Ca' Foscari
University of Venice

Master's degree in Conservation Science and Technology for Cultural Heritage

Final Thesis

# Human paleodiet reconstruction based on stable isotopes ($^{13}C$ and $^{15}N$) in bones at Equilus (Jesolo, Italy)

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# ABSTRACT

The aim of this thesis work was to reconstruct the diet of the human population that inhabited the medieval settlement of Equilus, in the municipality of present-day Jesolo (VE), in a period between the 6th and 9th centuries. For this purpose, $\delta^{13}C$ and $\delta^{15}N$ isotope fractionation analyses of 52 human and 5 animal bone specimens were performed. The specimens were collected from the burial site of the monastery of San Mauro during the period from 2018 to 2021, as part of the excavation campaign conducted by the Department of Humanistic Studies at Ca'Foscari University, which had previously been identified as a potential site of archaeological interest.

The study involved an initial part of research into the analytical method and sample preparation, in particular the procedure for extracting collagen from bones, the main site where the isotopic imprint of the individual is linked to his diet during his life span. The preparative methodology developed consists of an acid demineralisation of the bone sample, which involves long-time immersion in HCl in order to bring the inorganic component into solution and leave only the collagen residue. The specimens were then treated with NaOH to remove traces of lipids and other contaminating compounds that the bones may have included during their time underground. Subsequently, the samples were brought back to neutral pH, freeze-dried and finally pulverised, and analysed in analytical runs using a CN elemental analyser linked to a mass-spectrometer, constructing a calibration curve using external standards. Through the use of the RStudio calculation software, a statistical analysis model was constructed which, after obtaining the isotopic fractionation values of each individual sample, made it possible not only to establish which foods were most likely to have been eaten by the subject during his or her lifetime, but also to derive with a good degree of reliability in what proportion a person ate the different food sources.

Overall, it was found that the diet most likely to have been eaten by these individuals consisted of terrestrial herbivores, land plants of type C3, saltwater fish and other marine food such as crustaceans and molluscs generally referred to as seafood. Through the application of the model, it could be seen that the distribution of food was not uniform throughout the population, but rather there were three clusters of individuals with a well-defined diet composition. Some had a recurrent prevalence of a terrestrial diet, others appeared to be predominantly seafood

consumers despite the fact that their diets might not always be predominantly terrestrial in origin, and the third cluster contained individuals with a distinctly maritime diet. The results were also evaluated in the context of other anthropological data, such as age of the subjects, diseases they were afflicted with at the time of death, and gender, with the aim of finding correlations with diet composition. A strong correlation was found between the consumption of fish and terrestrial food sources in general, in which as the consumption of one increased, the presence of the other decreased, and vice versa. Seafood consumption, on the other hand, appears to be unrelated to these other two sources. In addition, there are strong correlations between the age and sex of the individuals and the prevalence of terrestrial or seafood diets, as individuals who died very young and belonging to a specific sex turn out to be those who consumed fish and seafood in the majority, while the opposite is true for older individuals and those of the other sex.

This work proposes an innovative approach to the study of the human diet in antiquity, which makes it possible to construct a detailed prediction of the consumption of a person's different food sources, which is of fundamental importance for studies of the archaeological contexts of the individual and how he or she related to his or her environment throughout life.

# 1. INTRODUCTION

## 1.1 BONE STRUCTURE AND COLLAGEN

Bone is composed of an organic matrix of structural proteins which is studded with crystals of calcium phosphate, largely in the form of hydroxyapatite (Figure 1). Dry bone is approximately 70% inorganic and 30% organic by weight. Most of the organic portion (85 – 90%) is an aminoacid polymer called collagen. The remainder includes noncollagenous proteins, proteoglycans, and lipids (Triffit, 1980). Collagen contains approximately 35% carbon and 11 – 16% nitrogen by weight (van Klinken, 1999), and is composed of a mixture of essential and nonessential amino acids. In teeth collagen is found in the dentin, located beneath the enamel and cement and forming the walls of the pulp chamber and root canals (Figure 2). The essential amino acids come from ingested protein (Ambrose, 1993; Lee-Thorp & Sponheimer, 2003, Ambrose and Norr, 1993; Froehle et al., 2010; Hedges, 2003; Jim et al., 2004; Krueger and Sullivan, 1984; Lee-Thorp et al., 1989; Tieszen and Fagre, 1993), while the nonessential amino acids may come from ingested protein, or they may be formed from other dietary sources and breakdown products within the body (Chisholm et al., 1982). In contrast, carbonate in bone mineral derives from blood bicarbonate which, in turn, reflects total dietary intake, including protein used as energy reserve, carbohydrates and lipids (Ambrose, 1993; Ambrose & Norr, 1993; Katzemberg, 2009; Hedges, 2003; Tieszen and Fagre, 1993; Zazzo et al., 2010). Because of these properties, collagen has become one of the main human tissues to be analysed for research into the diet of humans and animals in the past (Katsemberg, 2008; Slovak, 2009). Bone collagen and also hydroxyapatite remodel through life and maintain an average isotopic footprint that reflects food consumption throughout the lifespan of the subject (Fogel et al., 1997; Francillon-Vieillot

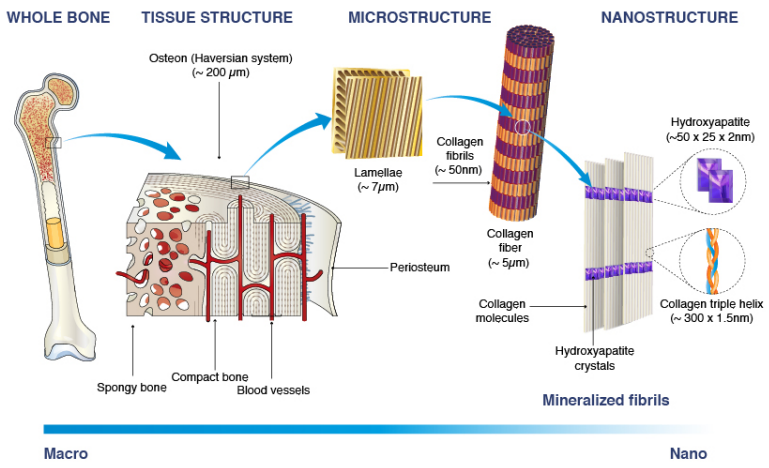


*Figure 1: bone structure, with emphasis placed on showing collagen (from Bases, 2021)*

et al., 1990; Katzenberg, 1993; Stenhouse and Baxter, 1979; Sehrawat et al, 2017). The idea that the carbon in the carbonate of bones and teeth comes from different dietary components than the carbon in collagen was first proposed by Krueger and Sullivan (1984). Ambrose and Norr (1993) and Tieszen and Fagre (1993), in two separate controlled feeding experiments, demonstrated that Krueger and Sullivan were correct in suggesting that collagen carbon comes mainly from ingested protein in the diet, whereas the carbon in biological apatite reflects whole diet.

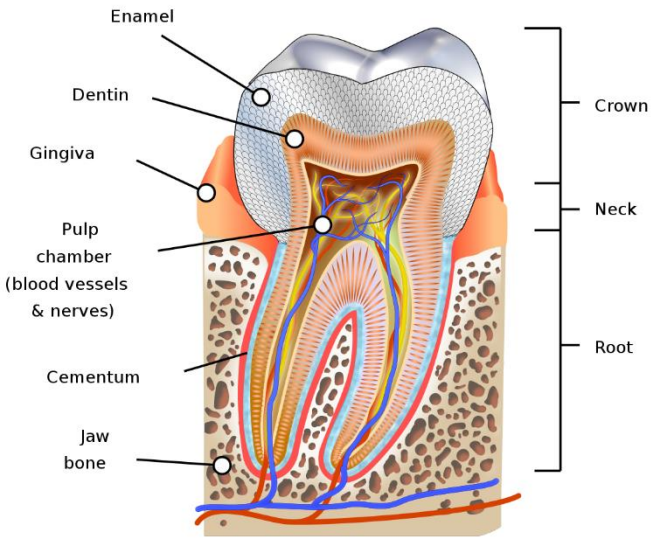


*Figure 2: tooth structure, it is possible to see dentin, where it is located tthe collagen, beneath the enamel (from wikipedia)*

Thanks of the intimate structural relationship between collagen and hydroxyapatite in the bones, collagen may survive relatively undamaged for thousands of years after the death of an individual if their bones are well preserved (Tuross et al., 1980). Some proteins that are probably degraded collagen has even been recovered from dinosaur fossils (Wyckoff, 1980). The detection of postmortem degradation of collagen has been an active area of research from decades now. Bones, and consequently also collagen after the death of the individual, can undergo tissue degradation (diagenetic alteration/breakdown) and contamination (presence of exogenous contaminants). The mechanisms of collagen degradation, leading to the loss of collagen from bone, involve the gradual breaking of collagen α-chains, the breaking of peptide bonds between amino acids. This is followed by the loss of larger or smaller peptides from the triple helix structure of collagen and loss from bone by leaching (Collins et al., 1993). The degradation does not necessarily include interactions between the bones and exogenous molecules, for example any contaminating molecules that may enter the bone matrix, but especially soil humic substances/melanoidins. Although degradation does not necessarily compromise isotopic integrity, it makes extractions more difficult or more marginal due to sample size effects. To extract a sufficient amount of collagen from a low-level sample, it is necessary to increase the amount and size of sample required for a measurement, thereby also increasing the background contamination present in the bone, which increases as the collagen yield decreases (van Klinken, 1998). Background contamination can be in situ, such as low-level contamination during burial that concentrates

during extractions, or laboratory-derived. The extent to which contaminants are able to bind with ''collagen'' in situ depends mainly on the ability of these substances to penetrate the bone matrix. The rate of reaction between collagen and humic substances is less relevant because it is so high that one can speak of an almost instantaneous reaction (van Klinken & Hedges, 1995). After this initial binding phase, many alterations can take place. Condensation and polymerisation can lead to much larger molecules than those initially present, including through the self-humification of collagen itself: i.e. condensation reactions of endogenous molecules, within the matrix bone, without the addition of exogenous substances, to form larger molecules similar to humic acids (van Klinken, 1998). If collagen condensation occurs with its own glycosylated residues, this would interfere with the $\delta^{13}C$ value, which for purified collagen includes these residues. In fact, autoumification could be considered a biologically mediated process under this definition, as it also occurs *in vivo* during the collagen maturation process (van Klinken, 1998). Condensation with fatty hydroxy acids or other bone lipids would also alter the $\delta^{13}C$ value. Exogenous contaminants may be both natural or man-made (e.g. preservatives such as resins).

In these cases, it is good to rely on the use of quality indicators, which are used to check whether a sample under analysis meets the minimum preservation requirements in order to guarantee reliable results. The collection of quality indicators should preferably be achieved without extra efforts beyond the chemical pre-treatment and combustion steps that are part of the normal preparative process (van Klinken, 1998).

The first of the quality indicators that can be used is the percentage collagen yield. Collagen yields are expressed as a weight percentage or weight ratio (mg/g). Modern, fresh bone contains about 22% collagen by weight; the collagen content decreases steadily during burial, the rate depending on climatic conditions. In Europe and other temperate to subtropical climatic zones, we find that losses are moderately slow. In warmer areas and areas with heavy rainfall, such as many parts of the United States and the tropics, bones lose collagen relatively quickly. Eventually, the collagen content drops below 0.5 per cent and contamination becomes difficult to remove. In routine practice, those bones with low collagen should be considered unsuitable for analysis (van Klinken, 1998). The carbon:nitrogen atomic weight ratio (C:N) of whole bone can also provide an indication of the general state of collagen preservation, the degree of deamination and/or the degree of contamination by exogenous carbon-containing materials such as humic acids. Modern fresh bone contains ~3.5-4.5% nitrogen (Stafford et al., 1988), of which ~90% is present as collagen and the remaining 10% as non-collagenic protein

(NCP) (Sillen and Parkington, 1996). However, the %N content of whole bone powder cannot distinguish between nitrogen present as collagen and NCP or that present in the soil, e.g. protein, nitrate or humic acids. It should also be noted that the measurement of %N content cannot specify the amount of non-nitrogenous soil-derived organic matter present in a sample (Hedges & van Klinken, 1992). Therefore, predicting the collagen content of some highly contaminated and/or degraded bones will always be problematic. DeNiro in 1985 was the first to develop a systematic indicator of collagen quality based on the observation of anomalies in the ratio of isotopic to elemental composition of ancient and modern bone collagen extracts. Specifically, he proposed that samples with atomic C:N ratios between 2.9 and 3.6 are less likely to have isotopic compositions that have been altered by post-mortem processes. Tis nérat-Laborde et al. in 2003 proposed that a C:N ratio >5 demonstrates extensive diagenesis and/or the presence of a high percentage of humic substances. Both values are the most widely reported and used as a reference in the literature for diet analysis by isotope fractionation.

## 1.2 CARBON AND NITROGEN ISOTOPES

Isotopes are atoms of the same element with the same number of protons but different numbers of neutrons. Since the atomic mass is determined by the number of protons and neutrons, the isotopes of the same element presents different masses. In contrast to unstable (radioactive) isotopes, stable isotopes do not decay over time. For example, $^{14}C$ in a dead organism decays to $^{14}N$, whereas the amounts of $^{12}C$ and $^{13}C$ in the same organism will remain constant (Katzemberg, 2008). Their abundance in nature is not the same. For the elements involved in human tissue analysis (nitrogen and carbon), even in stable isotopes alone, there is a great imbalance between the most abundant species at even mass numbers and the least abundant species at odd mass numbers (Table 1)

*Table 1: carbon and nitrogen stable isotopes*

| Element | Isotope | Abundance (%) |
|---|---|---|
| Carbon | $^{12}C$ | 98.89 |
| | $^{13}C$ | 1.11 |
| Nitrogen | $^{14}N$ | 99.63 |
| | $^{15}N$ | 0.37 |

In chemical reactions, such as the conversion of atmospheric $CO_2$ into glucose by plants, the relative amounts of $^{12}C$ and $^{13}C$ differ in plant tissue relative to that of atmospheric $CO_2$. This variation is due to the fact that isotopes vary in mass and therefore have slightly different chemical and physical properties (Bartelink et al., 2014). Isotopes with higher mass (heavier isotopes) such as $^{13}C$ usually react slightly more slowly than lighter isotopes such as $^{12}C$. Compared to $^{13}C$, $^{12}C$ in cellulose in wood from trees is enriched by a factor of about 2 % during this process (Katzemberg, 2008). Physical phenomena that occur during chemical reactions as a result of the mass differences in isotopes are referred to as "isotope effects." The resulting difference in the isotope ratio of the carbon in the plant tissues as compared with the carbon in atmospheric $CO_2$ caused by isotope effects is termed "fractionation." Fractionation is the basis for stable isotope variation in biological and geochemical systems, and gaining an understanding of the chemical reactions that result in stable isotope variation allows the biological anthropologist, archaeologist, geochemist, or ecologist to put stable isotope analysis to work to solve a wide range of interesting problems (Hoefs, 1997; Fry, 2006).

Stable isotope abundance ratios (δ) are determined relative to the ratios (R) of those same isotopes in standard materials. International standards are available through the National Bureau of Standards (NBS) and the International Atomic Energy Agency (IAEA), Vienna. The standard used for Carbon isotopes is called VPDB (Vienna Pee Dee Belemnite), which was manifactured from marble of unknown origins and present a $^{13}C/^{12}C$ ratio of 1.118%, where the Nitrogen standard is the Atmospheric Air Nitrogen.

$$\delta\ in\ ‰ = \frac{R_{sample} - R_{standard}}{R_{standard}} \cdot 1000 = \frac{\frac{^{13}C}{^{12}C}_{sample} - \frac{^{13}C}{^{12}C}_{standard}}{\frac{^{13}C}{^{12}C}_{standard}} \cdot 1000 \qquad (1)$$

The $\delta^{13}C$ isotopic values for most organisms are negative compared to the standard, while $\delta^{15}N$ isotopic values are generally positive in organisms, it is because atmospheric nitrogen is more depleted in $^{15}N$ than most living things (Bartelink et al., 2014).

# 1.3 ISOTOPIC FOOTPRINT

## 1.3.1 Food Sources

The main source of carbon for most marine organisms is dissolved carbonate, which has a $\delta^{13}C$ value of 0‰, whereas the main source of carbon for terrestrial organisms is atmospheric $CO_2$, which had a $\delta^{13}C$ value of 27‰ in pre-industrial times but nowadays it is assested around 28‰ (Santis et al., 2021). Many terrestrial plants use the Calvin cycle (C3) to fixate carbon from atmospheric $CO_2$ and will display a $\delta^{13}C$ range between −33 and −23‰ (Marshall et al., 2007; Sharp, 2017).
Maize is one of several tropical grasses that fixes carbon by a different photosynthetic pathway (referred to as the Hatch –Slack or C4 pathway) than most plants found in temperate regions. C4 plants, which also include sorghum, millet, and sugar cane, adapt to heat and aridity by minimizing the amount of time that the leaf pores (stomata) are open, thereby minimizing water loss. These plants discriminate less against the heavier isotope, $^{13}C$, than do temperate plant species, which use the C3 photosynthetic pathway. Because of this they display higher $\delta^{13}C$ values compared with terrestrial C3 plants, typically between − 16 and − 9‰ (Sharp, 2017; Santis et al., 2021). Some aquatic photosynthetic organisms generally follow a C3 photosynthetic pathway, but many types of marine algae and cyanobacteria utilize sources of carbon with higher $\delta^{13}C$ values, such as oceanic bicarbonate, which results in the correspondingly higher $\delta^{13}C$ values compared to terrestrial C3 plants, often between −22 and −17‰ (Fry et al., 1982; Keegan and DeNiro 1988; Schoeninger et al., 1983). fish, ranging between −25 and −13‰, and thus largely overlap in values with C4 plants (Katzenberg and Weber 1999)

Based on their nitrogen isotope ratios, organism are grouped into three major divisions: nitrogen fixing plants (N2-fixing), terrestrial plants that utilise nitrates from non-atmospheric sources, and marine foods that are not based on N2 fixation (Price et al., 1985). In almost all instances, marine vertebrates have higher $\delta^{15}N$ values than terrestrial vertebrates at similar trophic levels, permitting the differentiation between marine versus C4 protein in human and non-human animal diets (Schoeninger & DeNiro, 1984; Walker & DeNiro, 1986). Legumes have a symbiotic relationship with bacteria of the genus Rhizobium. The bacteria live in the roots and can fix nitrogen (combine it with other elements such as hydrogen or oxygen), thereby making it available to the plant (Brill, 1977). Other plants must get their nitrogen from decomposed organic matter, which breaks down to compounds such as ammonia or nitrate.

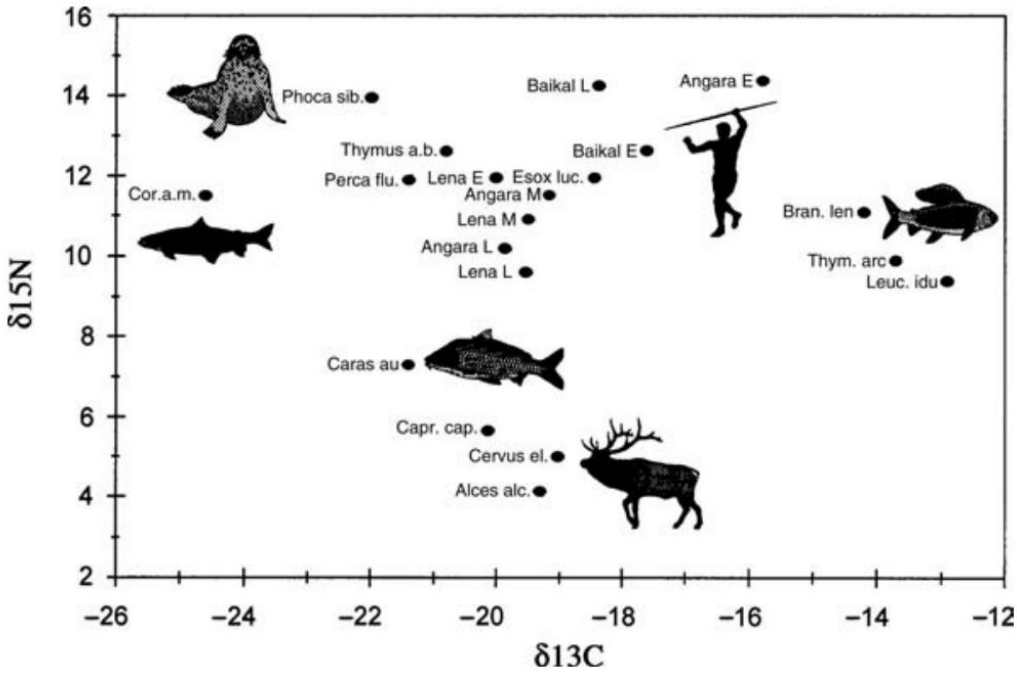


*Figure 3. representation of the isotopic footprints of certain animal species (from Katzenberg, 2008)*

Isotopic composition of human tissues is systematically enriched when compared to the values of what they eat (DeNiro & Epstein, 1978). Various studies have been proposed to determine the differences between the isotopic footprint of diet and various body tissues (Ambrose and Norr, 1993; Lyon and Baxter, 1978; Tieszen and Fagre, 1993; Vogel, 1978). Bone collagen has a carbon isotope value approximately 5‰ more positive than the values of its plant diet. This fact is the basis for the expression 'you are what you eat ±5‰'. This value was first proposed by van der Merwe and Vogel (1978), based on measurements made on mammals in the wild and their diet, and was later confirmed by Ambrose and Norr (1993). Even in controlled studies on the diet of rats, diet and bone collagen differed by 5‰ when protein, carbohydrate and fat all came solely from a similar source (plant C3 or C4). In situations where the source of protein differs from that of carbohydrates and fats, this difference is magnified (Ambrose and Butler, 1997. This situation can arise in humans if people consume the meat of C3 herbivores, such as deer, and C4 plants, such as millet, together. Again during the controlled rat study, this resulted in an interval between diet and collagen of less than 5‰. Further studies indicate the difference in carbon isotope values in collagen and bone carbonate to be 4‰ and 11‰ more positive, respectively, than in plants forming part of the diet (Koch et al, 1994). In addition, there is a fractionation factor in collagen values, which is linked to the trophic level between herbivore and carnivore, as the latter have approximately 1.5‰ more positive values than their prey (van der Merwe & Tschauner, 1999; Slovak, 2009). In contrast, the $^{15}N$ variation of herbivores is approximately 3‰ higher than the plants they feed on. Legumes are the plants with nitrogen

isotopic values closer to atmospheric air than non-legumes, which show higher fractionation. This results in a richer isotopic composition in animals eating non-leguminous plants than in those who prefer a legume diet (Fry & Sherr, 1984). For the acquatic food web the tissues of carnivores are again enriched in $^{15}N$ compared to their prey by approximately 3‰. Nevertheless, previous studies show that $\delta^{15}N$ values can increase by 1.3-5.3‰ per trophic level (Minagawa & Wada, 1984; Wada et al., 1991, 1993; Lajtha & Michener, 1994), with possible variations in enrichment. McCutchan et al. (2003) estimated an average trophic shift for aquatic environments of 2.3±0.2‰ for nitrogen and 0.4±0.2‰ for carbon. Due to the small trophic enrichment of carbon 13, such values can sometimes provide controversial or inconclusive results, and therefore require to be interpreted with caution (Grey et al. 2004; Balic et al 2014).
It should be remembered that such values should not be taken as absolute certainties, but only as guidelines for the interpretation of results (Katzemberg 2008). The reference isotopic ranges for food funds were taken from various sources in the literature, all of which, however, referred to analysed archaeological specimens. Excluding differences in the environment, it cannot be guaranteed that the isotopic values of modern specimens are directly comparable with those of pre-Industrial Revolution times. For this reason, it is important to use reference specimens that are as geographically and chronologically similar as possible to the studied individuals in question (Fischer 2007). In general, all the articles consulted agreed on the extent of isotopic contributions related to the individual food source.

Marine foods are dominantly protein-rich (Prowse, 2004), on the other hand, terrestrial plant-derived foods would be predominantly rich in carbohydrate and lipid (bread, wine, oil), which should contribute little carbon to the collagen molecules when adequate protein is present in the diet (Prowse, 2004). Increased consumption of marine resources guarantees an increase in $^{15}N$ and $^{13}C$ isotope values in human tissue (Walker and DeNiro, 1986; Richards et al., 2006). Conversely, diets rich in freshwater marine food at low trophic levels and shellfish will ensure relatively low $\delta^{13}C$ and $\delta^{15}N$ values in bone collagen (Fischer, 2007; Slovak, 2009). Medieval Western Europe demonstrated significant temporal and geographical variations in diets, due to factors such as climatic and socio-economic variations, and differences in customs and traditions (Weiss Adamson, 2002). Despite this variation, an important role of marine resources is frequently present. A wide variety of marine foods were consumed, including both aquatic mammals and other fish, molluscs or bivalves in the diet. While fish were often eaten fresh, a large proportion of the catch was salted, dried or smoked. An important motivation for

the use of marine resources in the diet may derive from the diets frequently imposed by Christian churches on their worshippers. Periods such as Lent or Fridays, where the faithful were forbidden to eat meat, stimulated the consumption of foods from other sources, including fish (Henisch, 1976; Bazell, 1997; Tanner and Watson, 2006). However, it is important to emphasise that the massive consumption of seafood was in use long before the advent of Christianity, as suggested by a study conducted by Prowse et al in 2004, on the diet in the Imperial Roman period at the archaeological site of Isola Sacra. Given the region of origin of our population, it is also worth noting that in areas with high seabird activity the soil can demonstrate significantly high levels of nitrogen and phosphorous due to the deposition of guano, which leads to a significant enrichment in $^{15}N$ (Commendador, 2013). This enrichment can manifest itself in food chains from the plants grown on those soils to all consumers along the chain.

A high $^{13}C$ value cannot only result from a maritime contribution, but also from a diet that includes many C4 plants. In the meantime, however, the nitrogen isotope value should remain unaffected by these, allowing C4 plants to be differentiated from the consumption of aquatic resources, which are richer in nitrogen isotopes despite similar $^{13}C$ values (Schoeninger & DeNiro, 1984; Schoeninger, 1995). Ancient literary sources refer to the use of millet, a C4 plant, as animal fodder, but it was considered less desirable for human consumption under normal conditions of food availability (Prowse, 2004). In contrast, individuals with lower $^{15}N$ and $^{13}C$ values rely much more heavily on terrestrial herbivores and C3 plants. Even within these herbivores, however, differences in isotope ratios can occur. The average $^{15}N$ value of deer and wild animals is more negative than that of domesticated livestock. This is due to the fact that deer eat grass in open spaces, whereas cattle have different diets in closed habitats. The different origin of their food results in slightly different footprints, as grass in open meadows has lower values of $^{15}N$ and $^{13}C$ than what is fed to domesticated animals. Pigs are a unique case, as they can also eat human food waste, including the remains of deer and cattle. This places them at a higher trophic level and therefore they should have higher values of $^{15}N$ and $^{13}C$ (Tian et al., 2011).

