of exchange with groups from the western and eastern lowlands, as well as the highlands (Zeidler, 2008).

In the central highlands, there are three sites to take into consideration. The first of these is Alausí, in the Río Alausí drainage. The second is Loma Pucará, situated in the Río Cebadas Valley; and the third, known as El Tingo, is situated in the Río Chimbo drainage (Zeidler, 2008: 476). Investigations at Alausí did not provide any radiocarbon dates; therefore, it was associated with the Middle Formative Period only after its ceramic material was seriated and linked to Machalilla's decorative techniques (Zeidler, 2008). Loma Pucará is a Late Formative occupation beginning around 670 BC (uncalibrated). It has been suggested that the people that lived at Loma Pucará were sedentary and in the process of transition from hunting and foraging to agriculture, despite no botanical evidence being available to support the existence of agriculture. El Tingo is a site that corresponds to the Late Formative, an association that was made based on the analysis of its ceramics, which are similar to those recovered in Cerro Narrío and Pirincay in the southern highlands (Zeidler, 2008: 475-476).

In the southern highlands the main Formative sites are Cerro Narrío in the Cañar Valley, Challuabamba in the Tomebamba Valley, Pirincay in the Paute Valley, and Putushío in southern Azuay Province, as well as other sites in the Catamayo area of Loja province. Cerro Narrío, Pirincay, and Putushío are located close to Challuabamba; and thus all exhibit some similarities. The first similarity lies in the presence of classic "eggshell"- thin ceramics in their ceramic assemblages. The architecture of these sites, and generally in the southern highlands, is represented by postholes frequently arranged in a rectangular shape. Their subsistence was similar to that of the northern highlands. The presence of maize, beans, and many other cultigens has been documented, as well as a variety of faunal remains including llamas, guinea pigs, and white-tailed deer (Zeidler, 2008: 478)

2.3.3. The Formative Period in the Eastern Lowlands

In the Amazon region, some of the sites that have been linked to the Formative Period are the Pastaza Phase on the Huasaga River, the Yasuní Phase on the Napo River, Pre-Upano and Upano I in the Upano Valley, and Cueva de Los Tayos (Zeidler, 2008; Torres, 2014). However, the radiocarbon dates from these sites seem to be problematic, especially after work by Athens (1990) in the proximity of the Huasaga River, which recovered material of the Pastaza Phase that could date to a more recent time, as well as the work of Duche and de Saulieu (2009). Following this research, Bruhns (2003) and Rostoker (2003) agreed that none of these phases should be assigned to the Formative Period (Zeidler, 2008: 479).

Given these uncertainties, the best known site in the eastern lowlands is Santa Ana La Florida, which was discovered in recent years. Excavations at this site have yielded architectural remnants, hearths and ceremonial offerings, as well as artifacts such as "a green stone mask, a polished stone bowl, a green stone anthropomorphic pendant, and several pieces of turquoise carved into zoomorphic shapes, most likely avian and serpent motifs" (Zeidler, 2008: 480). Other finds comprise human remains and funerary offerings, including a tall stirrup-spout bottle with human faces on either side, zoomorphic turquoise ornaments and malachite ornaments, stone bowls with various motifs, and a large amount of marine shell. Surprisingly, many of the elements recovered at Santa Ana La Florida have not been discovered in other sites in the coast or the highlands. A radiocarbon date has placed these offerings recovered from a pit between 2270 and 2260 BC (Zeidler, 2008: 480).

2.4. Available sources of information on Formative Diets

Most of the information related to Formative diets in Ecuador comes from three different sources. The first source is the data obtained through the analysis of pollen and carbonized seeds. The second source is linked to the zooarchaeological records from each site. The third source is carbon and nitrogen stable isotope analysis. Other lines of evidence include iconographic analysis and the use of ethnohistorical records, although the latter consist of Spanish chronicles of a later period, which should not always be extrapolated.

This section focuses on the archaeobotanical evidence, the zooarchaeological records, and the few stable isotope analyses that have been conducted so far. As McConnan (2013) points out, it is important to emphasize that although these methods and the information they provide have strengths and weaknesses on their own, when combined they can offer a better insight into ancient diets.

2.4.1. Archaeobotanical Evidence for Formative diets

Before discussing the information available through palaeobotanical or palynological analysis, it is important to highlight why the region under study does not have a robust database for these analyses as some others do. The explanation is due primarily to the fact that the recovery of archaeobotanical evidence from prehispanic sites in Ecuador and the neotropics represents a "formidable challenge", in the words of Pearsall (1995: 113). Some of the main reasons why this is so are explained below.

The first reason has to do with the preservation of macroremains (e.g., seeds, tubers, wood, corn cob fragments), which in Ecuadorean sites studied to date is limited to charred materials (Pearsall, 1995). The latter can be hard to detect and recover during excavation, as they may be highly fragmented due to post-depositional destruction (Pearsall, 2003). Even after being recovered, the identification provided by such remains becomes more problematic, once the wide range of flora species of the region is considered. To perform reliable identifications, a large botanical comparative collection and sometimes the application of specialized identification techniques (e.g., scanning electron microscopy) is required (Pearsall, 1989; Pearsall, 1995).

The second problem lies in establishing whether the quantities of well-identified remains are sufficient enough to assess their contribution to the diet, taking into consideration that measurements such as ratios or percentages of the presence of plants could lead to false inferences about subsistence if unrecovered and unidentified material is not taken into consideration (Pearsall, 2003). This factor is also related to a third challenge, which has to do with the recovery of charred plant remains as opposed to phytoliths. The former, as previously explained, can disappear from the archaeological records over time, while phytoliths are not subjected to the same patterned loss. This difference is relevant because in order to do a quantitative comparison between macroremain assemblages, their preservation and recovery should be equal; otherwise they should not be compared quantitatively (Pearsall, 2003).

The last main reason why Ecuador does not provide a robust archaeobotanical database is that not all sites have been sampled for phytoliths or charred remains, a fact that is associated with the limited number of researchers specializing in this area that work in the country. Moreover, in some cases in which these archaeobotanical remains were indeed collected, the process was done using an old flotation technique that could have led to certain gaps in the records; this situation is especially true in sites excavated during the 1960s and 1970s (Pearsall, 2003).

The paleoethnobotanist Deborah Pearsall, who has worked on several archaeological sites in Ecuador, has provided a list of the plants identified as present during the Formative period. The list can be found in the following Table 2.1.

Table 2.1 - Plants used in the Formative Period[†]

Common Name	**Scientific Name**	**Botanical family**
achira	*Canna edulis*	Cannaceae
amaranth	*Amaranthus* spp.	Amaranthaceae
arrowroot	*Maranta* spp.	Marantaceae
avocado	*Persea americana*	Lauraceae
bean, common bean	*Phaseolus vulgaris*	Fabaceae
bedstraw	*Galium* spp.	Rubiaceae
begonia	*Begonia geraniifolia*	Begoniaceae
bottle gourd	*Lagenaria siceraria*	Cucurbitaceae
chenopod	*Chenopodium* spp.	Chenopodiaceae
chili, *ají*	*Capsicum* spp	Solanaceae
ciruela de fraile	*Bunchosia armeniaca*	Malpighiaceae
cotton	*Gossypium barbadense*	Malvaceae
grass (wild)		Poaceae
guava	*Psidium guajava*	Myrtaceae
hackberry	*Celtis* spp.	Ulmaceae
jack bean	*Canavalia* spp.	Fabaceae
jicama	*Pachyrrhizus* spp.	Fabaceae
llerén	*Calathea* spp.	Marantaceae
lima bean	*Phaseolus lunatus*	Fabaceae
lucuma	*Pouteria lúcuma*	Sapotaceae
lupine	*Lupinus* spp.	Fabaceae

[†] (Pearsall, 2003: 216)

Table 2.1 (continued) - Plants used in the Formative Period[†]

Common Name	**Scientific Name**	**Botanical family**
maize	*Zea mays*	Poaceae
manioc, *yuca*	*Manihot esculenta*	Euphorbiaceae
oca	*Oxalis tuberosa*	Oxalidaceae
pacae	*Inga feuillei*	Fabaceae
palm		Arecaceae
peanut, *maní*	*Arachis hypogaea*	Fabaceae
potato	*Solanum tuberosum*	Solanaceae
quinoa	*Chenopodium quinoa*	Chenopodiaceae
sedge	*Cyperus* or *Scirpus*	Cyperaceae
soursop, *cherimoya, guanábana*	*Annona* spp.	Annonaceae
squash	*Cucurbita* spp.	Cucurbitaceae
squash/gourd		Cucurbitaceae
sweet potato	*Ipomoea batatas*	Convolvulaceae
tree legume	*Acacia, Prosopis,* or similar	Fabaceae
ullucu	*Ullucus tuberosus*	Basellaceae

[†] (Pearsall, 2003: 216)

Pearsall (2003) also presents the distribution of some of these plants in 27 Formative sites (or site components) where they were identified. A brief summary can be found in Table 2.2.

Table 2.2 - Botanical remains identified at Formative sites†

Region	Formative Site	Culture/Phase	Botanical evidence
Coast	Loma Alta	Valdivia 1, 2	Palm, *Annona* spp. (soursop), tree fruit, *Celtis* spp. (hackberry), Sapotaceae, dense cotyledon sedge, *Canna* (achira), root/tuber, maize, Jack bean
	Real Alto	Valdivia 1, 2 Valdivia 3 Valdivia 4, 5, 6, 7	Tree fruit, tree legume, dense cotyledon sedge, *Canna* (achira), root/tuber, maize, Jack bean, cotton
	Río Perdido	Machalilla	*Canna* (achira), maize
	La Ponga	Machalilla	Maize
	Anllulla	Valdivia D	Tree fruit, root/tuber, maize
	San Isidro	Valdivia/Piquigua Chorrera/Tabuchila	Palm, sedge, *Canna* (achira), arrowroot, maize, squash/gourd, gourd
	Capaperro	Valdivia/Piquigua	Tree fruit, dense cotyledon, porous endosperm
	Dos Caminos	Chorrera/Tabuchila	Tree fruit, dense cotyledon, maize, common bean, porous endosperm
	Finca Cueva	Chorrera/Tabuchila	Tree fruit, palm, sedge, *Canna* (achira), arrowroot, maize, porous endosperm
	El Mocorral	Chorrera/Tabuchila	Tree fruit, palm, sedge, *Canna* (achira), arrowroot, maize, porous endosperm
	Perdomo	Selva Alegre Guadual	Palm, sedge, *Canna* (achira), arrowroot, maize
	Herradura	Herradura	Palm, maize
	Selva Alegre	Selva Alegre Guadual	Palm, sedge, *Canna* (achira), arrowroot, maize
	R30	Selva Alegre Las Cruces	Palm, sedge, *Canna* (achira), Maize
	C69	Mafa Guadual	Palm, sedge, maize
Andes	Cotocollao	Formative	Root/tuber (monocotyledon, dicotyledon), maize, lupine
	Nueva Era	Formative	Root/tuber (monocotyledon), maize
	La Chimba	Early/ Middle/ Late Formative	Potato, oca, root/tuber, cotton

† Botanical remains identified in 27 Formative sites. Modified from Pearsall (2003)

The data show that the Formative people in the coast and the highlands used a combination of wild and domesticated plants. On one hand, in the coastal region, maize was integrated into a subsistence suite of local domesticates that included cotton, jack bean, squash, arrowroot, and achira. On the other hand, in the sierra, maize was incorporated into a subsistence suite that included common bean, potato, oca, and lupine. In the Amazon region, the use of plants during the Formative is still obscure (Pearsall, 2003); however, the data from Santa Ana La Florida are helpful in this regard. The palaeobotanical analysis conducted by Zarrillo and Valdéz (2013) at this site has recovered evidence of corn starch, chili, possibly cacao and other tubers not yet identified.

Nevertheless, from all the sites examined by Pearsall, the identification of botanical remains at the sites of Cotocollao and La Chimba is of particular interest to this study. The former is a site in the Pichincha province that is part of this research, while La Chimba is a site in the Imbabura province located relatively close to Las Orquídeas, a site that is also under study here.

In the case of Cotocollao, Pearsall (1984) examined 22 carbonized samples and 35 phytolith samples that included maize, tree fruit fragments, two types of root/tubers, and lupine. Here, the identification of maize is interesting for two reasons. First, a maize phytolith and a carbonized sample were discovered at the earliest level of the site, equivalent to the Early Formative Period. Second, the maize was similar to that identified at La Ponga, a Formative site in the coast (Lippi et al. 1984; Villalba, 1988); and was identical to that discovered in Nueva Era, another Formative site in the Pichincha province (highlands) (Pearsall, 2003). Additionally, this find is contemporaneous with the evidence of maize recovered at La Chimba. Thus, the archaeobotanical evidence indicates that maize could have been present in Preceramic and early ceramic sites in the highlands (Pearsall, 2003).

La Chimba is a site that was continuously occupied through three sequences: Early La Chimba (700 – 440 cal BC), Middle La Chimba (440 – 44 cal BC), and Late La Chimba (44 cal BC – cal AD 250) (Stahl and Athens, 2001). At this site, charred botanical materials and other remains recovered using flotation were in excellent condition. Here, large pieces of charred tubers and maize cob fragments were common, while cotton was also present although not that common (Stahl and Athens, 2001; Zarrillo, 2012). These finds are described as follows:

> A variety of small seeds from wild/weedy annuals, including wild legume, sedge, bedstraw, lupine, Malvaceae, chenopod, amaranth, and several grasses occurred throughout the La Chimba sequence. Carbon samples contained many of the same plant taxa as the flotation samples, with the addition of common bean in a Late Formative period sample. […] The La Chimba botanical data suggest that subsistence was quite stable over the 1,000 years of site occupation and that root crop and maize agriculture was supplemented by use of wild, gathered resources. The antiquity of this subsistence system is likely to be considerably earlier than the 2640 BP date for the beginning of occupation at La Chimba (Pearsall, 2003: 230-231)

Pearsall concludes that "the few data available to date suggests the Formative of Ecuador was characterized by a diversity in subsistence strategies, with maize playing a role even before the appearance of ceramics" (Pearsall, 1988: 156). The botanical remains also indicate that at least some of the subsistence systems had an agricultural component, which is more visible in the Late Formative Period. However, despite the fact that the presence of cultivated plants has been established, it remains impossible to assess how common cultivated crops were in relation to wild plant resources, especially since the preservation of these remains is poor; so only few databases can be examined quantitatively (Pearsall, 2003).

The research conducted by Zarrillo (2012) complements this information about Formative diets. Zarrillo analyzed starch granules collected from charred cooking ceramic and stone tools from sites of the Formative Period in the highlands, and one site from the eastern Andean slopes. The sites investigated included La Chimba, Tajamar, Cerro Narrío, Chaullabamba, La Vega, Trapichillo, and Santa Ana-La Florida (SALF).

The analysis revealed that domesticated crops, such as oca, potato, lupines (chocho/tarwi), quinoa, beans and maize, were widespread in the northern highlands of Ecuador at least since the end of the Early Formative to the Late Formative, as evidenced at La Chimba, Tajamar, Cotocollao, Cerro Narrío, and Chaullabamba. In contrast, the La Vega and Trapichillo sites located in the southern highlands, as well as the Santa Ana La Florida site (eastern Andean slopes), show the presence of crops more suited to a lowland tropical agricultural system, including manioc, sweet potato, yam, maize, beans, and probably cacao (Zarrillo, 2012).

Zarrillo (2012) also discusses other lines of evidence for an earlier presence of maize, likely prior to the Early Formative Period. This observation comes from the analysis of sediment core pollen from lakes. In the northern highlands, the presence of maize is suggested by pollen and phytolith analysis from Lago San Pablo sediment core (Athens et al., 2016) and sediment core pollen from Lago Yambo (Colinvaux et al. 1988). In the southern highlands, near Chaullabamba, maize cultivation is evidenced by the Laguna Chorrera sediment core pollen sequence. All these sequences suggest the cultivation of maize took place from the Middle to Late Formative Period (ca. 4000 cal BP), or even before the Middle Formative (Hansen et al. 2003; Zarrillo, 2012).

2.4.2. Zooarchaeological Evidence

In Ecuador, the recovery and analysis of faunal remains began only a few decades ago. According to Stahl (2003), the relevance of zooarchaeological data was not entirely recognized with a few exceptions until the late 1950s, when the work of the archaeologists Betty Meggers, Clifford Evans, and Emilio Estrada (1965) at the type Valdivia Formative site included the identification of taxa and a list of frequencies by excavation unit, as well as an interpretation of the site that incorporated these data. Later, during the late 1960s and early 1970s, when the archaeology of the Formative Period increased, the recovery, analysis and interpretation of faunal remains not only became more important but also more standardized (Stahl, 2003).

Since then, several researchers have looked in more depth at the faunal remains from different archaeological sites. Here, it is important to note the work of Peter Stahl, who has worked in Ecuador's four regions and has arguably been involved in the majority of archaeological projects incorporating zooarchaeology. His publication of 2003 titled "*The Zooarchaeological Record from Formative Ecuador*" reviews the zooarchaeological evidence from Formative contexts recovered in the western lowlands and highlands of Ecuador. His database compiles the information from 27 archaeological sites, which have been briefly summarized in Table 2.3. The coastal sites include La Emerenciana, El Encanto, Hormiga Shelter, OGCH-20, Real Alto, OGSE-62, OGSE-42, San Pablo, Valdivia, Buena Vista, Río Chico, Capaperro, La Cabuya, La Ponga, Guarnal, Punta Brava, OGSE-46, Loma Alta, Salango, Dos Caminos, San Isidro, Finca Cueva, and El Mocorral. The sites in the northern highlands include La Chimba, Cotocollao, Pirincay, and Putushío.

Table 2.3 - Formative Period zooarchaeological assemblages in Ecuador†

Region	Period	Fauna
Coast	Early / Middle / Late Formative Period	• Molluscs, gastropods, crustaceans, cartilaginous and bony fishes (Chondrichthyes & Osteichthyes). • Amphibians (salamanders, frogs, toads) • Reptiles (turtles, lizards, serpents). • Birds (pelicans, ducks, hawks, falcons, pigeons, doves, parrots). • Mammals: primates, marsupials (opossums), xenarthrans (sloths), chiropters (bats), carnivores (dogs), mustelids (weasels), felines (cats, puma, jaguar), tapirids (tapirs), tayassuids (peccary), cervids (deer), rodents (paca, etc) and leporids (rabbits/hares).
Highlands	Middle / Late Formative Period	• Birds (pigeons, doves, parrots). • Mammals: primates, marsupials (opossums), carnivores (bears, dogs, racoons), mustelids (weasels), felines (puma), tapirids (tapirs), camelids (llama, alpaca) and cervids (deer), rodents (guinea pigs, paca) and leporids (rabbits/hares).

† Brief summary based on Stahl (2003)

The summary presented in Table 2.3 does not do justice to Stahl's database, which originally depicts "55 orders, 134 families, 175 genera, and 193 species belonging to nine zoological classes" (Stahl, 2003:182). Although the information presented by Stahl (2003) does not include all the Formative sites discovered to this day, it gives a better idea of the striking richness of the archaeofaunal records from the Formative sites in Ecuador. In addition, the identification of faunal remains has provided valuable information about some of the activities that were performed by people in the Formative Period. An example is the introduction of domesticated species, such as camelids and guinea pigs, from other regions. The sites Cotocollao, Tajamar, and Pirincay, in the northern and southern highlands, provide evidence of the introduction of domesticated camelids (llama/alpaca) by the end of the

Formative Period (Villalba, 1988; Pennycook, 2013; Stahl, 2003). In the case of *Cavia* (*cuy*) remains, most of the evidence comes from archaeological sites in the coast; but the species appears at the sites Las Orquídeas and Putushío in the northern and southern highlands by the Late Formative Period (Dyrdahl, personal communication; Stahl, 2003). Also, the zooarchaeological record gives an account of the diverse strategies of exploitation of resources that were employed at that time. Stahl (2003) stated that these strategies may have ranged from simple collection to the use of sophisticated weaponry, the manipulation of domesticates, and the employment of ocean-going skills and long-distance trade. Moreover, the record is a source of information about the faunal remains that were used as tools in the manufacturing of different artifacts, adornments, and textiles; and about those animals that were part of ritual ceremonies and offerings (Stahl, 2003).

In terms of diet, the presence of certain species in an archaeological site does not guarantee that the people who lived in that settlement consumed the meat from those animals, nor does it indicate the frequency with which they may have consumed those food resources. However, the database presented by Stahl (2003) certainly shows that the Formative settlements in the highlands may have not consumed any marine resources, probably due to the long distance from the coast. This factor should be taken into consideration when examining the stable isotope values of the sites under study.

2.4.3. Stable Isotope Analysis of Formative Diets

There are only a limited number of studies that have conducted archaeological stable isotope analysis in Ecuador. Two studies were performed in the coastal region, and another three in the northern highlands.

The first study was carried out by van der Merwe, Lee-Thorp, and Raymond (1993) on samples collected from several coastal sites from the Preceramic Period (Las Vegas), the Early Formative (Valdivia culture), the Middle Formative (Machalilla), the Late Formative (Chorrera), the Regional Development Period (Guangala), and the Integration Period (Manteño phase). Las Vegas is a Preceramic site dated from 7000-5500 BC. From this site, a total of 51 human samples were collected, of which only two gave results. The $\delta^{13}C$ values were between -14.0‰ and -14.9‰, while the $\delta^{15}N$ fell between 11.0‰ and 7.9‰. These values suggest marine food consumption and a substantial intake of C_4 foods (see Chapter 4 for a discussion of the theory and interpretation of stable isotope values). However, van der Merwe et al. (1993) could not establish whether the C_4 component was due to grasses in the natural environment or to maize; or even whether these measurements were valid, due to the poor quality of the sample.

From the Formative Period, they examined 12 samples from Valdivia I and II contexts (4400-2800 B.C.) (Zeidler, 2003), of which eight samples were successful, providing a mean $\delta^{13}C$ value of -19.0‰ and a mean $\delta^{15}N$ value of 11.7‰, suggesting a predominantly C_3-based terrestrial subsistence. They also examined 40 samples from the Valdivia III context from Real Alto (2900-2700 B.C.), of which only one sample yielded a $\delta^{13}C$ value of -18.8‰, indicating a mainly C_3-based diet. However, this sample did not produce any $\delta^{15}N$ values; therefore, van der Merwe et al. (1993) took into consideration the $\delta^{15}N$ values provided by two other samples that failed to produce any $\delta^{13}C$ values. These two samples displayed a mean $\delta^{15}N$ value of 13.8‰, which might indicate some marine dietary input, although an alternative explanation is the inclusion of some riverine food resources in a diet with a C_3 and terrestrial focus. In the Machalilla phase at Salango (1700-1100 BC), van der Merwe et al. (1993) noticed a change in the diet. The ten individuals for whom $\delta^{13}C$ values could be obtained displayed a mean of -12.3‰, whereas the 11 individuals that produced $\delta^{15}N$ values displayed a mean of 14.0‰, suggesting a major shift to the inclusion of marine food and possibly some maize in the diet. However, there is high variability at the site in terms of both $\delta^{13}C$ and $\delta^{15}N$ values. The variability may reflect rapid dietary change within the phase or simply heterogeneity within the population. Also in Salango, the subsequent phase, which is Chorrera (1100-300 BC), exhibited a similar pattern to that seen in the Machalilla phase, although there is slightly less intra-site variability. Values for the Chorrera phase in Loma Alta suggest more C_4 consumption, slightly less marine food consumption, and high intra-site variability.

From the Integration Period, van der Merwe et al. (1993) examined the Guangala Phase (300 BC - l00 AD) at the Valdivia site (OGSEMA-172). Here, a shift towards maize is more obvious, since the remains of seven individuals analyzed displayed a mean $\delta^{13}C$ value of -8.0‰, indicating an overall reliance on maize as a staple. The mean $\delta^{15}N$ value is 14.4‰, suggesting a level of marine foods similar to that in the Chorrera phase. Finally, from the Integration Period they studied remains of eight individuals from the Bahía and Manteño Phase (1470 - 1580 AD) at the site OMJPSL-141b in Salango. In this phase, they observed essentially the same pattern, since the $\delta^{13}C$ values are similar, only slightly lower (mean value -9.0‰); and a similar mean $\delta^{15}N$ value (14.0‰) suggests that the marine contribution remained the same.

Tykot and Staller (2002) conducted stable isotope analysis at La Emerenciana (El Oro province). This is one of the largest Early Formative sites associated with the Valdivia culture on the coast. Excavations recovered four burials in which the individuals were placed in an upright and tightly flexed position inside a circular burial pit, along with ritual offerings consisting of remains of marine fauna, birds, and terrestrial mammals (Tykot and Staller, 2002: 668-669). This study conducted carbon and nitrogen stable isotope analysis of bone collagen and apatite for all the individuals, as well as carbon stable isotope analysis of tooth enamel from one of the individuals. Out of the four individuals sampled, one did not yield collagen with acceptable C:N values. The other three individuals consisted of two adult females and one juvenile between 5 and 7 years old. The researchers concluded that the individuals from La Emerenciana may have had a diet that relied more on the combination of terrestrial and freshwater fauna and C_3 plants rather than on a diet based on marine fauna, maize or other C_4 or CAM plants. Although maize phytoliths were identified inside pots at this site, confirming the consumption of maize, Tykot and Staller (2002) suggest that maize was not a staple crop in the coast of Ecuador during the Valdivia period (Early Formative). The researchers also compared their results to those obtained by van der Merwe et al. (1993) for other Valdivia sites (Loma Alta and Real Alto), and samples from the subsequent Machalilla and Chorrera phases (Tykot and Staller, 2002). This comparison showed "a long-term trend in coastal Ecuador toward increasingly enriched carbon isotope ratios and increased consumption of maize" (Tykot and Staller, 2002: 674).

The first study that conducted stable isotope analysis on a site from the northern highlands with the purpose of examining human diet was that published by Ubelaker, Katzenberg and Doyón (1995). The researchers sampled a total of 32 individuals from the site of La Florida in Quito, radiocarbon dated to 1610 BP (uncalibrated)[3] (Doyón, 1989); in other words, the site was dated to the Regional Development Period ("Desarrollo Regional"), subsequent to the Formative Period. These individuals were recovered from six deep shaft tombs with a depth between 12.5 to 15.1 m. Ribs from males and females aged between 7

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[3] Molestina (2006) published a different radiocarbon date of 600 AD, which suggests that La Florida belongs to the Integration Period.

and 50 years old were sampled; 9 of them likely represented high-status individuals, while the other 23 were considered low-status. The elite burials were characterized by the presence of artifacts made of metal, marine shell, textile, exotic wood, and stone, and emerald beads, as well as offerings that included ceramics and wild and domesticated faunal remains. Meanwhile, remains of human sacrifices, decapitated remains, and people probably buried alive that were also recovered at the site were considered to be low-status individuals (Doyón, 1991; Ubelaker et al., 1995; Ubelaker, 2000). Thus, La Florida offered a great opportunity for examining whether dietary differences based on status existed.

Katzenberg conducted carbon and nitrogen stable isotope analysis of bone collagen from those 32 samples and bioapatite stable carbon isotope analysis from five high-status and five low-status individuals. All samples provided usable collagen. The mean $\delta^{13}C_{collagen}$ value for the high-status group is -10.3 ± 1‰, while the mean $\delta^{13}C_{collagen}$ value for the low-status group is -11.6 ± 1.5‰. The mean $\delta^{13}C_{bioapatite}$ value for the high-status group is -4.2‰ ± 1‰ and the mean $\delta^{13}C_{bioapatite}$ value for the low-status group is -6.2‰ ± 1.5‰. The mean $\delta^{15}N$ value for the high-status group is 8.8 ± 5‰, and the mean $\delta^{15}N$ value for the low-status group 8.5 ± 1.1‰. No significant differences between ages or sexes were found. The values suggest that there was no differential consumption of terrestrial animal protein between high and low-status individuals. However, differences were observed in terms of maize consumption. Although both groups consumed a lot of maize, the high-status ones consumed a greater proportion of maize than the low-status individuals, a difference seen more strongly in the bioapatite $\delta^{13}C$ values. This patterning suggested to Ubelaker et al. (1995) that the differences in $\delta^{13}C$ values are not linked to differential access to a high protein resource such as the meat of maize-fed animals, or differential consumption of maize-fed vs. free ranging animals, as both of those dietary differences would be expected to show up more strongly in collagen

$\delta^{13}C$ values than in bioapatite $\delta^{13}C$ values. Instead, they suggest as an explanation that the high-status individuals had a greater consumption of maize beer (*chicha*), a low protein resource that preferentially impacted bioapatite $\delta^{13}C$ values.

Tykot, van der Merwe, and Athens (1996) also used carbon and nitrogen stable isotopes to examine the significance of maize in two sites from the northern highlands. The first site is La Chimba in Imbabura province, in the general proximity of Las Orquídeas which is part of the present study. The second site is Socapamba, also located in Imbabura province. The results of this study were presented at the 61st Annual Meeting of the Society for American Archaeology in 1996. Although a published version of this report unfortunately could not be located, some brief references to the results can be found in other publications. For example, Tykot et al. (2006) mention that the individuals from La Chimba displayed noticeable quantities of maize protein in their diet (Tykot et al., 2006: 194). Tykot and Staller (2002) mention that in highland Ecuador, maize is clearly important during the first half of the 1st millennium BC even at sites above 3,000 m like La Chimba, since individuals from this site display average collagen $\delta^{13}C$ values of -17‰. However, it becomes a staple in the 1st millennium AD., when collagen $\delta^{13}C$ values that fall between -15‰ and -9‰ are found (Tykot et al., 1996; Tykot and Staller, 2002: 670).

A third study was conducted by Pennycook (2013) on two sites from the Integration Period (500/600 AD – 1530) located in the Quito basin. Diet was examined using collagen carbon and nitrogen stable isotope analysis, as well as bioapatite $\delta^{13}C$ analysis. From the first site, Tajamar, Pennycook (2013) sampled 73 individuals that displayed a mean collagen $\delta^{13}C$ mean value of -9.1 ± 1.1‰ and a mean $\delta^{15}N$ value of 8.6 ± 1.1‰. These values were considered indicative of a C_4 diet, with minimal consumption of small mammals/small wild mammals. No age-based or burial-based dietary differences were observed. There were also no differences in terms of diet between those individuals that presented cranial modification and those that did not. However, males exhibited higher $\delta^{15}N$ values, perhaps due to the consumption of more protein or protein from a different source. Among other interesting finds, it seems that the people from Tajamar did not consume much camelid protein despite the fact that the camelid remains recovered from the site display cut marks and evidence of burning. If they did consume such protein, it was not on a daily basis. In addition, the camelid isotopic composition shows a mixed form of animal management that involved C_3 grazing and C_4 foddering. Moreover, canids show a similar diet to humans.

The second site studied was one of many sites discovered at the NAIQ (New Quito International Airport). Pennycook (2013) sampled 42 individuals from this site that exhibited a mean collagen $\delta^{13}C$ value of $-18.6 \pm 0.3‰$ and a $\delta^{15}N$ mean of $+8.0 \pm 0.7‰$ that are indicative of a C_3 diet that also involved protein from wild animals, and some maize consumption. No burial, age or sex-based differences were found. Camelids and *cuy* (guinea pig) did not contribute to the diet. Canids also exhibited a similar diet to humans.

2.4.4. Summary of the Dietary Evidence

In summary, the palaeobotanical record has confirmed the early presence of domesticated crops, such as oca, potato, lupines (chocho/tarwi), quinoa, beans and maize, in the highlands. Early sites from the lowlands show evidence of the presence of manioc, sweet potato, yam, maize, beans and achira, among other products. Most of the pollen, carbonized seeds, and phytoliths examined were obtained from habitation contexts, cooking ceramics, and hearths, which increase the probability that these food resources were in fact consumed during the Formative. The zooarchaeological record from this period includes an ample variety of remains from molluscs, fish, reptiles, amphibians, birds, and mammals, which have been recovered in sites located in the western lowlands. Meanwhile, in the highlands the remains of diverse species of birds and mammals were also recovered. These finds together with stable isotope analysis confirm that the diet of coastal groups included marine and freshwater resources, a pattern that is not mimicked by groups in the highlands. Although the palaeobotanical and zooarchaeological evidence can not tell us about the proportions of different plant and animal species consumed, it gives an account of the different resource exploitation strategies that may have been used in the sea coast and inland. In addition, the discovery of some species in the highlands that were first found in the coast region indicates the introduction of such species into other regions, as well as the existence of trade and exchange networks and mobility.

The evidence obtained through stable isotope analysis of coastal groups indicates that maize and/or other C_4 plants were part of the diet of Formative populations. On the coast, the consumption of maize apparently increased throughout this period. At the outset of the era it may not have played a significant role in the diet, but it was likely important later on. Throughout the Formative, coastal diets were apparently mixed, involving the consumption of C_3 and C_4 plants, as well as terrestrial, marine, and/or freshwater protein. In contrast, there is no direct isotopic information available about the diet of Formative groups in the highlands. The evidence from the subsequent periods (Regional Development and Integration) suggests that the consumption of maize may have not necessarily increased as in the coast region, or at least not in all communities. For instance, the site NAIQ from the Integration Period has isotopic signals that suggest that maize did not play an important role in the diet; however, the contemporary site Tajamar displays isotopic values that indicate that maize was a staple food (Pennycook, 2013). This difference suggests that different subsistence systems may have coexisted in the highlands, and that the importance of maize may have been different for each settlement at least until the Integration Period. In addition, the people of the highlands supplemented their diet with C_3 plants and apparently only terrestrial protein, as there is no evidence of the consumption of marine resources, probably due to the long distance from the coast. Another interesting find was that made by Ubelaker et al. (1995) at La Florida, where no differential access to meat between low and high status individuals was found. However, there were differences in terms of the consumption of maize apparently in the form of chicha, a fermented beverage. This difference may indicate that maize or chicha consumption may have been connected with status.

2.5. Conclusion

As seen in this chapter, the varied topography and climate of Ecuador has created different environments that support a wide variety of animals and plants. These resources were exploited by Formative groups, as seen in the paleobotanical and zooarchaeological record. Although such records only provide evidence of the presence of different species at each site, stable isotope analysis confirms the consumption of protein from different sources; and demonstrates the consumption of C_3 and C_4 plants as early as the beginning of the Formative Period, at least in the coastal region. Stable isotope evidence for diet, particularly maize consumption, at highland Formative era sites is clearly needed to improve our knowledge of the period.

Chapter 3: Sites

Since the purpose of this study is to examine the diet of human groups that lived during the Formative Period in the Northern Highlands of Ecuador, I decided to select the sites Rancho Bajo, Cotocollao, and Las Orquídeas, since they met these requirements. They were also chosen based on: 1) the number of individuals and the presence of faunal remains; 2) the availability of the remains for chemical analysis; 3) their radiocarbon dates, which placed them at the beginning of the Early Formative Period or at the end of the Late Formative Period; and 4) the amount of information available about the site. The latter was considered relevant, since the more data are available for a site, the better and more accurate the interpretations will be. As well, in the past I had the opportunity to collaborate with the group of archaeologists that studied Rancho Bajo and Las Orquídeas, not only during field work but also in the analysis of the materials at the laboratory.

Fig. 3.1 - Map locating the sites Rancho Bajo, Cotocollao, and Las Orquídeas[4]

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[4] Map modified after d-maps.com (https://d-maps.com/carte.php?num_car=3401&lang=es).

This chapter includes a brief description of the archaeological finds at the sites Rancho Bajo, Cotocollao (early and late settlement), and Las Orquídeas. Since this study will focus on the reconstruction of diet through the analysis of human remains, a special emphasis will be given to the results of the osteological analyses conducted at each site.

3.1. Archaeological Background

Reconstructing the diet of human groups involves more than the use of chemical analysis; it requires having as thorough an understanding as possible of factors including the surroundings of a settlement, the local plants and fauna, the community’s subsistence and economy, the site’s habitation and funerary contexts, the group’s demographics and health, and their mortuary practices and grave goods. These requirements highlight the importance of presenting the archaeological evidence from each site.

3.2. Rancho Bajo

This archaeological site was accidentally discovered in 2011 during construction work for a house in Rancho Bajo, a neighbourhood located in the northwest of Quito. The initial discovery was made by the owner of the property, who immediately contacted the police after machinery revealed the presence of human burials. Once it was established that the skeletal remains were archaeological, the archaeologist María Fernanda Ugalde was asked to rescue all the cultural materials that could be affected by construction in the area (Ugalde, 2012).

Since 2011, three field seasons have taken place at this site. The first season was considered a rescue project, while the second and third seasons have been cataloged as research projects. The third season was completed recently, and the results of its excavations are not yet available.

The rescue project carried out in 2012 focused on the recovery of the eight burials that were left exposed by earth-moving machinery and by police officers, who partially excavated the bodies in order to establish whether or not they represent recent deaths. However, after recovering the eight burials, Ugalde (2012) decided to excavate a new unit in order to establish the limits of the small cemetery; thus, the total number of individuals recovered rose to 18. Most of the individuals were recovered from primary burials (individuals: 1, 2, 3, 6, 8, 9, 10, 12, 14, 15, 16 and 18); three were registered as secondary burials (individuals: 7, 13, and 17), while in the last three burials it was not possible to establish if they were primary or secondary burials (individuals: 4, 5, and 11).

All of the burials were found about 2 m below the surface, located right above the *cangahua* stratum, which is considered by archaeologists to be a sterile level with no evidence of cultural material. The cangahua is a deposit of volcanic origin dated to the Pleistocene that covers the Quaternary sequence; it is characterized by its degree of consolidation, mineral content, distribution, and alteration (Villalba and Alvarado 1998; Ugalde; 2012). This find is relevant because it helped to identify the individuals buried at this cemetery as representing the earliest occupation of the site, since the only stratum below the burials is the cangahua and no other cultural artefacts can be found below it. It is also interesting that, although almost all the burials were individual and primary, they were arranged relatively close together as in a small cemetery (see Fig.3.2) (Ugalde, 2012[5]; Torres, 2014).

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[5] Statement about the burials made by the biological anthropologist María Guevara, which can be found in Ugalde (2012).

Fig. 3.2 - Burials excavated during the first field season at Rancho Bajo (Ugalde, 2013a: 8)

Four samples from this cemetery were extracted and submitted for radiocarbon dating at the Beta Analytic laboratory in Florida. The first sample was a charcoal fragment recovered in feature 14 (also known as burial 14), while the other samples were first molars from different individuals. The radiocarbon dates for these samples range between roughly 1660 cal BC and 1450 cal BC (calibrated with 95% probability) (Ugalde, 2012). For detailed information about the radiocarbon dates, see the table below.

Table 3.1 - Radiocarbon dates from the site Rancho Bajo[†]

Lab Code	Type of sample	Feature/burial	Date BP	Date BC (95% CI)
Beta-314968	Molar	12	3300±30	1643-1504 cal BC
Beta-314967	Charcoal	14	3250±30	1613-1451 cal BC
Beta-355317	Charcoal	19	3350±30	1737-1534 cal BC
Beta-363534	Molar	24	3260±30	1616-1454 cal BC
Beta-363536	Molar	30, individual 1	3210±30	1595-1418 cal BC
Beta-366957	Molar	30, individual 2	3230±30	1608-1432 cal BC

[†] (Ugalde 2012:75-76; 2013b: 132)

During the second field season, the skeletal remains from another nine individuals were discovered (see Fig. 3.3). Of these, eight individuals were recovered from primary burials, and only one individual was associated with a secondary burial, with only loose teeth recovered. Again, these burials were all found directly over the cangahua stratum, arranged as in a multiple grave without well-defined tombs. In addition, all of the burials appeared partially covered with cangahua blocks and small stones showing no evidence of modification (Ugalde, 2012).

Fig. 3.3 - Burials discovered during the second field season (Torres, 2014: 44)

Of the 27 burials, only one had clear evidence of what could be considered a grave good. The find was made during the rescue project, and consisted of a pendant made of polished basalt that was recovered from a juvenile burial (feature/burial 13) (see Fig.3.4). The age of this individual was estimated to be between 2 and 4 years, based on its dental development (Ugalde 2012; Torres, 2014). In the second field season, an obsidian core was found in the proximity of an adult male individual (feature/burial 24), who was also buried in a different position from that of the other individuals. The possibility that the obsidian core was also a type of grave good has not been dismissed (Torres, 2014).

Fig. 3.4 - Pendant made of basalt associated with juvenile (Ugalde, 2013a: 8)

At the site, obsidian flakes and some ceramic fragments were also recovered; however, the ceramic fragments correspond to a later period, and they do not appear to be associated with the burials in any way (Ugalde 2012; Torres, 2014).

The analysis of the lithic component of the site was conducted by the archaeologist Angelo Constantine. According to Constantine, the lithic technology from Rancho Bajo exhibits the typical characteristics seen in Formative industries, in which the use of the techniques of retouching or modification of tools and cores decreases in comparison to the lithic industries from the Preceramic Period. In Rancho Bajo, there is a intentionality in the elaboration of certain instruments, which demonstrates knowledge of the specific techniques for the manufacture of those artifacts (Ugalde, 2012)[6].

Constantine also conducted the analysis of the lithic material discovered during the second season. After examining how the artifacts were manufactured, he concluded that the lithic industry was based on 1) the manufacture of standardized tools also known as "formal tools", as well as 2) the manufacture of generalized artifacts or "informal tools" through the

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[6] The analysis of stone tools and artifacts within this study was performed by Angelo Constantine.

use of a pressure technique. In addition, there is evidence that flake-removal and a systematic retouching of flakes took place at the site with the purpose of using those flakes as tools. At the same time, the presence of tabular-shaped cores and flakes with rectangular and prismatic shapes suggests that the people of Rancho Bajo knew how to perform flake removal without wasting raw material. Finally, he suggests that the manufacture of formal tools might have been carried out only for specific actions like those in the spectrum of scraping and penetrating such as burins or spear points, perhaps linked to specialized activities like the processing of skins (Ugalde, 2013b: 112)[7].

In the case of ceramics, only small and fairly eroded fragments were recovered from all deposits later than deposit 6, which consists of an ash layer from the Pululahua eruption dated around 500 BC. This ash layer sealed the previous occupations of the site, and confirms that the ceramic sherds found at the side belonged to a different period of time and occupation from that of the burials (deposit 8). According to Ugalde, almost all the ceramics found in those upper deposits are consistent with eroded ceramic sherds displaced by rainfall, among other taphonomic processes, with the exception of the ceramic assemblage found in deposit 7, which corresponds in its entirety to material from Cotocollao, another site included in this study (see Villalba, 1988) (Ugalde, 2013a: 80; Torres, 2014).

