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

Mestrado em Ciência dos Materiais Arqueológicos (ARCHMAT)

Dissertação

Late Antiquity in Troia: an isotopic study to investigate husbandry practices and early childhood diet

Ayoola Bamidele Oladele

Orientador(es) | Cristina Barrocas Dias
Anne-France Maurer
Maria Inês Correia de Barros Vaz Pinto

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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor do Instituto de Investigação e Formação Avançada:

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Abstract

Eight human and seventeen faunal osteological samples from the Roman site of Tróia were analysed for their bone-stable carbon, nitrogen, sulphur, and oxygen isotopic compositions through EA-IRMS (Elemental Analyzer- Isotopic Mass Spectrometry), to ascertain the children's dietary patterns and animal husbandry practices on the site during Late Antiquity. The results obtained from the humans indicate a diet based mainly on C_3 plants, with addition of proteins of aquatic and terrestrial origins. Results are consistent with age, reflective of childhood diets based on C_3 plants with gradual incorporation of fish products and animal proteins as the children aged. There are distinctions between adult and childhood dietary patterns as the adult consumed more dietary protein such as fish and/or terrestrial animals. Fauna also yielded results of dietary strategies based on C_3 plant materials. Variability in their carbon isotopic compositions indicates less intensive to no animal management. Signals of non-local terrestrial faunal are detected which confirms historical, archaeological and zooarchaeological suggestions of animal resources from cities further away. Diagenetic alteration of the bone samples was assessed using ATR-FTIR, EA, and XRD, while the presence of calcite within the bone mineral phase was detected. Being a pilot study, the sample size is limited, and the hypothesis made will be contextualised within the results of an ongoing project from the same site.

Keywords: isotopes, childhood diet, animal husbandry, diagenesis, Late Antiquity, archaeometry, Portugal.

Resumo

Foram analisadas oito amostras osteológicas humanas e dezassete amostras faunísticas do sítio romano de Tróia para as suas composições isotópicas de carbono, azoto, enxofre e oxigénio estáveis através de EA-IRMS (Elemental Analyzer- Isotopic Mass Spectrometry), para determinar os padrões alimentares das crianças e as práticas de criação de animais no sítio durante a Antiguidade Antiga. Os resultados obtidos dos seres humanos indicam uma dieta baseada principalmente em plantas C_3, com adição de peixe, proteínas de água doce e de origem terrestre. Os resultados são consistentes com a idade, reflexo de dietas infantis baseadas em plantas C_3, com incorporação gradual de productos de peixe e proteínas animais à medida que as crianças envelhecem. Há distinções entre os padrões dietéticos para adultos e para a infância. A fauna também produziu resultados de estratégias dietéticas baseadas em plantas C_3. A variabilidade nas suas composições isotópicas de carbono indica uma gestão animal menos intensiva. São detectados sinais de fauna terrestre não-local que confirmam ainda mais afirmações históricas, arqueológicas e zooarqueológicas. A alteração diagenética das amostras ósseas foi avaliada utilizando ATR-FTIR, EA, e XRD, enquanto a presença de calcita dentro da fase mineral óssea foi detectada. Sendo um estudo piloto, o tamanho da amostra é limitado, e a hipótese feita será contextualizada pelos resultados de um projecto em curso a partir do mesmo local.

Palavras-chave: isótopos, dieta infantil, criação de animais, diagênese, Antiguidade tardia, arqueometria, Portugal

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List of Abbreviations and Terms

AD: Anno Domini

AIR: Ambient Inhalable Reservoir

Am/P: Amide I to phosphate ratio (relative collagen content)

ATR-FTIR: Attenuated Total Reflexion- Fourier Transmission Infrared Spectroscopy

BCE: Before Common Era

CI: Crystallinity index

C:N: Carbon to nitrogen ratio (relative carbonate content)

C/P: Carbonate to phosphate ratio

CAM: Crassulacean Acid Metabolism

EA-IRMS: Elemental Analyser- Isotope Ratio Mass Spectrometry

GNIP: Global Network of Isotopes in Precipitation

IAEA: International Atomic Energy Agency

IR-SF: Infrared Splitting Factor

Rpm: Revolutions per minute

VPDB: Vienna Pee DE Belemnite

VCDT: Vienna Canyon Diablo Troilite

WISER: Water Isotope System for data analysis, visualisation, and Electronic Retrieval

1. Introduction

Several archaeological excavations in the Roman fish-salting production centre of Tróia have yielded numerous materials including (but not limited to) human and zoo-osteological remains, which cut across interesting periods of Antiquity in ancient Lusitania (Portugal). The skeletons excavated from Tróia represent different biological and socio-cultural groups buried in such a tradition common during the Roman period. Extensive excavation seasons over the past 15 years have enabled the discovery of some late antique children's burials and archaeofaunal remains which can provide new insights into the past lifeways during the Late Antiquity from bioarchaeological and geochemical perspectives.

The Late antiquity period, spanning the end of the 3rd to 6th centuries CE, was a time characterized by a dynamic economy in Iberia. Archaeological and historical evidence suggests an ancient economy based on agriculture, fishing, sea trades, and salt exploitation which enabled the production of salted fish and fish sauces (e.g., *garum*), a very important industry in Roman Lusitania. Roman presence in Iberia brought significant changes in different spheres of life of the Iberian people. There were also drastic changes in the economy of the Iberians throughout the period of Roman rule from the 1st century BCE till the 5th century CE. The Roman province of Lusitania became fully incorporated into the economy of the vast Roman Empire in the 1st century BCE. Between 50 CE and the late 2nd century CE, important agricultural production capabilities and marine resource exploitation became extensive and played a very important role in the trade of the Iberian areas.

Tróia, one of the industrial complexes established in Lusitania (part of modern Portugal) during the Roman era, was a large production centre with numerous facilities. Based on its production capacities, it required lots of manpower which probably resulted in the evolution of the site to an urban centre clustered with workshops, houses, baths, and cemeteries amongst others (still unknown). High infant mortality in the Roman Empire has been documented in several written records and perhaps Tróia was not an exception. Its urbanism and location between the Atlantic coast and the Sado estuary are an indication of its probable proneness to the proliferation of infectious diseases which pregnant women and children would be easy targets. Furthermore, the unique location of the site is suggestive of a notable external dependency on construction materials for building purposes, ceramic vessels for shipping food products, and likely on food supplies as the sand would not be ideal for agricultural activities. Also recorded in the archaeology of this site are periods of abandonment with the exact cause yet to be fully ascertained. This episodic abandonment brought significant changes in the subsistence strategies of the people evidenced in the archaeological records up to the 5th century AD, thereby leading to three phases of occupational activities. As observed elsewhere in the Iberia, the presence of the Romans in Lusitania and this site saw the transition of funerary rituals from incineration to inhumation.

The unusual human foetal and children, as well as faunal remains recovered from this site, provide excellent grounds for further archaeometric investigation to understand cultural practices of food habits, subsistence, intra-population variation in diet that may be related to age, status, and sex (when compared with data from adult individuals), and even possible maternal and foetal/perinatal stress in the population during late antiquity. Stable isotopic analysis of carbon, nitrogen and sulphur in the bones can provide information on the dietary habits of humans (in this case, children) and animals within the context of the area under study. Palaeomobility proxies such as sulphur and oxygen are also explored to detect human and animal movement into this settlement.

Therefore, this study aims to investigate the dynamics of human-animal (environment) interactions by using the reconstructed dietary habits of animals to establish the pattern of husbandry practices and elucidate the diet and weaning pattern of children in this population during Late Antiquity. Data obtained from Tróia are also compared with other contemporaneous Roman sites of the Iberian Peninsula for further interpretation and contextualisation. For diagenetic assessment, ATR-FTIR, XRD and EA are also employed to pre-screen the osteological samples using different indices to evaluate collagen content and bone preservation.

The results of this study will be integrated into a project financed by Direçao Geral do Património Cultural DGPC, dedicated to the study of past diet, mobility, and husbandry practices in the fish-processing factory of Tróia and southwestern Lusitania, coordinated by Inês Vaz Pinto, the archaeologist from TROIA RESORT responsible for the site of Tróia, and Cristina Dias from the HERCULES Laboratory, University of Évora.

2. Historical Context

2.1. Iberian Peninsula

The name "*Iberia*" was coined by the Greeks, who referred to the ancient inhabitants as Iberians, probably because of Iber (Ebro) River, the second largest river after the Tagus, while the Romans referred to the peninsula as Hispania. Gradually, the name Iberia was replaced by Hispania, although both were synonymous and were used interchangeably. Over time, the term Hispania was corrupted to España and only applied to Spain, a country formed as a result of the fusion of the central and eastern medieval Kingdoms. While Portugal, a derivative of Portus Cale (the Roman name for Oporto) was formed in the 12th century, although lost independence and was finally liberated from the geographical unit of Spain in the 17th century (Loidi, 2017).

To understand the historical cum socio-cultural evolution of Iberia, it is perhaps important to briefly lay out the physical and geographical framework of the peninsula. Majorly occupied by Spain and Portugal, the Iberian Peninsula is located at the southwestern end of the continent of Europe.

Figure 1: Iberian Peninsula and its structural features

The peninsula covers a total extent of 583,834km^2, encompassing Spain, Portugal, the Principality of Andorra, the British colony of Gibraltar, some areas of France in the Pyrenees, in addition to the 4992km^2 of the Baleric Islands (Britannica, n.d.; Loidi, 2017). Its massive pentagonal shape with large centre areas of medium to high elevation, huge central plateau (Meseta), an average elevation of 600m above sea level, as well as its peculiar nature of an almost-island located between Europe and Africa, and between the Atlantic and the Mediterranean determines the complex dynamics of interaction with the rest of Europe, its independence, and isolation from the rest of the continent over time (Loidi, 2017). Due to its particular and varied geography, the peninsula is divided into its well-defined regions which play a crucial role in the processes of regionalization characterising the Roman Periods and beyond. Central Meseta is surrounded by high mountains on three sides and is crossed by three main rivers (Duero, Tagus, and Guadiana) from the east into Portugal and the ocean, thereby forming fertile valleys surrounded by drier high ground very rich in agricultural terms (Martínez Jiménez et al., 2018). The Pyrenees Mountain range forms an effective barrier in the northeast, separating the Iberian Peninsula from the rest of Europe, and in the south, at Gibraltar, the peninsula is separated from North Africa by the narrow strait of Gibraltar. The Atlantic Ocean washes the northern, western, and southwestern coasts, and the Mediterranean Sea washes the southern and eastern shores (Britannica, n.d.; WorldAtlas, n.d.).

Geologically, Iberia is a highly diverse territory with different rock types, relief models and materials of a long array of ages (Loidi, 2017). This peninsula was constructed around a primary old core to which successive portions were added over time. Its geological history is primarily conditioned by its position between the African and the Eurasia tectonic plates which joined with the Mediterranean with two momentous events (the Hercynian Orogeny at the end of the Palaeozoic and the Alpine Orogeny during the Tertiary) in the middle of the western Tethys Sea plate (Loidi, 2017; Wiley et al., 2009). Thus, the Peninsula is divided into two main sectors, the Hercynian cycle (the old core) and the Modern and post-Alpine stages. Regarding the origin and age of its rock types, the Iberia peninsula is built of a combination of emerged materials, some belonging to the old core (Hesperian Shield) with Pre-Cambrian and Palaeozoic siliceous materials (quartzite, silicious conglomerates or slate), and others belonging to the sedimentary cover, mostly Triassic, Jurassic, and Cretaceous periods such as limestone while some depressions were filled with eroded soft materials such as marls (Loidi, 2017).

Mostly mentioned in the political regions and administrative areas during the Late and post-Roman periods are the north-west region (Galaecia), south of Douro and the west edge of the Meseta and modern Portugal is Lusitania crossed by the Tagus and the Guadiana, and the seat of the last Roman capital of Hispania, Merida. The Guadalquivir valley roughly coincides with the Roman province of Baetica, Ebro valley mostly fits in Terraconensis, the last province under Roman control, while the eastern half of the Meseta and the south-eastern coast formed the Carthaginensis (Martínez Jiménez et al., 2018).

2.2. Romans in the Iberia

Towards the end of the second millennium BCE, new Indo-European populations had arrived in the Iberian Peninsula. Amongst them, the Lusitani tribes settled in the Beira region and parts of Spanish Extremadura, while the Conii tribes settled in the Alentejo, part of southern Spanish Extremadura and the Algarve. The introduction of new blood to Iberia occurred around 500 and 250 BCE with the waves of Celtic populations carrying iron weapons and tools. Along with this, the Tartessian, Phoenician, Greek, and Carthaginian colonists who settled along the southern Iberian shores had also brought new cultures with increasing contact with the Mediterranean (Corsi et al., 2013), while the Romans' arrival in the Iberia in the 3rd century BCE led to the transformation of the Hispanian administration, landscape and culture (Firnigl, 2013).

Roman permanent presence in the Iberian Peninsula was a result of Hannibal´s (Carthaginian general) use of Spain as a base and to recruit Hispanian allies to attack Rome. Successful campaigns of the Romans in the region during the second Punic war led by Scipio Africanus resulted in Rome´s decision to remain in Iberia after they must have seen the peninsula´s wealth and economic potential (Fear, 2009a; Firnigl, 2013; Houck, 1998a). The Roman conquest was the first step in the process of incorporating all the Iberian regions under the same political power (Carneiro, 2019). After the second Punic war (218-201BC), Roman presence in the Iberia Peninsula and the introduction of provincial governments (Hispania Ulterior and Citerior) in the early 2nd century BCE, brought about changes in all spheres of the indigenous society of the Iberia (Fear, 2009a; A. Figueiredo, 2001).

Figure 2: Provincial subdivision of Roman Iberia in the (a) Republic (b) Principate (c) and Diocletian´s reorganization in the 3rd c. CE (Firnigl, 2013)

By 19 BCE, the Asturias and Cantabria were incorporated into the empire during which the peninsula was divided into three provinces under Augustus. Hispania Ulterior got divided into two new provinces (Hispania Ulterior *Baetica* and Hispania Ulterior *Lusitania – Augusta Emerita*) while some parts of the ulterior and the rest of the region remained as the province of Hispania Citerior (Tarraconensis).

Under Diocletian, the Iberian Peninsula was further divided into five provinces in the early 3rd century CE (see *Figure 2*). The new provinces were created from the northwest – Gallaecia and the southeast – Hispania Carthaginensis (Fear, 2009a; Firnigl, 2013). Furthermore, unbroken peace and prosperity were enjoyed in Hispania in the first two centuries of the common era (CE) period until the 3rd century CE. Marked changes were also observed in the settlement patterns of the region. The indigenous Iron Age populations´ settlement patterns were gradually abandoned and replaced by large fortified towns as a response to the growing socio-economic, political, and military instability enhanced by Roman military campaigns in the Iberian Peninsula after the second Punic War (Mantas, 2004)

The region flourished both commercially and politically under the Roman rule which led to an influx of people including the Romans. Therefore, the Iberian population was made up of settlers and indigenous people with different legal statuses (Fear, 2009a; Mantas, 2014). Although, the Romanisation of the region did not happen without opposition and element of socio-cultural resistance in such a way that certain aspects of pre-roman indigenous culture such as language and religion were still retained in some parts of the Iberian areas. The indigenous Iberia religion did not change but took attributes that made it look Roman. Although, three main religion traditions (indigenous, Roman, and oriental) coexisted in the areas under Roman control in Iberia from the 2nd century BCE. A practice said to have occurred throughout the Roman world (A. Figueiredo, 2001). It is noteworthy to mention that Iberia experienced a process of transformation with widespread effects which were unevenly felt. The impact was only superficial in areas where indigenous cultural forms persisted while the Roman effects became deep-rooted in certain areas at the onset (Egan, 2013; A. Figueiredo, 2001). The Roman initial involvement in the Peninsula was restricted to the urbanized zones, as observed in the Romanization of other places (Cunliffe, 1995).

However, the peninsula was exploited for its richness in such minerals as gold, silver, copper, iron, tin etc. As asserted by Strabo in his writing of the *Geography*, "*the totality of the Iberia is full of metals in such quantity and quality not found anywhere else in the world*" (Erskine, 2009; Fear, 2009a). This assertion, however untrue, points to the importance of Iberian mineral resources in the early Roman periods. However, being a finite resource, there was depletion in the mineral reservoir of the peninsula in the 2nd century CE (Fear, 2009b). These economically important metals are distributed in the wide arch of the pyrite zone of Iberia (Cunliffe, 1995).

In addition to the minerals, a major contribution of the Iberia to the empire is agriculture underlining the production of olive oil and fish sauces. This region is also known as the main producer of oil and fermented fish sauces, *garum,* which has been said to be a Roman development of previous endeavours similar to the mining industry (Fear, 2009a). Indeed, evidence of increased agricultural and improved zootechnical practices has been seen in different Iberian areas and it has been said that important changes regarding livestock management practices materialised from the early Roman periods onward (Colominas, 2017; Houck, 1998a). New plant species and culinary traditions were introduced to the conquered territories of Roman Iberia (Peña-Chocarro et al., 2019). Classical literature also indicates the existence of fertile valleys, densely populated with evidence of intense agricultural production, which suggests the existence of an agrarian society that became well-developed in Iberia during Roman domination which certainly transformed large parts of the territory (Loidi, 2017).

Trade between the Roman Empire's domestic and international markets covered a variety of Iberian goods, such as wheat, wine, olive oil, timber, cattle, salted fish, fish sauce and other products, and precious metals, demonstrating the importance of the Peninsula to Rome (Houck, 1998a). Archaeological findings from the Peninsula also provide evidence of expanded and ongoing navigation and commercial exploitation of the coastal areas for importation and exportation during and after the Roman times, even up to the 5th and 6th centuries CE (Amato A & Bombico S., 2013; Fabião, 2009). The influence of the Romans also extends to the death treatment and burial practices of the Iberian people. The most common form of burial rite practised in the Peninsula was cremation until the 2nd century CE, although inhumation was a long-established tradition in some parts of the peninsula before the arrival of Roman practices. However, inhumation became the general funerary practice from the 2nd century onwards (A. Figueiredo, 2001; C. (Archaeologist) Pereira, 2015)

By the end of the 5th century CE, many parts of the peninsula were loosely under the control of the Visigoths and the North-western corner was ruled by the Suevic monarchy (A. Figueiredo, 2001; Jiménez & García, 2015). The arrival of the Germanic groups which led to the ultimate formation of the Christian kingdom also altered the Roman political structure. Therefore, these new circumstances enhanced the transformation of the Iberian Peninsula at many levels after the collapse of the Roman empire (Peña-Chocarro et al., 2019).

2.3. Roman Lusitania

The Roman province of Lusitania covered a great part of modern Portugal and some areas in western Spain. Based on the description of the physical settings of Lusitania, this region was divided by two major rivers, the Tagus (modern Tejo River) and bordered on the north by the Durius (modern River Douro) while the third major river was the Monda (modern River Mondego) that flows through the city of Coimbra. Some of the parts of the Serra da Estrela, the highest mountains in Central Portugal of about 2000 meters are bounded by the Tagus and Durius rivers while the south of the Tagus has plains and plateaus with alternating rocky and sandy coasts. Today, the climate of the coast in southern Lusitanian is Mediterranean with an annual mean temperature of 18^0C. From south Tejo, the annual rainfall is 500-700mm and 450mm in Algarve. From the coasts towards the middle of the country is where the continental climate type is stronger with increasing annual rainfall in the northernmost parts. It has also been observed that Roman dams were constructed where the average annual rainfall was relatively low in such areas under 800mm and under 600mm in southern Portugal (Butzer et al., 1985; Firnigl, 2013). With its relatively flat topography and strong sun radiation per unit area (irradiance), the vegetation of the Lusitania nowadays is consistent with thermo- and meso-Mediterranean bioclimatic categories (Nieto-Espinet et al., 2021).

The Roman conquest led to the end of a long-running process connecting the Southwest of the Iberian Peninsula to the Mediterranean circuits, ongoing since the beginning of the first-millennium BCE. Prior to Roman arrival, the Punics, Greeks, and Phoenicians with their trades, ships, and written cultures, brought new influences with different impacts to the indigenous communities of Iberia (Carneiro, 2019; Corsi et al., 2013). However, Roman presence in the western part of the Iberian Peninsula grew significantly between the middle of the 1st century BCE and the 1st century CE, as seen in the large corpus of archaeological records and classical literature (Alexandra & Bombico, 2017; Fabião, 2009; Fear, 2009a). Indeed, this did not occur without prior resistance and opposition. Of all the conflicts described in the literary sources, well-spoken about is that of the Lusitanian wars that occurred from 150 BCE and lasted until 134 BCE (Berrocal-Rangel, 2018).

The most famous Lusitanian leader, Viriatus, responded to grievances such as the betrayer by the *Praetor Servious Sulpicius Galba* who had invited the Lusitanian people to peace talks but massacred many of them in 150 BCE (L. D. Monteiro, 2008; UNRV, n.d.). This led to a series of brutal combats between the Lusitanians and the Romans during which the Lusitanians under the control of Viriatus, conquered some parts of the Roman territory in the Iberia (Berrocal-Rangel, 2018). Suffice it to say that the Lusitanians were accorded a certain degree of respect for their military ventures. They were autonomous and had no economic reasons to join the Romans, as they were not accustomed to paying tributes and certainly did not need the protection of the Roman military (Egan, 2013). They were said to have raided regions that cooperated with the Romans and burned their crops. Lusitania according to Strabo, was at war against the Romans for the longest period in the Iberia (Edmondson, 1992a). The assassination of Viriatus by three of his very close friends who were bribed by Caepio led to the decline of the Lusitanian campaign against the Romans and the eventual victory over the Lusitanians (Berrocal-Rangel, 2018; UNRV, n.d.).

The Roman´s victory over some areas in Lusitania such as Olisipo (Lisbon), and Moron (near Santarem) in the 2nd century BCE and the consolidated Roman presence in the south of the Tagus River and some towns of the Alentejo (e.g., Ebora) in the 1st century CE led to further occupation and expansion of the Roman territory in Iberia. This resulted in an extensive territorial and political reorganization under Augustus, which was further established by the construction of proper Roman towns and connecting road networks from 27 BCE onwards (Corsi et al., 2013).

Therefore, the Roman province of Lusitania was integrated into the Roman Empire in 25 BCE, with Augusta Emerita (modern-day Mérida) as the provincial capital under the Diocesis Hispaniarum (Carneiro, 2019; Quaresma, 2017). This Roman Province covered an area that takes in most of present-day Portugal excluding the North and a part of neighbouring Spanish Extremadura. In the 1st century CE, Lusitania was divided into three *conventus* with Augusta Emerita (modern Mérida, Spain), Pax Iulia (Beja, Portugal), and Scalabis as the centres. Many other important cities were also developed such as Olisipo (Lisbon), Norba Caesarina (Cáceres) and Ebora Liberialitas Iulia (Évora) (Carneiro, 2019; Corsi et al., 2013; Fabião, 2014b). A total of 45 urban communities were built, and they were connected by a sophisticated road system (Firnigl, 2013). It appears that the Roman roads in Lusitania were built to connect seaside ports to one another and to the territory's river networks (*Fig. 3)* (Bombico, 2015b; Mantas, 2004). The southern part of present-day Portugal and the Extremadura province of Spain, mostly in the Duro valley were the parts that the pre-Roman Lusitanians occupied. Based on Strabo's description, "there is Atlantic coast to the north and the west, to the south is the Tagus river, and mountains to the east (Fear, 2009b).

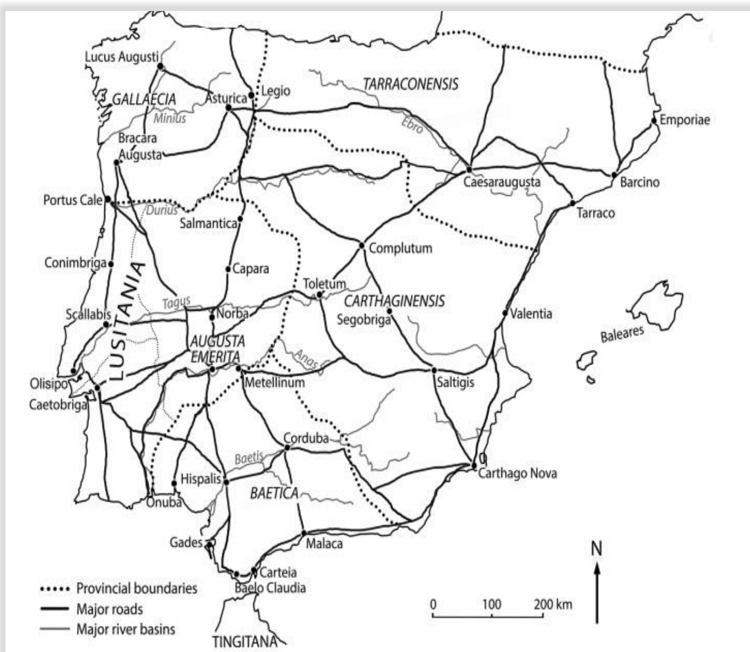

Figure 3: Late Roman Hispania and Routes (Osland, 2016)

Northern Lusitania was part of the last region to be incorporated into the Roman Empire, unlike Baetica and southern Lusitania (A. Figueiredo, 2001). Becoming a province under Augustus also resulted in a further division of Lusitania into *civitates* which extended the landscape of Roman rule in Iberia. The road network of the new province was also extended during which a major road connected Portus Cale to Olisipo with another one from the northern limit of the territory to the capital and beyond (Edmondson, 1992a). Due to this, Emerita became of economic importance due to its strategic position along a major artery of overland travel that connected the rich gold-mining regions and cities in Spain. Also, the Anas River (Guadiana) provided a shallow-draft water route connecting Emerita to the Straits of Gibraltar, and overland routes connected Emerita to both the Tagus River at Scallabis and the Atlantic coast at Caetobriga, south of Olisipo. These connections enabled the linkage of the western coast of Lusitania with cities such as Corduba, Hispalis, and Caesaraugusta in Baetica and Tarraconensis (Osland, 2016). This road network enabled the existence of frequent long-distance contact with other provinces and the Mediterranean. Roman influence on Lusitania's rural landscape resulted in changes in the province's landscape, particularly in the patterns of rural settlement, the nature of land usage and agrarian exploitation, and how Lusitania's residents observed and thought about their surroundings (Edmondson, 1992b, 1992a).

2.3.1. Life in the Roman Lusitania

Roman Lusitania was characterized by a high level of industrial and agricultural productions, trade and financial systems, advanced urban culture as well as arts (Firnigl, 2013). There was increased migration into the province after the Roman conquest of the region. Indeed the new colonies were created where land cultivation was possible, and through this, the social problem of the installation of its former soldiers was solved for Rome (Fabião, 2014b). Thus, some people moved due to their military or administrative obligations to the Roman Empire. It has been recorded that, soldiers came to Lusitania, particularly the capital (Augustus Emerita) where there was a military garrison during the early Empire and was finally settled by the discharged soldiers of the fifth and tenth legions (H. Stanley Jr, 1990). Furthermore, most people probably migrated for their economic and occupational improvement or due to newly freedman and slave status (Curchin, 2017; Farland H. Stanley Jr, 1990). The concomitant effects of this integration also led to the construction of new buildings and residences throughout urban and rural landscapes while Augusta Emerita became the radiating centre of the new architectural and urban models for the province (Fabião, 2014b).

Figure 4: The Roman Province of Lusitania (J. Edmondson, 2010)

The resultant Roman landscape formed by villas as the main units dominated most of the rural landscape around the 3rd and 4th centuries when the system began to reach its peak (Carneiro, 2016, 2019). Dams were also connected to the villas for the water supply of baths, irrigation of gardens and fields and watering animals (Butzer et al., 1985). People began to settle more along the coast in places like Balsa or Osonoba (Faro), Salacia (Alcacer do Sal) and Felicitas Iulia Olisipo (Lisbon), where construction of new buildings and public spaces, as well as extensive urban buildings restructuring, were evident (Edmondson, 1992a; Fabião, 2014b).

In terms of exploitation, the mineral richness of this province also exhibited the importance of Lusitania to the Roman Empire (Alarcão, 1989). Lusitania was very rich in auriferous resources (see *Figure 5*), especially the Tagus aurifer which has been revered in lots of classic literature. The naturalist, Pliny highlighted the gold wealth of Lusitania, when he stated that "*about twenty thousand pounds were produced in Asturias, Galeacia, and Lusitania annually"*. Due to its greater gold wealth than Lusitania, Asturias has, however, been the focus of prior studies (Fabio, 2014b). Nevertheless, recent scientific research on the Tagus and its tributaries has demonstrated the significance of gold mining in the Roman province of Lusitania. Other important mining complexes have also been documented on the River Alva and in Sierra de Francia, Mina da Presa, Covão do Urso, Conhal da Arneiro, etc. which further proved that Lusitania was an extraordinarily relevant mineral-production centre in the Roman Empire (Currás & Sánchez-Palencia, 2021). Additionally, a Latin inscription of Idanha-a-Velha discovered in the civitas Ignaeditanorum bears information about a person named Tiberius Claudius Rufo, who in the inscription, was thanking *Jupiter hecho* after extracting 120 pounds of gold from the Tagus basin (Fabião, 2014b).

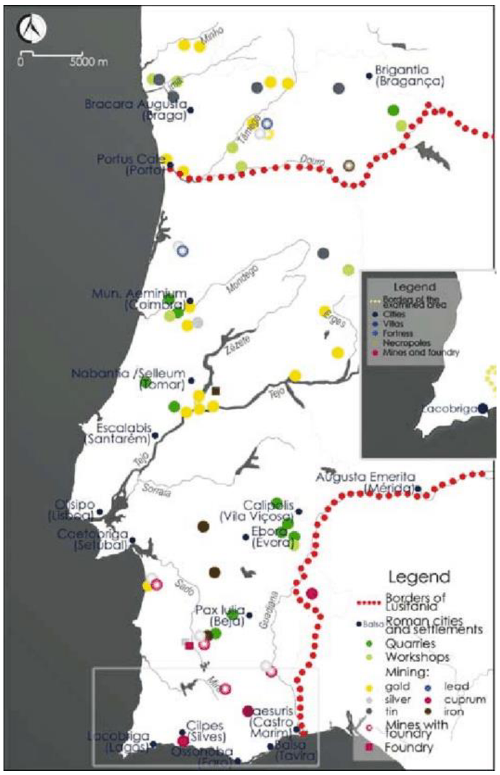

Figure 5: Mines and Quarries of the Roman Portugal (Butzer et al., 1985

The discovery of a partially destroyed Roman camp due to the encroachment of mining activities close to Mina da Presa suggests the involvement of the Roman troops in the mining activities (Currás & Sánchez-Palencia, 2021; Houck, 1998b). Furthermore, a lower number of mines have been discovered in the southern parts compared to northern Roman Portugal.

The agricultural endowment of Lusitania is also an added advantage to the Iberia and the Roman Empire. Some areas of Lusitania have evidence of a pre-existing agriculture type with origins in the Mediterranean (Fabião, 2014b; Loidi, 2017). The Romans were aware of the potential of Hispania's natural features, and as a result, they made use of them after conquering the region, particularly its countryside. In actuality, the expansion of Hispanic agriculture and the exploitation of maritime resources began with the establishment of the Lusitanian province and reached its pinnacle in a few decades (Firnigl, 2013). Some old notes of Pliny give insight into the diversity of Lusitanian agricultural prowess, which not only highlights the qualities of local products as demonstrated by their circulation but also their appreciation by the elites of the Roman Empire (Fabião, 2014a; Loidi, 2017). Archaeobotanical data from Lusitanian and the whole of Iberia suggest the existence of a diverse agriculture dominated by naked wheat and hulled barley in a more or less similar proportion at both rural and urban sites (Peña-Chocarro et al., 2019). Additionally, wheat, grape, and olive were the most suitable for the climatic and edaphic conditions in Roman Iberia, and their production became so advanced with the involvement of the labour of indigenous people in the Roman period that the products could be exported. Cereal cultivation further enhanced the development of the towns of Roman origins such as Santarém, Évora, Beja and Alcácer do Sal (Firnigl, 2013). Data from Northwest Portugal further corroborate the importance of naked wheat, millet, hulled barley, faba beans, oat, and hulled wheat in the agricultural cum food basket of Iberia during Roman times (Pedro & Tereso, 2012). Furthermore, legumes are important food for both humans and animals due to their high protein content. They are also significant in agriculture due to their ability to convert atmospheric nitrogen into ammonia which functions as natural fertiliser in symbiosis with the root bacterium *Rhizobium.* There are records of legumes cultivation in the Iberian Peninsula since the early Neolithic. However, the range of Legumes cultivated in Iberia during Roman times was very diverse. Cereal cultivation was common everywhere in Iberia during the Roman times, while legumes were only known in some parts including the E, NE, NW, and S. In addition, legumes are only found in the East and Northeast of the Peninsula during Late Antiquity (Peña-Chocarro et al., 2019).

Regarding animal husbandry practices, the growth in the body size of cattle and sheep, as well as a general change in the animal's slaughter patterns and taxonomic frequencies, are only a few of the modifications in animal husbandry methods that can be seen throughout the Roman Empire. In areas that Rome had conquered, including France, Britain, Belgium, the Netherlands, Germany, Switzerland, Slovenia, and even the Iberian Peninsula, this increase in cattle body size has been observed (Colominas, 2017; Trentacoste et al., 2021). Livestock management of Roman origin is characterised by improved feeding practices, change in sex ratios with a preference for male animals and the importation of new phenotypically large stock (Kron, 1937; Trentacoste et al., 2021). There is also evidence of diversion and regional reorganisation of animal production in many of these areas including Portugal after the Roman conquest. However, this information varies as we move from one site to another in the Iberian Peninsula. Faunal assemblages from some Iberian sites show evidence of improved husbandry practices after the Roman conquest, especially in cattle and sheep, while others suggest that no improvement in the size of livestock occurred after the Roman conquest

(Valenzuela-Lamas, 2014). Further evidence that the Romans did not significantly enhance the livestock herding in the western part of the Iberian Peninsula comes from the biometric data of faunal remains found at such sites as the Governor's house (Belém, Lisbon), Núcleo Arqueológico da Rua dos Correeiros, Conimbriga, Santarém, and Gallaecia. This is indicative of high diversification between Lusitanian sites in terms of meat consumption (Valenzuela-Lamas, 2014; E. Wright & Ginja, 2022).

According to several zooarchaeological investigations, cattle, sheep/goats, pigs, and red deer were the four dominant animal species in various Roman sites from Lusitania (Nieto-Espinet et al., 2021; Valenzuela-Lamas, 2014; E. Wright & Ginja, 2022). Furthermore, it has been demonstrated that small settlements, villae, and industrial sites tend to depend more on smaller animals (sheep, goat, and pig) in contrast to cities founded by the Romans which depended to a greater extent upon cattle (COLOMINAS, 2016; E. Wright & Ginja, 2022). Some cities founded before Roman arrival such as Santarém yielded cattle bones of smaller size than the cattle bones from new Roman cities like Ammaia, Augusta Emerita, and villa of Torre de Palma, among others. Whereas other data from Late Roman contexts at such sites as Ammaia indicate the continued practices and further improvement of the Roman techniques applied to cattle herding even when the city was in a process of abandonment (E. Wright & Ginja, 2022). Indeed, some archaeological sites exhibit diachronic traits, such as a noticeable increase in sheep and goat remains between the 1st and 2nd centuries CE and the 4th and 5th centuries CE. Additionally, the 4th and 5th centuries are characterized by a high proportion of sheep and goats throughout the Mediterranean, which may reflect changes in the economy following the 3rd century CE crisis and the 4th and 5th centuries Ulterior crises (Valenzuela-Lamas, 2014).

2.3.2. Marine Resources Exploitation in Lusitania

Due to its unique geographic location between the Mediterranean and the Atlantic Ocean, the Roman province of Lusitania is well suited for the exploitation of marine resources and the movement of goods within the Empire (Amato A & Bombico S., 2013; Fabião, 2009). The Atlantic front has abundant fish resources and a hot environment with protracted dry spells that were ideal for making salt. In contrast to the Mediterranean, the Atlantic has known fish potential. Indeed, salt exploitation linked to fishing activities resulting in the production of fish sauce (e.g., *garum*) and salted fish in addition to the agricultural prowess has been said to be one of the most important industries in Roman Lusitania (Bombico, 2015a; Edmondson, 1992a; Fabião, 2009). The species used in the preparation of fish sauces during the Roman times include clupeiform fishes such as sardines, *Sardinella* species, anchovies (*Engraulis encrasicolus*), and Sea breams (*Sparidae*). Spanish mackerel (*Scomber japonicus*) was most preferred for salsamenta, although scad *(Trachurus* sp.) was also used. In Lusitania, fish bones of sardines have been seen in several Lusitanian amphorae from shipwrecks, as well as in the tanks from Lusitanian factories such as Workshops I and II of Tróia, Rua dos Correeirios**,** Quinta do Marim, Travessa do Freire Gaspar, among others. Furthermore, analyses of fish remain from later phases in the use of fish vats, from the 3rd and the 5th centuries AD point to sardine as the most significant element in the making of Lusitanian fish products during Late Antiquity (Bombico, 2015b). The size and importance of these operations in the Roman Empire are confirmed by the vast concentration of ancient fish preparation and salt production facilities that have been found along the southern Portuguese coast (Fabião, 2009; Pinto, Magalhães, Brum, et al., 2020). The cities of Olisipo, Salacia, Mirobriga, and Cuncus, as well as the settlements of Ossonoba, Balsa, and Myrtilis, are only a few examples of those named by Pliny as being on the shore from the beginning of the Tagus. Notable are a few towns along the lower Tagus River, such as Olisipo, Salacia (Alcácer do Sal), Ossonoba, Balsa, and Scallabis, whose commercial functions dates back to the pre-Roman era. (Amato A & Bombico S., 2013; Bombico, 2015a).

