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ANCIENT FOOD HABITS OF A BRONZE AGE POPULATION LIVING IN SOUTHERN ITALY

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ABSTRACT

The aims of this study are to investigate the nutritional behavior of the Bronze Age communities living in Southern Italy, highlighting possible links between dietary intake and environments, culture heritage and health status of these ancient populations. The Bronze Age dated back to 2300 BC and was conventionally classified in Ancient Bronze Age (2300-1700 BC), Middle Bronze Age (1700-1350 BC) and Late Bronze Age (1350-950 BC). We have chosen this archaeological period because of the implications for nutritional behavior due to social changes and innovations in livelihood strategies. In particular, the specialization of agriculture and livestock reached the maximum increase during this Age. The investigated sites were selected on the basis of geographical location in order to point out differences depending on availability of resources and environment. For this purpose they were grouped as follows: Group 1, inland hilly/mountain sites of Calabria (Grotta della Monaca, Grotta du' Scuru, Grotta di Donna Marsilia, Grotta dell'Antenato, Grotta di Sant'Angelo); Group 2, inland hilly sites of Basilicata (Toppo d'Aguzzo, Murgia Timone and Grotta Funeraria); Group 3, inland plan of Apulia (Ipogeo dei Bronzi). The reconstruction of oral health and dietary patterns of ancient populations was carried out by means of traditional and innovative anthropological analyses: 1) the stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$ ratios) on bone collagen extracts; 2) analysis of microscopic patterns (microwear) of tooth surfaces; 3) analysis of macroscopic patterns diet-related on teeth.

Stable isotopes analysis (carbon and nitrogen) are useful to investigate the feeding habits of ancient communities, focusing on the protein intake of the diet. In particular, they allowed to define the ecosystem of origin of consumed foods (marine or terrestrial) and the type of diet (vegetarian or carnivorous). Furthermore, carbon ratios specify the type of vegetables consumed (C_3 plants or C_4 plants) and nitrogen ratios are appropriate to understand the animal intake (herbivores or carnivores). The analyses were carried out on 33 human collagen extracts and 12 faunal extracts using the Mass Spectrometry. Results pointed out a mixed diet, relied on the consumption of terrestrial resources: C_3 crops (cereals and legumes) and animal products (meat and dairy foodstuffs). The agricultural crops looked mostly consumed by individuals, while animal protein intake was moderate for all the communities examined. A slight difference in animal consumption was pointed out among the three Groups, showing that the individuals from Basilicata (Group 2) had a diet richer in animal products than those from Calabria and Apulia (Groups 1 and 3).

The analysis of microwear was undertaken on 24 human teeth, using the Scanning Electron Microscope (SEM). Two types of patterns were pointed out: scratches and pits. Scratches are directional and not chaotic *striae* that originate when abrasive particles of food are dragged between opposite enamel surfaces of the teeth. Pits are holes or small cavities of the enamel tooth surface and result from compression of food or contaminant particles with the enamel surface during the chewing. Our data indicate a higher occurrence of the scratches than pits, showing a diet characterized by the high intake of fiber foods.

The sample of Grotta della Monaca (Sant'Agata di Esaro, Cosenza) was then chosen as case-study in order to reconstruct the diet using three different methods: stable isotope analysis, examination of microwear and the **analysis of macroscopic tooth patterns diet-related**. Regarding the last method the frequency of caries, calculus, wear and chipping were analyzed on human tooth surfaces. Our results, based on the three methods, pointed out a mixed diet - mainly consisted of terrestrial resources (vegetables, cereals, legumes and animal products), and characterized by the

high intake of fibers derived from agricultural products. On the contrary, the amount of animal protein looked limited. No consumption of marine/freshwater resources was attested for Grotta della Monaca population. In addition to the "direct" patterns of paleo-nutrition, hypoplasia of the tooth enamel was observed as an indicator of stress diet-related. Our results demonstrated that the stress episodes occurred during the weaning because of the transition to an adult diet of poor quality, preferably represented by carbohydrates.

Concluding, this study has contributed to integrate different information by different scientific approaches to assess the feeding behavior of the Southern Italian populations during the Bronze Age. Our findings indicated a mixed diet for all the analyzed contexts, based on the higher consumption of agricultural resources than livestock products. Results pointed out a similar pattern in the three Groups, with a higher intake of animal protein in communities of Basilicata than in other contexts. Although some sites are close to the sea or to a river, no evidence of marine or freshwater consumption was recorded in human diet. This result could depend on cultural implications that mirrored in the nutritional behavior of Mediterranean and Southern European Bronze Age communities. Though social differences (gender-related and age-related) occurred in terms of dietary intake only in individuals from Ipogeo dei Bronzi (Apulia), owing to the samples size we cannot exclude similar differences also in individuals from other sites, where there is a clear evidence of these in the archaeological assemblages found in the graves. In perspective, this research will be continued and deepened by a more extensive sampling in the area, expanding the sample sizes of single site, the number of sites and environments. Age- and sex-specific differences in diet and in general oral health could possibly be shown if complete skeletons will be available in the near future. Other studies on Bronze Age populations are needed to confirm and clarify our findings and to further contribute to the archaeo-anthropological knowledge of past populations in Southern Italy.

CHAPTER 1: INTRODUCTION

1.1 Premise and purpose

The reconstruction of oral health and dietary patterns of ancient populations can be carried out by means of traditional and innovative anthropological analyses. Most previous studies have analyzed macroscopically the teeth, calculating the prevalence of lesions and other pathologies. Only few recent studies are based on microscopic features of teeth or on stable isotope of bone collagen in order to reconstruct dietary behaviors. All these approaches are important and can contribute, in an integrate manner, to clarify the lifestyle and health in the past, especially in areas such as Southern Italy during the Bronze Age, that has been almost totally neglected in this regard.

Stable isotope analyses are useful to give information about the proteins consumed during the life. In particular, carbon and nitrogen stable isotopes have been shown to have a great potential in paleonutritional studies (De Niro and Epstein, 1980; Shoeninger and De Niro, 1984; De Niro 1978, 1981, 1987, etc.). *Carbon* is present in the atmosphere as CO_2 (carbon dioxide), with a constant ratio ^{13}C:^{12}C (1:100). During photosynthesis, plants assimilate a quantity slightly greater of ^{12}C than ^{13}C, setting the relationship between the two elements. The plants that fix carbon dioxide in a molecule composed by three atoms of carbon (said C_3 plants) incorporate a percentage of ^{13}C higher than those that fix CO_2 in a molecule composed by four carbon atoms (C_4 plants). These differences in the resulting isotope ratios are useful to distinguish a diet based on vegetables of temperate environments (C_3 plants) from one based on vegetables of arid environments (C_4 plants). In marine waters, the amount of ^{13}C is higher and plants have a higher isotope ratio than those on dry land, allowing to discriminate food habits linked to the marine environments. Even *nitrogen* isotopes are present in greater proportion in the sea. Thus, the relationship ^{15}N:^{14}N becomes also important to establish the environment of the foods consumed: low values indicate a diet based on terrestrial products, high values indicate a diet based on aquatic nourishment (Price *et alii*, 1985). In addition to the distinction marine/terrestrial food, the nitrogen content allows the difference among terrestrial plants: legumes from "non-legumes". In fact, the first plants fix atmospheric nitrogen through symbiotic microorganisms, making them richest of ^{15}N, while the “non-legumes” absorb nitrogen by the ammonium and nitrate ions in the soil (Price *et alii*, 1985). Finally, nitrogen stable isotopes reflect, more clearly than carbon, differences related to trophic level: they increase about 3‰ in each step of the food chain, distinguishing the type of animal food consumed. In particular, nitrogen allows to understand if the diet depends on carnivores (meat) or on herbivores (meat and dairy products). For this purpose, human isotope values should be compared to faunal isotope ratio of the same environment (Vogel and Van der Merwe, 1977; Minawaga Wada, 1984; Schoeninger and De Niro, 1984).

Microscopic patterns diet-related (dental microwear) are observable on the occlusal surfaces of human teeth using SEM (Scanning Electron Microscope). The rate of these patterns is useful for paleonutritional investigations because it depends on the types of food consumed, in particular on the amount and kind of abrasive particles contained in the food (Molnar, 1972; Bermúdez de Castro *et alii,* 2003; Kaifu, 2000; Kaifu, Kasai, Townsend and Richards, 2003). Authors distinguish two types of tracks: pits and scratches. *Pits* are holes or small cavities of the enamel tooth surface and the result from compression of food or contaminant particles with the enamel surface during the chewing (Maas, 1994; Schmidt, 2010). *Scratches* are directional and not chaotic *striae* that originate

from abrasive particles of food when they are dragged between opposite enamel surfaces of the teeth (Ryan, 1979). Pits are believed to be characteristics of an omnivorous diet and scratches of a mostly vegetarian diet, characterized by the high intake of high-fiber foods (Butler, 1952; Walker *et alii*, 1978).

Macroscopic patterns diet-related are observable on human teeth. These dental features are useful to obtain information about nutritional behavior of past populations (Hillson 1986, 1996, 2001; Kelley and Larsen, 1991; Buikstra and Ubelaker, 1994). In particular, caries, calculus, wear and chipping have proven to be useful as diet-related markers. Dental *caries* of hard tissue indicate a diet rich in carbohydrates as they are generally the result of sugars fermentation (Hillson, 1986; Larsen, 1997; Caselitz, 1998; Hillson, 2001). *Calculus,* a mineralized plaque accumulated on the tooth surface, relates to the consumption of high-protein foods: these proteins increase the alkalinity in the mouth and cause the precipitation of minerals in the oral fluids (Sheie *et alii*, 1989; Hillson, 1996). Dental *wear* of occlusal enamel is associated with the consumption of hard fibrous foods or the use of processing techniques that introduce abrasive elements into the food, as powder from grinding stones (Smith and Knight, 1984; Larsen 1997). *Chipping* is an irregular crack involving the enamel or enamel and dentin, and it provides information about both masticatory and non-masticatory activities (Milner and Larsen, 1991). Regarding diet, these lesions are useful to recognize the consume of hard food that scrapes the tooth surfaces. In addition to the "direct" indicators of paleo-nutrition, *hypoplasia* of the tooth enamel is an "indirect" indicator of diet. In fact, it is a marker of stress that may depend, among various causes, on the diet (Pindborg, 1970; Winter and Brook, 1975). This defect is caused by an interruption of amalogenesis process during the childhood and may be the result of a deficient dietary status that decreases the individual's immune defenses up to non-optimal health conditions (Solomons and Keush, 1981; Goodman and Rose, 1991).

The **aims of this study** are to 1) investigate the nutritional behavior of the Bronze Age communities living in Southern Italy; 2) explore possible links between specific dietary patterns and environments; 3) gain some insight in health status and food habits of these ancient populations using different approaches (stable isotope analysis - $\delta^{13}C$ and $\delta^{15}N$ - on bone collagen extracts; analysis of microscopic -microwear - and macroscopic patterns diet-related on human teeth).

1.2 The Bronze Age sites investigated

Among the different approaches used to obtain a representation of the ancient nutritional behavior, stable isotope and dental microscopic analyses were carried out on different Bronze Age communities of South Italy; while the third approach was applied only on the Bronze Age site of Grotta della Monaca (Calabria). The Bronze Age dated from 2300 BC to 950 BC and was classified by archaeologists in three crono-cultural phases: Ancient Bronze Age (2300-1700 BC), Middle Bronze Age (1700-1350 BC), Late Bronze Age (1350-950 BC). The choice of the period was due to the interest towards different innovations and social changes characterizing this Age. Archaeological data indicated that the settlements multiplied during the Bronze Age; some sites were fortified and others assumed the role of satellite-sites of the largest settlements. The good graves found in burials (usually ornaments for females and weapons for males) reveled a complex society characterized by people from different social status (Pearce, 2004; Bietti Sestieri, 2011). Although introduced by the previous Neolithic culture with new subsistence strategies, agriculture

and livestock reached their maximum increment during the Bronze Age. Some sites of Southern Italy were mentioned in the literature for their archaeological and historical importance as examples of the innovations that characterized this period (Broglio di Trebisacce in Calabria, Toppo d'Aguzzo and Lavello in Basilicata, Coppa Nevigata and Ipogeo dei Bronzi e degli Avori in Apulia) (Peroni, 2004; Bietti Sestieri, 2011).
In order to obtain information on the impact of these innovations on food habits, the following nine sites were selected in this study: Grotta della Monaca, Grotta di Sant'Angelo, Grotta dell'Antenato, Grotta di Donna Marsilia, Grotta du' Scuru in Calabria; Toppo d'Aguzzo, Murgia Timone and Grotta Funeraria in Basilicata; Ipogeo dei Bronzi in Apulia. Their localization is reported in the map (Fig. 1). The selection was made in order to represent all Southern regions and the different environments of this area: inland hilly/mountain sites of Calabria, inland hilly sites of Basilicata, inland plan of Apulia. The main information about each site are summarized in the descriptions reported below. It is important to remark that published data are not available for all these sites and the information was often drawn from unpublished archaeological reports at the Museums or the regional Superintendency. Human bone samples from Grotta dell'Antenato, Grotta di Sant'Angelo, Grotta Funeraria and Grotta du' Scuru were prepared for ^{14}C dating. In the present study, we will refer only to the archaeological dating for these sites, as the dating analyses are still ongoing.

Fig. 1. The Southern Bronze Age sites investigated.

The inland mountain - hilly sites of Calabria

Regarding the nine sites examined in this study, five of them are located in the Calabria region. The skeletal remains analyzed were sampled through the collaboration of the Museo Archeologico Nazionale della Sibaritide and Centro Regionale di Speleologia "Enzo dei Medici", after obtaining the permission of Soprintendenza dei Beni Archeologici della Calabria. All skeletal remains analyzed were found in caves. They were in poor state of preservation and they were not connected anatomically. Although grave goods have been found in the caves, in most cases they were not directly related to the examined individuals.

Grotta della Monaca (GdM)

Grotta della Monaca is a karsic cave placed in Sant'Agata di Esaro (Cosenza), in North-Western Calabria. The cave entrance is located on the top of a very steep rocky peak in the Upper Esaro River Valley, at 600 m above sea level (Fig. 2 a, b). The cave is about 500 m long and it has been divided by archaeologists in three mainly sectors: Pregrotta, Sala dei Pipistrelli and Cuniculi Terminali (Fig. 3) (Larocca 2005, 2011). Archaeological research, started in 2000 and they still ongoing, dated back the human frequentation of the site from the Upper Palaeolithic to post-Medieval times. The long relationship between Man and the cave was due to its richness in metallic minerals, especially iron and copper ones (Larocca and Levato, 2013; Larocca and Breglia, 2014). During the Bronze Age the mining interest decreased and a burial ground was implanted in the final deepest sectors of the cave: "Sala dei Pipistrelli" and "Cuniculi terminali". Here, five burial areas were detected in niches and fractures of the rock (Fig. 4 a, b). The skeletal remains were commingled and not anatomically connected. The grave goods consisted of few elements in poor state of preservation and not clearly associated with individuals (Arena *et alii*, 2014; Arena and Gualdi, 2014). Besides many pottery vessels, ornaments and fossil shells of *Cypraea* sp. (probably used as necklace pendants) were found. Furthermore, a spindle-whorl, connected to weaving activities, were discovered (Larocca, 2005). Evidences of funerary rituals carried out in the cave were recorded, such as the intentional breaking of metal swords and the deposition of animal parts (Fig. 5a, b). In particular, burned skeletal remains of a *Sus scrofa* were found in the final part of the Pregrotta, near the necropolis (Larocca, 2005). Radiocarbon analyses performed on human skeletal remains dated the burials to the Middle Bronze Age, about half of the II millennium BC. Evidences indicate that the necropolis was used for 300 years, between 1700 and 1400 BC (Arena *et alii*, 2014).

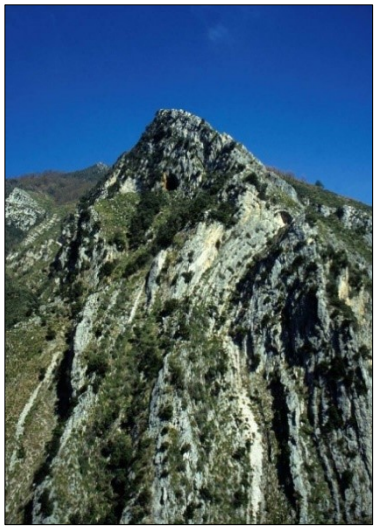


Fig. 2a. On the left, the cave's entrance.
Fig. 2b. On the right**,** the cave's entrance by internal view.

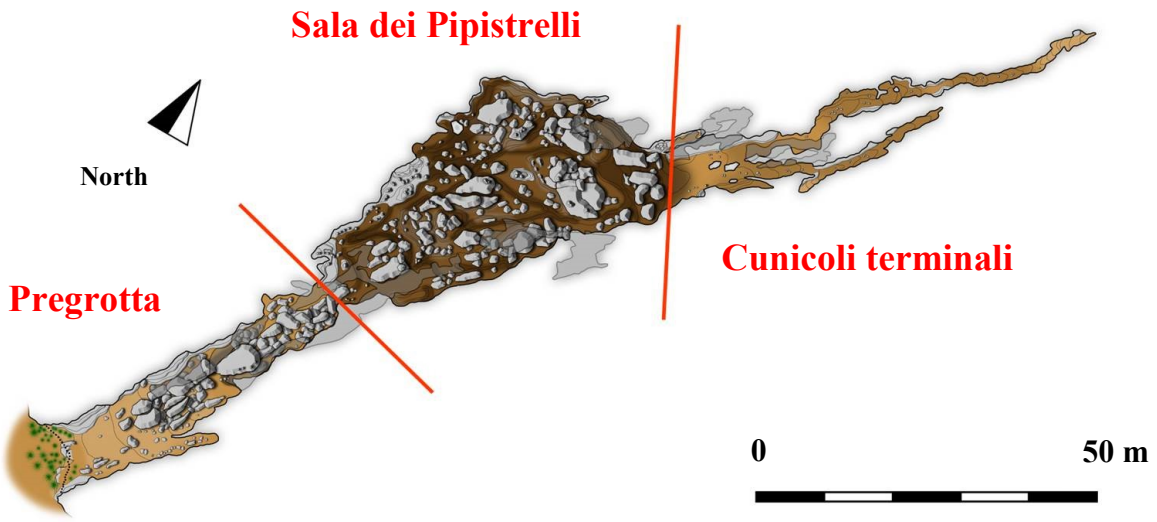


Fig. 3. The plan of the cave with the indication of the three main hypogeal sectors: Pregrotta, Sala dei Pipistrelli, Cunicoli terminali.

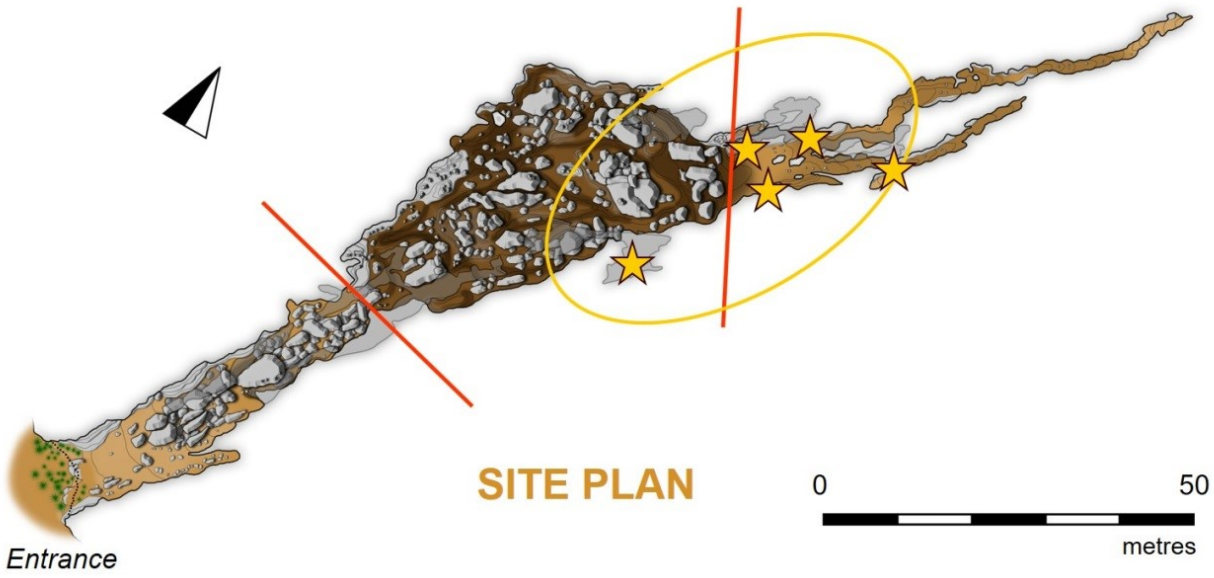


Fig. 4a. The five burial areas in the deepest sectors of the cave.

Fig. 4b. Sala dei Pipistrelli: a burial area inside a fracture of the cave's wall.

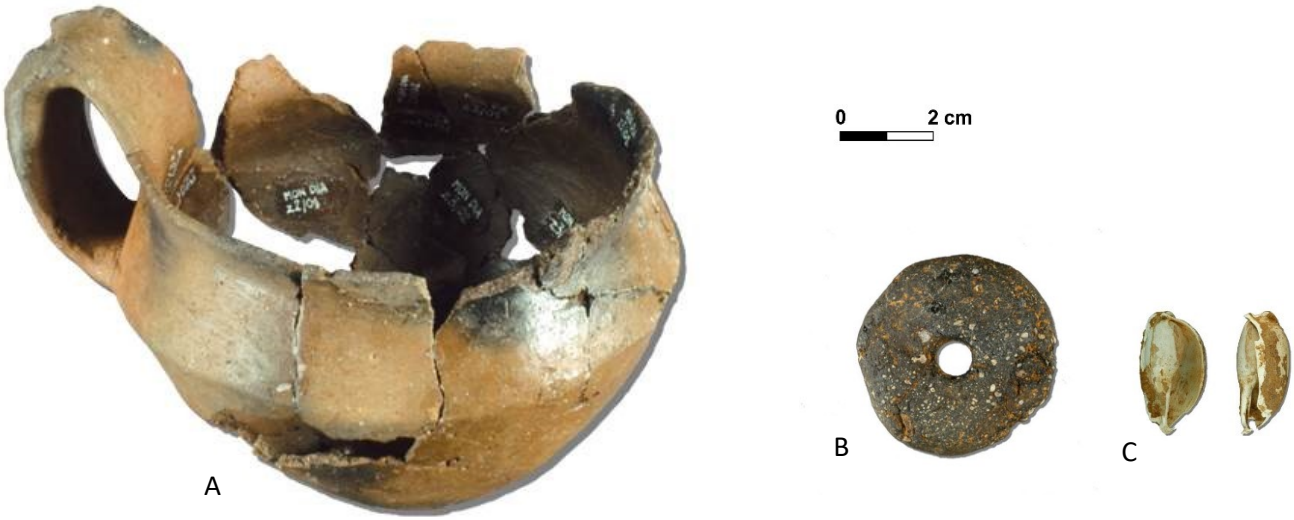


Fig. 5a. The grave goods found: A= Bronze Age vessel; B= spindle-whorl; C= a fossil shell *Cypraea* sp.

Fig. 5b. Evidences of funerary rituals: A= a burned humerus of *Sus scrofa*; B= broken swords.

Grotta di Sant'Angelo (GdSA)

The cave places in the North-Eastern Calabria (Cassano allo Ionio, Cosenza), in the area of the Mount St. Marco (502 m above the sea) and Muraglione (593 m above the sea) characterized by a series of small hills. The cave was discovered by a team of speleologists (Commissione Grotte E. Boegan) in 1977-78. It develops on two levels, one upper, called "Grotta di Sant'Angelo superiore", and the other lower, called "Grotta di Sant'Angelo inferiore" (Gasparo, 1979) (Fig. 6, 7).

Grotta di Sant'Angelo superiore opens with two entrances on the North-western side of the Mount St. Marco-Muraglione system. It is a cave extremely complex, characterized by a series of horizontal tunnels that develop for about 1000 m at different heights, in correspondence of two main levels: 440 m and 425-430 m above the sea level. The thin deposits contained clays, guano and gypsum. During the first investigation of the cavity (1978), the speleologists discovered human skeletal remains associated with numerous vessels dated back to the Ancient Bronze Age (Fig. 8 a, b) (Gasparo 1979, 1980).

Grotta di Sant'Angelo inferiore is located under the Southern entrance of the Grotta superiore. The initial tunnel opens in a large cave with the bottom occupied by clastic material and blocks of considerable size (up to several hundred cubic meters). No human remains were found by speleologists (Gasparo 1979, 1980).

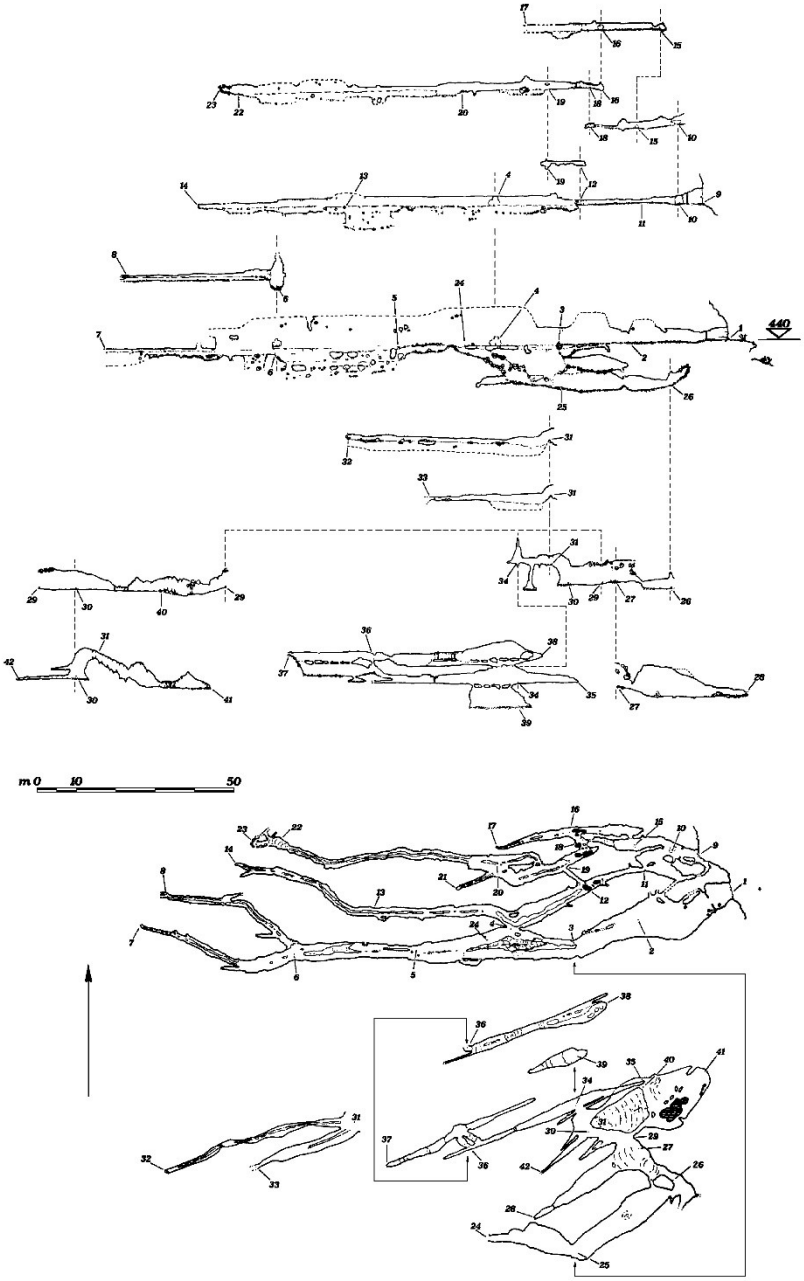


Fig. 6. Plan of Grotta di Sant'Angelo Superiore (Gasparo, 1979).

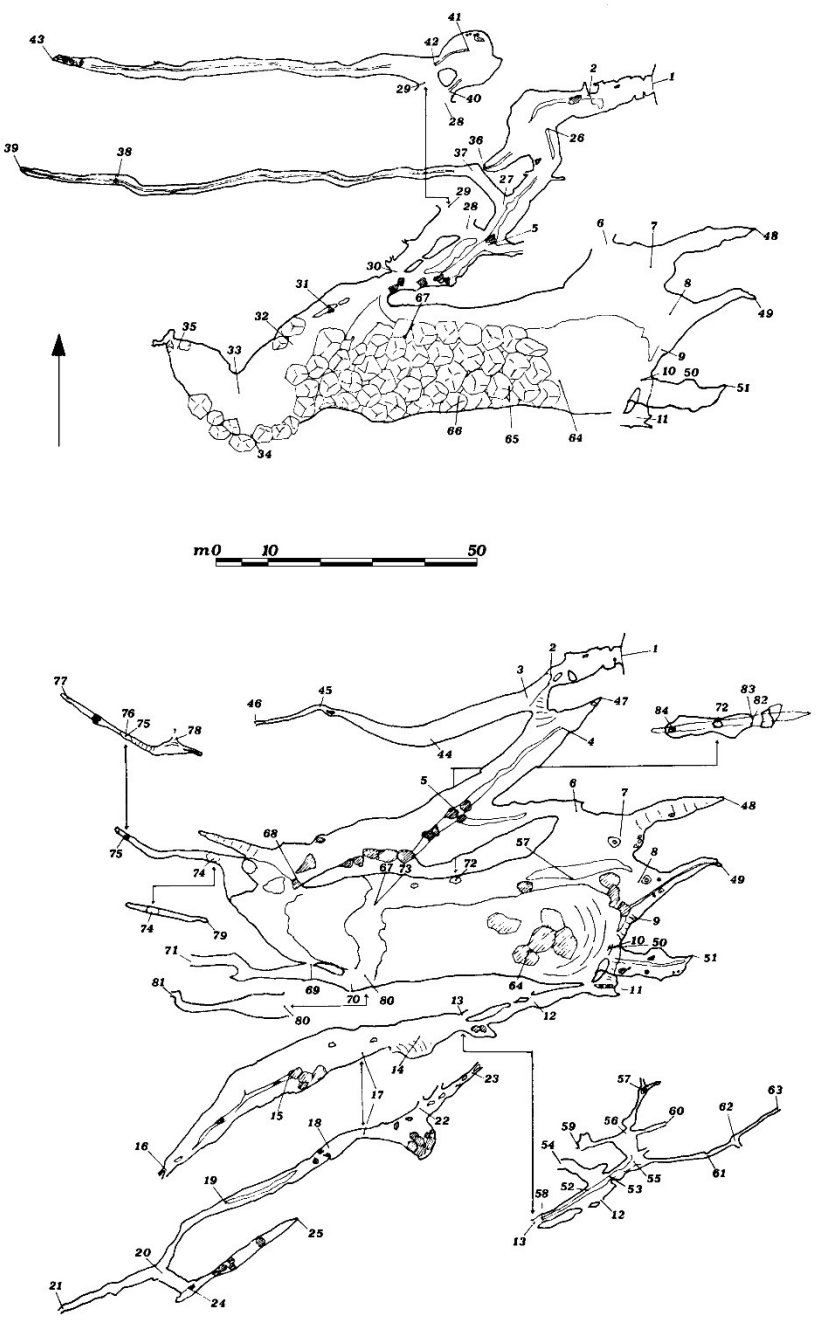
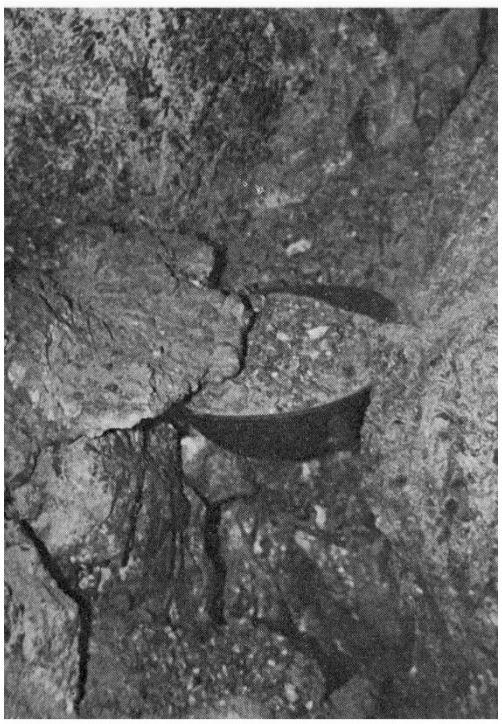
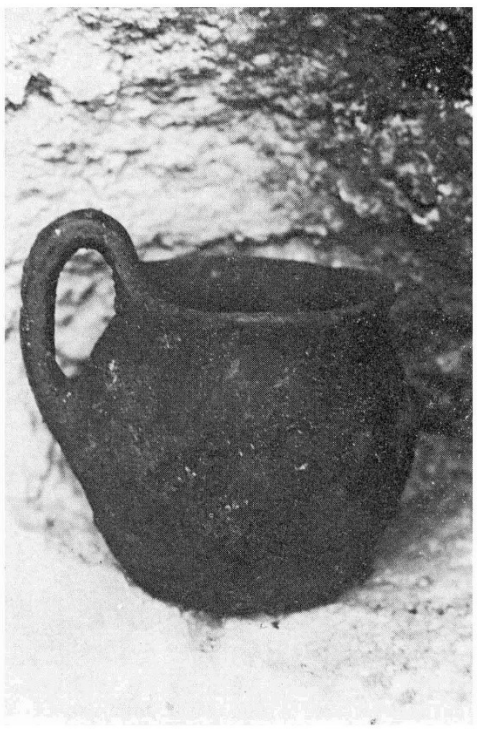


Fig. 7. Plan of Grotta di Sant'Angelo Inferiore (Gasparo, 1979).



A



B

Fig. 8a-b. Vessels discovered inside Grotta di Sant'Angelo Superiore by Commissione Grotte E. Boegan, between points 20 and 22 of the plan (Gasparo, 1980).

Grotta dell'Antenato (GdA)

The cave is placed in the North-eastern Calabria (Cassano allo Ionio, Cosenza) in the area of the Mount St. Marco-Muraglione system (as the Cave previously described). It was named "Grotta dell'Antenato" by Kleibrink (2006) and recently indicated as "Grotta Sant'Angelo IV" because of its topographical position (Ippolito, 2016): from the entrance of the Archaeological park, Cave IV is located to the left of Sant'Angelo caves (Fig. 9). The site was discovered by speleologists in 1998: archaeological findings indicate that the cave was used as burial ground from the Middle Eneolithic to the end of the Early Bronze Age (Ippolito, 2016). The patronage of the funerary site was classified in three different phases by archaeologists based on of the typology of pottery and the radiocarbon dates performed on human and faunal remains. Among anthropological findings, the presence of three individuals was recorded: a young subject (point A in the map) dated to the end of the Middle Eneolithic (1405-1295 BC); two individuals: one inhumed adult and a secondary burial in a ceramic jar containing some long bones (point F in the map), dated back to the Late Eneolithic-Early Bronze Age (Fig. 10). The animal bones (found at point G and, to a lesser extent, at points B-C-D) demonstrate that one sheep/goat, one pig, one bovine and one wild animal (boar or red deer) were carried out into the cave during the final phase of the Early Bronze Age (1690-1610 BC). The faunal and human skeletal remains sampled in this study belong to the Early Bronze Age. Archaeologists asserted that from this period the funerary use of caves was preserved only for some particular categories of individuals (Cocchi Genick, 1999; Bietti Sestieri, 2011). The demographic growth of communities, due to the development of agricultural practices, may have resulted in social stratification with individuals to which a more complex funerary ritual was reserved,

involving votive offerings and banquets, as suggested by the animal remains and ritual vessels found in Grotta dell'Antenato (Ippolito, 2016).

Fig. 9. Plan of Grotta dell'Antenato (Ippolito, 2016).

Fig. 10. The jar found in association with bones (Ippolito, 2016).

Grotta di Donna Marsilia (GdMR)

The cave is located close to Morano Calabro (Cosenza), in a hilly area called "contrada Sassone" (Fig. 11). It was discovered in 1960 by Agostino Miglio an later explored by Santo Tinè (Fig. 12). The archaeological and anthropological evidences were found during a survey in 1962. In 1983,

after they were examined by the archaeologist S. Luppino, they were stored in the Museo Archeologico Nazionale della Sibaritide. The human skeletal remains belong to a single individual: unpublished archaeological data indicate that they were associated with faunal remains and Eneolithic-Bronze Age vessels (reported by archaeologists of Museo Archeologico Nazionale della Sibaritide).

Fig. 11. Contrada Sassone (Morano Calabro - CS) (Google image).
Fig. 12. The entrance of the Grotta di Donna Marsilia (Google image).

Grotta du' Scuru (GdSc)

The cave is near Amantea (Cosenza). Recent surveys - carried out by speleologists and archaeologists of the Centro Regionale di Speleologia "Enzo dei Medici" in 2012 and still ongoing - pointed out the archaeological importance of the cavity (Fig. 13, 14). Human skeletal remains, discovered in the site, belonged to two individuals: one adult and one adolescent. Unpublished archaeological data referred that the remains lied in association with pottery of latest phases of Bronze Age (reported by archaeologists of Centro Regionale di Speleologia Enzo dei Medici).

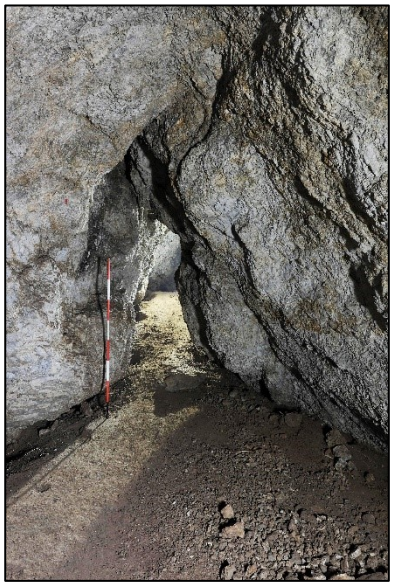


Fig. 13. The entrance of the Grotta du' Scuru (from archives of Centro Regionale di Speleologia "Enzo dei Medici").
Fig. 14. Grotta du' Scuru: internal view (from archives of Centro Regionale di Speleologia "Enzo dei Medici").