No evidence of habitation contexts or faunal remains has been recovered to date, an absence which could be due to incomplete excavation of the site, or could be because habitation activities took place at a location away from the cemetery. Another possible scenario is that some of these cultural materials have already been lost due to construction in the area, and will not be recovered in the future. Unfortunately, survey and excavation at

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[7] Comments on stone tools were made by Angelo Constantine, and can be found in Ugalde (2013b).

Rancho Bajo are limited to those few lots where nothing has been built. Hopefully if other field seasons are conducted in the area before more houses are built, the researchers will be able to get a better idea of the area originally covered by this settlement.

3.2.1. The Burials

The analysis of the skeletal remains was conducted by two researchers. The human remains recovered during the first field season were examined by the Colombian biological anthropologist María Isabel Guevara from the Universidad de Los Andes between 2011 and 2012. During that time, I participated as an assistant along with other undergraduate students from the Pontificia Universidad Católica del Ecuador. Later in 2013, during the second field season, I was personally in charge of the analysis of the nine burials recovered, research which included visiting the site and examining the internments *in situ*. It is important to emphasize that the analysis of the remains was carried out when I was still an undergraduate student.

Both analyses focused on estimation of sex, age, stature, and ancestry. The methods used for sex and age estimation are those described in Buikstra and Ubelaker (1994); the calculation of stature employed the regression formula published by del Ángel and Cisneros (2004), which consists of a corrected version of the formula presented by Genovés (1964). The estimation of ancestry was carried out using dental morphological characteristics following Krenzer (2006). These analyses also aimed to register any non-metric traits, pathologies, and occupational stress marks. The findings of both examinations were summarized in my undergraduate thesis (Torres, 2014), and have been included in this section.

Before presenting the results of the osteological analyses, it is important to clarify that almost all the remains recovered to date were found in individual primary burials. The majority of individuals were disposed in a seated position, with the upper and lower limbs tightly flexed towards the chest. Exceptions to this pattern were described for burials 24 and 30 (individual 2). The person in burial 24 was placed in a lateral decubitus position with flexed arms and legs, while the second skeleton found in burial 30 was found lying on its back with arms and legs flexed ventrally. In the proximity of its feet a mandible from a different individual was recovered. Near this disarticulated mandible a few fragments of long bone were found. However, all remains from this disturbed individual appeared inside the excavation wall; and were deliberately left *in situ* due to the proximity of the wall to a modern structure and the risk of its collapse.

Sex could be estimated for 15 of the 27 individuals buried at the site (see Table 3.2), of which 13 show traits consistent with males (burials 1, 3, 5, 6, 8, 9, 10, 17, 24, 26, 30 individual 1 and 2, and 35) and five show traits consistent with females (burials 2, 15, 16, 27, and 31). The sex of the remaining nine individuals was undetermined (burials 4, 7, 11, 12, 13, 14, 18, 29, 33); this category includes all subadults as well as poorly preserved adult burials in which only a few bone fragments were recovered and a sex estimation was not possible (Ugalde, 2012[8]; Ugalde, 2013b[9]; Torres, 2014). Age estimations indicated that six of the 27 individuals were skeletally immature, ranging from 2 to 21 years old (burials 12, 13, 14, 18, 29, 33), while 17 individuals were skeletally mature adults (burials 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 15, 16, 17, 24, 26, 27, 30 individual 1 and 2, 31, and 35). Only in two cases was it not possible to estimate the age. Stature estimations were only possible for a few

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[8] The osteological analysis within this publication was performed by María Guevara.

[9] The osteological analysis within this publication was performed by Paula Torres.

individuals, and indicate statures between 146 cm and 154 cm for both men and women (Ugalde, 2012; Ugalde, 2013b; Torres, 2014).

Table 3.2 - Detailed information from the burials at Rancho Bajo[†]

Burial No.	Type of internment	Position of the individual	Sex	Age
1	Primary	Seated and flexed	Male	35 - 45 years
2	Primary	Seated and flexed	Female	>30 years
3	Primary	Seated and flexed	Male	30-40 years
4	N/D	N/D	N/D	N/D
5	N/D	N/D	Male	N/D
6	Primary	Seated and flexed	Male	30-40 years
7	Secondary	N/D	N/D	N/D
8	Primary	Seated and flexed	Male	25-35 years
9	Primary	Seated and flexed	Male	35-45 years
10	Primary	Seated and flexed	Male	15-21 years
11	N/D	N/D	N/D	N/D
12	Primary	Seated and flexed	N/D	14-17 years
13	Secondary	N/D	N/D	2-4 years
14	Primary	Seated and flexed	N/D	5-7 years

[†] Based on Ugalde (2012) and Ugalde (2013b)

Table 3.2 (continued) – Detailed information from the burials at Rancho Bajo[†]

Burial No.	Type of internment	Position of the individual	Sex	Age
15	Primary	Seated and flexed	Female	16-20 years
16	Primary	Seated and flexed	Female	25-35 years
17	Secondary	N/D	Probably Male	N/D
18	Probably primary	Seated and flexed?	N/D	8-12 years
24	Primary	Lateral decubitus and flexed	Male	33-45 years
26	Primary	Seated and flexed	Male	25-35 years
27	Primary	Seated and flexed	Female	33-45 years
29	Secondary	Seated and flexed	N/D	2-4 years
30 (#1)	Primary	Seated and flexed	Male	25-35 years
30 (#2)	Primary	Flexed ventrally, lying on the back	Male	25-35 years
31	Primary	Seated and flexed	Female	40-60 years
33	Primary	Seated and flexed	N/D	10-15 years
35	Primary	Seated and flexed	Male	40-60 years

[†] Based on Ugalde (2012) and Ugalde (2013b)

Non-metric traits were also registered; and include shovel-shaped incisors, supernumerary bones in the skull, torus palatinus, and enamel pearls, all of which are non-metric traits that appear with more frequency in Native American populations (Ugalde, 2012[10]; Ugalde, 2013b[11]; Torres, 2014).

Some of the bone pathologies that were observed include periostitis, degenerative joint disease, button osteomas, and an unconfirmed case of possible porotic hyperostosis. Dental pathologies included linear enamel hypoplasias (LEH), dental caries, and dental calculus. The only evidence of a traumatic injury was found in the skull of the individual buried in feature 27, and consisted of a skull fracture located in the parieto-occipital region of the vault that could have been related to the cause of death (Ugalde, 2012[12]; Ugalde, 2013b[13]; Torres, 2014).

Two additional studies have studied the human remains from Rancho Bajo, each one from a different perspective. The first research was carried out as part of the second field season, and consisted of a palaeoparasitology study conducted by Nancy Orellana Halkyer from the Instituto de Investigaciones Antropológicas, Universidad Mayor de San Simón, Bolivia.

Halkyer examined soil samples extracted from the area of the pelvis and sacrum of three individuals of the eight that were recovered in the second field season (burials 26, 31 and 33). The researcher discovered several eggs (fertilized and non-fertilized) and two free larvae of *Ascaris lumbricoides* (roundworm) in the sample from individual 26 in the area surrounding the pelvis (Ugalde, 2013b)[14]. The fertilized eggs correspond to the infectious stage, while the larvae (worms) correspond to a stage in which the worms migrate to other

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[10] The osteological analysis within this study was performed by María Guevara.

[11] The osteological analysis within this study was performed by Paula Torres.

[12] Results of the osteological analysis performed by María Guevara can be found in this study.

[13] Results of the osteological analysis performed by Paula Torres can be found in Ugalde (2013b).

[14] The palaeoparasitology study conducted by Nancy Halkyer can be found in the work of Ugalde (2013b).

parts of the body, infecting organs such as the lungs, digestive system, or nervous system. According to Halkyer, this individual probably suffered from diarrhea and problems of nutrition due to the presence of these parasites (Ugalde, 2013b).

The presence of *Ascaris lumbricoides* does not only give insight into the state of health of the individual, but is indicative of some poor hygiene habits that may have led to parasitization. Halkyer suggests that open air defecation could have been one of these habits, which probably contaminated the soil, water, food, and finally humans. Although no parasites were found in the samples from individuals 31 and 33, Halkyer does not dismiss the possibility that those individuals could also have been parasitized; but that the enteroparasites may have yielded to desiccation, preventing their identification. This scenario is based in part on the consideration that the parasitized individual (individual 26) had a significant parasitosis, and could have infected other individuals by sharing food (Ugalde, 2013b).

The second study was conducted three years later by Pablo Morales (2016) as part of his doctoral dissertation from the Universidad de Alicante, Spain. He focused on examining dental pathologies at different archaeological sites from Ecuador, one of which was Rancho Bajo. Morales (2016) found that of all the teeth examined from Rancho Bajo, only 1% displayed dental caries; 15% displayed dental calculus, and 17% showed periodontal disease. No alveolar abscesses were detected. In addition, 12% of the teeth exhibited enamel hypoplasias.

Although the percentages of caries, dental calculus, and periodontal disease from Rancho Bajo are the lowest in comparison to those from the sites Las Vegas (Preceramic) and La Tolita (Regional Development Period) in the coast, or NAIQ (Integration) in the northern highlands as noted by Morales (2016) (see Table 3.3), these dental pathologies still reflect poor hygiene to some extent. The low percentage of caries found at Rancho Bajo also suggests that the people from this settlement probably did not have a diet that relied heavily on cariogenic foods, such as starches. Meanwhile, the presence of enamel hypoplasias, which are defined as deficiencies in enamel thickness which may be caused by systemic metabolic stress, hereditary anomalies, and localized trauma (Buikstra and Ubelaker, 1994: 56), suggests that these individuals could have experienced an episode of stress during childhood. This stress episode could have been related to nutritional factors, or infections and systemic illnesses (Waldron, 2009). In fact, the evidence of a parasitization with *Ascaris lumbricoides* (Ugalde, 2013b) suggests this roundworm could have been a potential stressor that led to the formation of the enamel hypoplasias. Other studies have emphasized the role that parasitic infections played in the health of prehispanic populations in Ecuador (Ubelaker and Newson, 2002; Alchon, 2004). For example, Ubelaker and Newson (2002) hypothesized that the high frequencies of porotic hyperostosis in coastal sites were associated with parasitism (Arriaza et al., 1998).

Table 3.3 - Percentages of dental pathologies in some Ecuadorian sites[†]

Sites	**Las Vegas (Preceramic)**	**Rancho Bajo (Formative)**	**La Tolita (Regional Development)**	**NAIQ[15] (Integration)**
Teeth examined	n= 155	n= 138	n=640	n=288
Caries	3%	1%	10%	37%
Dental calculus	41%	15%	43%	30%
Periodontal disease	48%	17%	44%	18%
Alveolar Abscesses	5%	0%	1%	0%
Hypoplasia	1%	12%	10%	3%

[†] Based on Morales (2016: 144-149)

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[15] Not to be confused with the NAIQ site examined by Pennycook (2013) discussed in Chapter 2. These are different settlements discovered under the New International Quito Airport.

3.3. Cotocollao

This archaeological site was accidentally discovered when urbanization works of the Housing Cooperative "23 de Junio" were taking place in Cotocollao in the late 1970s. What captured the interest of the researchers was the presence of habitation contexts and cemeteries that evidenced the existence of an early and late component of the site, or early and late settlements respectively. Other finds of note include ceramic and lithic artifacts, as well as a layer of volcanic ash that sealed the Formative occupation and confirms the absence of a new occupation after this volcanic event (Villalba, 1988).

3.3.1 Cotocollao's Early Settlement

The earliest radiocarbon date obtained from the early settlement of Cotocollao was 3495±210 BP, a date that resulted from the analysis of a piece of charcoal found in a post hole located in a habitation floor. More radiocarbon dates were obtained using carbon recovered from hearths and the cemetery (Villalba, 1988) (see Table 3.4).

Table 3.4 - Uncalibrated radiocarbon dates from the early settlement of Cotocollao[†]

Sample ID	Unit	Origin	Material	Date BP
GX-4768	F23 op. 1	Post hole	Charcoal	3495±210
GX-4766	F19	Cemetery	Charcoal	3135±165
GX-8323	F6 op. 2	Feature 19 Hearth floor	Charcoal	3310±150
GX-7210	F50	Hearth floor	Charcoal	3340±135

[†] (Villalba, 1988: 242-243; Torres, 2014: 62)

There is a second publication by Ziolkowski et al. (1994) (see Table 3.5) in which these dates were corrected and calibrated. This procedure resulted in a much earlier date than had been thought for the charcoal recovered from the post hole: 2405 – 1381 BC calibrated with 94-95% confidence.

Table 3.5 - Calibrated radiocarbon dates from the early settlement of Cotocollao[†]

Sample ID	Unit	Origin	Material	Date BP	Date BC (94-95% CI)
GX-4768	F23 op. 1	Post hole	Charcoal	3495±210	2405–1381 cal BC
GX-4766	F19	Cemetery	Charcoal	3135±165	1743–973 cal BC
GX-8323	F6 op. 2	Feature 19 Hearth floor	Charcoal	3310±150	1970–1255 cal BC
GX-7210	F50	Hearth floor	Charcoal	3340±135	1955–1313 cal BC

[†] (Ziolkowski et al. 1994: 121-124; Torres, 2014: 63)

The different implications of the dates presented by Villalba (1988) and Ziolkowski et al. (1994) are evident. The calibrated dates presented by Ziolkowski et al. (1994) indicate that the early component of Cotocollao may have been a much earlier occupation than previously thought, running about 2405 – 1255 cal BC. There is some overlapping between this date and the date of 1610 – 1450 cal BC obtained from Rancho Bajo, suggesting these two groups may have cohabited in the Quito area. However, any interpretations about possible contact between the two sites due to their proximity or similarities should be done carefully.

This early settlement was characterized by habitation areas that were formed by irregular agglutination of houses in groups, located on a slope close to ravines. The houses were built of "*bahareque*", a construction material similar to adobe commonly used in the Andes, which consists of clay or mud that has been reinforced with sticks or canes. Other elements registered as part of these habitation contexts are post holes, terraces, hearths, and the original rectangular shape of the houses. On each house floor several types of artifacts were recovered, including manos and metates, obsidian cores and other lithic artifacts, faunal remains, carbonized seeds of maize and beans, as well as fragments of ceramic utensils (Villalba, 1988). According to Villalba (1988), most of the faunal bones belonged to deer, rabbit, and cuy (guinea pig). However, the zooarchaeologist Peter Stahl has stated that the photographs of cuy remains that were published by Villalba (1988) and used as evidence to claim the early appearance of this taxon at Cotocollao between 1500 and 1100 BC[16] actually show remains of rabbits (Stahl, 2003).

Besides the domestic contexts, a cemetery was also discovered. Villalba (1988) observed that there was a certain intentionality in the way that the housing areas were distributed with respect to the cemetery. The Cotocollao cemetery was located directly over the cangahua stratum (Villalba, 1988) which is a culturally sterile stratum. A similar pattern was described at the cemetery of Rancho Bajo by Ugalde (2012).

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[16] Dates that follow the original publication of Villalba (1988).

3.3.1.1 The Burials

As previously stated, the cemetery was situated directly over the cangahua stratum, and was found in area F19 of the site, in which five tombs were discovered. Most of these tombs contained the skeletal remains of a single individual; in tombs 4 and 5 only human teeth were recovered. Once area F19 was expanded (the expansion being designated as F38), three more tombs were found, two of which were single burials, while the third tomb contained a multiple burial (Villalba, 1988).

The tombs from F19 were pits of oval or almost circular shape, with a diameter between 1 and 1.5 m and a depth between 0.2 and 0.3 m. In each pit a single individual was placed with the upper and lower limbs flexed, either seated or in a fetal position (for the two burials yielding only loose teeth, body position was unclear). The first two burials in unit F38 are similar to those in F19, as they consisted of individual burials; however, the third tomb was different, as it had five individuals. Large stones were found inside the tombs, either surrounding the body or close to the head or feet of the individual, a pattern similar to that observed in Rancho Bajo (Ugalde, 2012, 2013a). No grave goods have been recorded at this cemetery. Villalba (1988) and Torres (2014) have suggested that the presence of stones in some of the burials may indicate that these stones had a special significance for the group; alternatively, the stones could have been just an element in the construction of the tombs. In fact, of the 13 individuals recovered from this settlement, only one (tomb 27) was found without any stones in the burial.

Regarding the analysis of the human skeletal remains, Dr. Douglas Ubelaker performed the initial study in 1978 and 1979. However, his study analyzed only the first five individuals recovered (tombs: 1a, 2, 3, 4, and 5); he did not examine the individuals recovered from the Unit F38 (tombs: 26, 27, 28a, 28b, 28c, 28d, 28e).

Ubelaker (1978) stated that the poor preservation of these remains made it impossible in some cases to estimate the sex and/or age, and calculate the stature. In fact, a stature estimate was only possible for one individual, the adult male in tomb 1a, whose stature was estimated at 160 cm. In this initial study, Ubelaker did not observe any bone or dental pathologies. Details for each individual are presented in the table below.

Table 3.6 - Detailed information from the burials from the early settlement at Cotocollao[†]

Burial No.	Type of internment	Position of the individual	Sex	Age
1a	Primary	Flexed, right side	Male	30-40 years
1b*	Primary	N/D	N/D	Infant
2	Primary	Flexed, right side	Male	30-40 years
3	Primary	Flexed	N/D	N/D
4	Secondary	N/D	N/D	22-25 years
5	Secondary	N/D	N/D	20-25 years
T26*	N/D	Flexed, right side	N/D	N/D
T27*	Primary	Flexed, right side	N/D	Adult N/D
T28a*	Primary	Seated	N/D	Adult N/D
T28b*	Primary	Seated	N/D	Adult N/D
T28c*	Primary	Flexed, left side	N/D	Adult N/D
T28d*	Primary	Flexed, left side	N/D	Adult N/D
T28e*	Primary	N/D	N/D	Adult N/D

[†] Based on Villalba (1988). *Individuals not examined by Ubelaker

3.3.2. Cotocollao's Late Settlement

According to Villalba (1988), the late settlement runs approximately between 1100 – 500 BC. This date is the result of an average of 50 uncalibrated radiocarbon dates from samples extracted from different habitation and funerary contexts. The original radiocarbon dates presented by Villalba (1988) and the calibrated versions published by Ziólkowski et al. (1994) are presented in Table 3.7.

Table 3.7 - Calibrated radiocarbon dates from the late settlement of Cotocollao

Sample ID	Unit	Origin	Material	Date BP†	Date BC (95% CI)‡
GX-8334	Cut F23 op.2	Floor/ fireplace	Charcoal	2535±135	937-364 cal BC
GX-8336	Cut F23 op.2	Floor	Charcoal	2555±145	1009-361 cal BC
GX-8340-G	Cut F38	Burial 24	Human bone	2660±140	1119-405 cal BC
GX-8342-G	Cut F19	Burial 76	Human bone	2625±140	1065-396 cal BC
GX-8343-G	Cut F19	Burial 30	Human bone	2645±165	1206-389 cal BC
GX-8345-G	Cut F19	Burial 18	Human bone	2395±140	812-158 cal BC
GX-8347-G	Cut F5	Burial 1	Human bone	2595±145	1050-379 cal BC

†Villalba, 1988: 242-243; ‡Ziólkowski et al. 1994: 124-127

The evidence recovered for the late settlement is not very different from that of the early settlement; and includes elements such as hearths, habitation floors, post holes, stairs, and cemeteries. However, there are important differences in terms of the extent of the settlement as well as its apparent population density. The differences can be seen in the housing clusters that appear scattered without a regular pattern between the two ravines that limit the settlement, covering approximately 26 hectares. They can also be seen in the number and distribution of tombs, which were discovered in more than one communal burial area (Villalba, 1988).

Another difference between the early and late settlements is that the latter is not located directly over the cangahua stratum, but was built over the remains of earlier constructions. The ground was flattened before being reused as a new floor, each time creating a new layer of at least 4 or 5 cm (Villalba, 1988).

The burials were accidentally discovered after tractors moved ground as part of the construction works for a housing cooperative. Archaeologists intervened to rescue the skeletons that had been disturbed, and recovered 150 burials in an area of 5 x 6 m of extent and 1.80 m of depth. Later they decided to extend this cut by a further unit of 3 x 3 m (F38) from which another 29 burials were removed, which made a total of 179 burials (Villalba, 1988). Cotocollao's cemetery constitutes the largest cemetery from the Formative Period discovered in the Ecuadorian Northern Highlands.

Several elements make this cemetery interesting. The first of these is a large concentration of stones that appears to have intentionally covered the skeletons. The second element is a natural layer of ash between 10 and 15 cm thick that was found above the concentration of stones. This layer sealed the cemetery; and confirms that after the volcanic event that deposited it, the site was abandoned and not reoccupied until modern times. Another interesting trait of the cemetery is that the skeletons appear at different depths; and that while those that were buried closer to the layer of stones were not deposited in well-defined tombs, those buried deeper were found in well-defined tombs (Villalba, 1988).

Unfortunately, a number of factors adversely affected the preservation of both the skeletons and the cemetery. Firstly, the soil-removal machinery used in the initial construction project destroyed some of the skeletons as well as the artifacts that were placed next to the individuals as grave goods, which included ceramic or stone vessels and artifacts. Secondly, the concentration of stones that covered the remains did not exactly improve the state of preservation of the skeletal remains; on the contrary, the weight of the stones made the skeletons more fragmented (Ubelaker, 1978).

As stated above, many ceramic and lithic artifacts were recovered not only from the cemetery, but also from the habitation contexts. Villalba (1988) mentions the recovery of around 150,000 obsidian artifacts, as well as artifacts made of basalt, stone, quartz, and other lithic raw materials. Beads, pendants, and tools made of animal bones were also discovered.

Among the faunal bones identified at the site are deer, rabbits, guanta (a large rodent), cougars, wolves, weasels, cuy (guinea pig), llamas, mice, and parrots and other birds. Most of the remains belonged to deer, rabbit, and cuy, which were also present at the early settlement. Llamas, on the other hand, appear for the first time in the late settlement (in levels dated to 800-500 BC), although still in a limited amount. The presence of birds in the site was also minor.

Phytoliths, carbonized seeds, and pollen obtained from hearths and other contexts were analyzed (Pearsall, 1984; Villalba, 1988). The results include evidence of maize (*Zea mays*), quinoa (*Chenopodium* sp.), beans (*Phaseolus* sp.; *Phaseolus vulgaris*), oca tuber (*Oxalis tuberosa*), potato (*Solanum tuberosum*), and chocho (*Lupinus mutabilis*), as well as some other unidentified tubers and wild seeds. Interestingly, there are similarities between the maize found at Cotocollao and maize from site La Ponga, on the coast of Ecuador (Lippi, 1984; Villalba, 1988; Pearsall, 2003).

3.3.2.1. The Burials

Remains of around 200 individuals were discovered in the late cemetery; they were found in primary or secondary burials accompanied with ceramic or lithic artifacts that appeared complete or fragmented.

The analysis of the skeletal remains was conducted by Ubelaker (1978); and consisted of estimations of sex, age and stature, as well as comments on pathologies and cultural modifications. In terms of sex, of the almost 200 individuals that Ubelaker mentioned in his report 44 were men and 38 women, while for 124 individuals the sex was undetermined (see Table 3.8). All of the different age groups were present in the cemetery, although the age could not be estimated in some cases due to poor preservation of the skeletons. The average stature was 157 cm ± 8 cm for men and 149 cm ± 11 cm for women.

Table 3.8 - Detailed information from the burials from the late settlement at Cotocollao[†]

Type of internment	Primary burial (n=65)
	Secondary burial (n=129)
	N/D (n=12)
Sex	Male individuals (n=44)
	Female individuals (n=38)
	N/D (n=124)
Age	Juveniles/Skeletally immature individuals (n=37)
	Young adults 20-35 years (n=58)
	Middle age adults 35-45 years (n=29)
	Old adults 45-65 years (n=20)
	N/D (n=63)

[†] Based on Villalba (1988)

The palaeopathological examination revealed seven individuals with evidence of periostitis in their lower legs, as well as four individuals with signs of trauma, including one skull fracture, a fracture in a fibula, trauma to a foot phalanx, and a fracture of the skull accompanied by a tibia fracture. Dental pathologies were also observed, although in relatively low frequencies. The presence of caries was noted in only 19 teeth compared to a total of 1157 teeth examined, which represents 2% of all the teeth; and 87 teeth were missing due to ante-mortem tooth loss out of the 1244 teeth estimated from the dental alveoli present. Three adults displayed enamel hypoplasias.

Of the 27 skulls that were almost complete and in good enough condition for examining any cultural modifications, only five individuals (three adult males, one adult female, and one subadult) displayed cranial modification. Based on this finding, Villalba (1988) suggests that it is possible that cranial modification reflected social status.

3.4. Las Orquídeas

Las Orquídeas is an archaeological site located in Imbabura Province that was discovered in 2013 when public works were taking place in the *barrio* Las Orquídeas to construct sporting fields. While levelling a parcel of land to make terraces, workers discovered dense concentrations of artifacts, which required the intervention of the archaeologists Carlos Montalvo and Eric Dyrdahl (Montalvo and Dyrdahl, 2014; Dyrdahl, 2017).

However, before the intervention of these archaeologists in 2013, there were two other salvage projects conducted in the area. The first of these was carried out by Camino (1999), who documented the presence of artifacts from different periods, while the second was directed by Villalba (2014), who found a limited set of contexts in another space near Las Orquídeas (Camino, 1999; Villalba, 2014; Dyrdahl, 2017).

A total of 18 samples from Las Orquídeas were submitted for radiocarbon dating at the Human Palaeoecology and Isotope Geochemistry Laboratory in Pennsylvania State University. The table below displays the earliest and three latest dates following information published by Dyrdahl (2017).

Table 3.9 - Some radiocarbon dates from the site Las Orquídeas[†]

Sample ID	Unit	Origin	Material	BP Dates	BC Dates (95-100% CI)
6174	U1, N8	Phase 2	Charcoal	2825±20	1029 – 916 cal BC
6175	U2, N7	Phase 1-3	Burnt Mat	2595±25	806 – 776 cal BC
6171	U49, PT627	Phase 3	Charcoal	2480±20	767 – 536 cal BC
6177	U22, N6, M3	Phase 3	Charcoal	2415±20	542 – 406 cal BC

[†] (Dyrdahl, 2017: 215)

Since 2013, Montalvo and Dyrdahl have conducted three field excavation seasons that led to the discovery of at least four important finds. The first find consisted of an intact stratigraphic sequence dating to around 800 – 400 cal BC. What makes this find noteworthy is that the sequence was formed on the slope of a hill that was continually used as a trash dump over the course of 400 years by the people from Las Orquídeas. This event left an intact stratigraphic record of more than 5 m on its vertical axis (Dyrdahl, 2017).

The second important find consisted of the recovery of a large array of evidence related to craft production. Among all the raw materials employed to elaborate different artifacts, adornments, and crafts at the site, Dyrdahl (2017) has described the following:

> Gold, greenstone, bluestone, *Spondylus princeps*, *Spondylus calcifer*, mother-of-pearl (both *Pinctada mazatlanica* and *Pteria sterna*), small gastropod shells (*Conus* sp., *Olivella* sp., and *Columbella* sp., among others), land snails (*Megalobulimis* sp.), obsidian, basalt, andesite, sedimentary and metamorphic rock (limestone, slate, schist, etc.), animal bone (including sperm whale ivory), antler, quartz, clay, and pumice (Dyrdahl, 2017: 2).

Dyrdahl has interpreted the ample variety of artifacts and raw materials as evidence of interregional interaction during the Late Ecuadorian Formative Period. He suggests that Las Orquídeas was part of an exchange network that moved items along two different axes. The first was an exchange system that moved north-south, and provided the people from this settlement with obsidian as raw material. The second exchange system was based on an east-west axis, which linked populations located in the highlands to coastal groups and possibly societies from the Amazon region (Dyrdahl, 2017).

The third find comprises the eight burials that have been recovered to this day, which will be described in a section below. The fourth find of considerable interest at Las Orquídeas consisted of a hearth and oven, which were located upslope from the area of refuse disposal (Dyrdahl, 2017). This activity area appeared during the third excavation season, and changed the perception of the site based on what the archaeologists discovered during the first two field seasons. It is now recognized that the slope area of the site was not only used as a place for trash disposal, but that other activities were also taking place there. However, further research must be done to fully understand this area.

Other interesting finds at the site include almost 100,000 obsidian artifacts, of which more than 46,000 were examined by Dyrdahl (2017). His analysis revealed that almost all of the obsidian artifacts were produced by percussion flaking, that they show little evidence of use, and that they were not retouched. Dyrdahl also used XRF instrumentation to analyze obsidian samples in order to establish the original sources of the raw material. He identified a strong trend towards the use of two sources, "La Chimba type" and "Callejones", over time. A third source used was "Mullumica", whose importance gradually waned over time as the use of the other two sources increased (Dyrdahl, 2017). The 'La Chimba type' obsidian was given that name after the first obsidian artifacts with that geochemical signature were found at La Chimba, a Late Formative site located in the northern highlands between the Pichincha and Imbabura provinces (see Chapter 2). It appears that the 'La Chimba type' obsidian comes from a new source in Oyacachi (Dyrdahl, 2017). The presence of the 'La Chimba type' obsidian, along with other cultural materials similar to those from La Chimba at Las Orquídeas, suggest the two sites were part of the same culture.

Finally, the site contains a large collection of faunal remains; one reason why it was selected for this study. Although all the faunal remains have not yet been identified, certain taxa have been recognized. The undergraduate student Ibis Mery from the Pontificia Universidad Católica del Ecuador has conducted a partial study of the material; and has identified bones of camelids (llama), cavia (cuy), cervids (deer), leporids (rabbits), dasypodids (armadillos), tayassuids (peccary), and carnivores.

3.4.1. The Burials

Dyrdahl and Montalvo invited me in 2014 and 2015 to participate in field excavation seasons at Las Orquídeas, and to conduct analysis of the human osteological material from the site in the archaeology laboratory of the Pontificia Universidad Católica del Ecuador. My work on the human remains to this point has included sex and age estimations; calculations of stature, ancestry, and pathologies (Montalvo and Dyrdahl, 2014[17]; Torres, 2015[18]). The sex and age estimations were made following the methods described by Buikstra and Ubelaker (1994). A corrected version of the regression formula of Genovés (1964) presented by del Ángel and Cisneros (2004) was used for the estimation of stature. Meanwhile, the estimation of ancestry was carried out using the dental morphological characteristics described by Krenzer (2006). Dental pathologies were recorded according to the methods described in Buikstra and Ubelaker (1994); for the identification of bone pathologies I used the work of Waldron (2009).

I examined the remains of eight individuals that have been recovered to date, of which seven were found in primary burials and one was found in what appears to be a disturbed burial (see table 3.10). Some of the individuals were found in a seated position with the upper and lower limbs flexed. In a few other cases the body appears to have been originally deposited in this position, but shifted due to the decay of soft tissues and/or the weight of the surrounding soil. In some of the burials it was not possible to determine with certainty the original position of the skeleton.

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[17] The osteological analysis of the human remains recovered during the second field season at Las Orquídeas can be found in Montalvo and Dyrdahl (2014).

[18] Unpublished internal report on results from the osteological analysis of the human remains recovered during the first field season at Las Orquídeas.

Of the group, two individuals were identified as adult males, three as adult females, and three as juveniles. None of these juveniles were infants. The estimated statures of the male individuals were 153.01 cm ± 5.63 cm (burial 6) and 156.91cm ± 3.65 cm (burial 8), while the stature of one of the females (burial 2) was estimated at 148.18 cm ± 5.63cm.

Table 3.10 - Detailed information on the burials from Las Orquídeas

Burial No.	Type of internment	Position of the individual	Sex	Age
1	Primary	Seated and flexed	Female	14-23 years
2	N/D (Looted)	N/D	Female	30-34 years
3	Primary	N/D	N/D	14-18 years
4	Disturbed	Seated and flexed	Female	20+ years
5	Primary	N/D	N/D	11-12 years
6	Primary	Seated? and flexed, lying ventrally.	Male	50+ years
7	Primary	N/D	N/D	6-7 years
8	Primary	Seated and flexed	Male	24-29 years

A macroscopic examination of the skeletal remains contributed to the identification of bone pathologies in both male individuals. In the first individual, estimated to have been between 24 and 29 years old, the pathology consisted of a possible hip subluxation that needs to be confirmed. The second individual, probably over 50 years old, presents signs of osteochondritis dissecans, osteophytosis, Schmorl’s nodes, extra bone growth on the palmar surface of hand phalanges, fractures of some foot phalanges, and lytic lesions in both tibiae, which are located above the tibial tuberosity in the tibial plateau. Among the dental pathologies registered are dental caries, a dental abscess, and ante-mortem tooth loss (see Fig. 3.5).

Fig. 3.5 - +50 years old adult male recovered from Las Orquídeas (Dyrdahl, 2017: 231)

In terms of grave goods, only one individual who was a young female between 14 and 23 years old, was buried with two ceramic vessels (see Fig. 3.6 and Fig. 3.7).

Fig. 3.6 - Location of the two ceramic vessels in the burial (Dyrdahl, 2017: 234)

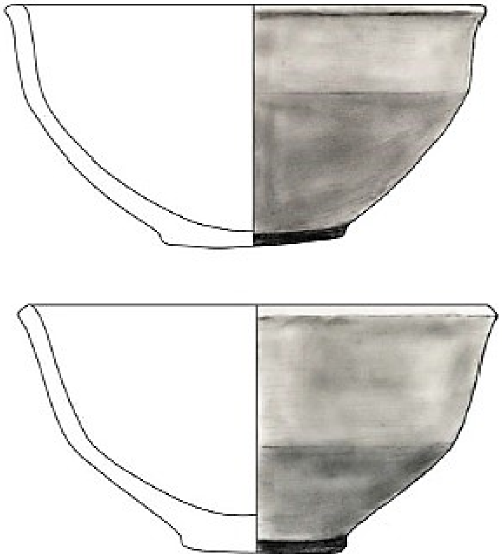

Fig. 3.7 - Drawings of the ceramic vessels. Scale for each drawing is 1:3.
Vessels drawn by Carlos Montalvo (Dyrdahl, 2017: 234)

Although the number of individuals discovered to this day is too small for drawing any conclusions about the funerary practices at Las Orquídeas, it is possible to say that some of the individuals were buried in a seated position with arms and legs flexed towards the chest, a pattern that was also identified at Rancho Bajo. The burials at Las Orquídeas, however, do not show the presence of stones or cangahua blocks as do the other sites in this study, an observation which suggests that those elements may have not been part of the burial construction technique, or that they did not have the same meaning for the group. It is also interesting that only one of the eight individuals was buried with grave goods. This occurance may indicate that the person (a young female) may have had a special rank or importance within the group. In terms of pathologies, although dental caries, dental abscesses, and ante-mortem tooth loss were observed at the site, they do not seem to appear in a high frequency or among all individuals. An explanation could lie in a diet that was not heavily dependent on starches, although an alternative explanation could be linked to the age at death of the individuals. Most of the individuals were young or middle age adults and did not exhibit many pathologies; it was the oldest individual from the group that displayed a larger number of pathologies. These comments are speculative, given the small sample size, and might be confirmed once more individuals are discovered and studied.

3.5. Conclusion

Rancho Bajo and Cotocollao are archaeological sites located in the Quito basin relatively close to each other. Rancho Bajo and the early settlement of Cotocollao share similarities in terms of having early radiocarbon dates that placed them in the Early Formative Period, and comprising a small group of individuals. Another similarity lies in the architecture of the burials, characterized by the use of stones and cangahua blocks to cover or surround the body of the individuals. Although Rancho Bajo does not offer evidence of habitation contexts, palaeobotanical studies, or zooarchaeological remains to give an insight into the diet of the group, the early settlement of Cotocollao has all of that evidence. This feature underscores the relevance of comparing both sites. The late settlement of Cotocollao is one of the most studied Late Formative sites from the northern highlands. It is different from the early settlement in terms of the number of individuals discovered at the site (n=~200), and the numerous habitation contexts found, spread along 26 ha. The fourth settlement examined by this study is Las Orquídeas, located in Imbabura province. The findings at this site comprise a large body of craft production that evidence trade and mobility, as well as a large collection of well-preserved faunal remains. Las Orquídeas may be located at a greater distance from Cotocollao than Rancho Bajo, but both sites are situated in the northern highlands and correspond to the Late Formative Period. These two reasons were sufficient for choosing these sites for comparison with the purpose of obtaining more information about diets at the end of the Formative Period.

Based on the evidence recovered at each of these sites, the hypothesis of this study is that the diet in Rancho Bajo probably did not include maize, or that the consumption of maize was very low. One reason for this prediction is that the site shows a very low percentages of caries when compared to agricultural sites from the subsequent periods. Additionally, it is suggested that the diet may have relied more on the consumption of animal resources, due to the presence of lithic artifacts at the site, some of which could have been used for the preparation of skins. A slightly similar hypothesis is held for the early settlement of Cotocollao, due to the presence of lithic artifacts and faunal remains, and the small number of ceramic fragments. However, the presence of some manos and metates and maize remains in this settlement (Pearsall, 1984), may support some consumption of this C_4 resource. For the late settlement of Cotocollao, it is expected to see stable isotope values that would confirm that maize may have played a major role in the diet of this group. The cultivation of maize at a larger scale could have supported the large number of individuals that were part of this settlement. They probably supplemented their diet with some terrestrial animal protein. In Las Orquídeas, the diet could have been similar to that of the late settlement of Cotocollao for three reasons. First, both settlements show evidence of the presence of maize; second, they have a large collection of faunal remains that mostly represents terrestrial animals of the region. The third reason has to do with the radiocarbon dates of these sites, which have placed them closer to the Regional Development Period in which there is evidence of higher consumption of maize, a differential consumption of this staple food between low-status and high-status individuals, as well as evidence of the consumption of maize in the form of chicha (see Chapter 2). This hypothesis suggests that maize, may have already had a certain importance in the diet by the end of the Formative Period.

Chapter 4: Stable Isotope Methodology and Background

This chapter gives a general introduction to the definition of stable isotopes; their measurement, notation, and the different processes that cause isotope fractionation; followed by a theoretical background on the use of both carbon and nitrogen stable isotopes. This includes information related to variation in $\delta^{13}C$ and $\delta^{15}N$ values in autotrophs and heterotrophs; and the variation seen between terrestrial, marine and freshwater environments. In addition, this chapter explains how human $\delta^{13}C$ and $\delta^{15}N$ values reflect the stable isotope values of food resources consumed, and how this information is used to reconstruct human diets. It also provides examples of the application of stable isotope analysis to address different questions in the archaeology of South America, specifically, the Andes, the region where this study takes place.

4.1. Stable Isotopes

The term "isotope" has its roots in the Greek word "iso" that means "same" and the word "topos" that means "place", meaning equal place, terms which indicate that isotopes occupy the same place as a particular element of the periodic table (Fry, 2006; Hoefs, 2018). Isotopes are atoms whose nuclei has the same number of protons but a different number of neutrons (Hoefs, 2018: 1). This feature means that the isotopes of an element have the same chemical properties but different masses (Kendall and Caldwell, 1998: 53). There are two types of isotopes: those that are stable and do not decay spontaneously, and those that are radioactive or unstable and undergo spontaneous decay (Hoefs, 2018: 1)

4.1.1. Measurement

For light elements such as carbon and nitrogen, stable isotope abundance measurements are made using isotope ratio mass spectrometers (IRMS). These mass spectrometers have four components: an inlet system, an ion source, a mass analyzer, and a series of ion detectors (Fry, 2006, Katzenberg, 2008; Hoefs, 2018). Several steps are followed in order to conduct the measurement. The first step is to weigh a small amount of sample and to introduce it into the machine. In modern automated equipment, this procedure is normally done by placing it into a tin capsule. The capsules are then placed in a tray that will drop each sample into a furnace (Katzenberg, 2008). The purpose of this step is to combust the samples to convert them into gases: hydrogen (H_2), carbon dioxide (CO_2), nitrogen (N_2), oxygen (O_2), and sulfur dioxide (SO_2) (Fry, 2006: 28). Once the gases are formed, they are introduced into an ion source through an inlet system. Here the sample is converted into an ion beam that is then directed to the mass analyzer zone through a flight tube (Katzenberg, 2008). There the positively charged ions pass through a magnetic field that separates them according to their atomic mass, with the resulting separated ion beams focused into collectors for counting. Then, computer software verifies the counts from the multiple collectors, and provides the final isotope values (Fry, 2006: 29).

4.1.2. Notation

Light stable isotope compositions of elements such as oxygen, hydrogen, carbon, nitrogen, and sulfur are normally reported using δ (delta) notation. The δ values are reported in units of parts per mil, which is denoted by the symbol ‰, relative to an international standard of known composition (Kendall and Caldwell, 1998). δ values are calculated using the formula:

$$\delta^{H}X = \frac{R_{SAMPLE} - R_{STANDARD}}{R_{STANDARD}} \times 1000$$

X represents an element (H, C, N, O or S). The superscript H indicates the heavy isotope mass of that element (^{2}H, ^{13}C, ^{15}N, ^{18}O, or ^{34}S), and R is the ratio of the heavy isotope to the light isotope for the element ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, or $^{34}S/^{32}S$). Multiplication by 1000 is done with the purpose of amplifying the small differences measured between samples and standards, making them easier to deal with in mathematical operations and writing (Fry, 2006: 23).

The international standards for the various elements are assigned a δ value of 0‰. When a sample displays a positive δ value it means that the isotopic ratio of the sample is enriched in the heavy isotope relative to the standard; thus it is “heavier”. Samples that exhibit negative δ values have less of the heavy isotope than in the standard; this also means they are relatively enriched in the light isotope and are referred to as “lighter” (Kendall and Caldwell, 1998; Fry, 2006)

4.1.3. Fractionation

Isotope fractionation results from processes that cause the isotopes in a substance to change in abundance. These changes often result from processes such as diffusion, evaporation, and biologically mediated reactions (Hoefs, 2018:10). Diffusion can alter the isotope abundances of any element, simply because the various isotopes have different masses and move at different speeds; thus, diffusion can lead to a separation of light isotopes from heavy isotopes (Criss, 1999, Hoefs, 2018). In the case of evaporation, molecules containing the lightest isotopes evaporate more easily. As a result, the residual liquid becomes more enriched in heavy isotopes (Criss, 1999). The biologically mediated reactions are those that are produced by organisms; these can lead to metabolic effects in which isotopic abundances change during processes like respiration or digestion (Criss, 1999).