### 1.3.2 Other Sources of Footprint Variations

In addition to diet, there are many other biological factors that can influence the isotopic footprint in human tissue. Many studies have shown how isotopic enrichment is a body tissue-

specific process (Yokoyama et al., 2005; Logan et al., 2006), linked to different tissue turnover rates (Tieszen et al., 1983; Frazer et al., 1997; MacAvoy, Macko & Garman, 2001, Cabanellas Reboredo, 2009). Each body tissue exhibits different rates of cell death and reconstruction, and also manifests preferential mechanisms towards lighter or heavier isotopes of the same element. De Niro in 1989 proposed a model based on the loss of body nitrogen through urea, which is present in urine. Urea has a depleted $^{15}N$ value compared to the diet. Under conditions of high water stress, due to frequent sweating from hot weather or dehydration, more urea is produced relative to the total urine volume, and consequently more of the $^{14}N$ isotope value is lost. Therefore more $^{15}N$ is retained by the body, where it can be used for the synthesis of new tissue. The result is that an increased value of $^{15}N$ will be present in that tissue. Another case of high $^{15}N$ values results from protein stress, linked to too low an intake of protein in the diet. This results in the breakdown and reuse of pre-existing tissue in the body, already enriched in $^{15}N$ due to the preferential excretion of $^{14}N$. Under conditions of nutritional stress new proteins are synthesised from the products of catabolism of pre-existing proteins (Hobson and Clark, 1992; Hobson et al, 1993). The $^{15}N$ increase in protein also results in an increase in bone collagen (Katzemberg, 2008). Studies of modern anorexic patients have shown that changes in nitrogen and carbon isotope ratios, previously interpreted as primarily related to dietary factors, can equally be the result of physiological stress, with nitrogen increasing and carbon decreasing during the extreme chronic malnutrition experienced during anorexia, and nitrogen decreasing and carbon increasing with increasing BMI (body mass index) if treatment and follow-up care is administered (Bakovi´c et al, 2017; Mekota et al., 2006; Neuberger et al., 2013).

Another factor to consider when looking at the isotopic values of immature people is the possible contribution that breastfeeding can make in children in the first few months of age. On roman and medieval times breastfeeding should last for the first six months of age and weaning should take place between 18 to 24 months, with a lower limit of 16 and an upper limit of 36 months (Garrison, 1965; Fildes, 1986; Prowse et al., 2008; Fulminante, 2015). There is a slight variability in $\delta^{15}N$ values during the first year of life, but the largest age range where this value changes is between 1-2 years (Prowse et al., 2008).

The isotopic value of nitrogen in infants, present a peak value approximately one trophic level above adult female levels (enriched by 2–3%), which represents an infant consuming exclusively breast milk, because the infants are in effect consuming their mother's tissues. This is followed by a progressive decline in $\delta^{15}N$ due to the removal of breast milk during transitional feeding, ultimately reaching levels representative of childhood diet once weaning has occurred (Romek et al., 2013, Prowse et al., 2008). The trends in Figure 4 show how the isotope profiles decline within the first year of life. Any variations in isotopic values between infants of the same age can be traced back to the mother's trophic level and dietary variations, which are reflected in the milk delivered to the child (Hedges and Reynard, 2007; Sehrawat et al., 2017). After the first few months of life, infants are gradually induced to wean, involving the inclusion of new foods complementary to milk. Studies have observed lower post-weaning $\delta^{15}N$ values than the reported average adult $\delta^{15}N$ values, which is hypothesised to indicate an infant diet that is somehow depleted in $\delta^{15}N$ (i.e. less animal protein), or due to an increased metabolic demand for nitrogen during the period of rapid infant growth (Tuross and Fogel, 1994; Schurr, 1997; Richards et al., 2002; Fuller et al., 2006). Some stillbirths and infants may have lower $\delta^{15}N$ values, similar to adults in the same sample, because these individuals died before breastfeeding could be recorded in the bone collagen (Katzenberg and Pfeiffer, 1995); however, Richards et al. (2002) observed high $\delta^{15}N$ (and $\delta^{13}C$) values in very young infants, suggesting that breast milk protein was rapidly incorporated into collagen. The range of variation observed in infant $\delta^{15}N$ values is not only due to the effect of trophic level, but is also related to variation in maternal diet. If the diet of the adult female shows a wide range of variation, we would expect to see a similar range of variation in breastfed infants, and $\delta^{15}N$ values may differ from the average for adult females by more or less than 3%. A recent study found considerable variation in the isotopic values of adult females over time, this variability was also reflected in the isotopic signatures of infants and juveniles at this site (Richards et al., 2006).

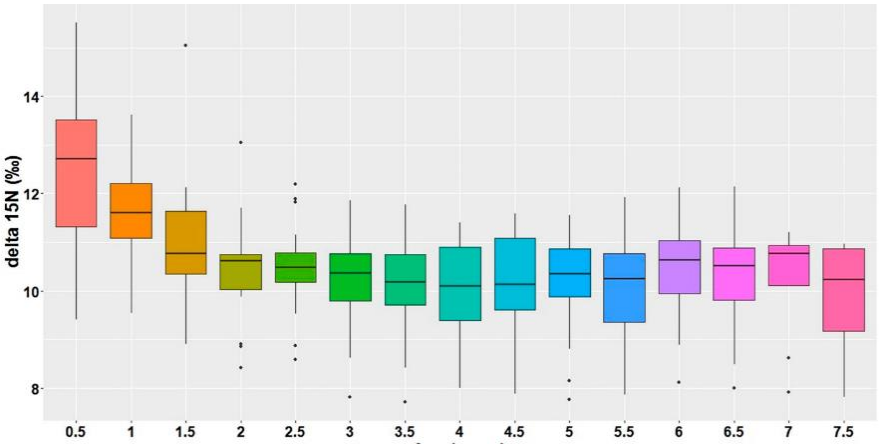


*Figure 4: boxplot showing the spread of $\delta^{15}N$ in the individuals per six-month intervals (from Gainatsou, 2022)*

The weaning diet reconstructed through a study of ancient archaeological samples (Ganiatsou, 2022) appears to have been largely based on terrestrial resources, C3 plants and animal protein, although there are some indications of the use of C4 plants and maritime resources. The most representative C4 plant in the ancient world is Millet, which was considered a cereal of low nutritional value, used to feed livestock (Garnsey, 1999; Kwok, 2015; Dotsika & Michael, 2018). Millet porridge is a dish known from antiquity, but also in modern societies it is used as nutritional food for children. Along with millet, small fish was also considered an inexpensive food with low nutritional value (Varro, On Agriculture, 3.17 and Columella, On Agriculture, 8.16.3-4). Fogel and colleagues (1989), in a study that included the analysis of stable nitrogen isotopes from the nails of mothers and infants, further confirmed this phenomenon, finding an increase in $\delta^{15}N$ in the protein of infants as soon after birth (after about three months), and declining when supplementary foods were added to their diet. Carbon isotopic trends also reflect the introduction of food and feed during weaning, increasing or decreasing depending on whether these are based on C3 or C4 plants (Ganiatsou, 2022).

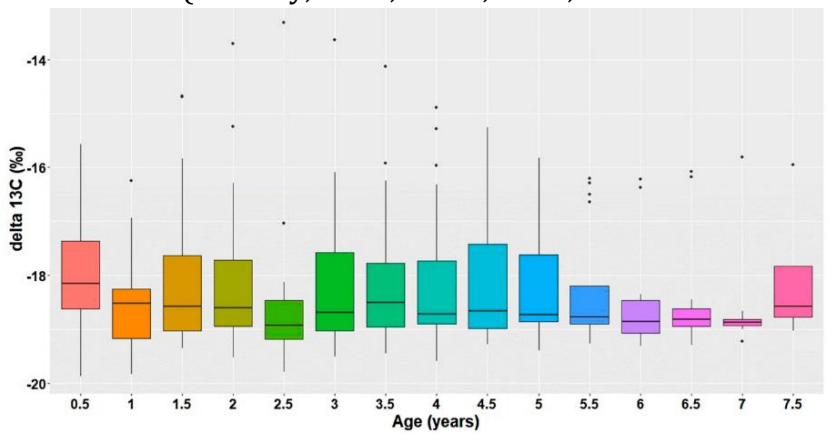


*Figure 5: boxplot showing the spread of $\delta^{13}C$ in the individuals per six-month intervals (from Gainatsou, 2022)*

## 1.4 ARCHAEOLOGICAL CONTEXT

The archaeological excavation complex from which the samples under analysis originate refers to the geographical area of the settlement of Equilus, located within the municipality of Jesolo, in the province of Venice. Historically, the area has undergone many changes, of natural or anthropic origin, to its morphology, which in alternating phases have caused the water level to rise or fall. The proximity of the area to the mouth of the river Piave resulted in the formation of a paralittoral lagoon a few metres above sea level, which represents the northern offshoot of

*Figure 6: aerial picture of the Venetian Lagoon*

the much larger Venetian Lagoon, with few small islands above sea (Gelchi 2013, Ciancinosi, 2022). Archaeological excavations have shown that the area was frequented from at least the middle imperial period (2nd-3rd century A.D.), when murex shells were harvested here to produce purple, an activity probably connected with the wool industry in the nearby Roman city of Altinum (Altino). The settlement of Equilus presents an initial nucleus dated between the 4th and 5th centuries, through the foundation of a large *mansio* within a network of connections within the lagoon, on one of these islets, immersed in a predominantly marshy environment (Gelchi 2013, Ciancinosi, 2022). From the 6th century onwards, important land reclamation works were carried out by the population that led to the drainage of the marsh waters, creating a system of neighbouring islands, divided by several internal canals. The expansion of the mainland area, and its important position due to its proximity to the mouth of the river Piave, led to an enlargement of the settlement, which resulted in the creation of new buildings exhumed from excavations, such as a monastery, a large boat pier and a cathedral (Gelchi 2013, Ciancinosi, 2022).

This process was not an isolated case within the Venetian lagoon landscape. During this period, many of the marsh settlements began to expand through similar reclamation works. As a result, several centres of commercial and aristocratic power, such as the settlements of Torcello and Cittanova, were formed within the Venetian lagoon at the time. These centres played the role of important institutional municipalities or even episcopal seats, including Equilus itself, thus exerting considerable influence on the inhabitants of the lagoon. The settlement of Rialto itself, the nucleus of what was to become the future Venice, originated through similar processes in this time interval. The presence of such locations within the same lagoon landscape inevitably led to competition between the different settlements. The centre of Rialto emerged victorious from this competition when, from the 10th century onwards, the ducal seat of the region was established there. The influence that this institution brought with it led to the annulment, even physical, of the power of the other, by then weaker lagoon centres, prompting even the population to migrate towards Rialto (Gelchi 2013, Ciancinosi, 2022). It is not known how much

the settlement of Equilus played a role in this lagoon competition, nor how much the birth of Venice influenced its inhabitants. Evidence of a community in Equilus derives from direct reports from written sources referring to this place, such as those appearing in the testament drafted by the Venetian duke Justinian Particiaco in 829, as well as from sculptural elements, such as the exceptional sarcophagus fragment of a certain Antoninus tribunus and his wife, also dating back to the 9th century. It is known, however, that from the 12th or 13th century onwards there was a general rise in the water level in the northern parts of the lagoon, due to the excrement of the river Piave, which caused a systematic silting up. The inhabitants were then forced to totally abandon the area, which became marshy and unhealthy again. This unhealthy condition continued until the land reclamations carried out during the 19th century, following which the new municipality of Jesolo was inaugurated. This settlement was therefore only established in contemporary times, without having any kind of relationship or continuity of memory with the ancient Equilus.

Archaeological excavations in the area focused in particular on two areas of the settlement: the San Mauro complex and the 'Le Antiche Mura' area. The former refers to the complex of structures that can be traced back to the Monastery of San Mauro, and must have been one of the cornerstones of the ancient settlement of Equilus. The 'Le Antiche Mura' area consists of the remains of a church, remains of the ancient cathedral, an adjoining structure with a cistern for collecting water, and a bell tower. The San Mauro complex had already been uncovered and excavated in 1954, but the structures were then buried again and only recovered by excavation operations as of 2018. The first traces of occupation in that area of the island date back to between the 7th and 8th centuries. The construction of a small one-nave church most probably dates back to this period, or shortly afterwards. In later times, perhaps between the 11th and 12th centuries, the church was extended with the construction of a three-nave

*Figure 7: aerial picture of the excavation sites*


*Figure 8: aerial picture of the San Mauro site*

building. Recent excavations have brought to light the perimeter of the church, and are exploring the cemetery that had developed inside and outside the church building. The burials excavated so far, around 150 inhumed bodies, are in association with the oldest and second oldest church. A C14 dating is currently awaited to better define the different phases of the cemetery and to associate the excavated graves into chronologically coherent sets. The two religious buildings were built on top of each other, as the newer structure seems to have used part of the pre-existing construction. Part of the new church, one of its three naves, was built outside of what was the older church, where some graves are located.

The place-name of the second area of archaeological interest, 'Le Antiche Mura', derives from the presence of the still visible remains of the cathedral, probably built in the 12th century, today reduced to a short raised section of the south-eastern façade apse and some masonry, the foundations of the aisles, the façade wall and the bell tower in front. The cathedral, already in ruins in 1800, had suffered final destruction during the First World War. And in 1944, in fear of an Allied landing in the Adriatic, a system of reinforced concrete bunkers had been built by the German army, which still exists today, and which damaged the archaeological area, destroying part of the presbytery and the crypt below.

*Figure 9: picture of the ancient Equilus catedral, before World War I*

The research on these excavations was coordinated by the Department of Humanistic Studies at Ca'Foscari University, with funding from the Municipality of Jesolo and the Fondo Scavi i Ateneo. PoliMedica S.r.l. collaborated with the Laboratory of Physical Anthropology of the University of Salento for the analysis of traumatic lesions found on skeletal remains, with the Laboratory of Physical Anthropology of the University of Salento for the excavation and study of osteological finds, with Harvard University for DNA analysis, and finally with the Department of Environmental Sciences, Informatics and Statistics for the study on the paleo-diet of the inhabitants, carried out on the exhumed remains of people buried in the San Mauro cemeteries.

# 2. MATERIALS AND METHODS

## 2.1 PRE-ANALYTICAL STAGE

### 2.1.1 Sample Selection

The samples granted for analysis came from the area of the burial sites in the San Mauro area, in the form of dental findings or from some other long bodily bone. All samples came from the excavations carried from 2019 to 2021, sent to the research lab on February 2022. At the same time as the paleodiet-analyses, parallel anthropological studies were carried out in order to report traits and characteristics of the subjects, such as gender, age at burial and possible pathologies. In Appendix I, the characteristics for each individual found in the analysis are summarised.

In addition to humans, faunal remains of various species were also found in the surrounding area. These faunal remains mainly fall into the category of herbivores, both domestic and wild, but there are also some specimens of fish and aquatic species. The analysis of such specimens can directly show the isotopic composition of the fauna directly involved in the feeding of the inhabitants of Equilus. As the number of animal specimens far exceeded the number of human individuals, it was decided to consider a limited number of samples for analysis, one for each animal species present in a relevant way in the catalogue, based also on the conservation status of the samples. Thus, a number of 6 animal samples were analyzed: a bovine, an equine, a pig, two cervids, a fish and a skate.


*Figure 10: image of a horse's tooth among the archaeological finds exhumed from excavations in*

Through a similar procedure the human samples were also skimmed, in this case solely on the basis of the state of preservation of the specimens. During this phase, some individuals were discarded, mainly because the degradation to which they were subjected corroded the inside of their bones, presenting a highly porous structure where there was abundant infiltration of sand and soil from the burial environment. As the tissue involved in this analysis, collagen in fact, likely resides predominantly on the inside of bones and teeth rather than on the surface, this condition would inevitably have presented heavy contamination during the analysis of

these specimens. Some of the subjects were presented with samples of both bone and teeth; for such individuals, it was decided to bring both dental and bones for analysis. This could allow to confirm the possible presence of a significant systematic deviation between the isotopic signal demonstrating a tooth versus a bone of the same individual.

### 2.1.2 Sample Preparation

A total of 63 humans and 6 animals entered the sample preparation step, which consisted in the external cleaning of the individual specimens. Using a mechanical cleaning method, the surface layer of dirt and soil that encrusted each specimen was removed, revealing cleaner layers of bone tissue or tooth enamel.

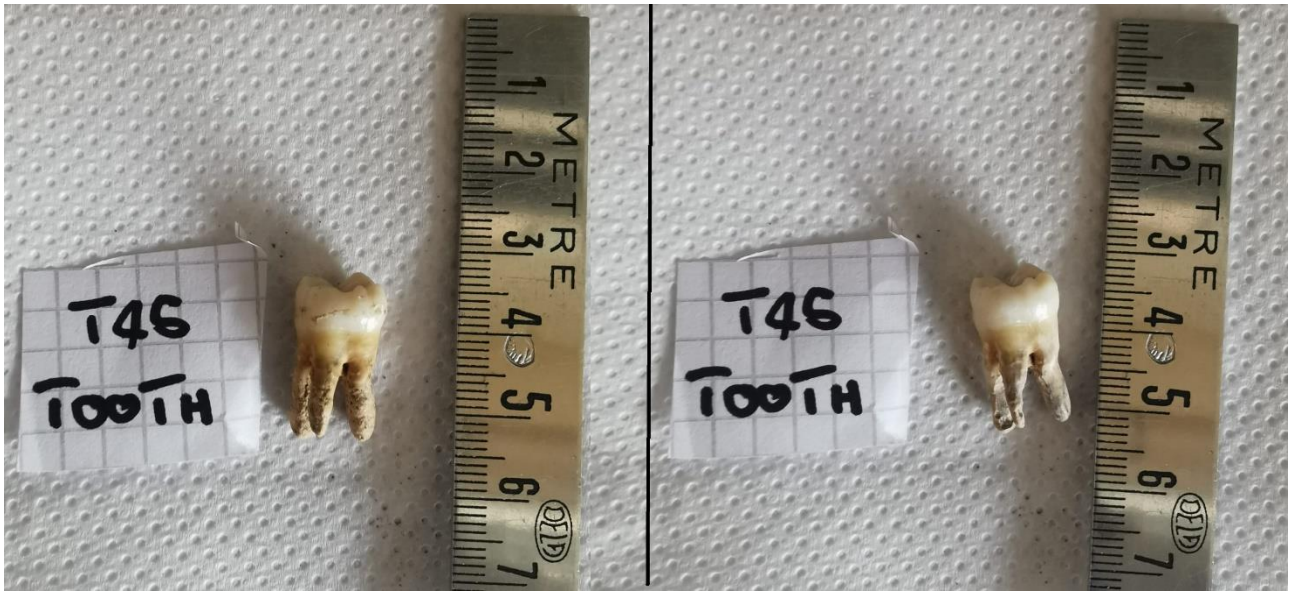


*Figure 11: example image of the mechanical cleaning of the same sample, before (left) and after (right) cleaning*

In the case of the teeth, although it was not always necessary, any dental caries were also removed. Their origin lies outside the tooth-forming tissue comprising the isotopic imprint of the subject, and therefore if left out they could have been a source of external contamination. After cleaning each sample was weighed. Many samples of animal origin, specifically teeth, were too large to fit into a test tube. In this case, a fragment of the appropriate size was cut from them so that it could both collect the most superficial part of the enamel and enter the interior where the dentine and collagen reside.

A good way to isolate bone collagen from the rest of the tissue suggested by involves two successive treatment steps with reagents: a first long acidification step in HCl solution (Berger et al., 1964; Sellstedt et al., 1966), followed by a second shorter immersion step in NaOH solution (Ambrose, 1990; Chisholm et al., 1983). What remained after the treatment was

subjected to freeze-drying and grinding in order to reduce the mall to powder or, at most, small hard grains.

The first step is crucial in order to demineralise the samples. Since the carbon of interest to our analysis is derived from the amino acid chains in the collagen, the simultaneous presence of structural carbon in the carbonate of the bone hard part and the enamel would inevitably interfere, leading to incorrect analytical results. Treatment with hydrochloric acid slowly dissolves the inorganic part of the sample into solution. In well-preserved samples, the result of this process leads to a pseudomorph of the organic part of the bone sample, whereas in less preserved samples, the residue will be more like a gelatinous amorphous (Sealy et al., 2014). This method is undoubtedly among the fastest for demineralisation, with the disadvantage that the acidic environment catalyses the decomposition of peptide bonds as well as some amino acid residues (including γ-carboxy-glutamic acid, aspargine and glutamine) (Collins, 1998). Prolonging the immersion in acid too long even after total demineralisation leads to the breakdown of the peptidic bonds and thus to a depolymerisation of the organic collagen molecule (Collins et al., 1995), a state to be avoided in order to conduct this research. The rate of demineralization appears to be proportional to the acid concentration itself, which is why processes with different acid concentrations were tried on other waste samples before proceeding with the test samples. Among these tests, the development of the demineralization process was recorded in order to be able to decide more clearly which solution would be the most suitable to use, depending on the nature of the samples themselves. As a result of these tests, it was seen that, with the same acid concentration, teeth take significantly longer than bones to show signs of demineralisation. In an attempt to obtain an equivalentprogression in all samples, it was decided to immerse cautiously under a fume hood the dental samples in a solution of 1M hydrochloric acid, while the bones would be subjected to 0.2M acid. The various samples were placed inside plastic falcons, and every two days the immersion acid was replaced. Despite being the fastest of the demineralization process, this

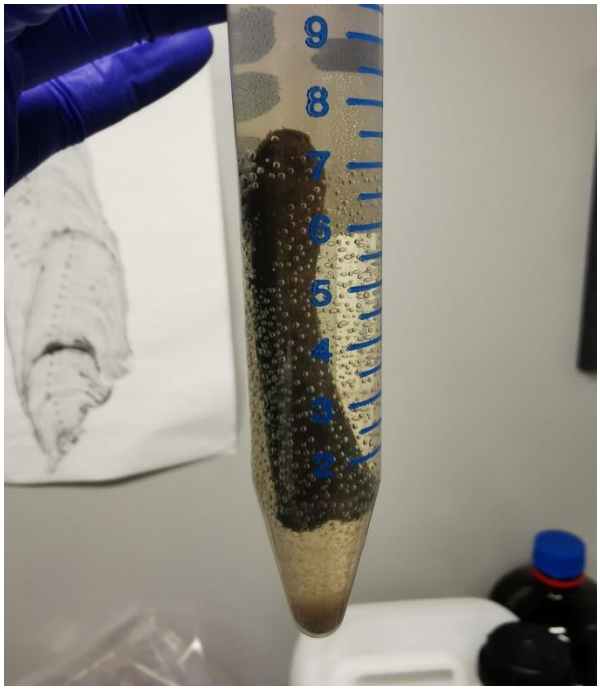


*Figure 12: image of a bone sample during the demineralisation process*

took a long time, as not only the nature of the sample determined its demineralisation time, but also, of course, its size. Smaller specimens took two weeks before being reduced to their collagen pseudomorph only, while many larger specimens required as much as a month of continuous immersion before reaching the end. When the process was complete, most of the teeth retained a morphology reminiscent of that of the original specimen, while the comparatively worse preserved bones possessed a more amorphous appearance. In both cases, what remained of the sample lost the rigidity and hardness typical of bone tissue, presenting a gelatinous appearance.

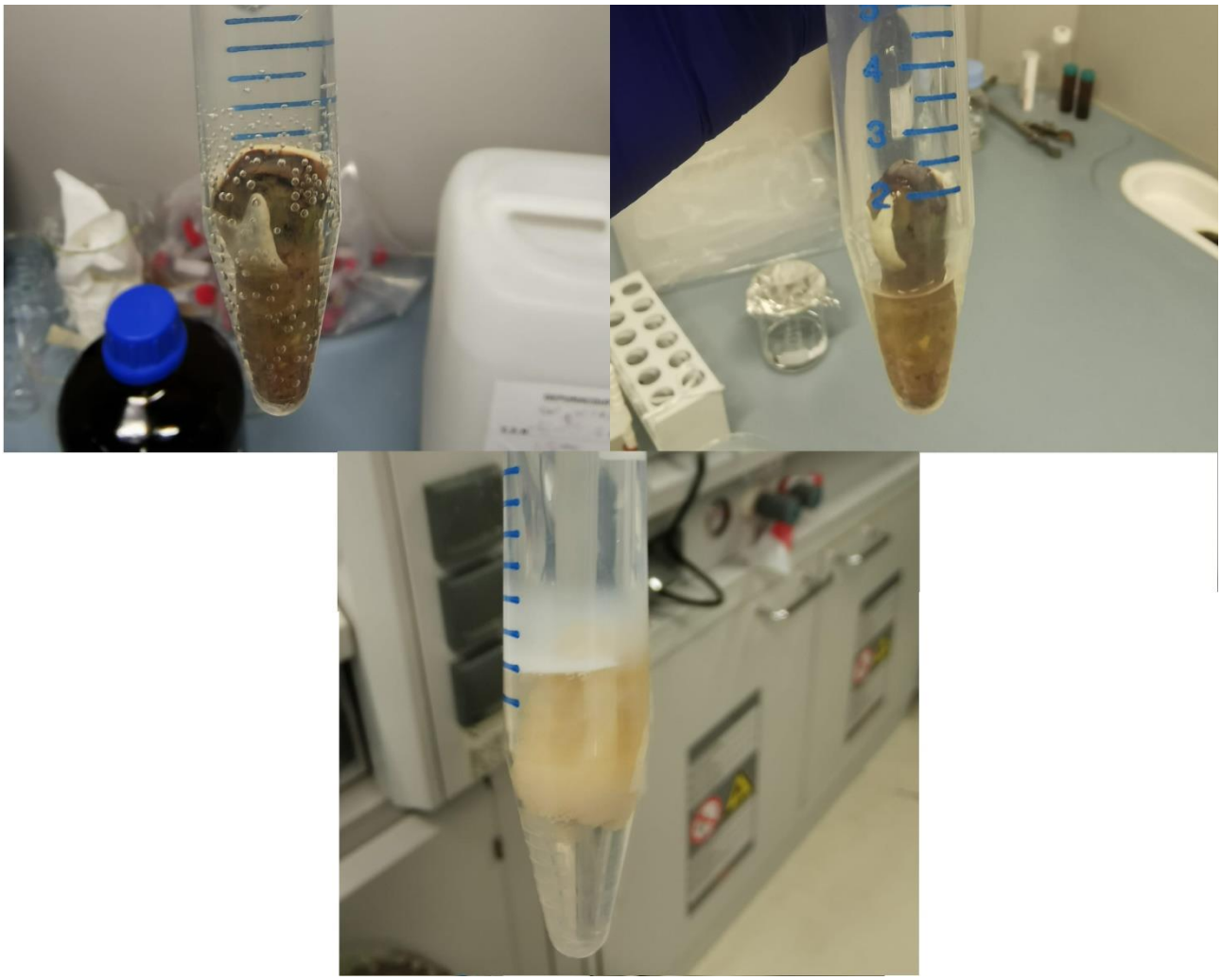


*Figure 13: image of the same specimen during different stages of demineralisation, you can see in the first two images the enamel receding leaving the underlying tissue exposed, the last image represents the pseudomorph composed solely of collagen*

Subsequent immersion in NaOH is required to remove humic acid, a product of organic tissue degradation, and other superfluous lipids, leaving only the amino acid chains that make up bone or dental collagen. This step is much quicker than the previous one, as using a 0.1M NaOH

solution only 24 hours are required to achieve this result. At the end of the process, a white patina containing all the waste was present on the surface of the solution. The sample was then removed and immersed in milliQ water for the purpose of restoring its pH to neutral values. This last step took a few days, during which the immersion water was changed daily for each sample.

About ten bone specimens failed during this part of the preparation. Many were already poorly preserved specimens to watch out for after the initial selection phase, while others only revealed their poor condition during demineralisation. Unfortunately, the skate sample was totally dissolved during the basic immersion day. A total of 52 human individuals and 5 animals remained. Following a period of a few days at -18°C, the samples were then all freeze-dried, following which they were again weighed in the balance. Finally, the last preparation step involved cutting and grinding the samples.