The inland hilly sites of Basilicata

A total of three sites from Basilicata region were included in this study. The osteological collections selected were sampled through the collaboration with the Museo Archeologico Nazionale "Domenico Ridola" of Matera and the Museo Archeologico Nazionale of Potenza, with the permission of the Soprintendenza dei Beni Archeologici della Basilicata. Also in these caves, the skeletal remains were in a poor state of preservation and not anatomically connected. Although good graves were found in the burials, they were not in clear association with the skeletons sampled in this study.

Murgia Timone (MT) and Grotta Funeraria (GF)

These two sites are located in the hilly area of Murgia (Matera), along the edges of the ravine of Bradano River. Here, several burials were discovered both at Murgia Timone and Grotta Funeraria. The graves consist in collective burials, typical of the Apennine Culture of Middle Bronze Age in Southern regions. These types of burials were probably reserved to elite as shown by the rich good graves. In particular, the presence of ornaments, weapons and vessels was recorded (Bietti Sestieri, 2011).

Published data and more detailed information regard only Murgia Timone. Archaeological excavations of the site - carried out between 1800 and 1900 by Ridola, Patroni and Rellini - discovered three graves (called Tomba 1, Tomba 2, Tomba 3), mentioned by archaeologists as "tombe a grotticella" that were typical of the Bronze Age Culture (Fig. 15). Tomba 1 contained 22 burials associated with 54 human skulls and burned bones. Tomba 2 was divided into two cells: cell A (with 2-3 individuals) and cell B (with 3 skeletons). Tomba 3 contained the highest number of depositions. The T1 and T2 dated back to the Middle Bronze Age, while T3 contained materials dated to the Iron Age. The anthropological samples from these graves were transferred to the Museo Archeologico Nazionale di Napoli in the last century by Patroni and, subsequently, they were sent to the Museo Archeologico ed Etnografico "L. Pigorini" in Rome (Quagliati, 1896; Patroni, 1897; Ridola 1901, 1912; Grifoni-Cremonesi 1976, 1999; Lo Porto, 1988; Tunzi Sisto, 1999; Matarese, 2014). We had the possibility to sample only the findings stored in Matera Museum: faunal remains of Murgia Timone and the human skeletal remains of Grotta Funeraria.

Fig. 15 a-b. Murgia Timone: the so called "Tombe a grotticella".

Toppo d'Aguzzo (TDT)

The ancient settlement of Toppo Daguzzo (Melfi) is located few kilometers away from Potenza, in the fertile farmlands of the Ofanto Valley. The systematic excavations conducted since 1980 discovered three burials along the slopes of the hill. The first grave, consisting of an underground structure with two compartments and a long access corridor, dated back to 16th-15th BC. The second burial was a pit grave placed chronologically in the same period and located few meters away from the former. The third consisted of a large underground chamber, dug into the tufa, with a long corridor (*dromos*) dated back to the Middle Bronze Age, more precisely, between 15th and 14th BC. Inside the room of the third burial, the remains in poor state of preservations of about ten individuals without good graves were found. A second layer with a situation completely different was discovered about twenty centimeters in depth: two rooms were recorded. In the first one, there were evidences of funerary rituals (with vessels and burned faunal remains); in the second room there were six males with weapons (connoted as warriors), four women (most of them with ornaments), and a child (Fig. 16 a, b). Different vessels were deposed close to the infant deposition (Fig.17, 18). It was interpreted as the burial of a warrior aristocracy (Cipolloni Sampo', 1986a-b; Bietti Sestieri, 2011). The skeletal remains analyzed in this study belonged to these individuals. Some of them were stored in the Museo Archeologico Nazionale of Venosa: they were not in anatomic connection.

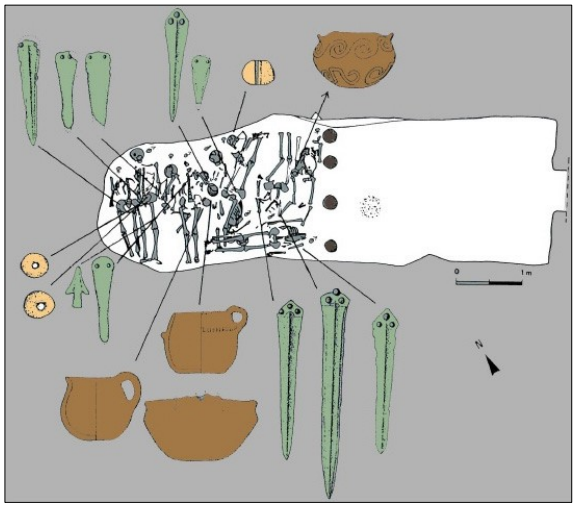


Fig. 16a. The burial n. 3 of Toppo d'Aguzzo (Goggle image).
Fig. 16b. Replica of a burial (Burial n. 3).

Fig. 17. The good graves of Burial n. 3: on the left, vessels.
Fig. 18. The good graves of Burial n. 3: on the right, a bronze blade.

Inland plan of Apulia

In this region, only one site has been examined. The skeletal remains of Ipogeo dei Bronzi were sampled through the collaboration with the Department of Biology (University of Pisa). They were in poor state of preservation and not anatomically connected. Althoug the presence of good graves was indicated by archaeological reports, the association with skeletons that we have sampled was not clear.

Ipogeo dei Bronzi (IpB)

The site is placed in the North of Apulia (Trinitapoli, Foggia), in a flat area of the promontory of Gargano (90 m above the sea level). It was a hypogeal burial ground dated back to the Middle Bronze Age (Fig. 15). Archaeological excavations recorded 200 individuals, both males and females of all age groups. Currently, some of the skeletons are lost and the remaining remains are in poor state of preservation (Bietti Sestieri, 2011). The archaeological record has recognized distinct groups of individuals, probably belonging to different family units. The male grave goods included weapons (swords and daggers), some knives and two razors. The female kits included bronze ornaments, bony objects and polished stones (counting amber, faïence and glass paste from the Aegean and the Eastern Mediterranean). Among pottery, cups, bowls, jugs and "pissidi" were found. Archaeologists assumed that the presence of several collective burials mirrored the social organization family-related; however, the record of grave goods richer than others allowed to assume different social status among individuals (Tunzi Sisto 1997, 1999; Vanzetti, 1999; Mazzei and Tunzi Sisto, 2005; Bietti Sestieri, 2011).

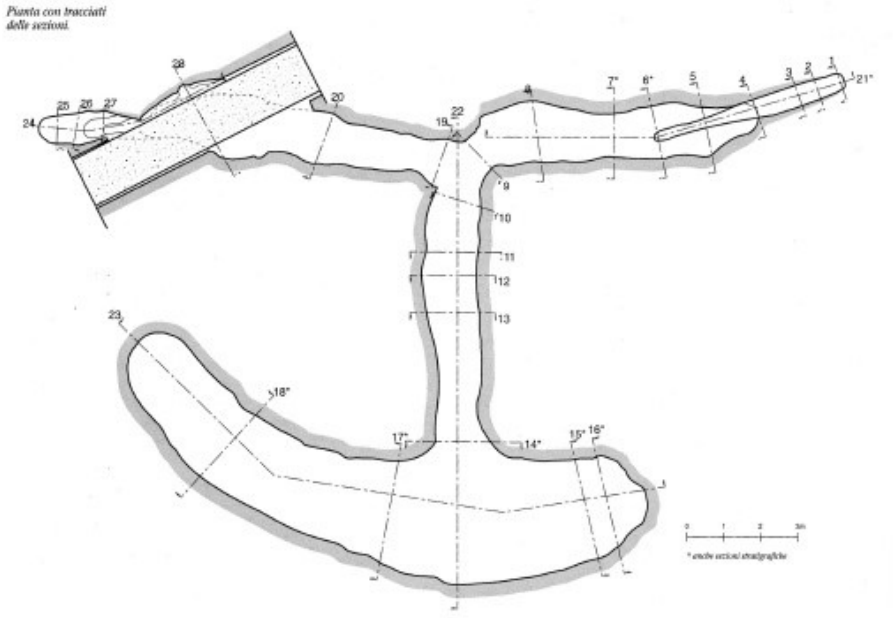


Fig. 15. Ipogeo dei Bronzi: plan (Google image).

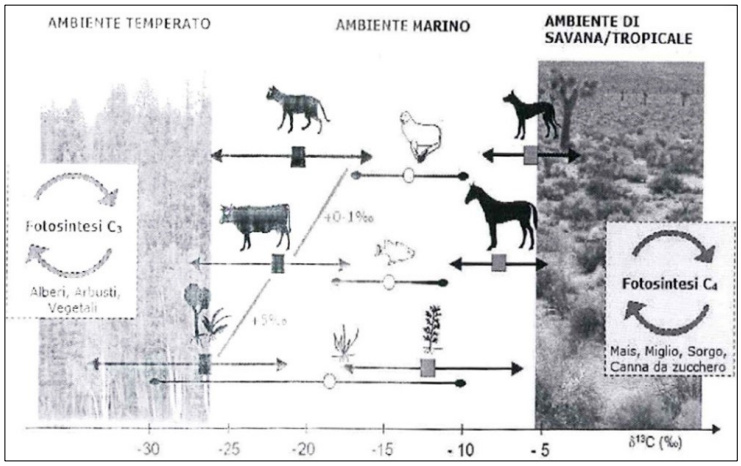
CHAPTER 2: STABLE ISOTOPES ANALYSIS AND DIET IN BRONZE AGE POPULATIONS LIVING IN SOUTHERN ITALY

2.1 Introduction

The use of stable isotopes for diet reconstruction is remarkable for the assumption that you are what you eat (Ambrose and Norr, 1993). Analysis of stable isotope ratios from mineralized tissues (bone or teeth) is a direct and widely-used technique for diet reconstruction (Reitsema, 2013) because it reflects a mixture of the isotopic signatures of foods consumed during an individual's lifetime (Shoeninger, 2011). In particular, stable isotope analysis on bone collagen reflects mainly the protein fraction of the diet. Carbon provides data on photosynthetic pathway (i.e. C_3, C_4 or CAM plants) and/or ecosystem of origin of the proteins consumed (terrestrial, freshwater, and/or marine). Carbon and, in particular, nitrogen isotopes ratios reflect the trophic level of the acquired dietary protein and increase by 0-1‰ for $\delta^{13}C$ and 3-5‰ for $\delta^{15}N$ every step of food chain (Shoeninger and De Niro, 1983; Bocherens and Drucker, 2003; Herrscher, 2003; Mannino, 2009; Mannino *et alii*, 2015) (Fig. 1, 2). Therefore, nitrogen ratios of faunal samples are useful to understand if proteins consumed by humans are derived by carnivores or herbivores (meat, milk and dairy products) consumption and/or their ecosystem of origin. In summary, stable isotope ratios give information on the type of diet, distinguishing broad food categories: for example, meat versus plants, terrestrial versus aquatic protein sources, C_4 plants versus C_3 plants (DeNiro 1978, 1981, 1987). The first applications of this method date back to 1977, when Vogel and van der Merwe analyzed carbon isotope ratios of Native American skeletons. These authors demonstrated that maize was not a significant component of the diet in the northeast United States until after 100 BC (Vogel and van der Merwe, 1977). Among subsequent studies, De Niro and Epstein (1980) used $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen to investigate the diet of population living in Tehucan Valley of Mexico over 7000 years span. Variation in the carbon and nitrogen values indicated that C_4 plants and legumes were introduced into the diet much earlier than suggested by conventional archaeological analyses. In 1983, Shoeninger and De Niro demonstrated that bone collagen $\delta^{15}N$ values were useful in determining relative dependence on marine and terrestrial food sources and, in investigation of trophic level relationships, among different animal species within an ecosystem (Shoeninger and De Niro, 1984; De Niro, 1985). The research of the dietary patterns in European prehistoric human populations has been widely studied (Triantaphyllou *et alii*, 2008; Lopez-Costas *et alii*, 2010; Goude *et alii*, 2011; McClure *et alii*, 2011; Nájera Colino *et alii*, 2012; Koch and Kupke, 2012; García Borja *et alii*, 2013; Salazar García *et alii*, 2013; Herrscher *et alii*, 2014), and only recently Italian prehistoric diet has been investigated through isotopic analyses. Lai *et alii* (2007, 2014) analyzed prehistoric communities of Sardinia; Lelli *et alii* (2012) performed stable isotopes analyses on earliest farmers of South-East Italy and Mannino *et alii* (2015) on Mesolithic hunters-gatherer diets in Sicily. However, regarding the Italian Bronze Age, nutritional investigations explored through isotopic approach were scarcely conducted. In particular, the first studies on stable isotopes analysis were carried out by Tafuri *et alii* (2009) on Northen (Olmo di Nogara, Sedegliano) and Southern Italy (Toppo d'Aguzzo and Lavello) and Lai *et alii* (2014) on Bronze Age communities of Sardinia. Recently, Varalli et *alii* (2016 a-b) carried out isotopic analyses on Early-Middle Bronze Age communities of Central-Northern Italy sites: Arano di Cellore (Southern Veneto), Grotta dello Scoglietto (Southern Tuscany), Grotta Misa and Falcetone

(Northern Latium). These authors demonstrated that the transition from Early Bronze Age to the Middle Bronze Age represents a moment of change, which is reflected by the presence of different dietary patterns. In particular, the Middle Bronze Age settlements of the Central Italy seem to be characterized by the introduction of new crops (such as millet). In contrast, the Southern Italian communities were characterized by a diet tied to Neolithic heritage based on C_3 plants consumption (i. e. wheat and barley) (Tafuri *et alii*, 2009). To date, studies on Bronze Age diet in Southern Italy are scarce, considering that only two contexts (Toppo d'Aguzzo and Lavello in Basilicata) were analyzed by isotopes analysis (Tafuri *et alii*, 2009).

This study aims to provide a more comprehensive representation about the diet of Bronze Age communities living in Southern Italy, in order to clarify the aspects emerged in previous research and possibly to add new information. In particular, we aim to extend the area of investigation, involving sites belonging to different geographical contexts and environments: the inland mountain-hilly sites of Calabria (Grotta della Monaca, Grotta du' Scuru, Grotta di Donna Marsilia, Grotta dell'Antenato e Grotta di Sant'Angelo), inland hilly sites of Basilicata (Toppo d'Aguzzo, Grotta Funeraria e Murgia Timone) and inland plain of Apulia (Ipogeo dei Bronzi). The explored period is the Bronze Age (2300-950 BC) because of the changes that occurred in relation to the subsistence strategies with specialized agriculture, becoming the main livelihood of the Mediterranean Bronze Age economy (Salvadei and Santandrea, 2003; Peroni, 2004; Bietti Sestieri, 2011).



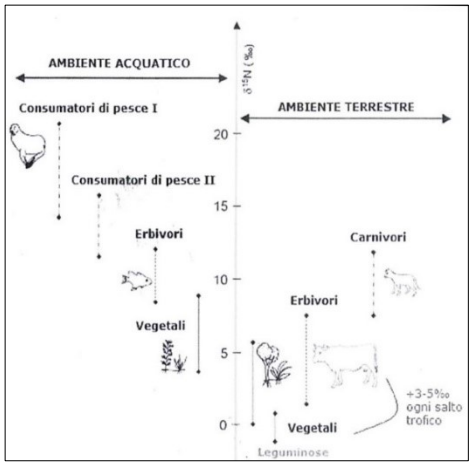


Fig. 1. Carbon fractionation according to trophic level (Herrscher, 2003).
Fig. 2. Nitrogen fractionation according to trophic level (Herrscher, 2003).

2.2 Materials and methods

2.2.1. The sample

A total of thirty-three human and twelve animal bone specimens were collected for isotope analyses from the sites described in the Chapter 1 (Introduction). Faunal samples are useful to compare human nitrogen ratios in order to understand whether proteins consumed derived by animal intake: carnivores or herbivores (meat, milk and dairy products). Given the funerary contexts, very few of the selected sites had associated fauna. Where animal bones were not available, specimens were collected in neighboring sites of the same area. Only adult bones were selected for both fauna and human specimens, in order to obtain information about a longer period of time, as bone turnover is

slower during the adulthood than adolescence and childhood. In fact the 20-50% of collagen of an adult femur, for example, aged back to adolescence, reporting isotope values of 10 years of the life of the examined subjects (Hedges, 2007). Both male and female bones were sampled for this study. The selection of anatomical parts to be cut was based on the bone state of preservation, according to criteria indicated by Schwarcz (1991). Moreover, when skeletons were not whole but commingled, only bones used for MNI (Minimum Number of Individuals) calculation were sampled. After selection, the cutting was carried out on bones by a micro drill (Dremel 3000), obtained sub-sampling approximately 2-3 grams of bone. Each specimen was then wrapped with pluriball paper and stored into a bag with an identification code.
In order to the subsequent statistical analyses, the specimens were grouped by geographical position and environment as follow: Group 1 (inland hilly/mountain sites of Calabria); Group 2 (inland hilly sites of Basilicata); Group 3 (inland plan of Apulia). All the remains dated back to the Middle Bronze Age, except those from Grotta di Donna Marsilia, Grotta dell'Antenato and Grotta di Sant'Angelo that dated back to Ancient Bronze Age and Grotta du' Scuru dated to Late Bronze Age.

2.2.2 Assessment of Minimum Number of Individuals (MNI) and biological profile of individuals: methods

The biological profile of humans was examined using the traditional anthropological methods. Conversely, faunal species were defined according to Barone (2006) and archaeological documentation available for consultation.

Minimum Number of Individuals (MNI)
When the reconstruction of skeletal identity was difficult, protocols known in literature (Cox *et alii*, 2008; Mallegni *et alii*, 1994; Pacciani, 1993; Mallegni and Rubini, 2002; Canci and Minozzi, 2005) were applied on commingled remains to estimate the Minimum Number of Individuals (MNI) of the examined necropolis. According to the cited authors, bones were classified by anatomical type (humerus, femur, tibia, etc.), side (left/right), sex (male/female), age classes (young adults/middle adults/old adults). Therefore, MNI was calculated on anatomical element more present *per* sex and age class than other parts of skeleton, using the following criteria:

- connection of skeletal remains belonging to the same anatomical element (e.g. diaphysis and epiphysis of femur)
- joints connection
- same bone maturation (criteria related to age of individuals)
- sex
- measurements: age-related differences or stature and strength of an individual (taking into account pathological asymmetries)
- symmetrical bones
- pathologies
- color of bones (independent from taphonomic processes)
- muscle insertions
- state of preservation (although bones of the same individual may have different levels of deterioration)

- archaeological information (with particular reference to position of bones in the archaeological stratigraphy)

The reconstruction of the skeletons (when possible) was done according to MNI bones at first, proceeding on the case of above criteria. The associations were based on unequivocal conformity. Therefore, in case of ambiguity, anatomical elements were not attributed to any skeleton.

Anthropological methods for sex diagnosis and age estimation in human skeletal remains

The poor state of preservation of ancient osteological materials made difficult the sex diagnosis and the age determination of human skeletal remains. For this reason, different methods were applied in order to a more truthful reconstruction.

Sex diagnosis was mainly performed according to Acsádi and Nemeskéri (1970) method. Authors provided five aspects of skull and pelvis morphology (X): iper-masculine (+2), male (+1), intermediate (0), female (-1), ultra-feminine (-2). In all cases, each anatomical district had a value (W). The degree of "femininity" and "masculinity" (M) was calculated with the following formula: $M = \Sigma WX/\Sigma W$

where M (degree of sexualization) = sum (Σ) of the product of the weight (W) of each character and its aspect (X), divided by the sum of the weights (ΣW) (Fig. 3-5).

The Ferembach *et alii* (1977-1979) standards were used for these assessments.

Carattere	Valore	Valutazioni				
		iperfemminile −2	femminile −1	intermedio 0	maschile +1	ipermaschile +2
CALVARIUM						
glabella (fig. 3)	3	molto debole (0)	leggermente marcata (1)	media (2)	marcata (3 - 4)	molto forte (4 - 5)
processus mastoideus	3	molto piccolo, appuntito	piccolo	medio	grande	molto grande, arrotondato
superficie del *planum nuchale*	3	liscia	linea nucale superiore debolmente indicata	linea nuc. sup. evidente, cresta occip. debolm. sviluppata	grande sviluppata	arrotondata molto forte
processus zygomaticus	3	molto basso, gracile	basso, mediamente gracile	intermedio	alto, forte	molto alto e forte
arcus superciliaris	2	molto debole	leggermente marcato	medio	marcato	molto forte
tubera frontalia et parietalia	2	marcate	mediamente marcate	intermedie	deboli	assenti
protuberantia occipitalis externa (schema di BROCA, fig. 3)	2	molto debole (0)	debole (1)	media (2)	forte (3)	molto forte (4 - 5)
inclinatio frontalis	1	verticale	quasi verticale	poco inclinata	leggerm. sfuggente	fortemente sfuggente
os zygomaticum	2	molto basso, liscio	basso, liscio	mediamente alto, con superficie irregolare	alto, con superficie irregolare	molto alto, con superficie irregolare
forma orbitae; margo supraorbitalis	1	circolare; molto acuto	circolare; acuto	intermedia; intermedio	leggermente quadrata; leggermente arrotondato	quadrata; fortem. arrotondato
MANDIBULA						
aspetto	3	gracile	mediamente gracile	medio	robusto	molto robusto
mentum	2	piccolo, arrotondato	piccolo	medio	prominente, forte, visto di faccia è angoloso	forte e con protuberanza bilaterale
angulus mandibulae	1	liscio	leggermente rugoso	con rugosità marcate	con rugosità marcate e leggera retroversione	con rugosità e retroversione notevoli
margo inferior		sottile	piuttosto sottile	medio	piuttosto spesso	spesso

Fig. 3. Predictive characters of skull according to Ferembach *et alii* (1977-1979).

Carattere	Valore	Valutazioni				
		iperfemminile −2	femminile −1	intermedio 0	maschile +1	ipermaschile +2
sulcus prae-auricularis	3	profondo, ben delimitato	più appiattito, meno ben delimitato	delineato	presente soltanto sotto forma di tracce	assente
incisura ischiadica maior	3	molto ampia, a forma di U	ampia, a forma di U	intermedia	a forma di V	molto stretta, a forma di V
angulus pubis	2	angolo fortemente ottuso e arrotondato	ottuso tendente all'angolo retto	sensibilmente ad angolo retto	debolmente acuto, a forma di A	fortemente acuto, a forma di A
arcus compositus	2		con doppia curva		con curva unica	
os coxae	2	basso, largo, con ala iliaca allargata e rilievi muscolari poco marcati	caratteri femminili un po' meno marcati	forma intermedia	caratteri maschili meno marcati	alto, stretto, rilievi muscolari marcati
foramen obturatum	2	triangolare, con margini acuti	triangolare	forma inclassificabile	ovalare	ovalare, con margini arrotondati
corpus ossis ischii	2	molto stretto, con tuberosità ischiatica poco impressa	stretto	medio	largo	molto largo con tuberosità ischiatica fortemente sviluppata
crista iliaca	1	a forma di S molto appiattita	a forma di S appiattita	forma intermedia	a forma di S netta	a forma di S accentuata
fossa iliaca	1	molto bassa, larga	bassa, larga	media per altezza e larghezza	alta, stretta	molto alta e stretta
pelvis maior	1	molto larga	larga	media	stretta	molto stretta
pelvis minor	1	molto larga	larga	media	stretta	molto stretta

Fig. 4. Predictive characters of *Pelvis* according to Ferembach *et alii* (1977-1979).

DETERMINAZIONE MORFOLOGICA DEL SESSO

CRANIO (Broca,1875; Acsadi e Nemeskeri,1970; WEA, 1980)

CARATTERE	IMPORTANZA (W)	VALORE (X)	W x X
Glabella	3		
Arcate	2		
Bozze front./pariet.	2		
Inclinazione del	1		
Tipo di mastoide	3		
Linee nucali	3		
Protuberanza	2		
Processo ziogomat.	3		
Osso zigomatico	2		
Cresta	2		
Orbita	1		
Σ Cranio	max. 24		
Mandibola (gener.)	3		
Mento	2		
Angolo goniaco	2		
Bordo inferiore	1		
Σ Mandibola	max. 8		
Σ Capo	max. 32		

Grade di sex. del Cranio: $\frac{(\Sigma WxX)}{\Sigma W} =$ (♂>0; ♀<0)

Grade di sex. del Mandibola: $\frac{(\Sigma WxX)}{\Sigma W} =$ (♂>0; ♀<0)

Grade di sex. del Capo: $\frac{(\Sigma WxX)}{\Sigma W} =$ (♂>0; ♀<0)

BACINO (Acsadi e Nemèskeri, 1970, WEA, 1980)

CARATTERE	IMPORTANZA (W)	VALORE (X)	W x X
Solco preauricolare	3		
Incisura ischiatica magg.	3		
Angolo sottopubico	2		
Arco composto(-1;+1)	2		
Morfologia gen.	2		
Forame otturato	2		
Corpo dell'ischio	2		
Cresta iliaca	1		
Fossa iliaca	1		
Grande pelvi	1		
Σ =	max.19		

Grado di sessualità: $\frac{\Sigma (W x X)}{\Sigma W} =$ (♂>0; ♀<0)

Fig. 5. Sex determination according to Acsádi e Nemeskeri (1970).

It was not possible to observe all districts involved in Acsádi & Nemeskéri (1970) method because of the poor state of preservation of skeletal remains. According to the availability of skeletal districts in the subjects examined, further anthropometric standards were applied:

- skull: height of the mastoid process (Giles and Elliot, 1964; Demoulin, 1972)
- mandible: thickness of the mandibular body between the first and the third molar (Piquet,1956)
- scapula: length and width of the glenoid fossa, length and thickness scapular spine (Olivier and Pineau, 1958).
- humerus: transverse diameter of the head (Dwight, 1905; Ferembach *et alii* 1977, 1979, 1980; Dittrick and Suchey, 1986; France, 1988);
- radius: vertical diameter of the head (Cattaneo and Grandi, 2008)
- ulna: anterior-posterior diameter and transverse diameter (France, 1998)
- femur: maximum diameter of the head (Iordanidis, 1961; Dittrick and Suchey, 1986)
- tibia: maximum sagittal diameter at the level of the tuberosity (Ferembach *et alii* 1977, 1979, 1980)
- talus: length and width (Steele, 1976)
- talus: length, width and height; calcaneus: maximus length, medial breadth, height of the body (Gualdi-Russo, 2006)

Although present in the examined osteological collections, was not possible to evaluate the diagnostic parameters of clavicle, sternum, vertebrae and sacrum because they were not clearly associated to a specific individual.

Age determination in ancient samples involves an estimation of the individual's age at the time of death by comparing morphological features in the skeletal remains with changes recorded for recent population of known age (Ubelaker, 1989).

In this study, different methods were considered:

- changes of the ileum auricular surface of the *os coxae* (Lovejoy *et alii*, 1985; Meindl and Lovejoy, 1985);
- pubic symphysis surface changes (Todd, 1921);
- synchondrosis of ectocranial suture (Meindl and Lovejoy, 1985; Buikstra and Ubelaker, 1994)
- tooth wear (Brothwell, 1981; Lovejoy *et alii*, 1985)

Authors classify changes of the ileum auricular surface according to 8 phases. Assessments were carried out using photographic plates of Ubelaker' (1989) and White and Folkens' (1991) standards. For a better understanding of the auricular surface changes, descriptions treated in detail by Bedford *et alii* (1989) were taken into account. The changes of auricolar surface are associated to 8 age classes, as it is summarily shown below:

1. 20-24 years: transverse billowing and very fine granularity. Articular surface displays fine granular texture and marked transverse organization.
2. 25-29 years: reduction of billowing but retention of youthful appearance.
3. 30-34 years: general loss of billowing, replacement by *striae*, and distinct coarsening of granularity. Loss of transverse organization and coarsening of granularity is evident.

4. 35-39 years: uniform, coarse granularity. Minimal changes are seen at the apex, micro-porosity is slight and there is no macro-porosity.
5. 40-44 years: transition from coarse granularity to dense surface.
6. 45-49 years: completion of densification with complete loss of granularity.
7. 50-59 years: dense irregular surface of rugged topography and moderate to marked activity in pre-auricular areas. Increased irregularity of margins is seen. Retro-auricular activity is from moderate to marked in most cases.
8. 60+: breakdown with marginal lipping, macroporosity, increased irregularity, and marked activity in pre-auricular area. Margins become lipped with degenerative joint changes. Retro-auricular area becomes well defined with osteophytes. There is clear destruction of subchondral bone.

The pubic symphysis surface metamorphosis was observed. Age-related changes on this surface continue during adulthood when other epiphyses of the limbs were merged. Young adult pubic symphysis has a rigged surface, traversed by horizontal ridges and intervening grooves. This surface changes with age: it loses reliefs, it is bounded by a rim and, progressively, it is characterized by erosion and general deterioration (White and Folkens, 1991). The assessment was performed using Todd method (1921), that pointed out 10 phases of pubic symphysis changes age-related (Fig. 6).

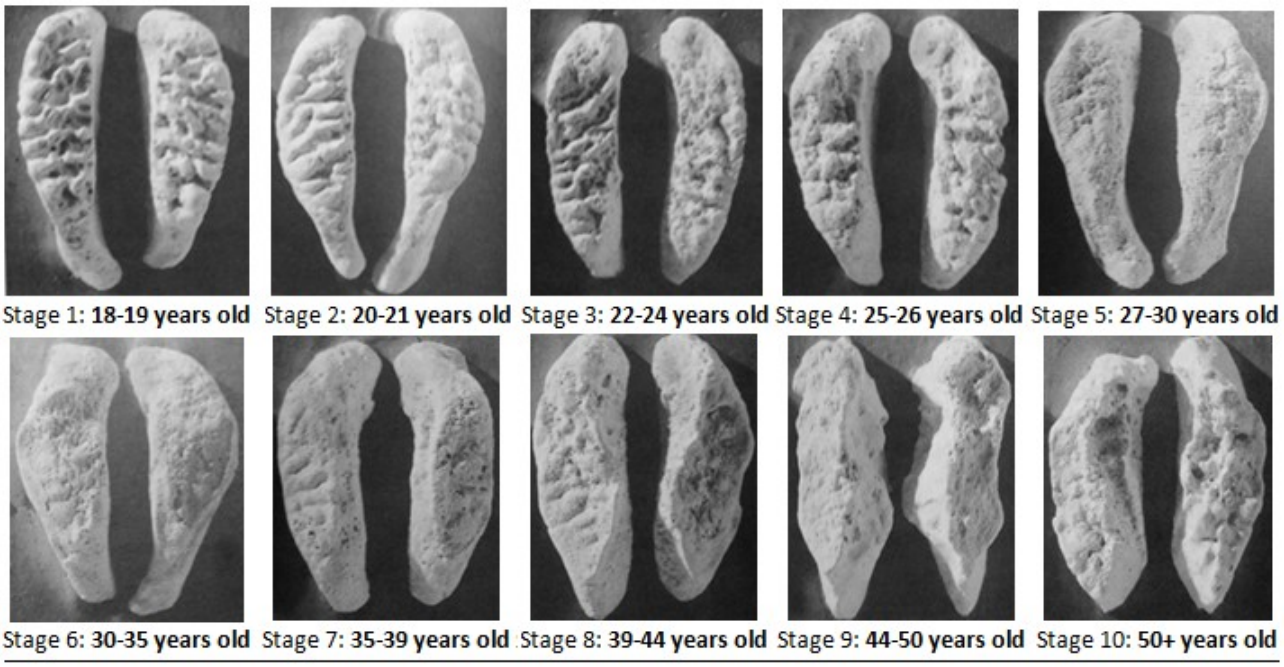


Fig. 6. Changes in pubic bone age-related (Todd, 1921).

Synchondrosis of cranial sutures begins generally at 20 years of age and continues, intermittently, until the complete obliteration in very advanced age (White and Folkens, 1991). Meindl and Lovejoy (1985) and Buikstra-Ubelaker (1994) standards were used for this assessment. The method selects a series of 1 cm segments of ectocranial sutures, assigning a score (0-3) age-related to each sites: 0= open, 1= minimal closure (< 50%); 2= significant closure (> 50%); 3= complete fusion (Fig. 7). Canci and Minozzi (2005) standard was used to define the age at death by assessments.

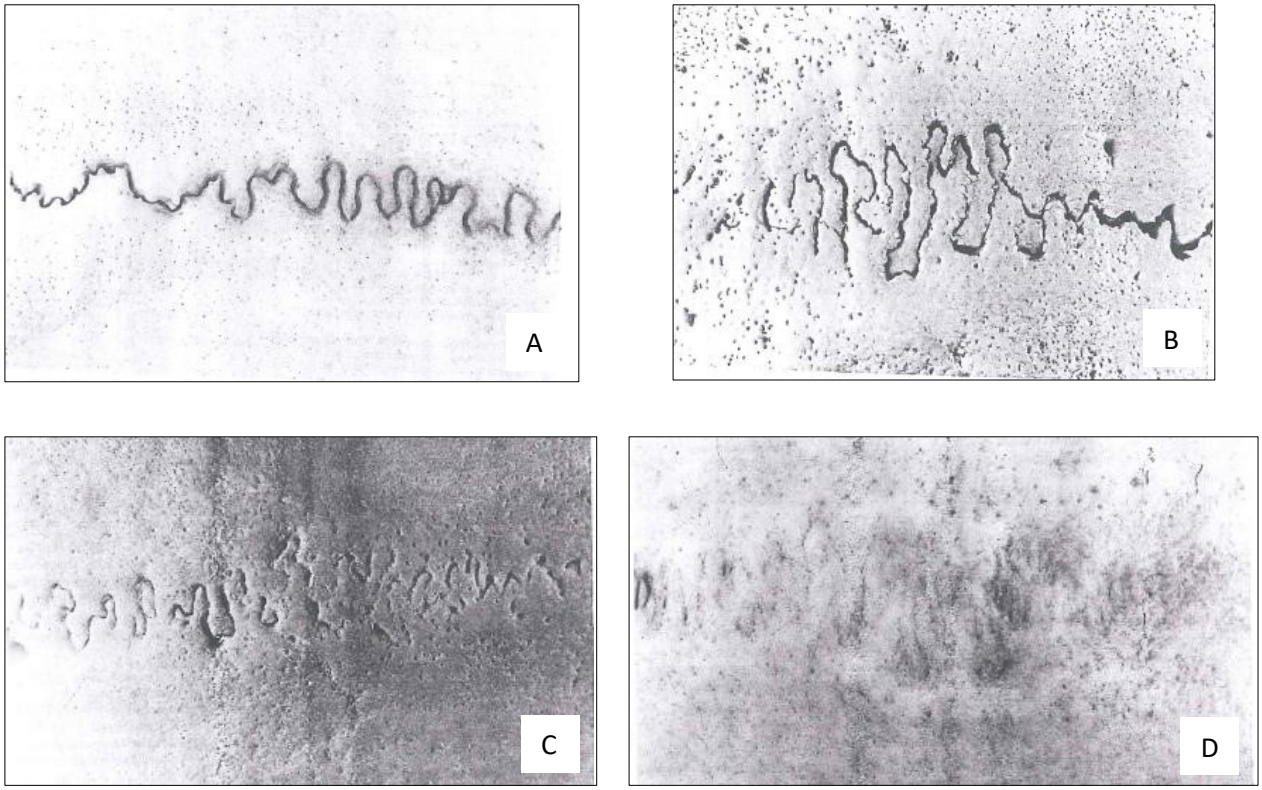


Fig. 7. Stages of obliteration of the cranial sutures: A= open suture; B= minimal closure; C= significant closure; D= complete fusion.

Wear of teeth occlusal surfaces gradually increases with aging. The assessments were carried out according to Brothwell (1981) standards and Lovejoy method (1985) (Fig. 8, 9).

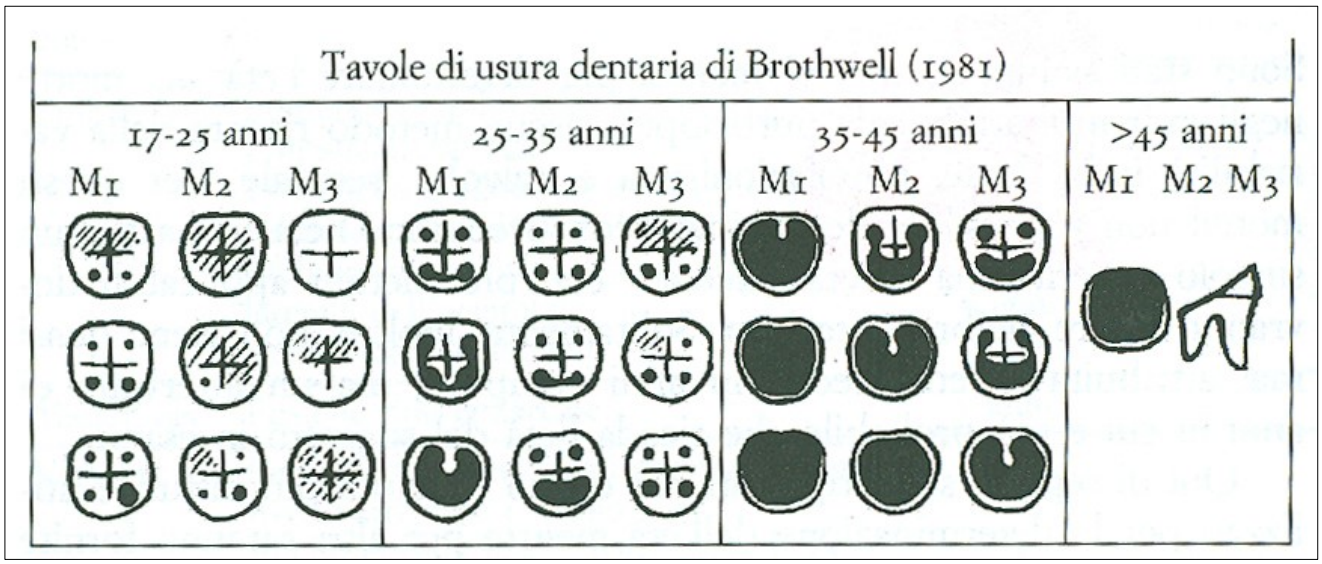


Fig. 8. Degrees of wear age-related according to Brothwell (1981).