Figure 6: Sites with vats for salting fish on the coast of Lusitania (Pinto, Magalhães, Brum, et al., 2020)

Historical and archaeological evidence discovered in recent decades points to the existence of a sizable marine resource exploitation activities (mostly the manufacturing of fish products), which was linked to exportation and amphorae production activities (see *Figure 6*). Throughout the Roman Empire, amphorae were be used to transport the sauce known as garum, which was made with salt and fish parts (E. Wright & Ginja, 2022). Research on Portuguese-made Roman amphorae reveals significant connection to the exportation of marine resources, which underpins the significance of fishing in the area during the Roman era and suggests a high level of fish preparation for export. In close proximity to the pottery centre geographically, several fish-salting workshops were created in navigable locations. About 18 ceramic centres that produced amphorae close to the fish-processing facilities have been found in the Peniche, Sado, and Tejo valleys, as well as on the Alentejo and Algarve coasts. There are signs that estuaries were preferred for the establishment of production centres, and seaside cities in

Lusitania developed along the Atlantic coast during the Roman rule. (Alexandra & Bombico, 2017; Amato A & Bombico S., 2013; Bombico, 2015a; Fabião, 2009, 2014b; Pinto, Magalhães, Brum, et al., 2020).

Suffice it to say that the construction of fish factories in Lusitania coincided with the establishment of a number of amphora workshops to facilitate the transportation of fish products across the Empire (Alexandra & Bombico, 2017; Fabião, 2009; Valenzuela-Lamas, 2014). In addition to the numerous artifacts related to fishing such as weights, hooks, and needles, huge cetary structures (vats for fish-salting) preserved in various sites also corroborate the idea of manufacturing of goods of fish origin. The fact that these foods were exported using the amphorae that were locally produced clearly illustrates how important this industry is to Lusitania's economy (Amato A & Bombico S., 2013; Fabião, 2009; Mantas, 2014).

Exploitation of these resources probably began before the Roman time. This has been suggested due to certain discovery of fish product related amphorae of pre-Roman typology in Sado estuary and Castro Marim area next to Guadiana estuary in Algarve (Fabião 2009). However, amphora production and probably site complexes with *cetariae* became widespread only during the 1st century CE. The period at which the exploitation of marine resource started in Lusitania is yet to be fully ascertained, but this exploitation became extensive during the Late Empire (Fabião, 2009, 2014b). It is said that the oldest fish products industry and its associated ceramic container dates from the beginning of the Principate, especially in the production centres of Abdul and Pinheiro, Tróia, and Morraçal da Ajuda, Núcleo Arqueológico da Rua dos Correeiros and Governor´s house in Torre de Belém. (Alexandra & Bombico, 2017; Valenzuela-Lamas, 2014). By the end of the 2nd century and the beginning of the 3rd century CE, there had been significant decrease in the manufacturing of fish products. This was distinguished by modifications and restructuring of fish processing facilities and pottery workshops, which were felt throughout Iberia and Roman world. Some production units were abandoned during this time, and when they were later reoccupied, the salting tanks were reduced and subdivided. In the Roman world, this occurred between the end of the second and the beginning of the third centuries CE, at a time when a broad range of economic and socio-political changes were taking place globally. Furthermore, in the major centres along the Sado and Tagus rivers, marine resource exploration resumed in the mid – third century and gradually developed reaching its peak during the 4th century CE with the emergence of new centres (Alexandra & Bombico, 2017; Bombico, 2015a; Fabião, 2009).

Evidence from amphorae studies further indicates that, the production and use of amphora type, Dressel 14, became dominant in the middle of 1st and the end of 2nd centuries CE. However, a new type called Lusitania 3, emerged during 2nd century. Between 3rd and 5th centuries CE, diverse amphora types became very rampant with Almagro 51c dominating the scene and other types said to be produced in the Tagus and Sado estuaries (Alexandra & Bombico, 2017; Bombico, 2015b; Bombico S, 2020). Although it has been said that most of these fish processing centres were abandoned from the end of the 4th to the beginning of the 5th century CE, when the economic circuits of the Empire collapsed (Valenzuela-Lamas, 2014). However, different research about amphora production centre and fish-processing factories, suggests that fish products and their exportation continued beyond the fall of the Roman Empire and even up to the 6th century CE (Bombico, 2015a; Fabião, 2009).

2.3.3. Economic interaction in the Late Roman Lusitania

Despite the fragility of Lusitanian trade, its goods, especially food, still reached the great interior sea´s far east. During the High Empire, there was a gradual development in the movement of Lusitania goods to Rome and the rest of the Roman world probably in the 1st century AD and stretched to the 3rd century AD. Lusitanian trading system became largely extensive during the Late Antiquity, especially by the late 4th and 5th centuries, a period characterised mostly by exportation of fish products (e.g., garum and salted fish) (Alexandra & Bombico, 2017; Quaresma, 2017).

In many literary works, Hispanic cereals were mentioned although without the stating a specific location. Albeit evidence of Roman warehouse (*horreae*) discovered so far in the Iberian Peninsula are mostly concentrated in Lusitania. The late 4th century AD was said to be a crucial period for Hispania in the supply of cereals to Rome. In fact, the flow of Hispanic cereal to Rome is said to be secondary to that of Africa and was mainly intended to make up for the times of African cereal crisis. Nonetheless, analyses of Lusitanian ceramic fabrics recovered from Ostia has shown an increase in Lusitanian salted fish sales towards the end of the 2nd century and late 4th century with indication of it becoming intensified in the second quarter of the 5th century AD, thus showing an extension of commercial capacity within the Mediterranean sphere until the end of and even beyond 5th century AD (Quaresma, 2017). In fact, most of the available data about the distribution of Lusitanian products are confined to the study of fish amphorae which led to the consideration that fish was the main food product produced and exported by this Roman Province. As a result of Lusitania's production and export of salted fish (salsamenta) and fish sauces (garum, hallex, liquamen, muria, etc.), the abundant marine life of the Atlantic Ocean was used to an economic advantage of the Empire. Evidence of these include the archaeological fish bone remains from processing tanks and shipwrecks in Lusitania and the Mediterranean, as well as a variety of amphora types found there (Alexandra & Bombico, 2017; Amato A & Bombico S., 2013; Bombico, 2015b; Fabião, 2009; Filipe, 2021).

High economic activity from Lusitania during the Roman period was highlighted by the rise in the number of archaeological records pertaining to the movement of goods by sea along the Atlantic coastline, such as the distribution pattern of some amphorae and terra sigillata, as well as the identification of archaeological remains of ancient navigation, such as anchor stocks, shipwrecks, and lighthouses, etc. (Bombico, 2015b). Along the Iberian Peninsula's Mediterranean and Atlantic coasts, numerous studies of shipwrecks with cargoes of African, Baetican, and some Lusitanian amphorae have been conducted (Quaresma, 2017). Numerous shipwreck sites containing Lusitanian type amphorae have been identified and even more than 40 shipwreck sites with Lusitanian container types are waiting to be studied. As discussed in previous section, research on amphorae are important in the study of Lusitanian maritime trade and even elsewhere, due to their specialised design suitable for maritime transport (Bombico, 2015b).

The trade networks along the Atlantic facades of the Iberian Peninsula have been said to supply the cities and fixed military camps located in the Iberian Peninsula. Preferential relationship with the Roman province of Baetica and the Gades´ Ports have been suggested, where the supply of cereals, wine and olive oil was controlled by the states (Bombico, 2015b). Olisipo became a large port city as it was a connecting point from where the local products were exported to Italy (Firnigl, 2013; Mantas, 2004, 2010).

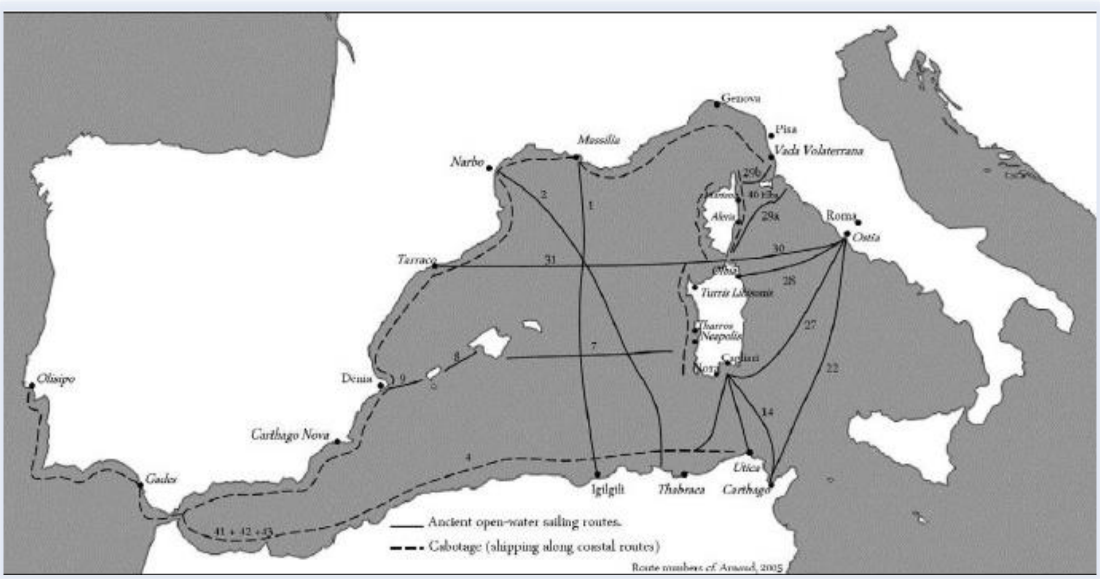

Figure 7: Sailing Routes for the Ports of Rome from Iberia (Bombico, 2015)

As suggested by Bombico (2015), the fish products from Lusitania would have probably been transported via uniform shipments that were loaded simultaneously in ports close to the production centres and then transported directly to another significant port (see *Figure 7*). For instance, the ports of Olisipo to Gades or Olisipo and Carthago Nova, as well as the infrequent travel between Olisipo and

Rome. Additionally, certain instances that fit another paradigm have been observed, where the homogenous shipment loaded simultaneously in a major port far away from the production centres of most of the goods were sent directly to another major port. Therefore, transportation models of Lusitanian goods were quite diverse in accordance with the series of economic and socio-political transformation of the province within the Roman Empire (Alexandra & Bombico, 2017; Bombico, 2015b).

The trade of Lusitanian wine, although not highly referred, occurred probably between 2nd and 4th century AD and was probably in a subsidiary position to fish and its products (Bombico, 2015b; Quaresma, 2017). Additionally, the Lusitanian amphorae trade into the Atlantic region became more intensive from the 3rd AD onwards as they have been seen in the North-western Hispania and the Britannia and Germania. Although, Lusitanian exports in the Northern Roman Empire was not as well represented as the Baetican olive oil, a smaller number of salted-fish amphorae from Lusitania were discovered there (Quaresma, 2017). Alongside Hispanic food products (olive oil, wine, fish sauce), copper or lead ingots from Baetica and Tarranconensis, amphorae of Lusitanian origin also circulated between the early 1st century and mid-2nd century AD, while North African products in African II amphorae became frequently transported alongside Lusitanian amphorae from mid-3rd century AD, thereby reflecting economic changes in the Late Antiquity during which the African province became great supplier of food products to Rome (Bombico, 2015a). In a similar vein, wine containers and salted-fish amphorae were represented in Britannia in smaller amounts (Quaresma, 2017). The importance of Lusitanian imports in South Gaul has been seen from Toulouse where Lusitanian amphorae were discovered in high numbers relating to the 5th century. Also, the presence of Lusitanian amphorae in Bordeaux and even Vigo further stresses the importance of food products transported in Lusitanian and South-Hispanic amphorae in the 6th and even the first half of the 7th centuries in those regions (Quaresma, 2017).

3. Archaeological Context

3.1. The Fish-salting Factories of Tróia.

Located on the southwestern coast of Portugal, the fish-salting production centre at Tróia is situated on a sandy peninsula separating Sado River´s estuary from the Atlantic Ocean (R. de Almeida et al., 2014; Nabais, 2015; Pinto, Magalhães, & Brum, 2020; Pinto et al., 2021) (Fig. 8) It is about 50km south of Lisbon, placed on the East side of the peninsula facing the Sado estuary and the Western part close to the Atlantic Ocean (Nabais, 2015). Geologically, this site is currently sitting on the Holocene sediments characterized by conglomerates, sandstones, gravel, sands, silts, and clays (Rebêlo et al., 2009). The Cenozoic system of the Tagus-Sado with high sedimentary concentration led to the accumulation of fluvial sands and dunes in this site, not very suitable for agricultural practice but only supporting the native vegetation of low-span species, bush types and patches of pine forest which accompanied the peninsula´s extension (J. P. Almeida, 2009; Brum et al., 2017).

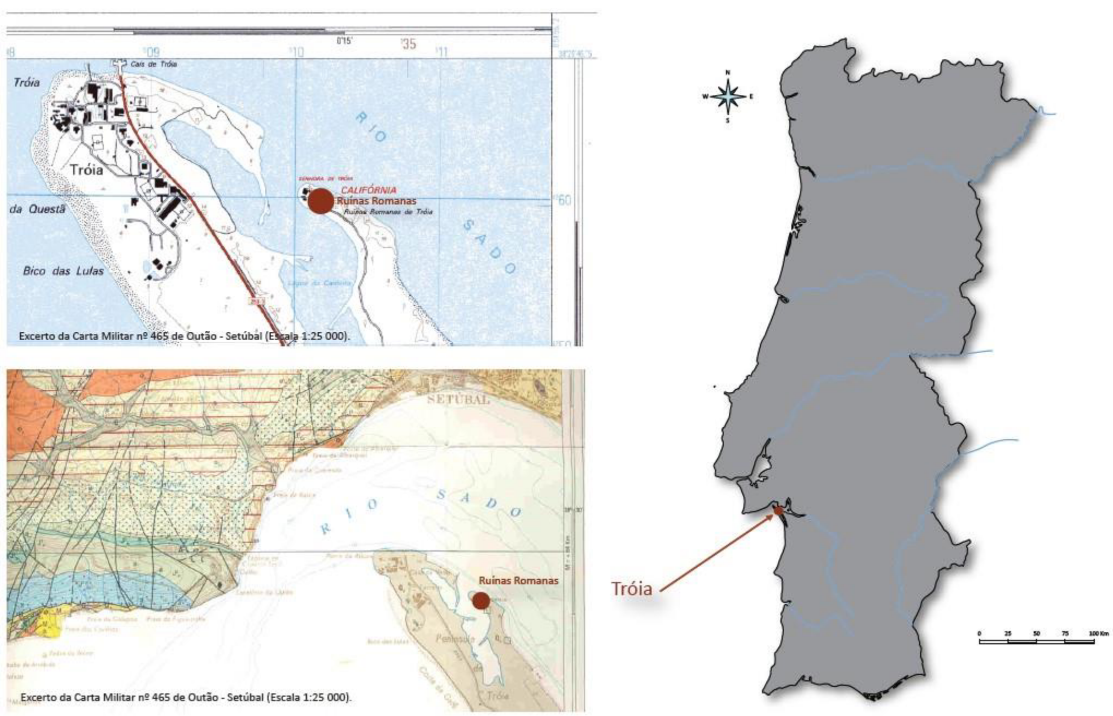

Figure 8: Geographical location and framework of the Roman Ruins of Tróia

Being part of the territory of the Roman city of Salacia (modern Alcácer do Sal) and across the river from the city of Setubal (ancient Caetobriga), this site was likely founded on the island of *Achale* as referred to in literature due to indications that the peninsula was probably a line of islands during the Roman period. This settlement later grew vigorously into one of the largest fish-salting production centres in the Roman world, becoming an urban-industrial centre with traces of occupation even up to the 6th century (R. de Almeida et al., 2014; Fabião, 2009; Pinto, 2009; Pinto, Magalhães, & Brum, 2020; Pinto et al., 2021; Rebêlo et al., 2009).

With about 27 known production workshops, the Roman site of Tróia is the largest known fish-salting factory of the Roman Empire (Figure 9). Its fish supplies came from the neighbouring ocean waters extremely rich in fish even up to this day and the high-quality salt from the saltmarshes of the Sado shores, showing the suitability of this site´s environment for fish-salting activities. The products from Tróia were transported in the amphorae produced in the several workshops on the right bank of the Sado River, where about nine workshops have been identified so far (Pinto et al., 2018; Pinto, Magalhães, & Brum, 2020).

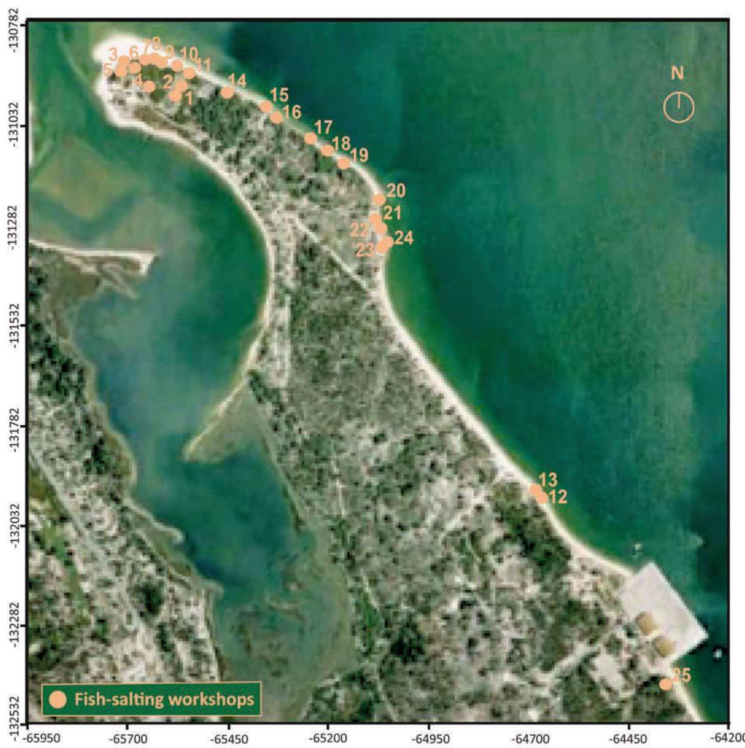

Figure 9: Location Fish-salting workshops at Tróia (Pinto, Magalhães, & Brum, 2020)

Covering an extension of about 2km, this industrial complex incorporates a considerable number of Roman constructions such as tanks (*cetariae*), wells, fish preparation areas, baths, graveyards, Basilica, a residential area with buildings of two floors with some covered with *opus tesselatum,* thereby showing a probable richness of the site. To further highlight the richness of this site, the Roman construction in the earlier periods was built with stone masonry mainly from the Setubal quarry in *opus incertum and quadratum* techniques, while brick was used in *opus testaceum* and *mixtum* in the building reformations between 4th and 5th centuries AD (M. Monteiro & Rodrigues, 2006).

Several archaeological studies of the workshops (I and II) since 1994 and recent research from 2007 to the present have yielded information about the periods of occupation on this site. Three phases of occupation have been identified through several studies of the fish-salting facilities of Tróia (see *Figure 10*). It has been said that this site began operation in the middle of the 1st century AD and lasted up to the second half of the 2nd century AD, while the second phase began from the early 3rd century AD to the early 4th century AD, and the last phase from the early 4th century to the second quarter of the 5th century AD (Pinto et al., 2018, 2021; Pinto, Magalhães, & Brum, 2020).

The archaeological amphora types such as Italic *terra sigillata*, Italic and Baetican Dressel 2-4 and regional Dressel 14 variant A found in workshop 2 suggest a starting date in the Tiberian period, being the earliest levels detected in Tróia. Thus, placing the founding date of this site in the Tiberian period and as the earliest fish-salting centre known so far in the low valley of the Sado river. Contemporaneous to this site are the amphora kilns in Largo da Misericórdia in Setubal, Abul in Alcácer do Sal on the other shore of the river with an abundance of clay, providing Tróia with the amphorae needed for storing and transportation of the fish products. The construction of several workshops during this period indicates a significant economic activity (Pinto et al., 2016; Pinto, Magalhães, & Brum, 2020).

Based on the finds recovered from workshop 1, a period of abandonment in the late 2nd century AD was observed marking the end of the first phase of occupation of this site. The presence of a violated and refilled tomb in the patio of workshop 1, further substantiates this interpretation. Despite the uncertainty about the cause of the abandonment, it has been suggested that this site was desolated probably due to an earthquake followed by a tidal wave, change in the route of migratory species of fish, disease outbreak, or economic crisis (J. P. Almeida, 2009; Pinto et al., 2016). However, this period of abandonment also coincided with the period of the 3rd-century crisis in the southwest of Hispania and Northwest Morocco, thereby showing a cross-regional trend of destruction and abandonment, something that could have been caused by a Tsunami or similar event, with a long-lasting negative effect on the economy of these regions (Pinto, Magalhães, & Brum, 2020).

The second phase of occupation was characterized by considerable socio-economic changes beginning from the 3rd century AD, due to the earlier period of abandonment. The fish-salting workshops became segmented into smaller ones while some built spaces were remodelled. Several studies of the workshops point to the reuse or reactivation of the vats in different areas of the settlement on a slightly reduced scale during the first half of the 3rd century AD, which may have been as a result of economic recession, thereby leading to an effective reduction in production capacity (Pinto et al., 2016, 2018; Pinto, Magalhães, & Brum, 2020). However, the amphorae used to transport fish products became diversified and there was a transition in the funerary practices in the late 2nd century AD during which inhumation was introduced (J. P. Almeida, 2009; Pinto et al., 2016, 2018; Pinto, Magalhães, & Brum, 2020). This phase continued until there were instances of abandonment (e.g., workshop six) and remodelling (e.g., workshop one) in the late 3rd and early 4th century AD with the possibility that different areas saw varying developmental periods. Some parts of the workshops (e.g., workshop 6) were also reused as a cemetery and a Christian basilica was later built over it (Pinto, Magalhães, & Brum, 2020).

The abandonment of workshop six and remodelling in other workshops marked the beginning of the 3rd and last phase of production on this site. This last phase came to an end in the first half of the 5th century. Despite the downscaling and other uses to which some of the fish-salting facilities were put in the 2nd phase of occupation, production continued in many of the workshops even with signs of intensive production of fish sauces and salted fish, as seen in workshop 2, thereby maintaining Tróia as an important production centre which generated a wealth of revenue until the middle of the 5th century AD. However, the discovery of ceramics (although in smaller numbers) belonging to the second half of the 5th, 6th, and even 7th centuries AD in workshops 1 and 2 suggests the continued functioning of this fish-salting centre until the second half quarter of the 5th century AD (Fabião, 2009; Pinto, 2009; Pinto et al., 2016, 2018; Pinto, Magalhães, & Brum, 2020).

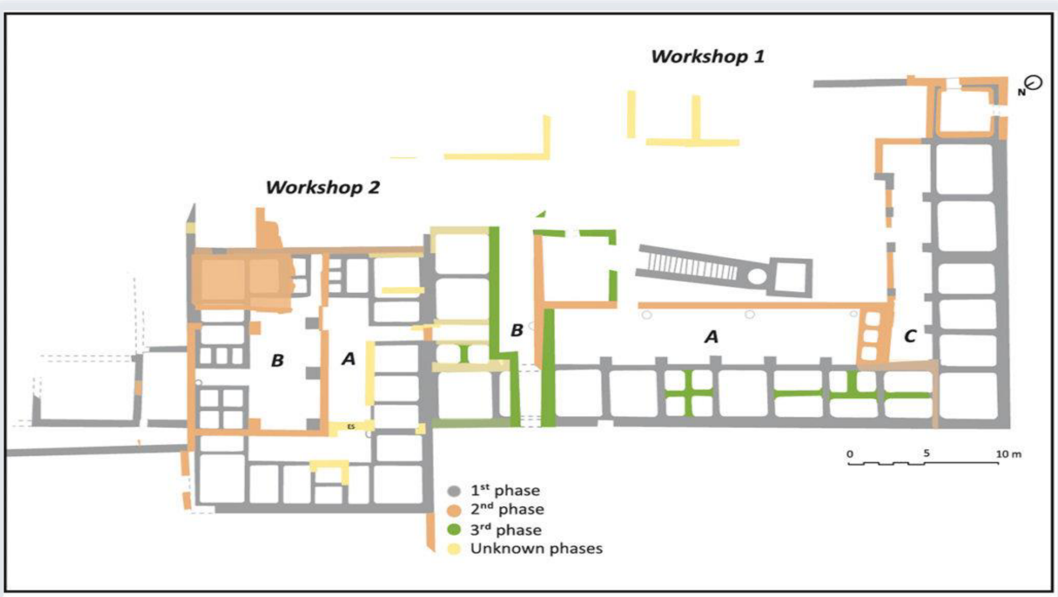

Figure 10: Plan of Workshops 1 and 2 showing their three phases(Pinto, Magalhães, & Brum, 2020)

Based on the studies of the vats in different workshops, it, therefore, became clear that people did not abandon the area for a considerable period as it is evidenced that some of the workshops (e.g., workshops 1 & 2) were still in use for other purposes such as cemetery and even garbage dumps (Pinto et al., 2016).

A residential area with traces of buildings on the ground floor and first floor (Rua de Princesa) was also constructed starting from the 4th century AD showing evidence of the wealth generated from fish-salting production. Similarly, this was also reflected by the construction of a palaeochristian basilica decorated with murals mostly of geometric and plant motifs, which was the most emblematic monument of Tróia in Late Antiquity (Pinto et al., 2016).

The fact that this site location was probably on an island, increasing its dependence on the urban agglomerate on the other shore, has led some authors to refer to it as "a secondary urban agglomerate," while others have referred to it as the city of Caetobriga's principal industrial district. Indeed, it is evident that Roman Tróia was well planned due to the general alignment of the building northeast-southwest with equidistant lots not allotted for cultivation as the white sandy soil of this site was not suitable for agriculture (*Figure* 11). The abundance of fish-salting workshops of Tróia at the mouth of the river, close to the ocean, in a location that is convenient for trade boats to pick up cargos as well as fishermen to deliver their catches, is an indication of well-thought-out planning of its establishment. Even the saltmarshes and pottery workshops probably delivered salt and amphorae to this site by boat (Pinto, Magalhães, & Brum, 2020).

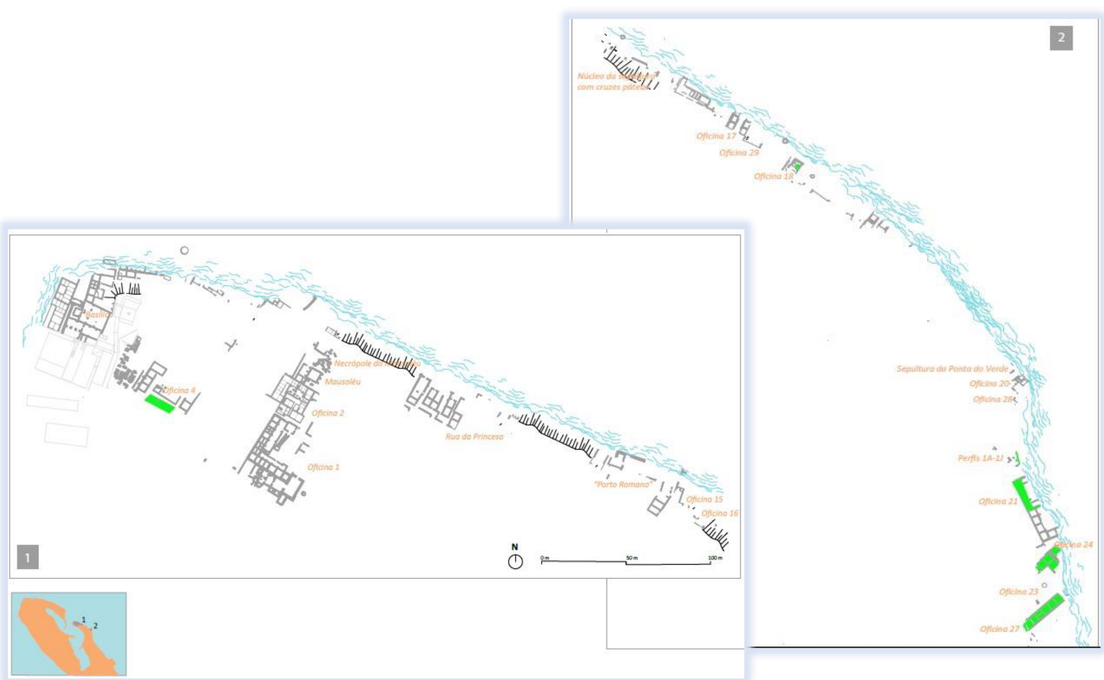

Figure 11: Plan of the fish-factories showing the orientation of the settlement (Pinto et al., 2020)

Contrastingly, this advantageous location is now causing loss of this important representation of the people´s industrial acuity and commercial ingenuity, because many of the fish-salting workshops are being degraded by the coastal erosion due to the rise of the sea level and periodic removal of the sediment by the tidal movement (Brum et al., 2017).

Suffice it to say that, for this type of industry to function, it would directly relate with several other subsidiary activities thereby creating a complex network of interdependent factories with the main objective of manufacturing certain products to be exported. The raw materials needed such as fish were obtained from the River Sado and the Atlantic Sea, thus ensuring the availability of this resource at certain periods of the year. Therefore, activities in this factory would have been conditioned by the quantity, quality, and availability of the marine resources which could have been a major or contributory factor that led to the reduction in capacity, or the abandonment experienced at different times in this fish preparation factory. Additionally, another activity directly related to this fish production is salt extraction which is well-known in the Sado estuary. Provision of the appropriate containers for the storage and transport of the fish derivatives was made possible by the pottery centres (such as Largo da Misericórdia, Quinta da Alegria, Pinheiro kilns, etc.) located in their overwhelming majority on the right bank of the Sado (R. de Almeida et al., 2014; J. P. Almeida, 2009; Fabião, 2009).

Despite the economic prosperity brought by the fish-salting business, Tróia also depended on building materials, foodstuffs, etc., and was probably in close relation with the city of Caetobriga. There are indications such as murals and mosaics, temples, grave types, funerary epigraphy, imported ceramics (terra sigillata) and amphorae, metallic and osseous objects that this production centre received new people and was resided by the diversity of the rich, common people, slaves, freedman and even foreigners, thus suggesting a dynamic and lively urban cluster (J. P. Almeida, 2009; Conejo, 2020; Pinto, 2009; Pinto et al., 2017; Pinto, Magalhães, & Brum, 2020).

The pre-Roman Iberia (Lusitania) people were known to have practised the funerary rite of cremation. While the cremated remains were usually deposited in a ceramic urn on the one hand, in some cases the remains of the deceased were buried in the place where the cremation rituals had occurred on the other. In some areas with Phoenician and Greek presence, inhumation was the main burial right even before the contact with the Romans (A. Figueiredo, 2001; Peyroteo-Stjernaje, 2015). However, inhumation became the common funerary practice during the Roman era in the Iberia and Roman province of Lusitania. More importantly, evidence of this transition has also been observed in the fish-processing factory of Tróia, as cremation was practised from the 1st century up to the first half of the 3rd century CE, and inhumation was progressively adopted from the end of the 2nd century and became the major funerary practice from the mid-3rd century CE onwards (J. P. Almeida, 2009; Pinto, 2009).

Despite its absence in several classical literatures, the extensive fish-salting vats in Tróia began to be significantly acknowledged in many literary works since the 16th century. Indeed, its discovery must have been strongly aided by the tide erosion from the Atlantic. This site was first excavated in the 18th century under the directorship of the then future Queen D. Maria I, and since then onwards, excavation continued until the present day. However, the first consistent publication dedicated to Tróia was made in 1994 by R. Etienne, Y. Makaroun, and F. Mayet highlighting the significance of this production centre and setting the chronological framework of the activities in the site (R. de Almeida et al., 2014; J. P. Almeida, 2009; Pinto, 2009; Pinto et al., 2016, 2018; Pinto, Magalhães, & Brum, 2020; Pinto, Magalhães, Brum, et al., 2020). Worthy of mention is also the contribution of archaeologists Inês Vaz Pinto, Ana Patrícia Magalhães, Patricia Brum, and many others, whose works since the last 2 decades have revolutionized and brought new insights into our understanding of the past in the Roman fish processing factory of Tróia.

Numerous archaeological excavations have enabled the discovery of different cemeteries in Tróia. More importantly, several late burials over the abandoned buildings such as the workshops and baths, as well as diverse tombs are known in Tróia. Various tomb types used in Tróia during the Late Antiquity include mensa tombs, plain graves, graves lined and covered with stones, bricks and tiles, amphorae with child burials, and stone sarcophagi (Pinto, 2009; Pinto et al., 2016).

3.2. Archaeological Excavations and contexts

As said earlier, several archaeological excavations have been carried out in Tróia since the time of its discovery. Excavation of workshop 1 was partially conducted between 1956 and 1958 during which 19 vats were studied and documented (Pinto et al., 2012). Pertinent to this study, however, are the series of excavations carried out in the areas of workshops 1 and 23 from the past 15 years led by the archaeologists Inês Vaz Pinto, Ana Patrícia Magalhães, Patricia Brum, others from the Tróia Resort and beyond. The excavations carried out by these people between 2008 – 2017 have led to the discovery of some late antique child burials and some interesting context rich in faunal remains from domestic refuse dumps.

3.2.1. 2008 Excavation

The excavation campaign of 2008 was conducted as part of the enhancement project in workshop 1 and to prepare the circuit for public visitation respectively. During this excavation session in workshop 1, three (3) funerary amphorae (amphorae 1, 2, & 3) were discovered, which further demonstrated the used of this area as a funerary space. Despite their discovery in the extension of the mausoleum located in the northeast of workshop 1, built in the 3rd century, many of the archaeomaterials collected and studied from this context showed indication of being deposited in a similar chronology of the first half of the 5th century. Of importance to this study, however, is amphora 2 found in the UE 487, a dune layer of whitish loose sands of fine and medium granulometry with fragments of shellfish. Other materials found include ceramics of different categories, blocks of mortars, and ichthyological and malacological faunal remains of consumption origin (Pinto et al., 2009).

Inside this amphora 2 (UE 525), was foetal remains (individual 525/T.OF1-L) of about 32 weeks packed in sands which were excavated in the laboratory by anthropologist Margarida Figueiredo. Ichthyological faunal remains and some seeds were discovered near the recorded skull fragment, which might be indicative of a funerary offering and/or celebration. The amphorae type used in this case is the Almagro 51c with osteological remains inside (*Figure 12*), this is a very common practice of inhumation used to bury infants in the Roman necropolises at the end of the Empire around the 4th century AD (M. Figueiredo, 2008).

The buried individual [525/T.OF1-L] was found in a good state of preservation showing partial anatomical connection with the lower limbs *in situ*. Furthermore, the arrangement of the bones is indicative of a primary deposition of the extended and parallel lower limbs even though the tibia and left fibula were found at an angle of almost 90° due to post-depositional and taphonomy processes. Generally, the foetal skeleton was in the lower part of the amphora with the skull pointed toward the mouth, and the feet towards the bottom (M. Figueiredo, 2008).

Figure 12: Amphora 2 in situ with individual [525/ T.OF1-L] (Pinto et al., 2009)

In this same session, a domestic dump was partially excavated leading to the discovery of some faunal remains from different stratigraphic units of workshop 1 and their chronology has been correlated to the last period of the abandonment of workshop 1 in the first half of the 5th century AD. Represented in these remains are cattle, goats/sheep, pigs, red deer, gallus sp., and rabbits. Other materials recovered include ceramic materials, metal and bone objects, shells, bones of fish and domestic animals, and fragments of mortar (Pinto et al., 2012).

3.2.2. 2010 Excavation

Following the earlier discussed excavations, the successive intervention works on this site in the year 2010 enabled the discovery of a diversified anthropological set of four (4) individuals, from which two (2) are involved in this study. Furthermore, the grave types discovered represent four different funerary types which correspond to existing frameworks of occupation in Tróia. They not only indicated a diachronic evolution in the treatment of death but also a structural differentiation in individual treatment (M. Figueiredo, 2010).

Individual [742/ T.OF1-SB] estimated to be between 9 - 11 years of age was excavated from sector 3 of workshop 1. The child was buried in a supine position with upper and lower limbs extended parallel within a simple pit-like structure with sub-rectangular contours, and walls covered in brick and mortar. This tomb typology is classified to fit group B3.4 described by Almeida (2008). This child whose skeletal remains only allowed age at death estimation was buried in a NW-SE orientation with an associated scallop on the left forearm and a coin next to the skull (see *appendix I*). The state of preservation of this individual is good with a high representation of most of the skeletal parts, except the skull, spine and thorax which were very fragmented due to the actions of taphonomic agents such as human violation of the grave, humidity and acidity of the deposit underlying the structures, which could have intensified the decomposition of fragile bones (M. Figueiredo, 2010).

Similarly, individual [680/T.BBT] was found in a structure with a base composed of well-compacted red clay and a cover of tiles arranged longitudinally. Anthropological analysis of the individual estimated the age at death to be between 6 and 7 months ± 2 months of gestation. The skeletal remains of this foetal individual were found dispersed and in random locations inside the grave. However, the minimal organization of the osteological remains suggests a NW-SE orientation with equally dispersed ichthyological faunal remains. There is also a lack of anatomical connections and a total absence of some bone pieces (see *appendix I*), which further indicate the mechanical actions of rodents and roots. Some bone fragments found on the grave edge further corroborated the continued effects of tidal waves on the structures and even on the osteological remains (M. Figueiredo, 2010).

Chronologically, the grave typology of individual [742] and orientation indicate a later period between the 4th and 5th century AD, while the associated archaeological evidence points to an earlier period of late 2nd and beginning of the 3rd centuries AD. Compared to the amphora (Almagro 51c) used in the burial of another foetal individual (not included in this study), burial [680] through the excavation data would fit in the chronological period between the 4th and 5th centuries AD (M. Figueiredo, 2010).