4.1.4. Stable Isotope Analysis and Ecology

Since the isotopic signatures of foods are passed on to their consumers, the analysis of stable isotopes has allowed researchers to trace ecological connections between organisms. In order to trace those connections, it is important to understand that as the carbon and nitrogen of foods are passed to the consumer, their isotopes are fractionated due to the process of digestion. Then, they undergo another fractionation as they are stored in the consumer's tissues, as each tissue is characterized by a particular fractionation factor. For instance, hair will display different stable carbon isotope values from bone, flesh, or fat (DeNiro and Epstein, 1978). Some of the factors involved in the variation of the abundances of carbon and nitrogen stable isotopes in the biosphere are detailed in the following sections.

4.2. Stable Carbon Isotopes: Characteristics and Measurements

Carbon has two stable isotopes ^{12}C (six neutrons) and ^{13}C (seven neutrons). The most abundant forms of carbon on Earth are carbon dioxide (CO_2) and carbonates (oxidized forms), as well as methane and organic matter (reduced forms) (Hoefs, 2018; Sharp, 2017). Carbon dioxide plays a key role in the carbon cycle, as it is actively exchanged between the atmosphere, terrestrial ecosystems, and oceans. Overall, about 98.89% of carbon is ^{12}C and 1.11% is ^{13}C, although the ratios of these stable isotopes in individual substances vary as a result of isotopic fractionation (O'Leary, 1981; Boutton, 1991; Fry, 2006). The $\delta^{13}C$ value of atmospheric CO_2 has suffered a decrease due to the burning of fossil fuels and biomass in the industrial era, a factor which has resulted in an input of CO_2 depleted in ^{13}C. It has been estimated that the decrease over the past 100 years has been almost 1‰, from about −7‰ to −8‰ (Fry, 2006: 45).

As previously explained, stable isotope abundance ratios are determined relative to the ratios of those same isotopes in an international standard. The standard used for estimating the abundance of carbon stable isotopes is carbon dioxide obtained from a limestone specimen (*Belemnitella americana*) obtained from the Upper Cretaceous Pee Dee formation in South Carolina. This standard was called PDB (PeeDee Belemnite) (Craig, 1953; O'Leary, 1981; Sharp, 2017). Although the original supply of this material became exhausted, and other standards have been prepared and calibrated against the isotopic composition of the original sample of PDB, PDB remains the standard used in reporting stable carbon isotope values. The $\delta^{13}C$ value of PDB is defined as 0‰ (Sharp, 2017). The values are expressed using the following notation (see Katzenberg, 2008):

$$\delta^{13}C\ ‰ = \frac{^{13}C/^{12}C_{\text{ SAMPLE}} - {}^{13}C/^{12}C_{\text{ STANDARD}}}{^{13}C/^{12}C_{\text{ STANDARD}}} \quad X\ 1000$$

4.3. Stable Carbon Isotopes in Autotrophs

Plants and microbes play an important role in the circulation of elements and isotopes in the biosphere, as many of the isotope signals in the biosphere are generated during fractionation (e.g., photosynthesis). Carbon fractionation takes place during photosynthesis, when terrestrial plants and marine plankton reduce the atmospheric CO_2 and convert carbon into organic matter. The reduced carbon in other organisms (e.g., animals) can trace its origins back to photosynthesis. Therefore, the isotopic fractionations that occur during photosynthesis should be the first to be studied (Fry, 2006; Sharp, 2017).

4.3.1. Photosynthesis

Three types of photosynthesis occur in the plant world, commonly referred to as the Calvin-Benson Cycle or C_3 photosynthetic pathway, the Hatch-Slack cycle or C_4 photosynthetic pathway, and the Crassulacean acid metabolism or CAM pathway (Lee-Thorp, 2008, Schwarcz and Schoeninger, 1991). The first two pathways are considered the most important photosynthetic pathways; they are named according to the number of carbon atoms that appear after carbon dioxide (CO_2) fixation. The CAM pathway is relatively rare, and typical of succulents (Waller and Lewis, 1979; Schwarcz and Schoeninger, 1991). In the late 1960s, it was discovered that terrestrial plants exhibit different $\delta^{13}C$ values, depending on their photosynthetic pathway (Fry, 2006).

4.3.1.a. The Calvin Cycle

The Calvin cycle was the first photosynthetic pathway to be described by Calvin and Benson (1948). Basically, during photosynthesis the plant converts the CO_2 from air (fixation) using the enzyme RUBISCO (an abbreviation for ribulose biphosphate carboxylase/oxygenase), and forms a phosphoglycerate compound with three carbon atoms (van der Merwe, 1982; Lee-Thorp, 2008).

The Calvin cycle was originally discovered when conducting experiments with algae, spinach, and barley (Calvin and Benson, 1948); however, now it is known that between 80% and 90% of terrestrial plants employ the C_3 pathway (Hoefs, 2018:334). The majority of cultivated and wild plants in temperate regions follow this photosynthetic pathway, as do trees, woody shrubs, herbs, and temperate or shade-loving grasses (Lee-Thorp, 2008; Schwarcz and Schoeninger, 1991).

C_3 plants have $\delta^{13}C$ values that are approximately 18‰ depleted in with respect to atmospheric CO_2 (Hoefs, 2018:334). Their $\delta^{13}C$ values average is -27‰ to -26‰, although they are subject to substantial variation depending on factors such as light, temperature, humidity, moisture, and recycling of CO_2 from decomposing plant material in the soil (Lee-Thorp, 2008; Schwarcz and Schoeninger, 1991; Sharp, 2017).

4.3.1.b. The Hatch–Slack Cycle

The Hatch–Slack cycle is a photosynthetic pathway that incorporates CO_2 by the carboxylation of the enzyme phosphoenolpyruvate (PEP), which converts the carbon dioxide into a four carbon compound, the dicarboxylic acid (Hoefs, 2018). For this reason, plants that follow this pathway became known as C_4 plants. These plants are more efficient at processing carbon, using less time and less water to convert a given amount of carbon dioxide into plant matter. Researchers agree that this photosynthetic pathway is advantageous for C_4 plants, as they can adapt themselves to hot climates, lack of water, and saline environments (van der Merwe, 1982; Hoefs, 2018).

After the initial discovery of the Hatch–Slack cycle in sugarcane in Hawaii and Australia by Kortschack et al. (1965) and Hatch and Slack (1966), respectively, the list of known C_4 species grew rapidly, to include some (but not all) members of 16 different families (Waller and Lewis, 1979; van der Merwe, 1982). These are the Aizoaceae, Amaranthaceae, Boraginaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cyperaceae, Euphorbiaceae, Gramineae, Nyctaginaceae, Portulacaceae, Zygophyllaceae, Acanthaceae, Capparidaceae, and Scrophulariaceae (Waller and Lewis, 1979: 12). The C_4 plants that became most important to humans, due to their role as staple foods, are maize, sorghum, and millet. Their average $\delta^{13}C$ values are -13‰ to -12‰ (Schwartz and Schoeninger, 1991; Fry, 2006; Sharp, 2017).

4.3.1.c. Crassulacean Acid Metabolism

Crassulacean acid metabolism, also known as CAM, is another photosynthetic pathway discovered after the Calvin cycle and the Hatch–Slack cycle. This pathway was found in succulents (e.g., cactus) (van der Merwe, 1982). Szarek and Troughton (1976) found that succulent plants suspected of being CAM showed a wide range of $\delta^{13}C$ values that ranged from -14‰ to -33‰. Something else that distinguishes CAM plants from C_3 and C_4 plants is their enzymic potential, which makes these succulents capable of assimilating CO_2 by different photosynthetic pathways, while C_3 and C_4 plants use strictly only one photosynthetic pathway. It has been suggested that CAM plants are environmentally sensitive, and may facultatively choose a photosynthetic carbon pathway according to their environmental growth conditions (Szarek and Troughton, 1976: 367). They constitute a small minority of plants, and more relevance has been given to C_3 and C_4 plants when interpreting human and animal diets (Lee-Thorp, 2008).

4.3.1.d. $\delta^{13}C$ variation within plant groups

In plants, the carbon stable isotope values can vary depending on the photosynthetic pathway. Plants with a C_3 photosynthetic pathway usually exhibit $\delta^{13}C$ values that range from −33‰ to −23‰, with an average of about -26‰ or 27%. In contrast, C_4 plants tend to exhibit $\delta^{13}C$ values that range from range from -16‰ to -9‰, with an average of -12‰ or -13‰ (Sharp, 2017). Since CAM plants can use both C_3 and C_4 photosynthetic pathways depending on the environmental conditions or pressures (Schwarcz and Schoeninger, 1991), their $\delta^{13}C$ values can range from -14‰ to -33‰ (van der Merwe, 1982).

4.3.1.e. Plants in marine and freshwater environments

Carbon sources in the oceans include dissolved carbon dioxide as well as bicarbonates and carbonates (van der Merwe, 1982: 599). Dissolved bicarbonate has a relatively high $\delta^{13}C$ value compared to atmospheric CO_2 (Lee-Thorp, 2008: 927); and carbonates have a $\delta^{13}C$ value around zero, which in consequence make marine organisms isotopically heavier than terrestrial organisms (van der Merwe, 1982: 599). Freshwater ecosystems display different $\delta^{13}C$ values due to their carbon sources. In freshwater ecosystems, carbon comes from 1) atmospheric CO_2, 2) CO_2 in the water, 3) bicarbonate and carbonate from rocks and soils, and 4) organic carbon as waste and decomposition products from plants and animals living in the water. The different carbon sources play a role in the variation of $\delta^{13}C$ values seen in organisms that live in different habitats within freshwater lakes (Katzenberg, 2008: 227). Aquatic plants have not been classified based on their photosynthetic pathway as in terrestrial environments; however, there are plants with isotopic compositions relative to their carbon source that resemble that of C_3 and C_4 plants (Roberts et al., 2007; Xu et al., 2012).

4.4. Stable Carbon Isotopes in Heterotrophs

The isotopic composition of heterotrophs is determined by the carbon isotope values of the plants and/or animals they have consumed. The differences in $\delta^{13}C$ values of their tissues will depend on the consumption of plants following a C_3 or C_4 photosynthetic pathway, or of animals that have consumed these plants (DeNiro and Epstein, 1978; Price, 2015). However, the $\delta^{13}C$ values will also vary according to the environment; thus, animals from terrestrial, marine, or freshwater environments will display $\delta^{13}C$ values that reflect their respective environments (Tykot, 2004).

The carbon isotopic signals of animals are also subject to change when they have been exploited by human groups and are fed with particular plants. For example, if animals from an environment dominated by C_3 plants are fed with C_4 plants (e.g., maize), their tissues will be enriched in the heavier isotope and they will display higher $\delta^{13}C$ values (Katzenberg, 2008). Humans consuming protein from animals that have been fed with C_4 plants will also show more enriched $\delta^{13}C$ values.

DeNiro and Epstein (1978) conducted a controlled experiment with animals grown in the laboratory with a diet of known carbon isotope composition. The study confirmed that the whole-body $\delta^{13}C$ value of animals is on average elevated by 1‰ relative to the diet. This type of elevation relative to the values of an organism's food is referred as a trophic level effect. So, organism $\delta^{13}C$ values generally increase from those exhibited by primary producers (e.g., plants) to those displayed by top consumers (e.g., carnivores). However, since l‰ represents such a small increment, the $\delta^{13}C$ values can not be used for tracing trophic levels (Schwarcz and Schoeninger, 1991). DeNiro and Epstein (1978) also found that the ^{13}C enrichment of the whole body relative to the diet is balanced by a ^{13}C depletion of the respired CO_2 or the excreted carbon, or both. In addition, the study found that the relationship between the $^{13}C/^{12}C$ ratio of a tissue and the $^{13}C/^{12}C$ of the diet depends on the type of tissue as well as on the diet (DeNiro and Epstein, 1978; Lee-Thorp, 2008).

Although it is best to study the heterotophs in a given area in detail, it is also possible to make generalizations about their $\delta^{13}C$ values based on certain types of diet. Their $\delta^{13}C$ values will tend to fall within certain ranges depending on whether they represent terrestrial herbivores eating C_3- or C_4- based diets, carnivores eating these herbivores, or animals in marine or freshwater environments. In one early study, Schoeninger and DeNiro (1984) found that bone collagen from 27 terrestrial animals they examined exhibited $\delta^{13}C$ values that ranged from -22.0‰ to -11.9‰, with an average $\delta^{13}C$ value of -18.9‰ ± 2.3‰; animals with pure C_3 diets displayed a mean $\delta^{13}C$ value of -21.5‰, while animals with pure C_4 diets displayed a mean $\delta^{13}C$ value of approximately -12.2‰. Based on more recent general surveys, Price (2015) also states that animals consuming terrestrial C_3 diets tend to have collagen $\delta^{13}C$ values of around -20‰, and that those consuming pure C_4 diets tend to exhibit $\delta^{13}C$ values of around -10‰.

Price's review (2015) states that marine diets tend to produce collagen $\delta^{13}C$ values of around -10‰. Higher $\delta^{13}C$ values for marine resources were also established early on by Schoeninger and DeNiro (1984) after examining 61 marine animals that included marine birds, mammals, and fish. These animals displayed $\delta^{13}C$ values that ranged from -16.4‰ to -9.6‰, with a mean $\delta^{13}C$ value of -13.5‰ ± 2.1‰. This mean $\delta^{13}C$ value was 5.5‰ higher than the mean $\delta^{13}C$ value of the terrestrial animals they studied (Schoeninger and DeNiro, 1984; Pate, 1994).

Schoeninger and DeNiro's study (1984) reported that carnivorous freshwater fish, as well as the aquatic migratory birds and anadromous fish, which spent part of the year on inland lakes, displayed $\delta^{13}C$ values similar to those of terrestrial animals. However, more recent studies show that the $\delta^{13}C$ values of freshwater organisms vary widely. For example, Katzenberg and Weber (1999) sampled seals and fish species from the Lake Baikal region of Russia. While seals displayed a low mean value of -22‰ ± 0.9‰ that was even isotopically lighter than local C_3 feeding terrestrial herbivores, the fish species studied showed widely varying mean $\delta^{13}C$ values, ranging from -24.6 to -12.9‰ depending on habitat and diet.

4.5. Stable Nitrogen Isotopes: Characteristics and Measurements

Nitrogen has two stable isotopes, ^{14}N (seven neutrons) and ^{15}N (eight neutrons). Almost 99% of Earth's nitrogen is present as atmospheric N_2 or as dissolved N_2 in the ocean. Atmospheric nitrogen comprises roughly 99.63% ^{14}N and 0.37% ^{15}N. A small amount of nitrogen also appears combined with other elements such as carbon, oxygen, and hydrogen. This small proportion of nitrogen includes the biological materials in ecosystems (Hoefs, 2018: 66-67).

Nitrogen isotope abundance ratios are expressed using atmospheric nitrogen as a standard. This standard is called AIR. The $\delta^{15}N$ value of N_2 is 0‰ by definition (Mariotti, 1983; Sharp, 2017).

$$\delta^{15}N\ ‰ = \frac{^{15}N/^{14}N_{\text{SAMPLE}} - {}^{15}N/^{14}N_{\text{STANDARD}}}{^{15}N/^{14}N_{\text{STANDARD}}} \quad \text{X } 1000$$

4.6. Stable Nitrogen Isotopes in Ecosystems

Nitrogen stable isotopes have proven to be useful for tracing trophic levels, understanding diet, and giving information about environmental conditions. As previously discussed, carbon stable isotopes cannot help to identify an individual's trophic level within a trophic system; however, $\delta^{15}N$ values show a larger enrichment across trophic levels that helps to trace trophic relationships (Schwarcz and Schoeninger, 1991; Ambrose, 1991; Katzenberg, 2008).

Stable nitrogen isotope values also make it possible to differentiate individuals from terrestrial environments from those from (or feeding in) marine or freshwater environments. They also provide information about breastfeeding and weaning practices (Schurr, 1998; Katzenberg, 2008).

4.6.1. Nitrogen in Diazotrophs (Nitrogen Fixers), Soil, and Water

Molecular nitrogen from the atmosphere (N_2) cannot be directly assimilated by plants. Nonetheless, nitrogen can enter foodwebs via atmospheric nitrogen-fixers, or diazotrophs, that have symbioses with plants or live independently in the soil (Ambrose, 1991; Franche et al., 2009).

The nitrogen fixation process involves the enzymatic conversion of molecular nitrogen to ammonia, which is catalyzed by nitrogenase (Franche et al., 2009: 36). Fixation commonly produces organic materials with $\delta^{15}N$ values slightly less than 0‰, ranging from −3‰ to +1‰ (Fogel and Cifuentes 1993; Hoefs, 2018).

The fixation of atmospheric N_2 adds ^{15}N-depleted nitrogen to the soil; then, denitrification and nitrification processes increase soil $\delta^{15}N$ values. Denitrification is the process in which NO_3^- and NO_2^- are converted to N_2O and ultimately N_2 gas by anaerobic bacteria, some fungi, and aerobic bacteria. This process accompanies degradation of organic matter. Nitrification is a process that involves the oxidation of ammonia to NO_3^- by nitrifying organisms, such as chemotrophic bacteria (Ambrose, 1991; Sharp, 2017).

Soil dryness and high temperatures inhibit soil nitrogen fixation. For this reason, cool and moist forest soils tend to have higher nitrogen fixation and in consequence lower $\delta^{15}N$ values, in comparison to hot, dry, and desert soils, which display higher $\delta^{15}N$ values (Delwiche and Steyn, 1970; Shearer and Kohl, 1989; Ambrose, 1991). According to Sharp (2017), the $\delta^{15}N$ values of soil range from -10 to +15‰, with most soils between +2 and +5‰.

In oceans, the average $\delta^{15}N$ value is close to 5‰ (Fry, 2006; Hoefs, 2018). Nitrogen in the ocean is present in forms, including nitrate, nitrite, and ammonium. Biological processes such as nitrogen fixation, nitrification, and denitrification cause nitrogen isotope fractionations. Local stable nitrogen isotope values in ocean water will depend on the isotopic composition of dissolved nitrate, on isotope fractionation during nitrogen uptake by phytoplankton, and on the relative contribution of nitrate versus N_2-assimilating primary producers (Hoefs, 2018).

4.6.2. Nitrogen in Plants

There are two ways in which plants can obtain nitrogen. The first way is to obtain it from inorganic ammonium and nitrate (NH_4^+ and NO_3^-) present in the soil. The second way is through symbiosis with atmospheric N_2-fixing bacteria. Because of these different paths, non-N-fixers display significantly higher $\delta^{15}N$ values than N-fixers (Ambrose, 1991: 296-297). Nitrogen-fixing plants (leguminous plants) have a nitrogen isotope composition similar to that of the atmosphere; thus their $\delta^{15}N$ values are usually close to zero (Delwiche and

Steyn, 1970; Sharp, 2017). Plants that cannot fix N_2 and incorporate nitrogen by assimilation of NH_4^+ or NO_3^- have $\delta^{15}N$ values that vary depending on the soil $\delta^{15}N$ values (Delwiche and Steyn, 1970; Sharp, 2017).

Because of the variation in soil $\delta^{15}N$ described above, plant $\delta^{15}N$ values are also subject to variation depending on their environments. Thus, for example, plants located in moist forests or montane areas display lower $\delta^{15}N$ values; while those found in arid environments, saline soils, and marine environments exhibit higher $\delta^{15}N$ values (Ambrose, 1991: 297). Plants grown in soils fertilized by manure can also have high $\delta^{15}N$ values, and this can be important for interpreting $\delta^{15}N$ values in agricultural communities (Fraser et al. 2011).

4.6.3. Nitrogen in Heterotrophs: The Trophic Level Effect

Like heterotroph $\delta^{13}C$ values, the $\delta^{15}N$ values of heterotrophs are a function of the diet. As discussed above, heterotroph $\delta^{13}C$ shows only a small trophic level elevation above the diet. In contrast, the $\delta^{15}N$ values of individuals from different trophic levels show an enrichment of 3‰ to 4‰, a larger difference which allows researchers to identify the organisms' trophic level (DeNiro and Epstein, 1981; Ambrose, 1991). As Ambrose (1991) explained, most of the ^{15}N enrichment that causes the trophic level effect results from the fractionation that takes place during transamination, deamination, and synthesis of amino acids. In connection with this process, research has confirmed that animal fluids and tissues such as blood and muscle tend to display $\delta^{15}N$ values higher than the $\delta^{15}N$ values of the diet, in contrast with urine and bile, which exhibit $\delta^{15}N$ values lower than the diet values. The above suggests that when amino acids are being metabolized, there is a preferential loss of

^{14}N that is excreted, thus leaving the nitrogen that form the tissues enriched in ^{15}N (Ambrose, 1991; Minagawa and Wada, 1984).

Unfortunately, as Schwarcz and Schoeninger (1991) demonstrated, $\delta^{15}N$ comparisons across systems or regions to determine trophic level are not valid. A terrestrial system has a nitrogen source (soil) with a different isotopic composition from that of a marine system or freshwater system; thus, they are not equivalent and can not be compared. This logic also applies to terrestrial trophic systems operating under different environmental conditions; for example, trophic systems in deserts should not be considered equivalent to those that can be found in well-watered areas (Schwarcz and Schoeninger, 1991: 299), as foodwebs in desertic areas tend to display enriched $\delta^{15}N$ values (Ambrose, 1991).

Another situation in which the $\delta^{15}N$ trophic level effect is seen is in breastfeeding human babies/infants (and in the nursing young of many other mammals). Since the babies are consuming a secretion of their mothers' body (milk), their $\delta^{15}N$ values exhibit an enrichment of 3‰ in comparison to the tissue $\delta^{15}N$ values of the mother. After weaning is complete, the baby's $\delta^{15}N$ values decrease and become more similar to the $\delta^{15}N$ values of the mother. This effect is discussed more thoroughly below in section 4.7.2.b.

4.6.4. Heterotroph $\delta^{15}N$ Values in Terrestrial and Aquatic Environments

As discussed above, varying local baseline $\delta^{15}N$ values make it impossible to directly compare organisms from different biomes; however, an early broad survey by Schoeninger and DeNiro (1984) provides some general values. Their study of nitrogen values in terrestrial animals (n = 27), which included mammals (herbivores and carnivores) and birds, produced a mean $\delta^{15}N$ value of 5.9 ± 2.2‰. According to a recent review by Price (2015), human consumers of both terrestrial animals and plants tend to display δ^{15}N values ranging from 6‰ to 10‰.

The 61 marine animals sampled by Schoeninger and DeNiro (1984) displayed δ^{15}N values that ranged from 9.4‰ to 23.0‰, with a mean δ^{15}N value of 14.8‰ ± 2.5‰. The animals from this study represented marine mammals, fish, and marine birds. Mammals exhibited a δ^{15}N value ranging from 11.7‰ to 22.9‰, with a mean δ^{15}N value of 15.6‰ ± 2.2‰. Fish had δ^{15}N values with a range between 11.4‰ and 16.0‰ and a mean δ^{15}N value of 13.8‰ ± 1.6‰; whereas birds had δ^{15}N values that range from 9.4‰ to 17.9‰, and have a mean δ^{15}N value of 12.9‰ ± 2.9‰ (Schoeninger and DeNiro, 1984).

Schoeninger and DeNiro (1984) noted further variation between animals eating fish and those that feed on invertebrates such as molluscs and arthropods. Fish eaters displayed a mean δ^{15}N value of 16.5‰ ± 1.9‰, while animals that do not feed on fish exhibited a mean δ^{15}N value of 13.3‰ ± 1.7‰. Generally, these marine results supported a difference of 3‰ between trophic levels.

Freshwater ecosystems can be considered similar to marine systems in terms of having high δ^{15}N values (Lee-Thorp, 2008: 928). Only five carnivorous fish from these environments were analyzed by Schoeninger and DeNiro (1984). These fish exhibited a mean δ^{15}N value of 8.0‰ ± 1.2‰, which is similar to that displayed by the terrestrial carnivores examined in the same study. More recent studies of freshwater environments, such as the large-scale survey work done in the Lake Baikal region (e.g., Katzenberg and Weber, 1999) have found similar elevated δ^{15}N values. For example, Katzenberg and Weber (1999) reported a mean δ^{15}N value of 14‰ ± 1‰ in seals and mean δ^{15}N values ranging from 9.4‰ to 13.7‰ in fish species.

4.7. Other Factors Influencing Heterotroph δ^{15}N

As previously discussed, heterotrophs δ^{15}N values are influenced by their diet and the ^{15}N enrichment proper to their trophic level. However, there are other factors that can cause variation in the nitrogen isotope values, including the arid environment effect, nursing, growth, and nutritional stress.

4.7.1. The Arid Environment Effect

As explained in the section discussing nitrogen in soil and water, hot, dry, and desert soils are characterized by inhibited nitrogen fixation, a higher volatilization of ammonia, lower mineralization, and higher δ^{15}N values. These conditions of dry and desert soils affect the δ^{15}N values of plants, since most of these obtain their nitrogen from the soil (Ambrose, 1991). According to Lee-Thorp (2008), environmental aridity causes a variation in the δ^{15}N values of soil and plants ranging from 1‰ to 4‰. For example, the mean δ^{15}N values of N_2-fixing plants in the Sonoran Desert ranged from +2.0‰ to +6.3‰, and the mean values of other plants ranged from +3.0 to +13.0‰ (Shearer et al., 1983; Ambrose, 1991). By affecting plants, the aridity of these soils also affects the δ^{15}N values of the animals that consume those plants. Consequently, animals in arid regions exhibit higher δ^{15}N values than those displayed by animals that live in moister environments (Ambrose, 1991). The elevation in δ^{15}N values of soils, plants, and animals caused by aridity is referred to as the arid environment effect.

4.7.2. Nursing, growth, and nutritional stress

Fogel et al. (1989) were the first researchers to investigate whether breast milk has a unique isotopic signature, and if it could be used to trace lactation. Through that research they discovered that nitrogen stable isotopes can be used for studying breastfeeding and weaning practices (Fogel et al. 1989; see also Fuller et al., 2006).

Because their tissues were formed in the mother's womb, new-borns have an identical nitrogen isotopic composition to that of their mothers until they are breast-fed. Once they start breast-feeding, breast-milk turns into their only source of dietary nitrogen, a source that is enriched in ^{15}N by 2 to 3‰ relative to the mother's diet. By the time the breast-feeding infant synthesizes new collagen, that collagen displays an increase in the ^{15}N content that reflects the trophic level effects associated with digestion and tissue synthesis. In other words, nursing infants appear one trophic level above their mothers. Later, when weaning foods are incorporated into the juvenile's diet, the juvenile starts displaying the ^{15}N values of the weaning food, following the principle that "you are what you eat". The ^{15}N content of weaning foods is usually lower than that of breast milk. In consequence, there is a progressive decline in $\delta^{15}N$ (Fogel et al., 1989; Schurr, 1998; Fuller et al., 2006). Based on this phenomenon, researchers have been able to estimate the timing of weaning and the nature of weaning foods in the past (Katzenberg et al., 1996; Schurr, 1997; Turner et al., 2007).

A frequent finding in these archaeological weaning studies is that the post-weaning values of children are lower than the $\delta^{15}N$ values of adults. Two main hypotheses have been developed in an attempt to explain these depleted childhood $\delta^{15}N$ values. These involve 1) the idea that childhood diets are often characterized by less consumption of animal protein than adult diets, and 2) the possibility that a higher metabolic demand for nitrogen during the period of rapid childhood growth depresses childhood $\delta^{15}N$ values (Prowse et al., 2008). This debate will be discussed further later on in this thesis in connection with the $\delta^{15}N$ value of one juvenile in the sample set.

There is another important cause of variation of $\delta^{15}N$ values. This cause is related to variation in physiology reflecting poor health; tissue $\delta^{15}N$ values sometimes display an increase under situations of nutritional stress (Turner et al., 2007; Katzenberg, 2008). This was first noted in a study conducted by Katzenberg and Lovell (1999). These researchers performed carbon and nitrogen stable isotope analysis in three normal human samples and four samples with pathological conditions (e.g., osteomyelitis, healing fracture), to examine if pathologies can cause changes in the isotope values. The values of the pathological samples varied, and were very high in some cases. This effect has since been further documented and used in research. For example, a more recent study conducted by Beaumont and Montgomery (2016) examined the remains of 20 workhouse inmates dated to the Great Irish Famine of the 19th century. The methods involved analyzing dentine samples, which represent diet at the time of tooth formation; and comparing their $\delta^{13}C$ and $\delta^{15}N$ values with the values obtained from bone collagen, which represent diet over the last few years of life. The researchers reported little variation in the $\delta^{13}C$ and $\delta^{15}N$ values of adult individuals during childhood; however, the dentine $\delta^{15}N$ values of children that died in the workhouse while their teeth were still forming rose shortly before death. According to Beaumont and Montgomery (2016) the rise in the $\delta^{15}N$ values most likely reflects recycling of body tissue stores, which occurs during starvation, suggesting that the $\delta^{15}N$ values increased under nutritional stress.

4.8. Stable Isotopes from Diet to Bone

Previous sections in this chapter have already discussed how carbon and nitrogen values in heterotrophs are subject to variation depending on the type of environment in which they live, their trophic level, their diet, as well as other factors such nursing, growth, and nutritional stress. However, the stable isotope values can also vary depending on the type of tissue used for analysis as different fractionations take place, and depending on the tissue's turnover rate.

4.8.1. Fractionation Values Between Diet and Bone

Because of the impracticality of determining the stable isotope values of the whole body of a large animal, and the need to study past animals using only their bones or teeth, researchers tried early on to determine the isotopic composition of a specific parts of an animal that would reflect the $\delta^{13}C$ value of the diet (DeNiro and Epstein, 1978). Their findings showed that when dietary carbon is incorporated into a particular tissue, a characteristic fractionation relative to diet occurs (Hobson and Clark, 1992).

Many studies have focused on examining the fractionations that occur between diet and bone collagen, as well as fractionations between diet and carbonate. To study the degree of isotopic fractionation that takes place between diet and bone collagen and between diet and carbonate, small and large animals with diets of known isotopic composition were analyzed. The results show that small animals display differences in the $\delta^{13}C$ values between bone collagen and diet that range from 1‰ to 3.7‰ (DeNiro and Epstein, 1978; Lee-Thorp et al, 1989; Ambrose and Norr, 1993; Pate, 1994). Greater differences were reported in large herbivores. Free-ranging ungulates show a mean difference of 5‰, and animals consuming C_4 plants a mean difference of 6‰ (Vogel, 1978; Krueger and Sullivan, 1984; Lee-Thorp et al., 1989; Pate, 1994). Based on these values, it has been established that the $\delta^{13}C$ value of bone collagen is 3-6‰ higher that that of an animal's dietary protein (Lee-Thorp et al., 1989; Ambrose and Norr, 1993). The difference estimated for humans is about +5‰ (van der Merwe and Vogel, 1978; Lee-Thorp et al., 1989). The reason why bone collagen $\delta^{13}C$ values reflect dietary protein is that collagen is preferentially synthesized from amino acids (protein); under conditions of poor protein nutrition, some of its carbon may come from carbohydrates or lipids (Ambrose and Norr, 1993).

In terms of bone or enamel carbonate, it is more enriched in ^{13}C than collagen; bone carbonate $\delta^{13}C$ values reflect average whole-diet $\delta^{13}C$ and are controlled by catabolic and respiratory processes (Pate, 1994; Lee-Thorp, 2008). The difference between whole-diet and bone carbonate $\delta^{13}C$ values varies between species, depending on body mass and dietary physiology (Lee-Thorp, 2008). In the study conducted by DeNiro and Epstein (1978) with mice fed with different diets, the researchers found that the bone carbonate $\delta^{13}C$ value relative to the diet was + 9.5‰ and +9.7‰ (Ambrose and Norr, 1993). In large wild herbivores, the difference between diet and carbonate $\delta^{13}C$ values is from +12‰ to +13‰ (Krueger and Sullivan, 1984; Pate, 1994). The exact value in humans is debated, but many recent studies argue for a separation of about +10‰ (e.g., Kellner and Schoeninger, 2007).

4.8.2. Tissue Turnover

As Tieszen et al. (1983) stated, each animal tissue has its own "isotopic memory", based on the carbon values of food consumed, fractionation during synthesis (see above), and the turnover rate of that particular tissue. Turnover rate refers to the rate in which older tissue protein and other tissue components that are degrading are being replaced by newer components (Tieszen et al., 1983). DeNiro and Epstein (1978) and Tieszen et al. (1983) conducted studies in mice and gerbils (*Meriones unguiculatus*) to establish the approximate turnover times of carbon in different tissues. The results of these studies showed that the values of tissues differed from that of the diet in the sequence hair > brain > muscle > liver

> fat (DeNiro and Epstein, 1978; Tieszen et al., 1983; Tieszen and Boutton, 1989). This suggested that those tissues that are more metabolically active, such as the liver, the pancreas, or fat tissue, have faster turnover rates in comparison to bone and connective tissue, which are less metabolically active (Tieszen et al., 1983). Tissues with rapid isotopic turnover tend to reflect recent diet, and tissues with slow turnover reflect longer-term diet (Hobson and Clark, 1992).

Examples of tissues with rapid turnover are hair and nails, as these have a mean growth rate of about 1.1 cm or 0.2 mm per month, respectively; thus, they provide information of the last years, months, and days of life. In contrast, bones provide information of the last decade or decades of life. However, there is variation depending on the age, sex, or health of the individual. In addition, the turnover rates will differ between bones; for example, ribs and vertebrae have faster turnover rates than long bones. In adults, the turnover rate of ribs was estimated to be 17% per year, while the turnover rate of a femur is estimated to be 3% per year; therefore, collagen extracted from ribs give information relative to the last ten years of life, and collagen from the femur gives information of the las 30 years of life. In contrast, teeth have no significant turnover during lifetime; thus teeth give information relative to the years of their formation, which occurred between birth and late adolescence (Lehn et al., 2015).

4.9. δ^{13}C and δ^{15}N Analysis in the Andes

Over the last decades, several studies have used carbon and nitrogen stable isotope analysis to gain an insight into the diet of different archaeological populations in the Andean region. Through these studies it has been possible to distinguish populations with a subsistence economy based on maize agriculture from human groups with a subsistence economy based on the agriculture of C_3 cultivars, which has also made it possible to trace when maize become a staple food. Some of these studies have also focused on examining the role played by marine, riverine, and terrestrial animal protein in the diet. This work has given researchers valuable information about husbandry and other animal management practices, or the use of manure for agriculture. Other studies have focused on examining whether there was a differential access to specific food resources based on age, sex, or status; or have employed stable isotope analysis as an approach for examining residential mobility. This section introduces some of the studies conducted in the Andean regions of Peru, Argentina, and Chile as well as the $\delta^{13}C$ and $\delta^{15}N$ values reported at the sites that were under study (unless otherwise noted, all stable isotope values are for bone collagen). The studies conducted in Ecuador were previously discussed in Chapter 2.

4.9.1. Peru

Tykot et al. (2006) examined several Peruvian sites dated to the Initial Period (ca. 1800-900 BC) and Early Horizon (Formative Period) (ca. 900-200 BC). Among these sites is Pacopampa, which is located in the northern highlands. The collagen mean $\delta^{13}C$ value in skeletal remains from this site is -19.3‰ ± 0.4‰, consistent with a pure C_3 diet with only a very small contribution from maize. The mean $\delta^{15}N$ value is 7.6‰ ± 0.5‰. The researchers suggested that llamas, wild deer, guinea pigs, and other animals were consumed regularly at this site. In contrast, all the coastal sites analyzed in Tykot et al.'s (2006) study, which include Cardal, which produced a mean $\delta^{13}C_{bioapatite}$ of -12.8‰ ± 1.4‰; Mina Perdida, where they obtained a mean $\delta^{13}C$ for hair of -18.1‰ ± 1.1‰ and a mean $\delta^{15}N$ of 10.6‰ ± 1.2‰, and La Tablada de Lurín, which displayed a mean $\delta^{13}C$ value of -11.2‰ ± 1.3‰ and a mean $\delta^{15}N$

value of 14.4‰ ± 0.7‰, suggest there was more maize consumption at these sites than in Pacopamba in the highlands. Based on these results, Tykot et al. (2006) stated that during the Initial Period and Early Horizon maize was not a dietary staple but a complement to the diet, even when the diet included a marine component. According to those authors, maize did become a staple in the Early Horizon when more complex societies such as Chavín de Huántar developed.

Finucane et al. (2006) examined diet of the Middle Horizon (ca. 600-1000 AD). The study analyzed samples from individuals at Conchopata, a site associated with the Wari polity, that is located in the central highlands of Peru. The mean $\delta^{13}C$ value was -10.7‰ ± 1.2‰, and the mean $\delta^{15}N$ value was 10.7‰ ± 1.2‰. These values were interpreted as consistent with a maize-based diet complemented with the consumption of a substantial quantity of animal protein. Finucane et al. (2006) also examined camelids, which had to be split into two groups due to their enriched and depleted $\delta^{13}C$ values. The first group displayed a mean $\delta^{13}C$ value of -10.4‰ ± 1.1‰; the second group exhibited a mean $\delta^{13}C$ value of -17.9‰ ± 1.5‰. The mean $\delta^{15}N$ value was 6.6‰ ± 1.5‰. Variation in the camelid values was considered to reflect two animal husbandry practices: 1) range stock grazing in puna pastures, and 2) maize foddering. Cavia (cuy) exhibited a mean $\delta^{13}C$ value of -11.0‰ ± 3.0‰ and a mean $\delta^{15}N$ value of 8.3‰ ± 0.9‰. Some of the Cavia values were considered to show some maize consumption. Phyllotis (mice) samples had a mean $\delta^{13}C$ value of -12.1‰ ± 0.8‰ and a mean $\delta^{15}N$ value of 11.4‰ ± 3.6‰ that indicate they consumed substantial quantities of C_4 plants or the tissue of animals foddered on C_4 plants.

Finucane (2007) used carbon and nitrogen stable isotopes to study diet from mummies of the Ayacucho Valley in the highlands. The mummies correspond to the late prehistoric-early colonial period (AD 1490-1640). The mean $\delta^{13}C$ of their bone collagen was -11.5‰ ±

1.4‰ and the mean $\delta^{15}N$ value was 11.1‰ ± 0.7‰. The results from this study indicate that maize was the base of the subsistence economy of the Ayacucho Valley. The author also suggests, based on the stable isotope values, that there was a preferential fertilization of maize with composted manure.

Somerville et al. (2015) examined the $\delta^{13}C$ and $\delta^{15}N$ of two Tiwanaku colonies (Río Muerto and Omo) from the Middle Horizon (AD 500-1000) in southern Peru in order to test whether there was a sex-based consumption of chicha (a fermented maize beverage), as reported in the Inca Empire. The mean $\delta^{13}C_{collagen}$ was -12.3‰ ± 1.5‰ and the mean $\delta^{15}N$ value 8.4‰ ± 1.6‰. The mean $\delta^{13}C_{bioapatite}$ was -7.3‰ ± 1.6‰. Somerville et al. (2015) found that maize was a staple crop in these colonies, and that males displayed significantly higher $\delta^{13}C_{collagen}$ values than females; this result was argued to indicate higher consumption of maize, probably in the form of chicha.

A study performed by Toyne et al. (2016) examined the Inca expansion into Chachapoyas territory in northern Peru using stable carbon, nitrogen, and oxygen isotope analysis of bone and dentin collagen and bone and enamel carbonate. It was hypothesized that some of the individuals buried at the Chachapoyas cemetery of Los Pinchudos (AD 1470 - 1535) may have been Inca. Bone $\delta^{13}C_{collagen}$ values have a mean of -15.38‰ ± 2.41‰; the dentine mean $\delta^{13}C_{collagen}$ value was -15.15‰ ± 1.73‰. Bone $\delta^{13}C_{apatite}$ had a mean of -9.78‰ ± 2.84‰, and tooth $\delta^{13}C_{apatite}$ had a mean of -9.01‰ ± 1.73‰. The bone mean $\delta^{15}N$ value was 7.76‰ ± 1.18‰, and the dentine mean $\delta^{15}N$ value 9.40‰ ± 0.93‰. Based on these values, Toyne et al. (2016) concluded that most of the individuals had a primarily C_3 diet, with some C_4 consumption and a limited portion of protein from terrestrial animals. Only two individuals displayed values consistent with a maize-based diet and higher consumption of terrestrial protein. These results, along with the data from oxygen stable isotopes, suggested that there was a minimal presence of non-local individuals at the cemetery (Toyne et al., 2016).

Turner et al. (2010) conducted a study to estimate the diet during infancy and childhood of 71 adults interred at the Inca site of Machu Picchu. The individuals in the category Infancy/Early Childhood displayed a mean $\delta^{13}C_{collagen}$ value of -13.6 ‰ ± 2.5‰ and a mean $\delta^{15}N$ value of -11.8‰ ± 2.2‰. Individuals in their middle childhood/adolescence exhibit a mean $\delta^{13}C$ value of -13.9‰ ± 2.2‰ and a mean $\delta^{15}N$ value of -11.8‰ ± 4.8‰. The mean $\delta^{13}C_{apatite}$ value for Infancy/Early Childhood was 6.8‰ ± 3.2‰; the mean $\delta^{13}C_{apatite}$ value of individuals in their middle childhood was -6.3‰ ± 3.2‰; while adolescents displayed a mean $\delta^{13}C$ value of -6.6‰ ± 3.3‰. According to Turner et al. (2010), there is a substantial variation in the $\delta^{13}C_{apatite}$ values among individuals. This variation indicates that while some individuals had an isotopically homogenous diet during infancy and childhood, others experienced a moderate change in their diets, and others experienced even more substantial shifts. The researchers noted that these findings were similar to those presented by Turner et al. (2009) that used oxygen stable isotope analysis to examine residential mobility in Machu Picchu. While some individuals show very little change in $\delta^{18}O$, other individuals displayed values consistent with a change in the source of water, which may have been in a different ecological zone. After comparing these results, Turner et al. (2010) suggested that the dietary variation seen within the individuals' infancy, childhood, or adolescence may reflect the time they lived in different ecological zones. Based on the above, they suggested that after the individuals were relocated to Machu Picchu, maize became a more important part of the diet; while protein sources such as meat, fish, or amaranth became less important.