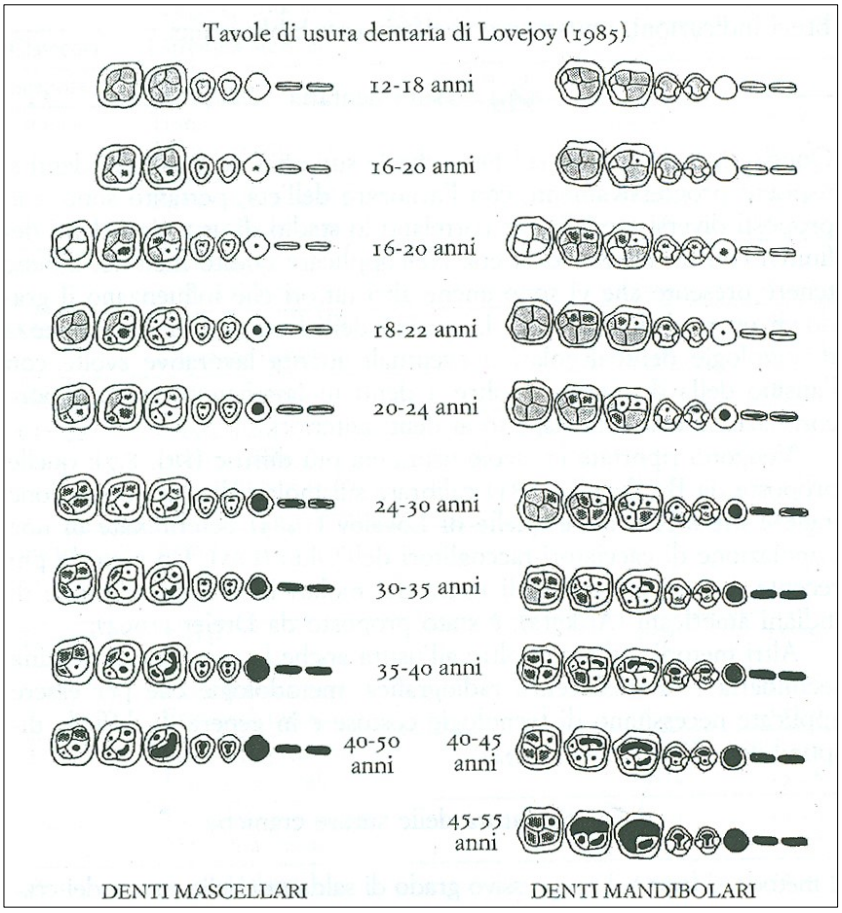


Fig. 9. Degrees of wear age-related according to Lovejoy (1985).

It was not possible to use anthropometric markers for age estimation because of the poor state of preservation of skeletal remains. The inability to associate with certainty some anatomical elements to specific individuals has led to discard other standards, such as age-related changes of vertebrae and sternal ends of ribs.

Age classes used in this study were composed according to Buikstra and Ubelaker (1994), as follows (Table 1):

Age class	Years of age
Young Adults	20-35
Middle Adults	35-50
Old Adults	50+

Table 1. Age classes according to Buikstra and Ubelaker (1994).

Concerning stable isotopes analyses, only two groups have been considered in order to compare data: young adults (< 35 years old) and adults (> 35 years old), because of the sample size.

2.2.3 Stable isotopes analysis: methods

Stable isotopes analysis on bone collagen was performed on all the 45 specimens selected (33 humans and 12 faunal skeletal remains). This approach consists of two phases:

1. the collagen extraction from both human and animal bone specimens
2. the mass spectrometry performed on collagen extracts

The analyses were carried out in collaboration with School of Culture and Society - Prehistoric Archaeology (University of Aarhus, Denmark). In particular, collagen extraction and spectrometry were carried out at the Aarhus AMS Centre - Institute of Physics and Astronomy (University of Aarhus, Denmark).

Collagen extraction

Bone collagen was extracted from 0.5 g of cleaned bone fragments (cortical bones), following Login (1971) method modified by Brown *et alii* (1988) and Jørkov *et alii* (2007). The ultrafiltration protocol, introduced by Brown and colleagues, separates high molecular weight (MW) components of the gelatinized "collagen" (>30kD) from low MW fractions (<30kD) (which will include broken-down/degraded collagen fragments, salts, soil derived amino-acids, other potential contaminants). The use of ultrafilters improves the quality of extracted collagen (evaluated by better C:N ratios) and eliminates salt components that could make gelatinization problematic (Higham *et alii*, 2006). Appendix 1 (attached to this chapter) shows in detail the operations performed.

Mass Spectrometry and stable isotopes outcome

Each collagen extract was then analyzed by elemental analyzer (EA) coupled to a continuous flow isotope ratio mass spectrometer (*EA-IRMS, Elemental Analysis - Isotope Ratio Mass Spectrometry*). We have analyzed 57 specimens: 44 human and faunal collagen extracts, 9 duplicates, 4 background bones' extracts and 10 standards (GEL-AD SID6 UDT: $^{15}/_{12\text{-}10}$). Each standard weighted 0.200-0.300 mg. The background bone (a whale bone aged 40.000 BP) was used as a background control sample.

The isotope $\delta^{13}C$ and $\delta^{15}N$ expressed the ratio of the heavy isotope (^{13}C, ^{15}N) to the light isotope (^{12}C, ^{14}N) in a sample, relative to international standards (International Atomic Energy Agency). Ratios were expressed using the delta (δ) notation, as part per thousand (‰). The $\delta^{13}C$ ratio has been reported relative to the V-PDB marine fossil limestone standard (Vienna Pee Dee *Belemnitella* fossil); $\delta^{15}N$ ratio was relative to atmospheric N_2 (AIR) standard (Ambrose and Norr, 1993; Sharp, 2007). The $\delta^{13}C$ ‰ was calculated, for instance, as follows (Ambrose and Norr, 1993):

$(^{13}C{:}^{12}C_{sample}/^{13}C{:}^{12}C_{standard} -1) \times 1000$.

The extracted collagen material was quality-checked in terms of collagen percentage and C:N values. For the purpose of this study, extracts were condidered well-preserved when they had yields of > 1%, C% ≥ 30% and N% ≥ 11% (wt%), as proposed by van Klinken (1999), and C/N molar ratios between 2.9 and 3.6, as proposed by De Niro (1985).

2.2.4 Statistical methods

Data were elaborated with STATISTICA for Windows - version 11 (StatSoft Italia srl, Vigonza, Padua, Italy). Mean and standard deviation (SD) were computed to describe the sample. Due to the sample size, the non-parametric tests were used to compare means: Mann-Whitney U-test (2 items) and Kruskal-Wallis test (3 items). For all statistical analyses, a significance level of $p < 0.05$ was used.

2.3 Results

The table below shows the samples selected for stable isotopes analyses, with indication of the bone considered, side and biological profile of human subjects (Table 2).

Sample ID	Species	Site	Archaeological context	Bone	Skeletal element	Side	Sex	Age class
Group 1								
Grotta di Donna Marsiglia								
GdMR1a	human	GdMR	Superficial layer	Cranial bone	parietal bone	R	F	adult
GdMR2	*Sus scrofa*	GdMR	Superficial layer	Humerus	diaphysis	L	ND	adult
GdMR3	*Sus scrofa*	GdMR	Superficial layer	Mandible	Corpus	L	ND	adult
GdMR4	*Sus scrofa*	GdMR	Superficial layer	3° Metatarsal	diaphysis	L	ND	adult
Grotta dell'Antenato								
GdA1	human	GdA	Superficial layer	Mandible	Corpus	ND	F	adult
GdA1C14	human	GdA	Superficial layer	Mandible	Corpus	ND	F	adult
GdA2	human	GdA	Superficial layer	Mandible	Corpus	L	M	adult
GdA4	*Sus scrofa*	GdA	Superficial layer	Mandible	Corpus	L	ND	adult
GdA5	*Sus scrofa*	GdA	Superficial layer	Humerus	diaphysis	L	ND	adult
Grotta di Sant'Angelo								
GdSA1	human	GdSA V	Superficial layer of gallery	Femur	diaphysis	R	M	adult
GdSA2	human	GdSA II	Superficial layer of gallery	Femur	diaphysis	L	F	adult
GdSA3	*Sus scrofa*	GdSA II	D22 US 1	Humerus	diaphysis	L	ND	adult
Grotta della Monaca								
GdM2	human	GdM	CTv/m5v/S/tg 1,2,3	Cranial bone	occipital bone	ND	F	young adult
GdM4	human	GdM	CTv/m5v/S/tg1/	Cranial bone	occipital bone	ND	F	young adult
GdM6	human	GdM	CT dx-fv/Z3	Radius	diaphysis	L	ND	adult
GdM9	human	GdM	CT dx-fv/Z4	3° Metatarsal	diaphysis	R	ND	adult
GdM10	human	GdM	CT dx-fv/Z6	Metatarsal phalanx	diaphysis	ND	ND	adult
GdM11	human	GdM	CT dx-fv/Z6	3° Metatarsal	diaphysis	L	ND	adult
GdM12	*Sus scrofa*	GdM	Pfs	Humerus	diaphysis	R	ND	young adult
Grotta du' Scuru								
GdSc1	human	GdSc	Superficial layer	Radius	distal metaphysis	R	ND	young adult
Group 2								
Toppo d'Aguzzo								
TDT13a	human	TDT	S' 17 150-180 cm 15	Mandible	Corpus	L	M	middle adult
TDT14a	human	TDT	S' 17 150-180 cm 3	Mandible	Corpus	L	F	old adult
TDT15a	human	TDT	S' 17 150-180	Mandible	Corpus	R	F	young

			cm 8					adult
TDT17a	human	TDT	S' 17 150-180 cm 12	Cranial bone	parietal-temporal bone	R	F	middle adult
TDT18a	human	TDT	S' 17 150-180 cm 12	Mandible	Corpus	R	M	young adult
TDT19a	human	TDT	S' 17 150-180 cm 11	Mandible	corpus	R	M	middle adult
TDT111	human	TDT	S' 17 150-180 cm 16	Cranial bone	parietal bone	L	ND	middle adult
Grotta Funeraria								
GF1a	human	GF	GF1	Mandible	Corpus	L	M	middle adult
GF2a	human	GF	GF2	Mandible	Corpus	L	M	middle adult
GF3	human	GF	GF12	Mandible	Corpus	R	M	middle adult
GF4	human	GF	GF11	Mandible	Corpus	L	M	young adult
GF5C14	human	GF	GF4	Mandible	Corpus	L	M	young adult
GF6C14	human	GF	GF7	Mandible	Corpus	L	M	middle adult
Murgia Timone								
MT1	*Ovis aries*	MT	3779	Metatarsus	metatarsal bone	ND	ND	adult
MT2	*Canis familiaris*	MT	3790/41	Mandible	Corpus	L	ND	adult
MT3	*Cattle*	MT	3792	Astragalus	astragalus	ND	ND	adult
MT4	*Sus scrofa*	MT	3787	Metatarsus	metatarsal bone	ND	ND	adult
MT5	Cattle	MT	3807/74	Metacarpus	metacarpal bone	ND	ND	adult
Group 3								
Ipogeo dei Bronzi								
IpB2	human	IpB	B2 (11)	Humerus	diaphysis	R	M	adult
IpB3	human	IpB	B2 (13)	Humerus	diaphysis	R	M	adult
IpB4	human	IpB	D7 (d)	Mandible	Corpus	R	M	adult
IpB5	human	IpB	D7 (c)	Mandible	Corpus	L	F	adult
IpB6	human	IpB	F8 (d)	Mandible	Corpus	L	F	adult
IpB7	human	IpB	C5 (d)	Mandible	Corpus	R	M	adult
IpB12	human	IpB	SFQ D6	Mandible	Corpus	L	M	adult

Table 2. The samples selected (R= right; L= left; M= male; F= female; ND= non determinable).

Among humans (N= 33), there were 16 males (48.5%) and 11 females (33.3%). The remaining 6 specimens (18.2%) were not determinable for sex (ND), because of their poor state of preservation (Fig. 10, 11):

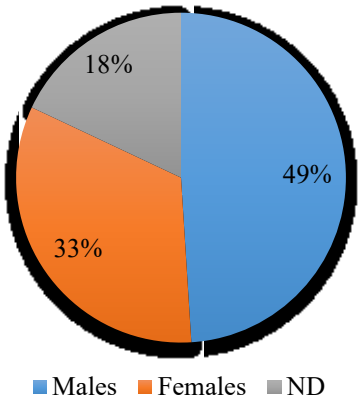


Fig. 10. On the left, human samples according to sex (N= 33).

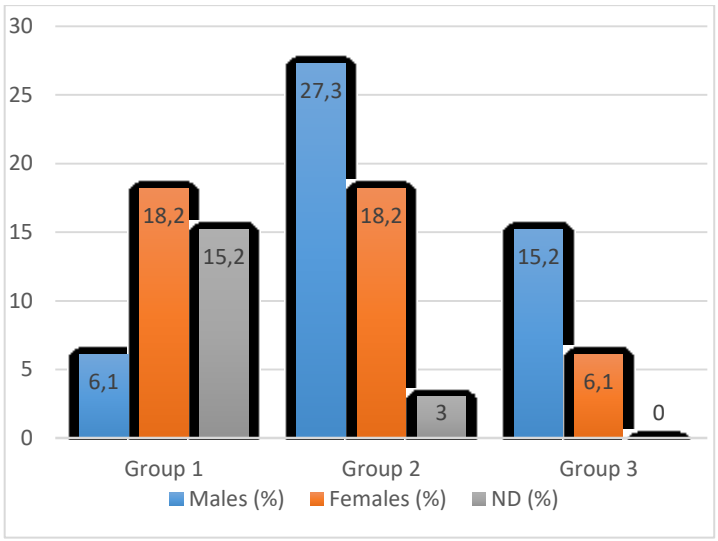
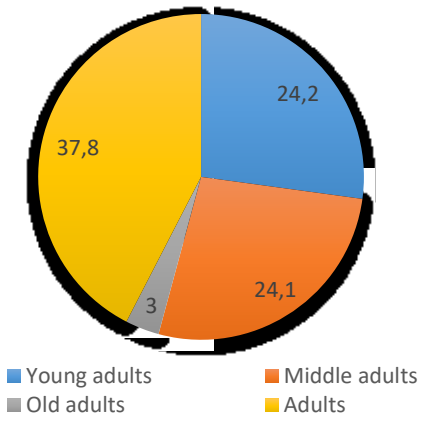


Fig. 11. On the right, human sample according to sex in each group (N= 33).

On 33 human specimens, there were 8 young adults (24.2%); 8 middle adults (24.2%); 1 old adult (3%); 17 generically defined as "adults" (52 %) (Fig. 12, 13).



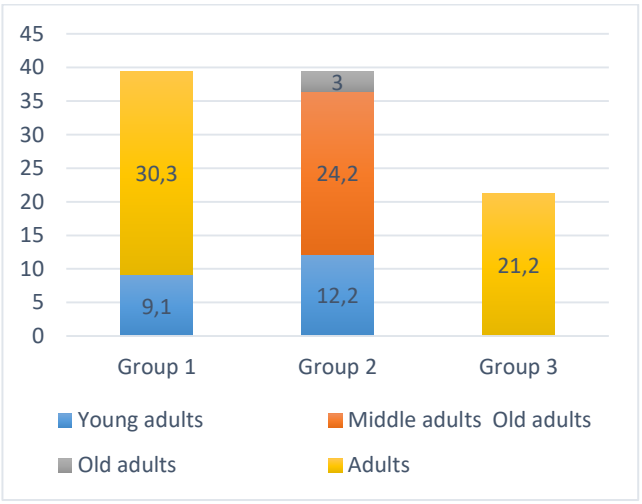


Fig. 12. On the left, the human sample according to the age (N= 33).
Fig. 13. On the right, human sample according to the age in each group (N= 33).

Concerning fauna, it was not given any indication regarding the sex of animals in the archaeological record. Faunal samples are generically indicated as "adults" by archaeologists, except for one young adult (cfr. Table 1: ID GdM12, *Sus scrofa*, according to Bux and Scintillani, 2005).

The results of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}C$) isotope analyses performed on collagen extracted are presented in Table 3.

Lab ID	S-ID	Site	Species	$\delta^{13}C_{V\text{-}PDB}$ (‰)	SD	$\delta^{15}N$ (‰)	SD	C (TCD)	SD	N_{AIR} (TDC)	SD	C:N	SD	% yield
Group 1														
28697	GdM09	GdM	Human	-19.67	0.3	9.46	0.45	45.48	0.01	16.33	0.01	3.249	0.01	7.3
28698	GdM02	GdM	Human	-19.61	0.3	8.98	0.45	45.08	0.01	16.27	0.01	3.232	0.01	7.1
28699	GdM04	GdM	Human	-20.31	0.14	7.69	0.23	43.66	0.007	16.09	0.007	3.166	0.007	5.4
28700	GdM10	GdM	Human	-19.81	0.3	8.75	0.45	46.91	0.01	17.34	0.01	3.156	0.01	7.2
28701	GdM11	GdM	Human	-19.87	0.3	7.37	0.45	44.31	0.01	15.97	0.01	3.237	0.01	6.5
28702	GdM06	GdM	Human	-19.93	0.3	7.01	0.45	43.05	0.01	15.44	0.01	3.253	0.01	2.2
28704	GdMR01 a	GdMR	Human	-19.68	0.3	10.13	0.45	44.45	0.01	16.33	0.01	3.176	0.01	2.8
28708	GdA01	GdA	Human	-19.17	0.3	10.82	0.45	43.42	0.01	15.91	0.01	3.184	0.01	2.6
28709	GdA01C 14	GdA	Human	-19.31	0.3	9.15	0.45	44.58	0.01	16.32	0.01	3.187	0.01	2.6
28710	GdA02	GdA	Human	-19.82	0.14	7.97	0.23	45.335	0.007	16.14	0.007	3.277	0.007	4.6
28713	GdSA01	GdSA	Human	-19.03	0.3	9	0.45	44.66	0.01	16.37	0.01	3.183	0.01	6.8
28714	GdSA02	GdSA	Human	-19.06	0.22	8.32	0.24	44.505	0.007	16.36	0.007	3.174	0.007	4.9
28741	GdSc01	GdSc	Human	-19.41	0.3	10.17	0.45	44.74	0.01	16.3	0.01	3.202	0.01	4.9
28705	GdMR02	GdMR	Sus scrofa	-21.42	0.13	4.06	0.21	3.24	0.006	44.893	0.006	3.24	0.006	7.3
28706	GdMR03	GdMR	Sus scrofa	-19.65	0.3	8.45	0.45	3.277	0.01	44.46	0.01	3.277	0.01	2.2
28707	GdMR04	GdMR	Sus scrofa	-20.71	0.3	2.96	0.45	3.184	0.01	44.38	0.01	3.184	0.01	5.1
28711	GdA04	GdA	Sus scrofa	-21.49	0.3	6.88	0.45	3.186	0.01	45.69	0.01	3.186	0.01	7.1
28712	GdA05	GdA	Sus scrofa	-20.52	0.3	5.3	0.45	3.206	0.01	44.74	0.01	3.206	0.01	7.9
28715	GdSA03	GdSA	Sus scrofa	-20.92	0.3	4.92	0.45	3.166	0.01	45.67	0.01	3.166	0.01	4.9
Group 2														
28716	TDT103a	TDT	Human	-19.39	0.3	10.23	0.45	45.02	0.01	16.46	0.01	3.191	0.01	3.0
28717	TDT104a	TDT	Human	-20.21	0.3	8.42	0.45	44.9	0.01	16.5	0.01	3.175	0.01	2.3
28718	TDT105a	TDT	Human	-19.71	0.3	9.16	0.45	44.76	0.01	16.32	0.01	3.2	0.01	3.4
28719	TDT107a	TDT	Human	-19.38	0.14	9.66	0.23	45.615	0.007	16.73	0.007	3.181	0.007	4.4
28720	TDT108a	TDT	Human	-20.24	0.3	8.79	0.45	44.21	0.01	15.77	0.01	3.271	0.01	3.2
28721	TDT109a	TDT	Human	-19.43	0.3	9.97	0.45	46.95	0.01	17.25	0.01	3.175	0.01	3.9
28722	TDT111	TDT	Human	-18.95	0.3	9.09	0.45	46.48	0.01	16.88	0.01	3.212	0.01	4.7
28723	GF01a	GF	Human	-19.89	0.3	10.21	0.45	43.98	0.01	15.99	0.01	3.209	0.01	2.8
28724	GF02a	GF	Human	-19.77	0.14	9.48	0.23	44.71	0.007	16.09	0.007	3.242	0.007	2.0
28725	GF03	GF	Human	-19.14	0.3	9.62	0.45	44.53	0.01	16.28	0.01	3.191	0.01	2.5
28726	GF04	GF	Human	-19.63	0.3	9.8	0.45	44.96	0.01	15.98	0.01	3.282	0.01	2.5
28727	GF05C14	GF	Human	-19.32	0.3	9.87	0.45	45.91	0.01	16.39	0.01	3.268	0.01	3.8
28728	GF06C14	GF	Human	-19.44	0.14	9.78	0.23	45.5	0.007	16.47	0.007	3.223	0.007	4.5
28729	MT01	MT	Ovis aries	**-20.66**	0.3	5.63	0.45	43.84	0.01	15.53	0.01	3.293	0.01	3.8
28730	MT02	MT	Canis familiaris	-19.08	0.3	8.01	0.45	43.99	0.01	15.88	0.01	3.232	0.01	2.8
28731	MT03	MT	Bos	**-19.2**	**0.3**	7.32	0.45	44.21	0.01	15.73	0.01	3.279	0.01	2.4
28732	MT04	MT	Sus scrofa	-20.38	0.3	7.68	0.45	44.75	0.01	16.23	0.01	3.217	0.01	3.4
28733	MT05	MT	Bos	**-19.13**	0.15	7.4	0.24	44.095	0.007	15.85	0.007	3.245	0.007	3.5
Group 3														
28734	IpB02	IpB	Human	-18.97	0.3	8.59	0.45	44.82	0.01	16.56	0.01	3.158	0.01	3.0
28735	IpB03	IpB	Human	-19.79	0.3	8.76	0.45	44.14	0.01	15.72	0.01	3.276	0.01	3.3
28736	IpB05	IpB	Human	-19.53	0.3	8.01	0.45	43.92	0.01	15.97	0.01	3.208	0.01	3.7
28737	IpB04	IpB	Human	-18.91	0.3	9.96	0.45	43.72	0.01	16.17	0.01	3.154	0.01	2.2
28738	IpB06	IpB	Human	-19.57	0.14	9.16	0.23	42.74	0.007	15.725	0.007	3.171	0.007	0.5
28739	IpB07	IpB	Human	-19.51	0.3	8.92	0.45	42.15	0.01	15.35	0.01	3.204	0.01	3.0
28740	IpB12	IpB	Human	-19.17	0.3	8.42	0.45	43.07	0.01	15.7	0.01	3.2	0.01	2.1

Table 3. Carbon ($\delta 1^3C$) and nitrogen ($\delta^{15}N$) isotope ratios in bone collagen extracted from faunal and human samples.

The pretreatment was carried out on the 45 skeletal remains (33 human bones and 12 faunal bones), however it was possible to extract collagen only from 44 samples as the sample ID GdM12 (*Sus scrofa*) was lost during the pre-treatment operations. All 44 extracts are considered well-preserved collagen because of their collagen yields > 1%. Specimen IpB 06 had a critical yield value (i.e. 0.5

%) and it, thus, considered with caution. In addition, C/N ratios obtained, for both animals and humans, fell in the range between 2.9-3.6, which indicated good collagen preservation.
The scatterplot below shows the spreading of faunal and human stable isotope ratios, according to Table 3 data (Fig. 14).

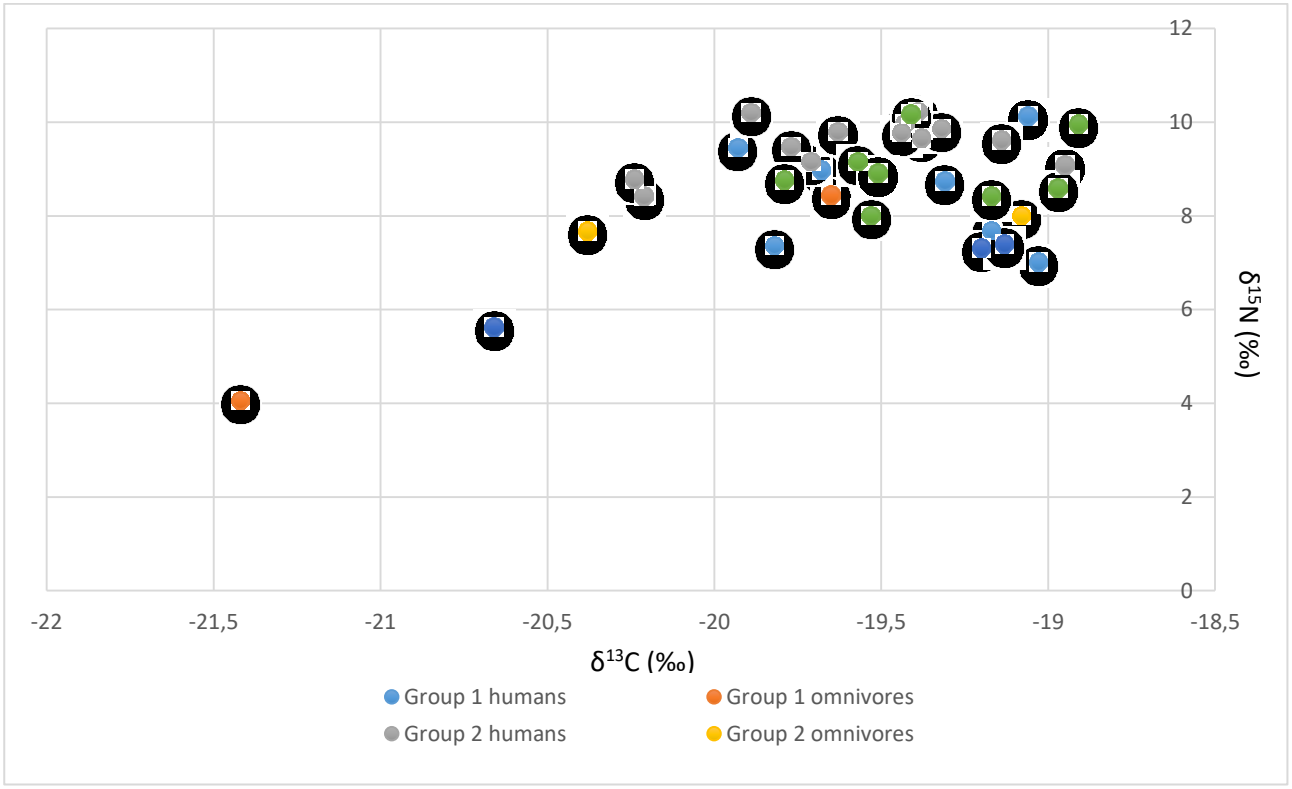


Fig. 14. Human and faunal $\delta^{13}C$ (x) and $\delta^{15}N$ (y) ratios scatterplot in the skeletal sample (N= 44).

The carbon and nitrogen analyses on the 6 animals of Group 1 (inland mountain-hilly sites of Calabria) resulted in bone collagen $\delta^{13}C$ values from -21.49 ‰ to -19.65 ‰ (-20.79 ‰ ±0.68 ‰) and $\delta^{15}N$ values from 2.96 ‰ to 8.45 ‰ (5.43 ‰ ±1.97 ‰). The 5 faunal extracts of Group 2 (inland hilly sites of Basilicata) showed bone collagen $\delta^{13}C$ values from -20.66 ‰ to -19.08 ‰ (-19.69‰ ±0.77‰) and $\delta^{15}N$ values from 5.63‰ to 8.01‰ (7.21‰ ± 0.92‰). Group 3 had no faunal specimens. The sample ID GdMR04 (*Sus scrofa* - Group 1) had different concentration of isotopes ratios ($\delta^{13}C$= -20.71‰ and $\delta^{15}N$= 2.96‰) pointing out a diet characterized by a prevalent intake of vegetal protein (C_3 plant derived). The faunal sample ID MT01 (*Ovis aries* - Group 2) had higher concentrations of $\delta^{13}C$ (-20.66‰) and slightly lower concentrations of $\delta^{15}N$ (5.63‰) than the other herbivores of Group 2 (cattle). No carnivores were present in the sample, therefore isotopes ratios (in particular $\delta^{15}N$ values) did not point out significant trophic level differences, with omnivores (*Sus scrofa* and *Canis familiaris*) ranged from -21.49‰ to -19.08‰ (-20.52‰ ±0.83‰) for $\delta^{13}C$ and from 2.96‰ to 8.45‰ (6.03‰ ±2.01‰) for $\delta^{15}N$ and herbivores (*Ovis aries* and cattle) ranged from -20.66‰ to -19.08‰ (-19.65‰ ±0.88‰) for $\delta^{13}C$ and from 5.63‰ to 8.01‰ (6.98‰ ±1.22‰) for $\delta^{15}N$.
The human specimens have a $\delta^{13}C$ mean value of -19.59‰ ±0.38‰ (from -20.31‰ to -19.03‰) and $\delta^{15}N$ mean value of 8.83‰ ±1.14‰ (from 7.01‰ to 10.82‰) in Group 1; $\delta^{13}C$ mean value of -19.58‰ ±0.38‰ (from -20.24‰ to -18.95‰) and $\delta^{15}N$ mean value of 9.54‰ ±0.54‰ (from 8.42‰ to 10.23‰) in Group 2; $\delta^{13}C$ mean value of -19.35‰ ±0.33‰ (from -19.79‰ to -18.91‰) and

$\delta^{15}N$ mean value of 8.83‰ ±0.62‰ (from 8.01‰ to 9.96‰) in Group 3. The humans in Group 1 had a $\delta^{13}C$ mean value by 1.2‰ higher than the omnivores mean value (-20.79‰ ±0.68‰) and by 3.4‰ higher than the omnivores $\delta^{15}N$ mean value (5.43‰ ±1.97‰). Humans in Group 2 had a $\delta^{13}C$ mean value by 0.2‰ higher than the omnivores mean value (-19.73‰ ±0.92‰) and by 0.1‰ than the $\delta^{13}C$ mean value of herbivores (19.66‰ ±0.86‰), as well as respectively by 1.7‰ higher than the $\delta^{15}N$ mean value for the omnivores (7.85‰ ±0.23‰) and by 2.8‰ higher than the $\delta^{15}N$ mean value for the herbivores (6.78‰ ±1.0‰). Applying the theoretical predator-prey scheme: $\delta^{15}N$= 3-5‰, $\delta^{13}C$= 0-1‰ (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984), the comparison between animal and human mean values showed that humans-group 1 was one trophic higher level than omnivores, therefore faunal resources (*Sus scrofa* meat) were consumed. Instead, humans-group 2 isotope values did not indicate an important animal protein intake. Regarding humans of Group 3 published data (Tafuri *et alii*, 2009) were used for comparison with faunal isotope ratios. Two reference animal bone samples of deer (*Cervus elaphus*) and sheep/goat (*Ovis vel Capra*), collected from the Middle Bronze Age site of Madonna di Loreto (Foggia, Apulia), showed the following isotope values: -20.4‰ of $\delta^{13}C$ and 7.2‰ of $\delta^{15}N$ for *Cervus elaphus*; -20.4‰ of $\delta^{13}C$ and 7.1‰ of $\delta^{15}N$ for *Ovis vel Capra*. The predator-prey scheme ($\delta_{\text{humans ratios}}$-$\delta_{\text{faunal ratios}}$) did not suggested an animal intake for Group 3 communities: humans had by 1.05‰ higher than the $\delta^{13}C$ value of herbivores and by 1.73‰ higher than $\delta^{15}N$ value of herbivores. In order to point out differences of diet according to archaeological period, Ancient Bronze Age sites (ABA) and Middle/Late Bronze Age sites (MLBA) were compared. The table below shows the mean values of humans belonged to Grotta di Donna Marsilia, Grotta Sant'Angelo e Grotta dell'Antenato (ABA) and Grotta della Monaca, Grotta du' Scuru, Toppo d'Aguzzo, Grotta Funeraria, Ipogeo dei Bronzi (MLBA) (Table 4).

	ABA		**MLBA**	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Min	-19.82	10.80	-20.31	7.00
Max	-19.03	7.90	-18.91	10.20
Mean	-19.35	9.20	-19.58	9.03
SD	±0.33	±1.09	±0.37	±0.89

Table 4. Human stable isotopes mean values (‰) of Ancient Bronze Age sites and Middle Bronze Age sites.

The Mann-Whitney U test results did not indicate a statistical significance between Ancient and Middle/Late Bronze age samples (U=53.50, p= 0.1993 for $\delta^{13}C$ and U= 77.00, p= 0.8519 for $\delta^{15}N$). After this assessment, it was possible to compare sites according to topography: Group 1, Group 2, Group 3. The human mean values of $\delta^{13}C$ and $\delta^{15}N$ are summarized in the following table (Table 5).

	Group 1		**Group 2**		**Group 3**	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Min	-20.31	7.01	-20.24	8.42	-19.79	8.01
Max	-19.03	10.82	-18.95	10.23	-18.91	9.96
Mean	-19.59	8.83	-19.58	9.54	-19.35	8.83
SD	±0.38	±1.14	±0.38	±0.54	±0.33	±0.62

Table 5. Human stable isotopes mean values (‰) *per* each group (1, 2, 3).

Non parametric tests were performed in order to test potential variability in diet among the three Groups. Results did not point out a statistical significance (Kruskal-Wallis test, H= 2.02, p= 0.3636

for $\delta^{13}C$ and H= 5.31, p= 0.0702 for $\delta^{15}N$). As shown by the standard deviation (±1.14‰), Group 1 nitrogen values have a greater variability than the other two groups.
In order to verify any difference according to the closeness to the sea, the Mann-Whitney U test was performed to compare the most inland sites (Group α: sites located more than 20 km from the sea) and those closest to the coast (Group β: sites located less than 20 km from the sea). The Group α includes TDT and GF (inland hilly sites of Basilicata); Group β includes GdM, GdMR, GdA, GdSA, GdSc (inland mountain-hilly sites of Calabria) and IpB (inland plan of Puglia). Table 6 shows the mean isotope values of the Group α (N= 13) and Group β (N= 20):

	Group α		**Group β**	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Min	-20.24	8.42	-20.31	7.01
Max	-18.95	10.23	-18.91	10.82
Mean	-19.58	9.55	-19.51	8.83
SD	±0.38	±0.54	±0.37	±0.98

Table 6. Mean $\delta^{13}C$ and $\delta^{15}N$ values (‰) in Group α and Group β.

The Mann-Whitney U test resulted a statistical significance for $\delta^{15}N$ (U= 122.00, p= 0.7682 for $\delta^{13}C$ and U= 68.00 p= 0.0224 for $\delta^{15}N$).
In order to point out differences in diet dependent on sex, the comparison between isotope mean ratios of males and females was undertaken. Stable isotopes mean values in males and females are shown in Table 7.

	Males		**Females**	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Min	-20.24	7.97	-20.31	7.69
Max	-18.91	10.23	-19.06	10.82
Mean	-19.47	9.34	-19.59	9.05
SD	±0.37	±0.70	±0.39	±0.93

Table 7. Stable isotopes mean values (‰) in humans according to sex determination.

The Mann-Whitney U test pointed out that there were not substantial differences between males and females (U= 74.50, p=0.5055 for $\delta^{13}C$ and U= 69.50, p= 0.3611 for $\delta^{15}N$). Evaluations concerning stable isotopes mean values between males and females in each group according to geographical location are the following (Table 8):

	Group 1			
	Males n= 2		**Females n= 6**	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Min	-18.82	7.97	-20.31	7.69
Max	-19.03	9.00	-19.06	10.82
Mean	-19.43	8.49	-19.52	9.18
SD	±0.56	±0.73	±0.46	±1.15
	Group 2			
	Males n= 9		**Females n= 3**	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Min	-20.24	8.79	-20.21	8.42
Max	-19.14	10.23	-19.38	9.66
Mean	-19.58	9.75	-19.77	9.08
SD	±0.34	±0.44	±0.42	±0.62
	Group 3			
	Males n= 5		**Females n= 2**	

	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Min	-19.79	8.42	-19.57	8.01
Max	-18.91	9.96	-19.53	9.96
Mean	-19.27	8.93	-19.55	8.83
SD	±0.37	±0.61	±0.03	±0.62

Table 8. Stable isotopes mean values (‰) in each group.

The Mann-Whitney U test demonstrated that there were not statistical differences between males and females in each group as shown by the following table. Only the $\delta^{15}N$ data in the Group 2 are close to the statistical significance (Table 9).

	Group 1		**Group 2**		**Group 3**	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
U	5.00	4.00	11.00	4.00	2.00	4.00
p-level	0.7389	0.5050	0.6439	0.0789	0.2453	0.6985

Table 9. Comparison between males and females in each group.

According to age classes, the isotope mean values of young adults was -19.75‰ ±0.39‰ (min. -20.31‰; max. -19.32‰) for $\delta^{13}C$ and 9.21‰ ±0.85‰ (min. 7.69‰; max. 10.17‰) for $\delta^{15}N$. The middle adults mean values were -19.42‰ ±0.30‰ (min. -19.89‰; max. -18.95‰) for $\delta^{13}C$ and 9.76‰ ±0.38‰ (min. 9.09‰; max. 10.23‰) for $\delta^{15}N$. Only one old adult was recorded (ID: TDT104a) with $\delta^{13}C$= -20.21‰ and $\delta^{15}N$= 8.42‰. In order to statistical comparisons, two age classes were created: young adults (< 35 years of age, N= 7) and adults (> 35 years of age, N= 9). The last one class concerned middle adults (N= 8) and one old adult (TDT104a). Mann-Whitney U test demonstrated not significant differences between age classes (U= 21.00, p= 0.2663 for $\delta^{13}C$ and U= 23.00, p= 0.3682 for $\delta^{15}N$). It was not possible to compare the age classes for each group with different geographical location because of the small sample's size.

2.4 Discussion

In order to analyze chemical markers of food habits, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis was performed on faunal and human collagen extracts belonged to the Bronze Age sites mentioned before. As expected, faunal isotope ratios were similar to data obtained for Italian environments by Varalli *et alii* (2016a). The following table shows mean values of domesticated animals obtained from site of Grotta Misa in Northern Latium and Grotta dello Scoglietto in Southern Tuscany and data obtained from inland hilly sites of Calabria (Group 1) and inland hilly sites of Basilicata (Group 2) (Table 10). Group 3 (Ipogeo dei Bronzi) was not included in the comparison since no faunal remains were associated with individuals examined in this study.

