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Stable Carbon and Nitrogen Isotope Analysis in Italy and Croatia: Bronze Age Food Practices Across the Adriatic

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Abstract

This research aims to look at dietary practices of separate populations from across the Adriatic Sea (Italy and Croatia). Paleodietary studies through stable isotope analysis is a means to look at possible food catchments chosen by past communities in order to make educated assumptions of economic and cultural practices. Stable carbon and nitrogen isotope analysis was carried out successfully on 22 humans and 28 animal bones from four separate Bronze Age sites. The sites analyzed are Coppa Nevigata (Apulia, Italy), Gusica Gomila, Jukica Gomila, and Brnjica (Dalmatia, Croatia), all dated to the Bronze Age (approximately XVIII-XII century BCE). The main objective is to investigate the contribution of different food sources (terrestrial and marine) and to observe distinctions on animal versus plant proteins in the diet to examine dietary differences within each site. This will allow for a greater understanding of dietary patterns in both Bronze Age Italy and Croatia and to possibly investigate any differences between the two areas. Collectively, the sites have presented carbon and nitrogen isotopic ranges that illustrate a diet dominated with C3 terrestrial plants and relative consumption of herbivore animal proteins. Compared to recent studies of Bronze Age Italy and Croatia, the results correlate well with a diet consisting mostly of cultivated C3 plants. Although, the Bronze Age is an important period for the introduction for a new crop, the C4 plant group of millets, only two individuals from Brnjica show signs of small consumption of C4 plants and/or marine foodstuffs. The individuals from Coppa Nevigata do not indicate any C4 plant consumption which supports recent studies that millet has only been proven to be consumed in North and Central Italy during the Middle to Late Bronze Age. Two individuals from Coppa Nevigata do however indicate small consumption (15-20%) of freshwater foodstuffs. No significant differences in stable isotope values in terms of intra-population variations such as sex, age, burial type, or period as far as the samples have provided. This study contributes to our understanding of dietary practices in prehistoric Italy and Croatia and provides new data on Southern and Eastern regions of the Italian Peninsula, all of which are generally under-represented in the Bronze Age. Further it adds interesting information on a clear distinction between Northern and Southern regions of Italy, which suggests that food practices are a good means to investigate on past cultural complexity.

Chapter 1: Introduction

The foundation of paleodietary studies through stable isotopes is the analysis of stable isotopic ratios in the tissue of a subject compared to that of foodstuffs that may have been consumed. It focuses on the stable isotopes as building blocks of tissues and follows fractionation and metabolic pathways from the consumed to the consumer. But it's not just a set of data representing chemical ratios. Paleodietary through stable isotopes sheds light on the relationship between environmentally available food catchments and the choice made by the consumers in a cultural and socioeconomic way. By understanding recently consumed foods and the parameters of how they were consumed, the analyzer can gain a better perspective on a wide scope of cultural exchanges. For instance, ecology of plants and animals, farming and cultivating strategies, animal husbandry, economics and trade, food technologies such as cooking and fishing, and ritual and status. Food can evidently lead to better ideas on past social identities. It's important to understand that the results of paleodietary studies is not a direct reconstruction of diet. Keegan et al. 1989 quotes "These signatures do not represent a 'reconstruction' of diet; rather, they facilitate the identification of consumption profiles of different foods eaten by past populations". In other words, the stable isotopic signatures are representative of recently consumed (up to 10 years or so) foods that may have been slightly altered due to preparation of foods, individual metabolism, and local environmental changes as well as potential bias depending on subject samples (Bumbsted et al. 1985). Compared with other paleodietary and archaeological studies such as skeletal pathology, dental analysis, fauna and flora analysis including pollens and coprolites, chemical residues in pottery, and even art can create a greater picture of past communities and even individuals at several levels such as sex, status, and age throughout time (Tykot et al 2004). Paleodietary though stable isotopes play a unique and important role as a primary source of information on individuals within a community. Bumbsted et al. 1985 distinguishes between the archaeological investigations, as mentioned, as the "menu" while stable isotopes help to understand a representation of the "meal". Furthermore, food is a necessity that becomes a main component in cultural decision making such as location and distribution of settlements, population densities, technological innovations, and economical and political organizations. This study will focus on the paleodietary investigation of Coppa Nevigata and Bronze Age Croatian Sites (Gusica Gomila, Jukica Gomila, and Brnjica) through stable isotopic carbon and nitrogen analysis on collagen with the intention to reveal not only recently consumed food through proteins, but the greater connection between the Adriatic Bronze Age populations.

1.1 Overview and Objectives

This study focuses on the Bronze Age populations of Coppa Nevigata located in Apulia, Italy and the Dalmatian Croatian sites; Gusic Gomila, Jukica Gomila, and Brnjica. The location of the sites was chosen purposely to build an understanding of the potential connection between the Bronze Age communities of Apulia and Dalmatia across the Adriatic. There has been

archaeological speculation due to the abundant material finds and strong trading routes along the Adriatic that the populations from the East and West Adriatic may be connected more than economically but culturally as well. This study aims to concentrate on the differences and similarities of the diet and food technologies to contribute to the greater goal of connecting or disconnecting these populations with their surrounding environments. This study uses skeletal materials to carry out stable isotope analysis for dietary investigation on bone collagen. There has not been many paleodietary isotopic studies conducted in either Apulia or Dalamatia during the Bronze Age aside from a handful of sites (Tafuri et al. 2009, Tafuri et al. 2018, Lightfoot et al. 2014) and not a single study that focused on the relationship between these two regions. Therefore, this study is of interest to increase isotopic studies and their foundations in Bronze Age Italy and Croatia in relation to the greater Mediterranean and Balkan histories. This study has the potential to create interesting connections between two populations of Bronze Age communities as well as contribute to the broader dietary dilemma in the Mediterranean and Adriatic such as the consumption of aquatic foodstuffs and the introduction of millet cultivation (Varalli et al. 2015). The primary objective is to discern the food catchments of each of the populations mentioned. This will include assessing the dietary importance of terrestrial and marine proteins in the diet as well as identifying the distinct contributions of plant and animal products in the diet. Intra-population variations concerning sex, age and burial types will be difficult for the study at hand due to the limited variations within the populations but still will be considered during the results. The isotopic analysis will be complemented by archaeological background of the sites to create a comprehensive understanding of the economical and perhaps cultural choices in terms of diet.

The layout of the paper is as follows. Chapter 2 and Chapter 3 will provide basic background knowledge on structures of bones, the macronutrients (proteins, carbohydrates, and fats), and the principle foundations of stable isotopes, carbon and nitrogen. These chapters serve the purpose to give the reader the basic knowledge to understand the study. Chapter 4 discusses in detail the isotopic histories in terms of plaoditerary of Italy and Croatia. This will assist in identifying dietary changes throughout history and to see how this study can contribute. Chapter 5 speaks of the archaeological background of the four sites in this study. This would also mention any food technologies (i.e silos, combustion structures) and present fauna and flora found on site as potential foodstuffs. Chapter 6 will discuss in detail the materials and method conducted for this study. Chapters 7 and 8 will go into detail about the results and the discussion of the identified food catchments, how these populations relate to each other and the greater surroundings, and what hypothesis are made followed by the conclusion in Chapter 9. This last chapter will briefly mention the limitations of this study and future goals and present the concluding thoughts.

Chapter 2: Anthropology of Bones

A basic foundation of bone structure, the macronutrients important to diet, and tissues of the body important for isotope studies is needed for the understanding of this paper. The questions to be answered in this chapter includes; what is the structure of bones and teeth; what are the macronutrients and their role in the body; and lastly what is the relationship of stable isotopes in body tissue and diet analysis?

2.1 Bone Structure

Bone is a hard, mineralized connective tissue with an extracellular matrix composed of an organic and inorganic phase (Price et al. 2014). Dry bone is approximately 30% organic and 70% inorganic by weight. The organic phase is mostly (~ 90%) collagen type I defined as a fibrous structural protein. The remainder is noncollagenous proteins like hormones such as osteocalcin, filler proteins like proteoglycans, and lipids (collectively known as 'ground substances' within the organic phase; Katzenberg et al. 2008, Leethrop et al. 2008). The inorganic phase is known as bone mineral and is composed of crystals of calcium phosphate in mostly the form of hydroxyapatite ($Ca_{10}(PO_4)_6OH_2$) with small variations in calcium and phosphate salts. Hydroxyapatite is a form of biological apatite as a crystalline calcium phosphate mineral, or the "mineral salts" of the bone (Tykot et al. 2004; Leethrop et al. 1989; Steele et al. 2007). Most of the body's calcium and phosphorus is stored in the inorganic portion of bone and they have important roles in bones being a structural component and for other metabolic functions in the body (Steele et al. 2007). Collagen itself is about 35% carbon and 11-16% nitrogen by weight (Van Klinken et al. 1999; Price et al. 1994; Ambrose et al. 1990 reports values 15.3 to 47% and 5.5 to 17.3% respectively). Carbonate ions (example: carbonate CO_3^{-2}- or bicarbonate HCO_3^-) contains a few percent carbon and are substituted in the biological apatite in two positions; structural carbonate (which is a substitute of phosphate in the crystal), and absorbed carbonate (found at the surface of the crystal). Koch et al. 1997 explains that carbonate substitutes at the hydroxyl and phosphate sites of hydroxyapatite within the crystal lattice accounting for structural carbonate and carbonate can settle in hydration or amorphous zones of the surface accounting for absorbed carbonates. Other biological apatites can form during diagenesis, such as fluorapatite, when ions in hydroxyapatite exchange with ions in the surrounding environment postmortem at the hydroxyl site. Nitrogen is only found in the organic portion of the bone and not in the inorganic. These basic chemical aspects of bone tissue pave the way for the overall structural foundation.

There are two types of bone tissue; cortical and cancellous (fig. 1). Cortical bone (also called compact) accounts for 80% of total bone mass and forms the dense and hard outer surface of bone. Cancellous bone (also called spongy or trabecular) is porous and less dense with a greater surface area and forms the inside of bones. The molecular and cellular composition of both types are similar although the two types have metabolic differences. For instance, cortical bone contains Haversian systems, which are composed of haversian canals accompanied by Volkmann's canals (right angled canals connecting the haversian canals) and canaliculi (small channels connecting lacunae) which are surrounded by concentric haversian lamellae (parallel collagen fibers) which

forms a recognizably organized pattern and is responsible for creating a passage system for blood cells and nerves. Within each haversian lamellae there are small cavities called lacunae (connected via canaliculi) that houses an osteocyte (fig. 1). These Harvarian systems, secondary osteons, lie on points of mechanical stress on the bone (Steele et al. 2007). Primary osteons form during the beginnings of new bone while secondary osteons are a result of remodeling discussed shortly. Cancellous bone, being more porous, functions via blood vessels in the surrounding bone marrow cavity. Bone marrow is a tissue within cancellous bones that is the site where new blood cells are produced, especially in younger bone, and is referred to as "red marrow" and gradually with age turns into "yellow marrow" with more fat cell deposits (White et al. 2005; Steele et al. 2007). In sum, different types of channel and cavities work together to pass along building cells and nutrients through bones to assist in metabolic processes in an otherwise heavy mineralized environment. The interlocking of the organic and inorganic structure of bone allows it to survive for thousands of years as well as creating both tensile and compressive strength respectively. This structure allows bone to function as supporting units, maintain homeostasis of calcium, bone repair and maintenance, producing red and white blood cells, storing minerals, and allowing mobility (Price et al. 1994; White et al. 2005). Now that there is the structure for the bone; what are the bone forming cells?

The outer surface of bone is covered in periosteum and the inner surface is lined with endosteum and they are both osteogenic tissues that hold bone forming cells. (White et al. 2005). (It's worth noting that the periosteum also holds fibroblasts which are cells that synthesize collagen; Bourne et al 1976). Bone tissue is made up of several bone cells with the three main cells being osteoblasts, osteocytes, and osteoclasts (White et al. 2005; Steele et al. 2007; Bourne et al. 1976). Osteoblasts are single nucleated cells and are bone forming cells that collect near the periosteum and endosteum. They are responsible for creating a protein matrix, the osteoid, a permineralized bone. Calcification or mineralization of hydroxyapatite crystals are deposited via apatites (calcium and phosphates) from blood serum. The calcification process builds the inorganic portion of bone and surrounds and traps the osteoblasts thus creating the osteocytes. Osteocytes are collected in lacunae and maintain the bone tissue while those osteoblasts not trapped form a protective lining on the surface (located on bone surface at Howship's lacunae or the resorption pits; Bourne et al. 1976). Osteoclasts are multinucleated cells and remove or resorb bone tissue. This is the basis of bone remodelling (Bourne et al. 1976, Price et al. 1994). Primary osteons are resorbed by osteoclasts at the site of remodelling and osteoblasts fill in emptied tunnels made by osteoclasts in concentric rings of bone - the lamellae- leaving a space for blood vessels thus creating the haversian system, the secondary osteon. Now the bone is fully formed, but what were the steps to get there?

There are two main paths bone can travel to become fully formed. There is the endochondral bone and the intramembranous bone. Endochondral bone forms from the foundation of cartilage (another dense connective tissue made of collagen type II and is not mineralized and functions as support for bones) which is eventually replaced by bone, while intramembranous bone forms from mesenchyme connective tissue that is gradually mineralized (Steele et al. 2007). Both

types basically begin with the differentiation of cells into osteoblasts to form ossification centers creating different parts of bones. Endochondral bone are mostly long bones and vertebrae (White et al. 2005). Intramembranous bone makes up most of the skull, clavicle, and mandible. These mechanisms of ossification are just different ways to create all bones, although endochondral is mostly utilized. Bones in humans have immature beginning as woven bone that matures into lamellar bone (primary osteons that have less lamellae than secondary osteons). Lamellar bone is characteristically organized by repeated lamellae (parallel stacks) while woven bond is characteristically unorganized due to its rapid formation. The formation of bones (in absence of teeth which is out of the scope of this paper) has been discussed but what is the connection with diet and the macronutrients?

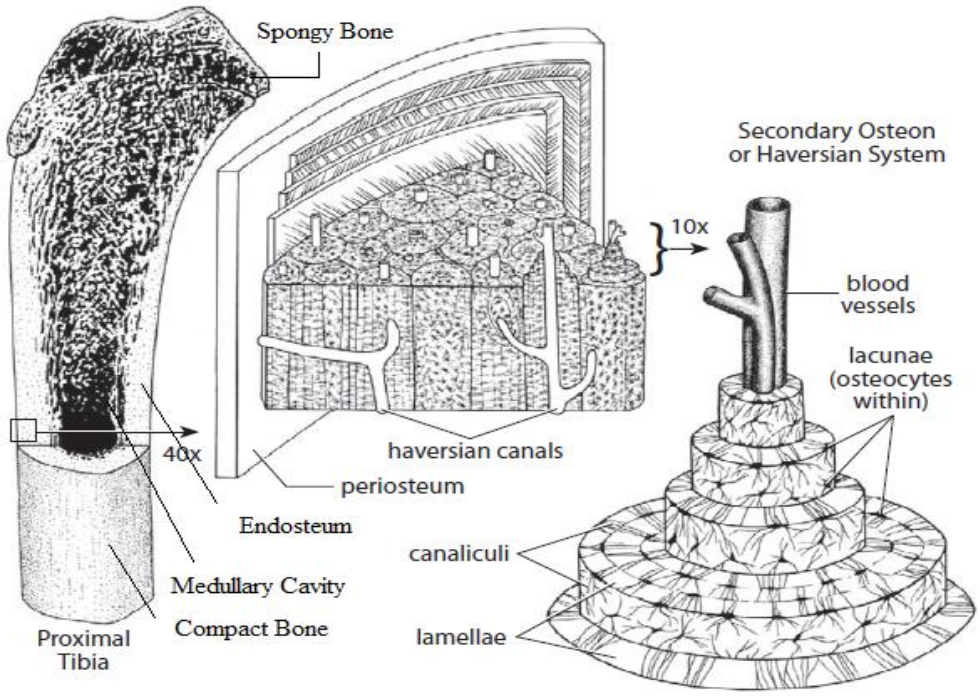


Figure 1: Structure of bones; Edited from White et al. 2005

2.2 The Macronutrients (Protein, Carbohydrates, and Fats)

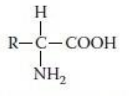
Digestion, absorption and metabolism of food converts chemicals of food into chemicals of the body. This energy exchange creates heat within the body and maintains body temperature (law of energy conservation; the body is dynamic and these process are never ending maintaining balance between energy used and energy gained). Energy is gained from oxidation and conversions of proteins, carbohydrates, and fats from foods and is transmitted via energy transfer compounds (like ATP) to essential macro and micro molecules that sustain all body functions and the remaining heat is radiated and excreted. Energy and metabolism follow similar steps in most mammals but difference occur due to length and capacity (especially in ruminants) of digestive systems, as well as different paths of synthesis (i.e fatty acid synthesis in humans occur in the liver while in a pig it occurs from fat depots) and can be understood better from this source; Hedges et al 2003. It is important to note, this is not a comprehensive review on metabolism (essentially every process in the body follows a metabolic path!) but an overview of some basic metabolic paths and how the macronutrients may be used in the body to better understand diet.

The three main macronutrients that play important roles in interpreting diet are the proteins, carbohydrates, and fats which are readily consumed when eating and contribute to the body in different metabolic ways. Proteins are the greatest contributors of the macronutrients and can be sourced from both plants and meats, with the highest sourcing derived from meats and animal by-products. In fact, proteins make up 18% by dry weight of the human body. Proteins are organic compounds consisting of Carbon, Hydrogen, Oxygen, sometimes Sulfur, and most importantly, Nitrogen. Nitrogen is only found in protein and can only be obtained by diet. In a metabolic steady state, nitrogen balance can reflect what may be happening within the body. For instance, nitrogen intake (from foods) should correlate evenly with nitrogen output (urea in urine or blood, sweat, etc). It could be assumed, that if there is a positive nitrogen balance, the body could be in growth or tissue repair and a negative nitrogen balance reflects a period of disease or malnutrition (see also Chapter 3.5.5). Proteins are synthesized in the cell nucleus via DNA that store information for the sequence of the particular protein. This information is read by mRNA and transported to the cytoplasm to react with ribosomes and tRNA where peptides are assembled and the protein forms. There are 20 types of amino acids in all. (fig. 2). There are essential and nonessential amino acids. What's important, is that essential amino acids cannot be produced by the body and must be gained through diet while nonessential amino acids can be synthesis in the body or consumed. These consumed amino acids, both essential and nonessential, are part of the basis for the overall cycle and synthesis of proteins. The basic formula for amino acids typically have a terminal carboxyl group (COOH) and an unsubstituted amino group ($-NH_2$) (proline is the exception with a substitute amino group) attached to the alpha-carbon as well as a functional group known as R that differs for each amino acid (fig. 2). Peptides are chain of amino acids folded in a particular sequence that creates a certain protein. These polypeptide chains determine the properties of the protein and are arranged by up to four different structures. The primary structure, determined by genes, is the basis of a polypeptide chain of amino acids connected via covalent peptide bonds. The secondary structure is linked by hydrogen bonds and can fold in several ways (alpha-helix or triple helix

helical arrays or pleated sheets). The tertiary structure is a combination of these folded chains and can be fibrous or globular and the quaternary structure is how these folded chains are arranged within a protein. Collagen is a fibrous protein which begins with 3 alpha-chain that assemble to form a procollagen triple helix that assembles together to form a collagen molecule apart of a greater collagen fiber (Fig. 6). These chains have over 1400 amino acids (every third being glycine, with the second and third most common being proline and hydroxyproline; Rose et al. 2008). Pate et al. 1998 mentions the atom to atom ratio of carbon to nitrogen in collagen is ~ 3:1 because of the frequency of glycine. Proteins have many functions (hormones, antibodies, carrier cells, etc), although they often are used as enzymes (catalysts that help create reactions). Proteins are digested in the stomach or small intestines (by gastrointestinal acids and enzymes) and they are absorbed by transport systems (protein carriers) into the intestinal tract to liberate the amino acids (hydrolyzed into amino acids or even further into keto-acids via transamination in the liver to generate energy for synthesis of glucose, fatty acids, and amino acids again; Berdanier et al. 2009; Rose et al. 2008). Although they are not technically stored in the body, amino acids not used for proteins can be deanimated and their carbon used for energy (i.e gluconeogenesis is an anaerobic process that creates glucose from noncarbohydrates like proteins such as alanine and glutamine. Thus some deaminated amino acids can serve as substrates for glucose synthesis effectively contributing to fuel and energy storage. This is important for sourcing discussed in Chapter 3). Because proteins are not stored, a low protein diet can lead to the breakdown of existing nitrogen in tissue which plays an essential role in diet sourcing, important for isotopic analysis discussed later (Chapter 3.3.2). What about carbohydrates?

Amino acid, carbon, and nitrogen contents of bone collagen

Amino acid	Percentage of amino acid in collagen	Percentage of carbon in collagen	Percentage of nitrogen in collagen
Aspartic acid[a]	4.4	4.61	3.69
Hydroxyproline[a]	8.9	11.65	7.47
Threonine[b]	1.7	1.78	1.43
Serine[a]	3.6	2.83	3.02
Glutamic acid[c]	7.4	9.69	6.21
Proline[a]	13.0	17.02	10.91
Glycine[a]	33.4	17.49	28.22
Alanine[a]	11.2	8.80	9.40
Valine[b]	2.5	3.27	2.10
Methionine[b]	0.5	0.65	0.42
Isoleucine[b]	0.9	1.41	0.76
Leucine[b]	2.3	3.61	1.93
Tyrosine[c]	0.3	0.71	0.25
Phenylalanine[b]	1.2	2.83	1.01
Hydroxylysine[a]	0.5	0.79	0.84
Lysine[b]	2.7	4.24	4.53
Histidine[b]	0.5	0.79	1.26
Arginine[c]	5.0	7.85	16.78



Basic Structure

Adapted from Ambrose (1993).
[a] Dispensable amino acid.
[b] Indispensable amino acid.
[c] Conditionally indispensable amino acid.

Figure 2: Amino Acids; Edited from Harrison et al. 2003

Carbohydrates in food are naturally occurring biomolecules formed by photosynthesis and for this reason are mostly sourced by plant material in the diet (also from milk containing disaccharide lactose). They are composed of Carbon, Hydrogen, and Oxygen. Their structure is in the form of polyhydroxy (hydroxyl and carbonyl groups COOH) aldehydes or ketones (fig. 3). Carbohydrates are either simple (monosaccharides) or complex (oligosaccharides or two to ten monosaccharides, and polysaccharides). Simple carbohydrates are commonly known as sugars, the most important being glucose, while complex carbohydrates include starches and cellulose (energy storage and structure for plants respectively). The structure depends on the degree of polymerization and each carbohydrate is connected by glycosidic linkage, a covalent bond. Carbohydrates can be a major source of fuel for the body and is readily consumed (energy or fuel is what is referred to as calories or kilocalorie that represents amount of heat required to make the temperature of one kilogram of water to 1C; Berdanier et al. 2009). Carbohydrates are also referred to as protein savers since they are used for energy instead of proteins (see also Chapter 3.3.2). Carbohydrates are digested starting from saliva in the mouth and broken down by enzymes in the intestines which results in the hydrolysis of the complex carbohydrates into its smaller components monosaccharides. Glucose($C_6H_{12}O_6$), the most abundant monosaccharide or simple sugar, eventually passes into the blood and acts as the body's preferred and universal fuel. Glucose has several metabolic paths. One main metabolic path is glycogenesis, anabolic reaction to create glycogen (polymer of glucose), a storage polysaccharide for animals (as starch is to plants). Glycogen is stored glucose in the muscle and liver. Once these storages are filled, excess glucose are converted to fatty acids and stored as triacylglycerols in adipose fat depots. Both these stored fats and glycogen can be oxidized later for energy. So what is the role of fats?

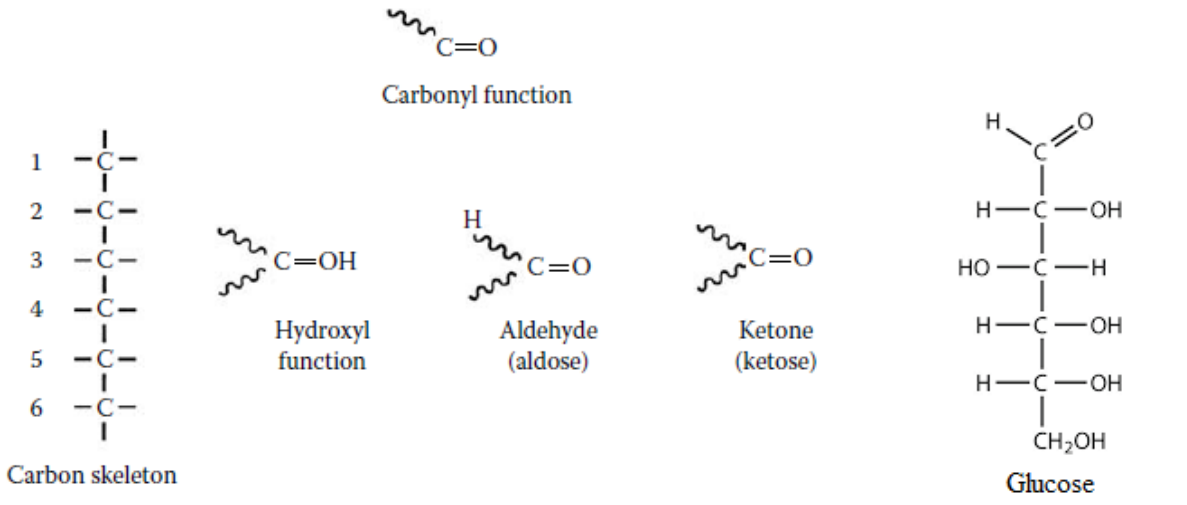


Figure 3: Structure of Carbohydrates; Edited from Berdanier et al. 2009

Fats are a form of lipids which are organic molecules composed of carbon, hydrogen, and oxygen. There are several lipids (cholesterol, phospholipids, glycolipids, etc.) but nutritional lipids are the simplest and are an ester of fatty acids with an alcohol which is a fat. Fat is triglycerides (also called tricylglycerols) which are an ester of a fatty acid and a glyceride (specifically three fatty acids linked to glycerol). Fatty acids are carboxylic acids with a carboxyl (COOH) and methyl group (CH_3) on either end of a hydrocarbon chain. (fig. 4) Fats can be unsaturated or saturated (animal fats are mostly saturated compared to plants and even less saturated is marine foods). Saturated or unsaturated depends on bonds (covalent single or double bonds respectively). Fats are a major source of fuel like carbohydrates and are both stored in the body for future use. Fats from diet are mostly derived from animal and animal product (although some nuts have high fat content; Berdanier et al. 2009, Rose et al. 2008). Fatty acid synthesis does not usually occur because diet provides the fatty acids but in a low fat diet, synthesis does occur in the liver and starts as acetyl-CoA that arises from oxidation of glucose or carbon skeletons of deaminated amino acids. Digestion of lipids begins in the mouth with saliva and is further separated by gastrointestinal acids and enzymes from the pancreas and bile acids from the gallbladder that breakdown lipids into free fatty acids (hydrolysis of triacylglycerol into free fatty acids and monoglycerides). They are further broken down and absorbed through a complex series of steps and eventually are transported by chylomicrons, a lipoprotein transport particle, which gets the dietary fats to the parts of the body needed (more on the breakdown of lipids in these sources, Berdanier et al. 2009; Rose et al. 2008). Their main metabolic path is beta oxidation. It begins with fatty acids converted into acyl-CoA (a coenzyme) through a catabolic reaction that releases acetyl-CoA used in the universally important citric acid cycle now discussed.

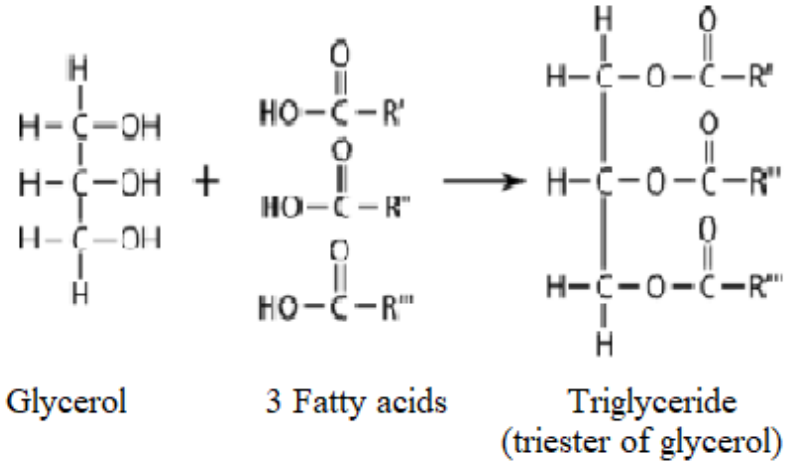


Figure 4: Structure of Fatty Acids; Edited from Berdanier et al. 2009

So how do the small components of the macronutrients (amino acids of proteins, glucose of carbohydrates, and fatty acids of fats) become metabolized in the body (Fig. 5)? Some individual metabolic pathways have already been discussed but ultimately all macronutrients contribute to the citric acid cycle. This cycle is simply a series of chemical reactions that releases energy that has been stored through the oxidation of acetyl-CoA derived from the metabolism of Proteins, Carbohydrates, and Fats into ATP (Adenosine triphosphate which is an organic chemical that mainly functions for energy transfer) and CO_2 (carbon dioxide). This cycle is important to all aerobic organisms. This is the catabolic path for all macronutrients and happens inside the mitochondria of a cell (oxidative phosphorylation occurs here where electrons that move from molecule to molecule during oxidation release energy in the form of ATP). The first step in the citric acid cycle must begin with acetyl-CoA (activated 2-carbon molecule mainly functions to deliver acetyl group to the citric acid cycle to be oxidized for energy) that is produced by glycolysis, fatty acid oxidations, and amino acid oxidation. Glycolysis is a catabolic reaction that converts glucose into pyruvate (a 6-carbon glucose broken into two 3-carbon molecules and produces ATP). The pyruvate is decarboxylated to acetyl-CoA and begins the cycle (Berdanier et al 2009; Rose et al 2008). The oxidation of fatty acids already discussed through beta oxidation. The oxidation of amino acids occurs in several ways. The first oxidative degradation of amino acids is the excess proteins not used for protein synthesis are catabolized or, during a poor diet, proteins are used for fuel. Both situations amino acids lose the amino groups and form keto-acids to produce CO_2 and water and the carbon skeletons of amino acids can be converted to glucose and enter the citric acid cycle (another metabolic path for excess deaminated amino acids is excretion through urine converting -NH_3 ammonia to urea and is important for isotope studies later on in chapter 3.5.5). The full cycle is not discussed here but can be better understood through these sources (Berdanier et al. 2009; Rose et al. 2008). In sum, all of these reactions, both catabolic and anabolic or energy producing and energy using, define the metabolic process and balance of the body to maintain function and cell life.

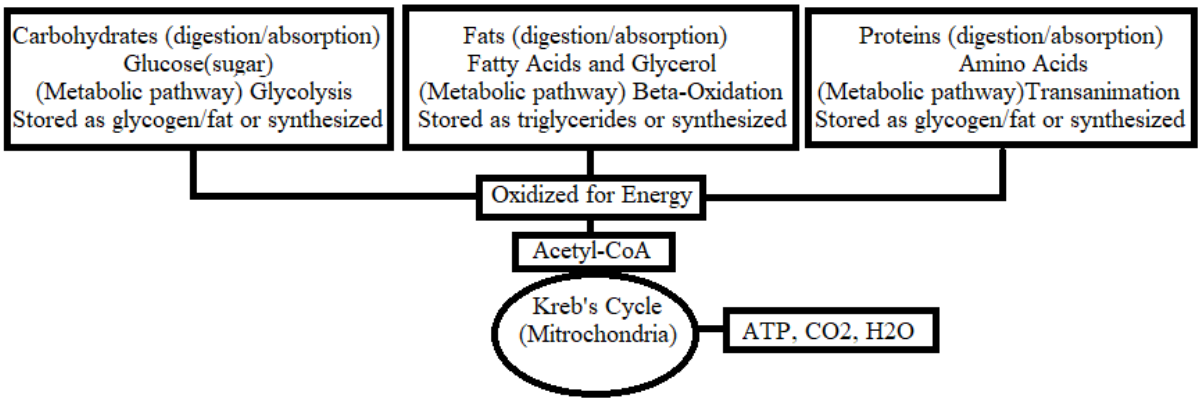


Figure 5: Summary of the metabolism of macronutrients

2.3 Tissues and Stable Isotopes

Isotopic values for various tissues in the same individual are affected by tissue composition, turnover rates, secondary fractionation, and synthesis of the tissue from different sources (Leethrop et al. 1989). Tissue selection of bones for paleodietary studies results by the ability of bone to preserve after long burial periods. The main tissue to be analyzed for stable isotope studies of human paleodiet is bone collagen. ("collagen" as in the proteinaceous residue from bone after treatments that might contain noncollagenous proteins Ambrose et al 1993). Due to degradation processes, bone mineral was taken upon paleodiet studies in the form of carbonates (CO_3), or biological apatite (Katzenberg et al. 2008). Some advantages of studying bone mineral include the analysis of much older materials where collagen is no longer observable and it has been understood that carbon for biological apatite records different dietary information than collagen alone. So the carbon in carbonate of bone has different information than carbon in collagen (nitrogen is only present in collagen). This idea was first proposed by Krueger and Sullivan et al. 1984 and was supported by Ambrose and Norr et al. 1993 and Tieszen and Fagre et al. 1993 in controlled feeding experiments. These researchers came to a conclusion that carbon in collagen is derived from ingested protein informing of dietary protein where carbon in bone mineral reflects the whole diet or the dietary energy sources. Collagen is composed of a mix of essential and non-essential amino acids from ingested proteins. The reason for this comes down to sourcing. Non-essential amino acids may come from ingested protein or formed from other dietary sources (collagen is disproportionately produced from protein of the diet; Tykot et al. 2004). Carbonate in bone is formed from dissolved bicarbonate in blood which comes from dietary carbohydrates, lipids, and protein. This is because almost all (~90%) of carbon atoms leave the body as respired CO_2 thus reflecting the ingested carbon. Respired CO_2 is said to be in equilibrium with blood bicarbonate (HCO_3^-) and assumed equilibrium with carbonate of bone (more about this distinction in chp. 3.3.2). Bone is a dynamic material that goes through remodelling. Remodelling is defined as the volume that has be resorbed or formed in a period of time. Collagen turnover is an effect of bone remodelling. While infants experience 100% turnover, adults experience about 18% turnover yearly (steele et al. 2007; Hedges et al. 2007). Collagen, especially for most long bones and ribs, have the slowest rate of turnover so ideally they can provide stable isotope information for the past 5-10 years of life. This study will only observe collagen for several reason. Collagen is well practiced, provides nitrogen values, and correlates with other isotopic studies done in Italy and Croatia. How are these tissues affected overtime?