Measurements with the instrument were performed using a calibration line constructed from external standards. In each rack sent for analysis were present four different standards with known elemental and isotopic composition: wheat flour, AP10 compound, sorghum flour and an organic sediment with a high carbon content (HCOS). The latter two are the standards used in the construction of the calibration curve, and were placed at the beginning of each rack in three increasing amounts from 1mg to 3mg. Quantities of AP10 and wheat flour weighing 2mg were instead inserted between each sample. For each sample under analysis, three different samples were taken, weighing approximately 2mg measured and recorded on an analytical balance.

*Figure 14: the falcons containing the pulverised samples after preparation*

# 2.2 INSTRUMENTAL ANALYSIS

All samples were analysed using a CN elemental analyser (EA Thermo Fisher Scientific) through an interface (Conflo IV) to a mass-spectrometer (a new Delta V Advantage IRMS Thermo Fisher Scientific). The analysisis carried out through two separate steps to perform two types of determinations: the mass quantification of elements C and N in the samples, and the isotopic ratio $\delta^{13}C$ and $\delta^{15}N$.

The CHNS elemental analysers provide a means for the rapid determination of carbon, hydrogen, nitrogen and sulphur in organic matrices and other types of materials. The analysers are often constructed in modular form such that they can be set up in a number of different configurations to determine, for example, CHN, CHNS, CNS or N depending on the application. For this work it was used the CHN set up. The principles of elemental analysis are based on the tendency of all atoms to prefer their oxidation states. In pure oxygenat high temperatures, all available carbon easily burns to carbon dioxide, all hydrogen into water and all nitrogen is converted to various nitric oxides. In the analyzer, a sample is moved from a 'cold zone' to a 'combustion train' filled with pure diatomic oxygen (minimum 99.9995%). Instruments are classified as either 'static' or 'dynamic' in terms of their combustion characteristics. In the 'static' type, a pre-set volume of oxygen is added to the combustion tube before the sample is introduced. In the 'dynamic' type, the oxygen is added to the tube at the same time as the sample introduction and continues to flow for a set time. In the majority of applications, either method is applicable. For slow burning materials such as coals and cokes, where multiple additions of oxygen are required for complete combustion, the 'static' system is preferred. The sample is heated to a temperature of 980 °C and the combustion described above occurs. After combustion, a stream of ultra-pure He moves the gases through the system A variety of

*Figure 15: the analysis instrument (proprietary image of Thermo Fisher)*

absorbents are used to remove these additional combustion products as well as some of the principal elements, sulphur for example, if no determination of these additional elements is required. The combustion products are swept out of the combustion chamber by inert carrier gas such as helium and passed over a heated quartz tube filled with reduced copper at 700°C. It removes all unreacted oxygen and converts nitric oxides to free nitrogen species that quickly form $N_2$. All species that are not $CO_2$, $H_2O$ or $N_2$ are adsorbed onto the copper. Consequently, the reduction tube must be replaced periodically. The combustion section of the analyser is designed to achieve both complete combustion of the sample and conversion of oxides of nitrogen to nitrogen gas ($N_2$). Although different approaches have been chosen by different manufacturers, the use of high purity copper is universal for the reduction stage. In some instruments, the combustion and reduction stages are housed in separate furnaces. In others, the reactions are combined in a single two-tier furnace. Catalysts are usually added to the combustion section to aid complete combustion and absorbents to remove potential contaminants. Various safeguards exist to ensure that the entire sample is gaseous and moves through the system. After the initial combustion, additional oxygen can be injected in bursts to try to achieve complete combustion. In addition, a 'high heat coil' vaporizes any condensate that may have settled on the chamber joints. After the reduction tube, the entire sample is pushed by the flow of He into the mixing volume chamber, where more He is added to ensure that all the gaseous sample is in this chamber. The flow continues until the chamber drops below 1500 mmHg. This chamber is designed to homogenize the sample gas and takes approximately 20-50 seconds depending on the flow rate. The gas expands to atmospheric pressure when a pair of valves opens to allow the sample gas to exit the mixing chamber through a tube of known volume. This becomes the volume of the sample and any excess gas can exit the system. The flow of He then pushes the sample gas through a series of thermal conductivity detectors and traps. The first couple is sensitive to the amount of water on the gas volume, and successfully traps it. These traps, just like the reduction tube, must also be replaced periodically to avoid contaminations or saturations. Both the catalysts/absorbents and copper metal are packed into readily exchangeable tubes made of ceramic material or high quality silica. The amount of the specie is determined by the difference of the volumes before and after the trap. The second detector is sensitive to carbon dioxide, followed by a trap (Ascarite) that removes all carbon dioxide. Finally, the gas, now composed only of $N_2$ and He, is pushed through a final detector that is coupled with a detector exposed only to He. A calculation of the volume difference will reveal the amount of nitrogen gas present in the sample volume.

The products of the CN elemental analyser are introduced into the mass spectrometer, where they are ionised in a targeted and accelerated manner. They are then introduced into a path subjected to a magnetic field orthogonal to the direction of movement. Due to the action of the Lorentz force, the electrically charged particles are subjected to a force whose direction, orthogonal to both the particle's velocity and the magnetic field it passes through, causes it to deviate its trajectory to perform a circular motion. Since this force acts as a centripetal force, it can be verified that the radius of curvature of the particle depends on the ratio of charge to mass of the particle. The heavier the charged particle, the greater the radius of curvature. In contrast, polyvalent ionised particles have a narrower radius of curvature than monovalent ones. For this reason, it is preferable to attribute energies to the analysed elements that do not induce second ionisations, since only particles with the same electrical charge have as their only distinction in the path the variation of the mass of the particle, i.e. the type of isotope of which it is composed. This energy for the elements we are interested in (C and N) is very similar, whereas greater care would have been required if samples containing alkali metals or halogens were analysed, the former being easily ionised and also susceptible to polyvalent ions, while the latter have a low ionisation efficiency. At the end of the run, these ions are detected by Faraday cups, positioned so that three different masses can be captured simultaneously. For nitrogen ($N_2$) the masses recorded are 28, 29 and 30uma and for carbon ($CO_2$) the masses are 44, 45 and 46uma.

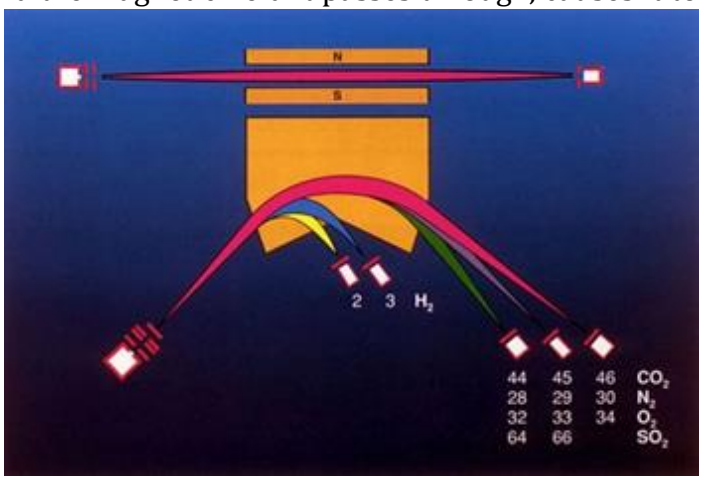


*Figure 16: example image of isotope curvature due to the Lorentz force, the radius of curvature depends on the mass of the isotopes*

## 2.3 STATYSTICAL PROCESSING

Computer processing of the data obtained from the analysis was carried out using the open software R-Studio. It has preset functions that automatically perform operations or statistical tests. The tests of interest for this study were the following: The Shapiro-Wilk test was used to check the normality the distribution of a population. The t-test, on the other hand, was used to determine how significantly different the distribution of two normal populations are or are not, while in the case of two non-normal distributions, the Wilcoxon test was used.

With the data available from the results of the analyses, it is possible to trace the isotopic composition of the samples back to general influences from certain categories of food sources, but without being able to go into detail as to how much they may have influenced the diet of the individual or the population as a whole. It was for this reason that it was decided to construct a prediction model that would lead to a quantified composition of the diet of the specimens analyzed. The basic idea behind this model is that a subject's isotopic footprint is the combination of the footprints of everything they have eaten, and depends proportionally on the amount of food of a certain nature ingested. If $\delta C_{observation}$is the $^{13}C$ isotope value of each individual sample, it will be given by the sum of the$\delta^{13}C_i$ of the individual food relative to their proportion $x_i$ in diet, where$\sum_i x_i = 1$. The same consideration can be made for the nitrogen isotope fraction of the sample.

$$\delta^{13}C_{observation} = x_1 \cdot \delta^{13}C_{source\ 1} + x_2 \cdot \delta^{13}C_{source\ 2} + \cdots + x_n \cdot \delta^{13}C_{source\ n} \quad (2)$$
$$\delta^{15}N_{observation} = x_1 \cdot \delta^{15}N_{source\ 1} + x_2 \cdot \delta^{15}N_{source\ 2} + \cdots + x_n \cdot \delta^{15}N_{source\ n}$$

The aim of this model is to derive the $x_i$ factors of proportionality of the various food sources. Once it has been determined which sources presumably contribute to the population's diet, the average isotopic footprint value of each source can be derived from the literature. Using these values, it is possible to construct a compositional space, *i.e.* a two-dimensional space formed by points, each representing a different combination of the different food sources, and whose coordinates of each point are the isotopic values of $\delta^{13}C$ and $\delta^{15}N$ resulting from its own combination. An individual with known isotopic fractionation but unknown composition can be placed within this compositional space. Its composition can be derived through the proximity of the points in the compositional space around it, the closert hese points, the more similar their composition will be to the unknown. The value of the unknown's composition can therefore be derived from the average of the composition values of its neighbouring points in compositional space, with the

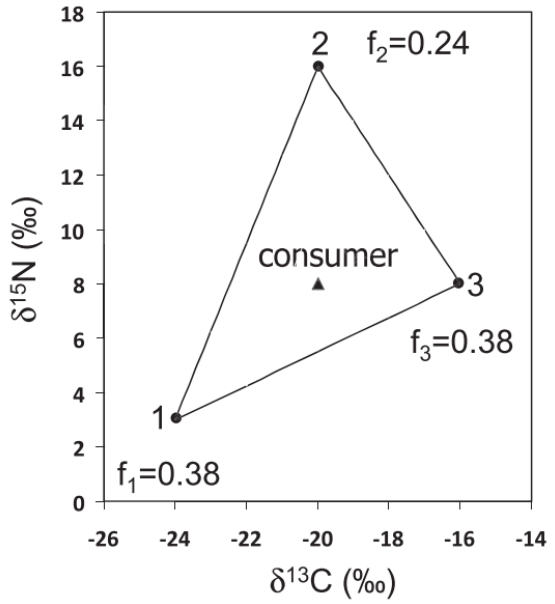


*Figure 17: the contribution of three food sources determine the isotopic value of the individual, the f values means the factor of proportionality of that single food source (from Philips, 2012)*

distance between the values being the Euclidean distance between the points in space. The choice of the number of first neighbors to be considered, as well as the standard deviation value to be presented in the final figure, is very delicate. This is because the various combinations considered by the first neighbors of the unknown are arranged according to a normal distribution, to increase to excess the number of points to be included in the mean would widen the distribution curve of the combinations considered too much, making the average obtained lose accuracy. At the same time, restricting to too few points increases the possibility of no longer including the true value of the unknown in the distribution curve of the combinations. This weighting is also valid for the standard deviation to be considered around the derived mean value: using, as is usually the case, a value of σ or 2σ of the normal distribution may not be a suitable choice for this model.

Validation of the model is necessary in order to be able to derive the most suitable values of these two parameters, before proceeding to use them with the unknown samples from the excavations. This validation was carried out by constructing a number of 500 test individuals of known composition, randomly extracted through the construction of a combination generator on R Studio. The isotopic footprint of each test individual was constructed through the above formula (2). However, instead of using the average isotopic footprint of a food source in the formula, to increase the stress of this test it was decided to use the footprint of a specific specimen found in the literature for each different food source considered. Each test individual then has a random combination and a set of reference food specimens, also randomly drawn. After placing them within the compositional space, using its resulting isotopic fractionation value in carbon and nitrogen as co-ordinates, the distance between each test individual and every other point making up the compositional space was derived. Subsequently, each test individual was placed within a matrix in which all points in the compositional space were ordered from the nearest to the furthest, and for each point the respective food source composition and isotopic fractionation parameters were listed. At that point, it was beginning to vary the mean and standard deviation of the compositions, varying the number of the first neighbors from 2 up to the maximum of the points making up the compositional space, and considering deviation values in a range from 0 to 2σ. For each time these values were changed, it was checked how many times all the true values of the composition of the test individuals were within the mean ± standard deviation range proposed by the model, and relating this to the total number of test individuals subjected to validation yielded a test success rate. In this way, a table was derived in which each pair of parameters to be selected, number of first

neighbors to be included in the mean and standard deviation to be considered, corresponds to a probability that the test returns the true value of composition of an unknown sample.

# 3. RESULTS

The data obtained from the analyses for the samples are shown in Appendix II. The %yield ratio was obtained prior to analysis, and refers to the mass of the sample once it had come out of freeze-drying compared to its mass after initial external cleaning. The quality of the extraction process was evaluated using the yield % and C/N ratio, after which the distribution in isotope fractionation values was evaluated, and finally these values were entered into the diet composition prediction model

## 3.1 EXTRACTION YIELD%

Through the chosen preparation procedure, the percentage yield is expected to be at a minimum of 0.5% to be acceptable (Van Klinken, 1999), up to upper limits of 20-25% (Sealy 2014; Brown 1998; Jørkov 2006). As it is possible to see in Figure 18, through these two parameters, two samples were found to be below the lower limit: T23 and T34.

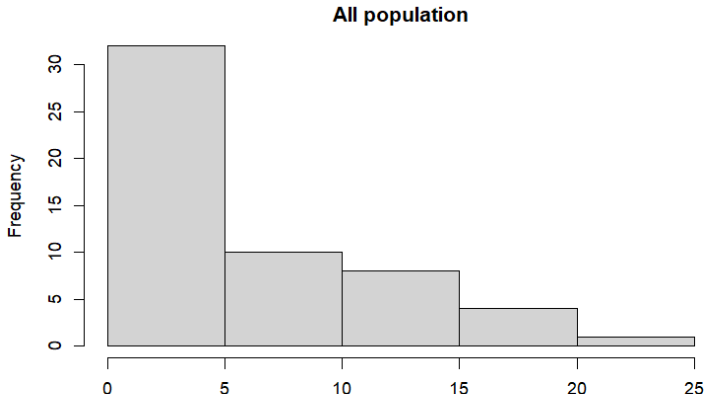


*Figure 18: extraction yield distribution of al sample analyzed*

The presence of significant differences among the populations was first examined between the main pairs of groups: Adult-Immature (snown in Figure 19) and Tooth-Bone (Figure 20). Subsequently, the same analysis was carried out with the cross-groups.

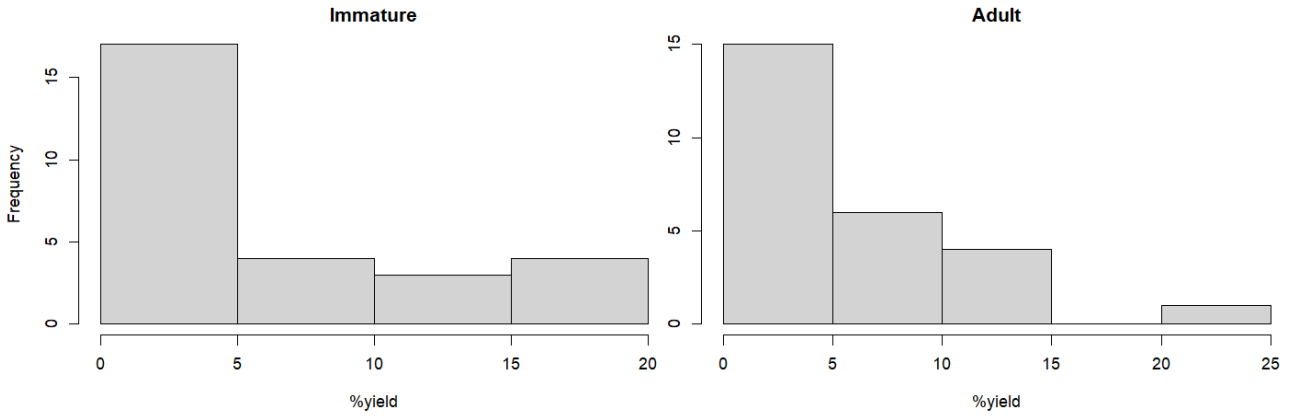


*Figure 19: extraction yield distribution divided among immature and adult samples*

The Shapiro Wilk test was used to check the normality of the distributions. As it could be expected from the positively skewed distribution, both p-values were far below the minimum value. The immature group had a mean value of 6.7% (sd=5.5) and a p-value=$2.40 \cdot 10^{-4}$, while the adults had 6.1(4.8) and p-value=$1.52 \cdot 10^{-3}$, respectively. Between the two non-normal populations, the Wilcoxon test used to evaluate possible difference between the means, and it provided a p-value=0.8154, suggesting that the exctraction yield is not affected by factor related to the age of the individuals. The same approach was used to evaluate any differences between tooth and bone samples.

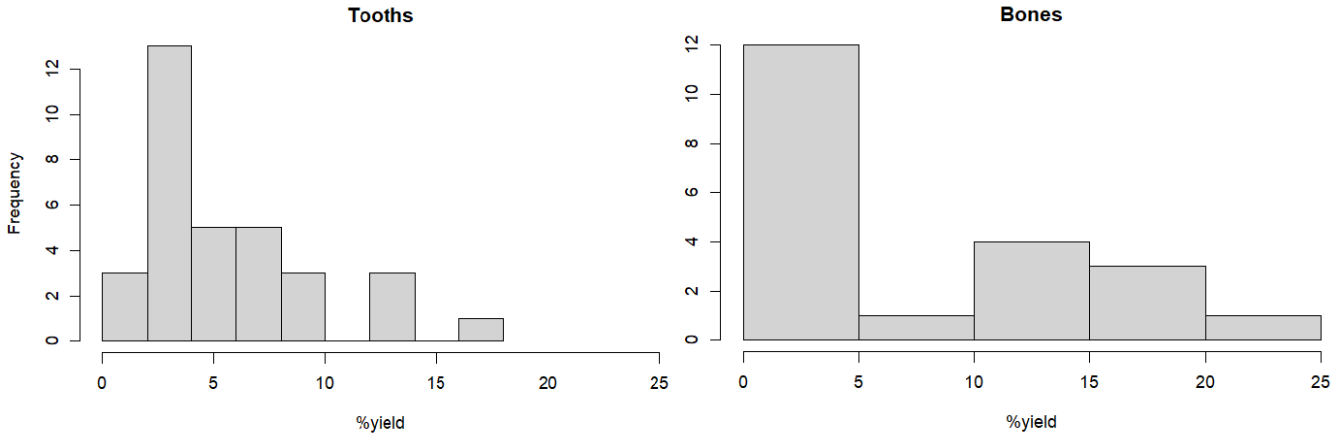


*Figure 20: extraction yield distribution divided among tooth and bone samples*

The teeth group shows a mean value of 5.5(3.8), with a p-value derived from the Shapiro Wilk test of $4.39 \cdot 10^{-4}$. The bone group reported a mean value of 7.8(6.7) and p-value=$8.05 \cdot 10^{-3}$. Since neither of them were found to be normal distributions, the test used to check for a significant difference was the Wilcoxon test. The p-value=0.30 suggests that even for these two groups, the difference in the nature of the sample did not result in significant variation in the mass yield of the procedure.

At this point, the same analysis procedure was carried out for the groups mixed between age and nature of the sample. In Figure 21 you can see the various distributions of the 4 groups.

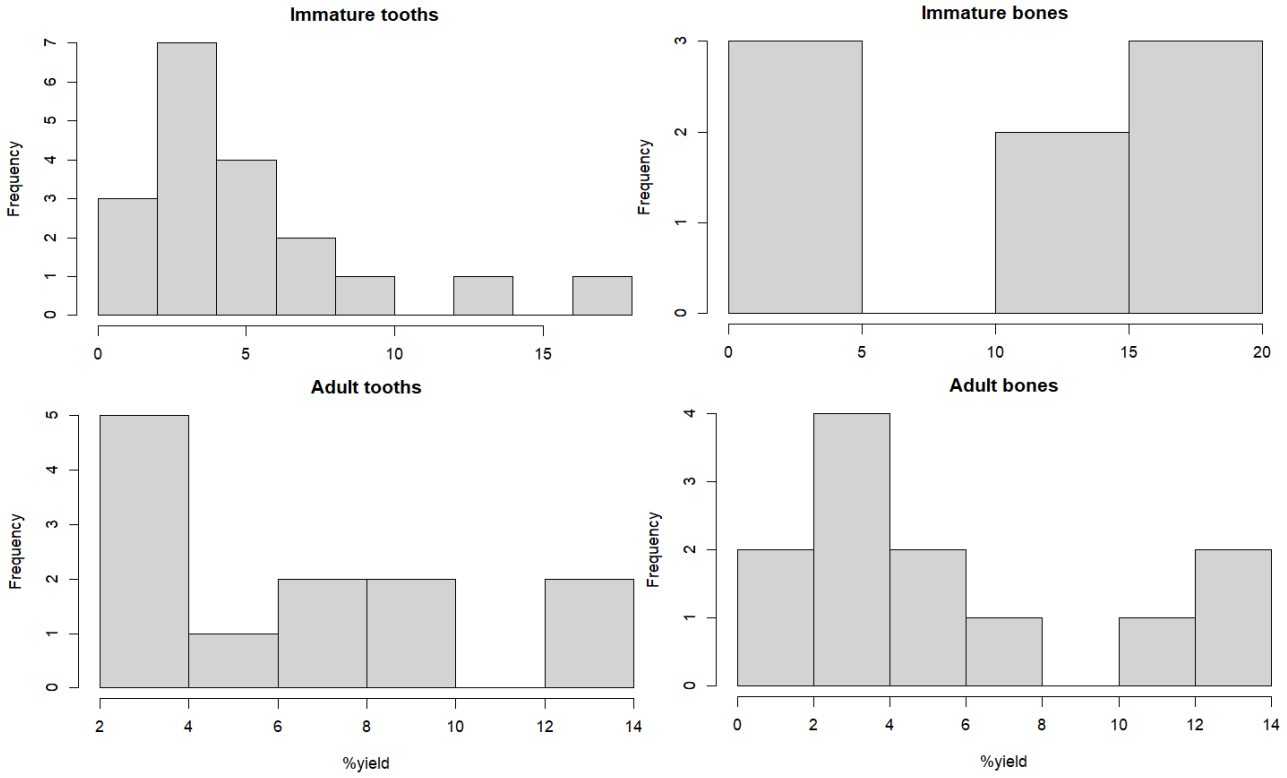


*Figure 21: extraction yield distribution divided both on age and nature of the samples*

Using the Shapiro Wilk test, it was found that only the 'Immature Tooth' group cannot be considered normally distributed, as it presented a p-value=$8.45 \cdot 10^{-4}$. The rest of the groups presented respectively: Immature bones p-value=0.13, Adult tooths p-value=0.11, Adult bones p-value=0.05, the latter being at the limit of acceptable values, as shown in Table 2.

*Table 2: minimum, maximum, p-vales of the Shapiro-Wilk and Wilcoxon-Mann Whitney tests of the %yield values obtained from adults, immature, tooth and bone samples.*

| *%yield* | **Adults (n=28)** | **Immatures (n=30)** | **Tooths (n=35)** | **Bones (n=23)** |
|---|---|---|---|---|
| **Min-max** | 0.5-21.1 | 0.4-18.6 | 0.4-16.8 | 0.5-21.1 |
| **Mean(sd)** | 6.1(4.8) | 6.7(5.5) | 5.5(3.8) | 7.8(6.7) |
| **Shapiro test** | $1.52 \cdot 10^{-3}$ | $2.40 \cdot 10^{-4}$ | $4.39 \cdot 10^{-4}$ | $8.05 \cdot 10^{-3}$ |
| **Student/Wilcoxon test*** | 0.82 | | 0.30 | |
| | **Bones** | | **Tooths** | |
| | **Immature (n=9)** | **Adult (n=14)** | **Immature (n=21)** | **Adult (n=14)** |
| **Min-max** | 1.0-18.6 | 0.5-13.7 | 1.3-16.8 | 2.1-13.0 |
| **Mean(sd)** | 10.5(7.4) | 5.5(4.5) | 5.3(3.9) | 6.2(3.8) |
| **Shapiro test** | 0.13 | 0.05 | $8.45 \cdot 10^{-4}$ | 0.11 |
| **Student/Wilcoxon test*** | 0.28 | | 0.41 | |

**Student t test was applied when distributions were normal; alternatively, Wilcoxon-Mann Whitney test was used.*

# 3.2 C:N RATIO

Human collagen extracted from archaeological remains is generally considered reliable for analysis when the molar C:N ratio falls within a range of 2.9-3.6 (Ambrose 1990, Deniro 1985; Deniro and Weiner 1988, van Klinken 1999). Figure 22 shows the results obtained from our population. Since almost one third of the samples would have been cut off by following these criteria, it was decided to proceed in a different way. Initially, the normality of the distribution was checked by means of the Shapiro-Wilk test and confirmed with a p-value=0.41. Therefore, it was decided to keep all the samples that fell within the 3σ interval of the Gaussian curve. In this case, having found a mean value of 3.0 and standard deviation of 0.2, the interval under consideration was 2.4-3.5. With this criterion there were no discarded samples. The comparison to be made now is of any difference in ratios between Adults and Immature, the two distributions being shown in Figure 23.

