



International Erasmus Mundus Master in

QUATERNARY AND PREHISTORY



**Dental Microwear Analysis of labial and buccal surfaces of Iberian populations
from the Late Prehistory in a complimentary methodological framework**

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Up to the next step!

ABSTRACT

Dental Microwear Analysis has proven to provide important insights concerning dietary patterns and behavioral levels of extinct and extant human populations. The specific discipline is laid in the examination of dental features on the enamel surfaces, such as striations, pits, grooves, and enamel chipping among others, which imply either the adoption of specific alimentation or the conduction of paramasticatory activities using the mouth. In this context, the Early Bronze Age of the Northeastern Iberian Peninsula demonstrates important interest in the examination of the subsistence strategies and cognitive patterns of the populations. The specific Master's Thesis aims in the examination of dental wear in the populations of Galls Carboners, El Bordellet, Els Pujols, and Porroig sites, which are located in Catalonia, Spain. The examined materials consist of both permanent and deciduous teeth, anterior and posterior dentition, thus including both sexes and all age groups, and providing an excellent opportunity for the obtainment of a holistic approach concerning the dietary habits and behavioral patterns of the studied populations; moreover, the methodological framework is comprised of the uses of different pieces of microscopic equipment (ZEISS Axioscope A1 metallographic microscope and Hirox KH8700 3D Digital microscope) aiming to explore the different applications respectively for the finest archaeological interpretation and provide insights concerning the level of complementation among the two microscopes. In this context, the specific Master's Thesis can be considered as a further contribution to the understanding of dietary habits and behavioral levels of the Early Bronze Age populations of the Northeastern Iberian Peninsula, providing methodological advances concerning the discipline of Dental Microwear Analysis through a complimentary methodological framework.

Keywords: Dental Microwear Analysis; Diet; Cultural Behavior; Early Bronze Age; Northeastern Iberian Peninsula

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1) Introduction

The specific study reflects the written part of the Trabajo Final de Máster for the International Erasmus Mundus Master in Quaternary and Prehistory (IMQP). In this context, the introductory part aims at the presentation of the structure of the specific Master's Thesis.

The second chapter functions as a presentation of the examined topic underlying the objectives and the planted hypotheses.

The third chapter presents the four studied cases, which correspond to the sites where the examined dental remains were retrieved, thus providing pieces of information concerning the retrieved archaeological record in total, along with insights concerning the Iberian Peninsula in the examined period.

The fourth chapter marks the research background of the specific Master's Thesis describing distinct aspects related both to the discipline of Dental Microwear Analysis and the study of dental remains generally. Explicitly, the discussed topics vary from issues of dental terminology and morphology to the understanding of chewing movements and a holistic overview of the existing methodologies concerning the analysis of dental wear. Furthermore, parts of the chapter are devoted to the understanding of taphonomic alterations on the enamel surfaces and the depiction of the results concerning the appliance of Dental Microwear Analysis on contemporaneous populations of the Iberian Peninsula and the rest of the globe.

In the fifth chapter, the examined materials and the following methodological framework are described and explained.

The sixth chapter includes the results of the described study. In this context, the results are separated concerning the casting procedures and methodological issues, the paleodietary reconstruction, the testing of the inter-microscopic variability, and the examination of the behavioral level of the studied cases.

The seventh chapter demonstrates the discussion and contextualization of the results of the previous chapter. In this chapter, the evaluation of the methodological framework holistically is carried out, along with the understanding of the level of complementation among the used pieces of microscopic equipment, the unraveling of the dietary habits of the studied populations through the obtained results and

comparison with other studies of the literature, and the contextualization of the paramasticatory behavior of our samples.

The eighth chapter demonstrates the conclusions of our study, while the ninth chapter includes the perspectives of the future, and the tenth chapter exhibits the cited bibliographic references.

The eleventh chapter includes the Supplementary Material, in which descriptions of the used materials and measurements of the dental features are demonstrated.

Finally, the twelfth chapter includes the Appendix. At this point, we present our publication “Correlation between dental microwear analysis and dietary habits of Neanderthal populations in the Iberian Peninsula” in the scientific journal *Radiocarbon*. Furthermore, two posters are included, which were presented at the 3rd *C¹⁴ and Diet Conference* and at the *Advances in Human Evolution, Adaptation and Diversity Conference*, along with three Abstracts of studies that are about to be presented in the coming months at the *XV Jornadas de Jóvenes en Investigación Arqueológica (JIA 2024)*, *Social Interactions in Mediterranean Prehistory (SIMEP 2024)*, and 8th *Archaeological Research and New Technologies Symposium (ARCH_RNT)* respectively.

2) Problematic, Objectives and Hypotheses

Dental Microwear Analysis has proven to provide important insights regarding the understanding of the dietary patterns and behavioral levels of extinct and extant human populations. The described discipline is laid in the examination of the dental features, which occur in the enamel surface and can be attributed to the consumption of specific foods or to the utilization of the mouth as a tool during the conduction of a para-masticatory activity.

In this context, the Chalcolithic and Early Bronze Age of the Iberian Peninsula forms an interesting temporal point for the understanding of the alimentary habits and behavioral patterns of the populations. Explicitly, examining populations, which are temporally placed after the point of the emergence of agriculture and implemented new technological means habitually, such as copper and bronze, can provide information regarding the understanding of the adaptive level of human societies during the Late Prehistory.

The main objectives of the specific Master's Thesis are:

- To infer dental wear patterns on the studied groups (Galls Carboners, El Bordellet, Porroig & Els Pujols), which are located in the Northeastern part of the Iberian Peninsula during the Chalcolithic period and Early Bronze Age, and
- To understand the level of complimentary use of different microscopic equipment.

From these main objectives, 3 complimentary objectives emerge:

- To determine possible differences among the dietary habits inside the groups of the same population,
- To attribute the conduction of behavioral activities on specific individuals, based on their sex and age, and
- To prove the suitability of using different microscopic equipment to obtain more precise views of the wear patterns.

2.1) Hypotheses

Considering the chronological framework of the studied populations (Chalcolithic and Early Bronze Age), we hypothesize that the analyzed groups adopted a mixed diet based on cereals and domesticated animals, similar to already studied contemporaneous populations (Quagliarello *et al.*, 2022; Kaupova *et al.*, 2023).

Moreover, we hypothesize that there are no differences in the results of dental microwear patterns by two different types of microscopy.

3) The samples studied

The examined dental remains come from 4 sites, which are located in Catalunya, Northeastern Iberian Peninsula, and are dated from the Chalcolithic Period to the Early Bronze Age (Figure 1). In this context, the sites are about to be explained based on their chronology, from the oldest site to the youngest one, along with the general occupational and socioeconomic statuses of the Iberian Peninsula during the examined periods.

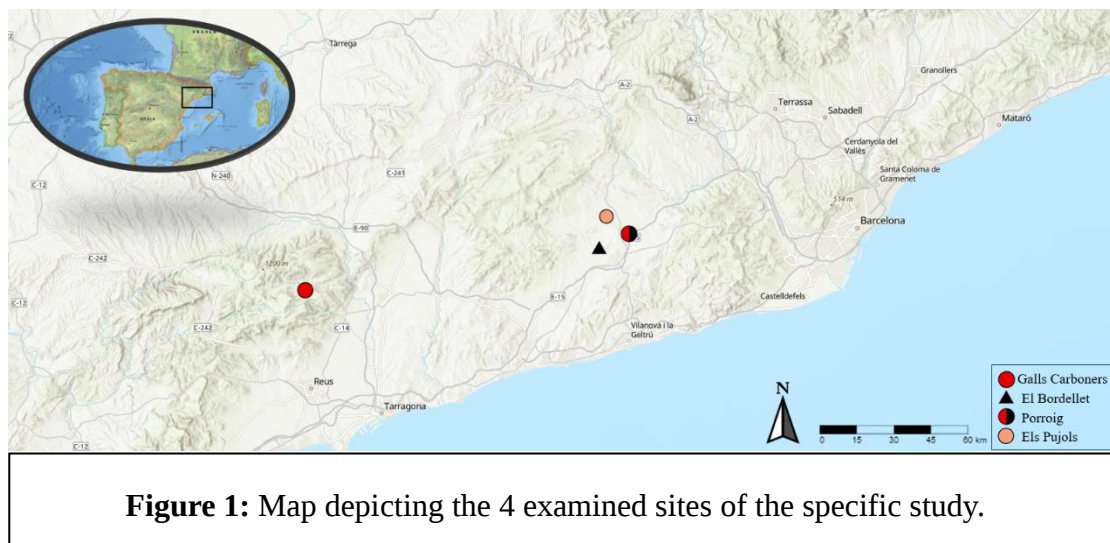


Figure 1: Map depicting the 4 examined sites of the specific study.

3.1) Information on the case studies

3.1.1) Galls Carboners

The Galls Carboners site is located in Montral (Tarragona province) in the Northeastern part of the Iberian Peninsula and exhibits important archaeological interest from the Late Pleistocene towards the Middle Bronze Age (Fernández-García *et al.*, 2016). The site is located at an altitude of 965 meters above sea level on the Els Motllats plateau within the Prades Mountains. The area is characterized by generally arid environmental conditions with water bodies found only on its perimeter and adjacent slopes, implying the prevalence of cold conditions during winter and warm ones during summer (Vergès *et al.*, 2017).

The first extraction of archaeological materials on the site occurred during the 1970s (however, without the excavational formalities of the archaeological discipline) from

the owner of the piece of land, in which the site is located, aiming to extract the archaeological materials from their depositional context. Subsequently, the donation of the private collection of archaeological materials from the owner in 1995 in the town hall of Santa Coloma de Queralt demonstrated the need for further research on the site based on the important number of the retrieved remains and their preservation state (Vergès *et al.*, 2017). In this framework, the archaeological interventions undergone by researchers of IPHES during 2009 and 2010, deciphering the topography and stratigraphy of the site, along with the recovery of archaeological materials.

The anthropological set consists of 1500 remains with a Minimum Number of Individuals (MNI) of 16 (9 adults, 3 adolescents, and 4 infants). Noteworthy, a human phalange was placed through the radiocarbon dating method in the Middle Bronze Age [3310 ± 30 BP (3620–3460 cal. BP)] (Vergès *et al.*, 2017). The dental record is comprised of 361 isolated teeth with a strong predominance of the anterior dentition (270 incisors and canines compared with 91 premolars and molars) (Hernando, 2022a). In this context, a study by Hernando *et al.* (2020b) combined the methodological frameworks of occlusal and buccal dental microwear aiming to extract pieces of information concerning issues of dietary maturation in children. The results demonstrated that the children from 0 to 4 years old from the site consumed a bigger number of processed foods due to the existence of less frequent occlusal pits. Noteworthy, the site has contributed to the understanding of the palaeoecological proxies and climatic fluctuations during the Late Pleistocene and Early Holocene (López García *et al.*, 2014; Fernández-García *et al.*, 2016).

3.1.2) El Bordellet

The site of El Bordellet is located in Vilafranca del Penedès in the area of Eix Diagonal in the northeastern part of the Iberian Peninsula. The site exhibits human occupation from the Middle Neolithic with continuity during the Early Bronze Age based on the existence of pit tombs on layers of the former and the use of structures on the latter (Esqué Ballesta, 2015). Concerning the Early Bronze Age, 19 structures have been identified, which are found in two different groups at a distance of approximately 15 meters. Noteworthy, the use of the specific constructions in both

domestic and funerary contexts, such as in the case of the El Bordellet site, is a characteristic of the Early Bronze Age of the Northeastern Iberian Peninsula and it has been documented in adjacent sites of the Catalanian plane, such as in the cases of Mas d'en Boixos and Cinc Ponts sites (Alfonso *et al.*, 2004; Esteve *et al.*, 2012).

Concerning the Early Bronze Age, the site has been dated through radiocarbon in 3 of the burial structures, indicating dates between $3515-3395 \pm 35$ years ago (1937-1613 cal. BP). However, the properties of deposits, which are characterized by similar characteristics among them, preserve the probability of contemporaneity among the different structures (Esqué Ballesta, 2015). Among others, the site exhibits important records of ceramic remains, which in one case is associated with a burial context (E5), and personal ornaments (burial association in E5 and E76). Typologically, the technological remains present important similarities with the general prevailing ceramic trends of the Early Bronze Age sites of the Catalunya with the prevalence of small- and medium-sized vessels with a ridged profile.

From the rest of the archaeological record of the El Bordellet site, a special mention should be made to the faunal and palynological remains. Explicitly, the excavation processes retrieved abundant faunal remains of domesticated species, such as ovicaprids, *Bos taurus*, *Sus domesticus*, and more. Furthermore, the carpological analysis exhibited traces of the conduction of agricultural practices, while the domesticated species were overlapping throughout the annual cycle, thus indicating a wide exploitation of plant resources. These elements are in conjunction with the general trends of the Iberian Bronze Age, which is characterized by a holistic conduction of a wide range of practices, aiming at the consumption of both animals and plants (Knipper *et al.*, 2020).

3.1.3) Porroig

The Porroig site is located in Vilafranca del Penedès in the area of Eix Diagonal in the northeastern part of the Iberian Peninsula. Its adjacent location to the El Bordellet site and its temporal placement permits direct comparisons between the two sites. Noteworthy, the retrieved human remains are all characterized by an important level of fragmentation based on the soil properties. The anthropological record is comprised

of one child burial (4 ± 1 years old), which presents an important level of bone fragmentation and is not correlated with any ceramic objects in a votive context (Armentano and Nociarová, 2012; Esqué Ballesta, 2015). In this framework, the dating of a human bone through the radiocarbon dating method revealed a date of 3485 ± 35 years BC (1896–1694 cal BC) and is placed in the same chronological framework with the burial E11 of El Bordellet site, as mentioned above.

From the rest of the archaeological record, a special mention should be made of the ceramic remains, which represent different typological types and sizes based on their utilization and follow the general trends of the Iberian Early Bronze Age. The lithic tools are underrepresented generally, which agrees with the general trends regarding the progressive preference toward the use of more developed technological means during the 3rd millennium cal. BC. Finally, the retrieved faunal remains, despite depicting a bad state of preservation, are attributed to domesticated species, such as bovids and ovicaprids, while the bone industry of Porroig represents affinities with the site of El Bordellet with the representation of an awl.

3.1.4) Els Pujols

The Els Pujols site is located in Vilafranca del Penedès in the area of Eix Diagonal in the northeastern part of the Iberian Peninsula. The human record is comprised of the remains of a minimum number of 5 individuals, which are characterized by important taphonomic alterations due to the acidic soil properties (Armentano and Nociarová, 2013). The human remains represent four adults (2 of them are probably males), and one child (2-3 years old). The site exhibits the existence of 3 funerary structures (2 of them represent a double burial). The morphology of the structures presents affinities, depicting circular shapes with correlation between the walls and the anatomical position of the individuals (Armentano and Nociarová, 2013). No pathologies or skeletal anomalies have been observed on the retrieved individuals.

3.2) The Iberian Peninsula during the Chalcolithic Period and the Early Bronze Age

The Iberian Peninsula during the Chalcolithic Period or Copper Age (3200-2250 BC) is characterized by the gradual widespread of new technological approaches and

genetic changes due to demographic movements. Explicitly, the starting point of the described temporal period in the Iberian Peninsula is considered to be the site of Los Millares in Almeria, the southern part of the Iberian Peninsula (3219–3124 cal BC, 68% probability) (Aranda Jiménez *et al.*, 2020), which has exhibited impressive aspects of architectural complexity based on the retrieved structures and rich votive offerings. A few centuries later (2823–2658 cal BC), the eastern part of the Iberian Peninsula (Tagus estuary) appeared to be the cradle of the Beaker phenomenon, which marked the ceramic tradition of Chalcolithic Europe during the 3rd millennium BC (Cardoso, 2014). Despite the ongoing discussion regarding the origin of the migratory waves (Reich *et al.*, 2018), which permitted the expansion of the technocomplex in Central Europe, it is without a doubt that the Iberian Peninsula experienced an important level of complexity concerning human occupations during the described temporal point.

Concerning the social statuses and occupation patterns, the majority of the retrieved sites imply the existence of occupations with improved building techniques (stone-based buildings), which provided further durability and can be interpreted as a hint toward more stable territory usage (Jover Maestre *et al.*, 2019). In the aftermath of the 4.2 ky climatic event, which decisively altered the balance of human societies on a global scale, the climate of the period is characterized by winter cooling and higher seasonality alternated with dry episodes (Weinelt *et al.*, 2021). Noteworthy, paleogenetic data have suggested population movements across the Iberian Peninsula suggesting high mobility of the populations in the specific geographic latitude (Szécsényi-Nagy *et al.*, 2017).

The Iberian Peninsula during the Early Bronze Age (2200–1900 cal BC) is indisputably marked by the El Argar social culture in its southwestern part. Explicitly, the archaeological record has revealed the existence of numerous hilltop occupations across the described geographic latitude, which are characterized by impressive social organization, the existence of complex networks, and distinctive votive behaviors (Lull *et al.*, 2014). The retrieved data imply that agricultural practices along with husbandry as the main economic activities of this temporal point in settlements with urban or proto-urban traits (Delgado-Raack and Risch, 2015). Isotopic studies have indicated populations' reliance on mixed diets, which were characterized by the

prevalence of cereals and animal proteins (both domesticated and wild animals) (Knipper *et al.*, 2020).

Noteworthy, the complex social networks and important mobility patterns are indicated by paleogenetic data, which imply contacts between the Iberian populations and the ones of North Africa and Northeastern Europe (Olalde *et al.*, 2019). Technologically, the Early Bronze Age is distinct from the Chalcolithic Period concerning the occupation patterns, along with the non-existence of Bell Beaker pottery and the implementation of metallurgy (Villalba-Mouco *et al.*, 2021). Finally, the climate of the Northeastern Iberian Peninsula was attributed to the continental Mediterranean one with warm summers and cold winters, however colder than the one of today (2.4 °C lower on average) and higher precipitated based on the conducted paleoenvironmental reconstructions (Bañuls-Cardona and López-García, 2016).

4) Research Background

4.1) Dental Terminology and Morphology

4.1.1) Fundamental information on the dental remains

The dentition is the most durable part of the human skeleton and the only anatomical structure, which can be partially seen by the naked eye. The human dentition is parted of the deciduous teeth, which are getting replaced by the permanent ones, along with the addition of premolars on the latter. Moreover, it is comprised of 32 teeth in total (20 teeth during the deciduous dentition), which are divided into incisors, canines, premolars, and molars. In this context, like many mammals, humans are characterized as diphyodonts, based on the replacement of the dentition throughout their lifetime. Furthermore, the dentition is divided between maxillary (upper) dentition and mandibular (lower) one based on the placement on the maxilla or the mandible of the individual.

The crown is the visible part of the human tooth and is overlayed with enamel, which consists of its vast majority (95 %) of apatite, the mineral that permeates to it its durable attributes (Vaissier Welborn, 2020). The biggest proportion of the interior part of the tooth is comprised of dentine. Noteworthy, during recent years many studies have highlighted the importance of the morphology of the point of connection between enamel and dentine (Enamel-dentine Junction or EDJ) for the understanding of genetic implications (Monson *et al.*, 2020; Gamarra *et al.*, 2022, for instance). The point of nerve and vessel connection with the mandible occurs through the apical foramen, which connects the pulp with the rest of the body entity and is located in the root of the tooth (Aiello and Dean, 1996), while the point of contact of the crown with the root is defined as cervical margin.

4.1.2) Dental Terminology

Concerning dental terminology, the mesial view is considered the point, in which the two central incisors contact, while the distal one is the opposite point of the mesial (White and Folkens, 2005). The labial view is a term used for the anterior dentition (incisors and canines) and signifies the part of the tooth, which is toward the lip. The lingual view, similarly, but contrary to the labial one, describes the part of the anterior

tooth, which is toward the tongue. The occlusal view refers to the part of the tooth, which is used during the masticatory process and mostly describes the posterior dentition (premolars and molars), even though a small part of the surfaces of the anterior tooth can be also described by this term (Sperduti *et al.*, 2018). Finally, the interproximal surfaces are the ones, in which the contact between two closely located teeth is detected.

The term eruption describes the emergence of the tooth from the gingiva. In this framework, the preliminary eruption of the deciduous teeth occurs from the sixth to the thirtieth months after birth, while the first process is completed at around 2 or 3 years old (Nelson, 2015). The replacement of the preliminary dentition with the permanent one, or exfoliation, follows and noteworthy, it is a process that can be asynchronous between the two jaws (Hovorakova *et al.*, 2018). Furthermore, the process or exfoliation includes a temporal point, at which the individual exhibits mixed (or transitional) dentition approximately from 7 to 10 years old $\pm$ 9 months, since the occlusion of all the dental remains doesn't occur on the same point, despite the important level of variability among the populations.

In the literature, specific abbreviations are used to simplify the described piece of information. In this framework, the use of the first letter in a capital case is qualified for the description of the permanent dentition, e.g. I for permanent incisors, C for permanent canines, etc., while the opposite process occurs for the deciduous teeth with the addition of the letter 'd' in lowercase, e.g. di for deciduous incisor, dm for deciduous molar, etc. Similarly, each tooth corresponds to a number based on how many teeth of the type can be found in each quartile. For example, the incisors are described as 1 or 2 (Central or Lateral), the canines can be only 1, the premolars can be 3 or 4, and the molars can be 1, 2, or 3. In this case, the laterality is described by the use of R or L for Right and Left respectively (Scheuer and Black, 2004). Finally, some anthropologists use the FDI nomenclature (World Dental Federation), in which both deciduous and permanent dentition are divided into hemi-arches, while one number is attributed to each hemi-arch respectively (e.g. 1: upper-right hemi-arch; 3: lower-left hemi-arch, etc.) (Moreno-Gómez, 2013).

4.1.3) Dental Morphology

In general, the identification of each type (incisors/canines/premolars/molars) of teeth is a relatively easy process, since the dental remains exhibit distinct morphologies, which derive from their role in the masticatory process. However, each tooth is comprised of different morphological characteristics individually, which contribute to its lateral allocation and identification since equifinality issues can occur during the process of the examination of a dental remain. In the framework of the specific Master's Thesis, the anatomical and morphological characteristics of each type of tooth will be explained separately to provide a holistic approach regarding the peculiarities of each one.

The incisors are the teeth that are characterized by a flat crown and a blade-like shape (White and Folkens, 2005) and are placed on the first and second position of the midline of the deciduous or permanent dentition in the dental arch (Scheuer and Black, 2004). The distinction between a deciduous or a permanent incisor is based mostly on the size and the crown margin since the deciduous ones are smaller and with a rounder shape due to the smaller properties of the crown. Each individual exhibits 8 incisors in total (4 maxillary and 4 mandibular ones), while the lateralization during the examination of isolated incisors in the field is undergone with the observation of landmarks, such as the crown size, the root shape, and the expression of the cingulum, which can be seen on the lingual surface and forms a curved protuberance.

The maxillary incisors are larger in shape than the lower ones, and their crowns are more square-shaped, while their root is more robust and coned-shape than their mandibular counterparts (Nelson, 2015). On the contrary, the lower incisors are smaller, with narrower roots. For the distinction between central or lateral incisor, the point of reference is the shape of the crown (more squared on the upper central incisor), and the cingulum (more centered on the lower central incisor).

The canines are placed on the corner of the dental arch between the incisors and premolars on the permanent dentition (between the incisors and molars during the deciduous dentition), while they demonstrate one cusp, along with a crown of a conical shape (White and Folkens, 2005). Each individual exhibits 4 canines (2 on each dental arch) and the distinction between a deciduous canine and a permanent one

is undergone through the tooth size and the crown margin; explicitly, the deciduous canines are smaller than the permanent ones, while the enamel is thicker on the former, thus providing the visual of a more constricted neck.