Turner and Armelagos (2012) compared the presence of criba orbitalia, porotic hyperostosis, and lineal enamel hypoplasias with the stable isotope data published by Turner et al. (2009) and Turner et al. (2010) from Machu Picchu in order to examine early-life diet and to examine if residential origins were associated with the presence of those pathologies. The researchers found that both criba orbitalia and porotic hyperostosis, which indicate anemia, were associated with where the individuals with these lesions were born and grew up, instead of being a result of what the individuals ate. In the case of linear enamel hypoplasia, they concluded that it is influenced by episodes of stress caused by an inadequate diet, along with environmental factors (e.g., contaminated water) from the place where the individuals grew up.

4.9.2. Chile

Petruzzelli et al. (2012) conducted stable carbon and nitrogen isotope analyses of bone collagen from 13 human samples from the northern coastal site Azapa-71, dated to the Early and Late Formative Period; and the site Pica-8, dated to the Late Intermediate Period. The Azapa-71 samples exhibit a mean $\delta^{13}C$ value of -14.5‰ ± 2.2‰ and a mean $\delta^{15}N$ value of 19.7‰ ± 3.5‰. These values suggested a diet consisting predominantly of marine resources from high trophic levels. The Pica-8 samples displayed a mean $\delta^{13}C$ value of -9.9‰ ± 1.7‰ and a mean $\delta^{15}N$ value of 18.4‰ ± 3.2‰. For Petruzzelli et al. (2012), these values indicated that the individuals from this site also consumed marine resources from high trophic levels; however, they also supplemented their diet with some maize.

Knudson et al. (2012) carried out stable carbon, nitrogen, oxygen, and strontium isotope analysis on enamel, bone, and hair from an individual found isolated in the middle of the Atacama Desert in northern Chile. The individual was dated to the Late Formative Period (AD 1 - 500). The results indicated a diet based on a mix of C_3 and C_4 foods and a substantial portion of marine protein ($\delta^{13}C_{collagen}$ -12.7‰; $\delta^{15}N_{collagen}$ 21.6‰), which was consumed during the first years of his life and frequently for approximately 10 to 30 years. The $\delta^{15}N_{collagen}$ and $\delta^{15}N_{keratin}$ values show that he switched between higher and lower trophic level sources, which has been considered as a result of frequent trips to and from the coast to the highlands.

Torres-Rouff et al. (2015) examined the relationship between the Tiwanaku polity and the individuals from the Middle Horizon (AD 500 - 1000) buried at the cemetery of Larache in San Pedro de Atacama in the interior northern Chilean desert. This study included an examination of cranial vault modifications and traumatic injury patterns, as well as conducting strontium isotope analysis and stable carbon and nitrogen isotope analyses. From the 18 individuals analyzed for strontium, only 5 displayed $^{87}Sr/^{86}Sr$ values in both early and late forming teeth well outside of the local range for San Pedro de Atacama. Three individuals had $^{87}Sr/^{86}Sr$ values consistent with the Lake Titicaca Basin, the other two had $^{87}Sr/^{86}Sr$ values consistent with the southern *altiplano*. The $\delta^{13}C$ values ranged from -21.4‰ to -14.7‰ which indicates the consumption of C_3 and C_4 resources of the region; however, three of the individuals with nonlocal $^{87}Sr/^{86}Sr$ values also exhibit different $\delta^{13}C$ signatures for both collagen and hydroxyapatite, which suggests these individuals consumed more C_4 plants and protein from maize-fed animals than the other individuals.

Santana et al. (2016) also studied paleomobility at Cementerio Oriente in the Atacama Desert. The individuals from this study have been dated to the Late Intermediate Period (AD 950 – 1400). Stable carbon, nitrogen, and oxygen isotope analysis were conducted for bone and dentine collagen, as well as bone and enamel apatite. The mean $\delta^{13}C_{collagen}$ of each of the two sectors of the cemetery under study was -12.5‰ ± 1.7‰. Their mean $\delta^{15}N$ values were 18.6‰ ± 4.1‰ and 17.9‰ ± 3.0‰. These values have been interpreted as indicators of a diet strongly influenced by the consumption of marine resources, with a contribution of maize. The $\delta^{15}N$ values are very high at the site, possibly reflecting the arid environment effect as well as marine resource use.

4.9.3. Argentina

Tessone et al. (2015) analyzed a total of 51 samples from individuals, ranging from ±36 weeks of age to 50+ years old adults, from a group of hunter-gatherers recovered from burials with dates ranging from ca. 800 BP to 350 BP at Lake Salitroso, Patagonia. The purpose of the study was to examine their breastfeeding and weaning practices. The researchers reported $\delta\ ^{13}C$ values that range from -19.8‰ to -17.1‰, with a mean $\delta\ ^{13}C$ value of -18.7‰ ± 0.5‰. They also reported $\delta^{15}N$ values that ranged from 10.1‰ to 14.8‰ with a mean $\delta^{15}N$ value of 11.9‰ ± 1.1‰. No age-based variation was observed in the $\delta^{13}C$ values. However, they noted variation in the $\delta^{15}N$ values. The results from this study indicate that this group of hunter-gatherers incorporated supplementary solid foods between 0.75 and 2 years of age, and that the cessation of breastfeeding occurred in children about 5 to 6 years old.

Gil et al. (2009) conducted stable carbon and nitrogen isotope analyses of collagen and apatite in remains of 20 individuals recovered from 14 different archaeological sites, with the purpose of studying the variation of human diet over the past 2500 years in central western Argentina. Especial emphasize was given to the consumption of maize. The sites studied were Viluco (ca. 200 BP), San Carlos (ca. 150 BP), Cápiz Alto (16th and 17th centuries), Barrancas (ca. 2000 BP), Upsallata Túmulo I (ca. 980 BP), Upsallata Túmulo II (ca. 1200 BP), Upsallata Túmulo III (ca. 670 BP), Potrero Las Colonias (ca. 570 BP), Upsallata Usina Sur (ca. 580 BP), an individual dated to 2480±50 from Agua de la Cueva, and Aconcagua (ca. 370 BP). The $\delta^{13}C_{collagen}$ values range from -18.7‰ to -10.8‰ with a mean $\delta^{13}C_{collagen}$ value of -14.6‰ (n=20); the $\delta^{13}C_{apatite}$ values range from -13.6‰ to -3.4‰ with a mean of -8.6‰ (n=14). The $\delta^{15}N$ values range between 7.8‰ and 11.7‰ with a mean $\delta^{15}N$ value of 9.5‰ (n=16). The results from this study indicates that the oldest samples, between ca. 2400 and 1750 BP, displayed the lowest values $\delta^{13}C$ values. Later, between 1250 and 300 BP, there is a higher consumption of C_4 plants as well as important variation. However, Gil et al. (2009) reported that the $\delta^{13}C$ values dropped significantly after 300 BP, which was associated to the introduction of European resources to the region that became staples.

Fernández et al. (1999) studied the diet of the mummy of a 7-year old child found on Mount Aconcagua, considered a human sacrifice of the Inca Imperial period dated between AD 1480 and 1532. The researchers conducted carbon and nitrogen stable isotopes of bone collagen; and compared the values to those from an individual in Upsallata, close to Mt. Aconcagua, from the same period. The $\delta^{13}C$ values were -10.8‰ and -13.5‰, respectively. The $\delta^{15}N$ values were 10.4‰ and 9.8‰. They also conducted carbon, nitrogen, and sulfur stable isotopes of hair from this mummy. The $\delta^{13}C$ values of hair samples varied between -14.5‰ and -10.0‰. Sulfur isotope analyses were conducted in root and tip sections of hair samples. The $\delta^{34}S$ values were 9.4‰ and 10.9‰, respectively. Fernández et al. (1999) considered that these values were consistent with either a marine diet or a diet based on C_4 plants. However, the hair analyses indicated a non-marine diet for the year and a half preceding the child's death.

4.9.4. The question of plant $\delta^{13}C$ values

As seen in this brief literature review, a recurrent element in the interpretation of $\delta^{13}C$ values in the Andes and South America is the assumption that the C_4 signals of humans and animals represent the consumption of maize. Cadwallader et al. (2012) examined a total of 89 modern plant samples from the south coast of Peru that may have played a significant role in diets of the region, with the purpose of demonstrating that other C_4 plants may have also contributed to the diet. Their data were combined with stable isotope values of 27 different species from the central coast and highlands of Peru determined by DeNiro and Hastorf (1985), and the results of a study by Tieszen and Chapman (1992) that studied plants from the coast and highlands in the Arica area in northern Chile. Of the total 144 plants considered by this study, almost one third displayed $\delta^{13}C$ values indicative of C_4 or CAM photosynthetic pathways. Identified within this group are many plants of the families Gramineae, Cyperaceae, Cactaceae, Chenopodiaceae, and Amaranthaceae. From these the authors highlight the domesticated amaranth (*kiwicha*), an important pseudo-cereal crop in the Andes. Although this study is an important caution for researchers, a look at the studies summarized here and in chapter 2 shows that maize is still considered the most likely C_4 crop in most dietary interpretations, which also take into consideration the archaeological and botanical evidence for maize at each site. It is also important to note that in temporal surveys such as that done in Peru by Tykot et al. (2006), human $\delta^{13}C$ values tend to stay more typical of C_3 consumption until they rise in eras when other evidence also suggests maize was important. Generally in Andean studies, groups concluded to have depended little or not at all on maize tend to show collagen $\delta^{13}C$ values around -19‰ or -18‰ (Pennycook, 2013;

Tykot et al., 2006; Burger and van der Merwe, 1990), while heavily maize-dependent societies show collagen $\delta^{13}C$ values around -10‰ or -8% (Pennycook, 2013; Finucane et al., 2006; Finucane, 2009).

4.10. Summary

Isotopes occupy the same place of a particular element in the periodic table, although they have different masses (same number of protons, but different number of neutrons). Variation in the abundances of stable isotopes are due to several processes that cause their fractionation, one of the best examples being photosynthesis. Given that plants can follow different photosynthetic pathways, their values $\delta^{13}C$ values can show variation. This variation in plants $\delta^{13}C$ values allows researchers to differentiate predominantly C_3 diets from diets with a heavy reliance on C_4 resources in humans. A similar principle lies behind the differences between terrestrial, riverine, and marine animal values. For this reason, it is also possible to establish what kind of protein was consumed by a population by using $\delta^{15}N$ values. However, examining diet through carbon and nitrogen stable isotopes is more difficult, as there are many other reasons for their variation; for example, nursing, growth, nutritional stress, or the aridity of the environment. In Ecuador and other Andean nations, stable isotope analysis has been used to address a number of questions, especially revolving around maize consumption.

Chapter 5: Samples and Methods

The purpose of this chapter is to present the details of the human and faunal skeletal samples that were collected for this study. The discussion of the human bone samples will outline the archaeological site, the bone elements that were selected, and the individual's sex and age if determined, as well as the sample identification number used in the laboratory. Additional information on the samples is presented in Appendix 1.

The discussion of the faunal samples that were analyzed in order to establish an isotopic baseline will include the bone elements sampled and the taxa to which the animal belonged to. Unfortunately, faunal remains were not available for sampling for all sites, so the majority of the faunal samples come from only one site. The reasoning involved in collecting samples from this site will be explained in further detail.

In addition, this chapter will describe the methods that were followed in the laboratory in order to process and prepare the bone collagen for carbon and nitrogen stable isotope analysis. A detailed explanation of the criteria employed to determine the sample quality will be also given.

5.1. Human Bone Samples

Human bone samples were collected from four settlements located in three different archaeological sites. These sites are Rancho Bajo, Cotocollao (early and late settlements), and Las Orquídeas, all of which are situated in the Northern Highlands of Ecuador; and have been described in detail in Chapter 3. A total number of 56 human bone samples were collected for this research.

5.1.1. Human Bone Samples from Rancho Bajo

The first set of samples were taken from the site of Rancho Bajo, located in Pichincha Province. This site was excavated in 2011, 2013, and 2018, producing a total of 27 human burials. The skeletal remains were cleaned and examined over the 2012 and 2013 field seasons, in which I participated. From these 27 burials, only 15 bone samples (representing 15 individuals) could be collected. The poor preservation of many of the skeletal remains made it impossible to sample all 27 individuals. Bone samples were taken exclusively from long bones, due to the poor preservation of the ribs. Fortunately, because almost all the individuals from Rancho Bajo were found in individual primary burials, there was no risk of sampling any of the individuals twice. This set of samples corresponds entirely to the Early Formative Period (see Table 5.1).

Obtaining these samples required visiting the storage area of the Instituto Metropolitano de Patrimonio and the Laboratory of Archaeology of the Pontificia Universidad Católica del Ecuador, both situated in the city of Quito. After the respective permits were issued and access was granted, the samples were extracted under the supervision of a representative of these institutions.

Table 5.1 - List of human samples from Rancho Bajo

Sample ID	Bone	Sex	Age[†]
PRB-01	Left femur	M	Middle Adult (35-45 years)
PRB-02	Left femur	M	Middle Adult (30-40 years)
PRB-03	Right femur	ND	Subadult (10-15 years)
PRB-04	Right femur	M	Middle Adult (30-40 years)
PRB-05	Right tibia	M	Young Adult (25-35 years)
PRB-06	Right humerus	M	Young Adult (25-35 years)
PRB-07	Left femur	F	Middle Adult (35-45 years)
PRB-08	Left femur	M	Adult (30-60 years)
PRB-09	Right tibia	F	Subadult (16-20 years)
PRB-10	Right tibia	M	Young Adult (25-35 years)
PRB-11	Right femur	M	Young Adult (25-35 years)
PRB-12	Right femur	M	Middle Adult (30-40 years)
PRB-13	Left femur	F	Adult (30-60 years)
PRB-14	Left tibia	F	Middle Adult (33-45 years)
PRB-15	Right humerus	ND	Subadult (14-17 years)

[†] Age ranges are those presented in Ugalde (2012) and Ugalde (2013b)

5.1.2. Human Bone Samples from Cotocollao

The second site to be sampled was Cotocollao, which is also located in Pichincha Province, relatively close to the site Rancho Bajo. As reviewed in Chapter 3, this site has two different components, both of interest to the present study. For the early settlement, which dates approximately between 2400–1200 cal BC, a total of 13 human burials were discovered. For the late settlement, whose dates run approximately between 1200 – 400 cal BC, about 179 burials have been found (Villalba, 1988; Ziólkowski et al., 1994).

It was the original plan to obtain as many human bone samples as possible from each period. However, once access to the collection was granted, it was discovered that not all of the material recovered from the excavation has been kept in a single location. Instead, the collection has been split, and is being curated by two different institutions. Every possible effort was made to gain access to the full collection; but in the end this was not possible, a circumstance that had a direct impact on the number of samples collected. A second limitation to sampling was the poor general preservation of the remains. Many individuals were represented only by chalky, badly degraded bone unlikely to yield usable collagen. These were avoided, and only those individuals that were in relatively good condition were sampled. These circumstances resulted in a final count of 33 samples for Cotocollao, far less than the total of ca. 179 burials recovered (see Table 5.2 and Table 5.3).

Table 5.2 - List of human samples from the early settlement of Cotocollao

Sample ID	Bone	Sex	Age[†]
PCO-14	Long bone	M	Middle Adult (30-40 years)

[†] Age range estimated by Ubelaker (1978)

Table 5.3 - List of human samples from the late settlement of Cotocollao

Sample ID	Bone	Sex	Age†
PCO-01	Femur	ND	Young Adult (20-30 years)
PCO-02	Femur	ND	Young Adult (23-30 years)
PCO-03	Femur	ND	Young Adult (25-30 years)
PCO-04	Humerus	F	Old Adult (40-60 years)
PCO-05	Humerus	M	Middle Adult (30-35 years)
PCO-06	Femur	M	Middle Adult (20-40 years)
PCO-07	Femur	M	Young Adult (25-35 years)
PCO-08	Long bone	ND	Young Adult (20-25 years)
PCO-09	Tibia	ND	Adult‡
PCO-10	Femur	ND	Young Adult (25-30 years)
PCO-11	Humerus	F	Young Adult (20-30 years)
PCO-12	Long bone	ND	Adult‡
PCO-13	Long bone	F	Young Adult (22-25 years)
PCO-15	Skull	M	Young Adult (20-30 years)
PCO-16	Skull	ND	Middle Adult (ca. 45 years)
PCO-17	Tibia	ND	Adult‡
PCO-18	Humerus	ND	Young Adult (18-25 years)[19]
PCO-19	Humerus	F	Old Adult (40-60 years)

†Age ranges are those presented by Ubelaker (1978). ‡ Ubelaker (1978) could not perform a sex and age estimation of these individuals.

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[19] Bone sample extracted from a multiple burial with two adults. Ubelaker (1978) could not perform a sex estimation of these individuals. To avoid sampling the same individual twice, only one sample was collected.

Table 5.3 (continued) – List of human samples from the late settlement of Cotocollao

Sample ID	Bone	Sex	Age†
PCO-20	Skull	M	Young Adult (25-30 years)
PCO-21	Skull	ND	Middle Adult (35-45 years)
PCO-22	Skull	M	Young Adult (ca. 25 years)
PCO-23	Rib	F	Old Adult (35-60 years)
PCO-24	Rib	ND	Young Adult (20-35 years)
PCO-25	Rib	M	Middle Adult (30-35 years)
PCO-26	Rib	ND	Subadult (ca. 17 years)
PCO-27	Rib	ND	Subadult (15-18 years)
PCO-28	Mandible	ND	Subadult (ca. 13 years)
PCO-29	Left clavicle	F	Middle Adult (35-45 years)
PCO-30	Humerus	M	Old Adult (40-60 years)
PCO-31	Radius	F	Young Adult (25-30 years)
PCO-32	Left humerus	M	Young Adult (22-25 years)
PCO-33	Left humerus	ND	Adult[20]

†Age ranges are those presented by Ubelaker (1978)

5.1.3. Human Bone Samples from Las Orquídeas

The third group of samples was collected from Las Orquídeas, the only archaeological site of this study situated in Imbabura Province. It has been dated to around 800 – 400 cal

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[20] Bone sample collected from a multiple burial with three adults. Ubelaker (1978) identified one of the individuals as a male of 22 - 25 years old. The sex or age of the other individuals could not be estimated. To avoid sampling an individual twice, a single sample was collected.

BC and thus corresponds to the Late Formative Period. A total of eight human burials have been discovered to date, all of which were sampled for the present study (see Table 5.4). Most of these individuals were found in single primary internments; therefore there was no risk of sampling an individual twice. The remains were almost complete and in good condition.

Table 5.4 - List of human samples from Las Orquídeas

Sample ID	Bone	Sex	Age
POR-01	Rib	F	Young Adult (14-23 years)
POR-02	Rib	F	Middle Adult (30-34 years)
POR-03	Rib	ND	Subadult (14-18 years)
POR-04	Rib	F	Young Adult (20+ years)
POR-05	Rib	ND	Subadult (11-12 years)
POR-06	Rib	M	Old Adult (50+ years)
POR-07	Rib	ND	Subadult (6-7 years)
POR-08	Rib	M	Young Adult (24-29 years)

5.2. Faunal Samples

As reviewed in Chapter 4, the stable isotope analysis of local fauna is important for establishing baseline values for the interpretation of human stable isotope values. To this end, a total of 19 faunal samples were collected for this study, all of them from the Formative Period.

Unfortunately, to this day no faunal remains have been recovered from Rancho Bajo; so faunal samples were only collected from Cotocollao and Las Orquídeas. The original study plan was to collect a large number of faunal samples from Cotocollao; but in the end only three were obtained from this site, due to complications encountered while sampling. Chief among them is that fact that many of the faunal remains were coated with varnish more than twenty years ago in an attempt to preserve them. This is a fairly common procedure that unfortunately made them unusable for stable isotope analysis.

Fortunately, Las Orquídeas, a Formative site in Imbabura Province about 100 kilometres from Cotocollao and Rancho Bajo, provided a good faunal sample. This site has a large faunal collection, most of which is in optimum condition. In order to avoid sampling the same animal, I collected 15 samples from different deposits. The Las Orquídeas faunal remains have not yet seen a full formal study; however, identification of the taxa represented by many of the bones (including those analysed here) was previously done by Ibis Mery, a student from the Pontificia Universidad Católica del Ecuador.

The faunal samples selected from Cotocollao and Las Orquídeas date to the Late Formative Period. For details of the faunal samples, see Table 5.5 and Table 5.6.

Table 5.5 - Cotocollao Faunal Sample Inventory

Sample ID	Site	Bone	Family	Common name
PCO-34	Cotocollao	Long bone	Cervidae	Possible deer
PCO-35	Cotocollao	Long bone	Cervidae	Possible deer
PCO-36	Cotocollao	Long bone	Cervidae	Possible deer

Table 5.6 - Las Orquídeas Faunal Sample Inventory

Sample ID	Site	Bone	Family	Common name
POR-09	Las Orquídeas	Long bone	Cervidae	Deer
POR-10	Las Orquídeas	Rib	Cervidae	Deer
POR-11	Las Orquídeas	Long bone	Cervidae	Deer
POR-12	Las Orquídeas	Long bone	Cervidae	Deer
POR-13	Las Orquídeas	Rib	Cervidae	Deer
POR-14	Las Orquídeas	Os coxae	Leporidae	Hare/Rabbit
POR-15	Las Orquídeas	Os coxae	Leporidae	Hare/Rabbit
POR-16	Las Orquídeas	Os coxae	Leporidae	Hare/Rabbit
POR-17	Las Orquídeas	Femur	Leporidae	Hare/Rabbit
POR-18	Las Orquídeas	Femur	Leporidae	Hare/Rabbit
POR-19	Las Orquídeas	Sternum	Camelidae	Llama/Alpaca
POR-20	Las Orquídeas	Sternum	Camelidae	Llama/Alpaca
POR-21	Las Orquídeas	Sternum	Camelidae	Llama/ Alpaca
POR-22	Las Orquídeas	Calcaneus	Camelidae	Llama/ Alpaca
POR-23	Las Orquídeas	Sternum	Caviidae	Guinea pig (Cuy)
POR-24	Las Orquídeas	Long bone	Caviidae	Guinea pig (Cuy)

5.3. Methods

In this section I will discuss the most common methods of collagen preparation used in stable isotope analysis. I will also describe how the human and faunal bone samples from Rancho Bajo, Cotocollao, and Las Orquídeas were processed for collagen extraction.

5.3.1. Commonly Used Collagen Preparation Methods

Since the introduction of bone collagen $\delta^{13}C$ and $\delta^{15}N$ analysis in the 1970s the technique has been widely used to examine human and animal diets (Van der Merwe and Vogel, 1978; DeNiro, 1985; Jørkov et al., 2007). Since then, several methods for collagen have been developed, involving collagen extraction from bone powders or small pieces or "chunks", as well as the variable use of gelatinization, filtration, and ultra-filtration (Sealy et al., 2014).

It has long been recognized that techniques for collagen isolation may have disadvantages in terms of failing to eliminate all the contaminants, or being too severe to the point of destroying all the original collagen (Longin,1971). Contaminants that can affect the stable isotope values of archaeological collagen include soil carbonates, solid sediments, humates, and microscopic plant matter, as well as naturally occurring fractions of bone such as hydroxyapatite, lipids, and non-collagenous proteins that were incompletely removed (Ambrose, 1990). In an attempt to eliminate these contaminants, several techniques have been developed. These include expensive and time-consuming techniques such as the purification of individual amino acids from collagen (Ambrose, 1990). However, there are also simple, robust and inexpensive techniques involving acidification of the bone sample to remove carbonates, phosphates, and fulvic acids. This procedure may be followed by solubilization in hot dilute acid, filtration or centrifugation to remove particulate contaminants, and/or treatment with NaOH to remove base-soluble contaminants such as humic acids and some lipids. However, after these techniques are applied, the collagen may still contain some bone proteins and extraneous organic and inorganic matter; or be degraded enough in itself to change its isotopic profile. Thus, quality checks should be used to confirm the suitability of the material for analysis (Ambrose, 1990).

The earliest commonly used method for archaeological bone collagen preparation was developed by Longin (1971) to extract collagen for radiocarbon dating. The original method involved crushing the bone sample and treating the powder in 8% HCl for around 15 minutes in order to demineralize it and remove some organic contaminants. This step was then followed by heating and stirring the residue in weakly acidic hot water (pH 3) at 90°C, to denature the collagen and form a gelatin solution. The gelatin solution was then centrifuged to eliminate some impurities (Longin, 1971). This method works well, especially with poorly preserved bone (Sealy et al. 2014). In its original formulation it may not eliminate some organic contaminants, so is commonly used with some modifications (Brown et al., 1988; Katzenberg, 2008). DeNiro and Epstein (1981), for example, proposed that in addition to the treatment with HCl, the sample should be soaked in 0.1M NaOH as an extra step for removing humic acids and some lipids. This process is a particularly common modification to the Longin procedure that has become fairly standard in the field (Brown et al. 1988; Ambrose, 1990; Jørkov et al., 2007).

An alternative approach for isolating collagen from archaeological bones and tooth dentine is to demineralize the sample without grinding it first. This approach was first described by Sealy (1986), who prepared bone collagen using slow demineralization of small (1 – 3 g) chunks of bone in 1% or 5% hydrochloric acid (HCl) solution depending on the density of the sample and its appearance. This procedure produced a collagen model or pseudomorph of the sample, which was then soaked in 0.1M sodium hydroxide (NaOH) to remove humic acids (Sealy, 1986). Since Sealy's initial publication, similar methods have been used by many researchers (Katzenberg, 2008). Some of these use other solutions for demineralization, such as ethylenediaminetetraacetic acid (EDTA), a chelating agent capable of removing the mineral fraction from the bone by binding to its calcium ions, leaving a collagen model of the sample behind (e.g., Tuross et al. 1988; Bocherens et al. 1995). Recently Sealy et al. (2014) compared the modified Longin preparation technique to the method described in Sealy (1986); and concluded that the two produce comparable results for well-preserved samples, though the modified Longin method produced better results for very poorly preserved material.

5.3.2. Diagenesis

Before developing any accurate dietary or environmental interpretations based on the isotopic δ^{13}C and δ^{15}N values of bone collagen, diagenesis should be taken into consideration as a factor that can alter samples to variable degrees, potentially affecting their stable isotope values and any dietary interpretations drawn from them (DeNiro, 1985; Ambrose, 1990).

Considering diagenetic alteration is relevant even for apparently well-preserved remains, since “all prehistoric bones have undergone diagenesis, which is the sum of the physical, chemical and biological processes that occur in the post-mortem depositional environment” (DeNiro, 1985: 808). For example, authors such as Piepenbrink (1986) and Hanson and Buikstra (1987) have demonstrated with histological analyses that there may be significant contamination of bone by bacteria, algae, or fungi (Ambrose, 1990). As Gillespie et al. (1984) noted, archaeological bones also often contain excess nitrogen originating from humic substances, soil amino acids, or fertilizers (Ambrose, 1990). The humic substances are frequently cited as a concern (Van Der Haas et al. 2018), and deserve specific attention. As characterized by van Klinken and Hedges (1995) these are dark-coloured, acidic materials classified into (1) humic acids, soluble in weak alkali; (2) fulvic acids; and (3) humin not extractable by weak alkali or acid. These substances are formed from plant and animal matter by a process known as humification. It has been suggested that in ancient bone (buried bone) there are two different reasons behind the presence of humic substances: (1) influx of humics from the soil, and (2) *in situ* humification of bone organic matter (predominantly collagen); these likely occur simultaneously, and may interact or amplify each other (van Klinken and Hedges, 1995).

Since stable isotope analysis was first adopted, experiments have led to the use of several different indicators for detecting the diagenetic alteration and contamination of collagen, including atomic carbon to nitrogen ratio, amino acid composition, and collagen concentration in whole bone (DeNiro, 1985; Ambrose, 1990; van Klinken and Hedges, 1995). The preservation indicators used in this study will be described in the following sections.

5.3.2.a. Collagen yield

Collagen yields are calculated as the weight of the dried prepared collagen divided by the starting dry bone weight, expressed as a percentage (van Klinken, 1999; Sealy et al., 2014)

According to van Klinken (1999) modern, fresh bone contains about 22 wt% collagen. However, in buried bones the collagen content drops gradually at a speed determined by climatic conditions and other aspects of the burial environment such as tomb construction (Hedges 2002; Holmes et al. 2006). Thus, for example, in a tropical region with high temperatures and substantial precipitation, bones will likely lose their collagen relatively quickly. In the end, the collagen content drops low enough to make the material problematic for analysis because the remaining collagen is often badly degraded, and the removal of contaminants is more difficult because these represent a larger proportion of the total organic material remaining. Researchers debate the exact yield value at which bones should be considered "low collagen" and not suitable for analysis, with proposed limits ranging from 0.5% to 5%; there is good support for a cut-off value of 1-2% (Ambrose, 1990; van Klinken, 1999; Dobberstein et al. 2009; Sealy et al. 2014).

5.3.2.b. Collagen model quality/texture

Since bone preservation can vary greatly within individuals or from one prehistoric site to another, the texture of the collagen models generated by a whole-chunk preparation method can also display great variability, ranging from solid or firm, through rubbery or soft, to fragmentary; a very poorly preserved bone will fail to form anything, dissolving into strawlike or sandy particles (Pennycook, 2008). The above information, along with any observations that suggest the possible presence of sediments or rootlets in the particular sample, is important to record because it contributes to a better understanding of the characteristics and quality of each sample.

As Sealy et al. (2014) have stated, the demineralization of well-preserved bone chunks tends to yield a piece of collagen of similar size and shape to that of the original piece of bone but translucent and flexible, which they refer to as a "pseudomorph". A pseudomorph of this quality strongly suggests good collagen preservation capable of keeping full structural integrity. In contrast, the demineralization of very poorly preserved bones does not yield a pseudomorph but what they have referred to as amorphous globules or threads that float. This occurs because the remaining collagen molecules are too degraded to hold together, since the bone has undergone significant diagenesis. Bones between the extremes of excellent and very poor preservation produce pseudomorphs of varying degrees of softness. In some cases, the bone sample may be contaminated by rootlets, requiring additional preparation in order to make it suitable for analysis (Sealy et al., 2014).

As Pennycook (2008:112) comments, many researchers do not describe the appearance of their collagen models or use this appearance as an indicator of diagenesis, apart from the basic precaution of rejecting samples that fail to form a pseudomorph at all. However, because the appearance of the prepared collagen does correspond to a degree with preservation (Garvie-Lok, personal communication), it is useful to track and was incorporated into this study.

5.3.2.c. Carbon to nitrogen ratio

The atomic carbon to nitrogen ratio of a collagen sample, usually expressed as the C/N ratio or C:N ratio, is a useful indicator of preservation. Pure intact collagen falls within a characteristic C/N range that reflects its molecular composition; departures from this range can indicate problems, including contamination with extraneous organic carbon (Van Klinken and Hedges, 1995).

DeNiro (1985) was the first to examine whether bone collagen stable isotope ratios could be altered by post-mortem processes. After conducting several experiments, he observed that archaeological collagen C/N ratios were influenced by the preservation of the sample. Based on his observations, he argued that bone collagen with atomic C/N ratios between 2.9 and 3.6 has not undergone diagenetic alteration that could cause significant isotopic shifts, so those samples can be useful for stable isotope analysis (DeNiro, 1985). After his research was published, C/N ratios came into common use for tracking collagen sample quality; and continue to be one of the most frequently consulted indicators, although some researchers argue for a narrower acceptable range of 3.1 to 3.5 (van Klinken 1999; see reviews by Ambrose, 1990; Katzenberg, 2008 and Lee-Thorp, 2008).

5.3.2.d. Carbon and nitrogen content

While the proportion of carbon to nitrogen (in the form of atomic C/N) is the most commonly used indicator of preservation, the sample's absolute carbon and nitrogen content is also a useful clue. The absolute carbon and nitrogen content of a collagen sample are expressed as the percentage of carbon and nitrogen (by % dry weight) of the combusted 'collagen', and are usually abbreviated as %C and %N (van Klinken, 1999).

Ambrose (1990) was the first to argue that %C and %N should be used as collagen sample quality indicators for stable isotope analysis. Based on an analysis of the composition of a large set of archaeological collagen samples, he argued that a %C range of 15% to 47% for carbon suggests the sample is in good enough condition for analysis. In terms of nitrogen content, he argued that samples with %N ranging from 5% to 17% should be considered as of good quality. Ambrose's discussion of %C and %N alteration largely focused on the tendency of both measures to decrease in very poorly preserved collagen.

The use of %C and %N as quality indicators was also discussed in depth by van Klinken (1999). Based on a large survey of collagen samples previously analysed for radiocarbon dating and stable isotope analysis, van Klinken (1999) argued that intact collagen samples display characteristic %C values of around 35%. Like Ambrose (1990), he suggests that lowered %C values are problematic, reflecting the presence of inorganic contaminants in the extracted collagen. He argued for a higher minimum acceptable %C value than Ambrose, suggesting that when %C values drop to under 30%, and appear along with low collagen yields and abnormal C:N ratios is it because the bone is poorly preserved and the sample should be rejected. For %N, he argues that acceptable collagen should show a %N value above 11%. In contrast to Ambrose, van Klinken also cautions that samples should be watched for excessive carbon or nitrogen content ranging much above the %C values of 34.8±8.8 and %N values of 11% - 16% typical of well-preserved collagen.

5.3.3. Bone Collagen Preparation

The collagen preparation method chosen for this thesis was the standard method used for stable isotope analyses in this department, which essentially follows the whole-chunk method described by Sealy et al. (2014). A small piece of dry bone from each of the human and faunal samples was cut using a Dremel tool with a diamond wheel. The size of each piece ranged between 200 mg and 1000 mg. The outer and inner surfaces were cleaned using the same tool with a grinding bit to remove contaminants such as soil. Then the bone chunks were placed in small glass jars that were partially filled with single distilled water, and sonicated for 10 minutes in an ultrasonic cleaner. This part of the process was repeated twice, changing the distilled water each time until it stayed clear. The accessories of the Dremel tool were also cleaned in the ultrasonic cleaner between samples in order to reduce the risk of cross-contamination. After being rinsed, the samples were left in small petri dishes to air dry for a few days. Once the samples were dry, their weight was recorded.

The next step consisted of placing each chunk of bone in a lidded glass jar filled with 80ml to 100 ml of 1% HCl solution. The solution was changed every two days, until the bone was demineralized. Due to the poor preservation of some of the samples, they began to fragment; and it was necessary to employ glass fiber filters to drain the HCl. Notes were taken every time the solution was changed; in order to document the changes observed in the sample and the solution in terms of color, the presence or absence of bubbles and fragments, the consistency of the sample and its translucency, as well as any additional information that could be relevant.

Once the bone was completely demineralized, the HCl was drained, the jar was rinsed, and the sample was soaked in two changes of distilled water with a wait of 24 hours between rinses, with the purpose of removing as much of the residual HCl as possible. Then the distilled water was drained, and the samples were soaked in 80ml of 0.125M NaOH for 20 hours in order to remove humic acids stemming from soil contaminants. After the 20 hours in NaOH solution, any changes observed in the color of the sample and solution were documented. Immediately after this process, the NaOH was drained and replaced with distilled water. As before, the sample was left in the distilled water to soak and remove as much of the NaOH as possible. This part of the process was repeated twice, with each water soak lasting 24 hours. When these rinses were done and the pH of the sample was neutral, the water was drained and the samples were placed in small weighed scintillation vials. At this point a final note of the collagen model texture was made for each sample. The collagen model texture was graded into one of four categories: solid/firm, deformable/rubbery, soft or fragmentary (Garvie-Lok, personal communication 2018). The purpose of these categories is to facilitate comparing the quality of the samples, and identifying samples in which the collagen quality might be dubious. After this final observation, the vials were placed in a freezer. Once the samples were frozen, they were freeze-dried for 24 hours. Once the samples were completely dry, the filled sampled vials that were weighed. This final weight was used along with the empty vial weights and the initial dry bone sample weight to determine the collagen yields (dry collagen weight divided by initial sample weight, expressed as a percentage).

The final step of the initial preparation process consisted of taking the samples to the Biogeochemical Analytical Service Laboratory (BASL) of the University of Alberta (Department of Biology, director Dr. Mingsheng Ma) and packing them for mass spectrometry. For each sample an amount of collagen ranging between 0.950 mg and 1.050 mg was weighed using a microbalance and packed into 3.5x5 mm tin foil capsules that were folded into a spherical shape. The stable isotope analysis was performed using an EuroVector EuroEA Elemental Analyzer coupled with an Isoprime Mass Spectrometer (Biogeochemical Analytical Services Laboratory, 2018). Readings from this equipment generated the stable carbon and nitrogen isotope values along with the mass %C and %N values. Atomic C/N values were calculated using the mass %C and %N values and a standard conversion factor.

5.3.4. Additional Step: Collagen Gelatinization

Once the carbon and nitrogen stable isotope values along with the %C, %N, C/N, and yield values were available, these were compared sample by sample, consulting the notes on the appearance and texture of the collagen models. After it was noted that a number of the more poorly preserved samples appeared to retain some organic contamination (based on high %C and %N values), it was decided to gelatinize and centrifuge the samples and repeat the $\delta^{13}C$ and $\delta^{15}N$ analyses.

As explained above, the purpose of this step is to eliminate possible contaminants present in the collagen; thus it was used in this study to confirm that the isotopic values obtained were not affected by possible contaminants. Collagen samples were gelatinized by heating in acidulated water, a method long used in collagen preparation (Longin, 1971; Sealy et al. 2014).

The freeze-dried collagen left from the first analytical run was reweighed and soaked in double distilled water for three days to rehydrate it. Then, using a pipet, I reduced the amount of water of the scintillation vials and transferred the samples with a small amount of water to 15 ml plastic centrifuge tube vials. These were filled with pH3 water and placed in a water bath for 48 hours at 80°C. After those 48 hours, the vials were taken out of the hot water and left in a fume hood for a few minutes until the vials reached room temperature. The next step consisted of centrifuging for 8 minutes in order to drive the insoluble impurities to the bottom of the vial. Pellets were left in the tube vials, and placed into the freezer for potential future analysis. Meanwhile, the collagen solution was transferred again to the scintillation vials, frozen, then freeze-dried and reweighed. The gelatinized collagen was then taken to the BASL, where from each sample an amount of collagen ranging between 0.950 mg and 1.050 mg was packed into 3.5x5 mm tin foil capsules folded in a spherical shape. The capsules were submitted again for stable isotope analysis using a Vario Pyrocube Elemental Analyzer coupled with an Isoprime Vision Mass Spectrometer (Kwan, personal communication). As reported in the results chapter (Chapter 6), comparing the $\delta^{13}C$ and $\delta^{15}N$ values of the first and second runs confirmed that in most cases the initial $\delta^{13}C$ and $\delta^{15}N$ values had been good even for poorly preserved samples; however, in some cases the additional treatment appears to have removed contaminants and improved the accuracy of the analysis.

5.4. Summary

This section detailed the context and number of human and faunal samples collected for this research. A total of 75 bone samples were selected from the archaeological sites Rancho Bajo, the Early and Late settlements of Cotocollao, and Las Orquídeas, of which 56 are human bone samples and 19 are faunal bone samples. Faunal samples could not be collected from Rancho Bajo, making a total of 15 human samples the only material analysed from this site. Another 33 human samples were taken from individuals buried in Cotocollao, along with three additional faunal samples. From Las Orquídeas eight human samples and 16 faunal bone samples were collected. This chapter also discussed the main methods used for collagen extraction for stable isotope analysis, and detailed the process that was followed at the laboratory to extract the collagen and determine its $\delta^{13}C$ and $\delta^{15}N$ values.

Chapter 6: Results and Assessment of Sample Preservation

This chapter presents the results of the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis of human and faunal samples from Rancho Bajo, Cotocollao, and Las Orquídeas. The values for each of the collagen preservation indicators (collagen yields, C:N atomic, %C, and %N) used in this study are also detailed in this section.

From the original 75 samples (56 human and 19 faunal) that were collected for this study, a total of 19 samples had to be discarded. Some of these samples had to be discarded due to their poor preservation that caused the preparation for the extraction of collagen to fail; while a few other samples had to be rejected after the collagen was analyzed, as they failed to meet acceptable quality indicator values. An explanation and discussion regarding why these samples did not meet the diagenesis indicators will also be presented.

As explained in Chapter 5, after examining the $\delta^{13}C$ and $\delta^{15}N$ values along with the %C, %N, C/N, and collagen yield it was noted that some of the samples apparently retained some contaminants, an observation that was based on their high %C and %N values. Thus it was decided to gelatinize and centrifuge the collagen and repeat the $\delta^{13}C$ and $\delta^{15}N$ analyses in order to confirm that the isotopic values obtained were not affected by these possible contaminants. After this additional step, the majority of the samples produced enough collagen for a second analysis. In the second set of measurements, a number of samples produced lower %C and %N values, suggesting that contaminating material was removed. In most cases, measurements before and after gelatinization and centrifuging changed very little, indicating that this material had not been distorting the $\delta^{13}C$ and $\delta^{15}N$ values. In a few cases, $\delta^{13}C$ and $\delta^{15}N$ values changed significantly. The repeat measurements are considered site by site in this chapter. Full tables of values for both the first and second analyses can be found in Appendix 2.

6.1. Bone Collagen Preservation

The bone collagen preservation assessments for each site are presented and discussed below.

6.1.1. Rancho Bajo Human Samples

The majority of human samples from Rancho Bajo were of bad apparent quality, with a chalky appearance; however, most of them displayed collagen yields and quality indicator values that can still be considered acceptable for analysis. Only two samples out of 15 that were collected from this site had to be excluded from the study.

The two samples that had to be excluded from the analysis are PRB-06 and PRB-07. These immediately dissolved after being left in the 1% HCl treatment, demonstrating that there was no longer usable collagen present in these samples (see Table 6.1). The other samples produced collagen that could be submitted for analysis. In several cases the amount produced was too small, making repeat analysis impossible. The stable isotope values did not change much (variation of 0.5‰ or less) for most samples that could be repeated, and the changes did not occur in a consistent direction. This result is consistent with typical re-runs of acceptably preserved material, suggesting that both the initial and repeat runs produced good values. The only exception is PRB-10, in which the %C and %N values rose; but the remainder of this sample used for the repeat run consisted of a very small amount of collagen of unusual appearance. For this reason, the value of the first run was used. For those samples that could not be analysed in the second run, the values of the first run were also used.

