SAPIENZA UNIVERSITÀ DI ROMA

ARCHMAT

ERASMUS MUNDUS MASTER in ARCHaeological MATerials Science

Isotopic Analysis of Paleo-diet on Skeletons from the Necropolis at Castel Sozzio, Viterbo, Italy.

Sonia Reyes Hidalgo

Supervisor, Alfredo Coppa

Cosupervisor, Emanuela Borgia

Rome, December 2021

Abstract

The discovery of a necropolis situated in the province of Viterbo in the municipality Civitella d'Agliano, at Castel Sozzio, has given way to gain knowledge on this region's role during the Late Roman Period and the Early Middle Ages. There is little known about this site as it has been excavated from 1997-1998 and then resumed for a couple of months in 2020 under the direction of prof Emanuela Borgia (Dipartamento di Scienze dell' Antichità, Sapienza Università di Roma). As well as lack of material evidence, as there is still so much more to discover, there have been no written records found about the site either. Therefore, multiple studies of the material found at the site are of great importance. My role and contribution to this project is that of performing Paleo-diet Analysis.

Nutrition is undoubtedly one of human beings' needs, and studying paleo-diet helps us to establish the key food resources and their evolution over time. The information gathered will provide us an understanding of the food and consumption of humans throughout that time period by evaluating carbon and nitrogen levels in human samples. Knowing what kind of diet they followed, provides insight into the resources available, as well as the climate, environment, food chemistry processes, trade, economy, as well as social status.

A total of 25 samples were studied; 7 animal bones and 18 humans. The human samples vary in age and include both females and males as well as children. These 25 samples were chosen and prepared at the Laboratory of Biology of Ancient Populations headed by Prof. Alfredo Coppa. After that, they were then chemically analyzed at the Laboratory of Paleoanthropology and Bioarchaeology led by Professor Giorgio Manzi and Mary-Anne Tafuri who runs the Isotopic Laboratory in Sapienza University, as well. 24 out of the 25 samples were ready for the isotopic mass spectrometry analyses which took place at Iso-Analytical Limited Laboratory in the United Kingdom.

The findings will provide us with information about a significant portion of Castel Sozzio's population. These discoveries will inevitably lead to new questions, revealing more and more about life in Civitella d'Agliano

Table of Contents

Chapter 1. Introduction

Background

In the municipality of Civitella d'Agliano, in the province of Viterbo, a necropolis was uncovered in the area that still bears the toponym of Castel Sozzio. It was assumed at the start of the first excavation season in 1997 that it had a significant extension. Excavation and research in the necropolis were resumed in 2020 under the direction of prof. Emanuela Borgia (Dipartamento di Science dell' Antichità, Università di Roma Sapienza) with a formal concession by the Istituto Centrale per l' Archeaologia and the *Soprintendenza Archaeologica Belle Arti e Paesaggio* per la provincia di Viterbo per *l' Etruria meridionale*[1] The necropolis is of particular importance as it is the only known funeral site of a specific entity in Lazio Teverina, and the necropolis' size does, however, allow for the existence of a rather big inhabited center. It is evident, through the excavation work that has been previously performed by the University of Sapienza, (as mentioned above), that people from different time periods have continuously inhabited the place. The necropolis has been constantly occupied, and this we know thanks to the differences in tomb styles that have been found, and in the numerous depositions in the graves (Borgia 2021). It also adds valuable information to the few sites known in the north of Lazio in a period between Late Antiquity and the early Middle Ages:

- Selvicola – Ischia di Castro [Toiatti – Pontacolone ,1985; Incitti,1992
- Bomarzo e Norchia [Raspi – Serra 1974b, 1976]
- Palombaro – Montalto di Castro [Corsini 1985]
- La Ficonaccia – Pian de'Gangani [Corsini 1985]
- Rossignolo e S. Lucia–Bagnoregio [Cagiano De Azevedo - Schemiedt,1974; Pompei ,1994].

The complex at Castel Sozzio is the only one at present under investigation in the Tiber Valley and for this, it is an important testimony in a context, or if you like, an area that has yet to be fully explored. The site is located in a zone known as the Teverina Viterbese, which covers the regions

---

[1] It is also worth stressing that the municipality of CIvitella D'Agliano (VT) is giving its full support to the research, hostinf the students and researchers and giving spaces for the laboratories.

of Lazio and Umbria. This area is centered between the east shore of Lake Bolsena and the western shore of the Tiber River.
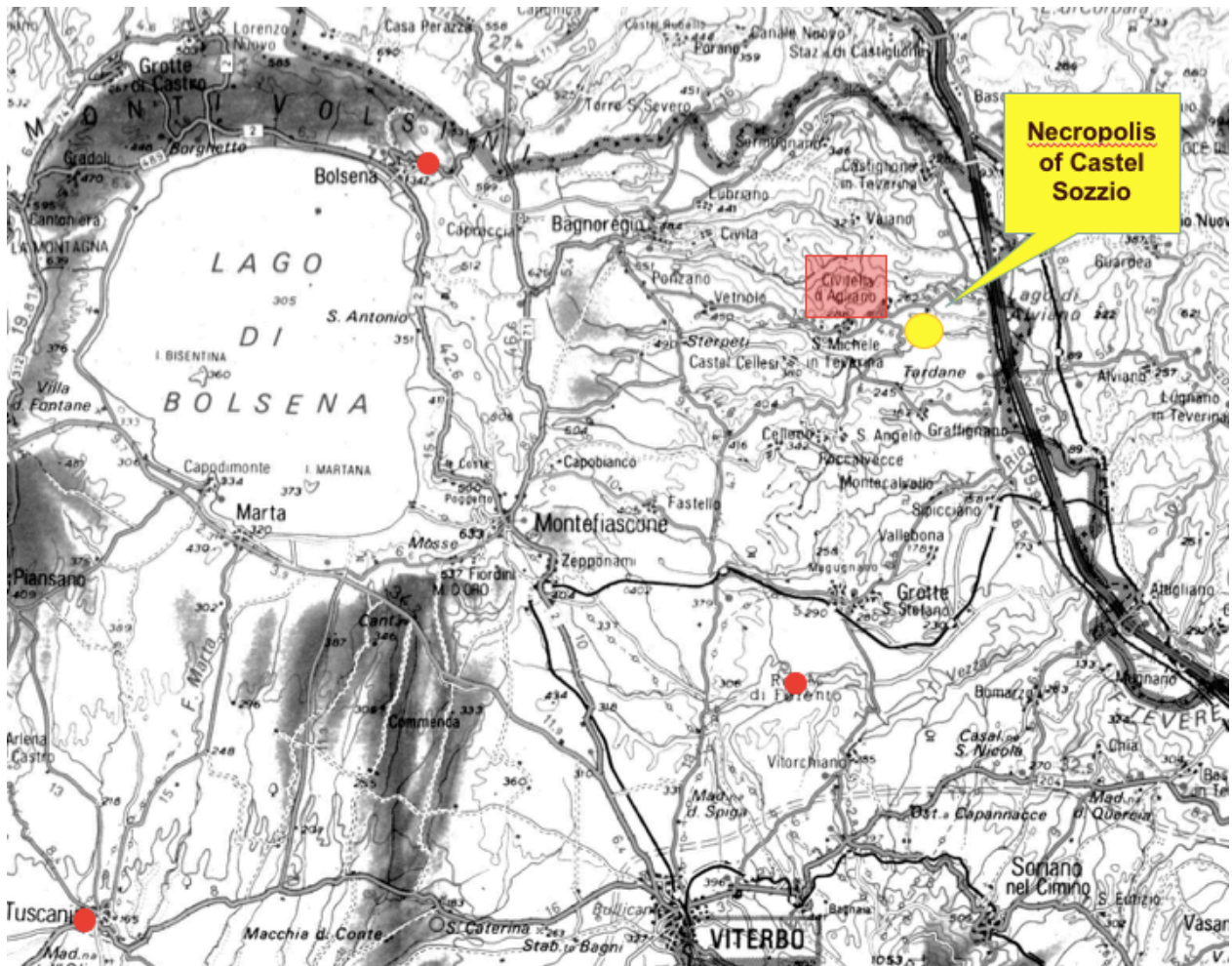
Figure 1.1 Map of Viterbo demonstrating Lake Bolsena, the Tiber River, and exact location of Civitella D'Agliano.
(courtesy Borgia 2021, unpublished material all rights reserved)

Knowing the type of land we are studying on, allows us to understand the conditions people lived in as well as gives us knowledge of the resources they had at hand. This area's geography and geology is constructed by valleys made up of limestone banks, volcanic rock, and green and yellow lithoid tuff (Pompei 1994). The hill slopes towards a broad alluvial plain adjacent to the Tiber, the northern part is marked by the characteristic gullies formed by the washout. The place is characterized by a series of travertine tuffs. It is composed by erosion that reveals sandy clay marked by the lack of arboreal and herbaceous crops as they decline near the Tiber river.

6

Denudation of the land began as soon as men removed the natural vegetation in the Etruscan era, giving space to a wide plain that is far better suited to agricultural purposes. (Spanu 2021).
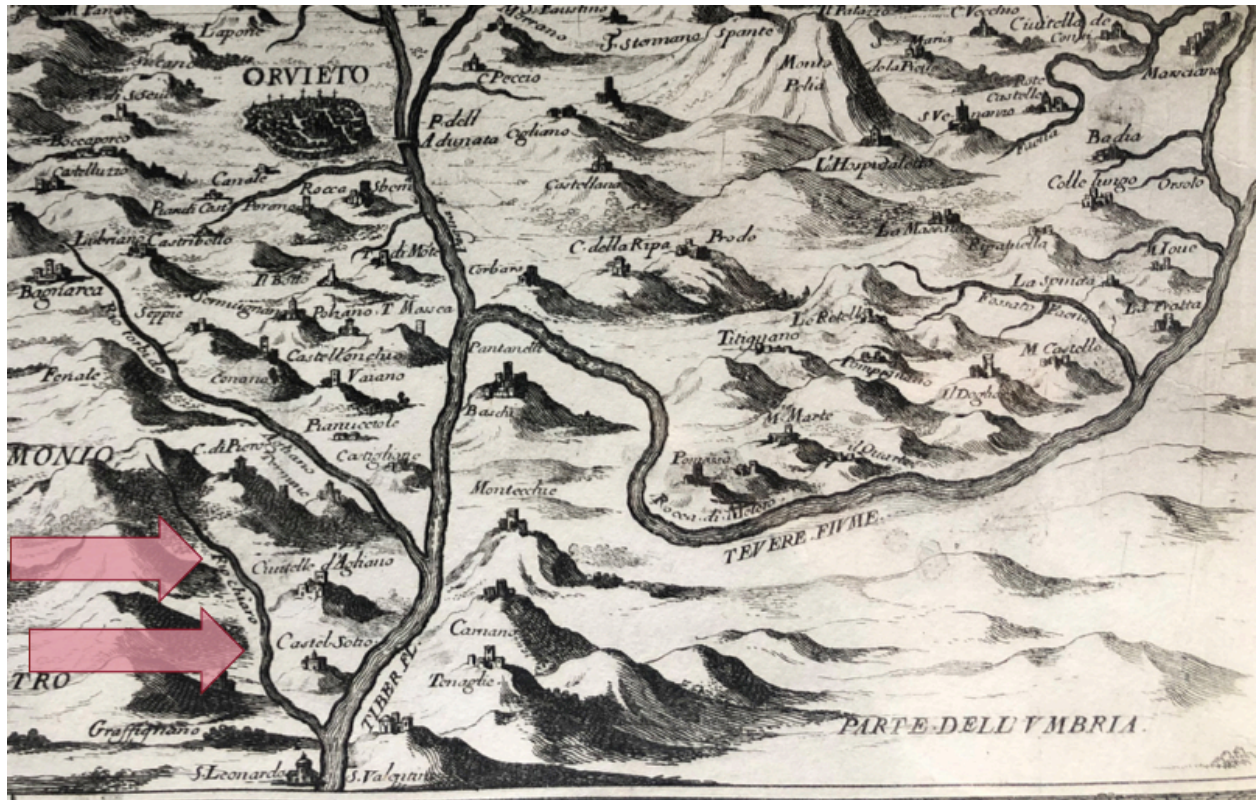

Figure 1.2 Close up of map pointing to Civitella D'Agliano and Castel Sozzio. Clearly showing the topography and its proximity to Orvieto and Umbria (courtesy Borgia 2021, unpublished material all rights reserved).

As mentioned above, the site pertains to the period of Late Antiquity which is the time of the fall of the Roman Empire. During the empire, the consul subjected the territory that currently makes up Civitella D'Agliano, as well as all of Etruria's possessions, to Roman sovereignty. The valley of the Tiber was split into various farms of the normal extension of the period of 120 roman acres, comparable to the present 25 hectares, in the late republican and early imperial eras. The toponymy provides us with important confirmations. The same toponym *Agliano* obviously stems from the Roman language substratum and denounces the presence of a *praedium*, which is a land property of stated dimensions that epigraphic sources indicate to the area and may have belonged to representatives of the *gens Allia* (Pompei 1994).

After the destruction of the Etruscan city in 265 B.C., Orvieto maintained its significance by becoming a central spot for transportation and the capital of pagan culture until the 4th century

A.D. At the turn of the 4th century, Christianity came to play part in the area of Viterbo. The remnants of the early Christian basilica that once stood on the forum of the Roman urban complex, as well as the exquisite catacomb of S. Cristina in Bolsena and the Christian hypogeum that once existed in Gratte; nonetheless, the first reference of a Volsinian bishop goes back to the end of the fifth century.

