

Universidade de Évora - Instituto de Investigação e Formação Avançada Università degli Studi di Roma "La Sapienza" Aristotle University of Thessaloniki

Mestrado em Ciência dos Materiais Arqueológicos (ARCHMAT)

Dissertação

Investigating diet of Nogarole Camponi (Northern Italy) through carbon, nitrogen and sulfur isotope analysis of animal bone collagen

Ana Davitashvili

Orientador(es) | Mary Anne Tafuri Silvia Soncin

Évora 2023



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Abstract

The archaeological site of Nogarole Camponi is located in Povegliano, south of the city of Verona, Italy. The site consists of a small settlement dated to the beginning of the Middle Bronze Age (2300 – 950 century BCE) and it is associated with the so-called Terramare culture. In this period, intensive agriculture, husbandry, and pastoralism became central in the subsistence of people living in northern Italy (from the southern slopes of the Alps to the central-eastern Po valley). In particular, the exploitation of secondary products (e.g., milk, wool) became predominant over primary products (e.g., meat), leading to important economic and social changes.

Stable isotope analysis of carbon, nitrogen, and sulfur of human and animal bone collagen has been applied in the last decades to a variety of archaeological contexts for the reconstruction of past dietary practices. The working principle consists in tracing back the isotopic signal of the specimen analyzed to the carbon, nitrogen, and sulfur cycles in nature, which allows discriminating between C_3 and C_4 plants, as well as producers from consumers and aquatic from terrestrial environments.

Recently, scholars have been building up the evidence for diet in Bronze Age northern Italy using stable isotope analysis, with a focus on humans. For example, the introduction and the role of C_4 plants (millets) have been outlined in some of these communities. However, the animal assemblage has a marginal role in these studies, used merely as a proxy for the baseline signal.

Here we add new evidence for the animal diet in Bronze Age northern Italy by analyzing cattle, sheep, goat, pig and dog remains from Nogarole Camponi, coupling the new data with those previously published and available in the literature. By doing so, we provide a reconstruction of the animal diet and link it back to the environment where they were raised, discussing husbandry practices and consequently, the human diet and economy.

Furthermore we present here a database of zooarchaeological isotopic data, which might contribute to better understanding patterns of consumption and environmental background in Bronze Age Italy.

By studying the diet of domestic animals from Nogarole Camponi, together with that from other sites of northern Italy, we explore the relationship between humans, the animals and the environment in an important period of major social changes.

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Chapter 1: Introduction

The Bronze Age period in Italy is the time of earliest urbanization, where small villages increase in size and number of occupants. Here, the domestication of animals became the main activity, with animals used not only for eating but also for secondary products such as wool, and dairy (Sherratt, A. 1981). Hunting was progressively abandoned, and people started harvesting and relying on specialized production of pottery (with the introduction of the wheel) and weapons (with the introduction of metallurgy) (Kirleis et al. 2022). This is also a period when new crops, such as millets, are introduced and exploited. Paleodietary analysis, such as stable isotope, and archaeobotany, are used by scientists to reconstruct domesticated plants in prehistory (Holloway, Lukesh, 2015).

Paleodietary analysis is a study of the diet of individuals in the past. It is a broad terminology and includes archaeology, zooarchaeology, archaeobotany, food residue analysis, and stable isotopic analysis. In fact, it is not only the study of past food consumption but also the study of interrelationships between animals and humans, ecology, culture, adaptation of crops, and animal husbandry (Pate, 1994). The best tool for studying the paleodiet of animals is stable isotopic analysis. Starting from the 1970s oxygen isotope analysis was used to better understand the climate and environment of the past. Nowadays other than oxygen, also carbon, nitrogen, sulfur and even strontium are used for the purpose of identifying the tropic levels, for studying the migrations of the past living creatures and their plant-based diets (DeNiro and Epstein, 1978).

Stable isotope analysis has become increasingly important for scientists who study animal husbandry, diet, and mobility.

The research presented here will rely on zooarchaeological data from an archaeological site Nogarole Camponi, near Verona, northern Italy. Zooarchaeology is a term that describes the study of faunal remains from the archaeological site to better understand the overall sociological and living conditions and patterns of civilizations from the past (Davis, 1987). It uses the qualitative osteological method, which includes the collection of taxonomic and bone modification data (Birch, 2013). The main aim of zooarchaeology is to identify species, sex, age, ecology, diet, mobility, taxonomic relationships, human choice, and actions related to animal use or secondary products (O'Connor, 2000). In our case, animal paleodietary analysis, which was carried out through stable isotopic investigation, is crucial for understanding the life of the Bronze Age settlements on the territory of Italy.

One of the main objectives of this research is to show the significant role of studying faunal remains through isotopic analysis to reconstruct past life. This research will lay out the main advantages of zooarchaeology as an independent science for examination of past diets, mobility, relationships between humans and animals, cultural events, and the environmental conditions of past. Furthermore, will help to understand the importance of stable isotopes analysis in today's archaeology and its importance as a tool for studying societies and prehistoric life.

Another important goal of this study is to better comprehend the lifestyle Bronze Age Italy population. As an author of this thesis, I can say that working with the materials supplied by Nogarole Camponi was enthralling because of the allure of the civilization that lived in this place. Furthermore, study of Nogarole Camponi is still ongoing and it encompasses several fields of research. This prompted me to accept the challenge and submit my research as an additional study to better understand this Bronze Age settlement, as to provide more analytical data to the scientific field and study of Bronze Age Italy.

Finally, because of its usefulness in future studies, creating the isotopic database of zooarchaeological remains from northern Italy Bronze age, should be considered as one of the

major aims of this study. The database presented in this study is built on prior research, and it includes archaeological sites from all around Italy. It includes data of geographical coordinates, locales, wild and domesticated animals, and isotope ratios of bone collagen. This database is the first attempt to summarize zooarchaeological isotopic data of Bronze Age Italy territory, in a single system.

As mentioned above, the research question of the study is to find out what the main diet of animals from Nogarole Camponi in the bronze age was, what was differences and similarities between different archaeological sites, how the database contributes to the interpretations of this study, if the consumption millet is confirmed for this region and how should this study help in future researches to reconstruct the paleodiet of the Bronze Age communities of Italy.

Chapter 2: Literature review

2.1 Zooarchaeology and the study of animals in the past

Zooarchaeology is study of faunal remains, which requires knowledge in zoology and paleontology (Davis, 1987). Nowadays the studies of faunal remains, which were very rare in the past, are increasingly more accessible. Such studies use qualitative osteological methods, which cover the collection of taxonomic data and bone modification (Kalanji, 2020). Among the aims of zooarchaeology is the identification of species, sex, and age, and the study of ancient ecological phenomena, economics, diet, taxonomic relationships, health status, human choices, and actions related to the use of animals as food and as for secondary use such as milk, wool or other purposes. Additionally, it helps to inform us about the ecological and socio-economical roles of animals in the past and reconstruct paleoethology, paleobiogeography, site formation processes, and religious and ritual patterns of population (Birch, 2013).

One of the first clear references to zooarchaeology was by Lubbock, in 1865. He mentioned the zooarchaeologists Steenstrup and Rutimeyer, who studied faunal remains. This fact influenced American zooarchaeology through Morlot and Wyman. Also, it was Lyman's idea to propose the term zooarchaeology as studies of paleoenvironmental conditions. A slightly different term - "Archaeozoology" was used on the territories of Eurasia and Africa. This term mostly emphasized the biological nature of studying animal bones (Giffond-Gonzalez, 2018).

Ultimately, animal remains can be studied via stable isotopes, DNA, and taxonomic analysis. Furthermore, studying animals gives us knowledge about site formation processes and animal consumption in religious and ritual practices (Birch, 2013). Studying animals has a unique advantage as it gives us the opportunity to study not only the environment of the past, but also, helps us to look into the social life of humans, by studying animals, we identify the religious features of societies, their diet and the meaning of animals for human beings. Animal remains, especially fossils, have been attractive to the human mind for centuries. From the 1700s, zooarchaeology relied on the combination of different kinds of disciplines for methods and explanations (Reitz, Wing, 1999). Biological research has heavily relied on faunal remains, exploration, and changes in the zooarchaeological distributions, morphology, population structure, paleoenvironment, and ecological conditions. According to this, many of these topics can be studied without references to humans, while faunal remnants are still very important tools for researching them. Additionally, a lot of research focuses on the domestication of animals from an agricultural point of view (J.M. Davis, 1999). However, the study of animals to understand their role in developing different complex cultures is undoubtedly an important characteristic for different time periods in the history of archaeology. Furthermore, different fields, like paleontology, ecology, biological anthropology, geology, etc. bring different perspectives to Zooarchaeology. According to Terry O'Conner, studying domestication of animals can provide different kinds of information. First and foremost, this elucidates human behavioral cooperation (O'Connor, 2000). This can be explored through the analysis of modifications in the shape and size of animal bones over time, as well as through an examination of shifts in their distribution. Conversely, it can be investigated as a phenomenon within human societies, offering valuable insights for ethnographic and cultural studies. Lastly, the field of zooarchaeology enables researchers to extend the temporal scope for observing interactions between human and animal populations. This affords a unique opportunity to investigate animal bones with direct contemporary relevance (Reitz & Wing, 1999).

One more important aspect of zooarchaeology is butchering. Paleolithic butchering evidence refers to the cut marks left by contact with stone tools. The butchering traces sometimes are mixed with natural cut marks, usually caused by the environment where the animal was living. For example, some samples from the cave sediments may have accidentally had contact with the stones. Other than location and orientation, the frequency of the marks is also an important aspect for consideration. The reasons for the butchering could be different. One can be the use of bones as a tool, or it can also be related to the consumption of marrow, which is inside the bones. So, the trace of butchering can tell us about its history by itself (Charles, 1995).

2.2 Bone structure and description

Bone is a connective, living, and growing tissue, with cells and blood supply. It is an essential mechanical component of the musculoskeletal system (O'Connor, 2000). Additionally, it derives information from many different levels such as isotopic, molecular, biochemical, and structural. Bones serve to protect and support soft tissues. They are made of a composite material, mostly in the form of proteins, around 90% of which is collagen, the rest of them are no collagenous proteins, like hormones (osteocalcin), filler proteins (proteoglycans), and lipids. The inorganic or mineral phase is formulated with crystals of hydroxyapatite (Ca10 (PO4)6OH2). The crystals of this mineral are calcium and phosphate salts (Pate, 1989). Calcium carbonate, magnesium ions, sodium ions, citrates, and trace amounts of potassium, chlorine, and fluorine ions are also present. These inorganic substances are bound to a matrix and give bones their hardness, rigidity, and fragility. Chemical information about the bone is crucial for understanding the diet of the individual. In some cases, chemical concentrations in bone reflect dietary concentrations. For example, nitrogen is only found in the organic portion of the bone, while carbon is located on the surface of the crystals. The chemical analysis of the bones gives us information about the food that was consumed, the diversity of the ecosystem, was it terrestrial or marine, about geological environments, and trophic levels (Pate, 1994). If we look into the gross anatomy of bones, all of them have two main structural components: compact and spongy bones. Compact bones make up the shafts of the bones in birds and mammals. The structure of the bone is simplest and concentrically about the longitudinal axis of the bone, filled by large and small channels. Osteocytes are responsible for the secretion and remodeling of the bone, they can be found in the lacunae. They are interconnecting with the branching and interdigitating of canaliculi. Finally, the bone system is connected to blood

vessels. With this, everything is transported through the body and bones are noticeably porous. In some mammals and dinosaurs, the remodeling of compact bone due to making secondary osteons is noticed. These kind of bones are sometimes called Haversian bones.

Cancellous bone, which is also called spongy bone, is at the two ends of compact bone in the limb part. Basically, ribs and shoulder blades consist of a thin layer of compact bone, and thickness is made up of spongy bone (Davis, 2002). (Figure 1)

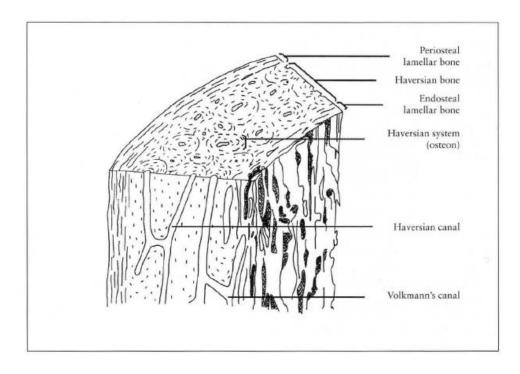


Figure 1: Microstructure of compact bone. (Edited from O'Conner, 2000).