As discussed in Chapter 5, samples with collagen yields below 2% should be treated with caution, and samples with collagen yields below 1% are truly not suitable for analysis. Most of the Rancho Bajo samples have collagen yields that fall between 3.41% and 8.24% with a mean of 5.77%, suggesting adequate preservation (see Table 6.2). The one exception is sample PRB-02. This sample presented a collagen yield of 41.45%, which is far above the collagen content of even fresh bone (van Klinken 1999). There are two possible explanations for this issue. The first may be that an error was made during the weighing of the initial chunk of bone; the second is that the presence of exogenous materials in the sample increased the final yield. However, the second explanation is unlikely. A yield of 41.45% would require most of the prepared sample to be contaminant; but the prepared material from PRB-02 was similar to other Rancho Bajo samples in appearance, and two measurements (before and after gelatinization) produced C/N, %C and %N values consistent with adequately preserved collagen. It was decided to retain the sample on the assumption that the problem was an error in the initial weighing step. With this decision, a set of 13 Rancho Bajo samples were left for further consideration (see Tables 6.1 and 6.2).

Table 6.1 - Rancho Bajo human samples rejected from study

Sample ID	Collagen Data				
	Yield (%)	C:N (atomic)	%C	%N	Reason
PRB-06	-	-	-	-	Sample preparation failed
PRB-07	-	-	-	-	Sample preparation failed

Table 6.2 - Rancho Bajo human collagen data

Sample ID	Collagen Data					
	Yield (%)	$\delta^{13}C$ (‰PDB)	$\delta^{15}N$ (‰AIR)	C:N (atomic)	%C	%N
PRB-01	3.41	-18.4	7.9	3.27	42.0	15.0
PRB-02	41.45	-18.4	8.5	3.25	42.3	15.2
PRB-03	4.63	-18.7	8.1	3.32	41.3	14.5
PRB-04	4.93	-18.3	7.8	3.27	42.4	15.1
PRB-05	5.17	-18.7	8.4	3.40	35.6	12.2
PRB-08*	8.24	-18.3	8.5	3.32	45.6	16.0
PRB-09*	7.07	-18.2	8.5	3.36	45.2	15.7
PRB-10*	8.00	-18.4	8.5	3.34	44.9	15.7
PRB-11*	5.89	-18.5	8.3	3.37	43.0	14.9
PRB-12*	5.56	-18.6	8.0	3.32	45.8	16.1
PRB-13*	4.46	-18.1	7.7	3.32	41.8	14.7
PRB-14*	5.45	-18.5	8.4	3.44	36.9	12.5
PRB-15*	6.44	-18.3	8.5	3.35	42.5	14.8

* Run 1 value.

The other preservation indicators (%C, %N, and C/N) were compared to literature values for acceptable collagen. Following Ambrose (1990), samples were also checked for any relationship between stable isotope and preservation indicator values that could reveal problems. These pairwise checks can also be found in Appendix 2.

All %C values fall above the 30% minimum recommended by Ambrose (1990). Most fall within van Klinken's (1999) range for acceptably preserved collagen (34.8±8.8); samples PRB-08, PRB-09, PRB-10 and PRB-12 fall slightly above it. All %N values fall above the 11% minimum recommended by Ambrose (1990) and van Klinken (1999). Most also fall under van Klinken's (1999) suggested maximum of 16%; PRB-12 falls very slightly above it. All sample C/N values fall into van Klinken's (1999) acceptable range of 3.1 - 3.5. With no relationships of concern identified between the preservation indicators and the stable isotope values (see Appendix 2), it was concluded that the 13 values in Table 6.2 are dependable and can be used.

6.1.2. Cotocollao Human and Faunal Samples

The Cotocollao human samples were characterized by fair to poor preservation. Of the 33 human samples collected, only samples PCO-07 and PCO-08 failed to produce collagen during the initial preparation for collagen extraction. Of the three faunal samples collected, samples PCO-34 and PCO-36 also failed to produce any collagen. Data for the rest of the samples are presented in Table 6.3 and Table 6.4.

Cotocollao was one of the sites whose samples provoked concern over high %C and %N values. After the samples were gelatinized, centrifuged, and rerun, most of the %C and %N values declined to more usual levels. In some cases, sample $\delta^{13}C$ and $\delta^{15}N$ values changed very little. However, 13 samples showed an increase in $\delta^{13}C$ of 1‰ or more in the second run. For some of those samples, $\delta^{15}N$ values also increased. These changes were strongly patterned by initial sample $\delta^{13}C$ value. Of the 11 samples whose $\delta^{13}C$ values changed little in the second run, 10 had initial $\delta^{13}C$ values of -18.5‰ or higher. Of the samples whose $\delta^{13}C$ values changed, 12 had $\delta^{13}C$ values in the initial run below -19‰. This pattern suggests very strongly that gelatinizing and centrifuging the samples removed a contaminant with low $\delta^{13}C$ values, perhaps fine particles of plant matter. Given this result, it is clear that the second run values should be used. Eight samples did not produce enough material to be analysed in the second run. After some consideration, it was decided to use the first run values for samples with δ^{13}C values above -19‰, but to mark them so that any unusual patterning could be tracked (see Table 6.3).

Table 6.3 - Cotocollao human collagen data

Sample ID	Collagen Data					
	Yield (%)	δ^{13}C (‰PDB)	δ^{15}N (‰AIR)	C:N (atomic)	%C	%N
PCO-01*	2.35	-18.2	7.5	3.31	46.2	16.3
PCO-02	1.64	-18.4	8.0	3.27	42.9	15.3
PCO-03	4.26	-18.4	8.8	3.25	42.9	15.4
PCO-04	8.48	-18.5	9.0	3.29	43.4	15.4
PCO-05*	4.66	-17.5	7.9	3.30	42.7	15.1
PCO-09	6.15	-18.5	9.6	3.29	43.4	15.4
PCO-10	6.94	-19.0	8.4	3.34	41.2	14.4
PCO-11	8.54	-18.5	9.8	3.25	42.9	15.4
PCO-13	46.07	-18.2	8.3	3.22	43.0	15.5
PCO-14	5.09	-18.8	9.0	3.41	40.0	13.7
PCO-15	4.35	-18.2	9.8	3.27	42.1	15.0
PCO-16	6.59	-19.0	9.1	3.28	43.3	15.4
PCO-17	5.75	-17.6	8.7	3.24	43.2	15.5
PCO-18	7.73	-18.4	7.8	3.20	42.7	15.6
PCO-21	3.87	-17.7	9.3	3.26	43.0	15.4
PCO-22	1.93	-18.5	9.6	3.25	42.7	15.3
PCO-23	8.37	-18.0	8.9	3.25	43.2	15.5
PCO-24	6.68	-18.1	8.2	3.27	44.0	15.7
PCO-25*	12.19	-18.4	8.9	3.28	46.7	16.6

* Run 1 values

Table 6.3 (continued) – Cotocollao human collagen data

Sample ID	Collagen Data					
	Yield (%)	δ^{13}C (‰PDB)	δ^{15}N (‰AIR)	C:N (atomic)	%C	%N
PCO-26*	14.76	-16.5	7.9	3.30	46.7	16.5
PCO-27	17.47	-18.2	8.5	3.28	41.3	14.7
PCO-29*	2.44	-18.5	9.4	3.28	46.4	16.5
PCO-30	4.28	-18.4	9.4	3.26	42.8	15.3
PCO-31	5.33	-18.2	9.3	3.24	42.8	15.4
PCO-32	5.82	-17.9	8.8	3.23	41.6	15.0
PCO-33	5.08	-18.3	8.6	3.26	41.9	15.0

* Run 1 values

Table 6.4 - Cotocollao faunal collagen data

Sample ID	Collagen Data					
	Yield (%)	δ^{13}C (‰PDB)	δ^{15}N (‰AIR)	C:N (atomic)	%C	%N
PCO-35	1.34	-19.6	5.0	3.30	40.2	14.2

Samples PCO-19 and PCO-20 had to be rejected, based on their particularly low collagen yields that fall below 1%, indicating their collagen was poorly preserved and are not suitable for analysis. PCO-28 showed a combination of low yield and high %C, and also had to be excluded (see Table 6.5). Two other samples also displayed low collagen yields. PCO-02 displayed 1.64% and PCO-22 displayed 1.92%. However, given that these values are still above the limit suggested by van Klinken (1999), and that these samples met all the other quality indicators, they were not rejected.

Table 6.5 - Cotocollao human and faunal samples rejected from study

Sample ID	Collagen Data				
	Yield (%)	C:N (atomic)	%C	%N	Reason
PCO-07	-	-	-	-	Sample preparation failed
PCO-08	-	-	-	-	Sample preparation failed
PCO-06	6.31	3.26	46.7	16.7	Possible contamination*
PCO-12	3.73	3.40	43.0	15.5	Possible contamination*
PCO-19	0.86	3.34	41.1	14.4	Yield
PCO-20	0.95	3.43	40.9	13.9	Yield
PCO-28	1.12	3.29	47.7	16.9	Yield and %C
Faunal samples					
PCO-34	-	-	-	-	Sample preparation failed
PCO-36	-	-	-	-	Sample preparation failed

* See detailed discussion in text

Sample PCO-13 displayed an extremely high collagen yield (46.07%) similar to that of PRB-02 from Rancho Bajo (41.45%), which also falls far above the collagen content of fresh bone (van Klinken, 1999). The most likely explanation for this issue is a measurement error made during the weighing of the initial chunk of bone. Given that the C:N, %C, and %N values obtained before and after gelatinization were always consistent with adequately preserved collagen, it was decided to include this sample in the study.

In the Cotocollao human samples, after excluding sample PCO-28 due to its high %C as mentioned above, the %C values range from 40.0% to 46.7%. All %C values fall above the 30% minimum recommended by Ambrose (1990). Most fall within van Klinken's (1999) range for acceptably preserved collagen (34.8±8.8); samples PCO-1, PCO-25, PCO-26 and PCO-29 fall slightly above it. The %N values range from 13.7% to 16.7%. All fall above the

11% minimum. Most also fall under 16%; again, PCO-1, PCO-25, PCO-26 and PCO-29 fall slightly above it. The C:N values range from 3.20 to 3.41; all of these, including PCO-1, PCO-25, PCO-26 and PCO-29, are within acceptable limits. Given these results and no apparent relationships between preservation indicators and stable isotope values (see Appendix 2), the data set in Tables 6.3 and 6.4 – 26 human samples and one faunal sample – is accepted.

6.1.3. Las Orquídeas Human and Faunal Samples

The human and faunal samples from Las Orquídeas were in an excellent state of preservation. All eight human samples produced collagen; and of the 24 faunal samples collected, only three failed to produce collagen. These were sample POR-21, a burnt camelid bone, and samples POR-23 and POR-24, burnt Cavia (cuy) bones. All of these disintegrated during collagen preparation, presumably due to heat damage to the collagen (see Table 6.6). Detailed information about the human samples can be found in Table 6.7, while information about the faunal samples can be found in Table 6.8. A full set of values for the first and second runs is in Appendix 2.

This sample series was the other that produced elevated %C and %N values, more so than Cotocollao. As with Cotocollao, gelatinizing and centrifuging the samples lowered the %C and %N values to more usual levels. Here, however, no significant changes were seen in sample stable isotope values. This result may reflect the fact that the Las Orquídeas samples were in a much better state of preservation, with higher collagen yields, so that any contaminants present did not have as much of an effect. For this site, all samples could be repeated, and all values from the second run were used. Details of the samples excluded from the study are presented in Table 6.8.

Table 6.6 - Las Orquídeas samples rejected from study

Sample ID	Collagen Data				
	Yield (%)	C:N (atomic)	%C	%N	Reason
Faunal Samples					
POR-21	-	-	-	-	Burnt bone, sample preparation failed
POR-23	-	-	-	-	Burnt bone, sample preparation failed
POR-24	-	-	-	-	Burnt bone, sample preparation failed

Table 6.7 - Las Orquídeas human collagen data

Sample ID	Collagen Data					
	Yield (%)	$\delta^{13}C$ (‰PDB)	$\delta^{15}N$ (‰AIR)	C:N (atomic)	%C	%N
POR-01	11.54	-11.8	9.0	3.23	43.5	15.7
POR-02	11.31	-15.8	6.9	3.26	42.7	15.3
POR-03	16.22	-15.7	8.1	3.22	43.6	15.8
POR-04	10.35	-16.2	6.7	3.27	43.1	15.4
POR-05	5.81	-15.5	7.3	3.26	43.1	15.4
POR-06	21.63	-15.8	9.2	3.21	43.5	15.8
POR-07	8.56	-16.4	5.7	3.26	43.2	15.5
POR-08	6.39	-15.7	7.3	3.26	43.7	15.6

Table 6.8 - Las Orquídeas faunal collagen data

Sample ID	Collagen Data					
	Yield (%)	$\delta^{13}C$ (‰PDB)	$\delta^{15}N$ (‰AIR)	C:N (atomic)	%C	%N
POR-09	11.57	-20.3	2.6	3.22	43.5	15.8
POR-10	12.68	-17.3	6.2	3.23	43.6	15.7
POR-11	5.92	-16.8	4.5	3.23	43.0	15.5
POR-12	10.18	-19.7	4.0	3.22	43.6	15.8
POR-13	13.81	-19.0	5.9	3.22	43.6	15.8
POR-14	11.05	-24.4	3.5	3.24	43.3	15.6
POR-15	15.55	-20.0	3.7	3.23	43.2	15.6
POR-16	11.32	-20.1	1.9	3.22	42.9	15.5
POR-17	10.98	-20.2	2.3	3.22	43.4	15.7
POR-18	6.16	-19.0	1.8	3.24	43.1	15.5
POR-19	12.82	-19.6	5.5	3.22	43.4	15.7
POR-20	15.57	-19.7	7.1	3.19	43.6	15.9
POR-22	15.51	-18.6	4.8	3.20	43.1	15.7

All the %C values fall above the 30% minimum recommended by Ambrose (1990), as these range from 42.7% to 43.7%. They also fall within the range recommended by van Klinken (1999). In terms of the %N values, these range from 15.3% to 15.9%; and thus fall within the 11% to 16% range recommended by Ambrose (1990) and van Klinken (1999). The C:N values range from 3.19 to 3.27, falling within the acceptable range of 3.1 - 3.5. The collagen yields of both humans and animals ranged from 6.2% to 21.6%, thus falling above the minimum acceptable value of 2% (Ambrose, 1990). With these values checked, and no relationships of concern between preservation indicators and stable isotope values (see

Appendix 2), all values in Tables 6.7 and 6.8 are accepted for a total of eight human and 13 faunal values.

6.2. Summary

Of the 15 human samples collected from Rancho Bajo, only two samples had to be rejected from this study. A total of 36 samples (33 human and 3 faunal) were collected from Cotocollao. From these samples, 10 had to excluded, including, unfortunately, two faunal samples. The samples from Las Orquídeas were the best preserved of the three sites. Of the 24 samples collected, only three could not be analyzed. Based on the $\delta^{13}C$ and $\delta^{15}N$ values of the samples presented in this chapter, the interpretation of the diet at Rancho Bajo, Cotocollao, and Las Orquídeas is presented in Chapter 7.

Chapter 7: Interpreting the Diet at Rancho Bajo, Cotocollao, and Las Orquídeas Using $\delta^{13}C$ and $\delta^{15}N$ Values

This chapter discusses the results of the $\delta^{13}C$ and $\delta^{15}N$ analyses. It has been structured to present the values for the human samples from Rancho Bajo, followed by the results from Cotocollao, and the values from the human and faunal samples from Las Orquídeas. The purpose of this chapter is to first identify general intra-site dietary patterns, as well as to observe any age or sex-based dietary differences. Later, the chapter compares and discusses the $\delta^{13}C$ and $\delta^{15}N$ values of the three sites examined by this study, in order to observe any dietary trends or changes that may have occurred over the Formative Period. In addition, the results from this analysis are compared to the results obtained by two prior stable isotope studies conducted in the region. The first study (Ubelaker et al., 1995) examined diet at La Florida, a site of the Regional Development Period; the second study was carried out by Pennycook (2013), who examined diet at the sites Tajamar and NAIQ from the Integration Period (see Chapter 2). These sites are all located in the northern highlands in the Quito Basin, where Rancho Bajo and Cotocollao are also situated. The Regional Development Period and the Integration Period followed the Formative (in that order). Examining all of their data together can reveal dietary patterns in Ecuador's northern highlands over three consecutive periods.

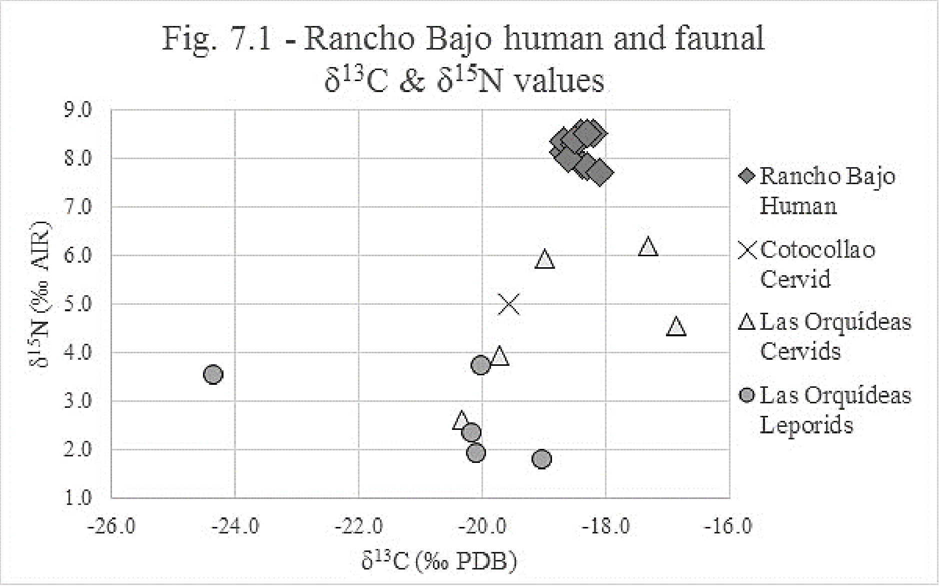
7.1. Rancho Bajo Human Values

Collagen $\delta^{13}C$ and $\delta^{15}N$ values for each sample can be found in Table 7.1, along with site means and standard deviations. Sex and age estimates of the individuals have also been included. Figure 7.1 displays a scatter plot of the values.

Table 7.1 - Rancho Bajo human data

Sample ID	Sex	Age	$\delta^{13}C$	$\delta^{15}N$
PRB-01	M	Middle Adult (35-45 years)	-18.4	7.9
PRB-02	M	Middle Adult (30-40 years)	-18.4	8.5
PRB-03	ND	Subadult (10-15 years)	-18.7	8.1
PRB-04	M	Middle Adult (30-40 years)	-18.3	7.8
PRB-05	M	Young Adult (25-35 years)	-18.7	8.4
PRB-08	M	Adult (30-60 years)	-18.3	8.5
PRB-09	F	Subadult (16-20 years)	-18.2	8.5
PRB-10	M	Young Adult (25-35 years)	-18.4	8.5
PRB-11	M	Young Adult (25-35 years)	-18.5	8.3
PRB-12	M	Middle Adult (30-40 years)	-18.6	8.0
PRB-13	F	Adult (30-60 years)	-18.1	7.7
PRB-14	F	Middle Adult (33-45 years)	-18.5	8.4
PRB-15	ND	Juvenile (14-17 years)	-18.3	8.5
		Mean	**-18.4**	**8.2**
		SD	**0.2**	**0.3**

The $\delta^{13}C$ values of this group range from -18.7‰ to -18.1‰, with a mean of -18.4‰ ± 0.2‰. The $\delta^{15}N$ values ranged from 7.7‰ to 8.5‰, with a mean of 8.2‰ ±0.3‰. Interestingly, all Rancho Bajo $\delta^{13}C$ and $\delta^{15}N$ values are tightly clustered together. The small differences in the $\delta^{13}C$ and $\delta^{15}N$ values between individuals fall within 1‰. These figures are consistent with the minor variations expected in a group eating an isotopically homogenous diet, probably reflecting small differences in individual metabolisms or health (DeNiro and Epstein, 1978; DeNiro and Schoeninger, 1983; Lovell et al., 1986; Ambrose and Norr, 1993). The similarities seen in the isotopic signals suggest that the individuals buried at this site probably shared a consistent set of food resources - basically, that they all consumed a very similar diet.



As mentioned in Chapter 4, collagen $\delta^{13}C$ values of around -20‰ are characteristic of terrestrial C_3 diets, and diets heavily dependent on C_4 resources exhibit $\delta^{13}C$ values of around -10‰ (Price, 2015). The $\delta^{13}C$ mean value of -18.4‰ ± 0.2‰ obtained from the Rancho Bajo human samples suggests that the individuals living at this site consumed mostly C_3 plants, probably wild resources from the area. This conclusion is suggested based on the presence of lithic artifacts and the complete absence of ceramics at the site, features which are characteristic of sites that may have depended less on agriculture (see discussion in Chapter 2). However, there is also the possibility that occasionally the people consumed C_4 plants, although not often enough to raise their $\delta^{13}C$ values very far. This possibility has support from evidence from other stable isotope studies conducted in the Andes and other regions, which show that pure C_3 diets tend to display $\delta^{13}C$ values of about -20‰; and C_3

diets with insignificant contributions from maize show $\delta^{13}C$ values of about -19‰ (Tykot et al., 2006). The $\delta^{13}C$ values seen in Rancho Bajo exhibit a slight elevation of -18.4‰ ± 0.2‰, which may indicate a small contribution of maize to the diet.

Recently some new evidence has been recovered that supports the scenario of a Rancho Bajo diet dominated by C_3 resources but with minor amounts of C_4 plants. The archaeologists Carlos Montalvo, Eric Dyrdahl, and María Fernanda Ugalde, who are currently working on the third field season at Rancho Bajo, recently collected charcoal and seed samples at the site and submitted them to the Center for Applied Isotope Studies of the University of Georgia. Although the results have not been published yet, the values are briefly summarized here with their permission. The stable isotope analysis revealed that most of the seeds collected fall within the ranges for C_3 plants; the mean $\delta^{13}C$ value was -24.7‰. However, other seeds display a mean $\delta^{13}C$ value of -9.8‰, typical of C_4 plants. Unfortunately, the seeds have not been identified through a palaeobotanical study; therefore it remains unknown whether they represent maize or other C_4 plants. Despite the fact that this analysis did not identify the plants, it certainly demonstrates that both C_3 and C_4 resources were available at Rancho Bajo.

To have a better understanding of the human diet, it is important to use the stable isotope values of the fauna from the area as a baseline for the comparison with the human values. Although it would be preferable to compare the Rancho Bajo human $\delta^{13}C$ and $\delta^{15}N$ values with values from immediately local fauna, as explained in Chapter 3, to this day no faunal remains have been recovered at this site, making it impossible to obtain such samples. Thus, the stable isotope values of the faunal remains from Cotocollao and Las Orquídeas had to be used as a baseline (see Figure 7.1; these faunal values are discussed more thoroughly in the following sections). Note that as explained in Chapter 2, camelid remains appear in the highlands only by the Late Formative Period (Stahl, 2003), which is why only the Las Orquídeas leporid and cervid values appear in Figure 7.1. Comparing the $\delta^{13}C$ values to these fauna also suggests that the individuals from Rancho Bajo had a predominantly C_3 diet with a small contribution of C_4 plants. Their values fall between the majority of cervid and leporid samples, whose values suggest pure C_3 diets; and a few cervid samples from Las Orquídeas whose values suggest a higher consumption of C_4 resources. The people from Rancho Bajo have $\delta^{15}N$ values about a trophic level above those of many of the cervid and leporid samples from Las Orquídeas, a comparison which suggests the people from this settlement could have consumed meat from similar animals.

As Pennycook (2013) reasoned for the Integration period sites Tajamar and NAIQ, also located in the Quito Basin, the difficulty of transporting marine resources into the basin makes it unlikely that pre-Columbian highland groups consumed marine protein, or at least unlikely that it played a major role in their diet. This reasoning is also supported by the zooarchaeological record of other Formative sites in the highlands, which consists exclusively of terrestrial animals and birds; and does not include any evidence of marine animal species (Stahl, 2003) (see Chapter 2). Although the presence of *Spondylus* and mother of pearl has linked some highland Formative sites with the coast (Dyrdahl, 2017), this feature has been linked to trade of exotic goods and not considered as evidence of inland marine consumption.

7.1.1. Human Variation by Age and Sex in Rancho Bajo

Figure 7.2 shows a scatter plot with the $\delta^{13}C$ and $\delta^{15}N$ values of the Rancho Bajo human samples differentiated between subadults (skeletally immature individuals) and young, middle, and old adult individuals. The scale has been adjusted to allow the minor differences between individuals to be seen. Because of the small sizes of the age categories, no statistical analyses were performed to compare the groups. In any event, overall variation in this sample is not much larger than analytical error (±0.1‰ for $\delta^{13}C$, ±0.2‰ for $\delta^{15}N$), a result which makes any attempt to discuss individual differences very speculative. Given that all of the samples cluster so tightly together, showing a range of less than 1‰, it appears that there were no age-based differences in diet.

Fig. 7.2 - Rancho Bajo human $\delta^{13}C$ & $\delta^{15}N$ values by age

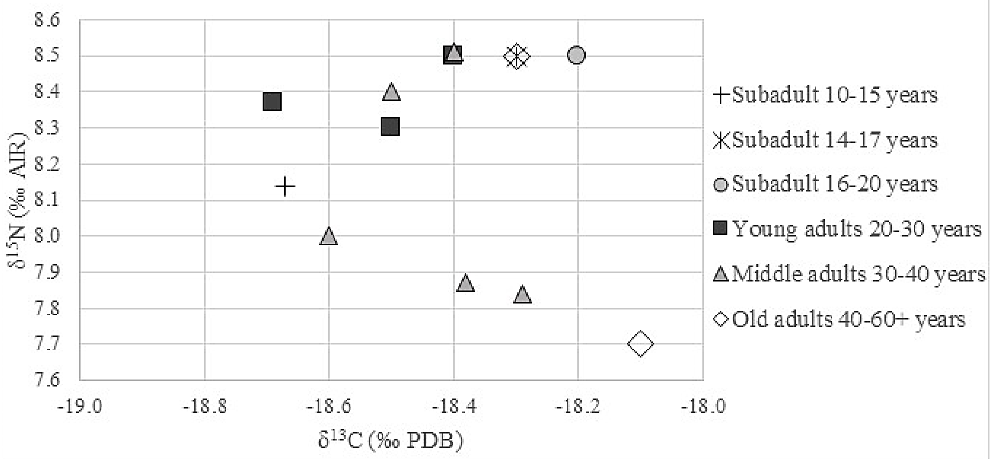
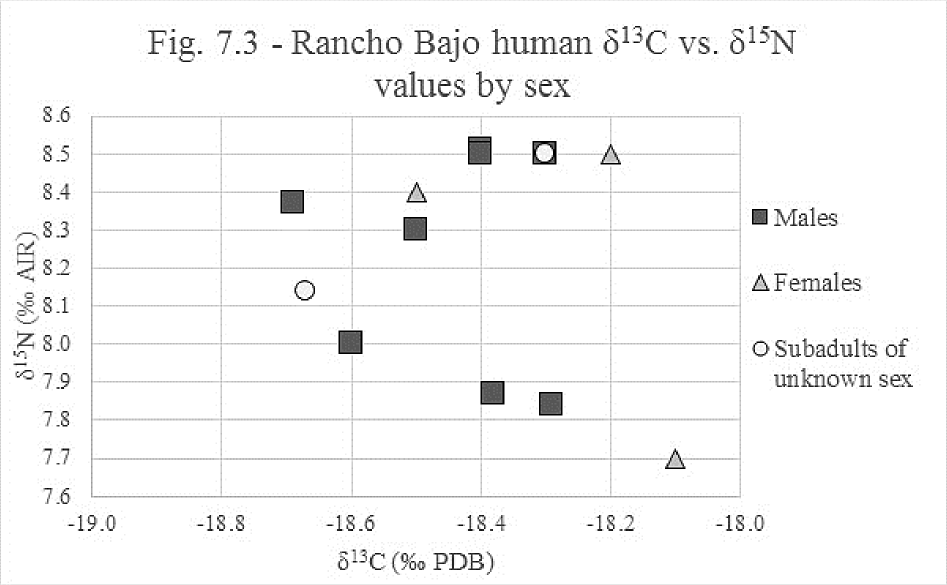


Figure 7.3 shows a scatter plot with the $\delta^{13}C$ and $\delta^{15}N$ values differentiated according to the estimated sex of the individuals. Figure 7.3 includes a ‘subadult’ category, which includes all the skeletally immature individuals, for which sex estimation was not possible. The purpose is to present the whole population.

The $\delta^{13}C$ values for males (=8) range from -18.7‰ to -18.3‰, with a mean $\delta^{13}C$ value of -18.4‰ and a SD of 0.1‰. Meanwhile, the $\delta^{13}C$ values for females (=3) range from -18.5‰ to -18.1‰, with a mean $\delta^{13}C$ value of -18.3‰ and a SD of 0.2‰. The $\delta^{15}N$ values for males range from 8.5‰ to 7.8‰, with a mean value of 8.2‰ and a SD of 0.3‰. The three females have $\delta^{15}N$ values that range from 8.5‰ to 7.7‰, with a mean $\delta^{15}N$ value of 8.2‰ and a SD of 0.4‰. For both isotopes, the male and female ranges overlap. Given the small range of values, and the fact that only three females are present, it is not useful to attempt statistical testing.



7.2. Cotocollao Human and Faunal Values

It was not possible to obtain significant information about the diet of the early settlement (Early Formative) from this study. Due to sample collection difficulties explained in Chapter 5, only one sample (PCO-14) was obtained for the early period. The $\delta^{13}C$ value obtained for this sample is -18.8‰; its $\delta^{15}N$ value is 9.0‰. Although the value of this single sample will be discussed, by itself it does not say much about diet at the site in the earlier phase. However, as explained below, some inferences about the diet of the early settlement can still be drawn based on the $\delta^{13}C$ and $\delta^{15}N$ values of the late settlement. The interpretation of the diet of both settlements was also developed based on the $\delta^{13}C$ and $\delta^{15}N$ values of a cervid sample of this site (PCO-35), along with the isotope values from the faunal samples from Las Orquídeas (see Fig. 7.4). PCO-35 exhibit a $\delta^{13}C$ value of -19.6‰ and a $\delta^{15}N$ value of 5.0‰, which show a diet that relied exclusively on C_3 plants.

The -18.8‰ $\delta^{13}C$ value exhibited by PCO-14 is relatively close to the ranges observed in predominantly C_3 diets (see Chapter 4). However, it is possible that this diet also included small amounts of C_4 plants, as the $\delta^{13}C$ value is slightly less negative than usual for full C_3 dependence. The consumption of maize is also supported by the evidence from the palaeobotanical study conducted by Deborah Pearsall (Villalba, 1988; Pearsall, 2003) that identified the presence of maize in the early phase of this settlement. However, the role maize played in people's diets will remain unknown unless further isotopic analyses on the early burials can be done. The 9.0‰ $\delta^{15}N$ value, along with the $\delta^{13}C$ value, suggests that the diet was also supplemented with protein from terrestrial animals, such as cervids and leporids. This reasoning is supported by two observations. First, PCO-14 exhibits an $\delta^{15}N$ value that appears about one trophic level above from the faunal samples of Cotocollao and Las Orquídeas (see Figure 7.4). Second, the zooarchaeological study conducted at the site identified the remains from these animals in significant amounts (Villalba, 1988). This study suggests that both cervids and leporids played an important role in the life of the people from this settlement. Similar to the case of Rancho Bajo, it is unlikely that they consumed protein from camelids, since the zooarchaeological record from highland sites suggests these animals were not present in the region during the Early Formative (Stahl, 2003).

It is interesting that PCO-14 shows a similar $\delta^{13}C$ value to that of the individuals from Rancho Bajo, but exhibits a higher $\delta^{15}N$ value. This difference suggests that the individuals from both Early Formative settlements may have had a similar diet, although PCO-14 probably consumed more protein from terrestrial animals. However, it is emphasized that this explanation is only a speculation, based on the values from a single sample.

The $\delta^{13}C$ values for the later period range from -19.0‰ to -16.5‰, with a mean of -18.2‰ ± 0.5‰. The $\delta^{15}N$ values range from 7.5‰ to 9.8‰, with a mean of 8.7‰ ± 0.7‰. Table 7.2 presents the $\delta^{13}C$ and $\delta^{15}N$ values of this group dated to the Late Formative Period.

Table 7.2 - Cotocollao Human Data (Later Period)

Sample ID	Sex	Age	$\delta^{13}C$	$\delta^{15}N$
PCO-01*	ND	Young Adult (20-30 years)	-18.2	7.5
PCO-02	ND	Young Adult (23-30 years)	-18.4	8.0
PCO-03	ND	Young Adult (25-30 years)	-18.4	8.8
PCO-04	F	Old Adult (40-60 years)	-18.5	9.0
PCO-05*	M	Middle Adult (30-35 years)	-17.5	7.9
PCO-09	ND	Adult†	-18.5	9.6
PCO-10	ND	Adult†	-19.0	8.4
PCO-11	F	Young Adult (20-30 years)	-18.5	9.8
PCO-13	F	Young Adult (22-25 years)	-18.2	8.3
PCO-15	M	Young Adult (20-30 years)	-18.2	9.8
PCO-16	ND	Middle Adult (ca. 45 years)	-19.0	9.1
PCO-17	ND	Adult†	-17.6	8.7
PCO-18	ND	Young Adult (18-25 years)	-18.4	7.8
PCO-21	ND	Middle Adult (35-45 years)	-17.7	9.3
PCO-22	M	Young Adult (ca. 25 years)	-18.5	9.6
PCO-23	F	Old Adult (35-60 years)	-18.0	8.9
PCO-24	ND	Young Adult (20-35 years)	-18.1	8.2

† Ubelaker (1978) could not estimate the age of these individuals.
* Run 1 values

Table 7.2 (continued) – Cotocollao Human Data (Later Period)

Sample ID	Sex	Age	$\delta^{13}C$	$\delta^{15}N$
PCO-25*	M	Middle Adult (30-35 years)	-18.4	8.9
PCO-26*	ND	Subadult (ca. 17 years)	-16.5	7.9
PCO-27	ND	Subadult (15-18 years)	-18.2	8.5
PCO-29*	F	Middle Adult (35-45 years)	-18.5	9.4
PCO-30	M	Old Adult (40-60 years)	-18.4	9.4
PCO-31	F	Young Adult (25-30 years)	-18.2	9.3
PCO-32	M	Young Adult (22-25 years)	-17.9	8.8
PCO-33	ND	Adult†	-18.3	8.6
		Mean	**-18.2**	**8.7**
		SD	**0.5**	**0.7**

† Ubelaker (1978) could not estimate the age of these individuals.
* Run 1 values

It can be observed that the $\delta^{13}C$ and $\delta^{15}N$ values do not differ much from the isotope values seen in the individual from the early settlement. They also indicate a diet that was primarily dependent on C_3 plants, either wild resources or C_3 cultivars like quinoa (*Chenopodium* sp.), beans (*Phaseolus* sp.; *Phaseolus vulgaris*), oca tuber (*Oxalis tuberosa*), potato (*Solanum tuberosum*), and chocho (*Lupinus mutabilis*), which were identified in Cotocollao as well as in other highland Formative sites (Villalba, 1988; Pearsall, 2003) (see Chapter 2 and Chapter 3).

The values also indicate that they apparently consumed a certain amount of C_4 plants, probably maize. This conclusion is supported, in the first place, by maize cobs that were recovered from the site (Villalba, 1988; Pearsall, 1984; Pearsall, 2003). It is also supported by the isotopic signals of the cervid sample PCO-35 and Las Orquídeas faunal samples that were used as a baseline. The individuals from Cotocollao's late settlement exhibit higher δ^{13}C values than most cervid, leporid, and camelid samples, which display isotope values expected for herbivores with a C_3 diet. However, they also display lower δ^{13}C values in comparison to two deer samples from Las Orquídeas that show more maize consumption (these will be discussed later in this chapter).

As well as in Rancho Bajo and Cotocollao's early settlement, the carbon stable isotope values indicate that although there was some maize consumption, this was probably not a very important food resource for the group. However, it seems that for some individuals this C_4 resource was more important than it was, at least, for the people in Rancho Bajo. This pattern can be seen in samples PCO-05, PCO-17, PCO-21, and PCO-26, which display a mean δ^{13}C value of -17.3‰ ± 0.6‰ and a mean δ^{15}N value of 8.4‰ ± 0.7‰. These values fall above the range seen at Rancho Bajo, and suggest these individuals had a diet that included a little more maize. This difference could be considered as evidence that this late settlement had a slightly more varied diet than the individuals from Rancho Bajo, who displayed more tightly clustered isotope values.

In addition, the individuals display δ^{15}N values that place them a trophic level above the faunal samples, which suggests they consumed protein from these animals. The camelid samples were not excluded from the diet interpretation, because many camelid remains were identified in the site and specifically associated with this settlement. The δ^{13}C and δ^{15}N values listed in Table 7.2 appear represented in a scatter graph in Figure 7.4.

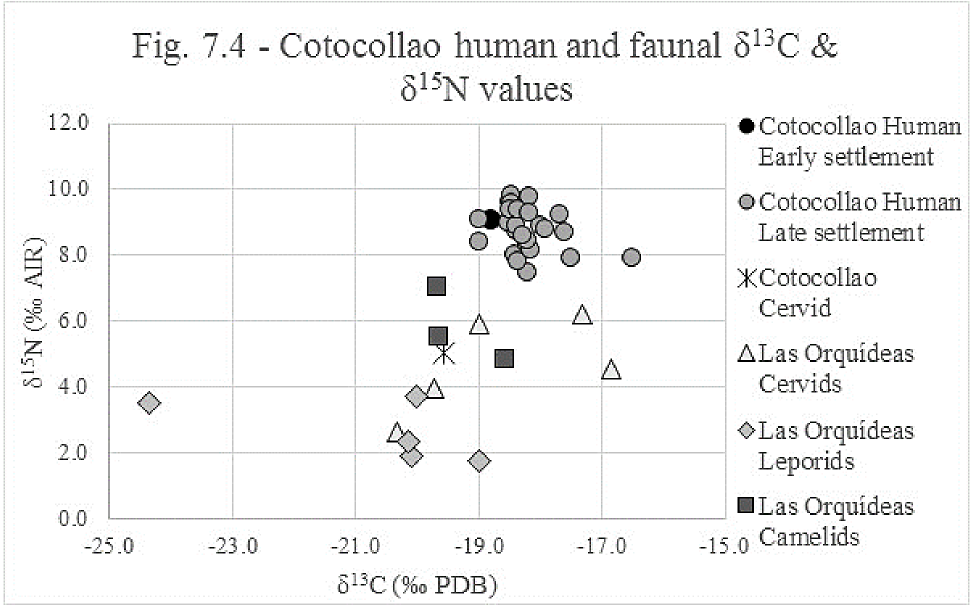


Fig. 7.4 - Cotocollao human and faunal $\delta^{13}C$ & $\delta^{15}N$ values

Based on the diet patterns of Rancho Bajo and the late settlement, it could be speculated that the diet of the early settlement might have been less varied than that of the late settlement, due to the small number of individuals that may have composed the population and been involved in the acquisition of food resources. Remains of only 13 individuals have been recovered from this settlement, while remains of around 200 individuals were recovered from the late settlement (Villalba, 1988). This reasoning suggests that the individuals from early Cotocollao might have had a fairly similar dietary pattern to the individuals of Rancho Bajo, consuming a strongly C_3 dominated diet and sharing all of their food resources. This pattern would place them in contrast to the late settlement of Cotocollao.

7.2.1. Human Variation by Age and Sex in Late Cotocollao

Figure 7.5 displays the $\delta^{13}C$ and $\delta^{15}N$ values for the late settlement, divided into four age categories: subadults, as well as young, middle, and old adults. A fifth category represents adults of unknown sex. As a result of the small size of the categories, no statistical tests were performed to detect age differences; however, the scatter diagram demonstrates that apparently there were no significant differences in diet related to the age of the individuals in the late settlement.

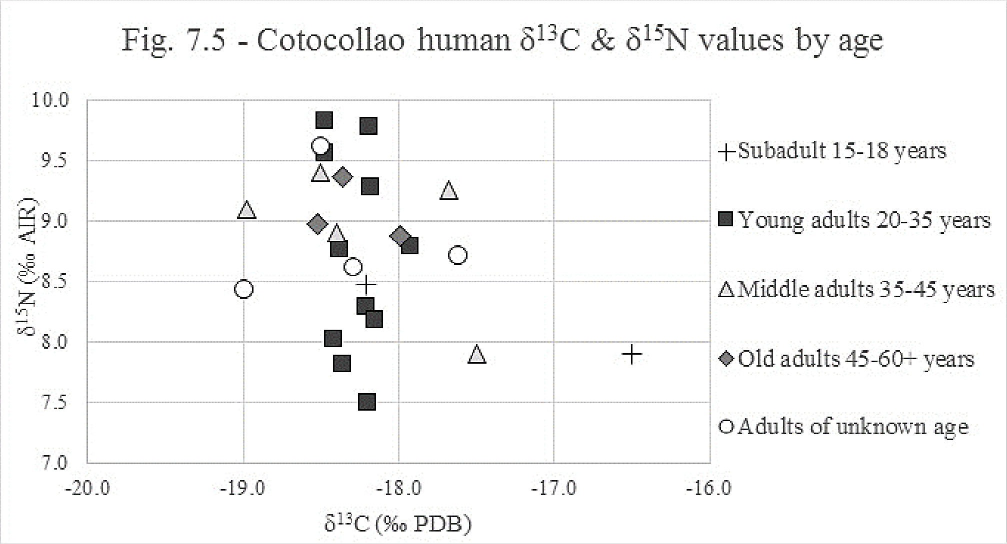
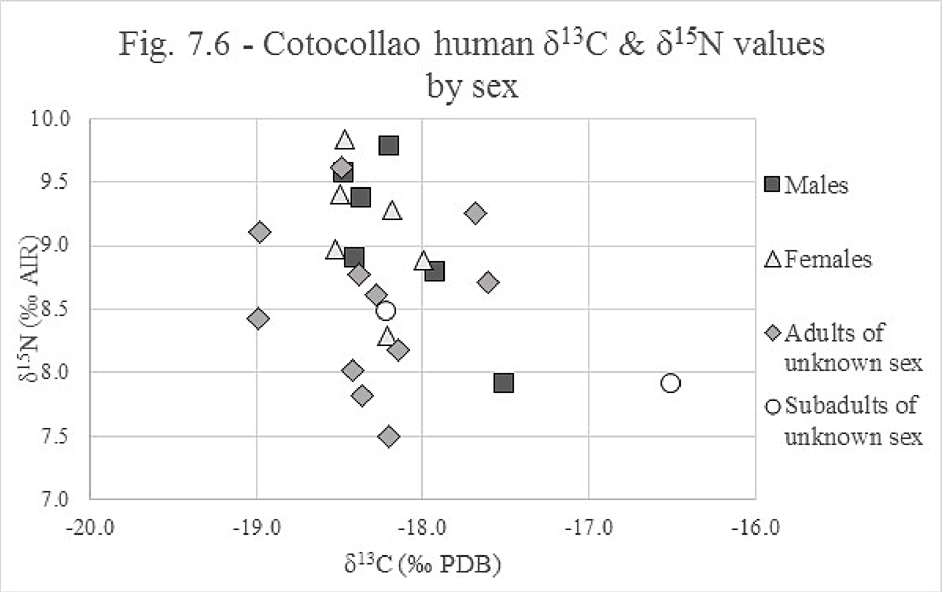


Figure 7.6 shows the δ^{13}C and δ^{15}N values according to sex. The scatter plot reveals no apparent dietary differences based on sex; however, it is important to emphasize that 14 of the 28 samples represent individuals of unknown sex.