2.4 Diagenesis

There are many different events that happen once a bone has been buried. It could be a chemical change, such as the exchange of ions from groundwater, or physical incorporations of material into the bone such as quartz in soil grains, charcoals, etc. (Price et al. 1992). These altering events are collectively termed diagenesis and they occur in bones post mortem. Hedges et al. 2002 lists several different diagenetic effects including, exchange of ions, leaching of collagen, microbial attack, infill with mineral deposits, increase crystallinity, dissolution and groundwater solute effects. After reviewing bone structure (chapter 2.1) it is understood that bones are porous, are composed of a matrix of collagen (organic, hydrophilic, and susceptible to decay) and hydroxyapatite (inorganic minerals that can be exchanged with the environment; Price et al 1992). These three factors are the main foundation of why diagenesis occurs.

As for collagen; Hedges et al. 2005 define diagenesis as the event when collagen is broken down and leached away leaving the remainder in a chemically degraded state mostly due to biological attack. Some contributors to diagenesis of bone include fungi, bacteria, and the exogenous organics like soluble humic acids. Collagen is hydrolyzed by acidic PH when mineral layer is solubilized and then actively degraded by microorganisms. Soil bacteria and fungi through collagenase destroy protein and mineral bonds and metabolizes specific amino acids which alter the $\delta 13C$ and $\delta 15N$ values (every amino acid has its own isotopic signature). In sum, there is selective biogenetic collagen break down by microorganisms (gradual breakup of collagen chain peptide bonds of amino acids; Van Klinken et al. 1999, DeNiro et al. 1984). Amino acids with high number of carbon atoms are lost preferentially from bone matrix (Ambrose et al. 2001). The most suitable conditions for good bone preservation are caves and temperate regions while arid and/or wet regions lead to worse bone preservation (Van Klinken et al. 1999; leethrop et al. 1989). Good collagen preservation was ultimately investigated by DeNiro et al. 1985 and Ambrose et al. 1990 who state the atomic C:N ratio should be within the range of 2.9 and 3.6. Anything outside this ratio indicates non-collageneous materials (Pate et al. 1994). Other conditional values include collagen yield and C and N content. Collagen yield is expressed as weight percent between 5-25% or higher than 10mg/g (Ambrose et al 1990; Ambrose et al 1993, Pate et al. 1994 says at least 1-2% of organics in original dry weight of bone is the cut off). C and N concentration in collagen expressed as weight percent is 13% for C and 4.8% for N (Ambrose et al. 1990, Ambrose et al. 1993, Van Klinken et al 1999 states content of %C 3-47% and %N 0.5-17% is the cut off range). These ranges refer to modern concentration so the closer to modern values, generally the better preserved. Ambrose et al 1990 explain how these values are determined. For instance, Collagen yields (weight % relative of whole bone) were calculated from weights of the dry bone samples against the freeze-dried collagen residues. Amounts of CO and N gas measured by the IRMS, provides C and N concentrations in "collagen" and the atomic C:N is determined by the C and N content.

The main instrument for assessing collagen preservation is the IRMS through the parameters mentioned (collagen yield, carbon and nitrogen content, C:N ratio). Two other instruments can potentially be used are AT-FTIR (Attenuated Total Reflectance-Fourier

Transform Infrared Spectroscopy) and SEM. DeNiro et al 1988 and Lebon et al 2016 notes by observing the amide I band (amide:phosphate) in AT-FTIR (through observing vibrational modes absorbed at different wavelengths revealing molecular structure), one can determine purity and presence of collagen. On the other hand, the use of IRMS for collagen preservation and quality is more well understood and reliable and AT-FTIR would require more bone sampling to produce powder. SEM (Scanning Electron Microscope; produces images and sometimes quantitative elemental results from measuring intensities of different particles after electron bombardment) can observe the presence of tunneling from microbial attack but it is not useful for isotopic analysis. This study is solely assessing collagen so IRMS and the usual parameters for diagenesis is suffice (details on methodology and instrumentation in chapter 6).

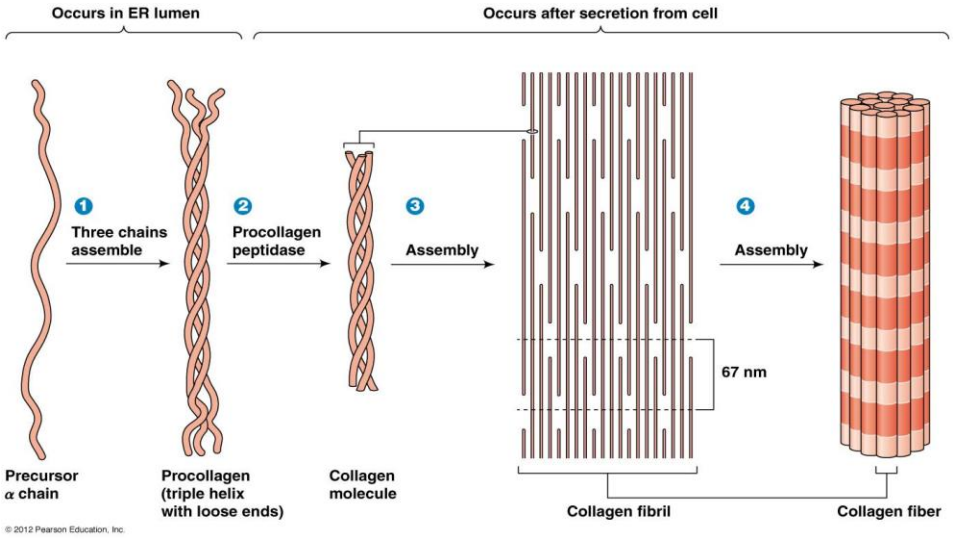


Figure 6: Structure of Collagen Fiber

Chapter 3: Carbon and Nitrogen Stable Isotope Analysis

Paleodietary studies relies on several fractionation paths of carbon and nitrogen taken throughout the food web. For instance, by comparing how plants and animals, the foodstuffs, obtain and process their carbon and nitrogen to human metabolism can help make inferences on diet. The first step is looking at how plants obtain carbon and nitrogen. Then, by looking at known herbivores who reflect isotopic values of local plants, assumingly, a basic stable isotope foundation is formed (i.e the baseline values). This is especially done at local and regional scales because the isotopic values of foodstuffs vary greatly. The following review will be solely on stable carbon and nitrogen isotopes and their role in paleodiet. Carbon was the first element in archaeological stable isotope variation studies due to familiarity with radiocarbon dating. Soon after, carbon studies were joined by stable nitrogen isotopes. The histories of this development of stable isotope studies of carbon and nitrogen reveals the path scientist took to help research like this one happen today, but what is a stable isotope?

3.1 Defining Stable Isotopes

Isotopes are atoms of the same element with the same number of protons but different number of neutrons in the atomic nucleus (fig. 7). Therefore, isotopes have the same atomic number since they have the same number of protons and share an elemental identity but differ in mass number. Mass is the number of protons and neutrons, so isotopes of an element vary in mass meaning different isotopes react slightly differently during physical and chemical reactions (Price et al. 2014). Chemical and physical properties are partly determined by the electrons so isotopes have the same properties since they have the same electrons (equivalent to number of protons in the neutral state where the negatively charged electrons are balanced with the positively charged nucleus), but mass in an atom controls some physical properties such as density and the vibrational energy of a nucleus which affects the reaction rate and bond strength, therefore accounting for the differences between isotopes (this is because of the unbalance between protons and neutrons changing the electrostatic forces in an atom causing a “mass effect”; Michener et al. 2008). Stable isotopes are called stable because they do not decay over time as unstable or radioactive elements do (this is because the shift in the number of neutrons is so great and the mass effect is high hence the atom decays trying to balance the number of neutrons or protons). For instance, a carbon isotope, carbon-14, is radioactive therefore unstable and decays into nitrogen-14 (this is the basis of radiocarbon dating). On the other hand, two isotopes of carbon, carbon-12 and carbon-13, do not decay and will remain constant in an organism. In chemical reactions such as photosynthesis (i.e. the conversion of atmospheric CO_2 and water into carbohydrates by plants) the amount of carbon-12 and carbon-13 differ in the plant tissue relative to the atmospheric CO_2. The difference is due to Isotopic Fractionation or a physical change that occurs during chemical reactions due to mass differences leading to the change of relative portions of isotopes (Katzenberg et al. 1989). In isotopic reactions (photosynthesis is a kinetic reaction that is unidirectional and is preferential of isotope forms) isotopically heavier forms will react more slowly and will be enriched in the isotopically lighter forms in the organism, just as plants are enriched in carbon-12 (Katzenberg et

al. 2008). As stated, the two stables isotopes of carbon are carbon-12 and carbon-13 while the stable isotopes for nitrogen are nitrogen-14 and nitrogen-15 with the lightest isotopes having the highest natural abundance. Carbon isotopes has a natural abundance of 98.89% for 12C and 1.1% for 13C. Nitrogen isotopes has a natural abundance of 99.63% for 14N and 0.37% for 15N. What kind of information can these isotopes reveal and how were they first used?

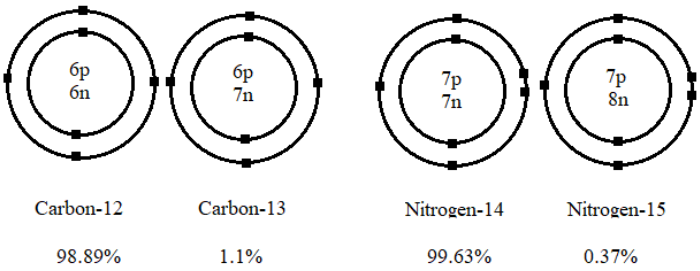


Figure 7: Carbon and Nitrogen Stable Isotopes (protons/neutrons) & Natural Abundance

3.2 Brief History

Stable isotopes were discovered in 1913 and most stable isotopes were identified by the 1930s. Throughout time, until the 1960s, isotope studies paired with mass spectrometry were used through various fields of chemistry and biology (Sharp et al. 2007). These studies focused on understanding the relative abundance of stable isotopes of various elements and the abundance ratios of these stable isotopes in different substances (Katzenberg et al. 2008, Price et al. 2014). Stable Isotope analysis accounts part of its origin in radiocarbon dating with studies being produced as early as 1960s (Bender (1968); carbon 13 variation in corn and grasses, Lowdon (1969); isotopic fractionation in corn). These studies opened up the pathway to paleodiet when variation in dates on organic remains such as maize were noted due to photosynthetic pathways (discussed Chp3. 3.3.1) producing varying quantities of carbon-14; by applying the same logic, similar behaviour was expected for carbon-13, with pioneering works on human ancient remains opening the way to a new line of investigation on paleodiet (Katzenberg et al. 2008). Stable carbon isotope variation in plants were first studied in the 1950s-1970s by researchers such as Craig (1954) who studied the relations between carbon-13 and carbon-14 variation in nature and Smith and Epstein (1971) who investigated 13C/12C ratios for plants. One of the first applications of stable isotope analysis for paleodiet reconstruction interpreted δ13C values of prehistoric peoples of North America in order to determine their consumption of maize (Vogel and Van der Merwe in 1977). Generally, the first studies for paleodiet using stable isotopes analysis were conducted around the 1970s-1980s including: DeNiro and Epstein (1978; 1981) who studied the influence of diet on distribution of carbon and nitrogen isotopes in animals through controlled feeding; Van der Merwe (1982) who focused on carbon isotopes, photosynthesis, and archaeology; Chisholm (1982) who studied carbon isotopes to differentiate marine and terrestrial based diet; Scoeninger and DeNiro (1984) who explored trophic level and regional variation of nitrogen isotopes; and Ambrose and DeNiro (1987) who investigated the trophic level variation in terms of diet in East Africa. (Katzenberg et al. 2008). The 1990s-2000s provided many different studies helping further

progress on ongoing questions on isotope fractionation, effects of metabolism, gender, age, difference between collagen and carbonate, isotopes and bone synthesis, etc. (Keegan et al 1989). How carbon and nitrogen are observed in paleodietary studies will now be discussed.

3.3 Carbon

3.3.1 Distinction Between C3 and C4 Plants

Plants follow different biochemical pathways of photosynthesis. This difference leads to a distinction known as C3 and C4 plant groups. A third plant group is referred to as CAM (Crassulacean Acid Metabolism) and has intermediate isotopic values compared to C3 and C4 because they tend to switch between the two photosynthetic pathways. The C4 (or Hatch-Slack) pathway metabolizes by diffusion of CO2 into the leaf by an initial conversion to a 4-carbon compound that incorporates 13C preferentially. C3 (or Calvin) pathway produces a 3-carbon compound and incorporates 12C preferentially (Van der Merwe et al. 1982). The outcome of either 3 or 4 carbon compounds once CO_2 has been incorporated into the leaf depends on how the plant fixes carbon through different enzymes. C3 and C4 plants use a carboxylating enzyme. C3 uses ribulose bisphosphate carboxylase and C4 plants uses phosphoenolpyruvate carboxylase (Price et al .1989; Michener et al. 2008). In sum, the different photosynthetic pathways evidently incorporate the heavier isotope, 13C, in C4 plant groups more so than in C3 plant groups. What are the plant groups? C3 plants include more temperate plants such as wheat, barley, rice, root crops, legumes, vegetables, trees and shrubs (woody, round-leafed species, 95% of all plants; Ambrose et al. 1993). CAM plants include cacti, agave, and bromeliads like pineapples. (Ambrose et al. 1990; Brothwell et al. 2005). C4 plants are mostly found in tropical regions, and includes maize, millets, sorghum, and sugar cane (grasses, sedges, and grains). Typically, C3 plants are browsed and C4 plants are grazed by animals (Hedges et al. 2007). What are the isotopic ranges typically found for these plant groups? The amounts of 13C and 12C in plant tissue is relative to the primary standard, atmospheric CO_2 (Fig. 8). In the past, atmospheric CO_2 had a δ13C value of -7‰ (with modern values of -8‰ due to burning of fossil fuels). C3 plants have an isotopic range from -20 to -35 per mil in δ13C values. C4 plants have an isotopic range from -9 to -14 per mil in δ13C values (Katzenberg et al. 2008; Brothwell et al. 2005; Hare et al. 1991). C4 plants have less negative values because they discriminate less against 13C. Alternate ranges are from DeNiro et al. 1978 that states δ13C isotopic values of -24 to -34 per mil for C3 plants and -6 to -19 per mil for C4 plants. Ambrose et al. 1993 claims and average of -26 and -12 per mil respectively. Furthermore, these values do not overlap and thus can be distinguished in diet. Another important distinction for carbon value is spacing which relates to how isotopes are affected in the body.

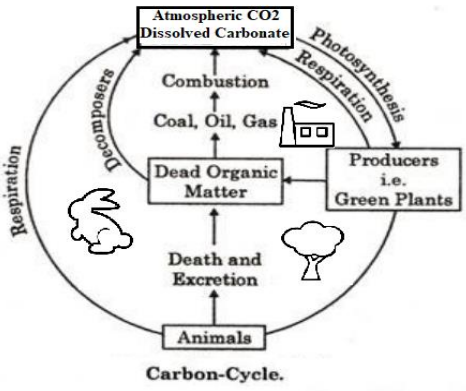


Figure 8: Simplified Carbon Cycle; Edited from Mondal et al. 2014.

3.3.2 Tissue Spacing

"You are what you eat (plus a few per mil)" is a common quote among diet studies that pertains to the diet and collagen spacing of carbon values. Bone collagen is around 5 per mil greater than δ13C values on account of secondary fractionation between collagen and the diet but why is that? Ambrose et al. 1993 discusses two possibilities for the carbon source in collagen. Carbon atoms of collagen may come from all parts of diet, referred to as the scrambled egg model, or only from dietary proteins, such as the essential amino acids, referred to as the routing model. If dietary protein is routed and is more negative (enriched) against the whole diet, the diet to collagen spacing will be smaller. Besides the diet to collagen (Δ13C d-col) spacing there is also the diet to carbonate spacing (Δ13C d-co) and collagen to carbonate spacing (Δ13C col-co). In the study mentioned by Ambrose and Norr (1993), the controlled feeding experiments showed the diet to collagen spacing was only 5 per mil when dietary protein, carbohydrates, and fats were from similar sources. Carbonate spacing will always be around 9 per mil regardless of sourcing. In other words, collagen and apatite are enriched by 5 per mil and about 9.4 per mil respectively when the sourcing for dietary protein and bulk diet are the same concluding that the apatite-collagen offset is 4.4 per mil (Price et al. 2014). Apatite is more enriched than diet ~ +9 per mil related to the equilibrium between gaseous CO2 and bicarbonates up until ~ +14 per mil with the larger value pertaining to larger herbivores in which fermentation is a part of their metabolic pathways such as rumaniants (Hedges et al 2003). The 5% per mil collagen rule can vary +1 to +5 per mil based on protein diets and animal size (Tykot et al. 2004; Hedges et al. 2005). But if the protein source differs from the carbohydrate and fat sources the spacing varies. So, spacing greater or less than 4.4 per mil between apatite-collagen indicates a dietary protein that is lighter or heavier of δ13C values to that of the whole diet (Price et al. 2014; Harrison et al. 2003). An example of a spacing higher than 4.4 per mil is a diet of C4 carbohydrates and C3 protein. C4 plants have higher δ13C values so therefore dietary protein would be less enriched in δ13C than the whole diet and the spacing will increase but if dietary protein derived from marine source, for example, and C3 carbohydrates, the spacing will get smaller because dietary protein would be more enriched in δ13C than the whole diet. Collagen carbonate spacing varies from 4.4 per mil up to 7 per mil between apatite and

collagen (price et al. 2015, Kruger & Sullivan et al. 1984). Spacing values are still a working theory and is based on a few assumptions (Tieszen et al. 1993). So how does this spacing relate back to the two source models? First, is the foundation of amino acids in the body. The routing of carbon to collagen is thought to be dependent on the relative proportions of essential to non-essential amino acids. Essential amino acids make up 12% of collagen but 18% of its carbon atoms (this is because essential amino acids have an average of 6 carbon atoms per molecule while non-essential amino acids, comprising of 44.6% of carbon in collagen, have 2 or 3 carbon atoms). The theory states, on low protein diets only 18% of carbon from diet can be obtained from dietary protein (or in other words this 18% is the minimum routing of carbon while the rest of carbon atoms can be synthesis from non-essential amino acids produced *de novo* from carbon of carbohydrates and lipids). On low protein diets carbon atoms can be taken from all macronutrients (the scrambled egg model) for collagen synthesis. But otherwise collagen carbon could just incorporate carbon from dietary proteins (routing model) and in this case the whole diet including the fuel (carbohydrates and fats) is not represented.

It's already been said that an average of +5 per mil for diet to collagen and +9 per mil for diet to carbonate spacing has been assumed and that it most likely relates to where the protein and energy (carbohydrates and fats) are coming from in terms of diet. Kruger and Sullivan (1984) and Lee-thorp (1989) attempt to explain collagen to carbonate spacing from a slightly different perspective. The argument is that collagen carbonate spacing changes with trophic level because different macronutrients have different importance. Herbivores derive protein from plant proteins and from carbohydrates through the transamination of keto acids. Herbivores derive energy from carbohydrates. Carnivores derive protein from meat and energy from lipids and excess proteins. In their experiment, they concluded that there is a +7 per mil collagen carbonate spacing for herbivores (assume the carbonate diet spacing is apatite +12 per mil minus collagen diet spacing +5 per mil) and +3 per mil spacing for carnivores (assume the diet to collagen spacing of the animal eaten, +5 per mil, against the negative value of lipids). These values fall within the purposed average spacing mentioned previously. In both the diet to collagen spacing and collagen to carbonate spacing it is a question whether carbon in protein is routed or scrambled and is carbon for carbonate routed or scrambled? It's assumed that bone carbonate derives from blood bicarbonate (produced during cellular metabolism). Almost all (~99%) of carbon atoms leave the body as respired CO_2 and if respired CO_2 is in equilibrium with Blood bicarbonate which is in equilibrium with carbonate in a metabolic steady state should have the same δ13C values (Jim et al. 2004). Therefore, carbonate assumes the scrambled model. Thus, carbonate takes from the total metabolic carbon pool which also means the diet (remember protein carbons as well can contribute to carbohydrate and lipid synthesis discussed in chapter 2.2). Carbohydrates and lipids are mostly used for fuel while protein is used for synthesis, but on low protein diets carbohydrates and fats can be used for tissue synthesis. This is juxtaposed to how trophic level plays a role in collagen and carbonate spacing. For herbivores, collagen and carbonate come from isotopically similar sources (isotopic makeup of carbohydrates and proteins are similar, while lipids are more depleted but make a small contribution. In terms of carbon values; protein>carbohydrates>fats.). Carnivores

on the other hand intake much more lipids and proteins. Since lipids are known to be more isotopically depleted (~6 per mil more negative) than proteins and play a major role in diet compared to herbivores, there spacing should be smaller hence the assumed percentile +7 per mil and +3 per mil. Although the metabolic understanding of these spacing is still not fully known there is a general consensus that collagen follows a routing path and reflect the protein portion of the diet while apatite carbonate follows a scrambled path and reflects the whole diet (Ambrose et al. 1993, Jim et al. 2006, Jim et al. 2004, Hare et al. 2004, Lee-thorp et al. 1989, Tieszen et al. 1993).

3.4 Nitrogen

3.4.1 Distinction Between Nitrogen Fixing and Non-fixing Plants

Nitrogen composition in plants varies on several levels including the type of nitrogen obtained, how it was obtained, and where it has been measured (fig.9). Firstly, there are two paths that plants obtain their nitrogen. The first is by symbiotic bacterial fixation using atmospheric nitrogen and second is directly from nitrates in the soil. Nitrogen fixing plants have a symbiotic relationship with bacteria, such as the genus *Rhizobium.* Bacteria live in the roots and fix nitrogen so the plant can access it in exchange for carbohydrates and produce ammonia for metabolic functions. Atmospheric nitrogen (N_2) is the primary standard (isotopic values are relative to this standard) and has a value of 0‰ Non-fixing plants get their nitrogen from decomposed organic matter in the soil as either ammonium (NH^{+4}), nitrite (NO_2^-) or nitrate (NO^3) and thus relies on soil conditions. This depends on the level of nitrification or the oxidation of these compounds by bacteria and result in more enriched δ15N values (Schoeninger & DeNiro et al. 1984). The main distinction is between legume and non-leguminous plants. Legumes (fixing) have δ15N values close to atmospheric nitrogen (0‰) and non-leguminous plants (non-fixing) have more enriched (higher) δ15N values. In other words, non-fixing plants who obtain nitrogen from soils are isotopically heavier or more enriched in 15N than nitrogen fixing plants who take directly from the atmosphere and have values closer to 0‰. Generally, terrestrial fixing plants range in δ15N values from -2 to +2‰ and non-fixing from 0 to +6‰ with a mean of +1‰ and +3‰ respectively (Pate et al 1994; Ambrose et al. 1991; Schoeninger & DeNiro et al. 1984). In terms of diet studies, an important occurrence to note is that nitrogen values are kept low by legumes (or any low nitrogen food source) due to their nitrogen intake (Fraser et al. 2013). This means that the isotopic values measured are a balance between the isotopes of the food sources and as such some foods can hide under isotopic radar if not consumed enough.

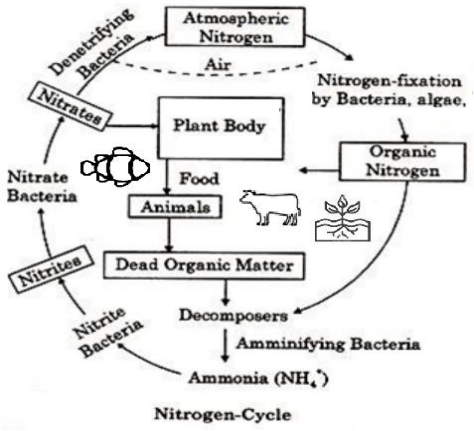


Figure 9: Simplified Nitrogen Cycle; Edited from Mondal et al. 2014.

3.5 Isotopic Theories Applied to Diet Studies

The basics of carbon and nitrogen stable isotopes and their role in diet has been explored. Next, the foundation of these values will be applied to several theories of how to determine diet.

3.5.1 Trophic Level Distinction

Nitrogen and Carbon isotopic values are related to trophic levels and can determine if humans ate mostly a plant based diet or animal based diet. The type of animal protein cannot be distinguished in terms of stable isotope studies and is defined as terrestrial animal protein (meat or milk; Hedges et al. 2007). The δ15N values increase, become more enriched, by about 3-5‰ up the food web (fig. 10). For instance, Herbivores δ15N values are roughly 3‰ higher than δ15N of their diet, the plants (keep in mind herbivores consuming legumes will have lower δ15N values than consuming non-leguminous plants due to nitrogen fixation). Similarly, Carnivores are enriched in δ15N by 3‰ than their diet, the herbivores. This is the principle of enrichment. Minagawa and Wada (1984) and Schoeninger and DeNiro (1984) explains that the principle of enrichment refers to the increase of nitrogen values while successively moving up the food web. Marine foodstuffs tend to reflect some of the highest nitrogen values and will be discussed in detail in 3.5.2. Nitrogen trophic level effect is believed to relate to metabolic process as a result of amino acid transamination and deamination (chemical reactions that creates and destroys new amino acids and involves transference of nitrogen from one amino acid to another). Basically there is δ15N enrichment in some amino acids and depletion in others involved in protein metabolic events although this is still not fully understood (Ambrose et al. 2001; Macko et a.l 1986; Hare et al. 1991).

Carbon isotopes can also reflect trophic level by shifts of ~1‰ (0‰ enrichment is assumingly vegetarian diet; Bocherens et al. 2003). From plants, to herbivores, to carnivores, then to marine, carbon values tend to become less negative as they become more enriched in the heavier isotope 13C (price et al. 2014). Why is there a general 1‰ δ13C increase in trophic level? Because of preferential uptake from tissue. For instance, animal tissues from brain, collagen, hair, fat, milk,

all have slightly different carbon ranges (DeNiro et al. 1978, Ambrose et al. 1991). This caution can be noted because variation occurs in the trophic level effect in different tissue within the same organism and among different taxa. Therefore, trophic level effect would be more pronounced in carnivores than herbivores due to the increase from tissue difference in biochemical composition. Plant parts, stems versus leaf for instance, also vary but it is said botanical elements average to the value of the carbohydrates δ13C content. These variations are not fully understood including their effect on diet studies. The connection between metabolism and secondary isotopic fraction patterns is not completely clear but there is a general assumption that the fractionation occurs kinetically and remains constant within humans (Tykot et al. 2004; DeNiro and Epstein et al. 1981; schoeninger et al 1984).

3.5.2 Distinction Between Marine and Terrestrial Foodstuffs

Different pathways taken by terrestrial and marine organism to obtain their carbon and nitrogen has been discussed, but is there a way to distinguish in the diet? The main carbon source for marine plants/animals is dissolved CO_2 which has a δ13C value close to 0‰ whereas the main source of carbon for terrestrial plants/animals is atmospheric CO_2 and has a δ13C of -7‰ (Pre-industrial; modern is -8‰). Therefore, its assumed that animals that consume only marine protein have a more enriched δ13C value (roughly 7% per mil heavier) than those that only consume terrestrial protein. Generally, diets based on C3 plants have δ13C values of around -20‰ and marines or C4 plants have values around -10‰ (price et al. 2014). There are various alternate ranges for pure diets reported which depends on the local study at hand. Chisholm et al 1982 reports pure feeders of a terrestrial diet with -20‰ and marine diet with -13‰. Schoeninger & DeNiro et al. 1984 reports pure feeders of terrestrial diet with -18.9‰ and marine diet with -13.0‰. Hedges et al. 2007 reports pure feeders of terrestrial diet with -20‰ and marine diet with -12‰. Because C4 plants overlap in isotopic values with marine food sources, carbon values alone should not be considered for this distinction in the presence of C4 plants and must to be paired with nitrogen values (Schoeninger & DeNiro et al. 1984). For Nitrogen, marine plants/animals have typically higher δ15N values than terrestrial due to denitrification and trophic level effect. Firstly, the fractionation of nitrogen is a balance between microbial fixation (fixing plants) and denitrification (non-fixing plants) in the biosphere (or the reverse, nitrification, which reduces back into atmospheric nitrogen N_2 by an anaerobic process by bacteria). While the δ15N values range between +1 to +6 per mil for terrestrial plants as mentioned, the values for marine or aquatic plants range from +5 to +10 per mil due to heavier denitrification in the water (aquatic plants usually follows fixation path with help of cyanobacteria and blue green algae). Secondly, It's understood that marine organisms have longer tropic chains which also accounts for the higher δ15N values depending on available food sources (example, carnivores fish have higher δ15N values than phytoplankton). Schoeninger & DeNiro (1984) reports collagen δ15N value of +1.9 to +10.0 per mil for terrestrial mammals and +11.7 to +22.9 per mil for marine organisms for example. Notice how the values do not overlap and can help distinguish marine versus terrestrial foodstuff in terms

of C4 plant presence with comparison of carbon values. For the information already discussed, it can be assumed that C4 plants, almost always non-leguminous, are non nitrogen fixing plants and therefore have lower δ15N values whereas marine foodstuffs have typically higher δ15N values. An assumption can then be made if the results of the mammals observed are enriched in δ15N than expected based on local vegetation then perhaps marine foods were consumed. Bocherens et al 2003 and Hedges et al 2007 is in support for this distinction and notes that fish protein is more enriched than meat/milk by 6‰ in δ15N values that were averaged between literature values of 2.3-8.1‰; with the lowest value pertaining to freshwater fish. In fact, there is an isotopic difference between marine and freshwater species as well.

3.5.3 Distinction Between freshwater and Marine Foodstuffs

Carbon and Nitrogen values can vary greatly depending on local freshwater and marine sources. This is because freshwater plants have many sources of carbon like atmospheric CO_2, bicarbonate (HCO_3^-) and carbonate (CO_3^{-2}) from rocks and soils, and organic carbon from decomposing animals plants and particulate matter (Zohary et al. 1994). Generally, aquatic plants/animals from fresh lakes and rivers have more terrestrial-like δ13C values than marine plants/animals although both would produce higher δ15N values than terrestrial plants/animals. (katzenberg et al. 2008) Although this is not taking into account naturally low nitrogen source plants/animals such as seaweeds or low trophic species and carnivorous fish that experience trophic level effect and have a slight increase of δ13C values (Hedges et al. 2007). One idea is that if C4 plants are not in the area of the humans under study but the δ13C values are higher (more enriched) than expected perhaps freshwater fish is consumed. Katzenberg et al 2008 speak in more detail about this distinction. It states that δ13C of fish bones ranges between -14.2 to -24.6 per mil with more negative values (depleted in δ13C) for shallow water fish than deep ocean fish which may account for the more terrestrial like values for freshwater fish compared to marine but this is highly variable and depends on local species and environment. An argument has been presented that at least 20-25% of the total protein diet must come from aquatic source to be seen isotopically in collagen because of the slight increase in δ15N (reports of 0.7 per mil increase to a diet pertaining 20% fish protein intake, Hedges et al. 2007; Katzenberg et al. 2008).

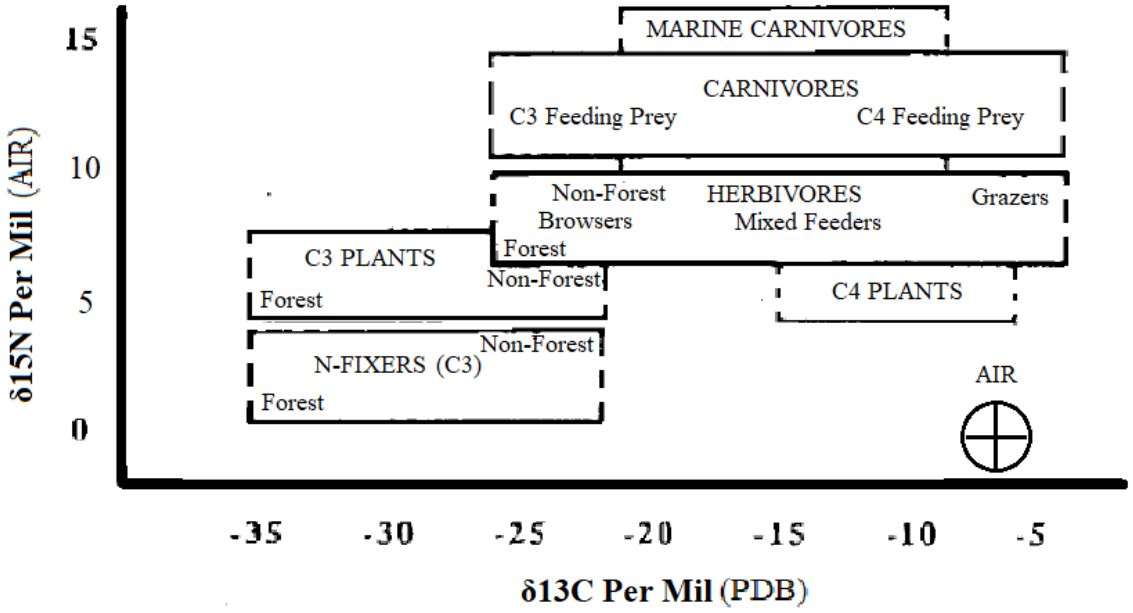


Figure 10: Averaged Values of Foodstuffs; Edited from Keegan et al. 1988

3.5.4 Effects of Breastfeeding and Weaning

Weaning is defined as consuming non-milk foods but not the complete cessation from breastfeeding. Breastfed children have δ15N values approximately 3‰ higher than their mothers. This is due to the trophic level effect. One way to utilize stable isotopic studies in order to assess breastfeeding in young children is by using nitrogen isotopes. The δ15N values reveal when there is a loss of breast milk in the diet and the transition to complete cessation. Fogel (1989) was one of the first studies to use nitrogen isotopes in this way and it was based on the assumption that nitrogen values increase by 2-3‰ through the trophic level. Through this, the age of weaning can be determined as well. Nitrogen values should decrease sharply once breastfeeding has stopped. Caution is noted that the ingestion of breastmilk does not show immediately and can take up to 3 months to show isotopically due to bone turnover rates (discussed in chapter 2.1 and 2.3; Katzenberg et al. 1996). Carbon isotopes can also contribute alongside nitrogen analysis to understand breastfeeding. For instance, a 1‰ increase of carbon values for children roughly 1 years old could be a sign of cessation due to introduction of solid foods. This is a gradual process and can take time to effect isotope values but the idea is the gradual decrease of nitrogen values and increase of carbon values in childhood. One caution for applying breastfeeding and weaning is that children who have died young could be affected by nutritional stress that can affect nitrogen levels. Nutritional stress in one of a few cautionary exceptions that may be considered.