One of the important aspects of bone is diagenesis. Bone diagenesis is the study of the processes that change the nature of bones after burial. Additionally, it explores how these processes are environmentally determined (O'Conner, 2000). There are numerous processes that occur to the bone under the ground. This can be circulation of organic matter, exchange of ions, breakdown of collagen, microbiological attack, etc. The reason for diagenesis is the nature of the bone, such as the porosity matrix of collagen and hydroxyapatite. This is because collagen is very easily hydrolyzed in an acidic environment and then degraded by microorganisms such as fungi or different kinds of bacteria. Protein and mineral bonds can be destroyed by bacteria as well (Hedges, 2002).

2.3 Macronutrients (carbon, protein, lipids)

While speaking about bones, for a better understanding of stable isotopic analysis, it is important to underline some aspects of macronutrients. Macronutrients are nutrients that every organism needs in order to provide energy for their daily activities. They are carbohydrates, proteins, and lipids (Shenkin, 2005).

Proteins are complex substances of both animals and plants. They are made up of carbon, hydrogen, oxygen, sulfur and nitrogen. They consist of long-chain peptides composed of amino acids, which are linked to each other by peptide bonds. Proteins are integrated with the cell nucleus via DNA, where the information is stored. This information is read by RNA and transported to the cytoplasm. Amino acids are commonly classified as "essential" and "non-essential". Essential amino acids cannot be produced by the body and need to be absorbed from the diet. Proteins are very important due to their functions. Except for carrier cells, amino acids are sometimes used for proteins and catalysts that speed up chemical reactions. As already mentioned, they are absorbed by the transport system into the intestinal tract, but they are not stored in the body. That is why when low protein consumption occurs, there is a danger of the breakdown of existing nitrogen in tissue, which is quite important for paleodietary analysis (Jim et. al. 2006).

Carbohydrates are biomolecules formed by photosynthesis in plants. They consist of carbon, hydrogen, and oxygen (Jim et al. 2006). Simple carbohydrates are known as sugars, while complex carbohydrates are starches and cellulose. They are considered as a source of fuel for the body and protein savers because they are used for energy instead of proteins. One of the simplest sugars is glucose, which is involved in several metabolic pathways. One of the most

important is glycogenesis, the storage of polysaccharides. They are stored in muscle and liver. So, to say it simply carbohydrates are one of the main sources of energy (White, 2012).

Lipids are composed of carbon, hydrogen, and oxygen. Chemically they are esters of glycerin and fatty acids. Fats have triacylglycerol because the glycerin molecule has three hydroxyl groups where the fatty acids are attached. Fats can be animal or plant based. Mostly, animal fats are unsaturated while plant-based are saturated (Jim et al. 2006). Fatty acid components can be identified in three ways – by their chain length, number and position of double bonds, and position of fatty acids within the glyceride molecule. After the consumption of fats, they are separated by gastrointestinal acids and enzymes from the pancreas. Then the lipids are broken down into free fatty acids, which are then absorbed and transported by chylomicrons, a lipoprotein transport particle. After this, they become dietary fats, which are fats that the body needs (Miller, 2020).

2.4 Paleodietary studies via stable isotope analysis

A paleodietary analysis is the study of past diets, which is mainly done by stable isotopic analysis. In fact, it also includes the study of archaeobotany, zooarchaeology, food residue analysis, and human bioarchaeology (Katzenberg, 2009). All together, these disciplines give us complementary information about past societies, in particular information about what people were eating in the past, how they adopted new crops, and what was their economy like. Indeed, the diet of past people is very connected to their everyday activities and experiences. It is a bridge between humans, fauna, and flora interactions (Pate, 1994). The basic premise in investigating paleodiet is "you are what you eat" (Schwarcz 1991; Parkington 1991; Beehr and Ambrose 2007). Paleodietary studies have variegated research outputs. It gave us diverse possibilities to study environmental archaeology, ancient societies, cultural adaptations, cultural beliefs, or in general everyday life of past populations. Additionally, dietary adaptations have featured some explanations of why some hominids are extinct, gave us answers to what their mobility was like and what was the migration processes of prehistoric human and animal beings. Additionally, understanding the diet of infants and the duration of nursing gives us the information about demographic variables and population growth (Katzenberg, 2008). Also, it apprises the transition from hunting/gathering to farming/breeding. Dietary patterns also play a crucial role in understanding how societies are developed and how they are structured (Guiry, Eric. 2015).

Stable isotope analysis remains the most efficient method of studying past people's diet. This method has the ability to measure dietary differences between or within human or animal populations and gives us information about what specific types of diets existed in the past. As an example, measuring the proportion of C₃ versus C₄ diets in the population is essential to productively investigate dietary dependence on different kinds of plants (Johnston, 2009).

2.5 Stable Isotopes

The first stable isotopes were discovered in 1913 by J.J. Thompson. By 1930 all stable isotopes were found, and scientists started experimenting with mass spectrometry. Stable isotope analysis was first applied to study ancient dietary practices in the late 70s and the pioneers were DeNiro and Epstein (1978). They studied the influence of animal diets with controlled feeding, using carbon and nitrogen-stable isotopes. After that, Van Der Merwe, mostly interested in photosynthesis, applied carbon isotope analysis to explore some archaeological questions (van der Merwe and Vogel 1978). In the last 15 years, the use of stable isotopes became more frequent, mostly thanks to reduced costs and analytical advancement (Katzenberg, 2009).

Isotopes are atoms where the number of protons is the same in the atomic nucleus, but the number of neutrons is different (Pate, 1984). So, they are the same in elemental identity but

different in mass number. In chemical properties, isotopes have the same assets while they have the same electron numbers. Physically, the density and vibrational energy of the nucleus is controlled by the mass number. This, in turn, affects the bond strength, which gives rise to differences between the isotopes. Stable isotopes do not decay into other elements, while unstable isotopes are radioactive and they do (A.W. Froehle, 2012). For example, stable isotopes of carbon are ¹³C and ¹²C while ¹⁴C is unstable, radioactive, and can decay in ¹⁴Nitrogen. Among the main elements that make up human, animal and vegetal tissues, carbon, nitrogen and sulfur are the ones that are mainly studied in archaeology, mostly for their abundance. The natural abundance is 98.89% for ¹²C and 1.1% for ¹³C. In the case of Nitrogen, the stable ones are ¹⁴N and ¹⁵N, with a natural abundance of 99.63% for ¹⁴N and 0.37% for ¹⁵N. Sulfur has 4 stable isotopes: ³²S (95.02%), ³³S (0.75%), ³⁴S (4.21%), and ³⁶S (0.02%) (Katzenberg, 2009).

A great part of the isotopic studies focuses on the reconstruction of past diets using collagen, as collagen is usually well preserved in archaeological contexts (Katzenberg, 2009) and is the most abundant protein in bones (Pate, 1994). Collagen however degrades over time under specific conditions. For example, collagen is usually badly preserved in hot and humid environments (Ambrose 1989). For stable isotope analysis, the preservation of collagen needs to be assessed using some quality parameters, specifically, it is relative content (collagen yield) and that of carbon and nitrogen (%C and %N) and the ratio between the two, expressed as (%C/%N)*(14/12) (Ambrose, DeNiro, 1986). Being composed of amino acids, the stable isotope composition of collagen mainly reflects the protein component of the diet. Scientists however also use other tissues for isotopic investigations (Ambrose, 1990). The stable isotope analysis of bone and tooth apatite, for example, are useful to trace back all components of the diet, specifically carbohydrates, lipids, and proteins, since apatite is formed from dissolved carbonates (HCO₃⁻) in the bloodstream, which derive from all the macronutrients ingested (Ambrose, 1989).

2.5.1 Carbon Stable Isotopes

Carbon is an element in periodic system, which has some isotopes. They can be stable and unstable. Unstable carbon isotopes are used for a dating method called radiocarbon dating, while stable carbon isotopes help scientists to determine diet of the individuals. (Leatherdale, 2014).

Different plants metabolize carbon differently from atmospheric carbon, in the form of carbon dioxide, in the process of photosynthesis. These plants are then eaten by animals or humans and by determining the isotopic composition of the animal or human tissue, it is possible to link the signal back to that of the plant ingested (Ambrose, Norr, 1993). Photosynthesis is a process used by plants to convert sunlight, carbon dioxide, and water into sugars, which are used as fuel by them. In this process, they are using the primary photosynthetic enzyme Rubisco. Three different types of photosynthetic pathways exist C₃ (Calvin-Benson), C₄ (Hatch-Slack), and CAM (Crassulacean Acid Metabolism). Stable isotope values of plants differ depending on their photosynthetic pathway (Nikolaas J. van der Merwe, 1982).

Most plants are C₃ plants, meaning that the first carbon compound produced contains three carbon atoms. During the process of photosynthesis carbon dioxide enters through the stomata and by the enzyme rubisco, it is converted into sugars. These plants are for example rice, wheat, soybean, cassava, and most of the trees. They are adaptive to cold environments and grow in either wet or dry environmental conditions. The average isotopic measurement values for C₃ plants are -35 to -20 ‰ (Deines, 1980). Or -34 to -24‰ (DeNiro et. al 1978).

 C_3 plants are losing water in the process of photosynthesis, but they do not need photosynthetic adaptation to reduce photorespiration (Walker, Berkeley, 2016). The method of photosynthesis of C_4 plants helps to reduce the water loss in hot and dry environments. In this case, 4 carbon compound is produced and carbon dioxide is localized in bundle sheath cells. The leaf anatomy allows carbon dioxide molecules to go directly to Rubisco, without any contact with oxygen. This helps plants to keep water and continue fixing carbon while the stomata are closed at the same time. C₄ plants are not many. They are maize, millet, crabgrass, corn, and sugarcane. They grow comfortably in sunny and hot climatic zones. Their isotopic value is between -14 to -9 per mil (DeNiro et. al. 1978). From the experiments, it is very clear that large and wild mammals have larger ¹³C values than small animals (Ambrose SH, Norr L. 1993). (Figure 2)

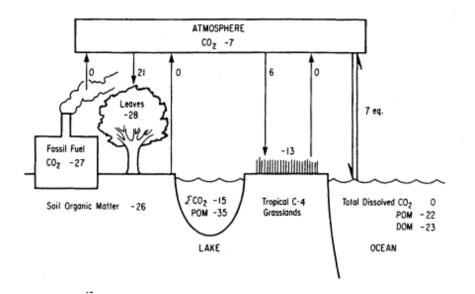


Figure 2: Carbon cycle Distribution in the Ecosystem. (Edited from Pate 1994).

2.5.2 Nitrogen Stable Isotopes

Nitrogen stable isotope analysis is another essential method for scientists to study paleodiet. Nitrogen has two stable isotopes: ¹⁴N and ¹⁵N. Mostly this method is used to determine the trophic level (herbivores/carnivores) of animals and to differentiate marine versus terrestrial source consumption presented in the bone collagen (Schoeninger et al., 1983; DeNiro, 1987). This method is also a good way to identify the habitat preference in the same environment. However, while studying diet through nitrogen isotopes, some limitations should be acknowledged. One of the most frequent limitations is related to climatic factors (Ambrose, 1989). Water plays a big role in nitrogen values, for example, forest-dwelling herbivores have the lowest nitrogen value, while savanna-dwelling individuals who preserve water and expel a big amount of urine have a higher value of nitrogen. This means that nitrogen isotopic composition relies on physiological adaptation to water (Heaton et al. 1986).

Nitrogen which is in the biosphere is presented as N₂ and exists as a gas in the atmosphere. N₂ is a primary standard, which means that isotopic values are relative to its value, which is 0‰. On the other hand, the value of dissolved N_2 in the ocean is +1.0 ∞ . There are some differences between nitrogen fixing and non-fixing plants. Legumes, which are fixing plants are closer to atmospheric nitrogen, while non-fixing plants are enriched in $\delta^{15}N$ (Virginia, 1982). There are different ways plants derive their nitrogen. For example, Nitrogen-fixing plants are ones whose roots are captured by bacteria, which extract nitrogen from the air and convert it for metabolic function, which helps these plants in growing. They are beans, lentils, soybeans, peanuts, and chickpeas. Non-fixing plants obtain their nitrogen by the decomposition of organic matter in the soil as ammonium (NH₄⁺), nitrite (NO₂⁻), or nitrate (NO₃) (Schoeninger & DeNiro et al. 1984). They depend on the soil conditions and environment. Examples of non-legumes plants are corn, wheat, tomato, and grasses. Fixing plants are closer to atmospheric nitrogen, while non-fixing ones are enriched in ¹⁵N. As isotopic ratios are different for each environmental condition, terrestrial fixing plant's isotopic ratios are from -2 to +2‰, while non-fixing plants' ratios are from 0 to +6% (by Pate 1994). Nitrogen is also useful in determining if the diet was based on plants or animal products. It was estimated that there is a trophic level enrichment of about 3‰ at each step of the trophic web. The mean of δ 15N decreased, as diet δ 15N increased (Alexander et. all 2015). The size of food chain is directly linked to the increase of nitrogen value. For example, the nitrogen value is higher in the sea creatures than in terrestrial ones as the food chain that functions within the marine environment is larger than the one that exists on the land.