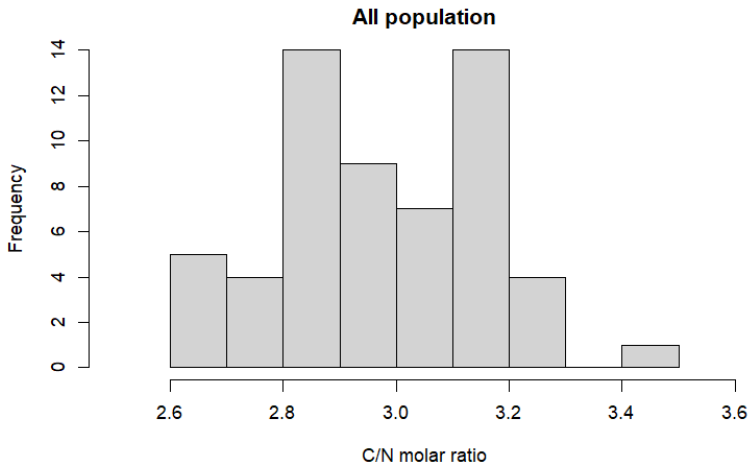


*Figure 22: C:N ratio distribution of all sample analyzed*

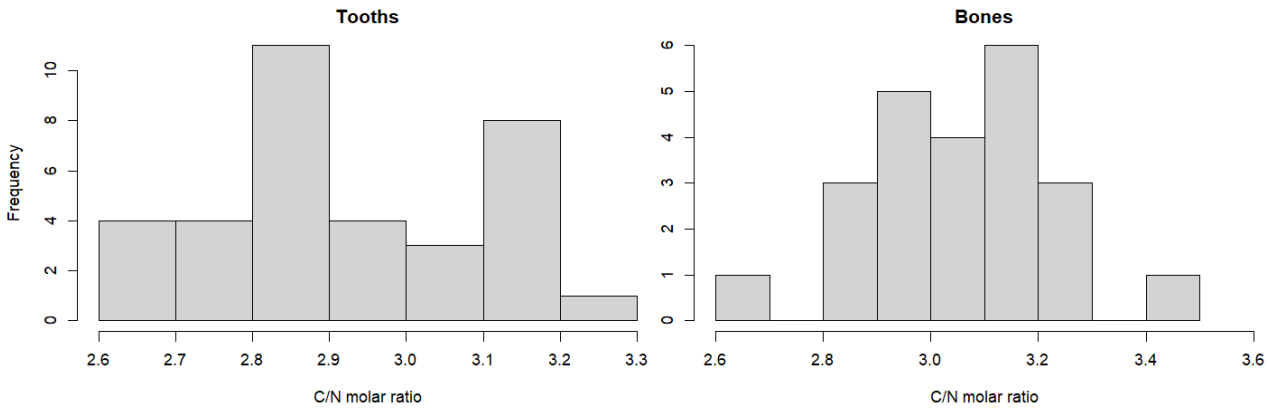


*Figure 23: C:N ratio distribution divided among tooth and bone samples*

The normality test returns a p-value of the first population of 0.024, while for the bones the value found is 0.80. The comparison between the two populations was again carried out using the Wilcoxon test, which returns a p-value of 0.0041. The two populations are therefore to be considered significantly different from each other. The mean C/N ratio value obtained for teeth is 2.9(0.2), while bones have 3.1(0.2). The distributions of adults and immatures are both

within the normality criteria for the shapiro test, the former having a p-value = 0.096, the latter a p-value = 0.12. Figure 24 shows the relevant histograms

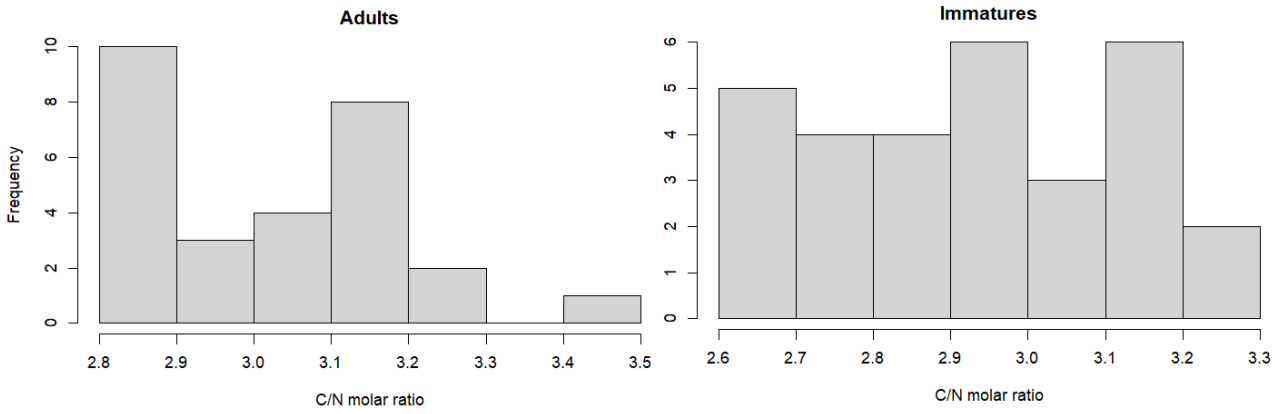


*Figure 24: C:N ratio distribution divided among tooth and bone samples*

The adult population had a mean ratio value, with standard deviation, of 3.0(0.2), the immature population 2.9(0.2). Since both distributions are normal, a t-test was performed to assess significant differences between the populations this time. The result p-value=0.04 suggests that the two distributions can be considered significantly different. The analysis now focused on cross-sectional groups, whose populations are distributed as shown in Figure 25.

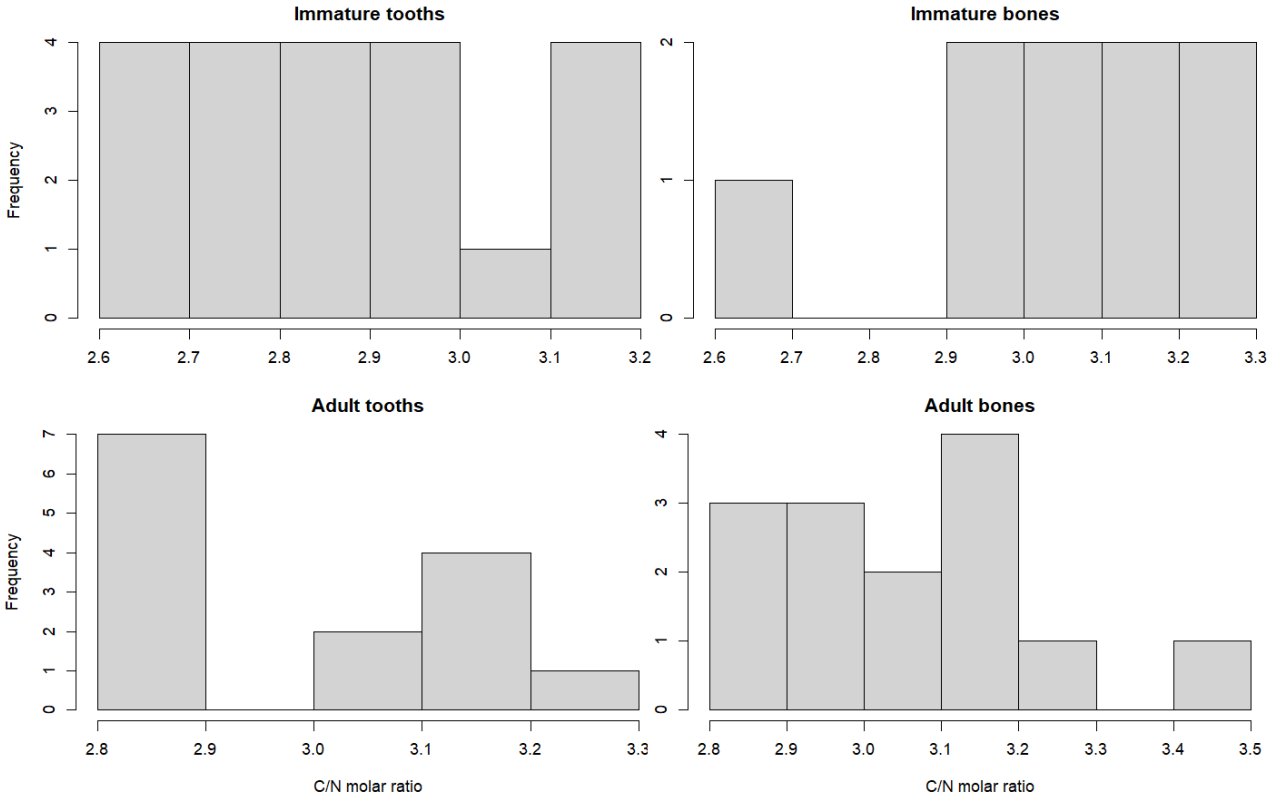


*Figure 25: C:N ratio distribution divided both on age and nature of the samples*

Of these, from the Shapiro test, only the Immature Tooth group is not considered normal, having a p-value of 0.020. The others are respectively Immature bones p-value=0.21, Adult tooths 0.058 and finally Adult bones 0.31. The comparison between the two groups of bones can be made with the t.test, which through a result p-value= 0.91 indicates a non-significance in the difference between the two groups. A different result was instead obtained between the two populations of teeth, since through the Wilcoxon test it was found that the difference in their distribution was significant, thanks to p-value=0.024. The results are summarised in Table 3.

*Table 3: minimum, maximum, p-vales of the Shapiro-Wilk and Wilcoxon-Mann Whitney tests of the C:N ratio values obtained from adults, immature, tooth and bone samples*

| *C/N molar ratio* | **Adults (n=28)** | **Immatures (n=30)** | **Tooths (n=35)** | **Bones (n=23)** |
|---|---|---|---|---|
| **Min-max** | 2.8-3.4 | 2.6-3.3 | 2.7-3.3 | 2.6-3.4 |
| **Mean(sd)** | 3.0(0.2) | 2.9(0.2) | 2.9(0.2) | 3.1(0.2) |
| **Shapiro test** | 0.096 | 0.12 | 0.024 | 0.80 |
| **Student/Wilcoxon test*** | 0.043 | | 0.0041 | |
| | **Bones** | | **Tooths** | |
| | **Immature (n=9)** | **Adult (n=14)** | **Immature (n=21)** | **Adult (n=14)** |
| **Min-max** | 2.6-3.3 | 2.8-3.4 | 2.7-3.1 | 2.8-3.3 |
| **Mean(sd)** | 3.1(0.2) | 3.1(0.2) | 2.9(0.2) | 3.0(0.2) |
| **Shapiro test** | 0.21 | 0.31 | 0.020 | 0.058 |
| **Student/Wilcoxon test*** | 0.91 | | 0.024 | |

## 3.3 $\delta^{13}C$

The samples obtained, all show negative isotopic values compared to the VPDB standard. The values reported can be traced back to the result of the formula (1). The mean value of the total population was calculated to be -16.4‰, with a standard deviation of 2.1‰. The distribution of the results obtained from the entire population is shown in Figure 26. As with the preliminary

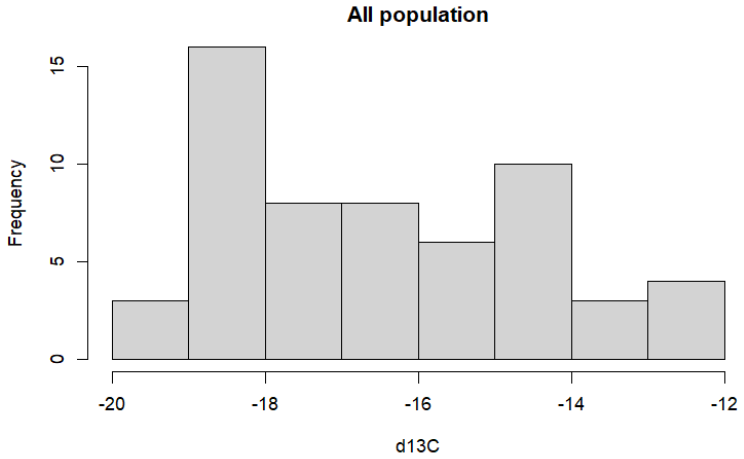


*Figure 26: $\delta^{13}C$ distribution of all sample analyzed*

analyses, the population was studied by dividing it into groups according to the age of the individuals and the type of samples.

The first division, as shown in Figure 27, was made according to the age of the samples. Neither distribution appears to be particularly symmetrical, with the adults being particularly right-skewed and the immatures more moderately left-skewed. In fact, the Shapiro Wilk test reports values below the 0.05 limit for both, the adults having p-value=0.018 while the immature ones 0.042. With a mean value of -17.6(1.4)‰ the adult population would appear to be isotopically poorer than the immature population, which has a mean value of -15.4(2.1)‰. How different the two populations may be is confirmed by the Wilcoxon test, which reports a result of p-value=$3.04 \cdot 10^{-4}$.

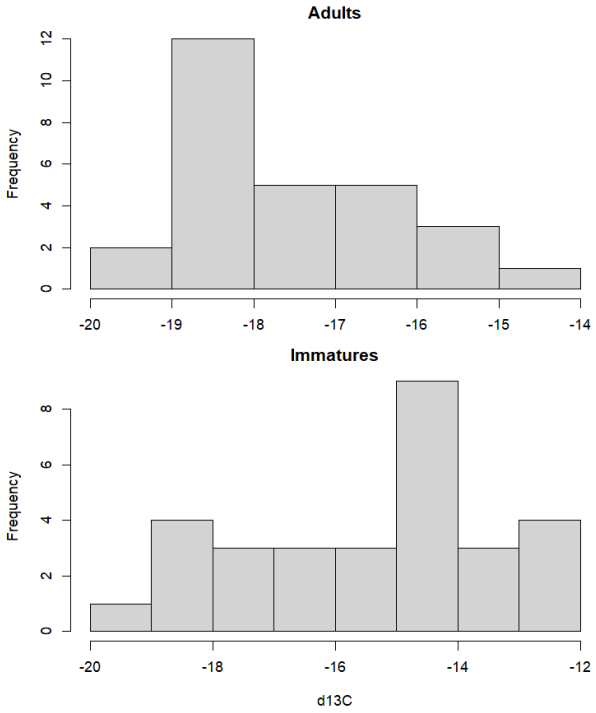


*Figure 27: $\delta^{13}C$ distribution divided among adult and immature samples*

Similar results can also be seen in the division of the samples between teeth and bones, in Figure 28. The first distribution seems to respect normality, and in fact the shapiro test returns an acceptable result of p-value=0.097. The second, on the other hand, is clearly right-skewed and far from a Gaussian shape, as confirmed by its p-value=0.0026. If we look at the mean values of the distributions, we see that teeth, with -15.9(2.1)‰. on average, appear to contain more Carbon13 isotope than bones, which show -17.3(1.7)‰. instead. At this point, it is necessary to confirm how significantly different the two populations may be. The Wilcoxon test calculates a p-value between the two distributions of 0.014, thus confirming this hypothesis.

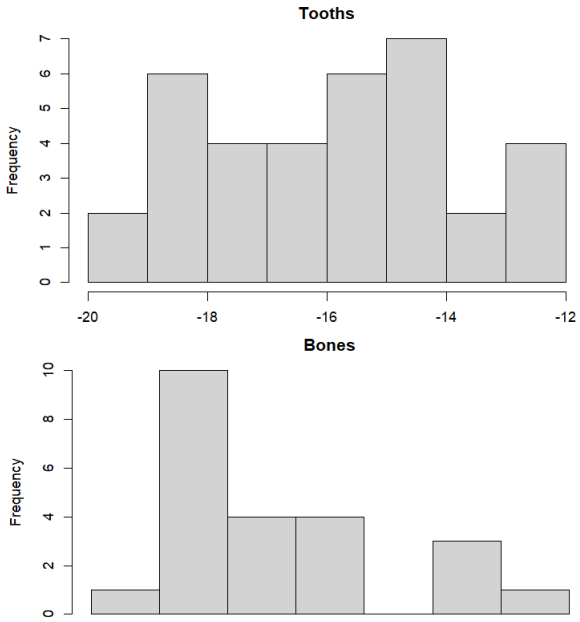


*Figure 28: $\delta^{13}C$ distribution divided among tooth and bone samples*

An additional step was performed for this group, as the difference in isotopic content between teeth and bones is a reflection of the body's metabolic processes. The results in isotopic content of the six control samples were plotted in a two-dimensional plot, placing them in abscissa according to their content in teeth and in ordinate according to their content in bone. Samples that were positioned above the bisector of the quadrant would have a higher $^{13}C$ isotopic content in bones than in teeth, and *vice versa*. This step can then be used to test whether the difference in content found in the population averages can also be systematic for the control samples, or whether they show a more random distribution. The results, shown in Figure 29, are in agreement with what has been explained by the mean values. All the means lie below the bisector, with the only exception of the error bar along the abscissa of sample T22B. This bar, however, turns out to be very large and exaggerated, therefore any contribution from it is not particularly reliable.

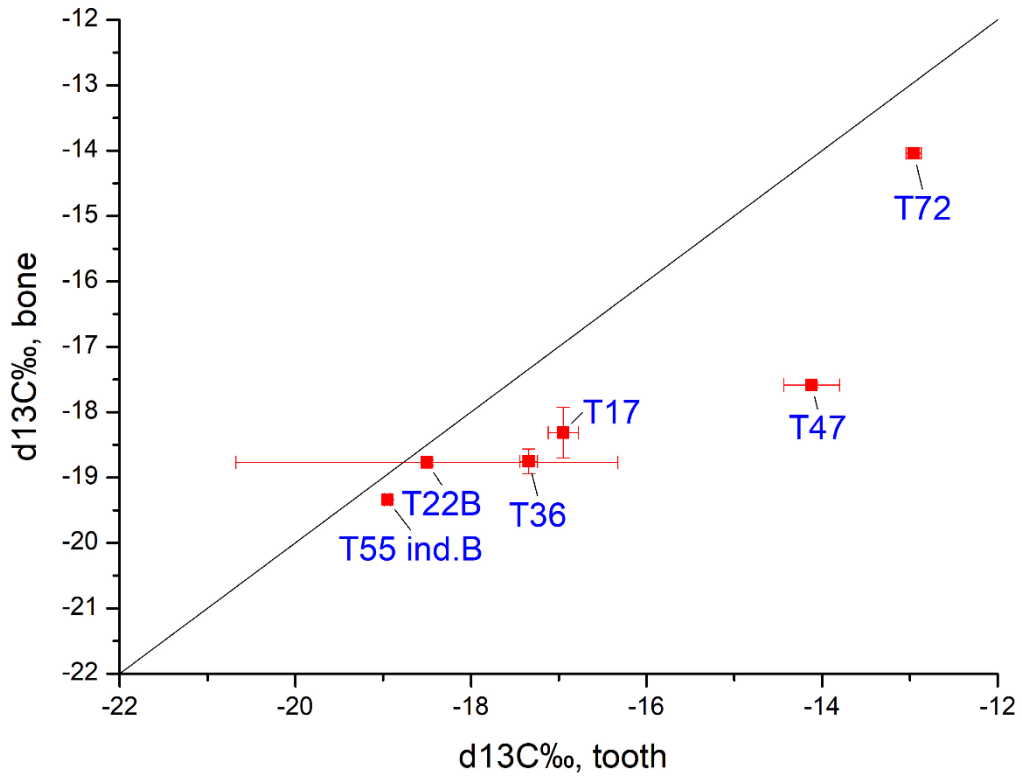


*Figure 29: $\delta^{13}C$ composition of bone and tooth samples from the same person*

Considering the distributions of the cross-samples, it can be seen that they, unlike the main groups from which they derive, exhibit normalised trends, as none of them have a p-value, via Shapiro's test, that is less than 0.05.

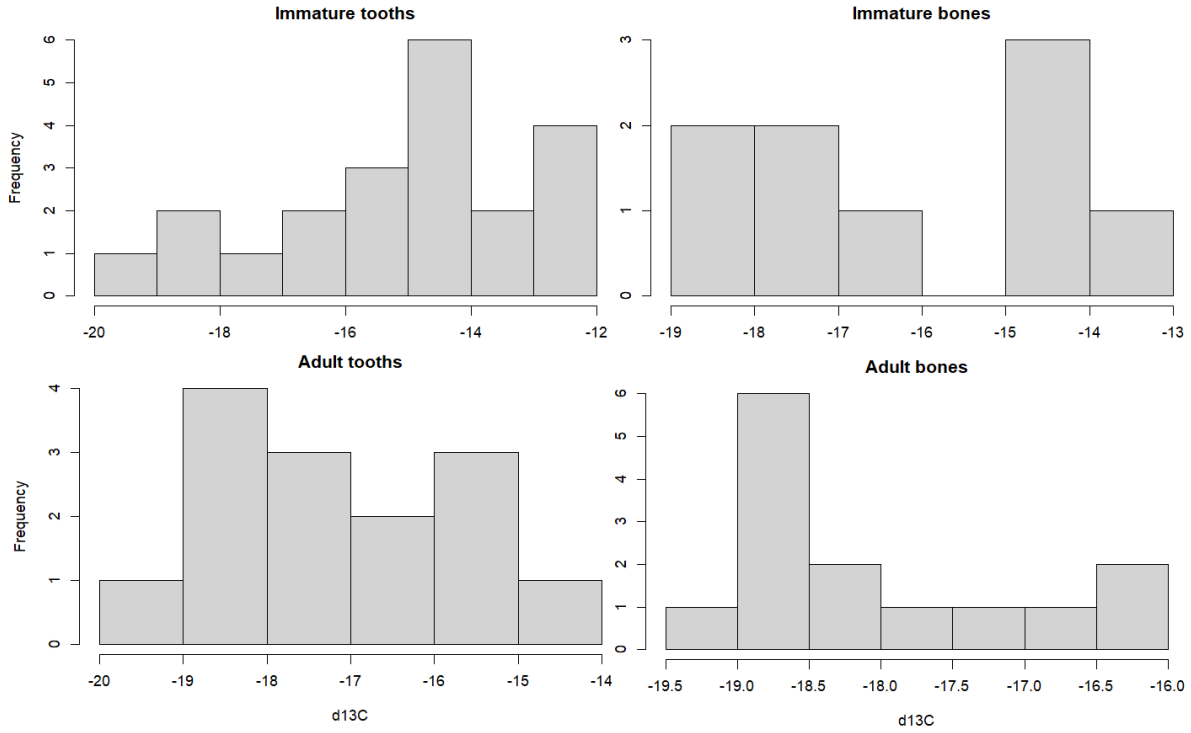


*Figure 30: $\delta^{13}C$ distribution divided both on age and nature of the samples*

In all cases, the results obtained reflect what has been observed previously, namely that in comparisons between adults and immatures the former are poorer in $^{13}C$, and in comparisons between teeth and bones the former are richer in $^{13}C$. Despite this, however, the differences between the cross-groups cannot be said to be non-significant, as for each of the comparisons carried out by means of a t-test, the p-values were always less than 0.05. All the results from these analyses have been collated in Table 4

*Table 4: minimum, maximum, p-vales of the Shapiro-Wilk and Wilcoxon-Mann Whitney tests of $\delta^{13}C$ values obtained from adults, immature, tooth and bone samples*

| $\delta^{13}C$ | **Adults (n=28)** | **Immatures (n=30)** | **Tooths (n=35)** | **Bones (n=23)** |
|---|---|---|---|---|
| **Min-max** | -19.3/-14.1 | -19.3/-12.1 | -19.3/-12.1 | -19.3/-13.4 |
| **Mean(sd)** | -17.6(1.4) | -15.4(2.1) | -15.9(2.1) | -17.3(1.8) |
| **Shapiro test** | 0.018 | 0.042 | 0.097 | 0.0026 |
| **Student/Wilcoxon test*** | $3.04 \cdot 10^{-4}$ | | 0.014 | |
| | **Bones** | | **Tooths** | |
| | **Immature (n=9)** | **Adult (n=14)** | **Immature (n=21)** | **Adult (n=14)** |
| **Min-max** | -18.5/-13.4 | -19.3/-16.0 | -19.3/-12.1 | -19.1/-14.1 |
| **Mean(sd)** | -16.2(2.1) | -18.0(1.0) | -15.0(2.0) | -17.1(1.6) |
| **Shapiro test** | 0.075 | 0.11 | 0.21 | 0.27 |
| **Student/Wilcoxon test*** | 0.032 | | 0.0019 | |

# 3.4 $\delta^{15}N$

Compared to the isotopic value of atmospheric nitrogen, all samples show higher values, derived from the same FORMULA (1). The mean value for the entire population is 9.6‰ with a standard deviation of 1.4‰. Figure 31 shows the distribution of all samples, which appears to follow a normalised pattern.

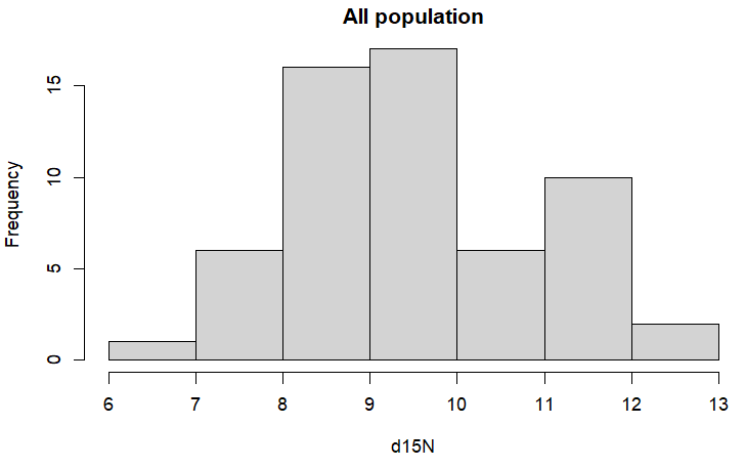


*Figure 31: $\delta^{15}N$ distribution of all sample analyzed*

The distribution that resulted less skewed is the adult population, which in fact reports a p-value from the Shapiro-Wilk test of 0.36. The immatures show higher and more scattered mean values, 10.1(1.6)‰ against 9.1(0.2)‰ for the adults, but a less normalised distribution with a p-value of 0.058, almost at the limit of the permitted values. The two populations are, significantly different from each other, as demonstraded by the Student t-test that returns a value of 0.0046.

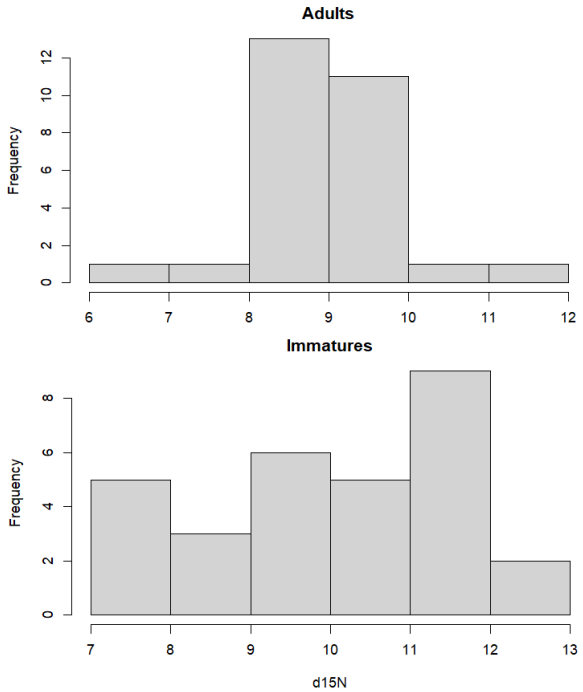


*Figure 32: $\delta^{15}N$ distribution distribution divided among adult and immature samples*

Even between teeth and bones, one distribution is less skewed than the other. The distribution of bone values, with a mean value of 9.3(1.2)‰, shows a p-value of 0.59 in the Shapiro Wilk test. The population of teeth shows a very similar mean value, 9.8(1.5)‰, but with a lower p-value of 0.085. Comparison of these two distributions, in contrast to the results between adults and

immature children, suggests that they do not differ significantly, as the obtained p-value in the t-test was 0.15.

The graphical plot comparing the isotopic levels of the same bone and tooth samples was also reproduced for the $^{15}N$ study. Unlike the results obtained for the plot for $^{13}C$, however, in this one the distribution does not seem to point systematically in one direction at all. The points are distributed in both halves of the quadrant bounded by the bisector. Thus it can be stated that only on average are bones poorer in the $\delta^{15}N$ isotope than teeth, but there is no systematic trend within the same samples to demonstrate this. The graphical plot can be observed in Figure 34.