3.2.3. 2011 Excavation

The study in 2011 by Inês Vaz Pinto and João Almeida to understand and categorize the mensa tomb types in the funerary nucleus, south of the palaeo-Christian Basilica and west of the necropolis, led to the discovery of a funerary space comprising a set of five graves corresponding to a period before the implantation of the mensae. During this excavation session, three (3) of these five graves were excavated and studied leading to the uncovering of three (3) non-adult individuals in a primary and individual deposition. The last two interventions at Ponta do Verde on the eastern edge of this peninsula also lead to the discovery of a Mensa grave and another violated grave inside which two adult individuals were exhumed. Despite the degree of fragmentation and preservation, these osteological remains allowed the correct assessment cum deduction of some palaeodemographic and pathological patterns. Additionally, some considerations were made within the scope of funerary anthropology and the treatment of individuals based on the observations of the tombs (M. Figueiredo, 2011b).

The sepulchre A contained a primary inhumation of a child (individual [855]/ T.ONM-A) buried in the supine position, with upper and lower limbs extended and parallel, skull facing upward, jaw in place and partially destroyed viscerocranium. The two-year-old individual was buried in a NW-SE orientation, in a hole (UE 857) with an anthropomorphic configuration of walls covered in ceramics forming a box (see *appendix I*). The lid was made up of fragments of bricks and tegulae horizontally arranged on the box, connected by clay and mortar. This grave was infiltrated by very fine-grained sediments with inclusions of gravel, roots, and fauna. The skeleton was met in a very good preservation condition with exception of deteriorated face area which was elevated and more exposed to the direct pressure of the lid and root action which penetrated between and inside the bones.

In Sepulchre B, a primary inhumation of foetus/stillbirth (individual [870]/ T.ONM-B) was found inside an amphora (Almagro 50). This type of funerary practice is already known to belong to the 4th century AD, within the context of foetal burial in the site complex of Tróia. Due to the fragmentary state of the amphora´s belly, the internal part was excavated *in situ* thereby revealing a few osteological remains of an individual between 38 and 40 weeks of gestation (M. Figueiredo, 2011b). The sarcophagus was buried in a SW-NE orientation with two brick fragments at each end and was probably infiltrated by very loose sand over time. High taphonomic processes were suggested because most of the appendicular skeleton was absent with the presence of malacological and ichthyological faunal remains inside the amphora (see *appendix I*). The orientation of the skeletal remains followed that of the amphora, as the skull was at the SW limit (the mouth of the container) and the tibia was at the opposite limit. It was deduced that the individual was deposited in a dorsal decubitus position with the lower limbs stretched out and parallel to each other, as observed in other amphora burials. The age of approximately 39 weeks of gestation led the anthropologist to consider a birth that probably ended in a stillbirth (M. Figueiredo, 2011b).

Sepulchre C also contained primary burial of a child (individual [868]/T.ONM-C) in a very poor state of preservation, buried in a wooden coffin and in a supine/decubitus position with NW-SE orientation. The best-preserved bones include the thorax with some vertebrae and ribs still connected and fragments of the skull, mandible, humerus, and tibia were found *in situ* while others were more dispersed. Some deciduous teeth (molars still in formation) were also found. Preferential preservation has been observed on the left side and the thoracic area, which is explained by the presence of another brick structure (probably a tomb) which cut this burial. Evidence of the wooden coffin is the lateral iron nails horizontally arranged in the four corners of the open trench. Based on the anthropological study of this skeleton, the age at death estimation placed the individual to be between 1.5 and 2.5 years of age with no associated pathologies. Indication of taphonomic agents (rodent) was observed on the shafts of the long bones (M. Figueiredo, 2011b).

About two months after the exhumation of the children's burials, archaeological rescue intervention began in two graves located at Ponta do Verde which were met with some degree of destruction due to tidal force and human violation (vandalism). The first one (disturbed on the outside by tides) was a mensa grave containing a primary deposition of an adult female individual [897]/T. PV-897 in a supine position inside an anthropomorphic box of brick covered in stucco, terracotta tiled base, and opus signinum walls oriented in a NW-SE direction (see *appendix 1b*). The second one (Inv. nº 8665) was a human-violated narrower (mensa) tomb box with walls, base, and cover made of plastered tiles juxtaposed in pyramidal or inverted V form. Inside it, an adult male individual (T. PVSV) buried in West-East was exhumed, with no anatomical connection due to the violation and destruction the grave suffered. Evidence of degenerative disease is distributed throughout the spine and pelvis of this individual while some degenerative, infectious, and oral pathological evidence were also observed on individual [897] (M. Figueiredo, 2011a).

Furthermore, individual [897] was found wrapped in roots inside the mensa grave, which somehow held the skeletal remains together, but also made them more fragile through the penetration actions. Direct action of water and humidity was also observed inside the structure, which destroyed the cortical tissues of long bones. Meanwhile, anthropic action was most evident in the violated grave, which led to partial destruction of the grave as well as scattering and destruction of the inhumed skeleton (see *appendix 1b*). Based on their locations, the funerary treatments, absence of associated grave goods, they were said to be deposited during the later occupational period in Tróia, between the 4th and 5th centuries AD (M. Figueiredo, 2011a; Pinto, 2009).

3.2.4. 2017 Excavation

During this excavation period, a set of large faunal bones which is now known to be of donkeys were found in the abandoned vat of workshop 23 in the stratigraphic unit (EU) 1355. The remains correspond to part of a jaw with teeth in connection, kneecap, femur, ribs, and part of the vertebrae still in connection (Pinto et. al., 2018).

Table 1: Summary of the Excavations and context of the individuals selected for this study based on the reports archaeological report

Year	Burial Type/ Context	Period	Exc. Code	Lab Code	Condition
2008	> Roman (Amphora)	Late Antiquity (IV century)	UE [525]-Amphora 2	T.OF1-L	Good state of Conservation
2010	> Roman > Rectangular-shaped structure with walls covered in bricks and mortar.	Late Antiquity (IV-V century)	UE [742]	T.OF1-SB	- Good state of preservation. - Scallop on the left forearm and coin next to the skull. -Taphonomic alteration due to grave violation by humans, humidity, and acidity.
	> Roman > Tile-covered burial	Late Antiquity (IV-V centuries)	UE [680]	T.BBT	- Poor preservation state - dispersed bones Anatomical connection absent. -Taphonomic action of Rodents, root and tidal wave.
2011	>Roman >Hole with anthropomorphic configuration, ceramic walls with lid made of brick fragments and tegulae	Late Antiquity	UE [855]	T.OMN-A	- Good preservation. - Taphonomic action of root

> Roman -Amphora (Almagro 50)	Late Antiquity (IV century)	UE [870]	T.ONM-B	-Poor preservation state. -High taphonomic processes. -Represented by a few fragments of the skull and left tibia.
-Roman - wooden coffin evidenced by the presence of nails. - cut by another brick tomb.	Late Antiquity	UE [868]	T.ONM-C	-Poor conservation state. -Preferential preservation on the left side (thoracic) -Presence of taphonomic marks from rodents on the diaphysis.
-Roman/Christian - Mensa tomb	Late Antiquity (IV-V centuries)	UE [897]	T. PV-897	- relatively good preservation. - Bones covered by a mesh of roots.
- Roman/Christian - Mensa tomb? - Violated	Late Antiquity (IV-V centuries)	UE 8665	T. PVSV	- Poor state of preservation. - fragmented and fragile skeletal remains -

Table 2: Anthropological and Pathological Information based on the reports

Context	Exc. code (individual)	Sample	Est. Age	Gender	Bone /Teeth Pathology
Oficina 1- amphora 2	525	T.OF1-L	32 uterine weeks	-	-
Oficnal 1 (rectangular grave)	742	T.OF1-SB	9 – 11year	-	-
Sector 5 Burial under tile	680	T.BBT	6 -7 ± 2 months of gestation	-	-
ONM burial A	855	T.ONM-A	Approx. 2 years	-	Tartar on the teeth
ONM burial B	870	T.ONM-B	38-40 uterine weeks	-	-
ONM burial C	868	T.ONM-C	1.5 – 2.5 years	-	-
Ponto do Verde (mensa tomb)	897	T. PV-897	> 30 years	Female	DJD - Joint arthrosis & enthesopathies overused knees & ankles, tooth wear, ante-mortem tooth loss, large caries and tartar suggesting a probable lack of oral hygiene
Ponto do Verde - violated tomb	8665	T. PVSV	> 30 years	Male	-Osteophytosis on the thoracic vertebrae, -DJD on the iliac auricular surface, -enthesopathies on the trochanter, digital fossa linked to overuse of the gluteus, obturators, & iliopsoas. >> prolonged walking -occlusal wear, antemortem tooth loss, caries, line of tartar

3.3. Funerary Archaeological Information

High infant/children mortality in antiquity has been inferred in the Roman world and the modalities of the funerary treatment employed depended on such factors as the age and social class of the dead within the society, and even the condition of the parents. These invariably led to the proliferation of different forms of deposition of the dead over time (C. Pereira & Albuquerque, 2018).

A common practice in antiquity was the use of amphorae as funerary containers for burying foetuses, neonates, and infants. The oldest child amphora burial in the Iberia was discovered in La Calle Quart (Valencia) in coexistence with both cremation and inhumation rites of the 1st century BCE. The dispersion of infant burials in amphora along the Mediterranean to the Atlantic coasts in the Iberian Peninsula has been documented by different researchers stretching from the 1st century BCE to the 5/6th century AD (C. Pereira & Albuquerque, 2018). In Tróia, the occurrence of this burial type increased progressively from the end of the 3rd and beginning of the 4th centuries AD (M. Figueiredo, 2008; C. Pereira & Albuquerque, 2018). These are known in other Roman necropolises in the Iberian Peninsula such as necropolises of Chipiona, Cadiz, Merida, Punta Umbria in Huelva, Ampurias, and Molino (Murcia), Tarragona, Malaga, Cordoba and many more. Even so, parallels of this burial type were also seen in other provinces of the Roman Empire including Africa (Norman, 2003; C. Pereira & Albuquerque, 2018).

The place of burials and type of the grave constitute the clues to decoding the mentalities and the diagnostic socio-cultural cum economic framework of the exhumed population. The choice of funerary space, the orientation of the dead, and the associated objects/materials are important in understanding the past funerary practices in certain locations over time (M. Figueiredo, 2008, 2011b). Burials in amphorae are perceived from classical sources to be representative of the womb, symbolizing a return of the baby to the intrauterine life, earth, or attempts at a chance of rebirth. Some authors also see the use of amphorae to differentiate neonates and infants from adolescents and adults in the same funerary space (Norman, 2003; C. Pereira & Albuquerque, 2018). The occurrence of ichthyological faunal remains and seeds inside the amphora burial of individual [525] may indicate the presence of funerary offerings and/or rituals which has been observed in similar contexts (M. Figueiredo, 2008).

The mensa tombs found in the archaeological site of Tróia are either plain or sigma shaped, and they have been found in different areas of this site. The absence of grave goods and the depositional orientation of both individuals from Ponta do Verde are suggestive of a Christian affiliation in which the body was buried according to the Jewish tradition (M. Figueiredo, 2011a; Pinto, 2009).

More so, there was diversity in the funerary practices of the Iberians during the Roman rule, as it involved less cremation, more inhumation, tiles-built graves, amphorae burials, and even prone burials with carelessly deposited human remains (Lopez-Costas, 2015). The identified grave types considered thus far in this study differ in their architectural structures and body treatment, which further indicates the existence of diversity in the community´s approaches to death during the Roman periods. Even so, a possible standardisation of funerary rituals has been suggested during the Late Antiquity (A. Figueiredo, 2001; Lopez-Costas, 2015). Based on different funerary studies of the Roman Era, a pattern of deposition was suggested where foetuses/stillbirths were often buried under houses, infants in necropolises or spaces deposited in coffins (including amphorae), children between 7 and 10 years in necropolises with graves architecture similar to those of adults (C. Pereira & Albuquerque, 2018).

4. Biochemical Approach

4.1. Bone Structure and its Diagenetic Alteration

Human and faunal remains represent direct evidence of past biological cum socio-cultural complexities of different locations of their origin and where they were deposited. Skeletal remains, in contrast to other archaeological resources, offer direct access to the individual level of investigation which can be contextualised within the population (Pate, 1994). And their study provides insight into the health and well-being, dietary history and migration pattern, lifestyle, violence and trauma, ancestry, demography, subsistence strategies and eventual economic needs, as well as other cultural practices of the past (Larsen, 1998; Reitz & Wing, 2009). On the one hand, understanding skeletal biology from a socio-cultural perspective is necessary to draw such conclusions. Having the knowledge of bone and teeth formation, their compositions, remodelling, as well as how discrete chemical signatures from the food and water ingested are incorporated during various stages of life, is crucial on the other hand (Moles, 2012; Pate, 1994, 2008). Also worthy of consideration are intrinsic and extrinsic factors that contribute to the degradation of the organic and mineral components of the bones after deposition, which would invariably affect the chemical elements of interest for this kind of study (Child, 1995; A. M. Katzenberg & Saunders, 2008). Interpretation of such data also requires understanding bone degradation phenomena from a physico-chemical point of view. In this chapter, attention will be paid to the structure and composition of the bones and how they can be diagenetically altered, while the elements from which the human and faunal dietary information can be inferred will also be discussed.

4.1.1. Structure and Composition of the Bone

Like any other connective tissue, the bone is made up of two major components – the cells, and the intercellular matrix where the cells are immersed. At the molecular level, the bone matrix is a highly specialized material of about 70 inorganic (mineral) and 30% organic components. The organic matrix comprises mostly structural protein (collagen), non-collagenous protein (proteoglycans, glycoproteins, phosphoproteins), lipids, carbohydrates, etc. while the inorganic fraction is calcium and phosphate in the form of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] also known as bioapatite (Child, 1995; A. M. Katzenberg & Saunders, 2008; Larsen, 1998). The collagen molecules in bones are intertwined to form flexible, slightly elastic fibres filled and stiffened by a dense inorganic material (hydroxyapatite), thereby serving a mutual relationship of flexibility and rigidity respectively and bones are made strong by this interplay of proteins and the mineral (White et al., 2019). Macroscopically, bone is of two types, compact (cortical) and spongy (cancellous/trabecular) bones (*Figure 13*). Compact bone is the solid, dense material in the walls of the bone shaft and on the external bone surface while the spongy type is composed of a more lightweight, porous, honeycomb structure found under the protuberance of tendon attachment, ends of the long and short bones, and even in-between the flat bones. Both are created by the layering and ring-shaping of collagen fibrils surrounding the osteon. They are cellularly and molecularly similar in compositions with porosity and structure as the distinguishing factor differentiating one from the other. (Child, 1995; White et al., 2019).

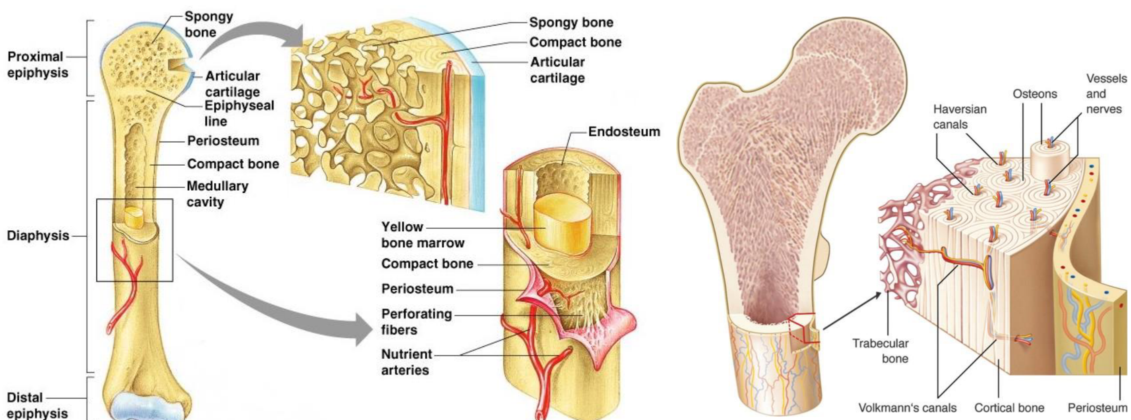

Figure 13: Structure and anatomy of the bone (sources: Bartl & Bartl, 2019 & https://onlinesciencenotes.com)

Within the bone, there are three primary living cells involved in the formation and maintenance of the bone tissue, such as the osteoblasts which are responsible for bone formation, osteocytes are groups of osteoblasts surrounded by a bony matrix and are involved in bone tissue maintenance, while osteoclasts are cells involved in bone resorption (White et al., 2019).

The most common protein in mammalian tissues is collagen, which is composed of about 28 distinct types. Of interest in this study is the Type I collagen having a triple-helix structure with the amino acid sequence of glycine-proline-hydroxyproline found in bones (Child, 1995; MacRoberts et al., 2020; White et al., 2019). In proportion to their amounts in the diet, the elements released from ingested food are absorbed into the organic collagen and inorganic hydroxyapatite components of bones (and teeth). The proteins ingested are broken down into their constituent amino acids and are used to synthesize collagen and other new proteins (Pate, 1994, 2008).

Skeletal development (modelling) is associated with bone turnover, which occurs rapidly in infants and declines during subsequent growth until the attainment of skeletal maturity at adulthood during which it becomes zero. Afterwards, bone remodelling is responsible for about 95% of human bone tissue turnover. Throughout lifetime, bone remodelling and changes in chemical composition occur within the bone structural units with skeletal parts having different turnover rates. The compact bone has an annual turnover rate average of 2.5%, while the trabecular bone has 10% (Pate, 1994). Therefore, it is imperative to mention that age and type of bone can determine the rate and process of remodelling and even the composition of the bone, as it has been observed that bone is remodelled more quickly in infants than in adults which means that chemical composition of normal adult human bone will reflect long-term dietary signals, as collagen turnover rate is estimated to be about 10 years, and this also varies with bone types (A. M. Katzenberg & Saunders, 2008; Pate, 1994; Zalaite et al., 2016).

Because the part of objectives of this study is to utilise the bone isotopic compositions for dietary reconstruction, it is noteworthy to mention that the isotopic values of different skeletal tissue vary, due to tissue compositional differences, secondary fractionation effects and chemical change from the different dietary component. The analysis of bone collagen will reflect the dietary proteins, while the analysis of bulk bone (apatite) will show the signal of the whole diet, although this reflects an average of the geochemical signature of the main foodstuffs ingested during the last 10-15 years before death, depending on the turnover rate of the bone part and age. Also, the availability and level of alteration allowed by the archaeological bone material should be considered (Cersoy et al., 2017; DeNiro & Weiner, 1988; Larsen, 2002; Malainey, 2010; Maurer et al., 2011; Pate, 1994). Despite the potential of archaeological bones in revealing information about palaeodietary habits, their isotopic compositions can be altered by diagenetic alteration and understanding these post-mortem processes are critical if we are to make reliable dietary reconstructions.

4.1.2. Bone Diagenetic Alteration

After deposition, a lot of changes occur to the bone underground which mostly depends on the chemistry and biochemistry of the burial environment (Child, 1995; Malainey, 2010). However, diagenetic studies also focus on how the bone compositions were transformed, in addition to the burial environment or activities that modify the character of the bones during burial. Due to their chemical and structural differences, the organic and inorganic components of bones degrade in very different ways (Lee-Thorp, 2008).

Archaeological bones can be altered by cation and circulating organics uptake, ion exchange, collagen breakdown and leaching, microbial attack, alteration and leaching of the mineral through post-mortem processes involving dissolution, precipitation, mineral replacement, recrystallization, crystal growth, and ionic substitution, amongst others (Hedges, 2002). These arise when microbial agents, plants, soils, and groundwater comes in contact with the bone's organic and mineral content in the deposition environment, along with other activities that physically modify or introduce impurities that speed up degradation during burial, exhumation, and storage. Collagen breakdown and chemical demineralization of bone apatite are the two main effects of bone deterioration (A. M. Katzenberg & Saunders, 2008; MacRoberts et al., 2020).

It is now understood that bone dissolution due to soil chemistry and groundwater are very important factors in bone degradation by demineralizing and increasing the pore size of bones. During this process known as hydrolysis, groundwater acts as the medium for mineral ion exchange between the bone and the surrounding soil, rock, or grave surface. The rate of dissolution is predetermined by the soil pH, degree of water infiltration, and concentration of the chelators (Biltz & Pellegrino, 1977; Child, 1995). These consequently further expose the bone to more microbial invasion, because this initial demineralization and increased pore size allow the large-size bacterial collagenases into the bone (Nielsen-Marsh & Hedges, 2000; Pate, 1994).

Bioapatite contains carbonates which are deposited from dissolved bicarbonate in the circulation that comes from all dietary components. This is due to the ionic substitution of carbonate, citrate, and other trace elements from consumed diets and water in the crystal lattice of the calcium, phosphate, and hydroxyl positions of the bone mineral phase (Pate, 1994). Specifically, carbonate (CO_3) or hydrogen carbonate (HCO_3^-) can be substituted for the phosphate group (forming structural carbonate), and in some cases, the hydroxyl sites (adsorbed carbonate) (Biltz & Pellegrino, 1977; Lee-Thorp et al., 1989; L. E. Wright & Schwarcz, 1996). For instance, the leaching of calcium and phosphate ions from the bone mineral by soil moisture can also lead to precipitation, recrystallization, and even hetero-ionic substitution resulting in the formation of such compounds as vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$], brushite ($CaHPO_4 \cdot 2H_2O$) within the bone. Because it occupies a larger space than apatite crystals, the presence of calcium carbonate ($CaCO_3$) in addition to soluble salts within the soil causes internal stress, brittleness, cracking, and damage to bone morphology in locations with alternating temperatures and humidity. All these are indicators of increased crystallinity as larger crystals are formed (Child, 1995; Hedges, 2002; Lee-Thorp, 2008; Nielsen-Marsh & Hedges, 2000).

Additionally, when body tissue decomposes after death due to microorganisms and enzymatic processes, cells are digested through their own hydrolytic enzymes (autolysis), and bacteria break down tissue proteins into ammonium compounds (putrefaction). This results in the production of collagenase and collagen breakdown by microorganisms that feed on the organic carbon and nitrogen from the collagen. Furthermore, the presence of protease (e.g., chymotrypsin) also contributes to this process of collagen degradation. Through these processes, an acidic microenvironment is created, thus promoting the dissolution of the inorganic phase of the bone mineral (Child, 1995; Pate, 1994).

Bones are better preserved in neutral slightly alkaline soils because of low tendencies of bone dissolution (Child, 1995).

Nevertheless, it is crucial to stress that bone diagenesis is a complex phenomenon that involves several processes and is dependent on a number of factors (such as pH, hydrology, redox condition, geology, microbial attack, temperature, humidity, etc.) as we move from one site to the other (Nielsen-Marsh & Hedges, 2000; Pate, 1994). Therefore, a multi-analytical approach is required to assess the degree of archaeological bone degradation and enhance the interpretation of bone geochemical analyses (Cersoy et al., 2017; Child, 1995; Hedges, 2002; Malainey, 2010). To explore these, chemical (EA-IRMS), mineralogical (XRD), and spectroscopic (FTIR) techniques are employed to ascertain the preservation status and extent of contamination of the human bone samples under study.

4.2. Analytical Techniques Employed for Diagenetic Assessment

4.2.1. Fourier Transform Infrared (FTIR) Spectroscopy

The term spectroscopy simply refers to the interaction of electromagnetic radiation (light) with matter which can occur in several forms such as absorption, emission, diffraction, transmission, and so on. Infrared spectroscopy is a technique used to measure the molecular vibrations resulting from the interaction of infrared (IR) radiation with matter. (El-Azazy, 2018; Monnier, 2018).

Summarily, IR spectroscopy is based on the fundamental principle that absorbed energy (IR) matching the vibrational frequency of a sample can initiate molecular vibrations because of the change in dipole moment by inducing continuous oscillations of the atom´s positions around their bonds in the molecules of the sample (El-Azazy, 2018; Monnier, 2018). The vibrating dipole moment creates a dipolar electric field that absorbs a discrete amount of IR energy unique to that transition (Monnier, 2018; Stuart, 2005). Therefore, when IR radiation is passed through a sample, part of the radiation is absorbed by the sample, and some are transmitted through. The excitation due to the absorption of light/photon (i.e., IR) causes molecular vibration of the bonded atoms in stretching bending, and torsional modes. Stretching affects the distance between two atoms i.e., bond length: and bending causes angular change between two atoms. The IR spectrometer (detector) measures the energy absorption and the resulting signal (spectrum) from the detector is like a fingerprint used for the sample identification, characterisation, structure elucidation, and quality assessment (El-Azazy, 2018; Monnier, 2018; Stuart, 2005).

In the earlier IR equipment (spectrophotometer), spectrum acquisition is time-consuming as the monochromator scans through all wavelengths, recording a single frequency at a time (Monnier, 2018). However, the Fourier transform spectrometer employs a Michelson interferometer composing a beam splitter and two mirrors. In this type, half of the beam from the source is transmitted and the other half is reflected to the moving and stationary mirrors respectively. After striking the mirrors, they are again reflected to the beam-splitter where they converge to irradiate the sample (figure 14). The emerging radiation from the sample is collected as an interferogram by the detector to be converted via a Fourier transform using a high-performance computer through which individual absorption frequencies are analysed simultaneously and a spectrum of intensity versus frequency is plotted (El-Azazy, 2018; Hesse et al., 2008; Leng, 2010; Monnier, 2018; Stuart, 2005; Theophanides, 2012). This innovation has resulted in a greater signal-to-noise ratio, improved resolution and sensitivity, and shorter analysis time in a matter of minutes (Monnier, 2018).

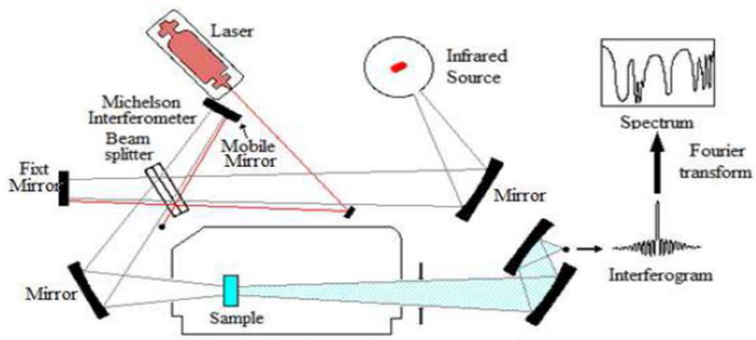

Figure 14: Modern FTIR schematic illustration (Theophanides, 2012)

Samples can be examined in two different modes, namely transmittance and reflectance techniques which allow analysis of a variety of samples and the derivation of different information. Attenuated total reflectance (ATR) was used in this study as it is based on the idea that reflection takes place at the interface of two media when radiation is transmitted from a higher refractive index medium to one with a lower refractive index. The reflected fraction of the beam increases as the angle of incidence increases. Depending on the refractive indices of the two media, If the angle of incidence is beyond the critical angle, a total internal reflection occurs as all radiations are completely reflected. This creates an evanescent wave that penetrates a few microns (about 2µm) into the samples which are then passed back to the sample surface opposite the region of incidence and collected by the IR detector (Monnier, 2018; Stuart, 2005). ATR mode allows the acquisition of structural information from the surface, and it is more favoured due to its short measurement time, suitability for all sample types, requires no sample preparation, ease of use, and even high-quality spectra databases for material identification and verification (Monnier, 2018). Therefore, FTIR spectroscopy is of high applicability to a variety of archaeological studies, as exemplified through this study.

4.2.2. X-Ray Diffraction (XRD)

X-ray diffraction is a technique which provides detailed information about the crystallographic structure and mineralogical composition of crystalline materials. It is used to characterise polymorphs and also to derive information about the presence of amorphous materials (Ali et al., 2022; Artioli, 2016; Bunaciu et al., 2015a).

This technique is based on the principle discovered by Max von Laue in 1912, that crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. In other words, light or radiation is diffracted when it impacted a three-dimensional array of atoms, molecules, or ions. This is expressed by Bragg´s law as $n\lambda = 2d\sin\theta$, where n is an integer, λ = radiation wavelength, d is the interplanar spacing generating the diffraction, and θ = diffraction angle (Ali et al., 2022; Bunaciu et al., 2015a; Day et al., 2016).

In this technique, X-rays (from the cathode ray tube, e.g., Cu) are filtered to produce monochromatic radiation which is then directed towards the sample (see *Figure 15)*. The interaction of the incidence rays with the sample produces constructive interference (and a diffracted ray) when the condition satisfies Bragg´s Law, which relates the wavelength of the radiation to the diffraction angle, and lattice spacing in a crystalline sample. The diffracted X-rays are then processed and counted by the detector (Bunaciu et al., 2015a), and plotted in the diffractogram as intensity vs 2θ (see *Figure 15)*, where 2θ is the sum of the incidence angle (θ) and reflection angle (θ)(Day et al., 2016). Based on the modes being utilised for analysis, the XRD instrumentation is used in a variety of geometry depending on the type of sample, size, and questions to be answered.

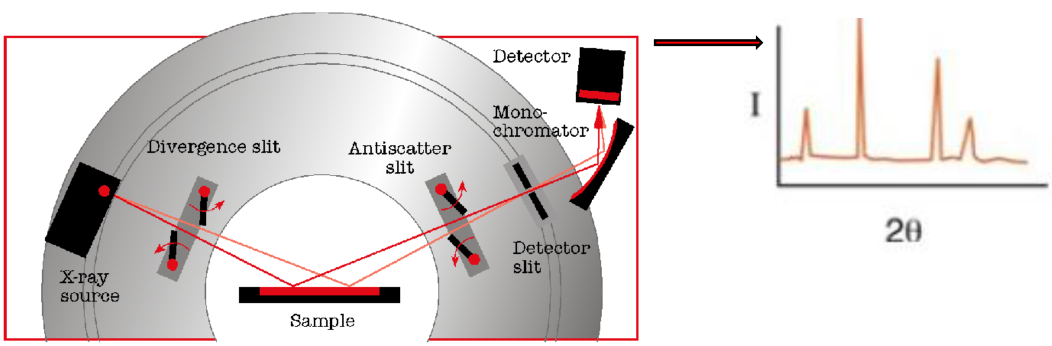

Figure 48: XRD goniometer in Bragg-Brentano geometry showing process of analysis (adapted from (He et al., 2003)

X-ray powder diffraction (XRPD) is usually employed due to the fact that most archaeological materials are polycrystalline and polyphasic (with few exceptions such as gem crystals), and most powder diffractometers use the Bragg-Brentano parafocusing geometry (Artioli, 2016; Bunaciu et al., 2015a). The Bragg-Brentano geometry allows the placement of the sample in the path of the collimated X-ray beam moving at an angle θ while the diffracted X-rays are collected by the detector which rotates at an angle of θ. Scanning the sample through a range of 2θ angles allows the measurement of the lattice in all possible diffraction directions (Bunaciu et al., 2015a; Day et al., 2016), and peaks are converted to the d-spacings used as fingerprints for identifying a compound (or crystalline phase or mixture of phases) because each compound has a set of unique d-spacings, which are compared with standard reference patterns. (Artioli, 2016; Bunaciu et al., 2015a).

Finally, XRD is advantageous for archaeological studies due to its fast, and easy sample preparation, high accuracy for mineral determination, as well as the availability of reference standards for thousands of materials (Bunaciu et al., 2015a; Schreiner et al., 2004), thereby making this method applicable for this study.

4.3. Dietary Reconstruction through Stable Isotopes.

Application of isotopic exposition in archaeology to understand past diets began in the late 1970s, although their discovery, studies, and applications in such fields as chemistry, physics, biology, and geochemistry started much earlier in the 20th century. Advancement in the instrumentation, improved resolution, detection, and protocols, as well as reduced time of analysis and cost, has made isotope analytical method more suitable in diverse archaeological aspects revealing previously unknown information regarding human, animal, and plant populations and ecological dynamics (A. M. Katzenberg & Saunders, 2008; Reitz & Wing, 2009; Vaiglova et al., 2014).

Any chemical element whose atoms possess the same number of protons, but a different number of neutrons is referred to as an isotope (A. M. Katzenberg & Saunders, 2008; Malainey, 2010; Schoeninger & Moore, 1992). They occur naturally in two forms – stable isotopes (e.g., ^{12}C) which remain fixed, and unstable/radiogenic isotopes (e.g.,^{14}C) which decay overtime (useful for dating) with stable isotopes having higher relative abundance. Based on their physical and chemical properties, stable isotopes can be categorised into light (e.g.,^{12}C) and heavy isotopes (e.g.,^{13}C), and the lighter one is usually favoured over the heavier in a chemical process. This usually results in the enrichment of the product with light isotopes because they react more rapidly than the heavier ones, which simply means they are not incorporated into living tissues at the same rate. (A. M. Katzenberg & Saunders, 2008; Lee-Thorp, 2008; Malainey, 2010).

Therefore, dietary reconstruction through stable isotope analysis relies on the principle that humans and animals retain the chemical signatures of ingested food and water in their body tissues, and the values of incorporated stable isotopes ratios remain more or less the same even after death

(Schoeninger & Moore, 1992; R. Tykot, 2006; Vaiglova et al., 2014). Isotopic ratios and compositions may vary due to several factors such as fractionation pathways, equilibrium and kinetic effects, chemical and biological processes, underlying geology, surrounding environment and soils, and post-mortem processes (Badeck et al., 2005; Bishop et al., 2020; Brugnoli & Farquhar, 2000; Garvie-Lok et al., 2004; Lee-Thorp, 2008; Van Der Merwe & Vogel, 1978).

Analysing the ratios of the stable isotopes in archaeological bones is a powerful approach for dietary investigation and assessment of the important food types and lifestyles in past populations because isotopic ratios of elements (e.g. carbon and nitrogen) vary in different food types (Brugnoli & Farquhar, 2000; Hunnis, 2007; Larsen, 2002; Lee-Thorp, 2008; R. Tykot, 2006). The information that such stable isotopes convey about nutrition indicates a period of time previous to an individual's death since bones are renewed and remodelled throughout life (see *section 4.1.1*), thus their composition represents an average of the time that depends on the bone type. (MacRoberts et al., 2020; Schoeninger & Moore, 1992). Furthermore, isotopic studies are being utilised in a variety of archaeological studies such as palaeoecology, palaeoclimatic reconstruction, provenance, mobility patterns, and forensics, amongst many others.

4.3.1. Stable Isotopes of Carbon

Of the three carbon isotopes, two exist naturally in stable forms, namely ^{12}C (which is about 100 times more abundant) and ^{13}C. (Larsen, 1998; Malainey, 2010). But the three are incorporated into aquatic and terrestrial plant tissues through photosynthesis, with the ratios of stable carbon isotopes in animal tissues (e.g., bone) coming from the diets consumed (Larsen, 1998). Put differently, due to the slight physico-chemical differences between the two stable carbon isotopes in mass, the lighter ^{12}C is more favoured than the heavier ^{13}C during the photosynthetic process in plants and the resultant $^{13}C/^{12}C$ ratios are passed to the animals and humans from the plants consumed (Caemmerer et al., 2014; Larsen, 1998; O`Leary, 1988; Pate, 1994). In a bid to adapt to the climatic conditions of the place they are grown, terrestrial plants photosynthesize (using atmospheric CO_2 whose $\delta^{13}C$ = -7 to -8‰) through three different mechanisms – including C_3, C_4, and CAM (Crassulacean Acid Metabolism) pathways which produce distinct $^{13}C/^{12}C$ values (Lee-Thorp, 2008; Pate, 1994; Schoeninger & DeNiro, 1984; Schoeninger & Moore, 1992). In contrast, aquatic plants, mostly C_3 photosynthesize using dissolved inorganic carbon with $\delta^{13}C$ values of 0‰ and -5 to -10‰ for marine and riverine water respectively (Pate, 1994). Most of the world´s vegetation and plants used as food by humans are in the C_3 group including barley, wheat, rice, beans, tubers, nuts and other tree species, shrubs, herbs and grasses found in cool-temperate environs, while C_4 plants include grasses found in tropical and hot/arid regions such as millet, sorghum, maize, sugarcane etc. (Larsen, 1998; Pate, 1994; Sealy et al., 1995). Whereas, plants in the CAM group are somewhat arid-land succulents such as cacti, agave, orchids, bromeliads with the ability to switch to the mechanism of the other two groups based on the prevailing environmental conditions (Pate, 1994; Van Der Merwe & Vogel, 1978).

Different proportions of C_3, C_4, and CAM plants in the diet will influence the $^{13}C/^{12}C$ ratios of the consumers, which also dictate the carbon isotope composition reflected in the human and faunal bones, teeth and other tissues found in archaeological context (Bell et al., 2001; Van Der Merwe & Vogel, 1978). The ratios of these isotopes are expressed as $\delta^{13}C$ which represents the relative difference between the measured stable carbon isotope of the sample and that of an international standard known as Vienna Pee Dee Belemnite (VPDB). High value than that of the VPDB denotes enrichment while a lower value simply indicates depletion of ^{13}C (Bell et al., 2001; Malainey, 2010; Schoeninger & Moore, 1992; Sealy et al., 1995). This is expressed using the following equation:

$$\delta^{13}C_{sample} = \left(\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{standard}} - 1 \right) \times 1{,}000‰ .$$

Equation 1: Calculation of $\delta\ ^{13}C$ values from $^{13}C/^{12}C$ ratios

Based on the equation above, the more negative the $\delta^{13}C$ values, the more ^{12}C is present and the lesser ^{13}C present, while a more positive $\delta^{13}C$ value indicates more ^{13}C in the sample (see *Figure 16* & 17). Higher $\delta^{13}C$ value has been observed in C_4 plants than C_3 plants – as C_3 plants exhibit $\delta^{13}C$ values ranging between ca. -20‰ to -35‰ with an average of -27‰ (A. Katzenberg, 2012; Malainey, 2010; O`Leary, 1988; Pate, 1994) while $C_4$ plants have values ranging between -9‰ to -19‰ with an average of around -13‰ (Caemmerer et al., 2014; Larsen, 1998; Macroberts, 2017; O`Leary, 1988). Furthermore, the $\delta^{13}C$ values of CAM and marine plants are overlapping the values in C_3 and C_4 plants (Larsen, 1998; Pate, 1994; Schoeninger & Moore, 1992; Sealy et al., 1995; Van Der Merwe & Vogel, 1978).