As a result, by analyzing the δ 15N levels of diverse creatures within an ecosystem, it may be feasible to deduce a portion of the ecosystem's trophic structure (Kling et al. 1992). However, Ecological studies suggest that mammals, fish, birds, reptiles, and insects all have similar enrichments (Caut et al. 2009). (Figure 3)

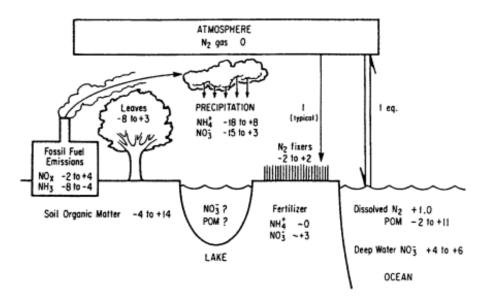


Figure 3: Nitrogen distribution in Ecosystem. (Edited from Pate, 1994).

2.5.3 Sulfur Stable Isotopes

The measurement of sulfur stable isotope is increasingly used for the reconstruction of dietary, ecological, and mobility patterns. Sulfur plays a crucial role in the protein structure of organisms (Nehlich, 2015). Mostly, it is used to investigate ecological relationships in different environments (e.g., Krouse and Grinenko 1991).

Sulfur is the 15th most abundant element on earth, and it ranks 7th in the organic human body, which is why it plays an important role in protein structure (Ingenbleek, 2006). Its reservoirs are localized in oceanic dissolved sulfates in the hydrosphere and evaporitic sulfates, and pyrite in the geosphere (Newton and Bottrell, 2007). Minor reservoirs include the atmosphere,

biomass, and soil. There are 4 stable isotopes of sulfur: ${}^{32}S$, ${}^{33}S$, ${}^{34}S$, and ${}^{36}S$, but the most abundant is the lightest isotope - ${}^{32}S$, with and abundance of 95.02%, followed by ${}^{34}S$ (4.21%), ${}^{33}S$ (0.75%), and ${}^{36}S$ (0.02%) (Faure, 1986).

Abundance of sulfur in organic tissue is lower than for example of carbon or nitrogen. Sulfur is mostly found in the amino acids cysteine and methionine and only a minor amount is available in the tissues and animal species (Nehlich, 2015). Additionally, the sulfur abundance is higher in muscle tissue than in bones (Eastoe, 1967).

The most abundant use of sulfur stable isotope in archaeological remains are bones and in special cases also hair, nails, and soft tissue (Hedges et al. 2007). Furthermore, sulfur isotopes in bone collagen, as other elements, represent a long-term dietary average over decay. Sulfur isotope values illustrate dietary protein rather than the whole diet. Due to this, this method is interdisciplinary and investigates the consumption of protein from different environments.

An experiment, which was conducted in England at the beginning of the 21st century, has shown that animals had highly depleted ³⁴S value as a result of pollution (Nehlich et al., 2011). Therefore, sulfur stable isotopes should be carefully evaluated considering environmental and geochemical circumstances. The certainty of sulfur isotopic analysis is influenced by the climate and the environment, however, due to multiple reason, the exact range of this is hardly identifiable, and therefore considering this influence in the analysis itself remains a difficult task. (Drucker et al., 2011, 2012).

In order for us to be able to understand the importance of sulfur in the diet, it's necessary that, the food products that were used by the animals, or humans for that matter, are rich in it, otherwise, the chance of sulfur being detected with the isotope analysis is low and we will not have the sufficient data to make any interpretations regarding its role in the food ration. So, for each environment, the ratios of sulfur will be different (Rend, Nehlich, 2018). According to a case study on the territory of Russia and Ukraine, (Privat et al. 2007), the organisms of freshwater lakes had similar sulfur isotopic value as terrestrial ones. Hence, it is often problematic to distinguish between terrestrial and freshwater consumers.

The study of the relationship between consumer and diet indicates a +0.5% increase in sulfur isotopic values (Nehlich, 2014). (Figure 4)

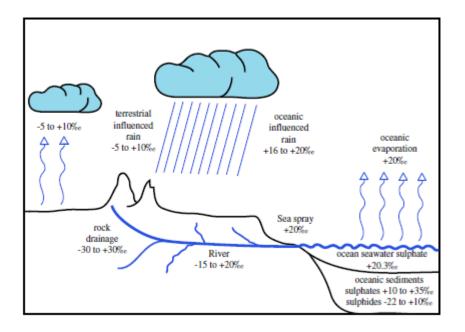


Figure 4: Sulfur isotope cycle in the environment. (Edited from Nehlich, 2015).

2.6 Stable isotope analysis of animal remains

While many articles are focused on analyzing human remains using stable isotopes, studying animals is also crucial for understanding prehistory on its own, as written sources are not available for that time and often preservation issues are also limiting. The application of stable isotopes gives us insight into the diversity of plants, their use, and their cultivation. Faunal results would reveal more details about the original population of animals exploited by humans. Furthermore, studying animal bones helps us to test the hypothesis about diverse aspects of human history (Connor, 2000). The early isotopic studies on animals started with oxygen to reconstruct the season of exploitation and season of site use. Around the 1980s, scientists started to analyze trophic levels and differences between marine and terrestrial environments through carbon and nitrogen isotopes of animal bone collagen (GiffordGonzalez, 2018). Besides climate and dietary factors, stable isotopes such as strontium are an applicable method to study mobility in animals, which is important to observe site formations, seasonal uses, and migration processes. One of the most important parts of discussing some topics with stable isotopes of animals is to have an integrated knowledge of ecology, physiology, and osteology in order to interpret isotopic data (O'Cooner, 2000). Also, the knowledge of zooarchaeology is important for sample selection and strategy. For a better understanding of the stable isotope analysis of animal remains, a case study will be discussed.

The publication about the diet of sled dogs with carbon and nitrogen analysis on dog bone and dentin, written by Harris and his colleagues shows the significance of stable isotope analysis (Harris et al. 2020). Sled dogs were a very fundamental part of the Labrador Inuit life. With their responsibilities to pull sleds and assist people, they needed a special diet with lots of protein and fat. The main objective of this study was to determine geographic variations between the south and north coast in the type of foods fed to dogs. An additional research question was - what kind of contribution can a diet of dogs have in zooarchaeological and archaeological evidence of human activities on the site? (Longing, 1971). Results showed that the main food ration was marine mammal protein, but codfish was not excluded because of the similarities between isotopic composition of codfish and marine mammals. The regional variation took place in the dog diets, as they probably used different geographic areas. Researchers think that this place may have been a temporary location for warm months when they were not working and were just eating caribou and fish. With the carbon and nitrogen values, respectively -14.2‰ and +16.7‰, they received half of their protein from marine mammals. The interesting part in comparison of the locations was that all dogs were eating marine mammals while dogs from the north coast were eating other mammals too, such as caribou and walrus. To the south diets of humans and dogs are closer. The main protein was the ringed seal. But researchers think that just ringed seal was not enough and probably they also were consumers of European goods such as salt pork. With this study, stable carbon and nitrogen analysis helped researchers to analyze the diet of sled dogs on Labrador Island. And

making the comparison between north and south coasts helped in understanding seasonal changes, geographical variations, food rations, and lifestyle (Harris et. al 2020).

Stable isotope analysis has been successfully used to understand domestication processes, such as in the case of the analysis of goat remains in the Near East (Makarewicz, Tuross, 2012), which used carbon, nitrogen, and oxygen stable isotope analysis of bone collagen. These isotopes in biogenic tissues reflect environmental and dietary inputs. Flora has represented C₃ plants around -26.0‰ and C₄ plants around 12.5‰. In these regions (southern Levant) both photosynthetic pathway plants are distributed in diverse proportions. Environmental and dietary information were also recorded by nitrogen isotopic analysis. Researchers also added oxygen analysis for a better understanding of hydrology, aridity, and the origin of food sources. The main intention of the study was to make a comparison between goat and gazelle diets, which is the key for understanding the transition from hunting to breeding. The result showed that there was a difference in feeding patterns. In comparison to domesticated goats, the isotopic variation of gazelles was much wider and the collagen greater. This study is about one of the earliest animal husbandry practices dating approximately to 8000 BCE, the period when feeding animals occurred in tandem with hunting in the region (Makarewicz, Tuross, 2012).

The isotopic studies are methods which can be used by archaeologists to document domestication processes and diverse approaches used by the first herders. The link between animal husbandry and previous practices with transformations in economic and cultural systems. It is also a favorable method to observe and study not only animal husbandry issues but also animal mobility, food ratio, site formation, or ecological and other cultural factors.

2.7 Background history of Bronze Age Italy

The Bronze Age period in Italy is the time of earliest urbanization, where small villages got bigger. Here domestication of animals became the main activity of the people. They started using animals not only for eating but for secondary products such as wool, and dairy. Hunting became more and more abandoned and people started harvesting and creating metallurgy (Kirleis et al. 2022). This is also a period when new crops are discovered, for example, millet. Through paleo-dietary analysis, such as stable isotope analysis, and archaeobotany, scientists tried to reconstruct the prehistoric domesticated plants. Generally speaking, Italy's territory through Bronze Age is divided into three regions: Po Valley and alpine valleys which is the northern part, the central part, and the southern part (Holloway, Lukesh, 2015).

In southern Italy the Bronze Age is characterized by the so-called Laterza culture, which sees the appearance of the sites such as Toppo Daguzzo (PZ), Coppa Navigata (FG), Tufariello (BN). Often sites showed the presence of defensive stone-built walls. By contrast, in Sicily, villages were totallynon-specific. Here rich tombs are very rare (Holloway, Lukesh, 2015). Additionally, it seems that they started cultivating fruit trees such as olives. Their pastoral economy is mainly based on sheep. Also, cattle, pigs, and dogs played important roles in their everyday life. Some of the sites mentioned, for example Toppo Daguzzo, starting from the Middle Bronze Age show the emergence of elite groups (Del Corso et al. 2022).

Central Territory of Italy in the Bronze Age is described with the Montemerano-Scoglietto-Palidoro culture. The economy relies on pastoralism which shows grazing camps both on the coast and the uplands. Settlements are presented in defended sites and caves. Social differentiation is shown in the example of the tomb omba della Vedova (Tomb of the Widow), at Ponte San Pietro, where the warrior was found with his sacrificed wife, bronze-age tools, and a dog (Dolfini, 2020).

In Northern Italy, the Bronze Age starts with the Polada culture, which was characterized by wetland settlements, and continued with the Terramare culture. It entailed moated and banked villages located in the alluvial plain of Po Valley. This is a key area for understanding the prehistory of Europe (Holloway H. et. al 1978).

2.7.1 Nogarole Camponi

The archaeological site of Nogarole Camponi, the object of this work, is located near Mozzecane, 30 km from Povegliano, south of Verona. (Figure 5) The settlement is very small with a diameter of 70m, and inhabited by approximately a hundred people (Salzani, 1991). In prehistoric times these territories were covered by forests, which created humid temperatures and climate. Archaeological surveys in the area were at first supervised by L. Salzani, from 1991 and as of now the research at the site is still ongoing under the supervision of P. Salzani of the SABAP of Veneto. The site yielded trace of human occupation, mostly characterized by the presence of several burials with mixed ritual (inhumation and cremation), together with the presence of fauna, which is dated with early Bronze Age period.

The fauna of Nogarole Camponi is presented by domesticated animals such as cattle, pigs, sheep, and goats and wild animals such as red and roe deer, hare, and fox. Domesticated animals played a huge role in the everyday life of Bronze Age people, although hunting is also attested, it was likely connected to the protection of the fields (Salzani, 1992). Sheep were abundant due to the type of environment of the archaeological site. They were most likely used for wool production, which was one of the essential added values to the economy. Pigs were used mostly for meat products (Salzani, 1992).