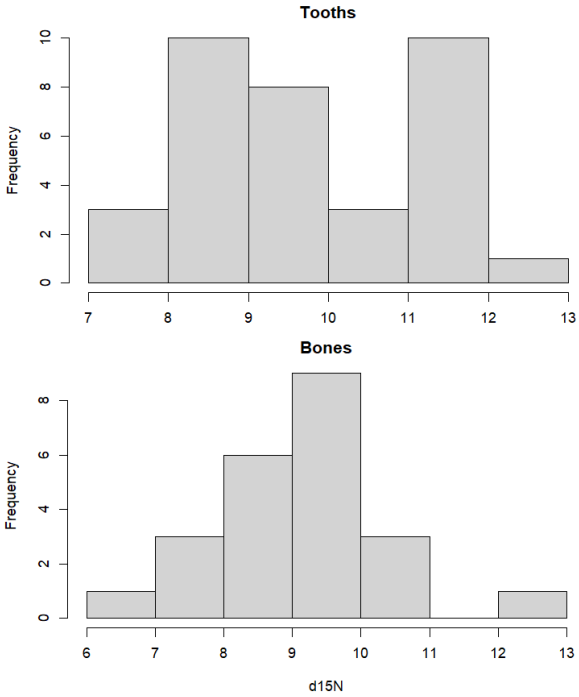


*Figure 33: $\delta^{15}N$ distribution divided among tooth and bone samples*

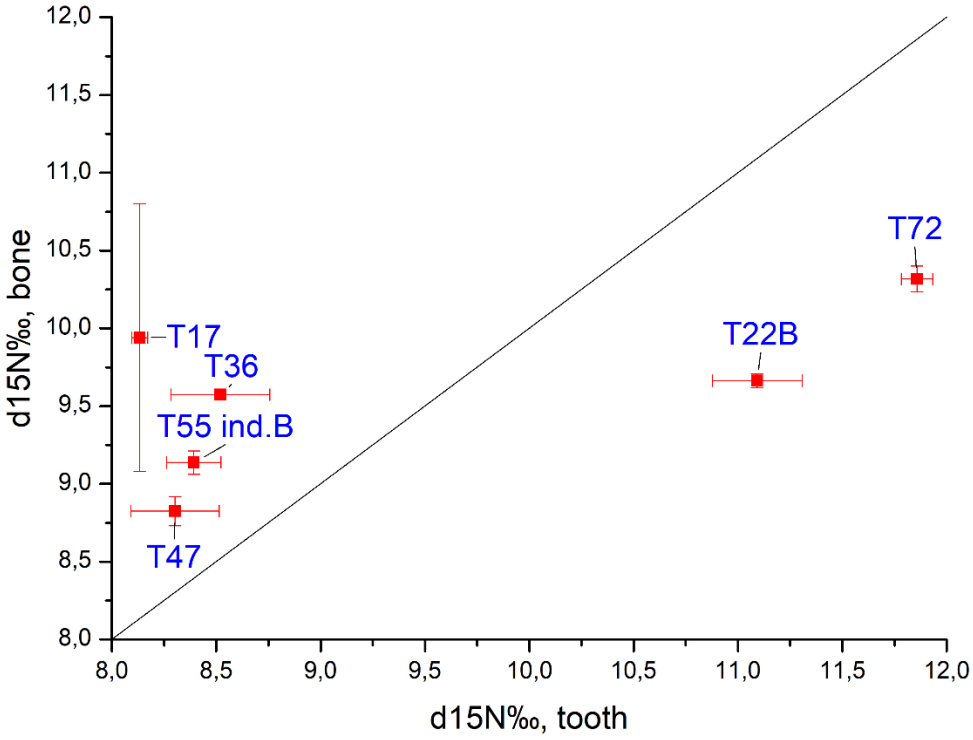


*Figure 34: $\delta^{15}N$ composition of bone and tooth samples from the same person*

The influence of the main groups is also very much felt in the cross-groups, as both adult and immature bone populations report high p-values in accordance with the macro group bones described above. The same can be said for teeth, both trends reflect little of normality by presenting obvious skewedness, on the left in the case of immatures and on the right in the case of adults. The different cross-population trends can be seen in Figure 35.

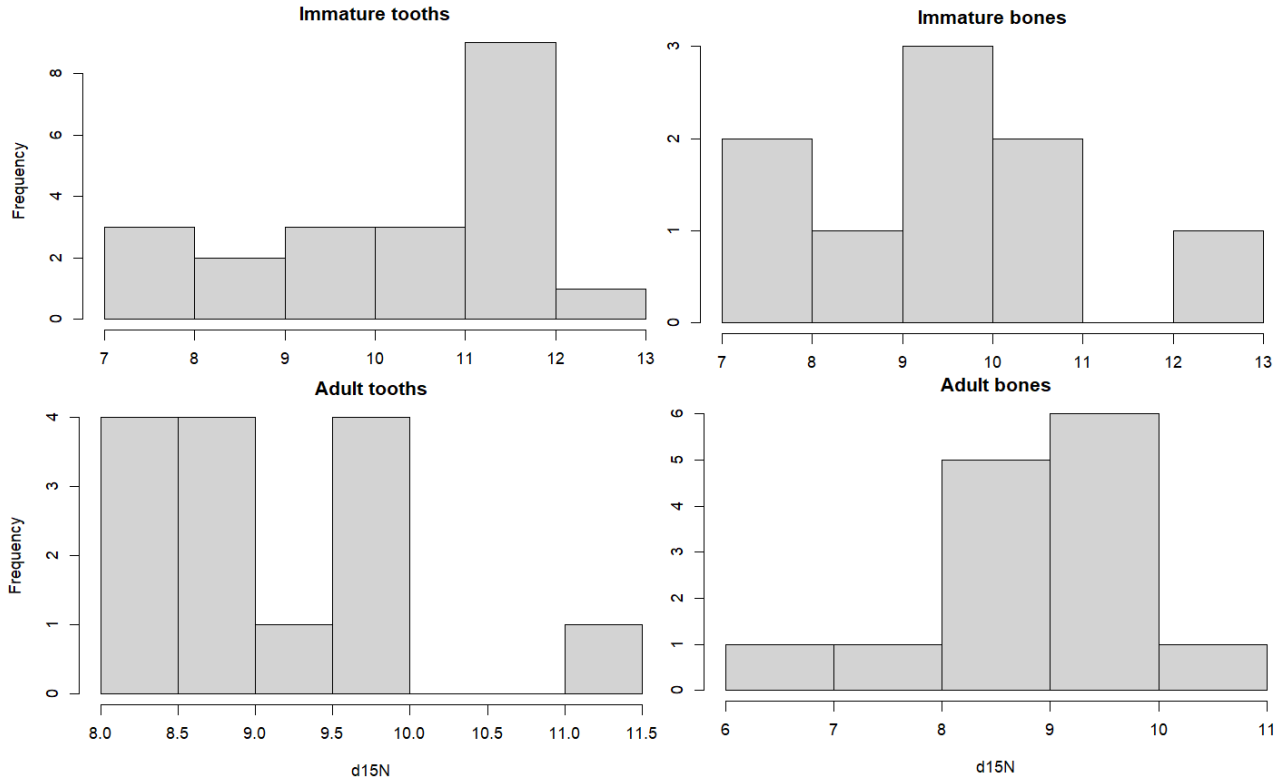


*Figure 35: $\delta^{15}N$ distribution divided both on age and nature of the samples*

The two tooth populations were significantly different from each other. The respective mean and standard deviation values were far apart, for adults 9.06(0.88)‰ while immature 10.28(1.65)‰. Finally on Wilcoxon's test their p-value showed a value of 0.026. In contrast, the two bone populations are more in agreement with each other, as their t-test confirmed a p-value of 0.35. Their mean values are also closer to each other, showing the immatures with 9.61(1.54)‰ and the adults with 9.04(1.01)‰. It should be emphasised, however, that the age differences found in the general populations are also perfectly mirrored in the cross-bred groups, with the adults on average always lower in the $^{15}N$ isotope than the immatures.

All the results discussed so far are summarised in Table 5

*Table 5: : minimum, maximum, p-vales of the Shapiro-Wilk and Wilcoxon-Mann Whitney tests of $\delta^{15}N$ values obtained from adults, immature, tooth and bone samples*

| $\delta^{15}N$ | **Adults (n=28)** | **Immatures (n=30)** | **Tooths (n=35)** | **Bones (n=23)** |
|---|---|---|---|---|
| **Min-max** | 6.5-11.1 | 7.2-12.4 | 7.2-12.4 | 6.5-12.3 |
| **Mean(sd)** | 9.1(0.2) | 10.1(1.6) | 9.8(1.5) | 9.3(1.2) |
| **Shapiro test** | 0.36 | 0.058 | 0.085 | 0.59 |
| **Student/Wilcoxon test*** | 0.0046 | | 0.15 | |
| | **Bones** | | **Tooths** | |
| | **Immature (n=9)** | **Adult (n=14)** | **Immature (n=21)** | **Adult (n=14)** |
| **Min-max** | 7.3-12.3 | 6.5-10.9 | 7.2-12.4 | 8.1-11.1 |
| **Mean(sd)** | 9.6(1.5) | 9.0(1.0) | 10.3(1.7) | 9.1(0.9) |
| **Shapiro test** | 0.94 | 0.14 | 0.026 | 0.088 |
| **Student/Wilcoxon test*** | 0.35 | | 0.026 | |

# 3.5 ANIMALS

The analysed samples of animals were only taken as examples and therefore no in-depth analysis was carried out on a large population of them. Studying their extraction yield%, C:N ratio and isotopic fractionation values as previously done with the human population, i.e. with a study of the distribution, mean, median, would not represent reliable statistical data, given the scarcity of samples present and their intrinsic diversity as they come from different animal species. For this reason, the parameters of interest for these animals have only been catalogued in Table 6.

*Table 6: collection of parameters of interest for the animals analyzed. the values of the isotopic fractionation instead of the standard deviation report the measure error*

| | **%yield** | **C:N ratio** | **$\delta^{13}C$** | **$\delta^{15}N$** |
|---|---|---|---|---|
| **Cattle** | 8.0 | 3.1 | -17.5(0.4) | 7.2(0.1) |
| **Deer bone** | 8.6 | 3.3 | -20.0(0.1) | 4.9(0.1) |
| **Deer tooth** | 1.6 | 3.1 | -21.4(0.3) | 4.0(0.3) |
| **Equine** | 3.3 | 3.2 | -18.7(0.1) | -5.5(0.1) |
| **Fish** | 16.2 | 3.0 | -12.0(0.1) | 7.5(0.1) |
| **Pig** | 0.7 | 3.0 | -21.2(0.4) | 2.0(0.2) |

Each of the animal samples falls within the analysis criteria dictated by the %yield and C:N ratio, synonymous with successful collagen extraction, and can all be included in subsequent considerations.

The values of the human population in isotopic fractionation were compared with those of the animal population in order to check whether and how far the fractionation values of the various animals were within them. Humans were divided into adults and immatures, and for both the comparison range consisted of the range from the respective mean value ±2σ. For animals, on the other hand, their single isotopic value and measure error given from the analysis instrumentation were used.

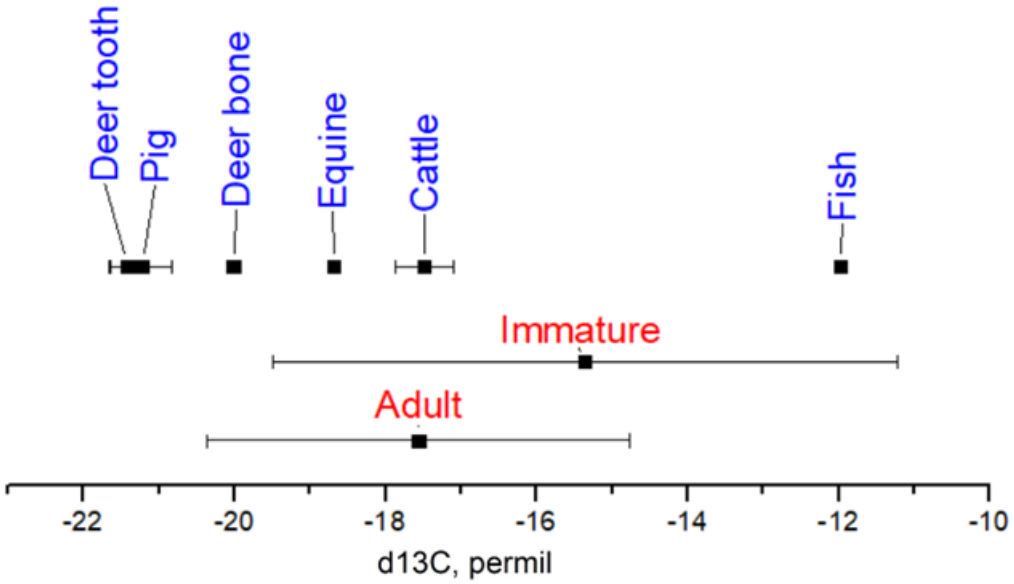


*Figure 36: comparison between $\delta^{13}C$ values of animals and human population*

For $\delta^{13}C$ not all animals fall within the human range, and there are also differences between what is understood by immatures and adults. The former are also the only ones who go as far as understanding the fish, which is considerably more detached from the rest of the animals. The deer tooth and the pig, on the other hand, are not understood by either human range. Different results come from the comparison on $\delta^{15}N$. The immature population manages to include the results obtained from cattle and fish. However, these are also the only two animals, for this isotope, to fall within the range of a human population. The rest of the animals have isotope values that are too low to overlap with human ranges. The adults even have such a narrow distribution that they do not include animals of any kind.

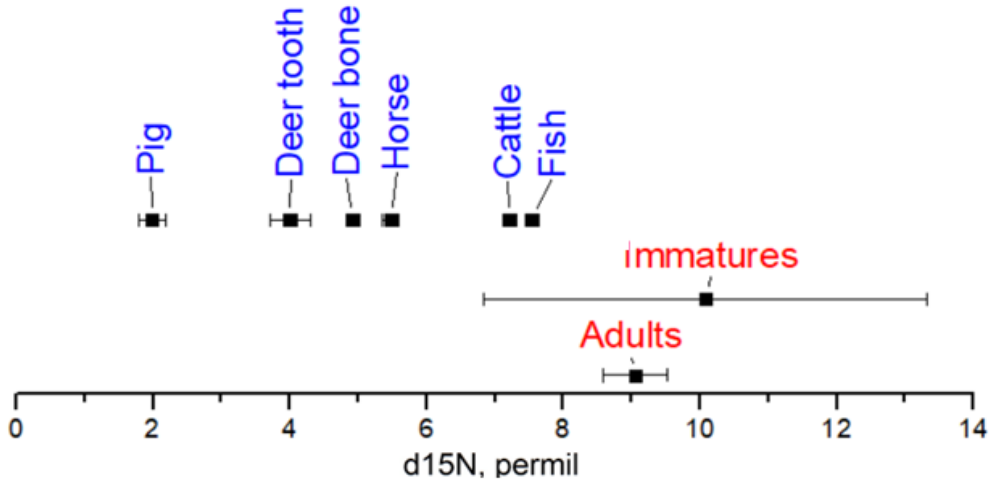


*Figure 37: comparison between $\delta^{15}N$ values of animals and human population*

# 3.6 DIET COMPOSITION MODEL

## 3.6.1 Development and Validation

You can see in the Figure 38 the plot enclosing all analysed samples, human and animal, according to their respective isotopic values.

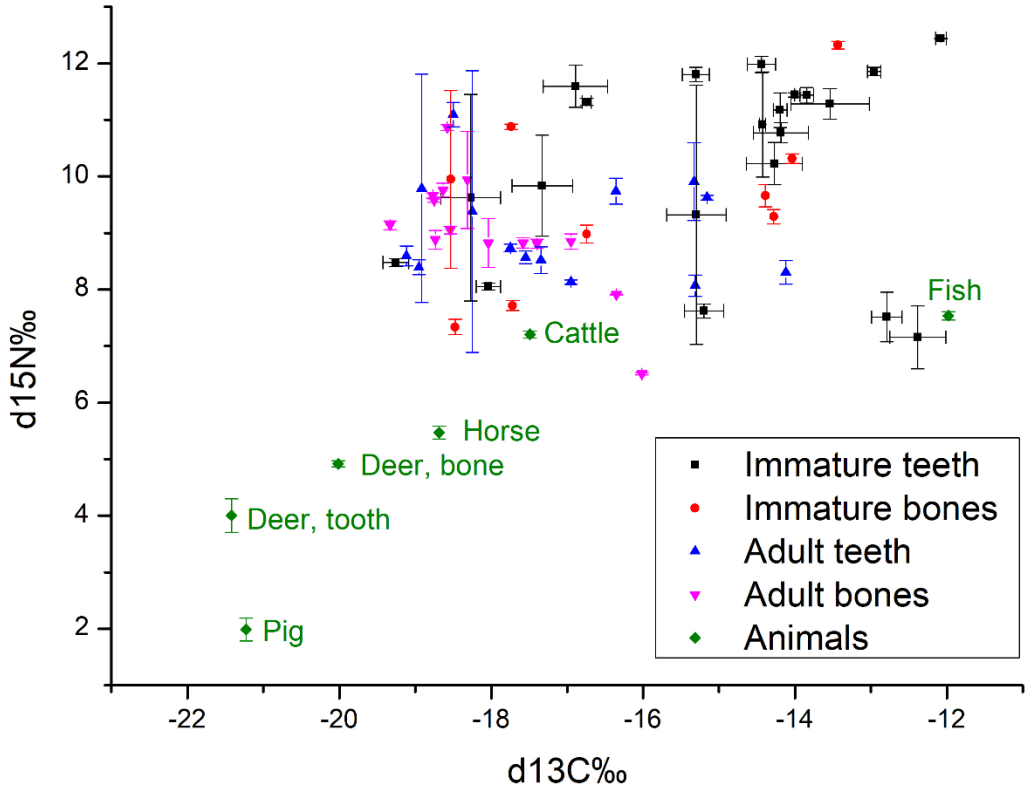


*Figure 38: depiction of the isotopic fractionation of all samples analyzed, with relative error bars*

The first thing one notices is how the animal group is clearly separated from the human group, tending to have fewer isotopes with the exception of the fish, which is on the far right of the plot and has higher $^{13}C$ values than any human individual. Immature individuals (black and red) in turn are more enriched in isotopes than adults, which are mainly present in the centre of the plot.

Areas of isotopic footprints from different food sources that match or are close to the values reported from human samples have been searched in the literature, along with what might have been the most likely food sources. The points in the plot appear to lie exactly in the middle of four precise food sources: C3 crops, C3-fed herbivores, marine or euryhaline fish, and other types of marine organisms such as crustaceans and molluscs, here generally referred to as seafood. All four of these food sources were very plausibly present within the diet of the Equilus inhabitants. In this same research, herbivores belonging to the consumers of C3 crops were analysed, and their presence may obviously imply the consumption of those same plants by humans as well. Finally, since Equilus is a maritime village, the presence of fish and seafood within the diet seems obvious. Within Figure 39 the footprints of the humans studied have again been shown, and the approximate footprint areas of the various food sources considered highlighted. None of those areas should be regarded as accurate, as the plot serves only as a general indication derived from data extracted from the literature. As mentioned above, the individuals are centred exactly in the middle of these four areas.

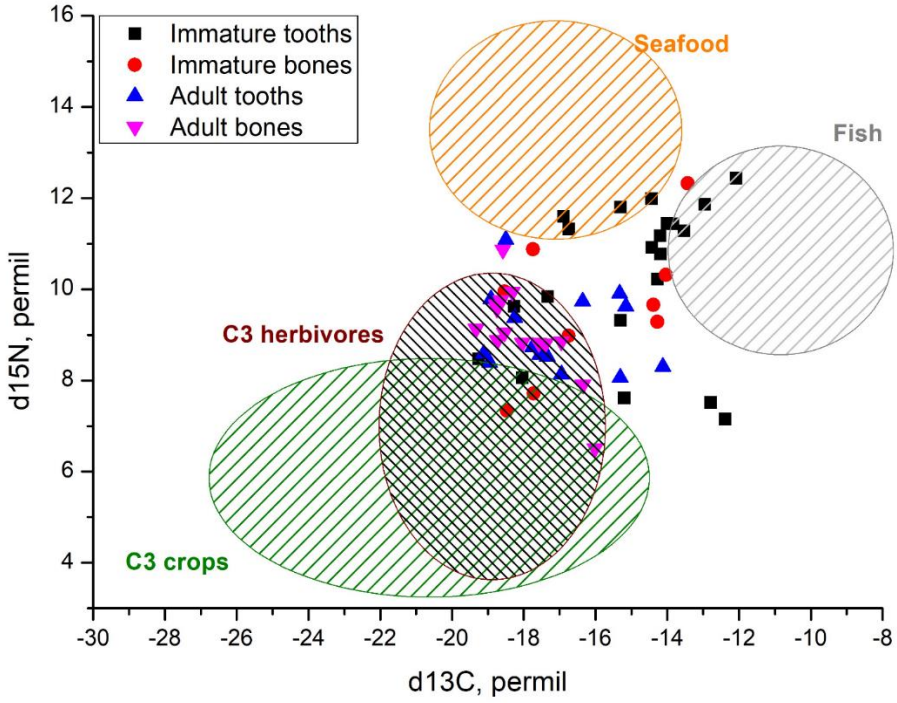


*Figure 39: representation of the isotopic value areas of plausible food sources as a possible diet for the analysed samples*

On the basis of this information, it is now possible to attempt to construct the model for reconstructing the dietary composition of individuals, using the four mentioned above as food sources. The literature search enabled the reconstruction of a database of isotopic footprints of each relative food (Vignola et al., 2017; Richards, 1999; Santis et al., 2021; Comendador, 2013; Katzenberg, 2008; Slovak, 2009; Bourbou, 2011; Tian et al., 2011; Fischer, 2007; Katzenberg et al., 2009; France, 2014, Pennycook, 2008). Using also the isotopic values obtained from the analysed animal samples, a total of 25 specimens make up the database used in this study and reported in Appendix III. This database is necessary to construct the compositional space on which the diet component prediction model is based. As a next step, the averages and standard deviations of each individual food source were derived, the results of which are shown in Table 7

*Table 7: collection of the average isotopic footprint values of the four food sources considered by the analysis*

| | **Crops** | **Herbivores** | **Fish** | **Seafood** |
|---|---|---|---|---|
| **Mean $\delta^{13}C$** | -19.7 | -18.8 | -10.4 | -16.5 |
| **Sd $\delta^{13}C$** | 0.7 | 0.4 | 0.9 | 1.5 |
| **Mean $\delta^{15}N$** | 7.4 | 8.2 | 11.7 | 14.7 |
| **Sd $\delta^{15}N$** | 0.6 | 1.0 | 1.7 | 0.6 |

It can be seen that the isotopic values of crops and herbivores are very similar to each other, due to trophic fractionation. This closeness, however, when compared to the distance to the isotopic values of the other two food sources, which are much further apart, creates problems within compositional space. Since every point $i$ in compositional space has isotopic composition:

$$\delta^{13}C_i = x_{1,i} \cdot \delta^{13}C_{crops} + x_{2,i} \cdot \delta^{13}C_{herbivores} + x_{3,i} \cdot \delta^{13}C_{fish} + x_{4,i} \cdot \delta^{13}C_{seafood} \quad (3)$$

$$\delta^{15}N_i = x_{1,i} \cdot \delta^{15}N_{crops} + x_{2,i} \cdot \delta^{15}N_{herbivores} + x_{3,i} \cdot \delta^{15}N_{fish} + x_{4,i} \cdot \delta^{15}N_{seafood}$$

the influence of two such similar factors unbalances the equation and causes the isotopic values of the points to be greatly influenced and shifted towards the region of space near the mean values of crops and herbivores. As a result, there is not a uniform distribution of points in compositional space, causing crowding in the region of the two similar sources while other areas are sparsely populated. This is evident by going to represent the created compositional space through a two-dimensional plot. A set of all possible combinations of $x_n$ proportionality factors was created through the model, such that in each combination the sum of the factors is

always equal to 1. In the Figure 40 shown below as an example, step 5 combinations were used, i.e. the proportionality factors can vary from one combination to the next in steps of 0.05.

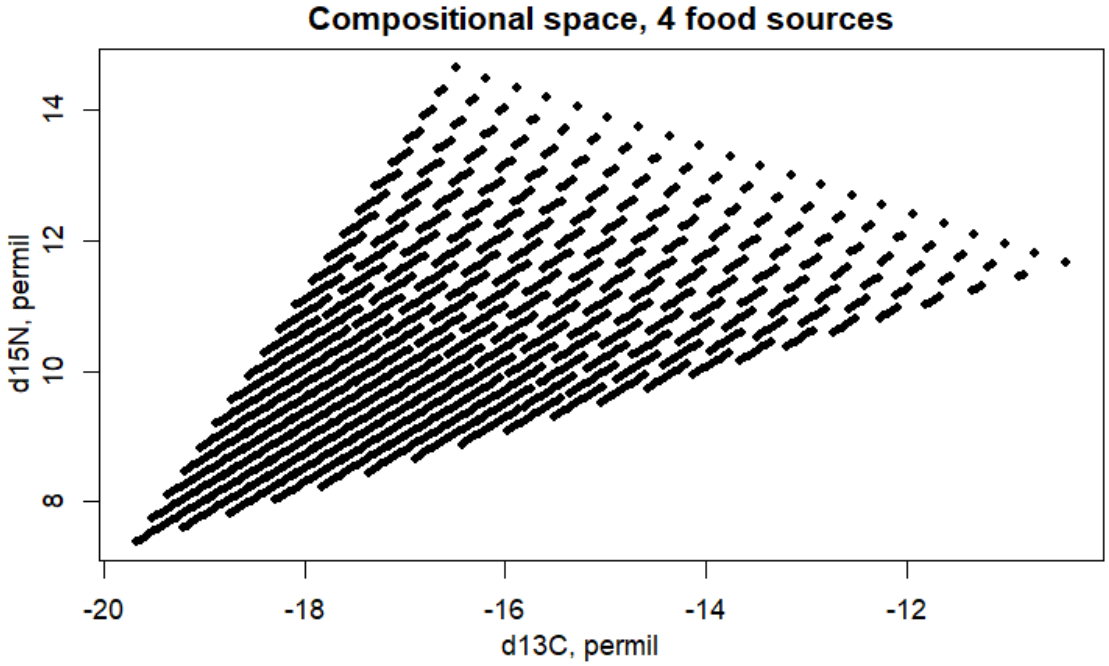


*Figure 40: compositional space in four food sources created by the model*

The picture above is the compositional working space of the model, each point in the plot representing the isotopic footprint of a specific combination. At the vertices of the triangle are the points with a 100% diet of a single food source. As pointed out earlier, there is a clear imbalance in the distribution of the points. Already the fact that the space is triangular in shape with three vertices when, having four different food sources, it should present four vertices, indicate the great similarity between crops and herbvores, since the vertex of the latter instead of extending into an area of its own lies hidden next to that of the crops. This inevitably leads to a problem in the model, since the position in which an unknown point lies within the space discriminates the even significantly different amount of first neighbours around it. Thus, the average isotopic values of the first neighbours located near the area of an unknown more influenced by crops and herbivores will always be more accurate than a point that is isotopically closer to fish or seafood. The proximity of the two terrestrial food sources to seafood also leads to great uncertainty in being able to correctly separate the influence of the two singles on each other.

In order to overcome these problems, it was decided to no longer consider the herbivores and crops food sources as two separate ones, but to combine them together into a single terrestrial food source, called *'terrestrial'*. In this way, the possibility of being able to distinguish how much

more influential the animal diet was than the plant diet was sacrificed, but the model was more reliable when a possible unknown sample was found to be more influenced by the marine food sources, which instead remained clearly separated from each other. At this point the table of averages and standard deviations of the food sources looks different (Table 8):

*Table 8: collection of the average isotopic footprint values of the now three food sources in consideration*

| | **Terrestrial** | **Fish** | **Seafood** |
|---|---|---|---|
| **Mean $\delta^{13}C$** | -19.2 | -10.4 | -16.5 |
| **Sd $\delta^{13}C$** | 0.7 | 0.9 | 1.5 |
| **Mean $\delta^{15}N$** | 7.8 | 11.7 | 14.7 |
| **Sd $\delta^{15}N$** | 0.9 | 1.7 | 0.6 |

The compositional space, when plotted as before in a two-dimensional graph, now forms a more homogeneous set of points.