During the long period of time examined, the rural territory had to receive a strong impulse to settle. Topographic studies on the land have demonstrated evidence of extensive agricultural practices. Today's landscape is characterized by extensive exploitation of the land, which is cultivated in the plains with cereals or alfalfa, and in the hilly slopes with vineyards and olive groves, leaving most areas internal to woods and scrubs (Spanu 2021). But even though this is an incredibly important explanation and motive for the area's ongoing population through Etruscan times to the Roman and the Early Middle Ages, it cannot, however, be restricted to agricultural exploitation opportunities, because the region's valuable features have always been its road connections. (Pompei 1994). Studies on the topography of Viterbo are being performed with techniques such as archaeological surveys and the in-depth research on archives historical maps, records, aerial photography, and, more recently, satellite images (Spanu 2016).

As Civitella D'Agliano is one of the small towns that are included in numerous towns belonging to Viterbo, not a lot of in-depth studies have been yet made about the town's history. The information stated above comes from the knowledge we have of the region as a whole, which focuses on the largest cities and towns. Thanks to the new archaeological excavation at Castel Sozzio, we will keep unearthing material that will. This will only add important information to the history of the region

The first investigations in the necropolis of Castel Sozzio began in 1997 and ended in 1998 by Margherita Cecchelli, Maria Consiglia Pompei, and Francesca Riganati, (Sapienza University), but the results were never published. In 2020 the excavation project was resumed by Professor Emanuela Borgia, also from Sapienza University of Rome: the first campaign was held in September of the year 2020 and the second campaign in September-October 2021. To the North West of the farmhouse of Castel Sozzio during the first years two trenches called Alpha and Beta were opened, the first (Alpha) most damaged by ploughing and agricultural activities in general. The majority of the tombs seemed to be arranged in parallel rows and are oriented East to West with the head of the deceased always to the West and the feet to the East. This is a common practice

used by Christians, though there were at least six tombs that are orientated north-south with the deceased's head positioned to the north (Borgia 2021).
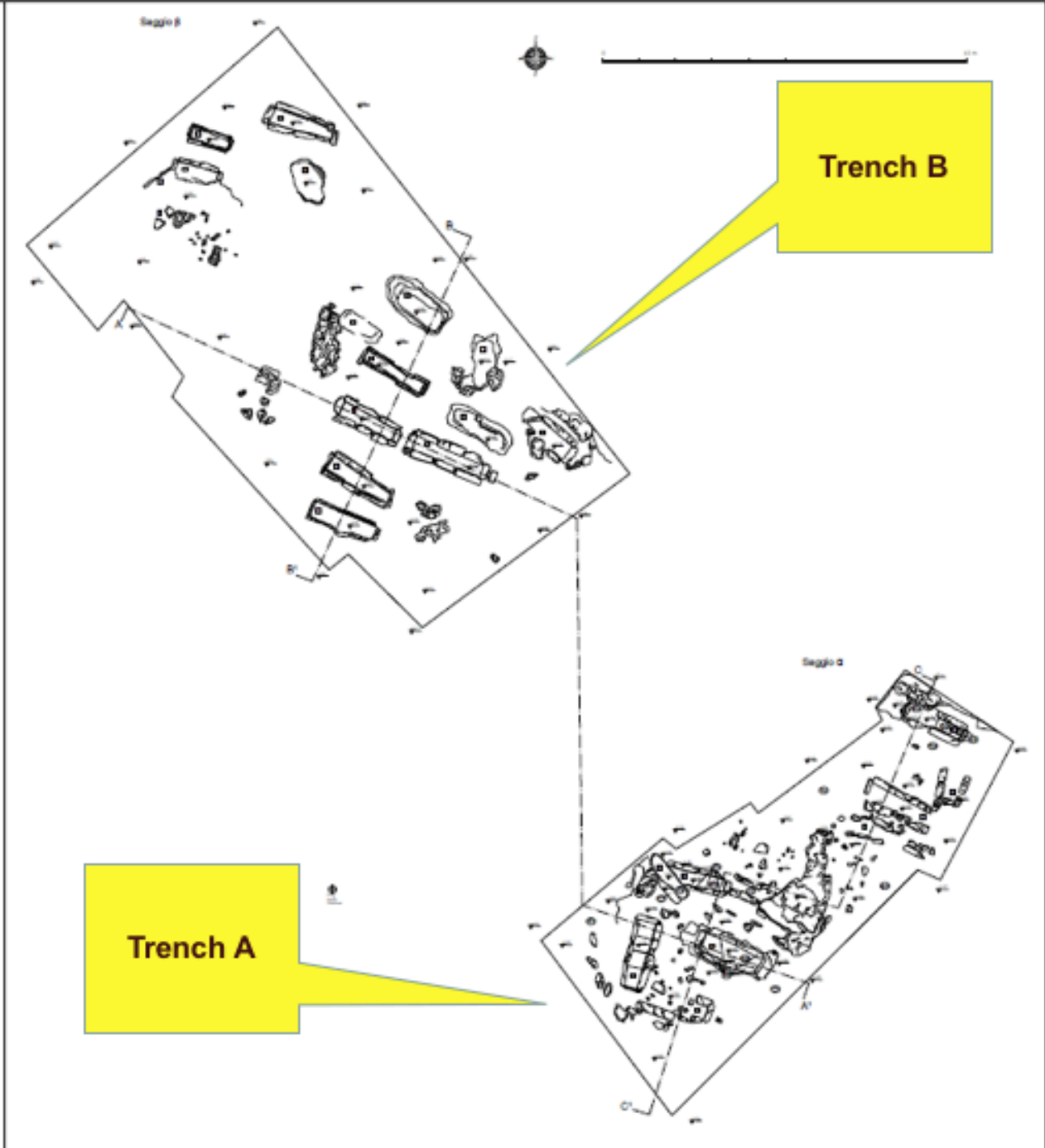

Figure 1.3 Archaeological plan of the excavated areas of the necropolis at the end of 2020 excavation. (Courtesy Borgia 2021, unpublished plan, all rights reserved).

Figure 1.4 Aerial photograph of site showing both excavated trenches 2020. (Courtesy Borgia 2021, unpublished image, all rights reserved).

A first chronological interpretation of the area dates the necropolis between the 5th and 6th century A.D. until at least the 7th century. AD. However, new elements emerged during the excavation campaign of 2020 that can suggest possibly a lowering of the chronological horizon.

The tombs unearthed show evidence that the necropolis has been used throughout different time periods. Apart from few "cappuccino" tombs, the majority of the graves are caissons, as well as several pit tombs/fossae (Pompei 1997). The majority of the tombs are of the lithic case type, with tuff or gray ignimbrite slabs lining the walls, beaten clay or travertine bench for the bottom, and three or four tuff slabs or ignimbrite slabs for the covering (Borgia 2021). It is also common to find various depositions, some of the graves with earthen ditches, filled with soil and simply covered with a layer of clay and small stones on the surface meaning that the graves were recycled or reused numerous times, maybe from the same familiar group or even by new occupants. What these tombs have in common is that they are extremely poor as there is a lack of material, which could aid in dating the graves precisely (Borgia 2021).

Figure 1.5 Photograph of a grave from 1997 Trench Beta (Archive of Castel Sozzio, courtesy Borgia 2021 all rights reserved).

In figure 1.5 we can see tomb T.30; its margins are made of; stone slabs, and inside it there are bones pertaining to different depositions. We can also see how there are bones outside of the tomb. This became more noticeable as the excavation proceeded all the way up to this year's campaign.
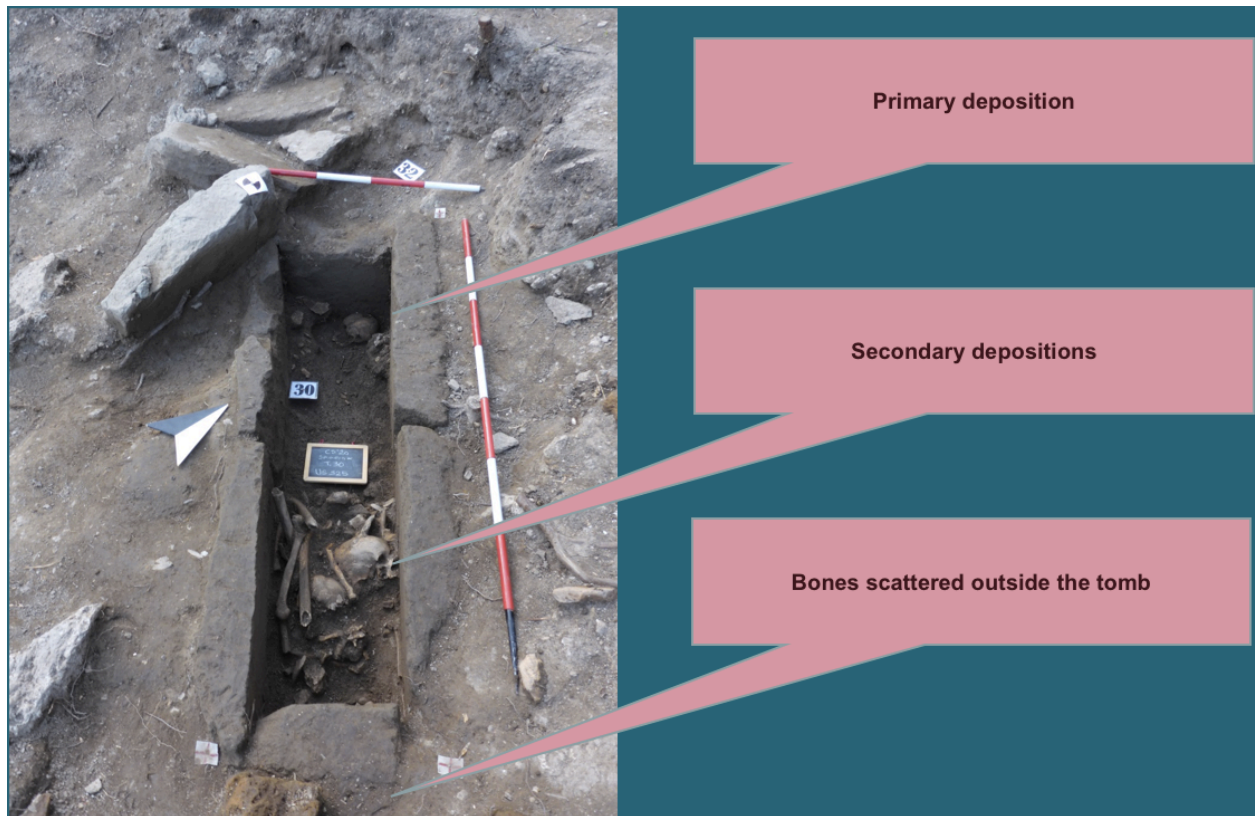

Figure 1.6 A clear example of a single tomb used in different times. 2020 photograph (Archive of Castel Sozzio, courtesy Borgia 2021, all rights reserved).

Due to the finding of the reuse of the tombs, it is possible to understand that this area had been occupied by the pass of the centuries. Finding out a concrete date is best done with the aid of artifacts. This year's findings, 2021, are yet to reveal more once they have been analyzed. As this campaign just concluded in October 2021, I do not have the new information to back up to the dating of the individuals found.

The material (bone remains) I based my analysis on was provided to me in the laboratory led by Professor Alfredo Coppa at Sapienza University. 70 individuals were identified as far as the 1997, 1998, and 2020 campaigns are concerned, comprising 22 males, 17 females, and 7 children (Borgia). This year, more individuals were uncovered by archaeologists from Sapienza, as well as analyzed by anthropologists at the laboratory at Castel Sozzio. Further in-depth analysis will only add to the collection that is slowly but surely building up. For now, I was able to perform analysis on individuals recovered in the 1997-1998 campaign and the one in 2020.

Chapter 2. Theoretical Framework Carbon and Nitrogen Analysis in Archaeology

This section will be covering the way chemical analysis aids in the study of archaeology and how it works.

The goal of the study of material is to understand it to the fullest, chemistry and physics supports in its doing. There is various analysis that may be applied to different archaeological material. Ceramic, pollen, glass, pigments, mortars, and bones are a couple of examples of materials that may undergo chemical analysis.

The analysis of bioarchaeological remains is a study that may become highly complete with the correct chemical analysis applied. When bone remains are found and taken to a laboratory, it is the anthropologist duty to sex, age, and identify pathologies. If given a number of non-articulated bones, it is his job to identify how many individuals. This analysis is done by observation. These aspects are of course crucial and basic of any bone remains, but with the right technological resources and knowledge in the field, the information that could be obtained would be incredibly valuable.

Bones and teeth are what remain from an individual. They are a record of the life an individual has lived, how it died, and what it went through after death. Of course, bones are usually

found in different conditions due to factors surrounding them such as soil, weather, exposure, and burial type. When these factors favor the remains, it is easier to perform chemical analysis. These studies reveal what may not be seen on the surface such as their diet, nutrition, where they come from, and where they lived.

Of course, the focus for this experiment is on Paleo diet. Paleo diet is done by doing an analysis on stable isotopes. Isotopes are distinct versions of the same chemical element with the same number of protons, same atomic number in each atom, but varying neutron numbers and mass numbers. Isotopes can be stable (12C & 13C) or radiogenic (14C), radiogenic not being considered a stable isotope. During their lives, the quantity of 14C in a living organism's tissues is equal to that in the atmosphere, the amount lost due to isotope decay is equal to the amount taken in. After an organism dies, the amount of 14 C isotope in the atmosphere continues to decline at a constant pace. It is possible to date an object by measuring the remaining amount of 14 C isotope in an organic based material, usually collagen.