3.5.5 Some Exceptions

This section is devoted to noted exceptions such as isotopic sensitivity to climate, precipitation, altitude, and landscape such as the "canopy effect" as well as to nutritional stress, either from lack of water or lack of nutrients, and anthropogenic effects of farming like the use of manure. To begin, "canopy effect" is known to produce more negative carbon isotope ratios compared to average values of C3 and C4 plants (mentioned in chapter 3.2). This is because of incomplete atmospheric mixing due to heavy forested area and less light intensity. There is a reuse of fractionated respired CO_2 among the plants, and decomposing plants at the ground of the canopy, which allows the δ13C of atmospheric CO_2 values to raise between -21 and -26 per mil (Ambrose et al. 2001; Keegan et al. 1989). There is typically a 3-4‰ more negative δ13C values near the ground thus reducing δ13C (Tykot et al. 2004; Heaton et al. 1999). In this case, different species from different ecosystems like a deer in a forested habitat vs cow in an open field should be considered with the isotopic values of local food webs. In fact, it has been noted that animals feeding on canopy floors have δ13C values up to 5 per mil lower than animals elsewhere or have access to the upper canopy (Ambrose & DeNiro et al. 1986).

In term of climate, the δ13C values change because of stomatal conductance in plants. Plants can either open or close their stomata on account of the climate affecting overall isotopic values (stomata, leaf pores, during high temperature and low humidity close to conserve water and conversely when stomata are open or have a high conductance there is low water use efficiency that lowers the rate of photosynthesis creating more negative values; Ambrose 2001; Pp 39-58). Nitrogen values plays an important role as well. Ambrose (1991) study illustrates that δ15N values are sensitive to climate and are typically elevated in arid regions. Generally, temperate forest soils have low δ15N values compared to desert type or tropical environments which have higher δ15N values. This is in relation to aridity and the N-cycle. It's understood that nitrogen fractionation such as nitrification, mineralization (organic nitrogen convert into ammonium by bacterias), denitrification, have great effects on N-loss leaving δ15N enrichment in the soil and plants. Furthermore, arid and hot ecosystems are prone to N-loss, therefore δ15N enrichment, then wet and cold environments that conserve and recycle nitrogen through the nitrogen pools more (Szpak et al. 2014; Ambrose et al. 1993; Martinelli et al. 1999) Ambrose et al. 1991 points out δ15N values in plants of more arid regions can change as well with values ranging as high as +13‰ probably because of how nitrate is distributed in the soil. Along with this come salinity of soils and presence of organic materials which would also have higher δ15N values (examples of areas with history of evaporation or have high animal residue like manure discussed shortly; Larsen et al. 2016). Saline environments also raise δ15N values related to higher content of soil nitrate and ammonium (Pate et al 1994). Juxtaposed to aridity and soil condition is Precipitation that also plays a role in changing nitrogen levels. Heaton et al. 1986 suggests a negative correlation of averaging less than 400mm of rain per year, can effectively increase δ15N values on the same assumption of water availability and aridity (~ δ15N 10 to 13 per mil). Lastly, altitude has shown to produce lower δ15N values by a few percent with increasing altitude on the assumption of the changes in

precipitation and temperatures with changing altitude. (Liu et al. 2010; Schoeninger & DeNiro et al. 1984).

Ambrose and DeNiro (1986) speak about the nitrogen loss in urea excreted in urine. Urea (an organic compound that yields from the breakdown of amino acids and is the main excretion of nitrogen from proteins and the body mostly via urine ~76%) is depleted in δ15N compared to diet (~2 to 5‰; Ambrose et al. 2001). When there is the condition of water stress, when the organism is not drinking enough water, more urea is excreted therefore lighter isotope 14N is lost (this is because the body tries to store remaining water in the kidney successful producing more urea). This leaves higher 15N values left in the body for tissue synthesis. This should be taken into consideration when observing if a human had a marine diet or it's due to water stress experienced by terrestrial animals in the environment. Elevated 15N can also be caused by protein stress. The idea here, is that there is more intense recycling of nitrogen already present in the body into the synthesis of new proteins that will evidently form collagen. Insufficient protein forces the body to breakdown and utilize existing nitrogen and becomes enriched in δ15N because of preferential transamination and deamination of isotopically lighter amino acids due to more intense recycling of nitrogen (Chapter 3.3.2; Ambrose et al. 2001, Ambrose et al. 1991). Both water stress and protein stress enrichment of δ15N is in relation to the nitrogen mass balance the body must retain (discussed in chapter 3.4) when the excretion is depleted in δ15N the body is enriched in δ15N.

The "standard model" proposed by Hedges et al. 2007 states that humans only eating plant proteins share the same δ15N values as local herbivores and those eating animal proteins have values 2-5 per mil more enriched than herbivores (trophic level effect). The limitation here is when human cultivate and consume crops other than the general vegetation shared with herbivores. In relation to this is the "manuring effect". Animal manure, or organic manure made from animal waste, is used to improve soil fertility and raises δ15N values of terrestrial food stuffs (~5 to 15 per mil increase is said by Ambrose et al. 1993 and DeNiro et al. 1985). Bogaard et al. 2007 state that δ15N values raise from manure and cultivation because of the preferential loss of the lighter isotope nitrogen-14 through volatile gaseous ammonia (nitrogen in urea from animal manure goes through volatilization and leave the soil by a metabolic reaction by bacteria in the soil) while residual ammonium and nitrate is left behind and is more enriched in the heavier isotope 15N (Fraser et al. 2013; Szpak et al. 2014). Ammonium goes through denitrification into nitrites and nitrates that are enriched in δ15N and is taken up by plants and account for the metabolic biosynthesis of amino acids in plants that evidently contribute to bone collagen in animals and humans (Ambrose et al. 2001). Szpak et al. 2014 also notes different types of animal manure or animals of the same species fed different fodder can produce different levels of δ15N by various metabolic fractionation reactions but generally in all cases δ15N values are higher. The caution to note here is that manuring effects can give the impression of trophic level shift in terms of diet studies and must be taken into consideration. Fraser et al. 2013 goes on to state that in mixed economy communities where there is both farming and pastoralism, there can arise a situation where humans consuming domesticated grains might provide fodder for the animals of the by products that are usually more enriched slightly in δ15N and can give the appearance of trophic

level shift. For instance, cereal grains like wheat and millet seeds tend to be more enriched in δ15N over whole leaves and stalks. Plant remains are not typically used for isotopic analysis due to limitations of finds but DeNiro et al. 1985 experiments with prehistoric and modern plants suggests that carbonized plants able to still be recognized morphologically varies roughly 3‰ from non-carbonized and is not as altered by diagenesis as non-carbonized plants. Isotopic values for both C3 and C4 plants are always beneficial for paleodietary to understand baseline values for the site and period.

Table 1: Summary of Values

Type	Range
Carbon and Nitrogen in Collagen	Carbon 35% (15.3 to 47%) Nitrogen 16% (5.5 to 17.3%)
C3 Plant Range	Carbon -35 to -20 Per mil
C4 Plant Range	Carbon -14 to -9 Per mil
Nitrogen Fixing Plant Range	Nitrogen -2 to +2 Per mil
Non-Fixing Nitrogen Plant Range	Nitrogen 0 to +6 Per mil
Terrestrial and Marine Plant Diets -Freshwater plants intermediate values -C4 plants and Marine values overlap	Terrestrial Nitrogen +1 to +6 Carbon -35 to -20 Per mil Marine Nitrogen +7 to 11 Carbon -15 to -4 Per mil
Terrestrial and Marine Animal Values -Freshwater animals intermediate values -C4 values do not overlap	Terrestrial Nitrogen +1.9 to +10.0 Carbon -22 to -11.9 Per mil Marine Nitrogen +11.7 to +22.9 Carbon -16.4 to -9.6 Per mil
Diet to Collagen Spacing	Carbon +2 to + 5 Per mil
Diet to Carbonate Spacing	Carbon +9 to +12 Per mil
Collagen to Carbonate Spacing	Carbon +4 to +7 Per mil
Trophic Level Effect	Carbon +1 Nitrogen +3 Per mil
Cautionary Values	Canopy Effect Depleted d13C Low Rainfall Elevated d15N Aridity and Salinity Elevated d15N Altitude Depleted d15N Water/Protein Stress Elevated d15N Anthropogenic Organic Manure Elevated d15N

Chapter 4: Isotopic History

This chapter will describe in some detail the previous paleodietary studies done in Italy and Croatia as well as some archaeological context of the Bronze Age period. This is to create a sense of familiarity of diet trends and cultural trends in these regions and to see what type of contributions this study can make in comparison to their neighboring communities.

4.1 Isotopic History of Italy

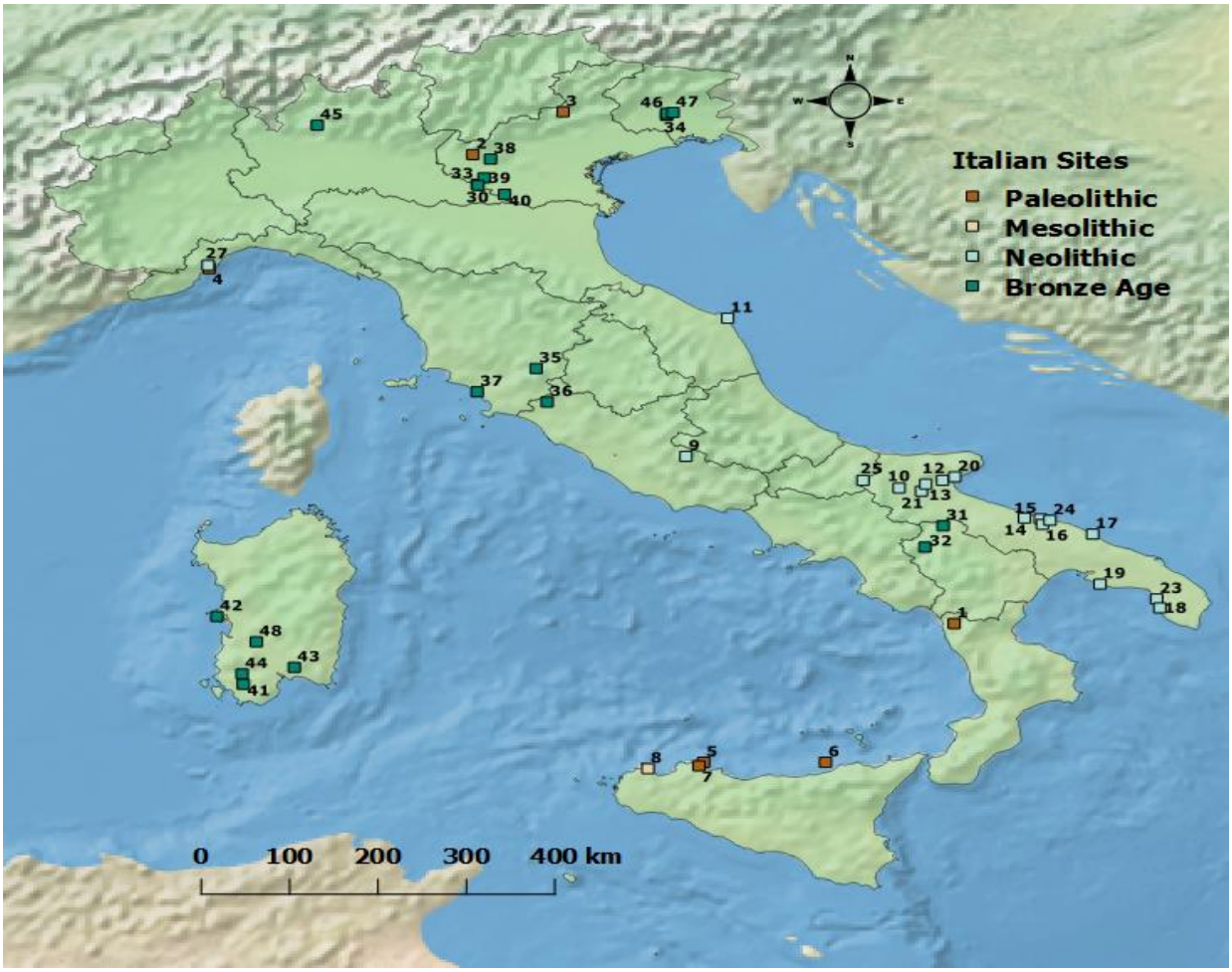


Figure 11: List of sites mentioned: 1.Grotta del Romito 2, Riparo Tagliente 3.Villabruna 4.Arene Candide 5.Addaura 6.San Teodoro 7.Grotta della Molara 8.Uzzo Cave 9. Grotta Mora-Cavorso 10.Rippa Tetta 11.Portonovo 12.Masseria Candelaro 13.Passo di Corvo 14. Palata 15.Masseria Maselli 16.Balsignano 17.Grotta delle Mura 18.Samari 19.Torre Castelluccia 20.Grotta Scaloria 21.Poggio Imperiale 23.Serra Cicora 24.Bari (S.Barbara, C.Colombo, Malerba, Cala Scizze) 25.Occhito 27.Liguria (Pollera, Pian del Giliegio, Gabru Surdu, Bergeggi) 30.Dossetto di Nogara 31.Lavello 32.Toppo Daguzzo 33.Olmo di Nogara 34.Sedegliano 35.Felcetone 36.Grotta Misa 37.Grotta dello Scoglietto 38.Arano di Cellore 39.Bovolone 40.Fondo Paviani 41.Montessu 42.Is Aruttas 43.Iscalitas 44.Concali Corongiu Acca 45.Ballabio 46.Gradisca di Codroipo 47.Merto di Tomba 48.Sedda sa Caudeba.

4.1.1 Before the Bronze Age (25,000-3,000 B.C)

This section will briefly mention the paleodietary studies conducted in Italy from the Paleolithic until the Neolithic. Although out of the scope of this paper, it provides a foundation of studies in Italy and also can shed light on trends in diet from transitioning periods into the Bronze Age. Stable Isotope analysis begins with the late Upper Paleolithic or the Epigravettian period (~21000-11,000 B.C) with some Mesolithic examples (~11,000-10,000 B.C) that includes studies conducted by Francaccli et al. 1988 (Mainland and Sicily), Pettitt et al. 2003, Vercellotti et al. 2008, Craig et al. 2010, Gazzoni et al. 2012 (Mainland) and Mannino et al. 2011 Mannino et al. 2011b, and Mannino et al. 2012 (Sicily).The Paleolithic and Mesolithic ages illustrated a heavy reliance on terrestrial animal proteins with some individuals consuming high amounts of marine proteins as well (Pettitt et al. 2003, Gazzoni et al. 2012, Mannino et al. 2012). Overall Mannino et al. 2012 agree that the subsistence strategies of the Hunter-Gathers in coastal Mediterranean and regional Italy during the closing of the Pleistocene and early Holocene is based on exploitation of terrestrial animals and minor contribution of marine resources. There is a supposed increase of marine diet during the Mesolithic (as it is noted in Atlantic regions; Richards et al. 1999, Richards et al. 2000, Spain; Garcia-Guixé et al. 2006, Garcia-Guixé et al. 2009, Garcia-Salazar et al. 2014 and France; Goude et al. 2017). This shift from Mesolithic to Neolithic exploitation of marine resources is not so clearly observable due to several reasons. Firstly, Mesolithic sites are sparse in Italy compared to other regions of Europe where this trend is noted. This is possibly due to the sea level rise during the transition into the Holocene mentioned. Several other hypotheses include, a low production of the Mediterranean due to limited tidal range and lack of specialized technology (Mannino et al. 2012, craig et al. 2010, Gazzoni et al. 2013).

The Neolithic Paleodietary studies are even more interesting and can help to reveal any dietary shifts from Neolithic into the Bronze Age. The “neoloitization” of Italy was introduced around 7,000-6,000 B.C and generally thought to have begun in the Mediterranean roughly 10,000 B.C in Greece and the surrounding Balkans (Blake et al. 2005). Definition in terms of the “neolithic package” may vary slightly but is generally accepted to include the introduction of domesticated animals such as sheep/goat, domesticated plants such as wheat and barley, use of ceramics and stone grinding tools, and a form of sedentarism involving villages and houses (In Italy it's usually compact settlements such as in Apulia discussed shortly; (Lelli et al.2012). The idea of “neoloitization” is that there is a shift from strategic hunting and foraging to communal agriculture and pastoralism but whether this is an abrupt shift, slow adaptation, or a complete or incomplete change is all in question. The spread of agriculture is supported by various radiocarbon dates from the Mediterranean that date Neolithic settlements earlier in the Balkans and Greece than Italy, and moreover earlier in Southern Italy and Apulia than Central and North Italy (Robb et al. 2007). Indeed, Apulia is a significant location for early agriculture in Italy and contains some of the most densely packed settlements (known as enclosed and C-ditched settlements; Skeates et al. 2000, Skeates et al. 2001) and could potentially be due to the spread of agriculture from the East. The Neolithic Stable Isotope analysis for Italy includes four main studies conducted by Rolfo et al. 2012, Lelli et al. 2012, Tafuri et al. 2014, and Tafuri et al. 2017 with additional research by Goude

et al. 2016 and Francalacci et al. 1988. Overall, the Neolithic diet in Italy comprises of mostly domesticated terrestrial herbivores (Rolfo et al. 2012), large amounts of cultivated C3 terrestrial plants (Tafuri et al. 2017), and various freshwater and marine consumption (Lelli et al. 2012). A diet trend during the transition into the Neolithic noted in neighboring regions (Richards et al. 2003, Mcculre et al. 2011) suggests a decrease in aquatic foodstuffs and a higher reliance on terrestrial herbivores. Aquatic foods have not been a major resource for Italian Prehistory except for a few individuals so this trend cannot be completely observed in Italy but there is a heavy reliance on domesticated terrestrial proteins. Malone et al. 2003 suggests that pastoralism may have played a more major role than farming in the earlier stages of 'neolithisation' with a general increase on C3 plant reliance. This paper notes the 'neolithization' of Italy and Apulia is one that is not homogenous and abrupt but gradual and experiences different food catchments and food practices throughout Italy at different moments as seen in the paleodietary studies. Specifics of the sites and values can be seen in the Appendix.

Table 2: Timeline of Italy

Selected Culture	Period	Time (B.C)	Geological Significance	Time (B.C)
Squared Mouth Pottery (North) Cardial Impressed (West) Impressed Ware; Red Band Painted Ware; *Figulina* (South);	Neolithic	~7,000	Dry Period (deforestation) Wet Period(South) Atlantic Period (warmest and most humid)	~5,000-4,600; 4,000-3,400 ~6,200-5,500; 4.400 ~8,000 - 3,000
Terramare (North) Castellieri (Istria) Appennine (Central/South) Nuragic (Sardinia) Castelluccio (Sicily)	Eneolithic Bronze Age EBA MBA RBA/LBA	~4,000 ~2,000 until ~1,000 ~2,000 until 1,600 ~1,600 until -1,300 ~1,300 until 950	Dry Period (North) Period of Aridity (deforestation) Subboreal Period (dry and cool)	~2,200-1,100 ~1,550-1,350 (1,590 to 1,500 and 1,390 to 1,250) ~3,000-500

4.1.2 Bronze Age (~2,000-1,000 B.C)

The Bronze Age marks the appearance of new technological innovations, chifley the making of Bronze. Bronze material, an alloy of tin and copper, first started to appears in archaeological contexts in Italy around 2,000 B.C. Some of the main forms of bronzes included daggers, axes, fibulae, swords, and pins (Blake et al. 2005). Although most of the bronzes seemed to be made for weaponry its not proven that they were used in such a manner but most likely as decorative pieces (Fokkens et al. 2013; Although the funerary rites of Olmo di Nogara cemetery illustrate burials with swords that could have been used for some form of weaponry). On the

contrary, the Bronze Age in Italy does present some well-established signs of the beginning of competitive communities. For instance, in terms of settlements, previous ages saw communities living in natural cave environments, open air settlements with housing of wattle and daub, ditches lined with stone, and so forth. These types of living environments continued but the Bronze Age brought about fortification walls made with stone and wood including major sites such as Coppa Nevigata and Rocca Vecchia in Apulia and the Nuraghi in Sardinia. Some also argue the wooden houses of the Terramare Culture, the wooden pile-dwellings or the *Palafitte*, in North Italy were also built above water tables to the point of defensive protection (Blake et al. 2005, Blake et al. 2017, Fokkens et al. 2013). Moreover, there seems to be some shifting of settlement from plains to hilltops, at least noted in Northern Italy (also mentions that some sites grew larger than surrounding sites perhaps becoming control centers; Blake et al. 2017). Burials in Bronze Age also suggest status through burial goods, with some burials having bronze or other materials, but more telling are the burials that had bipolar deposition between male and females with males being typically buried towards the left and women towards the right. This can be understood as burial ritual and also perhaps status (as seen in the site of Arano di Cellore; Varalli et al. 2016). Furthermore, collective burials and multiple burials were used continuously over time, perhaps depicted lineage (Varalli et al. 2015, Blake et al. 2017). Generally, burial types and ritual varied greatly in the Bronze Age with some structures including megalithic tombs, chamber tombs, urn-burials, cist graves, Tumuli, depositions, cave burials, etc (Recchia et al. 2011, Harding et al. 2004, Trump et al. 1958). Burials, which are typically single inhumations, increase in multiple and continued burials, and the use of cremation grew significantly especially in the North (Fokkens et al. 2013). Climate during the Bronze Age experienced a period of aridity around 2,000 B.C and signs of natural deforestation (Mercuri et al. 2012, Ravazzi et al. 2003, Sadori et al. 2011, Valsecchi et al. 2005). Other natural events that occurred during this period was the supposed lowering of the water table that could have affected the Terramare culture in the North and the eruption of Vesuvius of Avellino Pumices in Central Italy effectively lowering the population and settlement density for some time (Fokkens et al. 2013). From around 4,000 B.C there was an increase in Evergreen taxa that continues on into the Bronze age as well as signs of olive tree (*Olea europaea*) and pistachio tree (*Pistacia*) that could be early signs of 'Mediterranization' of the climate in Italy (Magri et al. 2009, Fiorentino et al. 2003). The sea level continued to raise gradually since deglaciation and the use of maritime travel during the Bronze age increase significantly. Trade was a major resource for Bronze age peoples and can be seen through material evidence of pottery types, as well as other foreign materials such as amber and ivory appearing throughout Italy. This shows a reliance on widespread trade with Greece and the East as well the trading within peninsular Italy between the North, South and Sicily and Sardinia. (Blake et al. 2005) Craft specialization is an important component of Bronze Age identities and trade and will be an important component of Coppa Nevigata discussed in the following chapter. Widespread trade affected material culture but also agriculture. For instance, some of the first signs of domesticated horse (*Equus Caballus L.*) can be seen in faunal deposits in North Italy that would evidently gradually spread throughout Italy and a rare deposit of domesticated donkey (*Equus*

asinus L.) in Apulia at Coppa Nevigata which more likely originated from trade from the East (Blake et al. 2005; Fokkens et al. 2013). Agriculture in the Bronze Age notably increased in Central Italy with use of fertile volcanic soils and mixed economies flourish throughout peninsular Italy with black earth soils in the North and alluvial soils in the South. Although agriculture is a major importance in Bronze age Italy, pastoralism is still much in use as well as transhumance migration (Blake et al. 2005). There is a general increase in ovicaprids and noted use of secondary sources especially from Bovines for milk, labor, and wool (Blake et al. 2017; Soncin et al. 2017 who studied chemical markers on teeth for direct milk consumption). Overall, the same types of animal assemblage from previous ages such as ovicaprids, goat/sheep, and pig are still in use (see section 4.1.2). In terms of agriculture and subsistence, the same domesticated foods used in Neolithic times continue (cereals-wheats *Triticum monococcum, T. aestivum, T. durum, T. dicoccum;* barleys-*hordeum distichum, H. Vulgare*; legumes-beans *Vicia faba minor* and lentils *Lens culinaris*; fruits *Figus caruca* and nuts *Quercus sp* and some signs of olive and grapes). It's important to note an increase in hearths or circular pits used for firing food or metalworking have been found throughout Italy (Blake et al. 2017). As the exploitation of aquatic resources has been a debate in previous periods, Bronze age continues this concern with an added question mark for the consumption of new and developing crop species. For Italian Bronze age the new crop species is typically Millet (Varalli et al. 2015). Several of the isotopic studies for Bronze age Italy follows the introduction and use of grasses such as domesticated Millets, particularly Broomcorn (*Panicum Miliaceum*) and Foxtail (*Setaria italica*) (There are rare finds of wild C4 plants of mainly tropical sedges and grasses but the use of them has not been noted so far, Tafuri et al. 2009). It's been agreed that these millets were introduced into central and Eastern Europe from the Steppe regions during the Neolithic (Taufri et al. 2009). How it was introduced into Mediterranean and Italy is less understood. There are millet seeds in the Po Plain Northern Italy but almost no botanical evidence in Southern Italy until classical times (Tafuri et al. 2009). Millet is known as a hearty species with a short growing season (about 3 months) and high yield and adaptability, so it is assumed to be a suitable crop for less tolerant environments. Due to this characterization of millet and the juxtaposed aridity and large scale deforestation of the time, millet could have been a go to crop that is illustrated by the isotopic studies on diet.

There are just a handful of stable isotopic studies conducted on Bronze Age Italian samples. The studies Include Lai et al. 2013 (Sardinia), Tafuri et al. 2009, Tafuri et al. 2018, Varalli et al. 2015, Varalli et al. 2016, and Mascotti et al. 2017 (Mainland). Lai et al. 2013 analysis on Bronze Age sites in Sardinia (Is Aruttas, Concali Corongiu'Acca (caves), Sedda sa Caudela (chamber Tomb), Is calitas (Pit), and Montessu (Rock-cut Tomb) correlates well with the scheme of the dominate terrestrial C3 diet with varying amount of animal protein seen thus far in the Mediterranean. While most values are fairly homogeneous; the sample from Monessu is an outlier relying more heavily on plant proteins. A few samples from Is Aruttas illustrate more enriched δ13C values and therefore it can be assumed that these people may have consumed minor amounts of aquatic food sources or perhaps even C4 based plants; the debate that leads the Bronze Age diet. Tafuri et al. 2009 examines four sites comparing North and South Italian Bronze age; Olmo di

Nogara (a cemetery in Northern Italy mostly inhumations that suggests social complexity), Sedegliano (fortified settlement in Northern Italy), Toppo Daguzzo and Lavello (two separated underground structures with group burials in Southern Italy). Fauna samples were taken by nearby coeval Bronze age sites of Mereto for the North and Middle Bronze Age site Madonna di Loreto from the South, Apulia. The results of this analysis concludes that the Northern Italian sites, more-so in Olmo di Nogara, most likely consumed domestic millet, provided there is no signs of wild millet and irregular consumption wouldn't be isotopically recorded. The values were compared to modern isotopic values of millet (reported by Tafuri et al. 2009 research to be δ13C -12 to -10 per mil and δ15N from 3 to 4 per mil) which led to the conclusion that the diet included some C4 plants but also an important contribution from C3 plants such as barley, legume, and wheat, and perhaps wild plants or the C3 values would have been more enriched. The question of freshwater fish consumption was also addressed and it has been concluded that due to the low δ15N values, fish consumption is less likely (Tafuri et al. 2009 research noted modern isotope values of freshwater Eurasian fish generally have lower δ13C values). It is not clear if the millet is consumed directly or indirectly via animals who ate millets due to isotopic evidence that the animals also consumed millets. For South Italy, the majority of diet (~65-70%) is based on C3 plants most likely wheat and barley perhaps due to the hypothesized introduction of domestic millet from the East through the Steppe region thus reaching Northern Italy before the South. No significant difference in diet compared to status or sex was reflected in the long term diet of the isotopic measurements but could have been occasional. Overall this study revealed millet consumption in Middle Bronze age Italy and was cultivated at least from Early Bronze Age as a direct food source and fodder for animals. The study by Tafuri et al. 2018 studies various northern Italian sites at various time interval during the Bronze Age as follows; cemetery of Valsera di Gazzo, pile dwelling of Dossetto di Nogara for EBA, cemeteries of Olmo di Nogara, Bovolone, Franzine Nuove, Scalvinetto, and embanked site and Terramare culture of Fondo Paviani for MBA/LBA all located in the Lower Verona Plain. For Friuli, in Northern Italy the following sites; cemeteries of Sedegliano, Tumulus of Mereto di Tomba for EBA/MBA, and embanked Gradisca di Codroipo (Castelliere culture) for LBA. The results can be summarized as a C4 diet most probable in Olma di Nogara, Bovolone and Dossetto di Nogara, while the sites from Friuli of sedegliano, and Mereto di Tomba have C3 plant consumption with some terrestrial animal proteins. The lack of isotopic evidence for C4 diet consumption in Friuli devalues the hypothesis for domesticated millets arriving from the East and thus raises interests to look towards the western Mediterranean for possible pathways but generally millet seems to gain importance in selected sites and not a complete dispersion. Some Bronze age studies from France and Spain (Goude et al. 2016, Van-Strydonck et al. 2005, Mcculre et al. 2011, and Lopez-Costas et al. 2015) suggests millet consumption in France but not in Spain so far. Interesting to note for Tafuri et al. 2018, is some of the first reported diet difference in terms of gender and status (by δ15N values only) possibly shown in Olma di Nogara where only some women would have access to more animal protein than other women (related to grave goods therefore status but all men with or without goods had similar δ15N values) and also in Bovolone where men seemed to have more access to animal proteins according the more enriched δ15N

values. Conversely from the Tafuri et al. 2009 study, this study is more certain that millet was directly consumed than indirectly through animals because the larger fauna dataset shows less δ13C value ranges. Varalli et al. 2015 looks at Central Italian Bronze Age sites in attempt to bridge the gap between North and Central of the new cultivation of millets. These sites include Grotta dello Scoglietto (described as a hospital due to high level of pathologies), Grotta Misa, and Felcetone. The results indicated, although sharing a similar environment, three chosen dietary patterns which are; Grotta dello Scoglietto indicative of an high animal protein diet with possible freshwater consumption for few individuals which may have been non locals according to Sulphur analysis, Grotta Misa indicative of less animal protein and probably consumption of C4 plants for one individual (this site had charcoal and seeds including *Panicum miliaceum* which could indicate that the millet was consumed cooked), and Felcetone indicative of mainly plant protein, particularly legumes (an important crop during Bronze Age), with probable C4 consumption of one individual. This shows that the cultivation of new crops such as millet is not only restricted in the North of Italy but also can be seen in Central Italy during the Bronze Age, particularly Middle Bronze age. In addition to this study, Lippi et al. 2017 studied the dental calculus from Grotta dello Scoglietto that found residual starch grains and phytoliths of *Hordeum* (barley) *Triticum* (wheat), *Avena* (oat) and millets (possibly *Panicum Miliaceum*). The isotopic analysis did not show C4 consumption for Grotta dello Scoglietto, but perhaps they were consumed in undetectable amounts. The next study by Varalli et al. 2016 looks at Arno di Cellore, Northern Italy. This is an extensive necropolis of multiple and single burials with evidence of bipolar burials separating males and females displaying both "ascribed status" determined by birth such as sex as well as "achieved status" determined by burial goods of which some of the chemically rare metal daggers have been found not of Bronze (alloy of copper and zinc) but of an alloy called Fahlerz (nickel, silver, arsenic, and copper). The diet reveals a dependence on mixed terrestrial foodstuffs with high consumptions of C3 plants. This site is excluded from the diffusion of millet and therefore it can be hypothesized that millet was introduced via a "leapfrog dispersion" only gaining importance in some sites. The diet shows no relevant signs of difference between gender, age, or status, on contrary to the importance of gender and status of the burials. It's interesting to note, in the last few studies by Tafuri and Varalli mentioned, pigs have shown similar isotopic signals as the humans and therefore could be hypothesized that pigs were fed the same diet as humans. Furthermore, values between domesticated and wild fauna are fairly homogeneous suggesting the domesticated fauna foraged the same areas as wild and gives some insight on animal care. Lastly, Masotti et al. 2017 continues studies in Northern Italy at Ballabio which contains separate primary and secondary burial with signs of social status in terms of burial goods. Ballabio displays a mixed terrestrial diet of C3 plants and some animal protein (besides a particular case for one woman with pathologies suggesting Periostitis showing freshwater exploitation or more animal protein due to enriched δ13C and δ15N values). Overall, the diet depends mostly on domesticated plants, with some domesticated animal protein during Bronze Age Italy. The pattern of food sources seems to be as follows; mostly hunted animal protein in the Paleolithic, increase interest in small game and gathered foodstuff in the Mesolithic, further reliance on domesticated crops in the Neolithic with

a decrease in animal protein, and continued reliance on domesticated crops in the Bronze Age with varying domesticated animal protein either from the meat or secondary sources such as the milk (social status playing a more significant role in diet during the Bronze Age). Marine foodstuffs seem to have had more significance in Paleolithic and Mesolithic times with decreasing use in Neolithic and Bronze Age. In terms of the introduction and adoption of new technologies such as the "Neolithic package" or millet of the Bronze age, one patterns seems to fit. New innovations spread sporadically perhaps not due to lack of introduction but gradual adaptation, or simply lack of communication or trade of some innovations in peninsular Italy. Can a similar pattern be seen in adjacent cultures that were in contact with Italy?