The maxillary canines are bigger than the mandibular ones, and their crowns are characterized by a more distinct cingulum and lingual fossae. On the contrary, the lower canines are smaller and depict a less prominent cingulum, lingual fossae, and ridges (Nelson, 2015). Finally, the definition of laterality in the canines is undergone through the examination of the distal bulge, which is the convex part of the labial view of the canine.

The premolars are part only of the permanent dentition and are placed posteriorly to the canines and anteriorly to the molars. Morphologically, they are characterized by the existence of 2 or 3 cusps and are placed on the position of the deciduous molars on the dental arch (succedaneous teeth), while the first premolar appears at the age of 10 years old (Nelson, 2015); morphologically are characterized by a well-formed lingual cusp, present and distinct buccal lobe, and sharp cusps, which are traits that attribute the shearing properties to the premolars. Each individual exhibits 8 premolars in the permanent dentition (4 on each dental arch).

As in all the dental remains, the distinction between the maxillary premolars and the mandibular ones is undergone based on specific landmarks; generally, the distinction of isolated premolars with a high level of wear can be a challenging process (Scheuer and Black, 2004). However, when examined from the occlusal view, the upper premolars depict a more oval shape with a clear distinction between the cusps, while the lower ones are rounder. Moreover, the maxillary premolars are characterized by two proportionate cusps, which are distinct by the existence of a central fissure, while the mandibular ones depict a bigger cusp towards the buccal view.

The distinction between the first premolar and the second one is undergone through the observation of the cusp size, and the root shape. Explicitly, the first premolar inherits morphological traits of the canines, such as a distinct buccal cusp, while the second premolar is characterized by generally similar-sized cusps, a trait inherited by the molars. Finally, the attribution to the right or the left side is undergone by the examination of the cusp height, along with the mesial crown surface and the root curvature. Explicitly, the first upper premolar presents a more concave root, and

higher buccal cusp, while the second upper premolar presents cusps, which are similar in height generally. The first lower premolar exhibits a central enamel ridge and a root, which is directed to the mesial surface in the majority of the cases. Similarly, the second lower premolar presents two pits on the occlusal view, which are connected by a fissure, along with a difference in the placement of the marginal ridge on the mesial side, being higher than the distal marginal one. Finally, the roots of the lower premolars show a tendency towards a distal placement (Scheuer and Black, 2004).

The molars are the dental remains, in which the first methodological frameworks for the appliance of Dental Microwear Analysis were formed during the 1980's. In this context, some specific characteristics are required to be explained in depth, since their terminology and anatomy are widely described in the literature and used for the determination of the dental features. Based on the placement of the molar maxillary or mandibular, the cusps on the occlusal surface present different suffixes for their determination in the literature; more specifically, the upper molars present cusps with a suffix of -cone, while the lower ones end with the suffix of -conid.

In this framework, the upper molars on the occlusal view exhibit the protocone (mesiolingual cusp), the hypocone (distolingual cusp), the paracone (mesiobuccal cusp), and the metacone (distobuccal cusp), while the existence of possible anatomical features on the mesiolingual view is attributed to Carabelli's effects. Similarly, the lower molars present the protoconid (mesiobuccal cusp), the hypoconid (distobuccal cusp), the metaconid (mesiolingual cusp), and the entoconid (distolingual cusp), while the hypoconulid can be described as the fifth and most distally located cusp of a lower molar (White and Folkens, 2005). Each individual exhibits 12 molars in total (6 on each dental arch), while the temporal point of the eruption of the third molar is placed in the framework of important variability among populations and individuals.

The distinction between the deciduous molars and the permanent ones is undergone through the examination of the crown size and shape, the thickness of the enamel at the crown margin, and the root size and shape (Scheuer and Black, 2004). Explicitly, the deciduous molars are characterized by smaller cusps and crowns, and by a distinct cervical enamel margin, providing the view of a bulbous crown, while the roots of the deciduous molars are generally more divergent (White and Folkens, 2005). The

attribution to lower or upper dentition is based on the arrangement of the cusps, the crown outline, and the root number and position. The permanent upper molars depict 3 or more cusps, along with an oblique enamel ridge, which can be observed from the mesiopalatal cusp to the distobuccal one (Scheuer and Black, 2004), while all the maxillary molars exhibit three roots -even though the phenomenon of root fusion can be observed (Aiello and Dean, 1996). On the contrary, the permanent lower molars retain the possibility of the exhibition of 5 cusps (primitive trait), and unlike the deciduous mandibular molars, the buccal cusps are sized disproportionately.

Regarding the distinction among the molars, the deciduous first molar is differentiated from the second one since its buccal cusps are wider mesiodistally than the lingual ones. Similarly, the deciduous first lower molar retains a more rectangular shape, along with a prominent mesiodistal width. In the cases of permanent dentition, the upper first molar is the bigger one out of the three, while it remains important similarities with the upper second molar, often providing difficulties in the identification when the examination is taking place on isolated dental remains. However, the distinct traits of the latter are the reduced distal cusps compared with the upper first molar, along with a bigger level of curvature on the roots (Scheuer and Black, 2004). The lower first molar is characterized by a rectangular shape on the occlusal surface and two roots with a distal curvature. Furthermore, the lower second molar is smaller and can be easily identified through the existence of four visibly distinct cusps due to the exhibited central fissures. Finally, the third molar is characterized by a big variability in shape among populations, however being smaller than the other molars, usually with three or more cusps, and a triangular outline.

4.2) The Chewing Movements and Dental Mechanics

As explained above, Dental Microwear Analysis examines the non-pathological loss of dental tissue, which occurs when a stronger particle than the enamel comes in contact with it either through the alimentary processes or during the behavioral utilization of the mouth during para-masticatory activities. In this framework, the understanding of chewing movements and dental mechanics is of vital importance since in the case of the detection of dietary patterns the examined dental features occur through the mastication process.

Each tooth in the human dentition reflects specific properties, which are adapted to the use of each dental remain during the chewing cycle respectively (Nelson, 2015). Explicitly, the incisors exhibit sharp edges for the cutting of the consumed particle into smaller pieces to facilitate the further process; the canines, which depict the sharpest edge of all dental remains and facilitate tearing and ripping of the consumed particle; the premolars exhibit cusps, which facilitate the shearing and grinding of the food; finally, the molars represent the bigger number of cusps on the occlusal surface since are used during the grinding and chewing of the food.

The first pieces of information on the chewing cycle of mammals appeared in the 1950s (Butler, 1952) with the comprehension of the deciduous molars occlusion of *Perissodactyla*. Noteworthy, this study highlighted the importance of the increase in the chewing area during the ontogeny of the examined species, since it implies morphological implications during the occlusion of the molars. Almost two decades later, the pioneering works of Hiiemae and partners (Hiiemae and Crompton, 1971; Kay and Hiiemae, 1974, for instance) centered the interest on the understanding of jaw movements and functional dental issues in primates. Based on their observations, dental functionality during the mastication process is divided into two phases (Phase I and Phase II), specifically into an initial “Buccal” phase followed by a “Lingual” one. Specifically, Phase I or “Buccal Phase” is initialized during the food-tooth-food contact or tooth-tooth contact and includes the anteromedial movement of the jaw. Likewise, Phase II, or “Lingual Phase” follows including the anteromedial movement, which is terminated when the masticated object is out of the occlusal contact.

The chewing cycle in humans occurs as a result of the coordination of different muscles and joints, which permits the complex movements of the mandible (Aiello and Dean, 1996). The described process implies strong craniofacial implications with subsequent evolutionary insights. In the case of humans, the activity between the temporalis muscles and the ones of the working side appears to be equal generally, while bigger mechanical values exist on the activity of the masseter and medial pterygoid muscles of the working side (Aiello and Dean, 1996). Subsequently, the appliance of mechanical forces during the masticatory activity is in accordance with the cranial development during the human ontogeny, such as in the case of the

complete fusion between ethmoid bone and metopic ridge only at the point of the appearance of the total deciduous dentition due to bigger masticatory processes (Scheuer and Black, 2004).

Despite the general adoption of the same chewing movements by all human populations, variability can be observed among sexes, masticatory practices, and chewing rhythmicity (Crane *et al.*, 2013). A study by Youssef *et al.* (1997) examined the mandibular movements during the process of habitual mastication among men and women, depicting differences in the values of the duration of the chewing cycle, along with the applied muscle forces. The results were interpreted through the existence of higher mandibular velocities in men, thus producing greater forces, and accelerating and/or bypassing parts of the chewing phases. Similarly, a study by Lepley *et al.* (2011) focused on the understanding of the masticatory processes of 30 individuals (balanced sex distribution) indicating that the chewing cycle is dependent on the size of the consumed bolus, the maximum bite force, and the occlusal contact between two dental remains, among other variables.

The described data above demonstrate that despite the understanding of the masticatory process on humans holistically, the chewing mechanism of each individual varies across human populations due to its dependence on several variables. However, it is without a doubt that the adoption of generally similar masticatory behavior is a fundamental element among lineages, while a small variation can be explained based on the existing pieces of information and peculiarities of each individual separately.

4.3) Research History of Dental Microwear Analysis

The appliance of Dental Microwear Analysis is laid in the detection of microtraces in the enamel surfaces of the dental remains, which can be attributed to both masticatory and para-masticatory activities. The first idea concerning the drawing of a conclusion from the dental remains for alimentation purposes is placed in the time frame between the decades 1920 and 1960, when for the first time the chewing process and its effects on dental wear were understood. Explicitly, the research of Simpson (1926) predicted the alimentary habits of Mesozoic mammals based on their dental morphology and

functionality; a subsequent work from the same author (Simpson, 1933) was focused on the understanding of the jaw movements, considering the tooth-to-tooth contact as a variable based on the properties of the consumed food on mammals of the Jurassic period; furthermore, the studies of Butler (1952) and Mills (1963) provided the scientific community with results on the attribution of specific features of dental facets on jaw movements in the cases of Perissodactyla and Primates correspondingly.

Despite the obtainment of the basic frame regarding the chewing cycle and the production of the dental features, it wasn't until the end of the 1970s for the appearance of a more stable methodological framework concerning Dental Microwear Analysis. Particularly, toward the end of this temporal point, the appearance of two pioneering works from Walker *et al.* (1978) and Rensberger (1978) exhibited the potential of the utilization of the Scanning Electron Microscope on the understanding of dental wear and its attribution to alimentary purposes on mammalian species. The described studies were laid in the confirmation of the correlation between diet and striations in the dental surfaces of species, whose dietary patterns were already known, while their results paved new possibilities regarding the appliance of the analysis on human dental remains.

However, equifinality issues appeared in the conduction of the analysis due to the absence of a widely accepted and applied methodological framework among researchers. Undoubtedly, the wear patterns are dependent on several factors, such as the position of the sample during the microscopic observation, the applied magnification, the used lenses, and more. From the 1980s and onwards, the further understanding of the potential of the use of Scanning Electron Microscope in the studies of dental wear brought a general tendency towards the obtainment of more quantitative approaches, aiming to obtain the maximum pieces of information from the observed dental features (Ungar *et al.*, 2009). In this context, the appearances of the first stable methodological frameworks are placed on the innovative works of Gordon (1982; 1984), who provided the founding ideas concerning the quantification of the dental features on each view of the dental remains.

4.4) Overview of the existing methodologies

Nowadays, the prevailing methodologies consist of the examination of the occlusal surfaces with the use of a Confocal Microscope, and the microscopic observations of the buccal surfaces with the use of a Scanning Electron Microscope. However, for the obtainment of a more holistic approach regarding the conduction of the preliminary observations, along with the understanding of the dental features and their determination in the surface of the dental remain, the use of an Optical Microscope and a 3D Digital one is recommended supplementary.

4.4.1) Optical Microscope

The Optical Microscope comprised one of the first tools ever used for the conduction of Dental Microwear Analysis, from as early as the decade of 1960's (Dahlberg and Kizney, 1962). Its accessibility to a wide range of institutions, its low cost, along with the possibility for the researcher to provide a proper description of the dental features using it, have maintained the importance of the Optical Microscope in the study of dental wear until today (Mihlbachler *et al.*, 2012). Despite the existence of more precise tools for the obtainment of images of dental views on a microscopic scale nowadays, the Optical Microscope is still playing a role of great importance in the preliminary localization of the dental features prior to the subsequent observation of the dental enamel surfaces through other means.

The current methodological framework for the use of an Optical Microscope in Dental Microwear is comprised of the appliance of low magnification, ranging from x30 to x100. A study by Hernando *et al.* (2020a) demonstrated the versatility of the use of an Optical Microscope for the detection of dietary striations; interestingly, when comparing the obtained results of an Optical Microscope (OM) with the ones of a Scanning Electron Microscope (SEM), an insignificant level of difference was observed among the results of the two microscopes on different variables. However, the non-existence of clear and widespread control regarding the use of different resolutions concerning each dental feature respectively could potentially lead to higher error possibilities in the quantification process (Mihlbachler and Beatty, 2012), while the first guidelines concerning the standardization of the protocol for its use on

the study of dental wear can be found in Hernando *et al.* (2020a). It is indisputable that further studies using the specific piece of equipment can provide more pieces of information concerning its use in a more concrete methodological framework in the future.

4.4.2) Scanning Electron Microscope (SEM)

The Scanning Electron Microscope is considered the equipment, in which the first methodological frameworks for the study of dental wear were established. It functions through the emission of electrons on the surface of the examined remain, aiming to define surface properties, which occur from the interaction among the electrons and the observed sample (Pereira-da-Silva and Ferri, 2017). Early studies with the appliance of a Scanning Electron Microscope predicted precisely specific dietary patterns of extinct human and ape populations (Gordon, 1982; Grine, 1987; Grine and Kay, 1988; Walker and Teaford, 1989, for instance).

The most widely used methodological framework for the use of Scanning Electron Microscope in the studies of dental wear is comprised of the appliance of magnification of x100 when examining the buccal surface and of x500 during the observation of the occlusal surface (Romero and de Juan, 2012). The described difference in the magnification is explained through the landmark properties of each surface since the latter (occlusal surface) can exhibit a potentially important level of peculiarity among tooth and enamel microwear features. In both cases, an important aspect of the methodological framework is the overlay of the replica with a very thin coating of gold layer (~15 nm) to prevent possible wear on the examined tooth surface due to the destructive properties of SEM (Pérez-Pérez *et al.*, 2017).

Based on its important level of resolution, the existence of stable methodologies on Dental Microwear Analysis through it, along with its stability in deciphering in depth the dental features of the examined teeth, the Scanning Electron Microscope remains today one of the most widely used tools for the understanding of alimentary habits of present and past animals (El-Zaatari, 2008; Alrousan, 2016). However, the inadequacy of its transfer from one laboratory to another, along with its high cost of use and

maintenance are the main restrictive factors for the undergone of Dental Microwear analysis through a Scanning Electron Microscope.

4.4.3) Confocal Microscope

During the last two decades, the technological development has provided the studies of wear with a new approach regarding the quantification of dental features to obtain the best possible objective view. In this context, the Confocal Microscope provides the possibility of conducting a topographic analysis due to its possibilities as a profilometer, which wasn't able to be obtained with an Optical Microscope or a Scanning Electron Microscope (Karriger *et al.*, 2016). It functions through the emission of a laser beam, which enables the subsequent formation of high-resolution images, along with 3D models of the examined surface (Boyde and Jones, 1995).

The potential of the use of the Confocal Microscope to understand dental wear patterns has been highlighted already from the beginning of the 1990s when Boyde and Fortelius (1991) detected two patterns of orientations on molars from the Miocene site of Pasalar, which were attributed to the jaw movements of *Listriodon* sp. and *Listriodon splendens*. Nowadays, the use of the Confocal Microscope has been correlated with the development of the Dental Microwear Texture Analysis (DMTA) for the comprehension of dental wear.

The Dental Microwear Texture Analysis (DMTA) was first introduced by Ungar (*et al.*, 2003) combining the scanning properties of a Confocal Microscope with a Scale-Sensitive Fractal Analysis aiming to confirm differences in the dental surfaces of animals, whose dietary patterns were already known. In recent years, the appliance of Dental Microwear Texture Analysis on a big scale has permitted the detection of alimentary habits of extinct animals through the extraction of descriptive data from their examined surfaces (Scott *et al.*, 2005; Prassack *et al.*, 2020; Hernando *et al.*, 2022).

The methodological framework of Dental Microwear Texture Analysis predicts the appliance of x100 magnification in the examined view of the dental remain, while the total examined area differs depending on the objective of the study (Winkler *et al.*, 2020; Xue *et al.*, 2021). Unlike other types of analysis, DMTA provides the

opportunity for the understanding of the tooth-food-tooth relationship and the subsequent masticatory dynamics in the view of the dental remain based on several different variables, such as Anisotropy (epLsar), Complexity (Asfc), Texture fill volume (Tfv), and more (Schmidt *et al.*, 2019). Finally, the obtainment of descriptive values from a microscopically automated approach permits the vast procedure of the data and accelerates the archaeological interpretation of the studied populations (El Zaatari *et al.*, 2011).

4.4.4) Occlusal Fingerprint Analysis (OFA)

The Occlusal Fingerprint Analysis (OFA) was first introduced by Kullmer *et al.* (2009) and is laid in the understanding of the opposing mechanical processes between two dental remains, which subsequently lead to occlusal wear through the tooth-food-tooth contact. In principle, OFA is based on the localization of the facets on the occlusal view of a dental remain, and their subsequent digitalization through the use of the principles of photogrammetry or 3D scanning systems (Kullmer *et al.*, 2009; Fiorenza *et al.*, 2023).

The appliance of Occlusal Fingerprint Analysis (OFA) is heavily dependent on the precise determination of the examined facets of the occlusal views of the dental remain, aiming to distinguish patterns of wear between the opposing facets. Inside this context, the methodology predicts the determination of the examined area of the dental surface based on the identification of Maier and Schneck, in which the occlusal surfaces of the upper and lower molars of the hominins are comprised of 13 opposing facets (1981; 1982). Recent studies have aimed the deciphering of the functional properties of each facet during the mastication processes, indicating that facets 9 and 11 are the ones that exhibit the biggest level of occlusal wear independently from the age of the examined individual (Bas *et al.*, 2020; Hernando *et al.*, 2021).

An important aspect regarding the interpretation of the piece of information, which is extracted through the undergone of Occlusal Fingerprint Analysis (OFA), is the independence of the result from the well-known “The Last Supper” effect since the level of wear on the respected occlusal sub facets occurs cumulatively (Fiorenza *et al.*, 2018). Explicitly, “The Last Supper” effect, as described by Grine (1986),

presents the perception that the examination of dental wear on the occlusal surfaces reflects the diet of the last weeks of the examined case study considering the period, which is required for the production of wear patterns on the dental surface. Inside this context, the measurement of the level of inclination on opposite facets provides the opportunity for the understanding of wear, which occurs throughout a bigger period of time, since it is a process that requires continuous mechanical appliances on the dental surface (Winkler *et al.*, 2020).

4.5) Factors that affect the final result of Dental Microwear Analysis

The appliance of Dental Microwear Analysis is dependent on several *ante*- and *post-mortem* factors, which can alterate the enamel surface and impede or reveal pieces of information regarding the understanding of alimentary habits in the studied populations. Based on the existing literature, the most important *post-mortem* changes occur either from mechanical processes (tumbling, trampling, and abrasion), from natural reasons (perikymata), or from chemical ones (erosion).

4.5.1) Enamel Alterations from Tumbling

As tumbling in the study of taphonomy is considered the process of the alteration of a bone remain, which occurs after the appliance of hyper-concentrated fluxes, such as in the cases of fluvial environments (Pineda *et al.*, 2019). The effect of tumbling on bone remains is identified through the recognition of cut marks on the surface, which was affected by the mechanical process. Recent experimental studies have aimed the distinguish between the produced cutmarks by the application of lithic tools on bones, and the ones by tumbling, indicating that the distinction between the two activities could potentially lead to equifinality issues (Gaudzinski-Windheuser *et al.*, 2010; Pineda *et al.*, 2023).

During the last few years, a number of experimental studies have aimed at the understanding of the taphonomic effect of tumbling on dental remains to determine the level of enamel alteration. Explicitly, a study by Böhm *et al.* (2019) targeted the determination of the level of resistance of microwear traces related to diet during fluvial transport on dental remains of small and large mammals, indicating that even if

the levels of alternation between the dental remains of different species is not identical, still a clear distinction between browsers and grazers could be done. Moreover, Weber *et al.* (2022) exposed dental remains of a variety of species on different levels of mechanical treatments concerning the type of soil in order to replicate taphonomic alternations; the results exhibited that the biggest level of enamel alteration occurred after the exposure of the tooth to fine to medium-grained gravel (2-8 mm), while the taphonomic results of fine sand (112-292 μm) and medium sand (221-513 μm) didn't differ significantly.

The comprehension of tumbling mechanics can provide important insights regarding the level of alteration and the possible hiding of archaeological information, especially in sites, which are located in fluvial environments. However, it is clear that the understanding of enamel alterations through tumbling is scarce, and the existing studies are limited. It is without consideration that the conduction of new studies and experimental works would contribute to the existing knowledge and provide more quantitative approaches to this specific taphonomic process.

4.5.2) Enamel Alterations through Trampling

The taphonomic phenomenon of trampling is described as a friction process between the bones and the substrate, related to the transit of hominins and animals (Fernández-Jalvo and Andrews, 2016), and effects widely described in the taphonomic studies of both human and faunal remains, and lithic industries (Indra *et al.*, 2022; Lev *et al.*, 2020; Balirán, 2014, for instance). The described taphonomic process can produce important equifinality issues in the archaeological interpretation since the main effects of this mechanical modification are superficial striae, breakage, and displacement of the bone. More specifically, experimental trampling has exhibited internal striae (Behrensmayer *et al.*, 1986), scratches, and scrapes (Andrews and Cook, 1985), indicating similar effects with cutmarks.

During recent years, a number of experimental studies, which were conducted by researchers of the Institut Català de Palaeoecologia Humana i Evolució Social (IPHES-CERCA) have aimed at the understanding the level of alteration of the enamel surface from trampling. Noteworthy, a study by Micó *et al.* (2023) intended to

identify the features caused by trampling and the ones, that imply dietary habits, on dental remains of ungulates. The results indicated that a morphological differentiation in the features from each activity respectively is available through the observation of specific variables on the produced striations, such as their shape and orientation, along with the existence (or not) of secondary striae, among others.

From the presented data, it can be easily inferred that the effect of trampling on the enamel surface is a topic of research, which has yet to be explored completely. It is without a doubt that the implementation of more quantitative statistical models on dental features and new experimental projects could provide insights into the effects of trampling on dental remains shortly.

4.5.3) Enamel Alterations through Abrasion

Abrasion is defined as the alteration of a bone surface, which occurs from the shoveling against the sediment during e.g. a fluvial or a water stream transportation (Fernández-Jalvo and Andrews, 2016). Based on the frequency of appearance in the archaeological record, this taphonomic process has been well-documented and studied in the literature (Gümrükçü and Pante, 2018; Fernández-Jalvo and Andrews, 2003, for instance). Concerning dental remains, abrasion occurs when an exogenous material, which is harder than the enamel, comes into contact with the tooth (Kaidonis, 2012a; Hua *et al.*, 2020), and can be divided into *ante-* and *post-mortem* enamel alteration.

Ante-mortem alteration occurs when a tougher particle than the enamel is squeezed into its surface during the masticatory activity or during the utilization of the mouth as a “third hand” for the conduction of an activity. For the former, experimental data have demonstrated the effect of the ingestion of sand particles, which are coherent on the food, during the chewing process (Uzunidis *et al.*, 2021; Louail *et al.*, 2022), since the underground storage of the meat in cold environments is of vital importance for its conservation (El-Zaatari, 2008), while phytoliths can also produce wear due to their siliceous properties (Rodríguez-Rojas *et al.*, 2020). Moreover, the use of the mouth as a tool has been documented in the ethnoarchaeological record and has been identified in dental remains dated from as early as the Middle Paleolithic (Lalueza Fox, 1992; Lozano-Ruiz *et al.*, 2004). Specifically, the alimentary apparatus provides the

possibility for the stabilization of an object during the conduction of an activity, which in turn results in the production of striations on the enamel surface.