Site	**Species**	**$\delta^{13}C$ (‰)**	**SD**	**$\delta^{15}N$ (‰)**	**SD**
Grotta Misa	herbivores	-20.15	±1.06	5.30	±1.41
Grotta dello Scoglietto	herbivores	-20.76	±1.24	5.34	±0.67
Group 2 sites	herbivores	-19.65	±0.88	6.98	±1.22
Grotta dello Scoglietto	omnivores	-20.60	±0.26	5.63	±0.25
Group 1 sites	omnivores	-20.79	±0.68	5.43	±1.97
Group 2 sites	omnivores	-20.52	±0.83	6.03	±2.01

Tab. 10. Stable isotopes mean values calculated using Varalli *et alii* (2016a) results and data obtained in this study.

Considering the ranges of $\delta^{13}C$ values recorded, it was possible to assert that they were consistent with the compositions expected for animals living in terrestrial and temperate ecosystem that consumed C_3 plants (Smith and Epstein, 1971; De Niro and Epstein, 1978; Schoeninger and DeNiro, 1984; Herrscher, 2003; Mannino *et alii*, 2015). The sample GdMR02 (*Sus scrofa* - Group 1) showed a different concentration of isotope ratios ($\delta^{13}C$= -20.71‰ and $\delta^{15}N$= 2.96‰) than the mean of other pigs (-20.80‰ ±0.75‰ for $\delta^{13}C$ and 9.41‰ ±6.89‰ for $\delta^{15}N$), pointing out a diet characterized by a prevalent intake of vegetal proteins (C_3 plant derived). This result could depend on the high variability of omnivorous diets: the standard deviation of nitrogen values confirmed a protein consumption that shifted from more vegetarian to more animal feeding. The sample MT01 (*Ovis aries* - Group 2) had higher concentrations of $\delta^{13}C$ (-20.66‰) and slightly lower concentrations of $\delta^{15}N$ (5.63‰) than the other herbivores of Group 2 (cattle: respectively -19.20‰ and -19.13‰ for $\delta^{13}C$, 7.32‰ and 7.40‰ for $\delta^{15}N$). This result could depend on a different pasture, considering that open-air environments increase the carbon concentrations (Mannino, 2009). It was interesting to note that, among omnivores of Group 2, the sample MT02 (*Canis familiaris*) had stable isotopes ratios more similar to humans (-19.8‰ for $\delta^{13}C$ and 8.01‰ for $\delta^{15}N$) than the sample MT04 (*Sus scrofa*), that probably had a more vegetarian diet (-20.38‰ for $\delta^{13}C$ and 7.68‰ for $\delta^{15}N$). Nitrogen isotope ratios reflected the trophic level of the acquired proteins and increase by 3-5‰ every step up the food chain (Schoeninger and De Niro, 1984; Bocherens and Drucker, 2003; Mannino *et alii*, 2015). No carnivores were present in the sample, therefore isotope ratios (in particular $\delta^{15}N$ values) did not point out significantly trophic level differences among faunal categories (herbivores and omnivores).

Regarding human isotopes ratios no statistical difference was indicated between Ancient Bronze Age sites and Middle/Late Bronze Age sites (U=53.50, p= 0.1993 for $\delta^{13}C$ and U= 77.00, p= 0.8519 for $\delta^{15}N$). This result contrasted with data obtained from Central-Northern Italy sites, where different diets were followed in these two periods. In fact, the Ancient Bronze Age sites of Sedegliano (Friuli Venezia Giulia) and Arano di Cellore (Veneto) showed a C_3 plants-related diet (-19.47‰ ±1.45‰ for carbon and 7.79‰ ±1.08‰ for nitrogen), while the Middle Bronze Age of Olmo di Nogara (Veneto), Falcetone and Grotta Misa (Northern Latium) showed that feeding intake based on C_4 plants (i.e. millet): -16.87‰ ±2.05‰ for carbon and 8.31‰ ±1.41‰ for nitrogen (isotopes mean values were elaborated using Tafuri *et alii*, 2009 and Varalli *et alii*, 2016 data). Comparison with our isotope values demonstrated a statistical difference between Northern and Southern Ancient Bronze Age communities (U= 0.00, p= 0.0000 for carbon and U= 41.00, p= 0.0025 for nitrogen) and between Central-northern and Southern Middle Bronze Age sites (U= 0.0000; p= 0.0000 for carbon and U= 348.00, p= 0.0281). These differences were probably due to a higher animal protein intake of Southern Ancient Bronze Age diet than Northern coeval communities, as demonstrated by nitrogen ratios (9.20‰ ±1.09‰). Moreover, regarding to Middle Bronze Age sites, differences could also depend on different vegetal proteins consumed (C_4 plants-related for Central-northern sites and C_3 plants-related for Southern sites). Millet was introduced in Central and Northern Italy during the Middle Bronze Age and it was not regularly consumed in Southern Italian communities of the same period (Tafuri *et alii* 2009; Varalli *et alii* 2016a). In Northern Italy *P. miliaceum* cultivation started during the Ancient Bronze Age as attested in Monte Covolo (Pals and Vorrips, 1979) and in Canár (Castiglioni *et alii*, 1998; Castelletti *et alii*, 2001), anyway the increase of this cereal's cultivation dated back to the Middle/Late Bronze Age, as attested in Terramara di Montale (Mercuri *et alii*, 2006). In fact, this period was characterized by

high economic development, that determined a significant increase of settlement in Central and Northern Italy (i.e. Terramara) resulting in environmental changes, such as deforestation (Peroni, 2004; Bietti Sestieri, 2011). The deforestation, which led to aridity of the soils, facilitated the introduction of millet (Valsecchi *et alii*, 2006; Cremaschi *et alii*, 2016). Moreover, new commercial trades with Europe transalpine could have promoted the diffusion of this new crop (Varalli *et alii*, 2016): paleobotanical record attested that millet was imported from Western Europe and indicated an increase in cultivation around 1700 BC (Lake de Bourget, South-East France) (Rösch, 1998; Jacob *et alii*, 2008). Although millet (C_4 plant) was sporadically recorded in South Italy by paleobotanical data (Bietti Sestieri, 2011), our data did not indicate a regular C_4 plant consumption in Southern communities. In fact, the human isotope composition of sites investigated in this study (Group 1, 2, 3) had a $\delta^{13}C$ mean value of 19.51‰ ±0.14‰ and a $\delta^{15}N$ mean value of 9.07‰ ±0.41‰. Data were coherent with mean values obtained by Tafuri *et alii* (2009) on humans of Southern coeval sites (Toppo d'Aguzzo and Lavello: 19.53‰ ±0.18‰ for $\delta^{13}C$ and 8.25‰ ±0.55‰ for $\delta^{15}N$), that indicated a diet C_3 plants-related. In order to point out the crops consumed, archaeo-botanical data of coeval sites were used as reference: Broglio di Trebisacce (Calabria, Cosenza); Lavello (Basilicata, Potenza); Coppa Nevigata (Apulia, Foggia), Piazza Palmieri (Apulia, Bari), Scoglio di Apani (Apulia, Brindisi), Roca and Scalo di Furno (Apulia, Lecce). Among C_3 species cultivated, findings showed the presence of einkorn weath (*Triticum monococcum*) and emmer weath (*Triticum dicoccum*), weath (*Triticum aestivum, compactum, durum*), barley (*Hordeum vulgare*, *distichum* and *exasticum),* scarcity of spelled, rye and oats, a small variety of broad bean (*Vicia faba* var. *minor*), lentil (*Lens culinaris*) and *Lathyrus cicera sativus* (Caldara *et alii*, 1999; Bietti Sestieri, 2011; Primavera and Fiorentino, 2014).

Concerning the animal protein intake, the comparison between faunal and human isotope ratios indicated it only for humans of Group 1, based on omnivores (*Sus scrofa*). Anyway, it is important to remake the high variability of faunal nitrogen ratios in Group 1 (omnivores mean value was 5.43‰ ±1.97‰ for nitrogen); therefore, this result must be considered with caution. On the contrary, results did not indicate the animal food consumption for humans-Group 2 (Toppo d'Aguzzo and Grotta Funeraria) and 3 (Ipogeo dei Bronzi). Although this result confirms previous assumption - Tafuri *et alii* (2009) estimated that C_3 plants provided around 65-70% of total dietary protein of Toppo d'Aguzzo human sample - it must be considered with caution. In fact, it is important to remark that it was not possible to obtain detailed baseline: we had not faunal remains of all species (herbivores and carnivores) and from each site examined. Comparisons between human and fauna were made using animal remains from neighboring sites. However, the nitrogen concentrations, resulted in human collagen extracts (around 9‰), indicated a moderate animal protein intake. Regarding the comparison with previous periods, our results ($\delta^{15}N$ ratios recorded) differed from the range of measurements made on human bones from the inland Neolithic site of Mora di Cavorso in Central Italy (Martinez-Labarga *et alii*, 2007) and from inland Neolithic sites of Central and Northern Europe, that showed a typically enrichment in ^{15}N (by 4-5‰) to local herbivores (Richards 2002, 2003; Dürrwächter *et alii*, 2006; Hedges and Reynard, 2007). This contrast may support the hypothesis that intensive widespread cereal cultivation and consumption did not occur before the Bronze Age and the notion that cereal cultivation (C_3 plants) was more important in Mediterranean Bronze Age economies than pastoralism (Salvadei and Santandrea, 2003; Tafuri *et alii*, 2009). Although Group 2 nitrogen values were slight higher than Group 1 and 3, the comparison between the isotope data among the three groups (Group 1, 2, 3) did not indicate a significant difference. Conversely, the evaluation between inland sites (Group α: sites located

more than 20 km from the sea) and those closest to the coast (Group β: sites located less than 20 km from the sea) indicated a statistical difference in human nitrogen concentration. This result could depend on a slightly higher consumption of animal proteins in communities of Group 2 (that showed the highest concentrations of nitrogen). This hypothesis is the most plausible, since our $\delta^{15}N$ values suggested to rule out other factors that could increase nitrogen concentrations, such as manuring (Bogaard *et alii*, 2007; Fraser *et alii*, 2011; Makarewicz, 2014; Makarewocz and Tuross, 2012; Makarewicz and Sealy, 2015). Moreover, they did not indicate a marine/freshwather intake (according to standard of Shoeninger and De Niro, 1984). The lack of marine/freshwater resources consumption in Mediterranean coastal sites was confirmed by Craig *et alii* (2006) studies. These authors affirmed that during the Bronze Age marine foods were only a minor component to an overall terrestrial diet (Craig *et alii*, 2006).

Although there was not archaeological information on grave goods related to the skeletons sampled, there was evidence of the presence of grave goods in the examined sites (Larocca, 2005; Cipolloni Sampò, 1986 a-b; Betti Sestieri, 2011). In particular, grave goods of Toppo d'Aguzzo (Tomba 3) and Ipogeo dei Bronzi showed the presence of weapons in male burials (Cipolloni Sampò, 1986; Borgognini Tarli *et alii*, 1991-1992; Tunzi Sisto 1999, 2005). In addition, a preferential differentiation among burials of males, females and sub-adults (which could correspond to roles or rank of individuals) appeared in Ipogeo dei Bronzi (Vanzetti, 1999). A reasonable hypothesis is that feeding practices followed social complexity. It was possible that certain foods (i. e. meat) were restricted to particular social groups (Schoeninger, 1979). However, our data did not give any evidence of "status" differences both from the stable isotope comparisons between males and females and between age groups. Although our results have been obtained on a small sample, we can assert that cultural differences did not extend to the diet, or at least, if any dietary differences existed, they were not isotopically measurable. Indeed, the occasional consumption of "prestige" foods which would not be reflected in the long-term stable isotopic dietary record (Tafuri *et alii*, 2009).

Concluding, the isotopic record suggests a similar nutritional behavior for all environments analyzed (Calabria, Basilicata and Apulia). Results indicate a terrestrial diet relied on the consumption of C_3 crops (cereals and legumes) and allow to assume a moderate animal protein intake (meat or dairy products), that was slightly higher for the communities of Group 2. Although the sites of Calabria and Apulia are next to the sea and Grotta Funeraria and Murgia Timone (Basilicata) are close to a river (Bradano), no evidence of marine or freshwater consumption was recorded.

Appendix 1. Bone pretreatment: data sheet.

Phases:

1. Take a subsample out
2. Decalcification
3. Removal of humates
4. Dissolution/gelatination of collagen
5. Cleaning of Ezee- and ultrafilters
6. Ultrafiltration
7. Freeze-drying
8. Weighing collagen and storing it in tin-capsules

The whole pretreatment took approximately a week, depending on the quality of the bone and chosen concentration of HCL (Table 11). The gelatination of the collagen took routinely 4 days. Each filter was checked (^{14}C dated) before the use.

Wednesday	0.5 M HCl
Thursday	1 M HCl, wash, 0.2 M NaOH, wash, 10^{-2} M HCl, adjust pH
Friday	Adjust Ph if needed
Saturday	
Sunday	
Monday	Clean ultrafilters
Tuesday	Ultracentrifuge, freeze-dry
Wednesday	Weighing collagen and storing in tin-capsules

Friday	0.2 M HCl
Saturday	
Sunday	
Monday	1 M HCl, wash, 0.2 M NaOH, wash, 10^{-2} M HCl, adjust pH
Tuesday	Adjust Ph if needed, Clean ultrafilters
Wednesday	
Thursday	
Friday	Ultracentrifuge, freeze-dry
Saturday	
Sunday	
Monday	Weighing collagen and storing it in tin-capsules

Table 11. Table shows the good days to start the procedures (according to protocols approved by AMS Laboratory).

1. Take a subsample out

Samples were cleaned with a small drill (Proxton Micromat 50/E, U=max, 18V). Pliers was used to make tiny pieces of each sample with size of 4-5 mm. Bone powder and too small bone remains were discarded because of fibers of collagen could be damaged. Bone fragments were put inside test tube (Pyrex type) with ID sample, the weight and the labcode. The weight of samples was 500/600 mg for human bones and 400/500 mg for fauna. One background bone (a whale bone aged 40.000

BP) *per* 15 test tubes was used as a background control sample. In order to prevent contaminations, all work surfaces and instruments were disinfected with pure ethanol *per* each sample. The thin paper and bowls used were changed *per* each sample. At the end of this first operation, drills were cleaned with a specific procedure:

a. cleaning drills with distillated H_2O
b. putting them inside a backer and immerse them with distillated H_2O
c. transfer backer with drills to ultrasound and wait for 10 m
d. putting ethanol inside the backer
e. repeat ultrasound for 10 m
f. remove ethanol and putting drills inside oven (95°)

2. Decalcification

HCl concentrations were in general:

- 0.5 M (1 M HCl + 200 ml demineralized H_2O) for a night/over the weekend/when it was not possible change the acid on one day
- 1 M (37% HCl + 900 ml H_2O) for short time (hours/during the workday, when it was possible to observe the samples)

Decalcification was carried out with cold HCl (4°C). It was used 1 M HCl during workday or when it was possible to observe the samples. We have shifted to 0.5 M HCl at the end of the workday and to 1 M HCl next morning. The following operations were conducted:

- add 0.5 M HCl to the test tubes, but only half full. In effect, sometimes developed CO_2 immediately;
- wait until there was no more CO_2 bobbles (it taked from 20 minutes to a couple of hours). After one hour at 5°C, it was possible to continue at room temperature. When samples did not decalcify at the same time, we continued the procedure with the non-decalcified ones and paused those calcified in water until the rest finished too;
- centrifuge (Thermo Electron Corporation "Holm & Halby" 1700 rpm) for 5 minutes. The supernatant was discarded;
- add fresh acid (1 M HCl) and put all samples inside the fridge for few hours (in the morning);
- centrifuge (1700 rpm) for 5 minutes. The supernatant was discarded;
- add fresh acid (0.5 M HCl) and put all samples inside the fridge for few hours (in the afternoon);
- continue the operation until the mineral fraction was dissolved completely: no bubbles, no density gradient. Generally, this step could last from 4 to 7 days;
- at last put demineralized H_2O inside test tubes.

3. Removal of humates

This operation was carried out after test tubes were centrifuged and the water was discarded. The operation was carried out with cold NaOH (5°C). The following steps were observed:

- add 0.2 M NaOH (800 ml demineralized H_20 + 200ml 1 M NaOH) into test tubes and wait 15 minutes;
- centrifuge (with small centrifuge 2200 rpm or big centrifuge 1700 rpm) in order to discard the supernatant;
- if NaOH was colored, add 0.2 M NaOH again and wait 5 minutes;

- centrifuge and discard supernatant again;
- repeat until NaOH stay clear/light colored;
- when activity was stopped, put Millipore H_2O in test tubes to clean samples;
- wash twice with Millipore H_2O and centrifuge in between.

4. Dissolution/gelatination of collagen

It was added 10^{-2} M HCl covering the bone fragments (test tubes were about 1/3 full) and stirred. After 30 minutes, pH was checked using specific standard. It should be between 1.6-2.2. If it was not, 2 drops of 1 M HCl were put inside test tubes. After 30 minutes, pH should be in range (if it was not, 3 drops of HCl 1 M were put inside test tubes). After 10 minutes, pH was checked. This last operation was repeated until pH was in the right range, therefore samples were placed in the oven (58 °C) over one night. A glass lid or a small beaker was put on each test tube. The next day, samples were stirred and centrifuged. The supernatant (= dissolved collagen) was saved and put into new test tubes. These ones were placed in the fridge for 3 days. 10^{-2} M HCl was put into the test tubes with bone fragments inside. They were left up to 3 more nights more at 58°C or until all was dissolved. After centrifuge, supernant was saved and added to the dissolved collagen in corresponding test tube. They were stored in the fridge for 3 days.

5. Cleaning of ezee- and ultrafilters

This operation was performed 3 days prior to use filters. Cleaning procedure was different according to the type of filter.

Ezee-filters © (5mm):

- it was put filters in a backer with Millipore H_2O
- filters were put in ultrasound-bath for 20 minutes (ultrasound bath BRONSON 2510)
- it was rinsed (Millipore H_2O) each filter for two times pressing with a test-tube
- filters were placed in bags with a handkerchief moistened
- bags were placed in the fridge

Ultra-filters (>30kDa Amicon© ultrafilters, code: R4CA36137 for human collagen, R5SA92347 for fauna):

- it was added Millipore H_2O
- Ultrafilters were centrifuged twice (10 min, 3500 rpm “ultrafilter program”).
- it was filled the filters with other distilled H_2O
- filters were put in ultrasound bat (Bronson 1200) for 1 hour
- after bath, filters were filled with distillated H_2O and centrifuged for 10 minutes, 3500 rpm (this operation was repeated twice)
- filters were filled with Millipore H_2O
- it was pressed a rinsed ezee-filter carefully into each glass. Sample ID was written on them
- filters were put in the fridge for 1 night

6. Ultrafiltration

- ezee- and ultrafilters were centrifuged (3500 rpm) for 10 minutes
- after centrifuge, H_2O was discarded

After 3 days, samples were centrifuged (1700 rpm) for 5 minutes. Supernatant (= dissolved collagen) was put into rinsed and labeled ultra-filters and centrifuged for 5 minutes (3500 rpm). It was added more supernatant and centrifuged for 5 minutes again. This step was repeated until all supernatant was used and the collagen extract was reduced to an amount similar to the spiked part of the ultra-filter. It was necessary be careful to have balance in the centrifuge.
The collagen extract was transferred into new labeled Pyrex glasses: it was used a 180 µl Finn-pipette to suck up and down for 5 times. A 800 µl pipette was used to put distillated water into the ultrafilter, therefore it was shifted to 180 µl Finn-pipette to suck up and down a couple of times and to put collagen into the Pyrex. This last step was repeated twice. Collagen extract was frozen in a tilted position 2-3 hours or overnight. Small lids with holes (or parafilm with holes) were put on Pyrex test tubes.

7. Freeze-drying

The frozen samples were washed with liquid nitrogen. Pyrex were blocked with caps and put inside the Freeze-dryer for one day.

9. Weighing collagen and storing it in tin-capsules

This last operation preceded the Mass Spectrometry. After pulling out samples from Freez-dryer, collagen was obtained. It was weighing and each value was put into database to calculate the collagen yield percentages. A collagen fragment was removed from each sample. Each collagen sub-sample, after being weighed (0.200 to 0.300 mg), was packaged in an aluminum tin-capsule (4x4x11 mm ELEMENTAR model). Each capsule was put in the box. A duplicate of a sample was prepared every 5 samples. For each series of the box was prepared a reference standard (0.200-0.300 mg): it was a gel with very thin granulometry (GEL-AD SID6 UDT: $^{15}/_{12\text{-}10}$). Ten standards were prepared. The ID gel/samples, the weights and the box position were written up in a sheet. In order to avoid contamination all work surfaces and instruments were disinfected with pure ethanol *per* each sample. For the same reason, thin paper and bowls used were changed *per* each sample.

CHAPTER 3: DIET-RELATED MICROWEAR PATTERNS IN BRONZE AGE POPULATIONS LIVING IN SOUTHERN ITALY

3.1 Introduction

Dental microwear analysis is a technique used to study microscopic features on the enamel of tooth surfaces (Teaford 1991, 1994). The rate of tooth wear is useful for paleo-nutritional investigations because it depends on both the masticatory frequency and the force produced by the upper and lower jaws during mastication and especially on the types of diet, specifically on the amount and kind of abrasive particles contained in the food (Molnar, 1972; Bermúdez de Castro *et alii,* 2003; Kaifu, 2000; Kaifu, Kasai, Townsend and Richards, 2003). In fact, the hard abrasive particles, such as the siliceous phytolites (present in plants, leaves, fruits or medullas), are able to produce microscopic damage (Galbany *et alii,* 2005). Researchers detected two types of dental microwear in order to reconstruct ancient diet: pits and scratches. *Pits* result from compression of food or contaminant particles on the enamel surface of teeth during the power stroke (Maas, 1994; Schmidt, 2010); *scratches* originate from the manner in which particles are dragged between opposite enamel surfaces of the teeth (Ryan, 1979). Therefore, changes in dietary habits can be evaluated through the frequency, size, orientation and morphology of these patterns (Mahoney, 2007). Studies on Paleolithic diet, performing on living populations, pointed out that people who consumed hard foods had a higher percentage of pits than those who eat soft foods. As a matter of fact they showed a higher percentage of scratches. Therefore, pits were characteristics of an omnivorous diet and scratches of a mostly vegetarian diet (Butler, 1952; Walker *et alii*, 1978). Many reviews on dental microwear (Teaford, 1988, 1994, 2006; Ungar, 1998; Rose and Ungar, 1998) showed the resulting advances over the past half century, such as the introduction of SEM (Scanning Electron Microscope) in order to investigate the full potential of microwear patterns as an analytical tool (Puech, 1977; Walker *et alii,* 1978; Rensberger, 1978; Puech and Prone, 1979; Ryan, 1979; Puech *et alii,* 1980; Walker, 1980, 1981; Grine, 1981; Puech *et alii,* 1981; Ryan, 1981; Rensberger, 1982). Standardization of imaging techniques and quantification of individual features were introduced as researchers began to count and measure scratches and pits on photomicrographs (Gordon 1982, 1984 a-c; Teaford and Walker, 1984). Ungar (1995) proposed a semi-automated procedure that required the identification of features by an observer, and it yielded computer-based tallies and measurements. These studies gave further evidence of the potential of dental microwear for reconstructing diet and subsistence practices of past peoples.

Very few studies on the dental wear of Italian Bronze Age populations have been published until now in international journals (Cucina, 2002; Dori and Moggi-Cecchi, 2014) and none of them examined tooth micro-wear patterns of Southern Italian Bronze Age population in order to diet's reconstruction. However, the research by Masotti *et alii* (2017) on Early Bronze Age inhabitants of Ballabio (Valsassina, Lombardy) should be mentioned. Authors found a mostly vegetarian diet for this community, although the high variability of microwear patterns suggested that it was necessary to analyze deeply these aspects in order to a better characterization of the diet.

This study aims to provide information on Southern Italian Bronze Age communities never investigated using this approach. Analysis was conducted on samples from different sites of Southern Italy, considering several regions (Calabria, Basilicata and Apulia) and environments

(mountain, hill, plain). In this way, inter- and intra-regional variations in food habits linked to the availability of resources and livelihood strategies were examined.

3.2 Materials and methods

3.2.1 The sample

This study was based on the analyses of the occlusal surfaces of 24 human permanent teeth from four Bronze Age sites of Calabria (Grotta della Monaca and Grotta dell'Antenato), Basilicata (Toppo d'Aguzzo) and Apulia (Ipogeo dei Bronzi) as listed in (Table 1):

Sample ID	Site	Archaeological information	Teeth
1	Grotta della Monaca	GdM/CTv/m5v/C/lv2/tg1/08	M2i
2	Grotta della Monaca	GdM /CTv/B/Z2/03	M1s
3	Grotta della Monaca	GdM/CTv S7/sC/D4I -12-23/08	M2i
4	Grotta della Monaca	GdM/CTsx.i/cap 12-13/00	M2s
5	Ipogeo dei Bronzi	IpB/SFQ D6	M2i
6	Ipogeo dei Bronzi	IpB/QE9 (e)	M3i
7	Grotta dell'Antenato	GdA/liv. sup.	M1s
8	Toppo d'Aguzzo	S' 17 150-180 cm 15	M1i
9	Toppo d'Aguzzo	S' 17 150-180 cm 8	M1i
10	Grotta della Monaca	GdM /Ctv/m5v/S/tg2/08	Cs
11	Grotta della Monaca	GdM /Ctv/m5v/S/tg2/08	Cs
12	Toppo d'Aguzzo	TDT109b	Ci
13	Toppo d'Aguzzo	TDT104b	M3i
14	Toppo d'Aguzzo	TDT110b	Pi
15	Toppo d'Aguzzo	TDT106b	M2i
16	Toppo d'Aguzzo	TDT107b	P2s
17	Toppo d'Aguzzo	TDT108b	M1i
18	Ipogeo dei Bronzi	IpB01d	M1i
19	Ipogeo dei Bronzi	IpB02d	M2i
20	Ipogeo dei Bronzi	IpB03d	I1i
21	Ipogeo dei Bronzi	IpB06d	P1i
22	Grotta della Monaca	GdM/CTdx-fv/Z3 (d) '02	M1s
23	Grotta della Monaca	GdM/Ctdx-fv/D12/'03	M1s
24	Grotta della Monaca	GdM/CTdx-fv/D11	M2i

Table 1. Dental samples analyzed by SEM and archaeological provenience (R= right; L= left; ND= non determined. Site abbreviations are mentioned in the Chapter 1).

Different types of teeth were selected, according to lower degree of macrowear (stage 2-3 by Smith 1984) on occlusal surfaces. The table below shows the total sample composition by tooth type, jaw and status (Table 2):

Tooth type	Total n= 24		Maxillary teeth				Mandibular teeth				ND for side		Isolated		Not isolated	
			Right		Left		Right		Left							
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
I	1	4.2	0	0	0	0	0	0	1	4.2	0	0	0	0	1	4.2
C	3	12.5	0	0	1	4.2	1	4.2	0	0	1	4.2	2	8.3	1	4.2
P	3	12.5	0	0	1	4.2	2	8.3	0	0	0	0	0	0	3	12.5
M	17	79.8	5	20.8	0	0	4	16.7	8	33.3	0	0	8	33.3	9	37.5

Table 2. Sample composition by tooth type, jaw and status (isolated and not isolated), N= 24.

In order to compare these data with those obtained through other analyses, the samples were grouped in three series according to geographical area (Group 1, 2, 3):

- Group 1: inland mountain-hilly sites of Calabria (Grotta della Monaca, Grotta dell'Antenato);
- Group 2: inland hilly sites of Basilicata (Toppo d'Aguzzo);
- Group 3: inland plan of Puglia (Ipogeo dei Bronzi).

It is necessary to remark that it was not possible to involve all the sites examined by stable isotopes, because of the unavailability of teeth in some sites.

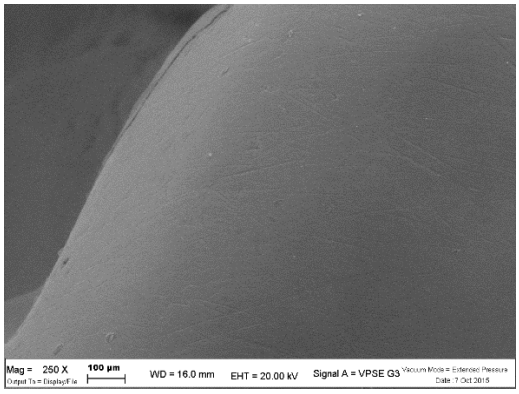
3.2.2 Biological profile of individuals: methods

When teeth are *in situ* (not isolated teeth), it was possible to associate them to the skeleton they belonged. In these cases, the sex and age at death assessments were performed to provide the biological profile of individuals. The methods and protocols used have been detailed previously in Chapter 2.
Age classes used in this study were composed according to Buikstra and Ubelaker (1994), as follows: Young Adults (20-35 years of age), Middle Adults (35-50 years of age), Old Adults (50+ years of age). Moreover, in order to compare results with stable isotopes data, two groups age-related have been also considered: young adults (< 35 years old) and adults (> 35 years old).

3.2.3 SEM analyses: diet-related microwear patterns and microanalyses of chemical elements

In order to define the different *microwear patterns* of this sample, tooth occlusal surfaces were examined using SEM Zeiss EVO 40 (LaB6 source, 30KV accelerating voltage) with magnification set at 250x at Electron Microscopy Center (Ferrara University). Specimens were observed at a variable depth of a few microns, with the possibility to operate in high vacuum and variable pressure (SEM XVP), with a maximum pressure of 6 Torr. Some of the specimens were cut by a hacksaw in order to insert them in the specimen chamber. Therefore, they were fixed on a specimen stub, perpendicularly to the support. SEM images (250x) of a selected area of tooth occlusal surfaces were obtained (Fig. 1). Digital micrographs were converted to bitmap file format and imported into a semi-automated microwear analysis software called Microwear 4.02 (Ungar, 2002). All features dimensions and frequencies were assessed by this program according to the examination protocol developed by Ungar (1995) and Ungar, Scott, Scott & Teaford (2008): pits and scratches were discriminated using a 4:1 length-to-width ratio and their frequency was calculated. The pits were identified with a value below 4:1 ratio, while the scratches with a value

above this threshold (Fig. 2). Microwear analysis included several variables: percentage of pits, percentage of scratches, pit length (microns), pit width (microns), scratch length (microns) and scratch width (microns), scratch tally and pit tally. Data were stored as ASCII files (Fig. 3).
SEM was equipped with a computerized *microanalysis* system (Link ISIS) and the spectrometry for the X-ray energy dispersive (EDS), that allowed to analyze the distribution and percentages of the inorganic chemical elements of dental hard tissues by comparing the X-ray emission of chemical elements with the specific standards (Fig. 4).



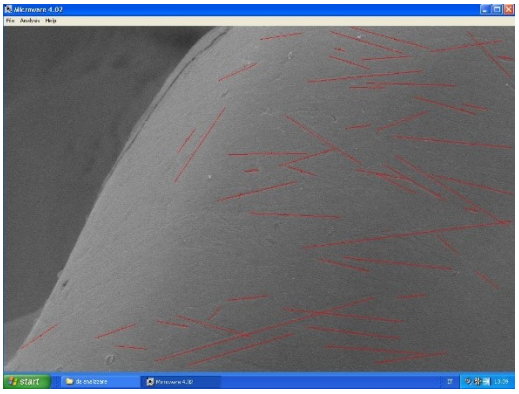
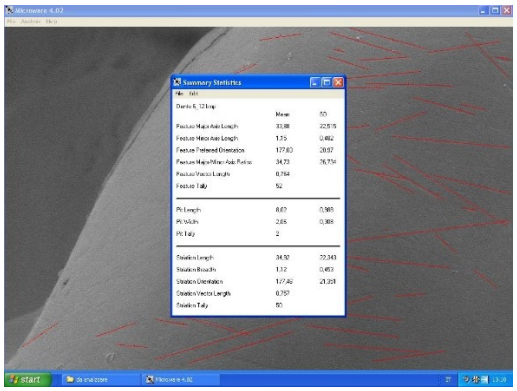


Fig. 1. SEM image at 250 x magnification. The area of the occlusal surface selected (area= 0.0441 mm^2).
Fig. 2. The image elaborated with Microwear 4.02 (Ungar, 2002).



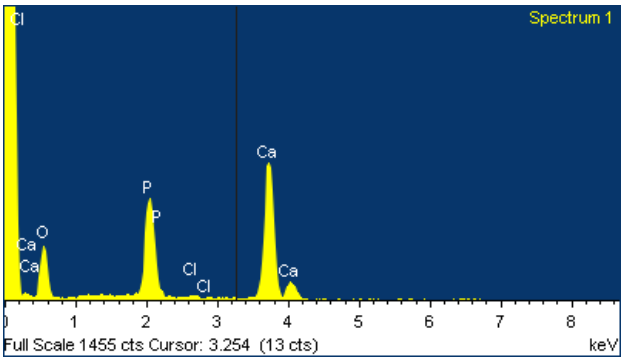


Fig. 3. Data set calculated by Microwear 4.02 (Ungar, 2002) automatically.
Fig. 4. Microanalyses on the dental occlusal surface selected (0.0441 mm^2) elaborated automatically by SEM.

3.2.4 Statistical methods

The results were expressed as means and standard deviation for quantitative variables and as absolute frequencies and percentages for qualitative variables. Chi-square test was used for comparisons between frequencies. Statistical comparisons were carried out for the following quantitative variables that have been shown in the past to be useful for distinguishing living species with different diets: 1) pits length, 2) pits width, 3) scatches length, 4) scratches width (Walker and Teaford, 1989; Ungar *et alii*, 1994; Teaford *et alii*, 2001; Rafferty *et alii,* 2002; Ungar, 2016). Non-parametric tests were used to compare quantitative variables: Mann-Whitney U-test (2 items) and Kruskal-Wallis test (3 items). The data were processed by Statistica for Windows, Version 11.0 (StatSoft Italia srl, Vigonza, Padua, Italy). A $p < 0.05$ was considered statistically significant.

3.3 Results

3.3.1 Biological profile

Biological characteristics of individuals to which the examined specimens belonged are presented in Table 3.

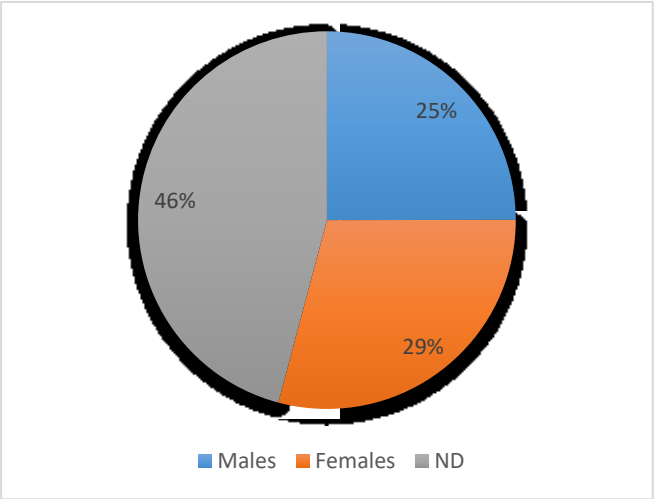
Sample ID	Site	Archaeological information	Teeth	Sex	Age class
1	GdM	GdM /CTv/m5v/C/lv2/tg1/08	M2i	ND	adult
2	GdM	GdM /CTv/B/Z2/03	M1s	ND	adult
3	GdM	GdM/CTv S7/sC/D4I -12-23/08	M2i	ND	adult
4	GdM	GdM/CTsx.i/cap 12-13/00	M2s	ND	adult
5	IpB	IpB/SFQ D6	M2i	M	adult
6	IpB	IpB/QE9 (e)	M3i	F	adult
7	GdA	GdA/liv. Sup.	M1s	ND	adult
8	TDT	S' 17 150-180 cm 15	M1i	M	young adult
9	TDT	S' 17 150-180 cm 8	M1i	F	young adult
10	GdM	GdM /Ctv/m5v/S/tg2/08	Cs	ND	adult
11	GdM	GdM /Ctv/m5v/S/tg2/08	Cs	ND	adult
12	TDT	TDT109b	Ci	M	middle adult
13	TDT	TDT104b	M3i	F	old adult
14	TDT	TDT110b	Pi	F	old adult
15	TDT	TDT106b	M2i	M	young adult
16	TDT	TDT107b	P2s	F	middle adult
17	TDT	TDT108b	M1i	M	young adult
18	IpB	IpB01d	M1i	ND	adult
19	IpB	IpB02d	M2i	F	adult
20	IpB	IpB03d	I1i	M	adult
21	IpB	IpB06d	P1i	F	adult
22	GdM	GdM/CTdx-fv/Z3 (d) '02	M1s	ND	adult
23	GdM	GdM/Ctdx-fv/D12/'03	M1s	ND	adult
24	GdM	GdM/CTdx-fv/D11	M2i	ND	adult

Table 3. Details of human teeth sampled for this analysis (i: inferior; s: superior; ND: non-determinable; M: male; F: female. Site abbreviations were specified in the Chapter 1). We use "adult" when it was not possible to specify the age at death of individuals.

According to sex diagnosis, 7 specimens (29.2%) belonged to females and 6 (25%) to males. The sex assessment was not possible for 11 specimens (45.9%) because 10 of them were isolated teeth (not associated to any skeletons) and, in one case, the skeleton did not preserve the anatomical districts needed for this diagnosis. Concerning the age classes, 4 specimens belonged to young adults, 2 to middle adults, 2 to old adults and 16 could only be referred generically as "adults" (Table 4; Fig. 5, 6).

Age class	Males	Females	ND	Total	% (n= 24)
YA	3	1	0	4	16.7
MA	1	1	0	2	8.3
OA	0	2	0	2	8.3
A	2	3	11	16	66.7
Total	6	7	11	24	100

Table 4. The total sample composition by sex and age at death (YA= young adults; MA= middle adults; OA= old adults; ND= non-determinable).



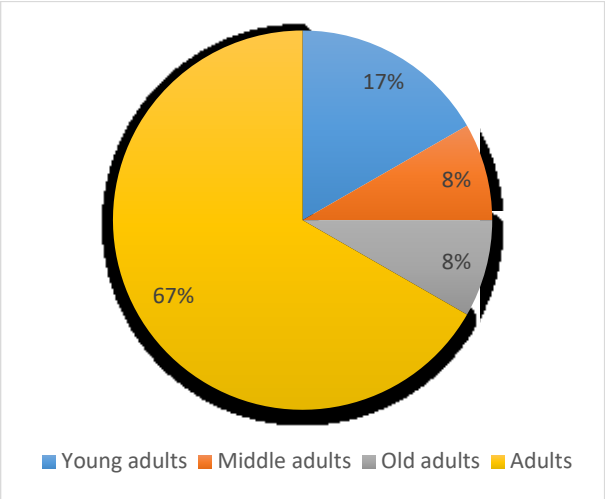


Fig. 5. Frequency of the samples by sex (N= 24).
Fig. 6. Frequency of the samples by age at death (N= 24).