Table 3: List of Stable Isotope Values of Italian Bronze Age Sites

Site *Burial Goods	n	δ15N per mil Mean/ Median/ Min/ Max value	δ13C per mil Mean/ Median/ Min/ Max value	Ref.
Arano di Cellore (Verona)*	Human 58 Animal 14	7.9 /7.9/6.9/8.9 4.5/4.7/2.8/ 6.1	−20.2/−20.3/−20.9/−19.7 −19.9/−20.3/−20.7/−18.2	Varalli et al. 2016
Grotta dello Scoglietto (Grosseto)	Human11 Animals 11	10.4 /10.3/ 9.0 /11.5 5.4 /5.6 /4.3 / 6.0	− 20.0/ − 20.0 /− 20.4/ − 19.5 − 20.6/ − 20.7/ − 22.0/ − 18.3	Varalli et al. 2015
Grotta Misa (Viterbo)*	Human 4 Animals 4	8.4/ 8.5/ 8.1/ 8.6 5.4/5.6/4.3/6.3	− 18.1/ − 18.2/ − 19.4/ − 16.5 − 20.7/ − 20.9 /− 21.7 /− 19.4	Varalli et al. 2015
Felcetone (Viterbo)*	Human 12	7.1/ 6.8/ 6.0/ 8.8	− 19.2/ − 19.1/ − 20.3/ − 17.3	Varalli et al. 2015
Sedegliano (Udine)	Human 2	8.3/8.3/ 8.1/8.4	−17.7/−17.7/−17.7/−17.6	Tafuri et al. 2009
Olmo di Nogara (Verona)*	Human 19 Animal 3	9.4/ 9.4/ 7.8/ 11.1 6.4/ 6.5 /5.4/ 7.3	− 17.7/ −17.7 /− 16.6/ − 13.9 − 19.6 /− 16.7 /− 17.8/ − 15.4	Tafuri et al. 2009
Toppo Daguzzo (Potenza)*	Humans 14	8.2/ 8.3/ 6.7/ 8.8	− 19.6 /− 19.6 /− 19.8 /−19.1	Tafuri et al. 2009
Lavello (Potenza)*	Humans 4 Animal 3 (Madonna di Loreto, Mereto)	8.5/8.3/ 8.2/ 9.3 6.2 /7.1/ 4.5 /7.2	− 19.5/ − 19.5/ − 19.6 /− 19.3 -20.4/ -20.4 / − 20.5/ -20.4	Tafuri et al. 2009

Dossetto di Nogara (Verona)	Human 1 Animal 3	8.5/-/-/- 4.8/-/-/-	-13.5/-/-/- -20.6-/-/-/	Tafuri et al 2018
Olmo di Nogara (Verona)	Human 64 Animal 5	9.3/-/7.5/11.1 6.9/-/-/-	-14.9/-/-17.8/-12.7 -20.3/-/-/-	Tafuri et al 2018
Bovolone (Verona)	Human 24	9.4/-/7.2/13.1	-15.2/-/-19.6/-10.6	Tafuri et al 2018
Fondo Paviani (Verona)	Animal 18 Aquatic 3	7.2/-/-/- -/-/7.9 to 12.7	-17.1/-/-/- -/-/-24.7 to -20.5	Tafuri et al 2018
Sedegliano (Udine)	Human 2	-/-/8.1/8.4	-/-/-17.7/ -17.6	Tafuri et al 2018
Mereto di Tomba (Udine)	Human 1 Animal 3	7.4 4.7/-/-/-	-20.2 -18.7/-/-/-	Tafuri et al 2018
Gradisca di Codroipo (Udine)	Animal 3	4.5/-/-/-	-20.5/-/-/-	Tafuri et al 2018
Concali Corongiu' Acca (Sardinia)	Human 4	11.4 ± 0.8/-/-/-	-18.9 ± 0.2/-/-/-	Lai et al 2007
Iscalitas (Sardinia)	Human 29	10.4 ±0.9/-/-/-	-19.1± 0.3/-/-/-	Lai et al 2007
Montessu (Sardinia)	Human 1	9.1	-20.3	Lai et al 2007
Is Aruttas (Sardinia)	Human 11	10.5 ± 0.9/-/-/-	-18.7 ± 0.3/-/-/-	Lai et al 2007
Sedda sa Caudeba (Sardinia)	Human 2	9.3 ± 0.3/-/-/-	-19.0 ± 0.1/-/-/-	Lai et al 2007
Ballabio*	Human 23 Animal 3	7.8/8.0/7.2/10.0 4.1/ 4.3/ 3.4/ 4.7	-18.6/-20.4/-20.8/-20.1 -20.6/-20.7/-20.8/-20.3	Masotti et al 2017
Toppo Daguzzo (Basilicata)	Human 2 Animal 2	-/-/8.3/ 8.5 -/-/2.1/ 8.4	-19.3/-/-/- -/-/-20.8/-19.1	Francalacci et al 1988

4.2 Isotopic History of Croatia

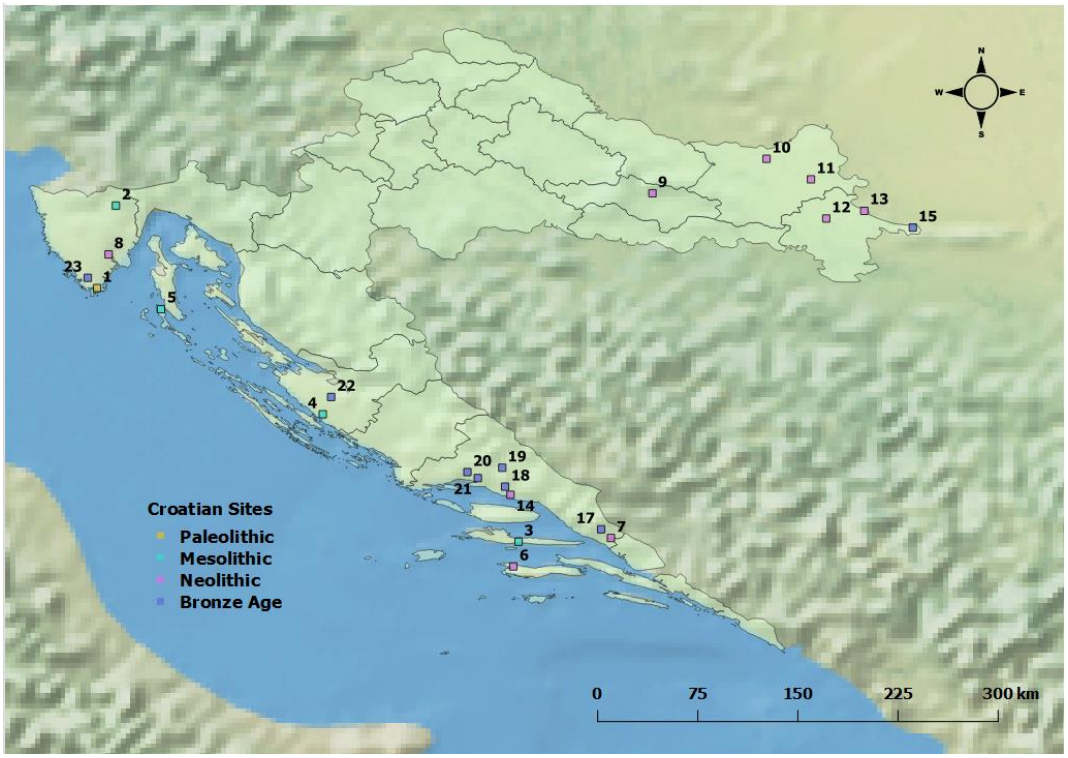


Figure 12: List of sites mentioned: 1. Sandalja II 2. Pupićina 3.Grapčeva 4.Crno Vrlo 5.Vela Spilja Lošinj 6.Vela Spilja-Vela Luka 7.Metaljka 8.Kargadur 9.Radovanci 10. Belišće Staro Valpovo 11.Osijek 12.VinkovciVinkovci 13.Vučedol 14.Zemunica 15.Ilok Dvor Knezova Iločkih 16.Matkovići & Veliki Vanik 17.Zavojane Ravča 18.Prosik & Koprivno 19.Radošić-Biluska 20.Vučevica 21.Konjsko Polje 22.Nadin-Gradina 23.Monte Orcino/Určin

For the following isotopic review, the main focus is on Croatia. This paper takes interest in the connection between the groups of people in Apulia and Dalmatian Coast. There is little published for Croatia in terms of paleodiet analysis using stable isotopes or zooarchaeological and archaeobotanical backgrounds of the specific sites so some detail about Croatia in general is provided here to fill in this gap. Croatia is focused on due to potential trade and demographic mixing between those of Dalmatia and of Apulia. There is materialistic evidence since the Neolithic times of trade between Italy and Croatia. Adding isotopic analysis such as Carbon and Nitrogen can further assess the connection between these cultures and if they mixed and on what level do they share similarities. Isotopic analysis summaries will reach until the end of the Bronze age for the purposes of this paper.

Archaeological evidence for Croatian prehistory follows a similar pattern as was discussed for Italian Prehistory up until the Bronze Age. Neolithic Age brought about increased open air sites, and innovations in permanent settlement such as pit dwellings and beginning of Tells; continued stacking of settlement buildings creating artificial mounds. Subsistence of Neolithic Age is similar to what was discussed in Italy with domesticated crops dominated by Wheats and Barley (einkorn (*Triticum monococcum*), emmer (*Triticum turgidum ssp. Dicoccum*), barley (*Hordeum vulgare ssp. vulgare*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), pea (*Pisum sativum*), and

flax *(Linum usitatissimum*); Reed et al. 2016b). Some main Neolithic Cultures of Croatia are the coastal cultures; Impresso, Danlio, and Hvar, and inland cultures; Korenovo and Sopot (Fokkens et al. 2013; Lightfoot et al. 2011). Earliest Trade across the Adriatic can be illustrated during the Neolithic. The Bronze Age sees more intensified widespread trade. Material finds of Cetina pottery (Cetina culture is a main culture of Bronze Age Croatia) has been found throughout Apulia. Bronze Age Croatia sees the continuation of Tells, ditch-settlements and houses, and fortified settlements especially in the Castelliere/Gradina culture, another important culture of Bronze Age Croatia, in Istria (Fokkens et al. 2013). Bronze Age Croatia has slightly different dating than Italian Bronze Age. Early Bronze Age correlates with 1,800-1,450, Middle Bronze Age is from 1,450 to 1,250, and Late Bronze Age is from 1,250 to 750 B.C (Gimbutas et al. 1965). Bronze Age Croatia also sees the growing importance of Millets (Broomcorn-*Panicum Milliaceuum*), spelt (*Triticum aestivum ssp, spelta*) and broad bean (*Vicia faba*) in their foods (Reed et al. 2016) and the continued pastoralism of cattle, sheep/goat, and pigs. Animal management in Croatia was studied through isotopic analysis by Zavodny et al. 2014 who concluded ovicaprid and cattle had consistent management while pigs were foddered differently in one case. The environment in Croatia from Neolithic to Bronze Age involved the replacement of Deciduous forest by Evergreen predominate by *Phillyrea* and *Juniperus* and forests dominated by *Quercus ilex.* A similar pattern was seen in Italian prehistory and can be described as the 'Mediterranization' of the area into more modern environment (Sostaric et al. 2005). Lastly, some burial types throughout prehistoric Croatia include deposits in caves, pit burials, single inhumation in tumuli lined with stone, and significant use of cremation (Fokkens et al. 2013).

The following isotopic studies for Croatia span from the Paleolithic (Richards et al. 2015), Mesolithic and Neolithic (Lightfoot et al. 2010, Lightfoot et al. 2011, Guiry et al. 2017), and Bronze Age (Lightfoot et al. 2014, Tafuri et al. 2018). The first isotopic study in this collection comes from Richards et al. 2015 of a Late Upper Paleolithic site of Sandalja II, Istria, Croatia. The results of 3 humans and 28 faunal samples state an overall freshwater fish consumption (a species of freshwater fish; *Pike*) as the main protein alongside terrestrial herbivores. This compares with other Late Upper Paleolithic analysis mentioned in Italy (San Teodoro, Addaura, Romito, Arene Candide, and Riparo Tagliente) as well as sites along the Atlantic, Spain, and France (section 4.1.1). Overall, this time has a diet dominated by large terrestrial animals and some fresh or marine water consumption with more examples along the Western Mediterranean. Lightfoot et al. 2010 and Lightfoot et al. 2011 explores Mesolithic to Neolithic transitions in Croatia. The first site being Vela Spila Cave, Korčula of 24 fauna and 4 humans from Mesolithic age and 1 infant from Neolithic age of which results shown that during the Mesolithic, diet was based on terrestrial resources and some marine protein (due to seasonal occupation its suggested marine foods, especially deep sea species like tuna, were ate at this site and terrestrial sources at another due to zooarchaeological evidence) and during the Neolithic shows a decrease in marine consumption. Lightfoot 2011 explores a range of sights from Mesolithic to Neolithic including coastal sites; Metaljka, Grapčeva, Vela Spilja-Vela Luka, Crno Vrlo, Vela Spilja Lošinj, Kargadur, Pupićina and inland sites; Radovanci, Belišće Staro Valpovo, Osijek, Vinkovci, Vučedol. 42 humans and

95 fauna Results show that Mesolithic individuals on the coast had a mixed diet of marine and terrestrial foodstuffs and Neolithic Inland had a terrestrial diet with no aquatic source. However, coastal Neolithic sites had wider dietary values which may indicate some marine protein (or lower trophic level marine animals). Overall terrestrial foods would have been the more important source of protein. Guiry et al. 2017 studied 10 humans and 63 fauna of the Neolithic site of Zemunica, Dalmatia, Croatia. Due to zooarchaeological evidence, this site was probably used as a "stable by mobile shepherds" and their diet focused homogeneously on domesticated terrestrial animal protein. For Bronze age, one study by Lightoot et al. 2014 examined 47 Bronze Age humans and no fauna from several sites ranging from Bronze age to Iron age. The sites include coastal Bronze Age sites; Nadin-Gradina (BA/IA), Radošić-Biluska Griža, Vučevica, Konjsko Polje, Zavojane Ravča, Prosik, Koprivno, Matkovići and Veliki Vanik, coastal Iron Age sites; Dragišić, Zadar-Relja, Gumance-Vela Luka (BA/IA), Zadar-Baziliža, Inland sites; Vinkovci-Nama (IA) Ilok Dvor Knezova Iločkih (BA). Results indicate notable C4 or marine foodstuff with C4 consumption being more likely on Iron Age Inland sites and C3 diet for both coastal and inland Bronze Age sites. (millet was much more consumed in Iron Age and its suggested that during Bronze age it was more of a weed and then fully cultivated later). Although some coastal Late Bronze Age sites indicate some C4 consumption including Nadin-Gradina possibly associated with burial type and status but it is unclear. In the Tafuri et al. 2018 study on Bronze Age Italy, there is one Croatian site from late Bronze Age collective burial at Monte Orcino/Určin, Istria which indicated a terrestrial diet and no C4 consumption of 19 individuals. The dietary patterns seem to correlate with Italian dietary patterns. In sum, diet consisted of mostly hunted animal protein in Paleolithic and Mesolithic, reliance on domesticated animals and plants during Neolithic and increase reliance on domesticated plants in Bronze Age. As millet seemed to gain importance in Italy around the Middle Bronze Age, millet doesn't seem to gain much importance until after the Bronze Age for Croatia. Lastly, fishing seems to play a greater role throughout prehistoric Croatia than it did in Italy, relating more to the sites from the Atlantic coast and Danube Gorge which probably was an important source of contact for Croatia in terms of agriculture and trade such as metals (Fokkens et al. 2013, Lightfoot et al. 2011).

Table 4: List of Stable Isotope Values of Bronze Age Croatia

Site	Sample	Mean d13C per mil	Mean d15N per mil	Period	Reference
Zemunica, Dalmatia	Human 10 Animal 63	−20.0 ± 0.1 -19.75	8.4 ± 0.6 5.6	Neolithic	Guiry et al 2017
coastal Bronze Age Nadin-Gradina, Radošić-Biluska Griža, Vučevica, Konjsko Polje, Zavojane Ravča, Prosik Koprivno, Matkovići Veliki Vanik Inland Bronze Age Ilok Dvor Knezova Iločkih	Human 47 8 7 7 6 1 6 1 4 5 2	Total 19.5 −18.5 −19.2 −19.4 −19.5 −19.8 −19.5 −20.0 −20.2 −20.2 Total −19.9	Total 8.8 9.5 8.6 8.9 9.2 9.0 8.6 91 8.6 8.6 Total 10.8	Bronze Age	Lightfoot et al 2014
Monte Orcino/Určin, Istria	Human 19	-19.6±0.3	8.7±0.7	Bronze Age	Tafuri et al 2018

Chapter 5: Archaeological Sites and Context

Figure 13: Location of Site under Investigation

This chapter is devoted to the archaeological background of Coppa Nevigata, Gusica Gomila, Jukica Gomila, and Brnjica. Coppa Nevigata has been extensively studied and well published. Therefore, severals sections would be devoted to Coppa Nevigata in terms of its geological background, archaeological contexts, fauna finds, floral finds, and osteological contexts. This is particularly important for paleodietary studies because this background information provides a comprehensive approach into understanding food practices especially in terms of fauna and flora finds as well as any cooking technologies found on site. Gusica Gomila, Jukica Gomila, and Brnjica are not well published and little information is known about these sites still under archaeological investigations. Although, the provided information does allow for a basic understanding of what types of bones were discovered and type of burials. Lastly, this chapter will briefly describe the archaeological connection between Apulia and Dalmatia in terms of why this study was interested in analyzing the diet.

5.1 Coppa Nevigata

5.1.1 Geological Background

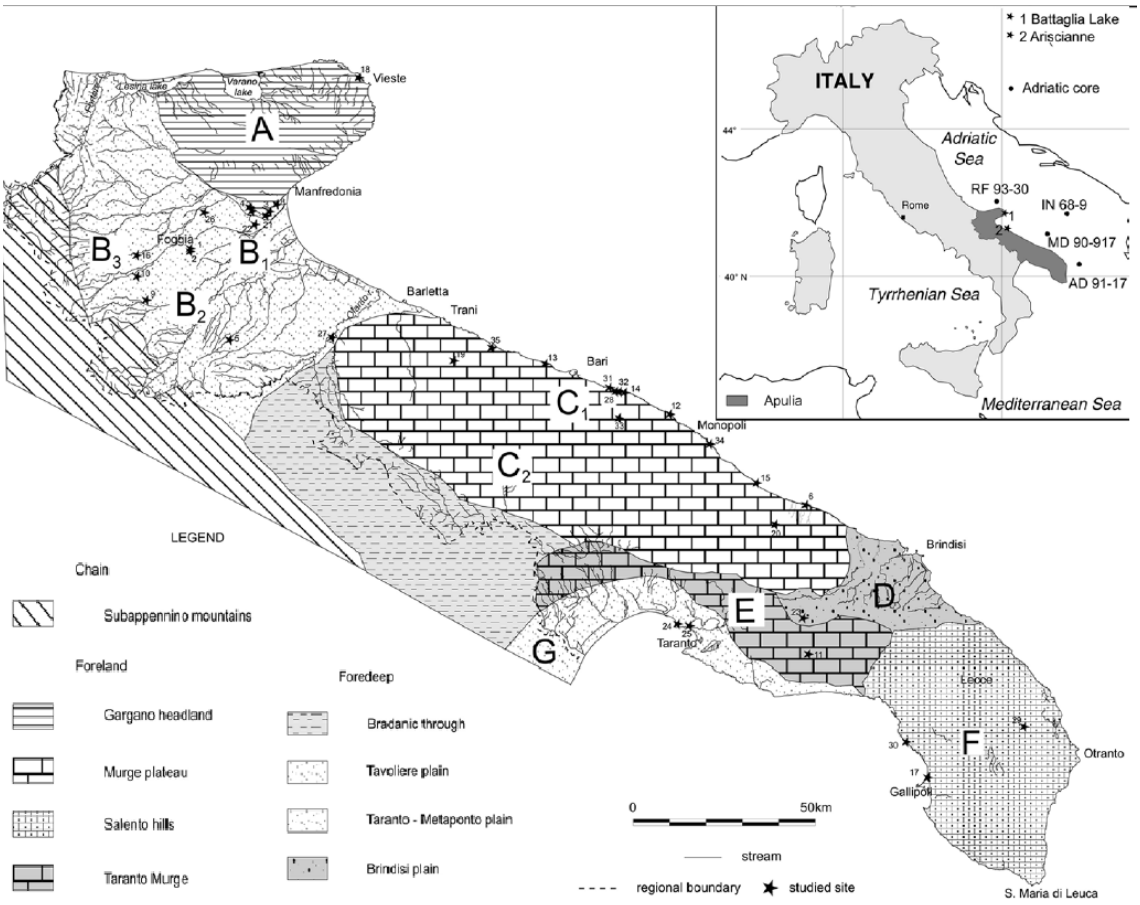


Figure 14: Geological Layout of Apulia; Fiorentino et al. 2003

Coppa Nevigata is located in the Apulia region of Southern Italy (Province of Foggia) in the vicinity of the Gargano promontory and the Tavoliere Plain. It is 5 km from the modern coast. It's in proximity to the shores of a Mid-Holocene lagoon (at its peak it expanded South of Manfredonia until the Ofranto river Delta). The Taviolere is boarded by Gargano in the North, Daunian Mountains in the West, Murge Hill in the South, and the Adriatic to the East. The Gargano consists of smooth limestone surface and the Taviolere is an alluvial coastal plain (Caldara et al. 2002; Caldara et al. 2004). The Apulia region has mild to cool winters, little rainfall (average is 600mm per year) and hot dry summers. Annual temperature averages between 15-18°C. Tavoliere plain is among the warmest area in Italy with a mean temperature of 26°C. On the contrary Central and North Italy are described as cooler and more humid (Fiorentino et al. 2013). There is a period of aridification that affected peninsular Italy (1,500-1,300 B.C) which accompanied a change in the environment of Coppa Neviata from open woodland dominated by deciduous species (deciduous oaks, hornbeam, hazel, elm and beech; Fiorentino et al. 2003) then evolved into woodland in favor of evergreens. Other noted patterns during Bronze Age Italy and Coppa Nevigata includes an overall decrease in *Olea* from 2,000 - 1,000B.C with a max peak at 1,100

B.C (increase in evergreen taxa, reflecting a typical Mediterranean climate based on the Alimini Piccolo pollen sequence; magri et al. 2009). Afterwards, there is a decrease in charcoal and increase in pollen of *Olea* that can suggest management practices (not that olive was being planted but perhaps selective exploitation that led to domestication). At Coppa Nevigata there is an abundance of deciduous oaks supposedly exploited from inland that is used for firewood and suggests the area near to the site was degraded and open. The agrarian societies managment of vegetation is still not fully understood in terms of firewood collection and livestock grazing (slash and burn tactics to clear land), but perhaps the land exploitation increased the climatic oscillation towards aridity that begun naturally between 1,500-1,300 B.C and prolonged it (Sadori et al. 2011). Plants can also tell about the climate. In Apulia 2,000-1,500 B.C, barley and wheat (especially emmer and free threshing wheat) were abundant and are sown during the Winter. There is a change by 1,500-1,300 B.C with small introduction of millets (the only crop that can be sowed and harvested in one season in Spring), increase in wild fruits and nuts, and decrease in free threshing wheat and no change in emmer (which is less sensitive to climatic oscillation and can be sown in the Spring as well as Winter). These pattern supports the natural aridity phase during this time and the adjustments to crop production taken by the agrarian societies in Apulia and Peninsular Italy. Lastly towards the end of Bronze age, 1,300-1,000 B.C, there seems to be need for surplus not related to climate with new cultivation of lands, double harvest and increase in free threshing grains that are higher yielding. Primavera et al. 2017 suggests this is due to increase in trade, local identities, specialization, and competition in a growing society and the need to produce more in Apulia representative of the Bronze Age. More specifics on the flora of Coppa Nevigata will be discussed shortly, but first a closer look at the surrounding environment.

There were several studies devoted to core analysis and the relationship between Coppa Nevigata and the nearby lagoon (Fiorentino et al. 2003, Caldara et al. 2002, et al. 2004, et al. 2005). From 'LGM' (Late Glacial Maximum) to the Neolithic period, the relationship between the site and coastal plain is briefly studied. Firstly, the environment is cold and wet with a sea level 170 m lower than present day and 70 km further than the moden coast. Sea level began to rise with the passing of the "LGM" and the Neolithic period had a sea level 10-15m lower than present day and evidently grew warmer. Core analysis discovered indirect evidence of the lagoon in correlation with the settlement. Not much information is known about the Coppa Nevigata Neolithic population, but it is suggested that there was a C-ditch village indicative of Neolithic sites in Apulia with the appearance of Impressed Ware ceramics and probably the lagoon existed for some time (but perhaps even as a salt marsh or wetland). Middle Neolithic there seem to be a decrease in population along the Apulian Coast most likely due to the aridity (~4,000B.C). At the end of Neolithic, the lagoon lost connection with the sea, due to increased temperature and low precipitation, and created a *Sabkha* indicative of gypsum in the cores. There is more information on the Bronze Age in terms of the lagoon succession and its relation to Coppa Nevigata. Early Bronze Age, Protoappennine, 1,800 -1,600 B.C, the base of the settlement nearest to the lagoon was submerged and the lagoon gradually retreated. The core discovered finds of Cetina pottery (recall chapter 4; Croatian form of pottery from Bronze Age Dalmatian Coast. Gradual sea rise

from Neolithic may have brought maritime travelers and their pottery as evidence of this find in Early Bronze Age). There were also some flints and accumulations of pumice (used in pottery temper found in Coppa Nevigata but it's good for dating in correlation with the Campanian eruptions). Middle Bronze Age, Ancient and Recent Appennine, 1,500-1,300 B.C, correlates with the expansion of the settlement outside the first fortification walls (built in the 16thC B.C) and signs of communal areas. There seems to be an increase interaction with the lagoon (contemporary with the arid phase and increase of evergreen taxa). There is an abundance of fragments of *Phyllonotus trunculus* shells on the site (importance explained in 5.1.3 for food and dying production), charred remains, fauna remains, and pyroclastic material from Somma-Vesuvius eruption of Avellino (because of the condition of the core and the great amount of settlement evidence its assumed that this area near the lagoon may have been used as a dumping ground). This period is characterized as *Hydrobiidae* and *Cerastoderma* lagoon environment from 1,800 to 1,500 B.C, named after the fauna found. Late Bronze Age, Ancient Subappenine 1,300-1,200 B.C, there is an infilling of the lagoon which evidently began to retreat. This infilling is thought to be anthropogenic due to increase settlement activity. Late Bronze Age, Recent Subappenine, 1,200-1,100 B.C, has small finds of Mycenaean spinned pottery illustrating decreasing trade. Other finds include *concotto* clay (heated clay from structures or ovens) and correlates with site expansion after lagoon infill. The lagoon begins to expand and is characterized as the *Cerastoderma* lagoon in the Final Bronze Age, Proto-Geometric, 1,100-1,000 B.C, with finds of some Proto-Geometric pottery important for dating. The lagoon retreats again and transformed into a salt marsh (brackish environment indicative of fauna finds in the core). The Iron Age is not in the scope of this thesis but Coppa Nevigata did continue into the Iron Age where there is a second event of a salt marsh formation and core finds indicative of pastoralism in which management of the land could have had an effect on the lagoon. So far, Coppa Nevigata seems to undergo settlement changes in connection with the lagaoon and in connection with trade.

5.1.2 Archaeological Contexts

Coppa Nevigata was occupied from the Neolithic until the Iron Age. There is very little published about Neolithic Coppa Nevigata. In a paper written by Skeates et al. 2001 Coppa Nevigata is mentioned to have been a C-ditch settlement (typically of Neolithic Apulia) around 5,750 until 5,500 B.C, accompanied by carbonized remains of barley grains, and some Impressed Ware and *Figulina* sherds which were radiocarbon dated. Additional radiocarbon dated material from Coppa Nevigata is from Whitehouse et al. 1994 that mentions carbonized grains as early as 7,000B.C. Cassano et al. 1987 and Cassano et al. 1995 discusses pottery analysis on Neolithic Coppa Nevigata impressed ware sherds (noting chert pieces used in the temper not uncommon in Southern Italy). Its mentioned the importance of microlith flints particularly found at Coppa Nevigata, and some residuals of wheat and barley grains. Forenbaher et al. 2012 speaks about Grapčeva burial cave from the Dalmatian Coast from the Neolithic which had stone blades referred to as 'shell-openers', which were found in this cave, Coppa Nevigata, and an Adriatic island Sušac. This can be important information for trade discussed further in 5.2.1. Skeates et al. 2000 mentions

Coppa Nevigata was a unique Neolithic ditch settlement compared to others in Southern Italy because it had some of the earliest finds dating back to the 7,000 B.C and it was near heavy alluvial soil where most Neolithic ditch sites were on lighter *crosta* soils easier for agriculture (Jarman et al. 2009). The site had a large stratified ditch with infilling of carbonized plant remains (this is typical of ditched settlements although the use of ditches are not completely understood). Because of plant finds and a grindstone it's understood that a mixed economy was practiced.

There are several levels of site occupation during the Bronze Age in Coppa Nevigata spanning from ProtoAppennine to Recent Subappenine. Firstly, Early Bronze Age corresponds to Protoappennine (2,000-1,650 B.C). During this time there were few signs of the settlement including flints, local *figulina* pottery, Cetina Pottery, and a bone tool described as an awl (Uncovered by coring; Caldara et al. 2004, Recchia et al. 2010). The first dry-stone fortification walls were built around 1,700-1,600 B.C along with secondary passageways, doorways, and towers. Shortly after, there was a fire (suggested due to a violent outbreak on account of a large concentration of arrowheads; Recchia et al. 2013b; Recchia et al. 2010b) that affected the settlement and another fortification of the 16thC B.C Roca in Southern Apulia (at least seven skeleton is said to have died from the fire in Roca). Cazzella et al. 2009 mentions Coppa Nevigata during the 18thC B.C, revealed the earliest examples of olive oil making in Italy through Gas Chromatography on residual fats in the pottery. This discovery predates the fortification walls. This is important because its believed Coppa Nevigata was first influenced from pre-Mycenaean people from the Aegean on account of olive oil making and the early fortification walls.

The Middle Bronze Age corresponds to Ancient and Recent Appenine (1,500-1,300 B.C) and brought about a flourishing settlement. There was an expansion of the settlement outside the first fortification walls which fell out of use. Communal areas developed including circular and rectangular structures, ovens, and a silo for food storage. There was a building of a new defensive wall, closing of some passageways, cobblestone path, and rectangular towers and ditches (Recchia et al. 2013). The Protoappenine wall was reused as burials within the walls and the passageways or doorways (5.1.4). This period reveals most finds of bones, fauna, and charcoals (increase in domistatced finds, especially sheep and goat; Recchia et al. 2009).

Late/Recent Bronze Age corresponds to Ancient and Recent Subappenine (1,300-1,000 B.C) with continued use of ditches, new roadways, and rectangular houses. Significant amount of yellow limestone filling inside the walls is noted (perhaps used to thicken the walls;Recchia et al. 2010, Rechia et al. 2013). Mycenaean type turned and painted pottery increased and new innovations like the donkey was found during this stage. Other finds during this period include Bronze dagger, arrowhead, and 2 spearheads, limestone furnace for pinheads, 39 bronze elements of pins and studs (these studs are similar to the cemetery of Olmo di Nogara, Verona that were associated with males but the context in Coppa Nevigata is not clear), a part of a necklace perhaps ivory (first ivory find in Coppa Nevigata) that was imported and worked locally, and a few decorated pieces of animal bone (Recchia et al. 2010). There is also a particular Bronze diadem with spirals similar to Eastern Adriatic type (12thC BC). Final Bronze Age corresponds to the Proto-Geometric (1,100-1,000 B.C) and had wheeled pottery of the proto-geometric type

accounting for the period. Little is said about this later stage and the Iron Age transition except signs of continued agriculture (Caldara et al. 2002). With the basic foundations of the settlement phases outlined, it's important to look at particular patterns, especially concerning trade.