Post-mortem enamel alteration occurs from the contact of the dental remain with sedimentary deposits after its final deposition, while the result of the described activity is the existence of parallel striations on a polished enamel surface. An experimental study by King *et al.* (1999) aimed at the reproduction of the specific patterns of enamel alterations from a variety of deposits. The results indicated that the biggest level of alteration was noticed after the exposition of the dental remains on medium-sized sand (grain size 250 to 500 μm), while interestingly not an important level of distortion of the dental features was described after the exposition on quartz pebbles and coarse sand (grain size 500 to 1000 μm).

4.5.4) Enamel Alterations due to the exposition of Perikymata

The perikymata or striae of Retzius appear as layers, which can be observed in the enamel surface as a result of enamel growth (Guatelli-Steinberg, 2008). The formation of these specific dental features requires a certain period of time, which can be however variable when examining population data, with an average of eight or nine days for modern humans and apes for each layer (Dean *et al.*, 2001). In this framework, perikymata can provide important insights concerning issues of the speed of the formation of tooth crowns, the age determination of the examined individual, and the attribution of the dental remain to a specific specie inside a bigger genus family (Elhechmi *et al.*, 2013).

However, perikymata can lead to important difficulties during the conduction of Dental Microwear Analysis as well. Explicitly, this physical process can conceal the dental features, which reveal dietary patterns, such as striations, thus not providing the opportunity for the extraction of suitable information. A study by Hernando *et al.* (2020), which was based on the methodological comparison of different microscopic approaches, as described above, exhibited the detection of perikymata through both an Optical Microscope and a Scanning Electron one, which prevented further microscopic observations on the specific dental remains. Furthermore, a study by McGrath *et al.* (2021) focused on the understanding of the growth rates of striae of

Retzius comparatively between teeth of *H. neanderthalensis* and *H. sapiens* using a Confocal profilometer (Sensofar S Neox), thus implying the possibility of the concealment of information during the conduction of Dental Microwear Texture Analysis as well.

In this context, despite their importance for the understanding of genetic implications and information on the examined populations and individuals, perikymata are considered a preventing factor for the appliance of Dental Microwear Analysis, leading inevitably to the exclusion of the dental remain from further analysis.

4.5.5) Enamel Alterations through Erosion

Dental erosion is described as the process of the alteration of the dental tissue due to a systematical exposure to acidic substances, which are characterized by a lower pH than the one of the oral cavity (Coupal and Sołtysiak, 2017). Concerning the *antemortem* erosive event on human teeth, even though it is considered a phenomenon of the last centuries due to the consumption of a bigger variety of processed foods and liquids (Hara *et al.*, 2021), the existence of erosive dental surfaces can be hypothesized from as early as the Neolithic period due to the familiarization with the production and consumption of fermented fruit beverages in some parts of the globe (McGovern, 2009).

The effect of the acidic etching on the enamel surface due to erosion is identified mostly by the existence of round pits. Specifically, an experimental study by King *et al.* (1999) aimed at the explanation of unusual wear patterns on dental remains of *Griphopithecus alpani* (King *et al.*, 1998). The results indicated that two dental remains of the described specie were more likely exposed to citric acid since the examined enamel surface was characterized by lighter edging, in a similar way as in the experiment. Concerning the macroscopic and microscopic observations, erosive teeth are generally characterized by occlusal grooving, reduced cusping on the occlusal surfaces, and smooth edges (Coupal and Sołtysiak, 2017).

Concluding and based on the existing literature, the need for the acceptance of a widely used protocol for the identification and characterization of erosive dental

surfaces can be characterized as imperative, since the attribution to the erosive processes remains scarce in many cases.

4.6) Dental Microwear Analysis in Populations of the Chalcolithic Period and Bronze Age

As described above, the appliance of Dental Microwear Analysis has proven to provide important insights into the dietary patterns and behavioral levels of the studied populations. In the framework of the specific Master's Thesis, the mentioned analyses are about to be explained chronologically with respect to the year of publication, from the oldest one to the youngest.

4.6.1) Unravelling of the dietary patterns in the Late Prehistory of the Iberian Peninsula

In general, the appliance of Dental Microwear Analysis on Iberian populations of the Chalcolithic Period and Bronze Age has yet to be explored on a large scale. The existing literature regarding this period is limited, thus highlighting the need for further research on these specific temporal points based on the abundant number of retrieved sites.

Concerning the examination of Iberian Bronze Age populations, the first Dental Microwear Analysis was conducted by Romero *et al.* (2004), who examined the buccal views of permanent left lower molars from the sites of Aspe (Alicante), Almansa (Albacete), and Cova dels Blaus (Castellón) with the use of a Scanning Electron Microscope at 100X magnification. The results demonstrated differences between the studied populations based on the number of striations and their orientation, with the authors however stating that the study remains preliminary.

A more detailed approach to Cova dels Blaus site (Castellón) was presented in a study from Polo-Cerda *et al.* (2007) aiming in the extraction of pieces of information for dietary patterns from the site using paleopathological approaches and Dental Microwear techniques. Explicitly, the buccal surfaces of permanent lower molars from six adults (4 women, 1 man, 1 unidentified) were examined with the use of a Scanning Electron Microscope at 100X magnification. Based on the level of wear, the

authors suggested a diet rich in carbohydrates from the population of the site (e.g. cereal), even if the percentage of the retrieved dental calculus remained low. Furthermore, the high number of observed striations was interpreted by the use of specific means during the food process, such as residues from grinding stones, which are harder than enamel and can subsequently wear it.

Furthermore, an analysis by Horwath *et al.* (2014) focused on the analysis of dental remains from Bolores site, Southwestern Iberian Peninsula, which is temporally placed on the transition between the Late Neolithic and Early Copper Age. Specifically, the examined sample included the left lower molars of 22 individuals (11 adults/ 11 children) with an applied magnification of 500X on the occlusal surfaces with the use of a Scanning Electron Microscope. The results demonstrated the consumption of soft and abrasive foods, which produced an important number of striations on the enamel surface due to the adhesion of particles, such as grit, originating from the used means for food process, as described above, or from the consumption of phytolith-rich plants.

Moreover, a study by García-González *et al.* (2018) examined the dental wear on remains of El Portalón de Cueva Mayor and El Alto de la Huesera sites. Explicitly, the study was based on the microscopic observation of both anterior and posterior teeth, including occlusal, buccal, and incisal views with the use of an Environmental Scanning Electron Microscope (ESEM). The results indicated the importance of the understanding of the different palaeoenvironmental variables for the archaeological interpretation. The analysis revealed similarities in the number of striations of the buccal surfaces from the examined sites with the values of the population from the medieval site of La Olmeda, implying the same level of dietary abrasiveness between the groups of the two chronological periods. Moreover, the existence of a higher number of horizontal striations than vertical ones on the buccal signatures of the Chalcolithic populations has been attributed to an important intake of meat protein.

An analysis by Hernando *et al.* (2019) examined the dental remains from the Middle Bronze Age site of Cova Valdavara (Becerreá, Lugo), describing their morphological characteristics and pathological conditions and applying Dental Microwear Analysis. Explicitly, the site is dated between 3250 ± 40 years BP (3.600-3360 years cal. BP) and 3270 ± 40 years BP (3.600-3.400 years cal. BP), while the samples were

comprised of 12 dental remains in total, which were attributed to 3 children, thus solely deciduous dentition. The DM Analysis was applied to the buccal surfaces of the dental remains using an Environmental Scanning Electron Microscope (FEI Quanta 600) at a magnification of x100. The results demonstrated the adoption of a generally abrasive diet based on the number of striations, which included poorly processed meat and cereals, and was close to the generally adopted by the adults of the same period ones.

The same author (Hernando *et al.*, 2022b) combined different proxies and methodological frameworks to unveil the dietary patterns of the population of the El Mirador Cave, which is located in Sierra de Atapuerca, Burgos, Spain. The study included the appliance of zooarchaeological and taphonomic approaches on the faunal remains, the examination of retrieved charred fruits and seeds, and the appliance of Dental Microwear Analysis and Isotopic Analysis on human remains of the site. The examined dental remains ($n=6$) are placed to the Bronze Age and were attributed to 6 individuals in total, including both deciduous and permanent dentition, while the microscopic observation underwent with the use of an Environmental Scanning Electron Microscope on the buccal surfaces. The results of the Dental Microwear Analysis indicated the adoption of a non-abrasive diet based on the low numbers of striations on the buccal surfaces revealing the adoption of a mixed diet with an important level of food processing. The described result comes in conjunction with the analysis of Lozano *et al.* (2022) regarding the frequencies of dental caries and dental calculus in the same individuals.

Moreover, a study by Gluitz *et al.* (2023) focused on the examination of the buccal views of molars and premolars from Bronze Age populations of Plaça de la Gardunya and Mercat de Santa Caterina (Barcelona plain) with the use of a Scanning Electron Microscope (Gluitz *et al.*, 2023). In total, dental features were identified in the dental remains of 21 individuals, while the results demonstrated the adoption of mixed diets, in which meat was the principal component. The attribution to this specific dietary pattern was based on the number and the length of striations, along with comparative approaches with existing data from the archaeological and ethnoarchaeological records.

The most recently published study consists of the appliance of Dental Microwear Analysis on dental remains from 8 sites of the NE Iberian Peninsula, from which 7 of them (Cova de Can Sadurní, Cova de la Guineu, Cova Foradada, Cova del Trader, Roc de les Orenetes, Cova del Gegant, and Cova dels Galls Carboners) are dated between the beginning of the Chalcolithic Period and the Middle Bronze Age (Hernando *et al.*, 2024). Explicitly, the analysis was undergone using an Optical Microscope on the buccal surfaces at a magnification of x100. The results demonstrated the adoption of generally mixed diets, which are indicated by the existence of high striation values, such as in the cases of Cova de la Guineu, Cova Foradada, and Cova del Galls Carboners. Interestingly, the microwear signatures of the dental remains from Roc de les Orenetes demonstrated a smaller number of striations, probably as a result of the appliance of food preparation techniques and/or consumption of secondary products of animal origin.

4.6.2) Understanding of the behavioral patterns in the Late Prehistory of the Iberian Peninsula

The study of Silva *et al.* (2016) examined the dental remains of 11 individuals from the Bronze Age Necropolis of Casas Velhas in the Southwest part of the Iberian Peninsula aiming to find wear patterns produced by non-alimentary behaviors. The analysis was performed macroscopically and with the use of hand lenses, revealing the existence of dental features, such as enamel chipping and grooves both in the anterior and posterior dentitions. The existence of chipping features in the posterior dentition was interpreted as an indicator of the adoption of a generally vegetarian diet since fruit stones and shells can potentially lead to such a result on the enamel surface due to the appliance of high mastication forces. Furthermore, the dental features, which were observed on the lingual surfaces of the anterior dentition, were attributed to the manipulation of vegetal fibres, a practice that was common in other Bronze Age populations as well, as described above.

An analysis by Lozano *et al.* (2017) examined both macro- and microscopically 382 dental remains from 58 individuals from 4 sites, which are located in Sierra de Atapuerca (Sima del Elefante, Gran Dolina, Sima de losHuesos, and Mirador Cave),

including 4 different *Homo* species. Concerning the examined temporal point of the specific TFM, the described study included 197 dental remains from 28 individuals of the Chalcolithic and Bronze Age layers of Mirador Cave, applying Dental Microwear Analysis on the anterior dentition with the use of a FEI QUANTA 600 Environmental Scanning Electron Microscope (ESEM). The results demonstrated the existence of cultural wear traits, such as enamel chipping, labial striations, and vestibular-lingual striations on the anterior dentition, indicating the possibility of the conduction of activities related to the manipulation of fibers.

Moreover, the study of Fidalgo *et al.* (2020) on 21 individuals from Torre Velha 3 site revealed unusual wear patterns, which indicated non-masticatory use of the feeding apparatus from the population of the site. The analysis was performed both macroscopically and with the use of hand lenses, detecting atypical wear patterns, such as chipping, the existence of anterior occlusal surface grooves (AOSG), and oblique wear planes. Explicitly, the detection of chipping on the anterior dentition provided a hint for the use of the tooth as a tool; interestingly, the recovery of *Ovis* bones in the framework of an offering in a burial context provided an implication for the potential familiarization of the populations with yaw, whose manipulation in some cases includes the use of the mouth, thus producing dental wear. Noteworthy, these patterns were not equally distributed to the examined individuals, thus suggesting task-related activities.

The analysis of Lozano *et al.* (2021) evaluated dental remains from the Castellón Alto site, which is located in the southeastern part of the Iberian Peninsula and was part of the El Argar culture. The examination consisted of macroscopic observations for the separation of the dental remains on 106 individuals, which exhibited non-alimentary dental features, with the subsequent microscopic observation on 5 of them with the use of an Environmental Scanning Electron Microscope (ESEM) on a variety of magnifications (30X to 300X). The dental features comprised of enamel chipping, grooves, and polished enamel, among others, and were interpreted through the repetitive utilization of the mouth on the manipulation of plant and animal fibers. Interestingly, the appearance of dental features was correlated with the sex of the individuals, since they appear solely in women, indicating task-division separation in the El Argar society concerning the elaboration of craft goods.

Furthermore, a publication from Willman *et al.* (2021) exhibited the existence of non-masticatory use of the feeding apparatus on two incisors of an individual from the Monte do Vale do Ouro 2 site. More specifically, the observation was conducted through a digital optical light microscope and revealed the existence of a deep groove in the labial surfaces of both incisors, which is asymmetrical among the two surfaces. Based on the morphology of the dental feature and on data from the literature, the authors attributed its production to activities, which are relevant to the manipulation of flax or/and wool.

The study of Díaz-Navarro *et al.* (2023) was focused on the macro- and microscopic examinations of the anterior dentition of 8 individuals from the Chalcolithic site of Camino del Molino in the Southeastern part of the Iberian Peninsula, which implied the conduction of para-masticatory activities based on their unusual wear pattern. The examined individuals are part of a bigger complex of funerary uses of the site, which counts more than 1300 individuals in total. The appliance of Dental Microwear Analysis was undergone with the use of a Scanning Electron Microscope on a range of magnifications (12X to 500X), while the dental features consisted of interproximal grooves, chipped enamel, and notches. Based on their morphology and information from the site, the authors attributed the production of these patterns to the manipulation of plant fibers with the use of the feeding apparatus for the making of yarns.

4.6.3) Other areas

As it can be easily understood, the record of the conducted Dental Microwear Analysis on a global scale includes numerous publications from almost every geographical latitude of the planet. In the framework of this specific Master's Thesis, the described analyses are selected in order to provide constructive comparisons with the explained Iberian sites above, along with the understanding of the dietary habits of contemporaneous but geographically distinct populations, and are presented based on the year of publication, from the oldest one to the youngest.

A study by Schmidt (2001) focused on the examination of 42 dental remains from 8 sites, which are dated at the Late Archaic (4500 to 4000 years BP) and Early/Middle

Woodland periods (2000 to 1500 years BP) of North America. In the framework of the appliance of Dental Microwear Analysis, lower second molars were the ones selected, which were examined with the use of a Scanning Electron Microscope at a magnification of 500X on the occlusal surface. Based on the important number of pits, the author proposed the adoption of hard foods (such as nuts and/or starchy seeds) on the diet of the studied populations, while the existing striations were attributed to exogenous adherent particles to the food, such as grit. Noteworthy, when comparing these results with the ones from the previous temporal point (Late Archaic period), the length of striations decreases, indicating the consumption of more processed foods from the populations of the Woodland period.

The analysis of Mahoney (2006a) focused on the lower molars of 10 individuals from the Tell es-Sa'idiyeh site in Jordan, which is dated between 3570- and 3000-years BP. The study included 28 molars in total, which were exposed to a magnification of 500X through a Scanning Electron Microscope on facet 9 of the occlusal surface since it is the most affected point of the enamel surface during the masticatory activity, as described above. Based on the aim of the article, which was the methodological overview of the examination of molars, along with the understanding of the different levels of mechanics among them, the author proposed a generally soft diet for the examined population due to the length of striations and the small frequency of pits.

Furthermore, a publication by Horňák *et al.* (2010) included the appliance of Dental Microwear Analysis on the dental remains of 11 individuals from the Early Bronze Age site of Melčice-Lieskové, which is located in Western Slovakia. The dental remains consisted of premolars and molars of both sexes, were examined through a Scanning Electron Microscope, and exhibited the adoption of a mixed diet with a prevalent intake of meat protein. The interpretation was extracted by the authors based on the similarities of the mean values of the measured variables with populations with known diets, such as Inuits, along with the temporal placement of the site, which implies the conduction of agricultural activities from the individuals.

An analysis from Sołtysiak (2011) examined microscopically 4 dental remains from the Early and Middle Bronze Age sites of Tell Ashara and Tell Masaikh, which are located in Euphrates Valley, Syria. Specifically, the analysis underwent on the occlusal surfaces of second molars with a good state of preservation using a Scanning

Electron Microscope at 300X magnification. The observed dental features consisted of striations, punctures, and pits, and implied the systematical use of cereal grinding techniques for the preparation of flour in a temporal point, which is placed centuries after the emergence point of agriculture in the same area.

The appliance of Dental Microwear Texture Analysis by Schmidt *et al.* (2015) examined the enamel surfaces of a variety of agriculturalists and pastoralist groups on a worldwide scale to extract dietary differences among them. Explicitly, the pastoralist groups included populations of the Late Bronze/Early Iron Age transition (Mongolia), while the agriculturalists were placed in the Early and Late Bronze Age (England and Greece), among others. Despite the general similarities in the subsistence strategies of the two groups (dependency both on meat and agricultural products in general), the differences in the values of Complexity (Asfc) and Anisotropy (epLsar) suggested the adoption of distinct dietary means. In this framework, the lower microwear texture values of pastoralists were interpreted through the adoption of a mostly meat-based diet, while the higher Anisotropy (epLsar) measurements of agriculturalists indicated a more abrasive diet depended on cereals and agricultural goods.

An analysis by Sperduti *et al.* (2018) focused on the macroscopic and microscopic examinations of a large dental sample (3014 dental remains from 217 individuals) from the Chalcolithic/ Early Bronze Age cemetery of Gricignano d' Aversa, Italy. The specific study aimed at the detection of possible indications of the conduction of paramasticatory activities in the examined population, such as grooves and striations on the labial surfaces of the anterior dentition. The dental remains were examined through a Scanning Electron Microscope revealing occlusal and para-occlusal grooves. Based on the morphology of the dental features, the authors proposed the use of canines and incisors during activities of fiber manipulation, such as the production of yarn. Noteworthy, the frequency of appearance of these dental features on the enamel surfaces of women in the majority provides similarities for the use of the feeding apparatus in the same context as the Argaric women of the Castellón Alto site, as described above.

Finally, a study by Bas *et al.* (2023) centered on deciduous teeth from the Early Bronze Age site (2050 to 1680 cal. BP) of Franzhausen I, Austria, applying Dental

Microwear Texture Analysis on the occlusal surfaces of 8 individuals. The specific site presents an impressive human record of 757 individuals in total with a continuous use of the cemetery for more than 3 centuries. The results were characterized by high Complexity levels (Asfc) and low Anisotropy ones (epLsar), implying the adoption of a coarse diet generally, which included harder elements, as a result of the food properties or/and of the possible existence of grit on it. Noteworthy, the non-existence of an important level of dental wear from a macroscopic view implies a small consumption of cariogenic goods, but a more abrasive diet than the ones of posterior periods.

5) Materials and Methods

The specific Master's Thesis is laid in the conduction of Dental Microwear Analysis on dental remains from the Late Prehistory of the Catalonia (Northeastern Iberian Peninsula). In this framework, we examined 145 dental remains from 26 individuals from the sites of Galls Carboners, El Bordellet, Els Pujols, and Porroig (Table 1). The dental samples belong in their vast majority to anterior dentition ($n= 141$), while we included both a premolar ($n= 1$) and molars ($n= 4$).

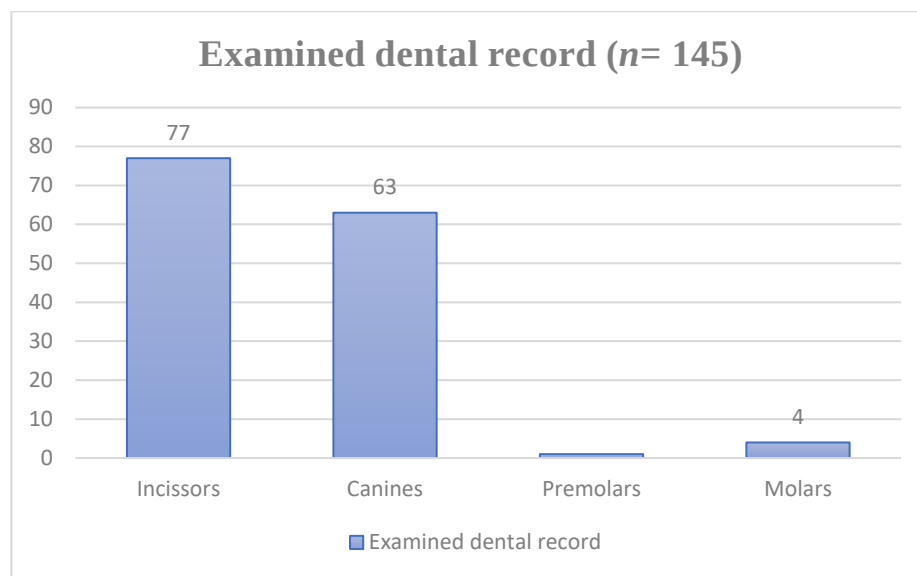


Table 1: The microscopically observed dental record.

In the case of Galls Carboners, in which the examined dental remains were recovered separately, loose from the mandibles and maxillas, the identification of the Minimum Number of Individuals (MNI) of the examined sample pool from the specific site underwent through the combination of the methodological frameworks of AlQahtani *et al.* (2010), along with the identification of the prevalent tooth, and the stratigraphic sequence when needed. The specific methodological step was decided due to the recovery of the human remains of the site in a conglomerate context due to its use for *postmortem* depositions of the individuals (Vergès *et al.*, 2017). On the contrary, the recovered individuals in the sites of El Bordellet, Els Pujols, and Porroig were found in funerary structures, thus making direct the attribution of the teeth to each individual (Esqué Ballesta, 2015). In this framework, the study of AlQahtani *et al.* (2010) provided a decisive approach to understanding the age of the individual concerning the examination of deciduous dentition based on the level of root resorption.

However, it should be noted that the MNI represents the lowest approximation for the number of people, who contributed to the sample, thus providing a relevant number generally (Adams and Byrd, 2008).

5.1) Casting Procedures

The appliance of Dental Microwear Analysis is facilitated by the production of replicas from the original dental remains, which provide precise imprints of the dental features on the examined tooth (Galbany *et al.*, 2006). The existence of already produced molds on the dental remains from the sites of El Bordellet, Porroig, and Els Pujols by Dra. Marina Lozano accelerated the casting procedures, thus requiring the production of casts to be able to continue the examinations macro- and microscopically (Figure 3). Notably, the used substance for the production of the molds is Polyvinylsiloxane, which is widely methodologically accepted and applied in the related disciplines to dental remains and dentistry (Mihlbachler *et al.*, 2019; Singer *et al.*, 2022).



Figure 2: The two catalysts (a) and the preparation of the epoxy resin (b), which permitted the production of high-resolution casts from the already produced molds. Photographs taken by: Marina Lozano.