The δ^{13}C values for males (=6) ranged from -18.5‰ to -17.5‰, with a mean δ^{13}C value of -18.1‰ ± 0.4‰. The δ^{13}C values for females (=6) range from -18.5‰ to -18.0‰, with a mean δ^{13}C value of -18.3‰ ± 0.2‰. A t-test was performed to test for significant differences in δ^{13}C values between males and females. The results confirmed no significant difference (t stat= 0.9800, t critical= 2.31, p-value= 0.36).

The δ^{15}N values for males range from 7.9‰ to 9.8‰, with a mean δ^{15}N value of 9.1‰ ± 0.7‰. The δ^{15}N values for females range from 8.3‰ to 9.8‰, with a mean δ^{15}N value of

9.1‰ ± 0.5‰. Again a t-test was performed to test for significant differences in $\delta^{15}N$ values between males and females. The results indicate no significant difference (t stat= -0.1665, t critical= 2.26, p-value= 0.87).



7.3. Las Orquídeas Human and Faunal Values

The Las Orquídeas faunal $\delta^{13}C$ and $\delta^{15}N$ values can be found in Table 7.3. Human values are listed in Table 7.4. The $\delta^{13}C$ and $\delta^{15}N$ values of both human and faunal samples are represented in Figure 7.7.

The faunal samples collected from this site, which represent animals from the family cervidae (e.g., deer), camelidae (e.g., llama or alpaca), and leporidae (e.g., hare or rabbit), have already been used as a baseline to examine human diets at Rancho Bajo and Cotocollao. They are discussed in detail here. The $\delta^{13}C$ values of the cervids range from -20.3‰ to -16.8‰, (mean = -18.6‰ ± 1.5‰). Their $\delta^{15}N$ values range from 2.6‰ to 6.2‰ (mean = 4.6‰ ± 1.5‰). Two of the cervid samples (POR-09 and POR-12) display a mean $\delta^{13}C$ value of -20.0‰ ± 0.4‰ and mean δ^{15}N value of 3.3‰ ± 0.9‰, as expected for herbivores. Therefore, they can be found in Figure 7.10 relatively close to the leporid samples. However, the other three cervid samples, especially POR-10 and POR-11, exhibit higher values. Together these three animals have a mean δ^{13}C value of -17.7‰ ± 1.1‰ and a mean δ^{15}N value of 5.6‰ ± 0.9‰, which are values closer to that exhibited by the human samples in this study**.** Based on the differences seen in δ^{13}C values and δ^{15}N values in the cervid samples, it is suggested that the isotopic signals of two of these deer (POR-10 and POR-11) reflect some consumption of C_4 plants (e.g., maize). The deer could have consumed maize leaves or other parts while feeding in areas close to the settlement. A possible parallel for this behavior is seen elsewhere in the stable isotope values of deer in Mayan sites. At some sites, deer have displayed δ^{13}C values ranging from -22‰ to -19‰ that are consistent with herbivores with a C_3 diet. However, other deer also displayed δ^{13}C values ranging from about -18‰ to -16‰ that show some inclusion of C_4 foods in their diets, considered to be consistent with occasional feeding from maize fields; and a few deer had δ^{13}C values of -7‰, showing they must have been feeding almost exclusively on maize (White et al., 2004; White et al., 2001).

The leporid samples display δ^{13}C values ranging from -24.4 to -19.0‰ (mean = -20.7‰ ± 2.1‰) and δ^{15}N values ranging between 1.8‰ and 3.7‰ (mean = 2.7‰ ± 0.9‰) that fall within values expected for hare/rabbit consuming only C_3 plants. Finally, camelids exhibit δ^{13}C values that range between -19.7‰ and -18.6‰ (mean = -19.3‰ ±0.6‰) and δ^{15}N values ranging from 4.8‰ to 7.1‰ (mean = 5.8‰ ± 1.1‰). These values are also in the ranges expected for an herbivore consuming C_3 plants.

The stable isotope values displayed by cervids, leporids, and camelids indicate that most animals' diets largely fall into a C_3 range, suggesting that most of the wild plants available in the area were C_3. This observation is support for the argument given below that the C_4 type values seen in humans are likely due to maize consumption.

Table 7.3 - Las Orquídeas faunal data

Family/Animal	Sample ID	$\delta^{13}C$	$\delta^{15}N$
Cervidae (e.g., deer)	POR-09	-20.3	2.6
	POR-10	-17.3	6.2
	POR-11	-16.8	4.5
	POR-12	-19.7	4.0
	POR-13	-19.0	5.9
	Mean	**-18.6**	**4.6**
	SD	**1.5**	**1.5**
Leporidae (e.g., hare/ rabbit)	POR-14	-24.4	3.5
	POR-15	-20.0	3.7
	POR-16	-20.1	1.9
	POR-17	-20.2	2.3
	POR-18	-19.0	1.8
	Mean	**-20.7**	**2.7**
	SD	**2.1**	**0.9**
Camelidae (e.g., llama/ alpaca)	POR-19	-19.6	5.5
	POR-20	-19.7	7.1
	POR-22	-18.6	4.8
	Mean	**-19.3**	**5.8**
	SD	**0.6**	**1.1**

In addition, the faunal $\delta^{15}N$ values appear to be very diverse. In consequence they are capable of providing some information about the animal protein that humans may have consumed. For instance, a human diet that was heavily dependent on leporids will exhibit different δ^{15}N values from a diet heavily dependent on cervids or camelids. A discussion on the animal protein possibly consumed by the people from Las Orquídeas can be found later in this section.

Table 7.4 - Las Orquídeas human data

Sample ID	**Sex**	**Age**	**δ^{13}C**	**δ^{15}N**
POR-01	F	Young Adult (14-23 years)	-11.8	9.0
POR-02	F	Middle Adult (30-34 years)	-15.8	6.9
POR-03	ND	Subadult (14-18 years)	-15.7	8.1
POR-04	F	Young Adult (20+ years)	-16.2	6.7
POR-05	ND	Subadult (11-12 years)	-15.5	7.3
POR-06	M	Old Adult (50+ years)	-15.8	9.2
POR-07	ND	Subadult (6-7 years)	-16.4	5.7
POR-08	M	Young Adult (24-29 years)	-15.7	7.3
		Mean	**-15.4**	**7.5**
		SD	**1.5**	**1.2**

The human δ^{13}C values range from -16.4‰ to -11.8‰ (mean = -15.4‰ ± 1.5‰). In terms of the δ^{15}N values, they range from 5.7‰ to 9.2‰ (mean = 7.5‰ ± 1.2‰); see Table 7.4. Of all the sites examined in this study, Las Orquídeas displays the highest δ^{13}C values, which is consistent with more consumption of C_4 resources. Nonetheless, the values do not indicate that maize played a dominant role in the diet of the people from Las Orquídeas. This interpretation is based on the carbon stable isotope values reported for Andean sites that had predominantly C_3 diets with only a small contribution of maize, and sites in which the reliance upon maize as a dietary staple was confirmed. Sites with predominantly C_3 diets display collagen $\delta^{13}C$ values that range approximately from -19‰ to -18‰ (e.g., Tykot et al., 2006; Pennycook, 2013). In contrast, sites where maize was a staple food exhibited collagen $\delta^{13}C$ values that range approximately from -13‰ to -8‰ (e.g., Finucane et al., 2006; Finucane, 2007; Pennycook, 2013). Most of the Las Orquídeas $\delta^{13}C$ values fall between these two groups of values, a result which can be considered indicative of a diet including both C_3 and C_4 plants. A different isotopic signal suggesting predominant C_4 consumption was observed in individual POR-01, which will be discussed below.

The comparison of human and faunal $\delta^{13}C$ values also confirms that the human diet is consistent with a mix of C_3 and C_4 resources. In addition, humans display $\delta^{15}N$ values that suggest that they supplemented their diet with protein from the terrestrial animals. As previously mentioned, the faunal $\delta^{15}N$ values for Las Orquídeas are very diverse; and as a result they can provide information about the animal protein consumed by humans. For instance, diets heavily dependent on leporid meat will exhibit different $\delta^{15}N$ values from diets heavily dependent on cervid or camelid meat. In Las Orquídeas, the human $\delta^{15}N$ values are about a trophic level above the overall faunal mean, which suggests a mixed diet consisting of some meat from all of these animals (see Figure 7.7). This observation is consistent with the large amount of faunal remains of varied taxa recovered at the site (Montalvo and Dyrdahl, 2014; Dyrdahl, 2017).

Based on the presence of burnt bones from guinea pigs (cuy) in the site, it is likely that the people consumed these animals at least occasionally. Unfortunately, the only two cuy samples that could be collected for this study failed during preparation for collagen extraction (see Chapter 6), so it is not possible to say what their isotopic contribution to the human diet might have been.

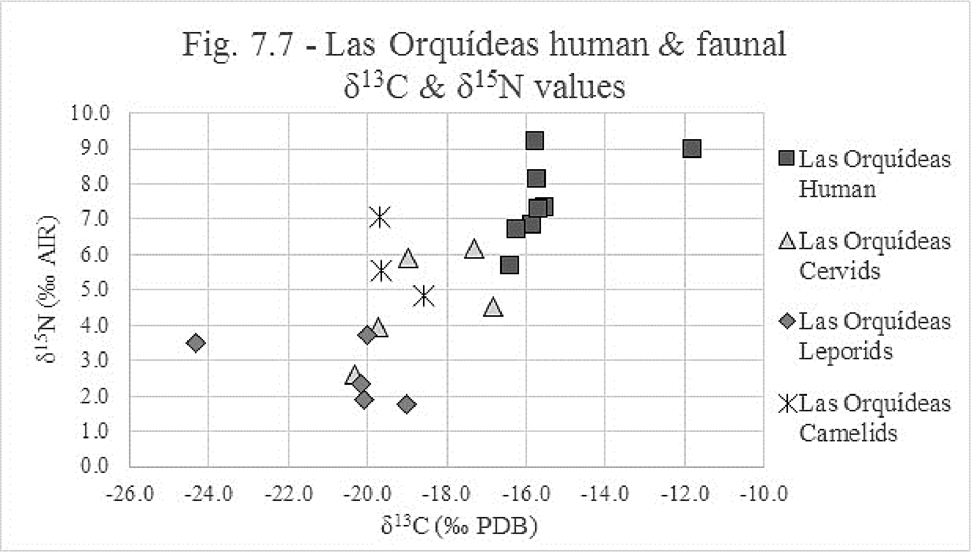


Fig. 7.7 - Las Orquídeas human & faunal $\delta^{13}C$ & $\delta^{15}N$ values

7.3.1. Variation of Las Orquídeas Human Values

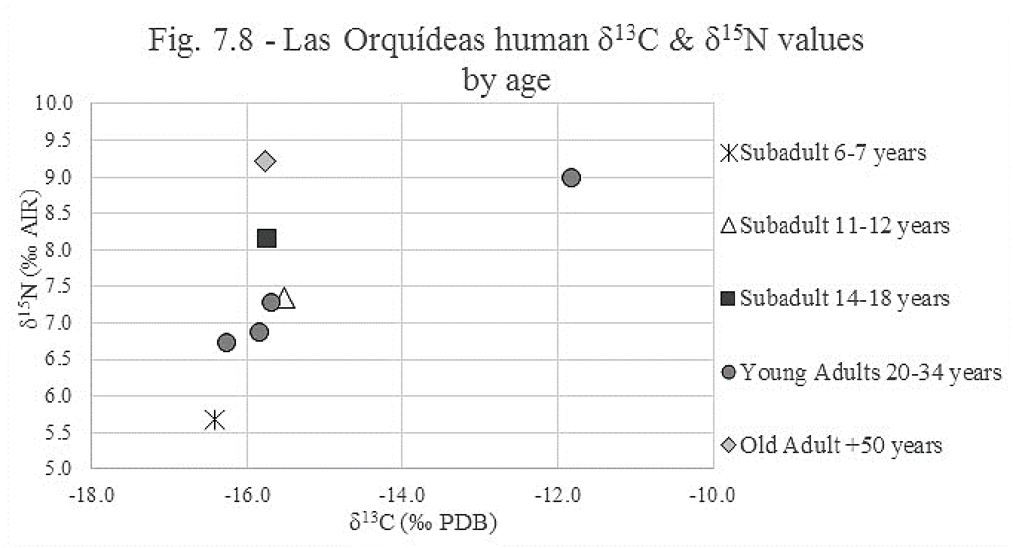
Figures 7.8 and 7.9 show the $\delta^{13}C$ and $\delta^{15}N$ values for the site organized into different age and sex categories. Due to the small size of the skeletal population discovered to date at Las Orquídeas, no statistical tests were performed to detect age or sex differences. Such analysis should be performed once remains of more individuals are discovered at this site and their remains analysed.

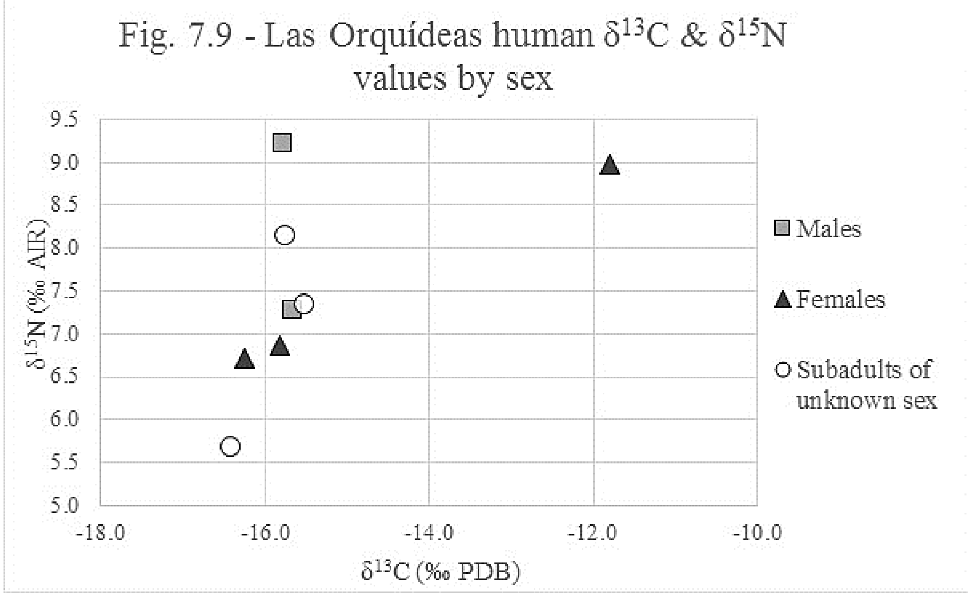
Although general patterning in this small sample cannot really be discussed, three of the eight individuals analyzed show interesting isotopic signals. The first individual (POR-06) is a male old adult of 50+ years, who displays the highest $\delta^{15}N$ value (9.2‰) of the group. An explanation of this higher $\delta^{15}N$ value could be greater consumption of animal protein in comparison with the rest of the group.

The second individual of interest (POR-07) is a juvenile between 6 and 7 years old, who displays the lowest $\delta^{15}N$ value (5.7‰), a pattern that has been observed in juveniles of similar age recovered from archaeological sites in other countries (White and Schwarcz, 1994; Schurr, 1997; Chinique de Armas et al., 2017). As detailed in chapter 4, nursing infants display higher stable nitrogen isotope values than their mothers. However, once weaning starts, new foods begin to replace breast milk; and the infants' tissues shift to reflect the isotopic compositions of those foods. During this period, juveniles exhibit a gradual decline in their δ^{15}N values. Although this decline would be expected to take them immediately to a typical adult δ^{15}N value, it is actually common for δ^{15}N values to dip below typical adult δ^{15}N values in early childhood; then increase slightly in later childhood until they reach typical adult mean δ^{15}N values (Schurr, 1997). This change may be because lower trophic level foods, such as legumes, cereals, and root cultigens are often preferred as weaning foods and young children's foods over higher trophic level foods such as meat from terrestrial animals (Chinique de Armas et al., 2017). The explanation for the low δ^{15}N values displayed by POR-07 is probably the consumption of similar lower trophic level foods. Other researchers have suggested a similar explanation after observing this pattern of low δ^{15}N values in juveniles (White and Schwarcz, 1994; Katzenberg and Pfeiffer, 1995; Richards et al., 2002; Turner et al., 2007). However, this pattern would be confirmed at Las Orquídeas only when more juvenile individuals are discovered and studied.

The third individual (POR-01) is an adolescent or adult female estimated to have been between 14 and 23 years old. This individual displays a higher δ^{13}C value (-11.8‰) than the rest of the group, as well the second highest δ^{15}N value (9.0‰). Taken alone, the δ^{13}C value would be interpreted as indicating probably more consumption of maize than the rest of the individuals at the site. However, it is quite a bit higher than other δ^{13}C values at the site, raising the possibility that this female individual belonged to another group or area – in other words, that this person could have been a non-local migrant from a community more reliant on maize. If this was actually a non-local individual, the possibility that the high δ^{13}C and

$\delta^{15}N$ values actually reflect a coastal signal should also be considered. However, only carrying out $\delta^{13}C$ and $\delta^{15}N$ analysis of more individuals from Las Orquídeas will confirm that her isotopic values do not fall within those of the group.





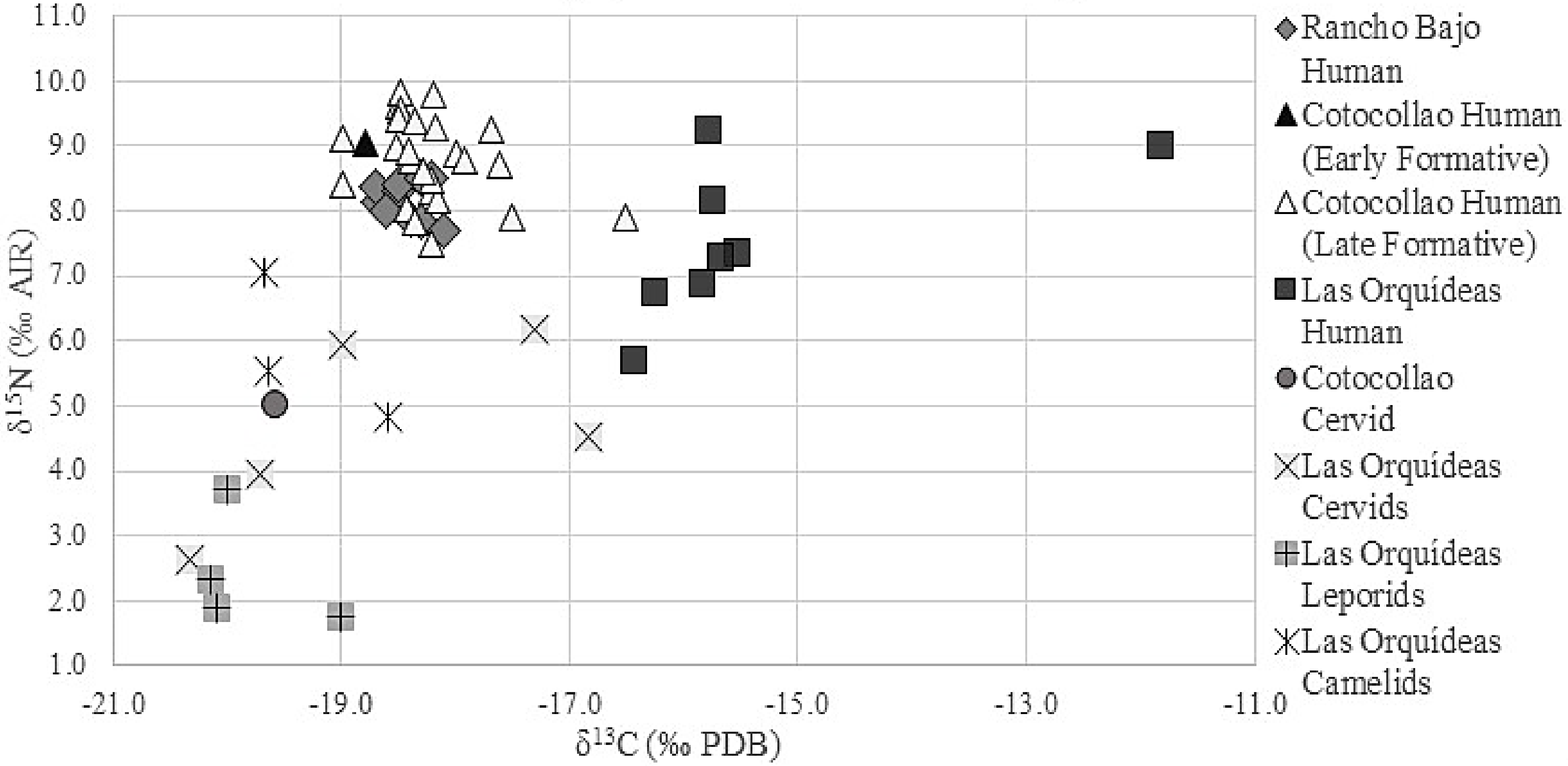
7.4. Patterning Between Sites

The isotopic signals from the individuals from Rancho Bajo, Cotocollao, and Las Orquídeas show that these Formative sites from the northern highlands did not have diets that were heavily dependent on C_4 resources. On the contrary, most of their diet relied on C_3 plants, which was supplemented with C_4 plants and protein from terrestrial animals. This situation is especially true at the sites Rancho Bajo and Cotocollao. At Las Orquídeas the consumption of C_4 resources (likely maize) is suggested by higher $\delta^{13}C$ values than the first two sites.

In order to arrive at that conclusion, the interpretation of the diet was based on $\delta^{13}C$ values commonly found in the stable isotope literature, as discussed in detail in Chapter 4. The literature has established that depending on whether the diet was predominatly C_3 or C_4, the $\delta^{13}C$ values will vary between -20‰ and -10‰ approximately. These values have also been described and confirmed by stable isotope studies at other sites in the Andes (Finucane et al, 2006; Tykot et al., 2006; Finucane, 2007; Pennycook, 2013). These values were then compared to the mean $\delta^{13}C$ values from each site under study. The mean $\delta^{13}C$ value at Rancho Bajo was -18.4‰ ± 0.2‰; the Cotocollao samples displayed a mean $\delta^{13}C$ value of -18.3‰ ± 0.7‰; while the samples from Las Orquídeas exhibited a mean $\delta^{13}C$ value of -15.4‰ ± 1.5‰. In general, the mean $\delta^{13}C$ values from Rancho Bajo and Cotocollao fall within the values reported in Andean sites with predominantly C_3 diets with a small contribution of C_4 resources (Finucane et al., 2006; Finucane, 2007; Pennycook, 2013). However, Las Orquídeas $\delta^{13}C$ values fall between the values reported for sites with primary C_3 diets, and those of sites with diets that were heavily dependent on C_4 plants (Tykot et al., 2006; Pennycook, 2013).

It was already mentioned that the people from the sites examined by this study consumed protein from terrestrial animals, based on their $\delta^{15}N$ values. It is possible to indicate the protein source by examining the $\delta^{15}N$ values of animals from the same region and period and comparing them to the $\delta^{15}N$ values of humans. In other words, the faunal samples collected from Las Orquídeas provide a baseline for understanding the human $\delta^{15}N$ values of all three sites (see Figure 7.10). Based on the presence of leporid, cervid, and camelid remains at the late settlement of Cotocollao and Las Orquídeas, and on the stable isotope values presented above, it is possible to suggest that the people from these Late Formative settlements consumed protein from these animals. Unfortunately, Rancho Bajo does not provide faunal remains that could confirm the presence of these animals at the site. The faunal remains documented in Formative sites of the highlands include birds and mammals such as primates, opossums, bears, dogs, racoons, weasels, pumas, tapirs, llama/alpaca, deer, guinea pigs, pacas, and rabbits/hares (Stahl, 2003) (see Chapter 2). All these animals were recovered in sites that correspond to the Middle and Late Formative Period; thus, it is not possible to assume that this entire range of animals was consumed by people in Rancho Bajo. However, based on the presence of leporids and cervids in the early settlement of Cotocollao (Early Formative), it can be suggested that the people of Rancho Bajo also consumed meat from these animals among other fauna. It is not suggested that they consumed meat from camelids, because according to Stahl (2003), evidence of camelids appears in the northern highlands only since the Late Formative Period, when they were apparently introduced to the region. This observation also applies to guinea pig remains in the highlands (see Chapter 2).

Fig. 7.10 - Human and faunal $\delta^{13}C$ & $\delta^{15}N$ values in Rancho Bajo, Cotocollao & Las Orquídeas



Comparing the stable isotope values for each site has made it also possible to state that during the Formative Period, human groups located in the northern highlands had variable subsistence practices. For example, the carbon and nitrogen stable isotope values presented for Cotocollao largely represent individuals from the late settlement, which, like Las Orquídeas, dates to the Late Formative Period. Although both sites represent the same period of time, their diets differed. The late settlement of Cotocollao has a diet that was heavily dependent on C_3 food resources, likely adding a small amount of maize to these staples. However, the individuals from Las Orquídeas display $\delta^{13}C$ values consistent with mixed dependence on C_3 and C_4 resources, representing the highest consumption of maize from all the sites under study. In addition, the young female individual represented by sample POR-01, who could have been a non-local migrant, displays $\delta^{13}C$ values consistent with a diet heavily dependent on maize. This result suggests that maize played even a more significant role in some other groups, at least during the Late Formative Period.

7.5. Patterning Between Periods

The results from Rancho Bajo, Cotocollao, and Las Orquídeas are compared to the stable isotope data available from the region of main interest for this study, the northern highlands. As discussed in Chapter 2, there are only two previous stable isotope studies in this region. The first study was conducted at the site La Florida of the Regional Development Period, which was published by Ubelaker et al. (1995). The second study was presented by Pennycook (2013), and involves the dietary interpretation for the sites Tajamar and NAIQ of the Integration Period.

7.5.1. Diet in the Formative and Regional Development Period

The Regional Development Period (500 BC –AD 500) has been defined as a time of increasing sociopolitical complexity that led to the emergence of chiefdoms or "*cacicazgos*" and elite kin groups, as a result of interaction and competition for the control of long distance exchange networks centered on the exchange of *Spondylus* (Meggers, 1966; Marcos, 1986; Massuci, 1995; Molestina, 1998; Masucci, 2008). It is a period also characterized by 1) the emergence of earthen platform mounds also known as "*tolas*" in urban centers (Guillaume-Gentil, 1998); 2); the emergence of distinctive regional ceramic stylistic traditions that included the elaboration of figurines that portrayed high-status individuals wearing symbols of authority and power; and 3) the elaboration of gold and silver luxury goods, which evidence the appearance of specialized craftsmen and elites (Massuci, 2008).

La Florida is located in the northwest of the city of Quito. After its discovery in 1983, several studies have been conducted at this archaeological site (Molestina, 2006). One of this studies was performed by Doyón (1989), who excavated six shaft tombs with a central chamber and multiple burials that have been radiocarbon dated to about AD 340±80 (uncalibrated) (Molestina, 2006). Some researchers have considered the radiocarbon dates presented by Doyón (1989) controversial; and believe this site may correspond to the Early Integration Period, based on the characteristics of the tombs (Ugalde, 2007; Castillo, 1999) and the ceramic assemblage, which is similar to that of the Integration sites Chaupicruz, Chilibulo, and Chillogallo dated to about 600 AD (Ugalde, 2007; Camino and Navarrete, 1995; Vásquez, 1999).

From these tombs Doyón recovered high-status individuals who were buried accompanied by human sacrifices, severed heads, or interments of representatives of other classes (Ubelaker, 2000:1).

Ubelaker et al. (1995) performed carbon and nitrogen stable isotope analysis on bone collagen from 32 individuals, as well as bioapatite stable isotope analysis on remains of ten individuals, of which five were high-status and five were low-status individuals (see Chapter 2). The mean $\delta^{13}C_{collagen}$ value for the high-status group is -10.3‰ ± 1‰, and the mean $\delta^{15}N$ value 8.8‰ ± 5‰; the mean $\delta^{13}C_{collagen}$ value for the low-status group is -11.6‰ ± 1.5‰, and the mean $\delta^{15}N$ value 8.5‰ ± 1.1‰. The $\delta^{13}C_{bioapatite}$ values range from -8.3‰ to -3.1‰, with an overall mean $\delta^{13}C_{bioapatite}$ value of -5.2‰. The mean $\delta^{13}C_{bioapatite}$ value for the high-status group is -4.2‰ and for the low-status group -6.2‰. The $\delta^{15}N$ results suggest that there were no differences in terms of access to animal protein between high and low status individuals, but the $\delta^{13}C$ results confirmed there was differential consumption of maize. The comparison between the collagen and the bioapatite stable isotope analysis was interpreted as indicating that the high-status individuals consumed more low protein C_4 resources (e.g., maize), not more high protein C_4 resources (e.g., meat from maize-fed animals). Combining this evidence with other archaeological and ethnographic information, they suggest that the high-status individuals probably consumed more maize in the form of chicha.

For instance, Doyón (1991) noted that the presence of decorated annular and pedestal base cups at La Florida, which are assumed to be used to serve liquids (likely chicha) (Doyón, 1991; Masucci, 2008), appeared limited to high-status tombs. In addition, these cups were placed apart from other ceramic vessels inside the tombs, suggesting these had a special significance for high social rank individuals. This evidence, along with the results obtained by Ubelaker et al. (1995), which confirm that high-status individuals consumed more maize probably in the form of chicha, confirm a link between higher maize consumption and higher social rank. The results from stable isotope analysis, along with the abundant carbonized maize remains, grinding stones for producing maize flour, and large ceramic jars suitable for brewing maize beer recovered at this site were cited by Ubelaker et al. (1995) as evidence that supports a correlation between an intensification of maize production and the development of social hierarchy.

Figure 7.11 shows a scatter plot comparing the human $\delta^{13}C$ and $\delta^{15}N$ values from the sites examined by this study to the collagen values presented by Ubelaker et al. (1995). Both low-status and high-status individuals from La Florida have collagen values suggesting a higher consumption of C_4 resources than all the Formative sites analysed for this study. There is only one individual (POR-01) who displays a very similar isotopic signal to that displayed by the individuals from La Florida. This female individual from Las Orquídeas displays $\delta^{13}C$ (-11.8‰) and $\delta^{15}N$ (9.0‰) values that differ from those of the group; thus, the most likely explanation is that she was a non-local migrant.

The comparison between the Formative sites from this study and the stable isotope values presented by Ubelaker et al. (1995) suggests that the consumption of maize increased from the Formative to the subsequent Regional Development Period in the northern highlands. This comparison also reflects that in the Regional Development Period there was an intensification of the production of maize, which may have sustained not only the population expansion, but the increasing socio-political complexity known for the period (see discussion about intensive maize agriculture and socio-political complexity later in this chapter). The comparison also provides evidence that during the Late Formative Period, there were groups that consumed almost the same amount of maize as groups in the Regional Development Period, as seen in POR-01's $\delta^{13}C$ value.

The higher consumption of maize seen in the Regional Development Period in comparison to the Formative is consistent with the archaeological evidence recovered at the sites. La Florida's assemblage includes notable amounts of manos and metates associated with processing maize flour. These artifacts appear in very small quantities at the early settlement of Cotocollao and Las Orquídeas, and are absent at Rancho Bajo. Although the late settlement of Cotocollao has a large number of these artifacts, it is probable, based on the results from this study, that these artifacts were mostly used for preparing C_3 cultivars and only occasionally for processing maize.

At La Florida large jars or "amphorae" and vessel forms used for the storage of liquids and solids appear in high frequencies. These are consistent with the intensification of food production to sustain a large population, and to support the chiefdom's need to over-produce with the purpose of trade (Doyón, 1989). These large jars are not present in any of the Formative sites from this study. In addition, La Florida finds included annular and pedestal cups that could be associated with the consumption of chicha, which have not been found in any of the Early or Late Formative sites from this study (Doyón, 1991; Villalba, 1988; Dyrdahl, 2017; Ugalde, 2012; 2013a, 2013b; Montalvo, 2017; Montalvo, personal communication).

Unfortunately, it is not possible to compare the abundance of maize phytoliths, starches, and carbonized seeds, given that there are no archaeobotanical studies at Rancho Bajo and Las Orquídeas, and that the botanical study that was done on La Florida could not be located. However, as discussed above, the seed samples collected at Rancho Bajo and submitted for stable isotope analysis have proven that most of the plants that the site's residents obtained from the area were in fact C_3 and that C_4 plants were present but in small quantities. At Las Orquídeas, a charred maize cob was recovered from a hearth; and the isotopic signals of humans and some deer have given clear confirmation of some maize consumption. From the early settlement of Cotocollao, the presence of maize was confirmed only by a few phytoliths and charred remains (Pearsall, 2003). However, Villalba (1988) reported that from the 3,118 pollen grains analyzed (most of them from the late settlement), a total of 1,422 grains were from *Solanum tuberosum* (potato) followed by 760 pollen grains from *Zea mays* (maize). The remaining pollen grains were from *Phaseolus vulgaris* (beans), *Chenopodium quinoa* (quinoa), *Lupinus mutabilis* (chocho), *Oxalis tuberosa* (oca) and other plants that could not be identified. This analysis confirms that the diet at Cotocollao was heavily reliant on C_3 plants and included maize but in lesser quantities. Meanwhile, both Doyón (1991) and Ubelaker et al. (1995) have mentioned that maize remains are abundant at La Florida.

In terms of caries frequencies, La Florida's caries rate (1.7%) is slightly higher than that of Rancho Bajo and Las Orquídeas (1% or less), and slightly lower than the caries rate at Cotocollao (2%). This comparison is surprising, as we would expect the percentage of caries at La Florida to be much higher than all three sites due to their higher consumption of maize. Instead, the percentages of caries at all sites are low, as is seen at most prehistoric sites in Ecuador (Ubelaker, 2000). In comparison to the data presented by Morales (2016) (see Chapter 3), La Florida's caries rate is much lower than the caries rate at La Tolita (10%), a coastal site of the Regional Development Period; and it is also lower than the caries rate he reported at one of the sites discovered at the NAIQ[21] (37%), dated to the Integration Period.

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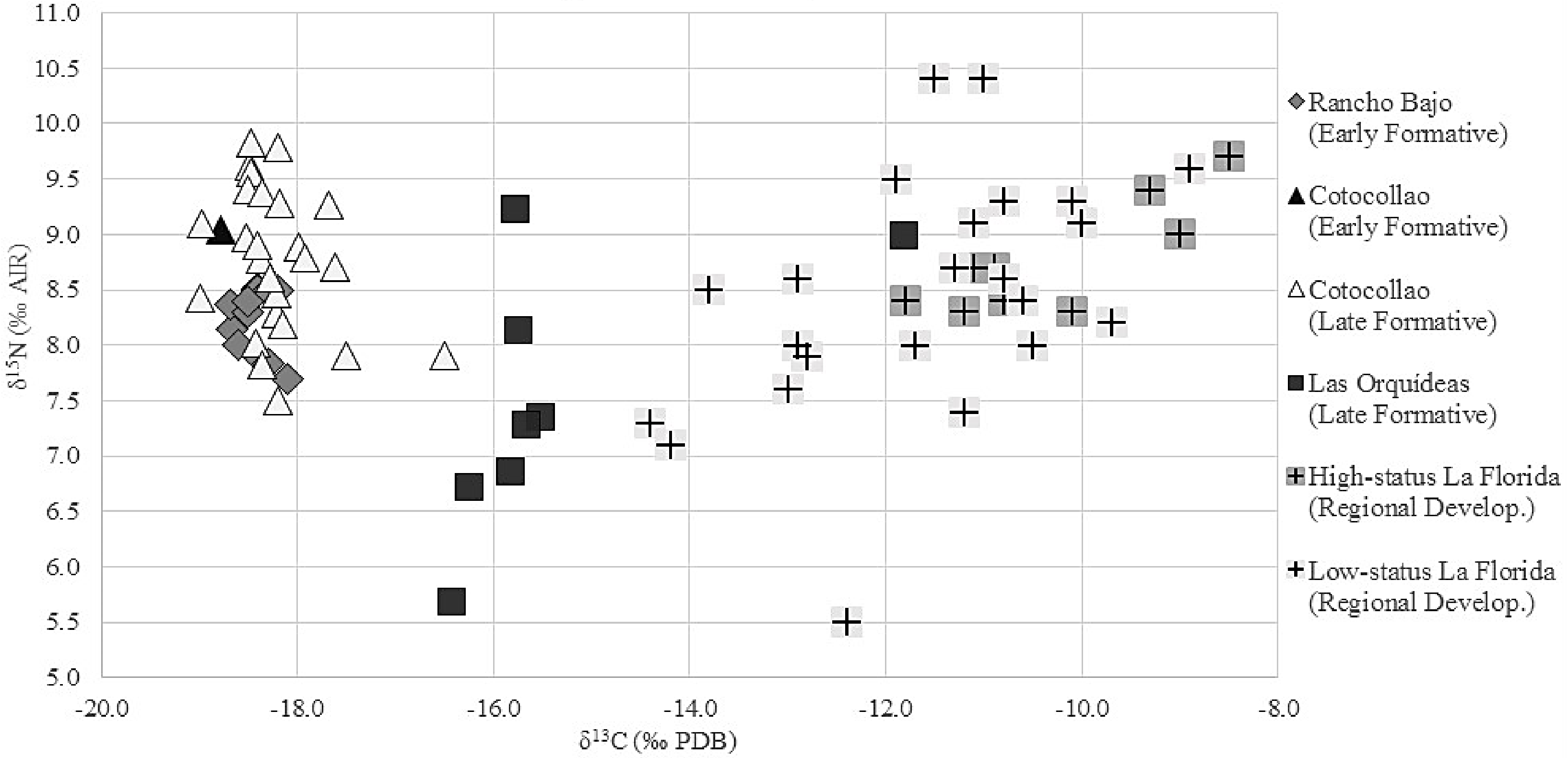
[21] Not to confuse with the site NAIQ studied by Pennycook. These represent different settlements discovered under the New International Quito Airport during different survey and rescue projects.

Fig. 7.11 - Human $\delta^{13}C$ & $\delta^{15}N$ values in the Formative & Regional Development Periods



7.5.2. Diet in the Formative and Integration Periods

In Ecuador, the Integration Period (AD 500–1500) followed the Regional Development Period and preceded the Inca conquest. Meggers and Evans (1963) described this period as characterized by large urban centers, the construction of large artificial earth mounds, and the mass production of pottery. These elements, along with a larger population size and the shift towards an economy based on agriculture and long-distance exchange, increased the sociopolitical complexity (Bray, 1992; Bray, 2008). As a result, during the Integration Period households and villages clustered around a central urban area also considered ceremonial, where a noble chief or "*cacique*" lived (the reason why these chiefdoms are commonly referred as "*cacicazgos*") (Salomon, 1986; Pennycook, 2013).

Pennycook (2013) examined the diet at the site of Tajamar, which is located in Quito in the Pomasqui valley. The finds at the site comprise structures, middens, hearths, local ceramics, plants and faunal remains, along with tombs and ceremonial structures. During the excavations supervised by the archaeologist Victoria Domínguez, over 100 tombs were discovered. The tombs varied in size and shape, as well as in the number of remains of individuals, their position in the burial, and grave goods. The more elaborate tombs were characterized by the presence of exotic goods such as beads made of *Spondylus* and ornaments made of "*tumbaga"* (gold-plated copper), articles which are considered to be evidence of high social/political status activities at the site (Domínguez, 2009; Pennycook, 2013). Pennycook (2013) sampled 73 human individuals and collected faunal samples from these tombs.

Pennycook (2013) also conducted stable isotope analysis of the site NAIQ, also located in Quito. The site was first discovered during field surveys in the construction site of the Nuevo Aeropuerto Internacional de Quito (New International Quito Airport). The archaeologists María Aguilera and Stefán Bohórquez conducted the excavation projects from 2004 to 2006 that led to the discovery of two necropolises that contained over 600 tombs dated to the Regional Development Period and the Integration Period. Among the grave goods recovered from these tombs are ceramic artifacts including non-local pottery, objects made of different metals as well as copper ornaments, and shell (e.g., mother of pearl) and faunal remains. No habitation areas were discovered (Aguilera, 2007; Pennycook, 2013). A total of 42 human samples (all from the Integration Period) along with some faunal samples were collected for stable isotope analysis (Pennycook, 2013).

The individuals from Tajamar displayed a mean collagen δ^{13}C value of -9.1 ± 1.1‰ and a mean δ^{15}N value of 8.6 ± 1.1‰, and the individuals from the NAIQ exhibited a mean collagen δ^{13}C value of -18.6 ±0.3‰ and a δ^{15}N mean of +8.0 ± 0.7‰ (Pennycook, 2013). Based on these values, Pennycook (2013) stated that the diet at Tajamar consisted primarily of C_4 resources such as maize, and that most of the protein may have derived from plants and meat from animals such as guinea pigs. In contrast, the diet at NAIQ consisted of a mix of C_3 and C_4 resources, but with a greater reliance on C_3 plants. The NAIQ diet was also supplemented with protein from wild and domestic terrestrial animals (see Chapter 2). Pennycook (2013) concluded that the differences seen between the isotopic signals of the individuals from Tajamar and NAIQ are likely the result of two populations that exploited different ecological zones within the same region (Pennycook, 2013). Figure 7.12 shows the isotopic signals from the Formative and Integration sites. A surprising finding is that the individuals from NAIQ exhibit similar isotopic signals to those from Rancho Bajo and the late settlement of Cotocollao, although they belong to very different periods. In contrast, Tajamar shows the expected isotopic signals for an agricultural site of the Integration Period in which maize was a staple food. This comparison suggests that households or villages had different dietary patterns as a result of different subsistence practices/strategies, and that they coexisted in the northern highlands during both the Formative and Integration periods.