There are different stable isotopes used to measure in bioarchaeology such as the Carbon 13 C/12 C, Nitrogen 15 N/14 N, Oxygen 18 O/16 O, Sulfur 34 S/32 S and Strontium 87 Sr/86 Sr. These isotopes may be analyzed from bone, soils, sediments, and pottery. They provide information on diet, environment, origin, climate, and source. The numbers mentioned with each element, the isotope ratio, present different masses within the element. The differences influence their specific behaviors during physical and chemical processes (Dias 2019).

These actions can cause isotope fractionation, a shift in the relative proportions of isotopes in reaction products compared to the proportions in the starting substrates. The isotopic concentration is always represented as a ratio between the two isotopes, with the heavier isotope always taking precedence over the lighter isotope, for example 13 C/ 12 C. Isotope fractionation can be divided into two types:

One is equilibrium fractionation which results from the differential exchange of isotopes between two physical phases that are in equilibrium with one another. Two differential fractionation results from the differential exchange of isotopes between two physical phases that are not in equilibrium with one another. The second is kinetic fractionation, which occurs during a chemical reaction, usually involves preferential reaction of lighter isotopes compared to heavier ones, which is important in biomolecular archaeology. The lighter isotopes become more abundant in the reaction products. The stable isotope concentration of ambient and biological fractions

differs by a few parts per thousand in most cases. It is consequently preferable to compare the sample's isotope ratio to that of widely recognized standards. The isotopic ratios of 13 C of the samples, 13 C/12 C sample, are represented in values ppm relative to a standard, 13 C/ 12 C standard, by convention.

$$\delta^{13}C = \left(\frac{({}^{13}C/{}^{12}C)_{sample}}{({}^{13}C/{}^{12}C)_{standard}} - 1 \right) \times 1000\%o$$

ViennaPeedeeBelemnite (VPDB)

Element	Isotope	Fractional abundance	1‰ change	International standard
Hydrogen	^{1}H	0·999844		Standard Mean Ocean
	^{2}H	0·000156	0·000000156	Water (SMOW)
Carbon	^{12}C	0·98889		PeeDee Belemnite
	^{13}C	0·01111	0·00001123	limestone (PDB)
Nitrogen	^{14}N	0·99634		Air
	^{15}N	0·00366	0·00000367	
Oxygen	^{16}O	0·99755		
	^{17}O	0·00039	0·00000039	Standard Mean Ocean
	^{18}O	0·00206	0·00000207	Water (SMOW)
Sulfur	^{32}S	0.9502		Canyon Diablo
	^{33}S	0.0075		Iron meteorite
	^{34}S	0.0421	0.0000450045	(CDT)
	^{36}S	0.0002		

Figure 2.1 Table with abundance of stable isotopes (Hoef 1996, Schoeller 1999).

Stable isotopes have standards which were created for there to be a guide, a reference that may be used internationally. Each element has its own standard based on their fractional abundance. The National Bureau of Standards (NBS) and the International Atomic Energy Agency (IAEA), both in Vienna, have international standards available, and their implementation yields equivalent results between laboratories (Katzenberg, 2008). The reference material for carbon is Peedee Belemnite (PDB), a dolomite fossil with a very high 13C / 12C ratio, hence the 13C readings are frequently negative. The standard for nitrogen is atmospheric nitrogen (AIR-Ambient

14

Inhalable Reservoir), thus the levels of 15 N in most species are higher than the standard, which is 0, and hence positive.

For archaeological analysis, the focus occurs on both Carbon and Nitrogen elements. During photosynthesis, carbon isotope fractionation occurs in plants, resulting in an enrichment in the 12 C isotope negative 13 C value. In all plants, there is an initial enrichment during the entry of atmospheric carbon dioxide into the plant, as the lighter isotope diffuses more quickly through the microscopic pores on the outer surfaces of the leaves. The exact value of 13 C varies dependent on environmental factors such as temperature, humidity, day length, and sunshine intensity. In C3 plants, some CO2 leaks out of plant tissues which then converts to sugar and then undergoes a second fractionation. C4 plants use all absorbed CO2 which means that no second fractionation takes place. Wheat, barley, rice, potato, and many other essential crops are naturally found in temperate regions of the world such as Europe, Asia, and North America. C4 plants utilize the C4 photosynthetic pathway, which is only found in a few tropical species such as sorghum and millet are two other examples. Because the 13 C value of a human or animal tissue is a mirror of the 13 C values of the food consumed, the distinctions between isotope fractionations happening in C3 and C4 plants are noteworthy (Dias 2019).

Just as the plants on land serve as food, so do the ones in the ocean. Photosynthesis in the oceans is largely carried out by organisms such as marine algae and phytoplankton, and is mostly via the C 3 pathway. The starting point for photosynthesis in the oceans is dissolved bicarbonate, which is enriched in 13 C compared to atmospheric CO 2 and thus has a less negative 13 C value. As a result, the tissues of marine photosynthesizers have higher 13 C values than those found in terrestrial C. Freshwater species on the other hand are a bit more difficult and varied.

The stable isotopes of nitrogen are 14 N and 15 N, with the former accounting for 64%of all nitrogen atoms in the biosphere. The balance between microbial fixation of atmospheric nitrogen into inorganic and organic molecules and its release through denitrification influences nitrogen isotope fractionation in the biosphere in a complex fashion.

$$\delta^{15}N = \left[\frac{(^{15}N/^{14}N)_{Sample}}{(^{15}N/^{14}N)_{Standard}} - 1 \right] \times 1000$$

All terrestrial plants cultivated in the same location have identical 15 N values, which indicate the quality of the soil from which they get their fixed nitrogen. When 15 N is measured, C 3 and C 4 plants are indistinguishable. The nitrogen content of the atmosphere is around 15 N 0, while soil nitrogen levels are typically around 14 N. Non-nitrogen-fixing plants, which get all of their nitrogen from soil nitrates, should be isotopically heavier than nitrogen-fixing plants, less negative 15 N, which get some of their nitrogen from the atmosphere. The coastal marine region has 15 N values in the range of 5 6 compared to 1 4 for terrestrial soils due to a greater degree of denitrification that happens in the oceans. As a result, when compared to terrestrial animals and plants of the same trophic level, marine resources have different and higher 15 N values. The trophic level in the food chain is reflected by stable nitrogen isotopes. In both ocean and terrestrial ecosystems, the amount of 15 N increases as it moves up the food chain.
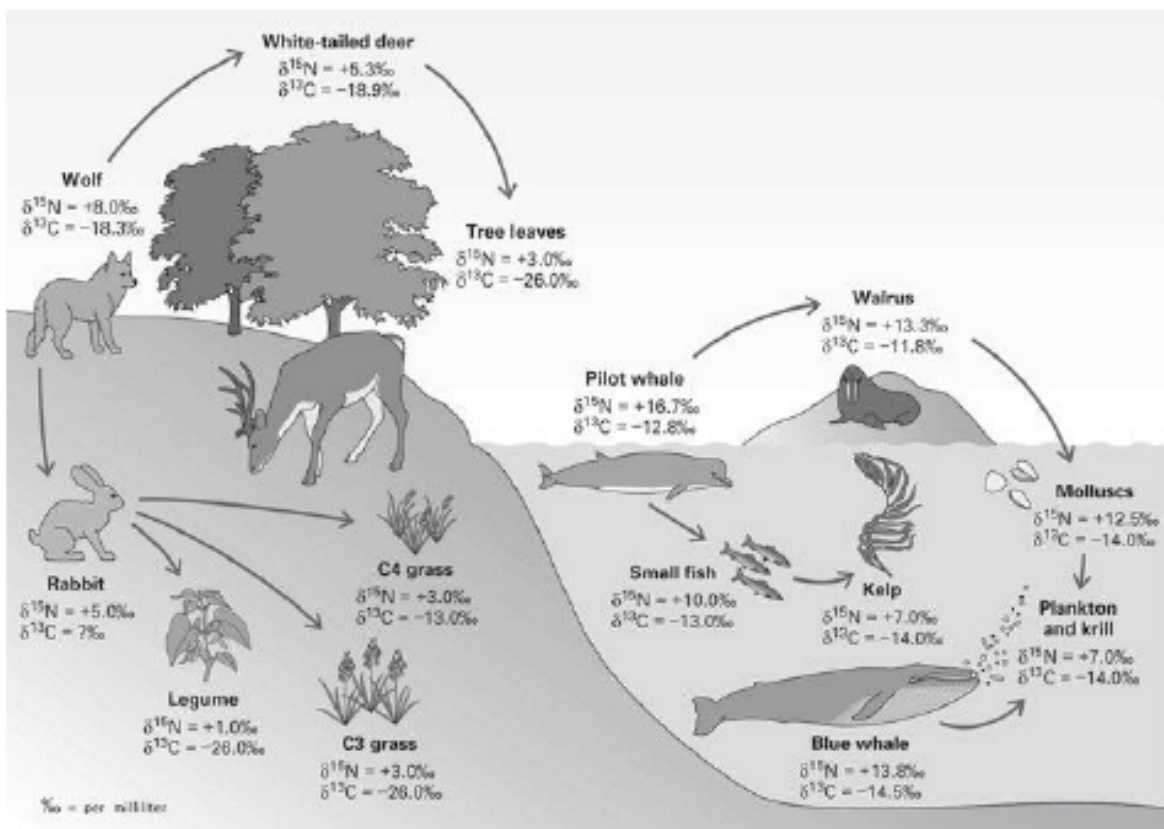

Figure 2.2 Depiction of Carbon and Nitrogen isotopes in terrestrial and marine food chains (Dias 2019).
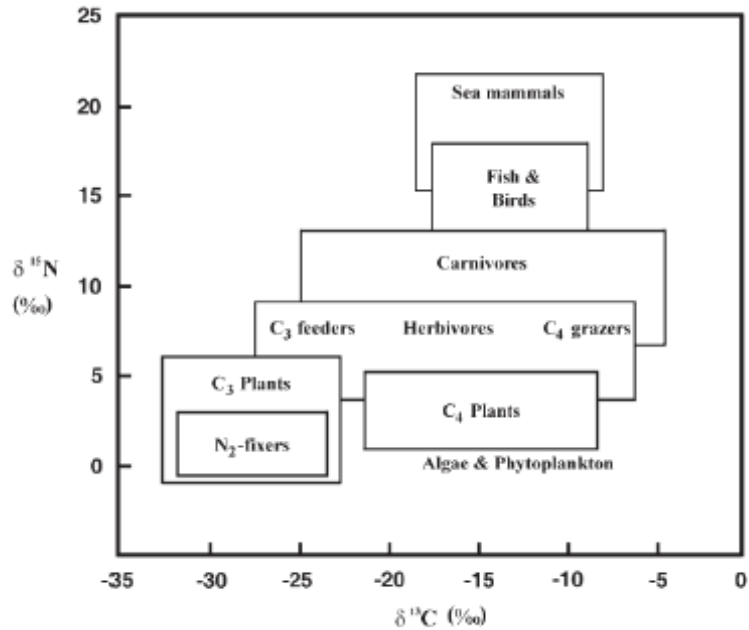
Figure 2.3 Graph showing the breakdown of C and N results (Dias 2019).

So, the body is a mineral deposit and reserve system that controls the absorption and possible release of nutrients obtained from food and water. Through the measurement of the concentration of some of these components in osteological results, it is possible to trace the sort of nutrition that a lived population had several millennia ago, based on this well-known concept (Ambrose, 1993). The analysis of bone samples allows you to get a nutritional picture of the individual by analyzing its last 5-10 years of life, which corresponds to the entire chemical remodeling time of the skeletal apparatus (Libby 1964).

The ratio of stable carbon and nitrogen isotopes contained in the bone collagen of the individuals from Castel Sozzio will be investigated in this study. In this situation, the concentration and relative ratio of these stable isotopes contained in bone collagen were used to determine the kind of diet. As the consumer assimilates a food, your atoms are integrated, resulting in an enrichment related to the food consumed, with varied properties based on the element considered.

Comparative investigations on animals with a known diet (Schoeninger, DeNiro 1984) shown that using this method, it is possible to recreate the protein portion of the food, with collagen being a protein. It is possible to reconstruct the animal or vegetable origin of these proteins, the relative environment of origin terrestrial or marine, and also the trophic level of belonging of the individual or animal analyzed using the characteristics of these isotopes and the studies carried out

17

over the years on both internal and external influences on the organism that undergoes these elements because of the characteristics of these isotopes and the studies carried out over the years on both internal and external influences on the organism that undergoes these elements.

To obtain a more complete picture than the surroundings and subsistence economy, a parallel analysis on animal bones and plant remains from the same context as the individuals analyzed is recommended; this allows you to get confirmations and feedback on the type of diet of the population analyzed, since, as will be seen, the isotopic variations between consumer and diet are well related precise. I will be analyzing 7 faunal remains pertaining to cow, sheep, dog, pig, and goat.

Even though this analysis is of incredible aid to understanding in an anthropological and archaeological perspective, it is difficult to affirm that your results will say more about the bigger picture of a site. The results obtained will provide the entire diet of an individual. As a whole, when working with a group of people from the same site, as we are doing in this analysis, we may compare each individual to understand and see them as a community. This will tell us how they ate, what they ate, give us an idea of the resources they were able to obtain, but we cannot assume this analysis will give us the answer to how a certain group or community in an area lived entirely. Paleo diet is an analysis that must be combined in an interdisciplinary way. Knowing the diet, the environment, the mobility, and DNA, is the only way to give a complete assessment of what life was truly like and how the people worked together.

Chapter 3. Methodology and Materials

In this chapter I will discuss the material I used and the methodology for the analysis of this project. As discussed before, this analysis is focused solely on the site of Castel Sozzio. Therefore, the material analyzed comes from a single site with bones pertaining to the graves mentioned. The next section contains my step-by-step process, which explains how the research was conducted and where, as well as the progress made and any difficulties encountered.