Roughly during the first stages of the Appennine period (18th to 14thC B.C) were the first signs of olive oil production and purple dye making. Not much to say about foreign ceramics but signs of foreign amber and Bronze material is noted. Most likely the Bronzes were made with Italian material from Etruria, Tuscany, or Calabria where metal sources were well known or taken from the Eastern Adriatic. Most of the Bronze material were discovered during the Subappennine phases with an exception of a few finds during the Protoappennine (Bronze axe most likely used as a tool no as a weapon; Recchia et al. 2010). There is an Increase in Italo-Mycenaean pottery (locally made Aegean type pottery in Coppa Nevigata) and purple dye in 14C B.C (et al. Cazzella 2005). This could be the peak dependency on Aegean technology for Coppa Nevigata. During the Apennine phase the amount of murex shells for dying reached its peak, then sharply decreasing in the Recent Subappennine phase (Minoan dying took place between the Middle Minoan and Late Minoan or 2,100-1,100 B.C). *Olea* seed reached its peak around 1,000 and decreased by the final stages of the Subappennine.The later half of the Subappenine (13th to 11thC B.C) showed an increase in Aegean type trade with Aegean doliums, internally thickened rimmed bowls, bossed-bone plaques (high concentrations in Dalmatian Coast as well), ivory, and vitreous paste in the Ancient Subappennine then decreasing in the Recent Subappenine. In sum, the trade connection between Apulia the Aegean sharply intestfies by the 3rd Millennium B.C peaking around 14thc B.C (Cazzella et al. 2007). Then, drastically dropped by the Recent Subappennine most likely due to the collapse of the Mycenaean period (evident by the fall of purple dye, olive oil making, and aegean finds; Minniti et al. 2002). Recchia et al. 2004 illustrates that Aegean ceramics were more concentrated in South Apulia where direct contact must have been made while Coppa Nevigata probably traded locally from the South with sites such as Roca Vecchia and indirectly with the Aegean (Cazzella et al. 2009). Amber, vitreous paste, and ivory were more concentrated in the Tavoliere plain which may have been traded from the Aegean or from Eastern Adriatic (although the paste and amber could come from Northern Italy reaching Southern Apulia by 13th C B.C such as the Trinito type of amber; Cazzella et al, 2005; Recchia et al. 2004b).

The influence of the type of fortification built at Coppa Nevigata is questionable. Recchia et al. 2013b mentions that the various fortifications in Southern Italy and Sicily during the Bronze Age were most likely influenced by Aegean fortifications which all spanned during the middle of the 2nd millenium (1,600 B.C) during the Mycenaean period. Coppa Nevigata predates these fortifications with the earliest walls built during the Protoappenine, 1,700 B.C. It's more likely that, at least for the first phases, the fortifications at Coppa Nevigata could have been influenced by Eastern Adriatic fortification such as Monkodonja, Istria of the Castelliere/Gradina Culture (Chapter 4). This hillfort site also had burials and deposits linked to the fortification walls uniquely seen in Coppa Nevigata in Italy as well as Cetina Pottery sherds (Recchia et al. 2011). Cazzella et al. 2013 states that there is no complete evidence for social elite in Coppa Nevigata, although by the 12thC B.C, due to new building structures and food storage circles, there may have been some

control over food storage or stocking but there is not enough evidence to make a clear claim. (an example is Italo-Mycenaean pottery found near bread oven and the open area so it can be related to only some nuclei groups; Recchia et al. 2009, Recchia et al. 1998). On the other hand, the formal burials (details in 5.1.4) could highlight social status. The building of the fortifications is understood to be a joint communal effort built by Coppa Nevigata with no foreign assistance and could hint at the raising importance of social identity in Bronze Age Mediterranean and the specialization in craft (more likely fortifications focused on specialization centers than focusing on war).

Table 5: Summary of Coppa Nevigata' archaeological features with relative chronology.

Period	Archaeological	Human Remains	Flora/Fauna Remains	Time (B.C)
Protoappenine	First walls, Fire, Cetina ware (some trade)	Few remains 4	Olive Oil, Purple Dye	2,000-1,650 EBA
Ancient Appenine	New walls, extended trade; foreign material local wheeled pottery	Most remains 278 Formal burials	Intro. of donkey; Increased in goat /sheep	1,500-1,425 MBA
Recent Appenine	Communal center (cooking, storing)	Some Remains 35	Purple dying peaks; Secondary resources	1,425-1,300 MBA
Ancient Subappennine	Limestone filling of wall, Trading increases	Some Remains 7	Increase in fish finds; first sign of Millet	1,300-1,200 RBA
Recent Subappenine	Trading decreases	Some Remains 10	Intro. Of horse; *Olea* seeds peak; drop in purple dying	1,200-1,100 RBA
Proto-geometric	Proto-geometric wheeled pottery (local)	N/A	Cont. pastoralism	1,100-1,000 FBA

5.1.3 Fauna and Flora

This section is devoted to the fauna and flora that have been archaeologically recovered from Coppa Nevigata during the Bronze age as well as some finds from the Neolithic period. Little has been published of Coppa Nevigata during the Neolithic occupation. Cassano et al. 1987 briefly mentions some archaeological finds including cereals (*Tritcum aestvum, T. compactum, T. spelta, Avena sativa, Hordeum*) attesting to the beginnings of agriculture (Cazzella et al. 2012). Fauna was even more rare but several finds of Mollusk shells have been noted and their continued used in the Bronze Age is important (Oxygen isotope analysis of these shells indicated summer harvest). Settlements as close as 2 km nearby Coppa Nevigata had signs of domesticated cattle, sheep, and pig which could translate over to economic practices of Neolithic Coppa Nevigata (Skeates et al. 2001 mentions the acidic soil near the lagoon could have destroyed fauna samples).

In terms of fauna for the Bronze Age occupation, archaeozoological collection found significant amounts of both wild and domesticated species. The wild species included ungulates, carnivores, rodents, birds, reptile, turtle (European Marsh Turtle and Greek Tortoise; *Chelonia,*

Testudo hermanni), and fish (*Dicentrachus labrax, Sparus auratus;* mostly concentrated in Subappenine). Hunting was still a significant economic resource for both ritual and perhaps sometimes food. Domesticated fauna included bovines, sheep/goat, pigs, dog, and horse. The predominant fauna is *Ovicaprid* (sheep more so than goats) followed by cattle. A rare example of the horse is not seen until the final stages of the Bronze Age (domesticated horse can be seen in Greece as early as the Middle Helladic, which may be a source of the domesticated horse seen in Italy. Earliest finds of horses in Italy are first found in the North with roots either from the East, but most likely, due to size of the specimen, are from the West such as France). A rare domesticated find of donkey is particular to Coppa Nevigata whose presence was confirmed by molars and is the earliest case of domesticated donkey in Italy during the Appenine period. Secondary use of animals for breeding, milk, wool, or labor can be understood by aging the animal bones. Animal used for meat typically are slaughtered before adulthood for taste as well as practicality (during the winter there are less animals are fed). Animals who were used for secondary resources died well into adulthood. In Coppa Nevigata, mostly cattle and *Ovicaprid* were used for secondary resources while pigs were used for meat. Dogs and horses were mostly used for labor and not their meat (Cassano et al. 1987, Siracusano et al. 1995). Minniti et al. 2002 conducted an in depth analysis on shell finds of Coppa Nevigata. There are over 50,000 finds, although most are probably washed from the lagoon, of which a significant amount has been worked. It's already been noted of the seasonally collection during the Summer and sometimes Fall of Mollusk shells in the Neolithic Age which continues into the Bronze Age. Particular, Murex (*Murex trunculus L.*), and shells used for food; Mytilus (*Mytilus galloprovinicalis Lam.*) and Cerastoderma (*Cerastoderma edule L.*) were mostly found (Middle Bronze Age or during the Appennine and SubAppeinne periods, particularly the Late Appenine). The predominate taxa shifted throughout the Bronze Age most likely due to the lagoons retreat from the sea in the beginning of the Late Appenine periods. Not only were the shells probably used as foods, such as throughout peninsular Italy, but also used as a secondary source to create dye particular only to Coppa Nevigata during the Bronze Age as mentioned.

Several studies have been conducted devoted to sorting the floral of Coppa Nevigata. It's noted that the Tavoliere plain in which Coppa Nevigata is near, has typically been dominated by herbaceous vegetation from the first half of the Holocene and could account for the intense signs of agriculture in Apulia (Cazzella et al. 2012). One study, by D'Oronzo et al. 2010 analyzed plant remains of mostly cereals (residual caryopses) with some legumes and few weeds, as well as charred remains (charcoals) through stereomicroscopy. In summary, cereal grains were mostly found (taxa of cereal grains; Oat-*Avena sp.,* hulled wheat (Einkorn-*Triticum cf. monococcum,*Emmer-*Triticum cf. diccoccum;* Spelt-*Triticum cf. spelta*) naked wheat (Bread-*Triticum cf. aestivum/durum,* Club-*Triticum cf, compactum*) hulled barley (*Hordeum vulgare cf subsp. distichum, Hordeum vulgare cf. subsp, vulgare*) discovered both as charred remains and seeds. The secondary predominate finds were legumes (taxa of legumes; Pulses-Leguminosae, rare finds of Lentil -*Lens culinaris,* and Faba bean-*Vicia faba var. Minor* in Late Bronze Age) and lastly weeds (taxa of weeds; Fat Hen-*Chenopodium cf. album,* Sun spurge - *Euphorbia cf.*

helioscopia, Bromus, Poppy - *Papaver,* Knotweed - *Polygonum*). Also worth mentioning are the anthracological finds of trees and shrubs typical of Mediterranean (Taxa; Wild Olive-*Olea europaea,* Pistachio-*Pistacia, Pistacia cfr. terebinthus,* Oak-*Quercus sp. Quercus robur.,* Grape Vine-*Vitis vinifera,* Juniper-*Juniperus sp.,* Pine-*Pinus Sp.,* and Beech Woods-*Fagus sp.*).These carbonized wood finds, and the residual plant charcoal are found near combustion structures (hearths and ovens) of which Coppa Nevigata has a notable collection around Middle Bronze Age and is proof of multistep cooking and storing (spatial analysis have discovered silos of food (one in particular due to highly fragmented cluster of barley caryopses and residual woven branches to form a basket). Between the floral remains and the spatial analysis conducted on pottery and combustion structures, its concluded that food was cooked, toasted, and stored near the fortification wall (the silo is indicative of long term storage, but Coppa Nevigata also practiced short term storage using ceramic vessels illustrating management practices; another note is that there is no definitive proof of control over food storages Cazzella et al. 2012). Recchia et al. 2001 studied residual fats on pottery varying from Protoappenine until Ancient Subappenine and concluded that there were vessels for storing, cooking, and eating with chemical signs of various cereals mentioned as well asl olive oil and animal fats (olive oil is particular to Coppa Nevigata and one of the earliest sites to have evidence of this in Italy). The range of taxa are typical of Bronze Age Italy has discussed in section 4.2.3. Fiorentino et al. 1998 and Primavera et al. 2017 compares archaeobotanical finds to other sites throughout Southern Italy which results indicated Coppa Nevigata having the overall majority of residual olive seeds during the Early Bronze Age with over 400 finds while the site of Rocca had around 200 finds during the Middle Bronze Age and the gradual drop of olive, in general, during the Final Bronze Age. *Pancium Sp.* or Millet is only found in very small amounts (<2) throughout the Bronze Age and just one find in Late Bronze Age in Coppa Nevigata. It's already been noted most millet seeds are found in North and Central Italy throughout Bronze Age in section 4.1.3.

5.1.4 Human Skeletal Remains

This section is devoted to human bones found in Coppa Nevigata throughout the Bronze Age. Osteological reports for human remains during the Neolithic occupation has not been published (most likely no remains have been found). Firstly, the most predominate bones at the site consisted of hands/feet (extremities 66%), vertebrae (15%), and some skull fragments (4%), and low finds of long bones (2%). This can be initially understood as burial manipulation which wasn't so uncommon in peninsular Italy even predating Bronze Age in some parts (skull manipulation in particular has been seen throughout the ages in Italy, and along the Adriatic as well; see chapter 4). On the other hand, it could be residual deposits from secondary placement of burials. Published reports come from two works; Recchia et al. 2007 and Cazzella et al. 2012 that sorted the osteological remains by period. During the first phase of Bronze Age (18thC) there were no human remains and no fortification walls. This point is significant because it seems the bones are mostly associated with the walls of the site but not in all cases. The following phase of

Protoapennine (2,000-1,650), when the settlement extended outside of the first fortification of the 16THC, bones are occasional (4 remains). The remains are mostly from Bronze Age 15thC (1,500-1,425) or Ancient Apennine (out of 350 bone finds; 278 of them are found during this period). For Recent Apennine, 14thC (1,425-1,300), there are 35 bones, some of which could have been initially deposited in Ancient Appenine and was mixed during excavation or beforehand but it's unclear. Regards to Ancient Subapennine (1,300-1,200 B.C), there were 10 bones that exhibit a different pattern in terms of the type of bones found not seen in other periods. Lastly, the Recent Subappenine (1,200-1,100 B.C) also had a few remains, 7 bones, to be discussed (roughly 13 bones could not be paired with a period). Please refer to the Appendix to understand the location of the bones.

The first period is the Protoappenine in which the oldest of the fortification walls were built and the bones were found external from the walls. 4 remains come from this period. This collection included a fragment of a calvaria and long bones (leg) of a single child (1-5 years old), a clavaria of an adult, and a single tooth of a young adult female (20-30 years old). It's unclear if this is a primary or secondary deposit. The position is noted to be away from residential areas and seems to be mostly bones of the skull during this phase but no assumptions can be made due to the limited remains.

The next area will focus around the Ancient Appenine. Most of the remains were found during this period and consists of 278 finds (74 feet, 69 hands, 30 vertebrae, 26 calvaria, 26 teeth, 16 hands or feet, 12 arms, 11 legs, 6 mandibles, 5 falange). The remains are found within the fortification of both East and West sides, and few finds of an area labeled as the doorway A and B. Bones were either in filling of wall sections or immediately external characterized by artificial accumulation of yellow limestone. 54 bones attempted to be sexed mostly of which are male with 30 males and 24 females, which is not a significant difference but this is uncertain, and the vast majority are adults with a significant concentration of infants and juveniles. The wall in which the bones were deposited seems to be lower in height of an estimated 80 cm compared to the juxtaposed towers. There is a reutilization of an old structure for burial deposits. Inside the filling of the wall were the remains as well as Bronze artifacts and worked animal bones (the artifacts also included worked stone, examples of worked bone including an awl and other elements, and Bronze hairpins). The focus on fragments could be ritualistic or due to burial conditions overtime (for instance, these fragments could be residual from removing the body into a secondary deposit). Mostly the bones of Coppa Nevigata are deposits but there are formal burials including an adult male (40 years old) in a crouched position (near doorway B internal West wall). Near the body was a ceramic bowl with raised handles near the head, and a worked bone arrow. Associated with this male was a child burial (0-1 years old) and residual remains of an unsexed person (13-15 years old) including a rib, leg bone (long bone) and a foot. Another burial of a young adult male (25-30 years old) was buried with worked bones, a small bowl with perforated raised handles, 4 Bronze studs, a Bronze 'cantilever', Bronze pin, and perforated bone disk (doorway A internal West wall). Further meaning behind these formal burials are not discussed but can be seen as examples of social status, hierarchy, and lineage which correlates with social patterns emerging in Bronze Age

Italy. There seems to be a pattern that skull fragments are related to the East and upper extremities (hands) are related to the West of the wall. Most remains were found within the wall on the West side (241 fragments of 278 within this period) and the formal burials near the doorway A and B on either side of this major grouping of remains. External to the West wall are a collection of a skull fragment, hands, legs, and teeth remains (1 fragment clavaria of male and 4 teeth of young adult, 2 fragments of the same leg bone of a young adult with a fresh bone fracture, 4 hand bones of an adult, 2 from the same individual, and one to a male and a leg bone of a male adult. Area East of the Protoappennine gate filling wall contained 4 human remains, 2 bones of an adult leg of a mature male and 1 tooth and 1 clavaria of an infant. (filling of wall is mixed ground and yellow limestone and was obliterated for the post). The remaining bones are East of the site adjacent to the walls but not within and include; Long bone of an adult and remains of skull of an infant, 9 are related to calvaria including 3 adults, 2 mature males, 2 juvenile and 2 infants. There was also 1 single tooth compared with a mature adult jaw male, 1 leg bone and 1 pelvic bone (the only pelvic bone noted throughout the collection) both related to an adult.

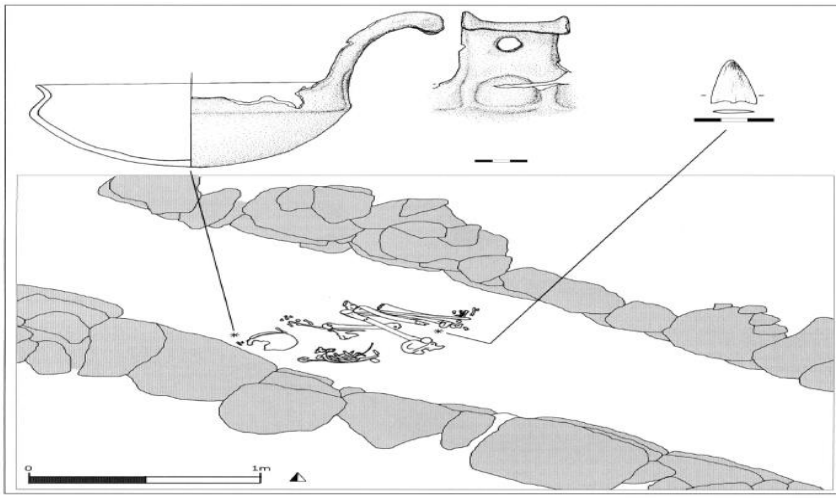


Figure 15: Image of Formal Burial at Coppa Nevigata; Cazzella et al. 2012

Lastly, the bones from the periods of Recent Appenine, Ancient Subappenine, and Recent Subappenine. The finds are associated with yellowish limestone deriving from the demolition and alteration of the fortification wall. In Recent Appenine the bones were found adjacent to the wall, inside the wall between the towers, in an open space, and outside of the wall including doorway G (East side of the wall). There were about 35 bones in total. (7 feet, 6 hands, 2 hands/feet, 6 calvaria, 4 arms, 3 teeth, 2 mandibles, and 2 vertebrae). 3 bones were attempted to be sex and seemed to be males. 6 bones were found superimposed from the Ancient Appenine remains adjacent to the internal West side of the wall and could be mixed with the Ancient Appenine finds. It includes 1 tooth of a juvenile (~15 years old), 1 bone of arm of an infant, bones of a vertebra, and 2 compatible bones of a hand, and 1 bone of a foot of which are adults. 3 bones are found along the same wall but to the East. This group includes 2 feet of an adult and 1 hand or foot from a young adult. 10 bones were found within structures of the Protoappenine towers. There is a fragment of clavaria of an adult, 4 hand bones which 3 relate to the same adult, 1 bone of hand or foot to an adult and 4 compatible bones of a foot to an adult (of which one might be male). They can be residual

remains from Ancient Appenine originally deposited like the others in the filling of the walled section and unintentionally retrieved in Recent Appennine excavation. Or the finds are primary and are evidence of continuity of the use of depositing selected human remains near fortification inside the filling of the wall (argues as unlikely and favors the accidental mixing of phases). 8 bones are found external to the wall. 4 fragments of clavaria which are not compatible, 3 of which is an adult, with possible traces of blows, and 2 bones of the leg both related to an adult and young adult. 2 compatible fragments of a mature adult, maybe male, the mandible and 1 tooth. External to the wall, there was an arm bone of an infant. Also here can be found 1 arm, 1 leg, and 1 vertebra, and 1 mandible element of an infant (6-9 months). This could be interpreted as an infant burial near posterior G (previous Protoappenine wall incorporated in the new fortification). There are two bones in the inhabited area. Tooth of an infant near an elliptical structure surrounded by stones, perhaps a combustion structures. The isolated teeth are most likely deposited randomly. There is a complete ulna (arm bone) of an adult deposited on cobblestones built up against the residue of the internal front of the Protoapennine wall (Could be a selected element or residual). Finally, from the open area paved with a cobblestone path, comes a fragment of clavaria of an adult and which is probably residual from a post deposition altercation. The remains are mostly adults (3 maybe are males) and infants.

The Ancient Subappenine has 10 remains (3 mandibles, 2 calvaria, 2 legs, 1 arm, 1 hand, and 1 vertebra). These bones were found near rectangular structures,burned structures, and open spaces. On the West, internal to the wall near a rectangular structure are bones of an adult male arm with a fresh cut and 2 mandibles, 1 infant and 1 juvenile. In the vicinity of the smaller second structure located farther East there is a leg bone of an individual adult female. The remaining are near a burnt structure internal to the site including 4 human remains, in particular 1 fragment of the clavaria related to a juvenile, 1 bone of the leg of infant, the bone of the hand and 1 of the vertebra of adults. In the area of the open space there were two further remains the fragment of the calvaria of the mature adult individual and 1 fragment of the jaw of adult (maybe female). The two bones found in open area, as in the case of Recent Appennine, may not be in primary position and may have been a result of secondary deposit transaction. All the other remains placed near structures are individual, and are hypothesized to probably be primary and intentional deposits to represent symbolic ritual. There were only 4 bones sexed, 2 of which male (arm and mandible) and 2 of which female (Leg and mandible maybe) with a total of 6 adults, 2 juveniles, and 2 infants.

Lastly, the Recent Subappenine had 7 remains (2 calvaria, 2 hands, 1 foot, 1 vertebra, and 1 tooth) mostly found by bicellular structures on the East side of the site. The last area is adjacent to the residential place (cooking plates, hearths, and daily activities were in concentration here). The remains are mostly adults of which 3 have been sexed (2 males and 1 female). There is an adult hand bone that's burnt in the levels above the collapse of a bicellular structure due to a fire. In the Eastern environment of the bicellular structure emerged near the doorway G to the inhabited area, are 3 human bones including a fragment of a clavaria of young adult male that has been burnt and with signs of cuts, a vertebra of a female individual with traces of combustion and 1 bone of foot of juvenile. Nearby, there are rich levels of fauna and artifacts and fragment of the claviaria

of an adult male and 1 hand bone of adult. Further to east there is an isolated tooth of an young adult (18-20yrs). These remains show multiple signs of alteration due to fire and cut marks. They are also in association with combustion structures and residual flora and fauna where it was probably a place to cook and eat. It's not certain if these bones were affected by fire on purpose as a ritual or were affected by the same fire that destroyed some building structures. There were noted artificial lumps of clayey soil that was probably from the nearby lagoon associated with the bones. It's possible that the bones and clay were taken from the lagoon and re deposited near the residential area where the bones were found.

There are generally a small amount of remains considering the size of Coppa Nevigata (estimates have been made for a community consisting of roughly 150 deaths every 50 years). Some differences can be seen throughout the periods. Protoappenine has limited remains and it's difficult to make assumptions. Ancient Appenine consists mostly of adult extremities with a considerably balanced amount of male and females. Recent Appenine follows a similar pattern of mostly fragmented adult extremities (mostly male sex has been determined but it's important to note the highly fragmented state of all the remains and sexing is difficult). The Ancient Subappenine has a different pattern consisting of mostly skull fragments of adults but not as significant a gap between adults and adolescent in previous phases (although the finds are limited). Finally, the Recent Subappenine consisted mostly of skull and extremities of adults. Overall, the bones were either left as fragments for ritualistic reasons, or are residual from secondary deposits. The formal burials hint at social status, lineage, and perhaps hierarchy and competition within a community. Some bones, especially in the Ancient and Recent Subappennine, seemed to gone through burning with some exhibiting fresh wounds.

5.2 Croatian Sites

5.2.1 Brnjica, Gusica Gomila, Jukica Gomila

There is very little published about these Croatian sites although all were Tumuli (a burial mound consisting of stone and earth covering graves of different types). The site of Gaj, village in Brnjica, is located on the Karst plain. It consisted of 18 tumuli. One tumulus has been excavated and published and its made of stone and red-brown soil placed on bedrock. On the Western half of the tumulus there is a large concentration of pottery sherds (a significant amount of Cetina pottery which seemed to be deliberately broken and assisted in dating the tumulus occupation around the Early Bronze Age) near an area with a small circular and ellipsoid structures along with a few fragments of burnt bone. (Menudisc et al. 1986). Guisca Gomila, a stone tumulus, is a part of a supposed clusters of tumuli near the Guisci village and most likely occupied during the Middle Bronze Age. Jukica Gomila is located near the Zagvozd and is a dry stone tumulus. There were at least two seperate Bronze Age graves (grave 3 and grave 4) that contained inhumations,burnt humans bones, 2 small Cetina jars, and a decorated Bronze sheet. More specifically, Grave 3 had inhumations consisting of two individuals along with seven burnt fragments of bone which were

unable to associate with the inhumations. Person A is poorly preserved but has been determined to be an adult male between 40-50 years old with evidence of Periostitis. (“upper and lower jaw, right shoulder blade, both pelvic bones, left patella, both tali, both calcanei, both humeri, both radii, both ulnae, both femora, both tibiae, both fibulae and a right rib. 12 teeth from the upper and 12 teeth from the lower jaw”). Person B is also in poor condition and determined to be a male skeleton fairly complete between the age of 35-45 years old with Periostitis (“frontal bone, two parietal bones, two temporal bones, the occipital bone, the left cheekbone, upper and lower jaw, both clavicles, left scapula, left pelvic bone, sacrum, both patellae, both tali, both calcanei, both humeri, both radii, both ulnae, both femora, both tibiae, right fibula, 5 cervical, 2 thoracic and 3 lumbar vertebrae, 3 right ribs and one left rib. 10 teeth of the upper and 11 from the lower jaw”). In particular interest are the burnt bones found alongside sherds of a Cetina vessel in dark soil in which the bones might have been contained. (Perhaps the sherds and spread burnt fragments concentrated in this dark soil have been damaged due to the destruction of the stone slabs). Grave 4 still had the stone cover intact. Little was found except some charcoals and bone fragments associated with the grave. These burials in mound one are roughly dated to the Bronze Age with Grave 4 slightly older (Grave 3: 2,030-1,880 B.C, Grave 4: 2,480-2,140B.C). In mound two was another prehistoric grave surrounded by stacked stones of a female 35-45 years old. (“frontal bone, two parietal bones, two temporal bones, the occipital bone, the upper and lower jaw, the left pelvic bone, sacrum, left humerus, left radius, both ulnae, left femur, left tibia, left fibula, one thoracic and one lumbar vertebra, and one left rib. Three teeth from the upper and seven from the lower jaw”). This burial is roughly dated from the Late Bronze Age (1,490-1,310 B.C). (These burials were juxtaposed with medieval burials. Olujić et al. 2012). A pattern can be seen throughout these prehistoric inhumations in which hands, feet, ribs, and spine elements were mostly missing. There are some elements of the skull and pelvis, but the long bones seem to dominate the burials. This has not been furthered researched if these were primary or secondary burials or if any ritual can be understood. The burnt bones are scattered near inhumations illustrating both practices were used during the Bronze Age in Dalmatia, Croatia.

5.2.2 Across the Adriatic

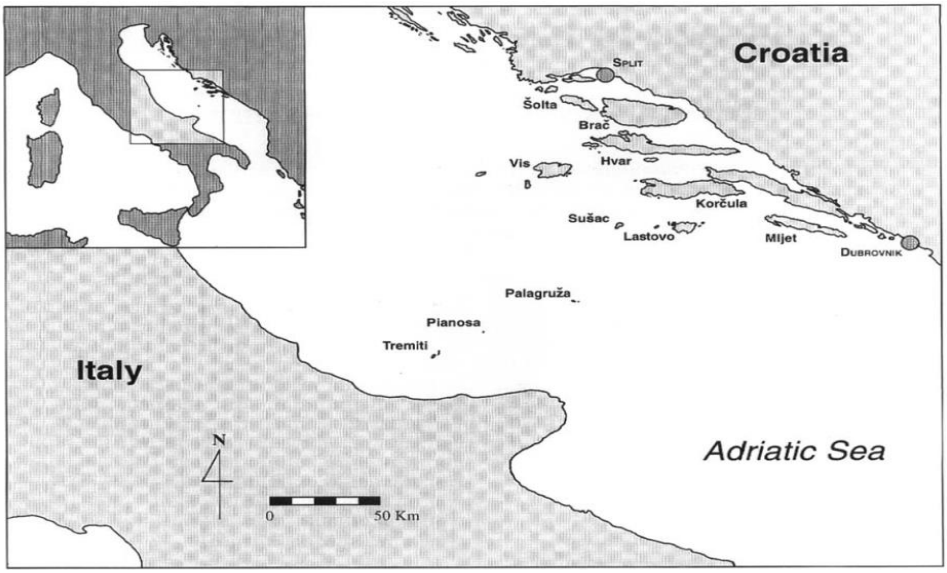


Figure 16: Island Chain of the Adriatic; Kaiser et al. 2016

The interest in the connection between Italy and Croatia arose from similar material finds across the Adriatic starting as early as the Neolithic period beginning with pottery, most importantly Impressed Ware pottery, that seemed to connect the earliest farming sites (Forenbaher et al. 2008). This correlates with several theories that the spread of agriculture originated from the East and moved in a sporadic North-West direction, most likely with the help of seafarers (recall chapter 4). Kaiser et al. 1999 and Kaiser et al. 2016 mentions islands connecting the Dalmatian coast and Apulia (Tremiti, Planosa, Palagruža, Sušac, Lastovo islands), in particular Palagruža, which consisted of anthropogenic evidence since the Neolithic period. These islands have been considered a chain to connect seafarers of the Neolithic and later from Dalmatia to Apulia. This idea is supported by several material finds on the island chain. The earliest finds are from the Neolithic on the islands Sušac and Tremiti (Forenbaher et al. 2008). Sušac had multiple open air Neolithic Sites with multiple finds of Impressed Ware and figulina pottery resembling Dalmatia (Vela Luka) and Apulia (Scaloria) types. There were also finds of Liparin obsidian (as well as a few examples of obsidian from Melos, Greece) and chert originating either from Gargano or Palagruža confirmed through petrographic analysis. Lastly, animal bones consisting of the domesticated taxa (sheep and goat) were found (use of the islands as a fishing center is attested towards the Late Bronze Age). At Tremiti Neolithic sites consisted of Impressed Ware pottery and decorative pottery indicative of Danilo Culture of Dalmatia (recall chapter 4). There were also human bone finds from a Middle Neolithic layer (roughly a dozen individuals placed in a pit) accompanied by Diana-style vessels near the heads (Diana-style is a type of pottery originating from Late Neolithic Central-Southern Italy; Malone et al. 2003). Signs of agriculture have been attested on both these islands (‘sickle gloss’ blades or blades that have grain residuals still intact, and stone axe-heads) suggesting semi-permanent to permanent settlement during the Neolithic. Neolithic pottery sherds also found in both directions with Dainlo and Hvar pottery in Italy and Serra d’alto and *figulina* sherds in Dalmatia. Perhaps one reason first anthropologic evidence

begins at the Neolithic is due to sea level rise and fall. For instance, Forenbaher et al. 2008 mentions that during the 'LGM' most of these islands were connected to the mainland with the exception of Palagruža and Sušac. After 12,000B.C the valleys were flooded until finally around 6,000 B.C the islands represent how they are now more or less.

Later finds are concentrated on the island of Palagruža, during the late Copper Age and Early Bronze Age. There are Cetina pottery finds (named after the Cetina river and concentrated finds along Spilt and Šibenik of Dalmatia; mentioned in Chapter 4). Some of these vessels have been found in Italy (Laterza, Rodi and Coppa Nevigata; Apulia). The Cetina cultures is associated with tumuli of both inhumations and cremations mostly in singular burials but multiple burials do occur. The 'Cetina Phenomena' (Gori et al. 2012) spread throughout the Mediterranean including Greece, West Balkans, and Italy reaching as far as Campania, Sicily, and Aeolian Islands. Most Cetina type pottery in Italy are found in Apulia in two concentrated areas; the North of the Gargano and Taviolere and the Inland near Laterza (Central-Southern). In the North, there are mostly surfaced sherds while inland there are complete incised jars. Gori et al. 2012 argues for two phases of contact of the Cetina type; first in the North from small groups and then extended into known maritime connection in the the Central-South. The Cetina phase is chronologically difficult to interpret but many finds are centered during the Early Bronze Age or the second half of the 3rd Millennium B.C. Further analysis on Palagruža revealed some decorated stone 'wristguards'(decorative stone or metal supposedly used by archers; Forenbaher et al. 2018) that resemble Late Copper Age Italian types from Central and North Italy and Eastern Adriatic (Kaiser et al. 2016). Chert is another main product that played an important trade role since the Neolithic. It has already been mentioned that the Gargano was an important center for chert. Palagruža also had a local chert quarry nearby. It's been noted that chert of Palagruža were found in Dalmatia (Vis and Hvar) and Gargano chert was found on Palagruža itself. Concluding that these island chains were used in both directions across the Adriatic. In support of this theory is the fact that these island chains were visible by eye from Gargano, Apulia and from vis, Dalmatia. There are no prehistoric shipwrecks within the Adriatic (the earliest example of seafaring in Italy comes from a Neolithic dugout in Lazio in which can be assumed similar paddle boats were used). Although there were multiple shipwrecks from Hellenistic and Roman times where material cultures have been found on these islands as well. This can be seen as continued use of a well-known connection in the Adriatic used since Neolithic and as late as Roman period. In fact, most materialistic finds are from the Neolithic period and the Hellenistic period which Forenbaher et al. 2008 argues are times of exploration and intensified trade.

Chapter 6: Material and Methods

This chapter will describe in detail the methodology and instrumentation used for this experiment as well at what material was analyzed. In terms of methodology, many studies vary in procedure and each study should account for the material at hand as well as access to equipment to decide the best procedure.

6.1 Materials

This study focuses on four Bronze Age sites along the Adriatic coast, one of which is in Italy, namely Coppa Nevigata, and three in Croatia, namely Gusica Gomila, Jukica Gomila, and Brnjica. The bone deposits of Coppa Nevigata were excavated between the late 1970s and the early 2000s by the Archaeological Mission at Coppa Nevigata, Sapienza University of Rome, under the direction of A. Cazzella and G. Recchia. The Croatian sites were excavated from the 1980s and the early 2000 by (Gusica Gomila by CeVaS Project directed by H. Tomas, and Jukica Gomila by Zagreb University directed by B.Olujić). In all, 15 individuals and 30 terrestrial fauna were sampled from Coppa Nevigata. In addition, 4 individuals from the site of Gusica Gomila, 2 individuals from Jukica Gomica, and 4 individuals from Brnjica. These values were juxtaposed to an already sampled individual from Croatia in from Jukica Gomica (JKG 3, students of Sapienza University). Fauna samples were not available from these sites so fauna already analyzed from nearby sites were used for comparison (65 Neolithic fauna from Zemunica, Dalmatia; Guiry et al 2017). Although not ideal, this can help provide base values for Croatian coastal sites that have no fauna. These samples are all from a Bronze Age period. Coppa Nevigata samples were specifically limited in choice. They were chosen to correlate with another study on Strontium stable isotopes to compare diet as well as mobility. Fauna samples and Croatian samples were chosen on availability. For the individuals of Coppa Nevigata, there were no signs of cremation, damage from animal scavenging, butchery, or root damaging. Although some samples were quiet brittle and yellow to color (can be from the surrounding soil the bones were deposited in as discussed in Chapter 5). The fauna samples were in similar condition to the individuals of Coppa Nevigata with only one sample showing signs of burning (CNF 21) and no obvious butchery marks. The individuals from Croatia exhibited some signs of charring and were in poor condition overall.