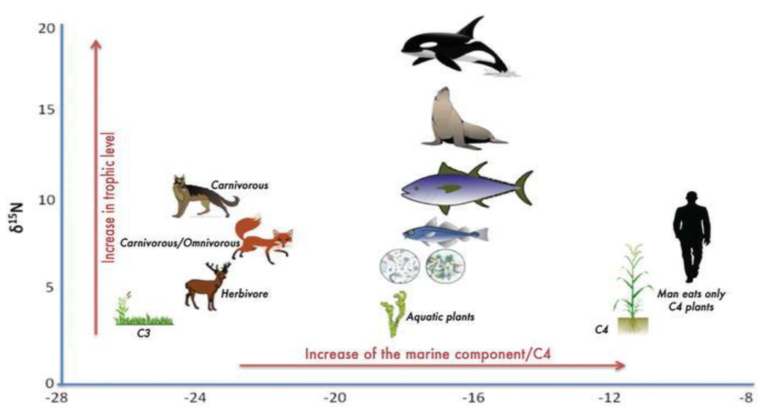

Figure 16: Carbon and Nitrogen Isotopic composition of different organisms (source: htt://chrono.qub.ac.uk – adapted from Schulting 1998)

The use of isotopic signals in animal tissues for dietary reconstruction purposes is not as straightforward as it seems, this is because of: (i) varying efficiencies of dietary components by the animals, (ii) further fractionation of dietary isotopes by animal tissues, and (iii) differential allocation of dietary nutrients to specific tissues, in addition to many digenetic factors (Gannes et al., 1997). However, the $\delta^{13}C$ values from a herbivore are typically less negative than the plants it consumes and the $\delta^{13}C$ values from a carnivore will differ from those of the herbivores it feeds on (Malainey, 2010), and this can be due to extra metabolic activities involved as itemized above. The extent of the variation detected largely depends on the tissue types being analysed because the process of nutrient assimilation varies from tissue-to-tissue intra-individual (A. Katzenberg, 2012). Therefore, a fractionation of +3 – 5‰ between plants (foods) and consumers values have been observed in the bone collagen of fauna and humans (Brugnoli & Farquhar, 2000; Gannes et al., 1997; Schoeninger & Moore, 1992; Van Der Merwe & Vogel, 1978), with an additional fractionation of +1‰ to +2‰ in omnivores and carnivores above the herbivore (primary consumers). Additionally, carbon isotopes in bone collagen are from diets of protein origin while the isotopes from bioapatite reflect the whole diet (protein, carbohydrate, lipids, etc) (Ambrose & Norr, 1993; A. M. Katzenberg & Saunders, 2008; Macroberts, 2017) Through this understanding, analysis of stable isotopes of carbon in faunal and human bones provide means of dietary reconstruction and gaining insights to the dietary contribution of food components (plants and animals) consumed. It has also been reported that the trophic level effect should be reflected between the mother and breastfeeding child, due to the tissue of the child reflecting elevated $\delta^{13}C$ values. Hence, an enrichment of about 1‰ of $\delta^{13}C$ has been recorded by Fuller et al. (2006).

4.3.2. Stable Isotopes of Nitrogen

Nitrogen is an essential element in protein synthesis which is made up of amino acids (the fundamental units of proteins). Typically examined is the stable isotope ^{14}N which makes up about 99.6% of the nitrogen found on earth and the ^{15}N which occurs in a less-abundant form (Malainey, 2010). By comparing the ($^{15}N/^{14}N$) ratio of the sample to those of the international standard (AIR – Ambient Inhalable Air), the stable isotopes of nitrogen present in the sample can be determined using the following equation:

$$\delta^{15}N = \left[\frac{^{15}N/^{14}N \text{ sample}}{^{15}N/^{14}N \text{ standard}} - 1\right] \times 1000$$

Equation 17: $\delta\ ^{15}N$ values from $^{15}N/^{14}N$ ratios

Due to the depletion of ^{15}N in the atmospheric nitrogen, the $\delta^{15}N$ of air will be close to 0‰ and as a result, most of the measured samples will have a positive $\delta^{15}N$ value (Malainey, 2010). Due to their atmospheric nitrogen fixing abilities, leguminous plants present lower $\delta^{15}N$ values than those of the non-nitrogen fixing plants which use nitrogen available from the decomposed organic matter and the addition of fertilisers further increases the $\delta^{15}N$ values depending on the manure types (Mora, 2022).

Therefore, nitrogen isotopic composition in organisms reflects the combination of those of their diet. In the same vein, the $\delta^{15}N$ values of tissues of fauna or humans are more positive than those of their diets (see *Figure 17*) (Mora, 2022; Schoeninger & DeNiro, 1984). This is due to the preferential incorporation of ^{15}N by metabolic reactions in an organism compared to ^{14}N, thus, comparison of $^{15}N/^{14}N$ ratios can be useful in reconstructing trophic levels and related dietary sources, they are also vital in answering questions about weaning practices and pattern within populations (A. M. Katzenberg & Saunders, 2008; Malainey, 2010; Pate, 1994; Schoeninger & DeNiro, 1984; Van Der Merwe & Vogel, 1978).

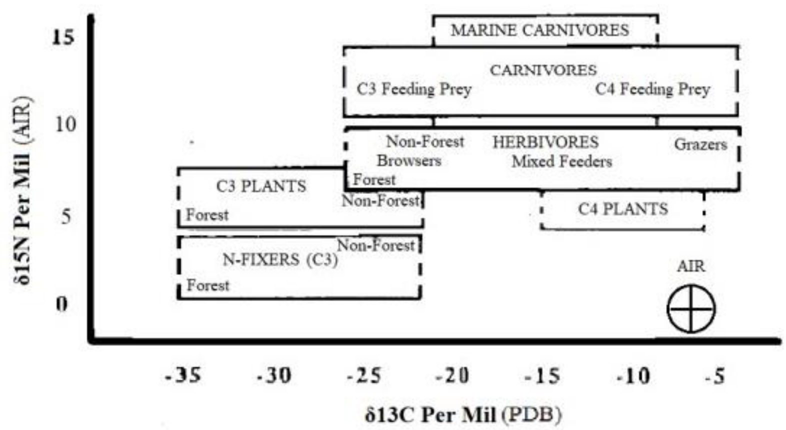

Figure 17: Average $\delta^{15}N$ and $\delta^{13}C$ values of Food sources (Miller, 2018)

When nitrogen isotopic compositions are compared, it can be possible to reconstruct the dietary intake of an individual relative to terrestrial versus aquatic food sources as well as legumes versus non-legume plants (A. Katzenberg, 2012; A. M. Katzenberg & Saunders, 2008; Schoeninger & DeNiro, 1984). As said earlier, since nitrogen fixers such as legumes have ratios lower than plants which assimilate other inorganic nitrogen (e.g., nitrate, ammonia), the consumption of these food sources can always affect the nitrogen isotopic ratio of the tissues of an individual (Mora, 2022; Schoeninger & DeNiro, 1984). Additionally, marine faunas such as fish have more positive $\delta^{15}N$ values than terrestrial counterparts which can be useful to distinguish food sources, although this depends on the trophic level of the fish and trophic chain in the aquatic ecosystem (Larsen, 1998, 2002; Schoeninger & DeNiro, 1984). Nevertheless, the bone collagen of consumers will show enrichment of $\delta^{15}N$ values by around 3-5‰ from the foods consumed, thereby showing an increase in the $\delta^{15}N$ values from one trophic level to the other (see figure 21 &22) (A. M. Katzenberg & Saunders, 2008; M. A. Katzenberg, 2007). In other words, nitrogen isotope ratios tend to reflect trophic level differences such that the higher an organism is in a food web, the more positive its $\delta^{15}N$ values become, and since the breastfed infants are consuming their mother´s tissue, they should be more enriched in $\delta^{15}N$ values than their mothers (M. A. Katzenberg et al., 1996). Children who are being breastfed will have $\delta\ ^{15}N$ values enrichment of around 2-4‰ compared to the weaned members of the same population. Based on the above, the age of weaning can be ascertained as there should be a sharp decrease in $\delta^{15}N$ values once breastfeeding has stopped. However, it should be noted that ingested breastmilk may not show immediately and can take up to 3 months to reflect isotopically due to bone turnover rates (A. M. Katzenberg & Saunders, 2008). Furthermore, individuals who died during childhood could be affected by nutritional stress which can affect nitrogen isotopic levels (Mora, 2022).

When considered separately, the interpretation of stable carbon and nitrogen isotope ratios is complicated by a number of factors such as precipitation, temperatures, altitude, soil salinity, the addition of fertilizers, preference of food type, and geographical range of food sourcing, additionally to other geological factors (Malainey, 2010; Mora, 2022; Schoeninger & DeNiro, 1984). Nevertheless, when their data are combined, they become an efficient method which provides a more comprehensive insight into dietary sources and behaviour (Keegan & DeNiro, 1988; Reitz & Wing, 2009). For instance, carbon isotopes alongside nitrogen analysis can enhance more understanding of breastfeeding practices, as an increase of even 1‰ carbon values for children of about 1 year old could be a sign of weaning due to solid foods introduction. The notion here is that there is a gradual decline in nitrogen values and an increase in carbon values during childhood (Fuller et al., 2006; A. M. Katzenberg & Saunders, 2008; M. A. Katzenberg et al., 1996; Siebke et al., 2019; L. E. Wright & Schwarcz, 1998). However, this is a gradual process as the reflection of isotope values within tissues can take time (Miller, 2018).

Furthermore, the period of elements incorporation into bone collagen of a growing foetus is based on nutrient availability in the mother´s womb and the rate of bone metabolism and turn over. A slight difference of about 0.4‰ increase in foetal δ^{13} C values compared to the maternal ones has been reported by de Luca et. al., (2012), Fuller et al., (2006) reported that there seems to be no difference (Tsutaya & Yoneda, 2015a). Herrscher et al. (2017) noted a difference of less than 0.5‰ between mother and child δ^{13} C and $\delta^{15}N$ values for the period close to birth since it takes about 24 and 12 weeks for nutrient incorporation to occur in mother and infant nails respectively, the change in isotopic values for mother and child during the last prenatal and first post-natal weeks was extrapolated (Herrscher et al., 2017). Therefore, the isotopic values in the foetus will reflect the mother´s diet to a certain extent (Siebke et al., 2019). Also, through comparative analysis of $\delta^{15}N$ values amongst infants, it is possible to detect the onset of breastfeeding signal in infants, which in the case of the study can be employed to discriminate between stillbirth and neonates who survived the birthing process (Siebke et al., 2019). Even so, such interpretation from δ^{13} C and $\delta^{15}N$ values can be challenging.

4.3.3. Stable Isotopes of Sulphur

Being the 15th most abundant element on earth, sulphur plays important role in the protein structure as sulphur-containing amino acids (e.g., cysteine and methionine) enhance protein folding. Methionine which is synthesized through diet also helps to initiate protein translation and methyl donation in humans and animals (Nehlich, 2015b), thereby reflecting the importance of sulphur in their growth and survival (Richards et al., 2003). There exist four stable isotopes of the element sulphur, namely ^{32}S which is the most abundant at around 95.02%, ^{33}S (0.75%), ^{34}S (4.21%), and ^{36}S (0.02%), among which the ratio between the lightest and heaviest sulphur isotopes (^{32}S and ^{34}S) is defined as $\delta^{34}S$ and measured relative to the meteorite standard known as Canyon Diablo Troilite (V-CDT) (Nehlich, 2015b; Richards et al., 2003), with $\delta^{34}S$ value expressed in per mil (‰) according to the following equation:

$$\delta^{34}S = \frac{\left({}^{34}S/{}^{32}S\right)_{sample} - \left({}^{34}S/{}^{32}S\right)_{standard}}{\left({}^{34}S/{}^{32}S\right)_{standard}} \times 10^{3}$$

Equation 33: $\delta^{14}S$ value from $^{32}S/^{34}S$ ratio

The oceanic dissolved sulphate (SO_4^{2-}), with a $\delta^{34}S$ value of near +20‰, evaporitic sulphates, and geospheric pyrite constitute the three major reservoirs of sulphur on earth. Sulphur is also present in the atmosphere, biomass (fossil fuel inclusive), and soils, and it is found in different forms e.g., dissolved sulphur dioxide ($SO_{2,}$), hydrogen sulphide (H_2S), minerals: pyrite ($FeS_{2)}$, barite ($BaSO_4$), anhydrite ($CaSO_4$), gypsum ($CaSO_4$ $2H_2O$) and several others bound by other elements etc (Nehlich, 2015b). With variable $\delta^{34}S$ isotope values based on age and rock type, sedimentary rocks are the largest reservoir of sulphur near the earth´s surface (Richards et al., 2003).

The bulk of sulphur in plants is absorbed through the roots as sulphate is produced from the weathering of local geological formations. Additionally, sulphur is incorporated into plants through wet deposition (precipitation of sea-sprayed sulphur or acid rain [H_2SO_4]) and dry deposition which occurs from SO_2 gas uptake. Absorption of atmospheric sulphur depends on the location and species of plants involved, and about 1/3 of plants' sulphur is atmospherically derived. Thus, resulting in variable values of $\delta^{34}S$ falling between extremes of -22 to +22‰ based on geology and location (Ebert et al., 2021; Richards et al., 2003). In the aquatic environment, there are marked variations among the $\delta^{34}S$ values of plant and animal samples from freshwater and marine ecosystems. Marine ecosystem typically has a mean $\delta^{34}S$ value of approximately +21‰ due to continuous oceanic mixing whereas freshwater organisms can have a range of $\delta^{14}S$ values between -20 to +14‰ due to sulphate ions reduction to hydrogen sulphide (that is depleted in $\delta^{14}S$) by anaerobic bacteria living in rivers and lake sediments (Ebert et al., 2021; Richards et al., 2003). Contrastingly, the $\delta^{34}S$ values in terrestrial environment range between +8 to +25‰ relating to the composition and age of local geology. Furthermore, the movement of sulphur through the sea spray effect can also lead to increased $\delta^{34}S$ values in the coastal terrestrial environment thus reflecting values like those of marine systems up to 30km inland (Ebert et al., 2021; Nehlich, 2015b; Richards et al., 2003). Put differently, humans and faunal remains from regions (sites) close to the sea (≤30km) will exhibit the sea spray effect, while fully terrestrial $\delta^{34}S$ values should be expected in regions that are about 40-50km away from the sea, where values are expected not to be lower than +14‰ (Nehlich, 2015b).

When sulphur is taken up by plants and animals (through diet), it is incorporated into the building blocks of proteins (amino acids - cysteine, methionine etc). In modern and archaeological bone, sulphur is incorporated in the inorganic matrix as calcium sulphate ($CaSO_4$) and within the collagen as methionine, while it is primarily derived from cysteine in hair with little contribution from methionine. Regarding the fractionation and trophic shift effects, there is a slight isotopic fractionation during sulphate incorporation and reduction in marine, freshwater, and terrestrial plants. That is, the total $\delta^{34}S$ value in plants has a closer range to the $\delta^{34}S$ value in local soil. In proportion with their sulphur sources, plants are depleted in $\delta^{34}S$ by ca. 1.5‰ while little enrichment occurs in consumers due to metabolic processing. The consumers (humans and animals) with natural diets tend to be more enriched in ^{34}S with average $\delta^{34}S$ values of +0.5±2.4‰, Therefore, the $\delta^{34}S$ values in the consumers reflect an average of soil bioavailable sulphur isotope ratios in a region (Ebert et al., 2021; Nehlich et al., 2011; Nehlich, 2015b; Richards et al., 2003). Terrestrial organisms usually have $\delta^{34}S$ values between 5-10‰ while freshwater organisms can have values ranging from -5 (or even lower) to 10-14‰(Britton et al., 2018; MacRoberts et al., 2020).

As a result of slightly differing $\delta^{34}S$ values between the consumers and their diet, the $\delta^{34}S$ values when used together with $\delta^{13}C$ and $\delta^{15}N$ values can provide information for dietary reconstruction and indicate possible marine resources exploitation, as well as indicate aquatic and marine protein contribution in subadults during the weaning period (Nehlich et al., 2011; Richards et al., 2003; Tsutaya & Yoneda, 2015b). Since the local food web $\delta^{34}S$ values depend on the geological, atmospheric, and soil microbial influences, $\delta^{34}S$ values from human and faunal remains can be used to identify local and extra-local (migrants) individuals in a region, provided they were from the locality with distinct environmental $\delta^{34}S$ values. (Richards et al., 2003)

When dealing with sulphur as well as other stable isotopes for dietary reconstruction, quality assessment is very crucial for reliable interpretation. In doing this, the quality and level of contamination of $\delta^{34}S$ values in bone collagen must be assessed through such criteria as the amount of C, N, S, as well as the ratios of C:S and N:S (Nehlich, 2015b; Nehlich & Richards, 2009).

4.3.4. Stable Isotopes of Oxygen

As one of the most prevalent elements on earth, oxygen (O) is a part of the hydrosphere, biosphere, and lithosphere's most frequent constituents. Three stable isotopes of oxygen with varying abundances exist naturally, namely ^{16}O (99.755%), ^{18}O (0.206%), and ^{17}O. Commonly analyse, however, is the ratio between ^{16}O and ^{18}O which is annotated as $\delta^{18}O$ values and measured against international reference materials Vienna Standard Mean Ocean Water (VSMOW) or Standard Light Antarctic Precipitation (SLAP), although SLAP is not available/used anymore, and values are expressed as per mil (‰) (Pederzani & Britton, 2019).

$$\delta^{18}O = \left(\frac{\left(\frac{18O}{16O}\right) sample}{\left(\frac{18O}{16O}\right) standard} - 1 \right) \times 1000$$

Equation 49: $\delta^{18}O$ values of $^{16}O/^{18}O$ ratios

Because the values for carbonates $\delta^{18}O$ values are often reported on the VPDB scale, conversion using the following equation by Coplen et. al (1983) is required (MacRoberts et al., 2020; Pederzani & Britton, 2019).

$$\delta^{18}O_{\ VSMOW} = 1.03091\ \delta^{18}O_{\ VPDB} + 30.91$$

Equation 65: Conversion of $\delta^{18}O$ VPDB values to VSMOW

The oxygen isotope ratio of the mammalian tissues is derived from the body water, and that of body water is determined by the oxygen isotopic composition of ingested water and food which, in turn, is also determined by the local rainfall (Lightfoot & O'Connell, 2016; Price, 2014). Secondary to precipitation as oxygen sources are food and atmospheric oxygen, and their reflection in skeletal tissues represents a mixture of sources subject to physiological and metabolic modifications, although consumed water has more influence with the reflection of local variability in the tissue oxygen values (Pederzani & Britton, 2019).

As an original water source, isotopes in rainfall can vary due to the enrichment or depletion of heavy $\delta^{18}O$ isotope relative to light $\delta^{16}O$ in water due to evaporation, condensation, and precipitation events. For instance, during the evaporation event in a water body, there is a preferential accumulation of lighter oxygen isotope ^{16}O to the vapour phase, thereby leaving the water body more enriched in heavier ^{18}O. As the vapour moves over land, it becomes enriched in lighter oxygen isotope such that the first rain contains heavier isotope, and as the clouds move more inland and even to higher elevations, the rain becomes comparatively depleted in ^{18}O than as it was in the first rain (see Figure 23) (Price, 2014; L. E. Wright & Schwarcz, 1998).

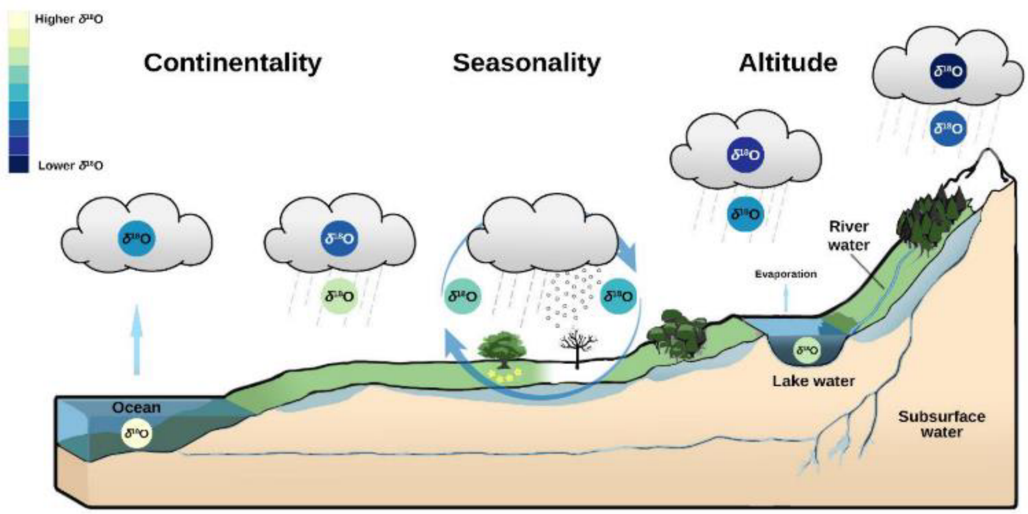

Figure 18: Geographical variation of oxygen isotopes in hydrological cycle (Pederzani & Britton, 2019)

The fractionation effects described above resulted from a number of geographical factors determining rainfall $\delta^{18}O$ values such as latitude, elevation, amount of rainfall, temperature, and the distance from the evaporation source (e.g., ocean), relative humidity, seasonality and so on (Pederzani & Britton, 2019; Price, 2014). Based on these, the oxygen isotope value of precipitation is strongly positively correlated with surface air temperature at mid- and high-latitude regions, and in tropical regions, it is negatively correlated with the amount of rainfall, as seasoner changes in rainfall $\delta^{18}O$ values are influenced by the amount of the rainfall (Pederzani & Britton, 2019). Higher oxygen isotope values may indicate a warmer period and while low values may be reflective of a colder period (MacRoberts et al., 2020). Therefore, the ratio of oxygen isotopes differs geographically and can be used to deduce information about human/animal movement (which is complicated) and palaeoclimatic/temperature reconstruction (Frantz et al., 2019; Lightfoot & O'Connell, 2016).

The use of oxygen isotope as a method of identifying migrants is based on the assumption that – with the existing geographical variations in the isotope ratios of environmental water, it is possible to identify individuals whose oxygen isotope ratios are different from those expected at the place of burial. In terms of application, this is also based on the assumptions that the oxygen isotope ratios of rainfall in different geographical areas vary, and these ratios are incorporated within human bodies in such a way that the differences in oxygen isotope ratios of rainfall between areas are preserved (Lightfoot & O'Connell, 2016). However, this is not always the case because drinking water is obtained from different water bodies (e.g., rivers, lakes, springs, and wells) and they generally reflect the average isotopic values/range of the precipitation within their localities. However, these water sources may have differing $\delta^{18}O$ values from that of local precipitation due to the influx or transport of non-local water (with low $\delta^{18}O$ values) from higher elevations, evaporative enrichment in standing water bodies, e.g., in lakes, (Pederzani & Britton, 2019), exchange between geological formation, past water body recharge when the isotopic composition of rainfall was different from the present, fractionation during water movement through soil or aquifer, and short-term isotope variation in rainfall, amongst others (Lightfoot & O'Connell, 2016).

Additionally, various cultural customs including cooking, brewing, boiling, diet, breastfeeding, and long-term water storage may also have an impact on the $\delta^{18}O$ values of the skeletal tissues (Price, 2014). For instance, because milk is enriched in $\delta^{18}O$ in proportion to local water, milk consumption may also influence consumer $\delta^{18}O$, and even foreign food and drink may have a different $\delta^{18}O$ value than the local water. However, the $\delta^{18}O$ in bone or teeth carbonate and phosphate are linked to that of body water and body temperature, although values may also vary as oxygen isotopic fractionation can be influenced by physiological factors such as metabolic rate and disease, etc. that occurs during tissue formation. The oxygen isotope values in mammalian skeletal tissues are, however, closely correlated with those of the local precipitation and thus reflect the local climate (Lightfoot & O'Connell, 2016; Pederzani & Britton, 2019). Therefore, it is assumed that irrespective of the varying oxygen isotope values of the water sources, the skeletal $\delta^{18}O$ values are reflective of the local precipitation $\delta^{18}O$ values. But if the use of a particular water source is suspected or obvious in an archaeological study, establishing baseline isotope variation of the environmental water can be crucial for an efficient interpretation of oxygen isotope data from the site under study (Pederzani & Britton, 2019).

Oxygen isotope can also be employed in the study of breastfeeding and weaning, and this is based on the principle of isotopic fractionation, where the breast milk of a lactating mother will be isotopically enriched in the heavier isotope (^{18}O) compared to ingested water. This is due to the discrimination against ^{18}O during the expiration of water vapour which usually leads to the enrichment of body water in ^{18}O, from which breastmilk is formed (Britton et al., 2015; Daniel Bryant & Froelich, 1995; L. E. Wright & Schwarcz, 1998). Since the main source of ingested water for infants before weaning is breast milk, mineral tissues formed before weaning (e.g., enamel bioapatite or bone apatite) should reflect this enrichment by showing higher $\delta^{18}O$ values until the child individual is completely weaned. Whereas the oxygen isotope values in tissues formed after complete cessation of breastfeeding should decrease (Britton et al., 2015; L. E. Wright & Schwarcz, 1998). Since the bone mineral $\delta^{18}O$ values may also indicate the average isotopic signature of water consumed over many years before death, the oxygen isotope values in subadults can also be a proxy for water sources contribution and liquid supplementation during childhood (Tsutaya & Yoneda, 2015b).

However, bone mineral is vulnerable to diagenetic alteration which can inhibit the accurate interpretation of biogenic isotopic signals obtained (Garvie-Lok et al., 2004; Maurer et al., 2014). Bioapatite phosphate is usually employed for dietary, paleoclimatic, and migration studies due to its high resistance to diagenesis, but the ease of sample preparation and analysis, better measurement precision, and the possibility to determine dietary input of other food components from its $\delta^{13}C$ values made bioapatite carbonate important for isotopic studies as well. However, carbonate analysis of the bone is usually avoided due to its vulnerability to diagenetic alteration (Britton et al., 2015; Lightfoot & O'Connell, 2016; Pederzani & Britton, 2019). For instance, the exchange of carbonates, phosphate, calcium, and strontium with groundwater or soil water after burial may affect the $\delta^{13}C$ values in bones, recrystallization during diagenesis often result in the formation of high-carbonated recrystallized apatite which is soluble than lower carbonated type, and adsorption of more soluble exogenous contaminants (e.g. calcite) may alter isotope values of both carbon and oxygen in apatite (Garvie-Lok et al., 2004; Hollund et al., 2013; L. E. Wright & Schwarcz, 1996). After death, the diagenetic increase of the apatite crystals will also incorporate the isotope signal of the local pore water (Trueman et al., 2003).

4.3.5. EA-IRMS (Elemental Analyser-Isotope Ratio Mass Spectrometer)

As employed in this study, measurement of isotopic abundance is carried out using a specialised instrument such as the multi-collector magnetic sector Mass Spectrometer (MS), also known as isotopic ratio mass spectrometer (IRMS). IRMS instrument is made up of five components, namely the sample introduction system, an electron ionization source, a magnetic sector analyser, a Faraday-multi-collector detector array, and a computer used for control and data acquisition (Muccio & Jackson 2008). In this type, the IRMS is coupled with an elemental analyser (EA) where the samples (e.g., bone collagen or apatite) are combusted via FLASH High Temperature (HT) or dynamic flash combustor to release gases such as H_2, CO, CO_2 or NO_x, and SO_2 (Ambrose, 1990a; Schoeninger & Moore, 1992). HT is employed for hydrogen and oxygen isotope analysis, while dynamic flash combustion is employed in the analysis of C, N, and S isotopes.

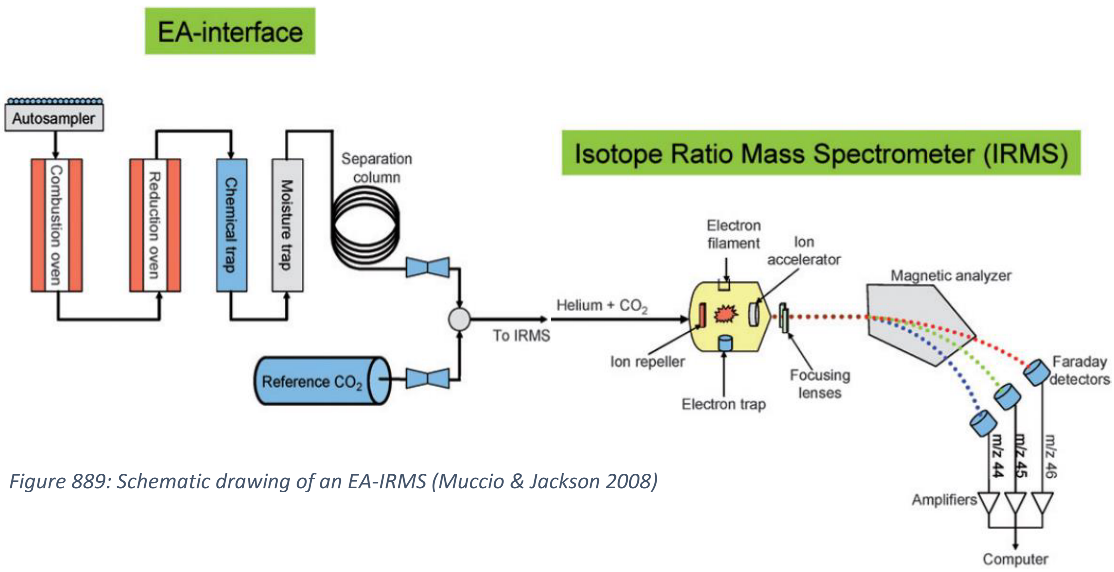

Figure 889: Schematic drawing of an EA-IRMS (Muccio & Jackson 2008)

Depending on the elements of interest, the combusted sample is carried by a helium gas stream (carrier gas) into the reduction chamber where the gases are separated in the EA and ionized by a beam of electrons produced from a heated filament before their entrance into the MS (see *Figure 19*). The high voltage within the ionizing chamber and ground potential of the rest of the mass spectrometer causes a potential difference through which the charged ions are accelerated into the mass analyser. Based on their mass and charged ratios, molecules are separated by magnetic sector and are measured using the electronic detector of the mass spectrometer (Muccio & Jackson 2008). For calibration to international standards, samples are measured along with laboratory standards. The EA-IRMS technique allows bulk measurement of non-volatile samples and the acquisition of representative data of the average isotopic signal of the entire sample, which is of importance in archaeological studies.

5. Materials and Methods

5.1. Bone sampling

Amongst the human and faunal remains recovered through the series of excavations conducted between 2008 and 2017 in the Roman fish preparation factory of Tróia, a total of 8 human skeletons and 17 animal bones were selected for this study. Of the selected human skeletons, 3 are children, 2 foetuses, 1 foetus/stillbirth/neonate, and 2 adults belonging to the Late Antiquity, and all were from the excavated sections of Workshop 1. Because this is a pilot study of an ongoing project, more adult bone samples (n=9) were included for diagenetic assessment (see table in appendix II). Most of the human and faunal bones sampled are long bones (humerus, femurs, tibia, and fibula) to obtain more cortical bone, while a few of the faunal bones ranging from the mandible, phalanx, scapula, pelvis, and ribs were also sampled.

Figure 20: individual T.OF1-SB bone fragment before sampling

More than 500mg of the bones were sampled due to their lightweight and fragility, as well as the series of analyses carried out required a higher amount than usual. Therefore, between 650-700mg of the bones were cut and the adhering dirt, discolouration and potential contaminants were cleaned using a DREMEL® rotary drill with a diamond-coated disc and a diamond-coated burr respectively, which were thoroughly cleaned in-between samples. Samples were carefully taken from the more compact bone areas, while the spongy bone was removed during the cleaning. Furthermore, the samples were photographed before the cutting and cleaning activities, to record the initial state of the samples, in case the macroscopic details are needed for further assessment.

It is noteworthy to also state that a parallel study of adult individuals from the same site and contexts are being conducted by another researcher from the Tróia Resort. Hence, in this study diagenetic alteration of the non-adult bones is compared to those of adult individuals, as will be seen in the following chapters.

Table 3: List of samples analysed (um = uterine months)

	Excavation number	Lab code	Age	Bone Type	Sample Type	Site
Humans	525	T.ONM-A	2yrs	Left Humerus	Humans	Tróia
	742	T.ONM-B	9 um	Left Tibia		Tróia
	680	T.ONM-C	2.5yrs	Left Tibia		Tróia
	855	T.OF1-L	8 um	Left Tibia		Tróia
	870	T.OF1-SB	10yrs	Right Fibula		Tróia
	868	T.BBT	7 um	Right Humerus		Tróia
	897	T. PVSV	>30	Right Humerus		Tróia
	8665	T. PV- 897	>30	Right Femure		Tróia
Fauna	554	T.554a	Herbivores	Right Mandible	O. cuniculus	Tróia
	554	T.554b		Left Mandible		Tróia
	488	T.488x		Left Humerus		Tróia
	488	T.488a		Phalanx	Bos	Tróia
	1355	T.1355a		Scapula	Equus asinus	Tróia
	1355	T.1355b		Long bone fragment		
	1355	T.1355c		Pelvis		
	1355	T.1355d		Ribs		
	471	T.471a		Right Radius	C. elaphus	Tróia
	471	T.471b		Left Tibia		
	488	T.488d		Right Tibia	Ovicaprid	Tróia
	488	T.488e		Right Tibia		Tróia
	488	T.488f		Right Humerus	O. cuniculus	Tróia
	518	T.518a	Omnivores	Right Humerus	Sus sp.	Tróia
	518	T.518b		Left Humerus		Tróia
	488	T.4888b		Right Humerus	Gallus sp.	Tróia
	488	T.488c		Right Humerus		Tróia

5.1.1. Analysis of Bone Preservation

As already outlined previously, bone consists of two major fractions, namely collagen and bioapatite, which are prone to the diagenetic alteration within and outside the soil. It is, therefore, crucial to assess the possible extent of bone degradation during burial, when performing the chemical analysis of bone materials. Since the sampling technique of isotope analysis is generally destructive, some methods can be employed to pre-screen the sample to minimise damage during the sampling. Furthermore, collagen integrity can also be assessed using the C/N ratio, % C and %N, and in some cases where the mineral matrix is also employed for obtaining dietary information, it is essential to ascertain the diagenetic status of the bone mineral for accurate interpretation of data (Ambrose, 1990a; Macroberts, 2017; Maurer et al., 2014). The molecular C/N ratio should be between 2.9 and 3.6 with %C and %N compositions between 26% and 11-16% respectively (Siebke et al., 2019)

Crystal growth within the bone structure is biologically regulated during life. But after death, reorganisation of the crystallites can occur as crystal defects are eliminated due to their increased size as collagen decomposes. Bioapatite can also be altered in a variety of processes (such as dissolution, recrystallization, and ionic substitution) as explained in the previous chapter. Exposure of the nanocrystals of the bone mineral to the environmental conditions of the burial environment (e.g., microbacteria, humidity, pH changes, alternating temperatures, etc) also contribute to further degradation of the bone (Beasley et al., 2014; Koch et al., 1997; Maurer et al., 2011, 2014; Thompson et al., 2011). Hence, assessment of changes in the crystal size can be done by measuring the infrared splitting factor (IRSF), while the carbonate content ratio can also be used for diagenetic evaluation of the bone, using the attenuated total reflection Fourier transform Infrared (ATR-FTIR) spectroscopy. Furthermore, the crystallinity index (CI) of the bone samples as well as the presence of calcite in the bone porosity can be evaluated by X-ray diffraction (XRD) (Saragoça et al., 2016).

Another diagenetic indicator is the nitrogen percent content of the bulk bone. Deterioration of the bone organic content (collagen) results in to decrease in %N because of the relative decrease in the amino acid content of the bone. This effect allows the assessment of sample integrity, but it does not distinguish the nitrogen source (either pedogenic, protein or non-collagenous protein). Hence, it should be used in concert with other techniques for more reliable results (Brock et al., 2010; Nielsen-Marsh & Hedges, 2000). Fresh bone contains between 3.5 – 4.5% (4% on average) of nitrogen and collagen is composed of about 90% nitrogen (Hedges, 2002; Pate, 1994). However, it has been suggested by Brock et al. (2013) that a nitrogen weight percent of about 0.7% can be used for determining if a bone sample will have sufficient collagen yield (at least 1%). It has also been argued that samples yielding about 0.5-0.7% N can yield enough collagen for chemical analysis if the sample is of importance to the study. Therefore, the samples in this study were pre-screened using the ATR-FTIR, elemental analysis by EA for %N of the bone powder, and crystallinity evaluation using XRD.

5.1.2. Elemental Analysis of bone powder for %N

Based on the macroscopic examination of the samples, it was determined that only the human bone samples should be pre-screened using the Elemental Analyzer (EA) since the fauna bones seemed much better preserved. A total of 17 human bones comprising both children (n= 6) and adults (n= 11) were sampled, of which 9 were incorporated from the adult study for comparative analysis. From the sampled bones, about 150mg was ground into a fine powder using an agate mortar and pestle. Between 0.5-07mg of the powdered samples were weighed into tin capsules and the %N content was measured using an elemental analyser (EA Flash 2000 HT, Thermo Fisher Scientific®). Nitrogen amount was obtained based on a calibration using aspartic acid (10.52%), nicotinamide (22.94%) and acetanilide (10.36%), while alanine (15.71%) was used as a check standard.

5.1.3. Fourier Transform – Infrared Spectroscopy

The results from the EA prompted the decision to assess 7 (excluding T.ONM-A) of the human samples with ATR-FTIR. Therefore, a total of 16 human bone samples (n= 5 children, and n= 11 adults) were used for this analysis done in the HERCULES Laboratory. Between 50-100mg of the powdered bones were analysed using a Bruker Alpha Spectrometer coupled with a single-reflection diamond ATR module (120 scans with a spectra resolution of 4 cm^{-1}, within 4000 to 375 cm^{-1} wavenumber range) to acquire the infrared spectra. The spectra and baseline were processed, normalised, and corrected using the OPUS/Mentor software (v. 6.5). Calculations of the Infrared Splitting Factor (IRSF), relative carbonate content (C/P) and relative collagen content (Am/P) were done using the peak heights at wavenumbers 565, 603, 1030, 1035, 1415, and 1640 cm^{-1} according to the established method (Garvie-Lok et al., 2004; Weiner & Bar-Yosef, 1990).