Sampling & Cleaning

The Laboratory of Biology of Ancient Populations houses the bone collection excavated in 1997, 1998, and in the recent 2020 campaign. This experiment took a total of 5 months to complete, beginning in March with the first stage which was looking into the inventory. The goal was to determine how many individuals possessed the most detailed information such as sex, age, DNA analysis, some identified pathologies, tomb number, survey, box number, and stratigraphic unit. I tried to choose evenly between males and females. Between them, I did not focus too much in separating them by age groups but instead looked for a couple of children to include in the analysis, as children are not sexed due to the lack of distinction on their bones because of their young age. All the samples were in a very good state of conservation.

Year	Trench	Burial	U.S Number	Age	Sex	DNA	Paleo Diet
1998	Beta	T. 7 Ind. 1	134	25-50	M	Petrosa sx	V
1997	Beta	T. 8	69	25-35 years	M	Petrosa sx	V
1997	Beta	T. 9 Ind A	121 dep 1			Petrosa sx	V
1998	Beta	T. 13/19 (Ind. A)	us 162	NR	M	Petrosa sx	V
1998	Beta	T. 13/19 Cranio 2	no us			Petrosa dx	V
1998	Alpha	T. 15 dep. Primaria (cranio 5)	210	40-50 years	M	Petrosa dx	V
1998	Alpha	T. 15 cranio 1	207	30-50 years	M	Petrosa dx	V
1998	Alpha	T. 15 cranio 2	207	NR	F	Petrosa dx	V
1998	Alpha	T. 15 cranio 3	207	35-45 years	F	Petrosa dx + sx	V
1998	Alpha	T. 15 cranio 4	207		M	Petrosa sx	V
1998	Alpha	T. 15 cranio 6	207	30-45 years	F	Petrosa dx	V
1998	Alpha	T. 15 cranio 7	207	25-30years	F	Petrosa sx	V
1998	Alpha	T. 15 cranio 8	207	30-50 years	M	Petrosa dx	V
1998	Alpha	T. 15 cranio 9	207	2-5 years		Petrosa sx	V
1998	Alpha	T. 16	208			Petrosa dx	V
1998	Beta	T. 20	no us	30-50 years	M	Petrosa dx	V
1998	Beta	T. 26	191	30-50 years	M	Petrosa dx	V
1998	Beta	T. 27	193	11-15 years		Petrosa sx	V
2020	Alpha	T. 24 Ind. D	310	11 - 13 years		no	V
2020	Alpha	T. 24 Ind. C	312	25-30 years	F	Petrosa dx	V
2020	Alpha	T. 24 Ind.a	306	40-50 years	F	Petrosa sx	V
2020	Alpha	T. 24 Ind.b	306	>50	M	Petrosa sx	V
2020	Alpha	T. 30 Ind.d	325	2-3 years		Petrosa sx	V

Table 3.1 All individuals chosen for the analysis with information obtained

Initially my idea was to examine 25 individuals. As I was searching for each individual I had sorted from the catalogue, I noticed some did not possess the additional information useful for the subsequent analysis. So, at the end I only had 18 samples that I believed were just right for

the next step. Fortunately, I was able to add animal bones into the research which were taken into the laboratory in April by Professor Emanuela Borgia straight from the storage at Castel Sozzio from the excavation done in 2020. The animal bones species included sheep, pig, canis, goat, and cow teeth.

Once I had all my samples set, I began the cutting and cleaning process. The cutting tool used for the bones was a Speed Rotary Dremel. I used a diamond wheel tool to cut/saw the bones at a maximum speed of 20,000 RPM. Each bone was weighed to a maximum of 3 g. Once they were cut, I polished them to get all the dirt and dust off them to prevent any type of contamination that may slow the next step which is demineralization. To polish, I used the Engraving Cutter Diamond Wheel Point, which I found worked perfectly when using it parallel to the bone at the lowest speed of 5,000 RPM.

After this process, I selected a piece that would weigh no more than 0.800g. and would be just big enough to fit into a test tube. The samples were all weighed on a precision balance. Even though I would only select a piece or pieces that would weigh 0.800g to be tested for the next step, I kept the rest as possible replica in case the process would not turn out as expected. For the entire operation, cleanliness was of vital importance. Before working with a sample, the table was sanitized with alcohol as well as the instruments. After it was cut and or polished, the dust was swept and once again the table, instruments, and scale were all sanitized.

Demineralization

After the sample preparation was finalized, I moved to the Isotopic Laboratory to extract the collagen. There, I was guided by Sara Bernardini who aided me through the process, following a modified protocol described by Longin (1971). The first thing to do was re-weigh the samples to make sure they were no more than .800g. After that, they were placed in test tubes which I marked with new identification codes for each sample. I named the samples CSF 1-7 for fauna and CSH 8-25 for the human samples. Once this was set, I proceeded to filling each test tube with hydrochloric acid of 0.5M which was previously prepared by Sara. When all the test tubes were filled, they were all covered with aluminum foil and placed inside the refrigerator at +4°C.

I went back to the laboratory to change the HCl every two days. This was done by taking out the HCl with a Pasteur pipette and disposing of it in a special beaker. Every time this was done,

I checked how hard the bones were. The hydrochloric acid removes the mineral part of the bone and purifies it of any pollutants during the demineralization phase, which is exactly how collagen extraction works. To determine when the sample process is complete, the bone sample must float and be soft. The samples were not ready at the same time. The first only took five days and they were already floating. These samples were rinsed 3 times with distilled water to remove all HCl residue, and were kept in distilled water until all the samples were ready.

Gelatinization

The next step the samples had to go through was gelatinization. In this procedure, the test tubes containing the demineralized bone samples were filled with roughly 8 ml of pH3 water, covered individually with caps, then all covered with aluminum, and heated to 75°C for 48 hours. The collagen should be dissolved after 48 hours. Once the time was met, the glass test tubes were taken out to be transferred into plastic test tubes that were previously weighed (without the cap to minimize errors). The whole process and weights were constantly documented in the laboratory and then digitized (Table 2).

812333 Extraction of Collagen Modified Longin method   812333 LABORATORY OF PALEOANTHROPOLOGY AND BIOARCHAEOLOGY

			0.5M HCl	pH 3 solution
		Date of solution preparation		

Project: Castel Sozio
Date: 6/24/21
Operator: Sonia Reyes

#		Sampling				Lyophilisation			EA-IRMS Results					
	ID	Tag name	Sample type	Weight (g)	Empty tube with cap (g)	Tube with collagen and cap (g)	Collagen (g)	Yield (%)	$\delta^{13}C$(‰) vs. VPDB	$\delta^{15}N$(‰) vs. Air	%C	%N	C/N	Notes
1	CSF-1	SB US 34		0.7932	4.3142	4.3556	0.0414	5.22%					#DIV/0!	
2	CSF-2	SB US 34 T.13		0.8901	4.3179	4.3914	0.0735	8.26%					#DIV/0!	
3	CSF-3	T.20		0.5921	4.3164	4.3304	0.014	2.36%					#DIV/0!	
4	CSF-4	SB US 12		0.5401	4.354	4.3728	0.0188	3.48%					#DIV/0!	
5	CSF-5	SB US 143		0.5995	4.3229	4.3508	0.0279	4.65%					#DIV/0!	
6	CSF-6	SB US 12 BAG IN BAG		0.5544	4.312	4.3452	0.0332	5.99%					#DIV/0!	
7	CSF-7	SB US 12		0.622	4.3107	4.3301	0.0194	3.12%					#DIV/0!	
8	CSH-8	T.27 US 193		0.9628	4.3009	4.3683	0.0674	7.00%					#DIV/0!	
9	CSH-9	T.15 CRANIO 3 US 207		0.7396	4.2964	4.3014	0.005	0.68%					#DIV/0!	
10	CSH-10	T.15 CRANIO 3 US 207		0.6936	4.2966	4.3254	0.0288	4.15%					#DIV/0!	
11	CSH-11	T.24 IND C US 312		0.6	4.3137	4.3545	0.0408	6.80%					#DIV/0!	
12	CSH-12	T.26 US 191		0.6617	4.2788	4.3267	0.0479	7.24%					#DIV/0!	
13	CSH-13	T.20 NO US		0.6086	4.3106	4.343	0.0324	5.32%					#DIV/0!	
14	CSH-14	T.13/19 CRANIO 2		0.8172	4.3252	4.3296	0.0044	0.54%					#DIV/0!	
15	CSH-15	T.15 CRANIO 1 US 207		0.743	4.2991	4.3147	0.0156	2.10%					#DIV/0!	
16	CSH-16	T.15 CRANIO1 US 207		0.6872	4.3035	4.3623	0.0588	8.56%					#DIV/0!	
17	CSH-17	T.15 CRANIO 7 US 207		0.9033	4.3181	4.3214	0.0033	0.37%					#DIV/0!	
18	CSH-18	T.24 IND D US 310		0.9589	4.3102	4.3172	0.007	0.73%					#DIV/0!	
19	CSH-19	T.15 CRANIO 4 US 207		0.6767	4.313	4.3358	0.0228	3.37%					#DIV/0!	
20	CSH-20	T.13/19 IND A US 207		0.75	4.3103	4.3662	0.0559	7.45%					#DIV/0!	
21	CSH-21	T.8 US 69		0.7144	4.2963	4.3266	0.0303	4.24%					#DIV/0!	
22	CSH-22	T.15 CRANIO 2 US 207		1.0037	4.3018	4.3944	0.0926	9.23%					#DIV/0!	
23	CSH-23	T.7 IND 1 US 134		0.7308	4.3445	4.4098	0.0653	8.94%					#DIV/0!	
24	CSH-24	T.15 CRANIO 5 US 207		0.7308	4.3114	4.3484	0.037	5.06%					#DIV/0!	
25	CSH-25	T.15 CRANIO 6 US 207		0.8999	4.2874	4.2895	0.0021	0.002333593						

Table 3.2 Collagen extraction sheet with list, weights and collagen yields of the analyzed samples.

The liquid is then placed into these plastic tubes with the aid of an Ezee filter. This filter consists of a graduated polypropylene tube with a sintered polyethylene filter, which works by placing the device's end into the tube and applying downward pressure until the filtered solution passes into the device's graduated barrel. Its purpose is to eliminate insoluble particles/residue from the bones so that only the liquid is kept. They are then covered with a parafilm that has been perforated, and then kept in the freezer overnight.

Freeze-drying

By keeping the samples in the freezer, the next step is lyophilization. This step involves freezing the substance, then lowering the pressure and increasing the heat to allow the frozen water to sublimate. Then the plastic tubes are frozen for 12 hours at -20 degrees Celsius, then transferred to a freezer at -80 degrees Celsius for at least 4 hours. Then they are put in a freeze-dryer for 3-4 days, which works by evaporating the liquid component, leaving only pure collagen filaments and any acid salts. The consistency of the end result is puffy, stringy but compact, and its color varies from white to beige. It is important to check the samples after the set time in case some are not ready. Once it is confirmed they are all ready, the tubes must be weighed again and noted down. The weight of the empty tube must be subtracted to that of the full one, this equation will give us the percentage value of the extracted collagen (Table 3.2).

Isotope Ratio Mass Spectrometry (IRMS)

The very final step is the introduction of the samples into the mass spectrometer. IRMS is used to obtain the composition of the collagen. This is a technique which is applied to indicate the composition, elemental or molecular, as well as the isotope ratio of each certain elements. The process of this analysis starts with the collagen sample preparation. For this, an amount of minimum 0.8 mg and a maximum of 1.1 mg must be placed in tin capsules. This is done by extracting a piece of collagen, placing it in the tin capsule, weighing it on a precision balance, and once you obtain the desired weight, close the tin capsule with the tweezers which are used through the entire process. All instruments must be sterilized after every sample has been done. The tin capsules are closed into a small ball and then placed into a labelled PCR plate. Out of the 25

samples, 24 met the collagen quality indicators, CSH-17 did not possess the sufficient weight to go to through to the next step (see chapter 4), the rest were analyzed by the mass spectrometer.

The 24 samples were sent to Iso-Analytical Laboratory in the United Kingdom to be analyzed using the EA-IRMS. The way this analysis works is by entering the weights into the mass spectrometer and comparing them to elements taken as a standard to evaluate the validity of analysis. A standard consists of substances that are known to contain carbon and nitrogen. This means that it will create a calibration curve when compared to the collagen samples we want to analyze, it basically measures the respective abundance of different isotopes of the same chemical element in a particular sample.

The isotope ratio mass spectrometer works with its different parts. An elemental analyzer, which is what the EA stands for, is coupled to a gas isotope ratio mass spectrometer and measured. In an elemental analyzer, the collagen sample is combusted at high temperatures. Resulting in the generation of several gases, which include the two of major interest, N2 and CO2. Which are then sent through a gas chromatograph, which separates the gases depending on their mass.

Once the gases are separated, they go into the mass spectrometer to be measured by isotope ratios. The spectrometer consists of an ion source, a mass analyzer and an ion detector. The collagen is combusted under high vacuum by means of special pumps, this is where the gases are collected. The spectrometer separates a mixture of ions according to their mass and charge ratio. The mixture is obtained by ionizing the molecules of each sample. They then pass through an electron beam a known energy.