The production of dental casts from the already existing dental molds underwent in the Biomarkers Laboratory of the Institut Català de Palaeoecologia Humana i Evolució Social (IPHES-CERCA) in Tarragona, Spain. For this purpose, we used an epoxy resin (Soloplast® R123 and R614 Hardener) to produce replicas of the examined dental facets. The use of epoxy resin for this specific purpose provides the possibility for the obtainment of a precise representation of the desired dental features, while it provides durable properties to the replica (Galbany *et al.*, 2004). In this context, the methodological framework predicts the mixing of the two components unevenly (70% Soloplast® R123-30% R614 Hardener) and the subsequent pouring of the resin on the molds. The process terminates around 48 hours later with the solidification and extraction of the dental casts.

5.2) Microscopic Observations

The methodological approach, which is followed on the conduction of the Dental Microwear Analysis in this Master's Thesis, is based on the utilization and combination of different microscopic approaches to extract the best possible optic on the quantitative and qualitative dental features on the examined enamel surfaces. In this framework, the described analyses aim in the understanding of the use of different microscopic applications, that can be combined and complement the archaeological information and interpretation, along with the understanding of the level of complementation among them.

The first microscopic observations are undergone through the use of an Optical Microscope (ZEISS Aixoscope A1 metallographic microscope), followed by a 3D Digital Microscope (Hirox KH8700). Explicitly, the observation of samples in an optical microscope of reflected light facilitates the detection and observation of the dental features on the enamel surface preliminarily, while the properties of a 3D digital microscope provide possibilities regarding the quantification of the features automatically, along with the digitalization of the data. The Optical Microscope (ZEISS Aixoscope A1 metallographic microscope) and the 3D Digital Microscope (Hirox KH8700) are both located in the Laboratory of Lithics in the Institut Català de Palaeoecologia Humana i Evolució Social (IPHES-CERCA), while the Environmental

Scanning Electron Microscope (Fei Quanta 600) is located in the Scientific and Technical Resources Service of the Universitat Rovira i Virgili (Tarragona, Spain).

During the examination of the dental remains, we followed the corresponding methodological framework to each microscope, setting as an aim the detection of the same dental view to be able to extract information on the dental features holistically. For this reason, we applied magnifications, whose measurements could be correlated with each other, thus permitting the direct comparison of the examined dental features and the extraction of pieces of information concerning the utilization of different microscopic equipment on the same dental view (Borel *et al.*, 2014).

Based on the properties of each microscope, its suitability in the discipline of Dental Microwear, and its accessibility for use, we hierarchized the microscopic observations differently. All 145 teeth were first examined using the ZEISS Aixoscope A1 metallographic microscope prior to the further use of the other two microscopic equipments. The specific choice was made based on the precise obtainment of a clear view regarding the existence or not of dental features, which could imply dietary habits, along with the detection of distorted dental views based on taphonomic factors or due to molding problems. As mentioned above, the use of the specific microscope in the studies of wear has been gaining ground in recent years, thus providing a reliable and accessible alternative (Hernando *et al.*, 2020a; Hernando *et al.*, 2024, for instance). The observations underwent through a preliminary magnification of x50 to obtain the first idea of the dental views, followed by a magnification of x100 on the points of interest.

The second part of the microscopic observations was performed using a 3D Digital Microscope (Hirox KH8700) on the dental remains, which exhibited dietary and behavioral dental features throughout the use of the ZEISS Aixoscope A1 metallographic microscope. The specific microscope has already proven to provide insights regarding quantitative approaches to dental wear of small mammals and identification of taphonomic features, among others (Courtenay *et al.*, 2019; Bañuls-Cardona *et al.*, 2022, for instance). In the specific study, we intended to find the exact dental views, that presented microwear patterns in the preliminary stage, thus providing a direct comparison between the two microscopes; moreover, we utilized its digital properties to examine aspects, that couldn't be undergone with the Optical

microscope, such as the quantification of the perimeter and the area of the non-alimentary dental features on the dental surfaces. In this context, we applied different magnifications, from x35 until x140, to obtain a varied view of the dental features, utilizing the different microscopic applications of the specific equipment for the extraction of the finest interpretation of our materials.

5.3) Image Processing and Quantification of the Dental Features

The obtainment of a further understanding concerning the level of complementation among two different microscopic equipments is heavily dependent on both the acquisition of the same dental view, along with the quantification of the dental features on images, whose measurements can be directly compared. The described necessity derives from the existence of different fields of view on each microscope based on each manufacturer's properties, which in turn sets in the first place the correct image processing and adjustment based on each microscope (Martín-Viveros and Ollé, 2020).

In the first place, we used an open-access software (GIMP - <https://gimp.es>) to maximize the efficiency of the quality of the obtained photos (Galbany *et al.*, 2004). The methodological framework regarding the quantification of features related to dental wear predicts the cropping of the obtained image on an area of 0.56 mm² (Pérez-Pérez *et al.*, 1994), which we acquired using the Adobe Photoshop software.

The mentioned quantification was undergone with the use of an open-access software (ImageJ). Noteworthy, the specific software has been already used in a variety of studies concerning the examination of dental wear patterns (Hernando *et al.*, 2019; Petraru *et al.*, 2022, for instance), and generally in the fields of Dental Anthropology (Modesto-Mata *et al.*, 2020), and Life Sciences (Abràmoff *et al.*, 2004) based on its abundance of possibilities and preciseness. In the context of the specific Master's Thesis, we used the mentioned program for the obtainment of the total number of striations (TN), the average length of striations (X, measured in µm), and the orientation, following the framework of Lalueza Fox and Pérez-Pérez (1993) (Figure 3). Based on the methodological frameworks of Lalueza Fox and Pérez-Pérez (1993) and Pérez-Pérez *et al.* (1994), which enriched the existing methodology of Puech *et*

al. (1980), the striations are categorized into 4 different orientations based on their inclination.

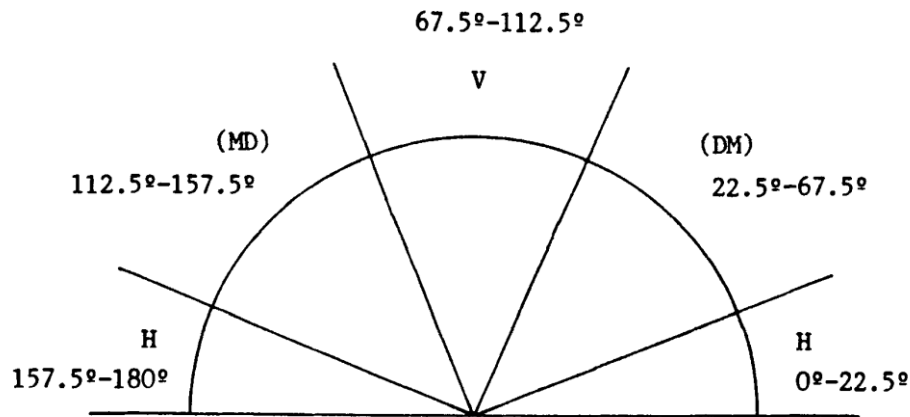


Figure 3: Diagram illustrating the 4 directions for the quantification of dietary striations on a left lower molar. H: horizontal, DM: disto-occlusal to mesio-cervical, V: vertical, and MD: mesio-occlusal to disto-cervical (Lalueza Fox and Pérez-Pérez, 1993).

Despite the establishment of the specific framework in studies of posterior dentition, we decided to follow the same method in our study since it provides the most stable ground during the quantification process of the dietary dental features. Explicitly, the striations are characterized either as horizontal (0° – 22.5° or 157.5° – 180°), disto-occlusal to mesio-cervical (22.5° – 67.5°), vertical (67.5° – 112.5°) or mesio-occlusal to disto-cervical (112.5° – 157.5°). It should be noted that the disto-occlusal to mesio-cervical and the mesio-occlusal to disto-cervical orientations are dependent on the position of the dental remain on the mandibular or the maxillary arch since the figure above describes the orientation of striations of a right lower molar (Hernando *et al.*, 2020a).

5.4) Data Analysis

The final step of the methodological framework was the conduction of statistical analysis on the obtained data, which were derived during the quantification processes. In the framework of the specific Master's Thesis, we selected specific descriptive

statistics to organize and summarize the information and to set the focus on variables, which can facilitate the extraction of answers in the discipline of Dental Microwear Analysis (Garrison, 2018). For this reason, we selected TN (Total Number of Striations), X (average striation's length, in μm), and their orientations. Finally, we calculated two indexes: The number of horizontal striations/ total number of striations (NH/TN), and the number of vertical striations/ total number of striations (NV/TN) for a better understanding of the relative frequencies among the studied groups (Lalueza *et al.*, 1996).

In the second step, we created different Box-and-Whisker Plots (Box Plots), setting as variables the Total Number of striations (TN) and the Average Length (X, in μm) to compare directly both the average number of striations among the studied groups, along with possible differences in values between the individuals of the same group, thus providing insights for possible intra-group dietary variability. In general, the use of the specific plots aims to compare between batches of data, describing properly their distribution (Banning, 2020), and contributing to the visualization of their statistical values (Krzywinski, 2014). Aiming to transfuse certainty to our results, we applied the Independent t-test to the results of different variables from the groups of El Bordellet and Galls Carboners, such as TN, XT, NV/TN, and NH/TN. The specific statistical test is considered ideal for comparing two different groups and by extension their distribution, based on the differences between their means (Kim, 2015). In the specific point of the study, the selected Null hypothesis was set as the non-existence of statistically significant differences among the different variables of the two selected groups.

Furthermore, we compared the values of different variables, which we obtained from the two microscopes (ZEISS Axioscope A1 Microscope and Hirox KH8700 3D Digital Microscope) to understand the level of complementation among the two equipments. Explicitly, we selected the total means of TN, DM, MD, V, H, NH/TN, and NV/TN from the dental remains of each site respectively, and we measured the Mean Error, the Mean Absolute Error (Figure 4), and the percentage (%) of increase. Considering the small sample's size ($n=16$), we applied a non-parametric statistical test (U Mann Whitney) to understand the level of inter-microscopic variability on the values of different variables, which were obtained from both microscopes. We measured Pearson's correlation coefficient (r) to understand and evaluate the

correlation between the values recorded by the Optical Microscope and the 3D Digital Hirox Microscope on each variable respectively, since we had to deal with two dependent variables (Van Pool and Leonard, 2010). Aiming to understand the representation of the distribution of the striations on the enamel surfaces, we created two scatter plots (NV/TN to NH/TN) based on the measurements of each microscope. Finally, we applied the Bland-Altman Analysis setting as a variable the Total Number of Striations (TN) to understand the level of agreement between the two microscopes; the specific type of graphic representation aims in the plotting of different measurements with a setting of a limit of 1.96% obtained from the values of the Standard Deviation (Bland and Altman, 1999). The specific statistical test underwent using the DATAtab Statistic Calculator.

$$\text{MAE} = \frac{1}{n} \sum_{i=1}^n |x_i - x|$$

Figure 4: The mathematical formula for the extraction of the Mean Absolute Error (MAE).

The final step of the Data Analysis underwent in the dental remains, which depicted an indication of the conduction of paramasticatory activity. We measured Pearson's r Correlation Coefficient in order to understand the relationship between the two variables. Finally, we calculated the Coefficient of Variation and conducted a Bivariate Regression Analysis to understand the relationship between the chipped area and the perimeter, since the apprehension of the relationship between the two variables (Chipped Area and Perimeter) can potentially provide insights into similarities of the values among our sample pool (Weisburd *et al.*, 2021).

6) Results

6.1) Casting Procedures and Methodological Issues

The use of Polyvinylsiloxane has been already widely used in the disciplines related to the understanding of wear on an artifact or dental remains (Ollé and Vergès, 2013; Rivals *et al.*, 2024, for instance). However, it should be noted that the specific methodological step is dependent on several factors, such as issues of improper mixing, incorrect use of the proportions of the components, or variations in the temperature of the laboratory, which can be mostly noted by the existence of air bubbles in the casts (Asryan and Rots, 2024). In the framework of the specific Master's Thesis, all the dental remains underwent a preliminary observation through the Optical Microscope to detect possible facets with interest regarding alimentary purposes, resulting inevitably in the exclusion of the ones affected by casting problems. The produced casting procedures were successful in their majority, thus permitting the production of high-quality casts from the existing molds. However, we also encountered dental casts, whose level of casting didn't permit further microscopic observations, thus leading to their exclusion from further observations (Figure 5).

From a methodological point of view, the biggest level of challenge was encountered in the detection of the same dental view on the two different microscopes (ZEISS Axioscope A1 Microscope and Hirox KH 8700 3D Digital Microscope). The existence of different properties between the two pieces of equipment (such as different field ranges, different types of light, etc.), along with the peculiarities of each material (original tooth and cast) made the process demanding and more time-consuming. However, we managed to detect precisely all correspondent facets (Figure 6) while the appliance of an x140 magnification on Hirox KH8700 permitted the direct comparison of the different quantified variables between the two microscopes based on the framework of Martin-Viveros and Ollé (2020) concerning the selected dental remains (Supplementary Material 1, page 110).

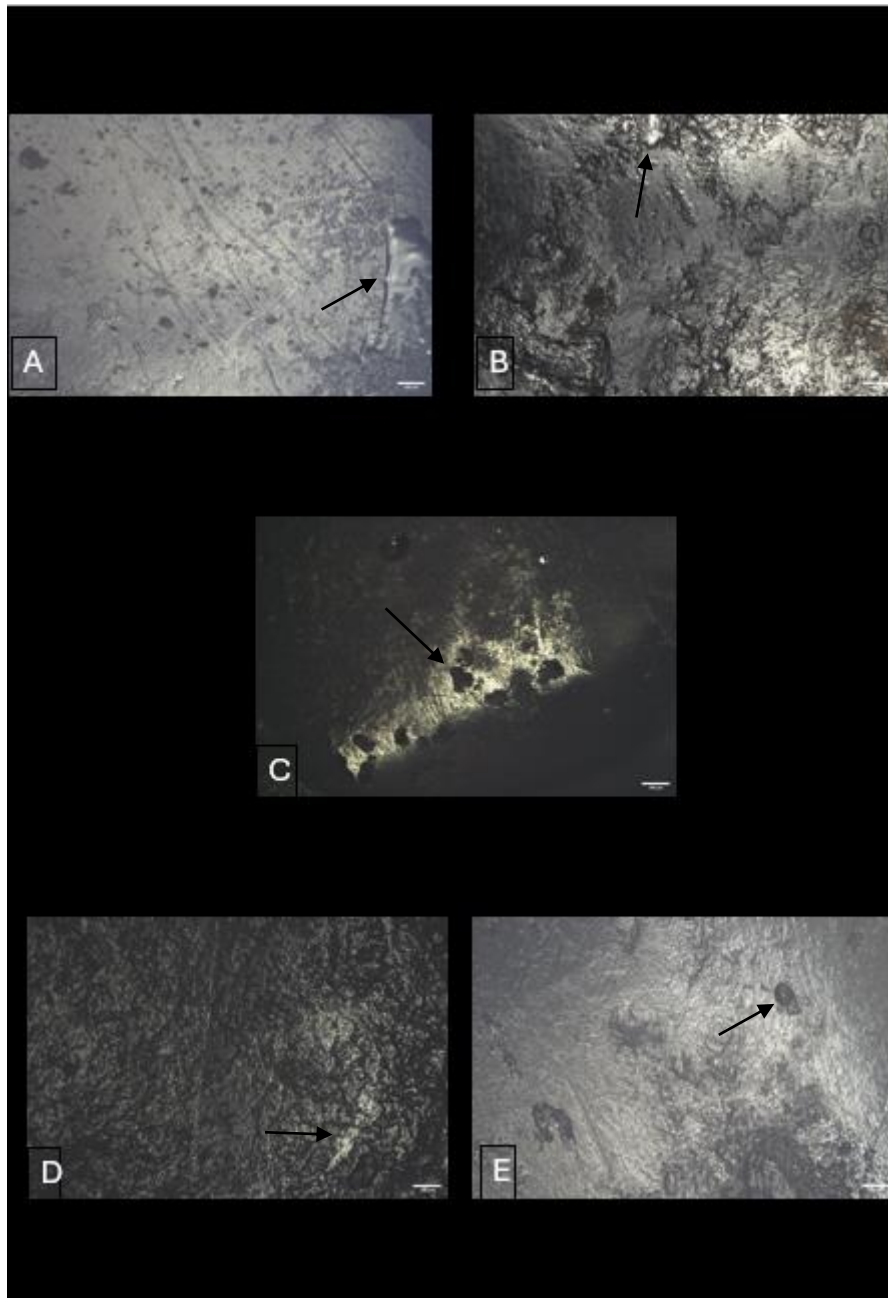


Figure 5: Dental surfaces with molding problems (scale: 100 μm). The arrows demonstrate evidence of insufficient molding, which conceals the information concerning the dental wear patterns.

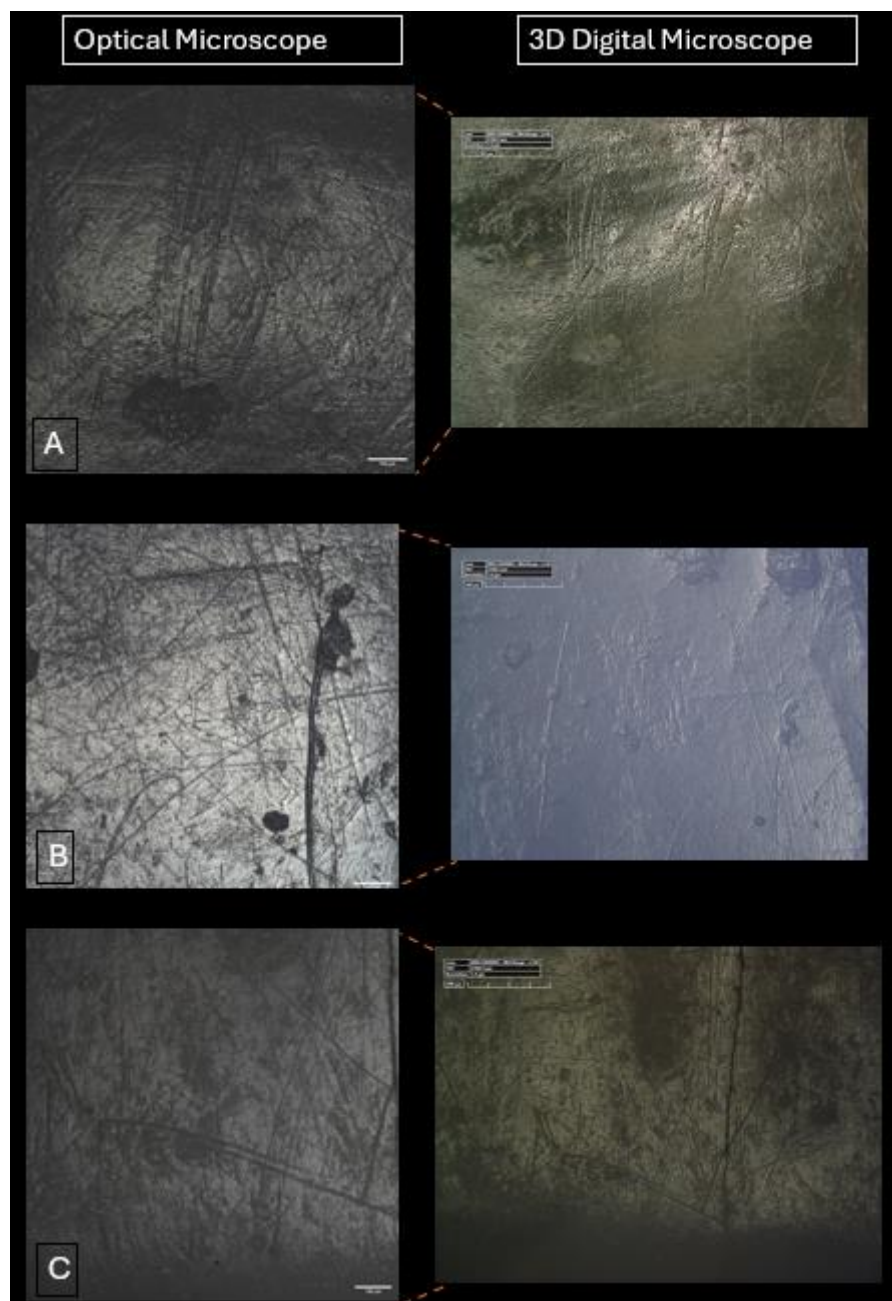


Figure 6: Examples of the finding of the exact dental surface on two different microscopes among our samples (A: El Bordellet 7, B: Els Pujols, C: Galls Carboners 1.98-118; Scale at the Optical Microscope: 100 μm ; Scale at the 3D Digital Microscope: 140 μm).

6.2) Paleodietary Reconstruction

From the 145 microscopically observed dental remains of 26 individuals, we qualified 16 for the extraction of palaeodietary information based on their microwear signatures. Moreover, considering that each of the dental remains corresponds to a different individual, we managed to extract dietary features from the majority of the available sample pool ($16/26 = 61.53\%$ of the individuals). However, the representation of two of the examined sites (Porroig and Els Pujols) by just one individual doesn't permit the extraction of pieces of information regarding the total population of the site but contributes to the further understanding of the general trends concerning the Bronze Age's diets on the Northeastern part of the Iberian Peninsula.

Variable	El Bordellet	Galls Carboners	Porroig	Els Pujols
Sample Size (n)	7	7	1	1
TN	111.28 ± 12.64	113.13 ± 14.78	96	110.66
XT (in μm)	120.78 ± 12.36	119.35 ± 8.46	114.02	147.9
NV/TN	0.46 ± 0.14	0.50 ± 0.06	0.53	0.39
NH/TN	0.13 ± 0.05	0.10 ± 0.03	0.07	0.19

Table 2: Descriptive statistics ($\pm$: Standard Deviation) on the results of the different groups based on a number of variables (TN: Total Number of Striations, XT: Average Length (in μm), NV/TN: Number of Vertical Striations to Total Number of Striations, NH/TN: Number of Horizontal Striations to Total Number of Striations).

The efforts for the understanding and reconstruction of the paleodiets of the groups underwent through the quantification of the dietary features on the enamel surfaces and the application of descriptive statistics based on a different number of variables (Table 2). For this purpose, we used the photos obtained from the ZEISS Axioscope A1 Microscope, since its utilization in the discipline of Dental Microwear Analysis has already been well known, as explained before, thus providing reliable results.

Notably, focusing the interest on the labial and buccal facets of our samples consequently leads to the acquirement of values regarding exclusively the striations, since it is the studied feature concerning the specific dental views (Pinilla Perez *et al.*, 2011).

The total number of striations (TN) of El Bordellet ranges between 111.28 ± 12.64 , while the one of Galls Carboners ranges between 113.13 ± 14.78 , thus providing very similar values. Similarly, the individual from Els Pujols represents an average TN of 110.66, while the buccal signature of the infant from Porroig demonstrates an average total number of striations of 96 (Figure 7).

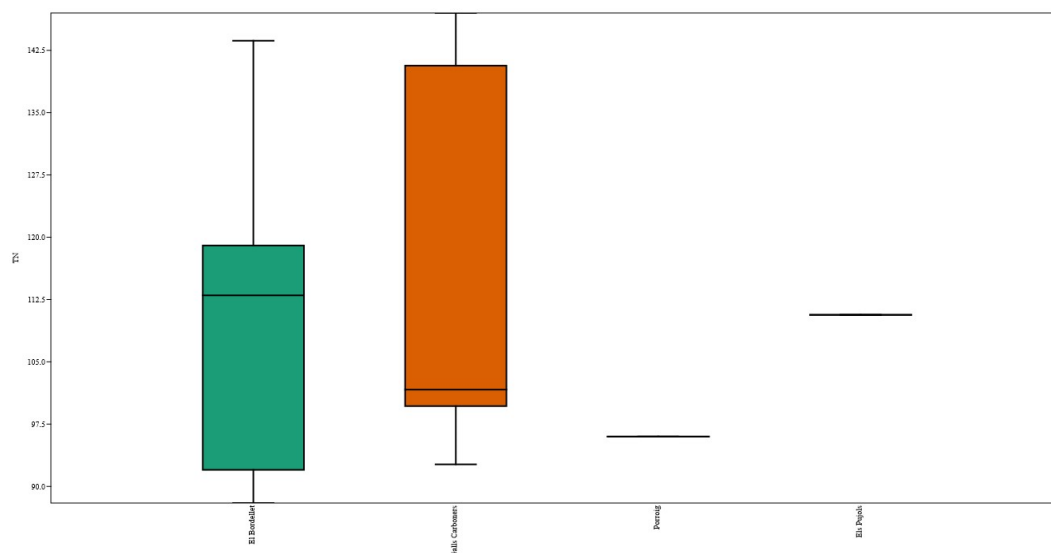


Figure 7: Boxplot representing the values concerning the Total Number of Observed Striations (TN) on the different sites.