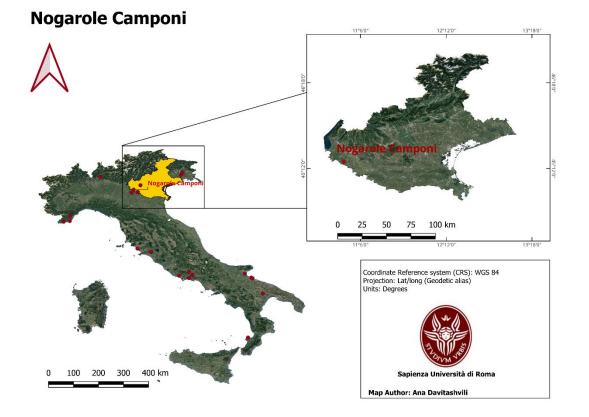


Figure 5: Map of Nogarole Camponi.

2.7.2 First trace of millet in Northern Italy

In the territory of Italy, through the Bronze Age period, mostly C₃ plants such as wheat, barley, and other cereals were most commonly in use. Archaeobotanical investigations, which are also held through isotopic analysis, are proving that in the northern parts of Italy C₄ plant such as millets were strongly in use (Kirleis, 2022). C₃ plants are more large-grained cereals, unlike broomcorn millet, which belongs to a small grain group. It is drought resistant and has a short growing cycle (Dal Corso, 2022). According to the dating, it seems that the first millest as a crop, In Europe appeared in the Bronze Age period in northern Italy and Ukraine. These two regions are one of the first places where millet cultivation was adopted. As Dal Corso et al.

(2022) report, the adoption of millet was probably due to two factors: i) the increasing diversity of cultivars and/or human adaptability; ii) the nature of the millets, a fast growing plant group , which is also well adapted to mobile settlement strategies (Baryeh, 2002).

One of the first researchers who studied millets adoption in Northern Italy were Tafuri et al. (2009). They used stable carbon and nitrogen isotopes for tracing millet consumption on animal and human bones. According to their analysis, millet should be the first domesticated C4 crop in Europe. Isotopic analysis is a handful and can distinguish diets with different photosynthetic pathways. It is affirmed that millet was not included in the Neolithic crop package and it was spread from Asia to Europe. However, the chronology and routine of this arrival are still under debate (Dal Corso 2022). On the territory of northern Italy, two species are mostly well known. They are broomcorn (Panicum miliaceum) and foxtail (Setaria italica) millet. From the Bronze Age period, we have both species in both parts of Italy, in the North and South, but significantly we meet it in the northern region. One of the first places in Italy, where millet consumption was confirmed is Olmo Di Nogara (Tafuri, 2009). The settlement is located near Verona, in the Adige valley. Olmo Di Nogara is one of the most important Bronze Age settlements in Italy. So it is interesting to see the evidence and the path consumption of millet on this archaeological site. Additionally, this thesis is mostly focused on the North part of Italy which is why I think for further interpretations this information will be necessary to better understand general situation of the diets from the Italy region in the Bronze Age. Millet has been detected charred carpological remains in phytoliths. Mostly it was found in ashes, which should be from litter-burning practices of domestic animals fed by millet (Dal Corso, 2022). According to their studies, the cereal spectrum shows that in RBA, different millet species were cultivated and used. The interesting fact of stable isotopic analysis showed that at Fondo Paviani, pigs were consuming millet, which makes us think that reason should be the habit of pigs when they are eating the waste of humans (Tafuri et. al 2018). On the other hand, there should be mentioned dietary importance of other plants, such as wheat and barley. Individuals from the southern Italian Bronze Age are depended on C₃ plants, which were a

significant nutritional source. Not only for the southern part of Italy but also for the Northern one. With quantitate analysis we see that millet is still a secondary crop to wheat and barley (Tafuri et al. 2009).

The interest in studying millet consumption in the Bronze Age is huge and more and more studies are focusing to have new insights into this new crop. First of all, it was one of the first C₄ plants released on the territories of Italy and generally in Europe. Additionally, the Bronze Age is the principal chronological period in the cultivation of new crops (Dalla Longa, 2019). It will be the key factor to study better the everyday life and environmental conditions of prehistoric settlements.

2.7.3 Animal exploitation in the Bronze Age

Animal exploitation was very popular in Bronze Age Europe. The population had some demographic changes and increasing settlements became important task to get diversification of animal resources and consumption (Del Corso et al. 2022). Hunting lost significance in the Bronze Age, while domesticated animals became more important not for only meat but for secondary products too. Some new materials and methods became known in everyday life such as wool production, dairying, and so on (Albarella et al. 2006). Due to this, zooarchaeology is one of the most important disciplines, which looks inside cultural and temporal divisions in archaeology. A quantitative and qualitative analysis should be made very precisely only in systematically coordinated datasets. This will avoid confusion and errors in future studies. For example, distinctions between the bones of sheep and goat are hardly identifiable, and this creates difficulties for using them in data. Therefore, most of the time they are written together, like sheep/goat. Also, incompatible reporting of renewable wild animal products can be confused with hunting (Del Corso et al. 2022). Despite some uncertainties, databases and

analyzing animal remains are very important for reconstructing the lifestyle of past populations and the development of the Bronze Age which is our main focus here.

The surplus of domesticated animals became an integral economic factor for Bronze Age societies. The versatility of using animals and exploitation of them changed over time and territory (Menotti, 2015). The use of different species is to different degrees in early Bronze Age period. For example, cattle (49%) dominated among other domesticated animals. Then are coming sheep and goats (31%) and lastly pigs (20%) (Del Corso et al. 2022). Those numbers are different for different territories, which supports the idea that the domestication of animals relies on the environment and settlement type. The condition of the environment plays a role in how animal distribution was, how they were adapted to the population, and how they were used in everyday life (Del Corso et al. 2022). Even small altitude differences influence the diversity of species. That is why the environment and climate contribute to the fact that mostly all kinds of animals were domesticated and used in Bronze Age European territories, especially in Italy. The cattle remains are more than 75% of the weight of all found bones in most settlements. Very interesting ideas are raised about the horse too. The horse is the animal that was fully domesticated in the Bronze Age, while all other animal exploitation processes started from the Neolithic period. In northern Europe, most of them were identified as wild horses. Only from 3500 BCE, was horse domestication widespread (Bertolini, Hohenstein, 2016). As an argument, we can bring up the horse milk residues on the Bronze Age pottery from northern Europe. The role of horse became more essential in the middle bronze ages, which might be connected to the mobile way of life. Sheep and goats are also one of the more important domesticated animals of this period. Their increase in the early Bronze Age period should be due to climatic changes as they thrive in dry grassland habitats. That is why pigs were less popular than caprine. Additionally, caprine herds are easier to merchandise and tax than pigs (Del Corso et al. 2022). On the Terramara plains (northern Italy) sheep/goat contribution rose to 40% in the middle bronze age. Using sheep/goats as meat was also very popular. According to the studies of Vartya culture (Hungary), only 15% of pig bone remains

were fully grown individuals. Others were killed before adult age. This is due to the fact that mutton was a big part of the human diet. Other than mutton, pig was very popular for consumption too. 62.5% of individuals were killed before their fourth year (Cremaschi, 2006). In the Terramara territory, which is the northern part of the Plain on Minco, capre (goat) are very well-spread animals, due to their habits and comfortability to the area of mountains. They also adapt very well to poor soils. While focusing on the territory of Italy, domesticated animals such as dogs should be mentioned as well, as they are very common in settlements or burials (Cremaschi, 2006).

To mention briefly, zooarchaeological analysis revealed the presence of some wild species too, such as red and roe deer, wild boar, fox, and hare (Del Corso et al. 2022). Their studies are also very significant to the study of botanical and ecological factors of past environments.

Chapter 3: Materials and Methods

3.1 Animals from Nogarole Camponi

The faunal remains investigated in this study come from Nogarole Camponi. Samples were selected from the collection of archaeological excavation, supervised by the Soprintendeza Archaeologica per il Veneto (Dr. P. Salzani). Samples were morphologically identified at the species level by Umberto Tecchiati from the University of Milan and sent to the Department of environmental biology of Sapienza University laboratory for the analysis of stable isotopes (Carbon, Nitrogen, Sulfur). A total of 25 faunal remains were selected considering the representation of different species of animals to characterize the economy of the Bronze Age site of Nogarole Camponi and the general northern part of Italy (Table 1). Each species was selected using a Minimum Number of Individual (MNI) criterion. Different domesticated species, such as cattle, dogs, sheep, goats, and pigs, were accountable for the Bronze Age animal package. This makes it possible to investigate animal dependence, especially when we are talking about the Bronze Age period when farming is largely attested. Further, the presence of studied animal stable isotope data from the Bronze Age northern Italy, in combination with this study, provides statistics that help us reconstruct the dietary background of humans living in the area.

The faunal remains showed a poor state of preservation. Some bones such as skull and jaws were broken. Bones showed butchering traces (Tecchiati pers. comm.), such as cut by chops on their ends. From the group of small domestic ruminants, only the sheep (*Ovis aries*) presented sufficient findings for a sampling of five individuals. The sampling is the result of the selection of 5 left distal tibias, in all cases adults. Five left shoulder blades were sampled from the ox, in all cases appearing as adults. For the goat, four female bone pins were sampled. As for *Sus*, it was possible to sample 2 right radii, whose proximal joints were fused. The other

three samples refer to the left radii whose proximal joints are not yet ossified, therefore they are young individuals. In the case of the dog, was possible to sample only 4 finds not necessarily referable to different individuals. (Appendix 2, 3, 4, 5, 6)

ID	Description	Species
NOG. S1	Ra prox. Right	Sus domesticus
NOG. S2	Ra prox. Right	Sus domesticus
NOG S3	Ra prox. Left	Sus domesticus
NOG S4	Ra prox. Left juveline.	Sus domesticus
NOG S5	Ra prox. Left juveline.	Sus domesticus
NOG B1	Scapula left	Bos taurus
NOG B2	Scapula left	Bos taurus
NOG B3	Scapula left	Bos taurus
NOG B4	Scapula left	Bos taurus
NOG B5	Scapula left	Bos taurus
NOG O1	Tibia Distale left adult	Ovis aries
NOG O2	Tibia Distale left adult	Ovis aries
NOG O3	Tibia Distale left adult	Ovis aries
NOG O4	Tibia Distale left adult	Ovis aries
NOG O5	Tibia Distale left adult	Ovis aries
NOG CF1	Hu distale Left adult	Canis lupus
NOG CF2	Tibia Distale left adult	Canis lupus
NOG CF3	Tibia prox. Left Juveline	Canis lupus
NOG CF4	Ulna	Dog
NOG CH1	Cavicchia Female	Capra hircus
NOG CH2	Cavicchia Female	Capra hircus

NOG CH3	Cavicchia ossea Female	Capra hircus
NOG CH4	Cavicchia ossea Female	Capra hircus

Table 1: List of samples and description from Nogarole Camponi.

3.2 creation of Database

To better contextualize the results obtained from Nogarole Camponi, we collected published stable isotopic measurements from the entire Bronze Age Italy. This would additionally be used to highlight differences and similarities across the Italian peninsula and islands. Firstly, this approach allows us to have an overview of the isotopic studies and secondly, it will be useful for future studies related to the territory of Italy in the Bronze Age. General background information plays a great role in analyzing data, allowing researchers to understand and interpret the information better.

The database used in this thesis relies on previously published isotopic studies of animals. It includes all the territory of Italy during the Bronze Age period from approximately the 2300 century to the 950 century BCE. Data is divided into different sections: it accounts for the reference to the original publication, geographical orientations (Longitude, Latitude), exact location, name of the archaeological sites, chronology of the archaeological site, animal groups (herbivore or carnivore), species, elements, and isotopic measurements as published in different studies. (Figure 6) Overall the database counts 230 entries, including samples from Nogarole Camponi with both wild and domesticated animals. Samples included in the database come from throughout the Peninsula (Figure 7), overall, they will contribute to the the discussion of the results obtained with this work.

For Database, please see the supplementary document, provided with this file.