Isotope Mass Spectrometry (IRMS)

Detection
Faraday collectors

$\{m/q\} = 46$
$\{m/q\} = 45$
$\{m/q\} = 44$

current

magnet

amplifiers

x

Ion source

ratio output

beam focussing
ion acelerator
electron trap
ion repeller
gas inflow (from behind)
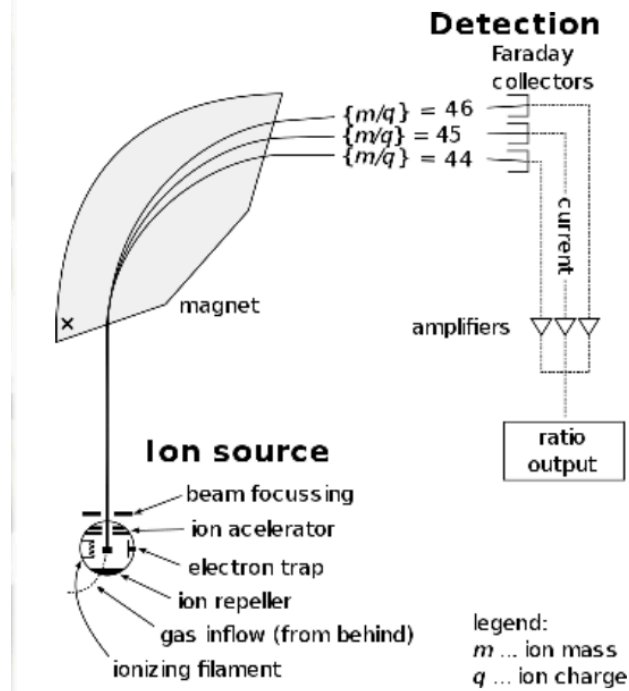ionizing filament

legend:
m ... ion mass
q ... ion charge

Figure 3.3 Diagram of IRMS function. (Dias 2019).

Ionized molecules are unstable and fragment into lighter ions according to typical schemes as their chemical structure. The mass spectrum reports the abundance of each ion as a function of the mass over charge ratio typical of each compound since it is directly related to its chemical structure and conditions ionization to which it has been subjected. Through the analysis of the generated ion signals it can reconstruct the original molecular structure backwards. The mass spectrometer does not directly measure the abundance of an isotope, but provides the ratio of two isotopes of an element with accurate precision. The stable isotope concentrations are measured as ratios of heavier isotopes on lighter isotopes with reference to an international reference scale, VPDB for carbon and AIR for nitrogen. The results are correct according to the entered standards, whose nitrogen and carbon values are known.

Chapter 4. Results

This section will cover the results obtained from the EA-IRMS measurement. The table below (table 3) demonstrates the weight of each sample that was used for the analysis, the carbon content percentage (δ13C), as well as the nitrogen content percentage (δ15N) which were provided by Iso-Analytical Lab. I added to the table the C/N ratio, as well biological and archaeological info as tomb, sex, age at death, and species. I also included and highlighted in pink, the samples excluded from the following analysis (CSH-14, CSH-18 and CSH-25) as they did not meet quality and reliability control criteria (i.e. respectively lower content of C and N, and C/N out of the acceptable range).

With the information presented in this table, we are able to break down the results and group them accordingly to better understand what the numbers are telling us. Every piece of data here serves as a step closer into deciphering how the people of Castel Sozzio lived.

Sample Code	Tag	Nitrogen Content (µg)	Nitrogen Content (%)	$\delta^{15}N_{AIR}$ (‰)	Carbon Content (µg)	Carbon Content (%)	$\delta^{13}C_{V-PDB}$ (‰)	C/N Ratio	Sex	Age	Species
CSF-1	SB US 34	110.7	11.07	7.19	322.2	32.22	-21.40	3.39			CANIS
CSF-2	SB US 34 T.13	97.0	10.78	4.93	264.8	29.42	-20.81	3.18			COW
CSF-3	T.20	127.5	11.59	5.19	354.2	32.20	-20.14	3.24			SHEEP
CSF-4	SB US 12 2	148.7	14.87	4.40	418.2	41.82	-21.07	3.28			SHEEP
CSF-5	SB US 143	115.3	11.53	5.36	312.3	31.23	-20.76	3.49			GOAT
CSF-6	SB US 12 BAG IN BAG	134.4	12.22	5.45	379.6	34.51	-20.43	3.29			SHEEP
CSF-7	SB US 12	120.4	13.37	3.85	337.6	37.52	-20.09	3.27			PIG
CSH-8	T.27 US 193	143.0	14.30	7.58	390.6	39.06	-18.82	3.18	NR	11-13 Y	
CSH-9	T.15 CRANIO 3 US 207	148.4	14.84	8.42	414.9	41.49	-19.50	3.26	F	30-45 Y	
CSH-10	T.15 CRANIO 9 US 207	120.7	12.07	7.62	327.7	32.77	-19.03	3.16	NR	2-5 Y	
CSH-11	T.24 IND C US 312	117.8	13.09	8.35	321.1	35.68	-19.58	3.17	F	25-30 Y	
CSH-12	T.26 US 191	166.3	16.63	7.76	452.4	45.24	-20.42	3.17	M	30-50 Y	
CSH-13	T.20 NO US	185.4	18.54	8.22	513.1	51.31	-19.81	3.22	M	30-50 Y	
CSH-14	T. 13/19 Cranio 2	1.3	0.42	---	7.1	2.38	---	---	---	---	
CSH-15	T.15 CRANIO 1 US 207	166.7	16.67	8.62	463.4	46.34	-19.38	3.24	NR	30-50 Y	
CSH-16	T.15 CRANIO 8 US 207	96.6	10.74	8.06	262.6	29.18	-19.11	3.16	M	30-50 Y	
CSH-18	T.24 IND D US 310	4.5	0.45	---	19.8	1.98	---	---	NR	11-13 Y	
CSH-19	T.15 CRANIO 4 US 207	138.3	12.57	8.75	377.4	34.31	-19.28	3.18	M	NR	
CSH-20	T. 13/19 IND A US 207	194.7	19.47	8.44	525.2	52.52	-18.42	3.14	M	NR	
CSH-21	T.8 US 69	159.9	14.53	8.46	434.7	39.52	-20.52	3.17	M	25-30 Y	
CSH-22	T.15 CRANIO 2 US 207	162.0	14.73	8.14	434.7	39.52	-18.72	3.12	F	NR	
CSH-23	T.7 IND 1 US 134	144.8	16.09	6.96	392.8	43.65	-20.09	3.16	M	25-50 Y	
CSH-24	T.15 CRANIO 5 US 207	153.4	17.05	8.37	421.5	46.83	-19.35	3.06	M	40-50 Y	
CSH-25	T.15 CRANIO 6 US 207	67.2	7.46	8.76	259.3	28.81	-22.24	3.86	F	30-45 Y	

Table 4.1. Results from mass spectrometry analysis of stable carbon and nitrogen isotopes of human and animal bone collagen, and relative quality indicators. M=male, F=female, NR= Not Determined In pink the samples excluded from the analysis as they did not meet quality indicators.

The first thing to do once the results from the mass spectrometer arrived, was to calculate the ratio between C/N. This calculation is an indicator of a measure of the quality of the combusted

protein. Collagen that has been isolated in the lab, as well as other protein samples, are more susceptible. The acceptable range of C/N of bone is 2.9-3.6. Any number outside of this range is a sign that you either do not have collagen in your sample or that it is has been contaminated, and of course, it cannot be used as part of the data (DeNiro 1985; van Klinken 1999).

The way the ratio is calculated is by the following formula: (Amt % C / Amt % N) x (14/12). This gives you the result of the ratio of the number of C atoms to the number of N atoms in the sample, the atomic number. Fortunately, out of all the samples that I sent and that had enough collagen to be analyzed, only one went above the set range, CSH-25 with a ratio of 3.86. The rest of the samples ranged from 3.06 to 3.49 (mean 3.24).

ID	TAG	SEX	$\delta^{15}N_{AIR}$ (‰)	$\delta^{13}C_{V\text{-}PDB}$	RATIO
CSF-1	SB US 34		7.19	-21.40	3.39
CSF-2	SB US 34 T.13		4.93	-20.81	3.18
CSF-3	T.20		5.19	-20.14	3.24
CSF-4	SB US 12 2		4.40	-21.07	3.28
CSF-5	SB US 143		5.36	-20.76	3.49
CSF-6	SB US 12 BAG IN BAG		5.45	-20.43	3.29
CSF-7	SB US 12		3.85	-20.09	3.27
CSH-8	T.27 US 193	NR	7.58	-18.82	3.18
CSH-9	T.15 CRANIO 3 US 207	F	8.42	-19.50	3.26
CSH-10	T.15 CRANIO 9 US 207	NR	7.62	-19.03	3.16
CSH-11	T.24 IND C US 312	F	8.35	-19.58	3.17
CSH-12	T.26 US 191	M	7.76	-20.42	3.17
CSH-13	T.20 NO US	M	8.22	-19.81	3.22
CSH-15	T.15 CRANIO 1 US 207	NR	8.62	-19.38	3.24
CSH-16	T.15 CRANIO 8 US 207	M	8.06	-19.11	3.16
CSH-19	T.15 CRANIO 4 US 207	M	8.75	-19.28	3.18
CSH-20	T. 13/19 IND A US 207	M	8.44	-18.42	3.14
CSH-21	T.8 US 69	M	8.46	-20.52	3.17
CSH-22	T.15 CRANIO 2 US 207	F	8.14	-18.72	3.12
CSH-23	T.7 IND 1 US 134	M	6.96	-20.09	3.16
CSH-24	T.15 CRANIO 5 US 207	M	8.37	-19.35	3.06
CSH-25	T.15 CRANIO 6 US 207	F	8.76	-22.24	3.86
	MEAN		7.15	-19.84	3.21571429
	STANDARD DEVIATION		1.577202068	0.82	0.09484574
	MAX VALUE		8.75	-18.42	3.49
	MIN VALUE		3.85	-21.4	3.06
	VALUE RANGE		4.9	2.98	0.43

Table 4.2 Table showing Carbon and Nitrogen results for each individual both animal (CSF) and human (CSH) as well as their C/N ratio, mean, standard deviation, max and min value, and value range.

It is important to look at the numbers closely before making an interpretation of the results. Therefore, we must keep an order to continue breaking down the results step by step.
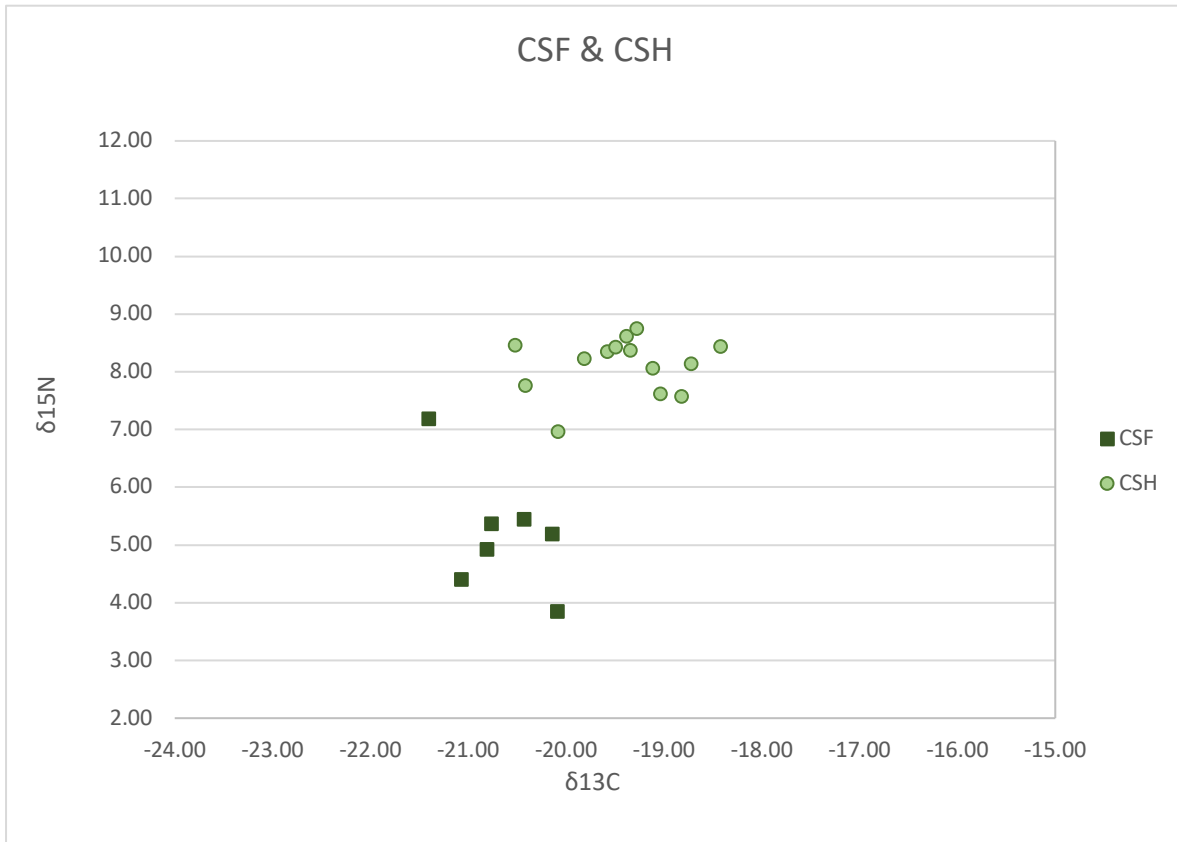
Figure 4.3 Graph representing all samples. CSF are the animal samples and CSH the human samples.

This graph (fig.4.3) presents all samples grouped together. We have both CSF (fauna) and CSH (humans). This gives us an idea of how the levels of C and N are distributed in a more general way. I start the breakdown by plotting by sex (fig 4.4).