3.3.2 SEM analysis

Microwear patterns diet-related

Analysis performed on occlusal selected areas of 24 tooth surfaces pointed out 580 markers: 500 scratches (86.2%) and 80 pits (13.8%). The table below shows results obtained for each specimen (Table 5):

Sample ID	Site	Teeth	I	ND	Side	Sex	Age class	Scratches		Pits	
								N	%	N	%
Group 1											
1	GdM	M2i	1		L	ND	adult	55	88.7	7	11.3
2	GdM	M1s	1		R	ND	adult	4	57.1	3	42.9
3	GdM	M2i	1		L	ND	adult	50	96.2	2	3.8
4	GdM	M2s	1		R	ND	adult	42	64.6	23	35.4
10	GdM	Cs	1		L	ND	adult	2	100	0	0
11	GdM	Cs	1		ND	ND	adult	31	93.9	2	6.1
22	GdM	M1s	1		R	ND	adult	2	18.2	9	81.8
23	GdM	M1s	1		R	ND	adult	19	100	0	0
24	GdM	M2i	1		L	ND	adult	17	100	0	0
7	GdA	M1s	1		R	ND	adult	10	100	0	0
								N	**%**	**N**	**%**
SUB TOTAL								232	83.5	46	16.5
Group 2											
8	TDT	M1i		1	L	M	young adult	14	93.3	1	6.7
9	TDT	M1i		1	L	F	young adult	0	0	6	100
12	TDT	Ci		1	R	M	middle adult	1	8.3	11	91.7
13	TDT	M3i		1	R	F	old adult	33	97.1	1	2.9
14	TDT	Pi		1	R	F	old adult	2	66.7	1	33.3
15	TDT	M2i		1	L	M	young adult	5	55.6	4	44.4

16	TDT	P2s		1	L	F	middle adult	17	100	0	0
17	TDT	M1i		1	R	M	young adult	99	99.9	1	1
								N	**%**	**N**	**%**
							SUB TOTAL	171	87.2	25	12.8
Group 3											
5	IpB	M2i		1	R	M	adult	20	100	0	0
6	IpB	M3i		1	L	F	adult	14	100	0	0
18	IpB	M1i		1	L	ND	adult	25	100	0	0
19	IpB	M2i		1	R	F	adult	2	18.2	9	81.8
20	IpB	I1i		1	L	M	adult	19	100	0	0
21	IpB	P1i		1	R	F	adult	17	100	0	0
								N	**%**	**N**	**%**
							SUB TOTAL	97	91.5	9	8.5
								N	**%**	**N**	**%**
							TOTAL	500	86.2	80	13.8

Table 5. Frequency of scratches and pits *per* tooth and *per* group 1, 2, 3 (ND= not determined. Site abbreviations were specified in the chapter 1).

In order to point out differences depending on different environments, Chi-square test was used to compare scratches and pits frequencies among the three groups (1, 2, 3). The results did not indicate significant differences: χ^2= 4.91, d.f.= 2, p= 0.0858.

The mean values of the variables (length, width and frequency) obtained *per* tooth are shown in the tables below (Table 6a, b). No statistical difference resulted by the comparison among quantitative features (scratches/pits length and width) in the total sample (Table 6a).

	Mean	**SD**	**H**	**p**
Number of pits	3.4	5.2		
Number of scratches	22.5	22.5		
Number of pits (%)	20.3	30.8		
Number of scratches (%)	77.0	21.4		
Pits length (µm)	6.8	3.5	3.83	0.1474
Pits width (µm)	3.1	1.5	3.25	0.1965
Scratches length (µm)	36.3	11.1	1.10	0.5765
Scratches width (µm)	1.8	2.6	1.95	0.3767

Table 6a. Mean values of length, width and frequency of scratches and pits *per* tooth in the total sample.

Group 1		
	Mean	**SD**
Number of pits	4.6	7.2
Number of scratches	23.3	20.2
Number of pits (%)	18.1	27.2
Number of scratches (%)	81.8	27.2
Pits length(µm)	6.5	2.5
Pits width (µm)	2.8	1.1
Scratches length (µm)	34.4	10.7
Scratches width (µm)	1.7	1.2
Group 2		
	Mean	**SD**
Number of pits	3.1	3.8
Number of scratches	21.3	32.9
Number of pits (%)	35.0	40.9
Number of scratches (%)	65.0	41.0

Pits length (μm)	8.4	4.1
Pits width (μm)	3.8	1.9
Scratches length (μm)	38.4	12.0
Scratches width (μm)	2.8	4.5
Group 3		
	Mean	**SD**
Number of pits	1.7	2.3
Number of scratches	22.7	10.6
Number of pits (%)	4.2	5.2
Number of scratches (%)	84.9	28.2
Pits length (μm)	3.7	1.0
Pits width (μm)	2.4	0.4
Scratches length (μm)	36.9	12.2
Scratches width (μm)	1.1	0.2

Table 6b. Mean values of length, width and frequency of scratches and pits *per* tooth in groups from different areas (1, 2, 3).

Taking sex into consideration, the analysis was possible only in Group 2 (TDT) and Group 3 (IpB), because of the availability of the sample. Scratches were much more frequent than pits in males and females (Table 7) with no significant difference in occurrence between the two sexes (χ^2= 3.05, d.f.= 1, p= 0.0806).

	Males		**Females**	
	N	**%**	**N**	**%**
Scratches	158	90.3	85	83.3
Pits	17	9.7	17	16.7
Total	175	100	102	100

Table 7. Frequency of scratches and pits in the total sample, according to the sex.

The analyses of frequency in each group showed that scratches were more present than pits in males and pits were more frequent in females with not statistical difference in Group 2 (χ^2= 0.03, d.f.= 1, p= 0.8719), but a substantial difference in Group 3 (χ^2= 9.40, d.f.= 1, p= 0.0022).

	Scratches		**Pits**	
Group 1				
	N	**%**	**N**	**%**
Males	0	0	0	0
Females	0	0	0	0
ND	232	83.5	46	16.5
	N	**%**	**N**	**%**
TOTAL	232	83.5	46	16.5
Group 2				
	N	**%**	**N**	**%**
Males	119	87.5	17	12.5
Females	52	86.7	8	13.3
ND	0	0	0	0
	N	**%**	**N**	**%**
TOTAL	171	87.2	25	12.8 12.8
Group 3				
	N	**%**	**N**	**%**
Males	39	100	0	0
Females	33	78.6	9	21.4
ND	25	100	0	0
	N	**%**	**N**	**%**

TOTAL	97	91.5	9	8.5

Table 8. Scratches and pits frequency in each group from different areas according to sex.

Mean values of length, width and frequency of scratches and pits (obtained *per* tooth) according to the sex, are shown in Tables 9 (a-b). No statistical difference resulted by comparison among quantitative variables (Table 9a-b).

Males			**Females**			
	Mean	**SD**	**Mean**	**SD**	**U**	**p**
Number of pits	3.5	4.0	2.0	2.4		
Number of scratches	27.2	35.8	16.9	15.2		
Number of pits (%)	24.7	36.8	22.4	36.1		
Number of scratches (%)	64.1	39.6	77.8	36.2		
Pits length (µm)	9.0	4.5	4.9	2.5	4.00	0.0758
Pits width (µm)	4.2	2.2	2.5	0.4	6.00	0.1745
Scratches length (µm)	40.2	16.1	37.2	5.0	14.00	0.5218
Scratches width (µm)	3.1	4.9	1.1	0.2	18.00	1.0000

Table 9a. Microwear evaluation according to the sex: length, width and frequency of scratches in the total sample.

Group 2						
Males			**Females**			
	Mean	**SD**	**Mean**	**SD**	**U**	**p**
Number of pits	5.3	5.1	2	2.7		
Number of scratches	6.7	6.7	13	15.3		
Number of pits (%)	47.6	42.5	34.1	46.5		
Number of scratches (%)	52.4	42.6	66	46.5		
Pits length (µm)	12.0	2.5	6	2.8	2.00	0.1573
Pits width (µm)	5.4	2.1	2.7	0.1	2.00	0.1536
Scratches length (µm)	40.9	18.5	39.9	3.0	3.00	0.2888
Scratches width (µm)	5.1	6.8	1.1	0.1	4.00	0.7071
Group 3						
Males			**Females**			
	Mean	**SD**	**Mean**	**SD**	**U**	**p**
Number of pits	2.0	2.8	2	2.7		
Number of scratches	22.5	3.5	22	16.5		
Number of pits (%)	2.3	3.2	6.8	6.3		
Number of scratches (%)	64.1	50.8	93.7	5.7		
Pits length (µm)	2.3	3.3	3.2	0.8	0.00	1.0000
Pits width (µm)	1.2	1.7	2.3	0.5	0.00	1.0000
Scratches length (µm)	46.1	18.8	34.6	5.8	2.00	0.5637
Scratches width (µm)	1.0	0.2	1.2	0.2	1.00	0.2582

Table 9b. Microwear evaluation according to the sex: length, width and frequency of scratches and pits in Group 2, 3.

According to age classes, this estimation was possible only for individuals of Group 2. The samples of Group 1 and 3 could only be generically defined as "adults". In Group 2, the highest frequency of scratches was shown in old adults and the highest frequency of pits in middle adults -the less numerous class- (Table 10a). The Chi-square test among the three age classes (young adults, middle adults, old adults) showed a statistical difference in scratches/pits occurrence (χ^2= 17.98, d.f.= 2, p= 0.0001). Moreover, in order to compare these results with those obtained by stable isotopes analysis, two age clusters were established: individuals < 35 years of age (young adults) and those > 35 years of age (middle and old adults) (Table 10b). Also in this case, the statistically significant

difference in scratches/pits frequency between the two age groups was confirmed: χ^2= 4.31, d.f.= 1, p= 0.0379.

	Scratches		Pits	
	N	**%**	**N**	**%**
Young adults	118	90.8	12	9.2
Middle adults	18	62.1	11	37.9
Old adults	35	94.6	2	5.4
	N	**%**	**N**	**%**
Total	171	87.2	25	12.8

Table 10a. Scratches and pits percentages in age classes of Group 2.

	Scratches		Pits	
	N	**%**	**N**	**%**
< 35 years of age	118	90.8	12	9.2
> 35 years of age	53	80.3	13	19.7
	N	**%**	**N**	**%**
Total	171	87.2	25	12.8

Table 10b. Scratches and pits percentages in two age clusters of Group 2.

Mean values of length, width and frequency of scratches and pits *per* each tooth, according to the age classes, are shown in Table 11 (a-b). No statistical difference resulted by comparison of quantitative variables among the three age classes (Table 11a); while a statistical difference resulted in scratches length by the comparison between the two clusters age-related (Table 11b).

Group 2								
Young adults			**Middle adults**		**Old adults**			
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**H**	**p**
Number of pits	3	2.5	5.5	7.8	1	0		
Number of scratches	29.5	46.2	9	11.3	17.5	21.9		
Number of pits (%)	38.3	45.6	45.8	64.8	18.1	21.5		
Number of scratches (%)	62	45.6	54.2	64.8	81.9	21.5		
Pits length (µm)	8.4	4.6	12.2	0	6.3	3.8	1.88	0.3916
Pits width (µm)	3.6	1.6	7.1	0	2.6	0.4	3.31	0.1907
Scratches length (µm)	29.1	3.5	52.3	13.8	38.5	1.9	5.36	0.0687
Scratches width (µm)	1.2	0.3	7.0	8.4	1	0.2	0.61	0.7382

Table 11. Microwear evaluation according to the age classes: length, width and frequency of scratches and pits in Group 2.

Group 2						
Young adults (< 35 years of age)			**Adults (> 35 years of age)**			
	Mean	**SD**	**Mean**	**SD**	**U**	**p**
Number of pits	3.0	2.5	3.3	5.2		
Number of scratches	29.3	46.2	13.3	15.1		
Number of pits (%)	38.0	45.6	32.0	42.5		
Number of scratches (%)	62.0	45.6	68.0	42.6		
Pits length (µm)	8.4	4.6	8.2	4.3	5.00	0.7236
Pits width (µm)	3.6	1.6	4.1	2.6	5.50	0.8596
Scratches length (µm)	29.1	3.5	45.4	11.3	0.00	0.0338
Scratches width (µm)	1.2	0.3	4.0	6.0	5.50	0.7236

Table 11b. Microwear evaluation according to the age clusters: length, width and frequency of scratches and pits in Group 2.

According to the side, scratches resulted more frequent on the left side and pits on the right side in the general sample (Table 12). The differences were statistically significant (χ^2= 11.47, d.f.= 1, p= 0.0007).

	Scratches		Pits	
	N	**%**	**N**	**%**
Right	251	81.2	58	18.8
Left	218	91.6	20	8.4
	N	**%**	**N**	**%**
Total	469	85.7	78	14.3

Table 12. Scratches and pits percentages by side.

The following table (Table 13) shows the percentages of scratches and pits in each group. Only one tooth (Group 1), with 31 scratches and 2 pits, was not determinable for side. Chi-square test showed a statistical difference in scratches/pits occurrence by side in Group 2 (χ^2= 6.28, d.f.= 1, p= 0.0122) (scratches were more frequent on the right and pits on the left) and Group 3 (χ^2= 12.29, d.f.= 1, p= 0.0005) (scratches were more frequent on the left and pits on the right), but not in Group 1 (χ^2= 26.62, d.f.= 1, p= 2.4766).

	Scratches		Pits	
Group 1				
	N	**%**	**N**	**%**
Right	77	68.7	35	31.3
Left	124	93.2	9	6.8
	N	**%**	**N**	**%**
TOTAL	201	82	44	18
Group 2				
	N	**%**	**N**	**%**
Right	135	90.6	14	9.4
Left	36	76.6	11	23.4
	N	**%**	**N**	**%**
TOTAL	171	87.2	25	12.8
Group 3				
	N	**%**	**N**	**%**
Right	39	81.3	9	18.8
Left	58	100	0	0
	N	**%**	**N**	**%**
TOTAL	97	91.5	9	8.5

Table 13. Scratches and pits percentages in each group.

Mean values of length, width and frequency of scratches and pits, according to the side, are shown in the tables below. The statistical difference of scratch length resulted by the comparison among quantitative variables in the total sample and in Group 3 (Table 14a-b):

	Right		Left			
	Mean	**SD**	**Mean**	**SD**	**U**	**p**
Number of pits	4.6	6.9	2.2	2.6		
Number of scratches	23.6	27.8	20.4	18.0		
Number of pits (%)	25.8	32.4	15.5	30.9		
Number of scratches (%)	74.3	32.5	78.4	34.9		
Pits length (µm)	5.6	3.2	8.0	3.7	12.00	0.0771
Pits width (µm)	3.2	1.7	3.1	1.5	24.50	0.7683
Scratches length (µm)	41.2	12.8	30.8	5.8	26.00	0.0250

Scratches width (µm)	2.4	3.5	1.3	0.4	58.00	0.8951

Table 14a. Microwear evaluation according to the side: length, width and frequency of scratches and pits in the total sample.

Group 1						
Right			**Left**			
	Mean	**SD**	**Mean**	**SD**	**U**	**p**
Number of pits	3.0	4.2	6.4	9.7		
Number of scratches	8.8	7.6	33.2	22.8		
Number of pits (%)	31.2	39.4	10.1	14.9		
Number of scratches (%)	68.8	39.4	89.9	14.9		
Pits length (µm)	6.0	2.6	5.7	2.4	1.00	0.2482
Pits width (µm)	3.8	1.5	2.0	0.2	2.00	0.5637
Scratches length (µm)	42.1	13.2	28.7	5.3	5.00	0.2208
Scratches width (µm)	2.1	1.8	1.5	0.5	10.00	1.0000
Group 2						
Right			**Left**			
	Mean	**SD**	**Mean**	**SD**	**U**	**p**
Number of pits	3.5	5.0	2.8	2.8		
Number of scratches	33.5	45.5	9.0	7.8		
Number of pits (%)	32.2	42.3	37.8	45.7		
Number of scratches (%)	67.8	42.3	62.2	45.7		
Pits length (µm)	7.3	4.0	9.8	4.5	3.00	0.2888
Pits width (µm)	3.7	2.3	4.0	1.7	3.50	0.3768
Scratches length (µm)	41.4	14.9	34.4	7.5	5.00	0.7237
Scratches width (µm)	3.9	6.0	1.2	0.2	5.00	0.7237
Group 3						
Right			**Left**			
	Mean	**SD**	**Mean**	**SD**	**U**	**p**
Number of pits	2.0	2.7	1.3	2.3		
Number of scratches	24.0	15.4	21.3	6.4		
Number of pits (%)	6.8	6.3	1.5	2.6		
Number of scratches (%)	93.7	5.7	76.0	41.5		
Pits length (µm)	3.2	0.8	4.6	0.0	0.00	1.0000
Pits width (µm)	2.3	0.5	2.5	0.0	0.00	1.0000
Scratches length (µm)	45.0	12.6	29.8	3.8	0.00	0.0495
Scratches width (µm)	1.1	0.2	1.1	0.1	3.00	0.5127

Table 14b. Microwear evaluation according to the side: length, width and frequency of scratches and pits in Group 1, 2, 3.

Microanalyses

Concerning chemical micro-analyses of enamel tissue, Ca and P are the elements present in all 24 specimens (100%). Fe was present in 6 of 24 specimens (25%) from two sites (GdM, TDT). The 25% of specimens (N= 24) from the same sites (GdM, TDT) contained C. Cl was present only in one specimen (4.2%, N= 24) of the sample 3 (GdM). Al was present in 8 of 24 specimens (33.3%) from three sites (GdM, TDT, IpB). The 45.8% of the total sample (N= 24) shows the presence of Si (GdM, IpB, TDT). Mg was present in 2 of 24 specimens (8.3%) from two sites (GdM, IpB). Na was present in only one of 24 specimens (4.2%) from IpB site. The table below gives the detailed results obtained (Table 15).

Sample ID	Ca	P	Fe	C	Cl	Al	Si	Mg	Na
1	P	P	P	P	0	0	0	0	0
2	32.0	12.2	3.6	0	0	0	0	0	0
3	15.6	14.8	0	0	0.4	0	0	0	0
4	34.6	16.2	0	0	0	0	0	0	0
5	33.9	18.9	0	0	0	0	0	0	0
6	30.6	17.5	0	0	0	0	0	0	0
7	27.6	10.9	0	0	0	1.7	3.7	2.3	0
8	27.6	13.9	0	0	0	0	0	0	0
9	23.4	8.0	0	0	0	0	4.0	0	0
10	27.9	15.0	1.6	0	0	0	0	0	0
11	35.1	14.3	3.9	0	0	0	0	0	0
12	24.5	11.1	0	18.1	0	0.6	0.8	0	0
13	17.6	8.9	0.9	11.2	0	1.5	3.2	0	0
14	3.8	0	0	71.8	0	0	1.7	0	0
15	21.1	10.0	0	17.7	0	1.0	1.5	0	0
16	20.1	10.2	0.6	11.1	0	1.1	1.7	0	0
17	20.7	11.0	0	8.2	0	1.7	2.9	0	0
18	55.8	15.2	0	0	0	0	0	0	0
19	30.9	15.8	0	0	0	0	1.5	0	0
20	30.3	13.2	0	0	0	1.4	3.6	0	0
21	27.5	13.0	0	0	0	1.4	4.2	0.6	0.5
22	35.5	16.0	0	0	0	0	0	0	0
23	33.6	14.8	0	0	0	0	0	0	0
24	23.7	11.8	4.9	0	0	0	0	0	0
Mean	27.5	12.7	0.5	6.0	0	0.3	1	0.1	0
SD	9.71	3.9	1.2	15.5	0	0.5	1.4	0.4	0

Table 15. Chemical elements percentages (%) in each sample (chemical components of specimens 1, available in small quantities, were generically indicated by the software as "P", present).

The figures below show the chemical element's ratios in each sample (Fig. 7) and the percentages of specimens *per* each chemical element (Fig. 8).

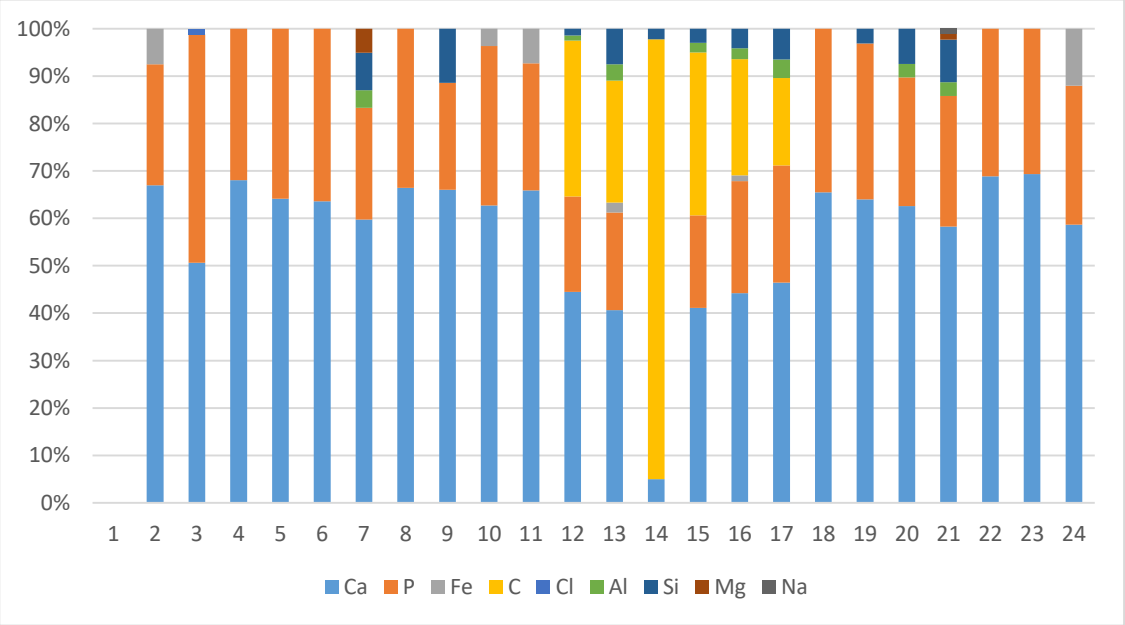


Fig. 7. Chemical element's ratios in each sample.

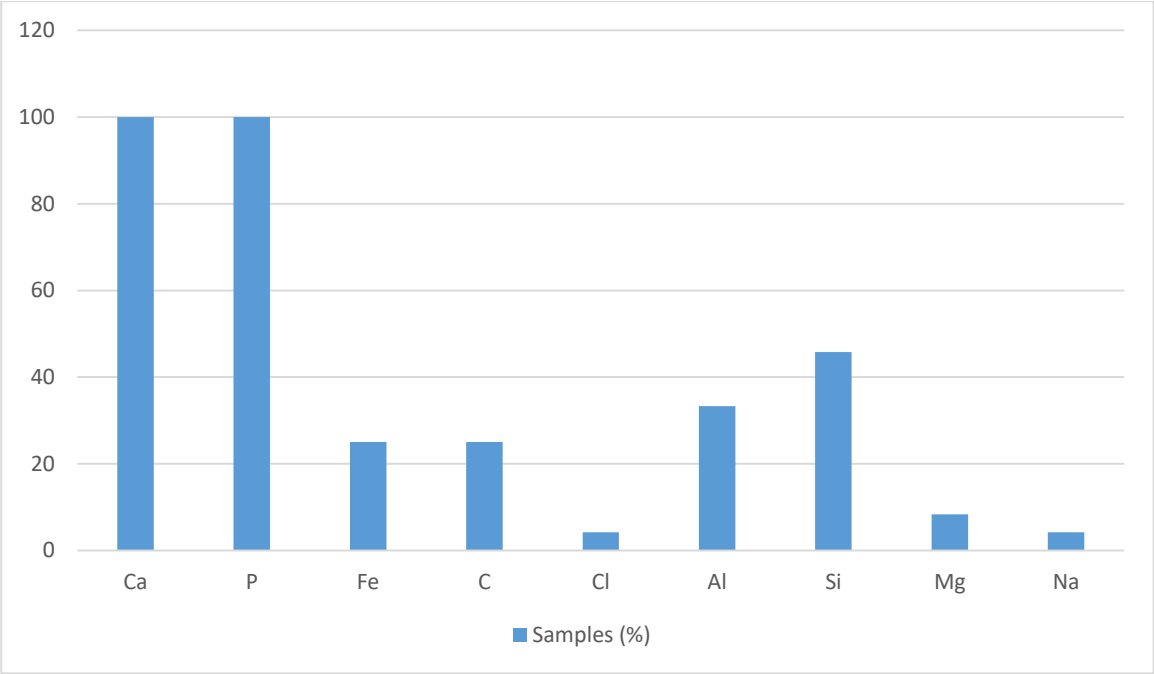


Fig. 8. Percentages of specimens *per* each chemical element (N= 24).

3.4 Discussion

Previous studies have suggested a close relationship between tooth wear and diet in various populations because the rate of tooth wear depends on the type of food, the amount and kind of abrasive particles contained in the food (Molnar, 1972; Bermúdez de Castro *et alii*, 2003; Kaifu, 2000; Kaifu, Kasai, Townsend, & Richards, 2003). In particular, occlusal microwear patterns reflect abrasive properties of ingested foodstuff caused by fragments of bone, phytoliths and microliths, sand, silt and clay deriving from plant roots or leaves (Pérez-Pérez *et alii*, 1994; Lalueza *et alii*, 1996; Polo Cerdá *et alii*, 2007; Schmidt, 2010). Therefore, the dental microwear formation seems to be strongly influenced by the amount of abrasive particles ingested with food and their intrinsic hardness (Lalueza *et alii*, 1994; Gügel *et alii*, 2001; Lucas, 2004; Lucas, 2013). The sample analyzed in this study showed the presence of both scratches and pits in different living environments: inland mountain/hilly sites of Calabria (Group 1), inland hilly site of Basilicata (Group 2) and inland plan of Apulia (Group 3). These two different dental patterns are representative of two distinct food habits: scratches indicate a mainly vegetarian diet (that includes highly abrasive particles), pits specify a mostly omnivorous diet (Romero and De Juan, 2012; Romero *et alii*, 2013). The highest frequency of scratches in comparison to pits suggested a predominantly abrasive diet that required shear forces in the masticatory process, as the scratches appear on teeth when particles are dragged or sheared between opposing enamel surfaces during the jaw movements (Smith, 1984). The high variability of the occlusal microwear patterns observed in this sample - as well as in ancient population (Romero *et alii*, 2013) - probably depends on the addition of agricultural products in human diet, which likely had a significantly higher content of abrasive particles than foraged food. Moreover, our data demonstrated a general higher prevalence of scratches on the left side and of pits on the right side of jaws. Considering the groups separately, this pattern is clearly confirmed only in Group 3. Probably, this result does not indicate a specific nutritional behavior of individuals in the group, but rather it highlights the variability of the tracks

in relation to the consumption of abrasive agricultural products. Nutritional studies on Bronze Age populations living in South Italy confirmed the assumption that diet was mainly based on agricultural products rather than on animal consumption. In particular, the Middle Bronze Age communities of Toppo d'Aguzzo and Lavello (placed in Basilicata) had a diet characterized by 60-70% of vegetarian protein intake (Tafuri *et alii*, 2009).

No studies were undertaken on Southern Italian Bronze Age populations using SEM analyses; conversely, Masotti *et alii* (2017) analyzed microwear patterns of the Early Bronze Age community of Ballabio (Emilia Romagna, North Italy). Authors indicated higher percentages of scratches than pits and suggested a diet relied on agricultural products, particularly cereals: wheat, barley and legumes. The data on crops cultivated during the Bronze Age by the Southern communities were provided by Fiorentino *et alii* (2004). The overall trend in the Southern regions showed the cultivation of traditional cereals of "Neolithic package" (*Hordeum vulgare*, *Triticum dicoccum*, *monococcum*, *durum* and *aestivum*) indicating also the emergence of new species of cereals and the progressive increase of the olive production. In order to understand the type of crops consumed, the archaeo-botanical records of the following sites were used as reference: Coppa Nevigata (Foggia, Apulia), Torre Castelluccia (Taranto, Apulia) and Broglio di Trebisacce (Sibari, Calabria). Paleobotanical data indicated the presence of different cereals: einkorn wheat (*Triticum monococcum*), spelled (*Triticum dicoccum*), wheat (*Triticum aestivum*, *compactum*, *durum*), barley (*Hordeum distichum* and *exasticum*), scarcity of millet, rye and oats were recorded. The cultivation of legumes attested an increase of peas (*Pisum sativum*), faba beans (*Vicia faba minor)*, lentils (*Lens culinaris*), and grass peas (*Lathyrus sativa cicera*) during the Bronze Age. Wild and cultivated fruits included acorns (*Quercus sp.*) and figs (*Figus carica*), walnut and olives. The profuse existence of olive trees indicated a highly modified landscape for the spread of agriculture, that was the mainly subsistence strategy (Kleibrink, 1996-1997; Vanzetti, 2000). The analyses of percentages of crops in each site indicated that the main grain crops cultivated were wheat and barley, with an apparent prevalence of *Hordeum vulgare* over *Triticum monococcum* (Nisbet and Rottoli, 1997; Rottoli, 2001 Carra *et alii*, 2003; Fiorentino *et alii*, 2004; Giachi *et alii*, 2010; Carra, 2012).

Pits generally indicate the consumption of "hard food" (such as seeds, nuts, etc.) and the chewing of bones deriving by the ingesting of meat or marrow. The low percentages observed in our study suggested a secondary intake of these foods. Archaeological record shows that the general trend of Bronze Age subsistence strategies was the prevalence of livestock compared to hunting (De Grossi *et alii*, 2004). In Italy, as in Mediterranean Europe, Bronze Age economy was mainly based on the consumption of sheep and goat for meat and dairy products (Arrighi *et alii*, 2007; De Grossi Mazzorin, 2013; Maini and Curci, 2013), although the evidence for animal exploitation was extremely variable (Harding, Fokkes, 2013). In fact, the animal economy showed a diversification in frequency at the regional level and sometimes even more locally, according to geography, environment and cultural models (Riedel, 1996; Battisti and Marconi, 2004). As in Northern Italian sites of Ledro (Bietti Sestieri, 2011) and Fiavè (Perini, 1987), the prevalence of sheep and goats among the domestic species was recorded also in Southern settlements, with an increase during the Late Bronze Age (De Grossi Mazzorin, 2013; Maini and Curci, 2013). In particular, Broglio di Trebisacce site (Calabria) was upward of sheep and goat of 42% (Tagliacozzo, 1994). At Coppa Nevigata (Apulia), sheep and goat intensified from 36.6% to 43.8% during the latest period of the Bronze Age (Bökönyi and Siracusano, 1987; Bietti Sestieri, 2011). Unlike livestock, hunting was generally poor during the Ancient and Middle Bronze Age as shown in the Broglio di Trebisacce

site, where wild species were completely absent (Tagliacozzo, 1994; De Grossi Mazzorin, 1987; De Grossi Mazzorin *et alii*, 2004).

Our data did not show significant differences according to the type of microwear (scratches/pits) among the three Groups. Cautiously, given the sample size, we can suggest that Bronze Age communities living in Southern Italy had the same diet system. It is, however, to point out that this result partially confirms those obtained from the analysis of stable isotopes on a larger sample. They indicated a common nutritional behavior for humans of the three groups: a terrestrial diet based on agricultural products with moderate consumption of animal protein that was slight higher for communities of Group 2. Conversely, Middle Bronze Age communities of Central Italy looked to have a diet characterized by three different food habits (Varalli *et alii*, 2016a). In particular, humans of Grotta dello Scoglietto (Tuscany) had a high-protein diet, and a probable consumption of marine/freshwater food was proposed for them. Community of Falcetone (Latium) had a diet mainly based on plant proteins. Humans of Grotta Misa (Latium) showed less animal protein intake and a probable consumption of different crops than Southern Italy, such as millet (Varalli *et alii*, 2016a). This difference between Northern and Southern Italy can be also explained by cultural reasons, mirrored in food practices: Early/Middle Bronze Age diet in Central and Northern Italy was influenced by relationships with Northwestern Europe (Varalli *et alii*, 2016a-b; Bietti Sestieri, 2011). Instead, Southern communities were characterized by intense trade with the Mycenaean Greek culture (Vagnetti, 1982; Marazzi, Tusa and Vagnetti, 1986; Laffineur and Greco, 2005; Guglielmino, 2005; Bietti Sestieri, 2011). Studies on the diet of the Mycenaean sites of Greece, in particular the site of Pylos (Messenia - Southwestern Peloponnese), pointed out a terrestrial diet, charachterized by cereals that would have provided the largest amount of carbohydrates; legumes as primary source of plant protein and domesticated animals (and their secondary products) supplied the majority of animal protein. Marine resource consumption was minimal: fish was notably absent in Mycenaean diet, even in coastal sites; hunting had not an important contribution to subsistence (Cosmopoulos *et alii*, 2003; Kotjabopoulou *et alii*, 2003¸Schepartz *et alii*, 2011).

Even though social differences (gender related) were evident in the archaeological assemblages found in the graves or in good graves of Southern Bronze Age sites (i.e. Toppo d'Aguzzo and Ipogeo dei Bronzi), such complexity did not occur in terms of dietary intake in skeletal remains from Basilicata (Group 2), in contrast to those from Apulia (Group 3). However, this difference can be influenced by the sample size and should therefore be considered with caution. In fact, it contrasts with results obtained by isotopic analysis on bone specimens from Ipogeo dei Bronzi (Group 3), indicating no difference in diet between males and females. The comparison between age classes was performed only for skeletal remains from Toppo d'Aguzzo site (Group 2), as a poor preservation state of specimens did not allow to estimate the age at death of individuals from other sites. Results pointed out a significant difference in the frequencies of scratches and pits between the age classes. In particular, the microwear' patterns were less developed in middle adults and old adults (> 35 years of age) than in young adults (< 35 years of age). This difference, not confirmed by the stable isotope analysis, could be explained by the effects of the increasing wear with age, that could have erased any signs of microwear. The results of microwear analysis, therefore, did not allow us to clearly delineate differences in feeding behavior with age and sex in the examined samples. Although scratches were less present in the oldest individuals, the evaluation of quantitative variables (length and width) showed that scratches were longer in middle and old adults (> 35 years of age) than in young adults (<35 years of age). This result could depend on a longer chewing due to *ante mortem* tooth loss and arthrosis of the jaw, typical of this age. Although they

were more present on the left side, scratches length was higher on the right side than the left side. This result confirms the high variability of the tracks in relation to the consumption of agricultural products.

The Analysis of Chemical Elements performed with SEM on the tooth samples could give information about the presence of food contaminants caused by the use of metallic tableware. As expected, data indicate that Ca and P were the elements mainly present in enamel tissues, followed by Fe, Al, Si and C. The Fe element was mostly present in Grotta della Monaca specimens, probably because the nature of soil. This cave was rich in iron oxides, in particular goethite and hematite, which were ubiquitously in the sediment where skeletal remains laid (Dimiuccio *et alii*, 2005; Larocca, 2005) (Fig. 9). Si was present in GdM and IpB specimens and Al on GdM, TDT and IpB tooth surfaces as concretions of clay soils contain these elements (Fig. 10). Carbon (C) was present in GdM and TDT remains as calcareous concretions: in particular, one specimen (ID: 14 from Toppo d'Aguzzo presented the highest percentages of limestone (Fig. 11). We therefore suppose that the presence of these elements could depend on soil concretions remaining on tooth surfaces even after cleaning, rather than food contaminants.

In conclusion, the examination of dental microwear on human teeth from Southern Italian Bronze Age sites confirms the stable isotopes results; at the same time, it allowed us to quantify the food intake: Bronze Age diet looked richer in vegetarian foodstuffs (mostly fibrous foods) than animal products, suggesting the large consumption of agricultural crops. No clear diversity in feeding behavior resulted among communities or between sexes and ages. Our findings on dietary patterns, expanding the current knowledge to Southern Italy, complete the framework for the Italian Bronze Age and may be of great importance for diachronic comparisons both in Italy and in Europe.

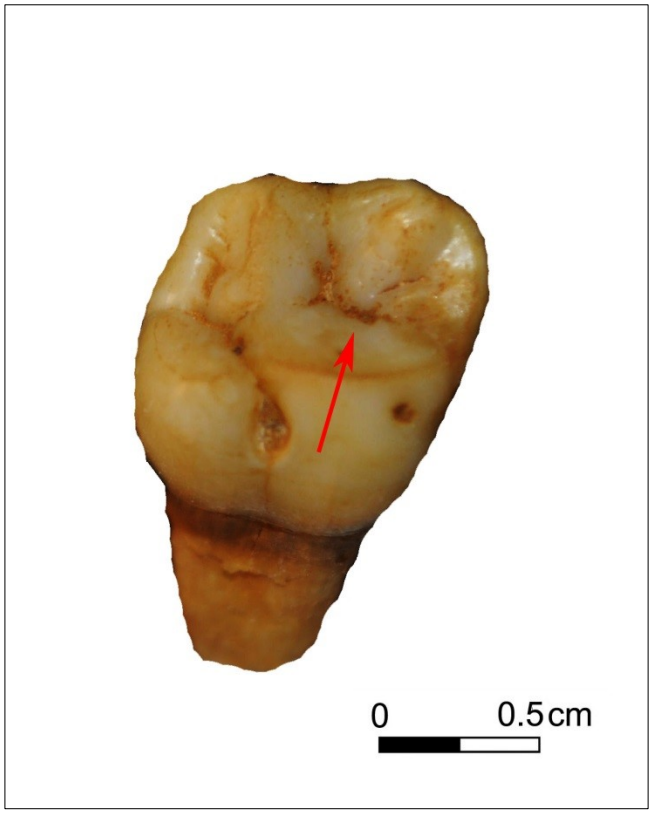


Fig. 9. Concretions of iron oxides (goethite and hematite) on the teeth of Grotta della Monaca.
Fig. 10. Clay concretions on the tooth occlusal surfaces of Ipogeo dei Bronzi.