For the construction of the workspace, decreasing the step value between the various combinations brings with it a greater number of points that make up the space, and with it greater precision in the model. However, decreasing it too much leads to an unsustainable lengthening of the software's working time during the model validation and distance computation phase. After several tests, the compromise level reached was to set the step to 2, i.e. the proportionality factors of the foods can vary by 0.02 between different combinations.

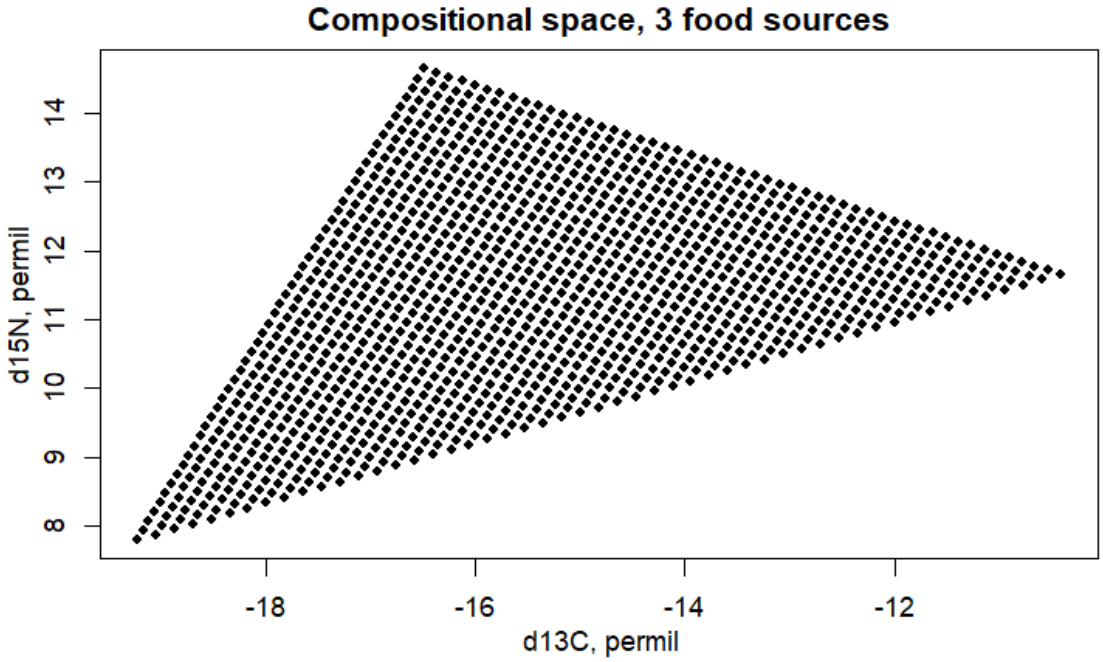


*Figure 41: compositional space in three food sources created by the model*

The validation phase was successfully performed by performing tests on 500 randomly selected specimens, as seen in Figure 42, and evaluated the value pairs, the number of neighbours to average between (dubbed *%th.v*) and how many times to consider the standard deviation (dubbed *sigma*), to achieve a test success rate of 80%. The number of first neighbours was not considered as an absolute value, but as a percentage of the total number of points making up the compositional space. The presence among the randomly extracted combinations of isotopic fractionations outside the compositional space itself allows the model to be tested with regard to the possibility that some human samples to be analysed later may also lie outside the compositional space.

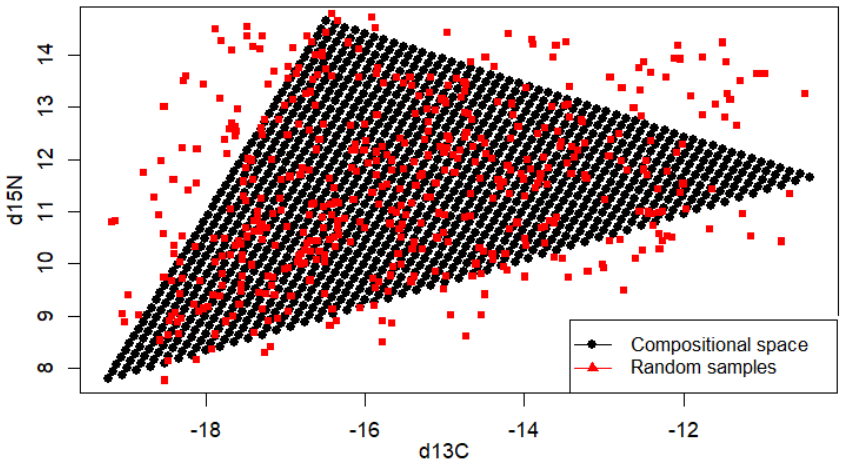


*Figure 42: positioning of the random control samples generated, compared to the compositional space of the model*

This can be explained in several ways. The first is that the compositional space uses reference values, to construct the mean isotope fractionation value for each food, which are also external to the Equilus environment itself. Despite being carefully selected from literature on environments as similar as possible to the typical lagoon environment of the upper Adriatic, it is inevitably impossible to use references that have exactly the same compositions of fauna and flora as the environment and time of study. In this way, even the constructed space will never reflect the true conditions in which any subject undergoing these analyses could have been found.Secondly, the food sources proposed here are the most likely to have been eaten based on the location of the various isotopic fractionations, but there is nothing to prevent the possibility that there may have been other foods that may have contributed in a minimal but undetectable way. As a third reason, it is necessary to remember that the degradation to which the samples have been subjected for centuries has certainly had a minimal influence on disrupting the original value derived from human collagen. Finally, the natural and high variability of isotopic footprints between individuals of the same species must also be taken into account.

Looking at the model's predictions for random samples outside of the computational space, it could be seen that there is no significant difference in the goodness of fit compared to samples lying inside the space. Thus, there will be no problems for human samples outside of space either.

The collected values can be found in the Apendix, while below is a two-dimensional plot of them, where the x-axis shows the %th.v value of the point and the y-axis the corresponding sigma value.

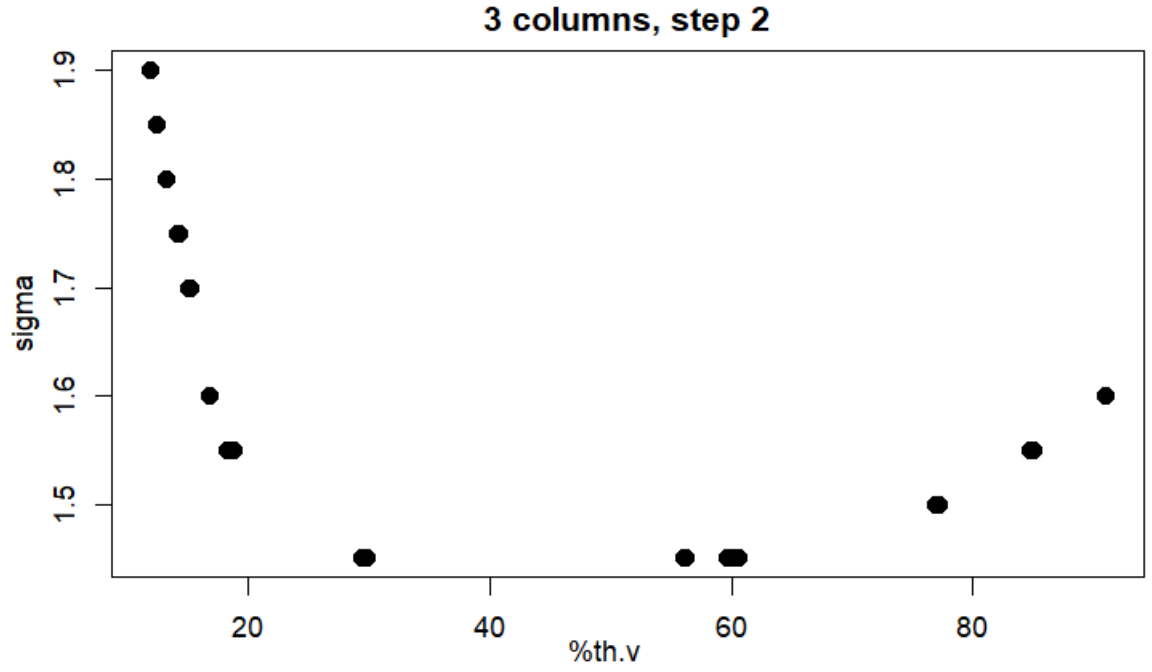


*Figure 43: %th.v vs sigma plot for the 80% correct predictions of the model*

The last step in the validation phase of the model is to find the right compromise so as not to simultaneously enlarge too much the number of first neighbours to be considered and the value of sigma. For this reason, those that make up the vertex of the paraboloid shape described by the points in the graph were chosen as suitable parameters for this study, with a *%th.v* value of 29.41 and a *sigma* value of 1.45. As mentioned above, applying these two values on the model it is possible to obtain an 80% success ratio on in establishing the correct diet composition of an individual.

## 3.6.2 Application

At the end of the validation the model was applied to the unknown samples from the excavations at Equilus, and the respective reports are summarised in Appendix IV. The results obtained demonstrate a singular correlation in which a large proportion of the population had a very similar diet, consisting of approximately 60% food of terrestrial origin. This result is evident from the histograms constructed on the frequency of the percentage of foods exhibited in Figure 45.

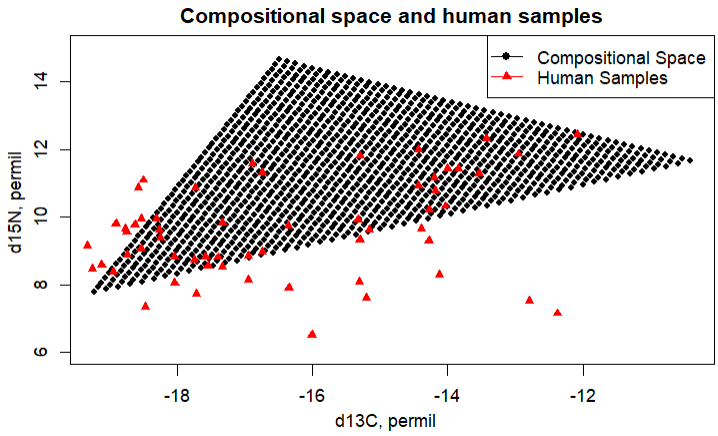


*Figure 44: positioning of the human samples analyzed in the compositional space of the model*

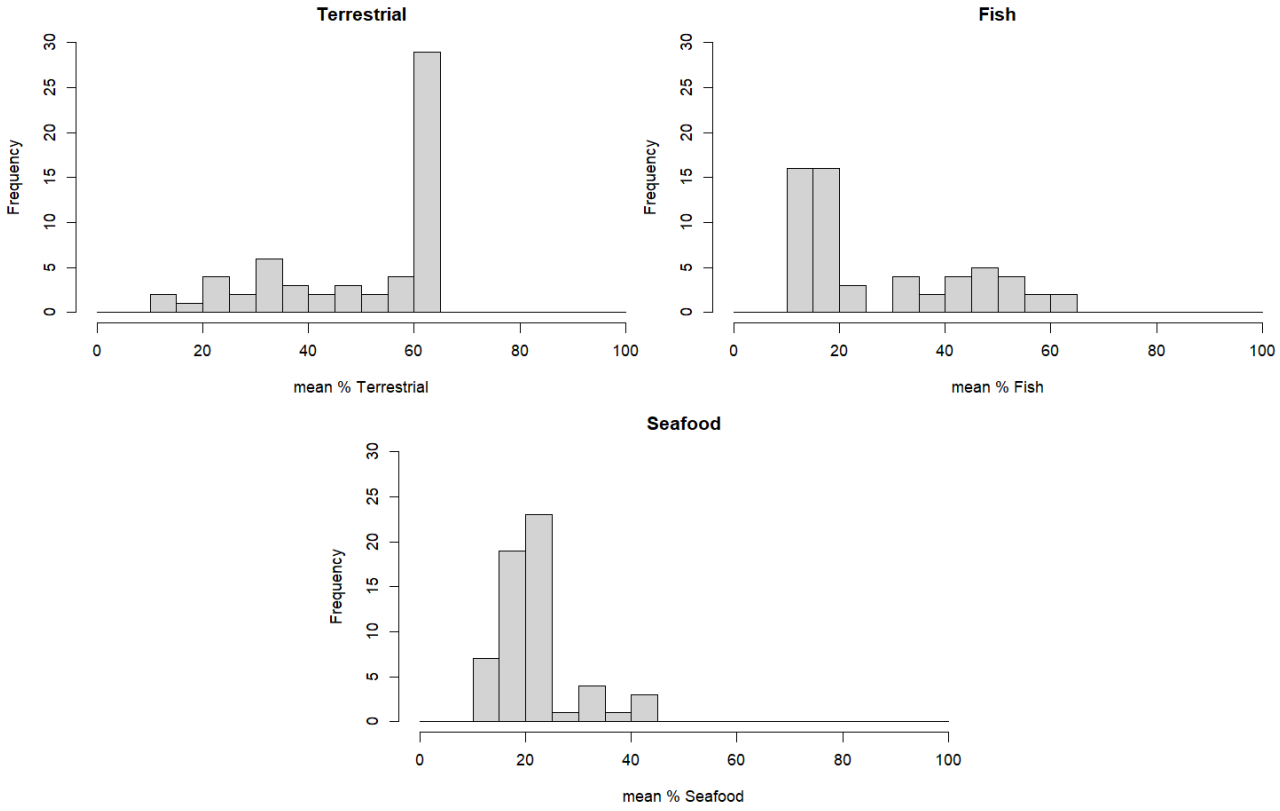


*Figure 45: distribution of the diet percentage for every food source considered on the sample population*

It is evident that there is no normal distribution at all among the three distributions. This seems to indicate the fact that a part of the population followed a distinct land-dominated diet compared to the rest of the inhabitants, who also spread out over very different values of diets.

A Shapiro-Wilk test was performed for each distribution, confirming a p-value much lower than necessary to demonstrate a normal trend, as shown in Table 9

*Table 9: mean, median, standard deviation and p-value for the distribution of the food source composition*

| | Mean Terrestrial % | Mean Fish % | Mean Seafood % |
|---|---|---|---|
| **Mean** | 50.1 | 28.0 | 21.9 |
| **Median** | 59.1 | 18.0 | 21.1 |
| **SD** | 16.9 | 16.3 | 7.0 |
| **p-value** | $3.05 \cdot 10^{-7}$ | $5.83 \cdot 10^{-7}$ | $1.44 \cdot 10^{-5}$ |

This distribution is also shown in a representation of the ratio of the percentage of terrestrial diet to the percentage of marine diet in each sample analysed. Along the abscissa, in order to improve the display of the plot, this ratio has been displayed on a logarithmic scale. The y-axis of the graph has no significance and was created solely to distribute the points in two dimensions instead of along a single line, a representation that would have been chaotic. Even more evident in this way is the accumulation of individuals with a very similar diet percentage among them, while the rest of the analysed individuals show a more scattered variation in diet composition.

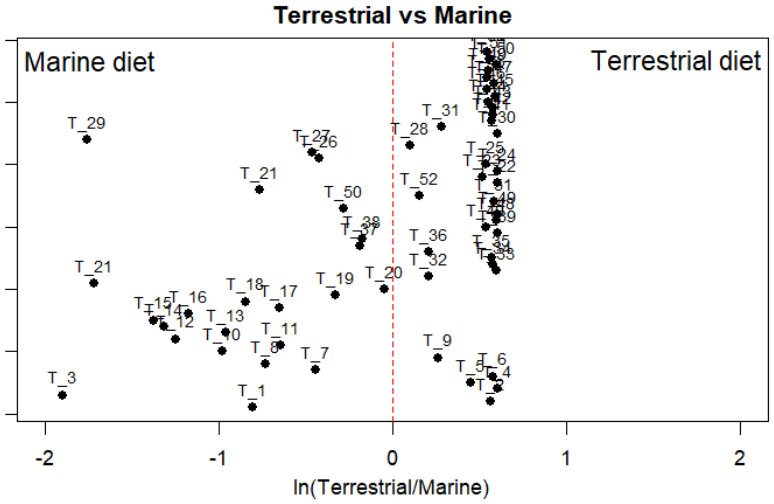


*Figure 46: distribution of the population according to the relevance of terrestrial or marine diet*

Following this finding, the presence of proportionality between the consumption of different food sources was investigated by analysing the values for each sample in pairs. Initially, a scatter plot was constructed in which the abscissa contained the percentages of feeding from one source and the y-axis the percentages of feeding from another source, using the pairs terrestrial-fish, terrestrial-seafood, fish-seafood. For all three source pairs, the distribution of points seemed to suggest, especially for the terrestrial-fish pair, a linear relationship between food consumption. Furthermore, in each graph it is interesting to note the presence of a precise set of samples, to be precise sample T-31, T-52, T-28, T-7, T-19, T-13, T-15, which deviate considerably from the distribution of the rest of the points. In order to confirm these

correlations, Pearson's test was performed for each food pair, setting the linear correlation between the two sources as a null hypothesis, as shown in Table 10. A linear regression was also attempted for each variable pair to construct a regression line within the scatter plot. Pearson's test and regression were repeated, also for the distribution of points from which the presence of the aforementioned discordant points was removed, to test how much the correlations, if any, might vary in the absence of these seemingly unrelated values in the main sequence.

*Table 10: collection of values resulting from the Pearson test for correlations between different food sources*

| | **Terrestrial vs Fish** | **Terrestrial vs Seafood** | **Food vs Seafood** |
|---|---|---|---|
| | **All Samples** | | |
| **r-Pearson** | -0.91 | -0.29 | -0.13 |
| **p-value** | $2.2 \cdot 10^{-16}$ | 0.027 | 0.33 |
| | **Removing the discordant points** | | |
| **r-Pearson** | -0.97 | -0.21 | -0.015 |
| **p-value** | $2.2 \cdot 10^{-16}$ | 0.134 | 0.92 |

For positive values of the correlation index r, the linearity is positive, while for negative indices the linearity is negative. The closer the value of r approaches 0, the less obvious the correlation between the two variables considered. The statistical test for the relationship coefficient will then return significance in the form of a p-value which, if less than 0.05, will indicate statistical evidence of a linear relationship between the two variables.

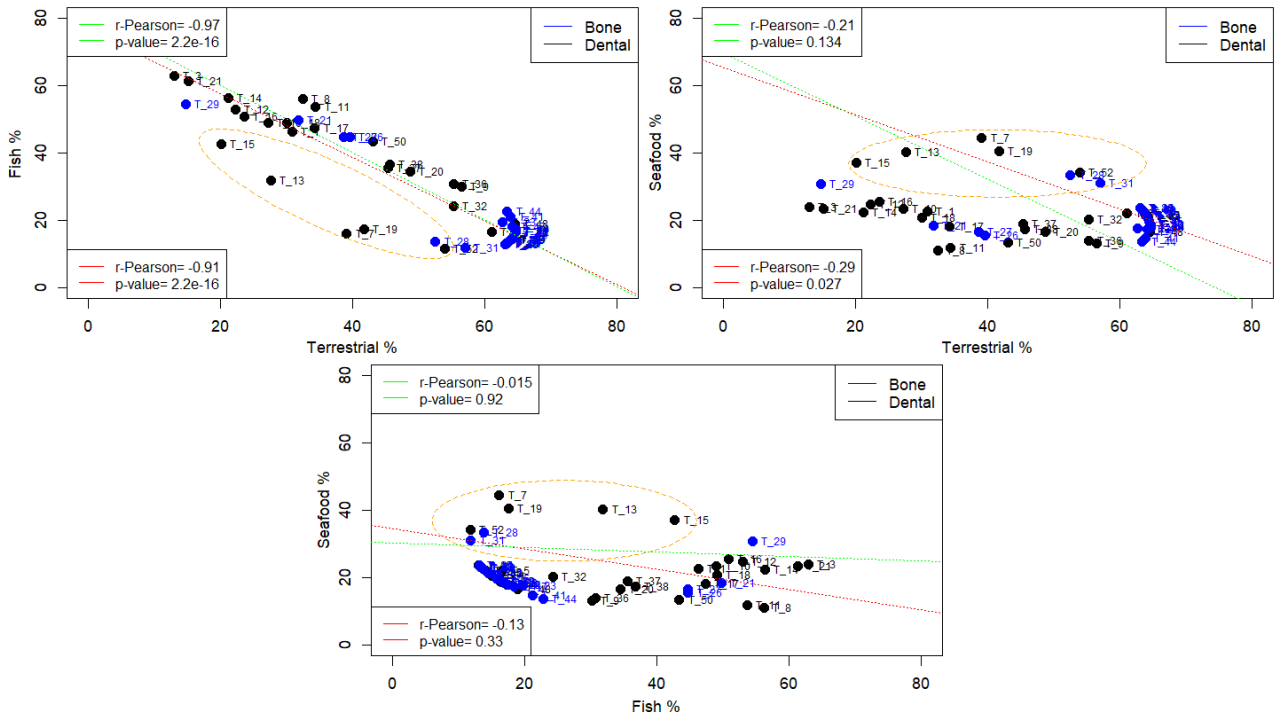


*Figure 47: linear regression for the correlation between different food sources, in red the regression with all the samples, in green the regression without the discordant points*

In the Figure 47 we also highlighted which samples correspond to a dental origin in black and which to a bony origin in blue. Of the three pairs of variables, the only one indicating a significant correlation is that between the percentage of terrestrial diet and fish diet, and this correlation is strictly negative. The more terrestrial foods were consumed in the diet, the less fish occupied the inhabitants' plates. In contrast, there appears to be no relationship between the other two pairs of food sources, suggesting that seafood consumption is more unrelated to the rest of the diet. The distribution of points in the three plots appears to be the same, rotated differently from plot to plot. Even in this representation the set of people with a high percentage of the terrestrial diet are distributed to form a well-defined cluster. Even in the plot comparing fish and seafood they are distinguishable from the rest of the subjects, presenting an equal composition of diet in these two food sources. It is worth emphasising that the absence of the set of points considered to be outermost, circled in orange, does not particularly affect the results of the correlation tests, although some regressions show clear differences (in red the regressions and tests considering all points, in green excluding the outermost points).

Following this step, it has become clear that it is necessary to perpetuate a hierarchical cluster analysis on the analysed population, in order to be able to identify which clusters of individuals may have correlations in diet. It is already expected that the cluster analysis will highlight the group of people with a 60% terrestrial diet, so the main objective is to check for possible

clusters. Clustering was performed by considering Euclidean distances between points and using the Ward method as criteria, and a dendrogram was then created to visualise the result of the cluster analysis. Figure 48 shows both the general dendrogram and three enlargements, each referring to a different cluster

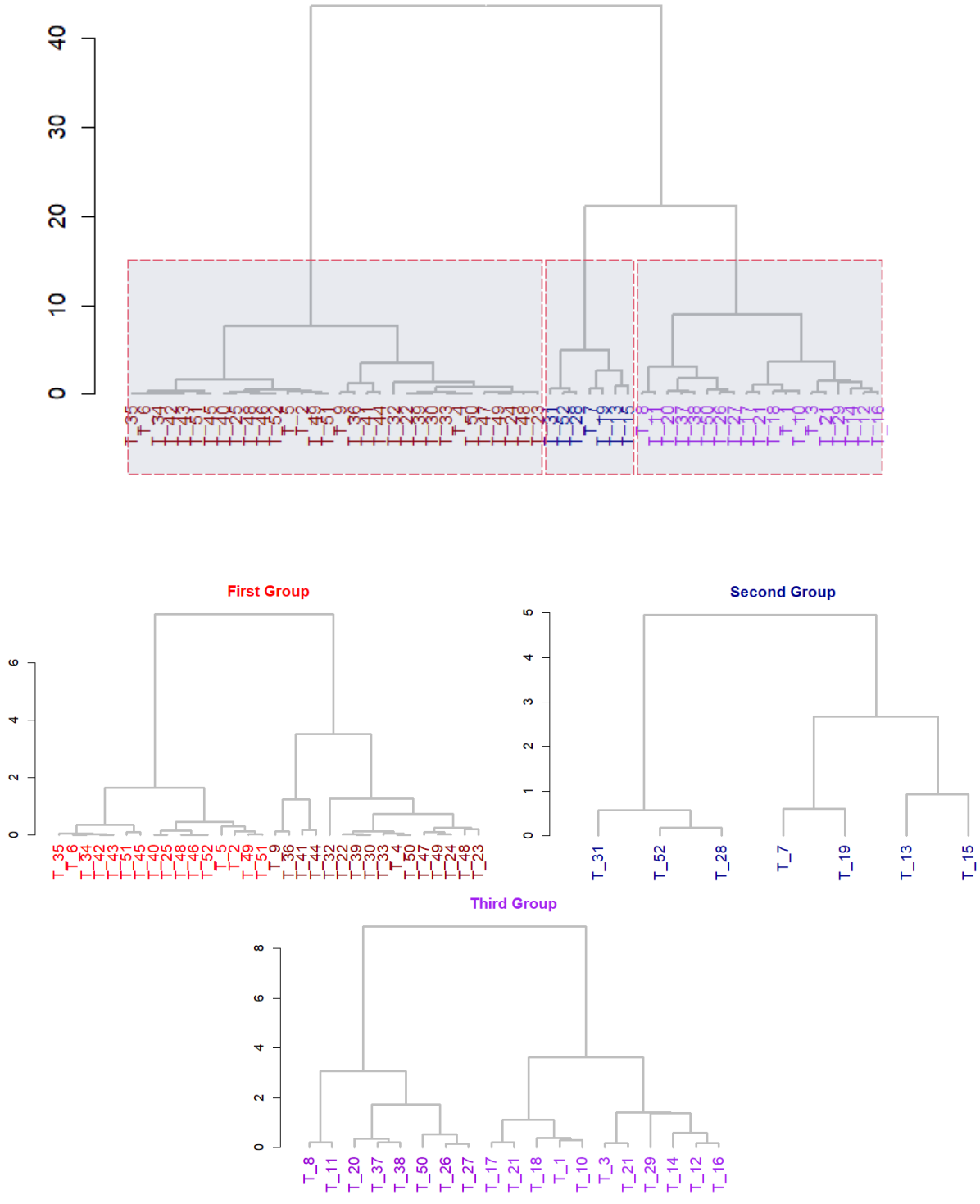


*Figure 48: dendrogram related to the cluster analysis performed on the samples, and the split dendrograms of the three different clusters*

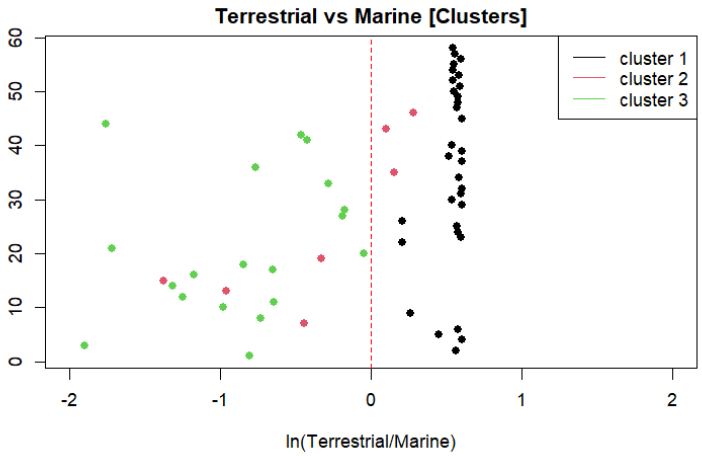


*Figure 49: highlited clusters on the distribution of the relevance of terrestrial or marine diet for each individual*

By setting the cut-off point as shown in the graph, the presence of three different clusters becomes evident. By taking the linear regression plots of the samples in Figure 46,47, the cluster to which each individual belongs can be highlighted as shown in Figure from 49 to 52. In this way, it can be seen that the distribution of the samples in these plots is separated into well-defined clusters. The first cluster in the dendrogram clearly corresponds to the cluster of individuals with 60% diet composition in terrestrial sources. The second cluster of the dendrogram are the points in the correlation plots between the food sources lying further out from the main sequence. The third cluster is the remaining points. Looking at how these clusters are positioned in relation to the preponderance of terrestrial or marine diet (Figure 49), the first cluster has a clear majority of terrestrial diet while the third is more towards a majority of marine diet. The second cluster, on the other hand, does not seem to be influenced by this factor at all, as it is divided almost equally between the two groups. This may indicate that what distinguishes this cluster from the rest of the population is not the preponderance of diet towards one food source over others.