The average length of striations (X , in μm) of El Bordellet's individuals ranges between $120.78 \pm 12.36 \mu\text{m}$, depicting similar values with the studied dental remains of Galls Carboners ($119.35 \pm 8.46 \mu\text{m}$). The specific variable contributes to the discipline of Dental Microwear for the addition of preciseness concerning the distinction of different types of diet (Grine, 1986; Teafor and Oyen, 1989b, for instance). In the same context, the buccal view of the deciduous molar from Porroig depicts an average length of striations of $114.02 \mu\text{m}$, while the individual of Els Pujols is characterized by higher values concerning the specific variable ($147.9 \mu\text{m}$) (Figure 8).

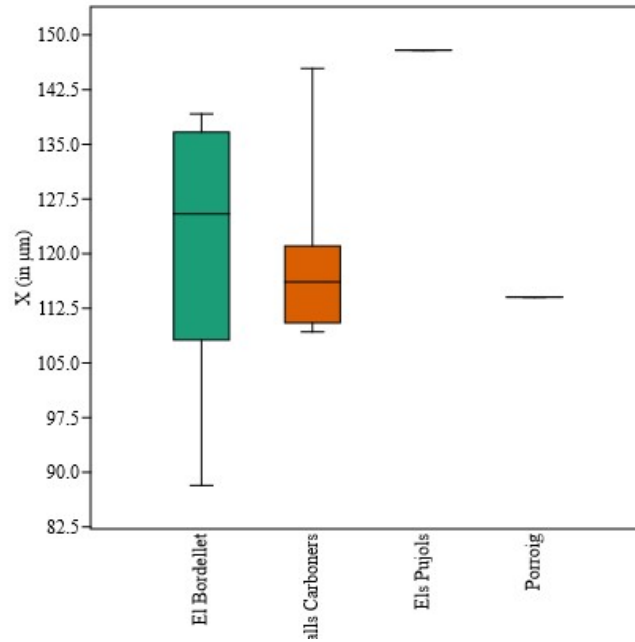


Figure 8: Boxplot representing the values concerning the Average Length of Observed Striations (X, in μm) on the different sites.

Both variables of NV/TN (Number of Vertical Striations to Total Number of Striations) and NH/TN (Number of Horizontal Striations to Total Number of Striations) contribute to the understanding of the distribution of striations among the examined surface (Lalueza Fox *et al.*, 1996). Concerning the former (NV/TN), the samples from the El Bordellet site demonstrate a value of 0.46 ± 0.14 , which is smaller than the one of the dental remains from Galls Carboners (0.50 ± 0.06) and Porroig (0.53), but bigger than the one of the individual from Els Pujols (0.39). At the same time, the NH/TN values of the El Bordellet site range between 0.13 ± 0.05 , which is a slightly bigger number than the one of Galls Carboners site (0.10 ± 0.03) and Porroig (0.07), but smaller than the one of Els Pujols (0.19). The specific values demonstrate the existence of a higher variability concerning the distribution of striations among the dental surfaces of the individuals from the El Bordellet site based on the big ranges of standard deviation when compared with the relevant values from the other examined sites.

Aiming to understand further the existence or absence of significant differences among the results of different variables from our samples, we applied an independent

t-test to understand the distribution of our values (Table 3). Explicitly, the results of different labial microwear variables (TN, XT, NV/TN, and NH/TN) from the two bigger groups of our sample pool (El Bordellet and Galls Carboners sites) depicted statistically insignificant differences (all critical t values lower than the t values) indicating similarities among the enamel properties of the two groups.

Variable	Test	Critical t value	t value	p value
TN	Independent t-test	2.178	0.141	0.889
XT	Independent t-test	2.178	0.029	0.865
NV/TN	Independent t-test	2.178	0.511	0.618
NH/TN	Independent t-test	2.178	0.974	0.348

Table 3: Results of the Independent t-test concerning intragroup differences (El Bordellet and Galls Carboners) based on different labial microwear variables.

6.3) Testing the inter-microscopic variability

Aiming to understand the level of complementation among the two used pieces of microscopic equipment (ZEISS Axioscope A1 Microscope and Hirox KH8700 3D Digital Microscope) we compared several values, which were obtained from the quantification of the same parts of the dental surfaces (Table 4).

Comparing the Total Number of Striations (TN), the Hirox KH8700 3D Digital Microscope presents bigger values than the Optical Microscope (129.66 to 111.24) with a 14.21% Mean Increase and a 14.67% increase in the Mean Absolute Error. The existence of a non-normal distribution among the two pieces of equipment ($U = 61.5$; $p = 0.004$) can be explained due to the properties of the former, which potentially doesn't permit a precise distinction between the taphonomic features and the ones correlated to masticatory processes. However, the existence of a strong correlation ($r = 0.728$) among the values of the two microscopes underlines the possibility of the

Hirox KH8700 3D Digital Microscope to detect dental features on enamel surfaces precisely.

Variable	Total Mean OM	Total Mean Hirox	Mean Error	Mean Absolute Error	% Mean Increase	% MAE	Test U Mann Whitney	Pearsons's r
TN	111.24	129.66	18.42	19.12	14.21%	14.67%	U= 61.5; p= 0.004	0.728; p < 0.000
DM	19.50	20.83	1.33	7.72	6.39%	28.36%	U= 128.5; p= 0.593	-0.491; p= 0.045
MD	23.08	22.49	0.59	6.73	2.62%	22.58%	U= 131.5; p= 0.666	0.540; p= 0.025
V	55.06	75.27	20.21	21.54	26.84%	28.12%	U= 66; p= 0.007	0.788; p< 0.000
H	13.60	11.07	2.53	5.75	22.97%	29.72%	U= 122.5; p= 0.458	0.378; p= 0.133
NH/TN	0.12	0.08	0.04	0.05	50%	71.43%	U= 101; p= 0.137	0.511; p= 0.035
NV/TN	0.47	0.57	0.10	0.11	17.54%	18.97%	U= 88.5; p= 0.055	0.649; p= 0.004

Table 4: Results of different variables obtained from the quantification of the same dental surfaces on our samples using different pieces of microscopic equipment (OM: ZEISS Axioscope A1 Microscope; Hirox: Hirox KH8700 3D Digital Microscope). The **bold** fields indicate a non-normal distribution among the compared values.

The Total Number of disto-occlusal to mesio-cervical striations (DM) present similar values among the 2 microscopes; explicitly, the Optical Microscope demonstrates an

average of 19.50, while the average value for the same variable on the 3D Digital Microscope is 20.83 (Mean Increase: 6.39 %), both being not statistically significant distributed among them ($U= 128.5$; $p= 0.593$). Notably, despite having a negative value on the correlation (r), the relationship between the two microscopes can only be interpreted as weak (Coefficient of Determination= $r^2= 0.241$), since the nearer to the 0 a value is placed, the weaker the relationship is among two groups of measurements.

Furthermore, the Total Number of mesio-occlusal to disto-cervical striations (MD) and the Total Number of Horizontal striations (H), both variables present bigger values on the ZEISS Axioscope A1 Microscope than the ones of the Hirox KH8700 Digital Microscope (MD: 23.08 to 22.49; H: 13.60 to 11.06) with a Mean Increase of 2.62 % for the MD and 22.97 % for the H. Interestingly, in both variables the values are not statistically significant (both p values ≥ 0.05), while both are characterized by positive correlations (MD's $r= 0.540$; H's $r= 0.378$). This element indicates the ability of the digital microscope to detect similarly to the Optical Microscope the striations of the specific orientations since a positive correlation indicates consistency among the tendency to document in both microscopes related values.

The biggest difference concerning the values of the Total Number of striations derives from the vertical ones (V : 67.5° to 112.5°). Explicitly, the Optical Microscope presents a Mean Value of 55.06, while the 3D Digital one has a value of 75.26 (26.84 % Increase). As a consequence of the specific difference among the results, there is a non-normal distribution among the values ($U= 66$; $p= 0.007$). However, there is a strong positive correlation between the values of the two microscopes ($r= 0.788$), indicating the consistency of detecting the vertical striations in both microscopes.

As it has been mentioned above, the values of NH/TN (Number of Horizontal Striations to Total Number of Striations) and NV/TN (Number of Vertical Striations to Total Number of Striations) are two ratios, which provide pieces of information concerning the distribution of the striations on the examined area of the enamel surface. In the framework of the specific Master's Thesis, we measured both indexes concerning each microscope respectively. Explicitly, the Optical Microscope presents higher values concerning the former (NH/TN) with 0.12 compared with the 0.08 (50 % Mean Increase) of the Digital Microscope with the values being normally distributed ($U= 101$; $p= 0.137$) and importantly correlated (0.511 ; $p= 0.035$). On the

other side, the Total Mean of NV/TN in the Digital Microscope is bigger, 0.57 compared to 0.47 in the Optical Microscope (17.54 % Mean Increase). Notably, in the specific case, the values are slightly normally distributed ($U= 88.5$; $p= 0.055$), while a strong correlation is noticed (0.649 ; $p= 0.004$) concerning the specific ratio among the two microscopes.

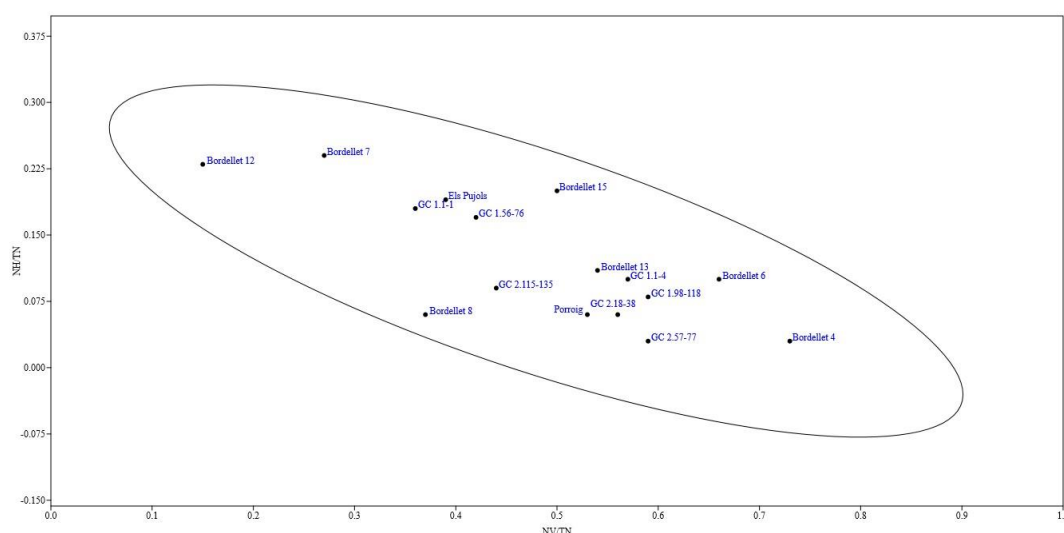


Figure 9a: Scatter plot of the obtained NV/TN to NH/TN values on the Optical Microscope. Each black dot represents a dental remain, while the black line represents the set concentration ellipse (95 %).

Aiming to understand better the distribution of the obtained values from the two microscopes concerning each examined dental remain, we created two different scatter plots with a concentration ellipse of 95 %. The specific type of plot is considered ideal for the depiction of relative frequencies, such as NV/TN and NH/TN, and their values are placed against each other (García-González *et al.*, 2015; Hernando *et al.*, 2020a, for instance).

When comparing the scatter plot obtained by the values of the Optical Microscope (Figure 9a) with the one obtained by the values of the 3D Digital Microscope (Figure 9b), a big change can't be observed directly. The most important one reflects the wider distribution of the values among the teeth observed with the Optical Microscope. However, the specific result confirms the normality of the distribution among both of the values obtained by the two microscopes, as explained above, along

with the strong correlations, that exist in both variables (NV/TN and NH/TN), concerning the finding of similar values among the two equipments.

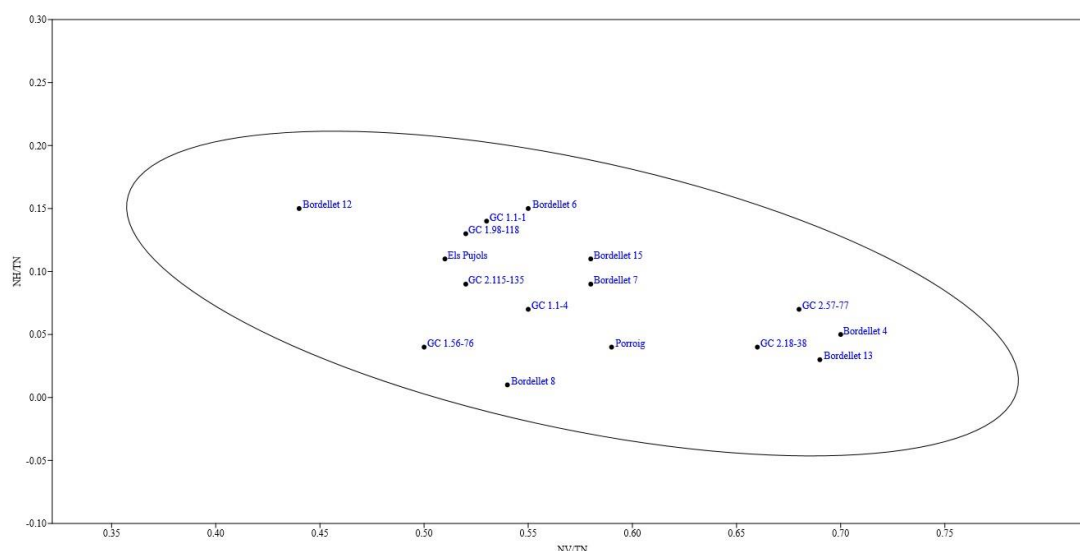


Figure 9b: Scatter plot of the obtained NV/TN to NH/TN values on the 3D Digital Microscope. Each black dot represents a dental remain, while the black circle represents the set concentration ellipse (95 %).

The last step for the understanding of the microscopic variability was the conduction of a Bland-Altman plot to further understand the differences in the measurements between the two pieces of equipment. In the specific point, we set as a variable the Total Number of Striations (TN) to investigate the level of agreement among the obtained values (Figure 9). In this case, the application of Bland-Altman analysis occurs from the set of a bias of -18.42 (Mean Error between the TN values from both microscopes), a bias of 13.47 as a Standard Error (1.96 SE with a 95% Confidence Interval), an Upper-Level Agreement of 8.54, and a Lower Level Agreement of -45.37. The graph below (Figure 10) depicts all the obtained values concerning the variable of TN concerning the two microscopes. Notably, all the values lie inside the 95 % interval (Upper Limit: 8.54; Lower Limit: -45.37), while half (8/16= 50 %) of the measurements lie between the limits of the Standard Error (-11.68 to -25.15), which confirms the consistency of the 3D Digital Microscope to detect the striations on a similar way with the Optical Microscope.

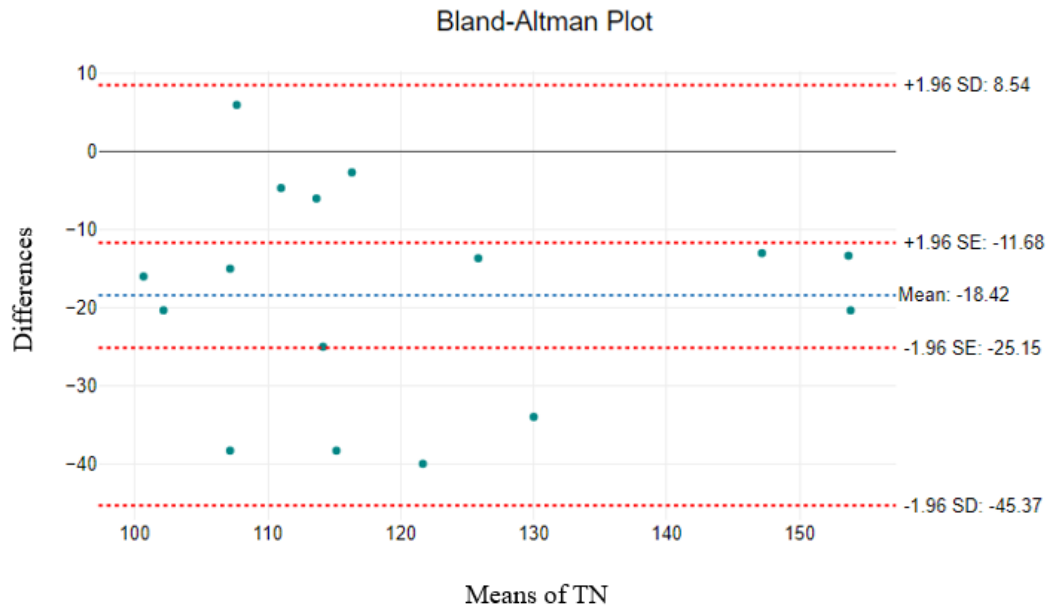


Figure 10: Graphic representation of a Bland-Altman Plot concerning the values obtained from the two pieces of microscopic equipment concerning the variable of the Total Number of Striations (TN).

6.4) Understanding the behavioral level of the studied populations

The macro- and microscopic observations revealed 17 teeth, which were characterized by indications of paramasticatory activity (enamel chipping) (Supplementary Material 2, page 111). Antemortem dental chipping is the result of a tooth coming into contact with a solid/hard object with sufficient force to break the enamel (Chai and Lawn, 2007). In the appliance of Dental Microwear Analysis, such patterns are usually encountered on the anterior dentition (Lozano *et al.*, 2017), and mostly indicate the conduction of tearing or shearing of an object with the use of the mouth (Fiorenza *et al.*, 2011; Krueger, 2015).

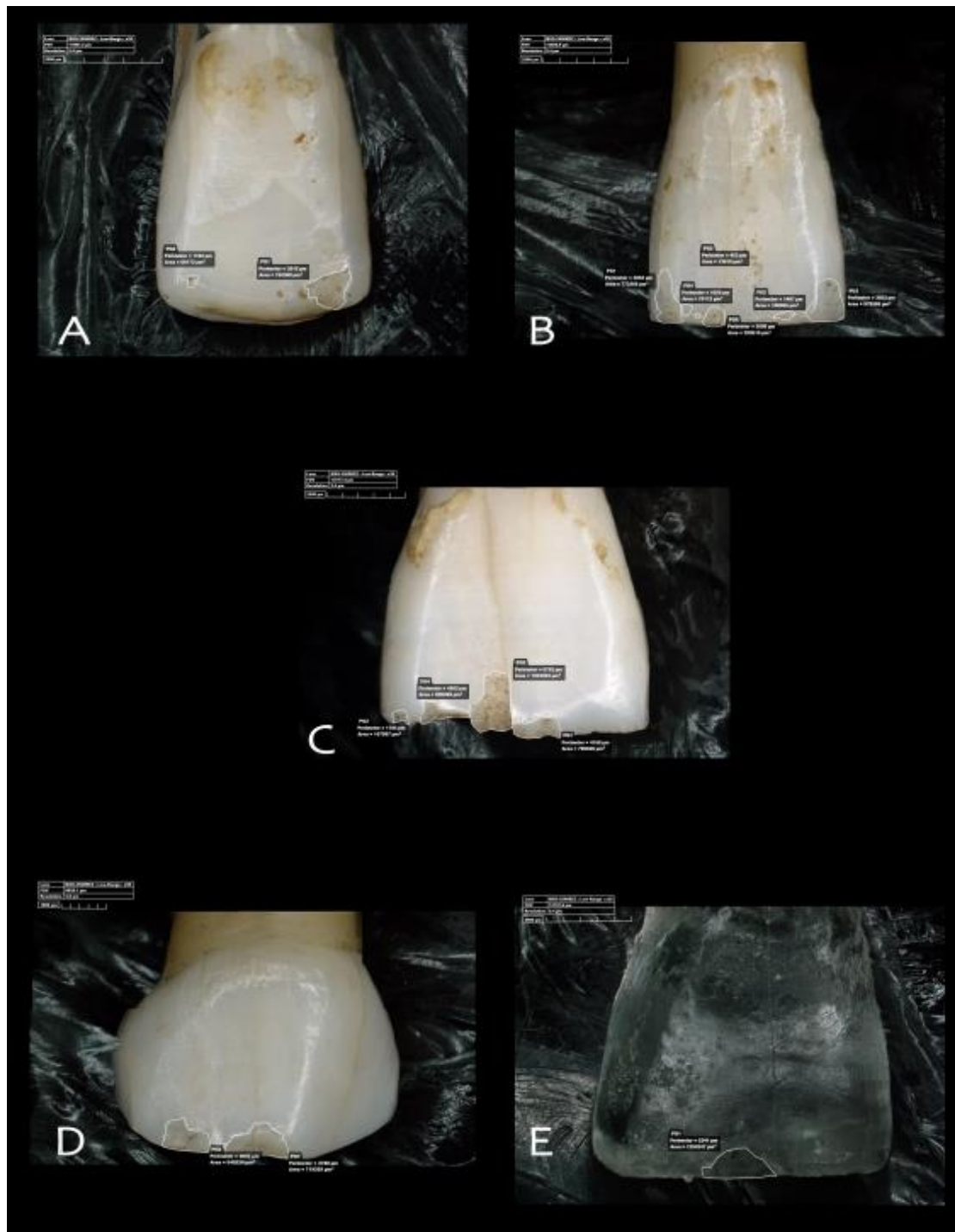


Figure 11: Detection and quantification of the chipped areas among our samples using the KH 8700 3D Digital Microscope (A: Galls Carboners 2.20-40; B: Galls Carboners 2.38-58; C: Galls Carboners 1.3-15; D: Galls Carboners 2.115-135; E: B10E5-2; Scale: 2500 μm).

From the obtained data (Supplementary Material 3, page 112), we measured different descriptive statistics based on the measurements of the area and perimeter of each one (Figure 11). Explicitly, we measured the Mean (M) and the Standard Deviation (SD) to find the Coefficient of Variation (CV) among the different dental remains ($CV=SD/M$). The usefulness of the specific measurement is based on the understanding of proportional changes in variables, thus permitting a further understanding concerning their properties (Pélabon *et al.*, 2020). An important level of Coefficient of Variance regarding the area (0.92) was expected due to the examination of both incisors and canines and the deciduous and permanent dentition of both sexes; notably, the value for the same measurement concerning the chipped enamel surface is much smaller (0.45), implying further implications (Table 5).

Measurement	Perimeter	Area
Mean	9614.706	2332367
Standard Deviation (SD)	4346.92	2165313
Coefficient of Variance (CV)	0.45	0.92

Table 5: Results of different measurements on the variables of perimeter and area on the enamel chipped areas.

The results of Pearson's r correlation test between the perimeter and the chipped area presented a strong correlation (0.695) between the two variables; furthermore, the existence of outliers on the plot of the Bivariate Regression can be explained due to different factors, such as repetitive use of the tooth during the activity, non-standardization of the practice among the populations, or/and different techniques on some individuals (Figure 12). This can be the explanation for the placement of 4 of the examined dental remains ($4/17= 23.52\%$) outside the 95 % confidence interval in our plot.