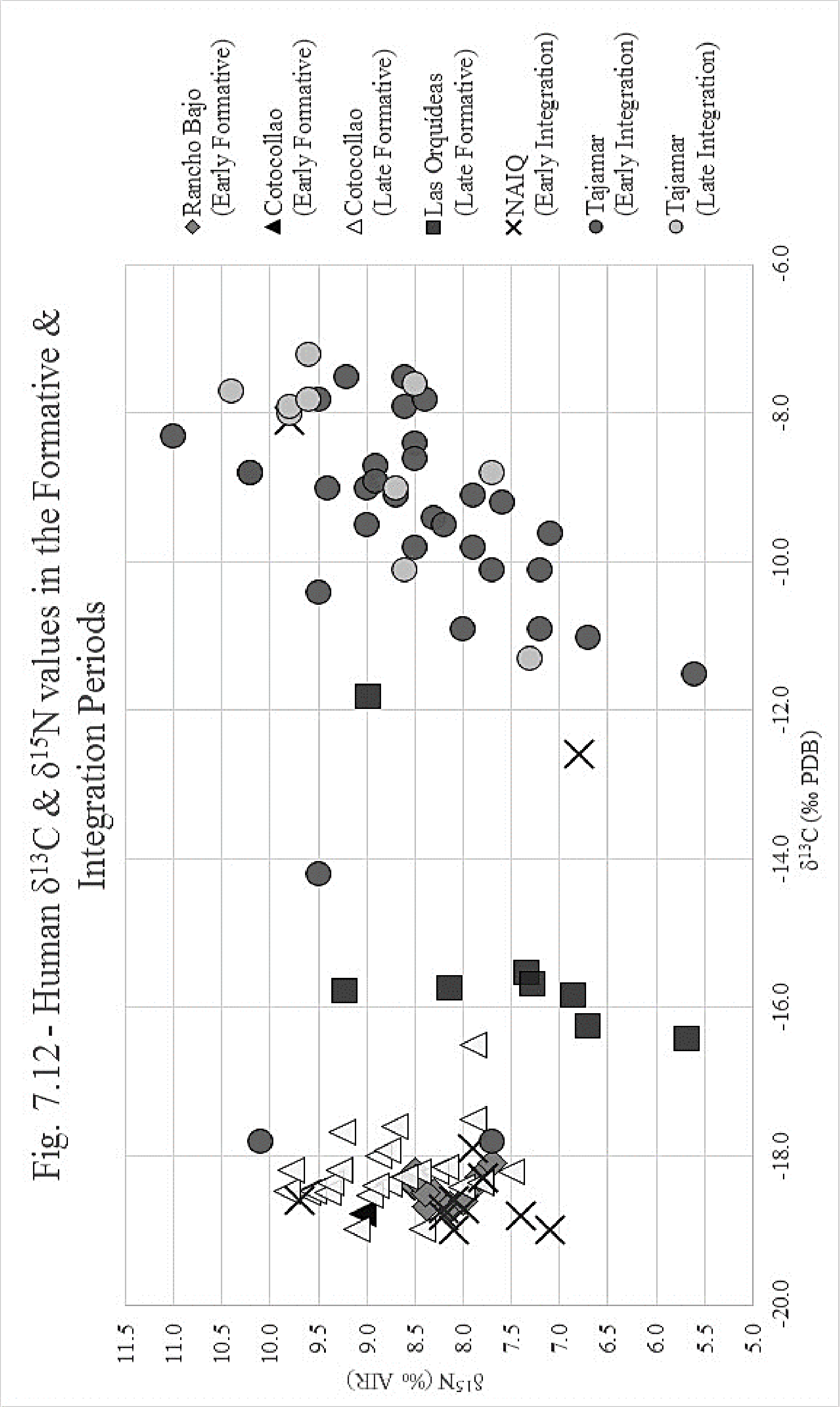


Fig. 7.12 - Human $\delta^{13}C$ & $\delta^{15}N$ values in the Formative & Integration Periods

A comparison of the faunal values recovered for the Formative and Integration periods is useful. Pennycook (2013) analyzed samples of cervids (n=1), camelids (n=13), and canids (n=11) from Tajamar. The cervid sample displayed a $\delta^{13}C$ value of -21.9‰ and $\delta^{15}N$ value of 3.4‰. The camelids' mean $\delta^{13}C$ value was -14.9‰ ± 1.9‰; their mean $\delta^{15}N$ value was 7.0‰ ± 0.9‰. The canids' mean $\delta^{13}C$ value was - 8.9‰ ± 1.3‰ and their mean $\delta^{15}N$ value was 8.5‰ ± 0.9‰.

From NAIQ, Pennycook (2013) sampled cervids (n=9), rodents/leporids (n=15), camelids (n=7), and a canid (n=1). The cervids' mean $\delta^{13}C$ value was -17.1‰ ± 3.5‰; their mean $\delta^{15}N$ value was 4.6‰ ± 2.2‰. Rodents and leporids (probably including Cavia) were considered under the same category, due to difficulties in identifying the bones. The mean $\delta^{13}C$ value was -17.2‰ ± 5.2‰; the mean $\delta^{15}N$ value was 4.2‰ ± 2.0‰. The camelids' mean $\delta^{13}C$ value was -12.2‰ ± 0.8‰; their mean $\delta^{15}N$ value was 8.2‰ ± 1.2‰. The canid sample displayed a $\delta^{13}C$ value of -8.3‰ and a $\delta^{15}N$ value of 7.3‰.

Based on these $\delta^{13}C$ and $\delta^{15}N$ values, Pennycook (2013) distinguished two groups of cervids. The first group had a pure C_3 diet, as seen in a mean $\delta^{13}C$ value of -20.2‰ ± 0.9‰, mean $\delta^{15}N$ value of 3.0‰ ± 1.1‰, and mean $\delta^{13}C_{bioapatite}$ value (n=6) of -10.7‰ ± 1.0‰; this group included the only cervid sample from Tajamar. The second group reflected a mixed diet of C_3 and C_4 resources; the mean $\delta^{13}C$ value was -13.6‰ ± 1.8‰, the mean $\delta^{15}N$ value was 6.5‰ ± 0.5‰, and their mean $\delta^{13}C_{bioapatite}$ value (n=2) was -6.4‰ ± 0.6‰. Although the $\delta^{13}C$ values of the mixed diet group are higher, this result is similar to the pattern discussed above for Las Orquídeas.

The rodent/leporid samples were divided into three categories according to their diet. The first group displayed a mean $\delta^{13}C$ value of -20.7‰ ± 1.6‰, a mean $\delta^{15}N$ value of 2.8‰ ± 1.1‰, and a mean $\delta^{13}C_{bioapatite}$ value (n=8) of -9.2‰ ± 2.9‰, consistent with a pure C_3 diet.

The second group showed a mixed diet with a mean δ^{13}C value of -15.4‰ ± 1.4‰, a mean δ^{15}N value of 5.7‰ ± 0.6‰; and a $\delta^{13}C_{bioapatite}$ value (n=1) of -10.0‰. The last group exhibited a mean δ^{13}C value of -8.4‰ ± 0.3‰, a mean δ^{15}N value of 7.0‰ ± 0.8‰ and a mean $\delta^{13}C_{bioapatite}$ value (n=1) of -3.3‰, consistent with a C_4 diet.

Camelids from Tajamar had a mixed diet of C_3 and C_4 resources. Their mean $\delta^{13}C_{bioapatite}$ value (n=12) was -6.7‰ ± 1.7‰. The camelids from NAIQ also had a mixed diet, although they consumed more maize. Their mean $\delta^{13}C_{bioapatite}$ value was -4.8‰ ± 0.8‰.

Canids at Tajamar display similar values to humans, which suggests a similar diet. The mean $\delta^{13}C_{bioapatite}$ value was -3.9‰ ± 1.0‰. In contrast, the canid from NAIQ has a very different diet from the NAIQ humans; but a similar isotopic signal to the canids from Tajamar, showing more maize consumption. Pennycook (2013) suggests it could have been traded by another group, as reported for the highlands. Its $\delta^{13}C_{bioapatite}$ value was -3.6‰.

A scatter graph in Figure 7.13 shows the δ^{13}C and δ^{15}N values from the faunal samples of Cotocollao, Las Orquídeas, Tajamar, and NAIQ. The cervids from Cotocollao and Las Orquídeas with a predominantly C_3 diet (mean δ^{13}C= -19.9‰ ± 0.4‰; mean δ^{15}N= 3.9‰ ± 1.2‰) have values close to those interpreted by Pennycook (2013) as consistent with a pure C_3 diet. The cervids from Las Orquídeas that consumed mostly C_3 plants and some maize (mean δ^{13}C= -17.7‰ ± 1.1‰; mean δ^{15}N= 5.6‰ ± 0.9‰) show less maize consumption in comparison to the cervids with a mixed C_3/C_4 diet reported by Pennycook (2013), an interesting parallel to the difference between humans at the two sites. The Las Orquídeas leporid samples (mean δ^{13}C= -20.7‰ ± 2.1‰; mean δ^{15}N= 2.7‰ ± 0.9‰) have almost identical values as the rodents/leporids with pure C_3 diets from NAIQ. While the camelid samples from Las Orquídeas (mean δ^{13}C= -19.3‰ ± 0.6‰; mean δ^{15}N= 5.8‰ ± 1.1‰) had a predominantly C$_3$ diet, the camelids from both Tajamar and NAIQ have values consistent with a higher consumption of maize.

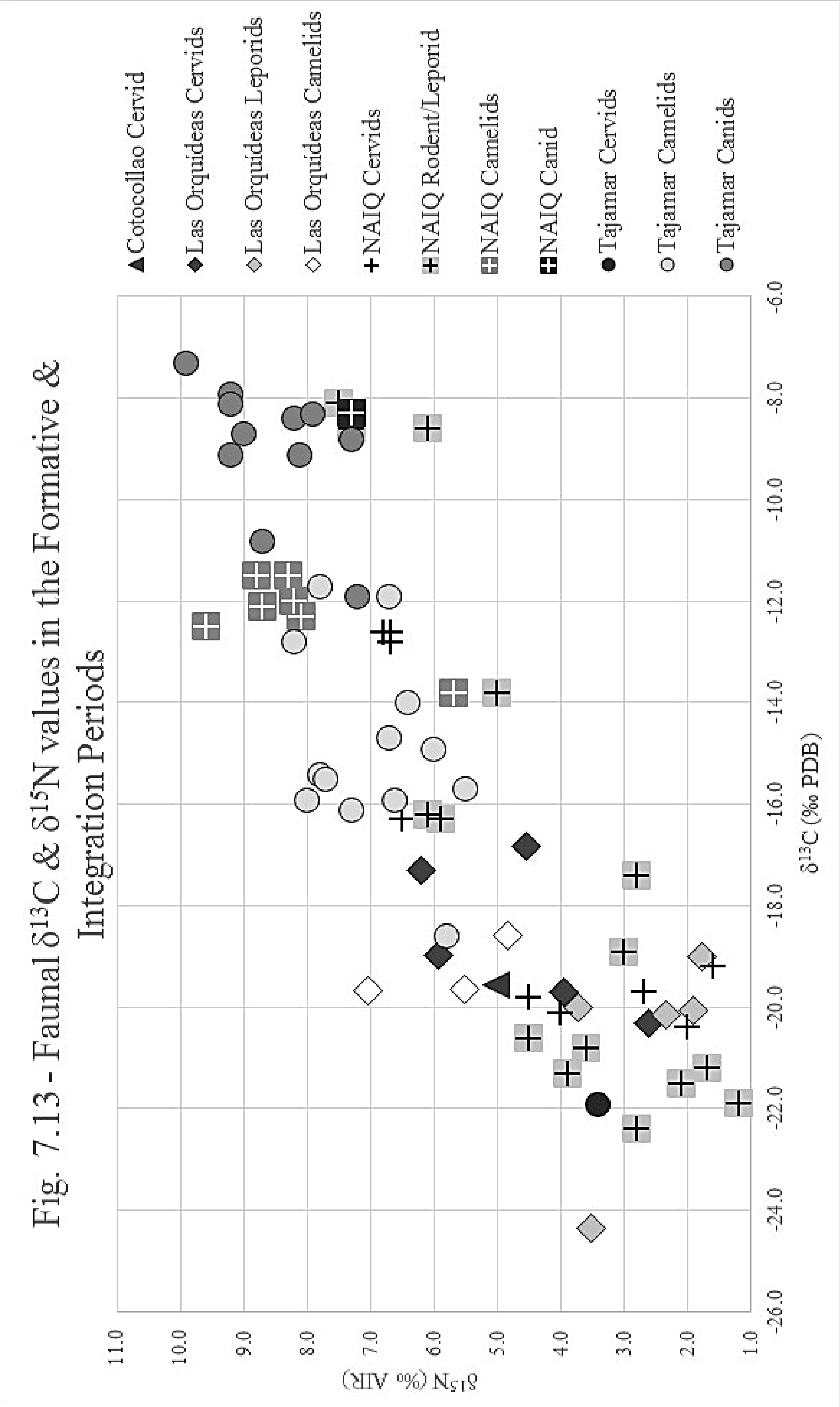


Fig. 7.13 - Faunal δ^{13}C & δ^{15}N values in the Formative & Integration Periods

Thus, both human and faunal $\delta^{13}C$ and $\delta^{15}N$ values confirm an increase in the consumption of maize during the Integration Period. However, this is more the case for the people and fauna from Tajamar. Other archaeological findings at Tajamar include a large number of tall jars and some "*aríbalos*" (Inca tall-necked jar forms) known for being mainly used to ferment chicha, but which could have been also used for the storage of solids (Domínguez, 2009).

In terms of NAIQ, although the faunal remains generally display stable isotope values consistent with a mixed diet of C_3 and C_4 resources, some animal species exhibit values that indicate that maize played a more significant role in their diet. In contrast, the human values from the site suggest their diet was heavily dependent on C_3 resources; and had only a small contribution of maize. Unfortunately, site reports from NAIQ could not be obtained by the time my study was completed, so any other archaeological evidence at the site that could reflect greater consumption of C_3 resources than maize cannot be included in this interpretation.

7.5.3. Diet in the Formative, Regional Development, and Integration Periods

To this day, our understanding of the diet of early prehispanic groups in Ecuador has been linked to the archaeobotanical and zooarchaeological records and a few stable isotope studies, while a frequent approach to understanding the diet of late prehispanic chiefdoms has been based on etnohistoric sources such as Early Spanish accounts, although these give little information related to plants and animals (Pennycook, 2013; Salomon, 1986).

Although archaeobotanical and zooarchaeological studies are fundamental to identify the presence of flora and faunal species and to quantify their abundance in archaeological sites, they cannot provide more precise information relative to the human and animal diets unless the remains have been recovered from secure contexts (e.g., starches found in pottery). Thus, only stable isotope analysis can give a better insight into a population's diet.

By conducting carbon and nitrogen stable isotope analysis on Formative sites, this study has obtained evidence that some settlements from the Early Formative period had a diet heavily dependent on C_3 wild resources and C_3 cultivars that also included small quantities of maize. The people from these settlements obtained most of their protein from cervids or leporids and other animals. It has also confirmed that the Late Formative sites had a predominantly C_3 diet accompanied with some maize consumption. However, the proportion in which maize was consumed differs between settlements, which suggests that the importance of maize was also different. In addition to the animals that were consumed during the early Formative, it is possible that the people from these settlements consumed meat from camelids and guinea pigs.

The comparison of the human and faunal $\delta^{13}C$ and $\delta^{15}N$ values from the Formative, Regional Development, and Integration periods presented by this study makes it possible to observe a general dietary trend in which maize consumption gradually increases over time in the northern highlands. This observation is not unexpected, given that many researchers have found a similar trend in North America, Mesoamerica, and the Andes. This evidence has been the basis for the discussion about the role played by the intensification of maize agriculture in catalyzing the evolution of sociopolitical complexity (Finucane, 2009; Burger and van der Merwe 1990; Tykot and Staller 2002). This role of maize has also been a subject of discussion by researchers who work in Ecuador (Pearsall, 2002; Staller and Thompson, 2002; Bruhns, 2003; Zarrillo and Valdéz, 2013).

The evidence commonly cited to support this assumption includes the abundant presence of maize botanical remains; and the large number of manos, grinding stones, and more specialized ceramic pots necessary for cooking maize and other hard seeds, as these involved a more difficult process of grinding before cooking (Pearsall, 2002; Athens et al, 2016; Piperno and Pearsall, 1998).

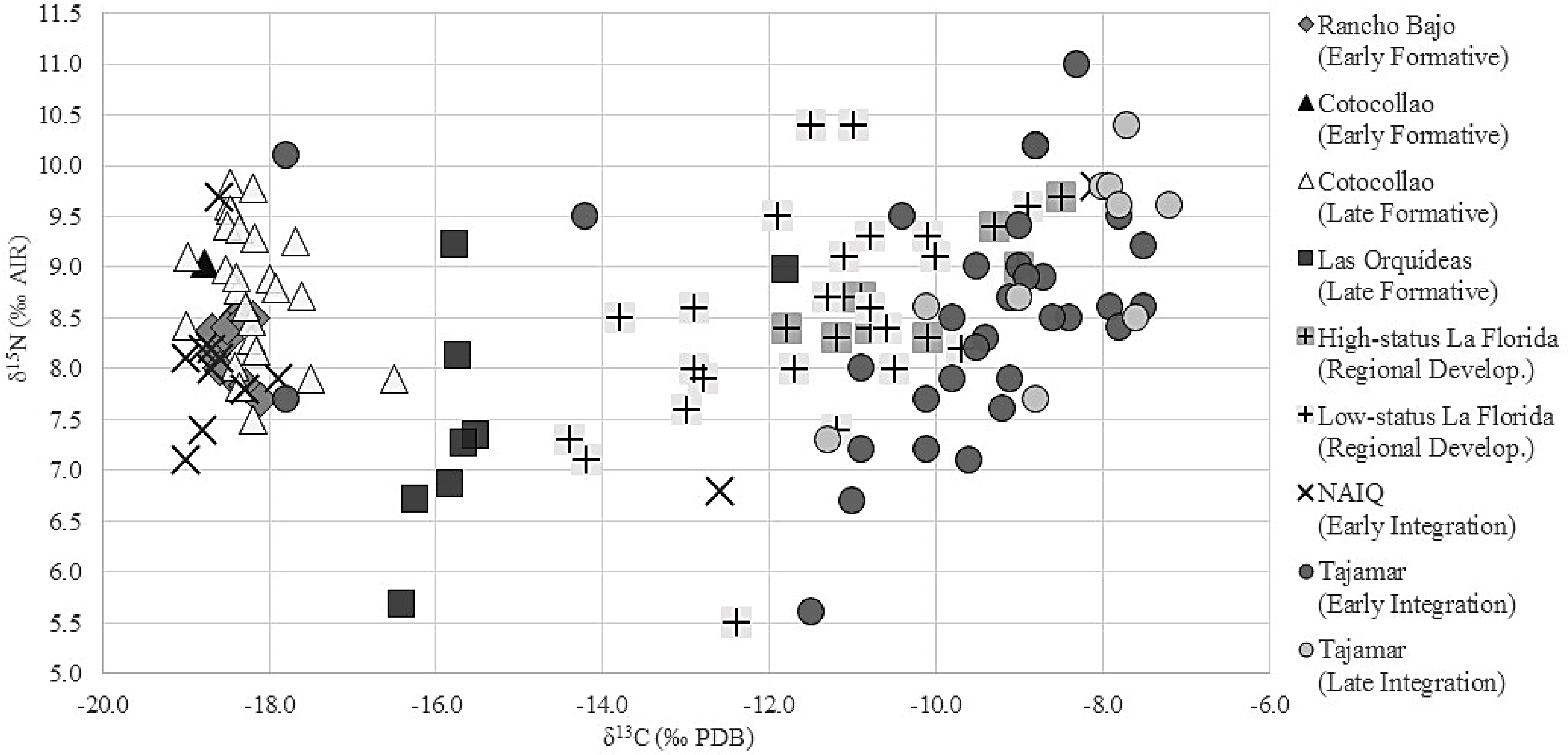
Other researchers cite the results from stable isotope analysis (Ubelaker et al. 1995; Staller and Thompson, 2002; Athens et al. 2016), and the abundant presence of large ceramic jars and *aríbalos* for brewing maize beer and feasting cups likely used for chicha. This conclusion includes the fact that the presence of these cups have been limited to high-status tombs (Doyón, 1991; Ubelaker et al. 1995; Masucci, 2008; Bray, 2008; Domínguez, 2009; Pazmiño et al., 2010).

In making this argument, researchers have also considered the construction of irrigation canals and raised fields known as "*camellones*" as they appear in maize-based polities (Doyón, 1991; Ubelaker et al., 1995; Pearsall, 2002; Bray, 2008; Damp and Brito, 2014; Athens et al., 2016). The importance of *camellones* is that they increase the soil productivity, modifying the water flow, the availability of humus and silt, and the microclimate on planted surfaces (Salomon, 1986).

All the evidence mentioned above shows that complex chiefdoms and polities had a subsistence economy based on maize agriculture. Regardless of this trend, the results from this study also show that in each of these periods the consumption of maize varied according to the settlement. During the Late Formative at least three human groups with different degrees of maize consumption were identified. The first pattern was observed in the late settlement of Cotocollao, which has $\delta^{13}C$ values consistent with a predominantly C_3 diet with a small contribution of maize. The second was observed in Las Orquídeas, with $\delta^{13}C$ values that have been interpreted as a mixed diet of C_3 and C_4 resources in which there was a slightly higher consumption of maize than in Cotocollao. The third group is represented by the single individual buried in Las Orquídeas, whose values suggest a heavy reliance on maize consumption. As mentioned before, this is also the only individual discovered to this day that was buried with grave goods in Las Orquídeas. The different isotopic signal of this female along with the presence of grave goods have been considered as evidence that she may have belonged to a different settlement. In terms of the $\delta^{15}N$ values, these suggest that all the Late Formative sites consumed protein from terrestrial animals such as leporids, cervids, camelids, and other fauna. There is certainly some variation in the $\delta^{15}N$ values that may depend on the animals that were the source of protein, the amount of protein consumed, as well as based on the diet of these animals (e.g., grazing animals, maize-fed animals).

Unfortunately, to this day little is known about the Regional Development Period in the highlands of Ecuador, including the diet. This situation is partially due to the discovery of only a few sites, as well as to the fact that many of these studies have focused on the cultural material and have not conducted archaeobotanical or zooarchaeological analyses. Moreover, most of the information available exists only in the form of grey literature. Therefore, not much can be added to the information provided by the stable isotope analysis conducted by Ubelaker et al. (1995) in order to develop an interpretation of the diet of this period. Yet, since the $\delta^{13}C$ and $\delta^{15}N$ values from La Florida are consistent with more reliance on maize consumption, they are useful to confirm the increase in C_4 consumption from the Formative to its subsequent period.

Fig. 7.14 - Human $\delta^{13}C$ & $\delta^{15}N$ values in the Formative, Regional Development & Integration Periods



The stable isotope values also give valuable information about the differential consumption of maize between high-status and low-status individuals. This also applies to the Integration site Tajamar, in which the stable isotope values have confirmed that maize was a staple crop. However, NAIQ, the other Integration site examined by Pennycook (2013), shows very different stable isotope values of those expected for sites from the Regional Development and Integration periods. In fact, the people from the site NAIQ exhibited an almost identical isotopic signal to the individuals from the Early Formative. This comparison again suggests that the importance of maize was not equal in all settlements, even during the Integration Period, close to the Inca conquest. No significant isotopic variation was seen in terms of age or sex at any of these sites. However, this could be partially due to the uneven male and female sample sizes examined by these studies. Figure 7.14 shows a scatter plot with the $\delta^{13}C$ and $\delta^{15}N$ values from all of the sites analyzed and discussed in this study.

7.6. Summary

The $\delta^{13}C$ and $\delta^{15}N$ values indicate that the Formative groups examined by this study had a diet predominantly based on C_3 resources and protein from terrestrial animals. The values also confirm that there was some maize consumption. The amount in which this C_4 resource was consumed varied between sites, showing that different subsistence practices took place during the Formative Period. The individuals that lived in Rancho Bajo display very tightly clustered isotopic signals, which suggests that all the food resources that were obtained may have been shared by the entire group. As a result of sharing the food, there is low variability in terms of $\delta^{13}C$ and $\delta^{15}N$ values between individuals. These small differences fall within 1‰, and could have been caused by individual variation in metabolism or health. Cotocollao is a site with $\delta^{13}C$ and $\delta^{15}N$ values suggesting that the diet of the individuals was similar to that of Rancho Bajo; in other words, its inhabitants too were heavily dependent on C_3 resources and consumed only modest amounts of C_4 resources. Finally, in Las Orquídeas the consumption of C_4 plants is more evident. However, the isotopic values from this site still fall quite far from those obtained from sites dated to the Regional Development Period or the Integration Period, when people appear to have consumed diets that were heavily dependent on maize. After comparing the diet of human groups from the Formative, Regional Development Period, and Integration Period, it is possible to say that, in general, the consumption of maize increased throughout these periods. However, Cotocollao and NAIQ are exceptions that document the existence of sites in which maize did not play a significant role in human diets, not even during the Integration Period close to Inca Conquest. Thus, it can be assumed that maize did not have equal importance along the different sites located in the region or during the Formative, Regional Development, and Integration periods. At the same time, this indicates that certain groups were characterized by subsistence activities centered on gathering C_3 wild resources and producing C_3 cultivars, as well as obtaining meat from terrestrial animals; while other groups, besides participating in these activities, also produced and consumed maize. The fact that, in general, no age or sex dietary variation was seen in the Formative sites suggests that most individuals had a similar diet, with only small differences in terms of proportions of C_3 and C_4 resources.

Chapter 8: Conclusions

The purpose of this research was to contribute new information about the plant and animal resources consumed by humans during the Formative Period in Ecuador's northern highlands. Through the stable carbon and nitrogen isotope analysis of 47 human samples and 14 faunal samples from the archaeological sites of Rancho Bajo, Cotocollao, and Las Orquídeas, this goal was achieved. Based on the $\delta^{13}C$ and $\delta^{15}N$ results, the diet of most of these human settlements was characterized by a heavy reliance on C_3 wild resources and C_3 cultivars, and the consumption of terrestrial animal protein. The isotopic signals also confirmed that maize was present in all these sites and that it was part of the diet. However, the carbon isotope values show that the contribution of this crop to the diet varied between sites, especially during the Late Formative, in which the consumption of maize ranged from an almost insignificant contribution to the diet up to the point in which it became a staple. The above was also confirmed by the $\delta^{13}C$ values of cervids, leporids, and camelids, since some of these animals exhibited the expected $\delta^{13}C$ values for herbivores with predominantly C_3 diets, while others had $\delta^{13}C$ values that indicate some maize consumption.

8.1. Revisiting the Hypotheses

The hypothesis of this study was that the $\delta^{13}C$ and $\delta^{15}N$ values of the individuals from Rancho Bajo would be consistent with the consumption of C_3 wild resources and terrestrial animal protein. As discussed in Chapter 3, this hypothesis was based on the complete absence of ceramics at the site; and the fact that only obsidian and basalt artifacts made with the purpose of cutting, scraping, and perforating were recovered, artifacts which suggest that this settlement had a hunter-gatherer economý. It was also hypothesized that if maize was consumed, it may have been only in very small quantities, given that dental caries occurred in very low frequencies, which usually indicates a diet that was not rich in starches. The stable isotope values are certainly consistent with this hypothesis.

Regarding the early phase of Cotocollao, the hypothesis was that the individuals probably had a predominantly C_3 diet supplemented with some maize, and that they consumed terrestrial animal protein. Part of the hypothesis was also that they consumed C_3 cultivars, based on the facts that 1) maize was identified at the site as well as many other C_3 cultivars; 2) the presence of some ceramic fragments was noted; 3) prior research on the period has argued for a link between the appeareance of ceramics and the appearance of agriculture (see Chapter 2); 4) some manos and metates were recovered, which indicate the grinding of seeds; 5) obsidian and basalt artifacts were found in large quantities at the site as along with many faunal remains, suggesting that hunting and the preparation of skins were also important activities at the site. The results from this study are consistent with this hypothesis. It is important to note that the archaeological evidence seems to indicate that this settlement had a slightly different subsistence economy from that of Rancho Bajo; yet the individuals from both settlements display similar $\delta^{13}C$ values. However, only one individual could be analyzed from this settlement. Thus, it would be necessary to analyze more samples in the future to confirm that the diets were actually isotopically similar.

In terms of the late settlement of Cotocollao, after taking into consideration 1) the presence of maize and C_3 cultivars, documented even since the early phase; 2) the large number of ceramic artifacts, manos, and metates; 3) the size of the population and settlement, and 4) the radiocarbon dates that place this settlement closer to the Regional Development Period in which there is also greater evidence of maize consumption, the hypothesis of this study was that maize may have been a staple food and that the diet was supplemented with some C_3 cultivars and terrestrial animal protein. The stable isotope values suggest that this hypothesis should be rejected in part. Although the diet included both C_3 and C_4 resources, maize was not one of the staple foods. The diet was heavily reliant on C_3 cultivars and included only modest amounts of maize, as well as terrestrial animal protein.

A similar hypothesis of maize dependence was posed for Las Orquídeas, since this settlement has physical evidence of maize, as well as large quantities of ceramic and lithic artifacts and faunal remains; and the radiocarbon dates are also close to those from the Regional Development period. Although maize was also not the core staple food for this settlement, it was certainly consumed in greater quantities in comparison to the other sites. In addition, one individual buried at the site may have come from a community more dependent on maize. Thus Las Orquídeas, dated toward the end of the Formative, does show the strongest evidence for maize consumption although the pattern is not exactly the same as originally hypothesized.

8.2. Contributions to the Literature

This is the first study that has conducted stable isotope analysis to examine diet in the northern highlands during the Formative Period. Thus, the information presented in this thesis is the first direct evidence of diet, given that prior interpretations were solely based on palaeobotanical and zooarchaeological studies if available.

Besides adding new information about the diet at Rancho Bajo, Cotocollao, and Las Orquídeas, this research has provided a diachronic perspective on diet, by comparing the results from this study to the values from the two subsequent periods. This comparison has made it possible to observe dietary trends, especially related to the consumption of maize, over three consecutive periods in the same region.

Moreover, by analyzing some faunal samples from the region, this study has provided a baseline that now is available for future studies, and may be consulted and added to as research in the area develops further.

8.3. Future Research

Given the small number of stable isotope studies that have been conducted in the country, it is possible to say that there are plenty of avenues for future research. For example, starting with the sites from this study, future research should focus on conducting strontium stable isotope analyisis in the female individual from Las Orquídeas, who was apparently a non-local migrant. This analysis will give valuable information about residential mobility. If a different place of origin is confirmed, then the stable isotope values will become a new line of evidence that would support the connections of other settlements with Las Orquídeas. This would be interesting, given the abundant evidence of trade present at the site, which has been used to suggest that Las Orquídeas was part of an exchange-system that worked on a north-south and east-west axis. Moreover, if a more detailed zooarchaeological analysis is conducted at Las Orquídeas, and all the other species are examined using stable isotope analysis, then those values will help to create a more complete baseline for the northern highlands.

In addition, it would be useful to contact the researchers that conducted carbon and nitrogen stable isotopes at La Chimba, a site dated to the end of the Formative and Regional Development Period (Tykot et al., 2006; see Chapter 2), to see if they would be willing to share their raw isotope values in order to compare them to the sites studied here. It would also be interesting to conduct stable isotope analysis on the individuals from *tola* Huataviro (Pazmiño et al., 2010). Although only a few individuals were recovered at this site, the fact that it has been dated to the Regional Development and Integration periods, and that it is located relatively close to Las Orquídeas and has high-status burials makes it a good opportunity for expanding our knowledge about diet in the region and during these periods. It would be a good opportunity for testing if there was differential consumption of maize between low-status and high-status individuals as seen in La Florida.

8.4. Summary

This study shows that stable isotope analysis can give an inmense contribution to our general knowledge of diet and life of prehispanic populations in Ecuador. Hopefully in the coming years more research of this kind will be carried out in the country.

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Appendix 1

Table A.1.1 - Human samples

Sample ID	Site	Feature/Burial/ Context	Time Period	Sex	Age	Bone sampled
PRB-01	Rancho Bajo	Feature 9	Early Formative	M	Middle Adult (35 - 45 years)	Left femur
PRB-02	Rancho Bajo	Feature 3	Early Formative	M	Middle Adult (30 - 40 years)	Left femur
PRB-03	Rancho Bajo	Feature 33	Early Formative	ND	Subadult (10 - 15 years)	Right femur
PRB-04	Rancho Bajo	Feature 5	Early Formative	M	Middle Adult (30 - 40 years)	Right femur
PRB-05	Rancho Bajo	Feature 26	Early Formative	M	Young Adult (25 - 35 years)	Right tibia
PRB-06	Rancho Bajo	Feature 8	Early Formative	M	Young Adult (25 - 35 years)	Right humerus
PRB-07	Rancho Bajo	Feature 1	Early Formative	F	Middle Adult (35 - 45 years)	Left femur
PRB-08	Rancho Bajo	Feature 35	Early Formative	M	Adult (30 - 60 years)	Left femur
PRB-09	Rancho Bajo	Feature 15	Early Formative	F	Subadult (16 - 20 years)	Right tibia
PRB-10	Rancho Bajo	Feature 30 Individual 1	Early Formative	M	Young Adult (25 - 35 years)	Right tibia
PRB-11	Rancho Bajo	Feature 30 Individual 2	Early Formative	M	Young Adult (25 - 35 years)	Right femur
PRB-12	Rancho Bajo	Feature 6	Early Formative	M	Middle Adult (30 - 40 years)	Right femur
PRB-13	Rancho Bajo	Feature 31	Early Formative	F	Adult (30 - 60 years)	Left femur
PRB-14	Rancho Bajo	Feature 27	Early Formative	F	Middle Adult (33 - 45 years)	Left tibia
PRB-15	Rancho Bajo	Feature 12	Early Formative	ND	Subadult (14 - 17 years)	Right humerus

Table A.1.1 (continued) - Human Samples

Sample ID	Site	Feature/Burial/ Context	Time Period	Sex	Age	Bone sampled
PCO-01	Cotocollao	52 F19	Late Formative	ND	Young Adult (20-30 years)	Femur
PCO-02	Cotocollao	134 9c13	Late Formative	ND	Young Adult (23-30 years)	Femur
PCO-03	Cotocollao	Feature 15 F19 Individual A	Late Formative	ND	Young Adult (25-30 years)	Femur
PCO-04	Cotocollao	144 F19	Late Formative	F	Old Adult (40-60 years)	Humerus
PCO-05	Cotocollao	T6 F38 5475	Late Formative	M	Middle Adult (30-35 years)	Humerus
PCO-06	Cotocollao	117 F19	Late Formative	M	Middle Adult (20-40 years)	Femur
PCO-07	Cotocollao	Feature 3 F19 9d17	Late Formative	M	Young Adult (25-35 years)	Femur
PCO-08	Cotocollao	113 F19	Late Formative	ND	Young Adult (20-25 years)	Long bone
PCO-09	Cotocollao	T21 F38	Late Formative	ND	Adult	Tibia
PCO-10	Cotocollao	17 F19 F3 1d17	Late Formative	ND	Adult	Femur
PCO-11	Cotocollao	137 F19	Late Formative	F	Young Adult (20-30 years)	Humerus
PCO-12	Cotocollao	123 5c12	Late Formative	ND	Adult	Long bone
PCO-13	Cotocollao	112/121 F19 4c10	Late Formative	F	Young Adult (22-25 years)	Long bone
PCO-14	Cotocollao	147 F19	Early Formative	M	Middle Adult (30-40 years)	Long bone
PCO-15	Cotocollao	51 F19	Late Formative	M	Young Adult (20-30 years)	Skull
PCO-16	Cotocollao	119 F19 5c12	Late Formative	ND	Adult (ca. 45 years)	Skull
PCO-17	Cotocollao	50 F19	Late Formative	ND	Adult	Tibia

Table A.1.1 (continued) - Human samples

Sample ID	Site	Feature/Burial/ Context	Time Period	Sex	Age	Bone sampled
PCO-18	Cotocollao	136 F19	Late Formative	ND	Young adult (18-25 years)	Humerus
PCO-19	Cotocollao	110/135 F19 1c11	Late Formative	F	Old Adult (40-60 years)	Humerus
PCO-20	Cotocollao	Feature 15 F19 Individual B	Late Formative	M	Young Adult (25-30 years)	Skull
PCO-21	Cotocollao	120 F19 5c13	Late Formative	ND	Middle Adult (35-45 years)	Skull
PCO-22	Cotocollao	142 8c13	Late Formative	M	Young Adult (ca. 25 years)	Skull
PCO-23	Cotocollao	122 5c12	Late Formative	F	Old Adult (35-60 years)	Rib
PCO-24	Cotocollao	T21 F38	Late Formative	ND	Young Adult (20-35 years)	Rib
PCO-25	Cotocollao	131 F19	Late Formative	M	Middle Adult (30-35 years)	Rib
PCO-26	Cotocollao	T6 F38 5475	Late Formative	ND	Subadult (ca. 17 years)	Rib
PCO-27	Cotocollao	141 4c13	Late Formative	ND	Subadult (15-18 years)	Rib
PCO-28	Cotocollao	53 F19	Late Formative	ND	Subadult (ca. 13 years)	Mandible
PCO-29	Cotocollao	130 7c13	Late Formative	F	Middle Adult (35-45 years)	Left clavicle
PCO-30	Cotocollao	111 F19 2c11	Late Formative	M	Old Adult (40-60 years)	Humerus
PCO-31	Cotocollao	17 Part of T2 F19 F3	Late Formative	F	Young Adult (25-30 years)	Radius
PCO-32	Cotocollao	143 8c13	Late Formative	M	Young Adult (22-25 years)	Left humerus
PCO-33	Cotocollao	143 8c13	Late Formative	ND	Adult	Left humerus

Table A.1.1 (continued) - Human samples

Sample ID	Site	Feature/Burial/ Context	Time Period	Sex	Age	Bone sampled
POR-01	Las Orquídeas	Unit 68, Level 2 Feature 1	Late Formative	F	Young Adult (14-23 years)	Rib
POR-02	Las Orquídeas	Superior terrace Looted	Late Formative	F	Middle Adult (30-34 years)	Rib
POR-03	Las Orquídeas	Season I Without context	Late Formative	ND	Subadult (14-18 years)	Rib
POR-04	Las Orquídeas	Unit 49 Feature 4	Late Formative	F	Young Adult (20+ years)	Rib
POR-05	Las Orquídeas	Unit 41 Profile 3m b/s	Late Formative	ND	Subadult (11-12 years)	Rib
POR-06	Las Orquídeas	Unit 34 Level 2, Meter 3	Late Formative	M	Old Adult (50+ years)	Rib
POR-07	Las Orquídeas	Unit 1, Level 14	Late Formative	ND	Subadult (6-7 years)	Rib
POR-08	Las Orquídeas	Unit 41 Cangahua level	Late Formative	M	Young Adult (24-29 years)	Rib

Table A.1.2 - Faunal Samples

Sample ID	Site	Feature/Burial/ Context	Time Period	Bone sampled	Family	Common name
PCO-34	Cotocollao	Co.1 F23 Op.2	Formative	Long bone	Cervidae	Deer?
PCO-35	Cotocollao	Co.1 F6 Op.2	Formative	Long bone	Cervidae	Deer
PCO-36	Cotocollao	“Hni”	Formative	Long bone	Cervidae	Deer?
POR-09	Las Orquídeas	Unit 51, Level 4 Feature 1	Late Formative	Long bone	Cervidae	Deer
POR-10	Las Orquídeas	Unit 53 Meter 1, Level 3b	Late Formative	Rib	Cervidae	Deer
POR-11	Las Orquídeas	Unit 54, Level 6	Late Formative	Long bone	Cervidae	Deer
POR-12	Las Orquídeas	Unit 52, Level 8	Late Formative	Long bone	Cervidae	Deer
POR-13	Las Orquídeas	Unit 53, Level 4	Late Formative	Rib	Cervidae	Deer
POR-14	Las Orquídeas	Unit 51, Level 4	Late Formative	Os coxae	Leporid	Hare/Rabbit
POR-15	Las Orquídeas	Unit 54, Level 3	Late Formative	Os coxae	Leporid	Hare/Rabbit
POR-16	Las Orquídeas	Unit 53, Level 4	Late Formative	Os coxae	Leporid	Hare/Rabbit
POR-17	Las Orquídeas	Unit 53 Meter 1, Level 3b	Late Formative	Femur	Leporid	Hare/Rabbit
POR-18	Las Orquídeas	Unit 52, Level 8	Late Formative	Femur	Leporid	Hare/Rabbit
POR-19	Las Orquídeas	Unit 53 Meter 3, Level 3B	Late Formative	Sternum	Camelidae	Llama/Alpaca
POR-20	Las Orquídeas	Unit 54, Level 2	Late Formative	Sternum	Camelidae	Llama/Alpaca