Reference	Varali et.	Varali et.	Romboni	Romboni	Romboni	Varalli et.	Varalli et.	Skeates e	Skeates e	Skeates e	Tafuri et a
Chronology	EBA	EBA	MBA	MBA	MBA	MBA	MBA	MBA	MBA	MBA	MBA-LBA
Archaeological Site	Grotta De	Grotta De	La Sassa c	La Sassa c	La Sassa c	Grotta Vit	Grotta Vit	Grotta Re	Grotta Re	Grotta Re	Olmo di N
Latitude	42.67292	42.67292	41.35909	41.35909	41.35909	41.46757	41.46757	41.6396	41.6396	41.6396	45.17893
Longitude	11.05105	11.05105	13.35327	13.35327	13.35327	12.9036	12.9036	13.34263	13.34263	13.34263	11.06374
Orientation	Central	Central	Central	Central	Central	Central	Central	Central	Central	Central	North
sample name	SC 05 F	SC 014 F	LS481	LS793	LS794	GVV f 4	GVV f 11				ODNC
Latin names	Bos	Bos	Bos	Bos	Bos	Bos	Bos	Bos	Bos	Bos	Bos
species	cattle	cattle	cattle	cattle	cattle	cattle	cattle	cattle	cattle	cattle	cattle
Group	Domestic	Domestic	Domestic	Domestic	Domestic	Domestic	Domestic	Domestic	Domestic	Domestic	Domestic
element											
d13CV-PDB (%)	-18.3	-20.7	-20.5	-22.5	-21.8	-20.5	-20.1	-21.6	-19.8	-22.4	-17.8
d15NAIR (%)	6	4.3	9	5.5	5.5	4.6	5.4	4.5	4.3	5.6	6.5
C (%)	40.4	36.7				40	35.8				46
N (%)	14.9	13.5				14.4	12.7				16.5
C:N	3.2	3.2	3.3	3.3	3.2	3.2	3.3	3.4	3.4	4.6	3.3
Rdt (mg/g)						39.8	39.7				
% collagen yield	6.2	4.9						7.3	5.6	0.4	11.5
S (%)	0.17	0.16									
δ34SV-CDT(‰)	10.9	-1.6									
Uncertainty δ34S	0.93	0.93									
C:S	611	606									
N:S	195	192									

Figure 6: Example of database, from Bronze Age Italy territory.



Figure 7: Map of the settlements on territory of Bronze Age Italy.

3.3 Methods

Over the years, several methods have been proposed for collagen extraction. Three are the most used ones to this date. The first method was provided by Sealy (1986). This method yields decalcified small chunks of bones in a hydrochloric acid solution (1%-5%), then bones are dissolved into sodium hydroxide to remove organic matter, and lastly, they are freeze-dried (Sealy, 1986). Another method was described by Bocherens (1995) and Tuross (1988) with the difference that a small chunk of bone is demineralized into EDTA (ethylenediaminetetracetic acid), a sodium salt. It isolates collagen from bone minerals. The third method was proposed by Longin (1970), DeNiro (1984) and Brown (1988). Here we have powdered bone

demineralized in 8% hydrochloride acid for just 18 minutes. Before it is powdered, the bone is in acidic hot water (ph. 3) for some weeks. This method is mostly used when we have poorly preserved bone. These methods are modified throughout time in protocols by different laboratories and researchers (for a review see Katzenberg et al. 2008).



Figure 8: Demineralization process of bone (photo A. Davitashvili).

Samples from Nogarole Camponi were prepared in the Department of Environmental Biology of Sapienza University of Rome, at the Paleoanthropology and Bioarchaeology Laboratory.

Collagen extraction for isotopic analysis followed a modified Longin (1970) method. Briefly, cortical bone (0.5-1.0) was mechanically cleaned using a Dremel. Then they were demineralized in 0.5 m HCL for up to 4 weeks at +4°C. (Figure 8) The samples were checked for structure to observe the demineralization process. After the samples became soft and translucent, the solution was removed, and samples were rinsed three times with pure water. Then samples were gelatinized in pH3 hydrochloric acid at 70°C for 48 hours. The collagen solution was filtered with 5–8 μ m Ezee filters, was frozen, and then freeze-dried. (Figure 9) Lyophilized collagen was weighed (ca. 1.2-1.6 mg) into tin capsules,

some of the samples were taken twice to check the accuracy of the EA-IRM. (Figure 10) Finally, stable carbon, nitrogen, and sulfur isotope ratios were measured using an automated elemental analyzer coupled in continuous-flow mode to an isotope-ratio-monitoring mass-spectrometer (Costech Elemental Analyzer coupled to a Thermo Finnigan MAT253 Mass

Spectrometer). Analysis was carried out in Scottish Universities Environmental research Centre (SUERC), UK.



Figure 9: Freeze-dried samples (photo A. Davitashvili).

3.4 EA-IRMS



Figure 10: Samples prepared in tin capsules (photo A. Davitashvili).

An Isotope Ratio Mass Spectrometer, IRMS, measures ionized gaseous molecules to find the abundance of isotopes. (Muccio, Jackson, 2009) The equipment consists of four components: an inlet system, an ion source, a mass analyzer, and sets of ion detectors. The sample is introduced to the mass spectrometer as a gas for some of the elements. When analyzing collagen, the combustion of the samples in tubes by EA is required. For carbon, nitrogen, and sulfur flash combustion temperature should be around 1,800C° to produce gases (Kelly et al. 2018). Combusted samples are broken down into the wanted gases in the chemical traps. The gas is let

into the ion source. Here the molecules are ionized by bombardment, which allows them to be controlled through a beam. This beam is by flight tube and is localized in the mass analyzer zone. A mass analyzer separates ions into smaller beams. The separation of the ion into smaller beams is desired in the mass spectrum. Beam intensities should be measured in the ion collector section because different beams have different intensities. Individual isotope ion beams are reported as isotope ratios. One of the big advantages of IRMS is that it needs just a small sample and can measure multiple isotopes very precisely (Sharp et al. 2007) (Figure 11)

3.5 Statistics

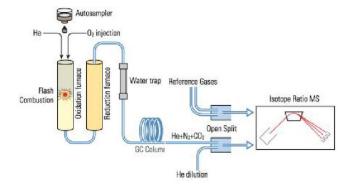


Figure 11: Process of EA-IRMS (edited from S. G. Karapurkar 2008).

Isotopic results from the animals of Nogarole Camponi and the data collected in the database for Bronze Age Italy were plotted and statistically explored. Data were filtered by quality and preservation of collagen according to Ambrose's (1990) and DeNiro's (1985) ranges. Descriptive statistics were calculated (standard deviation,

mean, median, minimum, and maximum for each species for the qualitative and quantitative analysis) with Excel 2013. A Chi-square test for comparisons between frequencies was run to check the type of distribution (normal vs non normal). The ideal uncertainty for repeated should be <0.2‰ for both carbon and nitrogen (Ambrose, 1990).

Scatter plots were used to represent values for two different numeric variables, in order to observe relationships between them. Normal Distribution, also called Gaussian distribution is a symmetric mean, showing that data near the mean are more frequent than data far from the mean (Patel, Read, 1996).

A Box Plot is a method for descriptive statistics, where numerical data with their quartiles are presented graphically. Each plot is divided into four quartiles. Quartiles are cut points and they divide the range of probability distribution into continuous intervals. For the Box plot, Nitrogen, Carbon and Sulfur values were filtered according to species to see the consumption of food in each of the animals. This helps to analyze the data more precisely and facilitates possible interpretations (Nuzzo, 2016).

Following the plotting of data through a Box plot, we ran some statistical tests. Firstly, it was necessary to identify whether the distribution was normal or not using the Shapiro –Wilk test. The aim of the test is to understand if the sample is normally distributed. If the p-value is less than 0.05 then the null hypothesis is rejected, and data is not normally distributed. The samples should not be less than 2 for the Shapiro-Wilk test to work.

In the case of the Database, which is presented in this thesis, carbon values distribution was not normal, while nitrogen's was. To compare distributions for statistical analysis we used different tests: for carbon, a Kruskall Wallis H test (multiple groups) and for nitrogen Oneway ANOVA.

ANOVA is a test for the analysis of Variance. It is the quality way to distinguish the difference between the means of more than two groups. The null hypothesis (H0) of ANOVA is used when there is no difference among group means, and the alternative hypothesis (HA) is used when one sample mean is not equal to others (Kim, 2017).

Kruskall Wallis test is an alternative non-parametric test for ANOVA. It indicates that at least one sample with a chance of probability dominates over other samples. It assesses the difference against the average lines in order to determine if the samples drawn come from the same population or not (McKnight, 2010).

Finally, all these statistical tools helped us make pairwise comparisons between each species group. It gave us information about which species are close in their diet to each other, and what similarities and differences are present between them. Additionally, statistical tools helped us to make visualization of the data, which is a better way to understand and present the information.

Chapter 4: Results

Results collected from bone collagen will be reported in this chapter, and they will be discussed in the next one. The stable isotope results and quality parameters are reported in the tables.

A total of 25 samples of domesticated animals' collagen (i.e., dogs, pigs, cattle, goats, and sheep) were analyzed. Stable isotope data are provided in <u>table 2</u> and plotted in <u>figure 12</u>, with descriptive statistics reported in <u>table 3</u>. Mean (average), median (midpoint), and mode (most frequent observation) are all equal to one another. For the normal distribution, the values less than one standard deviation away from the mean account for 68.27% of the set; while two standard deviations from the mean account for 95.45%; and three standard deviations account for 99.73% (Chen, 2023). All these are reported in the chapter of discussion. Scatter plots were done in the software - Excel 2013, while box plots and statistical tests were done in the analytical software called SPSS IBMS (29.0.1).

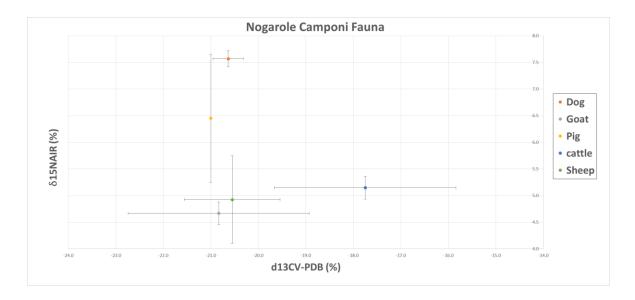


Figure 12: Scatter plot of carbon and nitrogen isotope data of Nogarole Camponi faunal samples.

Three of the samples identified by the code NOG CH2 (goat), NOG O2 (sheep), and NOG B2 (cattle) were run in duplicates to check the reproducibility of results. First, the quality of the data was assessed by referring to specific quality parameters. As Van Klinken (1999) reports, good quality collagen yield has to be at least 0.5%. Other quality parameters are %C 13-47%, %N 4.8-17.3% (Ambrose, 1990), %S 0.15–0.35% (Nehlich and Richards 2009), C: N 2.9-3.6 (Ambrose, 1990), C: S 300-900, C: N (Nehlich and Richards 2009).

Furthermore, it is important to have a look at quality criteria standards for carbon, nitrogen, and sulfur values to understand if data from IRMS has good quality criteria. The first important point is that the mean and standard deviation of laboratory standards and sample materials should be around $\pm 0.1\%$ (Ambrose,1990). As reported by SUERC, normalization was checked using the marine collagen USGS88 (δ^{13} C VPDB = $-16.06 \pm 0.07\%$, δ 15 N AIR = $14.96 \pm 0.14\%$, and δ 34 S VCTD = $17.10 \pm 0.44\%$) and the porcine collagen USGS89 (δ 13 C VPDB = $-18.13 \pm 0.11\%$, δ 15 N AIR = $6.25 \pm 0.12\%$, and δ 34 S VCTD = $3.86 \pm 0.56\%$), which gave the values reported in the <u>table 3</u>.

Additionally, it is important to check the status of preservation of bone collagen in the sample, which can be assessed using the C/N ratio. This should be between 2.9 to 3.6 according to Ambrose (1990). In most cases, the C/N ratio falls within the expected range for a good-quality sample. Only the samples NOG CH1, NOG S1, and NOG S2 were slightly higher. "The carbon to nitrogen (C/N) ratio is an important parameter, which will relate the composting reactions to the relative concentrations of essential chemical constituents required for the growth and metabolic reactions of the microbial population" (Martin, 2007). The C/N ratio can be calculated as the percentage of C divided by the percentage of N and multiplied by 14/12 due to the atomic weight difference (%C/%Nx14/12).

As shown in <u>table 2</u>, where all samples are presented, only the samples between 2.9 to 3.6 meet quality criteria standards. Others which do not meet quality criteria were excluded from the discussion part. Regarding Ambrose's (1990) quality criteria for C%, it should be from 13% to 47%, all samples were acceptable, except NOG S5, and B4, which had very low values and were excluded. Other results were considered reliable.