Unfortunately, I was not able to study a more even number of males and females, but even so, they do have quite varied numbers (fig). There are also three samples which are not sexed as they are two children and three unidentified adults.
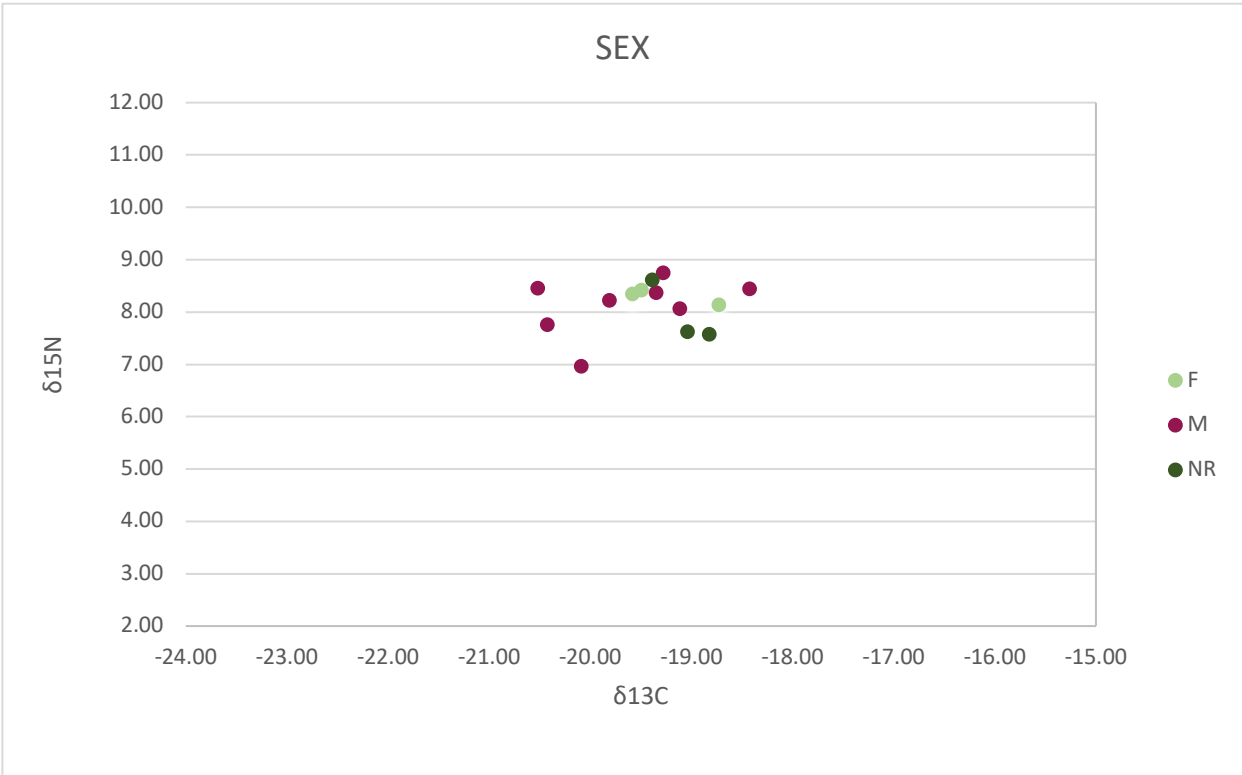
Figure 4.4 Graph depicting sex of samples. F= females, M= males, NR= not recorded.

I also checked if there were any differences in age at death, so I made a graph that included both males and females grouped by ages (fig. 4.4). Males (n= 8) present mean values of δ13C -19.62‰, and δ15N 8.13‰. With their minimum being δ13C -20.52‰, δ15N 6.96‰ and a maximum of δ13C -18.42‰, δ15N 8.75‰, ranging δ13C 2.1‰ and δ15N 1.8‰. While females (n= 3) present mean values of δ13C -19.27‰ and δ15N 8.30‰. With a minimum of δ13C -19.58‰, δ15N 8.14‰ and a maximum of δ13C -18.72‰, δ15N 8.42‰, ranging δ13C 0.86‰ and δ15N 0.28‰.
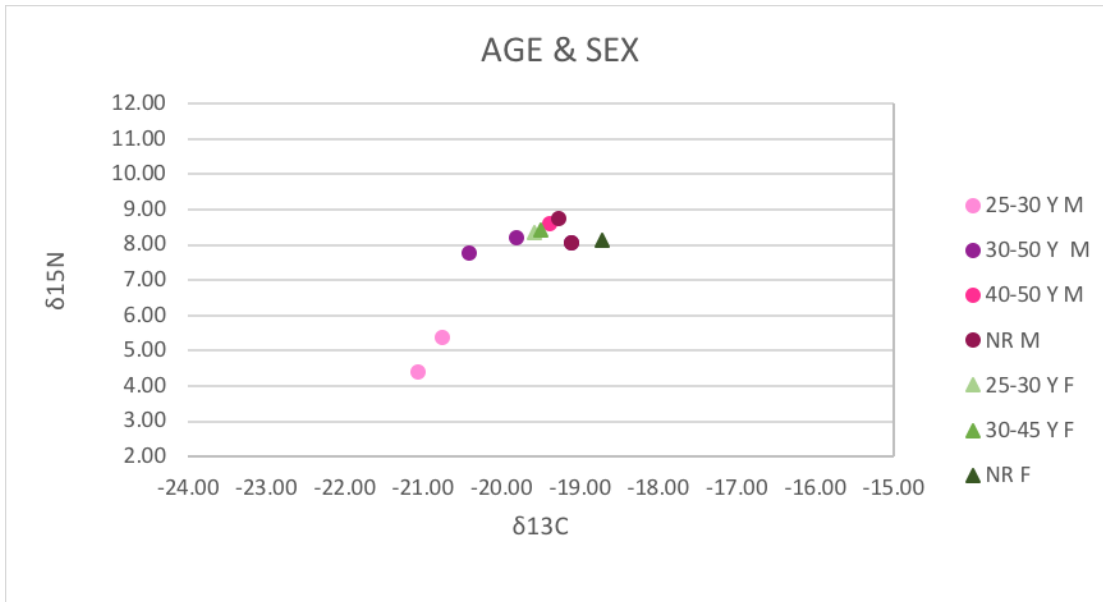
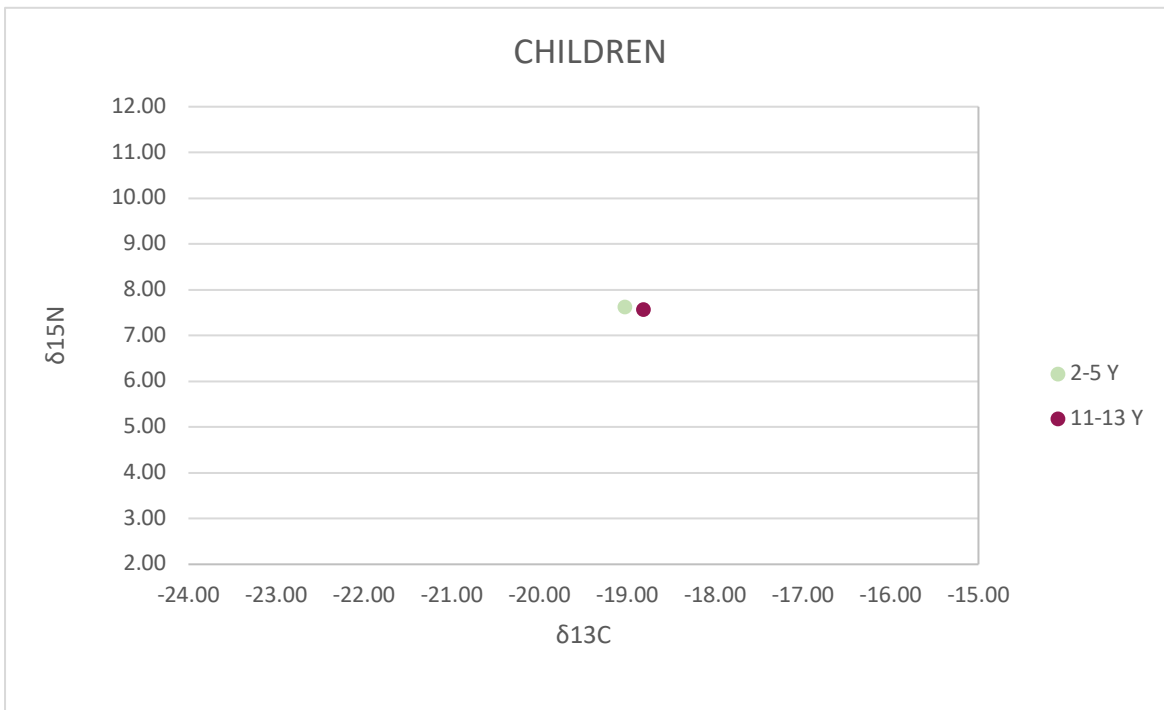**Figure 4.5** Graph depicting human (CSH) ages and sex.

Figure 4.6 Graph showing both children of different age.

There were also two children included which show similar C and N values, though there is an age difference. Younger children/infants that are breastfeeding, tend to have high $\delta^{15}N$ values, even higher than their mothers. This is due to the fact that their bodies process their mother's milk

29

and fractionate the values by 3-5% being in a trophic level higher than their mothers. (Beaumont et al. 2015; Richards et al. 2002; Schurr 1998).
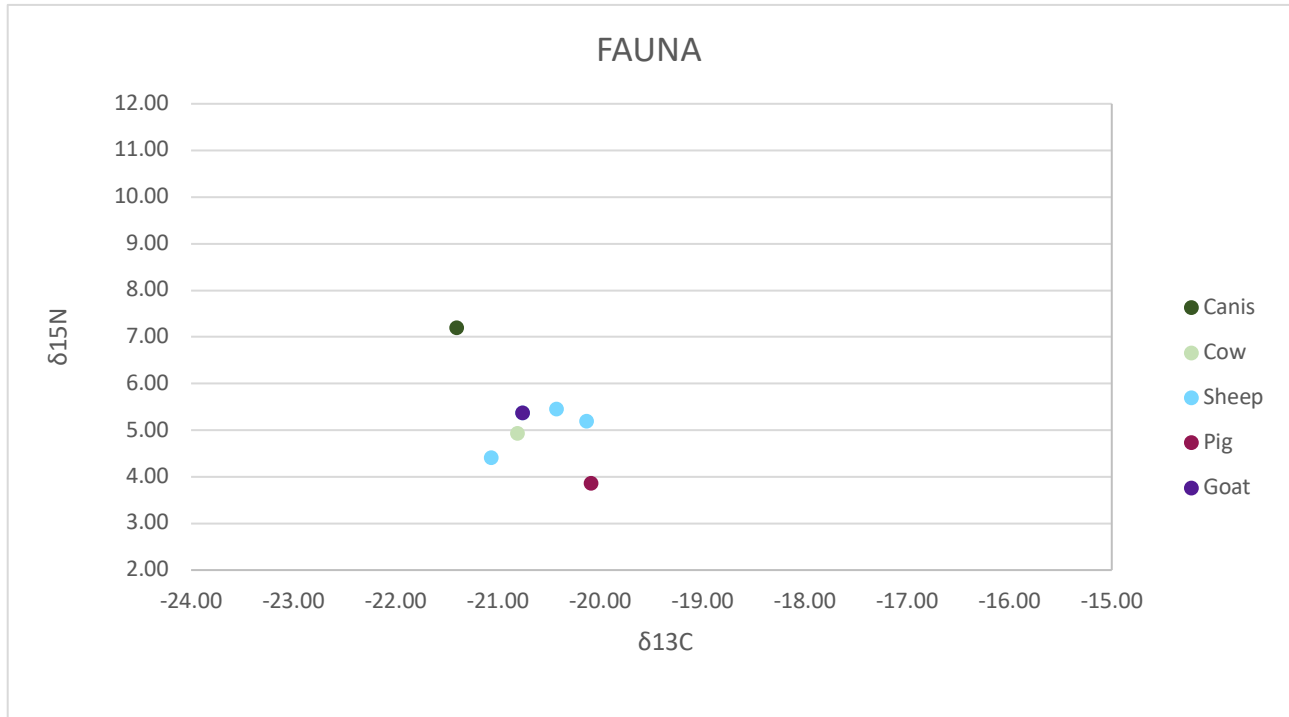