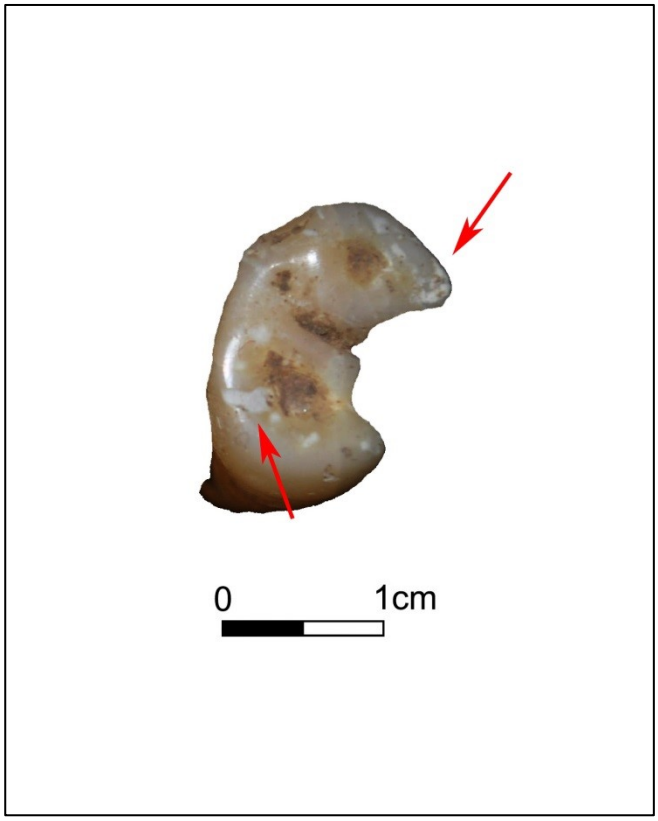


Fig. 11. Sample 14 (right mandibular premolar) with calcareous concretions on the occlusal surface.

CHAPTER 4: MULTIPLE APPROACH FOR DIET'S RECONSTRUCTION. THE CASE-STUDY OF GROTTA DELLA MONACA (CALABRIA)

4.1 Introduction

Information regarding the nutritional behavior of ancient populations may be gleaned to traditional anthropological analysis of teeth or from stable isotopes.

Dento-alveolar features are useful to obtain information about diet of past populations, as highlighted by many researchers (Hillson 1986, 1996, 2001; Kelley and Larsen, 1991; Buikstra and Ubelaker, 1994). The alterations of tooth surfaces (caries, calculus, wear, chipping) are particularly significant of the diet of individuals examined, as demonstrated since the early 1900s when different studies were performed to point out the relationship between these patterns and diet, in particular:

- caries (Mummery, 1870; Colyer, 1922; Leigh, 1925; Stewart, 1931; Goldstein, 1948; Brothwell, l959; Hardwick, 1960; Brinch and Moller Christiansen, 1949; Lunt, 1974; Moore and Corbett l97I, 1973, 1975; Corbett and Moore, 1976; Buikstra, 1977; Turner, 1979; Manchester, 1983; Milner, 1984; Powell, 1985; Lukacs, 1989; Larsen *et alii*, 1991);
- wear (Nicholls, 1914; Campbell, 1925; Leigh, 1925, Rabkin, 1943; Pedersen, 1949; Smith, 1984);
- calculus (Baaregaard, 1949; Hughes, 1963; Koritzer, 1968; Harris and Ponitz, 1980; Strouhal, 1983; Leek, 1986; Caselitz, 1986; Ibrahim, 1987; Davis and Janssen, 1991; Lukacs, 1992; Littleton and Frohlich, 1993; Lalueza *et alii*, 1996);
- chipping (Turner and Cadien, 1969; Lukacs and Hemphill, 1990; Bonfiglioli *et alii,* 2004; Belcastro *et alii*, 2007);

Caries is a process characterized by focal, irreversible and progressive demineralization of dental hard tissue. It is the result of fermentation of dietary carbohydrates especially sugars by the oral bacteria in dental plaque (Hillson, 1986; Larsen, 1997; Caselitz, 1998; Hillson, 2001). As Powell (1985) affirmed, different factors are involved in the etiology of caries: environmental factors (minerals in food and water), exogenous factors (chemical composition, texture, methods of preparation of foods or oral hygiene) and endogenous factors (morphology of the tooth, bacteria in the oral cavity, enamel integrity, saliva flow rate and chemical composition). Therefore, dental caries is a complex multifactorial disease caused by the interaction between the presence of cariogenic microorganisms and a suitable oral environment (Patterson, 1984). *Calculus* is mineralized plaque accumulated on the tooth surface, especially near the salivary gland ducts (lingual surfaces of the anterior lower teeth and buccal surfaces of the upper molars) (Hillson, 1986). The consumption of high-protein foods increases alkalinity in the mouth favoring precipitation of minerals in the oral fluids. However, the beginning of mineralization is related to the amount of plaque (the microorganisms in dental plaque destroy the inhibitors of mineralization), and to factors that increase its accumulation, such as poor oral hygiene and the consumption of carbohydrates (Sheie *et alii*, 1989; Hillson, 1996). Therefore, a diet rich in carbohydrates facilitates a plaque accumulation and leads to the development of caries and calculus (Hillson, 1996). Dental *wear* is a physiological process that removes the occlusal enamel, damaging the primary dentin (Smith and Knight, 1984; Larsen, 1997). Sometimes, a heavy wear causes the deposition of secondary dentin that protects the pulp chamber, but when the wear is faster than the deposition, the

pulp chamber will be exposed. Wear can provide information about dietary behavior because of its association with the consumption of hard fibrous foods or the use of processing techniques that introduce abrasive elements into the food, like powder from grinding stones (Larsen, 1997). It is necessary take into account that there is a negative correlation between caries and the degree of wear (Powell, 1985; Maat and Vand der Velt, 1987). In effect wear gradually smooths the occlusal cusps, eliminating fissures and pits on the crown and reducing the presence of cariogenic substances on the tooth. Dental wear also provides information about activities related to non-masticatory tooth use (Turner and Cheuiche Machado, 1983; Larsen, 1985; Lucaks and Pastor, 1988; Milner and Larsen, 1991; Alt and Pilcher, 1998; Belcastro *et alii*, 2001; Bonfiglioli *et alii,* 2004). For this reason, it is important to observe the futures of the wear traces that, when they are characterized by a specific morphology, can be referred to extra-masticatory activities. *Chipping* is an *ante mortem* irregular crack involving the enamel or enamel and dentin. It can be on the buccal, lingual, interproximal edge or on the crest of the tooth. Chipping provides information about both masticatory and non-masticatory activities (Milner and Larsen, 1991). In addition to the "direct" indicators of paleo-nutrition, it was observed *hypoplasia* of the tooth enamel, which is an "indirect" indicator of diet. The hypoplasia, in fact, is a marker of stress that may depend, among various causes, on the diet. Hypoplasia of tooth enamel occurs during the development of the tooth, caused by disorders that stop ameloblastic activity and it persists because the enamel is not remodeled after its formation (Sarnat and Shour, 1989). Enamel hypoplasia depends on the following main etiological factors: hereditary anomalies (involving the whole crown at birth), local trauma (on a single tooth or on adjacent teeth) and metabolic stress (Pindborg, 1970; Winter and Brook, 1975). This last etiological factor may be the result of malnutrition and infectious diseases during childhood that decrease the individual's immune defenses (Solomons and Keush, 1981). Goodman and Rose affirmed that the dietary intake is decisive for the onset of hypoplastic defect (Goodman and Rose, 1991). The first research on hypoplasia dated back to the end of 1900s (Blakey and Armelagos, 1985; Duray, 1996; Goodman and Armelagos, 1988; Rose *et alii*, 1978; Simpson *et alii*, 1990; Ensor and Irish, 1995; Stodder, 1997). Since then several studies have been conducted on this pathological marker. Some of them introduced regression equations and formulas that used the position of enamel hypoplasia on tooth surfaces to estimate the age of onset of this defect (Blakey and Armelagos, 1985; Ensor and Irish, 1995; Goodman *et alii*, 1980, 1984, 1987; Lanphear, 1990; Rose *et alii*, 1985; Saunders and Keenleyside, 1999).

Concerning the Italian Bronze Age, although there are several studies that analyze the macroscopic tooth markers diet-related (such as the most recent Varalli *et alii,* 2016b and Masotti *et alii*, 2017), few *compendia* are recorded in literature. The only synthesis performed on macroscopic tooth markers was carried out by Minozzi *et alii* (1994). This study analyzed different Bronze Age sites: Franzine Nuove (Veneto), Grotta dello Scoglietto (Tuscany), Grotta Vecchi (Latium) in Central-Northen Italy; Toppo d'Aguzzo (Basilicata), Madonna di Loreto (Apulia), Marcita and Ponte della Paolina (Sicily) in Southern Italy. On the basis of dental markers, authors highlighted low incidence of stress and malnutrition. This result could be explained with the technological improvements in the economic system that characterized the Bronze Age communities, in particular, the increasing agriculture (Minozzi *et alii*, 1994). The intensification of farming mirrored even in food habits: the high rate of wear on the occlusal surfaces showed a more vegetarian diet, linked to the consumption of fibrous foods, cereals and legumes (Varalli *et alii*, 2016b; Masotti *et alii*, 2017).

As shown above, little data are available for the Bronze Age of Southern Italy. Grotta della Monaca (GdM) is a karstic cave placed in the North-western Calabria (Sant'Agata di Esaro - CS). This site

was a hypogeal burial ground during the Middle Bronze Age, around the half of the II millennium BC (cfr. Chapter 1). This interesting necropolis was chosen as case-study in order to analyze the dietary behavior of Southern Italian Bronze Age population by different approaches. The size of the tooth sample allowed to carry out the examination of macroscopic diet-related markers observable on dental surfaces. In addition, the analysis of stable isotopes on bone collagen extracts, as well as the investigation of microwear on enamel of teeth were performed. *Carbon* and *nitrogen* stable isotope analysis of skeletal remains can give explanation of diet of ancient populations, indicating differences with regard to age, sex, social status, geographic landscape, as shown in Chapter 2. This approach is based on the principle that the isotopic composition of body tissue reflects the diet of the individual (Cheung *et alii*, 2012). In fact, as mentioned previously, the analysis of the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is useful to specify the proteins assumed by individuals during their life and the environment of origin of resources consumed (Mannino, 2009). The stable isotopes allow to determine whether the proteins derived from a vegetarian diet or from animal foodstuffs, specifying the type of protein: C_3 plants (such as barley, wheat, legumes) or C_4 plants (such as millet and sorghum), carnivores or herbivores (meat and dairy products) (De Niro and Epstein 1978, 1981). In addition, stable isotopes give information on the origin of foods (terrestrial or marine/freshwater) (Schoeninger and De Niro, 1984). Furthermore, the microwear of teeth surface is useful to investigate the rate of abrasive particles assumed during the chewing (Molnar, 1972; Bermúdez de Castro *et alii,* 2003; Kaifu, 2000; Kaifu, Kasai, Townsend and Richards, 2003) and the type of diet followed during the life of individuals examined. In particular, *pits* result from compression of food particles on the tooth surface during the chewing and indicate the consumption of hard foods typical of an omnivorous diet; *scratches* originate when abrasive particles are dragged between opposite tooth surfaces and indicate the consumption of soft food, recurring in a mostly vegetarian diet (Butler, 1952; Walker *et alii*, 1978; Ryan, 1979; Maas, 1994; Schmidt, 2010).

The purpose of this study was to investigate the nutritional behavior of Grotta della Monaca community and, at the same time, we wanted to point out the limits and the advantages of the different methods applied in diet's reconstruction. Moreover, our data aimed to enrich the information regarding nutritional indicators (macroscopic, microscopic and chemical markers) of the Southern Italian populations.

4.2 Materials and methods

As mentioned above, Grotta della Monaca sample was analyzed by three different approaches: macroscopic analysis of diet indicators on tooth surfaces, dental microscopic analysis (microwear), stable isotopes analysis of bone collagen extracts. In the following section, the methods used for macroscopic examination will be indicate; those used for the other two analyses have been previously reported in detail (Chapters 2 and 3).

Analysis of macroscopic diet-related indicators

This analysis was carried out on 351 permanent teeth belonging to the burial areas of Grotta della Monaca (Table 1, 2).

Maxillary		**Mandibular**		**ND**		**Right**		**Left**		**ND**		**Isolated**		**Not isolated**	
N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
207	59	141	40.2	3	0.9	148	42.2	171	48.7	32	9.1	290	82.6	61	17.4

Table 1. Grotta della Monaca sample composition by anatomical part, side and status (N= 351).

Tooth type		
	N	**% n= 75**
Iu	1	1.3
I1	42	56
I2	32	43
	N	**% n= 351**
Total incisors	75	21.4
	N	**% n= 57**
C	57	100
	N	**% n=351**
Total canines	57	16.2
	N	**% n= 80**
Pu	1	1.3
P1	32	40
P2	47	58.8
	N	**% n= 351**
Total premolars	80	22.8
	N	**% n= 139**
Mu	1	0.7
M1	62	44.6
M2	41	29.5
M3	35	25.2
	N	**% n= 351**
Total molars	139	39.6

Table 2. Grotta della Monaca sample composition by tooth type (Iu= undeterminable incisors; Pu= indeterminable premolars; Mu= undeterminable molars).

Although the sample consisted of teeth *in situ* (in alveolar bone), in addition to isolated teeth, it was not possible to determine the sex and specify the age at death of individuals because of the lack of diagnostic anatomical parts for this analysis. The Minimum Number of Individuals (MNI) was calculated on molars because of they were more present than the other teeth (40%, N= 351). In particular, among 62 M1 (35 maxillary and 27 mandibular; 19 right maxillary and 14 left maxillary), the right maxillary M1 was chosen as representative of MNI.

According to Belcastro *et alii* (2004), we considered the following nutritional indicators: caries, calculus, wear, chipping and hypoplastic defect.

Information was gathered on the tooth *status* distinguishing its presence and state of preservation, as follow: completely erupted tooth included in the alveolus (P); isolated and complete tooth (when the alveolus was not preserved) (I); *post mortem* tooth loss (X); non recordable (tooth or alveolus damaged) (9).

Caries were recognized according to Powell (1985) remark: "only those cavities that would admit the tip of a dental explorer were scored as actual caries, to eliminate false scoring of discolored but intact enamel" (Powell 1981). They were classified following Belcastro *et alii* (2004):

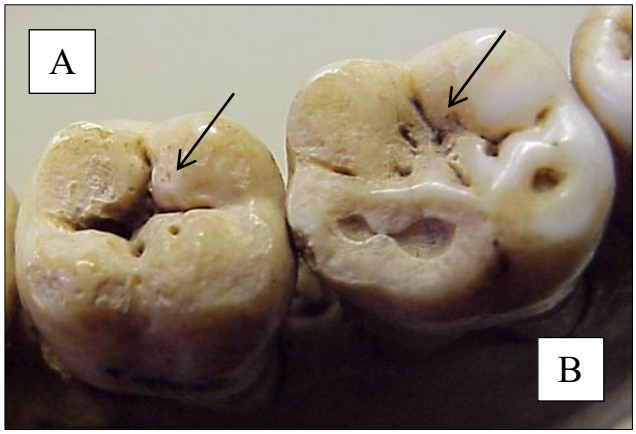
1. on the basis of their *severity* as non-penetrating (involving only the enamel); penetrating (involving the enamel and dentin); destructive (if the tooth crown was destroyed and it was not possible to identify the location of the lesion);
2. on the basis of their *location* (occlusal, coronal, cervical, radical) and *position* (buccal, lingual, interproximal mesial, interproximal distal):

In order to record these lesions, specific codes were used that took into account the absence and all the possible combinations of categories (severity, location, position). The codes used in this study are the following:

1: penetrating occlusal caries (Fig. 1-A)
2: penetrating coronal/lingual caries
3: penetrating coronal/buccal caries
4: penetrating coronal/ inter proximal-mesial caries
5: penetrating coronal/ inter proximal-distal caries
8: penetrating cervical/ inter proximal-mesial caries
15: non penetrating occlusal caries (Fig. 1-B)
16: non penetrating coronal/lingual caries
17: non penetrating coronal/buccal caries
28: destructive caries (Fig. 2)

The undetermined condition has to be recorded when the tooth was *post mortem* damaged, in the case of heavy wear and for any reason that made impossible to record the lesion. In case of multiple caries on the same tooth all the caries were recorded in the same cell of Excel listing, according to the following remarks (Belcastro *et alii*, 2004):

- more caries of the same severity, location and position: they were recorded as present only once;
- caries of different severity in the same location and position: only the penetrating one was recorded;
- same severity in different locations and positions and different severity in different locations and positions: all caries had to be recorded;
- when the pulp chamber was exposed as a result of caries or severe wear, it must be indicated in the caries and wear rows by a symbol (*).



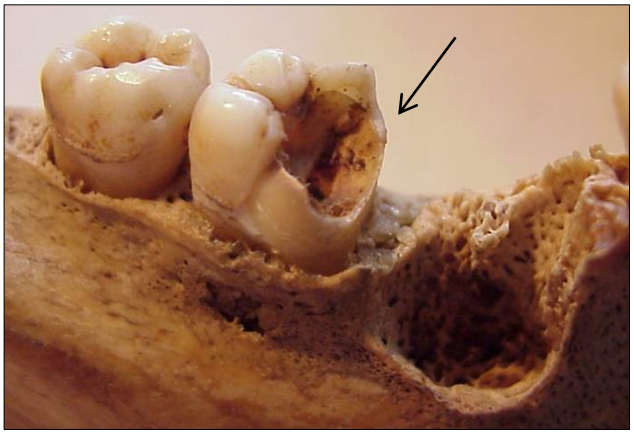
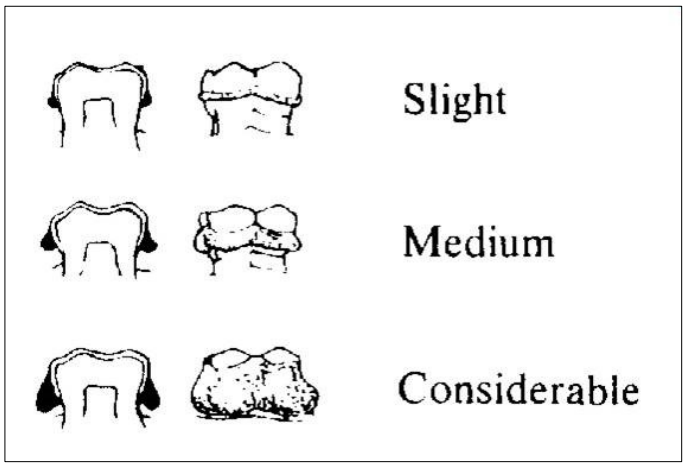


Fig. 1. On the left, occlusal penetrating caries (A) and occlusal non penetrating caries (B) (foto by Belcastro, 2004).
Fig 2. On the right, destructive caries (foto by Belcastro, 2004).

Calculus was scored according to Brothwell's scale of gravity (Brothwell, 1981), as follow: absent (0), low deposition (1), moderate deposition (2), severe deposition (3), non-recordable (9). When calculus covered entirely or almost all the surfaces of the tooth, it was recorded as 4 (Belcastro *et alii*, 2004) (Fig. 3, 4).



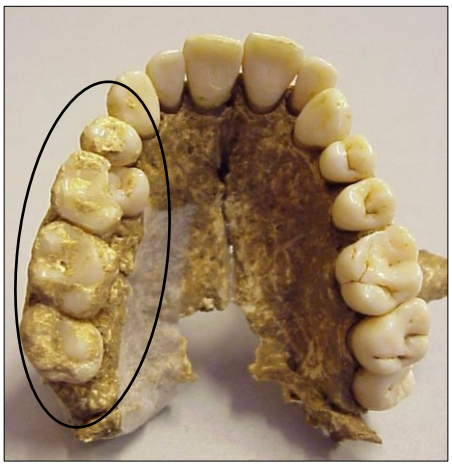


Fig. 3. On the left, the degrees of calculus (stages 1-3), according to Brothwell (1981).
Fig. 4. On the right, the degree of calculus: stage 4, according to Belcastro *et alii* (2004).

Dental *wear* was recorded for each tooth in terms of occurrence and location (anterior or posterior; maxillary or mandibular; right or left). The level of wear was determined by Smith's eight-grade scale system for anterior teeth and premolars (Smith, 1984). The non-recordable condition was scored as 9 (Fig. 5).

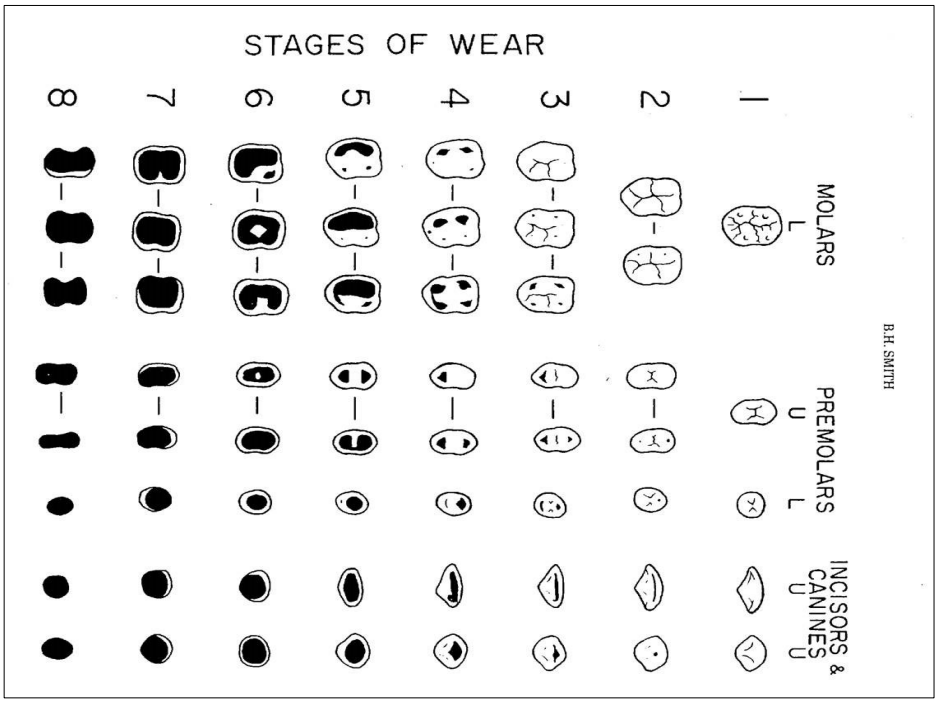


Fig. 5. Stages of wear (1-8), according to Smith (1984).

Hypoplastic defects appear as pits and grooves on the tooth, more frequently found on the buccal surface. Hypoplasia was recorded as follow according to the Fédération Dentaire Internationale (1982, 1992) standard: 1- enamel opacities colored white or cream; 2- enamel opacities colored yellow or brown; 3- pits (enamel pitting); 4 - horizontal grooves (linear enamel hypoplasia: LEH); 5 - vertical grooves; 6 - missing enamel; 9 - not recordable (Fig. 6). Goodman and Rose (1990) regression equation has been used to calculate the age of onset of hypoplastic defects. These Authors suggested to select maxillary first incisor (I1) and mandibular canine (C), because of they are more predictive than other teeth (Goodman and Rose, 1990).

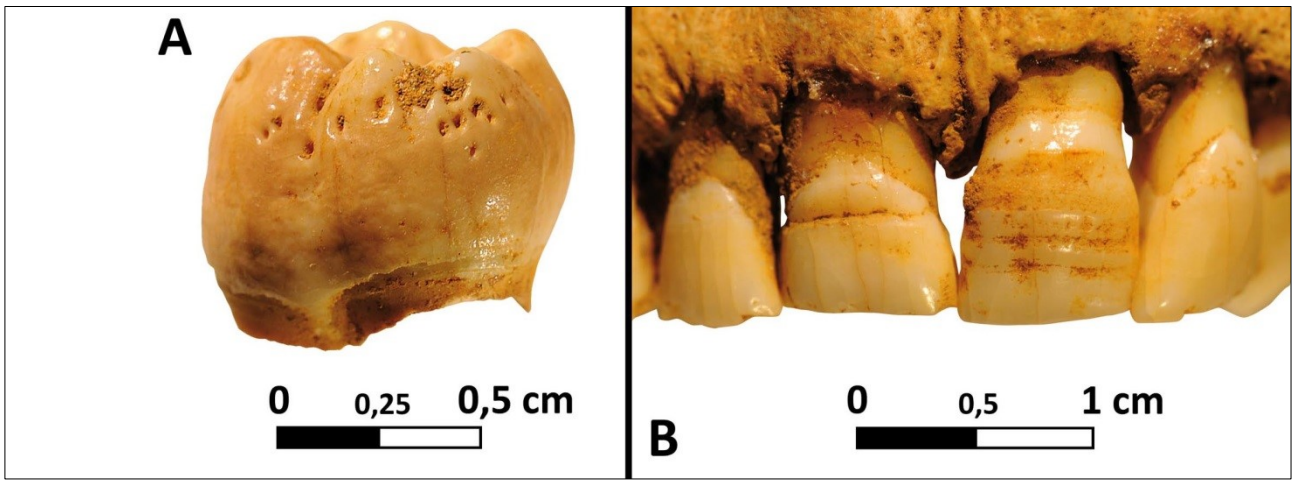
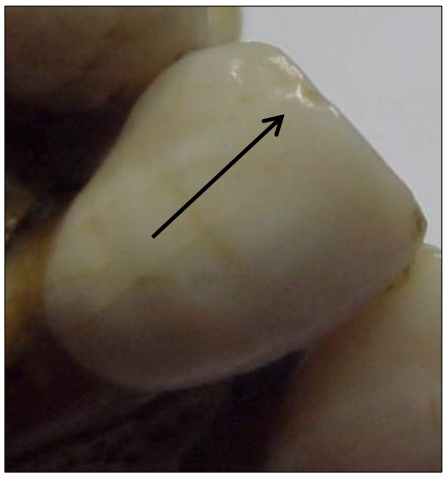


Fig. 6. Type 3 (A) and 4 (B) of hypoplasia (Arena *et alii*, 2014).

Chipping was classified according to Bonfiglioli standard (2002, 2004). The method proposed three-degree scale of gravity: 1= slight crack or fracture (0.5 mm), or larger but superficial enamel flake loss; 2= square irregular lesion (1mm) with the enamel more deeply involved; 3= crack (>1

mm) involving enamel and dentine or large fracture -very irregular in shape - that could destroy the tooth (Fig. 7, 8). The chipping was recorded in relation to the position (buccal - B, lingual - L, interproximal mesial - M, interproximal distal - D) and grade (0, 1, 2, 3). The non-recordable condition (9) was indicated when the tooth was *post mortem* damaged, in the case of heavy wear and for any reason that made impossible to record the trait (Belcastro *et alii*, 2004). In this case, the position of the damage was recorded in the notes. In case of multiple chipping on the same tooth, all them were recorded in the same cell, as follow:

- more chipping of the same degree in the same position was recorded as present only once;
- in case of chipping of different degrees in the same position, only the highest grade was recorded;
- in case of the same degree in different positions and different grades in different positions, all conditions were recorded;
- in order to encoding, the degree (1/2/3) preceded the position.



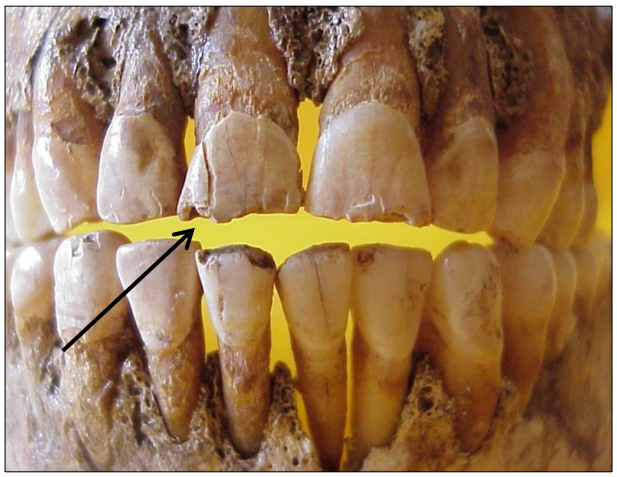


Fig. 7. Degree of chupping (stage 1), according to Bonfiglioli *et alii* (2004).
Fig. 8. Degree of chupping (stage 3), according to Bonfiglioli *et alii* (2004).

Analysis of microscopic diet-related indicators (microwear)

The samples selected in order to analyze dental microwear consisted of 9 isolated permanent teeth. The specimens were selected on the basis of both a good state of preservation and the degree of macrowear, selecting the teeth with slight degree of wear: stage 3, according to Smith (1984). The selected teeth were all isolated, therefore it was not possible to determine the sex and age at death of individuals to which they belonged. Anyway, in order to select teeth belonging to different individuals, they were sampled in different areas of the site and, when they were from the same archaeological contest, the same tooth was sampled (Table 3).

The identification of the microwear patterns diet-related on tooth surfaces was carried out using the Scanning Electron Microscope (SEM). This method has been described previously in Chapter 3.

Sample ID	Site	Archaeological contest	Tooth type	I	Side
1	GdM	GdM /CTv/m5v/C/lv2/tg1/08	M2i	1	L
2	GdM	GdM /CTv/B/Z2/03	M1s	1	R
3	GdM	GdM/CTv S7/sC/D4I -12-23/08	M2i	1	L
4	GdM	GdM/CTsx.i/cap 12-13/00	M2s	1	R
10	GdM	GdM /Ctv/m5v/S/tg2/08	Cs	1	L
11	GdM	GdM /Ctv/m5v/S/tg2/08	Cs	1	ND
22	GdM	GdM/CTdx-fv/Z3 (d) '02	M1s	1	R
23	GdM	GdM/Ctdx-fv/D12/'03	M1s	1	R
24	GdM	GdM/CTdx-fv/D11	M2i	1	L

Table 3. The samples selected for SEM analyses (i: inferior; s: superior; R: right; L: left; ND: not determinable).

Stable isotopes analysis

In total, we analyzed human bone collagen specimens from 7 individuals and 1 faunal specimen (*Sus scrofa*). The table below shows the specimens selected for pretreatment (Table 4):

Sample ID	Species	Archaeological contest	Bone	Anatomical part	Side
GdM02	human	GdM/CTv/m5v/S/tg 1,2,3	Cranial bone	occipital bone	ND
GdM04	human	GdM/CTv/m5v/S/tg1/	Cranial bone	occipital bone	ND
GdM06	human	GdM/CT dx-fv/Z3	Radius	diaphysis	L
GdM09	human	GdM/CT dx-fv/Z4	3° Metatarsal	diaphysis	R
GdM10	human	GdM/CT dx-fv/Z6	Metatarsal phalanx	diaphysis	ND
GdM11	human	GdM/CT dx-fv/Z6	3° Metatarsal	diaphysis	L
GdM12	*Sus scrofa*	GdM/Pfs	Humerus	diaphysis	R

Table 4. Bones sampled for stable isotopes analyses (R= right; L= left; ND= non determinable).

All individuals selected for stable isotope analyses were adults because of the bone turnover is slower during the adulthood than the childhood, allowing to obtain information on a longer period of individual's lifetime. The age at death estimation and sex determination were carried out on individuals sampled using traditional anthropological methods, as described in Chapter 2.

The information regarding faunal remains (species, sex and age) were obtained by literature (Bux and Scintillani, 2005).

The bone specimens were taken from different bones, as reported in Table 4. Bone collagen was extracted from 0.5 g of cleaned bone fragments (cortical bones), following standard procedures described in Login (1979) method modified by Brown *et alii* (1988) and Jørcov *et alii* (2007), as reported in detail in the Chapter 2.

4.2.1 Statistical methods

The results of macroscopic patterns diet-related and microwear analyses were expressed as means and standard deviation for quantitative variables and as absolute frequencies and percentages for qualitative variables. Statistical differences were tested by Chi-square test for comparison between frequencies. Regarding stable isotope analysis, mean and standard deviation (SD) were computed to describe the sample. Due to the sample size, the non-parametric Mann-Whitney U-test was used to

compare means (2 items). The data were processed by Statistica for Windows, Version 11.0 (StatSoft Italia srl, Vigonza, Padua, Italy). For all statistical analyses, a significance level of $p < 0.05$ was used.

4.3 Results

4.3.1 Macroscopic diet-related markers

Results of the analysis of macroscopic nutritional indicators pointed out different dental markers useful to reconstruct the diet of individuals during their life. The following table shows their frequency in the total sample (Table 5, Fig. 9).

	Wear	Calculus	Caries	Chipping	Hypoplasia
N	215	72	30	0	96
%	61.3	20.5	8.5	0	24.4

Table 5. Frequency of nutritional indicators in the total sample analyzed (N= 351).

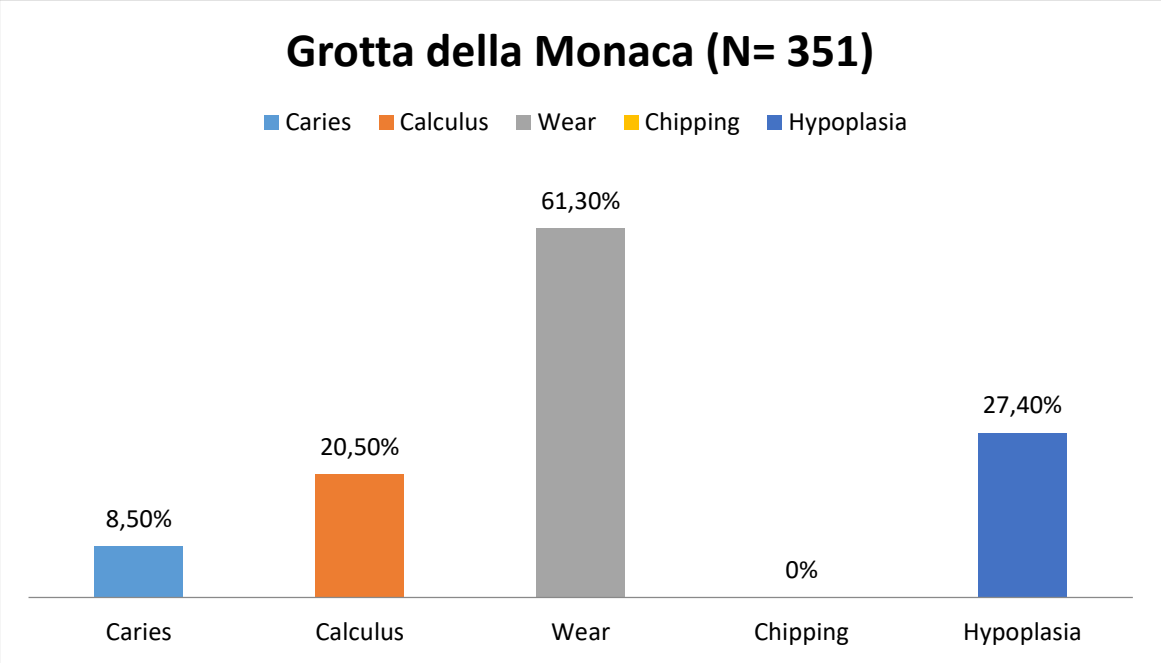


Fig. 9. Frequency of nutritional indicators in the total sample analyzed (N= 351).

DENTAL WEAR

The more present feature on dental surfaces was the wear that affected 215 teeth on 351 (61.3%), (Table 6, 7):

Maxillary		Mandibular		ND		Right		Left		ND		Anterior		Posterior		ND	
N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
136	63.3	77	35.8	2	1	84	39.1	111	51.6	20	9.3	86	40	111	51.6	18	8.4

Table 6. Frequency of wear according to the anatomical part, the side and the position (N= 215) (ND= non determined).

I		I1		I2		C		P1		P2		M		M1		M2		M3	
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
1	0.5	29	13.5	19	8.8	37	17.2	16	7.4	30	14	1	0.5	39	18.1	25	11.6	18	8.4

Table 7. Frequency of wear according to the type of teeth (N= 215).

Concerning the gravity, six degree of gravity-types were recorded according to Smith, 1984 (Table 8, 9; Fig 10-12):

Gravity	Maxillary		Mandibular		Right		Left		Anterior		Posterior	
	N	%	N	%	N	%	N	%	N	%	N	%
1	25	11.6	15	7	16	7.4	24	11.2	13	6	27	12.6
2	42	19.5	21	9.8	25	11.6	36	16.7	22	10.2	42	19.5
3	34	15.8	17	7.9	18	8.4	26	12.1	24	11.2	27	12.6
4	20	9.3	13	6	13	6	15	7	16	7.4	18	8.4
5	12	5.6	7	3.3	7	3.3	8	3.7	7	3.3	12	5.6
6	3	1.4	4	2	5	2.3	2	0.9	4	2	3	1.4

Table 8. Frequency of the degree of gravity according to the anatomical part, the side and the position of teeth (N= 215).

Gravity	Iu		I1		I2		C		P1		P2		Mu		M1		M2		M3	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
1	0	0	3	1.4	2	0.9	8	3.7	3	1.4	6	2.8	0	0	6	2.8	5	2.3	7	3.3
2	1	0.5	2	1	5	2.3	3	1.4	5	2.3	4	1.9	0	0	5	2.3	4	1.9	4	1.9
3	0	0	6	2.8	5	2.3	13	6	3	1.4	8	3.7	0	0	8	3.7	7	3.3	1	0.5
4	0	0	9	4.2	2	1	5	2.3	3	1.4	2	1	1	0.5	9	4.2	2	1	1	0.5
5	0	0	3	1.4	2	1	2	1	1	0.5	5	2.3	0	0	3	1.4	2	1	1	0.5
6	0	0	1	0.5	2	1	1	0.5	0	0	0	0	0	0	1	0.5	0	0	2	1

Table 9. Frequency of the degree of gravity according to the type of teeth, N= 215 (Iu= undetermined incisors; Mu=undetermined molars).