LabNo	Site	ContextID	SampleID	SampleType	Species	δ¹⁵N	δ¹³C	δ³4S	%N	%С	%S	CNMolar	CSMolar	NSMolar
GUsi15286	Nogarole Camponi	NOG	NOG CF1	collagen	Dog	7.7	-21.0	4.2	9.6	28.1	0.24	3.4	306	90
GUsi15287	Nogarole Camponi	NOG	NOG CF2	collagen	Dog	6.6	-27.7	7.9	0.9	2.6	0.17	3.3	42	13
GUsi15288	Nogarole Camponi	NOG	NOG CF3	collagen	Dog	7.6	-20.5	4.8	12.3	34.5	0.20	3.3	460	141
GUsi15289	Nogarole Camponi	NOG	NOG CF4	collagen	Dog	7.4	-20.4	4.8	13.4	38.1	0.23	3.3	433	130
GUsi15290	Nogarole Camponi	NOG	NOG CH1	collagen	Goat	4.8	-22.8	3.6	7.8	26.3	0.43	3.9	164	42
GUsi15291	Nogarole Camponi	NOG	NOG CH2 (A)	collagen	Goat	4.3	-21.1	0.5	13.3	37.4	0.23	3.3	440	134
GUsi15292	Nogarole Camponi	NOG	NOG CH2 (B)	collagen	Goat	4.3	-21.1	3.3	11.0	31.1	0.17	3.3	495	151
GUsi15293	Nogarole Camponi	NOG	NOG CH3	collagen	Goat	5.6	-20.2	3.8	11.0	32.8	0.21	3.5	423	122
GUsi15294	Nogarole Camponi	NOG	NOG S1	collagen	Pig	6.3	-23.7	0.9	3.6	13.0	0.27	4.3	127	30
GUsi15295	Nogarole Camponi	NOG	NOG 05	collagen	Sheep	5.3	-20.7	5.1	12.1	35.0	0.21	3.4	454	135
GUsi15296	Nogarole Camponi	NOG	NOG S4	collagen	Pig	5.6	-21.0	2.6	9.8	28.2	0.21	3.3	365	109
GUsi15297	Nogarole Camponi	NOG	NOG 03	collagen	Sheep	4.9	-20.6	5.6	5.4	15.7	0.26	3.4	161	47
GUsi15298	Nogarole Camponi	NOG	NOG S3	collagen	Pig	7.3	-21.0	6.1	12.3	34.3	0.19	3.3	485	149
GUsi15299	Nogarole Camponi	NOG	NOG O4	collagen	Sheep	4.9	-19.1	5.8	12.7	35.5	0.20	3.3	462	142
GUsi15300	Nogarole Camponi	NOG	NOG 02 (A)	collagen	Sheep	3.8	-21.0	1.9	12.0	34.0	0.22	3.3	421	127
GUsi15301	Nogarole Camponi	NOG	NOG O2 (B)	collagen	Sheep	3.8	-21.1	1.5	10.8	30.2	0.20	3.3	401	123
GUsi15302	Nogarole Camponi	NOG	NOG CH4	collagen	Goat	4.1	-21.2	1.3	14.0	39.7	0.21	3.3	503	152
GUsi15303	Nogarole Camponi	NOG	NOG B1	collagen	Cattle	5.0	-19.1	3.3	7.8	22.0	0.17	3.3	354	108
GUsi15304	Nogarole Camponi	NOG	NOG O1	collagen	Sheep	5.7	-21.4	4.7	10.5	29.4	0.20	3.3	389	120
GUsi15305	Nogarole Camponi	NOG	NOG S5	collagen	Pig	7.0	-22.3	3.6	2.8	7.9	0.16	3.3	132	40
GUsi15306	Nogarole Camponi	NOG	NOG B4	collagen	Cattle	4.8	-20.8	2.5	3.4	10.1	0.19	3.4	142	41
GUsi15307	Nogarole Camponi	NOG	NOG B2 (A)	collagen	Cattle	5.3	-16.4	5.5	12.8	36.7	0.27	3.4	365	109
GUsi15308	Nogarole Camponi	NOG	NOG B2 (B)	collagen	Cattle	5.3	-16.4	5.7	12.5	35.8	0.26	3.4	370	110
GUsi15309	Nogarole Camponi	NOG	NOG S2	collagen	Pig	6.4	-22.4	0.3	1.9	6.3	0.14	3.8	117	31

Table 2: Stable Isotope data of the faunal remains from Nogarole Camponi.

For the N%, Ambrose (1990) suggested us the criteria between 4.8-17.3%, all samples were acceptable, except CF2, S1, 03, S5, B4, and S2 which were excluded. Finally, the quality criteria for sulfur given by Nehlich and Richards (2009), is mostly upheld, with the exception of NOG S5 having lower value, this sample was excluded as well.

We analyzed statistically the data, by grouping them according to species or dietary category (i.e., herbivore, omnivore, and carnivore). In <u>figure 13</u> the boxplot shows much higher δ^{13} C values in the cattle while the others present quite similar values. In <u>figure 14</u> the boxplot shows that, unsurprisingly, the dog has a much higher δ^{15} N value, following pig values, while other species are mostly on the same level at lower values. In <u>figure 15</u> the boxplot shows that all species except goat has high value of stable sulfur isotope.

Tables – 4 and 5 report the descriptive statistics for each species. According to the results, the mean, standard deviation, median, minimum, and maximum are shown here. (A description of these statistical analyses can be found in Chapter 3). The mean δ^{13} C for all fauna is -20.2 ‰ with a range from -16.4 ‰ to -21.4 ‰. The mean δ^{15} N value for all fauna is 6.1 ‰ with the range from 4.9 ‰ to 7.7 ‰. The mean δ^{34} S for all fauna is 4.2 ‰ with the range from 1.9 ‰ to 5.2 ‰ (Table 3). On the diagram, all of these are put together. Scatter plots demonstrate the importance of species connections. The scatter plots in figure 12 show that the average nitrogen and carbon values of dogs and pigs are greater, while others are on the same level. Furthermore, the largest distribution has cattle with a carbon isotope value and pigs with a nitrogen isotope value, according to the error bars.

When comparing sulfur and nitrogen values, as shown in <u>figure 16</u>, dogs and pigs are still high in nitrogen. Cattle and sheep have lower nitrogen values, but they are high in sulfur. Goat samples represent an intriguing case because sulfur and nitrogen values are both relatively low.

We also created scatter plots to examine the interrelationships between carbon and sulfur, which provides us with additional information about the ecosystem. As seen in figure 17, cattle have a lower δ^{13} C value (-18.0 ‰) but a greater δ^{13} C ratio (6.0 ‰), whereas sheep and pigs have a lower carbon value but a larger sulfur ratio. The only exception is goat, which has relatively low sulfur and carbon levels (for a discussion see Chapter 6).

Descriptive tests, which were performed in SPSS Statistics, are an important component of statistical tools. The multiple comparison analysis was provided by the Anova test. The

relevance of stable carbon isotope abundance is more than 0.5, and each species consumed C₃ plants in a comparable manner. The contrast is in nitrogen abundance, where the significant difference between species dog and cow, dog and sheep, and dog and goat are demonstrated. Dogs and pigs, (sig. 0.5) as well as other herbivore animals share commonalities among each other.

			mean d1	mean d13 C			
	Ν	mean	1SD	2SD	median	minimum	maximum
Dog	4	-20.6	0.3	0.6	-20.5	-21.0	-20.4
Goat	4	-20.8	0.6	1.1	-21.1	-21.2	-20.2
Pig	5	-21.0	0	0	-21.0	-21.0	-21.0
Cattle	5	-17.8	1.9	3.8	-17.8	-19.1	-16.4
Sheep	5	-20.6	1.0	2.0	-20.9	-21.4	-19.1
			mean d1	5N			
		mean	1SD	2SD	median	minnimum	maximum
Dog	4	7.6	0.2	0.3	7.6	7.4	7.7
Goat	4	4.7	0.8	1.6	7.4	4.1	5.6
Pig	5	6.5	1.2	2.4	5.6	5.6	7.3
Cattle	5	5.2	0.2	0.4	4.3	5.0	5.3
Sheep	5	4.9	0.8	1.6	5.6	3.8	5.7
			mean d3	mean d34SV			
		mean	1SD	2SD	median	minnimum	maximum

Dog	4	4.6	0.34641	0.69282	4.8	4.2	4.8
Goat	4	1.9	1.721434	3.442867	1.3	0.5	3.8
Pig	5	4.4	2.474874	4.949747	4.4	2.6	6.1
Cattle	5	5.2	1.555635	3.11127	4.4	3.3	5.5
Sheep	5	4.9	1.711481	3.422962	4.9	1.9	5.8

Table 3: Descriptive statistics of the faunal samples at Nogarole Camponi.

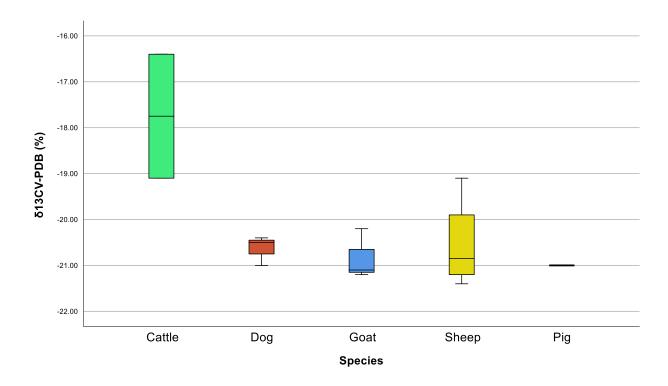


Figure 13: Box plot of $\delta^{13}C$ value for Nogarole Camponi (Cattle (n=2); dog (n=3); goat (n=3); sheep (n=4); pig (n=2)).

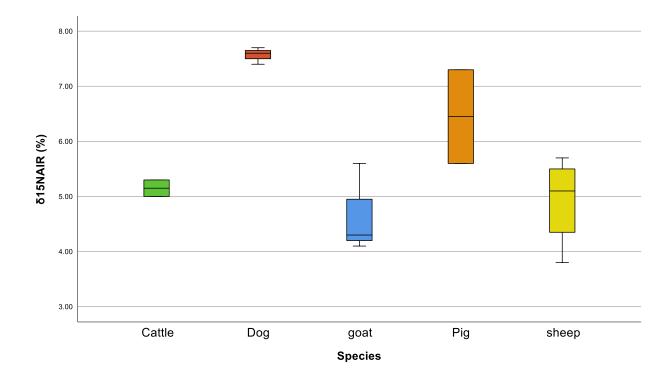


Figure 14: Box plot of $\delta^{15}N$ for Nogarole Camponi (Cattle (n=2); dog (n=3); goat (n=3); sheep (n=4); pig (n=2)).

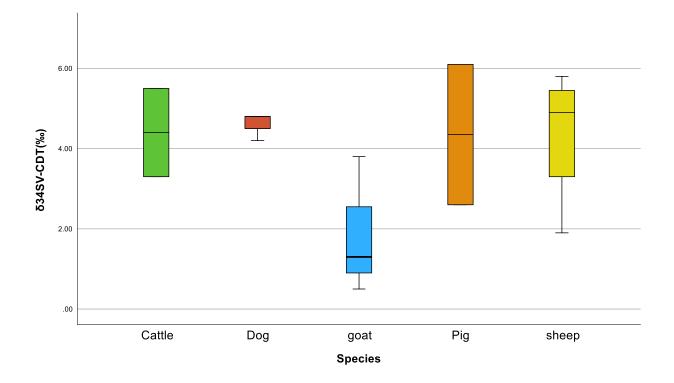


Figure 15: Box plot of $\delta^{34}S$ for Nogarole Camponi (Cattle (n=2); dog (n=3); goat (n=3); sheep (n=4); pig (n=2)).

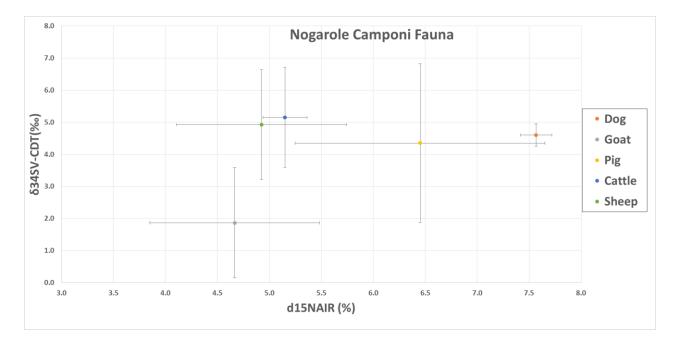


Figure 16: Scatter plot of nitrogen and sulfur of Nogarole Camponi.