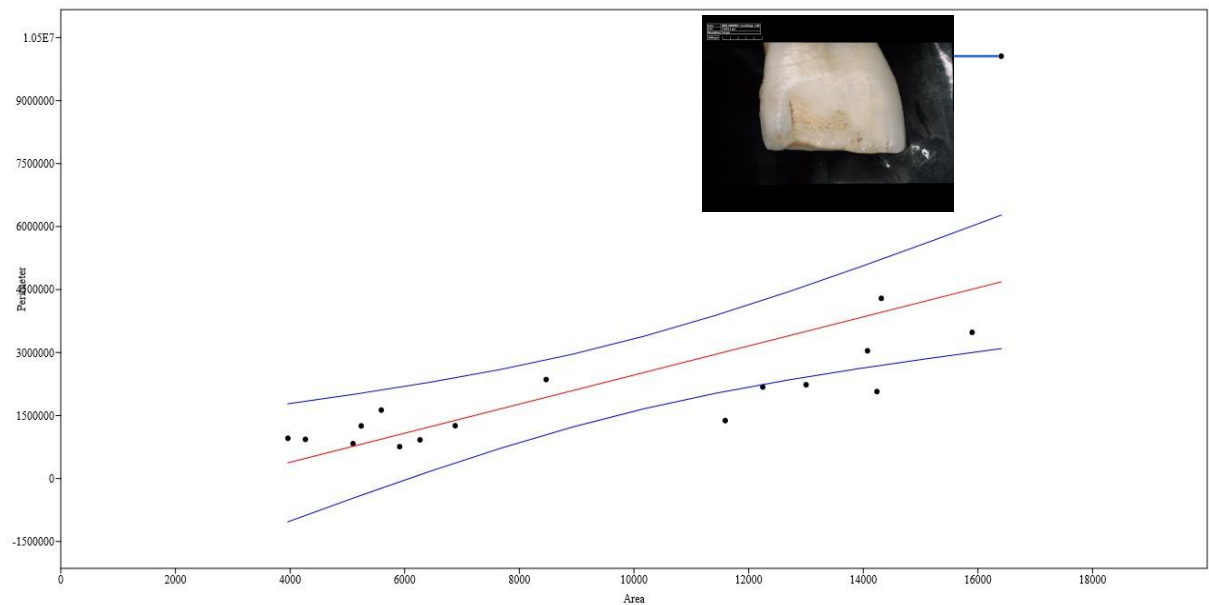


Figure 12: Plot of a Bivariate Regression concerning the Area and the Perimeter of the chipped parts of the enamel on the examined dental remains with a set of 95 % confidence intervals (blue lines). The photo represents the most heavily chipped tooth of our sample pool, Galls Carboners 1.2-12 (Scale: 140 μm).

7) Discussion

The specific Master's Thesis is laid in the understanding of the dietary habits and the paramasticatory behaviors of Early and Middle Bronze Age populations of the Northeastern Iberian Peninsula through the examination of dental wear patterns; moreover, we aim to further investigate the relationship between the ZEISS Axioscope A1 metallographic microscope and the Hirox KH8700 3D Digital Microscope for a further understanding of the utilization of the latter during the conduction of Dental Microwear Analysis, along with the level of complementation between the two pieces of equipment. In this context, it is necessary to contextualize our results to acquire further answers concerning our study and to test their quality.

7.1) Evaluation of the methodological framework

The adopted methodological framework consists of the appliance of the basic principles of the Dental Microwear Analysis concerning the replication processes. As mentioned above, the production of high-quality molds and casts facilitates the procedure of the examination of dental wear, providing the possibility for easier transportation of the samples among laboratories, and their examination independently of where the original tooth is located.

In the framework of the specific Master's Thesis, the used material for the production of molds was Polyvinylsiloxane. The specific material has already been proven to provide precise impressions in a wide range of disciplines, such as Prosthetic and Restorative Dentistry (Mandikos, 1998; Dogan *et al.*, 2015, for instance). Concerning the discipline of Dental Microwear, studies have already shown that materials with medium viscosities (such as the used Coltene President Plus Jet®) provide reliable results concerning the impression of the original enamel (Goodall *et al.*, 2015; Sawaura *et al.*, 2022). In our sample pool, the production of molds from Dra. Marina Lozano facilitated the process, thus requiring the subsequent production of casts for the conduction of the microscopic observations. From the total number of 74 molds, problems in the production of casts occurred only in 2 of them, which were discarded from further observations. In this context, it can be observed that the molding process successfully provided the preliminary ground for the conduction of the analysis.

In the second step, the production of casts was done using an epoxy resin (Epo-Tek 301, Epoxy Technology, Inc). The use of epoxy resin has been adopted methodologically from the beginning of the systematic conduction of the discipline of Dental Microwear Analysis since it is a material with durable properties and provides reliable imprinted results (Rose, 1983; Teaford and Oyen, 1989a, for instance). However, the production of high-quality casts depends on several factors, as they have already been mentioned above (see Asryan and Rots, 2024). Despite the existence of other techniques concerning the production of casts, such as the use of Triafol dissolved in Chloroform or other bicomponent products of polyurethane (Galbany *et al.*, 2004), the use of epoxy resin during dental casting procedures remains predominant today. Concerning the framework of the specific Master's Thesis, part of the produced casts demonstrated problems, such as contamination from air bubbles. However, the obtainment of a total of 9 highly accurate casts, which represent 9 individuals (out of a total of 14 individuals, whose teeth were replicated) indicates success on the specific methodological step.

Consequently, we used the ZEISS Axioscope A1 metallographic microscope for the conduction of the first microscopic observations to precisely detect the dental remains, which demonstrated well-preserved facets for further examination. The specific step was chosen due to the accessibility of the Optical Microscope, its low cost of maintenance, and the reliable results concerning the depiction of dietary features during the conduction of Dental Microwear Analysis (Hernando *et al.*, 2020b; Hernando *et al.*, 2024, for instance). For these specific reasons, we decided to use the obtained photos of the specific microscope in our effort to reconstruct the dietary habits of the studied populations.

Finally, we utilized the Hirox KH8700 3D Digital Microscope for the second step of our microscopic observations. In this case, the specific piece of equipment was chosen based on two factors: firstly, due to its digital applications, which permit the quantification of variables, such as the chipped enamel area and the perimeter, which couldn't be undergone with the use of the Optical Microscope; secondly, despite the important use of the specific microscope during a variety of disciplines, such as Taphonomy and Use-Wear Analysis of lithic artifacts (Courtenay *et al.*, 2019; Martín-Viveros and Ollé, 2020, for instance), its usefulness concerning the study of wear on

human dental remains has yet to be explored completely. In this context, we acquired to extract pieces of information concerning the dental features of the studied populations following a holistic methodological framework, while at the same time, we explored further methodological possibilities concerning the conduction of Dental Microwear Analysis.

7.2) Understanding the inter-microscopic variability

The appliance of different microscopic approaches has proven to be a valuable tool in the in-depth understanding of dental features since the phenomenon of inter-microscope variability on the measurements of the same dental features has been observed (Arman *et al.*, 2016). Explicitly, the obtained result is heavily dependent on the type of microscope, the applied magnification, and the used lenses, among others. In this framework and taking into account the variety of microscopic equipment, which is used nowadays for the examination of dental wear, the utilization of two different microscopic approaches and the understanding of the complimentary relationship between the Optical Microscope and the Hirox 3D Digital Microscope can provide insights into the methodological context of Dental Microwear Analysis in total.

The Total Number of striations (TN) demonstrates upward trends in the Hirox KH8700 3D Digital Microscope (129.66) with a mean increase of 14.21 % when compared with the values obtained from the Optical Microscope (111.24). This can be explained by the properties of the specific equipment, which despite being ideal for the examination and quantification of properties of taphonomic features, such as cutmarks (Valtierra *et al.*, 2020), and of ones related to conservation (Valtierra *et al.*, 2023), it can provide equifinalities in the quantification of the dental features that needed to be quantified separately, such as striations associated with the diet. The specific assumption is confirmed by the existence of non-normally distributed data concerning the specific variable among the two microscopes (U Mann Whitney: $U=61.5$; $p=0.004$).

Concerning the rest of the measured variables, an interest is focused on the number of observed vertical (V) striations among the two microscopes. More specifically, the

total observed number on the ZEISS A1 Axioscope Microscope (55.06) is considerably lower than the one on the Hirox KH8700 3D Digital Microscope (75.27), demonstrating a Mean Increase of 26.84 % and non-normally distributed data (U Mann Whitney: $U = 66$; $p = 0.007$). Notably, since the quantification was performed in photos, which can be directly compared based on the protocol of the calibration of magnification of Martín-Viveros and Ollé (2020), the results underline the ability of Hirox KH8700 3D Digital Microscope to detect a higher number of vertical striations comparing to the Optical Microscope. Indeed, Maté-González *et al.* (2017) reported the possibility for the obtainment of higher values and possibly less detail through the Hirox KH8700 3D Digital Microscope concerning quantitative variables in cutmarks when compared with other techniques, such as Laser Scanning Confocal Microscope and Microphotogrammetry. These results can be due to the smaller image resolution of the specific microscope, which can potentially provide equifinalities in the conduction of Dental Microwear Analysis (Table 6).

Features	Optical Microscope	3D Digital Microscope
Total Processing Time	Fast	Depending the application
Cost	Less expensive to purchase and less maintenance	More expensive to purchase, but has the same level of maintenance
Accessibility	Bigger	Smaller
Image Resolution	(3072 px x 2048 px)	(1600 px x 1200 px)
Comparative Databases	Few databases yet	No databases yet
Nature of the Image	Three-dimensional appearance, more definition	More depth of field, images with larger surface areas
Original and High-resolution cast	Suitable for both materials	Better Resolution on the original tooth

Table 6: Comparative Table concerning the ZEISS Axioscope A1 metallographic microscope (Optical Microscope) and the Hirox KH8700 3D Digital Microscope.

Furthermore, the comparison between the two scatter plots concerning the indexes NV/TN versus NH/TN obtained from the measurements of each microscope respectively (Figures 9a and 9b, Pages 66-67) represents similarities, thus highlighting

the possibilities of Hirox KH8700 to detect on a similar way with the Optical Microscope the specific patterns of wear. As discussed above, the specific indexes are widely used in the discipline of Dental Microwear Analysis since they provide important information concerning the distribution of striations on the examined surface.

Overall, the Optical Microscope accelerates the microscopic observations, while the ones with the 3D Digital Microscope are dependent on the selected application of the specific microscope (e.g. tilling is more time-consuming). Both microscopes are generally less expensive to purchase and require less maintenance compared with the Scanning Electron Microscope (SEM), while both require a small physical place in the laboratory; however, the Optical Microscope can be found in a bigger number of laboratories and, thus can be characterized as more accessible for the conduction of Dental Microwear Analysis. Concerning the nature of the image, the different properties of the microscopes seem ideal for the obtainment of a holistic approach regarding the same dental facet, thus enabling the complementation during the quantification processes and the understanding of different aspects of the microwear patterns. However, the non-existence of databases concerning the Hirox KH8700 3D Digital Microscope underlines the need for the conduction of further analyses of dental wear using the specific equipment.

7.3) Unravelling the dietary habits of the studied populations

The appliance of Dental Microwear analysis aims the understanding of dietary habits of populations of the past, along with deciphering the behavioral level of the studied cases. As a corollary to the goals of the conducted analysis, it is without a doubt that the specific study aims to unravel the dental features, which could be attributed to the adoption of specific dietary habits of the North-eastern Iberian populations during the Chalcolithic Period and the Early Bronze Age, furtherly contributing to the discussion concerning the alimentation of the populations of the Iberian Peninsula during the Late Prehistory.

In the framework of the specific Master's Thesis, the efforts for the paleodietary reconstructions underwent quantifying the dental features (striations) on the examined

enamel surfaces. Despite the establishment of most methodological frameworks on posterior dentition concerning the conduction of Dental Microwear Analysis, our study is based on its vast majority on examining incisors and canines. Notably, the anterior dentition has already proven to provide answers concerning the alimentation of the studied individuals/populations during their participation in the masticatory process (Ungar, 1994; Delezene *et al.*, 2016, for instance), despite depicting a smaller level of wear when comparing with the posterior dentition due to the appliance of bigger masticatory forces to the latter. However, it has been also observed the possibility of the existence of a higher level of wear in the anterior dentition than in the posterior one, possibly due to peculiarities in the craniofacial morphology in some cases (Kaidonis *et al.*, 2012b).

Concerning the Total Number of Striations (TN), the Mean number of the individuals from the El Bordellet site demonstrates very similar values when compared with the individuals of the Galls Carboners site (111.28 ± 12.64 to 113.13 ± 14.78), and the individual from Els Pujols (110.66). The specific variable reflects the abrasiveness of the diet, which can be either a result of the consumption of vegetables due to their high concentrations of phytoliths (Gügel *et al.*, 2001), or the ingestion of exogenous particles, which are adherent to the consumed food, such as grit, and sand (Pérez-Pérez *et al.*, 1994). The specific results demonstrate similarities with the results obtained from the buccal surfaces (TN: 121.83 ± 31.44) of modern hunter-gatherer groups (Babongo group, Central Africa), whose alimentary habits are characterized as mixed, including both plants and meat, with however a bigger inclusion of animal protein (Romero *et al.*, 2013). Moreover, taking into account the fact that the individuals of our sample pool are coming from a related chronological framework, the existence of similar values potentially represents the consumption of food with similar properties during the Bronze Age of the Northeastern Iberian Peninsula.

In the discipline of Dental Microwear Analysis, the variable of the Average Length of Striations (X, in μm) provides pieces of information concerning the texture of the consumed food depending on the harder or softer characteristics of the latter. The specific statement is explained by the application of harder or softer forces during the masticatory process, which results in variability in the length of striations (Teaford and Walker, 1984). In our samples, the average length of striations between the

populations of the El Bordellet site and Galls Carboners sites are almost identically (120.78 ± 12.36 to 119.35 ± 8.46) indicating the consumption of foods with similar textures among the two populations. Interestingly, the value for the same variable on the buccal surface of the molar of the child from Porroig demonstrates a closely related value (114.02), possibly due to the non-existence of differences in the alimentation among children and adults. However, the retrieval of only the child's burial from the site of Porroig doesn't permit the extrapolation and contextualization of the results through comparison with individuals of the same population; noteworthy, similarities between the diets of children and adults of the same group can be influenced by different factors, such as the death of the mother, and cultural and social structure among others (Hernando *et al.*, 2020b). The highest average length of striations is recorded on the individual from Els Pujols site (147.90), presenting the inclusion of the most abrasive diet among the four studied cases.

Moreover, the two calculated indexes (NV/TN and NH/TN) can contribute to the understanding of the distribution of the striations in the enamel surface, which in response provides pieces of information concerning the texture of the consumed food. Explicitly, the predominance of a carnivorous diet is implied by the existence of high values concerning the vertical striations (Lalueza Fox and Pérez-Pérez, 1993; Lalueza *et al.*, 1996), while the consumption of plants and/or hard materials is implied by the predominance of horizontal striations (Hinton, 1981). In our study, the dental remains from the El Bordellet site present intermediate values concerning the NV/TN index (0.46 ± 0.14), indicating the predominance of animal protein in the dietary habits with an important consumption of plants (NH/TN: 0.13 ± 0.05). Related values are obtained from the dental remains of Galls Carboners site (NV/TN: 0.50 ± 0.06) indicating a higher consumption of meat with the inclusion of plants and/or vegetables in the diet (NH/TN: 0.10 ± 0.03). Concerning the specific site, the results come in absolute accordance with the ones presented by Hernando *et al.* (2024) concerning the molar microwear of dental remains of Galls Carboners, highlighting the possibilities of microwear on anterior dentition for the efforts of precise paleodietary reconstructions.

The highest value concerning the NV/TN index is demonstrated by the buccal view of the child from the Porroig site (0.53). Taking into account the obtained values and its

age (3-4 years old), its diet can be characterized by the predominance of soft foods, such as meat, with a notably smaller consumption of harder objects. Furthermore, based on the age of the child and the examined period, the specific microwear pattern could also occur from the consumption of porridge of cereals (Sołtysiak, 2011), a technique that reduces significantly the abrasiveness of the specific food (Haaland, 2007). Interestingly, the individual from the Els Pujols site demonstrates the lowest value concerning the NV/TN index (0.39), and the highest one concerning the NH/TN index (0.19), indicating a mixed diet, in which meat and plants were almost of equal frequency. The two indexes confirm the obtained Average Length of striations on the same dental remain (147.90 μm), indicating the application of big forces during the mastication processes, which can be possibly attributed to the consumption of plants and/or fibrous objects.

Towards the beginning of the Holocene, the dental remains presented a shift towards the appearance of more abrasive patterns (Ungar, 2017). The appliance of Dental Microwear analysis on Natufian populations, which are placed chronologically just before the emergence point of the Neolithic period, suggested a relied diet on stone-ground plant foods, which require processing before consumption (Mahoney, 2006b). The described procedure introduced tools in the process, that are harder than the enamel and could introduce exogenous particles in the aliment, thus producing wear on the dental facets (Hua *et al.*, 2020). Similarly, an analysis from Nystrom (2008) demonstrated high frequencies of pits on the occlusal surfaces of dental remains from the Neolithic period of the Moravian plain, which implies the consumption of both hard and soft foods. However, the generalization of results towards the attribution of specific microwear patterns to specific dietary habits should be faced with caution, since the implementation of new technologies in the process of food preparation has been identified, resulting in a smaller level of abrasiveness of the enamel surfaces (Nava *et al.*, 2021).

Finally, the temporal placement of the populations permitted the existence of husbandry practices from the individuals. This economic strategy characterized the Iberian populations during the Bronze Age, supplying them with all the necessary resources for consumption (Knipper *et al.*, 2020). Interestingly, the archaeological record of the North-eastern part of the Iberian Peninsula presents a notable trend

towards livestock occupation from as early as the Early Neolithic with the prevalence of taxa such as *Sus domesticus*, *Ovis aries*, and *Capra hircus* (Navarrete *et al.*, 2017). These results demonstrate the adoption of a mixed diet, which was characterized by a variety of foods and plants and was facilitated by the inclusion of tools in the cooking process for the manipulation of possible abrasive elements.

7.4) Contextualizing the paramasticatory behavior

The conduction of paramasticatory activities using the mouth has been an important element of human behavior from as early as the Early Pleistocene (Frayer *et al.*, 2016), reported as well in remains of the Middle Pleistocene (Lozano *et al.*, 2008; Sun *et al.*, 2014), while the specific practices have been widely documented in the lineage of *H. neanderthalensis* (Krueger *et al.*, 2019). From a functional perspective, the utilization of the mouth provides stabilization on the object during the conduction of an activity, operating as a ‘‘third hand’’, while the specific activity is implied by the existence of cutmark-like striations in the enamel surface (Lozano-Ruiz *et al.*, 2004). Tooth-picking behavior is indicated by the existence of grooves in the morph of parallel striations, which in turn implies the conduction of actions correlated with the cleaning of the mouth (Frayer and Rusell, 1987), while the specific grooves can be polished within them in some cases (Krueger and Ungar, 2012). Finally, chipped enamel surfaces occur during the conduction of tearing or shearing practices with the use of the mouth (Krueger, 2015).

Concerning our sample pool, the observed dental feature attributed to paramasticatory activities was enamel chipping. The microscopic observations and quantification of the specific feature were performed using the Hirox KH8700 3D Digital Microscope. The specific choice was taken due to the properties of the specific equipment, which permits the direct evaluation of the quantitative properties of the chipped surface. In this context, the specific Master’s Thesis can be considered a further contribution to the enlargement of the methodological framework of dental microwear analysis, combining different microscopes to find distinct aspects of the discipline.

The results for the Coefficient of Variance (CV) demonstrated interesting values with respect to the perimeter of the chipped areas. Explicitly, the notably small value (0.45)

can potentially result from the conduction of similar paramasticatory activities among the populations of El Bordellet and Galls Carboners sites. At the same time, the high level of the CV of the chipped area (0.92) can be attributed to several factors, which are correlated with each individual respectively, as discussed above. Concerning the former, the relative uniformity of the paramasticatory activities during the Late Prehistory of the Northeastern Iberian Peninsula reveals aspects of similar social behavior and environmental constraints in the aftermath of the 4.2 ky BP climatic event, which contributed to the forging of new living conditions in the specific geographic latitude (Blanco-González *et al.*, 2018).

The values of the Coefficient of Variance concerning the perimeter can be confirmed further by the results of Pearson's r correlation test, which revealed a relatively strong correlation (0.695) between the area and the perimeter concerning the values of the specific variables, possibly as an effect of the use of the mouth during similar activities. Notably, the plot of Bivariate Regression placed 4 of the examined dental remains outside of the 95 % confidence interval, indicating possibly the conduction of the same paramasticatory activity in a different way inside the same examined population.

In this context, a special mention should be given to the sample GC 2.115-135. The specific deciduous Upper Left Canine exhibited enamel chipping, thus indicating the conduction of paramasticatory activities by children as well in our studied populations. However, the described behavior doesn't appear to have been found exclusively in adults. Estalrich and Krueger (2022) reported the existence of analogous behavioral strategies between the deciduous teeth of Neanderthal and Inuit populations; similarly, Estalrich and Marín-Arroyo (2021) noticed evidence of toothpicking behavior on a Neanderthal premolar from Axló site, which is attributed to a juvenile, and chipped enamel on the premolar of a juvenile individual from Las Caldas site, which is placed in the Aurignacian period. Despite the existence of the chipped enamel on only one of the examined deciduous teeth on our sample pool, which subsequently doesn't permit the extrapolation of the specific result concerning all the children in our studied cases, the specific result demonstrates the general inclusion of all age groups in the behavioral use of the mouth during the Bronze Age of the Northeastern Iberian Peninsula.

As discussed above, behavioral patterns on studied teeth of the Late Prehistory of the Iberian Peninsula have already been found in several sites across the specific geographic latitude. Regarding the dental feature, which has been observed in our sample pool (enamel chipping), similar patterns appear in the sites of Casas Velhas (Silva *et al.*, 2016), El Mirador (Lozano *et al.*, 2017), Torre Velha 3 (Fidalgo *et al.*, 2020), and Castellón Alto (Lozano *et al.*, 2021), which have been however attributed to different uses of the mouth concerning each case respectively. Contextualizing and comparing the results of the mentioned sites, with the ones of our case studies, the existence of enamel chipping in our samples could be attributed to the adoption of an abrasive diet based on hard fruits and nuts, such as in the case of Casas Velhas site (Silva *et al.*, 2016). Similarly, we can't dismiss but we don't have enough evidence to prove the existence of chipped enamel in our samples due to the manipulation of yaw solely, such as in the case of Torre Velha 3 (Fidalgo *et al.*, 2020).

However, we can't exclude the utilization of the mouth for the manipulation of animal fibers and/or plants as well, such as in the cases of El Mirador Cave and Castellón Alto site (Lozano *et al.*, 2017; Lozano *et al.*, 2021). The specific practices have already been documented in a wide range of geographical latitudes and chronological frameworks, from subarctic Aleuts and Inuit populations (Turner II and Cadien, 1969) to Epipaleolithic populations of Morocco (Bonfiglioli *et al.*, 2004) and Medieval populations of the Italian Peninsula (Belcastro *et al.*, 2001). Notably, the archaeological remains of the El Bordellet site consist of pendants, prismatic buttons, and remains of *Ovis aries* among others (Esqué Ballesta, 2015). The specific remains imply the conduction of activities concerning personal adornment, which in turn can potentially justify the use of the mouth as a "third hand" during the processes of the manipulation of the animal fibers. Finally, a similar assumption can't be made in the case of the Galls Carboners due to the recovery of the archaeological remains in a conglomerate context, since the site functioned as a deposit for the *postmortem* placement of the individuals (Vergès *et al.*, 2017).