Figure 4.7 Graph showing the fauna samples

As mentioned before, I was able to include animal bones in my analysis. Seven samples underwent the collagen extraction process just as the human bones. The animals studied are three sheep, one cow, one dog, one pig and one goat. All the samples came from the animals' teeth, and even though dentin is known to take a long time to demineralize, these samples were ready in a short while. The graph above shows the results and we can see how out of the seven animals, only one, the dog, is evidently different from the group (fig.4.7) Their mean values are δ13C 20.67‰ and δ15N 5.19‰. With a minimum of δ13C -21.40‰, δ15N 3.8‰, and a maximum of δ13C -20.08‰, δ15N 7.19‰, ranging on δ13C -1.31‰ and δ15N 3.34‰.
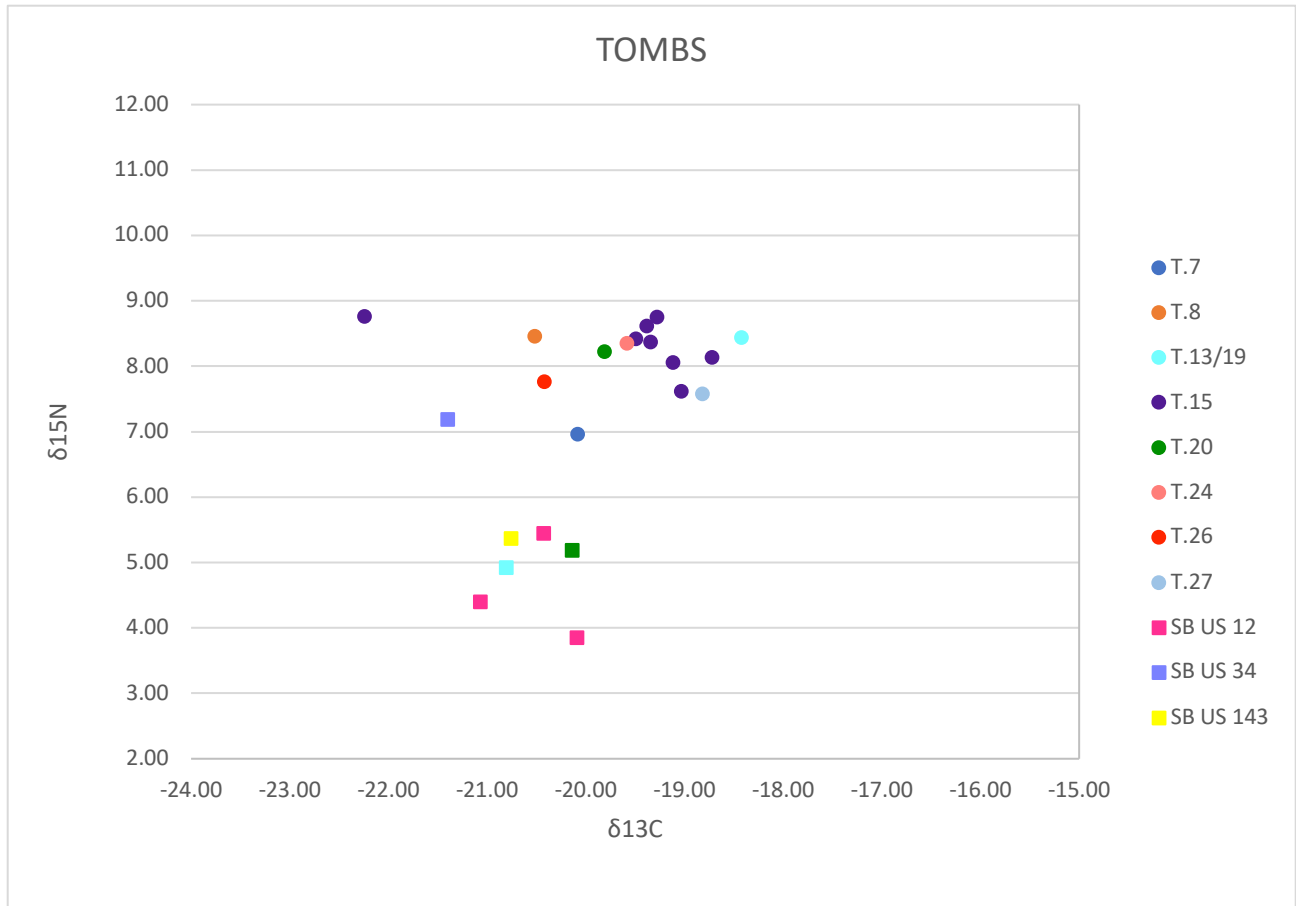
Figure 4.8 human and fauna data plotted according tombs of provenance (human are circles, fauna squares). Tombs T.13/19 and T.20 provided both human and fauna remains.

This graph (fig 4.8) shows stable C and N isotope values a to the tombs of provenance, both for humans and animal samples to see the distribution of burial places. Analyzing the distribution and comparing it with the levels of C and N, may give us an idea of the humans that were buried there.

Chapter 5. Discussion & Conclusion

The data acquired from the analysis has been displayed in the previous chapter. With the aid of graphs, the information obtained has been broken down in order to better explain what the results tell us about Castel Sozzio.

The atomic C:N ratio demonstrates a high degree of collagen preservation, with values in the predicted range of 2.9–3.6 for all humans and animals (DeNiro 1985; van Klinken 1999). From the 24 samples that underwent analysis, 3 human samples did not meet the criteria and were not included in the data results and interpretation. Both fauna and humans present isotopic values pertaining to a C3 and C4 diet.

Fauna

The isotopic data for the faunal remains were a mean of C -20.67‰ and N 5.19‰. A minimum of C -21.40‰ and N 3.8‰. A maximum of C -20.08‰ and N 7.19‰ Ranging at C 1.31‰ and N 3.34‰ The animals in the analysis included one goat, one pig, one dog, one cow and three sheep. Two of the three sheep were very close both in C and N values while one of them had both lower C and N, this could depend on the usually wide grazing area of the herbivorous species, ranging also in altitude (transhumance practices) which influence C and N values. Between them, the cow and the goat show close values in both elements. Between them the sheep had both the smallest and highest amounts of C and N; CSF- 4 C -21.07‰ and N 4.40‰, CSF-3 C -20.14‰ and N 5.19‰, and CSF-6 C -20.43‰ and N 5.45‰.

From all the animals, the dog had the highest Nitrogen value as I expected, given its usual omnivorous diet. CSF- 1 C -21.40‰ and N 7.19‰. This could be due to the fact that dogs tend to be fed mainly meat compared to the other omnivores. This diet could either indicate a domestic dog belonging to a family with good social status that was able to feed it meat, or it could be a stray dog eating what he found in the fields/vegetation. I was expecting the other high nitrogen level in the group to be the pig, given the fact that it is usually fed with leftovers.

The pig, CSF- 7 had C -20.09‰ and N 3.85‰, both values falling in the lower end, placing it as being part of a C3 diet. Pigs are known to be fed the leftovers humans do not eat. They are known to be animals that can even eat bone. There are domestic pigs that are used as they are fed a combination of plants, nuts, or seeds such as oaks and chestnuts (. These pigs are mainly fed with this grazing diet because they are grown to be eaten. In general, these animals fall within C3 feeders. The goat, cow, pig and sheep lived on C3 plants while the dog falls within human values,

which is indicative of a diet based also on leftovers, similar to the human diet which is commonly found in domestic dogs.

Humans

Stable isotopes from humans had a mean of C -19.43 and N 8.17‰ a range of C -20.52‰ to C-18.42‰ and N of N 6.96‰ to 8.75‰. A C/N ratio of 3.06 to -3.26.

Human data must be analyzed in relation to faunal data. Performing this analysis will give us an idea of the enrichment the humans received from the animals when being consumed. The human mean of C -19.43‰ minus the fauna mean of C -20.8‰ gives us a value of 1.37‰. The human mean of N 8.17‰ minus the fauna mean of N 4.92‰ gives us a value of 3.25‰. The known trophic enrichment shift is of +1‰ for and +3-5‰ for N (DeNiro and Epstein, 1978). Both C and N results fall into the range meaning It is important to mention that in the fauna mean, I did not include the values of the dog as it is a domestic animal which is not commonly known to be eaten, and since no proof of this exists (for example written record) of this practice with the people of Castel Sozzio, I decided to not include it in the enrichment analysis.

What these results (of +1.37‰ for and 3.25‰ for N) indicate is a terrestrial diet consisting of both plants and animal protein. This is so because for example, a high offset; 4-5‰ of N is indicative that animal protein was an important contribution. Since the result was of 3.25‰, it is safe to say that it is proof of the diet being balanced between plants and animal protein. The way to discern whether the plant diet is a C3 or C4 is by looking at the C values. High C values of about -17, -16 C pertain to C4 plants. C3 plant diets usually lie between -20 and -19 C. The highest C value in the analysis is of -18.42 which falls right between C3 and C4 plant diets. The contribution of freshwater resources may be the reason for that value (Fry 1991).

In the study three out of the eleven sexed humans were females. These females had a mean of C -19.27‰ and N 8.30‰. A minimum of C-19.58‰ and N 8.14‰ and a maximum of C -18.72‰ and N 8.42‰. With a range of C 0.86‰ and N 0.15 ‰ and a standard deviation of C 0.47‰ and N 0.15‰. From these three females, two of them share similar C and N values. CSH-9 and CSH-11 share similar C and N values, lower when compared to CSH- 22 ones. In fact, CSH-

22 shows a higher value of N. As well as being close in both elements, the three females belong to the same trench, Trench Alpha.

CSH-9 and CSH-22 belong to the same tomb, T.15. This tomb, which is surrounded on all four sides by local stone slabs, features an individual in the main position at the center of the tomb, as well as the skulls of eight additional people. CSH-9 and CSH-22 were two of the skulls that were added to the grave.

Figure 5.1 Photograph of T.15 in Trench Alpha opened on 1998 campaign. (Archive of Castel Sozzio, courtesy Borgia 2021 all rights reserved).

CSH-11 belonged as well to Trench Alpha in the tomb T. 24. T.24 was a tomb that was excavated during the 2020 campaign. CSH-11 presented a higher level of C and lower level of N

compared to CSH-9 and CSH-22. This suggests a diet which leans a little bit more towards C4 based on the C level. The N does not permit us to state that this female was considered a carnivore, fully, but falls more in the herbivore category leaning towards a more C4 plant diet than C3 plants. A terrestrial diet based mostly on animals or a diet inclined to C3 diet. Another possibility that could explain these C and N values, could be the possibility of having consumed freshwater fish, as the C is not as high as to be a full C4 consumer. Consuming fresh water fish is a possibility as the site is in vicinity to Lake Bolsena and he Tiber River. If for some reason the before mentioned chance was not the case, he slight difference in isotopic values and tombs, opens the possibility that these women lived during a different period of time which would explain the variation.

The eight males presented a mean of C -19.62‰ and N 8.13‰ a minimum 0f C -20.52‰ and N 6.96‰ and a maximum of C -18.42‰ and N 8.75‰ with a range of C 2.1‰ and N 1.8‰ and a standard deviation of C 0.71‰ and N 0.55‰. The males studied were dispersed between the two trenches; three in Trench Alpha and five in Trench Beta. From Trench Alpha, they belong to tombs T.15, comprising three of the eight skulls added. On Trench Beta the males come from tombs T.7, T. 8, T. 13/19. T. 20, and T.26. All these tombs were excavated during the 1997 and 1998 campaigns.

Starting with the males in Trench Alpha, all three of them belong to the before mentioned T.15. They are part of the skulls that we placed in the grave and have very similar isotopic values to those of the females from the same tomb. This makes sense as it was believed that the eight skulls that were found in that grave as being added to it, were added at the same time, all belonging to the same time period. In Trench Beta, the five males pertain to different graves. The N values are not as closely related as the individuals on Trench Alpha, but four out of the five are located the lower part of the graph CSH-23= N 6.96‰ CSH-12= N 7.76‰ CSH-13= N 8.22‰ AND CSH-21= N 8.46‰, also seen below on figure 5.3.
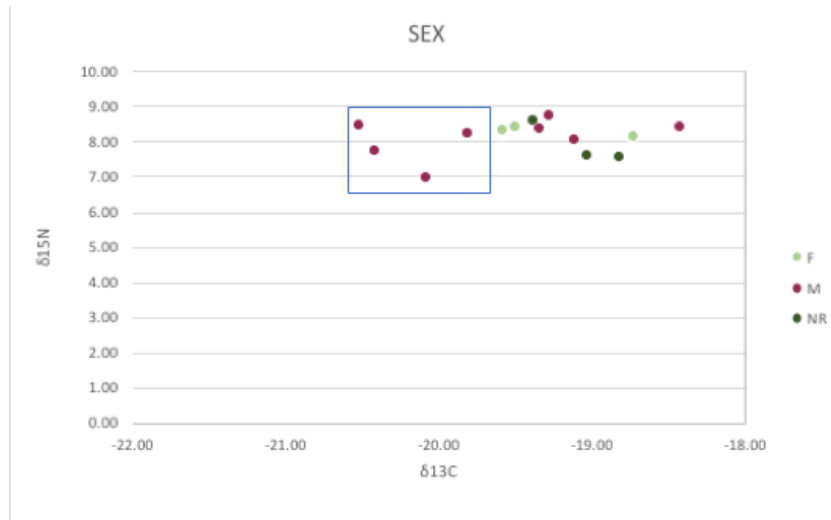
Figure 5.2 Sex graph enclosing four male individuals from Trench Beta.

The separation is clearly visible from one trench to the other. The curious thing about this is that the missing male, male number 5 is at the opposite side of the graph. Individual CSH-20 has isotopic levels that match up best to the individuals in Trench Alpha (see figure 5.3 below). The graves were both uncovered during the 1998 campaign. This just gets me thinking about the possibility that the first four males discussed belong to a different time period than the fifth male because of its isotopic level similarities with Trench Alpha individuals. Trench Beta individuals lean more towards a C3 diet while Trench Alpha and CSH-20, are closer to border lining C3 and C4 diets.