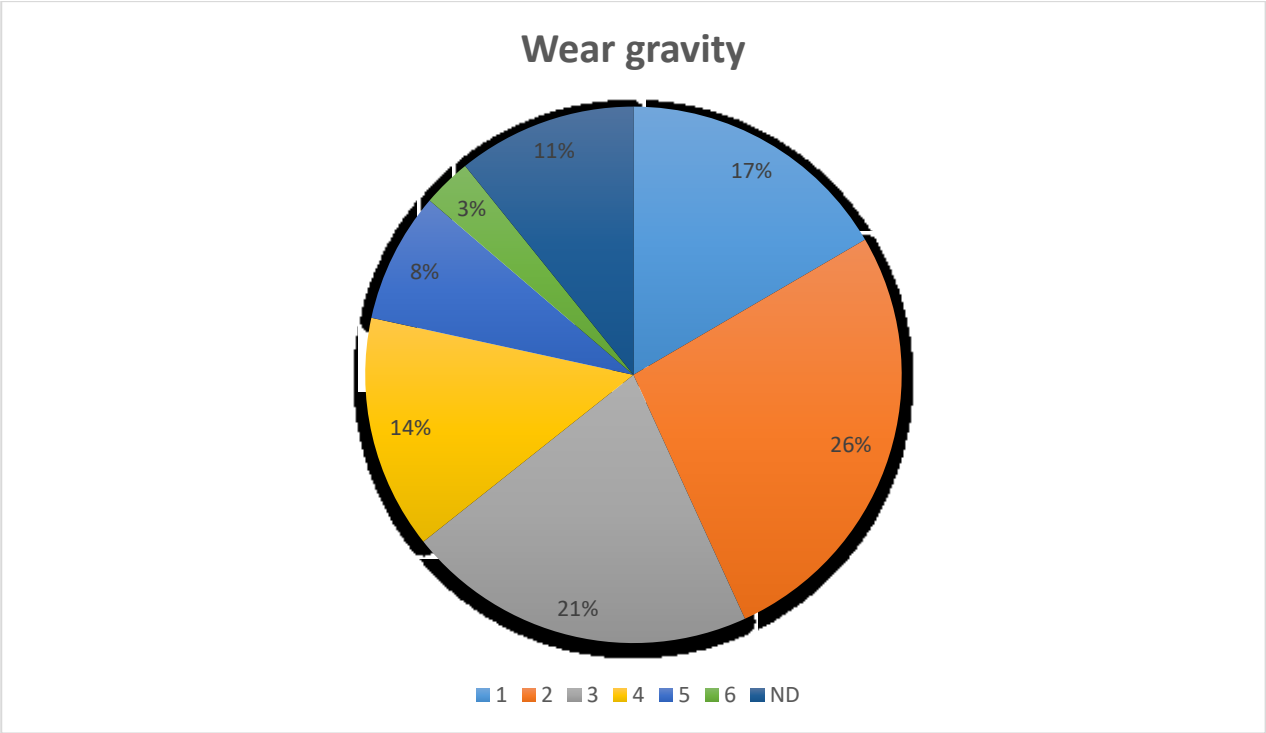


Fig. 10. Frequency of wear (%) according to the degree of gravity (N= 215).

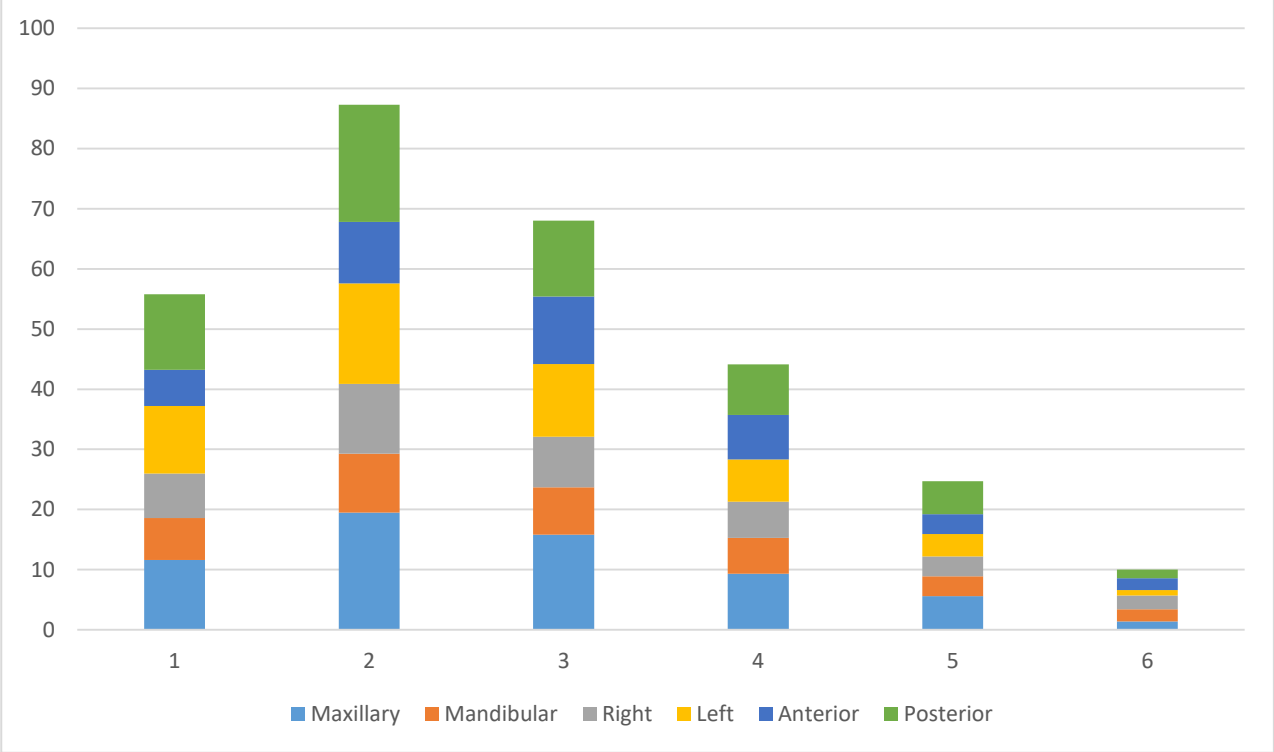


Fig. 11. Frequency of the gravity's degree according to the anatomical part (maxillary/mandibular), the side (right/left) and the position (anterior/posterior) (N= 215).

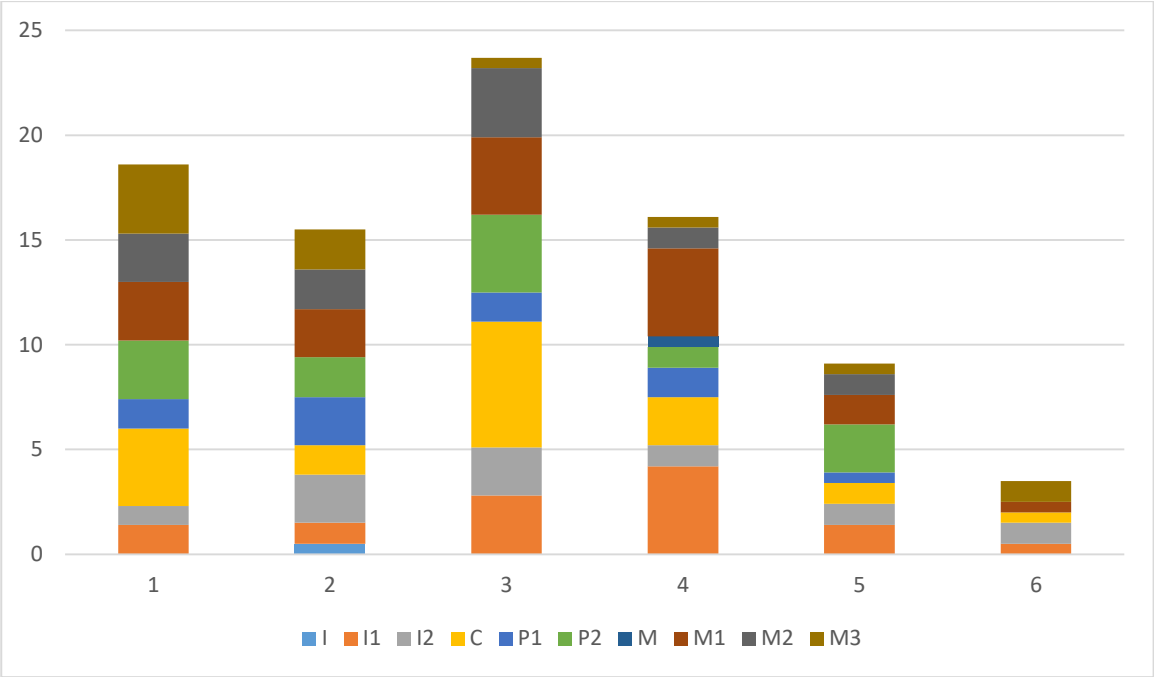


Fig. 12. Frequency of the degree of gravity according to the type of teeth (N= 215).

Of 215 teeth, heavy wear (type 5 and 6) was present in 12% of teeth; intermediate wear (type 3, 4) was present in 39.5% of teeth and slight wear (type 1, 2) was present on 48.4%. Chi-square test did not point out differences for the incidence of the gravity-types in the sample examined (χ^2= 66.82; d.f.= 5; p= 4.6962).

CALCULUS

The calculus was present on 72/351 teeth (20.6%). The tables below show data in detail (Table 10, 11):

Maxillary		Mandibular		ND		Right		Left		ND		Anterior		Posterior		ND	
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
35	50	35	50	2	2.8	28	38.9	41	57	3	4.2	31	43	41	57	0	0

Table 10. Frequency of calculus according to the anatomical part (maxillary/mandibular), the side (right/left) and the position (anterior/posterior) (N= 72; ND= undetemined).

Iu		I1		I2		C		P1		P2		Mu		M1		M2		M3	
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
1	1.4	11	15.3	10	13.9	9	12.5	3	4.2	11	15.3	1	1.4	11	15.3	8	11.1	7	9.7

Table 11. Frequency of calculus according to the type of teeth, N= 72 (Iu= undetermined incisors; Mu= undetermined molars).

Concerning the gravity, two degree of gravity-types were recorded according to Brothwell's scale of gravity (Brothwell, 1981) (Table 12, 13; Fig. 13-15):

Gravity	Maxillary		Mandibular		Right		Left		Anterior		Posterior	
Degree	N	%	N	%	N	%	N	%	N	%	N	%
1	23	31.9	30	41.6	21	29.2	32	44.4	23	31.9	31	43
2	12	16.7	5	6.9	7	9.7	9	12.5	8	11.1	7	9.7

Table 12. Frequency of gravity according to anatomical part, side and the position of teeth (N= 72).

Gravity	Iu		I1		I2		C		P1		P2		Mu		M1		M2		M3	
Degree	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
1	1	1.4	9	12.5	9	12.5	4	5.6	3	4.1	10	13.9	0	13.9	7	9.7	6	8.3	5	6.9
2	0	0	2	2.7	1	1.4	5	6.9	0	0	1	1.4	1	1.4	4	5.6	2	2.8	2	2.8

Table 13. Frequency of gravity according to type of teeth, N= 72 (Iu= undetermined incisors; Mu=undetermined molars).

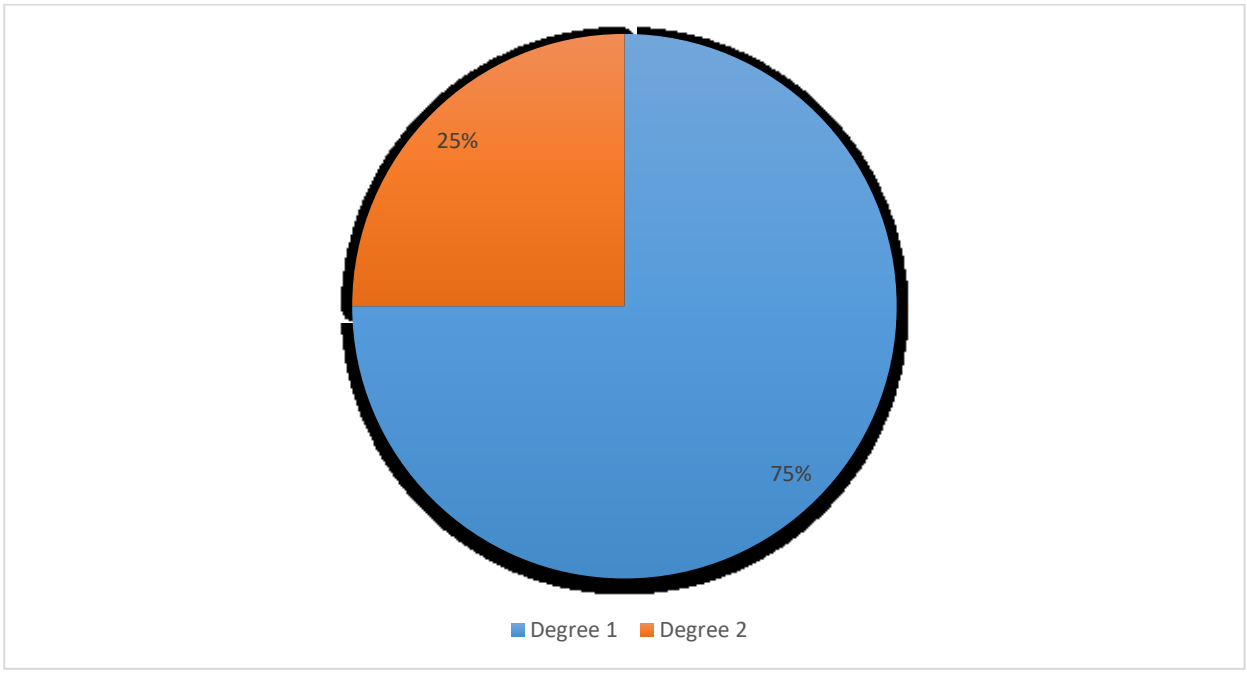


Fig. 13. Frequency of the degree of gravity (N= 72).

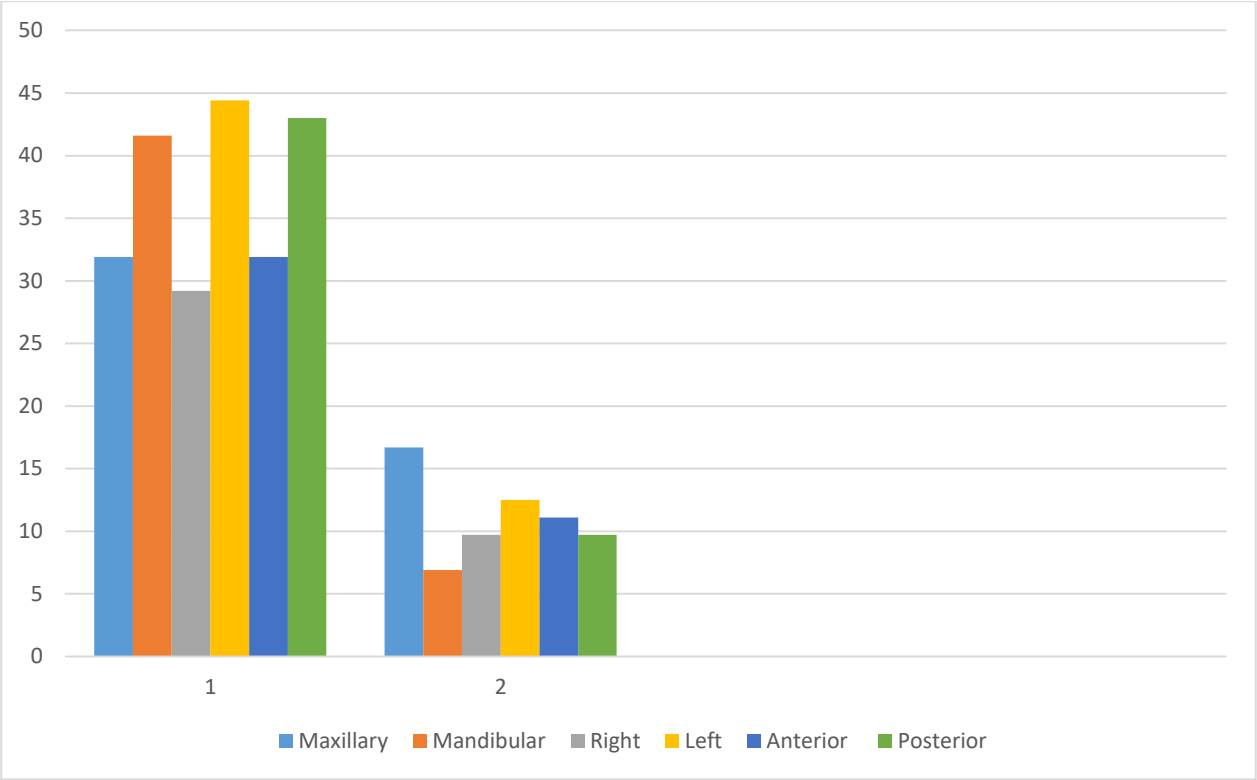


Fig. 14. Frequency of gravity according to the anatomical part, the side and the position of teeth (N= 72).

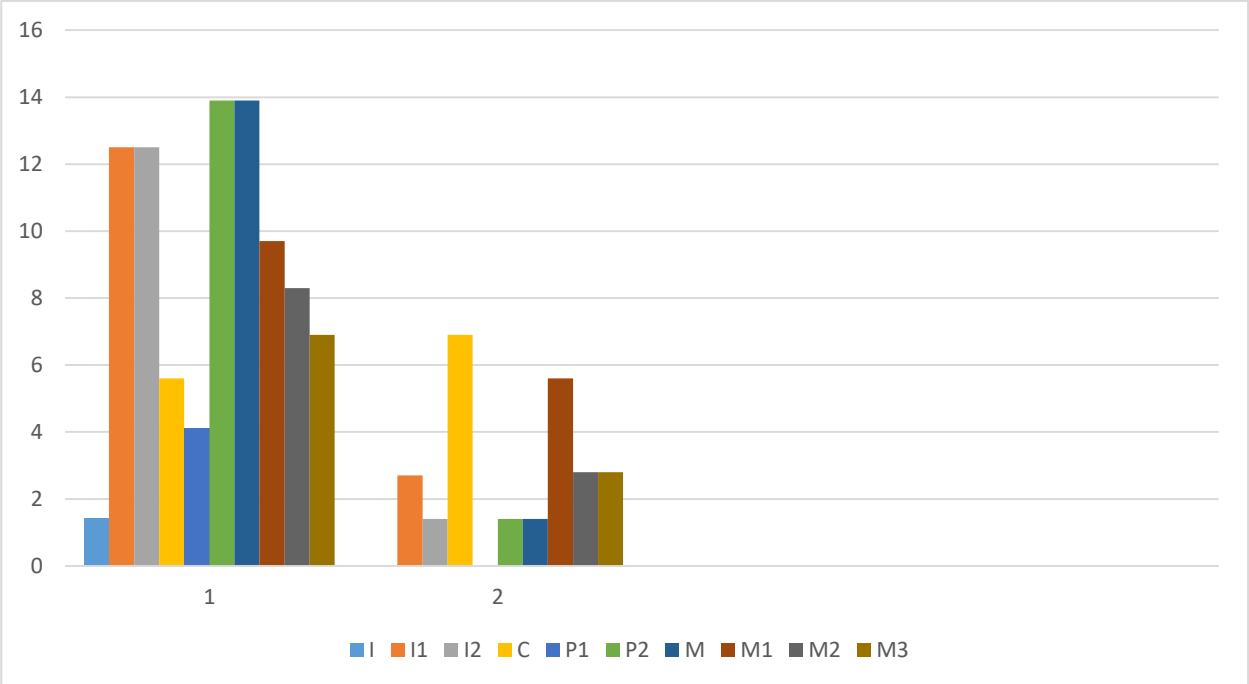


Fig. 15. Frequency of gravity according to the type of teeth (N= 72).

Of 72 teeth affected, 54 teeth were degree 1-type (75%, N= 72) and 18 teeth were degree 2-type (25%, N= 72). Chi-square test showed a different incidence between type 1 and 2 in the sample examined (χ^2= 20.06; d.f.= 1; p= 0.0000).

CARIES

Caries were present in 30/351 teeth (8.6%). Multiple caries were recognized in 3.3% of cases (1/30): three caries type 3 were present on M3. The following table details the results obtained (Table 14, 15):

Maxillary		Mandibular		ND		Right		Left		ND		Anterior		Posterior		ND	
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
14	46.7	13	43.3	3	10	14	46.7	11	36.7	5	16.7	2	6.7	25	83.3	3	10

Table 14. Frequency of caries according to the anatomical part (maxillary/mandibular), the side (left/right) and the position (anterior/posterior) (N= 30; ND= undetermined).

I2		C		P1		P2		M1		M2		M3	
N	%	N	%	N	%	N	%	N	%	N	%	N	%
1	3.3	1	3.3	1	3.3	5	16.7	5	16.7	7	23.3	7	23.3

Table 15. Frequency of caries according to the type of teeth (N= 30).

Different type of gravity were recorded according to Belcastro *et alii* 2004 (cfr. 4.2.3) (Table 16, Fig. 16-19):

	Type of caries									
	1	2	3	4	5	8	15	16	17	28
N	7	1	5	4	2	1	3	1	5	1
%	23.3	3.3	16.7	13.3	6.7	3.3	10	3.3	16.7	3.3

Table 16. Frequency of caries according to the type (N= 30).

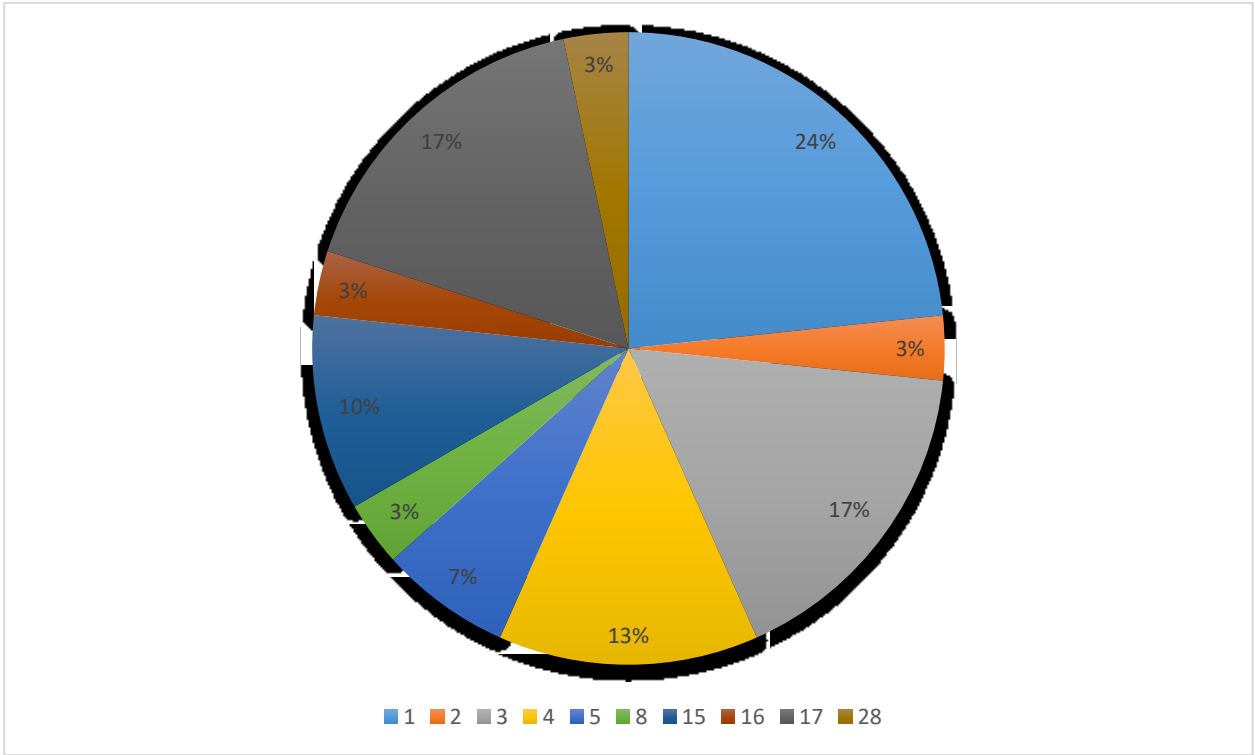


Fig. 16. Frequency of caries according to the type (N= 30).

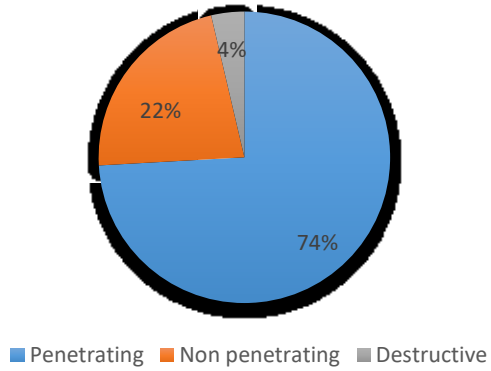


Fig. 17. On the left, frequency of caries according to the type (N= 30).

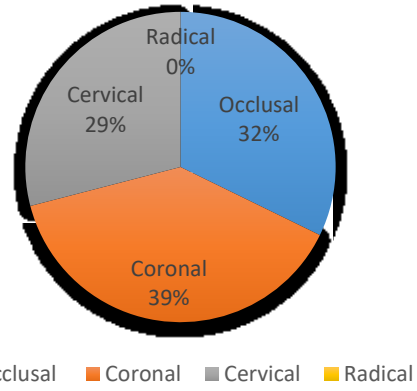


Fig. 18. On the right, frequency of caries according to the position (N= 30).

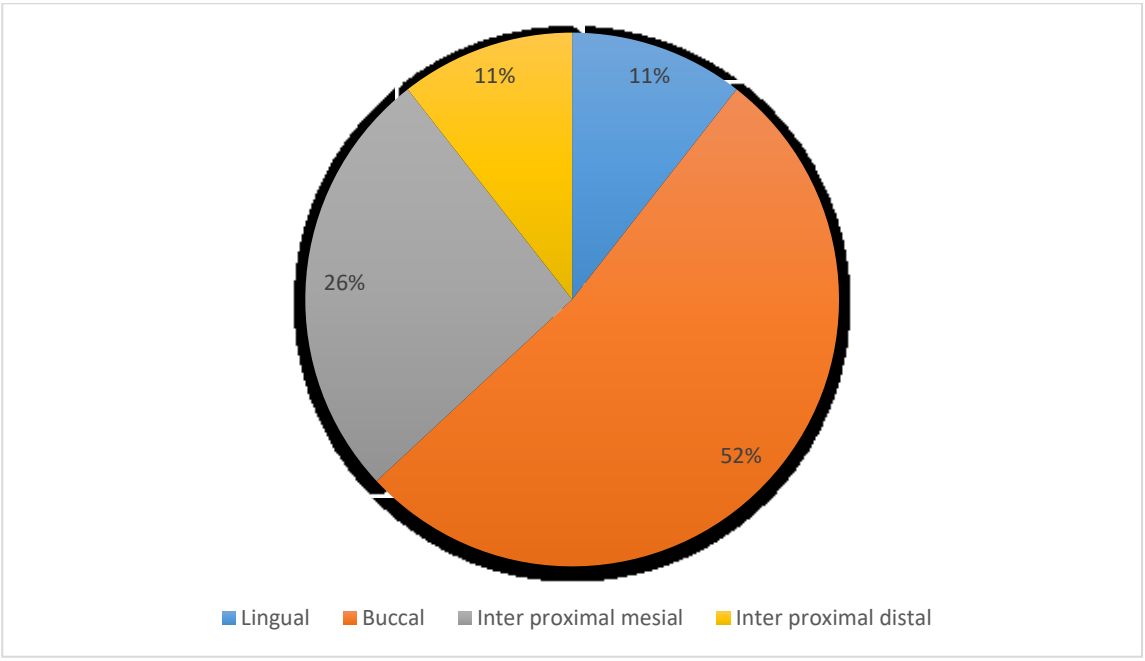


Fig. 19. Frequency of caries according to the side (N= 30).

Concerning gravity, three degrees were recorded following Belcastro *et alii* (2004): penetrating (type 1, 2, 3, 4, 5, 8), non penetrating (type 15, 16, 17) and destructive caries (28). Penetrating caries were present on 20 teeth (66.7%), non penetrating caries were present on 9 teeth (30%). Only one destructive caries (3.3%) was recognized. In order to compare data using statistical tests, these sub-samples were grouped in two clusters, according to the gravity:

1. penetrating caries and destructive caries (N= 21 teeth)
2. non penetrating caries (N= 9 teeth)

Chi-square test showed a statistical difference: χ^2=5.01; d.f.= 1; p= 0.0251.

LINEAR ENAMEL HYPOPLASIA

The hypoplastic defect was recordable on 96 teeth/351 (27.4%). The following tables show the distribution according to anatomical part (maxilla/mandibula), side (right/left), anatomical position (anterior/posterior) (Table 17, 18; Fig. 20, 21):

Maxillary		Mandibular		ND		Right		Left		ND		Anterior		Posterior		ND	
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
52	54.2	44	45.8	0	0	33	34.4	58	60.4	5	5.2	37	38.5	59	61.5	0	0

Table 17. Frequency of hypoplasia according to the anatomical part (maxillary/mandibular), the side (right/left) and the position (anterior/posterior) (N= 96; ND= undetermined).

I1		I2		C		P1		P2		M1		M2		M3	
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
8	8.3	6	6.25	23	24	8	8.3	17	17.7	11	11.5	11	11.5	12	12.5

Table 18. Frequency of hypoplasia according to the type of teeth (N= 96).

Two types of hypoplasia were detected according to the Fédération Dentaire Internationale method (1959, 1992) standard: type 3 (PITS) and 4 (LEH). LEH was recordable on 93 teeth (96.9%) and pits on 3 teeth (3.1%). It was not possible to use statistical test to compare type 3 and 4 for the discrepancy between the two types (Fig. 13).

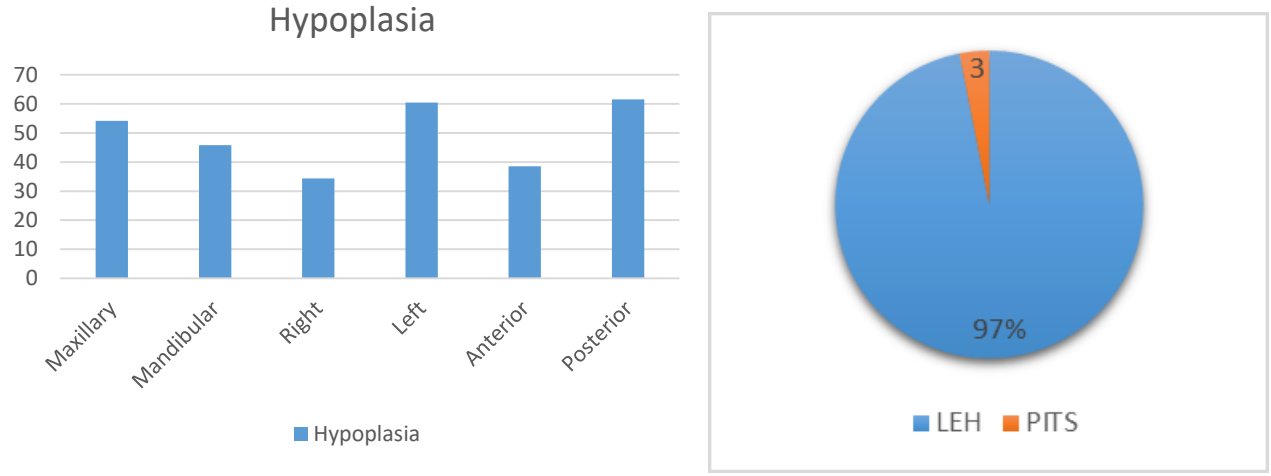


Fig. 20. Frequency of hypoplasia according to the anatomical part (maxillary/mandibular), the side (right/left) and the position (anterior/posterior) (N= 96).
Fig. 21. Frequency of hypoplasia according to the type of defect (N= 96).

In order to define the age of onset of hypoplastic defect, Goodmann and Rose (1980) equation was calculated on mandibular canines and maxillary I1. The age of onset of hypoplastic defect was 3.8 ± 1.3 years.

4.3.2 Microwear analysis

Of 268 markers observed by SEM, 222 were scratches (82.8%) and 46 were pits (17.2%) (Table 19). According to the type of teeth, 189 scratches (89.2%, N= 222) were on molars and 33 scratches (14.9%, N= 222) on canines; 44 pits (8.7%, N= 46) were on molars and 2 were on canines (4.4%, N= 46). With reference to anatomical part, 122 scratches were mandibular (55%, N= 222) and 100 were maxillary (45.1%, N= 222); 9 pits were mandibular (19.6%, N= 46) and 37 (80.4%, N= 46) were maxillary. According to the side, 124 were left scratches (55.9%; N=222), 67 were right scratches (30.2%, N= 222), 31 were ND for side (20%, N= 222); 9 were left pits (20%, N= 46), 35 were right pits (76.1%, N= 46) and 2 were ND for side (4.4%, N= 46) (Fig. 22, 23).

Sample ID	Site	Teeth	I	Side	Sex	Age class	Scratches	Pits
1	GdM	M2i	1	L	ND	adult	55	7
2	GdM	M1s	1	R	ND	adult	4	3
3	GdM	M2i	1	L	ND	adult	50	2
4	GdM	M2s	1	R	ND	adult	42	23
10	GdM	Cs	1	L	ND	adult	2	0
11	GdM	Cs	1	ND	ND	adult	31	2
22	GdM	M1s	1	R	ND	adult	2	9
23	GdM	M1s	1	R	ND	adult	19	0
24	GdM	M2i	1	L	ND	adult	17	0
						SUB TOTAL	222	46
						TOTAL	268	

Table 19. Scratches and pits in the total sample (i: inferior; s: superior; R: right; L: left; ND: non determinable)

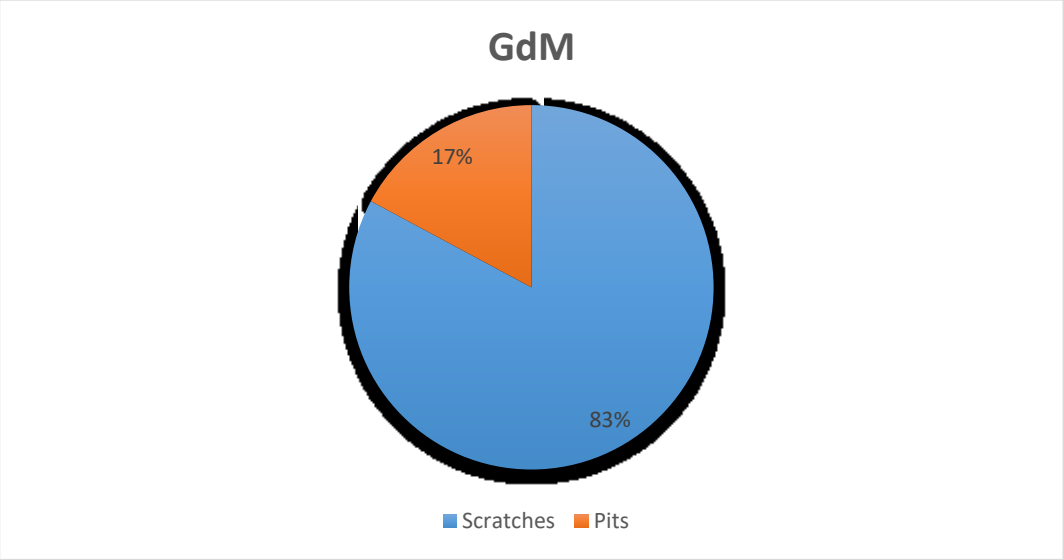


Fig. 22. The frequency of the scratches and pits in the total sample (N= 268).

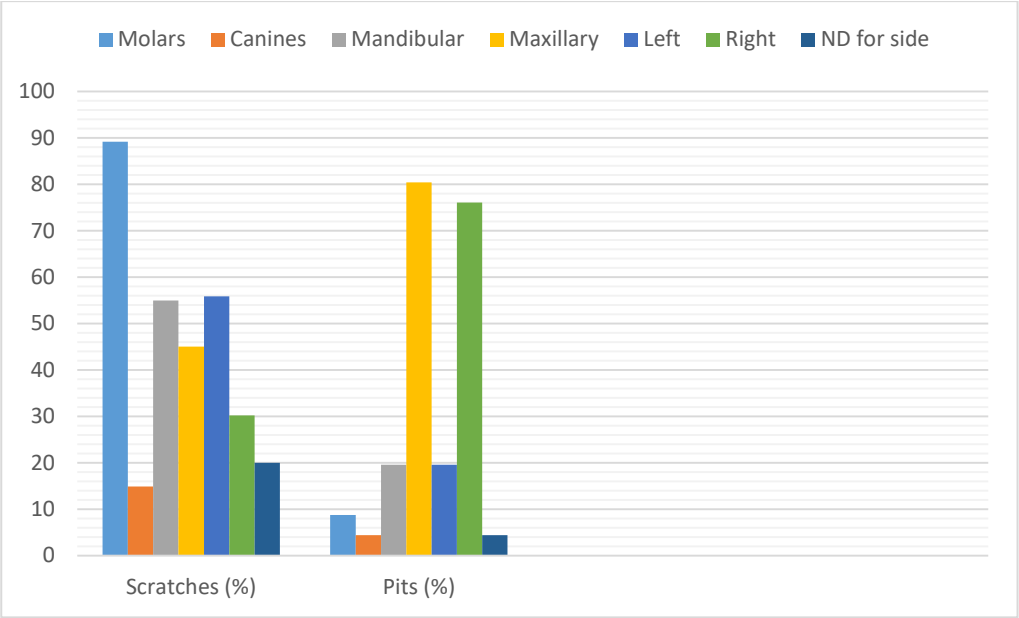


Fig. 23. The frequency of the scratches and pits according to the type of teeth, the anatomical part and the side (N scratches= 222; N pits= 46).

The following table shows length, width and frequency mean values of scratches and pits recorded in GdM sample (Table 20):

	GdM	
	Mean	**SD**
Number of pits	5.11	7.42
Number of scratches	24.78	20.80
Number of pits (%)	20.14	28.09
Number of scratches (%)	79.86	28.09
Pits length (µm)	6.49	2.47
Pits width (µm)	2.76	1.14
Scratches length (µm)	31.65	6.51
Scratches width (µm)	1.72	1.22

Table 20. Microwear evaluation: length, width and frequency of scratches and pits in Grotta della Monaca.

The microanalyses of chemical elements on dental enamel shows that Ca and P were present in the all the specimens (100%), Fe was present in 4 specimens (44.4%, N= 9), Cl was present in one specimen (11.1%, N= 9) (Table 21).