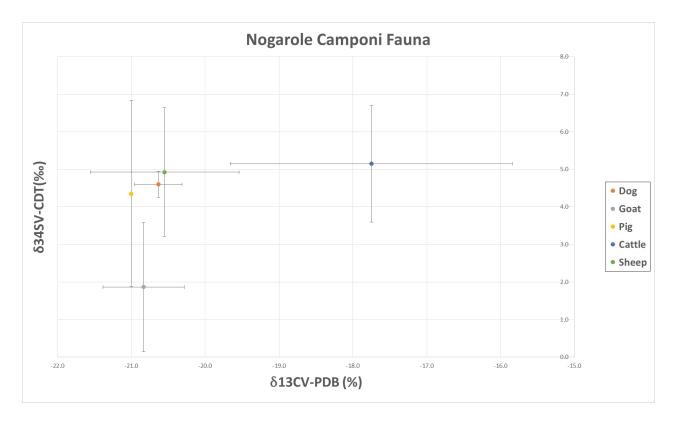


Figure 17: Scatter plot of carbon and sulfur isotopes of Nogarole Camponi.

Chapter 5: Discussion

This chapter will go over the findings in further depth. It will include extra statistical analysis, particularly in terms of food proportions in the diet, and will emphasize any minor differences among species.

As previously stated, in this work, we created a database of stable isotopes of animals from Bronze Age Italy, in order to compare and evaluate it with the samples from Nogarole Camponi. It is employed at various archaeological sites in Italy's north, south, and center regions, and includes both wild and domesticated animals. There are 9 species in total at 23 archaeological sites reported in the literature. One of the key points of this chapter is to create geographical comparisons. For example, since Nogarole Camponi is an archaeological site located in northern Italy, it will be interesting to see what the diet was like in other parts of the northern Italy. It will also provide an opportunity to observe the similarities and make some contrasts between the north, south and central regions of the peninsula.

To sum up the data from Nogarole Camponi, as figure 12 shows, the majority of the animals from this site were consuming C₃ plants as their main diet. According to carbon values (around $-20.0 \ \infty$) one sample of cattle can be considered as the only exception, as its carbon value is greater than $-16.4 \ \infty$. The carbon values of other herbivore animals are different from that of cattle and are measured in-between $-20\ \infty$ to $-25\ \infty$. The main contributor to this dissimilarity is the different lifestyles of animals. Some domesticated herbivores do not require location change, so they can be kept at a certain place for feeding which makes them easy to maintain and control. However, others are more mobile, need to often change pastures and are usually left grazing. Grazing in animal agriculture refers to letting animals consume wild flora outside. In other words, instead of having food provided to them as it does on feedlots, the limited areas

of industrial farms, the animals are able to search for and consume the items they choose within a specific space (R.H. Hart et al. 1993). Under these definition cattle can be qualified as animal which, if the necessary food is provided, can be maintained at a place without it having to travel distances. Goats and sheep on the other hand, have the necessity to change the pastures. If we observe the results from Nogarole Camponi, we will see that although they are all herbivores, cattle's carbon values are different. This suggests that goats and sheep were more likely left grazing, while the cattle might have been fed by humans themselves. This also contrasts with the high carbon value of cattle, which, although we can't say for certain, can be connected to the consumption of millet.

According to Smith and Epstein (1971), the δ^{13} C value of C₃ plants ranges from approximately 36 ‰ to 19 ‰ (global mean 26.5 ‰), whereas C₄ values vary from approximately 17 ‰ to 9 ‰ (global mean 12 ‰). With a δ^{13} C mean value of -20.5 ‰ from Nogarole Camponi, we can deduce that C₃ plants were commonly consumed. On the other hand, one of the objectives of this study was to determine if millets, which is C₄ plant was consumed at Nogarole Camponi, as attested elsewhere in the Bronze Age Po Plain (Tafuri et al., 2009, 1018). In fact, at Olmo Di Nogara, a large cemetery near Nogarole Camponi the use of C₄ crops is largely attested.

Despite the fact that millet played a vital role in human life at the time, at least for this region, it is possible that millet was not yet widespread, and its consumption was not shown in animals. Furthermore, one of the samples, NOG B3, had a higher carbon isotope level (-16.4‰), indicating that it is possible that a mixed consumption of both C_3 and C_4 plants occurred at least for this animal. The possible reasons of why the millet consumption is not confirmed unequivocaly will be discussed in the last part.

For all faunal data, the mean nitrogen isotope abundance is 6.1 ‰. It informs us about the trophic level of the various species. Figure 12 clearly shows that dogs (7.7‰) and pigs (7.3‰) have the highest values. This is not surprising given that dogs are omnivore, and might have also consumed human leftovers. The same might apply to pigs, animals that consume almost

everything, particularly garbage. While both species have greater nitrogen values than other herbivores like sheep, goats, and cattle, we can assume that they were predominantly omnivores that ate both animal and plant-based foods. As a result, we can say the same for the humans as well, their food ratio was most likely similar to that of pigs and the dogs, therefore, other than plants, animal products were also an essential part of the human diet at this site.

It is also interesting to explore sulfur isotope ratios to supplement carbon and nitrogen dietary data. Figure 17 shows that the sulfur stable isotopic ratio is very low, with a mean of 6.7‰ ranging from 0.5‰ to 6.1‰. They are especially low in goats. This difference highlights the above underlined argument about goats being able to move more freely as their food ratio was different from other animals the sulfur values of which are higher.

It is interesting here to compare other data from other archaeological sites from the database to Nogarole Camponi. To begin, I would like to point out that, with the exception of the archaeological sites of Mereto, Fondo Paviani, and Olmo Di Nogara, the carbon isotopic abundance is roughly the same throughout Italy (Figure 18). Carbon value of Nogarole Camponi faunal remains are unusually low as compared to others, ranging from -21.0‰ to - 17.0‰. Fondo Paviani and Olmo Di Nogara are archaeological sites that are thought to be one of the firsts in Europe where millet was a significant crop. Mostly, others from south and central part of the Italy are consuming C₃ plants such as wheat and barley, which explains why their mean of carbon value is -20.2‰ similar to Nogarole Camponi. Also for more insights box plots were done for the faunal data of whole Italy. (Table 4, Table 5)

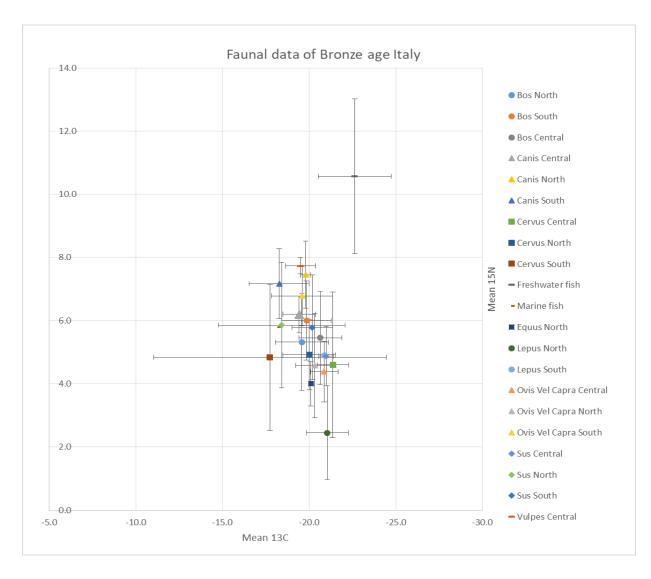


Figure 18: Scatter plot of faunal database (Bronze Age Italy territory).

There are countless species in the database and the Nogarole Camponi only provided 5 of them. Consequently, making a comparison regarding the fauna between the Nogarole Camponi and other sites will be difficult, however, the matter will be discussed in this chapter.

Carbon and nitrogen levels for dogs, sheep, goats, and cattle are the same as illustrated in figures 18 with very tiny differences towards the south. However, there is a big disparity in pig values. Pig nitrogen abundance is higher (7.3‰) at Nogarole Camponi than in other

archaeological sites in Italy's north, center, and south (5.5‰). This seems to suggest that consumption of animal proteins was higher at Nogarole Camponi than at other sites. Also, if we compare the sulfur values from Grotta Missa, which is also located in northern Italy we can see that they are much higher (9‰ -11‰) than what was recorded at Nogarole Camponi.

If we go deeper in sulfur values of goat samples, we can detect that, although the carbon values are same for goats and sheep and different for cattle, the sulfur values, which informs us about the environmental factors, are different for goats and same for sheep and cattle. The reason for this might be that goats were given more freedom in their movements, meaning that they traveled more and to different locations than the other animals. This might have multiple reasons. First and foremost, goats are browser animals, meaning that they prefer eating tree leaves and shrubs, so their food preference is different than that of sheep or cattle. Another reason might be that goats are more defensive in their approach; they can travel well and can take on steep hills without any issues, this makes them harder targets for predators and also makes them hard to control as they need more space for searching proper and preferred plant products. Sheep on the other hand are much easier targets and need constant attention, also, they might have had more value for the people of Nogarole Camponi than the goat, as they are not only usable for milk or meat products but can also provide wool. All of these together might have contributed to developing different herding methods for cattle, sheep and goats, which can be read through the aforementioned differences in sulfur values.

			mean d13				
	NT 1		C	200	1.		
	Numbe	mean	1SD	2SD	median	minimu	maximu
	r of					m	m
	samples	20.6	1.0	25	20.5	00 F	10.0
Bos Central	10	-20.6	1.2	2.5	-20.5	-22.5	-18.3
Bos North	35	-19.6	1.5	3.1	-19.4	-22.1	-15.5
Bos South	12	-19.9	1.4	2.9	-20.52	-21.21	-17
Canis Central	5	-19.4	0.9	1.9	-19.5	-20.5	-18.2
Canis North	5	-19.8	0.3	0.6	-19.8	-20	-19.6
Canis South	6	-18.3	1.7	3.5	-18.8	-20.04	-16.1
Cervus Central	4	-21.4	0.9	1.8	-20.9	-22.4	-20.8
Cervus North	4	-20.0	1.5	3.0	-20.9	-20.9	-17.4
Cervus South	12	-17.7	6.7	13.4	-20.23	-21.23	0
Freshwater fish	3	-22.6	2.1	4.2	-22.7	-24.7	-20.5
Marine fish	1	-18.2			-18.2	-18.2	-18.2
Equus North	2	-20.1	0.1	0.3	-20.1	-20.2	-20
Lepus North	3	-21.1	1.2	2.4	-21.05	-21.9	-20.2
Lepus South	1	-20.9			-20.9	-20.9	-20.9
Ovis Vel Capra	17	-20.9	0.8	1.6	-21.05	-22	-19.2
Central							
Ovis Vel Capra	40	-20.3	1.1	2.2	-20.5	-22	-16.7
North							
Ovis Vel Capra	10	-19.6	1.8	3.5	-20.17	-20.7	-14.9
South							
Sus Central	9	-21.0	0.4	0.8	-20.95	-21.7	-20.4
Sus North	25	-18.4	3.7	7.3	-20.4	-21.4	-11
Sus South	9	-20.2	1.2	2.4	-20.44	-21.49	-16.5
Vulpes Central	3	-19.5	0.9	1.7	-19	-20.5	-19

Table 4: Descriptive Statistics of Carbon from database (Bronze Age Italy).

			mean d15N				
	Numbe		1SD	2SD	median	minnimu	maximu
	r of	mean	15D	25D	median		
	samples					m	m
Bos Central	10	5.5	1.5	2.9	5.4	4.3	9
Bos North	35	5.3	1.5	3.1	5.1	2.9	9
Bos South	12	6.0	1.3	2.5	6.2	3.4	7.7
Canis Central	5	6.2	0.6	1.2	6.7	5.6	6.7
Canis North	5	7.5	1.1	2.1	7.5	6.7	8.2
Canis South	6	7.2	1.1	2.2	7.5	5.3	8.3
Cervus Central	4	4.6	0.6	1.2	4.3	4.2	5.3
Cervus North	4	4.9	1.1	2.2	4.9	3.2	6.3
Cervus South	12	4.8	2.3	4.6	5.2	0	7.2
Cyprinidae Ind.	3	10.6	2.4	4.9	11.1	7.9	12.7
North	0	10.0	2.1	1.7	11.1	1.5	12.7
Epinephelus	1	5.8			5.8	5.8	5.8
South							
Equus North	2	4	0.7	1.4	4	3.5	4.5
Lepus North	3	2.5	1.5	3.0	2.5	1.4	3.5
Lepus South	1	4.9			4.9	4.9	4.9
Ovis Vel Capra	17	4.4	1.0	1.9	4.5	1.9	5.8
Central							
Ovis Vel Capra	40	4.6	1.6	3.3	4.4	2.3	8.3
North							
Ovis Vel Capra	10	6.8	0.9	1.9	6.9	5.6	8.5
South							
Sus Central	9	4.9	0.9	1.9	5.1	2.9	5.9
Sus North	25	5.9	2.0	4.0	5.2	3.2	9.7
Sus South	9	5.8	1.7	3.3	5.8	3.0	8.7
Vulpes Central	3	7.7	0.3	0.5	7.7	7.5	8

Table 5: Descriptive statistics of nitrogen from the database (Bronze Age Italy).