IRSF, according to Weiner & Bar-Yosef (1990), was calculated by summing up the absorbance values for the phosphate peaks (at 565 and 603 cm^{-1}) and dividing the result by the height of the valley between them. Furthermore, the C/P ratio is acquired when the height of the carbonate peak at 1415 cm^{-1} is divided by that of the phosphate peak at 1035 cm^{-1}. Also, by dividing the height of the amide peak at 1640 cm^{-1} with that phosphate peak at 1035 cm^{-1}, the relative collagen content (Am/P) can be obtained. By using the Am/P ratio with standard deviations, it is possible to also estimate N wt% and collage wt%. Despite the usefulness of these calculations for the detection of viable samples (with > 0.5 wt% N which is around 3wt% of collagen) to be analysed, ATR-FTIR is known to overestimate the N wt% by 0.5-1wt%. Therefore, it is advised that these values should be utilised with caution (Beasley et al., 2014; Cersoy et al., 2017; Garvie-Lok et al., 2004; Macroberts, 2017; L. E. Wright & Schwarcz, 1996). Measurement of crystallinity index (i.e., crystal length) resulting from crystal growth and loss of smaller crystals, was done using IRSF values based on the formula from Trueman et al. (2004). The formulae for the calculation of these indices are itemized below:

IRSF = (565ht+605ht)/590valley… (Equation 6) (Weinger and Bar-Yosef, 1990)

C/P = 1415ht/1035ht… (Equation 7) (Schwarz 1996; Beasley et al. 2014)

Am/P= 1640ht/1035ht… (Equation 8) (Lebon et al., 2016)

N %wt= 20.6 Am/P +0.31… (Equation 9) (Lebon et al., 2016)

Collagen wt%= 113.13 Am/P + 1.69… (Equation 10) (Cersoy et al., 2016)

Mean Crystal Length (nm) = (IRSF-0.822)/0.048… (Equation 11) (Trueman et al., 2004)

5.1.4. X-Ray Diffraction

The results obtained from the IR analysis also prompted the need to assess the crystallinity index as well as the presence of other secondary minerals in the bone samples using the powder X-ray diffraction technique. A total of three human bone samples, composed of individuals T.ONM-C (2years old child), D3S5101, and D3S3148 (adults) were selected for this analysis. The crystallinity index (CI) values of the bone allow the semi-quantitative estimation of diagenetic change in archaeological bones (Person et al., 1995).

About 1g of the bone samples was ground to fine powder using agate mortar and pestle which were cleaned in between samples. These powdered samples were placed in the sample holders and analysed at the HERCULES lab using a BRUKER D8 Discovery X-Ray Diffractometer with a $CuK\alpha$ radiation source operating at 40kV and 40mA. The diffractograms were obtained at an angular range 2θ between 3° and 75° with a step size of 0.05° at 1 s per step, and between 20° - 40° with an increment of 0.02° and a step time of 6 s/step, for high resolution. The diffractograms were treated and the mineral phases were identified using the DIFFRA.SUITE EVA software package combined with the specific Powder Diffraction Database and International Center for Diffraction Data (PDF2-ICDD). The crystallinity index was calculated using the DIFFRA.SUITE EVA software package and with the equation from Person et al. (1996)

CI= Σ {H[202], H[300], H[112]}/H[211]=(a+b+c)/h… (Equation 11) (Person et al. 1996)

A high CI value is indicative of an increase in crystallinity which is correlated to the loss of organic matter in archaeological bones. However, this is not as straightforward as it seems because the preservation quality of organic matter in bones is not solely dependent on crystallinity increase. Nevertheless, this semi-quantitative crystallinity estimation can be employed as a proxy for diagenetic alteration and the taphonomic effect of the burial environment on bones (Bunaciu et al., 2015b; Maurer et al., 2014; Person et al., 1995; Schreiner et al., 2004).

5.2. Bone preparation for Bioapatite Carbon and Oxygen analysis

Based on the results obtained from the EA measurement of %N, 6 human samples (excluding T.ONM-A and T. PV-897) with relatively good nitrogen values were selected for carbon and oxygen isotope analysis from bioapatite. Approximately 20mg of the pulverised bone samples were treated following Sponheimer and Lee-Thorp´s (1999) protocol. The samples were placed into 1mL of NaOCl (2-3%) for 24 hours to remove organic matrix and other biogenic contaminants, followed by rinsing up to four times with deionised water, and then placed into 1 mL (1M) acetic acid buffered with Li-acetate solution for 24 hours (instead of 12hrs for teeth) to remove soluble diagenetic carbonates. The samples were rinsed again four times with deionised water and oven-dried at 50°C. Between 800-850µg of the resultant dried apatite powder was then weighed into crucibles and analysed at the Institute of Geosciences, the University of Mainz. The analysis was performed using a Continuous Flow – Isotope Ratio Mass Spectrometer (Thermo Scientific® 253) coupled to a GasBench II. The stable isotope ratios were obtained using a 2-point calibration of IVA Carrara Marble ($\delta^{13}C$ = -2.01‰, $\delta^{18}O$ = -1.91‰) with average precision error (1σ) better than 0.07‰ for $\delta^{13}C$ and 0.18 for $\delta^{18}O$, and long-term accuracy of the mass spectrometer between 0.05‰ and -0.02‰ respectively. The results are expressed in parts per thousands (‰) relative to VPDB.

5.2.1. Collage Extraction

Irrespective of the results obtained through EA and FTIR, all human and faunal osteological samples (n=8 human, 17 fauna: total n= 25) were subjected to the collagen extraction treatment. A variation of Longin's (1971) method was employed to extract collagen from the sampled bones. Approximately 650mg of sampled bones were broken into smaller pieces and placed in 10mL 0.5M HCl for about 14 days to remove the mineral component of the bones, although, the acid was changed after one week. The samples were vortexed at least thrice daily and were in alternating temperature conditions by placing them inside the refrigerator (4°C) during the night while spending the day at room temperature. Afterwards, the acid was removed, and samples were rinsed with deionised water between 5-7 times during which the samples were vortexed and centrifuged for 5 minutes at 6000 rpm between each rinsing until the pH was neutral. Following this, the samples were placed in 10mL 0.125M NaOH for 20 hours to remove potential contaminants such as humic and fulvic acids that may have accumulated during burial. This was followed by the removal of NaOH while the pseudomorphs were rinsed with deionised water, vortexed and centrifuged between 3-5 times until neutral, similar to the same rinsing procedure mentioned earlier. Furthermore, 10mL of 0.01M HCl was added to each of the samples, and they were placed in an oven at 70°C for 48 hours and were regularly vortexed. Samples were then filtered (using Ezee-Filter™ separator—Elkay Lab Product) into the pre-weighed plastic vials. Due to logistic issues and the ongoing construction works in the lab, freezing the collagen in liquid nitrogen was not possible, hence, the collagen was placed in the -80 industrial freezer, and then lyophilised for 48 hours. Upon removal from the lyoph, the collagen vials were weighed to ascertain the collagen yield of the samples, after which the vials were tightly wrapped with parafilm to keep the moisture out until they could be analysed.

5.2.2. EA-IRMS Analysis

Around 0.7mg of the lyophilised collagen was cautiously weighed into tin capsules. Analysis was performed at the Laboratório de Isótopos Estáveis (Stable Isotopes Analysis Facility, SIIAF), Faculdade de Ciências de Universidade de Lisboa (LIE-FCUL) as the ongoing construction works constrained analysing the samples at the HERCULES laboratory. Approximately 1.0mg of the lyophilised collagen samples were analysed in a Sercon 20 Hydra IRMS coupled with EuroEA elemental analyzer (EA3000) to measure the carbon and nitrogen isotopes, while an IsoPrime mass spectrometer was used for the sulphur isotope analysis. Carbon and nitrogen values were calibrated using the international standards IAEA-C-3 ($\delta^{13}C$= -24.19‰), IAEA-CH-7 ($\delta^{13}C$= -32.15‰) and IAEA-N-1 ($\delta^{15}N$= +0.4‰), IAEA-N-2 ($\delta^{15}N$= +20.3‰) respectively, as well as in-house laboratory standards protein ($\delta^{13}C$= -20.81‰, $\delta^{15}N$= +5.60‰), Glucose ($\delta^{13}C$= -10,93‰), IAEA-600 (($\delta^{15}N$ = +1.6‰), with values relative to VPDB and AIR, and measurement uncertainty of 0.03‰ and 0.08‰ for nitrogen and carbon respectively. While sulphur was calibrated using the inorganic international reference standards NBS127 (20.3‰), IAEA S1 (-0.3‰) and an internal standard casein protein (+4.24‰) with a measurement uncertainty of 0.12‰ and $\delta^{34}S$ values are given in part per thousand (‰) relative to Vienna-Canyon Diablo Troilite (VCDT).

6. Result and Discussion

6.1. Evaluation of Bone Preservation

The results obtained from the FTIR and XRD indices, elemental analysis, collagen quality and yield are used as preservation indicators of the bone samples, to determine the extent of degradation that bones have suffered over time. Collagen content as we know deteriorates continuously over time during burial, and the rate of degradation depends on a number of factors controlled by the immediate climate and environment of burial. Similarly, the mineral fraction of the bone suffers alteration due to these processes which invariable degrade the structural integrity of archaeological bones. Therefore, the results obtained from the series of analyses conducted are itemised and the possible causes are discussed in this chapter. The results of the stable isotope analysis are also enumerated and interpreted.

6.1.1. ATR-FTIR estimation of bone crystallinity, carbonate, and protein content

The indices calculated from the ATR-FTIR spectra including IRSF, C/P, Am/P, mean crystal length, N wt%, and collagen wt%, were used to estimate the preservation status of the bone samples. The values obtained through ATR-FTIR are summarised in table 4 with values from other studies for comparative analysis, and the full results (Table 8) are attached in appendix III.

Table 4: Summary of the diagenetic indices from ATR-FTIR in comparison with other studies

	IRSF	C/P	Am/P	Mean Crystal Length (nm)	N wt%	Collagen wt %
Mean	3.3	0.24	0.6	53.8	3.6	20.4
Min	3.0	0.14	0.3	45.4	1.1	5.9
Max	3.8	0.36	1.0	66.2	16.8	92.2
Std. Dev.	0.3	0.1	0.2	6.2	3.7	20.3
Modern Bone	**2.5-3.5 (Beasley et al. 2014)**	**0.5 (0.23-0.34) (Wright & Schwarz, 1996).**			**3.5-4.5 (Brock et al., 2012) 4-4.5 (Lebon et al,. 2016)**	**20-25% (Sealy et al., 2014)**
MacRoberts 2017 (medieval)	4.63	0.153	0.073	79.34	1.82	9.97
Zalaite 2016 (chalcolithic)	3.4	0.23	0.055	54.4		

Hence, according to the infrared splitting factor (IR-SF) values, the bone samples showed relatively good preservation values ranging between 3.0 to 3.8, with a mean value of 3.3. Comparatively, the children samples exhibited good preservation (from 3.1 to 3.4) except individual T.ONM-A which was excluded from this analysis based on poor N wt% value from the EA pre-screening (*table 8* in appendix). Similarly, the adult samples are also relatively preserved as their IRSF values ranged from 3.0 to 3.8 (*table 8*, appendix). Fresh bone IRSF values have been reported to be between 2.5 and 3.25 by Beasley et al. (2014) with an average value of 2.9 (Garvie-Lok et al., 2004; L. E. Wright & Schwarcz, 1996). Furthermore, archaeological bones with values less than 3.3 within the set range will yield a sufficient amount of collagen for analysis (Beasley et al., 2014), and a broader range of ≤ 5 can be explored because of some samples, especially those of adults present values as high as 3.8. There is an overlap between the samples (adult vs children) as their values follow the same trend with crystal-size values close to those of fresh bones., while the values of three adult samples tend to be more crystallised than the others (see *Figure 21*). With this overlap, it can thus be said that the bone samples are moderately crystallised as high IRSF values in archaeological bones may be an indication of increased crystal growth resulting from diagenetic processes (e.g., dissolution of more soluble, less ordered crystals) during burial (Weiner & Bar-Yosef, 1990; L. E. Wright & Schwarcz, 1996).

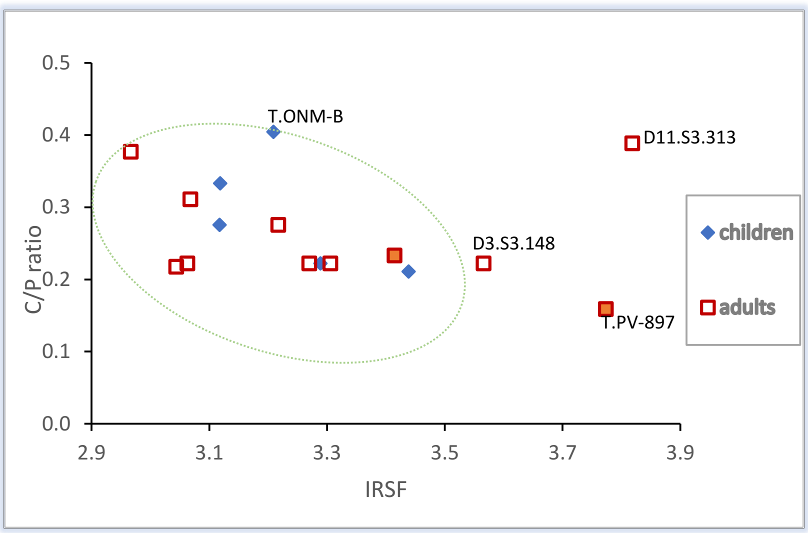

Figure 21: C/P ratios and IRSF values of the human bone samples from ATR-FTIR (unshaded squares = adults samples from Troia adapted from the ongoing project; shaded squares = adult samples in this study; blue diamonds = children samples in this

The relative carbonate content, (C/P), is used to determine the presence of secondary calcite within the bone microstructure or carbonate loss during burial. The C/P values of the bone samples ranged between 0.14 - 0.4 with a mean value of 0.24. The C/P values of the modern bone fall between 0.23 and 0.34 (Beasley et al., 2014; Garvie-Lok et al., 2004; L. E. Wright & Schwarcz, 1996), and values below or above this range are reflective of diagenetic alteration. Therefore, all children samples show good

C/P values between 0.24 – 0.36, with sample (T.ONM-B) exhibiting a C/P ratio of 0.36 and splitting factor value of 3.2 which is still good but could be a sign of the starting point of diagenetic alteration indicating the presence of calcite. Similarly, the adult samples also show good C/P ratios from 0.1 – 0.35, amongst which two (D11.S3.148, T. PV-897) are with values (0.35, 0.1 respectively) above and below the preservation threshold, as also indicated in their IRSF values of 3.8 (*Figure 21*), thereby showing increased crystallinity and relatively slight degradation if compared with values from other studies.

The IRSF values can be affected by the amount of carbonate (CO_3) present in the apatite. Apatite with high carbonate content tends to show lower IRSF values beyond the accepted range. Bone samples with good preservation will reflect a negative correlation between C/P and IRSF, whereas, a positive correlation in the values of these indices connotes bad preservation (L. E. Wright & Schwarcz, 1996). Put differently, an increase in IRSF values and decreasing C/P ratios indicate increasing crystallinity in the bone apatite. As seen in *Figure 21*, when samples are considered together, the C/P ratio of the samples decreases as IRSF increases, thus showing crystal growth trends of the samples accompanied by loss of carbonate. The samples within the circle are expected to give sufficient collagen while the outliers (which are all adult samples) exhibit signals of diagenetically altered bones. However, the human bone samples are generally well preserved compared to values from other studies as seen in table 4.

As calculated using the formula by Trueman et al., (2004), the mean crystal length of the analysed bone samples averaged 53.8nm with a standard deviation of 6.2nm (see *table 4*). The extent of diagenetic alteration of bones is reflected in increased crystal length. The more the bones are degraded the more crystal size increases with direct correlation to IRSF (R^2=0.9309), and three adult

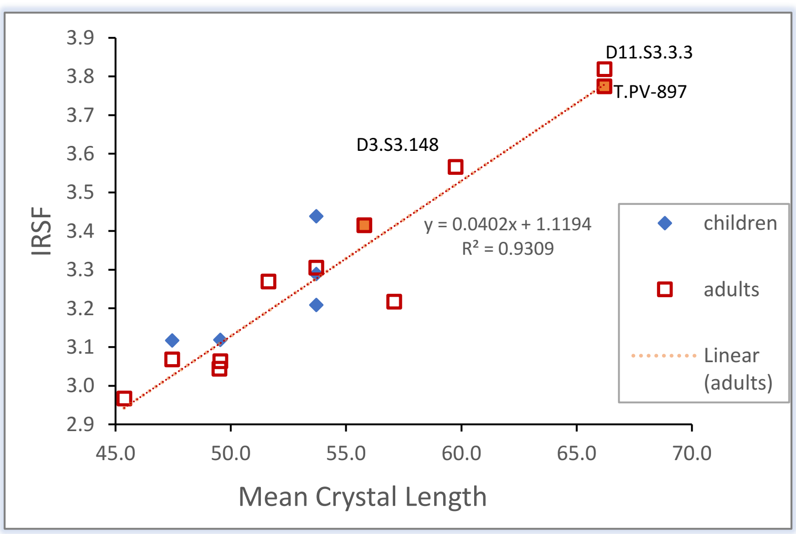

Figure 22: Connection between Crystal length (nm) and Splitting Factor ratios of the Samples (unshaded squares = adults samples from Troia adapted from the ongoing project; shaded squares = adult samples in this study; blue diamonds = children samples

bone samples (which are the outliers in *Figure 21)* reflect the largest mean crystal size as seen in *Figure 22.* The degradation pattern between adults and children is similar, while no trend was observed relative to age and rate of degradation.

The relative collagen content was also deduced using the Am/P index from the ATR-FTIR. The Am/P index of the bone samples was obtained by dividing the height of amide peak I (1640 cm^{-1}) by that of the phosphate peak (1035 cm^{-1}). The samples exhibited values ranging between 0.3 and 1.0. A pattern can be observed as the adult bone samples (T. PV-897, D11.S3.313) with high IRSF can be seen to have low relative collagen content (see red circle in *Figure 23*) thus indicating poor preservation. Sample D4.S7.168 showed a very high relative collagen content and well-preserved crystallites, as illustrated in the green circle of figure 23. This is, therefore, suggestive that the samples (children and adults) in-between are relatively well-preserved, due to their intermediate Am/P and IRSF values. If the clustering of samples within a certain region of the graph is to be considered (see *Figure 23*), it is possible that the outliers (in red circle) were more exposed to the burial environmental conditions than the others. For instance, the bone remains of T. PV-879 were found wrapped in plant roots in a grave where direct action of water and humidity was evident. These are signs that the sample experienced some diagenetic processes (such as hydrolysis, mineral dissolution and recrystallization, among others) leading to collagen and carbonate loss, as well as increased crystallinity (Smith et al., 2007). Generally, moving from left to the right of the plotted graph (Figure 23), it is observable that the bone crystals (IRSF) increase with decreasing relative collagen in the bones, although there is no strong correlation (R^2=0.3392) of such assertion from the values of Tróia samples as seen in Figure 23.

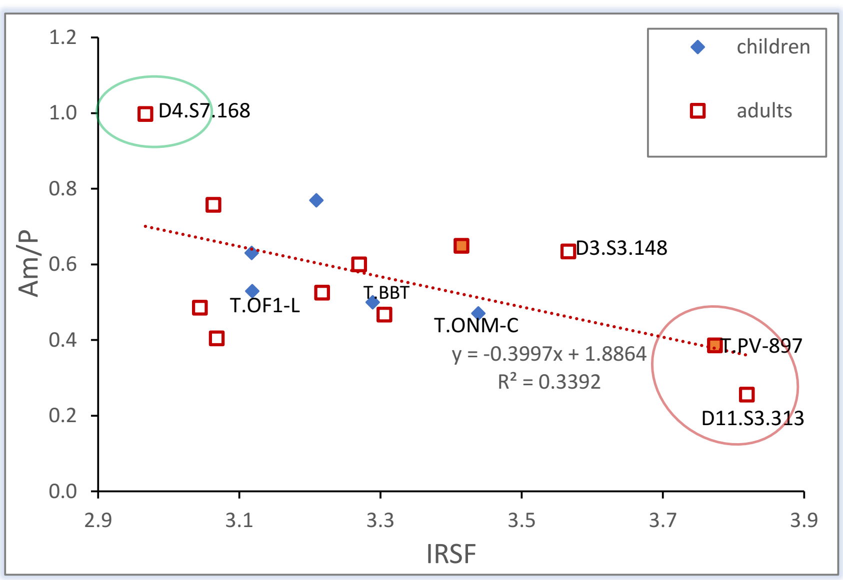

Figure 23: Relationship between relative collagen content (Am/P) and IRSF values of the samples (unshaded squares = adults samples from Troia adapted from the ongoing project; shaded squares = adult samples in this study; blue diamonds = children samples in this study.

Therefore, considering all the indices calculated from ATR-FITR spectra, the human bone samples analysed reflect signals of good preservation with exception of about three which are relatively affected by diagenetic processes. If these values are compared with values extracted from other studies, it can be said that the human samples from Tróia are relatively well preserved (see *Figure 24)*.

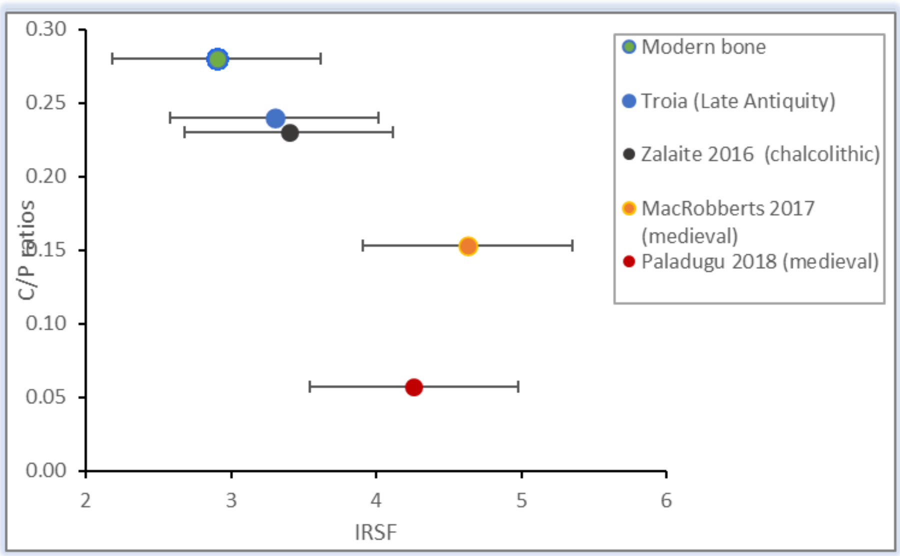

Figure 24: Comparison of IRSF and CP averages with modern bone and other studies

6.1.2. Diagenetic evaluation of %N content of the bone samples by EA

Results obtained from the EA analysis of 17 human bone powdered samples reveal a range of values between 0.0 and 3.1. Among the bone samples, two (T.ONM-A, T. PV-897) yield a value of 0.0, therefore, indicating the complete absence of organic fraction (collagen). As indicated in the previous chapter, fresh modern bone contains between 3.5 – 4.5% (4% on average) of nitrogen, and collagen is composed of about 90% nitrogen and 10% non-collagenous proteins (Brock et al., 2010; Hedges, 2002; Pate, 1994). Archaeological samples with a value of 0.7 %N should contain relatively sufficient collagen, while samples with values between 0.5-0.7 %N can still be used when necessary (Brock et al., 2010). If the two samples (TONM-A and T.PV.897) with poor %N are excluded, all samples contain sufficient nitrogen, irrespective of their mineral preservation. The children's bone samples are seen to contain enough organic fraction, based on the %N results, while the two adult bones are at the lower limit. (see *Figure 25)*.

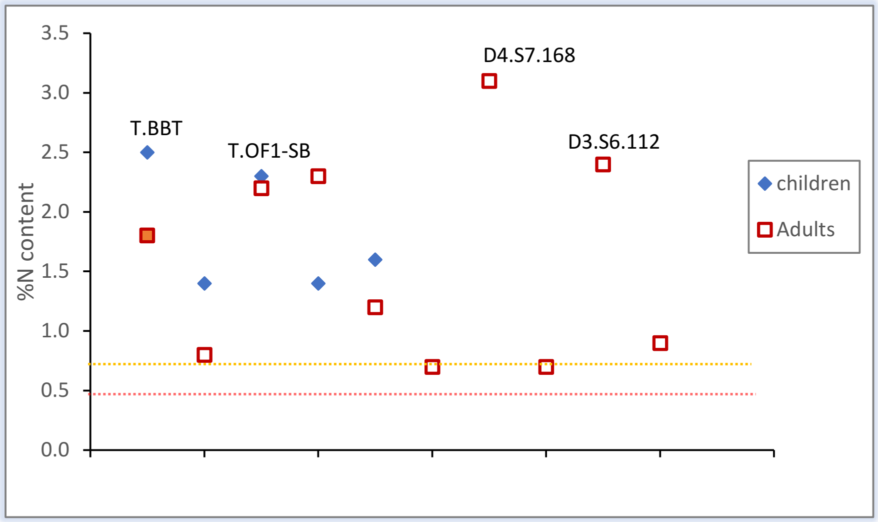

Figure 25: Bulk bone %N content of human samples from Tróia (samples above yellow line = good preservation and collagen yield; samples below red = badly preserved; unshaded squares = adults' samples from Troia adapted from the ongoing project; shaded square = adult sample in this study; blue diamond= children samples in this study)

6.1.3. Connections between ATR-FTIR and EA %N results

Correlation between data obtained by ATR-FTIR and EA analyses can enhance accurate data interpretation for isotopic studies, and these techniques are advantageous because they require only small amounts of sample and minimum to no sample preparation. The IR indices (IRSF, C/P, and Am/P) were compared with %N values from the EA to ascertain their relatedness to the diagenetic alteration of the bone samples. From *Figure 26,* the comparison of IRSF and %N data shows no correlation between increasing crystallinity and nitrogen content (excluding samples with a value of 0). The children's bone samples are within the range indicating well-preserved collagen content with slightly increased crystallinity (*Figure 26)*. Comparatively, the children's bone samples are less diagenetically altered while one adult bone (D4.S7.168) appears to be the most preserved of the samples. Some adult bones (*in the yellow circle, Figure 26*) contained low nitrogen values, and not-so-high IRSF showing that the collagen content of the bones was depleted without a significant increase in mineral crystals, which can occur due to microbial attack and/or the decomposition of collagen itself (Hedges, 2002). Samples inside the blue circle should yield enough collagen thus showing good preservation.

Irrespective of the very high IRSF of the two outliers (see *Figure 26)*, the %N values indicated their collagen yield potential despite their increased crystal size.

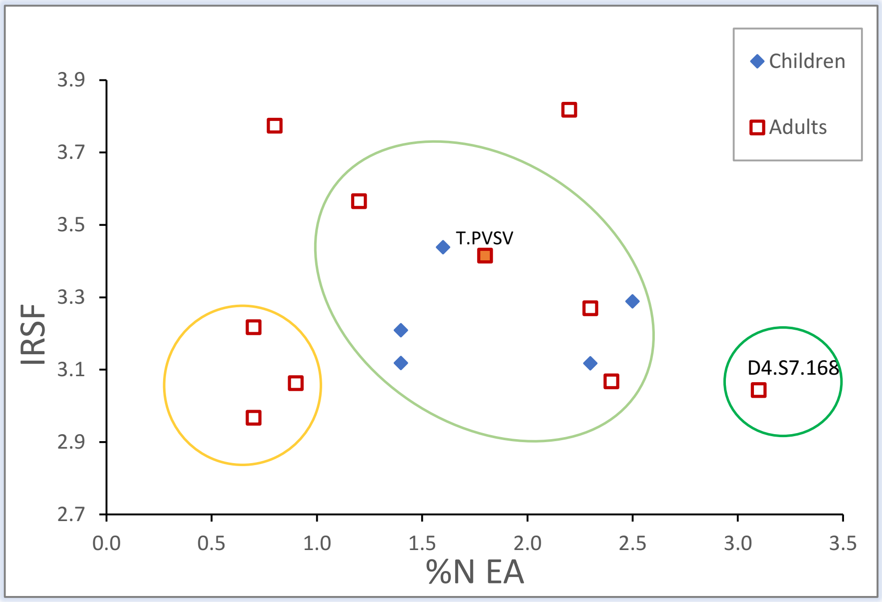

Figure 26: Comparison of IRSF and %N values of the samples. (Unshaded squares represent adult samples from the same sites adapted from the ongoing study; shaded square = adult sample in this study; blue diamond = children's samples in this study

There seems to be no direct correlation between the relative carbonate content (C/P) and the %N of the bone samples. The C/P values of the samples generally are below that of fresh bone (0.5), and any sample with higher values than modern bone is reflecting the adsorption of external carbonate from groundwater onto the bone crystallites surface (Nielsen-Marsh & Hedges, 2000). Since the samples in this study reflected lower C/P values than those of modern bone, it is, therefore, probable that the bone carbonates were eliminated through dissolution/recrystallization processes, accompanied by protein loss (%N) and reduction in surface area (Nielsen-Marsh & Hedges, 2000). it should also be noted that a sample (T. PV-897) reflected a C/P value of 0.1 which seems to be the baseline level of complete carbonate loss. When this value is reached in a bone, the splitting factor is no longer controlled by carbonate loss in a similar way as when IRSF values are low (Smith et al., 2007). But, there is a correlation (R^2=0.8229) between the Am/P ratios (relative collagen content) and %N, of the analysed bone samples (*Figure 27)*. Sample D4.S7.168 stands out with very high Am/P and %N values, which makes it the most preserved of the samples, while the least preserved are adult samples (see *Figure 26*), again the children samples have intermediate values in this trend thus showing relatively good preservation.

Hence, the values obtained through the series of analyses conducted thus far show that the human bones from Tróia are slightly well preserved except a very few which appeared to be more altered.

Therefore, other indices and techniques such as XRD were further explored to deduce the possible alterations that might have occurred to the samples during the burial. In this study, XRD was employed to further check the crystallinity of the samples, as well as the presence and how much exogenous carbon-containing compounds have contaminated the samples.

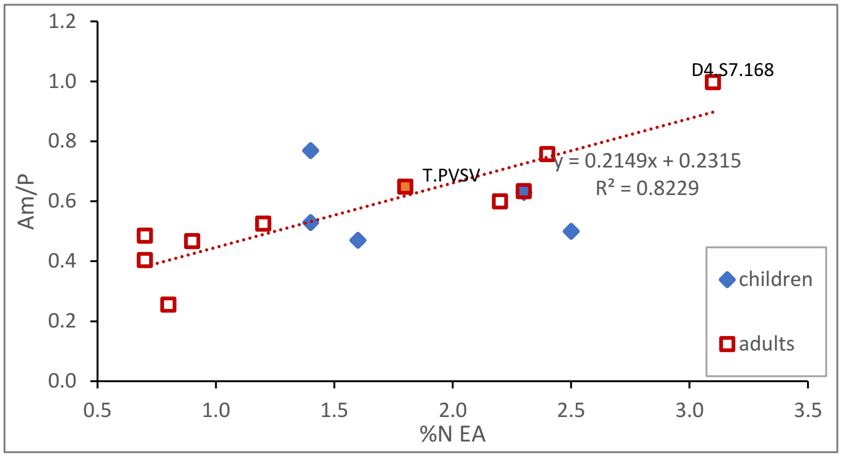

Figure 27: Comparison of Am/P ratios with %N from EA (unshaded squares = adult samples from the same site adapted from the ongoing project; shade square = adult sample in this study; blue diamond = children in this study)

6.1.4. Crystallinity Assessment through X-Ray Diffraction

Following the ATR-FTIR assessment, three samples, T. ONM-C (2years old child), D3.S5. 101, D3. S3.148 (adults) were assessed for possible post-mortem mineral inclusions and crystallinity increase. The values obtained through the DIFFRA.SUITE EVA software indicated crystallinity of 57.8%, 58.9%, and 65.8% for the three samples respectively. The crystallinity index values obtained using Person et al. (1996) method are in the order 0.04, 0.16, and 0.19, for the samples respectively. Samples with CI values greater than zero are understood to be diagenetically transformed (Person et al., 1995).

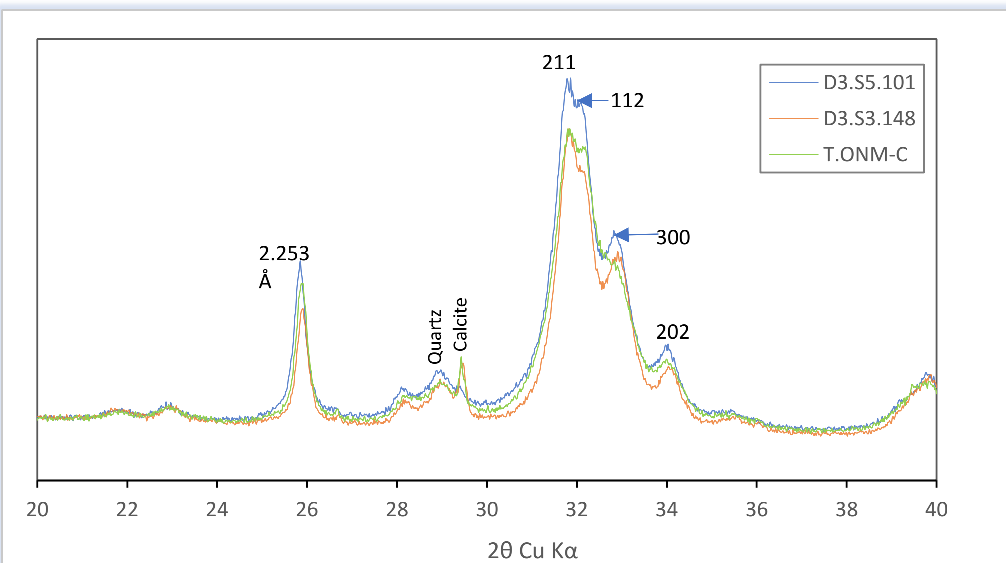

Figure 28: X-ray Diffractograms of the 3 samples showing the presence of calcite and quartz within the hydroxyapatites of the samples

These results correspond and indicate increasing crystallinity between the children and adult bone samples, although this may not be the case in the whole samples from Tróia. As stated earlier in the previous chapter, high CI values are an indication of increased bone crystallinity due to diagenetic processes. Hence it can be said the bone samples analysed are relatively altered**.** An increase in crystallinity has been associated with the loss of organic matter in archaeological bone, while it might also be due to the loss of CO_3 in the carbonate hydroxyapatite (Person et al., 1995). An estimate of post-mortem incorporation of about 3.2% and 4.6 % calcite was also observed in samples T.ONM and D3.S3.148 respectively (see *Figure 28*).

The increase in crystallinity is also in tandem with the diagenetic process of bone dissolution-recrystallisation which often affects the dietary signal. The contact of bone with groundwater usually results in the precipitation of calcite (Maurer et al., 2011; Person et al., 1995). In calcitic environments, the carbonate radicals are usually deposited as calcite on the bone surface, within cracks and pore spaces (Nielsen-Marsh & Hedges, 2000). Perhaps, samples from Tróia are also affected by this sort of process. The soil geochemical atlas of Portugal indicates the high concentration of calcium in the lithology of Setúbal and Lisbon, thus, spreading to Tróia and its environment (Inácio et al., 2007), thereby revealing the possibility of calcite incorporation into the bone samples. However, from the quantity indicated by the XRD as well as the intensity of the calcite peaks in the diffractogram (see *Figure 28*), the adsorbed calcite was probably removed through acetic acid treatment of the samples before the analysis of the isotopic composition of the mineral part of the bones.

6.1.5. Collagen yield of the bone samples

The preservation status as well as the extent of apatite and collagen alteration could be ascertained after the series of evaluations by ATR-FTIR and XRD. Generally, the bone samples are relatively well preserved with the exception of samples (T.ONM-A, and T. PV-897) whose %N and IRSF values indicated poor preservation. The slight alteration and increased crystallinity observed are random and show no relationship with age. The adult samples from the separate study also showed indications of yielding sufficient collagen. However, discussion from this section onwards will be centred around the bone samples of the 8 humans (n=3 children; n=2 foetuses, n=1 foetus/stillbirth/neonate, and n=2 adults) and 17 fauna whose collagen was extracted for this study, as mentioned in the previous chapter. The %N values of human bone samples in this study ranged between 0 and 2.5. Following extraction, the collagen yield of the samples was calculated over the initial raw bone weight.

The collagen yield of the human samples ranged between 3.23 – 14.19% with an average yield of 9.0% and a standard deviation of 3.8%, while those of faunal bone yielded between 0.59 – 15.34% with an average of 8.0% and a standard deviation of 5.1%. Among the fauna, 5 bone samples exhibited collagen yield below 4%, among which 2 samples (T.1355b =1.9%, 1355c = 0.59) came back with insufficient collagen for analysis after lyophilisation, and 5 samples (T.1355a =3.35%, T.554b = 6.94%, T.471a = 5.37%, T1355a =3.35%, T.1355d =3.77, T.488e =5.86%) yielded a low quantity of collagen sufficient for C and N isotope analysis, but not enough for sulphur analysis. As a result, 6 human and 10 fauna bone collagen samples were analysed for C, N, and S isotopes composition, while C and N isotopes were analysed in the 6 collagen samples: 1 human (T.ONM-A) and 5 fauna (T.554b, T.1355a&d, T.471a, T.488e) due to the small collagen amount. Being a pilot study, these data and the results obtained can be part of the baselines for the ongoing project where a larger set of adult skeletal remains from Tróia are being studied.