Table 6: List of samples for isotopic analysis

Sample ID	Description	Sex/Age of Death	Location
Coppa Nevigata Homo			
CN1	Rib	M 40+	Ancient Appenine
CN2	Mandible	F Adult	SubAppennine
CN3	Mandible	Juv. 15-20	Ancient Appenine
CN4	Mandible	M. Adult	Recent Appenine
CN5	Skull		ProtoAppenine
CN6	Mandible	M. 30-35	Ancient Appenine
CN7	Mandible	Adult	Ancient Appenine
CN8	Mandible	M. 15	SubAppennine
CN9	Mandible	Juv. 3-4	Surface
CN10	Mandible	M 16-18	Ancient Appenine
CN11	Mandible	Juv. 7	SubAppenine
CN12	Mandible	M. Adult	Ancient Appenine
CN13	Mandible	F 20-25	Ancient Appenine
CN14	Mandible	M. Adult 20	Ancient Appenine
CN15	Mandible	M. Adult 40	Ancient Appenine
Coppa Nevigata Fauna			
CNF1	Bos taurus Tibia	F	SubAppenine
CNF2	Bos taurus Cranium		Ancient Appenine
CNF3	Bos taurus Femur		SubAppenine
CNF4	Bos taurus Metacarpal		SubAppenine
CNF5	Bos taurus Mandible		

CNF6	Bos taurus Metacarpal		Ancient Appenine
CNF7	Bos taurus Falange	F	
CNF8	Bos taurus Metacarpal		
CNF9	Sus domesticus Tibia		SubAppennine
CNF10	Sus domesticus Antler		Ancient Appenine
CNF11	Sus domesticus Coxal	F	SubAppennine
CNF12	Sus domesticus Radius		
CNF13	Sus domesticus Calcaneus		
CNF14	Sus domesticus Metacarpal		Ancient Appenine
CNF15	Sus domesticus Ulna		
CNF16	Sus domesticus Coxal	F	Recent Appenine
CNF17	Cervus elaphus Calcaneus		SubAppennine
CNF18	Cervus elaphus Calcaneus		Ancient Appenine
CNF19	Cervus elaphus Tibia		SubAppennine
CNF20	Cervus elaphus		

	Ulna		
CNF21	Cervus elaphus Femur	F	Ancient Appenine
CNF22	Cervus elaphus Falange	F	
CNF23	Ovis/Capra Humerus		SubAppennine
CNF24	Ovis/Capra Humerus		SubAppennine
CNF25	Ovis/Capra Scapula		
CNF26	Ovis/Capra Femur		Ancient Appenine
CNF27	Ovis/Capra Metacarpal		
CNF28	Capra hircus Falange	F	
CNF29	Capreolus capreolus Falange	F	
CNF30	Canis familiaris Metacarpal	F	
Croatian Homo			
Gusica Gomila			
GG1	Femur	Adult	Middle Bronze Age
GG2	Femur	Adolescent	Middle Bronze Age
GG3	Femur		Middle Bronze Age
GG4	Femur	Adolescent	Middle Bronze Age
Jukica Gomila			
JKG1	Long Bone		Tomb 3 G1: Indv. B Early Bronze Age
JKG2	Long Bone		Tomb 3 G1: Indv. A Early Bronze Age

JKG3*	Fragment		Tomb 3: Gomila 2 Late Bronze Age
Brnjica			
BRN1	Rib		Early Bronze Age?
BRN2	Phalange		Early Bronze Age?
BRN3	Long Bone		Early Bronze Age?
BRN4	Mandible		Early Bronze Age?

6.2 Methods

Samples were cleaned and prepared in the Laboratory of Paleoanthropology and Bioarchaeology of the Department of Environmental Biology of Sapienza University of Rome. Collagen extraction followed a known procedure (Longin et al. 1971; partly adapted by Brown et al. 1988). Samples were cut by using a Dremel(™) Tool and the outer surface was mechanically cleaned by abrasion and weighed. Bone chunks were used for this study. This paper stands by the argument that bone chunks are preferred to bone powder when the bones could be in poor condition and provide better results (i.e., Jrkov et al. 2007 state that collagen fiber structure is preserved better in chunks rather than powder in poorly conserved bones). Next, the bone chunks (roughly around 600 mg to 1g) began demineralisation in approximately 8ml cold 0.5 HCl solution and covered with aluminum foil to prevent contamination and stored in a +4°C fridge (the acid is diluted with demineralized water to be less harsh for the samples and let the samples sit longer in the solution for more dependable results; Katzenberg et al 2008). Samples were shaken every few days and the acid was changed once no longer reactive. This process took a few weeks for all samples although the human samples took roughly a week longer than the fauna samples. This step is to insure the inorganic portion of the bone has been removed to only extract the collagen. It also assists in removing acid soluble organics like carbonates (which are more soluble than whole apatite) and calcites and water soluble contaminants such as free amino acids that can affect isotope values (Price et al. 1989; DeNiro et al. 1988; Price et al. 1992; Koch et al. 1997). When sample became soft, translucent, or floated (tested by touching with pasteur pipette), the solution was removed and the samples were rinsed with distilled water three time (with use of Ezee(™) filters; 60-90µm). The appearance of the samples indicates collagen is still intact but the mineral portion has dissolved (Sealy et al. 2014). Lee-thorp et al. 2008 mentions collagen denatures once the hydrogen bonds are broken allowing the fibrils to dissolve and this is why certain temperature, PH conditions, and moisture environments are chosen after experimentation to prevent this. At this point, some studies subject the samples to NaOH treatment to remove humic acids but it has been debated this step can produce sample lost due to the vulnerability of collagen to NaOH (Szpak et al. 2017; Van der Haas et al. 2018; Stafford et al. 1988). For this study the samples then went

through gelatinisation by heating in PH 3.0 water (about 7-8ml) at 75°C for 48 hours tightly sealed. This leaves behind all acid insoluble materials like humic or fulvic acids from soils (Longin et al. 1971; Stafford et al. 1988; Price et al. 1989). Then, with use of ezee filters, the supernatant liquor - that is the collagen solution - was placed into labelled test tubes. The samples were then placed into -20°C fridge to freeze overnight. The samples then went through freeze-drying by first transferring the plastic tubes to a -80°C freezer for at least 4 hours then into the freeze drier and left there between 1-4 days until dry. This material will contain collagen and possibly some acid salts (see Chapter 2.3). The collagen yield was determined and sample with at least 5% of collagen to dry bone weight were used for MS analyses (this is done by dividing the weight of the freeze dried sample to the original dry weight of the sample). The collagen samples were then placed into tin capsules (about 0.8-0.1 mg max 1mg) to be combusted in the EA-IRMS. The samples were done in single runs with every 5th sample or so in duplicate. This is to insure accuracy of the equipment.

The isotopic results were analyzed statistically by the following methodology. To better understand the results, the isotopic values were run against two statistical methods; The Bayesian Mixing Model and a Mann–Whitney U-test when applicable. Briefly, the Bayesian Mixing Model is based on the assumption that proxy signals (δ13C and δ15N) are directly representative of a mixture of food contributors (i.e. terrestrial fauna, marine fauna, freshwater fauna, C3 and C4 plant groups) of which are directly related to the catchment of recently assimilated consumed diet by the target consumers (I.e. Coppa Nevigata and the Croatian sites; Phillips et al. 2012). The proxy signals will represent only the chosen fraction of the food groups in relation to consumer tissue (Hopkins et al. 2012). In the case of collagen analysis, the protein fraction of the food group was chosen based on the generally accepted notion that collagen reflects the proteins of the diet (Chapter 2). Carbon can be assimilated in different parts of the tissue depending on diet routing (Chapter 2) so therefore the outcome can lead to overestimation or underestimation of food groups and should be read carefully. This Bayesian Mixing Model also accounts for metabolic offset of isotopic values due to isotopic fractionation. This study used the offset values for δ13C of 2 with an uncertainty of 1 and an offset for δ15N of 5 with an uncertainty of 1 due to trophic level shift. The Mann-Whitney U-test is a statistical method that compares independent variables that is not normally distributed and is performed on ranked (ordinal) data, such as a group of isotopic values of females versus isotopic values of males. The two groups to be compared would be the females and males, the ordinal data is the isotopic values which would be ranked and statistically analyzed to understand if there is a difference between the group data or not. More details will follow in the results.

6.3 Instrumentation: EA-IRMS

Stable carbon and nitrogen isotope ratios were measured using an automated Elemental Analyzer interfaced to a continuous-flow Isotope Ratio Mass Spectrometer (EA-IRMS). Analysis was carried out by the UK Iso-Analytical team with a Europa Scientific Elemental Analyzer.

The basic components of an EA-IRMS can be seen in (fig. 17). Generally, this equipment measures ionized gaseous molecules that have been separated by their mass-to-charge ratio via electric and magnetic fields to find the relative abundance of isotopes in a sample which is the measured current produced by the ion beams. How carbon and nitrogen are measured will now be summarized. The Elemental Analyzer is composed of the automatic sampler, combustion mechanism and several traps and filters. The IRMS is composed of an inlet system, ion source, mass analyzer, and ion detector or the Faraday cups, and lastly a computer. Samples are introduced into the Elemental Analyzer as solid samples and into the IRMS as a pure gas (N_2 and CO_2 for nitrogen and carbon respectively) that is achieved by combustion following sample preparation described in chapter 6.2 (solid sample into tin containers into automatic sampler of the EA). The EA is responsible for combustion of the samples. For carbon and nitrogen, Flash Combustion is used at a temperature around 1,800C° to produce N_2, CO_2 and other gases. Helium is an inert carrier gas that carries the products through a column where it can go through several steps. The presence of the He carrier indicates the "continuous flow" instrumentation that helps the produced gases flow directly to the ion source. There are reduction centers and chemical traps that allows the combusted sample to breakdown into the wanted gases (for instance nitrous oxides NOx from the combusted sample are reduced into N_2 and excess O_2 is removed while the chemical trap removes water). Lastly, N_2 and CO_2 go through a gas chromatography column (this is a stationary phase, and the mobile phase which is the gas reacts with the column and the gases are separated by how fast they leave or elute from the column) to separate and prepare to enter the IRMS (all the while reference gas like CO_2 for instance is simultaneously being imputed for better accuracy). The IRMS is responsible for ionizing the products produced by the EA, accelerating them and separating by mass, and lastly detected by Faraday cups which are positioned in a way that masses can be caught simultaneously (N_2 masses are 28,29,30 and for CO_2 masses are 44,45,46; for example, a mass of 28 could indicate N14N14 N_2 or mass 29 could indicate N14N15 N_2). Samples are ionized (an ion is an atom or molecule that has lost or gained an electron resulting in a charge) via electron bombardment produced by a heated filament which allows them to be focused into an ion beam. The ion source works at a high potential voltage while the rest of the IRMS is at ground potential allowing the acceleration of the ions out to the magnet. The ion beam travels through a tube into the mass analyzer. Here, a magnet separates the ion beam into several beams resulting in a mass spectrum according to their mass to charge ratio (m:z lighter versus heavier beams bend at the radius of the magnet differently while the light ions are deflected more easily than heavier ions of the same charge). The different beams have different intensities and can be measured in the ion detector which is the faraday cups (conductive metal cups that have an electric field to force the secondary electrons into the cups). The IRMS works under a vacuum system the helps reduce collision between ions. (Katzenberg et al. 2008; Sharp et al. 2007; Muccio et al. 2009). The benefits of the IRMS is its sensitivity and precision in measuring multiple masses of isotopes simultaneously and the use of small sample size. Limitation of this machine includes the need for high sample purity (although the GC column makes this less of a limitation), price, and portability.

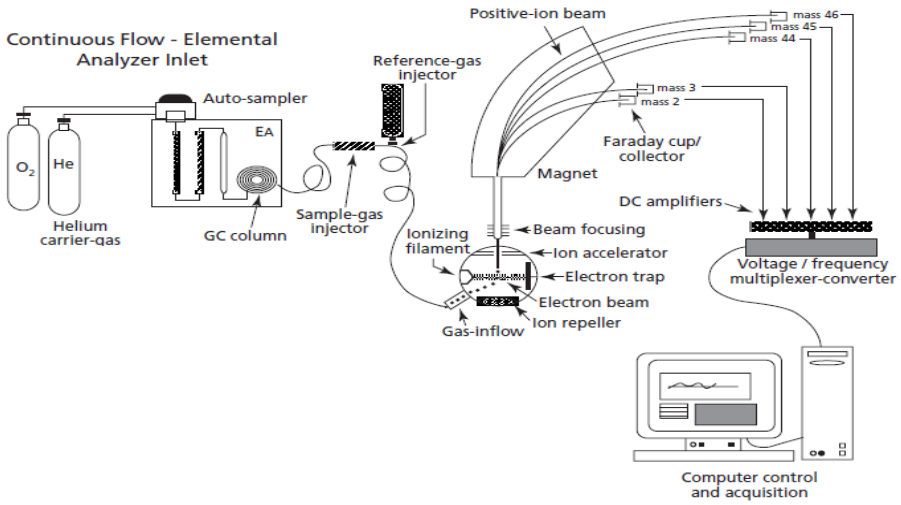


Figure 17: EA-IRMS; Edited from Michener et al. 2008

6.4 Standards, Precision, Accuracy

For this measurement, based on international (PDB; AIR) and laboratory standards (IA-R068 (soy protein, 13CV-PDB = -25.22 ‰, 15NAIR = 0.99 ‰)), measurement errors are less than ±0.1‰ and ±0.2‰ for δ13C and δ15N respectively.

Carbon and nitrogen stable isotopes in biological material is reported as ratios of heaviest isotope to lighter (13C:12C, 15N:14N). These ratios are compared to a standard and reported as "delta" notation which can be defined as the very small relative difference in isotopic ratios. The sum of the sample ratio and standard ratio are divided by the standard ratio and multiplied by a thousand reported as parts per mil or parts per thousand (‰). In other words, if there is a negative value of δ13C, -20.0, then the sample has a 13C/12C ratio that is 20.0 per mil or 2.00% lower than the standard (Sharp et al. 2007). This is not an absolute isotope abundance but the expression of a sample difference to a standard. The isotopic values of the sample are measured relative to either a reference gas or a local working standard local of a lab and an international standard. Working standards are usually CO_2, N_2, H_2 and the difference between working and international standard must be calculated (Sharp et al. 2007). The stable isotope ratios of international standards are well known. The international standard for carbon is calcite from a mineral deposit PeeDee Belemnite (PDB). Biological material produces negative values of δ13C‰ generally ranging from -25 to 0.0 per mil (PDB is rich in 13C so most biological materials are negative to it; Kruger et al. 1984). Negative values mean the resulting ratio is lower than the standard or ppm lighter than PDB (Price at al. 2014). The international standard for Nitrogen is atmospheric nitrogen (N_2), simply AIR. Biological material produces positive values of δ15N‰ generally ranging from 0.0 to +25 per mil. Positive values mean the resulting ratio is higher than the standard or ppm heavier than AIR (Katzenberg et al. 2008; Sharp et al. 2007).

$$\delta^{13}C‰\,\mathrm{PDB} = \frac{\left[{}^{13}C/{}^{12}C_{\mathrm{sample}} - {}^{13}C/{}^{12}C_{\mathrm{standard}}\right]}{\left[{}^{13}C/{}^{12}C_{\mathrm{standard}}\right]} \times 1000$$

Chapter 7: Results

This chapter will discuss the results of the analysis. Firstly, the collagen preservation of the samples will be explained followed by first observations of Coppa Nevigata animals, Coppa Nevigata humans, and the Croatian humans.

7.1 Collagen Yield, C:N, and C and N content

The stable isotope results for all samples and the quality parameters are reported in table 7. Firstly, the collagen quality controls were examined. These parameters include collagen yield, carbon and nitrogen content (%), and the C:N ratio (Chapter 2). In terms of collagen yield all samples fell within the accepted range between 5-25% except for CNF 6, CNF 21, and BRN 3 which had low values. Ambrose et al. 1993 and Pate et al. 1994 mention at least 2% would be acceptable. All samples had acceptable carbon content (13-50%) except for CNF 18, CNF 21, and BRN 3 which had low values. Ambrose et al. 1993 and Van Klinken et al. 1999 accept values as low as 3%. All samples had acceptable nitrogen content (4-18%) except for CNF 18, CNF 21 which had low values. Van Klinken et al. 1999 and Ambrose et al. 1993 mention values as low as 0.5 % is acceptable. All samples fell into the accepted range for C:N ratio of 2.9 and 3.6 except for CNF 21 and BRN 3, both with higher values. Due to the good C:N ratios, good collagen yields, and seemingly averaged δ13C and δ15N values, the slightly anomalous samples mentioned were deemed acceptable but to be interpreted with caution that they may cause inconsistencies in the results. On the other hand, BRN 3 and CNF 21 were removed due to the highly inadequate values mentioned. Upon visual analysis of the “collagen” residue of CN 2, CN 10, CN 12 and CN 25, they were excluded from MS analysis. They were sticky and dark in color and most likely only resulted in residual dirt and did not present adequate collagen yields in some cases like CN 10. In the majority of the samples, good collagen yields resulted (low yields ranged from 0.7 to 3.6% and good yields ranged from 4.4 to 17.6 %) even without pretreatment by NaOH and ultrafiltration in this sample set (Chapter 6). The references standard deviation values for the Mass Spectrometer fell within 0.01 to the expected mean values, therefore the sample runs were accepted. 13 duplicates ran alongside the samples and provided accuracy for the Mass Spectrometer (see Appendix). In total 22 human and 28 animal samples were considered for further analysis.

Table 7: List of Results

Sample ID	Code	Collagen Yield (%)	C:N	N Content (%)	C Content (%)	δ15N (‰)	δ13C (‰)
Coppa Nevigata Homo							
CN1	CN 95 CNV D4E SII	8.10	3.12	12.73	34.01	9.20	-19.51
CN2	CN 09 F3H 2IV	9.88	0.0	0.0	0.0	0.0	0.0
CN3	CN 07 CNV G2D	8.47	3.20	9.63	26.38	10.06	-19.38
CN4	CNV G2A	13.16	3.15	12.38	33.39	8.66	-19.80
CN5	CN 10 CNV B	12.59	3.16	14.79	40.04	7.46	-20.17
CN6	CN 11 CNV H2L 5.1	12.53	3.10	16.59	44.15	8.11	-20.26
CN7	CNV Cγ 2e	13.78	3.14	19.52	52.56	8.99	-20.06
CN8	CNV G1	10.38	3.17	18.99	51.57	8.88	-19.71
CN9	CN CR	11.91	3.21	20.29	55.78	10.63	-19.86
CN10	CNV F5 A	0.74	0.0	0.0	0.0	0.0	0.0
CN11	CNV H 1C	11.64		18.75	51.44	9.31	-19.59
CN12	CNV F5 B	6.43	0.0	0.0	0.0	0.0	0.0
CN13	CNV F5 C	5.71	3.30	5.60	15.83	8.99	-20.09
CN14	CNV F5 D	6.90	3.25	10.33	28.81	10.56	-19.55
CN15	CNV F5 E	10.00	3.21	11.27	31.04	9.55	-19.96
Coppa Nevigata Fauna							
CNF1	CN 09 F3 H2	12.61	3.14	15.40	41.40	5.43	-21.21
CNF2	Cn 1971 F5	5.33	3.30	3.85	10.88	7.72	-20.14
CNF3	CN 1971 G1	6.45	3.21	7.47	20.58	6.37	-20.51
CNF4	CN 1971 HIC	7.49	3.23	11.29	31.22	6.58	-20.62

CNF5	CN 1994 D3R 2II	4.58	3.24	6.86	19.03	5.63	-20.53
CNF6	CN 11 H2L 5Iia	3.11	3.20	8.79	24.14	6.52	-20.63
CNF7	CN11 G2p 10a	16.28	3.14	17.81	47.97	3.38	-21.18
CNF8	CN95 D43 1V Ia	10.68	3.14	9.61	25.83	5.02	-20.85
CNF9	CN 09 F3H2	9.03	3.12	13.37	35.75	6.03	-19.85
CNF10	CN 1971 F5	9.99	3.19	7.67	20.94	4.97	-20.63
CNF11	CN 1971 G1	9.10	3.23	8.85	24.47	4.25	-20.37
CNF12	CN 1971 HC1	4.94	3.23	10.94	30.32	6.29	-20.27
CNF13	CN94 D3R 2II	11.53	3.18	6.62	18.05	4.10	-20.71
CNF14	CN11 H2L 52II	12.44	3.20	9.91	27.22	7.15	-20.60
CNF15	CN11 G2P 10	11.82	3.14	9.95	26.78	8.69	-20.31
CNF16	CN 07 G2A Iva	12.05	3.3	13.95	37.95	6.68	-20.44
CNF17	CN 09 F3H 2	10.76	3.15	9.11	24.61	5.18	-20.27
CNF18	CN 1971 F5	8.97	3.27	0.76	2.13	6.86	-21.23
CNF19	CN 1971 G1	9.81	3.19	14.09	38.47	7.09	-19.66
CNF20	CN 1971 H1C	10.21	3.22	11.52	31.81	5.57	-21.23
CNF21	CN 11 H2L	3.64	9.5	0.21	1.71	0.0	0.0
CNF22	CN 11G2P 10	13.90	3.16	12.86	34.87	6.97	-20.47
CNF23	CN 09 F3H 2	8.82	3.16	14.16	38.34	7.23	-19.39
CNF24	CN 1971 G1	7.44	3.0	9.26	25.76	5.87	-19.94
CNF25	CN 1971 H1C	11.51	0.0	0.0	0.0	0.0	0.0
CNF26	CN 11 H2L 5II	12.12	3.15	15.19	41.06	7.64	-20.69
CNF27	CN94 D3R 2II	8.03	3.16	15.64	42.42	6.69	-20.44
CNF28	CN95 D4E 1 IIa 23	17.65	3.17	12.30	33.38	7.29	-19.94
CNF29	CN 95 D4E 1	12.23	3.15	15.34	41.45	8.50	-18.87

	IIIa 22						
CNF30	CN G2P 10	14.40	3.19	11.09	30.32	7.33	-20.04
Croatian Homo							
Gusica Gomila							
GG1	GII Grave 1	6.31	3.42	9.91	29.06	9.34	-20.21
GG2	GII Grave 2	4.38	3.33	11.97	34.13	8.43	-20.17
GG3	GI Grave 3	14.02	3.26	13.96	38.99	8.65	-20.04
GG4	GII Grave 3	4.74	3.48	7.60	22.65	8.12	-20.25
Jukica Gomila							
JKG1	Bag 2	10.22	3.29	10.02	28.22	8.73	-19.94
JKG2	Bag 4	6.33	3.50	4.71	14.11	9.19	-20.30
JKG3*	Bag T3	5.57	3.34	8.65	24.74	8.70	-19.43
Brnjica							
BRN1	Bag25	4.79	3.32	9.99	28.47	8.14	-18.66
BRN2	Bag24	6.70	3.59	7.56	23.27	9.04	-18.91
BRN3	Bag30	1.75	3.84	2.59	8.52	8.19	-20.64
BRN4	Bag BRN	6.36	3.29	6.75	19.06	9.75	-19.92

Descriptive statistics of δ13C (‰) of human and animal samples (N values in brackets). Abbreviations: CNF = Coppa Nevigata Fauna; CNH (Coppa Nevigata Herbivores); CNO (Coppa Nevigata Omnivores).

Sample Group	Min Value(‰)	Max Value (‰)	Median (‰)	Mean (‰)
CN (12)	-20.26	-19.38	-19.83	-19.83 ± .29
GG (4)	-20.25	-20.04	-20.19	-20.17 ± .09
JKG (3)	-20.3	-19.43	-19.94	-19.89 ± .44
BRN (3)	-19.92	-18.91	-18.91	-19.16 ± .67
CNF (28) CNF H (19) CNF O (8)	-21.23 -21.23 -20.71	-18.87 -18.87 -19.85	-20.46 -20.51 -20.40	-20.39 ± .55 -20.41 ± .63 -20.39 ± .27

Descriptive statistics of δ15N (‰) of human and animal samples (N values in brackets). Abbreviations: CNF = Coppa Nevigata Fauna; CNH (Coppa Nevigata Herbivores); CNO (Coppa Nevigata Omnivores).

Sample Group	Min Value(‰)	Max Value (‰)	Median (‰)	Mean (‰)
CN (12)	7.46	10.63	9.10	9.20 ± .93
GG (4)	8.12	9.34	8.54	8.64 ± .52
JKG (3)	8.7	9.19	8.73	8.87 ± .27
BRN (3)	8.14	9.75	9.04	8.98 ± .81
CNF (28) CNF H (19) CNF O (8)	3.38 3.38 4.10	8.69 8.50 8.69	6.55 6.58 6.16	6.32 ± 1.28 6.40 ± 1.17 6.02 ± 1.55

7.2 Fauna Results CN

Fauna results for Coppa Nevigata included all samples except for CNF 21 and CNF 25 making a total of 28 samples (Fig. 18). It includes 8 domesticated cattle (*Bos taurus*), 8 domesticated pigs (*Sus domesticus*), 5 deer (*Cervus elaphus*), 4 Ovicaprid (*Ovis vel Capra*), 1 goat (*Capra hircus*), 1 Roe deer (*Capreolus*), and 1 dog (*Canis familiaris*). The mean δ13C value for all the fauna is -20.39‰ with a range from -21.23 to -18.87‰. The mean δ15N value for all the fauna is 6.32‰ with a range from 3.38 to 8.69 ‰. If the pigs are considered omnivorous species, they will have separate values from the herbivores.The mean δ13C value for omnivores is -20.39‰ with a range from -20.71 to -19.85‰. The mean δ15N value for omnivores is 6.02‰ with range from 4.10 to 8.69‰. The mean δ13C value for herbivores is -20.41‰ with a range from -21.23 to -18.87‰. The mean δ15N value for herbivores is 6.40‰ with a range from 3.38 to 8.50‰. The δ13C values for both groups are uniform and generally seem consistent with a terrestrial diet. The *Sus* show values that are in line with those of the herbivores, which seems to suggest a plant-reliant diet for this group, which is unsurprising for prehistoric contexts (Albarella et al. 2006). Lastly, the dog is considered carnivorous (δ13C: -20.04, δ15N:7.33 ‰) but its value is fairly similar with the pigs and cows and may indicate a plant-reliant diet as well (similar to Goude et al. 2016 of a dog mostly consuming plants). When comparing the mean to the median values for each of the sample grouping (table 7) and observing the narrow range, it's clear that that the collective fauna results are homogeneous with no striking differences between herbivores/omnivores or browsers/grazers in terms of δ13C values. This is not the case in terms of nitrogen values which seem to have a greater range. At first glance, the δ15N values for the fauna seem high but comparing to other fauna data from Southern and Northern Italy (see Chapter 3), these values correlate well with Southern Italian δ15N values which are typically higher than Northern Italy. This data illustrates that some of the lowest δ15N values fall within the strictly herbivores (the cattle) and some of the highest values reflect supposed omnivores (the pigs). Furthermore, the range between domesticated cattle (δ13C 1.0, δ15N 4.3) and domesticated pigs (δ13C .86, δ15N 4.5) are similar and suggest that the pigs did not have different fodder practices than the cattle and

mainly had a vegetarian diet as noted elsewhere (Varalli et al. 2016). Moreover, due to the narrower range of the pigs in term of carbon values, its likely they fed on a similar diet as the humans of Coppa Nevigata (range δ13C .88, δ15N 3.2) and probably consumed higher amounts of vegetal proteins. Nitrogen values for the collective fauna supports that domesticated and wild species fed in similar niches as well as on fodder. Some fodder may also have included leguminous plants (pigs CNF 10,11,13 and cows CNF 7,8) accounting for the high range of nitrogen values. The Roe deer, *Capreolus*, has the most amonolous isotopic values from the rest of the fauna which is expected of this species which usually browsed in different environmental niches including open areas perhaps avoiding some of the 'canopy effect' (Chapter 2).

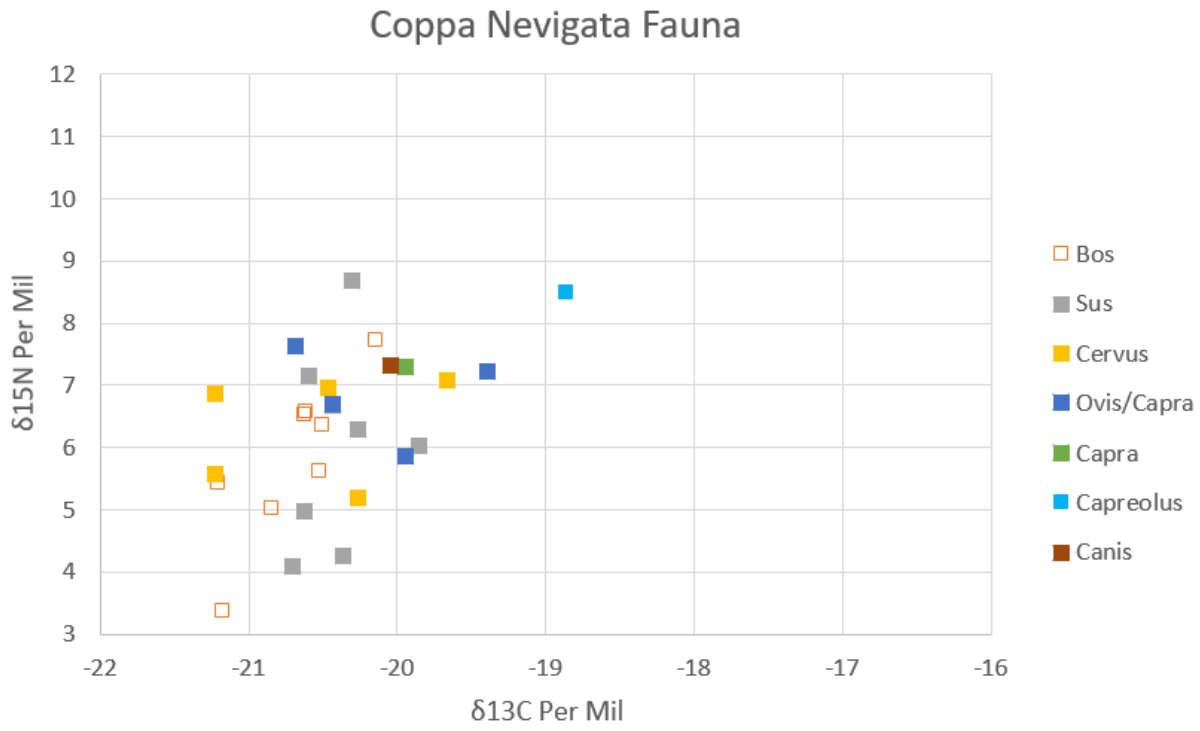


Figure 18: Scatter Plot of CN Fauna Categorized by Species.

7.3 Human Results CN

Human results for Coppa Nevigata included all samples except for CN 2, CN 10, and CN 12 making a total of 12 samples (Fig. 19). The description for sex and age can be seen in table 6**.** The sample analyzed is predominately of adult males. There is not enough information to make any assumptions based on sex for this sample set. The mean δ13C value is -19.83‰ with a range from -20.26 to -19.38‰. The mean δ15N is 9.20‰ with a range from 7.46 to 10.63‰. The sample collection seems fairly homogeneous in terms of δ13C values but do seem to vary for the δ15N. There is no statistical outliers as seen in figure 21, so there are no formal outliers but there is small

variances. For instance, 3 samples go beyond a nitrogen δ15N value of 10‰. CN 3 and CN 9 are both juveniles while CN 14 is an adult male. Perhaps CN 9, estimated age to be 3, could have more enriched δ15N values than the rest due to breastfeeding. CN 3 is estimated to be 15 and 20 years of age and CN 14 being an adult could result in a different diet perhaps pertaining to more animal proteins. Higher δ15N values usually correlate with marine or C4 consumption however the δ13C values do not suggest this. Referring back to Chapter 3; terrestrial diets have δ13C values close to -20‰ and marine diet (which overlaps with C4 consumption) have δ13C values closer to -12 ‰. All Coppa Nevigata humans fall within the terrestrial values. Although, a range of 2.0 in terms of δ15N values are indicative of omnivores diets (Varalli et al. 2016) and in this case the range is δ15N 3.2 which is slightly above (range δ13C .88, δ15N 3.2). It is tempting to then say perhaps some freshwater fish may have been consumed (recall freshwater species have similar δ13C values as terrestrial fauna but slightly elevated δ15N values). This idea is further supported due to the location of Coppa Nevigata near the lagoon (Chapter 5). Although, if this were the case then more individuals should show isotopic signs of consuming aquatic species or perhaps the consumption was limited and fell under the isotopic radar. Future analysis of more individuals from Coppa Nevigata would help to clarify. It is interesting to note that CN 14 was a part of the formal burial and therefore the diet might be associated with cultural differences such as status. Although, CN 15 is also a part of the formal burial and does not show this diet shift and CN 3 does show the diet shift and is not a part of the formal burial so it's unlikely that diet in this case is related to age or status. The difference between mean humans and fauna values (Δ13C ho-fa=.56, Δ15N ho-fa=2.88) indicates a mixed terrestrial diet due to trophic level shifts of +1‰ for carbon values and +3‰ for nitrogen values but in this case it seems like a heavier reliance on terrestrial plants than animal proteins. This also illustrates that marine foods were not consumed which typically leads to a greater difference in δ15N values but does not exclude possible consumption of freshwater fish. According to zooarchaeological data from Coppa Nevigata, pig is most likely the main source of meat while cattle and ovicaprid were used for secondary resources (as seen elsewhere in Bronze Age Italy; Varalli et al. 2016). Nitrogen values also do not predict any manure use which usually leads to highly enriched δ14N values (on average -15‰; Tafuri et al. 2014). Reports of carbon values of -16‰ is indicative of C4 plant ranges (which are more enriched in carbon, recall chapter 2) which is not seen here. Two more individuals stand out: CN 5 and CN 6 have fairly low nitrogen values. This could mean they consumed less animal proteins or they consumed more vegetables that are low in nitrogen such as legumes (i.e., fixing plants, Chapter 2). Legumes were considered an important staple in Bronze Age diet in the Mediterranean and is the second most highly found archaeobotanical remain in Coppa Nevigata with as much as 253 finds after barleys and wheats with over 1,300 finds (Primavera et al. 2015, Varalli et al. 2015). Higher consumption of plants with low nitrogen content such as legumes would typically show nitrogen values closer to 6‰ while consuming cereals (non-fixing plants) would show values closer to 9‰. Depending on the environment and fauna, isotopic fractionation can vary (legumes on average have values around 2‰ plus trophic shift 3-4‰ increase creates the assumption stated here). In this sample, the two individuals seemingly consuming legumes have nitrogen values around 7‰ while the majority

have values around 9‰ indicative of C3 plant consumption and some animal proteins. Furthermore, these two individuals, had isotopic values overlapping with domesticated herbivores with no trophic shift suggesting these individuals were mainly vegetarian. This could be the case, or they consumed small amounts of animal protein with larger amounts of legumes as mentioned. Interesting to note, the individual who have the anomalous nitrogen values (CN 3,5,6,9,14) are spread throughout the Appenine period and age which suggests this does not factor in for the difference and could be choice of diet although cultural indicators such as status is not clear. A Mann–Whitney U-test demonstrates there is no significant statistical difference in the carbon (U=6, p=.48) or nitrogen (U=9, p=1.0) isotopic values of the individuals throughout the Appenine period in Coppa Nevigata as well as no significant statistical difference in the carbon (U=4, p=.10) or nitrogen (U=8, p=.41) isotopic values of individuals under 20 years of age (excluding the 3-year-old, CN 9) and those over 20 years of age.