Sample ID	Ca	P	Fe	Cl
1	P	P	P	0
2	31.97	12.2	3.57	0
3	15.6	14.82	0	0.37
4	34.64	16.24	0	0
10	27.93	15	1.61	0
11	35.12	14.28	3.86	0
22	35.53	16.04	0	0
23	33.55	14.84	0	0
24	23.72	11.84	4.86	0
Mean	29.76	14.41	1.74	0.04
SD	7.02	1.61	2.06	0.12

Table 21. Chemical elements percentages in the total sample (N= 9). The software did not elaborate percentages of chemical components in sample 1, but the chemical elements that were present by the spectrum were generically indicated as "P" (present).

According to elaborations performed by a computerized microanalysis system (Link ISIS) for each specimen, the chemical element's ratios in each specimen are shown in figure 24. The figure 25 shows the percentages of teeth *per* each chemical element.

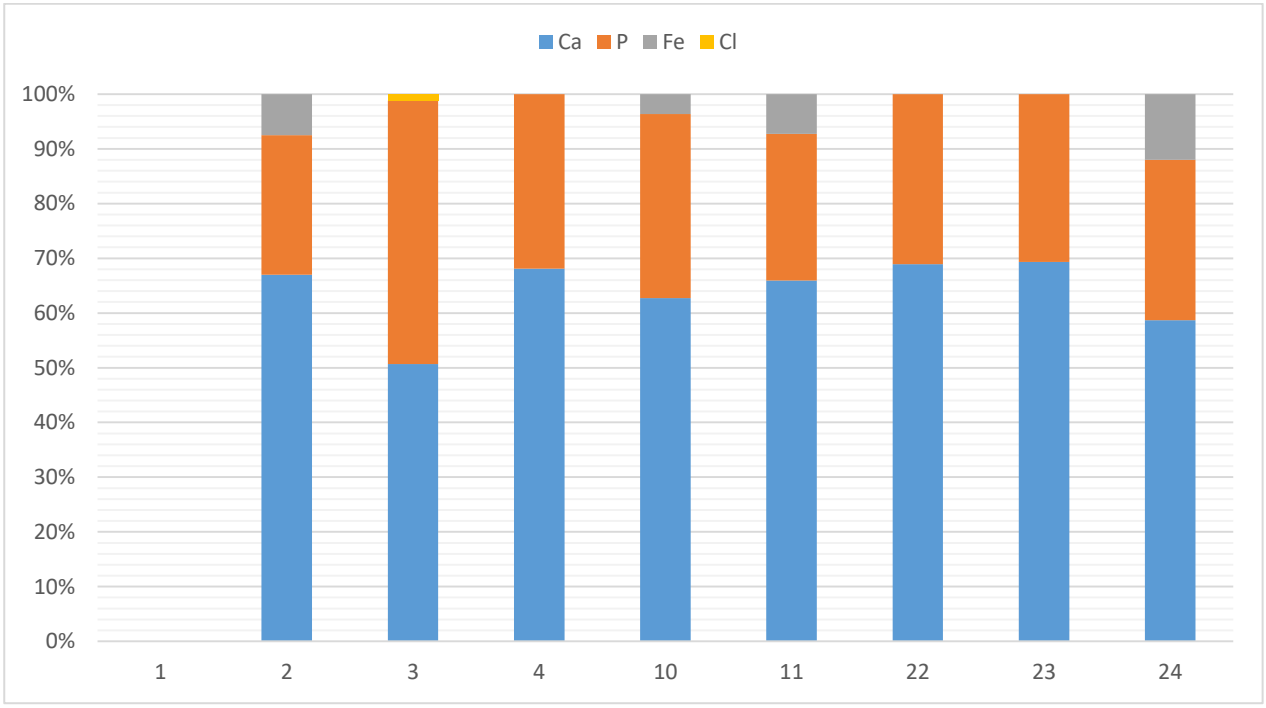


Fig. 24. Chemical element's ratios in each sample.

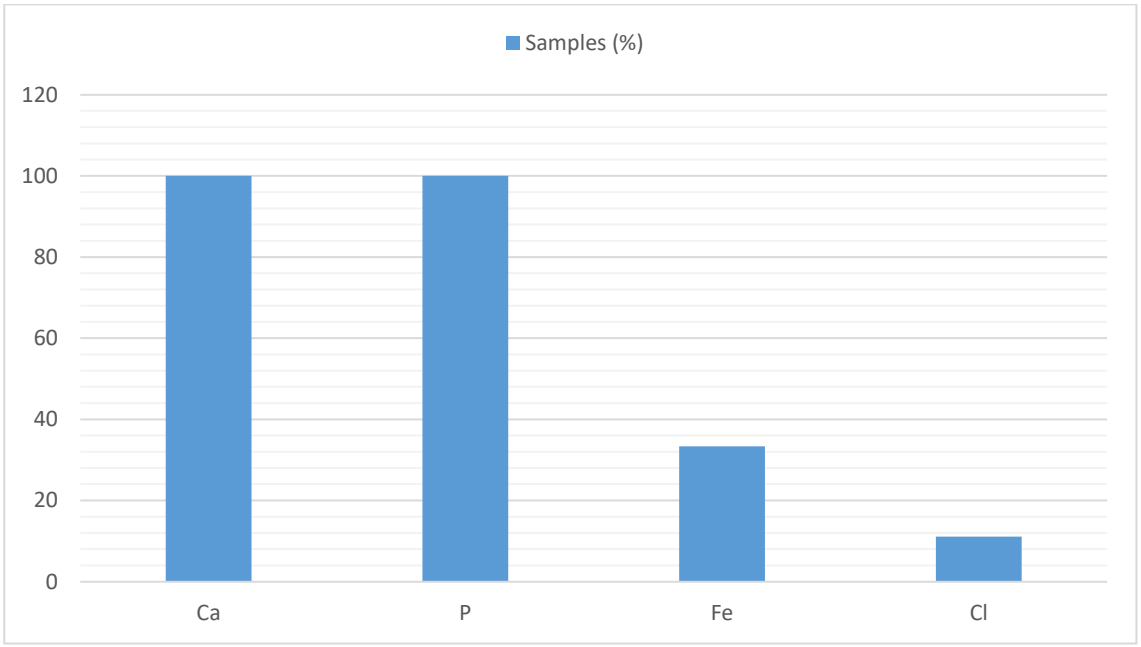


Fig. 25. Percentages of teeth *per* each chemical element (N= 9).

4.3.3 Stable isotopes analysis

With regard to chemical investigations, the table below shows the specimens selected for pretreatment and the biological profile of individuals to which they belong (Table 22):

Sample ID	Species	Site	Archaeological contest	Bone	Anatomical part	Sex	Age class
GdM02	human	GdM	GdM/CTv/m5v/S/tg 1,2,3	Cranial bone	occipital bone	F	young adult
GdM04	human	GdM	GdM/CTv/m5v/S/tg1	Cranial bone	occipital bone	F	young adult
GdM06	human	GdM	GdM/CT dx-fv/Z3	Radius	diaphysis	ND	adult
GdM09	human	GdM	GdM/CT dx-fv/Z4	3° Metatarsal	diaphysis	ND	adult
GdM10	human	GdM	GdM/CT dx-fv/Z6	Metatarsal phalanx	diaphysis	ND	adult
GdM11	human	GdM	GdM/CT dx-fv/Z6	3° Metatarsal	diaphysis	ND	adult
GdM12	*Sus scrofa*	GdM	GdM/Pfs	Humerus	diaphysis	ND	young adult

Table 22. The biological profile of individuals (F= female; R= right; L= left; ND= non determinable).

Isotopes investigations were carried out on 7 bone specimens from Grotta della Monaca. Of them, 6 were human skeletal remains and 1 faunal remain (*Sus scrofa*). During the pretreatment the faunal specimen (ID: GdM 12) was lost, probably because its poor state of preservation. Therefore, stable isotopes analyses were carried out on human samples exclusively. The table below shows results obtained (Table 23).

Lab ID	S-ID	Species	$\delta^{13}C_{V\text{-}PDB}$ (‰)	SD	$\delta^{15}N$ (‰)	SD	C (TCD)	SD	N_{AIR} (TDC)	SD	C:N	SD	% yield
28697	GdM09	Human	-19.67	0.3	9.46	0.45	45.48	0.01	16.33	0.01	3.249	0.01	7.3
28698	GdM02	Human	-19.61	0.3	8.98	0.45	45.08	0.01	16.27	0.01	3.232	0.01	7.1
28699	GdM04	Human	-20.31	0.14	7.69	0.23	43.66	0.007	16.09	0.007	3.166	0.007	5.4
28700	GdM10	Human	-19.81	0.3	8.75	0.45	46.91	0.01	17.34	0.01	3.156	0.01	7.2
28701	GdM11	Human	-19.87	0.3	7.37	0.45	44.31	0.01	15.97	0.01	3.237	0.01	6.5
28702	GdM06	Human	-19.93	0.3	7.01	0.45	43.05	0.01	15.44	0.01	3.253	0.01	2.2

Table 23. Carbon ($\delta 1^3C$) and nitrogen ($\delta^{15}N$) isotope ratios in bone collagen extracts from human specimens.

All The extracts were considered well-preserved according to collagen yield > 1% and C/N (between 2.9-3.6), as detailed by Table 23. The graphic below shows the scatterplot of human stable isotope ratios (Fig. 26).

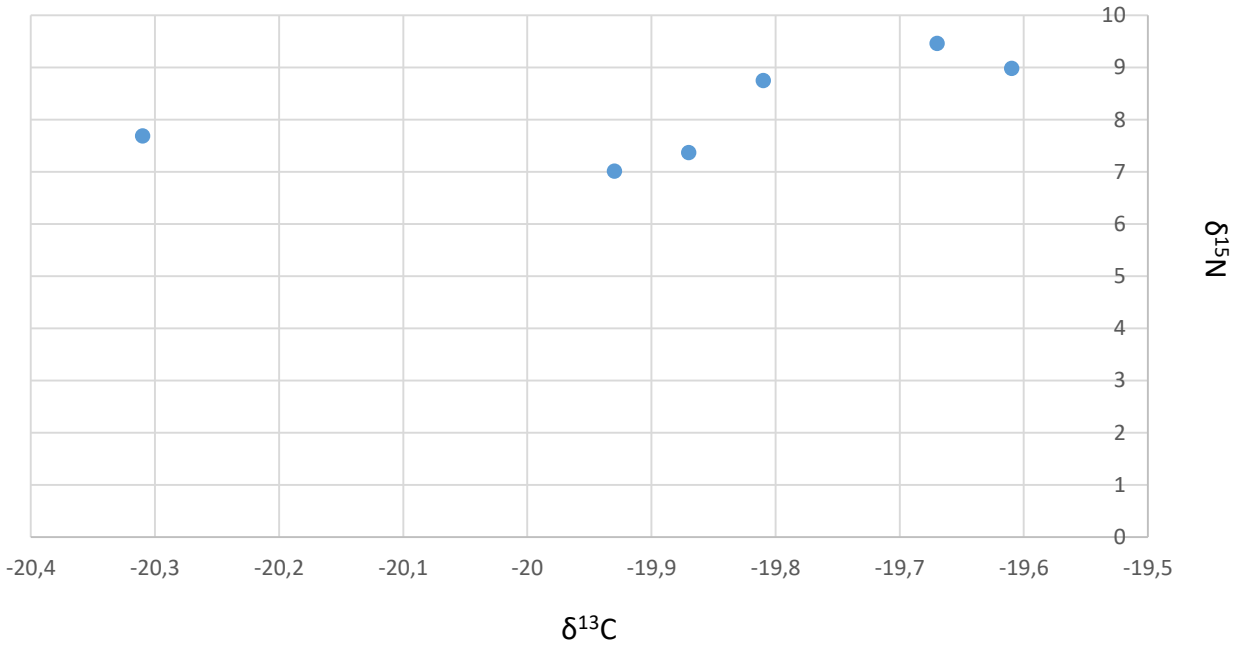


Fig. 26. Human $\delta^{13}C$ (x) and $\delta^{15}N$ (y) ratios scatterplot (N= 6).

The carbon and nitrogen analyses on the human skeletal remains resulted in bone collagen $\delta^{13}C$ values from -20.3‰ to -19.6‰ (-19.9‰ ±0.3‰) and $\delta^{15}N$ values from 7.0‰ to 9.5‰ (8.2‰ ±1‰). As shown by the scatterplot, the stable isotopes ratios of specimen GdM04 slightly deviated from the average, but not significantly (U= 12.50, p= 0.6443 for $\delta^{13}C$ and U= 14.50, p= 0.9264 for $\delta^{15}N$). Considering the $\delta^{13}C$ and $\delta^{15}N$ mean values obtained, it was possible to assert that human feeding of Grotta della Monaca people was characterized by vegetal proteins intake, based on C_3 plants (DeNiro and Epstein 1978, 1981). Although there were not animal bones in the sample, human $\delta^{15}N$ ratios were compatible with a moderate terrestrial faunal consumption, mostly herbivores (meat and/or dairy products), and did not suggest a regular marine/freshwater protein consumption.

4.4 Discussion

Grotta della Monaca is a karsic cave places in North-western Calabria (Sant'Agata di Esaro - CS). It opens at 600 m above sea level, in the Upper Valley of the Esaro River. During the Middle Bronze Age, between 1700 and 1400 BC, the cave was used as burial ground: skeletal remains were found by archaeologists inside cracks and niches of the rock, in the most hypogeal sectors of the cave: "Sala dei Pipistrelli" and the "Cuniculi terminali" (Larocca, 2005; Arena *et alii*, 2014) (cfr. Chapter 1).

In order to reconstruct ancient food habits of individuals inhumed in Grotta della Monaca, three different approaches were used in this study: the macroscopic examination of nutritional markers of teeth, the microwear analysis of tooth surfaces and the stable isotopes analysis on bone collagen extracts.

The examination of macroscopic markers diet-related was performed on 351 permanent teeth (from the mainly burial areas) belonged to 19 individuals. The analyses showed a higher frequency of enamel *wear* (61.3%) (Fig. 27). According to the gravity, intermediate and slight wear were more present than heavy wear, but statistical differences in the incidence of gravity-types did not occur.

This result allowed us to assume the same nutritional behavior of GdM individuals: the high frequency of dental wear indicated a diet consisting of abrasive foods that required vigorous mastication, as suggested by analogous situations for ancient populations (Molnar, 1972; Smith, 1984; Kaifu, 2000; Bermúdez de Castro *et alii*, 2003; Kaifu, Kasai, Townsend & Richards, 2003;). As it is known, agricultural populations often demonstrated a high dental wear frequency because of the consumption of abrasive textured grains and cereals. In addition, the millstones used to grind the grains could release abrasives into the food and promote the increasing of tooth wear (Larsen, 1997). The diet of Middle Bronze Age Southern communities was mainly based on agricultural products, particularly cereals, such as wheat, barley and legumes (Fiorentino, 2004; Tafuri, 2009; Bietti Sestieri, 2011). The comparison with the Northern populations of Italy demonstrates that agricultural products were manly consumed during this Age: in fact, analyses performed on 357 teeth belonged to the site of Ballabio (Lecco, Lombardy) recorded 96.6% of tooth wear in the total sample, that was related to a mostly vegetarian diet (Masotti *et alii*, 2017).

Calculus was present in lower percentages than wear (20.5%) with manly slight deposit (type 1) than moderate deposits (type 2) (Fig. 28). Different studies connected this dental marker with the consumption of high-protein foods such as poor oral hygiene (Sheie *et alii*, 1989; Hillson, 1996). The low incidence of calculus and the high incidence of wear confirm the general trend of the Bronze Age communities that consumed more vegetables than animal proteins (Tafuri *et alii*, 2009; Varalli *et alii*, 2016a-b). Furthermore, this result reflected the general trend of subsistence strategies that was mostly based on agriculture in this Age (Bietti Sestieri, 2011). In fact, archaeological studies pointed out that the consumption of meat, coming mainly from animal husbandry, spread in the Late Bronze Age (De Grossi Mazzorin, 2013; Maini and Curci, 2013). In particular, at Broglio di Trebisacce (Calabria) sheep and goat increased of 42% during the Late Bronze Age compared to the Ancient and Middle Bronze Age (Tagliacozzo, 1994). Furthermore, wild species, that were absent during the first phases of the Bronze Age, increased at the end of this Age (De Grossi Mazzorin, 1987; Tagliacozzo, 1994; De Grossi Mazzorin *et alii*, 2004). Coppa Nevigata faunal sample (Apulia) showed higher percentages of sheep and goat than cattle with the intensification during the Middle-Late Bronze Age: cattle increased from 30.3% to 18.6%, sheep and goat intensified from 36.6% to 43.8% (Bökönyi and Siracusano, 1987; Bietti Sestieri, 2011). However, it is important to remark that the low incidence of calculus in Grotta della Monaca sample could depend on the poor state of preservation of teeth that might have affected the results: in fact, the calculus presence in the coeval North Italian sites - such as Ballabio (79.1%, N=354) - and other Bronze Age Mediterranean sites - such as Castellets Cave (26.4%, N=227), Vinalopó Valley (40.5%, N=446) and Cova dels Blaus (17.3%, N=110) in Spanish - was higher than at Grotta della Monaca (Masotti *et alii*, in press). *Caries* are the result of fermentation of dietary carbohydrates (especially sugars) by the oral bacteria in dental plaque (Hillson, 1986; Larsen, 1997; Caselitz, 1998; Hillson, 2001). This alteration of tooth enamel depends on a complex multifactorial disease caused by the interaction between the presence of cariogenic microorganisms and a suitable oral environment (Patterson, 1984; Powell, 1985). In the sample of Grotta della Monaca the presence of caries was very low (8.5%) in comparison to the other alterations of tooth enamel (wear and calculus) (Fig. 29). They were mostly penetrating than non-penetrating. This result demonstrated that, although the low frequency, there were more frequently the most severe types (penetrating caries) in the sample examined. The lower percentages of caries would seem to contrast with the general trend recorded for the Bronze Age communities: the high consumption of grains and legumes should increase the onset of caries. As in other European sites (Lukacs, 1992), an increase

of carious lesions from the Neolithic to the Iron Age was observed also in Italy in relation to the consumption of plant foods with new food preparation techniques linked with a more sedentary lifestyle (Masotti *et alii*, in press). Studies conducted on the Central-Northern Bronze Age Italian populations indicated higher frequency of caries: Grotta dello Scoglietto (Grosseto, Central Italy) (11.2%; N=178), Arano di Cellore (Verona, North Italy) (13.4%; N=1388), Grotta Vecchi (Latina, Central Italy) (18%; N=128), Ballabio (Lecco, North Italy) (16.7%; N= 354). (Varalli *et alii*, 2016b; Masotti *et alii*, in press). However, a research performed on several sites in Southern Italy showed an overall low frequency of tooth caries and a clear degree of variation between sites depending on the resources present in the different environments, in particular on their sugary content (Minozzi *et alii*, 1994). General results indicated a very low frequency of caries in Southern Bronze Age communities: Marcita (Trapani, South Italy) (3.3%; N= 998), Toppo Daguzzo (Potenza, South Italy) (5.9%; N=204), Madonna di Loreto (Bari, South Italy) (5.4%; N= 572) (Minozzi *et alii*, 1994). These values were lower or similar to results obtained in some Mediterranean Bronze Age environments, in particular in Spanish sites of Vinalopó Valley (8.9%, N=446) and Cova dels Blaus (5.4%, N=110) (Masotti *et alii*, in press). In addition, it is important to point out that the wear affects the preservation of tooth caries: there is a negative correlation between caries and the degree of wear (Powell, 1985; Maat and Vand der Velt, 1988). In case of high enamel wear rates, such as Grotta della Monaca, tooth caries may have been "erased": wear gradually smooths the occlusal cusps, eliminating fissures and pits on the crown and reducing the presence of cariogenic substances on the tooth.

The same reason could have influenced the preservation of *chipping*. Chipping is an *ante mortem* irregular crack involving the enamel or enamel and dentin and provides information about both masticatory and non-masticatory activities (Milner and Larsen, 1991). In this study the complete absence of chipping may have several causes: 1) the high frequency of wear; 2) the consumption of foods not particularly hard (then cooked); 3) the absence of extra masticatory use of teeth (in relation to specific work activities). Given the high incidence of wear, we support the first hypothesis.

Dental enamel *hypoplasia* was present with a low frequency (24.4%) in the Grotta della Monaca sample, more often as linear grooves (LEH) than as pits (Fig. 30 a-b). This feature is usually considered to be a non-specific indicator of stress (Nikiforuk and Fraser, 1981) with different origin, such as nutritional deficiencies, infections, metabolic disorders and diseases (Brothwell, 1963; El-Najjar and Mc Williams, 1978; Rose, 1977; Skinner and Goodman, 1992). Our findings demonstrated that the stress episodes causing them occurred between 3 and 4 years of age, analogously to other studies that indicated the occurrence of these defects during the weaning because the transition to an adult diet of poor quality, preferably represented by carbohydrates (Varalli *et alii*, 2016b). Preliminary analyses, performed on individuals of Grotta della Monaca (included in the current sample), indicated the same result and allowed to assume a precarious health state for an inadequate nutritional supply during early childhood (Scattarella *et alii*, 2005). From the comparisons of Grotta della Monaca (Middle Bronze Age) with other Bronze Age Italian sites - placed in different environments and in different periods (Ballabio - Lecco, Lombardy, from Ancient Bronze Age and Castello del Tartaro - Verona, Veneto, from Late Bronze Age) - a different incidence of hypoplastic defects (p= 0.0000) resulted with the highest frequency at Castello del Tartaro (60%, N= 584) vs Ballabio (48%, N= 255) and Grotta della Monaca (33%, N= 310). On the base of this tooth indicator, the Grotta della Monaca population seemed to have enjoyed a better state of health than Ballabio and Castello del Tartaro. This result could depend on

environmental factors: Castello del Tartaro was a *Terramara* located in a marshy flat land that could improve the spread of infections and parasitosis. The onset of hypoplasia in the third year of life, probably during the weaning, is confirmed in all the three samples. Moreover, the comparison with other Mediterranean Bronze Age sites shows that the frequencies of LEH in the Grotta della Monaca sample were slightly higher than in the Spanish samples from Castellets Cave (22.9%, N=227), Vinalopó Valley (23.3%, N=446) and Cova dels Blaus (20.9%, N=110) (Masotti *et alii*, in press). These data pointed out that food habits as well as environmental factors could have an important influence on the incidence of hypoplasia (Arena *et alii*, 2016).

The analysis of microwear confirms data of macroscopic examination, suggesting that the prevalence of scratches (82.8%) on pits (17.2%) was due to a mostly vegetarian diet. In fact, these two different patterns are representative of two distinct food habits: scratches indicate a mainly vegetarian diet (including highly abrasive particles); pits are specific of a higher content of "hard food" (such as seeds, nuts, etc.) and indicate the chewing of bones deriving by the ingesting of meat or marrow (Romero *et alii*, 2013). No paleobotanical data (useful to detect the plants consumed) have been recorded for Grotta della Monaca, but the documentation of the next site of Broglio di Trebisacce (Sibari, Calabria) showed the presence of einkorn wheat (*Triticum monococcum*), spelled (*Triticum dicoccum*), wheat (*Triticum aestivum*, *compactum*, *durum*), barley (*Hordeum distichum* and *exasticum*), faba beans (*Vicia faba minor)*, lentils (*Lens culinaris*), grass peas (*Lathyrus sativa cicera*) and scarcity of millet, rye and oats (Fiorentino *et alii*, 2004). Paleobotanical and environmental analyses at Broglio di Trebisacce proved intensive anthropogenic use of the landscape: the profuse existence of olive trees indicated a highly modified landscape for the spread of agriculture, that was the mainly subsistence strategy (Kleibrink, 1996 -1997; Vanzetti, 2000). Given the proximity between sites (located in the Northern area of Cosenza's district), we can assume that these observations can also be valid for Grotta della Monaca.

Although the diet was mostly vegetarian, the presence of pits demonstrated the secondary consumption of animal products. Also in this case, no faunal data were recorded on Grotta della Monaca. Anyway, the archaeological record of Broglio di Trebisacce indicated mostly goats and sheep among domesticated species. Wild species were not consumed during the Ancient and Middle Bronze Age of the Southern communities because of the hunting was generally poor: wild species were absent at Broglio di Trebisacce, but an increase of them was recorded in the next Late Bronze Age (De Grossi Mazzorin, 1987; Tagliacozzo 1994; De Grossi Mazzorin *et alii* 2004).

The stable isotopes analyses performed on Grotta della Monaca confirme this result. Findings obtained are compatible with a terrestrial diet, based on vegetal proteins (C_3 plants related), since the carbon isotope values of the sample were about -19.9‰ ±0.3‰ and nitrogen values ranged on a mean value of 8.2‰ ±1‰ (according to De Niro and Epstein 1978, 1981; Schoeninger and De Niro, 1984). Even though all examined individuals were buried close to the Esaro River and not far from the Tyrrhenian Coast (about 20 km), nitrogen concentration did not indicate marine/freshwater food consumption. Although there were not animal bones in the examined sample, human $\delta^{15}N$ ratios were compatible with a limited amount of terrestrial animal products, mostly derived from herbivores (meat and/or dairy products). Stable isotopes ratios of specimen GdM04 slightly deviated in $\delta^{13}C$ rates, from the average. This result could indicate a more vegetarian intake for this individual, a young adult, probably a female, found in the so-called "Vestibolo of m5", as well as the GdM02 individual who, however, did not differ from the average. No good graves - which may indicate a different social status - were found directly associated with this alleged woman. The presence of a spindle-whorl (related to the spinning work) has been recorded in the burial area and

was compatible with a female good grave. Anyway, GdM04 was not the only female found in the same grave: the "Vestibolo di m5" was an area of the cave with several inhumed individuals in poor state of preservation, found as commingled skeletal remains by archaeologists (Arena *et alii*, 2014; Arena and Gualdi-Russo, 2014). Paleopathological examination performed on the woman GdM04 as well as the other individuals inhumed in the same burial area, showing the presence of *cribra orbitalia* (Fig. 31), can support the hypothesis of a diet low in animal intake (Arena *et alii*, in press). As it is known from literature (Stuart-Macadam 1982, 1985, 1987a-c, 1991, 1992a-b; Stuart-Macadam and Kent, 1992; Miquel-Feuch *et alii*, 1999a-b; Rothschild, 2000, 2012; Rothschild *et alii*, 2004; Djuric *et alii,* 2008; Walker *et alii*, 2009; Oxenham and Cavil, 2010; etc.), cribra - among other causes - may also be associated with a diet low in iron and vitamins such as B_{12} and folic acid.

In conclusion, different nutritional markers and, in some cases, the presence of non-specific indicators of stress (*cribra orbitalia*) suggested an association with a diet lower in animal proteins and higher in vegetables. It is likely that the weaning was preferably represented by carbohydrates during the Bronze Age (Varalli *et alii*, 2016), as demonstrated by the high rate of tooth wear recorded on deciduous teeth of Grotta della Monaca (Arena *et alii*, 2016). The passage to poor nutrient foods did not adequately meet the nutritional demands of infants and could result in iron and vitamins deficiency, causing the appearance of hypoplasia.

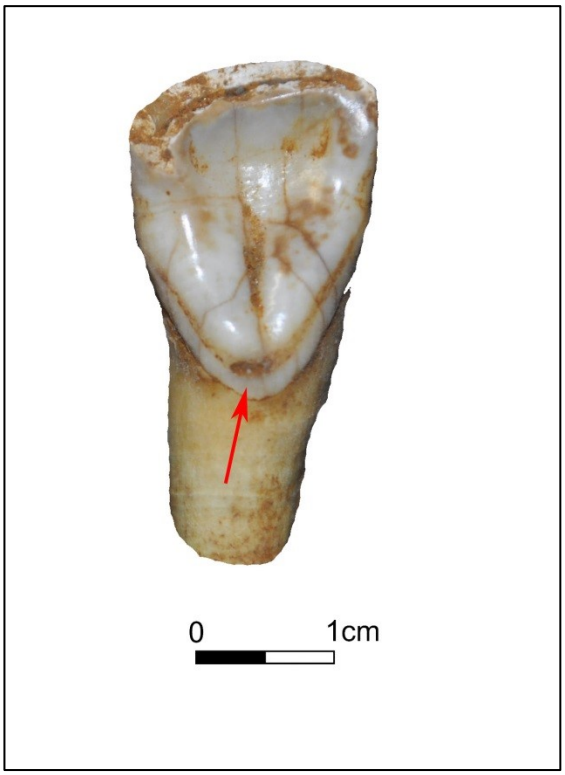


Fig. 27. On the left, heavy dental wear on a molar.
Fig. 28. On the right, slight calculus deposits.

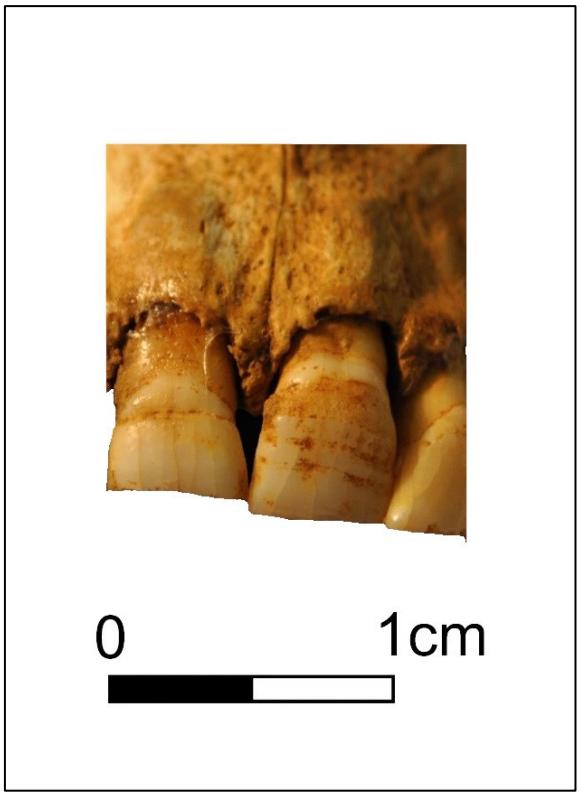


Fig. 29. On the left, destructive caries on a P2.
Fig. 30a. On the right, enamel hypoplasia as LEH.

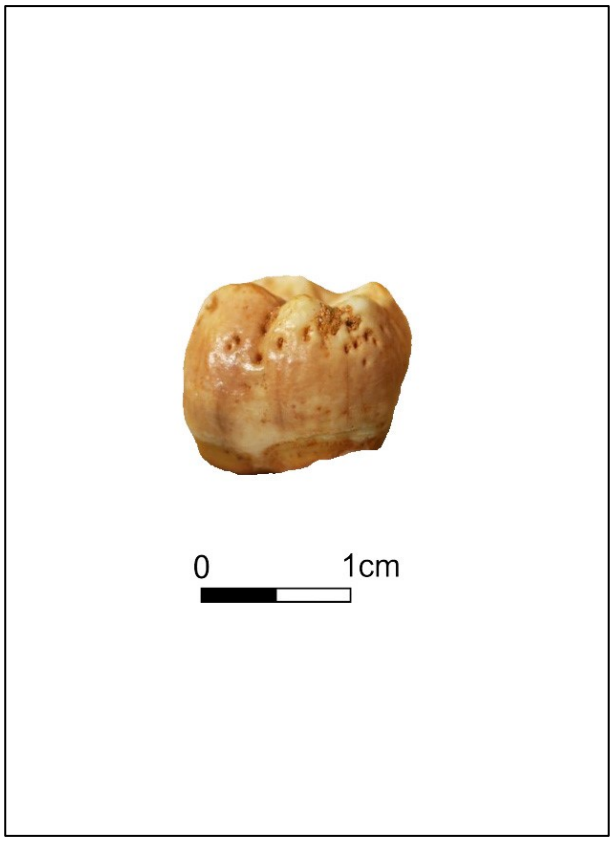


Fig. 30b. On the left, enamel hypoplasia as PITS.
Fig. 31. On the right, cribra orbitalia (individual GdM04).

CHAPTER 5: CONCLUSIONS

This study presents an extensive investigation on dietary practices of Bronze Age communities of Southern Italy through different approaches. The selected sites are located in three different environments: inland hilly/mountain sites of Calabria, inland hilly sites of Basilicata, inland plan of Apulia. The applied methods highlighted different aspects of the diet. In particular, stable isotopes analyses have found indications about the type of protein consumed during the life, specifying if they derived from vegetal or animal products, terrestrial or marine/freshwater foods. Microscopic analyses of microwear (scratches and pits) allowed to quantify the amount of the different foods ingested, indicating if the diet was mostly vegetarian or based on the consumption of "hard food" (such as seeds, nuts or bones connected to the chewing of meat and marrow). Finally, the site of Grotta della Monaca (Calabria) has been used as case-study in order to apply not only these methods, but also the analyses of macroscopic patterns diet-related on teeth.

The **stable isotopes** values ($\delta^{13}C$ and $\delta^{15}N$) indicated a diet relied on the consumption of vegetal proteins (C_3 crops-related) derived from agricultural products: cereals and legumes. The animal protein intake (meat or dairy products) was moderate for all the sites examined and slightly higher for the humans of Group 2 (Basilicata). Although some sites are next to the sea or to a river, no evidence of marine or freshwater consumption was recorded in human diet in the examined remains. For example, the community of Grotta della Monaca or Grotta Funeraria - close to Esaro River and Bradano River, respectively - did not show the consumption of marine/freshwater resources. Although social differences (gender-related and age-related) were recorded in the good graves by archaeologists, such complexity did not occur in terms of dietary intake, at least not in a way recordable through nitrogen and carbon analyses, in examined individuals.

The examination of **microwear** revealed a higher incidence of scratches than pits, thereby indicating a diet rich in foods with high fibers content (i. e. agricultural products such as vegetables, cereals, etc.). Despite the isotopic approach, the analyses of microwear pointed out some possible gender-related difference in Ipogeo dei Bronzi population. However, given the small sample size from this site, further analyses of this and other populations will be necessary to investigate this aspect. The comparison between age classes was performed only on Toppo d'Aguzzo human remains because of the poor state of preservation of the other samples that did not allow the estimation of the age at death of individuals. Results pointed out the decrease of microwear frequency with adulthood as a consequence of the increasing wear that smoothed the occlusal surfaces of teeth.

The analyses of **macroscopic patterns diet-related** of Grotta della Monaca tooth specimens showed a high frequency of tooth *wear* and a low occurrence of *calculus* with a significant prevalence of slight deposit. This result confirms a diet mostly vegetarian (based on high fibrous food) and a moderate intake of animal protein (meat and dairy products). *Caries* rate was very low, although the hypothesized vegetarian diet (usually high in carbohydrates) should cause high rate of caries. The poor sugar content of the available resources can justify this unexpected result. In addition, it could depend on the high frequency of wear that affected the preservation of tooth caries, as mentioned by literature. The absence of *chipping* can be explained in the same way. The occurrence of *hypoplasia* appeared to be in analyzed teeth, giving information not only on health status of individuals, but also on their diet of the early years of life. In particular, our results

demonstrated that systemic stresses, such as malnutrition or diseases, occurred between the third and the fourth year of age, on average. Moreover, our data confirmed the possibility of an occurrence of hypoplasia during the weaning because of a transition to an adult diet of poor quality, mostly represented by carbohydrates, as suggested in other Bronze Age communities. The hypothesis of a mainly vegetarian diet was confirmed for Grotta della Monaca community on the basis of stable isotopes analysis on bone collagen extracts and of microwear on dental surfaces.

Our results demonstrated a common behavior in food practices for Southern Italian populations: a mixed diet, relied on the consumption of terrestrial resources and mostly characterized by a vegetal protein intake (C_3 plants-related). The consumption of animal protein (meat or dairy products) was moderate for all the sites. On the basis of the comparison with Bronze Age sites of Mediterranean and Southern Europe it was possible to assume that animal consumption was secondary (compared with agricultural products intake) and depended on domesticated animals that supplied the majority of animal protein. As confirmed by our data, marine/freshwater resources' consumption was minimal during the Bronze Age. The comparison with Neolithic sites - showing a difference in animal feeding (that was lower during the Bronze Age) - supported the hypothesis that intensive widespread cereals' cultivation and consumption occurred during the Bronze Age.

This study was useful to integrate different information by different scientific approaches to assess the feeding behavior of the Southern Italian populations during the Bronze Age, given that the research carried out until now were few. In particular, the sample from Grotta della Monaca provided the possibility for a more detailed study of these populations. In general, the characteristics, that have emerged, allowed to clarify aspects related to livelihood strategies, such as the increasing agriculture that had a strong influence on the life-style of Bronze Age communities. The selection of foods seemed influenced by cultural choices. In particular, the observed lack of marine and freshwater resources consumption mirrored a cultural behavior: it was an "atypical" food choice since the sites are close to the sea or rivers.

Concluding, as pointed out in a recent study (Masotti *et alii*, in press), the Italian Bronze Age was a critical period due to the modification of dietary strategies. The integration of different methodologies may help to clarify the diets of Bronze Age communities, allowing to overcome any limitation. For example, the impact of agriculture on $\delta^{15}N$ human values should be evaluated in the interpretation of stable isotope data as animal manuring and, presumably, use of human waste as fertiliser (Sallares, 2013) with consequent $\delta^{15}N$ enrichment in cereal crops (Fraser *et alii*, 2011). These factors have led to an overestimation of meat/dairy contribution to diet in isotopic analyses carried out on Romano-British populations (Bonsall *et alii*, 2015). In general, as completion of the various methods used, these studies on diet need to be supported by accurate botanical investigations to define uses and practices of soil, that were not often well defined in the Italian Bronze Age contexts. Moreover, sex differences recorded in stable isotopes values can reflect different mobility of male and female individuals rather than diet differences (Muldner, 2013). In this perspective, the dental patterns diet-related are useful to clarify stable isotopes results. At the same time, stable isotopes analyses give information about aspects of diet compromised by the poor state of preservation of dental patterns diet-related (such as calculus or wear). Moreover, stable isotopes outcome integrates results obtained by SEM analyses of microwear that examine only a selected area of tooth surface. Therefore, only the integration of different methods allows us to reconstruct the complexity of the Bronze Age food behaviour.

It is important to remark that the poor state of preservation of skeletal remains and the availability of samples (in particular of faunal remains) have strongly restricted and conditioned our research.

However, to the best of our knowledge, this is the first study carried out on so many Bronze Age sites of Southern Italy. Therefore, the future prospective could be to improve the information potential of this research by a more extensive sampling, expanding the sample sizes, the number of sites and environments. Age- and sex-specific differences in diet and general oral health could possibly be shown if complete skeletons are available in the near future. Similar studies on Bronze Age populations are needed to clarify our findings and to further contribute to the archaeo-anthropological knowledge of past populations in Southern Italy.

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