6.1.6. Quality of Bone Collagen

The ratios of carbon to nitrogen (C:N) in the collagen samples were employed to assess the quality of the collagen extracted from the bone samples. For the human samples, the C:N ratios ranged between 3.2 and 4.0. with an average of 3.2, while those of fauna ranged between 3.2 and 3.4. It is understood that C:N ratios that fall between 2.9 and 3.6. are reflective of well-preserved samples (Deniro, 1985), because fresh modern bone contains a ratio of 3.2, and any deviation from this value is believed to be caused by the processes that alter the physico-chemical properties of the bone in the burial environment, and variability might also occur due to the extraction procedure employed (Ambrose, 1990b; A. M. Katzenberg & Saunders, 2008). As mentioned in the previous section, bone samples with degraded collagen often contain low nitrogen content, which would invariably have effects on the stable nitrogen isotopes of the samples. In cases where there is an elevation of C:N ratio above 3.5, the $\delta^{13}C$ value of such sample would be more negative thereby indicating excess carbon contamination from humic acid in the burial environment (Van Klinken, 1999; Zalaite et al., 2016). The C:N ratio of the bone collagen confirmed the contamination of bone sample T.BBT with a value of 4.0, despite being one of the samples with a high collagen yield. Therefore, this contamination will invariably lead to an increase in the carbon isotope value of this individual. Other samples, including human and faunal shows C:N ratios within the established range, thereby indicating good quality collagen that will provide isotopic composition formed during life. The bone sample T.PV-897 has no collagen yield, hence its exclusion. The human bone collagen and apatite carbon, nitrogen and oxygen results are itemised in table 5 while table 6 contain results of faunal bone collagen carbon and nitrogen stable isotopic compositions.

Table 5: Collagen quality indicators with Carbon, Nitrogen, Oxygen, & Sulphur stable isotope results (um = uterine months)

	Sample ID	Age (yrs)	Collagen Yield	%C	%N	C:N	%S	C/S	N/S	$\delta^{34}S$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Humans	**T.OF1-SB**	10	11.26	40.39	14.52	3.2	0.2	539	166	12.3	-17.5	11.6
	T.ONM-A	2	7.62	4.45	1.54	3.4	-			-	-18.9	14.0
	T.ONM-B	9um	11..69	38.09	13.60	3.3	0.2	508	155	13.2	-18.1	12.9
	T.ONM-C	2.5	8.94	40.20	14.27	3.3	0.22	487	148	12.3	-17.7	11.7
	T.OF1-L	8um	7.62	31.96	11.61	3.2	0.19	449	140	12.4	-18.0	12.7
	TBBT	7um	11.12	49.76	14.46	4.0	0.22	603	150	12.9	-21.0	5.8
	T. PVSV	>30	14.9	37.99	13.69	3.2	0.2	507	156	12.4	-17.2	13.1
	T.PV-897	>30	3.23	-	-	-	-	-	-	-	-	-
	Mean		9.0	34.7	12.0	3.4	0.2	515	153	12.6	-18.3	11.7
	Std. Dev.		3.8	14.3	4.7	0.3	0.0	57	7	0.4	1.3	3.7

6.2. C, N and S, stable isotopes in bone collagen

The focus of this study is to reconstruct the children and animal dietary habits during the Late Antiquity in Tróia and southwestern Iberia. Samples also include one adult individual which would enhance the interpretation and contextualisation of data among different human groups. Mobility indicators such as sulphur stable isotope will be used to identify locals and non-locals as well as animals raised or imported into the site, while apatite oxygen isotope will be utilised to assess the source of dietary water, as well as their correlation to human mobility. Analysing a combination of human and faunal bone remains is then advantageous to reconstruct the trophic level as well as the contextualisation of the human values within those of their probable food sources. The isotope compositions obtained thus far will be compared with those from contemporaneous sites in the Iberia to ascertain patterns of husbandry practices and childhood care during the Late Antiquity in southwestern Iberia. This is a pilot study which prepares the ground for the analysis of a larger set of adult samples from the same site (Tróia) to have a clearer picture of lifeways during the Late Antiquity.

6.2.1. Human Dietary Reconstruction of Tróia

For the human, the $\delta^{13}C$ values ranged between -21.0 ‰ and -17.2 ‰ with an average of -18.3 ±1.3‰. Consumers who feed mainly on C_3 plants have $\delta^{13}C$ values between -20‰ and -18‰ while more positive values of around -7.5‰ is pointing to consumers with C_4 plants as major food sources (Schoeninger & Moore, 1992; R. H. Tykot, 2006; Van Der Merwe & Vogel, 1978). As was previously discussed in chapter 5, these human samples are composed of different age groups, hence, the need to examine their values according to age for a better understanding. Figure 29 illustrates the carbon values of the samples according to age.

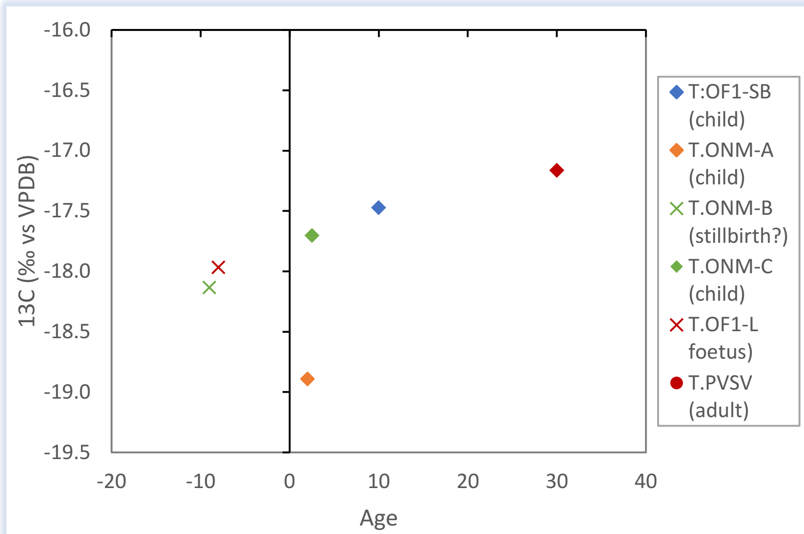

Figure 29: δ13C values according to the age (in years after birth and months before)

As mentioned in chapter 3, isotopic values of the foetus and breastfeeding child will reflect the mother´s diet, as a slight difference of about 0.4‰ increase in foetal and 1‰ in breastfeeding children $\delta^{13}C$ values were reported (Fuller et al., 2006; Herrscher et al., 2017; Tsutaya & Yoneda, 2015b). As illustrated in *Figure 29,* there is a difference of 0.8‰ and 0.9‰ in the values of T.ONM-B and T.OF1-L compared to the 2 years old child individual (T.ONM-A) which shows that the child individual is depleted in ^{13}C. These two foetal individuals reflect the values of the mothers. However, sample TBBT exhibited a more negative $\delta^{13}C$ value (-21.0) beyond the rest of the samples, with a C:N ratio of 4% which is out of the acceptable range (2.9 – 3.6), hence, this sample is diagenetically altered. (Deniro, 1985). Excluding individual TBBT (not present int the plots), the mean $\delta^{13}C$ value (-18.5‰ ±0.6) obtained from the Tróia human skeletons falls close to the values anticipated from consumers of predominantly C_3 plants. The foetal individual reflects values very close to that of the unique adult analysed.

As stated earlier in chapter 2, cereals such as wheat, barley, millet, oat etc. were more cultivated in the Iberia during the Roman period (Peña-Chocarro et al., 2019). But much cannot be said about the cultivation of plant food materials in this site due to the absence of archaeobotanical data. The sandy soil type of the present-day Roman Tróia is indicative of its unsuitability for food crop production. However, some cities of Roman origin such as Alcácer do Sal, Évora, and Beja, among others, were further developed by cereal cultivation (Firnigl, 2013). Hence, it is probable that these were the major food sources consumed by the inhabitants of Tróia during Late Antiquity. Nevertheless, making such inferences is impossible without a larger population set. The interpretation being made here should be considered as a tentative discussion that will be confirmed and contextualised with more data from adults and children's skeletal samples being studied in a larger project from the same site.

It is, therefore, imperative to also check the $\delta^{15}N$ values of these individuals relative to age. The connection of nitrogen isotopic composition with age is illustrated in *Figure 30*. The $\delta^{15}N$ value of the human skeletal remains from Tróia falls between 14.0‰ and 5.8‰ with an average of 11.7‰. As can be seen in *Figure 30*, the $\delta^{15}N$ values of the foetal individuals (T.OF1-L and T.ONM-B) are close to that of the adult sample (T. PVSV) thereby reflecting the values of the mothers. Sample T. ONM-A (2 years of age) had the highest $\delta^{15}N$ value (14.0‰) (see *Figure 30),* thereby showing a signal of breastfeeding at the time of death. Furthermore, individual TBBT (7 uterine months foetus) reflected the lowest nitrogen stable isotope signal with a $\delta^{15}N$ value of 5.8, a value close to those of herbivores. However, this value is improbable considering the C:N ratio of 4.0 of this sample.

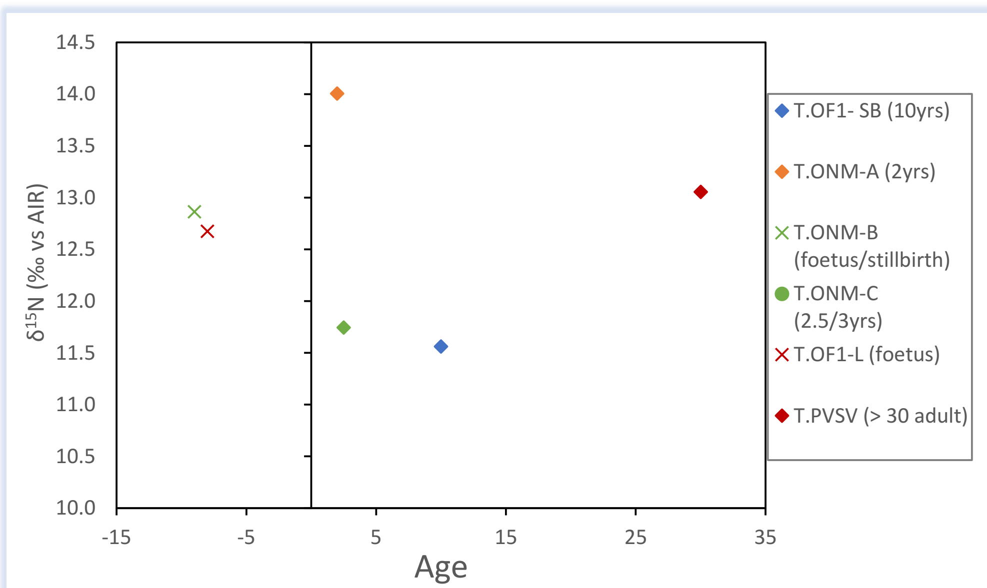

Figure 30: Correlation between $\delta^{15}N$ and age of human skeletons from Tróia (in years after and month before birth

Moving from sample T.ONM-A to the 2.5 years old individual (T.ONM-C), there is a sharp decrease in $\delta^{15}N$ value that might relate to weaning. A further depletion of 0.1‰ was observed in T.OF1-SB (10years old child), while the adult sample is enriched in ^{15}N with a difference of about 1.5‰, indicative of more protein food (animal) consumption by adults compared to children in this settlement (see *Figure 29 & 30)*.

Interpreting the stable isotopes of carbon and nitrogen separately can be complicated by some factors such as the preference of food type, the geographical range of food sourcing, temperature, and precipitation, amongst others (Malainey, 2010; Mora, 2022; Saragoça et al., 2016). By combining the $\delta^{15}N$ and $\delta^{13}C$ values, it is possible to gain a more comprehensive insight into dietary sources and behaviour over time (Keegan & DeNiro, 1988). The $\delta^{15}N$ and $\delta^{13}C$ values further confirmed some of the hypotheses made earlier. As seen in *Figure 31*, individual T.ONM-A shows more enrichment of $\delta^{15}N$ values which is suggestive of a breastfeeding child (Fuller et al., 2006; M. A. Katzenberg et al., 1996).

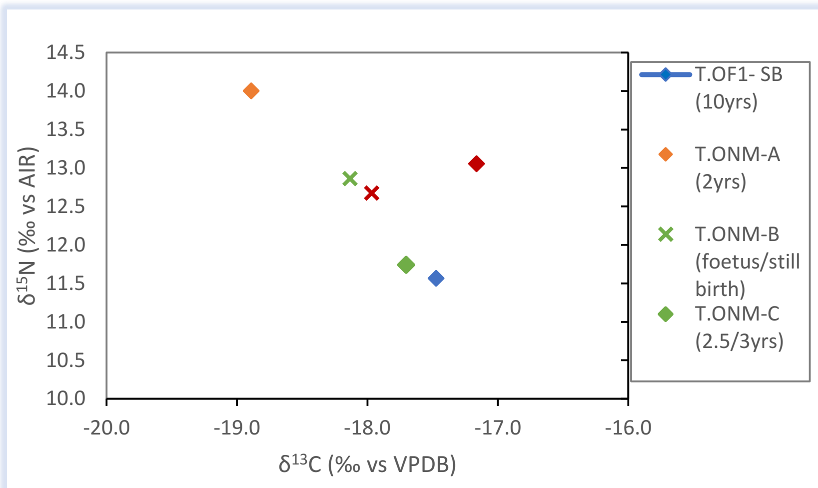

Figure 31: $\delta^{13}C$ and $\delta^{15}N$ values of the humans from Tróia

An enrichment of around 1.7‰ has been suggested for foetal $\delta^{13}C$ values during gestation and can be 1.0±0.5‰ higher than the mother´s by the end of pregnancy, whereas $\delta^{15}N$ values can be between 0.9 – 2.7‰ higher than the mother´s (Lübcker et al., 2020). The foetal and/or stillbirth individuals (T.OF1-L and TONM-B) yielded close stable nitrogen and carbon isotopic compositions. The difference (of about 1.2‰ and 1.1‰) in their $\delta^{13}C$ values to that of the male adult individual (T. PVSV), is suggestive of a probable "antenatal" diet of C_3 plant sources. Their $\delta^{15}N$ values are also close to that of adults individual. This means that their mothers probably had a lower $\delta^{15}N$ than the unique adult individual analysed. The sex of the latter is male and only future analysis within the frame of the larger ongoing project will show whether a difference in diet between males and females exist.

Regarding their stable sulphur isotopic compositions, their $\delta^{34}S$ values ranged between 12.3‰ and 13.2‰ with a mean of 12.6±0.4‰, and all the analysed samples met the quality criteria established by Nehlich and Richards (2009) (*table 5)*. These individuals exhibit consistent values all through thereby indicating the effect of sea spray or their common origin/context except one individual (T.ONM-B) showing a more enriched value (13.2‰) (*Figure 32)*. Sulphur stable isotopes in freshwater environments vary between -22‰ to +20‰; modern freshwater animals typically display values between -20‰ and +14‰ while terrestrial organisms typically have $\delta^{34}S$ values ranging from -5‰ and 10‰ but may be enriched due to evaporitic rocks filled geological settings or due to the effect of sea spray. Modern marine mammals have a range between +14‰ to +19‰, similar to marine fish (Nehlich, 2015b; Nehlich & Richards, 2009). Comparing the $\delta^{34}S$ values obtained from these individuals to the general ecosystem baselines eliminate the major influence of marine animal food source. This might be indicative of their consumption of other dietary proteins with lower $\delta^{34}S$ values in association, however, with some marine input as evidenced by the closeness of the values to the marine ecosystem range (see *Figure 32*). Interestingly, this is a coastal site where the possible sea spray effect is most likely.

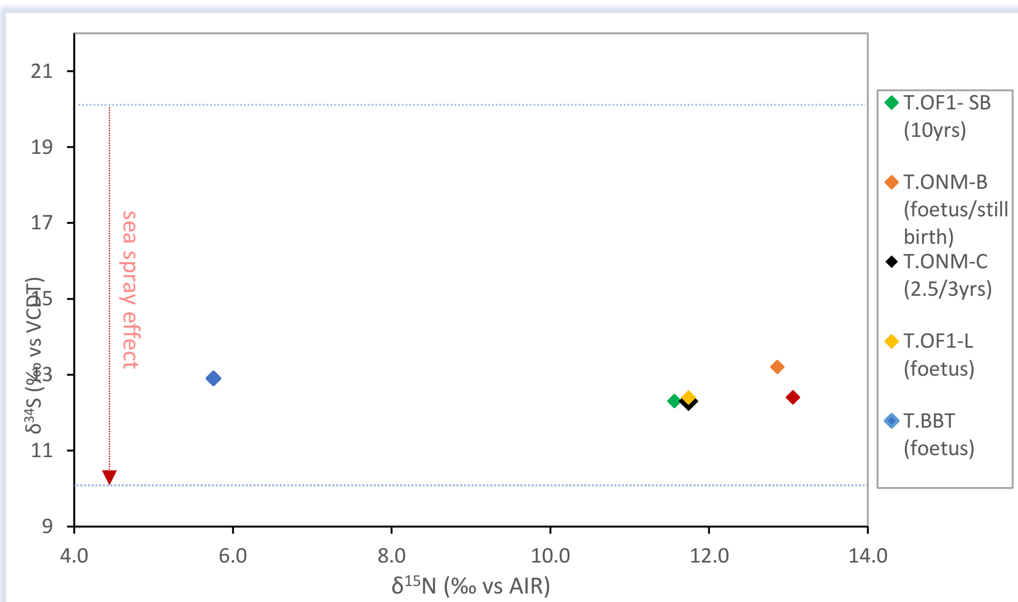

Figure 32: $\delta^{34}S$ and δ15N isotopic compositions of the samples

Also, most of the fish types explored to produce fish sauces (*Salsmenta* and *Liquamen*) are of marine origin. Sardines (*Sardinella* sp.) have been said to be the most exploited in the making of Lusitanian fish products during the Late Antiquity (Bombico, 2015a). Does this mean the people were not consuming much of the fish products being produced in their factories? The zooarchaeological remains from the site are rich in domesticated fauna with very few fish bones, although fish bones are usually not well preserved in archaeological contexts. Zooarchaeological studies in different parts of the Iberia have also indicated more livestock consumption than marine products in the late antique periods in contrast with earlier periods when fish was more common (García-Moreno et al., 2022). And it has been said that $\delta^{34}S$ values in coastal environments can be high (>14‰) due to sea spray effects (Britton et al., 2018; Nehlich & Richards, 2009). Their almost homogenous $\delta^{34}S$ values are then indicative of this effect, although this might also be pointing to their consumption of marine food resources.

6.2.2. Bioapatite Carbon and Oxygen isotopic composition and dietary implications

As mentioned in chapter 4, analysing the isotopic composition of the bone apatite helps to have a clearer picture of the dietary compositions of the individuals under study. The dietary influence of all the food sources (carbohydrate and lipids, protein) is reflected in the $\delta^{13}C$ values of the bone apatite (Garvie-Lok et al., 2004; M. A. Katzenberg, 2007), while it also incorporates the signal of the oxygen stable isotope in water consumed (Pederzani & Britton, 2019). The carbon and oxygen isotopic measurements of the bone apatite are presented in *table 6*, while those of the 9 individuals incorporated from an ongoing study of the same site are presented in *table 9, appendix III.* The decision to exclude the faunal samples is because this kind of analysis from faunal apatite is complicated as the carbon value of bone apatite and collagen can vary inter and intraspecies-wisely (Cersoy et al., 2017), as well as the limited timeline which may inhibit the completion of the study.

Table 6: C apatite-collagen spacing and oxygen stable isotopic compositions of the bone apatite

Sample ID	$\delta^{13}C_{apa}$ (‰) VPDB	$\delta^{13}C_{apa-col}$ (‰)	$\delta^{18}O_{apa}$ VPDB	$\delta^{18}O_{bc}$ VSMOW	$\delta^{18}O_{en}$ VSMOW	$\delta^{18}O_{p}$ VSMOW	$\delta^{18}O_{en}$ Phos.	$\delta^{18}O_{Dw}$ VSMOW
T.OF1-SB	-11.52	6	-6.33	24.4	26.1	-7.2	17.1	-6.0
T.ONM-A	-	-	-	-	-			
T.ONM-B	-12.87	5	-5.68	25.1	26.8	-6.1	17.8	-5.0
T.ONM-C	-12.68	5	-4.77	26.0	27.7	-4.6	18.7	-3.5
T.OF1-L	-11.10	7	-4.86	25.9	27.6	-4.8	18.6	-3.7
TBBT	-13.74	7.2	-6.30	24.4	26.1	-7.1	17.1	-6.0
T. PVSV	-13.62	4	-5.06	25.7	27.4	-5.1	18.4	-4.0
T. PV-897	-	-	-	-	-			
Mean	**-12-50**	**-**	**-5.22**	**25.23**	**27.23**	**-5.34**	**18.23**	**-5.1**
Std. Dev.	**1.0**	**-**	**0.7**	**0.7**	**0.7**	**1.1**	**0.7**	**1.0**

Analysis of the children apatite samples yielded $\delta^{13}C_{apa}$ values ranging from -13.7‰ to -11.1‰ with an average of -12.6 ± 1.1‰., excluding individual T.ONM-A whose %N by EA was low, and as such was not included for bioapatite analysis. As asserted by Lee-Thorp (2008), there is a difference of +12‰ between diet and bone apatite $\delta^{13}C$ values. In a situation where the people feed entirely on C_3 plants, values around -14‰ could be expected, while values close to 0‰ would be observed in a purely C_4-based diet. Hence, the values obtained are well within this range, thus, suggesting a diet (lipid carbohydrate and protein) majored on C_3 plants during the bone formation of these individuals from Tróia.

To have a better idea of the influence of different dietary sources, the stable carbon apatite-collagen ($\delta^{13}C_{apa\text{-}col}$) spacing values can be employed to discriminate trophic levels, as the trophic level decreases with increasing $\delta^{13}C_{apa\text{-}col}$ spacing (Saragoça et al., 2016). For a better understanding of this relationship, the stable carbon apatite-collagen ($\delta^{13}C_{apa\text{-}col}$) spacing values were plotted against the $\delta^{15}N$ values of these individuals as seen in *Figure 33*. The average $\delta^{13}C_{apa\text{-}col}$ obtained for the children individual is 6‰. The idea here is that any individual with $\delta^{13}C_{apa\text{-}col}$ value less than 7‰ is ^{13}C enriched which can be indicative of fish consumption and/or diets comprising of C_4, while an individual with values more than 7‰ indicates a dietary intake of ^{13}C depleted food resources, where 7‰ represent monoisotopic individuals as illustrated in *Figure 33* (Salesse et al., 2013).To understand the adult $\delta^{13}C_{apa\text{-}col}$ values obtained, values of three late antique individuals were extracted from Saragoça et al., (2016), and plotted in Figure 33, thereby showing that the $\delta^{13}C_{apa\text{-}col}$ obtained is consistent with the interpretation.

Most of the samples analysed present values of $\delta^{13}C_{apa\text{-}col} < 7$, indicating a probable ^{13}C-enrichment in the protein component of their diet, and that the presence of lipids in their diet reduced the spacing between collagen and apatite stable carbon isotopes (Salesse et al., 2013; Saragoça et al., 2016). Generally, the carbon and nitrogen isotopic compositions of the analysed human bone samples indicate a population with a mixed diet composed majorly of C_3 plants as well as high proteinous food consumption. This is a coastal site with evidence of fish processing (salted fish) and fish sauce production, the people likely consumed some of their fish products, thereby indicating the influence of marine and/or freshwater fish in their diet. Their sulphur-stable isotopic composition shows the influence of protein dietary sources depleted in $\delta^{34}S$ as compared to a population that would rely mainly on marine fish (Nehlich, 2015a), and their $\delta^{34}S$ values are also higher than those expected from a pure terrestrial environment. This could be a result of the consumption of fish and/or plants cultivated under the sea spray effect. The coincidence of this depleted $\delta^{34}S$ average and high $\delta^{15}N$

values is suggestive of their consumption of low trophic level fish (e.g., sardine), which was the kind used in fish sauce production (Nehlich et al., 2011),.

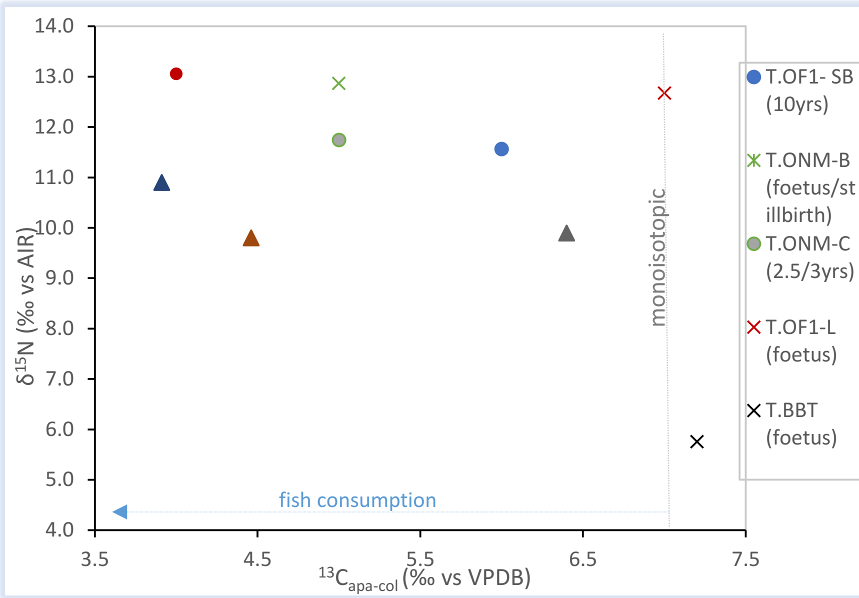

Figure 33: $\delta^{13}C_{apa\text{-}col}$ spacing and $\delta^{15}N$ values of the human samples compared with 3 adults samples (triangles) from Saragoça et al., (2016)

The dietary difference between the adult individuals and the children might be indicative of access due to status/age group. However, the fish used for the fish sauce production was likely available to the local population. The zooarchaeological studies from this site also recorded the presence of some animals (e.g., ovicaprids, pigs, chicken cattle, etc) used as food in the site (Nabais, 2015), which further establish the influence of animal dietary protein in the food consumption of the people of Tróia. The study of the faunal diets is needed before any conclusion can be drawn regarding human diets.

Regarding weaning and childhood diet, it is understandable that the small sample size limits this interpretation, but from the $\delta^{13}C$ and $\delta^{15}N$ values obtained, it is likely that the 2 years old individual was still being breastfed up to the time of death, while the individual of 2.5 years of age was almost/completely weaned and was even consuming solid food almost similar to that of the 10 years old child. The question now is: was this the general weaning pattern in this site and other Roman sites in Iberia during Late Antiquity? To answer this question, a larger dataset of children is required. Weaning practices during the Roman period were reported to contain supplementary foods (boiled honey, or a mixture of honey and goat´s milk) introduced gradually from the age of six months and complete termination of breastfeeding at three years of age. Supplementary foods introduced during the weaning process often result in the gradual depletion of $\delta^{15}N$ and reaching the adult values when the child is completely weaned (Fuller et al., 2006; Saragoça et al., 2016). There is a significant enrichment (of 1.5‰) in the adult $\delta^{15}N$, thereby indicating a clear difference in the food types consumed between adults and children, which can be suggestive of a gradual incorporation of more protein or foods of higher trophic levels as individuals mature. Again, the low sample amount makes these interpretations a tentative possibility of the data obtained, which would be further explored and contextualised in the results of the ongoing adult project.

6.2.3. Mobility and Oxygen data

For the oxygen analysis, nine adult samples from the larger project were included to check their variability. The oxygen values were converted from VPDB to VSMOW using the equation established by Coplen (1988). The resultant values were then converted to teeth VSMOW through an equation by Warinner and Turos (2009), while the teeth carbonate ($\delta^{18}O_c$) values obtained were converted again to teeth phosphate ($\delta^{18}O_p$) because of the diagenetic vulnerability of carbonate ions and a better understanding of the connection between $\delta^{18}O_p$ values in bioapatite and drinking water ($\delta^{18}O_{Dw}$) (Chenery et al., 2012; Daux et al., 2008; MacRoberts et al., 2020). Conversion to drinking water ($\delta^{18}O_{Dw}$) was then carried out to compare oxygen bioapatite relative to consumed water based on the equation (6) $\delta^{18}ODw = 1.590 \times \delta 18OC - 48.634$) from Daux et al., (2008), as applied by Chenery et al., (2012). These conversions were necessary as the oxygen isotope data for Portugal is relatively recent and the range for $\delta^{18}O_p$ of the population is yet to be fully ascertained (MacRoberts et al., 2020). The values obtained are presented in *Table 6.*

The precipitation $\delta^{18}O$ values for Tróia were established by acquiring data from the Global Network of Isotopes in Precipitation (GNIP) from IAEA (WISER-Water Isotope System for data analysis, visualization and Electronic Retrieval, 2022). The annual $\delta^{18}O$ mean obtained for 2014 and 2017 varies between -4.31 and -5.32‰, however, as oxygen isotope data is known to fluctuate within short period (Lightfoot & O'Connell, 2016), a cautious range was established between the months of May and August which had the highest records of -1.46‰ and -2.92‰ while the lowest values were recorded in the month of December with a value of -6.65‰. Illustrated in *Figure 34* are the oxygen isotopic compositions of the human samples analysed in this study, with those of nine adult individuals from the larger project (*data in table 9, Appendix III*) for comparison.

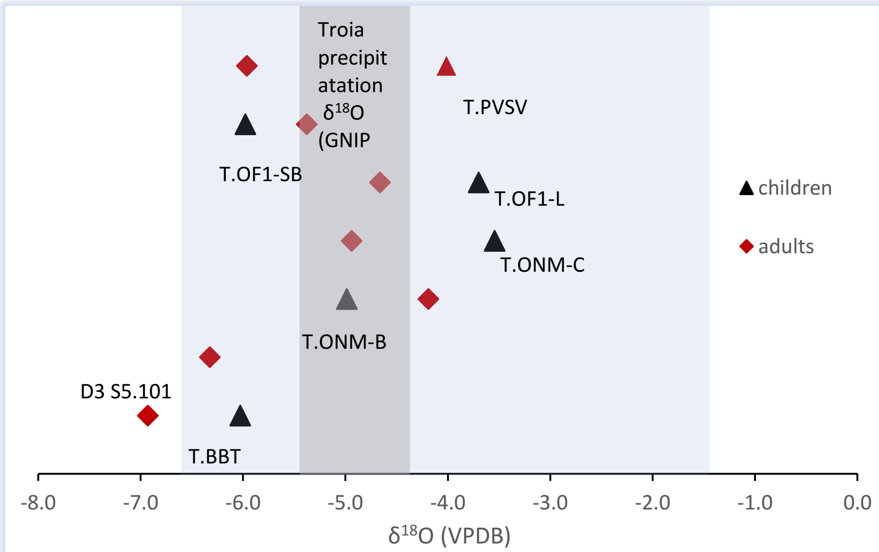

Figure 34: Tróia human bones GNIP precipitation data ($\delta^{18}O$) - recorded annual averages are shaded in grey, and seasonal variability in lighter blue (red diamonds = adult samples from the ongoing project; black triangles = children samples in this study; red triangle = adult sample in this study.

Variation between the $\delta^{18}O$ values of the analysed individuals can give mobility signals which can be further explored using such mobility indicators as strontium. The $\delta^{18}O$ values of the children individuals were plotted with those of the adults from the larger project to show variability, as seen in *Figure 34.* All the children appear to have consumed precipitation water within the variability established. Individual T.ONM-B and two adult samples had values that tally exactly within the GNIP range. Two of the children samples seem to have had $\delta^{18}O_{Dw}$ enriched in ^{18}O but they are within the variation range. Of all the samples analysed, an adult individual D3.S5.101 is the only outlier, which is suggestive of mobility, since s/he consumed water with isotopic precipitation from other environments (see *Figure 34)*.

As highlighted in chapter four, human body water is enriched with heavy $\delta^{18}O$ relative to water consumed from food and as drinking water. The preferential removal of the lighter oxygen stable isotope through different processes occurring within the human body usually leads to the presence and high concentration of heavy ^{18}O (Bryant and Froelich, 1995; Kohn, 1996). Breastmilk is derived from the water pool of the body and is heavier in ^{18}O than the water consumed by the lactating mother, which usually leads to the presence of heavier ^{18}O in breastfeeding children. Unfortunately, the individual T.ONM-A that shows a signal of breastfeeding was not included in this study due to its digenetic signal detected through elemental analysis. However, individual T.ONM-C also shows an indication of containing heavier $\delta^{18}O$, which might be suggestive of breastfeeding, since s/he seems to be one the with heavier $\delta^{18}O$ values (*Figure 34).* The sample size of children analysed in this study limits what can be inferred since one individual cannot represent a category of people within a population.

6.2.4. Faunal Dietary Reconstruction

The isotopic compositions of all faunal remains analysed are listed in *table 6*. The animals analysed are of two categories, namely herbivores (*Bos taurus, O. cuniculus,* ovicaprids, *C. elaphus, Equus asinus)* and omnivores (Sus sp. and Gallus sp.) (*table 3*). As can be seen in *table 7,* they exhibited varying carbon and nitrogen isotopic compositions with a reflection of consuming diets rich in C_3 plants. For better understanding, this relationship is illustrated in *Figure 35.* Some of the analysed faunal samples (T.488e, T.471b, T.1355a&d) did not meet the collagen quality control criteria, and as such are excluded from the following discussion.

Table 7:Faunal carbon and nitrogen isotope results and quality indicator of collagen (empty = no collagen)

	Sample ID	Type	Collagen Yield	%C	%N	C:N	C:S	N:S	%S	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Fauna	**T.554a**	O. cuniculus	5.94	36.67	12.93	3.3	407	123	0.24	-20.1	5.4	17.4
	T.554b		6.94	39.52	14.30	3.2				-21.2	7.8	
	T.488x		7.19	40.75	14.75	3.2	418	130	0.26	-22.5	5.1	16.2
	T.488f		2.11	39.65	13.94	3.3			0.08	-22.0	3.6	14.9
	T.488a	Bos	12.97	36.53	13.32	3.2	541	169	0.18	-22.0	5.2	11.6
	T.4888b	Gallus sp.	14.31	39.48	14.25	3.2	421	130	0.25	-19.4	9.0	15.0
	T.488c		13.45	40.34	14.40	3.3	489	150	0.22	-19.4	9.2	14.4
	T.488d	ovicaprid	15.35	39.98	14.45	3.2	561	174	0.19	-21.3	9.2	9.8
	T.488e		5.86	11.55	4.15	3.2	-	-	-	-23.6	5.4	
	T.518a	Sus sp.	12.84	38.22	13.80	3.2	536	166	019	-19.6	8.0	16.3
	T.518b		14.92	38.24	13.82	3.2	566	176	0.18	-20.1	8.1	15.4
	T.471a	C. elaphus	5.37	22.02	8.12	3.2	-	-	-	-21.7	4.5	
	T.471b		9.65	36.90	13.06	3.3	518	157	0.19	-21.3	4.1	10.5
	T.1355a	Equus asinus	3.35	16.89	5.95	3.3	-	-	-	-21.5	9.2	
	T.1355b		1.19	5.36	1.86	3.4	-	-	-	-22.3	8.6	
	T.1355c		0.59	-	-	-	-	-	-	-	-	
	T.1355d		3.77	-	-	-	-	-	-	-	-	

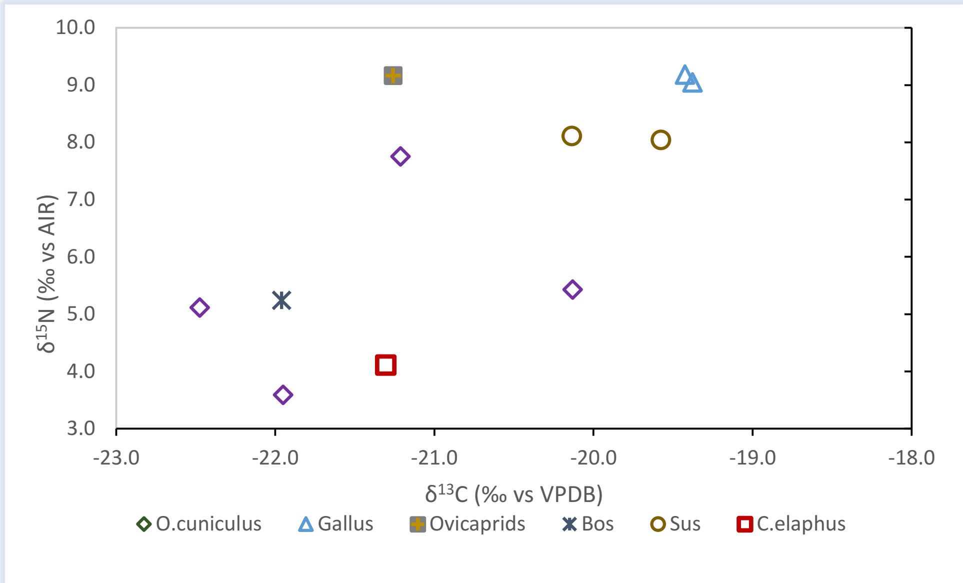

Figure 35: $\delta^{13}C$ and $\delta^{15}N$ values for fauna from Tróia.

As seen in *Figure 35*, the faunal analysed presents different values of $\delta^{15}N$ and $\delta^{13}C$. Regarding the wild animals, *C elaphus* present a typical signal of a wild C_3 grazer, but the rabbits (O. cuniculus) show a wide range of $\delta^{15}N$ and $\delta^{13}C$ values which might be indicative of mixed food ingestion (wild and manured). The *Sus* and *Gallus* are omnivorous animals and, as expected, present high values of $\delta^{15}N$ (mean= 8.4‰) and ^{13}C (mean= -19.6‰), because they were probably fed with human food leftovers and by-products, which likely included for some low trophic level fish remains (reflected by a higher $\delta^{13}C$ value). The chickens present a higher $\delta^{15}N$ value when compared to the pigs, likely because they complemented their diet with insects and other small animals, thereby increasing the amount of protein in their diet.