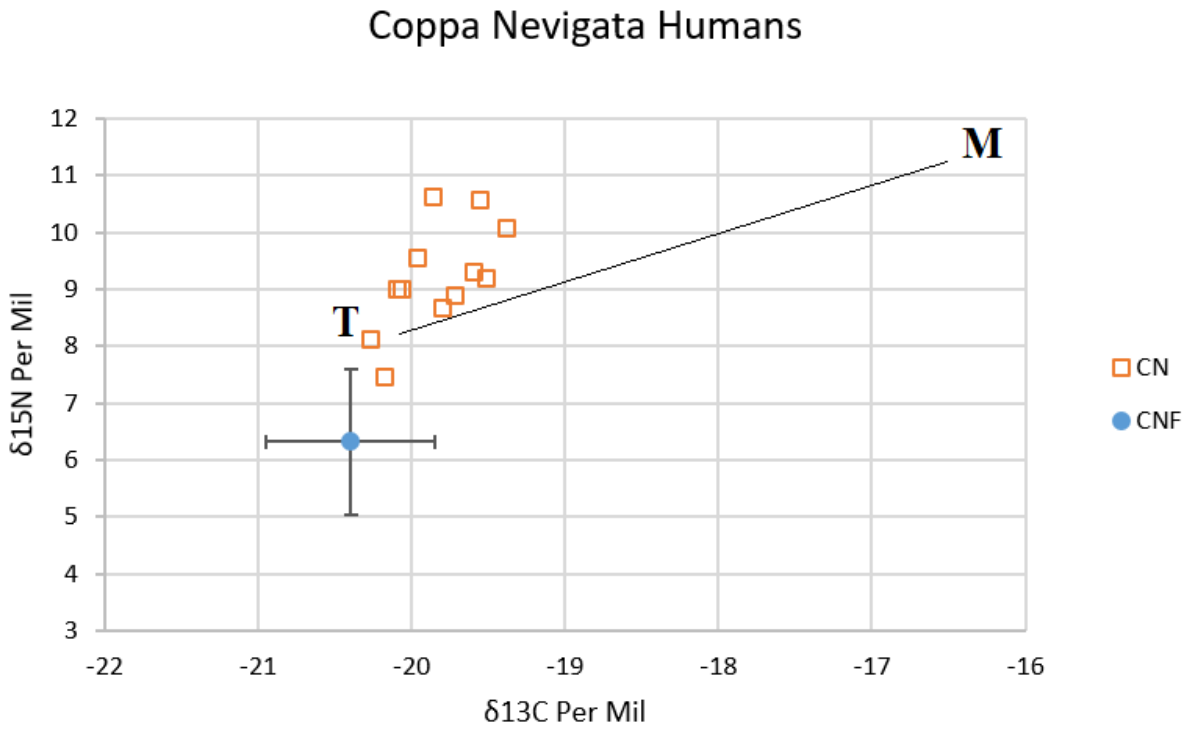


Figure 19: Scatter Plot of CN Humans. CNF is the mean value of CN Fauna with standard deviation. T represents 100% terrestrial diet and M represents 100% marine diet values (taken from Lubell et al. 1994).

7.4 Human Results Croatia

Human results for Croatia included all the samples except BRN 3, making a total of 10 (Fig. 20). This included 4 individuals from Gusica Gomila (GG), 3 individuals from Jukica Gomila (JKG), and 3 individuals from Brnjica (BRN). There is not enough information to make assumptions on sex or age but the limited information can be seen in (table 6). The mean δ13C value for Gusica Gomila is -20.17‰ with a range from -20.25 to -20.04‰. The mean δ15N value for Gusica Gomila is 8.64‰ with a range from 8.12 to 9.34‰. The mean δ13C value for Jukica Gomila is -19.89‰ with a range from -20.3 to -19.43‰. The mean δ15N value for Jukica Gomila is 8.87‰ with a range from 9.70 to 9.19‰. The mean δ13C value for Brnjica is -19.16‰ from -19.92 to -18.91‰. The mean δ15N value from Brnjica is 8.98‰ with a range from 8.14 to 9.75‰. The range for Gusica Gomila is δ13C 0.2 and δ15N 1.2. The range for Jukica Gomila is δ13C 0.87 and δ15N is 4.9. The range for Brnjica is δ13C is 1.26 and δ15N is 1.61. While the Gusica Gomila values seem rather homogenous comparing the median and mean values as well as the narrow range (see table 7); the samples from Jukica Gomila and Brnjica do not. There seems to be two sets of groups within Jukica Gomila (JKG 1,2 and JKG 3) and Brnjica (BRN 1,2 and BRN 4). According to figure 21, there are no formal outliers, but just slight variance interesting to note. For instance, BRN 1 and 2 have carbon values around -18‰ but nitrogen values indicative of C3 terrestrial diet. This could indicate probable consumption of mainly C3 plants and small amounts of C4 plants also seen in Italian Bronze Age sites (Felcetone: Varalli et al. 2015). Although, this could indicate some consumption of marine foodstuffs, it's more likely the carbon values are reflecting C4 input when compared to the nitrogen values (8.14‰ and 9.04 ‰ respectively). The difference between JKG 1 and 2 and 3 may be related to burial. JKG 1 and 2 come from the same mound in separate tombs and JKG 3 comes from a different mound but the difference is negligible and there is no indication of separate diet between the two mounds based on these samples. Croatian Bronze Age fauna is not available, so Neolithic fauna was used from Zeuminca, Croatia (Guiry et al. 2017). The Neolithic fauna values are much lower than the Bronze Age fauna values and correlate more with nitrogen values of Northern Italy. Similarly, nitrogen ranges are much lower than Coppa Nevigata perhaps due to this reason. The difference between the mean values from the Croatian humans and Neolithic fauna are as follows; Gusica Gomila (Δ13C ho-fa=.13, Δ15N ho-fa=4.5), Jukica Gomila (Δ13C ho-fa=.43, Δ15N ho-fa=4.7), Brnjica (Δ13C ho-fa=1.2, Δ15N ho-fa=4.8). It's important to note that these values could be misleading since the fauna is not site specific or time specific. Looking at these differences, it seems the Croatian population did not rely heavily on terrestrial animal protein but perhaps instead on terrestrial plant proteins. Brnjica individuals on the other hand show a heavier reliance on a mixed terrestrial diet than Gusica Gomila and Jukica Gomila. Although, some of the nitrogen differences are higher than typical +3‰ trophic shift for animal protein and may suggest marine consumption. When looking at the individual values this does not seem to be the case. The highest reported nitrogen value for Croatian sites is 9.75‰ which is not indicative of marine consumption. The carbon values do not show any trophic level shift and could be because different types of plants including wild plants were consumed affecting the carbon values (variation in carbon trophic shift, Chapter 3.5.1). To

help clarify, animal bones were analyzed from the site of Jukica Gomila (JKG 9,10,11 conducted by students of Sapienza University; Appendix). These samples were not used in any statistical models because they may not be time specific and the type of animal is unknown. Basically there is not enough information to formally use these samples although the comparison resulted in a similar result (Δ13C ho-fa=.26 Δ15N ho-fa=3.7). It appears, mostly the diet consisted on terrestrial plants with fewer individual consuming more or less animal protein. One way to clarify if aquatic or C4 resources were consumed is to run the samples through statistical analysis juxtaposed to reference values.

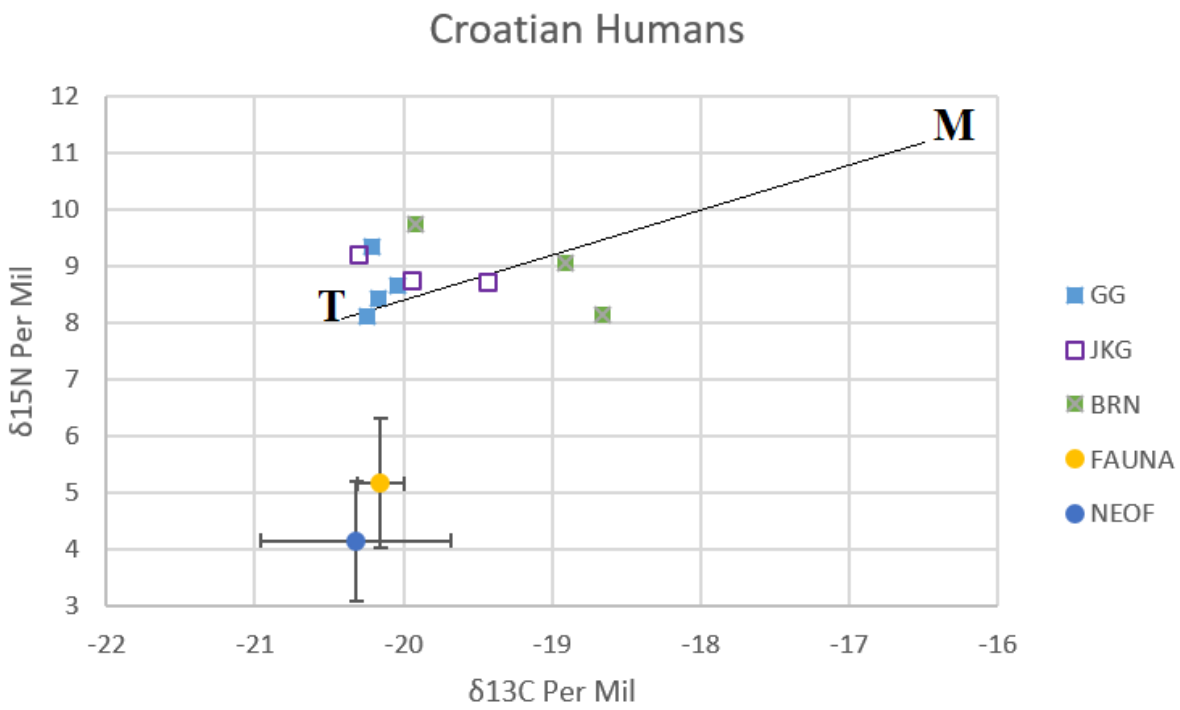


Figure 20: Scatter Plot of Croatian Humans by site. FAUNA is the mean value for the fauna analyzed on JKG (no information on type of bone or period, perhaps Bronze Age?). NEOF is the Neolithic fauna from Zemunica used a reference. Both mean values are accompanied with standard deviations.

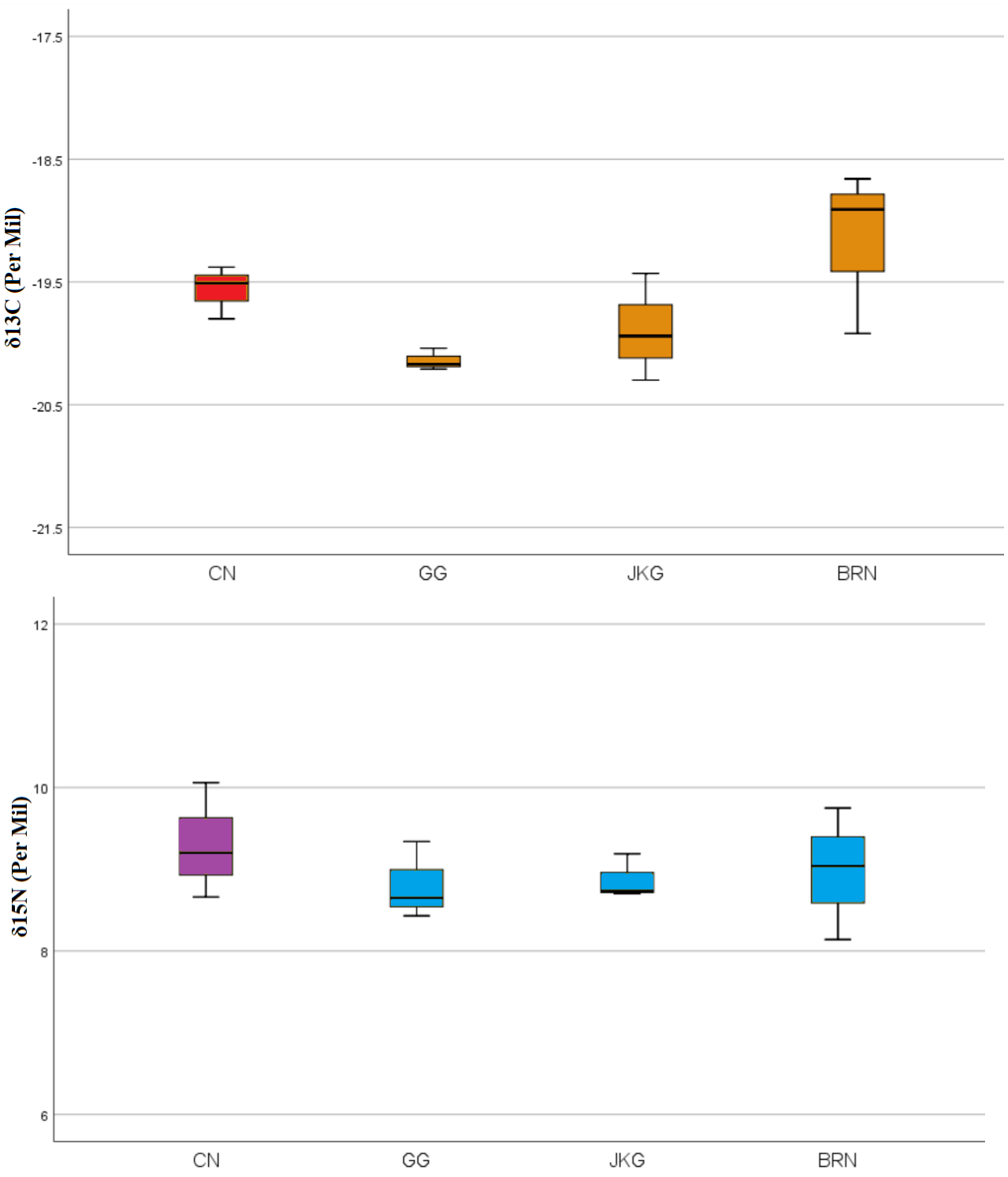


Figure 21: Boxplots of Coppa Nevigata (CN) humans and the Croatian humans δ13C and δ14N values by site (GG=Gusica Gomila, JKG=Jukica Gomila, BRN=Brnjica). Notice no formal outliers (ran by SPSS™).

Chapter 8: Discussion

This chapter will explore the results in greater detail. It will add additional statistical analysis especially in terms of proportions of foodstuffs in the diet and highlight any slight variances (there are no formal outliers). The results will then be compared to dietary patterns seen in the greater Mediterranean and Adriatic and juxtaposed populations during the Bronze Age and examine how these studies correspond.

8.1 Probable diet

After the initial analysis of the individual isotopic values, each group (CN, CNF, GG, JKG, BRN) was run through a Bayesian Mixing Model program (FRUITS ™ - Food Reconstruction Using Isotopic Transferred Signals; Fernandes et al. 2014) to create food catchment assumptions. In other words, this is a mathematical tool (probability and statistics) to estimate source proportions, this case, the relative importance of animal and vegetal proteins (Chapter 6.2). The groups were run against reference values for different foodstuffs. Since the Croatian sites did not have available zooarchaeological finds, fauna from the nearby (no more than a 100 km from each Bronze Age Croatian site in question) Neolithic site of Zemunica, Dalmatia were used (Guiry et al. 2017). The references for aquatic foodstuffs were taken from Bronze Age Italian site, Fondo Paviani, for freshwater fish (Tafuri et al. 2018). Bronze Age marine fish values were taken from Spanish site, Balearic Islands, Cova des Riuets (Guixe et al. 2010) and the Bronze Age Greek site, Archontiko (vika et al. 2012). Plant samples, both C3 and C4 (millets) were taken from Greek Bronze Age sites, Archontiko and Thessaloniki Toumba (Nitsch et al. 2017). These references were chosen to create a foundation for a variety of foodscapes possibly consumed by the target consumers from Coppa Nevigata and the Bronze Age Croatian sites.

According to the Bayesian Mixing Model, the humans of Coppa Nevigata consumed roughly 40-80% on average of C3 terrestrial plants and 10-35% terrestrial animal protein. CN 3, CN 9 and CN 14 consumed on average 30-60% of C3 terrestrial protein 10-40% terrestrial animal protein. According to this model, CN 9 and CN 14 may have consumed 20% freshwater fish (Recall Chapter 2 at least 20% of aquatic sources need to be consumed regularly to be isotopically seen). CN 9 is excluded because of age (the individual was estimated to have an age at death of 3-4) and most likely is reflecting breastfeeding values. In term of the Croatian humans, individuals consumed roughly 30-70% C3 terrestrial plants and 10-40% terrestrial animal proteins. The exception of JKG 3 and BRN 1 and 2 which had slightly lower percentile of C3 terrestrial diet. Furthermore, terrestrial animal protein for JKG 3 and BRN 1 and 2 had values closer to 50% but the variance is not statistically significant and is negligible. BRN 1 and 2 show little to no C4 plant consumption (roughly 10%) that supports the prior assumptions made. Unexpectedly, there may be 10% freshwater fish consumption throughout the Croatian samples. Coppa Nevigata fauna consumed over 90% C3 terrestrial diet and no signs of C4 plant consumption. There is no fauna available for the Croatian sites so the assumption if C4 was eaten directly or through animals who ate C4 cannot be made. Overall, both Coppa Nevigata and the Croatian sites relied heavily on C3 terrestrial plants. Looking more closely at the individual isotopic nitrogen values for Coppa

Nevigata and Coppa Nevigata fauna, there is a homogenous enrichment around 3% from fauna (especially domesticated cattle and pig) to human which supports that there was a mixed diet (if just plants were eaten the nitrogen values would be the same between humans and the herbivores who reflect the plant environment; Hedges et al. 2007). Applying the same logic, the Croatian humans on average share similar nitrogen values with the Croatian Neolithic herbivores which supports the Bayesian Model that they relied almost entirely on C3 terrestrial plants with some animal proteins. Because there is not enough isotopic data on Neolithic and Bronze age fauna in Croatia, this theory should be approached with caution and should be clarified that these values could misrepresent the animal protein intake of the Croatian humans if compared to Bronze age fauna.

8.2 Sustenance Patterns (Italy and Croatia)

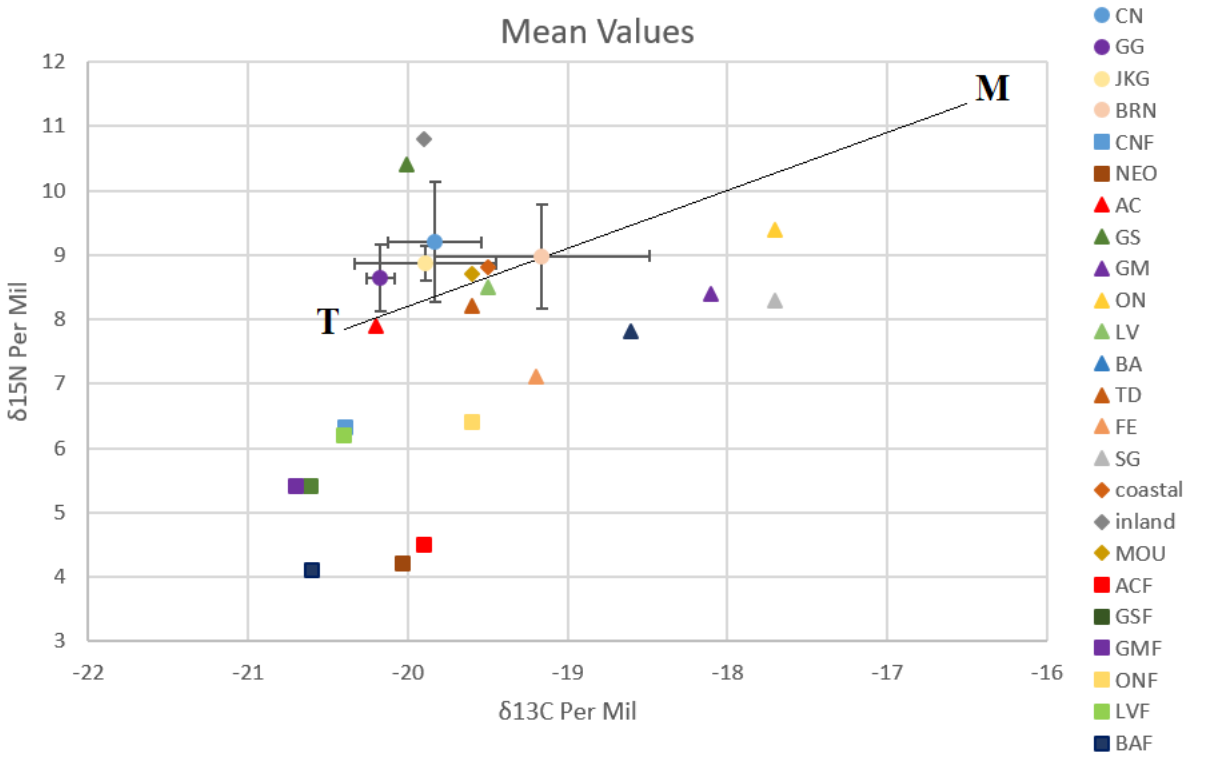


Figure 22: Scatter Plot of mean values of this study,Coppa Nevigata humans and fauna & Croatian sites (GG, JKG, BRN) along with NEO (Neolithic fauna reference from Zemunica). Standard Deviations of the human sites of this study is included. Also plotted are the mean values for the corresponding Bronze Age sites in Italy (AC-Arano di Cellore, GS-Grotta dello Scoglietto, GM-Grotta Misa, FE-Felcetone, SG-Sedgliano, ON-Olmo di Nogara, TD-Toppo Daguzzo, LV-Lavello, BA-Ballabio; ACFauna, LVFauna, GSFauna, GMFauna, ONFauna, BAFauna) and Croatian sites (Inland & Coastal sites listed in Chapter 4.2) and MOU-Monte/ Orcino/Určin.

Diet of Bronze Age Italy and Croatia vary in some degree (recall Chapter 3), let's now take a look in better detail comparing this studies population to previous studied populations. Firstly, to understand if supposed diet catchments are related due to choice in foodstuffs or environmental difference, it's good to compare the isotopic values of the fauna. Fauna values, mostly herbivores, reported from peninsular Italy (Arano di Cellore; Varalli et al. 2016, Grotta dello Scoglietto, Grotta Misa; Varalli et al. 2015, Olmo di Nogara, Lavello; Tafuri et al. 2009, Ballabio; Masotti et al. 2017). Comparative mean values for human and fauna from this study and Bronze age sites from Italy and Croatia can be seen in tables 3 and 4. The first striking result from comparing the Bronze Age Italian fauna is the difference of δ15N ratios in Italy. It is clear the fauna from Central and South Italy have more enriched δ15N isotopic values than North Italy most likely due to environmental difference in altitude, aridity, or precipitation (Chapter 2). The fauna collectively represents a dominant diet in C3 terrestrial plants except for Olmo di Nogara of North Italy which illustrated some C4 consumption. The human comparative data will be from the same sites in addition with Felcetone; Varalli et al. 2015, Sedgliano and Toppo Daguzzo; Tafuri et al. 2009, and Dossetto di Nogara, Bovolone, Gradisca di Codroipo (Castelliere culture); Tafuri et al. 2018. Firstly, majority of the sites represent a mixed terrestrial diet with heavier reliance on C3 plants (Arano di Cellore (EBA), Grotta dello Scoglietto (EBA), Lavello (MBA), Toppo Daguzzo (MBA), Ballabio (EBA), Gradisca di Codroipo (LBA)). Some individuals from Grotta dello Scoglietto, however, have high δ15N values reported as higher consumption of animal protein, perhaps including fish (perhaps freshwater fish because the carbon values do not represent marine intake but generally freshwater fish have closer carbon values to terrestrial animals). Secondly, some sites indicated mixed C3 and C4 plant consumption between individuals (Grotta Misa (MBA), Sedgliano (EBA), and Felcetone (MBA)-1 individual). Lastly, Dossetto di Nogara (EBA), Bovolone (MBA), Olmo di Nogara (MBA) had considerable C4 terrestrial plant consumption indicative of the higher δ13C values. The apparent pattern is a shift in C4 consumption from Early Bronze Age to Middle Bronze Age with sporadic events in North and Central Italy and not in South Italy. The data from Coppa Nevigata supports this. The reason for the shift in diet is unclear, but recall that there is a period aridity throughout peninsular Italy that may have caused the change to C4 millets that are resistant and have a short growing season (perhaps South Italy didn't feel this shift because its naturally more arid than North Italy). The values of CN 9 and CN 14 also closely resemble Grotta dello Scoglietto, Italy which reportedly consumed aquatic foodstuffs (Varalli et al. 2015). Throughout the Neolithic and the Bronze age, there seems to be an overall decrease in aquatic food sources and increase in domesticated plants. Recall Chapter 2 which mentions an overall terrestrial diet with some instance of marine consumption during the Neolithic sites (Apulia, Marche; Lelli et al. 2012) and heavier reliance on animal proteins (herbivores). Neolithic Italy showed an increase in domesticated C3 plants which then seems to intensify in the Bronze Age. Overall, the Bronze Age illustrates a heavy reliance on domesticated terrestrial plants rather than animal proteins and Coppa Nevigata supports this pattern.

In terms of the Croatian sites, this study can be compared to Bronze Age Croatian sites described in detail in Chapter 3 conducted by Lightfoot et al. 2014 and one site by Tafuri et al.

2018. While the Croatian site, Monte Orcino/Určin (Tafuri et al. 2018) illustrated a C3 terrestrial diet, the inland and coastal Bronze Age sites from the study of Lightfoot (2014) varies. It is unclear if the slightly higher δ13C values of Bronze Age sites in Croatia are due to marine or C4 consumption (marine and C4 nitrogen values tend to overlap) but considering the δ15N values are not as high to represent marine food, it could be slight consumption of C4 plants, most likely Broomcorn (*Panicum Miliaceum*) or Foxtail (*Setaria italica*) according to archaeobotanical remains in the general Mediterranean but no charcoals or seeds were reported for the site. The same situation is seen for BRN 1 and 2 of this study. It is not until Late Bronze/ Early Iron Age consistent millet consumption is seen in Croatian sites described by Lightfoot (2014). Especially telling is the Coastal site Nadin-Gradina that shows slight elevation in δ13C values during Late Bronze age into what seems like a full transition to C4 plants in the Early Iron Age with δ13C values as high as -16‰. The Bronze Age Croatian sites from this study supports a C3 terrestrial diet consistent with the previously studied sites. The archaeological reports for the Croatian sites are very limited. Brnjica is known to have been occupied during the Early Bronze Age due to the Cetina pottery finds (Menudisc et al. 1986). If so, this could be some of the earliest finds of partial C4 consumption in Croatia (comparative to Prosik (EBA) which may have had some C4; Lightfoot et al. 2014). Of course this is just a theory because there is no fauna to confirm if C4 was directly consumed or not and also the dates of the bones are not for certain. There is no confirmed marine diet in Bronze Age Croatia although Lightfoot et al. 2014 highly suggested the possibility. The consumption of freshwater fish in this study is unique but not strange since the sites are near the Cetina River and Čikola River (less than 20km). Although, considering the nitrogen values (highest value 9.75‰), it then must be argued the little freshwater consumption most likely came from a low trophic species of aquatic foodstuffs with low nitrogen content. Although, there is not much archaeological background of these sites, archaeobotanical context of Bronze Age Croatia (see 4.2) illustrates reliance on the cultivation of C3 plants which correlates here in terms of diet. From the Neolithic to Bronze Age Croatian Isotopic studies show various levels of marine consumption in the Neolithic and heavier reliance on animal proteins. Recall, Croatia consistently showed marine consumption also in the Neolithic (similar to areas like the Danube Gorge but contrasts Italy; Chapter 2). In Croatia, reliance on domesticated plants doesn't seem to play an important role until the Bronze Age and is mostly C3 reliant. This study supports a C3 plant based diet in Croatia.

These results contribute to the understanding of broader Bronze Age Mediterranean and Adriatic diet questions such as the movement from millets into the Mediterranean. Comparatively, Italy seems to show some of the earliest C4 consumption in North and Central Italy (EBA-MBA) followed by Sardinia (MBA-Is Aruttas; Lai et al. 2013), Nadin-Gradina, Croatia (LBA), Barbuise, France (LBA -Goude et al. 2016), and several Greek sites (Aghia Triada, Almyri, Rhymino, and Korinos (LBA); Pertousta et al. 2010). It is hard to make assumptions on the path to C4 domestication except it gains popularity in Southern Europe around the Mediterranean and Adriatic during later periods of the Bronze Age and most likely was introduced from prehistoric Chinese sites of which millet was a principle cultivated crop following the Steppe Plain and down

into Central Europe (Zohary et al. 2013, Lightfoot et al. 2013). More isotopic data is needed for further assumption. Several theories are proposed of the shift of diet between Neolithic and Bronze Age; decrease in aquatic resources and increase in plant consumption (Richards et al. 2003) or decrease in animal proteins and increase in domesticated plant consumption (Malone et al. 2003, Tafuri et al. 2009). Comparing isotopic data in Chapter 2 and this study there is no clear indication of either argument and more isotopic data is needed. So far in the Mediterranean it may be proposed that the Bronze Age does show intensified interest in domesticated plants, even if during the Neolithic it was available, especially with the introduction of C4 plants. This is supported by this study. Aquatic resources have continuously varied throughout the Mediterranean since the Neolithic period and no assumptions can be made but this study suggests low level freshwater fish consumption for Italy and Croatia. This study did not contribute in the understanding of building social identities in the Bronze Age (I.e sex and animal protein in Olmo di Nogara, Italy; Tafuri et al. 2018, millet consumption in terms of burial type in Nadin-Gradina, Croatia; Lightfoot et al. 2014, and marine food and status in Mycenae, Greece; Papathanasiou et al. 2015), but Coppa Nevigata did show some indication of differential diet in relation to formal burials but nothing statistically significant compared to the studied population.

Chapter 9: Final Remarks

9.1 Limitations and Further Study

This study experienced some limitations. Firstly, the small sample size for both Coppa Nevigata and the Croatian sites were greatly limiting. Coppa Nevigata comprised of over 300 bones from the site and this study was able to fully analyze just 12 individuals. For sure this will bring a level of bias in terms of representing the food catchment of the entirety of the population. Furthermore, the intra-population variation was significantly limiting. Most of the sample consisted of adult males from Ancient Appenine which did not allow for proper comparison between age, sex, and period within the Bronze Age which could result in interesting data. Especially worth noting is that only 2 out of the 4 individuals from the only formal burials of Coppa Nevigata were analyzed and it would be of great significance to analyze all to see if there is a pattern between diet and burial type and perhaps even status. Stable isotope analysis indeed is a costly and time consuming technique that can take a few months to process, but future studies would benefit by increasing the sample size even slightly including the formal burials, and some variation in sex, age, and period. Samples were chosen in relation to an ongoing stable isotopic study of Strontium of the same individuals in hopes to understand the mobility of the population of Coppa Nevigata and Bronze Age Croatian population of Dalmatia to see if there are any similarities or differences similar to the aims of this study (Ongoing study by Dr. Mary Anne Tafuri and Sapienza University, Rome). In terms of the Croatian sites, the sample was also very small with just 3 to 4 individuals from each site. Although, these sites are still in the preliminary stages of excavation and publication with very little information on the sites in general. Future studies will benefit once the sites have been published and more samples are available for analysis. The Croatian sites would also greatly benefit from site-specific fauna in terms of location and time period to establish more accurate baseline values. Both Coppa Nevigata and the Croatian sites would benefit from site-specific archaeobotanical analysis for this same reason. Fish bones are some of the rarest zooarchaeological finds and rarely survive or even get collected so the availability of both freshwater and marine reference values from Greece, Spain, and elsewhere in Italy during the Bronze Age was a plus.