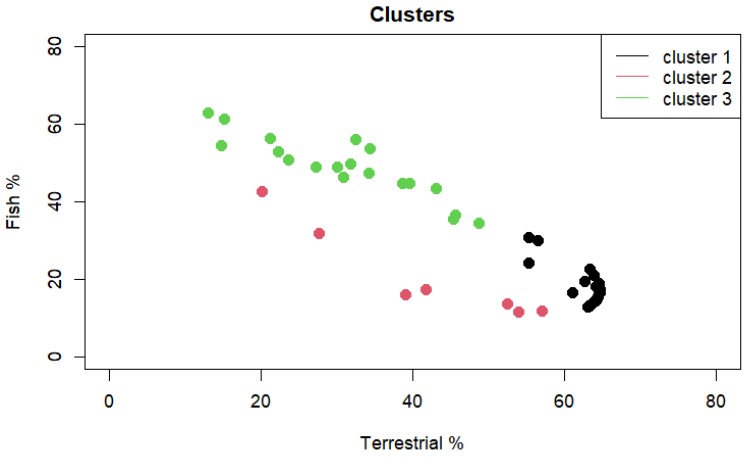


*Figure 50: highlited clusters on the Terrestrial % vs Fish % plot*

It is also worth visualising the cluster division in the correlation plot between the percentage of fish and seafood in the population. These two were previously shown to have no statistically relevant correlation index, however the distribution of the clusters closely resembles that observed in the previous correlation plot. In particular, clusters 1 and 3 having a similar percentage of seafood consumption are distinguished by consumption instead of fish, the first cluster by a lower consumption and the third by a higher consumption. Cluster 2 has a fairly distributed consumption of fish, but stands out for higher consumption of seafood than the others. This finding is in concordance with what was observed earlier, as seafood consumption does not appear to be closely related to terrestrial or fish consumption, and therefore an individual with a high seafood consumption may be in either the highest terrestrial or the highest seafood consumption cluster.

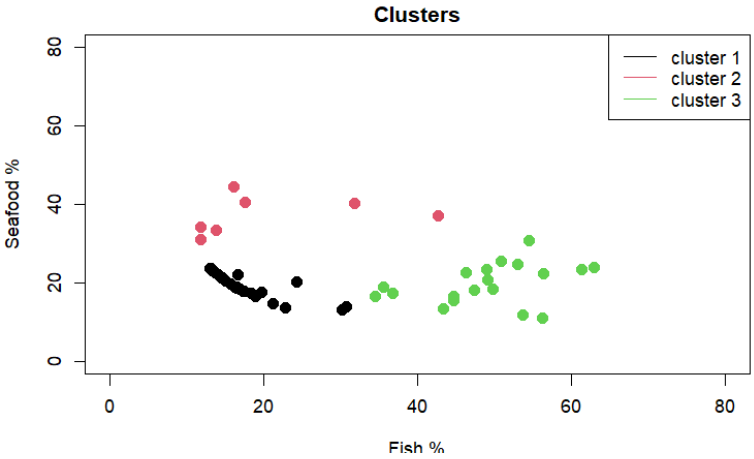


*Figure 51: highlited clusters on the Terrestrial % vs Seafood % plot*

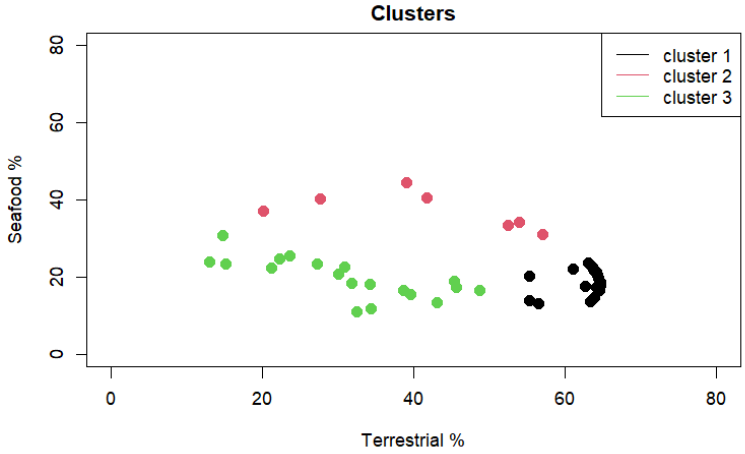


*Figure 52: highlited clusters on the Fish % vs Seafood% plot*

# 4. DISCUSSION

The quality indicators initially allowed very few specimens to be removed. Both the percentage yield and the C:N ratio returned values representing an acceptable state of preservation for almost all specimens that arrived at the end of the preparation. For the percentage yield, no particular differences were reported that linked more or less degradation to age or type of body tissue under analysis. However, different results are offered by the analysis of C:N ratios, where it appears that the immature population has a lower relative carbon composition than the adult population. The same difference can also be observed when comparing dental and bone samples, the latter being much more enriched in ratio than the former. When assessing the differences in C:N ratio between the adult and immature populations, it was found that although the values of the two averages including the standard deviations were superimposable, 3.0(0.2) and 2.9(0.2) respectively, through the t-test the two populations were significantly different, p-value=0.042. The explanation for this has to do with the fact that by making a large number of observations, as in this case, the range of each mean becomes increasingly narrower due to the very nature of the confidence interval, which is inversely proportional to radqn. This is why the t-test gives that result, reporting that they are significantly different at the 95% significance level. The situation represents a borderline case, because at a significance level of 99%, the two populations are no longer significantly different possessing a p-value=0.19.

The isotopic enrichment of $\delta^{13}C$ of dental collagen compared with bone collagen reflects the information set out during the introduction, about the preference towards lighter o heavier isotopes of the same element of a human tissue. Probably teeths, despite being similar in stucture to the bones, present a formation mechanism which includes heavier carbon isotopes. The non-correspondence of this relationship for $\delta^{15}N$ could suggest that the different nitrogen isotopes are not discriminated by the different formation mechanisms of these two tissues. On the other hand, it is impossible to totally exclude the impact that some contaminations of the samples could have had on these results. In general, the results obtained from the analysis of the distribution of nitrogen differ in some respects from that of carbon, showing significant similarities between branches of the population where they did not previously appear to be present. In both cases, however, the most important comparison, that between the isotopic content of adults and immatures in general, reports a significant difference between the two. This finding may already suggest a different diet composition based on the age of the subjects.

Anthropological studies have made it possible to link not only the age to each individual, but also information concerning the sex and state of health of the individuals at the time of death. In addition, precise knowledge of age has made it possible to identify which of these individuals are very young children, still breastfeeding or recently weaned, thus those with a very high nitrogen isotope fractionation signal.

Among the most recurrent diseases, anaemia and its hereditary form thalassaemia (also called Mediterranean anaemia or sickle-cell anaemia), recur very frequently among the population. Medical studies have confirmed that anaemia is a protection against malaria, as the structural changes in haemoglobin typical of sickle cell anaemia prevent the exploitation of cell resources by *Plasmodium falciparum*, the parasite that carries malaria (Lanzer & Ouagadougou, 2011). The Equilus area, being a marshy and lagoon area at the time, certainly presented the right environmental conditions for malaria to flourish. In this way, the inhabitants sick with thalassaemia would have formed a resilient core population to the disease. Through the generations, anaemic people had a better chance of surviving because of this, and consequently a better chance of contracting marriages and transmitting thalassaemia to subsequent generations.

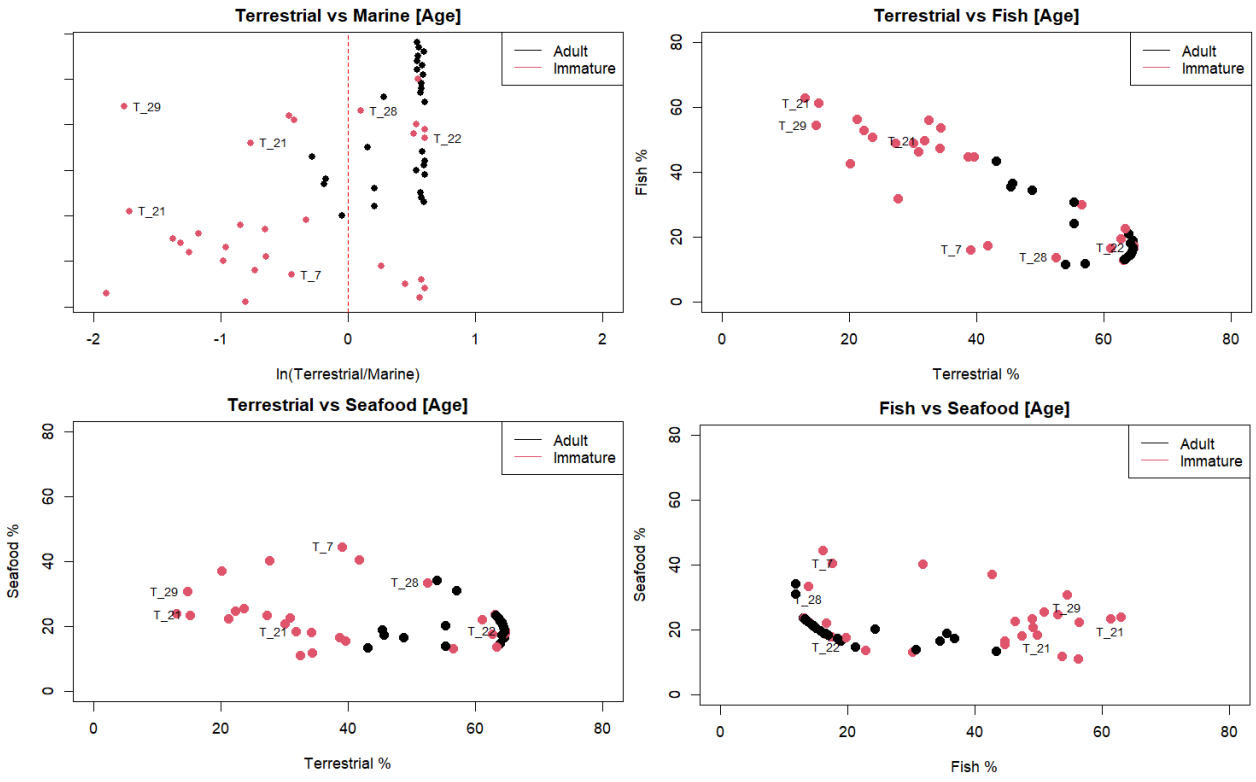


*Figure 53: diet composition and food sources correlations highlithing age of the individuals*

Through the representations in Figure 53, one can see how the age parameter appears to greatly influence the diet component of individuals. Immature individuals appear to have a diet imbalance more inclined towards the consumption of marine food, while the vast majority of adults make up the population cluster that feeds predominantly from terrestrial sources. In particular, the population cluster with a 60% terrestrial diet composition is composed almost entirely of adult members of the population. In the plots, individuals whose age at death was less than 2 years were highlighted and labelled. This is because such individuals have a higher level of $\delta^{15}N$ due to the imprint of breast milk still included in their diet or recently weaned, as exposed on the introduction. The higher levels of the nitrogen isotope may result in a false reading by the model used, of a diet more composed of marine than terrestrial foods. In fact, it can be verified that two of the very young dead subjects (T29, T21) represent some of the most relevant marine dietary points. Another of these children is also included in the group of the most marine-dominated diet, however two individuals are nonetheless within the group of the most terrestrial-dominated diet. Seafood consumption does not appear to be related in any way to the age of the individuals analysed, so what is discriminating is the higher average fish consumption by the younger individuals. Even allowing for the shift in nitrogen levels of around 2-3‰ in each case, the only individual that would be affected by a change in diet from a marine majority to a terrestrial majority is Individual 7, as they already have very similar carbon isotope levels to the rest of the individuals on a terrestrial diet. In all cases of very young dead subjects, however, their isotopic footprint is clearly outside the area of influence of possible C4 plant consumption, as they should have had high carbon isotope values but very low nitrogen, even considering lactation and the weaning period. We can therefore assume that, contrary to the introductions, a diet of millet or other C4 plants i is unlikely to have been used as weaning food for infants in this village.

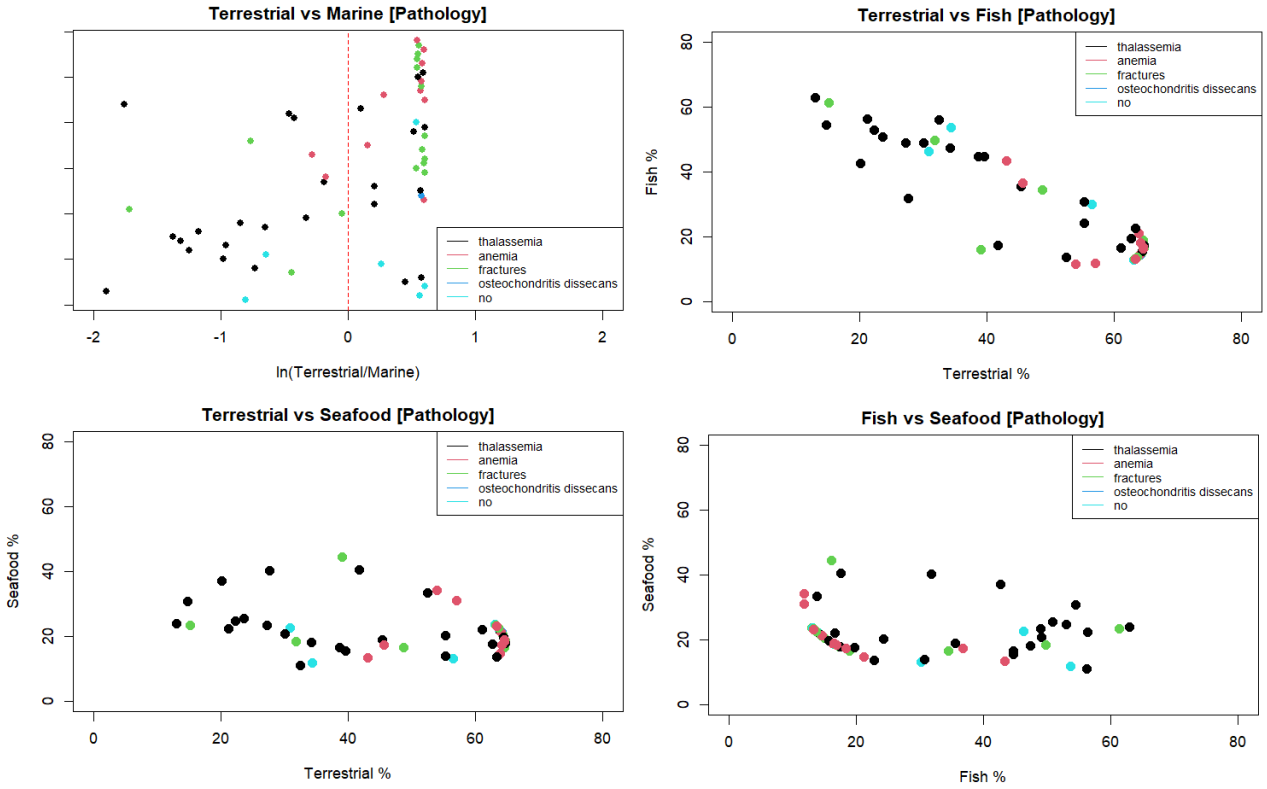


*Figure 54: diet composition and food sources correlations highlithing pathologies of the individuals*

The distribution of diseases, on the other hand, does not seem to reflect any of the trends derived for diet composition. Thalassaemia as expected is by far the most prevalent disease within the population, but it is distributed evenly between the different dietary groups. The tendency for fractures to be present among the terrestrial diet population seems to be more related to the fact that individuals who died young were less likely to cause fractures to themselves, and the link between age and diet composition has already been established. Turning instead to the fact that protein stress can develop an increase in $^{15}N$ isotopic values, as described earlier in the introduction, the presence of so many sick individuals within our population sample may have led to an average rise in $^{15}N$ levels. Indeed, the healthy dead individuals have a lower average nitrogen footprint than the rest of the population. This factor could shift the preponderance of the diet more towards terrestrial sources than maritime ones. Classic anemia also seems to have a tendency towards people on a terrestrial diet, with low consumption of both seafood and fish at the same time.

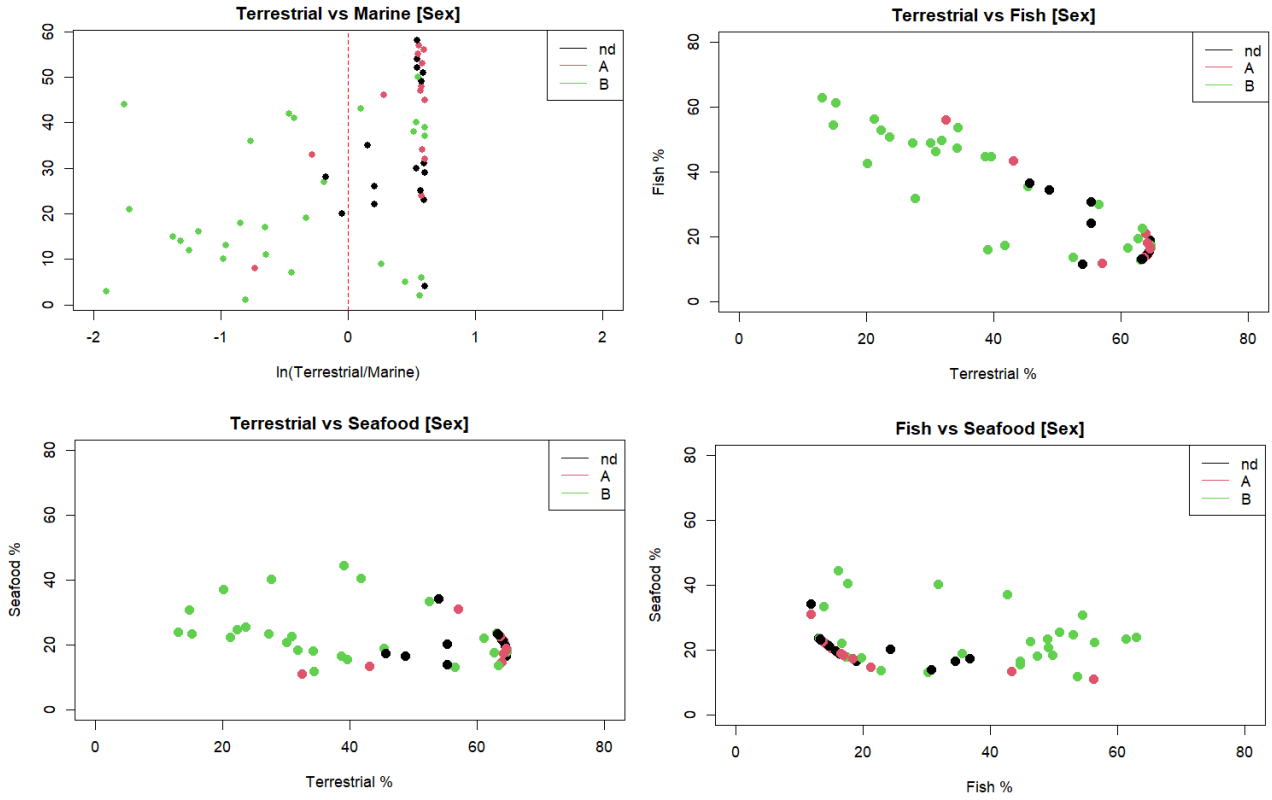


*Figure 55: diet composition and food sources correlations highlithing sex of the individuals*

Anthropological research did not allow the sex of all the individuals to be found with certainty. Furthermore, in order to avoid bias when reading the results of the analyses, it was preferred to categorise the two sexes as A and B, not knowing which one corresponds to male and which to female. With the data available, one can see a preponderance of sex B towards the seafood diet, while the preponderance of the terrestrial diet seems to be more oriented towards sex A individuals and those of unknown sex. Again, the consumption of seafood is not related to either of these two categories, the only discriminator of such within the marine diet being the amount of fish in the diet.

Finally, one can also construct these groups here detected by the model within the isotopic footprint plot initially studied.

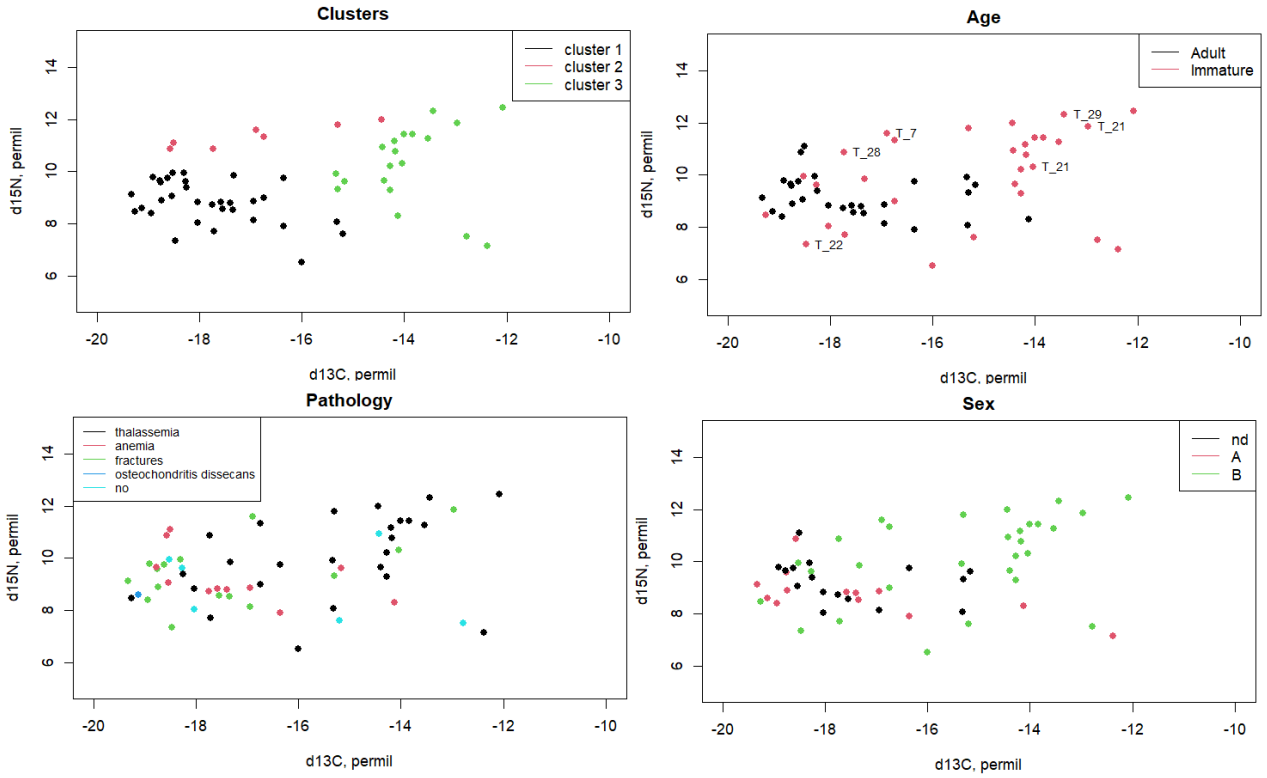


*Figure 56: isotopic fractionation plot, highlighting cluster, age, pathologies and sex of each individual*

The first thing one notices is how the clusters are very distinct even within this plot. Cluster 3 represented those individuals with a predominantly marine diet percentage and in fact is represented by those individuals with an isotopic footprint lower in carbon and tending to be higher in nitrogen, shifted precisely towards the marine food bands. While cluster 1, which is in fact the predominantly terrestrial cluster, comprises those individuals with a footprint tending towards the C3 plant and herbivore related zone. Cluster 2 at last resembles the footprint of people with high consumption of seafood. And as noted above, this arrangement mirrors well within the evidence of age and sex, while that of disease does not seem to correlate with them. It is readily observable in Figure 56 how the individuals of sex B coincide very much with the immature individuals. This begs the question of whether the preponderant terrestrial diet is derived from the age factor or the sex factor of the individual. It is certain that this overlapping of factors represents an unfortunate coincidence that makes the interpretation of the data obtained more uncertain. In both cases, it is unclear how either age or gender can discriminate the dietary components of individuals.

It is curious to point out a difference in consumption between seafood and fish that existed until a few years ago in the human maritime communities of the Upper Adriatic. Fishing requires the use of a boat to travel away from the coast and reach sea areas where fish are more likely to be

found. Seafood harvesting, on the other hand, can also be carried out directly on the beach with bare hands, targeting organisms such as crustaceans and bivalves, both of which are readily available without straying too far from the coast. Although these maritime settlements may also be very poor, the faculty of owning a fishing boat denotes a relatively affluent economic status by the standards of such villages. Conversely, until recently, seafood was considered to be the food of very poor people, as it was mainly consumed by those who did not have the means to deliver fish themselves and therefore had to make do with what the coast had to offer. Although it has been shown that they are not correlated with each other, the difference in seafood consumption between individuals in cluster 2 and 3 can be assumed to be an economic difference between those individuals, considering those in cluster 2 to be relatively poorer. A further diversification that may arise based on the economic and social status of individuals at the time is that in medieval maritime communities, monasteries generally fed their monks using mainly seafood instead of any land-based sources (Muldner & Richards, 2004). This finding, however, does not appear to be of any interest to this study, as the population group found to be more likely to be on a maritime diet here comprises immature individuals, hardly part of the monastic complex.

The difference in the percentage of land versus sea diet could also depend on the origin of the individuals in the burials. In those times it was not uncommon for a person who had spent a good part of their life inland to migrate to the coast. Either because the lagoon areas offered better protection for the population against invasion raids (remember that in the centuries covered by the burials first the Lombards descended into Italy and later also the Franks and Magyars), or because trade routes and exchanges by sea remained safer than overland roads, which had been neglected since the fall of the Western Roman Empire, and this meant that those areas were relatively richer than the immediate hinterland without large towns nearby. Consequently, a person could live in a maritime village who spent a good part of his life consuming land products, and this caused a marked isotopic imprint in his collagen. This may partly tie in with the higher frequency of maritime diet in immature compared to adults. Since it is more likely that a child or young person spent most of his or her life in the Equilus village at the time of death, and thus had the opportunity to consume more maritime food than land-based food. However, it remains unexplained why so few adults have a prevalence of the maritime diet, while almost all are more inclined towards the terrestrial diet.