Conclusion

The goal of this study was to evaluate the diet of the faunal remains from Nogarole Camponi, through stable carbon, nitrogen and sulfur isotope analysis, as well as to identify the possibility of millet consumption, as attested by earlier studies near this location. Furthermore, one of the most significant task of the research was to demonstrate the importance of disciplines such as zooarchaeology and creating a database of faunal remains of Bronze Age Italy region.

Like most research, this study had some limitations. Firstly, the small sample size of Nogarole Camponi was greatly limiting. Due to the standards of the stable isotope analysis from a total of 25 samples, only 14 were used. This is the main reason why we could not confirm with certainty the consumption of millet.

This research tried to better understand the diet of not only animals, but of humans as well, however, the study at Nogarole Camponi is still ongoing, therefore this research has its limit in bringing out the necessary archaeological information, which in turn limits the interpretation process. As the study of the site goes on further, the findings of this research will also provide some new insights into the currently unanswered questions.

This study was an examination of stable isotopic ratio measured in a sample of animal data, in relation to the faunal isoscape of Bronze Age Italy and the archaeological contexts of the sites, which aided in developing new insights into the diet at Nogarole Camponi.

After analyzing the results of stable isotopic analysis of samples from Nogarole Camponi, it was discovered that the predominant food of the domesticated animals was C₃ terrestrial

plants, most likely wheat and barley, which seems similar to other archaeological sites from Italy in the Bronze Age period.

Considering the data, the chances of animal consumption of C_4 plants are quite minimal at this site, but it's not unsubstantiated as in the case of sample NOGB3 we can confirm that the mixed consumption of C_4 and C_3 plants took place. This is also supported by the idea that only the cattle were kept at the site in which case, they might have been fed millet together with C_3 plants by humans. The fact that, unlike cattle, sheep and goats were never fed any C_4 plants, gives us another argument for the idea that, the different husbandry practices took place for different animal species, which could also be interpreted by analyzing differences and similarities in sulfur isotope values for goats, sheep and cattle. All of this is just a theory that would confirm the millet domestication at the site, but more research, especially on human remains, is needed to develop a clearer understanding of usage of C_4 plants at Nogarole Camponi.

Currently there are no isotopic studies of human individuals from Nogarole Camponi. Additionally, the nitrogen abundance of the dog and pig indicates that their diet contained meaty goods. Unsurprisingly, there was no evidence of fish consumption at the site, which is in line with the isotopic evidence from other coeval sites in the region. Besides, the sulfur isotope analysis results give us interesting insights into the importance of goats and sheep for residents of Nogarole Camponi. The lower sulfur values in goats suggest that, goats were most likely very mobile in their search for consumables and they were most likely left browsing by humans. And same sulfur values in cattle and sheep speak about the importance of these two species for the people as they were mostly kept close to the site due to their worthiness as animals with valuable provisions.

The goal of this research was not only to examine the fauna of Nogarole Camponi but also to develop a faunal database for the entire Italy region during the Bronze Age period. This database might represent a source for researchers working on stable isotopes in the Peninsula. Database will be useful for future studies on specific archaeological sites to compare and contrast current interpretations regarding human dietary practices.

Lastly, it's important to mention that more research is needed not only for the faunal but also for human remains on the site. The archaeological expedition is currently ongoing, providing future researchers with fresh materials for laboratory study. The northern Italian region remains an intriguing and contentious location, owing to the presence of millet as an important crop on this territory and, more generally in Europe.

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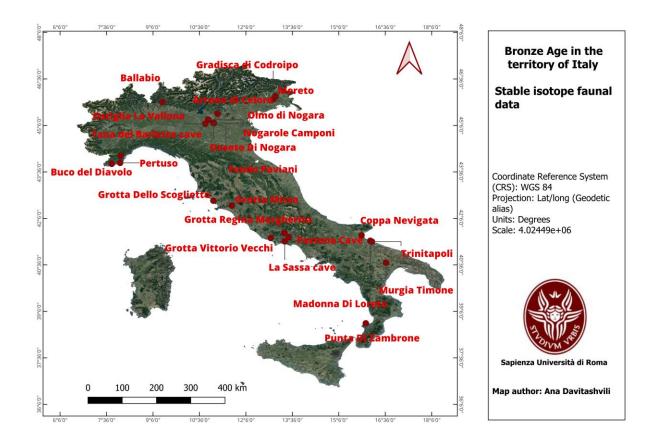
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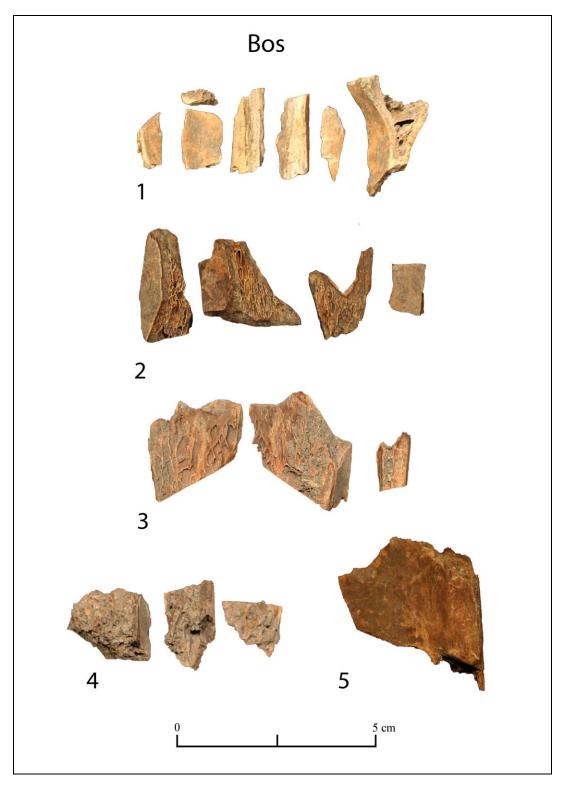
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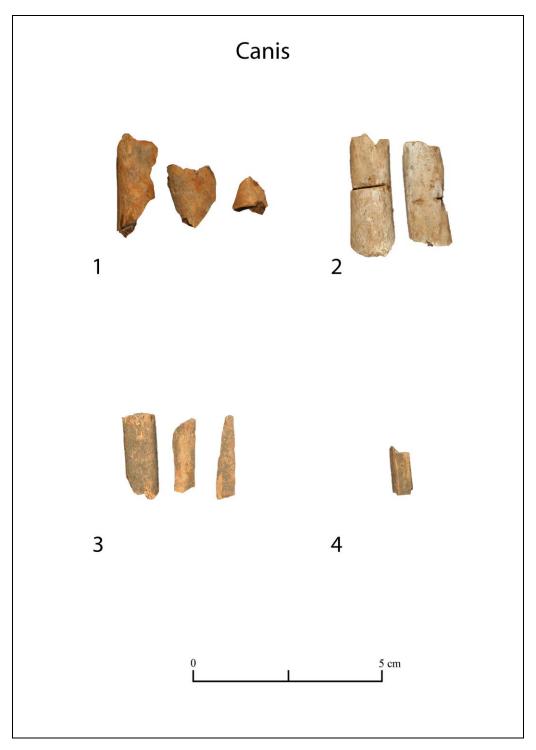
Appendixes



Appendix 1: List of sites mentioned: Ballabio, Gradisca di Codroipo, Arrano di Celore, Mereto, Olmo di Nogara, Ostiglia La Vallona, Tana del Barletta cave, Nogarole camponi, Doseto Di Nogara, Pertuso, Buco del Diavolo, Fondo Paviani, Grotta Dello Scogliato, Grotta Missa, Grotta Regina Margherita, Coppa Navigata, Pastena cave, Grotta VITTORIO Vecchi, La Sassa cave, Trinitapoli, Murgia Timone, Madonna Di lorento, Punta Di Zambrone.



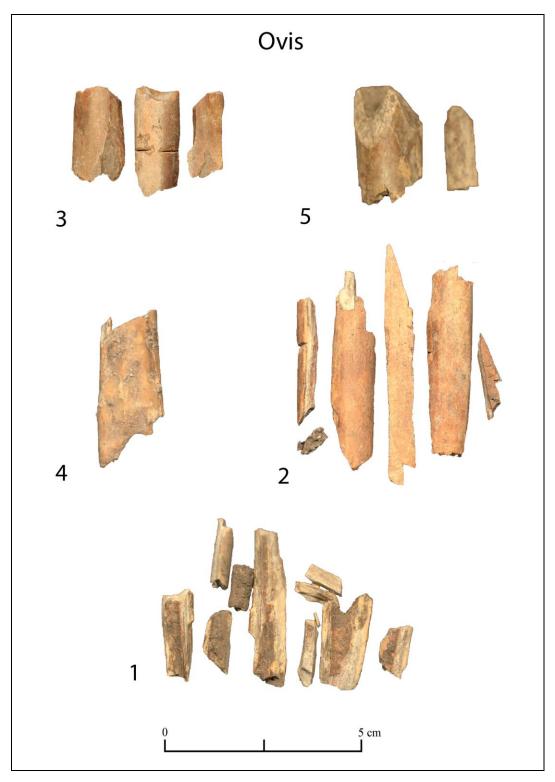
Appendix 2: Bos (cattle) bones from Nogarole Camponi. 1-NOGB1, 2-NOGB2, 3-NOGB3, 4-NOGB4, 5-NOGB5.



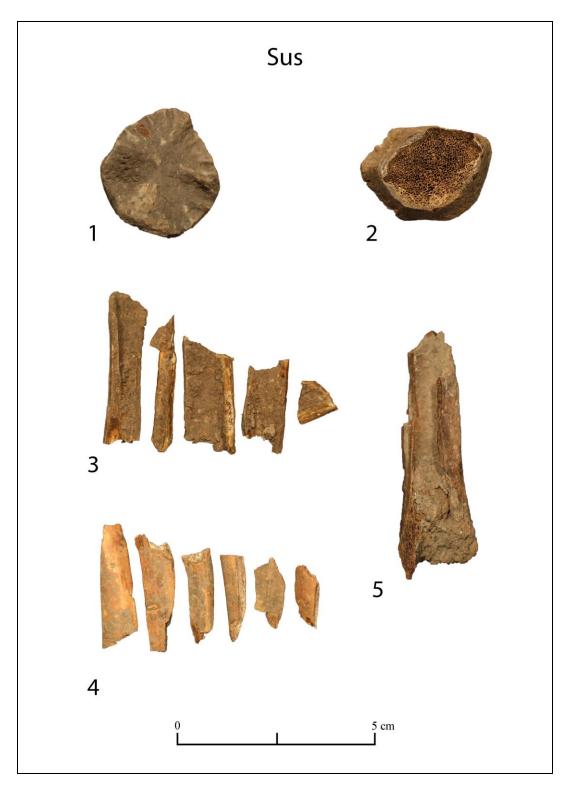
Appendix 3: Canis (dog) bones from Nogarole Camponi 1-NOGCF1, 2-NOGCF2, 3-NOGCF3, 4-NOGCF4.



Appendix 4: Hircus (goat) bones from Nogarole Camponi 1-NOGCH1, 2-NOGCH2, 3-NOGCH3, 4-NOGCH4.



Appendix 5: Ovis (sheep) bones from Nogarole Camponi. 1-NOGO1, 2-NOG02, 3-NOG03, 4-NOG04, 5-NOG05.



Appendix 6: Sus (pig) bones from Nogarole Camponi 1-NOGS1, 2-NOGS2, 3-NOGS3, 4-NOGS4, 5-NOGS5.