9.2 Conclusion

This study was a comprehensive review of the interpretation of the stable isotopic ratio results juxtaposed to the stable isotopic histories of the neighboring Bronze Age communities and the archaeological contexts of the sites that assisted in creating a well hypothesized view of the assimilated diet in Coppa Nevigata and the Croatian sites. Overall, both Coppa Nevigata and the Croatian sites relied on a mainly terrestrial C3 diet with some animal (herbivore) proteins while the Croatian sites generally relied more heavily on plant proteins than Coppa Nevigata. Coppa Nevigata also had two individuals who most likely consumed leguminous plants. Brnjica shows two individuals who may have consumed some C4 plant proteins, most likely millets. Fauna and flora data is not available for the Croatian sites unfortunately so it cannot be understood if they directly consumed C4 plants or if there were any seeds or charcoals found on site. This mixed diet was accompanied by at least 10% of freshwater fish of all populations in the Croatian sites and perhaps at least 15% for two individuals from Coppa Nevigata. Aquatic resources may not have not been a main source of protein for either Italy or Croatia, but have been consumed in either area since the Neolithic, although more so in Croatia. More isotopic analysis should be done within each population to gain a wider perspective on assimilated diet to see if the consumption of freshwater fish is an anomaly in either population. There were no statistical significant intra-population variations in terms of sex, age, period, or burial type. In terms of the Croatian sites there was not enough information to observes these variables. Statistical analysis for period and age were run for Coppa Nevigata individuals but there were no significant differences although the samples were limited. In terms of the greater Mediterranean and Adriatic Bronze Age diet; Coppa Nevigata and the Croatian sites fits the general pattern of heavy reliance on C3 terrestrial plants with some animal proteins which seems to differ from the Neolithic isotopic studies done previously in both areas where the Italian Neolithic sites relied more heavily on animal proteins and C3 plants and the Croatian sites relied more heavily on animal proteins, C3 plants, and aquatic resources. Coppa Nevigata supports the previous studies that claim the introduction of millets arrived in the Middle Bronze Age only in North and Central Italy, thus far. This study also raises the question for the introduction of millets in Croatia and pushes for more analysis on the subject in the future. Of course, no pattern can be fully understood or determined without further isotopic analysis in both areas throughout the Neolithic and Bronze Age.

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Appendix

App. 1: List of Stable Isotope Values for Italy (Paleo-Neo)

Site	n	δ15N‰ Mean/ Median/ Min/ Max value	δ13C‰ Mean/ Median/ Min/ Max value	Period	Ref.
Grotta del Romito (Cosenza)*	Humans 8 Animals 21	10.1/ 9.5/ 12.4/8.9 5.3/5.2 /3.7/7.2	-19.5/-19.5/-20.0/-18.9 -21.1/-/-21.3/-19.0	Late Epigravettian	Craig et al. 2010
Riparo Tagliente (Verona)*	Human 1 Animal 11	13.0 4.1/ 4.8/1.2/9.4	-18.4 -19.6/-19.6/-20.6/-18.4	Late Epigravettian	Gazzoni et al 2013
Villabruna*	Human 1	8.0	-19.7	Late Epigravettian	Vercellotti et al 2008
Arene Candide*	Human 1	12.6	−17.6	Gravettian	Pettitt et al 2003
Arene Candide	Human 2 Animal 9	-/-/8.9/9.1 4.7/-/3.4/6.5	-/-/-20.0/-18.9 -19.4 -/ -20.3/-18.0	Epigravettian	Francalacci et al 1988
Uzzo Cave (Sicily)	Human 2 Animal 4 Marine 4	-/-/10.4/10.7 -/-/6.0/10.6 -/-//9.8/11.8	-21.0/-/-/- -/-/-20.7/-10.2 -/-/-17.4/-10.6	Mesolithic	Francalacci et al 1988
Addaura*	Human 1 Animal 9(Grotta delle Incisioni)	9.6 -/-/5.7/9.9	−19.7 -/-/-2.4/-19.2	Late Epigravettian	Mannino et al 2011
San Teodoro*	Human 4 Animal 8	11.5/11.6/11.4/12.5 -/-/5.2/8.2	19.8/-20.0/-20.2/-19.1 -/-/-24.0/20.9	Late Epigravettian	Mannino et al 2011
Grotta d'Oriente*	Human 4 Animal 16	11.1/11.2/10.6/11.6 -/-/4.2/9.9	-18.6/-18.9/-19.0/-17.8 -/-/-21.7/-8.9	Late Epigravettian	Mannino et al 2012
Grotta Addaura Caprara*	Human 2 Animal 4	-/-/8.7/ 9.7 -/-/4.9/8.6	-/-/-19.6/-19.3 -/-/-21.6/-20.3	Late Epigravettian	Mannino et al 2011b
Grotta della Molara*	Human 2 Animal 13	-/-/7.1/10.4 -/-/5.1 / 8.9	-/-/-20.2/-19.5 -22.0/-19.7	Late Epigravettian	Mannino et al 2011b

Ripa Tetta (Tavoliere)	Human 4 Animal 4	10.6/10.7/9.3/11.7 -/-/6.2/8.0	-20.0/-20.0/-20.1/-19.8 -/-/-20.7/-20.0	Neolithic	Lelli et al 2012
Fosso Fontanaccia Portonovo (Marche)	Human 4 Animal 11	12.9/12.9/12.6/13.3 8.9/9.1/5.8/11.0	-20.6/-20.6/-20.6/-20.5 -22.0/-21.4/-24.1/-21.0	Neolithic	Lelli et al 2012
Torre Castelluccia (Apulia)	Human 1	8.4	-18.8	Neolithic	Lelli et al 2012
Samari (Apulia)	Human 4	10.0/9.9/9.8/10.5	-19.0/-19.5/-19.2/-18.7	Neolithic	Lelli et al 2012
Grotta delle Mura (Apulia)	Human 2 Animal 12 Marine 6	-/-/7.8/8.0 -/-/4.5/6.5 -/-/9.0/14.0	-/-/-19.6/-17.7 -/-/-21.7/-19.3 -/-/-18.9/-10.8	Neolithic Epigravettian	Lelli et al 2012
Balsignano (Murge)	Human 1 Animal 1	8.1 6.2	-19.3 -20.0	Neolithic	Lelli et al 2012
Masseria Maselli (Murge)	Human 1	8.1	-16.6	Neolithic	Lelli et al 2012
Palata (Murge)	Human 2 Animal 3	8.6/9.6 -/-/6.7/8.1	-19.5/-19.1 -/-/-20.4/-18.5	Neolithic	Lelli et al 2012
Passo di Corvo (Apulia)*	Human 14 Animal 5	13.3/-/11.2/15.4 10.2/-/9.3/11.7	-19.3/-/-20.9/19.0 -19.7/-/-20.5/18.3	Neolithic	Tafuri et al 2014
Masseria Candelaro*	Human 24 Animal 2	9.3/-/8.2/11.4 6.3/-/6.3/6.4	-19.2/-/-19.9/-18.2 -21.1/-/-21.5/-20.8	Neolithic	Tafuri et al 2014
Grotta Scaloria*	Human 46 Animal 20	8.4/-/6.8/10.6 6.0/-/4.1/7.9	-19.3/-/-19.9/-18.9 -19.9/-/-21.2/-17.6	Neolithic	Tafuri et al 2014
Grotta Scaloria	Human 43 Animal 22	8.4/-/-/- 6.0/-/-/-	-19.3/-/-/- -19.9/-/-/-	Neolithic	Tafuri et al 2017
Passo di Corvo	Human 13 Animal 5	13.4/-/-/- 10.2/-/-/-	-19.1/-/-/- -19.7/-/-/-	Neolithic	Tafuri et al 2017
Masseria Candelaro	Human 24	9.3/-/-/-	-19.2/-/-/-	Neolithic	Tafuri et al 2017

Ripa Tetta	Human 2	10.0/-/-/-	-19.7/-/-/-	Neolithic	Tafuri et al 2017
Poggio Imperiale	Human 4 Animal 3	8.4/-/-/- 7.3/-/-/-	-19.4/-/-/- -20.3/-/-/-	Neolithic	Tafuri et al 2017
Occhito	Human 9	9.0/-/-/-	-19.9/-/-/-	Neolithic	Tafuri et al 2017
Samari	Human 8	9.6/-/-/-	-19.1/-/-/-	Neolithic	Tafuri et al 2017
Bari (S.Barbara, C.Colombo, Malerba, Cala Scizze)	Human 6	8.9/-/-/-	-19.4/-/-/-	Neolithic	Tafuri et al 2017
Serra Cicora	Human 16 Animal 10	9.2/-/-/- 6.1/-/-/-	-19.4/-/-/- -20.8/-/-/-	Neolithic	Tafuri et al 2017
Pian del Giliegio (Liguria)	Human 2 Animal 10	-/8.4/8.1/ 8.8 -/-/3.8/5.6	-/-20.5/-20.8 /-20.2 -/-/-22.1/-17.4	Neolithic	Goude et al 2016
Bergeggi (Liguria)	Human 5	-/8.8/8.6/9.1	-/-20.0/-20.3/-19.6	Neolithic	Goude et al 2016
Pollera (Liguria)	Human 12 Animal 13	-/8.5/7.1/9.4 -/-/3.6/6.2	-/-20.3/-20.6/-19.9 -/-/-21.8/-19.5	Neolithic	Goude et al 2016
Gabru du Surdu (Liguria)	Human 1	9.4	-20.2	Neolithic	Goude et al 2016
Arene Candide (Liguria)	Human 8 Animal 13	-/8.9/6.2/9.4 -/-/3.8/6.8	-/-20.0/-21.1/-19.4 -/-/-22.4/19.6	Neolithic	Goude et al 2016
GrottaMora - Cavorso*	Human 8 Animal 3	Refer to Article	Refer to Article	Neolithic	Rolfo et al 2012
Arene Candide	Human 4 Animal 9	9.5/9.2/9.1/10.3 4.7/-/3.4/6.5	-20.1/-20.1/-20.3/-19.7 -19.4/-/ -20.3/-18.0	Neolithic	Francalacci et al 1988

Selected Culture	Period	Time (B.C)	Geological Significance	Time (B.C)
Gravettian	Paleolithic Pleistocene Epoch	~28,000-24,000	Ice Age	~2,000,000
Early Epigravettian	Paleolithic	~21,000	Late Glacial Period	~17,000-10,000
Late Epigravettian	Paleolithic	~17,000-12,000	Late Glacial Maximum (LGM)	~17,000-15,000
Sauveterrian	Mesolithic Holocene Epoch	~11,000	Early Late Glacial Late Glacial Interstadial Bølling-Allerød Dryas III Younger Dryas Boreal Period (warm, sea rise)	~15,000-14,000 ~12,000-12,000 ~15,000-12,000 ~12,000 ~12,000 ~9,000

App 2: List of Stable Isotope Value of Croatia (Paleo-Neo)

Site	Sample	Mean d13C‰	Mean d15N‰	Period	Reference
Šandalja II, Istria	Human 3 Animal 24 Marine 4	-20.6 -20.1 -23.2	13.6 7.9 9.0	Paleolithic	Richards et al 2015
Vela Spila Cave, Korčula	Human 4 Animal 24 Marine 4 Human 1	Refer to Article	Refer to Article	Mesolithic Neolithic	Lightfoot et al 2010
coastal sites; Metaljka, Grapčeva, Vela Spilja-Vela Luka, Crno Vrlo, Vela Spilja Lošinj, Kargadur, Pupićina inland sites; Radovanci, Belišće Staro Valpovo, Osijek, Vinkovci, Vučedol	Human 42 Animal 95	Refer to Article Humans: Mesolithic coastal sites -19.0. Neolithic coastal sites -19.6 Neolithic inland sites -20.3	Refer to Article Humans: Mesolithic coastal sites 10.0. Neolithic coastal sites 9.3 Neolithic inland sites 10.3	Mesolithic Neolithic	Lightfoot et al 2011

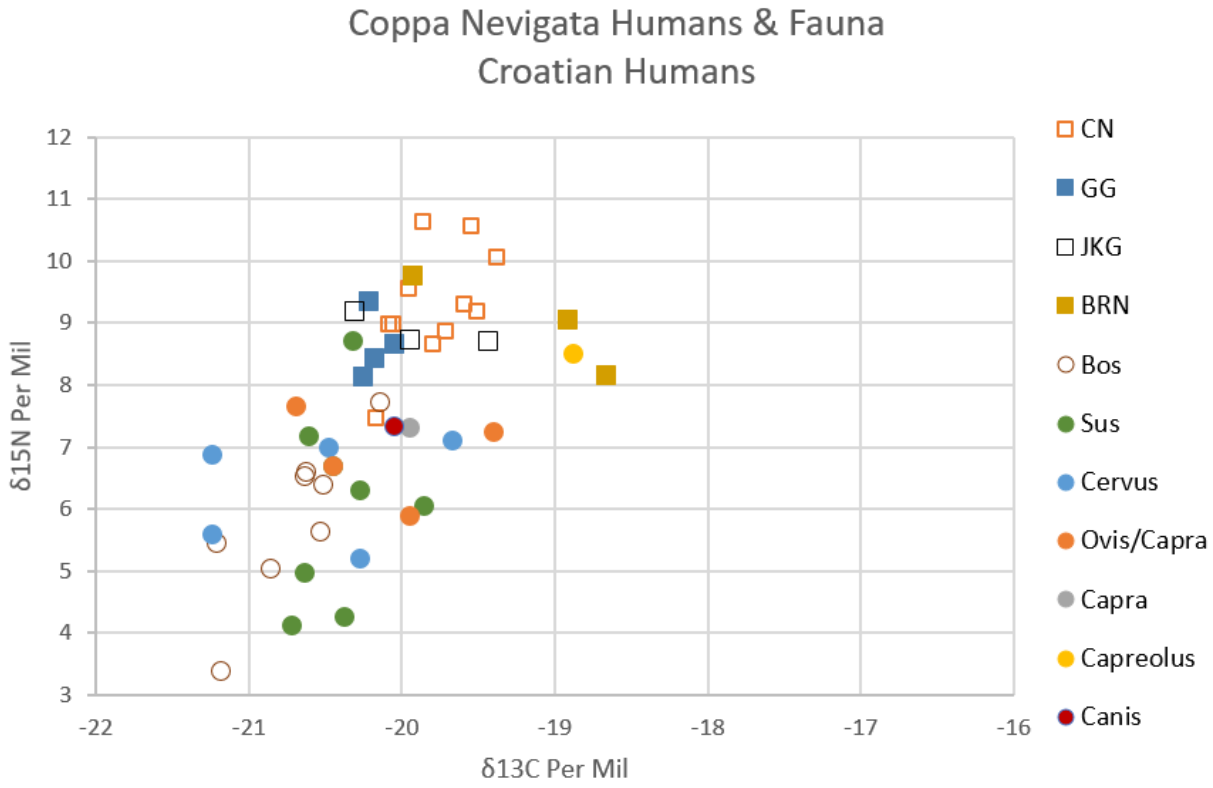
App.3: Duplicate Stable Isotope Values

Duplicate ID	Code	Collagen Yield %	C:N	N (%)	C (%)	δ15N ‰	δ13C ‰
CN7B	CNV Cγ 2e	13.78	3.18	14.11	38.51	8.91	-19.95
CN11B	CNV H1C	11.64	3.19	14.09	38.58	9.26	-19.43
CN15B	CNV F5 E	10.00	3.20	12.21	33.51	9.61	-19.73
CNF5B	CN 1994 D3R 2II	4.58	3.18	12.71	34.66	5.63	-20.59
CNF10B	CN 1971 F5	9.99	3.19	9.26	25.29	4.95	-20.64
CNF15B	CN 11 G2P 10	11.82	3.23	14.88	39.83	8.77	-20.26
CNF20B	CN 1971 H1C	10.21	3.20	14.39	39.42	5.65	-21.24
CNF27B	CN94 D3R 2II	8.03	3.14	15.30	41.19	6.66	-20.38
CNF30B	CN G2P 10	14.40	3.17	11.32	30.74	7.33	-20.04
GG3B	GI Grave 3	14.02	3.27	14.00	39.24	8.69	-19.99
BRN4B	Bag BRN	6.36	3.29	8.34	23.52	9.84	-19.98

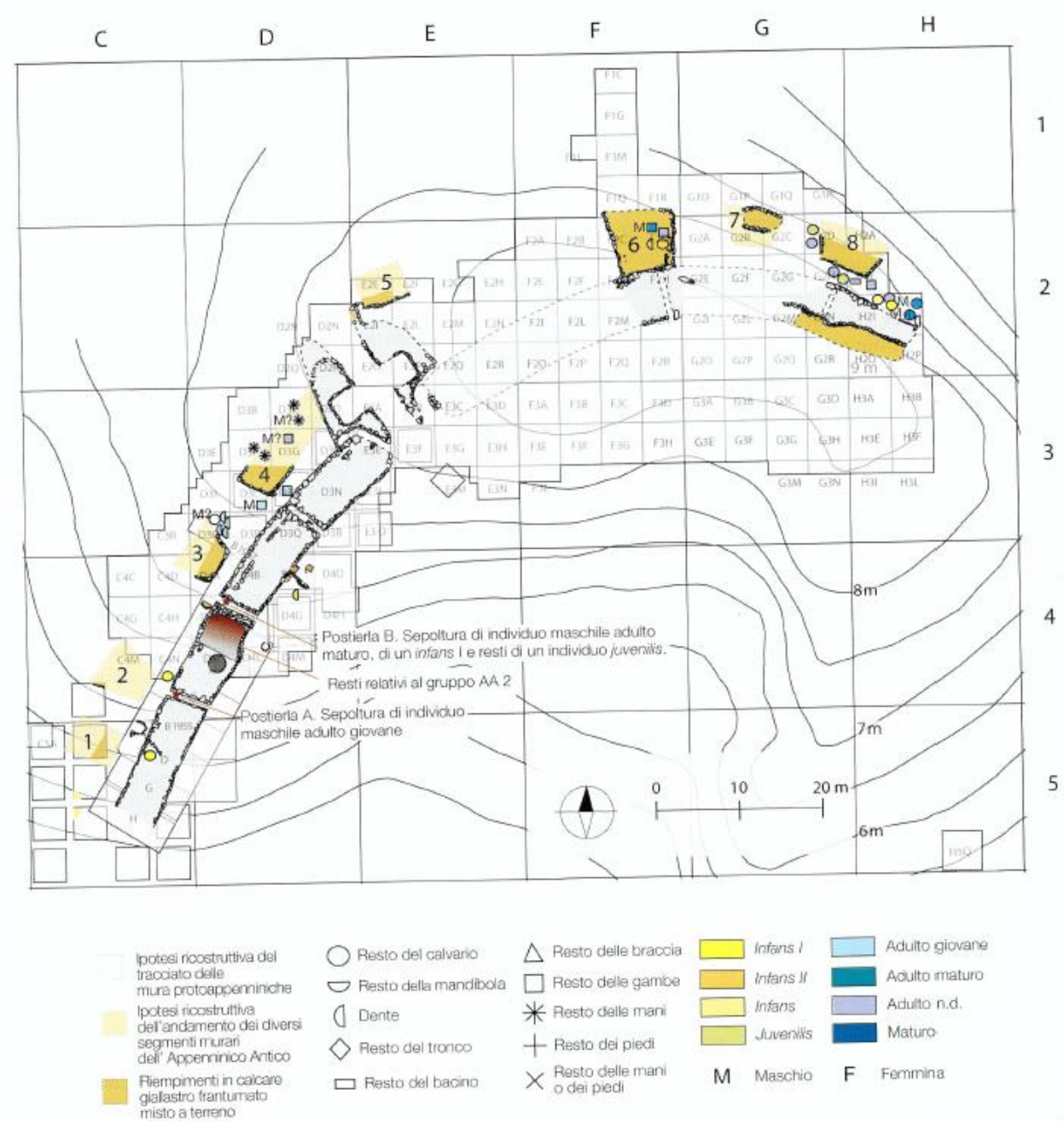
App. 4: Isotope Values for the 3 Unidentified animal bones found in Jukica Gomila, undated.

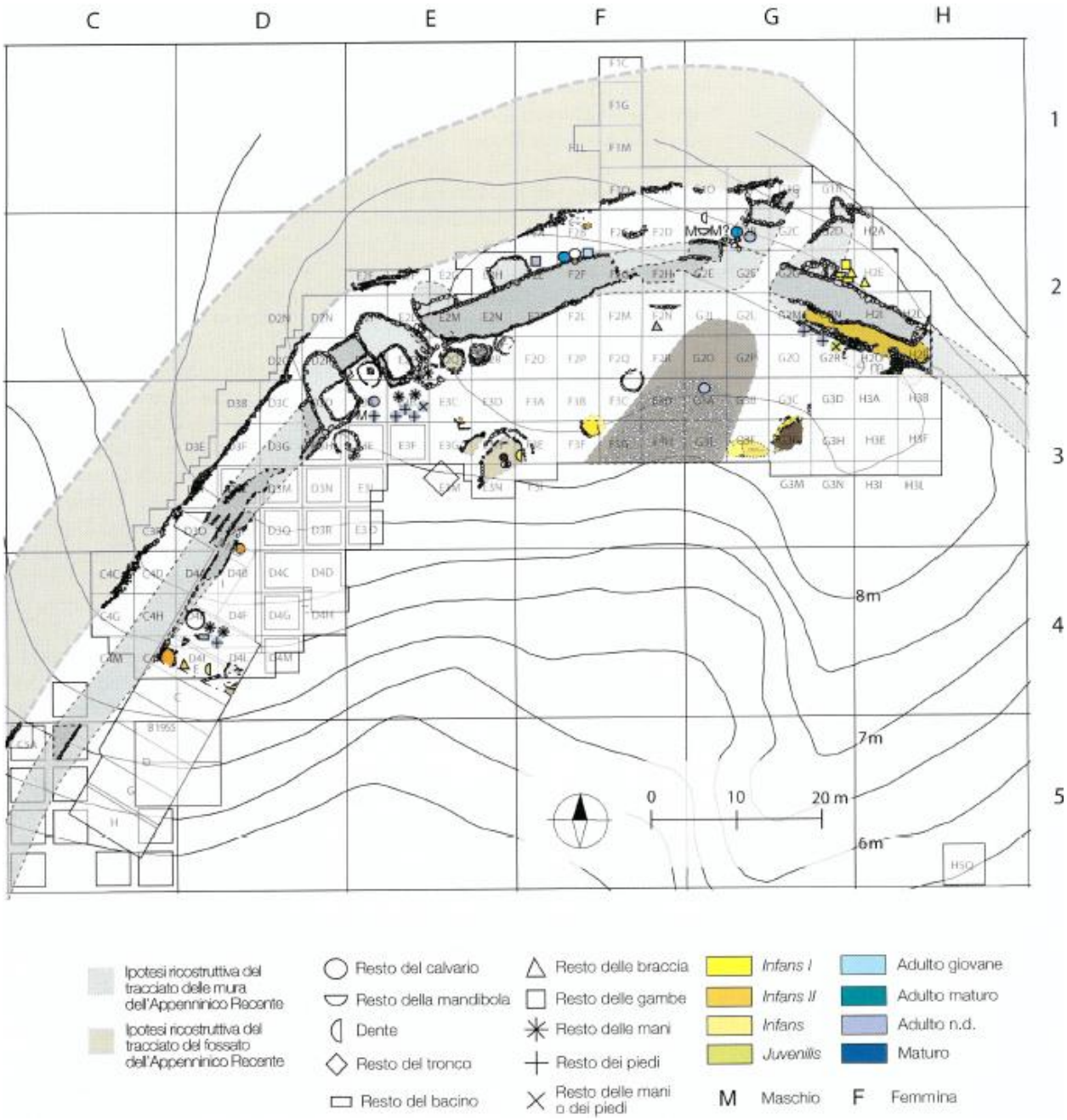
Sample ID	Code	Collagen Yield %	C:N	N (%)	C (%)	δ15N ‰	δ13C ‰
JKG9*	Bag10	17.47	3.15	16.51	44.59	6.49	-20.08
JKG10*	Bag11	16.75	3.17	14.43	39.27	4.39	-20.05
JKG11*	Bag12	11.83	3.26	4.31	12.05	4.63	-20.33

App.5: Scatter Plot of all Samples Investigated in this Study



App. 6: Layout of Coppa Nevigata structures and bones from 1. Ancient Appenine 2. Recent Appenine 3. Ancient SubAppenine 4. Recent SubAppenine; more about the structures and the bones from Cazzella et al. 2012




C
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1
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3
4
5
8m
7m
6m
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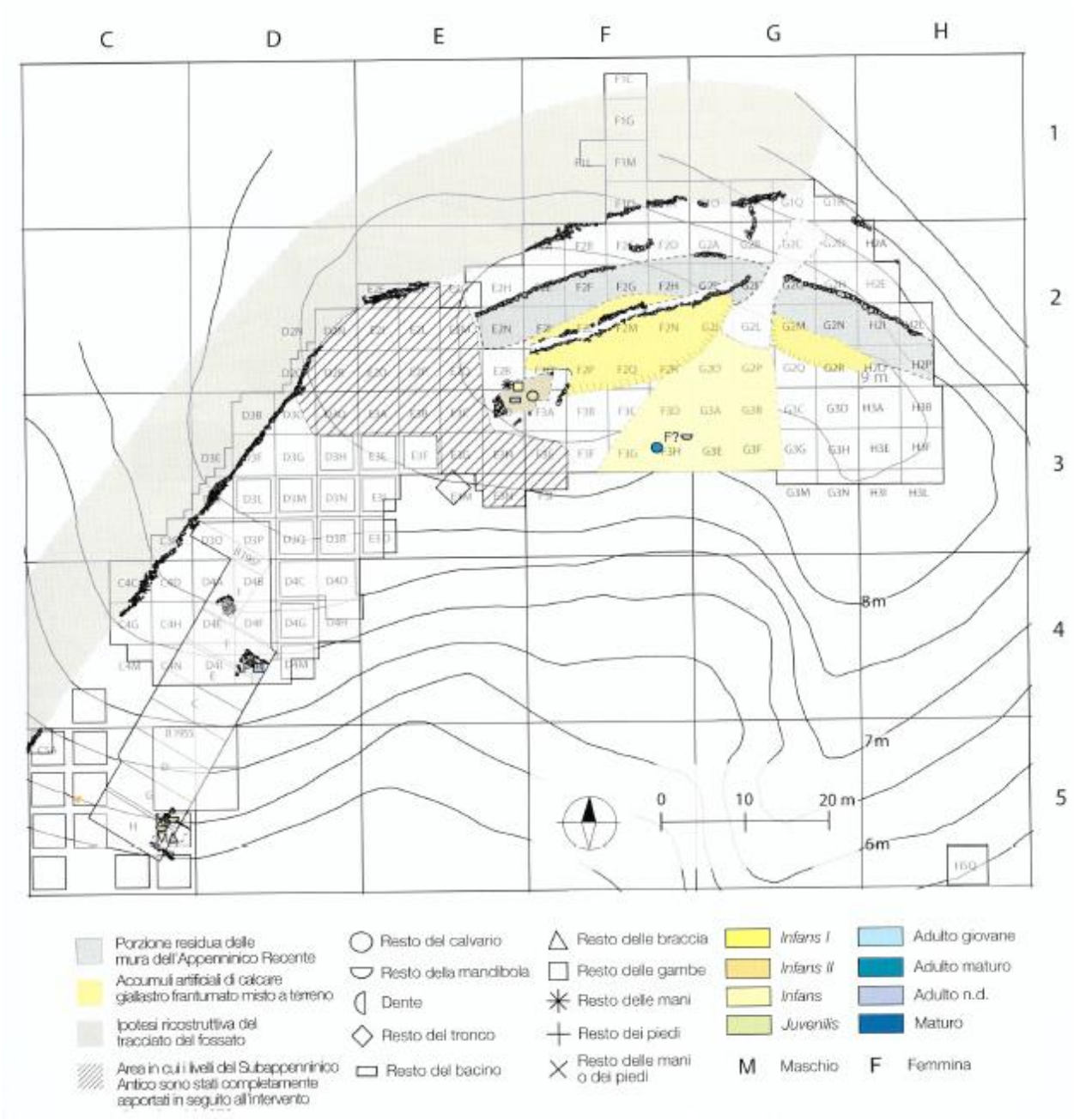
10
20 m
Ipotesi ricostruttiva del tracciato delle mura dell'Appenninico Recente
Ipotesi ricostruttiva del tracciato del fossato dell'Appenninico Recente
Resto del calvario
Resto della mandibola
Dente
Resto del tronco
Resto del bacino
Resto delle braccia
Resto delle gambe
Resto delle mani
Resto dei piedi
Resto delle mani o dei piedi
Infans I
Infans II
Infans
Juvenilis
Adulto giovane
Adulto maturo
Adulto n.d.
Maturo
M Maschio
F Femmina


C
D
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8m
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6m
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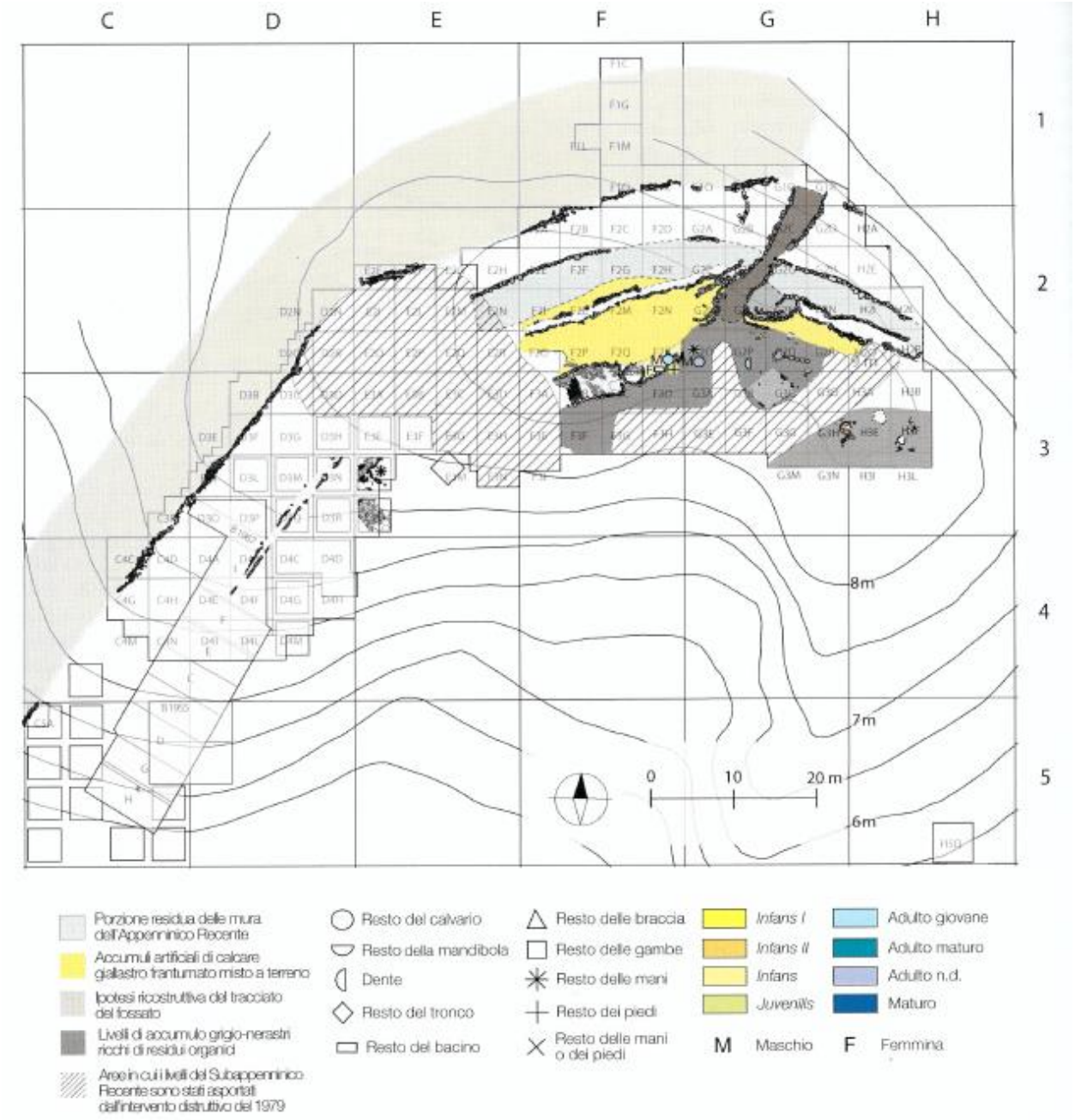
10
20 m
F?
Porzione residua delle mura dell'Appenninico Recente
Accumuli artificiali di calcare giallastro frantumato misto a terreno
Ipotesi ricostruttiva del tracciato del fossato
Area in cui i livelli del Subappenninico Antico sono stati completamente asportati in seguito all'intervento
Resto del calvario
Resto della mandibola
Dente
Resto del tronco
Resto del bacino
Resto delle braccia
Resto delle gambe
Resto delle mani
Resto dei piedi
Resto delle mani o dei piedi
Infans I
Infans II
Infans
Juvenilis
Adulto giovane
Adulto maturo
Adulto n.d.
Maturo
M Maschio
F Femmina


C
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Porzione residua delle mura dell'Appenninico Recente
Accumuli artificiali di calcare giallastro frantumato misto a terreno
Ipotesi ricostruttiva del tracciato del fossato
Livelli di accumulo grigio-nerastri ricchi di residui organici
Aree in cui i livelli del Subappenninico Recente sono stati asportati dall'intervento distruttivo del 1979
Resto del calvario
Resto della mandibola
Dente
Resto del tronco
Resto del bacino
Resto delle braccia
Resto delle gambe
Resto delle mani
Resto dei piedi
Resto delle mani o dei piedi
Infans I
Infans II
Infans
Juvenilis
Adulto giovane
Adulto maturo
Adulto n.d.
Maturo
M Maschio
F Femmina