An important piece of information that is not known is the temporal location of the various samples. The inhumed individuals do not all derive from a single archaeological context, but

were buried over a period of centuries, in which the ecological and environmental environment of the settlement may have varied even considerably. It cannot therefore be ruled out that these changes impacted the way of life of the inhabitants of Equilus, and consequently also their diet. The absence of this anthropological data unfortunately leaves this possibility open only to speculation.

# 5. CONCLUSIONS

This thesis work sought to test a new approach to determining the foods consumed by past subjects, with more specific results concerning the composition of the diet itself. Overall, the results allowed for the reconstruction of the dietary routines of people belonging to the settlement of Equilus during the time window of the early Middle Ages. Through a continuous search of the data available in the literature, it was possible to successfully collect and compare the isotopic fingerprints of the samples under analysis, both human and animal, and to determine their eating habits with good reliability.

In general, the analysis made it possible to divide the human population into three distinct groups:

1) a macrogroup of people consuming predominantly terrestrial food sources, all with a ratio of around 60%. These individuals also almost all fall within the same sex category, and most are reported to have died in adulthood

2) a group of predominantly immature individuals whose primary consumption consisted of seafood and who placed no particular preference in terrestrial foods over fish consumption

3) a macrogroup of individuals with consumption of predominantly seafood sources, who also had no obvious predilection for fish or seafood. These persons represent the opposite of the first macrogroup, as they predominantly belong to the other sex category and died at an immature age

A correspondence has also been found that inversely links terrestrial food consumption with fish consumption. As the presence of one source in the diet increases, the presence of the other decreases, and vice versa. This correlation was only verified for these two food sources, as seafood consumption does not appear to be particularly related to either of the former two food sources. Other anthropological information on the samples, however, does not seem to have found any particular links with the diet of the individuals under analysis. This work also reconfirmed the importance and reliability of bone collagen analysis when studying the diet of individuals.

In conclusion, therefore, although lacking specific anthropological information regarding the year of the individual's burial, which would have made it possible to perhaps establish

additional links regarding the consumption of food linked to the historical period experienced by the village, this study has made it possible to highlight the cognitive potential provided by a statistical prediction model applied to the isotopic footprint of individuals, the use and perhaps further refinement of which it is hoped can be exploited in subsequent studies in the field of archaeometric studies.

# 6. APPENDIX

## *Appendix I*

Table containing the anthropological data for each human subject.

For ease of reading, the table has been divided between the bone and dental samples.

| ID | species | Class | Age | Age of death (years) | Sex | Pathology | sample |
|---|---|---|---|---|---|---|---|
| T_21 | Human | Human | Immature | 1.5 | nd | | Bone |
| T_22 | Human | Human | Immature | 0-1 | nd | | Bone |
| T_23 | Human | Human | Immature | 7.5 | nd | severe anemia | Bone |
| T_24 | Human | Human | Immature | 3.5 | nd | severe anemia | Bone |
| T_25 | Human | Human | Immature | 9 | nd | thalassemia | Bone |
| T_26 | Human | Human | Immature | 6.5 | nd | severe anemia | Bone |
| T_27 | Human | Human | Immature | 3.5 | nd | rickets; severe anemia | Bone |
| T_28 | Human | Human | Immature | 1 | nd | anemia | Bone |
| T_29 | Human | Human | Immature | 1.5 | nd | severe anemia | Bone |
| T_30 | Human | Human | Adult | 41.5 | B | fractures | Bone |
| T_31 | Human | Human | Adult | 50+ | B | fractures | Bone |
| T_41 | Human | Human | Adult | 60 | B | fractures | Bone |
| T_42 | Human | Human | Adult | 40 | B | | Bone |
| T_43 | Human | Human | Adult | 60+ | A | fractures | Bone |
| T_44 | Human | Human | Immature | 10.5 | nd | slight anemia | Bone |
| T_45 | Human | Human | Adult | 19.5 | A | severe anemia | Bone |
| T_46 | Human | Human | Adult | 22.5 | A | | Bone |
| T_47 | Human | Human | Adult | 20 | B | fracture | Bone |
| T_48 | Human | Human | Adult | 20 | A | | Bone |
| T_49 | Human | Human | Adult | 37 | B | | Bone |
| T_50 | Human | Human | Adult | 38 | B | fracture | Bone |
| T_51 | Human | Human | Adult | 22.5 | B | | Bone |
| T_52 | Human | Human | Adult | 47 | A | fractures | Bone |

| ID | species | Class | Age | Age of death (years) | Sex | Pathology | sample |
|---|---|---|---|---|---|---|---|
| T_1 | Human | Human | Immature | 6.5 | nd | prob. thalassemia | Dental |
| T_2 | Human | Human | Immature | 5.5 | nd | prob. thalassemia | Dental |
| T_3 | Human | Human | Immature | 7.5 | nd | severe anemia | Dental |
| T_4 | Human | Human | Immature | 19.5 | A | prob. thalassemia | Dental |
| T_5 | Human | Human | Immature | 6.5 | nd | severe anemia | Dental |
| T_6 | Human | Human | Immature | 9.5 | nd | anemia | Dental |
| T_7 | Human | Human | Immature | 2.5 | nd | | Dental |
| T_8 | Human | Human | Immature | 14 | B | anemia | Dental |
| T_9 | Human | Human | Immature | 4.5 | nd | prob. thalassemia | Dental |
| T_10 | Human | Human | Immature | 4.5 | nd | severe anemia | Dental |
| T_11 | Human | Human | Immature | 11 | nd | thalassemia | Dental |
| T_12 | Human | Human | Immature | 4.5 | nd | severe anemia | Dental |
| T_13 | Human | Human | Immature | 3.5 | nd | severe anemia | Dental |
| T_14 | Human | Human | Immature | 4.5 | nd | severe anemia | Dental |
| T_15 | Human | Human | Immature | 5.5 | nd | severe anemia | Dental |
| T_16 | Human | Human | Immature | 3.5 | nd | severe anemia | Dental |
| T_17 | Human | Human | Immature | 4.5 | nd | severe anemia | Dental |
| T_18 | Human | Human | Immature | 3.5 | nd | severe anemia | Dental |
| T_19 | Human | Human | Immature | 3.5 | nd | severe anemia | Dental |
| T_20 | Human | Human | Adult | 50+ | A | | Dental |
| T_21 | Human | Human | Immature | 1.5 | nd | | Dental |
| T_32 | Human | Human | Adult | 19.5 | A | anemia | Dental |
| T_33 | Human | Human | Adult | 20 | A | fractures | Dental |
| T_34 | Human | Human | Adult | 51 | B | osteochondritis dissecans | Dental |
| T_35 | Human | Human | Adult | 21 | A | severe anemia | Dental |
| T_36 | Human | Human | Adult | 38 | A | anemia | Dental |
| T_37 | Human | Human | Adult | 20 | nd | anemia | Dental |
| T_38 | Human | Human | Adult | 22.5 | A | fractures | Dental |
| T_39 | Human | Human | Adult | 22.5 | A | | Dental |
| T_40 | Human | Human | Adult | 50+ | A | | Dental |
| T_48 | Human | Human | Adult | 20 | A | | Dental |
| T_49 | Human | Human | Adult | 37 | B | | Dental |
| T_50 | Human | Human | Adult | 38 | B | fracture | Dental |
| T_51 | Human | Human | Adult | 22.5 | B | | Dental |
| T_52 | Human | Human | Adult | 47 | A | fractures | Dental |

# *Appendix II*

Table containing the isotopic fractionation, C:N ratio and yield% obtaine from the analysis for each human subject.

For ease of reading, the table has been divided between the bone and dental samples.

| ID | $\delta^{13}C$ | sd ($\delta^{13}C$) | $\delta^{15}N$ | sd ($\delta^{15}N$) | C% | sd(C%) | N% | sd(N%) | C:N | yield% |
|---|---|---|---|---|---|---|---|---|---|---|
| T_21 | -14,04 | 0,07 | 10,32 | 0,08 | 39,85 | 0,53 | 15,92 | 0,20 | 2,92 | 1,64 |
| T_22 | -18,47 | 0,13 | 7,34 | 0,14 | 37,55 | 2,08 | 14,42 | 0,84 | 3,04 | 18,49 |
| T_23 | -16,74 | 0,07 | 8,98 | 0,16 | 38,05 | 0,50 | 13,88 | 0,11 | 3,20 | 18,58 |
| T_24 | -17,72 | 0,09 | 7,72 | 0,09 | 38,72 | 0,29 | 15,28 | 0,12 | 2,96 | 0,96 |
| T_25 | -18,53 | 0,22 | 9,95 | 1,57 | 33,92 | 1,40 | 12,49 | 0,60 | 3,17 | 14,10 |
| T_26 | -14,28 | 0,01 | 9,29 | 0,13 | 22,91 | 0,64 | 8,20 | 0,17 | 3,26 | 11,58 |
| T_27 | -14,39 | 0,13 | 9,66 | 0,20 | 15,91 | 4,18 | 7,08 | 1,18 | 2,62 | 3,61 |
| T_28 | -17,74 | 0,08 | 10,88 | 0,05 | 38,94 | 0,29 | 14,97 | 0,10 | 3,03 | 15,21 |
| T_29 | -13,43 | 0,03 | 12,32 | 0,07 | 31,03 | 0,31 | 11,27 | 0,10 | 3,21 | 3,83 |
| T_30 | -17,40 | 0,31 | 8,81 | 0,08 | 38,04 | 0,49 | 15,06 | 0,18 | 2,95 | 2,40 |
| T_31 | -18,58 | 0,07 | 10,86 | 0,06 | 37,54 | 0,71 | 14,02 | 0,31 | 3,12 | 10,71 |
| T_41 | -16,35 | 0,10 | 7,91 | 0,01 | 40,97 | 0,27 | 16,51 | 0,18 | 2,89 | 1,78 |
| T_42 | -18,74 | 0,02 | 8,88 | 0,17 | 35,67 | 0,78 | 14,07 | 0,32 | 2,96 | 13,74 |
| T_43 | -18,54 | 0,31 | 9,05 | 0,07 | 9,91 | 0,10 | 3,64 | 0,23 | 3,18 | 12,45 |
| T_44 | -16,01 | 0,10 | 6,51 | 0,02 | 38,84 | 0,54 | 15,94 | 0,20 | 2,84 | 2,23 |
| T_45 | -18,04 | 0,32 | 8,82 | 0,43 | 42,95 | 0,28 | 16,15 | 0,13 | 3,10 | 4,54 |
| T_46 | -18,63 | 0,12 | 9,76 | 0,12 | 34,40 | 0,97 | 13,84 | 0,29 | 2,90 | 3,83 |
| T_47 | -16,95 | 0,26 | 8,85 | 0,13 | 3,81 | 0,45 | 1,30 | 0,17 | 3,43 | 21,05 |
| T_48 | -18,31 | 0,39 | 9,94 | 0,86 | 40,68 | 0,83 | 14,92 | 0,35 | 3,18 | 2,30 |
| T_49 | -18,75 | 0,19 | 9,57 | 0,03 | 33,88 | 0,90 | 13,17 | 0,01 | 3,00 | 4,55 |
| T_50 | -17,58 | 0,04 | 8,82 | 0,09 | 33,39 | 1,91 | 11,91 | 0,64 | 3,27 | 0,56 |
| T_51 | -19,33 | 0,01 | 9,14 | 0,08 | 33,11 | 0,54 | 13,29 | 0,15 | 2,91 | 7,04 |
| T_52 | -18,77 | 0,05 | 9,66 | 0,04 | 34,62 | 0,90 | 13,35 | 0,42 | 3,03 | 4,68 |

| ID | $\delta^{13}C$ | sd ($\delta^{13}C$) | $\delta^{15}N$ | sd ($\delta^{15}N$) | C% | sd(C%) | N% | sd(N%) | C:N | yield% |
|---|---|---|---|---|---|---|---|---|---|---|
| T_1 | -14,43 | 0,04 | 10,92 | 0,93 | 40,67 | 0,56 | 17,60 | 0,21 | 2,70 | 3,64 |
| T_2 | -18,27 | 0,40 | 9,62 | 1,82 | 41,17 | 0,22 | 17,13 | 0,11 | 2,80 | 6,93 |
| T_3 | -12,08 | 0,07 | 12,44 | 0,00 | 40,06 | 0,18 | 15,91 | 0,03 | 2,94 | 3,28 |
| T_4 | -18,04 | 0,16 | 8,05 | 0,06 | 41,77 | 0,19 | 17,37 | 0,09 | 2,81 | 5,02 |
| T_5 | -17,33 | 0,40 | 9,84 | 0,89 | 42,65 | 0,54 | 18,57 | 0,20 | 2,68 | 3,89 |
| T_6 | -19,26 | 0,17 | 8,47 | 0,07 | 42,38 | 0,44 | 17,50 | 0,14 | 2,82 | 2,92 |
| T_7 | -16,89 | 0,42 | 11,59 | 0,37 | 40,80 | 0,40 | 17,36 | 0,17 | 2,74 | 1,26 |
| T_8 | -12,38 | 0,37 | 7,15 | 0,56 | 41,15 | 0,22 | 16,54 | 0,10 | 2,90 | 6,28 |
| T_9 | -15,20 | 0,26 | 7,62 | 0,13 | 41,41 | 0,26 | 15,57 | 0,08 | 3,10 | 0,43 |
| T_10 | -14,20 | 0,09 | 11,17 | 0,30 | 40,57 | 0,36 | 17,64 | 0,10 | 2,68 | 9,59 |
| T_11 | -12,79 | 0,20 | 7,52 | 0,44 | 41,56 | 0,34 | 17,28 | 0,10 | 2,81 | 1,53 |
| T_12 | -13,84 | 0,09 | 11,43 | 0,14 | 41,67 | 0,04 | 18,00 | 0,03 | 2,70 | 4,22 |
| T_13 | -15,30 | 0,18 | 11,80 | 0,13 | 40,85 | 0,07 | 15,17 | 0,04 | 3,14 | 3,85 |
| T_14 | -13,54 | 0,52 | 11,28 | 0,27 | 41,54 | 0,74 | 15,77 | 0,24 | 3,07 | 0,39 |
| T_15 | -14,44 | 0,19 | 11,98 | 0,15 | 40,75 | 0,26 | 17,31 | 0,17 | 2,75 | 3,44 |
| T_16 | -14,00 | 0,00 | 11,44 | 0,04 | 39,98 | 0,13 | 15,75 | 0,01 | 2,96 | 3,97 |
| T_17 | -14,27 | 0,37 | 10,22 | 0,37 | 41,67 | 0,38 | 17,95 | 0,14 | 2,71 | 1,86 |
| T_18 | -14,18 | 0,36 | 10,77 | 0,18 | 39,92 | 0,34 | 15,74 | 0,07 | 2,96 | 12,47 |
| T_19 | -16,74 | 0,06 | 11,32 | 0,06 | 39,73 | 0,70 | 14,95 | 0,06 | 3,10 | 4,60 |
| T_20 | -15,30 | 0,39 | 9,32 | 2,29 | 42,59 | 0,17 | 16,03 | 0,06 | 3,10 | 4,71 |
| T_21 | -12,96 | 0,09 | 11,86 | 0,07 | 38,28 | 0,44 | 16,14 | 0,16 | 2,77 | 16,76 |
| T_32 | -16,36 | 0,71 | 9,74 | 0,23 | 42,02 | 0,34 | 17,19 | 0,04 | 2,85 | 2,12 |
| T_33 | -17,75 | 0,09 | 8,73 | 0,07 | 42,12 | 0,38 | 17,28 | 0,15 | 2,84 | 6,21 |
| T_34 | -19,12 | 0,68 | 8,59 | 0,17 | 41,22 | 0,19 | 16,82 | 0,09 | 2,86 | 12,30 |
| T_35 | -18,25 | 0,16 | 9,38 | 2,49 | 40,83 | 0,37 | 15,21 | 0,08 | 3,13 | 2,37 |
| T_36 | -15,31 | 0,19 | 8,07 | 0,19 | 41,50 | 0,34 | 17,25 | 0,07 | 2,81 | 6,00 |
| T_37 | -15,33 | 0,06 | 9,91 | 0,69 | 42,78 | 0,16 | 17,25 | 0,30 | 2,89 | 3,57 |
| T_38 | -15,16 | 0,11 | 9,62 | 0,04 | 41,01 | 0,02 | 15,23 | 0,05 | 3,14 | 4,45 |
| T_39 | -17,55 | 0,27 | 8,57 | 0,11 | 41,89 | 0,19 | 15,70 | 0,03 | 3,11 | 12,96 |
| T_40 | -18,91 | 0,38 | 9,79 | 2,02 | 43,13 | 0,13 | 16,21 | 0,03 | 3,10 | 6,51 |
| T_48 | -16,95 | 0,17 | 8,13 | 0,04 | 42,18 | 0,44 | 16,16 | 0,13 | 3,05 | 2,67 |
| T_49 | -17,34 | 0,10 | 8,52 | 0,24 | 42,36 | 0,22 | 17,59 | 0,03 | 2,81 | 3,41 |
| T_50 | -14,12 | 0,32 | 8,30 | 0,21 | 42,24 | 0,88 | 17,49 | 0,35 | 2,82 | 8,46 |
| T_51 | -18,95 | 0,04 | 8,39 | 0,13 | 41,92 | 0,32 | 16,10 | 0,17 | 3,04 | 9,38 |
| T_52 | -18,50 | 2,17 | 11,09 | 0,22 | 43,76 | 4,82 | 15,59 | 0,97 | 3,27 | 2,12 |

# *Appendix III*

Table containing the isotope fractionation values of the animal species used, during the validation process and when using the diet prediction model, to derive the average value to associate with each food source considered in this study.

| Sample | Food Class | $\delta^{13}C$ | $\delta^{15}N$ | Reference |
|---|---|---|---|---|
| Wheat | Terrestrial | -20.5 | 7.7 | Burbou 2011 |
| Wheat | Terrestrial | -18.9 | 6.7 | Burbou 2011 |
| Wheat | Terrestrial | -19.1 | 8.2 | Burbou 2011 |
| Wheat | Terrestrial | -20.2 | 7.7 | Burbou 2011 |
| Barley | Terrestrial | -19.2 | 6.5 | Burbou 2011 |
| Barley | Terrestrial | -19.5 | 7.3 | Burbou 2011 |
| Barley | Terrestrial | -20.3 | 7.5 | Burbou 2011 |
| Cattle | Terrestrial | -18.7 | 9.0 | Burbou 2011 |
| Cattle | Terrestrial | -18.9 | 7.2 | Burbou 2011 |
| Deere | Terrestrial | -18.52 | 7.92 | Burbou 2011 |
| Pig | Terrestrial | -18.8 | 9.1 | Burbou 2011 |
| Pig | Terrestrial | -19.2 | 8.7 | Burbou 2011 |
| Pig | Terrestrial | -18.1 | 6.6 | Burbou 2011 |
| Pig | Terrestrial | -19.3 | 8.9 | Tian et al 2011 |
| Fish | Fish | -10.48 | 10.54 | Pennycook 2008 |
| Mugil_Vika | Fish | -8.65 | 13.84 | Garvie-Look 2001 |
| Mugil_Vika | Fish | -10.66 | 11.33 | Garvie-look 2001 |
| Mugil_Vika | Fish | -11.49 | 10.12 | Burbou 2011 |
| sea bream | Fish | -10.6 | 10.5 | Burbou 2011 |
| White sea bream | Fish | -10.6 | 13.6 | Burbou 2011 |
| sepia officinalis | Seafood | -14.76 | 13.88 | Balic et al 2014 |
| Nardoto | Seafood | -17.7 | 15.1 | Balic et al 2014 |
| Nardoto | Seafood | -18.1 | 15.2 | Balic et al 2014 |
| Moles | Seafood | -16.6 | 14.95 | Balic et al 2014 |
| Moles | Seafood | -15.3 | 14.1 | Balic et al 2014 |

# *Appendix IV*

Table containing the values of each individual's diet composition returned by the application of the model.

Again for ease of reading, the table has been divided between the dental and bone samples

| source | name | mean Terrestrial % | mean Fish % | mean Seafood % | mean Sum | sd Terrestrial | sd Fish | sd Seafood |
|---|---|---|---|---|---|---|---|---|
| dental | T_1 | 30,87 | 46,34 | 22,79 | 100,00 | 0,11 | 0,12 | 0,14 |
| dental | T_2 | 63,78 | 14,11 | 22,11 | 100,00 | 0,14 | 0,10 | 0,15 |
| dental | T_3 | 13,01 | 63,01 | 23,98 | 100,00 | 0,09 | 0,15 | 0,16 |
| dental | T_4 | 64,61 | 16,54 | 18,86 | 100,00 | 0,13 | 0,12 | 0,13 |
| dental | T_5 | 61,10 | 16,59 | 22,31 | 100,00 | 0,13 | 0,11 | 0,14 |
| dental | T_6 | 64,12 | 14,59 | 21,29 | 100,00 | 0,14 | 0,11 | 0,15 |
| dental | T_7 | 39,12 | 16,17 | 44,71 | 100,00 | 0,13 | 0,10 | 0,16 |
| dental | T_8 | 32,52 | 56,23 | 11,25 | 100,00 | 0,18 | 0,18 | 0,08 |
| dental | T_9 | 56,50 | 30,22 | 13,28 | 100,00 | 0,15 | 0,15 | 0,09 |
| dental | T_10 | 27,30 | 49,07 | 23,63 | 100,00 | 0,11 | 0,12 | 0,14 |
| dental | T_11 | 34,44 | 53,71 | 11,86 | 100,00 | 0,17 | 0,17 | 0,08 |
| dental | T_12 | 22,26 | 52,98 | 24,76 | 100,00 | 0,11 | 0,12 | 0,14 |
| dental | T_13 | 27,67 | 31,87 | 40,46 | 100,00 | 0,11 | 0,12 | 0,15 |
| dental | T_14 | 21,22 | 56,34 | 22,44 | 100,00 | 0,11 | 0,12 | 0,14 |
| dental | T_15 | 20,14 | 42,76 | 37,10 | 100,00 | 0,11 | 0,12 | 0,15 |
| dental | T_16 | 23,58 | 50,83 | 25,59 | 100,00 | 0,11 | 0,12 | 0,15 |
| dental | T_17 | 34,30 | 47,37 | 18,33 | 100,00 | 0,12 | 0,13 | 0,12 |
| dental | T_18 | 30,03 | 49,08 | 20,89 | 100,00 | 0,12 | 0,12 | 0,13 |
| dental | T_19 | 41,79 | 17,54 | 40,67 | 100,00 | 0,12 | 0,11 | 0,15 |
| dental | T_20 | 48,77 | 34,46 | 16,77 | 100,00 | 0,13 | 0,13 | 0,11 |
| dental | T_21 | 15,18 | 61,31 | 23,51 | 100,00 | 0,10 | 0,13 | 0,15 |
| dental | T_32 | 55,26 | 24,28 | 20,46 | 100,00 | 0,12 | 0,12 | 0,13 |
| dental | T_33 | 64,55 | 16,30 | 19,14 | 100,00 | 0,13 | 0,12 | 0,14 |
| dental | T_34 | 64,12 | 14,59 | 21,29 | 100,00 | 0,14 | 0,11 | 0,15 |
| dental | T_35 | 63,96 | 14,42 | 21,62 | 100,00 | 0,14 | 0,10 | 0,15 |
| dental | T_36 | 55,28 | 30,77 | 13,95 | 100,00 | 0,15 | 0,15 | 0,10 |
| dental | T_37 | 45,39 | 35,62 | 18,99 | 100,00 | 0,12 | 0,13 | 0,12 |
| dental | T_38 | 45,64 | 36,79 | 17,57 | 100,00 | 0,13 | 0,13 | 0,12 |
| dental | T_39 | 64,62 | 16,86 | 18,52 | 100,00 | 0,13 | 0,12 | 0,13 |
| dental | T_40 | 63,10 | 13,03 | 23,87 | 100,00 | 0,15 | 0,09 | 0,17 |
| dental | T_48 | 64,53 | 18,91 | 16,56 | 100,00 | 0,13 | 0,13 | 0,12 |
| dental | T_49 | 64,63 | 17,35 | 18,02 | 100,00 | 0,13 | 0,12 | 0,13 |
| dental | T_50 | 43,06 | 43,42 | 13,52 | 100,00 | 0,15 | 0,15 | 0,09 |
| dental | T_51 | 64,28 | 15,06 | 20,66 | 100,00 | 0,14 | 0,11 | 0,15 |
| dental | T_52 | 53,95 | 11,78 | 34,27 | 100,00 | 0,16 | 0,08 | 0,18 |

| source | name | mean Terrestrial % | mean Fish % | mean Seafood % | mean Sum | sd Terrestrial | sd Fish | sd Seafood |
|---|---|---|---|---|---|---|---|---|
| bone | T_21 | 31,78 | 49,76 | 18,46 | 100,00 | 0,12 | 0,13 | 0,12 |
| bone | T_22 | 64,66 | 16,67 | 18,68 | 100,00 | 0,13 | 0,12 | 0,13 |
| bone | T_23 | 62,65 | 19,69 | 17,66 | 100,00 | 0,13 | 0,13 | 0,12 |
| bone | T_24 | 64,70 | 17,45 | 17,86 | 100,00 | 0,13 | 0,13 | 0,13 |
| bone | T_25 | 63,10 | 13,10 | 23,80 | 100,00 | 0,15 | 0,09 | 0,16 |
| bone | T_26 | 39,64 | 44,77 | 15,59 | 100,00 | 0,13 | 0,14 | 0,10 |
| bone | T_27 | 38,62 | 44,74 | 16,65 | 100,00 | 0,13 | 0,13 | 0,11 |
| bone | T_28 | 52,55 | 13,89 | 33,56 | 100,00 | 0,14 | 0,09 | 0,17 |
| bone | T_29 | 14,71 | 54,48 | 30,81 | 100,00 | 0,09 | 0,14 | 0,16 |
| bone | T_30 | 64,61 | 16,93 | 18,46 | 100,00 | 0,13 | 0,12 | 0,13 |
| bone | T_31 | 57,04 | 11,88 | 31,08 | 100,00 | 0,16 | 0,08 | 0,18 |
| bone | T_41 | 63,88 | 21,24 | 14,88 | 100,00 | 0,14 | 0,14 | 0,10 |
| bone | T_42 | 64,08 | 14,55 | 21,37 | 100,00 | 0,14 | 0,11 | 0,15 |
| bone | T_43 | 64,07 | 14,58 | 21,35 | 100,00 | 0,14 | 0,11 | 0,15 |
| bone | T_44 | 63,42 | 22,84 | 13,74 | 100,00 | 0,15 | 0,15 | 0,10 |
| bone | T_45 | 64,43 | 15,67 | 19,90 | 100,00 | 0,14 | 0,11 | 0,14 |
| bone | T_46 | 63,33 | 13,33 | 23,34 | 100,00 | 0,15 | 0,10 | 0,16 |
| bone | T_47 | 64,16 | 18,37 | 17,47 | 100,00 | 0,13 | 0,13 | 0,12 |
| bone | T_48 | 63,26 | 13,33 | 23,41 | 100,00 | 0,15 | 0,09 | 0,16 |
| bone | T_49 | 63,50 | 13,54 | 22,96 | 100,00 | 0,15 | 0,10 | 0,16 |
| bone | T_50 | 64,58 | 16,49 | 18,92 | 100,00 | 0,13 | 0,12 | 0,13 |
| bone | T_51 | 63,64 | 13,70 | 22,67 | 100,00 | 0,15 | 0,10 | 0,16 |
| bone | T_52 | 63,33 | 13,33 | 23,34 | 100,00 | 0,15 | 0,10 | 0,16 |

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