Table A.1.2 (continued) - Faunal Samples

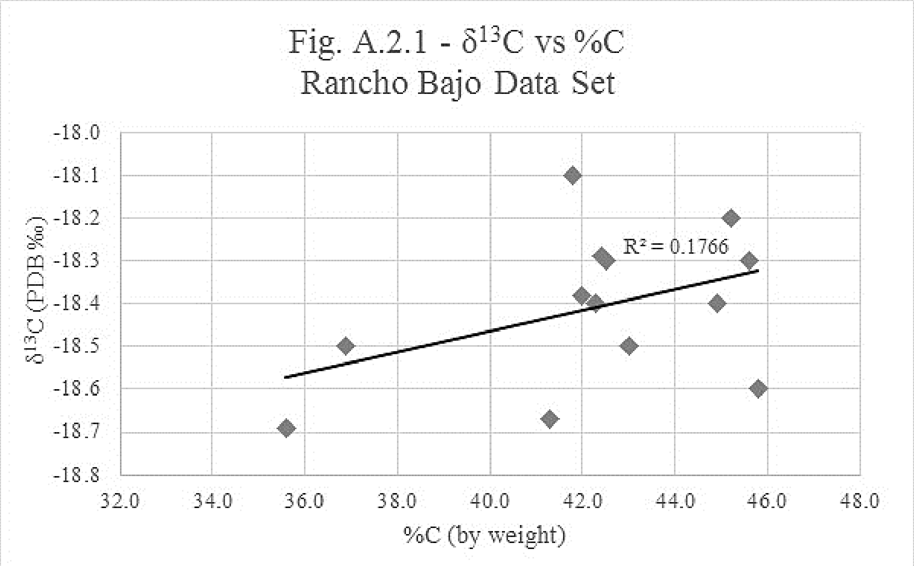
Sample ID	Site	Feature/Burial/ Context	Time Period	Bone sampled	Family	Common name
POR-21	Las Orquídeas	Unit 54, Level 4	Late Formative	Sternum	Camelidae	Llama/Alpaca
POR-22	Las Orquídeas	Unit 2, Level 8	Late Formative	Calcaneus	Camelidae	Llama/Alpaca
POR-23	Las Orquídeas	Unit 54, Level 3	Late Formative	Sternum	Caviidae	Guinea pig (Cuy)
POR-24	Las Orquídeas	Unit 54. Level 3	Late Formative	Long bone	Caviidae	Guinea pig (Cuy)

Appendix 2

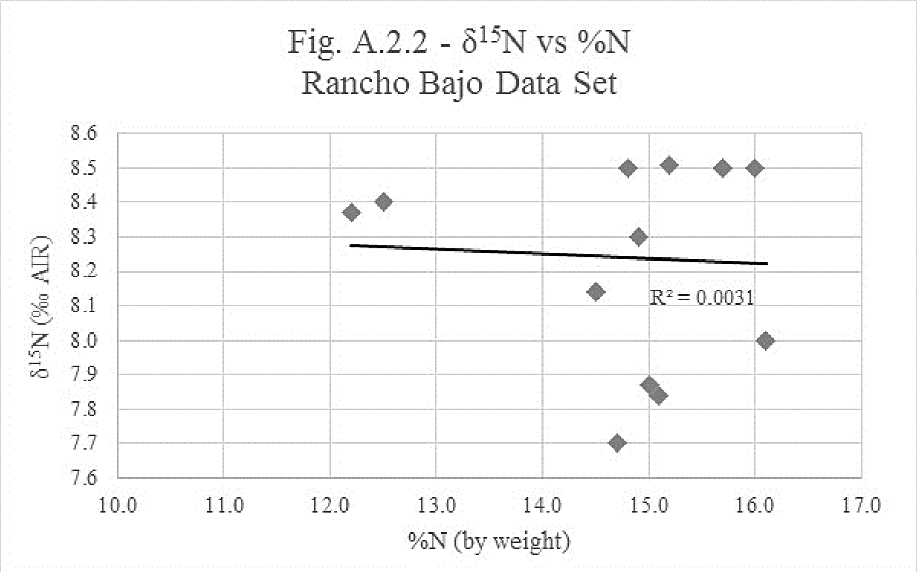
Table A.2.1 - Rancho Bajo pre and post gelatinization values

Sample	$\delta^{13}C$ Run 1	$\delta^{13}C$ Run 2	Diff.	$\delta^{15}N$ Run 1	$\delta^{15}N$ Run 2	Diff.	%C Run 1	%C Run 2	Diff.	%N Run 1	%N Run 2	Diff.	C/N atomic Run 1	C/N atomic Run 2	Diff.
PRB-01	-18,7	-18,4	0,3	8,3	7,9	-0,4	45,7	42,0	-3,7	15,9	15,0	-0,9	3,35	3,27	-0,09
PRB-02	-18,2	-18,4	-0,2	8,4	8,5	0,1	45,9	42,3	-3,6	16,2	15,2	-1,0	3,30	3,25	-0,06
PRB-03	-18,3	-18,7	-0,4	7,9	8,1	0,2	46,6	41,3	-5,3	16,5	14,5	-2,0	3,29	3,32	0,03
PRB-04	-18,3	-18,3	0,0	8,5	7,8	-0,7	46,7	42,4	-4,3	16,5	15,1	-1,4	3,30	3,27	-0,03
PRB-05	-18,5	-18,7	-0,2	7,9	8,4	0,5	45,8	35,6	-10,2	16,2	12,2	-4,0	3,30	3,40	0,11
PRB-08	-18,3			8,5			45,6			16,0			3,32		
PRB-09	-18,2			8,5			45,2			15,7			3,36		
PRB-10	-18,4	-17,0	**1,4**	8,5	8,5	0,0	44,9	47,1	2,2	15,7	16,9	1,2	3,34	3,25	-0,09
PRB-11	-18,5			8,3			43,0			14,9			3,37		
PRB-12	-18,6			8,0			45,8			16,1			3,32		
PRB-13	-18,1			7,7			41,8			14,7			3,32		
PRB-14	-18,5			8,4			36,9			12,5			3,44		
PRB-15	-18,3			8,5			42,5			14,8			3,35		

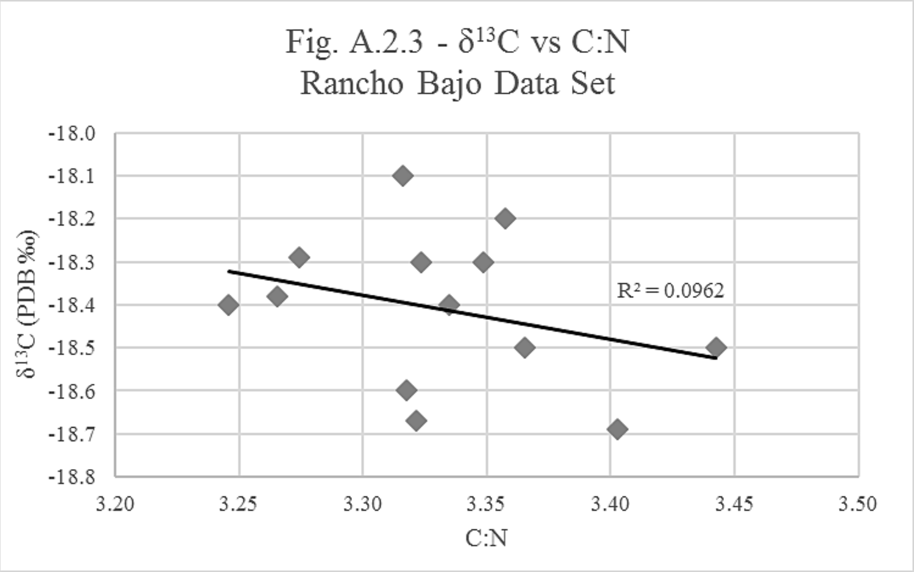
Comparison of $\delta^{13}C$ and %C in the Rancho Bajo data set shows no strong relationship between them ($R^2 = 0.1766$, see Fig. A.2.1).

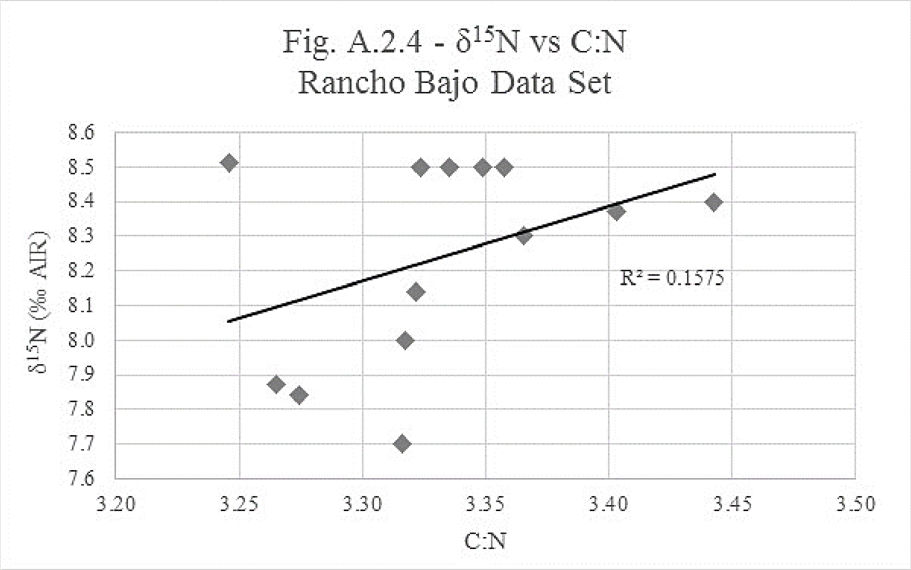


When the relationship between $\delta^{15}N$ and %N values was tested, the results also show no relationship ($R^2 = 0.0031$, see Fig. A.2.2).

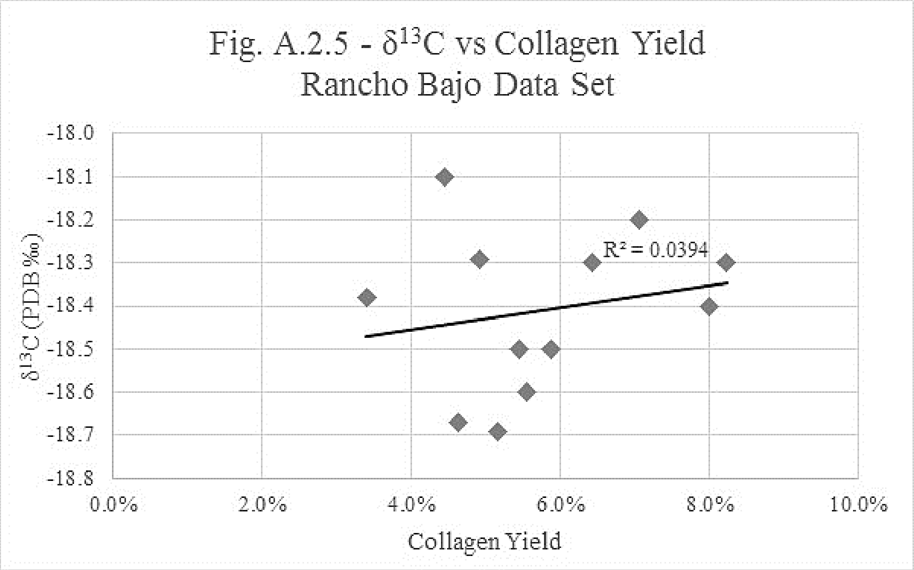


Comparison of the $\delta^{13}C$ and C:N values shows no relationship between these variables ($R^2 = 0.0962$, Fig. A.2.3). The comparison of the $\delta^{15}N$ and C:N values indicates a modest positive relationship ($R^2 = 0.1575$, Fig. A.2.4) but not one of concern.





Returning to collagen yield, in figure A.2.5 it can be observed that there is no relationship between $\delta^{13}C$ and the collagen yield ($R^2 = 0.0394$).



However, after testing for a relationship between $\delta^{15}N$ and the collagen yield (Fig. A.2.6), a definite positive relationship was seen ($R^2 = 0.6127$). This result could potentially indicate problems, most likely with some samples at the upper end of the $\delta^{15}N$ range which also showed higher C/N values. However, given that the overall range of $\delta^{15}N$ values is very narrow and most measures indicate that these samples are acceptable, it was decided to keep them in the data set.

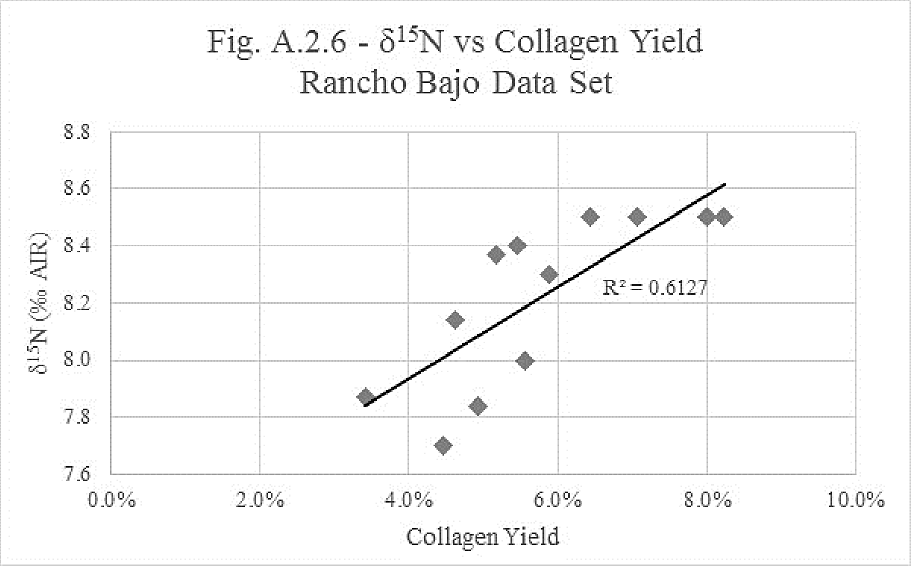


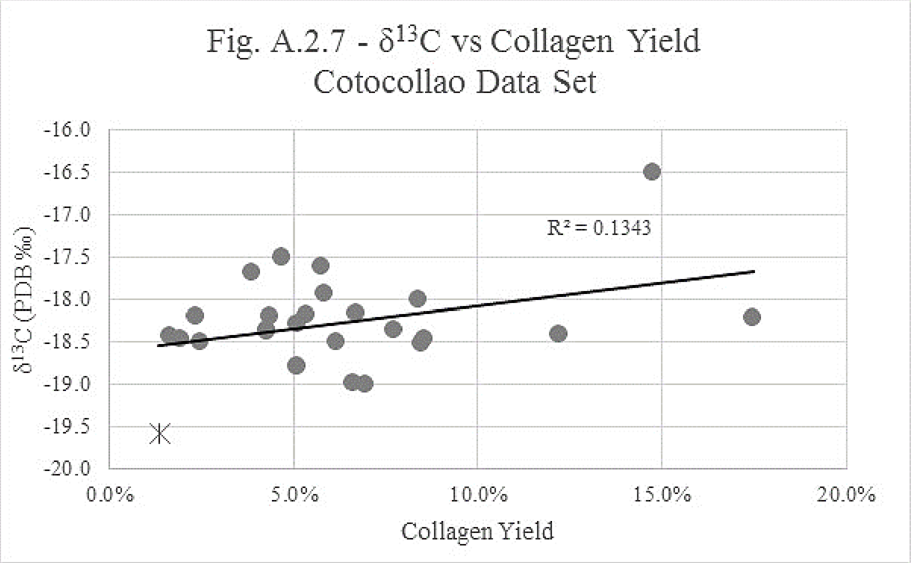
Fig. A.2.6 - δ15N vs Collagen Yield
Rancho Bajo Data Set
δ15N (‰ AIR)
8.8
8.6
8.4
8.2
8.0
7.8
7.6
R² = 0.6127
0.0%
2.0%
4.0%
6.0%
8.0%
10.0%
Collagen Yield

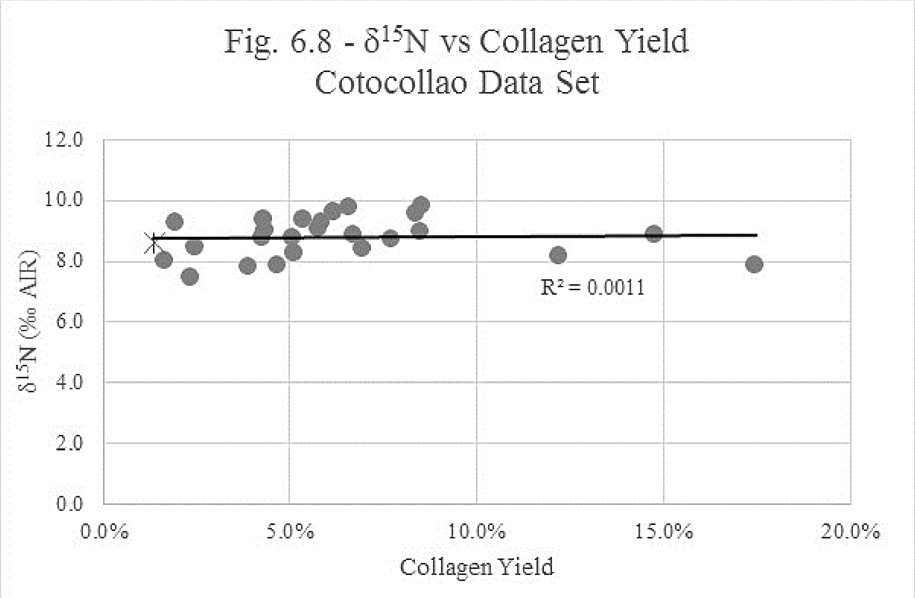
Table A.2.2 - Cotocollao human and faunal pre and post gelatinization values

Sample	$\delta^{13}C$ Run 1	$\delta^{13}C$ Run 2	Diff.	$\delta^{15}N$ Run 1	$\delta^{15}N$ Run 2	Diff.	%C Run 1	%C Run 2	Diff.	%N Run 1	%N Run 2	Diff.	C/N atomic Run 1	C/N atomic Run 2	Diff.
PCO-01	-18,2			7,5			46,2			16,3			3,31		
PCO-02	-18,1	-18,4	-0,3	8,4	8,0	-0,4	45,7	42,9	-2,8	16,1	15,3	-0,8	3,31	3,27	-0,04
PCO-03	-17,7	-18,4	-0,7	7,3	8,8	1,5	46,2	42,9	-3,3	16,3	15,4	-0,9	3,31	3,25	-0,06
PCO-04	-20,2	-18,5	1,7	8,4	9,0	0,6	45,9	43,4	-2,5	16,4	15,4	-1,0	3,26	3,29	0,02
PCO-05	-17,5			7,9			42,7			15,1			3,30		
PCO-06	-20,8			8,4			46,7			16,7			3,26		
PCO-09	-20,7	-18,5	2,2	8,2	9,6	1,4	46,2	43,4	-2,8	16,3	15,4	-0,9	3,31	3,29	-0,02
PCO-10	-21,0	-19,0	2,0	9,4	8,4	-1,0	47,6	41,2	-6,4	16,9	14,4	-2,5	3,28	3,34	0,05
PCO-11	-21,3	-18,5	2,8	9,7	9,8	0,1	46,5	42,9	-3,6	16,6	15,4	-1,2	3,27	3,25	-0,02
PCO-12	-21,1			8,7			39,7			13,6			3,40		
PCO-13	-20,9	-18,2	2,7	8,0	8,3	0,3	46,6	43,0	-3,6	16,7	15,5	-1,2	3,25	3,22	-0,03
PCO-14	-20,2	-18,8	1,4	9,3	9,0	-0,3	39,1	40,0	0,9	12,1	13,7	1,6	3,77	3,41	-0,36
PCO-15	-21,2	-18,2	3,0	8,1	9,8	1,7	46,3	42,1	-4,2	16,5	15,0	-1,5	3,27	3,27	0,00
PCO-16	-20,5	-19,0	1,5	9,5	9,1	-0,4	46,7	43,3	-3,4	16,7	15,4	-1,3	3,26	3,28	0,02
PCO-17	-19,7	-17,6	2,1	8,4	8,7	0,3	47,4	43,2	-4,2	17,0	15,5	-1,5	3,25	3,24	-0,01
PCO-18	-20,4	-18,4	2,0	7,5	7,8	0,3	46,9	42,7	-4,2	16,8	15,6	-1,2	3,26	3,20	-0,06
PCO-19	-20,4	-18,5	1,9	8,3	8,4	0,1	46,5	41,1	-5,4	16,7	14,4	-2,3	3,25	3,34	0,09
PCO-20	-20,0	-18,7	1,3	7,1	7,6	0,5	46,0	40,9	-5,1	16,4	13,9	-2,5	3,27	3,43	0,16
PCO-21	-18,9	-17,7	1,2	8,9	9,3	0,4	46,1	43,0	-3,1	16,4	15,4	-1,0	3,28	3,26	-0,02
PCO-22	-18,3	-18,5	-0,2	9,3	9,6	0,3	46,8	42,7	-4,1	16,7	15,3	-1,4	3,27	3,25	-0,02
PCO-23	-17,7	-18,0	-0,3	8,7	8,9	0,2	46,4	43,2	-3,2	16,6	15,5	-1,1	3,26	3,25	-0,01
PCO-24	-17,6	-18,1	-0,5	8,9	8,2	-0,7	46,2	44,0	-2,2	16,3	15,7	-0,6	3,31	3,27	-0,04
PCO-25	-18,4			8,9			46,7			16,6			3,28		
PCO-26	-16,5			7,9			46,7			16,5			3,30		
PCO-27	-18,1	-18,2	-0,1	8,0	8,5	0,5	47,0	41,3	-5,7	16,8	14,7	-2,1	3,26	3,28	0,01
PCO-28	-18,3			8,8			47,7			16,9			3,29		
PCO-29	-18,5			9,4			46,4			16,5			3,28		
PCO-30	-18,2	-18,4	-0,2	8,3	9,4	1,1	46,1	42,8	-3,3	16,5	15,3	-1,2	3,26	3,26	0,00
PCO-31	-18,5	-18,2	0,3	9,3	9,3	0,0	46,1	42,8	-3,3	16,5	15,4	-1,1	3,26	3,24	-0,02
PCO-32	-18,2	-17,9	0,3	8,9	8,8	-0,1	47,1	41,6	-5,5	16,9	15,0	-1,9	3,25	3,23	-0,02
PCO-33	-18,2	-18,3	-0,1	8,9	8,6	-0,3	43,4	41,9	-1,5	15,2	15,0	-0,2	3,33	3,26	-0,07
PCO-35	-19,4	-19,6	-0,2	4,9	5,0	0,1	43,2	40,2	-3,0	15,0	14,2	-0,8	3,36	3,30	-0,06

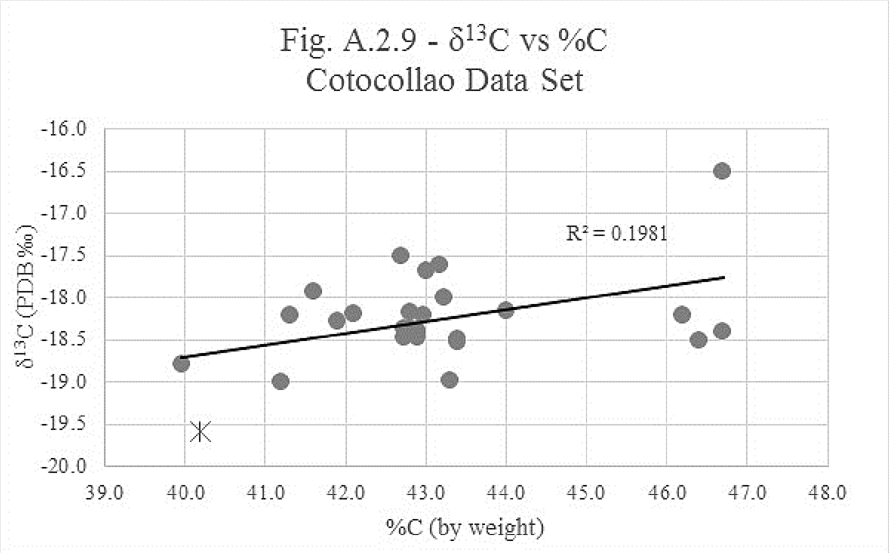
* PCO-35 represents the only faunal sample of the site.

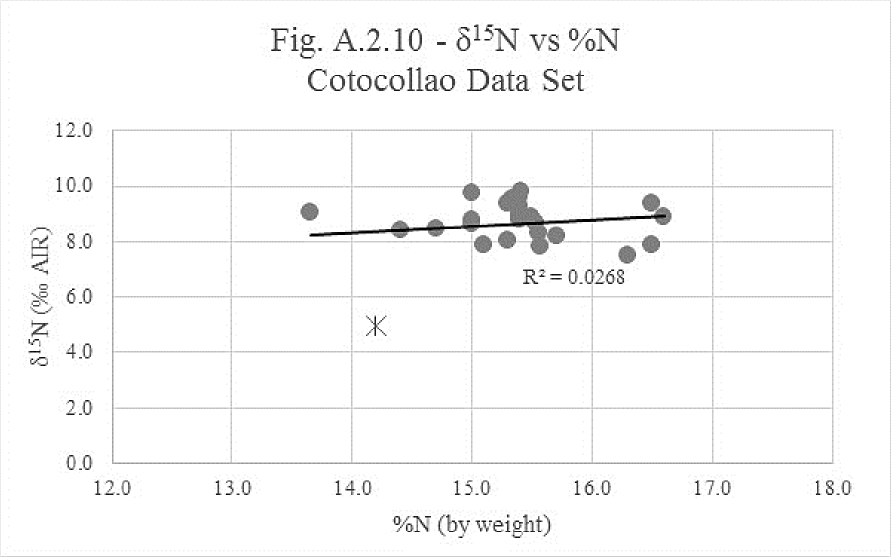
The collagen yields for accepted samples at Cotocollao range from 1.64% to 17.47%, with a mean of 6.43%. When the relationship between the δ^{13}C values and the collagen yield was tested, the results show a slight positive relationship ($R^2 = 0.1343$, see Fig. A.2.7). When the relationship between the δ^{15}N values and collagen yields is examined, no relationship is seen ($R^2 = 0.0011$, see Fig. A.2.8). The faunal sample appears represented with an asterisk.



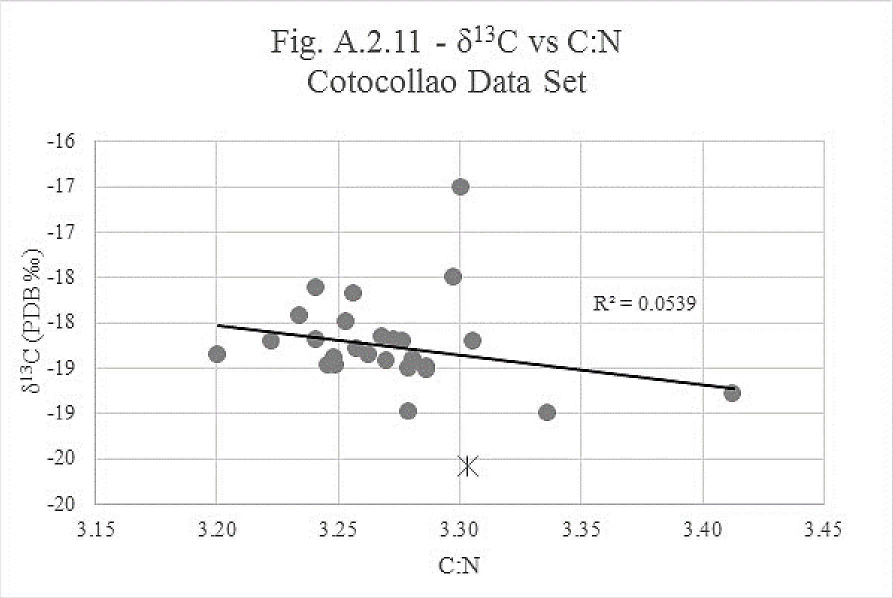


Once the relationship between δ^{13}C and %C values is tested, it was observed that there is a modest positive linear relationship ($R^2 = 0.1981$, see Fig. A.2.9). Visual examination shows that this relationship is primarily due to the high δ^{13}C and %C of sample PCO-26, and is incidental rather than indicating systematic problems with the sample. The relationship between δ^{15}N and %N values was tested; the results confirm there is no relationship between these variables ($R^2 = 0.0268$, see Fig. A.2.10).





The relationship between $\delta^{13}C$ and C:N values was tested. The results showed that there is very weak negative relationship between both variables (R^2 = 0.0539, see Fig. A.2.11). When the relationship between $\delta^{15}N$ and C:N values was tested, the results confirmed that there is no relationship between them (R^2 = 0.0202, see Fig. A.2.12).



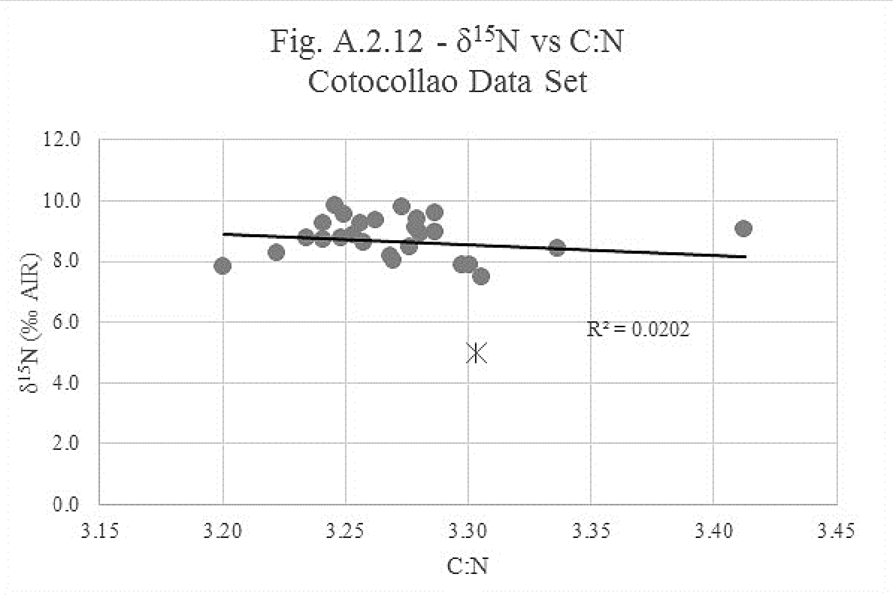


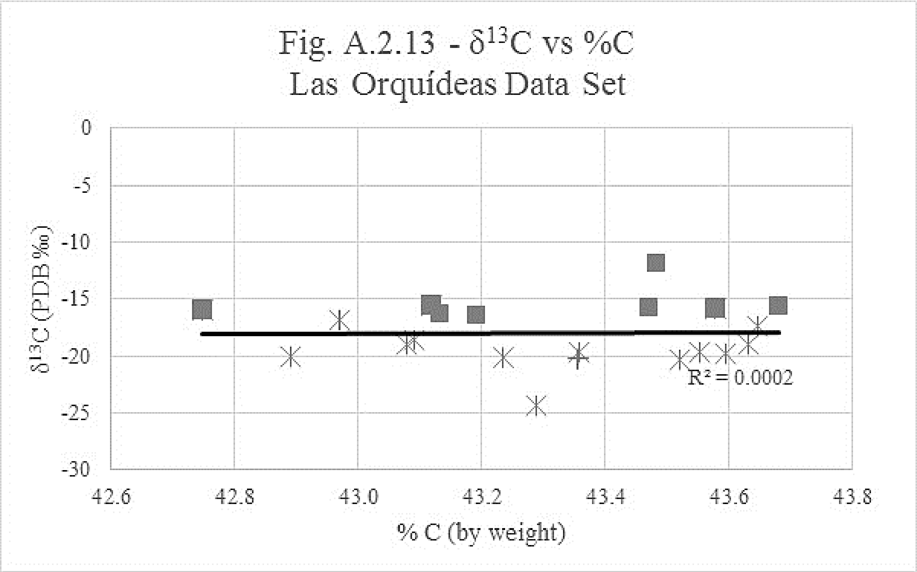
Table A.2.3 - Las Orquídeas human pre and post gelatinization values

Sample	$\delta^{13}C$ Run 1	$\delta^{13}C$ Run 2	Diff.	$\delta^{15}N$ Run 1	$\delta^{15}N$ Run 2	Diff.	%C Run 1	%C Run 2	Diff.	%N Run 1	%N Run 2	Diff.	C/N atomic Run 1	C/N atomic Run 2	Diff.
POR-01	-11,7	-11,8	-0,1	8,8	9,0	0,2	47,5	43,5	-4,0	16,9	15,7	-1,2	3,28	3,23	-0,05
POR-02	-15,4	-15,8	-0,4	6,7	6,9	0,2	47,2	42,7	-4,5	16,8	15,3	-1,5	3,28	3,26	-0,02
POR-03	-15,5	-15,7	-0,2	7,9	8,1	0,2	48,5	43,6	-4,9	17,4	15,8	-1,6	3,25	3,22	-0,04
POR-04	-16,2	-16,2	0,0	6,4	6,7	0,3	47,0	43,1	-3,9	16,7	15,4	-1,3	3,28	3,27	-0,02
POR-05	-15,0	-15,5	-0,5	7,2	7,3	0,1	46,8	43,1	-3,7	16,7	15,4	-1,3	3,27	3,26	0,00
POR-06	-15,6	-15,8	-0,2	9,1	9,2	0,1	47,4	43,5	-3,9	16,8	15,8	-1,0	3,29	3,21	-0,08
POR-07	-16,2	-16,4	-0,2	5,6	5,7	0,1	47,0	43,2	-3,8	16,7	15,5	-1,2	3,28	3,26	-0,02
POR-08	-15,7	-15,7	0,0	7,1	7,3	0,2	47,4	43,7	-3,7	16,9	15,6	-1,3	3,27	3,26	-0,01

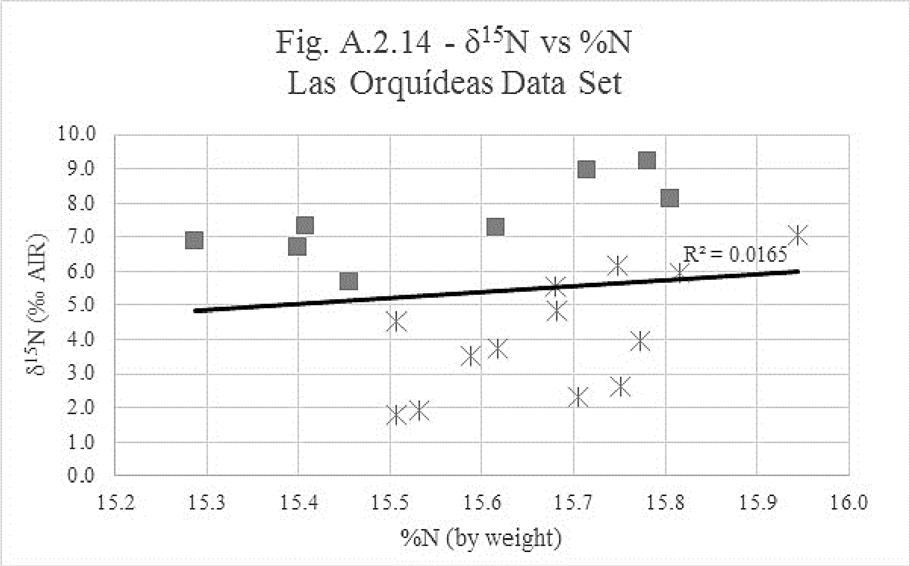
Table A.2.4 - Las Orquídeas faunal pre and post gelatinization values

Sample	$\delta^{13}C$ Run 1	$\delta^{13}C$ Run 2	Diff.	$\delta^{15}N$ Run 1	$\delta^{15}N$ Run 2	Diff.	%C Run 1	%C Run 2	Diff.	%N Run 1	%N Run 2	Diff.	C/N atomic Run 1	C/N atomic Run 2	Diff.
POR-09	-20,3	-20,3	0,0	2,5	2,6	0,1	46,6	43,5	-3,1	16,5	15,8	-0,7	3,29	3,22	-0,07
POR-10	-17,2	-17,3	-0,1	6,1	6,2	0,1	47,5	43,6	-3,9	17,0	15,7	-1,3	3,26	3,23	-0,03
POR-11	-16,5	-16,8	-0,3	4,4	4,5	0,1	45,4	43,0	-2,4	16,0	15,5	-0,5	3,31	3,23	-0,08
POR-12	-19,7	-19,7	0,0	3,9	4,0	0,1	48,3	43,6	-4,7	17,3	15,8	-1,5	3,26	3,22	-0,03
POR-13	-19,0	-19,0	0,0	5,7	5,9	0,2	48,5	43,6	-4,9	17,4	15,8	-1,6	3,25	3,22	-0,03
POR-14	-24,4	-24,4	0,0	3,0	3,5	0,5	46,8	43,3	-3,5	16,6	15,6	-1,0	3,29	3,24	-0,05
POR-15	-20,1	-20,0	0,1	3,4	3,7	0,3	47,2	43,2	-4,0	16,8	15,6	-1,2	3,28	3,23	-0,05
POR-16	-20,3	-20,1	0,2	1,7	1,9	0,2	46,9	42,9	-4,0	16,5	15,5	-1,0	3,31	3,22	-0,09
POR-17	-20,2	-20,2	0,1	2,0	2,3	0,3	47,2	43,4	-3,8	16,8	15,7	-1,1	3,28	3,22	-0,06
POR-18	-18,7	-19,0	-0,3	2,0	1,8	-0,2	46,1	43,1	-3,0	16,4	15,5	-0,9	3,28	3,24	-0,04
POR-19	-19,5	-19,6	-0,1	5,6	5,5	-0,1	47,4	43,4	-4,0	16,9	15,7	-1,2	3,27	3,22	-0,05
POR-20	-19,7	-19,7	0,0	7,0	7,1	0,0	47,9	43,6	-4,3	17,2	15,9	-1,3	3,25	3,19	-0,06
POR-22	-18,8	-18,6	0,2	4,8	4,8	0,0	49,1	43,1	-6,0	17,7	15,7	-2,0	3,23	3,20	-0,03

After testing the relationship between δ^{13}C and %C values, the results show that there is no relationship between these variables ($R^2 = 0.0002$, see Fig. A.2.13). In all figures, the human samples appear represented with squares and the faunal samples with an asterisk.

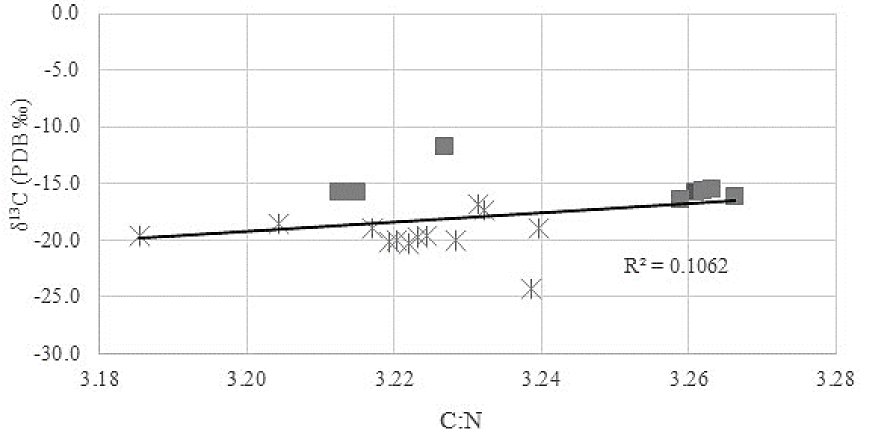


Once the relationship between the δ^{15}N and the %N values was tested, the results confirmed that there is no relationship between these variables ($R^2 = 0.0165$, see Fig. A.2.14).



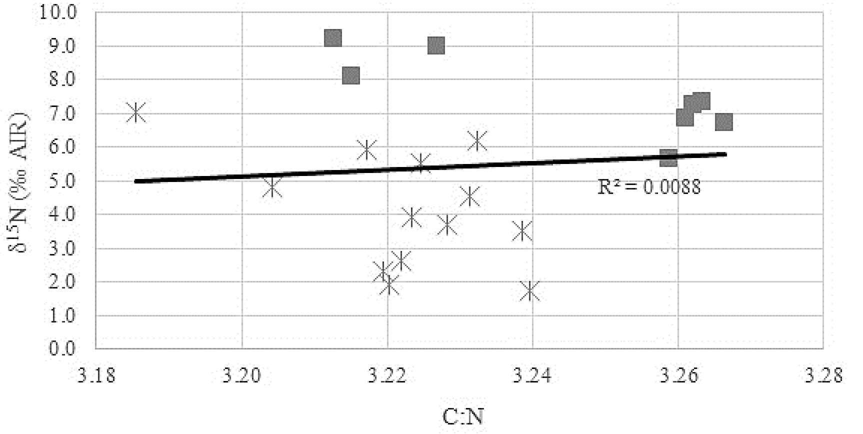
When the relationship between the $\delta^{13}C$ values and the C:N values was tested, the results confirmed that there is a slight positive relationship between these two variables ($R^2 = 0.1062$, see Fig. A.2.15).

Fig. A.2.15 - $\delta^{13}C$ vs C:N
Las Orquídeas Data Set



After testing the relationship between the $\delta^{15}N$ and C:N values, it was confirmed that these variables have almost no relationship ($R^2 = 0.0088$, see Fig. A.2.16).

Fig. A.2.16 - $\delta^{15}N$ vs C:N
Las Orquídeas Data Set



When the relationship between the $\delta^{13}C$ and collagen yield was subject to testing, the results confirmed that there is almost no relationship between these variables in Las Orquídeas (R^2 = 0.053, see Fig. A.2.17). However, when the relationship between $\delta^{15}N$ values and collagen yield was examined, it was found that there is a weak positive relationship, although it is not enough to be considered an issue (R^2 = 0.1037, see Fig. A.2.18).

Fig. A.2.17 - $\delta^{13}C$ vs Collagen Yield
Las Orquídeas Data Set

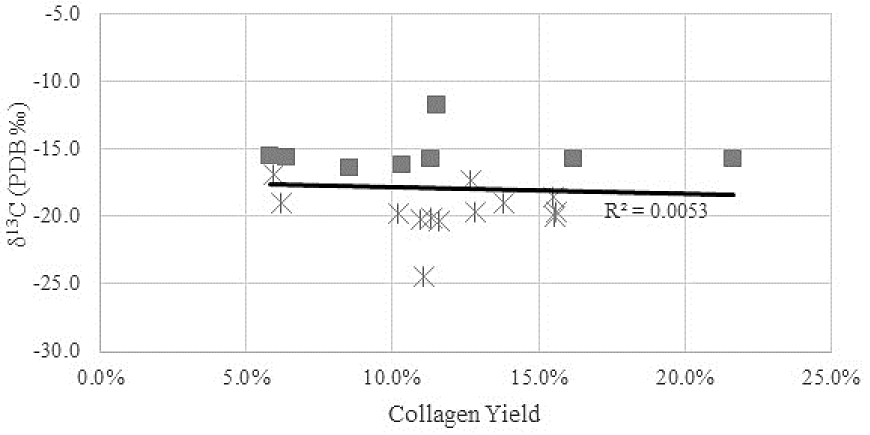


Fig. A.2.18 - $\delta^{15}N$ vs Collagen Yield
Las Orquídeas Data Set