In this framework, the existence of enamel chipping in our samples can be attributed to the consumption of foods with hard textures, such as fruits and nuts, or/and to the utilization of the mouth during the conduction of a paramasticatory activity, such as the manipulation of fibers of animal or/and plant origin.

8) Conclusions

In the specific study, the analysis of dental wear on individuals' teeth from 4 sites of the Northeastern Iberian Peninsula during the Late Prehistory was performed in a comparative methodological framework. The results provided insights both into the dietary and behavioral habits of the populations in the studied cases, along with the importance of utilizing different microscopic equipment for the finest archaeological interpretation.

1. The present study is based on its vast majority (140/145= 96.55 %) on examining dental wear through the study of anterior dentition (incisors and canines). In this framework, the specific work highlights the potential of anterior dentition for the extraction of information concerning the alimentation of populations of the past.
2. The inclusion of different methodological frameworks for the microscopic observations (ZEISS Axioscope A1 metallographic microscope and Hirox KH8700 3D Digital Microscope) permitted the study of different aspects of dental wear and the understanding of the complementation among the two pieces of microscopic equipment.
3. The efforts for the paleodietary reconstruction were undertaken using the Optical (metallographic) microscope due to its reliability in detecting and quantifying dental features associated with the diet. In this framework, the results of the specific Master's Thesis can be characterized as a further enrichment of the existing databases concerning the specific equipment.
4. Based on the microwear variables (TN, X, NV/TN, and NH/TN), the diets of the individuals of the El Bordellet site and Galls Carboners site can be characterized as homogeneous and mixed, with the intake of both plants and meat.
5. The most abrasive microwear pattern was reported in the individual of Els Pujols site. The specific statement implies either the adoption of a mixed diet with a notable increase in the intake of plants and fruits or the intake of exogenous particles correlated with the food preparation processes (e.g. grinding stones).

6. The microscopic observations and the quantification processes of the paramasticatory dental features (enamel chipping) were performed using the Hirox KH8700 3D Digital Microscope. Exploring its different applications, we acquired direct values on different variables, which couldn't be taken using the Optical (metallographic) Microscope, thus providing new aspects of the archaeological interpretation and complimenting the obtained information using different microscopic approaches.
7. The conduction of Dental Microwear Analysis revealed features of paramasticatory behavior (enamel chipping) in two of the studied cases (El Bordellet and Galls Carboners). The specific dental feature can be attributed either to the manipulation of animal and plant fibers from the individuals or/ and to the consumption of hard foods, such as fruits and nuts.
8. The statistical analysis of the descriptive variables from the features of enamel chipping (Chipped Area and Perimeter) revealed the conduction of possibly the same activity with the use of the mouth among the two populations; however, the detection of intra-variability among the individuals of the populations can potentially reveal aspects of social behavior and structure concerning the studied populations.
9. The detection of enamel chipping on a deciduous tooth (GC 2.115-135) demonstrates the inclusion of all age groups in manipulating animal and plant fibers using the mouth.
10. Concerning the inter-microscopic comparison, the biggest differences were detected in the values of TN (Total Number of Striations), and V (Total Number of Vertical Striations). Considering that the use of Hirox KH8700 in the studies of wear has been implemented recently, the specific study can be considered as a further step towards the understanding of the utilization of the specific equipment in the discipline of Dental Microwear Analysis, providing a new database concerning the quantification of dental features attributed to the diet and the behavior.

9) Perspectives of the Future

The study of dental remains is considered a vital part of the studies of the anthropological and archaeological fields. Explicitly, the frequency of the retrieval of teeth in the archaeological record due to their durable properties and the possibilities for the extraction of reliable results in a wide range of disciplines highlight the teeth as a means for the extraction of different results, which contribute significantly to the archaeological interpretation.

Concerning the discipline of Dental Microwear Analysis, the combination of methodological frameworks and microscopic approaches can be the only way to obtain a holistic point of view regarding the examined dental views. In the framework of the specific Master's Thesis, the examined teeth can be observed microscopically on a further level using both an Environmental Scanning Electron Microscope (ESEM) and a Confocal Microscope; in this context, we can acquire a comprehensive understanding concerning the level of complementation of different microscopic equipment and to measure aspects, which couldn't be examined using the Optical Microscope and the 3D Digital Microscope.

Finally, the specific Master's Thesis can be considered a closer step toward the persuasion of a career in investigation, in which the study of dental wear would play a role of primary interest. Explicitly, the importance of the conduction of Dental Microwear Analysis is laid in the acquisition of pieces of information concerning some of the most vital elements for the dominance of the lineage of *Homo*, such as the alimentation and behavioral strategies, unraveling aspects of the human adaption and prevalence in diverse conditions and environments. For this reason, the next step would potentially be the prosecution of a PhD degree, in which different methodological aspects of the discipline would be discussed and applied, such as the exploration of the digital applications concerning the study of dental wear using the Hirox KH8700 3D Digital Microscope, along with the understanding of the complementation of the Optical Microscope with the Confocal Microscope based on the combination and interpretation of the different obtained variables and values.

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11) Supplementary Material

Site	Chronology	Specimen	Material	Tooth	Observation
El Bordellet	Early Bronze Age	B10E5-7	Cast	LRM2	Buccal Microwear
El Bordellet	Early Bronze Age	B10E5-4	Cast	LLC	Labial Microwear
El Bordellet	Early Bronze Age	B10E5-1	Cast	ULI1	Labial Microwear
El Bordellet	Early Bronze Age	B10E5-2	Cast	URC	Labial Microwear
El Bordellet	Early Bronze Age	B10E76-1	Cast	ULI2	Labial Microwear
El Bordellet	Early Bronze Age	B10E19-1	Cast	LRI1	Labial Microwear
El Bordellet	Early Bronze Age	B10E5-8	Cast	LLC	Labial Microwear
Galls Carboners	Middle Bronze Age	1.1-1	Original	ULI1	Labial Microwear
Galls Carboners	Middle Bronze Age	1.1-4	Original	ULI1	Labial Microwear
Galls Carboners	Middle Bronze Age	1.56-76	Original	ULC	Labial Microwear
Galls Carboners	Middle Bronze Age	1.98-118	Original	dULI1	Labial Microwear
Galls Carboners	Middle Bronze Age	2.18-38	Original	LLI1	Labial Microwear
Galls Carboners	Middle Bronze Age	2.57-77	Original	LLC	Labial Microwear
Galls Carboners	Middle Bronze Age	2.115-135	Original	dURC	Labial Microwear
Els Pujols	Early Bronze Age	P10-1	Cast	URI1	Labial Microwear
Porroig	Early Bronze Age		Cast	dLLM1	Buccal Microwear

Supplementary Material 1: The selected dental remains for the extraction of dietary information in the framework of the specific Master's Thesis.

Site	Chronology	Specimen	Material	Tooth	Observation
El Bordellet	Early Bronze Age	T1-S4	Cast	ULI1	Enamel Chipping
El Bordellet	Early Bronze Age	B10E5-4	Cast	URI1	Enamel Chipping
El Bordellet	Early Bronze Age	B10E5-1	Cast	LRI1	Enamel Chipping
El Bordellet	Early Bronze Age	B10E5-2	Cast	ULI2	Enamel Chipping
El Bordellet	Early Bronze Age	B10E5-8	Cast	LRI2	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.2-8	Original	ULI1	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.2-11	Original	ULI1	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.2-12	Original	ULI1	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.3-13	Original	ULI1	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.3-15	Original	LRI2	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.5-17	Original	ULI2	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.5-18	Original	ULI2	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.5-19	Original	ULI2	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.56-76	Original	ULC	Enamel Chipping
Galls Carboners	Middle Bronze Age	1.70-90	Original	URC	Enamel Chipping
Galls Carboners	Middle Bronze Age	2.20-40	Original	LLI1	Enamel Chipping
Galls Carboners	Middle Bronze Age	2.31-51	Original	LLI2	Enamel Chipping
Galls Carboners	Middle Bronze Age	2.38-58	Original	LRI2	Enamel Chipping
Galls Carboners	Middle Bronze Age	2.45-65	Original	LLC	Enamel Chipping
Galls Carboners	Middle Bronze Age	2.115-135	Original	dULC	Enamel Chipping

Supplementary Material 2: The dental remains, which demonstrated signs of paramasticatory behavior (enamel chipping).

Site	Sample	Perimeter (in μm)	Area (in μm^2)	Pearson's r
El Bordellet	B10E5-4	3962	960426	r= 0.695; p= 0.001
El Bordellet	B10E5-2	5241	1254247	
El Bordellet	B10E5-8	5911	760174	
Galls Carboners	GC 1.2-8	4267	934498	
Galls Carboners	GC 1.2-12	16406	10057699	
Galls Carboners	GC 1.3-13	14237	2072056	
Galls Carboners	GC 1.3-15	15898	3480977	
Galls Carboners	GC 1.5-17	6266	921243	
Galls Carboners	GC 1.5-18	14073	3042576	
Galls Carboners	GC 1.5-19	12246	2179196	
Galls Carboners	GC 1.56-76	11591	1380411	
Galls Carboners	GC 1.70-90	14314	4291324	
Galls Carboners	GC 2.20-40	5098	831552	
Galls Carboners	GC 2.31-51	5591	1631511	
Galls Carboners	GC 2.38-58	13002	2235136	
Galls Carboners	GC 2.45-65	8467	2357733	
Galls Carboners	GC 2.115- 135	6880	1259483	

Supplementary Material 3: Results of the quantification processes on the variables of perimeter and area on the enamel chipped areas.

12) Appendix

Radiocarbon (2024), pp. 1–13
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Selected Papers from the 3rd International Radiocarbon and Diet Conference, Oxford, 20–23 June 2023

CONFERENCE PAPER

Correlation between dental microwear analysis and dietary habits of Neanderthal populations in the Iberian Peninsula

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Abstract

The dietary habits of Neanderthals are considered an issue of great interest in the literature and have opened an important number of fruitful debates. Indeed, understanding diets can provide important information regarding issues of palaeoenvironmental reconstructions and subsistence strategies. In this respect, dental remains can play a vital role in the conducted efforts to reconstruct the palaeoecological niches securely and accurately since dental microwear analyses have precisely detected dietary patterns of the populations in the past. In this context, the Iberian Peninsula forms an interesting model for examining Neanderthal populations, their subsistence strategies, and adaptive skills. This study aims the examination of already published data in order to provide a holistic approach regarding the dietary habits of *H. neanderthalensis* populations in the Iberian Peninsula, along with the importance of the utilization of dental microwear analysis in the archaeological record.

Introduction

The Iberian Peninsula is located in the southwestern part of the European continent and covers an extent of more than 583 km². The area is characterized by an abundance of geomorphological and environmental diversities, constituting a unique mosaic in every manifestation. Exhibiting sites such as Fuente Nueva 3, the Atapuerca sites, El Sidrón, and many more, it can be easily inferred that this specific geographical latitude played a major role in every aspect of human evolution from as early as the Early Pleistocene (Blain et al 2011; Espigares et al 2019). The abundance of well-dated sites and preserved remains regarding the lineage of *H. neanderthalensis* places the peninsula as a point of primary importance regarding hotly debated issues, such as the contacts between AMHs and Neanderthals (Yravedra et al 2015) and the temporal determination of the extinction of the latter, since it appears that the area functioned as a late refugium for the last populations of our closest ancestors (Zilhao 2000; 2006).

The dental microwear analysis is based on the detection of specific alterations on the enamel surface, which originate either from food consumption or by behavioral utilization of the teeth from the populations. These features, which are related to diet, mostly consist of striations and pits and are heavily dependent on several variables, such as the examined individual, the post-depositional processes, and the type of food, elements that can significantly disrupt the enamel profile (Espurz et al 2004). The possibility of intragroup variation regarding the microwear signatures has already been

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exhibited in previous studies, exposing sexual and age separation with respect to masticatory activities (Pérez-Pérez et al 1994; Romero and de Juan 2007, for instance).

The first idea regarding the extraction of pieces of information to infer dietary habits from dental records is placed in the 1920s, when enamel striations were interpreted as indicators of chewing processes (Calandra et al 2019). However, it was only until the end of 1970s, that two pioneering works managed to provide a more stable methodological framework using scanning electron microscope on the occlusal views of mammalian teeth (Rensberger 1978; Walker et al 1978). During recent decades, technological development and the implementation of more enhanced microscopic applications have provided the opportunity to construct more stable methodologies and quantitative approaches. Nowadays, the most widespread methodology consists of examining the occlusal and buccal surfaces of the dental remains using a confocal microscope and a scanning electron microscope respectively, while at the same time, new types of microscopes are explored and utilized for the determination of dietary patterns, such as the digital microscope (Firmat et al 2010; Mühlbacher and Beatty 2012).

The microwear traces, which are produced during masticatory activities, such as pits and striations, can be detected on the occlusal and buccal surfaces of the dental remains, and occur from a variety of factors. Pits occur either when a particle is squeezed into the enamel surface (Mahoney 2006b), or as a consequence of acidic etching (King et al 1999), while the striations are the result of the tooth-food-tooth contact during the chewing cycle (Mills 1966). Regarding the latter, the quantification process predicts the delineation of the orientation of the observed striae on the enamel surface since studies have already depicted the importance of the comprehension of the jaw mechanics for the understanding of alimentary habits (Grine and Kay 1988; Williams et al 2009). Based on the proposed methodology by Lalueza Fox and Pérez-Pérez (1993), which enriched the existing framework of Puech et al (1980), the striations are attributed to four different orientations concerning their inclination, and more specifically are described either as horizontal (0° – 22.5° or 157.5° – 180°), disto-occlusal to mesio-cervical (22.5° – 67.5°), vertical (67.5° – 112.5°) or mesio-occlusal to disto-cervical (112.5° – 157.5°).

The effect of non-alimentary activities on dental remains is revealed by the presence of striations, grooves, or chipping on the enamel surface. Explicitly, striations with characteristics of cut marks occur on the enamel tissue through the use of the mouth as a “third hand” for the stabilization of an object during the conduction of an activity, a practice, that has been identified in both the archaeological and ethnoarchaeological records (Lalueza Fox 1992; Lozano-Ruiz et al 2004). Grooves appear in the enamel surface in the morph of parallel striations (Frayer and Russell 1987), while their attribution to para-masticatory activities can be confirmed by the existence of polish within them, indicating tooth-picking behavior (Krueger and Ungar 2012). Finally, chipping on the dental tissue occurs through the process of holding or tearing different objects with the use of the alimentary apparatus (Fiorenza et al 2011a; Krueger 2015).

The dental features differ with respect to the view of the dental remain, since the examination of the buccal surfaces aims the detection of scratches, while the one of occlusal surfaces extracts information from scratches and pits. Raw meat is not hard enough to scratch dental enamel (Organ et al 2005). The microwear turnover, which can be attributed to the consumption of meat, can be better explained since the dried and tough meat demands high chewing forces for a longer time, subsequently producing dental microwear. In some cases, individuals with a mainly based diet of meat show a high number of striations due to exogenous grit adhered to dental surfaces.

Furthermore, the use of grasses and plants in the diet is indicated by more worn facets with respect to the occlusal surfaces due to the abrasive composition of phytoliths, which is characterized by the existence of high levels of silica (Karriger et al 2016; Rodríguez-Rojas et al 2020). On buccal surfaces, this element can be recognized in the number and the orientation of scratches, since the specific part of the tooth is not directly exposed to the masticatory processes (Dritikolova Kaupova et al 2023). Important changes in the occlusal views regarding the tooth tissue can be attributed to tooth-to-tooth contact during the mastication process, as it has also been highlighted by experimental studies (Winkler et al 2020; Krueger et al 2021). Finally, microwear can also occur from the consumption of exogenous particles, such as sand, generic dirt, and ashes, which can be included in the food; this is the case mostly

when examining populations, which are placed in cold climates where the underground storage of meat is considered of vital importance for its long-term preservation (El-Zaatari 2008).

An important aspect for the undergone of dental microwear analysis is the production of replicas and the subsequent microscopic observations on the impression molds and casts. The production of the former is undergone with the use of silicone compound, while a study by Sawaura et al (2022) demonstrated that the most accurate result originates from the use of materials with medium viscosity, even though previous analyses have exhibited the importance of the use of compounds with low viscosities for the prevent of damage on the enamel and precise replications (Galbany et al 2006; Goodall et al 2015). For the latter, epoxy resins are the ones preferred due to their durable properties and their good possibilities regarding the production of precise imprints of the enamel surfaces of the tooth (Galbany et al 2004; Ungar et al 2019). Furthermore, the produced positives and negatives permit easy transportation among laboratories, providing convenience in the efforts for transmission and transfusion of knowledge in the scientific community. In the case of the examination of the buccal surface, the use of a conductive material such as a gold coat for the coverage and the subsequent non-exposure of the examined replica to the radiation of the scanning electron microscope prevents possible wear of the surface and loss of the archaeological information and permits a rational use of the SEM.

The examination of the buccal surface aims the detection of wear patterns, which suggest feeding habits and dietary hints (Pinilla Perez et al 2011; Pérez-Pérez et al 2017). An important aspect regarding the buccal surface is the understanding of dietary patterns throughout the lifetime of the individual since this specific view is not exposed to non-masticatory activities (Alrousan 2016). In this case, the microscopic observation is being held with the utilization of a scanning electron microscope, while the methodology predicts the coverage of the replicas in gold-coating for the avoidance of any alternation of the original enamel surface during the examination process.

Dental microwear analyses on the occlusal surfaces of dental remains have proven to provide important insights regarding issues of dietary reconstructions on both extinct and extant human or mammal populations (Arman et al 2016; Bas et al 2021; El-Zaatari 2008, 2010). Since the occlusal surface of the tooth is heavily exposed to masticatory activities, an important level of wear can be observed on this specific surface (Grine 1986). At the beginning of the discipline, the analysis was performed using a scanning electron microscope (SEM); subsequently, the confocal microscope was introduced, which eventually replaced SEM for the examination of the occlusal surfaces since it provides the opportunity for the obtainment of 2D and 3D proxies of the enamel surface (DeSantis et al 2013). Based on the functional identifications of Maier and Schneck, the upper and lower molars of hominins are characterized by 13 corresponding pairs of facets on their occlusal surfaces (1981; 1982). Previous studies have aimed the understanding of the mechanical processes on occlusal surfaces, indicating that the most heavily affected parts are the facets 9 and 11 in the cases of both deciduous and permanent molars (Bas et al 2020; Mahoney 2006a).

The aim of this study is the examination of Neanderthal sites from the Iberian Peninsula, in which dental microwear analysis has been conducted, along with the explanation of general issues concerning the dental microwear methodologies (Figure 1).

Materials and Methods

This research is based on bibliographic data, which pertain to operated dental microwear analyses with respect to neanderthal remains of the iberian record to date. For this reason, the described sites have been sorted based on their chronology, from the oldest site to the youngest one (Table 1).

The Devil's Tower site in the southern part of the Iberian Peninsula presents impressive findings with decisive paleoanthropological interest. More specifically, the discovery of human remains identified as Neanderthals in 1926 in the Gibraltar karstic system, along with the extraction of ancient DNA from the fossils during the last years has placed the site in the spotlight and has created important debates regarding palaeoecological issues. However, the non-existence of scientific formalities and excavation

Table 1. Dental microwear analyses applied on Neanderthal populations of the Iberian record

Site	Dating	Examined dental remains	<i>n</i>	Reference
Devil's Tower	130,000–30,000 BP	L dM ₁ /R dM ₂	2	Lalueza Fox and Pérez-Pérez 1993
Figueira Brava	106,000–86,000 BP	LP ⁴	1	Egocheaga et al 2004
Axlor	49,300 BP	L dPM ⁴ /L dI ¹	2	Estalrich and Marin-Arroyo 2021
El Sidrón	49,000 BP	LM ₂ /RM ¹ /LM ₂ /LM ₂ /LM ₁ /LM ₂ /LM ₁ /RM ¹	8	Estalrich et al 2017
El Salt	47,200–45,200 BP	UPM	1	Pérez-Pérez et al 2003
Zafarraya	46,700 BP	Undetermined	3	Krueger et al 2017
Sima de las Palomas	43,000–40,000 BP	RM ₂ /LM ₁	2	Pérez-Pérez et al 2017
Total			19	

**Figure 1.** Geographic determination of the examined sites.

methods during the fieldwork has prevented a precise temporal specification of the findings, but a conventional one between 130 and 30 kya (Bokelmann et al 2019). The human remains at the site consist of a cranium and a mandible, which are attributed to a Neanderthal male child. Inside this context, the analysis of Lalueza-Fox and Pérez-Pérez (1993) focused on the buccal surfaces of the deciduous molars, aiming to extract pieces of information regarding dietary proxies.

Another Late Middle Pleistocene site with paleoanthropological interest attributed to Neanderthals, which includes dental remains as well, is Gruta da Figueira Brava on the eastern coast of the Peninsula. The site is dated between 106 and 86 kya and preserves an impressive record, which includes the

exploitation of marine resources (Nabais et al 2023; Zilhao et al 2020). Indeed, it appears that the location of the site on the coastline and its placement inside the Mediterranean vegetation contributed to the provision of all the decisive elements to the Neanderthal populations, which occupied the area. The dental remain of the site, which has undergone a dental microwear analysis is an LP⁴, while the microscopic observation focused on the buccal surface with the use of a scanning electron microscope (Egocheaga et al 2004).

Axlor Cave in the Cantabrian region has provided important insights regarding the subsistence strategies of Neanderthals, along with the development of the Mousterian knapping technocomplex (García-Díez et al 2013). The Neanderthal occupation on the site appears in layers III and IV, with the latter to have been dated at >49,300 through the usage of new protocols for the extraction and the ultrafiltration of collagen from deer remains, which experienced anthropogenic modification (Gómez-Olivencia et al 2018). The human remains of Level IV have produced a debate regarding their attribution to a specific lineage, with González-Urquijo et al (2021) supporting a clear taxonomical allocation on Neanderthals based on the understanding of the morphologies of the occlusal surfaces of the Axlor's dental remains and the sedimentary processes of the site. In this context, a study from Estalrich and Marin-Arroyo (2021) focused on detecting patterns of enamel alternation using a digital microscope.

El Sidrón site has provided the scientific community with some of the most important human remains with respect to the lineage of *H. neanderthalensis* on a global scale. The site is located in the northern part of the Iberian Peninsula and is dated at ca. 49 kya through AMS dating method, thus providing the opportunity for the extraction of pieces of information just before the beginning of the end of Neanderthals (Ríos et al 2019). Explicitly, the existence of anatomical anomalies in an important number of individuals indicates an alternation in the behavioral levels and subsistence strategies of Neanderthals just in the temporal point, when the arrival of AMHs in Europe is clearly documented (Hublin et al 2020; Slimak et al 2022). The study of Estalrich et al (2017) included the analysis of the occlusal surfaces of the dental remains from 11 individuals and their subsequent microscopic observation with a confocal microscope.

El Salt site is located in the southeastern part of the Iberian Peninsula and is characterized by important findings for the understanding of the existence of Late Neanderthals. Explicitly, the site is placed between 47.2 ± 4.4 and 45.2 ± 3.4 kya through the TL dating method, while the dental record is comprised of six teeth (Garraleta et al 2014). The recovery of human-modified faunal bones, a human coprolite, and a rich lithic record, which is associated with the human remains provides a rare opportunity for the undergone of a precise dietary reconstruction (Rampelli et al 2021; Sistiaga et al 2014). In this context, the study of Pérez-Pérez et al (2003) focused on the examination of the buccal surface of an upper premolar using a scanning electron microscope.