The ovicaprids analysed present very different values of $\delta^{15}N$ and $\delta^{13}C$, indicative of a different diet. This difference could be due to the different husbandry practices among the analysed animal species, but ZooMS is needed to be able to identify and distinguish the osteological remains of these animals to the genus level. The single Bos sample analyzed presents a diet mostly of non-manured C_3 plants. It is important to refer that the zooarchaeological data from the site suggested the rarity of large mammal consumption in the site, but smaller animals such as pigs, chickens, and goats/sheep were common (Nabais, 2015).

To better reconstruct the human diets, consideration of their isotopic values should be done relative to the local faunal rather than isolating the data (MacRoberts et al., 2020; Saragoça et al., 2016). Hence, the carbon and nitrogen stable isotopic compositions of the humans and faunal analysed are illustrated in *Figure 36*. It can be observed that the animal protein used by humans is mainly derived from sus and chicken (*figure 36*), which comes in agreement with the available archaeozoological data (Nabais, 2015). However, the slightly more positive human $\delta^{15}N$ and $\delta^{13}C$ average values are likely due to the consumption of small amounts of low trophic fish (Chen et al., 2012).

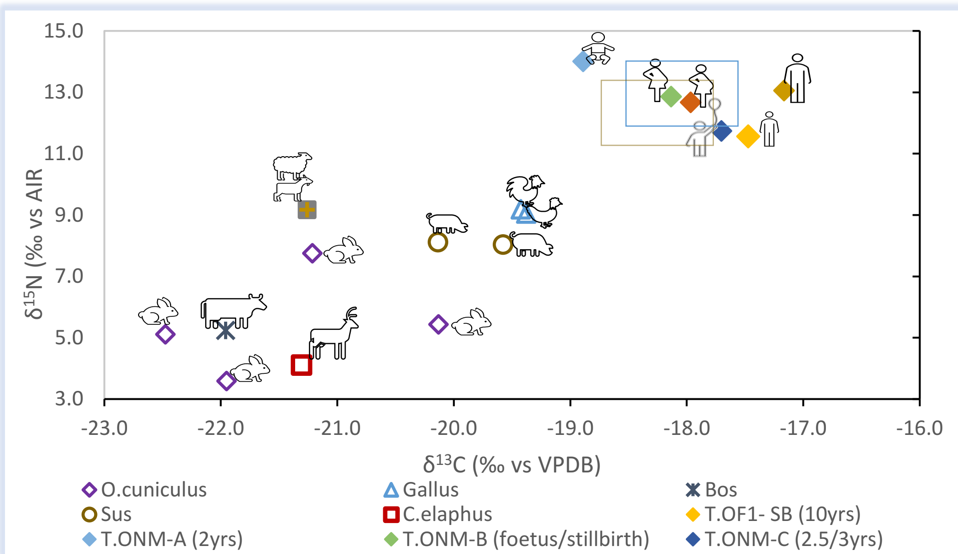

Figure 36: $\delta^{13}C$ and $\delta^{15}N$ values for human and fauna from Tróia

Due to little or no bone collagen yielded by some faunal samples, analysing $\delta^{34}S$ compositions was only possible for ten of them. The $\delta^{34}S$ values in the faunal sample also ranged between 9.8 ‰ and 17.4‰ with a mean of 14.15 ‰. Only one (ovicaprids-T.488d) of the ten samples shows values within the range. Others are more enriched in $\delta^{34}S$ values than the accepted values. Terrestrial animals typically have $\delta^{34}S$ values between -5‰ to +10‰, the enrichment of the sulphur isotopic compositions in the majority of the samples is indicative of the sea spray effect (Britton et al., 2018). T.488d exhibits values within the acceptable range (9.8‰), thereby showing its consumption of diet depleted in $\delta^{34}S$ characteristics of the terrestrial diet. Similarly, the red deer exhibited a 0.05‰ $\delta^{34}S$ enrichment while Bos shows a value that is 1.5‰ than the acceptable range for terrestrial habitat. These animals likely arose from locations further away from this archaeological site. Red deer usually live in forest environments which were not located in the immediate vicinity of the site. The enrichment seen in the terrestrial faunal values here is due to the sea spray effect since this is a coastal site (Nehlich, 2015b; Nehlich et al., 2011; Nehlich & Richards, 2009). This relationship is illustrated in *Figure 37,* based on the different ecosystem values exhibited by the fauna and humans from Tróia.

Hence, as indicated by $\delta^{34}S$ values obtained from the human and faunal analysed, effect of sea spray is evident. However, the humans had diets relatively depleted in ^{34}S probably due to freshwater fish and/or animal diet consumption, while the $\delta^{34}S$ values of the fauna are masked by the sea spray effect except for one ovicaprid whose $\delta^{34}S$ value shows terrestrial food influence, and two samples (cow and red deer) which are within the freshwater range (see *figure 37*). Considering how close the human values are to the marine diet range, this is an indication of their marine animal diet consumption although not as high as the freshwater and terrestrial food diets.

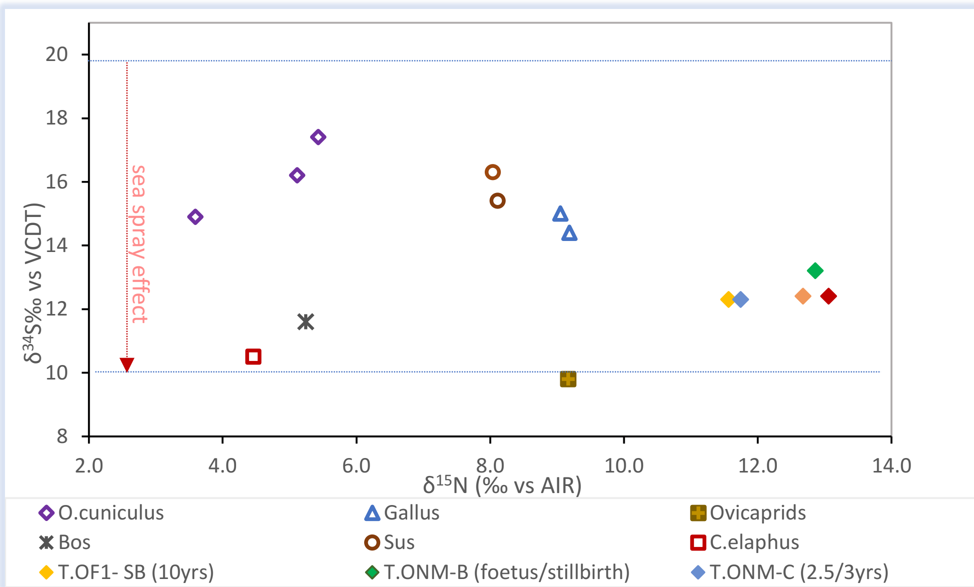

Figure 37: The $\delta^{34}S$ values exhibited by the Humans and fauna from Tróia (blue lines represent the range of sea spray)

6.2.5. Animal Husbandry Implications.

Based on the stable carbon, nitrogen, and sulphur isotopic compositions assessed from the faunal samples from Tróia, it became evident that this was a husbandry practice that was based on C_3 plant consumption. The variability between the $\delta^{15}N$ values can be a result of manuring soils with fertilizers such as algae or faecal matter where the animals were kept or keeping the animals in enclosures, thereby increasing the $\delta^{15}N$ values (García-Moreno et al., 2022; Saragoça et al., 2016). Clustering can also indicate the faunal consumption of similar plant diets (see *Figure 33).* But in the case of the faunal from Tróia, the same faunal type (e.g., ovicaprids, *O. cuniculus, Equus)* shows some variabilities in their $\delta^{13}C$ values scattered around the plot, therefore indicating their consumption of different C_3 plants or different parts of C_3. Based on the zooarchaeological data of the site, large mammals such as cattle and horses are rare, which is indicative of the absence of large animal husbandry. However, pigs and caprines are well represented in the zooarchaeological assemblage of the site, as they can be easily maintained, which are indications of small-sized animal husbandry on a relatively small scale and a probable short-term basis.

With the obtained faunal $\delta^{34}S$ values, it is apparent that some of the animals (e.g., red deer and sheep/goat) were from a more terrestrial ecosystem. Most of the faunal samples exhibited higher values than humans, therefore indicating the presence of evaporated marine sulphates within the ecosystem of the site. However, the variability in their $\delta^{34}S$ values can also be used to extrapolate and identify the probable animal importation to the site. Faunal sample T.488d (ovicaprid) shows the value that is very distinct from the other faunal, and even to those of the humans, which is suggestive of being newly brought to the site and was fed less with plants from the marine ecosystem before it was killed (see *Figure 37)*. Two others (*Bos* sp. and *C. elaphus*) reflect values very different from other faunal samples (*Figure 37)*. Although they are enriched in $\delta^{34}S$ than the terrestrial range, the fact that bone remodels over a period of time simply indicates that their bone collagen $\delta^{34}S$ values were yet to be fully equilibrated with the isotopic values of the coastal environment, therefore suggesting that they were more recent to the area than the ones reflecting marine $\delta^{34}S$ values (Nehlich, 2015a). The red deer is a wild animal which was probably hunted from a different ecosystem. Therefore, through this sulphur isotope analysis of the faunal samples, it can be deduced that there is an influence of probable external animal food materials in this site.

6.3. Comparison with other Late Antique sites in the Iberian Peninsula

The average value of the herbivores was compared to those of the herbivores from late antique Roman sites across the Iberia. All the sites compared present a mainly C_3 plant-based diet husbandry with varying $\delta^{15}N$ values (Alaica et al., 2019; Alexander et al., 2019; Fernández-Martinez et al., 2020; García-Collado et al., 2019; García-Collado et al 2016; Garcia, Subira, and Richards 2004; Saragoça et al., 2020). The values obtained from Tróia show a very distinct pattern as the herbivores appear to be more depleted in $\delta^{13}C$ than any of the sites (see *Figure 38)*. This is indicative of husbandry practices more based on C_3 plants than any of the sites. It might also be a probable confirmation of the zooarchaeological perspective which indicates little to no agricultural activities were taking place on the site (Nabais, 2015), because large animal remains such as cattle are very few. The inclusion of values from the omnivores such as pigs and chickens does not yield any significant change, due to the low sample size. Hence, to fully elucidate this dietary difference of Tróia from other Late Antique sites, there is a need for a larger sample size. Representation of a group by two individuals cannot be used to fully distinguish this trend.

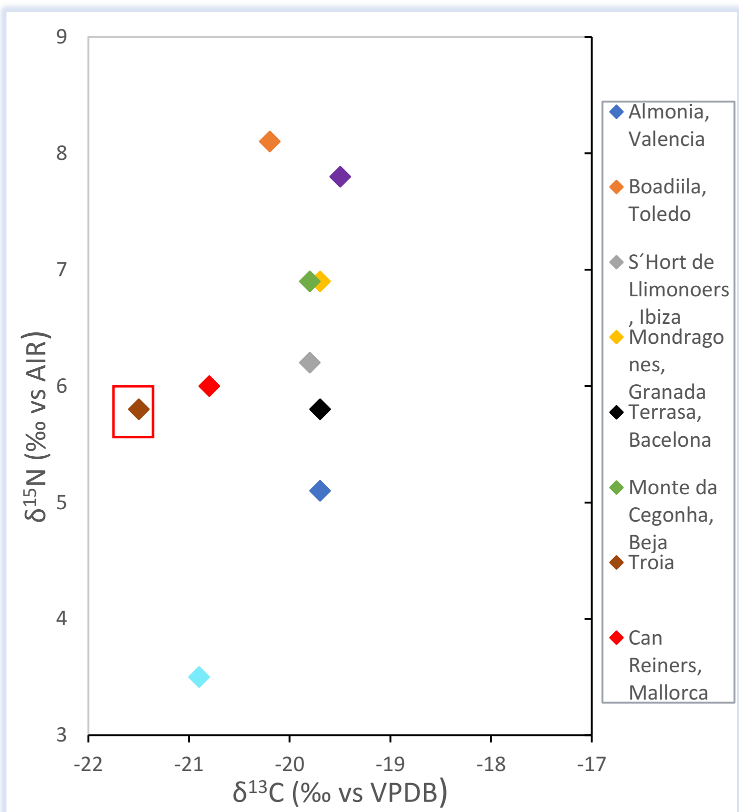

Figure 38: $\delta^{13}C$ and $\delta^{15}N$ mean values for Late antique herbivorous faunal in Iberia

As can be observed in *Figure 38,* it is obvious that animal feeds were based on C_3 plants during Late Antiquity. The use of C_3 plants is also evident in Tróia according to their $\delta^{13}C$ values, but with limited usage of manured crops when compared with $\delta^{15}N$ values of the other sites, which the Troia´s value seems to average (*figure 38*). This is indicative of a very different animal management or the absence of specialised husbandry practice in Troia.

One of the objectives of this study is to ascertain the early childhood dietary pattern in this site and southwestern Lusitania. Very few late antique children's skeletal remains have been studied through geochemical exposition in the Iberia peninsula. Only one is compared in this study due to its well-established date which is exactly contemporaneous to the materials being studied. Fernandez-Martinez et al., (2020) studied the Late Roman population from Mondragones (Granada, Spain) including 24 non-adults, where it was deduced that non-adults aged 0-3 consumed diets that were significantly different from adults, possibly due to breastfeeding, while individuals between the age of 9 and 18 had diets not so different from adults. Hence, the diets of the people on the site were rich in C_3 plants, with some meat in-take from terrestrial herbivores (Fernandez-Martinez et al., 2020). The average values for the children and young adult individuals from Mondragones were extracted and compared with the values obtained from the children samples with the inclusion of one adult (TPSV) to establish a trend, as illustrated in *Figure 39*.

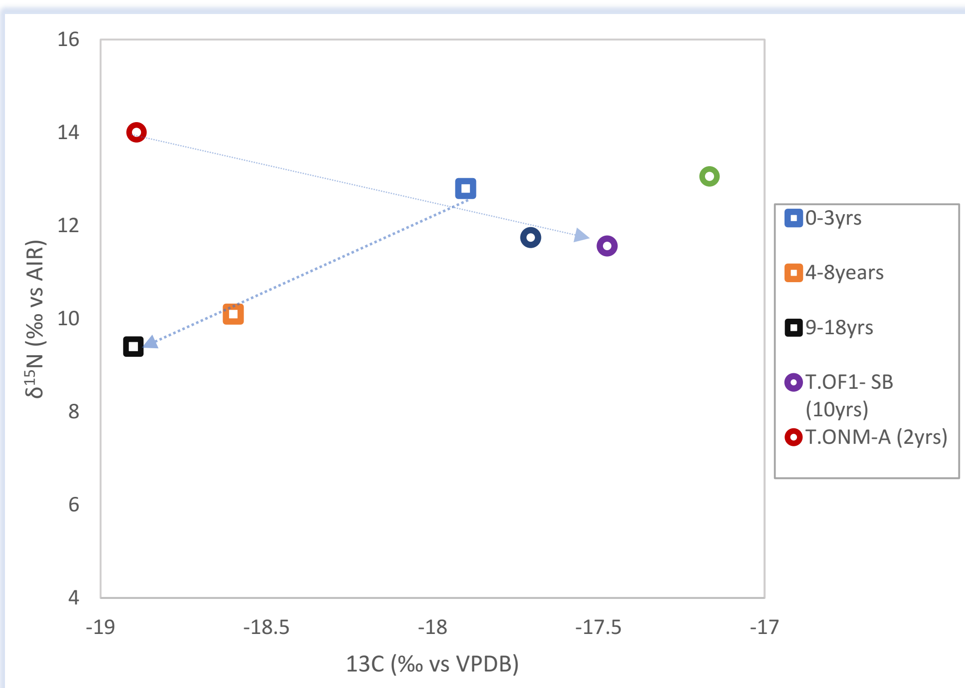

Figure 39: $\delta^{13}C$ and $\delta^{15}N$ mean values of children and young adults from Mondragones with samples from Tróia. Square= Mondragones` samples, circle represents the samples from Tróia

From the plotted graph, two interesting trends are observable. The similarity between the two sites is the consumption of more C_3 than C_4 plants, and the influence of terrestrial faunal is also evidenced by the isotopic values and the proportion of zooarchaeological materials recovered from Tróia. However, there is an inverse relationship in the diets of the sites relative to age. The children from Mondaragones were weaned with diets that were more ^{13}C depleted, which is reflective of being fed with more C_3-rich solid food. Tróia on the hand shows a signal reflective of more fish product consumption as the children aged. Put differently, as the children grew in Tróia, more $\delta^{13}C$ depleted solid food materials were incorporated into their food. The ten years old child (T.OF1-SB) shows a signal of ^{13}C enrichment and depletion in ^{15}N which can be indicative of restricted access to other dietary proteins compared to the adults. One possible reason for the inverse relationship is that the population of Mondragones were known for their farming activities, and as such lived mainly from their agricultural production (Fernandez-Martinez et al., 2020). Tróia on the other hand was known for its fish processing activities, with no evidence of agriculture. Hence, they probably introduced some fish-based products into their diet. While it is true that the diet of the adults from both sites is different from those of age 9/10 downwards, the age at which young adults' isotopic values would be similar to those of adults cannot be ascertained in this study due to the small sample size. One adult and three children cannot be used as representatives of the population. This is a pilot study of a much larger project within which these results and hypotheses would be properly contextualised. This also leaves room for future study to determine the age at which the children from Tróia attained cultural adulthood, at least in terms of diet.

7. Conclusions

Archaeological bones are often with certain alterations that usually limit the isotopic interpretation of the data obtained. This usually leads to the unnecessary destruction of osteological samples without yielding meaningful information which more often than not wastes resources, manpower, and time. Analysing bone samples with ATR-FTIR, EA, and XRD helps to identify diagenetically altered bones from the ones that are well preserved, which is very advantageous as it prevents the destruction of such archaeological materials with vital information that can be lost forever when destroyed unnecessarily. These methods were employed to assess the preservation status of the analysed bone remains.

About 9 adult samples from mother-project of this study were incorporated to check bone integrity relative to age and the spectra and elemental compositions obtained were used to exclude some samples (TONM-A, and T. PV-897) from further analyses, although an attempt was made to extract their collagen due to the importance of their age-group in this study. Some samples showed increased crystallinity, but they yielded enough collagen for analysis, however, T. PV-897 came back with empty vials, thereby confirming the results of EA. Whereas, T.ONM-A yielded enough collagen for nitrogen and carbon isotope analysis but shows indication of carbon contamination which therefore establishes the EA and FTIR as a pre-screening protocol before isotope analysis of human and faunal osteological remains. The results obtained from these analyses also prompted the need to check the samples with XRD. Three samples comprising one child and two adults were analysed with XRD, and two samples (one adult, one child) indicated the presence of calcite in their mineral phase with moderately increased crystallinity. The alteration indices calculated through these physico-chemical techniques point to a slightly degraded group of bone samples. Comparison with values from other studies shows that most of the samples analysed in this study are relatively well preserved.

The dietary implications inferred from stable carbon and nitrogen indicate that dietary habits for this group of people from Tróia were based on major consumption of C_3 plants with some addition of animal protein and fish products, complemented with terrestrial faunal. The collagen-apatite spacing δ^{13}C values of the human individual point to the consumption of some fish products. The location of Tróia between the Atlantic coast and Sado River´s estuary is an indication that the people probably exploited the freshwater Sado River for fish. It has been stated in chapter two that the fish from both Sado and the Atlantic were exploited to ensure resource availability over certain periods of the year (R. de Almeida et al., 2014; Pinto et al., 2021). The values of δ^{34}S for the individuals studied are lower than those of most of the animals gathered from the site. To gain more insight into the dietary habits of the Tróia inhabitants, there is a need to analyse the fish bones from freshwater environment as their sulphur values can be very variable, and in this way investigate a possible contribution of these food products.

Regarding childhood diet, it is difficult to centre our interpretation of this aspect around five to six individuals. But the implication of the results obtained point to predominantly C_3 diets used in weaning children from about the age of two upwards. Although the 2.5/3 years old individual (T.ONM-C) shows values very close to those of the ten years old and indicated their consumption of similar diets gradually enriched in fish products. The adult diets were very different from those of children, as more fish products likely incorporated into the diet caused the enrichment in his $\delta^{13}C$ value. The high $\delta^{15}N$ value and his less enriched $\delta^{13}C_{apa\text{-}col}$ are indicative of the high protein diet consumption of fish in concert with proteins from terrestrial fauna. While a lower protein diet in children is evidenced. Most of the individuals analysed from Troia also consumed similar precipitation water based on the oxygen isotope data extracted from IAEA WISER, reflective of a coastal_environment with an outlier (D3.S5.101) indicating more depletion of stable oxygen due to consuming water different from Troia´s precipitation values. This can be checked in future studies with more mobility indicators such as strontium isotopic ratios of these individuals. The clustering of the analysed humans with almost perfectly matched $\delta^{34}S$ values can also be indicative of similar geographical origin since sulphur values in organisms are environmentally and geologically controlled, however, such homogeneity in their values is also reflective of the effect of sea spray.

Animal husbandry practices in the Roman site of Troia were on C_3 plant-based feeds. Such animals as pigs and chickens appear to be fed with human food scraps. Enrichment in the nitrogen isotopic compositions of some wild and domesticated herbivorous animals is indicative of manure application to the plants or field in which the animals grazed or highly saline environment, while the animals that required less caring such as pigs and chicken might have been kept for the dietary purpose. Most of the faunal analysed for $\delta^{34}S$ values reflect signs of sea spray as their values are significantly enriched in $\delta^{34}S$ above 10. An ovicaprid (T.488d), the Bos and red deer reflect $\delta^{15}N$ and $\delta^{34}S$ values of terrestrial fauna, a signal that they were raised far from the effect of the sea spray. it can then be concluded that some of the terrestrial animals on the site are of external origin. Therefore, corroborating the zooarchaeological inferences, although the low sample size analysed in this study has put a limit to what can be said. Hence, this is a pilot study of a much larger project where these hypotheses would be fully explored and elucidated.

In conclusion, this multi-isotopic study of the children and faunal bone remains from Tróia has improved the understanding of diet and animal management in southwestern Lusitania. There are chemical indications of the external influence of animal food resources from other places or nearby cities. The variability in the $\delta^{13}C$ values of some herbivores has indicated possible consumption of C_3 plants from different environments. Not much can be said about the possible childhood diet for the general population, all that is done in this study is to take a quick peep at what the dietary trend could be. Based on the analysed samples, the children were probably still being breastfed with the addition of solid food until they were completely weaned at a probable age of three, which is consistent with other Roman sites. The ongoing adult study from the same site will help to contextualise this snapshot of life about childhood in Late Antique Tróia and southwestern Lusitania.

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References

Alarcão, J. de. (1989). [Recensão a] J. C. Edmondson, Two industries in Roman Lusitania: Mining and garum production. *Conimbriga: Revista de Arqueologia*, *28*, 236–243. https://doi.org/10.14195/1647-8657_28_13

Alexandra, S., & Bombico, R. (2017). *ECONOMIA MARITÍMA DA LUSITÂNIA ROMANA : EXPORTAÇÃO E. I.*

Ali, A., Chiang, Y. W., & Santos, R. M. (2022). X-Ray Diffraction Techniques for Mineral Characterization: A Review for Engineers of the Fundamentals, Applications, and Research Directions. *Minerals*, *12*(2). https://doi.org/10.3390/min12020205

Almeida, R. de, Pinto, V. I., Magalhães, A. P., & Brum, P. (2014). *Which amphorae carried the fish products from Tróia (Portugal)?* https://www.academia.edu/es/8345053/Which_amphorae_carried_the_fish_products_from_Tróia_Portugal_

Almeida, J. P. (2009). *A necrópole romana da Caldeira, Troia de Setubal: Escavações de Manuel Heleno nas décadas de 40-60 do século XX*. Repositório Da Universidade de Lisboa. https://repositorio.ul.pt/handle/10451/362

Amato A & Bombico S. (2013). (14) Romans and Muslims in the Portuguese Atlantic | Sónia Bombico and Alessia Amato—Academia.edu. In Marie-Yvane Daire, Catherine Dupont, Anna Baudry, Cyrille Billard, Jean-Marc Large, Laurent Lespez, Eric Normand, & Chris Scarre (Eds.), *BAR International Series 25702013 Anciens peuplements littoraux etrelations Homme/Milieu sur les côtesde l'Europe atlantiqueAncient Maritime Communities andthe Relationship between People andEnvironment along the EuropeanAtlantic Coasts*. Archaeopress Publishers of Bri�sh Archaeological Reports Gordon House 276 Banbury Road Oxford OX2 7ED England. https://www.academia.edu/1368772/Romans_and_Muslims_in_the_Portuguese_Atlantic

Ambrose, S. H. (1990a). Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Science*, *17*(4), 431–451. https://doi.org/10.1016/0305-4403(90)90007-R

Ambrose, S. H. (1990b). Preparation and characterization of bone and tooth collagen for isotopic analysis. *J Archaeol Sci*, *17*(4), 431–451. https://doi.org/10.1016/0305-4403(90)90007-r

Ambrose, S. H., & Norr, L. (1993). Given the isotopic composition of a specific tissue, that of the diet or of other tissues may be calculated if the. *Prehistoric Human Bone*, 1–37.

Artioli, G. (2016). X-Ray Diffraction. In *Encyclopedia of Earth Sciences Series* (pp. 1019–1025). https://doi.org/10.1007/978-1-4020-4409-0

Badeck, F. W., Tcherkez, G., Nogués, S., Piel, C., & Ghashghaie, J. (2005). Post-photosynthetic fractionation of stable carbon isotopes between plant organs—A widespread phenomenon. *Rapid Communications in Mass Spectrometry*, *19*(11), 1381–1391. https://doi.org/10.1002/rcm.1912

Beasley, M. M., Bartelink, E. J., Taylor, L., & Miller, R. M. (2014). Comparison of transmission FTIR, ATR, and DRIFT spectra: Implications for assessment of bone bioapatite diagenesis. *Journal of Archaeological Science*, *46*(1), 16–22. https://doi.org/10.1016/j.jas.2014.03.008

Bell, L. S., Cox, G., & Sealy, J. (2001). Determining isotopic life history trajectories using bone density fractionation and stable isotope measurements: A new approach. *American Journal of Physical Anthropology*, *116*(1), 66–79. https://doi.org/10.1002/ajpa.1103

Berrocal-Rangel, L. (2018). The Lusitanian Wars, a faceless conflict from the Archaeology of the 2nd century BC. *Fields of Conflict: Conference Proceedings Vol 4 Pequot Museum and Research Center*.

Biltz, R. M., & Pellegrino, E. D. (1977). The nature of bone carbonate. *Clinical Orthopaedics and Related Research*, *129*, 279–292. https://doi.org/10.1097/00003086-197711000-00040

Bishop, K. G., Garvie-Lok, S., Haagsma, M., MacKinnon, M., & Karapanou, S. (2020). Mobile animal management in the Mediterranean: Investigating Hellenistic (323-31 BCE) husbandry practices in Thessaly, Greece using δ13C, δ18O, and 87Sr/86Sr recorded from sheep and goat tooth enamel. *Journal of Archaeological Science: Reports*, *31*(April). https://doi.org/10.1016/j.jasrep.2020.102331

Bombico, S. (2015a). Salted fish industry in Roman Lusinatia: Trade memories between Oceanus and Mare Nostrum. *Heritages and Memories from the Sea Conference Proceedings. 1st International Conference of the UNESCO Chair in Intangible Heritage and Traditional Know-How: Linking Heritage 14-16 January 2015. Évora. Portugal Conference Proceedings*, *January*, 229.

Bombico, S. (2015b). Salted Fish Industry in Roman Lusitania: Trade Memories Between Oceanusand Mare Nostrum. *1st International Conference of the UNESCO Chair in Intangible Heritage and Traditional Know-How: Linking Heritage*, 19–39.

Bombico S. (2020). Anciens peuplements littoraux et relations Homme/Milieu sur les côtes de l'Europe atlantique / Ancient Maritime Communities and the Relationship between People and Environment along the European Atlantic Coasts. *Anciens Peuplements Littoraux et Relations Homme/Milieu Sur Les Côtes de l'Europe Atlantique / Ancient Maritime Communities and the Relationship between People and Environment along the European Atlantic Coasts*. https://doi.org/10.30861/9781407311913

Britannica. (n.d.). *Gulf of Cádiz | gulf, Atlantic Ocean | Britannica*. Retrieved 3 August 2022, from https://www.britannica.com/place/Gulf-of-Cadiz

Britton, K., Fuller, B. T., Tütken, T., Mays, S., & Richards, M. P. (2015). Oxygen isotope analysis of human bone phosphate evidences weaning age in archaeological populations. *American Journal of Physical Anthropology*, *157*(2), 226–241. https://doi.org/10.1002/ajpa.22704

Britton, K., McManus-Fry, E., Nehlich, O., Richards, M., Ledger, P. M., & Knecht, R. (2018). Stable carbon, nitrogen and sulphur isotope analysis of permafrost preserved human hair from rescue excavations (2009, 2010) at the precontact site of Nunalleq, Alaska. *Journal of Archaeological Science: Reports*, *17*, 950–963. https://doi.org/10.1016/J.JASREP.2016.04.015

Brock, F., Higham, T., & Ramsey, C. B. (2010). Pre-screening techniques for identification of samples suitable for radiocarbon dating of poorly preserved bones. *Journal of Archaeological Science*, *37*(4), 855–865. https://doi.org/10.1016/j.jas.2009.11.015

Brugnoli, E., & Farquhar, G. D. (2000). *Photosynthetic Fractionation of Carbon Isotopes*. 399–434. https://doi.org/10.1007/0-306-48137-5_17

Brum, P., Pinto, V. I., Magalhães, A. P., Santos, F., & Muller, J. (2017). The STORM Project and Coastal Erosion The case of Troia (Portugal). *Skyllis Zeitschrift Fur Maritime Und Liminische Archaologie Und Kulturgeschichte*, *1*.

Bunaciu, A. A., Udriştioiu, E. gabriela, & Aboul-Enein, H. Y. (2015a). X-Ray Diffraction: Instrumentation and Applications. *Critical Reviews in Analytical Chemistry*, *45*(4), 289–299. https://doi.org/10.1080/10408347.2014.949616

Bunaciu, A. A., Udriştioiu, E. gabriela, & Aboul-Enein, H. Y. (2015b). X-Ray Diffraction: Instrumentation and Applications. *Critical Reviews in Analytical Chemistry*, *45*(4), 289–299. https://doi.org/10.1080/10408347.2014.949616

Butzer, K. W., Mateu, J. F., Butzer, E. K., & Kraus, P. (1985). Irrigation Agrosystems in Eastern Spain: Roman or Islamic Origins? *Annals of the Association of American Geographers*, *75*(4), 479–509. https://doi.org/10.1111/j.1467-8306.1985.tb00089.x

Caemmerer, S. von, Ghannoum, O., Pengelly, J. J. L., & Cousins, A. B. (2014). Photosynthesis, Carbon isototope discrimination as a tool to explaore C4 photosynthesis. *Journal of Experimental Botany*, *65*(13), 3459–3470. https://doi.org/10.1093/jxb/eru127

Carneiro, A. (2016). Mudança e Continuidade no Povoamento Rural no Alto Alentejo Durante a Antiguidade Tardia. *A Lusitânia Entre Romanos e Bárbaros*, 281–307.

Carneiro, A. (2019). Fields of Rome. Lusitania, the Mediterranean connectivity, the Roman Empire and the loss of knowledge. In Teresa Ferreira, Maria de Fátima Nunes, Ana Cardoso de Matos, & António Candeias (Eds.), *Web of knowledge: A look into the past, embrancing the future.* (pp. 8–11). Sílabas & Desafios. http://dspace.uevora.pt/rdpc/handle/10174/26544

Cersoy, S., Zazzo, A., Lebon, M., Rofes, J., & Zirah, S. (2017). Collagen Extraction and Stable Isotope Analysis of Small Vertebrate Bones: A Comparative Approach. *Radiocarbon*, *59*(3), 679–694. https://doi.org/10.1017/RDC.2016.82

Chen, G., Zhou, H., Ji, D., & Gu, B. (2012). Stable isotope enrichment in muscle, liver, and whole fish tissues of brown-marbled groupers (Epinephelus fuscoguttatus). *Ecological Processes*, *1*(1), 7. https://doi.org/10.1186/2192-1709-1-7

Chenery, C. A., Pashley, V., Lamb, A. L., Sloane, H. J., & Evans, J. A. (2012). The oxygen isotope relationship between the phosphate and structural carbonate fractions of human bioapatite. *Rapid Communications in Mass Spectrometry*, *26*(3), 309–319. https://doi.org/10.1002/rcm.5331

Child, A. M. (1995). *Microbial Taphonomy of Archaeological Bone Author (s): A. M. Child Source: Studies in Conservation , Feb ., 1995 , Vol. 40 , No. 1 (Feb ., 1995), pp. 19-30 Published by: Taylor & Francis , Ltd. On behalf of the International Institute for Cons*. *40*(1), 19–30.

COLOMINAS, L. (2016). Roman Conquest and Changes in Animal Husbandry in the North-East of the Iberian Peninsula: Searching for Patterns, Rates and Singularities LÍDIA. *Archaeofauna International Journal of Archaeozoology: La Romanizacion de La Peninsula Iberica, Una Vision Desde La Arqueozoologia*, *15*(2), 1–23.

Colominas, L. (2017). Roman conquest and changes in animal husbandry in the north-east of the Iberian Peninsula: Searching for patterns, rates and singularities. *Archaeofauna*, *26*, 9–22. https://doi.org/10.15366/archaeofauna2017.26.001

Conejo, N. (2020). *Coins and villae in late roman lusitania: Collapse of the roman currency economy?* European Journal of Post-Classical Archaeologies. https://www.researchgate.net/publication/342013821_Coins_and_villae_in_late_Roman_Lusitania_collapse_of_the_Roman_currency_economy

Corsi, C., Klein, M., Johnson, P. S., Taelman, D., & Vermeulen, F. (2013). *Ammaia: A Roman town in Lusitania Ammaia: Uma cidade romana na Lusitânia*.

Cunliffe, B. (1995). Diversity in the Landscape: The Geographical Background to Urbanism in Iberia. *Proceedings of the British Academy*, *86*, 5–28.

Curchin, L. A. (2017). Slaves in Lusitania: Identity , demography and social relations. *Conimbriga*, *LVI*, 75–108. https://doi.org/10.14195/1647-8657_56_3

Currás, B. X., & Sánchez-Palencia, F. J. (2021). Landscape archaeology of Roman gold mining in Lusitania: The ‘Aurifer Tagus’ project. *Antiquity*, *95*(382). https://doi.org/10.15184/AQY.2021.82

Daniel Bryant, J., & Froelich, P. N. (1995). A model of oxygen isotope fractionation in body water of large mammals. *Geochimica et Cosmochimica Acta*, *59*(21), 4523–4537. https://doi.org/10.1016/0016-7037(95)00250-4

Daux, V., Lécuyer, C., Héran, M. A., Amiot, R., Simon, L., Fourel, F., Martineau, F., Lynnerup, N., Reychler, H., & Escarguel, G. (2008). Oxygen isotope fractionation between human phosphate and water revisited. *Journal of Human Evolution*, *55*(6), 1138–1147. https://doi.org/10.1016/j.jhevol.2008.06.006

Day, Z., Wandsnider, L., Douglass, M. J., & University of Nebraska--Lincoln. (2016). *Preliminary studies in using X-ray diffraction for analyzing the atomic structure of Central Plains tradition constituents*. University of Nebraska-Lincoln.

Deniro, M. J. (1985). Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature*, *317*(6040), 806–809. https://doi.org/10.1038/317806a0

DeNiro, M. J., & Weiner, S. (1988). Chemical, enzymatic and spectroscopic characterization of ‘collagen’ and other organic fractions from prehistoric bones. *Geochimica et Cosmochimica Acta*, *52*(9), 2197–2206. https://doi.org/10.1016/0016-7037(88)90122-6

Ebert, C. E., Rand, A. J., Green-Mink, K., Hoggarth, J. A., Freiwald, C., Awe, J. J., Trask, W. R., Yaeger, J., Brown, M. K., Helmke, C., Guerra, R. A., Danforth, M., & Kennett, D. J. (2021). Sulfur isotopes as a proxy for human diet and mobility from the preclassic through colonial periods in the

Eastern Maya lowlands. *PLoS ONE*, *16*(8 August). https://doi.org/10.1371/JOURNAL.PONE.0254992

Edmondson, J. C. (1992a). Creating a Provincial Landscape: Roman Imperialism and Rural Change in Lusitania. *Studia Historica. Historia Antigua*, *10–11*, 13–30.

Edmondson, J. C. (1992b). Creating a Provincial Landscape: Roman Imperialism and Rural Change in Lusitania. *Studia Historica. Historia Antigua*, *10–11*, 13–30.

Egan, J. M. (2013). Comparative Resistance in Iberia: The Turdetanians and the Lusitanians. *Studies in Mediterranean Antiquity and Classics*, *3*. http://digitalcommons.macalester.edu/classicsjournalhttp://digitalcommons.macalester.edu/classicsjournal/vol3/iss1/2

El-Azazy. (2018). Infrared Spectroscopy—Principles, Advances, and Applications. *Infrared Spectroscopy - Principles, Advances, and Applications*. https://doi.org/10.5772/intechopen.73071

Erskine, A. (2009). A Companion to Ancient History. *A Companion to Ancient History*, 1–693. https://doi.org/10.1002/9781444308372

Fabião, C. (2009). Cetárias, ânforas e sal: A exploração de recursos marinhos na Lusitania. *Volume Comemorativo Do XX Aniversário Do Centro de Estudos Arqueológicos Do Concelho de Oeiras (Câmara Municipal de Oeiras) 1988-2008*, *June*, 555–594.

Fabião, C. (2014a). La creación de la provincia romana de Lusitania. *Augusto y Emerita (Catálogo de Exposición). Museo Nacional de Arte Romano (Mérida)*, *July 2014*, 25–39.