Also, the only animal bone in which the tomb was shared with a human is CSH-3. CSH-3 is a sheep bone that was found in tomb T.20 in Trench Beta as well. I decided to add it to the Alpha and Beta Trenches graph as the discovery of this being the only animal bone in a human grave was really interesting.
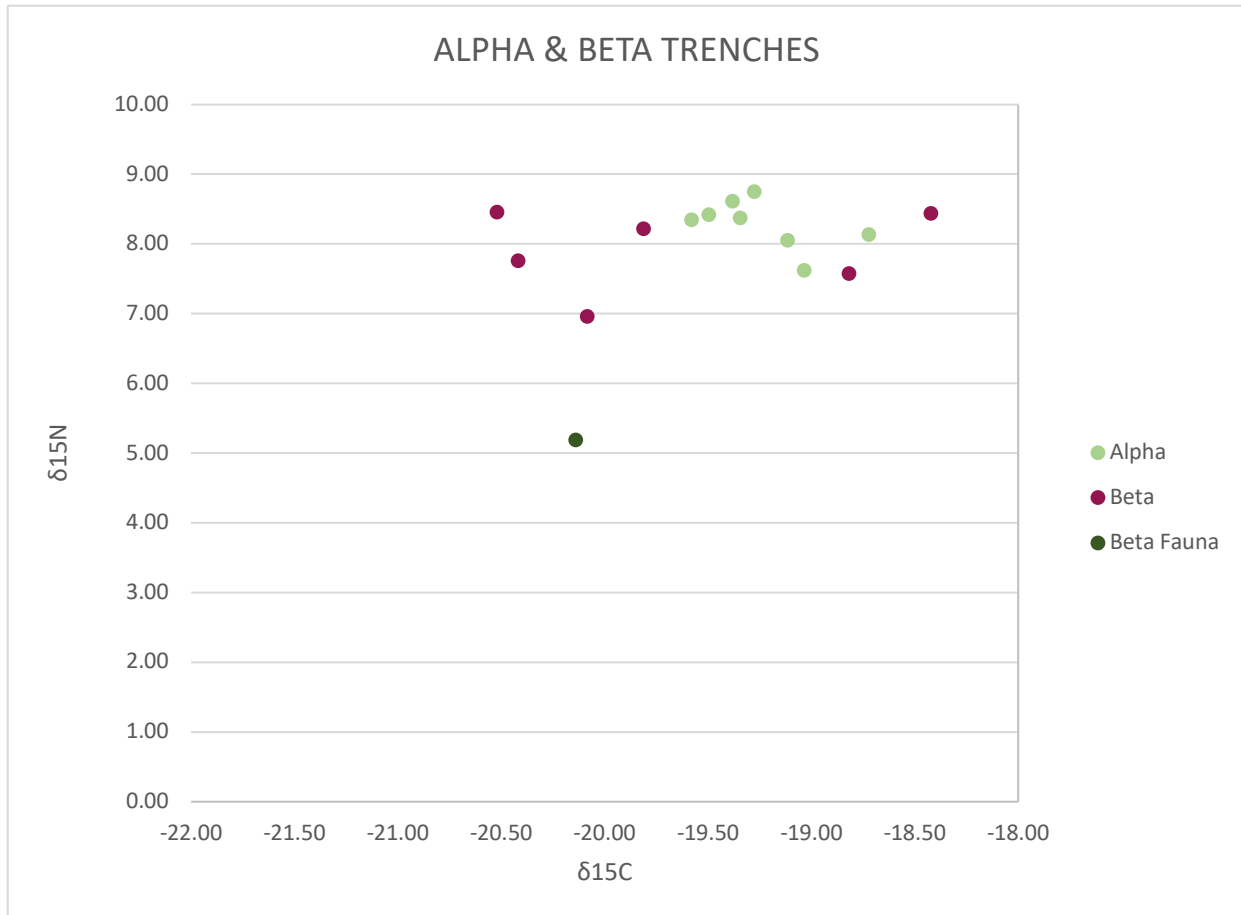
Table 5.3 Graph showing both Alpha and Beta trenches distribution of humans with the exception of the only animal found in these graves.

The individuals which were not sexed, NR, were three. These three individuals presented a mean of C -19.08‰ and N 7.94‰. They have a minimum of C -19.38‰ and N 7.58‰ and a maximum of C -18.82‰ and N 8.62‰. A range of C 0.56‰ to N 1.04‰ and a standard deviation of C 0.28‰ and N 0.58‰. Two of them were located in Trench Alpha, CSH-10 and CSH-15, and one of them in Trench Beta, CSH-8. The Trench Alpha individuals have higher C levels and lower N. They both belong to T.15 making them the last two craniums in the grave. Their levels are consistent with their grave partners although they both do present that point (CSH-10 N= 7.62‰ AND CSH-15 N=7.58‰) lower in N. Trench Beta individual belongs to T.27 and is placed in the middle of Trench Alpha and Trench Beta. CSH-8 seems to be related to the levels of two of the

three females found in Trench Alpha. These results and the positioning of the NR group could indicate that they belong to the C3 diet.

When learning about this analysis, I came across numerous articles and books that would verse me in work that I was performing. I found two which were of great aid as they were the ones closer to my area and time period. I studied "Stable isotopic reconstruction of dietary changes across Late Antiquity and the Middle Ages in Tuscany." From the Journal of Archaeological Science, written by Ricomi, G. Minozzi, S., Zech, J., Cantini, F., Giuffra, V., Roberts, P. on 2020. Also, 'Stable Isotope Evidence for the Consumption of Millet and other Plants in Bronze Age Italy." From the American Journal of Physical Anthropology, by Tafuri, M. A., Craig, O. E., & Canci, A., in 2009.

Riccomi's search was in the same time period as Castel Sozzio, Late Antiquity and Early Medieval, except it was located in Tuscany. Tafuri's search was Classical and Post Classical Times and the location was in Central Italy and the province of Viterbo. They both made comparisons between sites as well. Of course, since either of them overlapped in both crucial aspects of my research, I focused on understanding and using both papers as guides, but did not use them as set in stone references.

Riccomi's results for the comparison of $\delta13C$ and $\delta15N$ of human bone collagen between the Late Antique and the Medieval period where the following: "Late Antique urban necropolis of VM (n = 32) and the Medieval rural site of SG (n = 44) display a clear shift in isotopic values. Humans at VM have a range of −20.5‰ to −18.0‰ (mean = −19.6‰ ± 0.5) and $\delta15N$ range of between 6.8‰ and 12.0‰ (mean = 9.6‰ ± 1.1). By contrast, at the Medieval site of SG $\delta13C$ ranges from −19.9‰ to −14.6‰ (mean = −17.1‰ ± 1.1) and $\delta15N$ ranging between 9.0‰ to 12.2‰ (mean = 10.8‰ ± 0.7)" The conclusion based on the data obtained was that of a C3 diet with increased access to C4 resources. They suggest that the consumption of freshwater resources had significance during the Medieval Period. An interesting theory for this is fasting which is a common Church practice. This then places C3 plant diets leaning more towards Late Antiquity while the drift towards C4 plant diet happened starting in the Medieval.

Tafuri's human stable isotope values comparing the two sites were: "Human stable isotope values from LFR range between −20.5‰and −17.9‰ (mean−19.6‰ ± 0.5, n=32) for carbon and 6.7‰ and 2.1‰ (mean 10.0‰ ± 1.3) for nitrogen. At SLV, stable isotope values range between −20.0‰ and −18.6‰ (mean−19.4‰ ± 0.3, n=31) for carbon and 7.6‰ and 10.4‰ (mean 9.1‰ ± 0.7). The average isotopic difference between faunal and human remains is 1.1‰ for carbon and 5.6‰ for nitrogen at LFR and 1.5‰ for carbon and 5.1‰ for nitrogen at SLV." The final result is that of a C3 terrestrial environment.

Now, comparing these researches to mine (Stable isotopes from humans had a mean of C -19.43 and N 8.17‰ a range of C -20.52‰ to C-18.42‰ and N of N 6.96‰ to 8.75‰. A C/N ratio of 3.06 to -3.26.), Tafuri's more closely relates to the Late Antiquity diet of C3 plants and Riccomi's results to both Late Antiquity and Medieval.

Being able to find isotopic work that has been done close in time period and location, yet not exactly the same has been interesting to do. This work that has been uncovered in Castel Sozzio will serve by informing and aiding, just as the work above mentioned, has done for this research.

Conclusion

The findings of the stable C and N isotope study on human and animal bone collagen proves that the people from Castel Sozzio had a terrestrial diet as animal proteins and C3 plants were consumed. As we know, the location and route evidence, this gave the opportunity to consume freshwater animals as a lake and a river were in proximity, to import food and have a flux of people, have technology introduced into the city all the way from the Roman Period until the Early Middle Ages. The people of the city had access to both C3 and C4 diets throughout time, and they took care of their livestock by feeding them accordingly. With the aid of more studies like this, in depth chemical analysis, we will be able to discover new information. As well as with the ongoing campaigns, slowly but surely the past of Castel Sozzio will be known as its own history just as its neighboring cities which make part of the historically rich Viterbo.

References

Ambrose, S. H. (1990). Preparation and characterization of bone and tooth collagen for isotopic analysis. Journal of Archaeological Science, 17(4), 431–451.

Ambrose S.H. (1993), Isotopic analysis of palaeodiets: methodological and interpretive considerations, in: K. Sandford (ed.), Investigations of Ancient Human Tissues: Chemical Analyses in Anthropology, Gordon and Breach, Langhorne, PA, pp. 1-37

Ambrose SH. (2002). Controlled diet and climate experiments on nitrogen isotope ratios of rats. In: Ambrose SH and Katzenberg MA, editors. Biogeochemical Approaches to Paleodietary Analyses. NewYork: Plenum Press. pp. 243–259

Ambrose SH, DeNiro MJ. (1986) Reconstruction of African human diet using bone collagen carbon and nitrogen isotope ratios. Nature 319, pp. 321–324.

Borgia, E. La necropoli di Castel Sozzio, Civitella D'Agliano (Viterbo): note preliminari, in C. Valdambrini (ed.), "Una terra di mezzo. I Longobardi e la nascita della Toscana. Catalogo della mostra". pp. 435-439 (in print 2021)

Cagiano de Azevedo, M.; Schmiedt, E., (1974): Tra Bagnoregio e Ferento, Roma.

Cecchelli M.; Pompei M. C.; Riganati F.; (1997) Sviluppo turistico-culturale della Teverina Viterbese. Il sito archeologico di Castel Sozzio in La Gestione del Patrimonio Culturale, Atti del II colloquio internazionale sistemi di beni culturali e ambientali

Corsini A., (1985): "Gli insediamenti minori", in Mostra: `La Romanizzazione dell'Etruria', Firenze, p. 53-55.

DeNiro, M. J. (1985). Post mortem preservation and alteration of in vivo bone collagen

isotope ratios in relation to paleodietary reconstruction. Nature, 317(6040), 806–809.

DeNiro, M. J., & Schoeninger, M. J. (1983). Stable carbon and nitrogen isotopes of bone collagen: Variation within individuals, between sexes, and within populations raised on monotonous diets. J. Archaeol. Sci., 10(3), 199–203

De Niro, M, Epstein, S., DeNiro, M. J., & Epstein S. (1978). Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta, 42, 495–506.

DeNiro, M. J., & Epstein, S. (1978). Carbon isotopic evidence for different feeding patterns in two hyrax species occupying the same habitat. Science, 201(4359), 906–908.

DeNiro, M. J., & Epstein, S. (1981). Isotopic composition of cellulose from aquatic organisms. Geochimica et Cosmochimica Acta, 45(10), 1885–1894.

Dias, C. (2019) Stable Isotopes in Archaeology, Univesidade de Évora.

Fry, B. (1991): Stable isotope diagrams of freshwater food webs. Ecology 72, pp. 2293–2297.

Incitti, M., (1992): "La necropoli 'longobarda' della Selvicciola", in Papers of the Fourth Conference of Italian Archaeology (held in the University of London, January 1990), p. 213-217.

Katzenberg, M. A. (2008). Stable Isotope Analysis: A Tool for Studying Past Diet, Demography, and Life History. In Biological Anthropology of the Human Skeletonpp. 413–442.
La Pastina, F. (2020) La Necropoli di Castel Sozzio, Civitella D'Agliano popwerpoint presentation slides 5-7.

Longin R. (1971), New method of collagen extraction for radiocarbon dating, in "Nature" 230, pp. 241-242.

Pompei, M.C., 1994: "I territori di Bagnoregio, Lubriano, Civitella d'Agliano e Castiglione in Teverina nell'antichità: nuove acquisizioni e nuove prospettive", Biblioteca e Società, a. XIII, 1, p. 9-16.

Ricomi, G. Minozzi, S., Zech, J., Cantini, F., Giuffra, V., Roberts, P. (2020). Stable isotopic reconstruction of dietary changes across Late Antiquity and the Middle Ages in Tuscany. Journal of Archaeological Science: Reports, Volume 33, 2020, 102546, ISSN 2352-409X,

Spanu, M. (2021). Breve rassegna sull'occupazione della Teverina laziale in epoca romana. Orizzonti Rassegna di archeologia

Spanu, M. (2016). Ancient Topography in Southern Etruria: An Appraisal of Twenty Years of Research

Tafuri, M. A., Craig, O. E., & Canci, A. (2009). Stable Isotope evidence for the consumption of millet and other plants in bronze age Italy. American Journal of Physical Anthropology, 139(2), 146–153.

Van Klinken, G. J. (1999). Bone collagen quality indicators for palaeodietary andradiocarbon measurements. Journal of Archaeological Science, 26(6), 687–695.

Acknowledgments

I would like to thank Professor Alfredo Coppa, Professor Emanuela Borgia, Professor Donatella Magri, Professor Tafuri, Sara Bernardini, and Francesco La Pastina for teaching me, guiding me, including me and supporting me throughout this experiment. Working at Sapienza always felt welcoming, thank you all for your trust. I want to thank my mom and sister for supporting me 100% the past couple of years especially, I would have not experienced any of this if it weren't for them. My father, who is not here to see this but I know would be very proud of me. And lastly, I want to thank all my friends from back home who kept cheering me on and helping in any way that they could. And a super special thanks to my Archmatians, you guys made this crazy journey the absolute best. Carlitos, Agucha, Felipe, Carlos, and Abu thanks for all the love!