Cueva del Boquete de Zafarraya in the south of the Iberian Peninsula depicts decisive evidence regarding the late appearance of *H. neanderthalensis*. This specific site has been at the epicenter of important debates with respect to its temporal allocation; specifically, the first placement between 31 and 28 kya has been discarded with the use of new protocols and methodological approaches, such as the ultrafiltration of bone collagen for the minimization of the contamination factor (Mellars 2006). Nowadays, the site is placed at >46,700 BP for the Neanderthal occupation, exposing important difference with the preliminary dating (Wood et al 2013). The human remains consist of a mandible with clear Neanderthal morphologies based on a biometric analysis (Sanchez 1999), while the dental microwear analysis in a study from Krueger et al (2017) focused on the labial surfaces with the use of a white-light confocal microscope.

The Middle Palaeolithic site of Sima de las Palomas is dated between 43 and 40 thousand years BP and exhibits important lithic and faunal records, indicating an important presence of Neanderthals (Walker et al., 2008;). The excavation processes began during the '90s and revealed impressive findings, which are dated in a decisive temporal point for the understanding of the Middle to Upper Palaeolithic transition in the Iberian Peninsula. The human remains consist of a partial skull and skeletons, along with several isolated remains as an aftereffect of the unique formation processes of the site (Walker et al 2012; Zapata et al 2017). The extraction of pieces of information regarding dietary

Table 2. Determination of the alimentary habits of the Neanderthal populations based on the exhibited dental features

Site	Observations	Diet	Reference
Devil's Tower	Predominance of vertical striations	Camivorous	Lalueza Fox and Pérez-Pérez 1993
Figueira Brava	Existence of subvertical grooves	Mixed	Egocheaga et al 2004
Axlor	Indication of para-masticatory activities (chipped enamel)	Undetermined	Estalrich and Marin-Arroyo 2021
El Sidrón	Complex occlusal facets	Mixed	Estalrich et al 2017
El Salt	Low number of striations	Camivorous	Pérez-Pérez et al 2003
Zafaraya	High anisotropy level (epLsar)	Mixed	Krueger et al 2017
Sima de las Palomas	Long striations and low scratch density	Camivorous	Pérez-Pérez et al 2017

habits from dental remains has been gone through by Pérez-Pérez et al (2017) on an RM₂ and an LM₁ concerning the buccal surfaces.

The Neanderthal record of the Iberian Peninsula is comprised of several sites, which exhibit important interest regarding the dental remains, such as Bolomor Cave (Arsuaga et al 2012b), Mollet Cave (Maroto et al 2012), Cova Negra (Arsuaga et al 2007), Cueva del Camino (Arsuaga et al 2012a), among others. It is without a doubt that the appliance of dental microwear analysis on a further scale on Neanderthal dental remains from this specific geographic latitude could provide pieces of information of major importance regarding palaeoecological issues and palaeoenvironmental reconstructions.

Results

The reported results include analyses, which have been conducted on both the occlusal surfaces and the buccal ones of dental remains using the protocols for their examination with the use of a confocal microscope and a scanning electron microscope respectively (Table 2). In the case of the examination of the labial surfaces from the dental remains of Zafaraya site, the analysis was undergone with the use of a white-light Confocal Microscope, while the non-alimentary microwear traces of the dental remains of Axlor site were revealed with the use of a digital microscope.

The analysis of Lalueza-Fox and Pérez-Pérez (1993) exhibited that the diet of the Neanderthal male child from Devil's Tower can be characterized as a camivorous one, based on the number and the direction of the striations in the buccal surface of the examined dental remain. Specifically, both examined deciduous molars exhibited the predominance of vertical striations on the buccal surfaces, along with a tendency towards lower values concerning the horizontal striations. When compared with data from the ethnoarchaeological record, the results indicate similarities with respect to the latter variable with groups of Fuegians and Inuit, while an important difference is observed in the number of striations and the level of wear (Lalueza-Fox and Pérez-Pérez, 1993). Previous studies on the dental remains of these groups have revealed mostly meat-based dietary habits, that can be explained by the unique environmental proxies, in which they are located (El Zaatari, 2010). In the case of Devil's Tower, the interpreted data are characterized by relative abrasive turnovers due to the intake of exogenous particles, which can be included in meat-based diets as described above.

In the case of Gruta da Figueira Brava, dental microwear analysis demonstrated the existence of interproximal grooves, which are the results of the appliance of heavy mastication forces during the chewing processes (Egocheaga et al 2004). Explicitly, the results indicated the inclusion of both meat and hard plants in the diets of the populations from the site due to the high frequency of subvertical grooves in the examined facet; inside this context, previous studies on the buccal surface of dental remains attributed to *H. neanderthalensis* have depicted the consumption of mixed diets, which can be

more readily apparent due to the existence of a noticeable level of wear on the buccal facet (Fiorenza et al 2011b).

Based on the microwear signatures, the dental remains of the populations from Axlor Cave are characterized by subvertical grooves, chipped enamel, and hints of tooth picking, which are alterations caused as an aftereffect of specific actions and consequently indicate behavioral processes (Estalrich and Marin-Arroyo 2021). The alteration of the enamel due to non-alimentary activities has been documented from as early as the beginning of our lineage (Estalrich et al 2020); concerning the Iberian Peninsula, behavioral traits have been extracted from the dental remains of the pre-Neanderthals from Sima de los Huesos (Lozano et al 2008; Lozano-Ruiz et al 2004), Cova Negra (Bermudez de Castro et al 1988), and Cova Forada (Lozano et al 2013) among others.

The occlusal dental microwear signatures of the El Sidrón site displayed the espousal of a mixed diet, which included the consumption of both meat and plants (Estalrich et al 2017). More specifically, the occlusal surfaces of the molars of 11 individuals from the site are characterized by important levels of complexity, which couldn't occur from the intake of only meat proteins; these specific results confirm the study of Egocheaga et al (2004), who attribute the existence of interproximal grooves on the distal facet of a lower right M_2 from El Sidrón to the inclusion of hard plants in the diet among other foods. Moreover, when comparing the results with relevant data from the ethnoarchaeological record, important similarities can be observed between the examined materials and the results from the populations of Khoe-san and Chumash, thus suggesting mixed dietary habits. Interestingly, a study by Radini et al (2016) focused on the analysis of retrieved dental calculus from five individuals of El Sidrón and suggested a general familiarization with the use of vegetal remains for both para-alimentary and behavioral reasons.

The analysis of Pérez-Pérez et al (2003) demonstrated a low number of striations in the buccal surface of the upper premolar from El Salt, which is however bigger when compared with contemporaneous sites from OIS 3 and less than the ones from OIS 4. The former temporal point (OIS 3) is characterized as a generally warm event, which still includes both cold and warm intervals (Barron and Pollard 2002). Inside this context, the microwear signature from El Salt indicates the dependence on mostly meat-based diets, which comes as a response to the abundance of faunal remains for exploitation during this warm period principally. Indeed, the density of striations increases significantly when examining populations, who were placed in cold environmental contexts or inhabited cold geographical latitudes, since the consumption of meat overlaps with the use of hard roots or/and plants, as a response to the confined number of available faunal resources for exploitation (Rivals and Deniaux 2005).

The examination of the labial surfaces from Zafarraya exhibited the existence of a medium level of wear concerning the volumes of anisotropy (epLsar) and texture fill volume (Tfv) (Krueger et al 2017). The volume of anisotropy (epLsar) as a variable is laid in the understanding of the determination of the roughness in the respective surface of the dental remain (Scott et al 2005), while the texture fill volume (Tfv) quantifies the dental surface in square cuboids for the comprehension of different volumes (Scott et al 2006). Indeed, when comparing different sites and obtained values, it can be inferred that the samples of Zafarraya are placed in the general context of populations, which inhabited deciduous forests or woodlands, such as the ones of Krapina, Tabun, and Shanidar; the diets of these populations can be characterized as mixed, with the incorporation of both meat and plants in the alimentary process. In the case of Zafarraya, a significant anisotropy level (epLsar) indicates the consumption of foods with adherent endogenous or/and exogenous particles, which subsequently lead to more worn facets regarding the labial surfaces.

The analysis of the buccal surfaces of the molars from Sima de las Palomas suggested a low scratch density and longer striations of the buccal surfaces when compared with species, which inhabited the Iberian Peninsula during the Early and Middle Pleistocene, such as *H. antecessor* and *H. heidelbergensis* (Pérez-Pérez et al 2017). As described above, the analysis of the buccal surface can provide a general approach to the alimentary composition throughout the lifetime of an individual (Pérez-Pérez 2004). Inside this context, the results from Sima de las Palomas underline the consumption of processed foods from the Neanderthal population of the site, which lead to less worn facets on dental remains due to the use of fire and more developed lithic technocomplexes in the preparation of food.

Discussion

The majority of the described analyses include conducted examinations on the buccal surfaces of the dental remains using scanning electron microscopes, such as in the cases of Devil's Tower, Figueira Brava, El Salt, and Sima de las Palomas sites. Based on the results above, it can be easily extracted that the diets of Neanderthal populations of the Iberian Peninsula are totally correlated to the environmental context, in which they inhabited. However, concerning the relatively small number of examined Neanderthal sites of the Iberian Peninsula through dental microwear analysis, along with the scarce pieces of information regarding demographic dynamics on the majority of them, it should be clear that the described results reflect the dietary habits of the populations in the studied cases with the existing data up to date.

Neanderthal populations on sites, which are located in a coastal context or/and a low elevation, are characterized by relatively less worn facets, whose possible level of abrasiveness is justified by the intake of adherent particles to the meat, such as in the cases of Devil's Tower, and Zafarraya, since the coastal or nearly coastal environments imply the existence of sand. The effect of the ingestion of exogenous particles during the masticatory activity has been already highlighted by experimental studies on mammalian dental remains (Louail et al 2022; Uzunidis et al 2021). On the contrary, the inhabitation of sites, which were located in environments with the presence of deciduous forests or/and woodlands, resulted in a bigger level of wear to the feeding apparatus due to the implementation of plants or/and hard roots in the alimentary processes, such as in the cases of Gruta da Figueira Brava, El Sidrón, and El Salt. Interestingly, when comparing the sites in chronological terms, a shift in the dietary patterns cannot be observed; this shift only occurs when considering the different palaeoecological placement of the sites, confirming the total correlation between the diets of the populations and the palaeoenvironmental context.

When comparing the dental microwear signatures between different sites from Europe and Western Eurasia, it can be easily understood that the Neanderthal behavior didn't change significantly with respect to different Marine Isotope Stages; on the contrary, the climatic fluctuations led to the adoption of different dietary habits, with the implementation of hard roots or/and plants in the cases of the cold-stepped environments (El Zaatari et al 2011). Indeed, dental microwear texture analysis on hunted ungulates has exhibited these differences in the palaeoecological statuses between MIS 5 and MIS 3, with the prevalence of more open environments and lower temperatures in the European continent (Berlioz et al 2023). Inside this context, it doesn't appear that the environmental conditions led to the gradual demise of the Neanderthal populations, which only occurred after the arrival of the AMHs (Timmermann 2020).

To obtain a holistic approach regarding the dietary habits of the populations of a site, the contextualization, and verification between the results of different types of analyses are required, imparting certainty regarding the archaeological interpretation. Inside this context, the low anisotropy levels on the dental remains of Vindija site, Croatia, indicate a mostly meat-based diet, which has been confirmed by the stable isotopic analysis from the site (Karriger et al 2016; Richards et al 2000). Specifically, the high values of $\delta^{13}\text{C}$ on the Neanderthal remains of the site have suggested the intake of animal protein on a similar level with carnivores of the Croatian plateau, such as the Arctic fox and the Wolf. It is without consideration that the appliance of both dental microwear analysis and stable isotopic one on sites of the Iberian Peninsula can provide comprehensive approaches concerning the alimentary tendencies of Neanderthal populations, such as in the case of Moros de Gabasa site (Jaouen et al 2022).

In the last decades, the appliance of analyses on dental remains on a further scale has reversed the perception of the total dependence of Neanderthal populations on the consumption of meat in their diets (Binford 1981, 1984). These assumptions were based on the correlation between faunal remains on Neanderthal levels, indicating a wide and—in some cases—solely exploitation of faunal resources. However, studies on dental calculus have demonstrated the introduction of plants for alimentary purposes in Neanderthal populations from a wide range of geographical latitudes, from the Iberian Peninsula (El Sidrón) to Northern Europe (Spy I and II), and Western Asia (Shanidar III and Douara

Cave) (Akazawa 1987; Henry et al 2010; Radini et al 2016). The above statement was confirmed by a subsequent metagenomic analysis of the dental calculus from two individuals of the El Sidrón site, which exhibited the consumption of pine nuts (*Pinus koraiensis*), and forest moss (*Physcomitrella patens*), among others (Weyrich et al 2017), even though a study by Dickson et al (2017) attributed the presence of the latter (*Physcomitrella patens*) to contamination or taphonomic reasons. The described elements indicate the high cognitive level, which characterizes the populations of *H. neanderthalensis*, along with endured adaptive skills.

Conclusions

From the described data above, it can be understood that the applied dental microwear analyses on dental remains of the Iberian Neanderthals have provided important insights regarding palaeoecological issues and have enhanced the scientific community with data regarding efforts of palaeoenvironmental reconstructions. Explicitly, the diets of the referred populations are characterized by a general consumption of meat, with an opportunistic implementation of plants and/or hard roots in the dietary habits in the cases of Figueira Brava, El Sidrón, and Zafarraya. The adoption of the exposed alimentary manners underlines the total correlation between the environmental context and the dietary habits of the populations, along with the behavioral complexity, which characterizes the Neanderthal populations of the Iberian record.

In general, the importance of dental microwear analysis in the discipline of Archaeology is laid in the understanding of one of the most vital elements for the survival and dominance of the genus *Homo* throughout our evolutionary line, which is the obtainment of pieces of information with respect to the subsistence strategies and dietary habits of extinct populations. It is without any consideration that technological development, along with the appliance of new means of research could provide more stable methodological frameworks, and quantitative approaches, and enhance our knowledge regarding the alimentary behaviors of populations of the past.

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Preliminary results of the appliance of Dental Microwear Analysis on Iberian Early Bronze Age populations.

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Introduction

The dental microwear analysis has been proven to provide important insights regarding issues of paleodietary habits, behavioral and cognitive patterns, and palaeoecological proxies (Krueger et al., 2017). This study is focused on the analysis of two dental remains from the sites of El Bordellet and El Porroig, which are located in the Eix Diagonal (Barcelona province, Catalonia) (Fig. 1). Our aim is to provide a methodological approach comparing Optical and 3D microscopy for the conduction of Dental Microwear Analysis on buccal surfaces.



Fig. 1: Geographic placement of Eix Diagonal (Barcelona Province, Catalonia).

Methods

Prior to the microscopic observations, molds and casts were created from the retrieved dental remains. Polyvinylsiloxane was used for the creation of the molds, while an Epoxy resin (Epo-Tek 301) was used to produce high-resolution casts from the molds. Subsequently, the high-resolution replicas were primarily observed with the use of a ZEISS AxioScope A1 optical microscope with a Differential Interference Contrast system (DIC) at a magnification of x50 and x100 (Fig. 2), followed by a second observation with a Hirox KH8700 3D digital microscope at a magnification of x35, x50 and, x140 (Fig. 3). The quantification and the comparison of the striations were undergone with the use of ImageJ software on a surface of 0.56 mm² based on the obtained images from x100 magnification from Optical microscope, and from x140 from the 3D digital one (Hernando et al., 2020).

Materials

The total sample consisted of 17 individuals from El Bordellet site, along with one individual from El Porroig site. We decided to include a deciduous tooth in this study aiming to examine possible differences between permanent and deciduous enamel during chewing processes. We chose permanent lower second molars from two individuals from El Bordellet site and one deciduous lower molar (dm¹) from El Porroig site to test differences on dental microwear signals due to their mechanical properties during the mastication processes. The teeth were retrieved during the excavation processes of 2010 on both sites; both individuals from El Bordellet site are adults, while the dental remain from El Porroig site is attributed to a child of about three years old.

Results & Conclusions

The described analysis faced methodological issues due to the differences in the angle of the obtained images on the sample from El Bordellet site due to the different type of lens or microscopes. The comparison between both types of microscopes demonstrates that in the case of the dm¹, we are able to observe a clear difference between the obtained image from the Optical Microscope, in which the scratches are visible, and the one from the 3D Hirox digital microscope (Fig. 4). The enamel patterns from El Bordellet site demonstrate the adoption of a generally abrasive diet, even though the results remain preliminary. Moreover, due to the soil properties, some dental remains from the sites have experienced post-mortem enamel alternations, thus not permitting the extraction of pieces of information through Dental Microwear Analysis (Armentano and Nociarova, 2012). The results demonstrate the need for the appliance of observations through different types of microscopes in order to acquire a holistic approach regarding issues of enamel alternations (Table 1).



Fig. 2: Observation of dental features with the Optical microscope.

	OM	3D
Total Number of Scratches	36	47
Average Length	146.48 μm	111.79 μm
Mean error	34.69 μm	

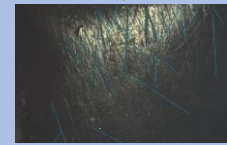


Table 1: Comparison between the measurements of each microscope.



Fig. 3: Observation of dental features with the Hirox 3D digital microscope.

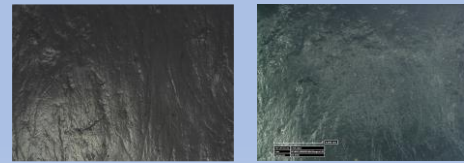


Fig. 4: Clear differentiation between the results of the microscopes regarding the sample from El Porroig site (left: Optical Microscope – right: 3D Hirox digital microscope).

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Aquí presentamos mediante una aproximación bibliográfica cuál es la teoría y método actualmente disponible para crear modelos predictivos utilizando el registro arqueológico como cuerpo de datos, y resolver así preguntas cruciales acerca de la evolución de las poblaciones humanas en la Prehistoria. Como conclusión, plantearemos nuevos horizontes de trabajo para una Arqueología teóricamente fundada y con una metodología testada en otras disciplinas satisfactoriamente, que aspire finalmente a la síntesis de las ciencias que estudian.

2. Experimental approach provides new insights regarding the morphology of paramasticatory dental features in human teeth.

Aristeidis Strimenopoulos, Erica Rosolani y Carlos Villa Sola (Universitat Rovira i Virgili)

Palabras clave: Dental Microwear Analysis, Experimentation, Lithic Flakes, Paramasticatory activities, Raw Materials.

Experimental approaches have proven to provide important insights concerning the understanding of human practices and behaviors in the past. Dental Microwear Analysis (DMA) is defined as the examination of the non-pathological loss of the enamel, which can be attributed either to the consumption of specific foods or to the usage of the feeding apparatus during the conduction of a paramasticatory activity. Concerning the understanding of behavioral patterns, DMA aims in the examination of dental features, such as enamel chipping and striations, which imply the usage of the mouth for the stabilization of an object during an activity, as it has been identified in the archaeological and ethnoarchaeological records. The specific study aims in the examination of the morphological differentiation of the paramasticatory dental features, which are produced in the framework of an experimental approach. The examined samples consist of 17 dental remains, which include both deciduous and permanent dentition, while the applied different raw materials are comprised of flint and quartzite in order to examine a possible morphological influence of the raw material concerning the production of dental features. Furthermore, the methodological framework consists by the utilization of two different microscopes (Optical and 3D Digital) for the obtainment of a holistic understanding of the produced dental features. In this framework, we anticipate our participation to provide a new contribution concerning the morphological differentiation of the non-alimentary dental features on the enamel surfaces of human teeth through the combination of different microscopic applications.



Title:

Unraveling the behavioral patterns of past populations through the study of dental wear:
The examination of 2 case studies from the recent Prehistory of the Iberian Peninsula

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Abstract (250-300 words maximum)

The study of dental wear has been proven to give valuable information about the alimentary and behavioral habits of extinct and current populations. Dental Microwear Analysis (DMA) is laid in the detection and examination of dental features, such as striations, pits, grooves, and enamel chipping among others, which can be traced in the enamel surface and imply either the adoption of specific alimentary habits or/and the utilization of the mouth during the conduction of a paramasticatory activity. Concerning the latter, both the archaeological and ethnoarchaeological records have confirmed the practice of the manipulation of different items in the mouth, which in turn reveals specific wear turnover. This study is laid in the detection of wear patterns in the enamel surfaces, which are attributed to paramasticatory activities, thus revealing the behavioral patterns of the studied populations. The examined dental samples are derived from the sites of El Bordellet and Galls Carboners, which are located in Catalonia (Northeastern Iberian Peninsula) and are dated in the Early Bronze Age and Middle Bronze Age respectively. Moreover, the methodological framework consists of the production of replicas and the subsequent application of different microscopic equipment (Optical Zeiss and 3D Digital Hirox microscopes) on both retrieved archaeological materials and produced replicas, thus providing the opportunity for the extraction of information through the combination of different approaches. In this context, we anticipate our participation to contribute to the further understanding of the behavioral patterns of the Iberian populations during the Late Prehistory through the study of wear patterns in a holistic methodological framework for the finest interpretation of the examined dental features.



ACTIONS WITH AN IMPACT: EXPERIMENTAL APPROACH FOR THE UNDERSTANDING OF PARAMASTICATORY STRIATIONS ON DENTAL REMAINS

Aristeidis Strimenopoulos^{1,2}, Erica Rosolani^{1,2}, Carlos Villa Sola^{1,2}, Constanza Arecheta Baratelli^{1,2} & Marina Lozano^{2,1}¹Facultat de Lletres, Universitat Rovira i Virgili, Tarragona, Spain²Institut Català de Paleoeologia Humana i Evolució Social (IPHES-CERCA), Campus Sescelades URV (Edifici W3), Zona Educacional 4, Tarragona, 43007, Spain

Experimental approaches have proven to provide insights into the understanding of past human practices, along with answers about the conduction of activities with subsequent cognitive implications. Dental Microwear Analysis (DMA) is defined as the examination of the non-pathological loss of dental tissue, which reveals potentially both information on the dietary habits and the behavioural level of the examined individual (Estalrich *et al.*, 2017). Concerning the latter, the use of the mouth for the stabilization of an object during the conduction of an activity has been a well-documented practice during the evolutionary line of the lineage of *Homo* (Lozano-Ruiz *et al.*, 2004; Lozano *et al.*, 2017), while the described practice has been documented as well in the ethnoarchaeological record (Lalueza Fox, 1992), producing a cutmark-like feature on the enamel surface. This study aims to the examination of the morphological differentiation of the paramasticatory dental striations, which are produced in the framework of an experimental approach with the use of different raw materials. The examined dental record consists of both deciduous and permanent dentition, while the applied raw materials derive from diverse geographical latitudes (United Kingdom and Spain) and are characterized by different mineral properties (flint, limestone, and quartzite) (Eixea *et al.*, 2016). Furthermore, the following methodological framework is comprised of the use of different microscopic equipments (ZEISS Axioscope A1 Microscope and Hirox KH8700 3D Digital Microscope) aiming to measure distinct aspects of the produced paramasticatory striations and to understand the level of complementation among the different methodological frameworks for the archaeological interpretation. In this context, we anticipate our study to provide new insights into the morphological properties of the non-alimentary striations on dental remains, along with a further understanding concerning differences in the enamel properties of deciduous and permanent dentition following a holistic methodological framework.

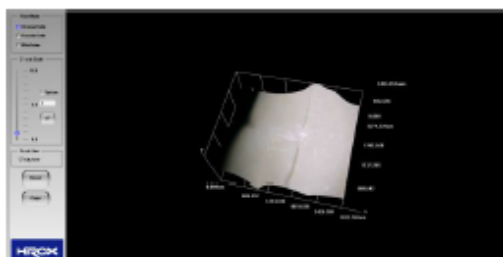


Figure 1: 3D model of a paramasticatory dental striation produced with a quartzite flake, x100, Hirox KH8700 3D Digital Microscope.

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