Fabião, C. (2014b). *Lusitania: An Atlantic Roman Province*. https://www.researchgate.net/publication/283711120_La_Lusitania_Una_Provincia_Romana_Atlantica

Farland H. Stanley Jr. (1990). Geographical Mobility in Roman Lusitania: An Epigraphical Perspective on JSTOR. *Zeitschrift Für Papyrologie Und Epigraphik*, *82*, 249–269.

Fear, A. T. (2009a). The Iberian Peninsula in the Roman Period. *A Companion to Ancient History*, 273–283. https://doi.org/10.1002/9781444308372.CH25

Fear, A. T. (2009b). The Iberian Peninsula in the Roman Period. *A Companion to Ancient History*, 273–283. https://doi.org/10.1002/9781444308372.CH25

Fernandez-Martinez, P., Maurer, A. F., Jiménez-Morillo, N. T., Botella, M., Lopez, B., & Barrocas Dias, C. (2020). Bone stable isotope data of the Late Roman population (4th–7th centuries CE) from Mondragones (Granada): A dietary reconstruction in a Roman villa context of south-eastern Spain. *Journal of Archaeological Science: Reports*, *33*(August), 102566. https://doi.org/10.1016/j.jasrep.2020.102566

Figueiredo, A. (2001). *(14) Death in Roman Iberia: Acculturation, resistance and diversity of beliefs and pratices (ERA 3/2001) |*. Era Arqueologia - Academia.Edu. https://www.academia.edu/7321583/Death_in_Roman_Iberia_Acculturation_resistance_and_diversity_of_beliefs_and_pratices_ERA_3_2001_

Figueiredo, M. (2008). Anthropological Report of Workshop 1. In *Troiaresort* (Vol. 6, Issue 2).

Figueiredo, M. (2010). Relatório Antropológico Estação Arqueológica de Tróia. In *Troiaresort*.

Figueiredo, M. (2011a). *Relatório de antropologia (Intervenção Anthropologica na Ponta do Verde, Tróia)*.

Figueiredo, M. (2011b). *Relatório de Antropologia, Troia ONM*.

Filipe, V. M. S. (2021). Olisipo (Lisbon, Portugal) and its place in the Roman trade. *SPAL*, *2*(30), 189–215. https://doi.org/10.12795/SPAL.2021.I30.22

Firnigl, A. (2013). The settling factors of Roman villas in southern Lusitania. *Acta Universitatis Sapientiae Agriculture and Environment*, *5*, 40–55. https://doi.org/10.2478/ausae-2014-0003

Frantz, L. A. F., Haile, J., Lin, A. T., Scheu, A., Geörg, C., Benecke, N., Alexander, M., Linderholm, A., Mullin, V. E., Daly, K. G., Battista, V. M., Price, M., Gron, K. J., Alexandri, P., Arbogast, R. M., Arbuckle, B., Bălăşescu, A., Barnett, R., Bartosiewicz, L., … Larson, G. (2019). Ancient pigs reveal a near-complete genomic turnover following their introduction to Europe. *Proceedings* *of the National Academy of Sciences of the United States of America*, *116*(35), 17231–17238. https://doi.org/10.1073/PNAS.1901169116

Fuller, B. T., Fuller, J. L., Harris, D. A., & Hedges, R. E. M. (2006). Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios. *American Journal of Physical Anthropology*, *129*(2), 279–293. https://doi.org/10.1002/ajpa.20249

Gannes, L. Z., O'Brien, D. M., & Del Rio, M. C. (1997). Stable Isotopes in Animal Ecology: Assumptions , Caveats , and a Call for More Laboratory Experiments Author (s): Leonard Z. Gannes , Diane M. O ' Brien and Carlos Martínez del Rio Published by: Wiley on behalf of the Ecological Society of America S. *Ecology*, *78*(4), 1271–1276.

García-Moreno, C. D., Martínez Cortizas, A., & López-Costas, O. (2022). A Stable Isotope Approach to Roman Diet and Its Legacy in Late Antiquity in Hispania and the Western Empire. *Environmental Archaeology*, 1–16. https://doi.org/10.1080/14614103.2022.2128158

Garvie-Lok, S. J., Varney, T. L., & Katzenberg, M. A. (2004). Preparation of bone carbonate for stable isotope analysis: The effects of treatment time and acid concentration. *Journal of Archaeological Science*, *31*(6), 763–776. https://doi.org/10.1016/j.jas.2003.10.014

Hedges, R. E. M. (2002). Bone diagenesis: An overview of processes. *Archaeometry*, *44*(3), 319–328. https://doi.org/10.1111/1475-4754.00064

Herrscher, E., Goude, G., & Metz, L. (2017). Étude Longitudinale De La Composition Isotopique Du Lait Maternel Et Implications Pour La Reconstitution De La Paléoalimentation Infantile. *Bulletins et Memoires de La Societe d'Anthropologie de Paris*, *29*(3–4), 131–139. https://doi.org/10.1007/s13219-017-0190-4

Hesse, M., Meier, H., Zeeh, B., Dunmur, R., & Murray, M. (2008). Spectroscopic Methods in Organic Chemistry. *Spectroscopic Methods in Organic Chemistry*. https://doi.org/10.1055/B-003-108602

Hollund, H. I., Ariese, F., Fernandes, R., Jans, M. M. E., & Kars, H. (2013). Testing an alternative high-throughput tool for investigating bone diagenesis: Ftir in attenuated total reflection (atr) mode. *Archaeometry*, *55*(3), 507–532. https://doi.org/10.1111/j.1475-4754.2012.00695.x

Houck, M. L. (1998a). *ROMAN CONQUEST OF SPAIN: THE ECONOMIC MOTIVE*.

Houck, M. L. (1998b). Roman conquest of spain: The economic motive. In *History*.

Hunnis. (2007). First Human. *American Journal of Physical Anthropology*, *132*(4), 535–544. https://doi.org/10.1002/ajpa

Inácio, M., Pereira, V., & Pinto, M. (2007). The Soil Geochemical Atlas of Portugal: Overview and applications. *Journal of Geochemical Exploration*, *98*, 22–33.

Jiménez, J. M., & García, C. T. (2015). Central places in the post-Roman Mediterranean: Regional models for the Iberian peninsula. *Journal of Mediterranean Archaeology*, *28*(1), 81–103. https://doi.org/10.1558/JMEA.V28I1.27502

Katzenberg, A. (2012). The Ecological Approach: Understanding Past Diet and the Relationship Between Diet and Disease. *A Companion to Paleopathology*, 97–113. https://doi.org/10.1002/9781444345940.ch6

Katzenberg, A. M., & Saunders, S. R. (2008). Biological Anthropology of the Human Skeleton: Second Edition. In M. A. Katzenberg & S. R. Saunders (Eds.), *Biological Anthropology of the Human Skeleton: Second Edition* (2nd ed.). John Wiley and Sons Inc. https://doi.org/10.1002/9780470245842

Katzenberg, M. A. (2007). Stable Isotope Analysis: A Tool for Studying Past Diet, Demography, and Life History. *Biological Anthropology of the Human Skeleton: Second Edition*, 411–441. https://doi.org/10.1002/9780470245842.ch13

Katzenberg, M. A., Herring, D. A., & Saunders, S. R. (1996). Weaning and infant mortality: Evaluating the skeletal evidence. *American Journal of Physical Anthropology*, *101*(S23), 177–199. https://doi.org/10.1002/(sici)1096-8644(1996)23+<177::aid-ajpa7>3.0.co;2-2

Keegan, W. F., & DeNiro, M. J. (1988). Stable Carbon- and Nitrogen-Isotope Ratios of Bone Collagen Used to Study Coral-Reef and Terrestrial Components of Prehistoric Bahamian Diet. *American Antiquity*, *53*(2), 320–336. https://doi.org/10.2307/281022

Koch, P. L., Tuross, N., & Fogel, M. L. (1997). The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. *Journal of Archaeological Science*, *24*(5), 417–429. https://doi.org/10.1006/jasc.1996.0126

Kron, G. (1937). Animal Husbandry. *Australian Veterinary Journal*, *13*(4), 135–137. https://doi.org/10.1111/j.1751-0813.1937.tb04106.x

Larsen, C. S. (1998). Bioarchaeology: Interpreting behavior from the human skeleton. In *Choice Reviews Online* (Vol. 35, Issue 11). https://doi.org/10.5860/choice.35-6311

Larsen, C. S. (2002). Bioarchaeology: The lives and lifestyles of past people. *Journal of Archaeological Research*, *10*(2), 119–166. https://doi.org/10.1023/A:1015267705803

Lee-Thorp, J. A. (2008). On isotopes and old bones. *Archaeometry*, *50*(6), 925–950. https://doi.org/10.1111/j.1475-4754.2008.00441.x

Lee-Thorp, J. A., Sealy, C. J., & Van Der Merwe, N. J. (1989). Stable Carbon Isotope Ratio Differences Between Bone Collagen and Bone Apatite, and their Relationship to Diet. *Journal of Archaeological Science*, *16*, 585–599.

Leng, Y. (2010). Materials Characterization: Introduction to Microscopic and Spectroscopic Methods. *Materials Characterization: Introduction to Microscopic and Spectroscopic Methods*. https://doi.org/10.1002/9780470823002

Lightfoot, E., & O'Connell, T. C. (2016). On the use of biomineral oxygen isotope data to identify human migrants in the archaeological record: Intra-sample variation, statistical methods and geographical considerations. *PLoS ONE*, *11*(4). https://doi.org/10.1371/journal.pone.0153850

Loidi, J. (2017). *Introduction to the Iberian Peninsula, General Features: Geography, Geology, Name, Brief History, Land Use and Conservation*. 3–27. https://doi.org/10.1007/978-3-319-54784-8_1

Lopez-Costas, O. (2015). Taphonomy and burial context of the Roman/post-Roman funerary areas (2nd to 6th centuries AD) of A Lanzada, NW Spain. *Estudos Do Quaternário / Quaternary Studies*, *0*(12), 55–67.

Macroberts, R. A. (2017). *Diet and dynamic of Christains and Muslim—A picture of Évora in the 8th—13th AD*. Universidade de Evora, Aristotle University of Thessaloniki, Sapienza Unversità de Roma.

MacRoberts, R. A., Barrocas Dias, C. M., Matos Fernandes, T., Santos, A. L., Umbelino, C., Gonçalves, A., Santos, J., Ribeiro, S., Schöne, B. R., Barros, F., Correia, F., Vasconcelos Vilar, H., & Maurer, A. F. (2020). Diet and mobility during the Christian conquest of Iberia: The multi-isotopic investigation of a 12th–13th century military order in Évora, Portugal. *Journal of Archaeological Science: Reports*, *30*. https://doi.org/10.1016/J.JASREP.2020.102210

Malainey, M. E. (2010). *A Consumer's Guide to Archaeological Science: Analytical Techniques (Manuals in Archaeological Method, Theory and Technique)*.

Mantas, V. G. (2004). A LUSITÂNIA E O MEDITERRÂNEO: IDENTIDADE E DIVERSIDADE NUMA PROVÍNCIA ROMANA (2004) p. *Conimbriga*, *XLIII*.

Mantas, V. G. (2010). A romanização da paisagem na Lusitânia. *Espaços e Paisagens: Antiguidade Clássica e Heranças Contemporâneas: Vol.3 História, Arqueologia e Arte*, *III*, 121–134. https://doi.org/10.14195/978-989-8281-69-2_9

Mantas, V. G. (2014). Atlântico e Mediterrâneo nos portos romanos do Sado Autor (es): Publicado por: URL persistente: DOI : Accessed: Mantas , Vasco Gil Instituto de História Económica e Social , Faculdade de Letras da Universidade de Coimbra URI : http://hdl.handle.net/1. *Instituto de História Económica e Social, Faculdade de Letras Da Universidade de Coimbra*. https://doi.org/DOI:http://dx.doi.org/10.14195/0870-4147_41_8

Martínez Jiménez, Sastre de Diego, I., & Tejerizo, C. (2018). The Iberian Peninsula between 300 and 850. *The Iberian Peninsula between 300 and 850*. https://doi.org/10.5117/9789089647771

Maurer, A. F., Gerard, M., Person, A., Barrientos, I., del Carmen Ruiz, P., Darras, V., Durlet, C., Zeitoun, V., Renard, M., & Faugère, B. (2011). Intra-skeletal variability in trace elemental content of Precolumbian Chupicuaro human bones: The record of post-mortem alteration and a tool for palaeodietary reconstruction. *Journal of Archaeological Science*, *38*(8), 1784–1797. https://doi.org/10.1016/j.jas.2011.03.008

Maurer, A. F., Person, A., Tütken, T., Amblard-Pison, S., & Ségalen, L. (2014). Bone diagenesis in arid environments: An intra-skeletal approach. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *416*, 17–29. https://doi.org/10.1016/j.palaeo.2014.08.020

Miller, D. (2018). *Stable Carbon and Nitrogen Isotope Analysis in Italy and Croatia: Bronze Age Food Practices Across the Adriatic Author* (Issue December). Universidade de Evora, Aristotle University of Thessaloniki, Sapienza University of Rome.

Moles, A. (2012). *A Stable Isotope Analysis Study for Dietary Reconstruction at the Multi-Period Site of Mesembria on the Black Sea*. The University of Edinburgh.

Monnier, G. F. (2018). A review of infrared spectroscopy in microarchaeology: Methods, applications, and recent trends. *Journal of Archaeological Science: Reports*, *18*(April 2017), 806–823. https://doi.org/10.1016/j.jasrep.2017.12.029

Monteiro, L. D. (2008). Ethnicity and Conflict in the Roman Conquest of Spain. *Theoretical Roman Archaeology Journal*, *0*(2007), 53. https://doi.org/10.16995/TRAC2007_53_61

Monteiro, M., & Rodrigues, P. F. (2006). Troia Roman baths (Portugal). Assessment of history of interventions. *International Seminar in Conservation. A Tribute to Cesari Brandi*, 313–322.

Mora, A. (2022). Stable carbon and nitrogen isotope analysis of archaeological human hair: Reconstructing diet and health of ancient individuals. *Journal of Archaeological Science: Reports*, *43*(April), 103439. https://doi.org/10.1016/j.jasrep.2022.103439

Nabais, M. (2015). *Proceedings of the First Zooarchaeology Conference in Portugal Held at the Faculty of Letters , Edited by Cleia Detry BAR International Series 2662*. *2*(March 2012).

Nehlich, O. (2015a). The application of sulphur isotope analyses in archaeological research: A review. *Earth-Science Reviews*, *142*, 1–17. https://doi.org/10.1016/j.earscirev.2014.12.002

Nehlich, O. (2015b). The application of sulphur isotope analyses in archaeological research: A review. *Earth-Science Reviews*, *142*, 1–17. https://doi.org/10.1016/J.EARSCIREV.2014.12.002

Nehlich, O., Fuller, B. T., Jay, M., Mora, A., Nicholson, R. A., Smith, C. I., & Richards, M. P. (2011). Application of sulphur isotope ratios to examine weaning patterns and freshwater fish consumption in Roman Oxfordshire, UK. *Geochimica et Cosmochimica Acta*, *75*(17), 4963–4977. https://doi.org/10.1016/j.gca.2011.06.009

Nehlich, O., & Richards, M. P. (2009). Establishing collagen quality criteria for sulphur isotope analysis of archaeological bone collagen. *Archaeological and Anthropological Sciences*, *1*(1), 59–75. https://doi.org/10.1007/S12520-009-0003-6/TABLES/9

Nielsen-Marsh, C. M., & Hedges, R. E. M. (2000). Patterns of Diagenesis in Bone I: The Effects of Site Environments. *Journal of Archaeological Science*, *27*(12), 1139–1150. https://doi.org/10.1006/JASC.1999.0537

Nieto-Espinet, A., Trentacoste, A., Guimarães, S., & Valenzuela-Lamas, S. (2021). Cattle from the East, cattle from the West: Diversity of Bos morphotypes in the Iberian Peninsula during the late prehistoric and Roman times. *Themes in Old World Zooarchaeology : From the Mediterranean to the Atlantic*, 226.

Norman, N. J. (2003). Death and burial of Roman children: The case of the Yasmina cemetery at Carthage-Part II, The archaeological evidence. *Mortality*, *8*(1), 36–47. https://doi.org/10.1080/1357627021000063115

O`Leary, M. H. (1988). Carbon Isotopes in Photosynthesis: Fractionation techniques may reveal new aspects of carbon dynamics in plants. *Bioscience*, *38*(5).

Osland, D. (2016). Abuse or reuse? Public space in Late Antique Emerita. *American Journal of Archaeology*, *120*(1), 67–97. https://doi.org/10.3764/aja.120.1.0067

Pate, F. D. (1994). Bone chemistry and paleodiet. *Journal of Archaeological Method and Theory*, *1*(2), 161–209. https://doi.org/10.1007/BF02231415

Pate, F. D. (2008). The use of human skeletal remains in landscape archaeology. *Handbook of Landscape Archaeology*, *January 2008*, 502–520. https://doi.org/10.4324/9781315427737-63

Pederzani, S., & Britton, K. (2019). Oxygen isotopes in bioarchaeology: Principles and applications, challenges and opportunities. *Earth-Science Reviews*, *188*, 77–107. https://doi.org/10.1016/j.earscirev.2018.11.005

Pedro, J., & Tereso, V. (2012). *Environmental Change , agricultural development and social trends in NW Iberia from the Late Prehistory to the Late Antiquity*. 269.

Peña-Chocarro, L., Pérez-Jordà, G., Alonso, N., Antolín, F., Teira-Brión, A., Tereso, J. P., Montes Moya, E. M., & López Reyes, D. (2019). Roman and medieval crops in the Iberian Peninsula: A first overview of seeds and fruits from archaeological sites. *Quaternary International*, *499*, 49–66. https://doi.org/10.1016/j.quaint.2017.09.037

Pereira, C., & Albuquerque, P. (2018). INUMAÇÕES INFANTIS EM ÂNFORA NA PENÍNSULA IBÉRICA DURANTE A ÉPOCA ROMANA : A PRÁTICA E O RITO. *Spal 27*, *1*, 89–118. https://doi.org/DOI: http://dx.doi.org/10.12795/spal.2018i27.04

Pereira, C. (Archaeologist). (2015). *The Roman necropolis of Algarve (Portugal): About the spaces of death in the south of Lusitania*. 21.

Person, A., Bocherens, H., Saliège, J. F., Paris, F., Zeitoun, V., & Gérard, M. (1995). Early Diagenetic Evolution of Bone Phosphate: An X-ray Diffractometry Analysis. *Journal of Archaeological Science*, *22*(2), 211–221. https://doi.org/10.1006/jasc.1995.0023

Peyroteo-Stjernaje, R. (2015). Death in Place: Rituals in Practice. *ANCIENT DEATH WAYS Proceedings of the Workshop on Archaeology and Mortuary Practices*, 204.

Pinto, I. V. (2009). Late Roman Tombs at Tróia (Portugal): The Mensae. *Journal of Ancient Egyptian Interconnections*, *1*(December), 8–17.

Pinto, I. V., Magalhães, A. P., & Brum, P. (2009). *Relatório final do Desafogamento das Oficinas de Salga 1 e 2 de Tróia (2008-2009)*.

Pinto, I. V., Magalhães, A. P., & Brum, P. (2012). UN DEPOTOIR DU VE SIECLE DANS L'OFFICINE DE SALAISONS 1 DE TROIA (PORTUGAL. *Rei Cretariæ Romanæ Favtorvm (R.C.R.F)*, *42*.

Pinto, I. V., Magalhães, A. P., & Brum, P. (2016). Tróia na Antiguidade Tardia. *A Lusitânia Entre Romanos e Bárbaros*, 309–334.

Pinto, I. V., Magalhães, A. P., & Brum, P. (2018). A reassessment of the fish-salting workshops 1 and 2 of Tróia (Portugal): The ceramic contexts. *Rei Cretariae Romanae Fautorum Acta 45 (Lisboa, 25 de Setembro a 2 de Outubro de 2016).* https://www.academia.edu/67682445/A_reassessment_of_the_fish_salting_workshops_1_and_2_of_Tróia_Portugal_the_ceramic_contexts

Pinto, I. V., Magalhães, A. P., & Brum, P. (2020). An overview of the fish-salting production centre at Tróia (Portugal). *Fish & Ships*, 145–157. https://doi.org/10.4000/books.pccj.1696

Pinto, I. V., Magalhães, A. P., Brum, P., & Santos, F. A. dos. (2017). *Projecto de Investigação Plurianual em Arqueologia Projecto: Valorização das Ruínas Romanas de Tróia*.

Pinto, I. V., Magalhães, A. P., Brum, P., & Santos, F. A. dos. (2020). CERAMIC EVIDENCE IN THE FISH-SALTING WORKSHOP 23 AT TROIA (PORTUGAL): AMPHORAE AND PITCHERS. In *REI CRETARIÆ ROMANÆ FAVTORVM ACTA* (pp. 105–114). https://doi.org/doi: http://dx.doi.org/10.32028/9781789697483-11

Pinto, I. V., Morais, R., Fabião, C., Oliveira, C., & Gabriel, S. (2021). Lusitanian Amphora Contents. *Roman Amphora Contents: Reflecting on the Maritime Trade of Foodstuffs in Antiquity (In Honour of Miguel Beltrán Lloris)*, *January*, 183–204.

Price, T. D. (2014). An Introduction to the Isotopic Studies of Ancient Human Remains. *Journal of the North Atlantic*, *7*(sp7), 71–87. https://doi.org/10.3721/037.002.sp708

Quaresma, J. C. (2017). Thoughts on Lusitania's economic interaction between c 50 and c 550+ a.D.: An analysis of exportable goods. *Spal*, *26*(26), 135–150. https://doi.org/10.12795/spal.2017i26.06

Rebêlo, L. P., Ferraz, M., & Brito, P. O. (2009). Tróia peninsula evolution: The dune morphology record. *Journal of Coastal Research*, *SPEC. ISSUE 56*, 352–355.

Reitz & Wing. (2009). Zooarchaeology. In *Art and Archaeology of Challuabamba, Ecuador* (Vol. 59). https://doi.org/10.7560/718920-013

Richards, M. P., Fuller, B. T., Sponheimer, M., Robinson, T., & Ayliffe, L. (2003). Sulphur isotopes in palaeodietary studies: A review and results from a controlled feeding experiment. *International Journal of Osteoarchaeology*, *13*(1–2), 37–45. https://doi.org/10.1002/oa.654

Salesse, K., Dufour, É., Castex, D., Velemínský, P., Santos, F., Kuchařová, H., Jun, L., & Brůžek, J. (2013). Life history of the individuals buried in the St. Benedict Cemetery (Prague, 15th-18th Centuries): Insights from 14C dating and stable isotope (δ13C, δ15N, δ18O) analysis. *American Journal of Physical Anthropology*, *151*(2), 202–214. https://doi.org/10.1002/ajpa.22267

Saragoça, P., Maurer, A. F., Šoberl, L., Lopes, M. da C., Alfenim, R., Leandro, I., Umbelino, C., Fernandes, T., Valente, M. J., Ribeiro, S., Santos, J. F., Janeiro, A. I., & Barrocas, C. D. (2016). Stable isotope and multi-analytical investigation of Monte da Cegonha: A Late Antiquity population in southern Portugal. *Journal of Archaeological Science: Reports*, *9*, 728–742. https://doi.org/10.1016/j.jasrep.2016.07.010

Schoeninger, M. J., & DeNiro, M. J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta*, *48*(4), 625–639. https://doi.org/10.1016/0016-7037(84)90091-7

Schoeninger, M. J., & Moore, K. (1992). Bone Stable Isotope Studies in Archaeology Stable URL : http://www.jstor.org/stable/25800615 Linked references are available on JSTOR for this article: Bone Stable Isotope Studies in Archaeology. *Journal of World Prehistory*, *6*(2), 247–296.

Schreiner, M., Frühmann, B., Jembrih-Simbürger, D., & Linke, R. (2004). X-rays in art and archaeology: An overview. *Powder Diffraction*, *19*(1), 3–11. https://doi.org/10.1154/1.1649963

Sealy, J., Amstrong, R., & Schrire, C. (1995). Beyond lifetime averages: Tracing life histories through isotopic analysis of different calcified tissues from archaeological human skeletons. *Antiquity*, *69*, 290–300. https://doi.org/10.1017/S0003598X00064693

Siebke, I., Moghaddam, N., Cunningham, C. A., Witzel, C., & Lösch, S. (2019). Those who died very young—Inferences from δ15N and δ13C in bone collagen and the absence of a neonatal line in enamel related to the possible onset of breastfeeding. *American Journal of Physical Anthropology*, *169*(4), 664–677. https://doi.org/10.1002/ajpa.23847

Smith, C. I., Nielsen-Marsh, C. M., Jans, M. M. E., & Collins, M. J. (2007). Bone diagenesis in the European Holocene I: patterns and mechanisms. *Journal of Archaeological Science*, *34*(9), 1485–1493. https://doi.org/10.1016/j.jas.2006.11.006

Stuart, B. H. (2005). Infrared Spectroscopy: Fundamentals and Applications. In *Infrared Spectroscopy: Fundamentals and Applications*. https://doi.org/10.1002/0470011149

Theophanides, T. (2012). Introduction to Infrared Spectroscopy. *Infrared Spectroscopy - Materials Science, Engineering and Technology*, *April 2012*. https://doi.org/10.5772/49106

Thompson, T. J. U., Islam, M., Piduru, K., & Marcel, A. (2011). An investigation into the internal and external variables acting on crystallinity index using Fourier Transform Infrared Spectroscopy on unaltered and burned bone. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *299*(1–2), 168–174. https://doi.org/10.1016/j.palaeo.2010.10.044

Trentacoste, A., Nieto-Espinet, A., Guimarães, S., Wilkens, B., Petrucci, G., & Valenzuela-Lamas, S. (2021). New trajectories or accelerating change? Zooarchaeological evidence for Roman transformation of animal husbandry in Northern Italy. *Archaeological and Anthropological Sciences*, *13*(1). https://doi.org/10.1007/S12520-020-01251-7

Trueman, C., Chenery, C., Eberth, D. A., & Spiro, B. (2003). Diagenetic effects on the oxygen isotope composition of bones of dinosaurs and other vertebrates recovered from terrestrial and marine sediments. *Journal of the Geological Society*, *160*(6), 895–901. https://doi.org/10.1144/0016-764903-019

Tsutaya, T., & Yoneda, M. (2015a). Reconstruction of breastfeeding and weaning practices using stable isotope and trace element analyses: A review. *American Journal of Physical Anthropology*, *156*(S59), 2–21. https://doi.org/10.1002/ajpa.22657

Tsutaya, T., & Yoneda, M. (2015b). Reconstruction of breastfeeding and weaning practices using stable isotope and trace element analyses: A review. *American Journal of Physical Anthropology*, *156*(S59), 2–21. https://doi.org/10.1002/ajpa.22657

Tykot, R. (2006). Isotope analyses and the histories of maize. *Histories of Maize: Multidisciplinary Approaches to the …*. https://www.academia.edu/767810/Isotope_analyses_and_the_histories_of_maize

Tykot, R. H. (2006). P A R T II STABLE ISOTOPE ANALYSIS: Chapter 10: Human diet and Maize. *History of Maize*, 131–142.

UNRV. (n.d.). *Viriathus And The Lusitanian War*. UNRV.Com Roman History. Retrieved 28 July 2022, from https://www.unrv.com/bio/viriathus.php

Vaiglova, P., Bogaard, A., Collins, M., Cavanagh, W., Mee, C., Renard, J., Lamb, A., Gardeisen, A., & Fraser, R. (2014). An integrated stable isotope study of plants and animals from Kouphovouno, southern Greece: A new look at Neolithic farming. *Journal of Archaeological Science*, *42*(1), 201–215. https://doi.org/10.1016/j.jas.2013.10.023

Valenzuela-Lamas, S. (2014). *Mammal remains from the Governor ' s House (Belém Tower , Lisbon) and Rua dos Correeiros (Baixa , Lisbon) in the context of fish processing factories in Lusitania Lusitania*. ResearchGate. https://www.researchgate.net/publication/277017512_Mammal_remains_from_the_Governor's_House_Belem_Tower_Lisbon_and_Rua_dos_Correeiros_Baixa_Lisbon_in_the_context_of_fish_processing_factories_in_Lusitania

Van Der Merwe, N. J., & Vogel, J. C. (1978). 13C Content of human collagen as a measure of prehistoric diet in woodland North America [11]. In *Nature* (Vol. 276, Issue 5690, pp. 815–816). https://doi.org/10.1038/276815a0

Van Klinken, G. J. (1999). Bone collagen quality indicators for palaeodietary and radiocarbon measurements. *Journal of Archaeological Science*, *26*(6), 687–695. https://doi.org/10.1006/jasc.1998.0385

Weiner, S., & Bar-Yosef, O. (1990). States of preservation of bones from prehistoric sites in the Near East: A survey. *Journal of Archaeological Science*, *17*(2), 187–196. https://doi.org/10.1016/0305-4403(90)90058-D

White, T. D., Black, M. T., & Folkens, P. A. (2019). *Human Osteology*. Elsevier Academic Press. https://doi.org/10.1017/9781139013826.007

Wiley, J., Benito-Calvo, A., Pérez-González, A., Magri, O., & Meza, P. (2009). Assessing regional geodiversity: The Iberian Peninsula. *EARTH SURFACE PROCESSES AND LANDFORMS Earth Surf. Process. Landforms*, *34*, 197–9337. https://doi.org/10.1002/esp.1840

WorldAtlas. (n.d.). *Iberian Peninsula Africa*. Retrieved 4 August 2022, from https://www.worldatlas.com/peninsulas/iberian-peninsula.html

Wright, E., & Ginja, C. (2022). *Cattle and people. Interdisciplinary approaches to an ancient relationship*. https://books.google.pt/books?id=lj5zEAAAQBAJ&pg=PA98&lpg=PA98&dq=diets+in+Roman+lusitania&source=bl&ots=fvNeQf1jpj&sig=ACfU3U1hCgM8O59bb4GAzPZDU5Jj37qC9A&hl=en&sa=X&ved=2ahUKEwj97Yykl9j5AhU-_7sIHcl3AfQQ6AF6BAgkEAM#v=onepage&q=diets in Roman lusitania&f=f

Wright, L. E., & Schwarcz, H. P. (1996). Infrared and isotopic evidence for diagenesis of bone apatite at Dos Pilas, Guatemala: Palaeodietary implications. *Journal of Archaeological Science*, *23*(6), 933–944. https://doi.org/10.1006/jasc.1996.0087

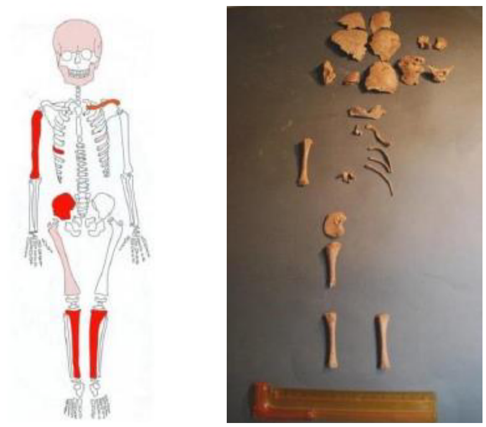
Wright, L. E., & Schwarcz, H. P. (1998). Stable carbon and oxygen isotopes in human tooth enamel: Identifying breastfeeding and weaning in prehistory. *American Journal of Physical Anthropology*, *106*(1), 1–18. https://doi.org/10.1002/(SICI)1096-8644(199805)106:1<1::AID-AJPA1>3.0.CO;2-W

Zalaite, I., Maria Barrocas Dias, C., Maurer, A.-F., & Carlos Valera, A. (2016). *Exploring Chalcolithic diet and mobility of humans and animals from Perdigões site , m34318*. *September*.

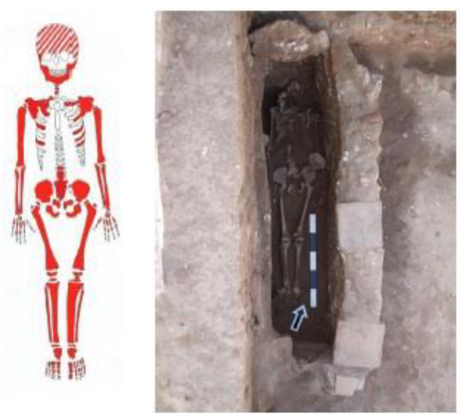
Appendices

Appendix I: Bone representation of the individuals

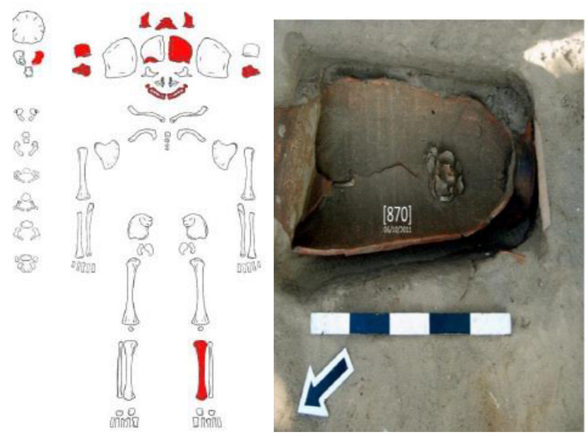
Individual [525] - T.OF1-L



Individual [680] - TBBT

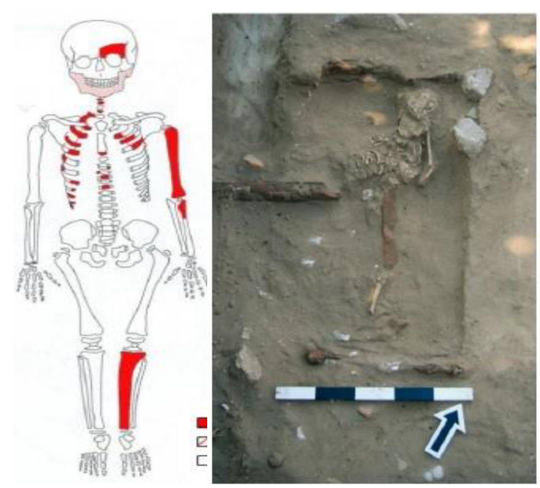


Individual [742] and in situ - T.OF1-SB



Bone rep-Individual [870]/ T.ONM-B in situ in amphora

Bone rep of individual [855]- T.ONM-A & in situ



Bone rep of individual [868]- T.ONM-C and in situ photo b.

Mensa grave, individual [897], and skeletal details in situ

Remains of the individual 8665 and the violate grave

Appendix II

Human bone samples before sampling

T. PVSV

T. ONM-C

T. ONM-B

T. OF1-L

T. BBT

T.ONM-A

T. PV-897

T. PVSV

Appendix III

Table 8: Data calculated from ATR-FTIR spectral for the human samples (* represents adult samples from the larger project)

Sample ID	IRSF	C/P	Am/P	Mean Crys Length (nm)	Collagen wt%	N% EA	CrystaLlinity index (CI) XRD
T.ONM-A	-						
T.ONM-B	3.2	0.2	0.8	53.7	24.0	1.4	
T.ONM-C	3.4	0.14	0.5	53.7	11.9	1.6	0.04
T.OF1-L	3.1	0.1	0.5	49.5	13.0	1.4	
T.OF1-SB	3.1	0.2	0.6	47.5	18.5	2.3	
T.BBT	3.3	0.2	0.5	53.7	11.6	2.5	
T. PVSV	3.4	0.2	0.6	55.8	18.7	1.8	
T. PV- 897	3.8	0.1	0.4	66.2	9.1	-	
D3-S6.112*	3.1	0.2	0.8	49.5	20.9	2.4	
D3. S19.398*	3.3	0.2	0.6	51.6	21.4	2.2	
D11.S3.313*	3.8	0.4	0.3	66.2	5.9	0.8	
D3. S5.101*	3.0	0.2	0.5	49.5	9.1	0.7	0.16
D3. S5.163*	3.1	0.3	0.4	47.5	92.2	0.7	
D4. S3.120*	3.3	0.2	0.5	53.7	12.9	0.9	
D3. S3.148*	3.6	0.2	0.6	59.8	17.0	2.3	0.19
D4. S7.168	3.0	0.3	1.0	45.4	31.0	3.1	
D3. S4.104*	3.2	0.2	0.5	57.1	9.1	1.2	
Mean	**3.3**	**0.2**	**0.6**	**53.8**	**3.6**	**1.6**	
Std Dev.	**0.3**	**0.1**	**0.2**	**6.2**	**3.7**	**0.9**	

Table 9:

Carbon and oxygen isotopic composition of the adult bone apatite (* = samples from the larger project)

Sample ID	$\delta^{13}C_{apa}$ (‰) VPDB	$\delta^{13}C_{apa-col}$ (‰)	$\delta^{18}O_{apa}$ VPDB	$\delta^{18}O_{bc}$ VSMOW	$\delta^{18}O_{en}$ VSMOW	$\delta^{18}O_{p}$ VSMOW	$\delta^{18}O_{en}$ Phos.	$\delta^{18}O_{Dw}$ VSMOW
D3-S6.122*	-12.79	-	-6.03	24.7	26.4	-6.7	17.4	-6.9
D3. S19.398*	-13.21	-	-5.65	25.1	26.8	-6.0	17.8	-6.3
D11.S3.313*	-12.67	-	-4.30	26.5	28.2	-3.8	19.2	-4.2
D3. S5.101*	-10.92	-	-4.77	26.0	27.7	-4.6	18.7	-4.9
D3. S5.163*	-11.03	-	-4.60	26.2	27.9	-4.3	18.9	-4.7
D4. S3.120*	-11.58	-	-5.05	25.7	27.4	-5.1	18.4	-5.4
D3. S3.148*	-13.81	-	-5.42	25.3	27.0	-5.7	18.0	-6.0
D3. S13.190*	-12.99	-	-4.30	26.5	28.2	-3.8	19.2	-4.2
D3. S4.104*	-12.99	-	-5.17	25.6	27.3	-5.3	18.3	-5.6